

Agilent VnmrJ 4.2

Familiarization Guide



Agilent Technologies

Notices

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Manual Part Number

G7446-90569

Edition

Revision A, May 2014

This guide is valid for 4.2 and later revisions of the Agilent VnmrJ software, until superseded.

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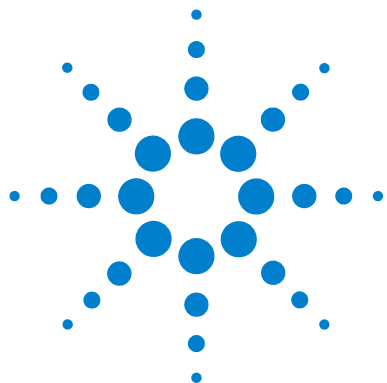
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1

Introduction

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This guide provides an overview of the Agilent VnmrJ software and how you use it to acquire and process NMR spectra using Agilent NMR Spectrometers. Descriptions of the VnmrJ program user interface, toolbars, and menu items are included, along with general overview and description of the VnmrJ workflow. For more detailed information on the various workflow steps, see the *Agilent VnmrJ Spectroscopy User Guide* provided with your system.



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Introduction to VnmrJ Workflow

The VnmrJ program is used to acquire and process data from your Agilent NMR spectrometer. Collecting an NMR spectrum requires the following steps. These steps are described in the following sections. For information on the VnmrJ program window, toolbars, and menu items, see “[VnmrJ Interface](#)” on page 15.

- [Preparing for an Experiment](#) – prepare and load the sample, tune the probe, and shim.
- [Acquiring Data](#) – select experiment to be run on the sample and enter sample information, or create a study containing multiple experiments to run on one or more samples.
- [Processing Data](#) – use the data processing tools to optimize the spectrum display.
- [Plotting Data](#) – use the plotting tools and Graphics Toolbar to adjust the displayed spectrum for the desired output.

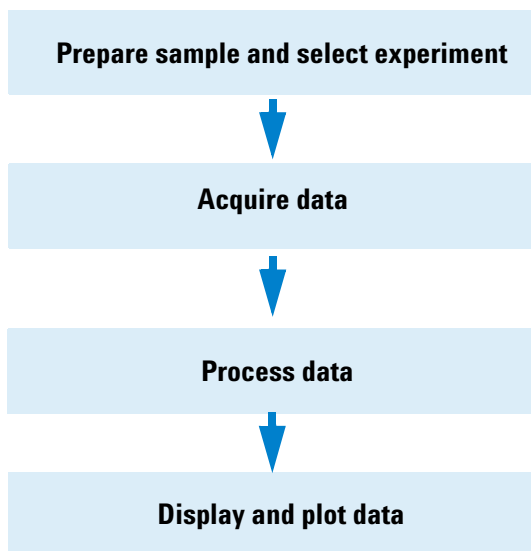


Figure 1 VnmrJ workflow

Commonly Used VnmrJ Terms

The following table lists some common VnmrJ terms that are used in this guide.

Table 1 Commonly used VnmrJ terms

Item	Description
Experiment	Combination of a pulse sequence, a parameter set, and possibly a data set.
Experiment Protocol	Software device that creates an experiment by executing a macro to set up parameters for a given pulse sequence. In Review Mode, a protocol operates in the current workspace and is typically followed by data acquisition. In Submit Mode, a protocol adds a node to the Study Queue for subsequent execution at run-time, followed by the start of data acquisition.
Sample	A physical object, either a tube with liquid or a solid sample in a rotor.
Study	Collection of one or more nodes in the Study Queue. In general, each node represents an experiment. A study is a list of operations to perform; it is not necessarily associated with a specific sample or any other physical object.
Study Queue	Interface feature that is used to display all the various types of queues that are available in VnmrJ. The Study Queue can be configured to show information in several different ways.

Table 1 Commonly used VnmrJ terms (continued)

Item	Description
Submit Mode and Review Mode	When you are interacting with data, processing/plotting data, or collecting data in manual mode, you interact with the software in Review Mode. When you use the Study Queue to load, build, edit, customize, or otherwise work with a study, the software is in Submit Mode. Submit Mode is entered by clicking the New Study , Edit Study , or Continue Study buttons. The interface is moved into Review Mode when you click the Done button in the Study Queue window (or it happens automatically upon sample submission).
Viewport	Interface feature that is used to display the contents of a workspace.
Workspace	Directories that can be thought of as digital objects to hold an experiment and/or data set. Equivalent to the idea of exp1, exp2, exp3, exp(n) in older versions of Vnmr.

Where to Find Help

Agilent provides a complete set of documentation to get you started generating quality data as quickly as possible. The following table contains a summary of the manuals and user guides provided, and what kind of information they contain.

Table 2 VnmrJ manuals and their uses

Manual title	Provided as	Information in this manual
VnmrJ Installation Guide	Printed, PDF, and in online help	Instructions on how to install Linux and the VnmrJ software
VnmrJ Administration Guide	PDF, and in online help	How to administer the VnmrJ system, including adding and changing users, and setting permissions and preferences
VnmrJ QuickStart	Printed, PDF, and in online help	Step-by-step overview of how to use Agilent VnmrJ software to collect an NMR spectrum on NMR systems with or without a Robot Sample Changer
VnmrJ Familiarization Guide (this document)	Printed, PDF, and in online help	Overview of the VnmrJ software, including description of the interface, menus, and commonly used tasks
VnmrJ Spectroscopy User Guide	PDF, and in online help	A more in-depth description of using the VnmrJ software to set up studies, perform shimming, and acquire, process, display, and output data
NMR System Calibrations Guide	PDF, and in online help	Use of VeriPulse, Autotest, and system calibrations are described
Basic NMR Experiments Familiarization Guide	PDF, and in online help	Provides a more detailed discussion about some of the most commonly used pulse sequences available in the VnmrJ Experiment Selector
Experiment Reference Guide	PDF, and in online help	Complete descriptions of VnmrJ experiments
Command and Parameter Reference Guide	PDF, and in online help	Comprehensive listing of VnmrJ commands and parameters
User Programming Guide	PDF, and in online help	Comprehensive pulse sequence programming and customization reference

Table 2 VnmrJ manuals and their uses (continued)

Manual title	Provided as	Information in this manual
CRAFT User Guide	PDF, and in online help	Overview and step-by-step instructions for using the CRAFT (Complete Reduction to Amplitude Frequency Table) application within VnmrJ
BioPack Quick Start Guide	Printed, PDF, and in online help	Step-by-step overview of using the BioPack option
BioPack User Guide	PDF, and in online help	Complete information on how to use the BioPack option
BioPack Experiment Guides	PDF, and in online help	Descriptions of BioPack experiments
SolidsPack User Guide	PDF, and in online help	Describes the use of SolidsPack, used to run a solids experiment and control solids console accessories such as shims, variable temperature, and magic- angle spinning
3D Gradient Shimming User Guide	PDF, and in online help	Description of how to use the Agilent 3D Gradient shimming software, used to map and optimize room- temperature, shim systems without additional hardware

Contact information

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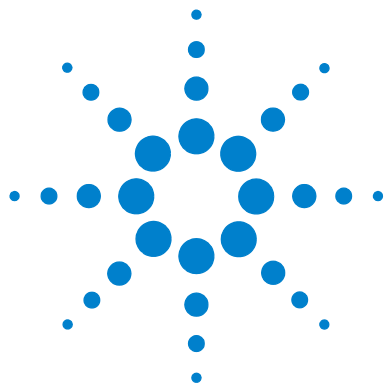
Telephone Number:

Toll Free: +1 (800) 227 9770

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<http://www.chem.agilent.com/en-us/ContactUS/Pages/ContactUs.aspx>



2

VnmrJ Interface

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This section contains descriptions of the main features of the VnmrJ program interface.



VnmrJ User Interface

The main areas of the user interface for VnmrJ are shown in Figure 2. These areas are described in more detail in other sections of this guide.

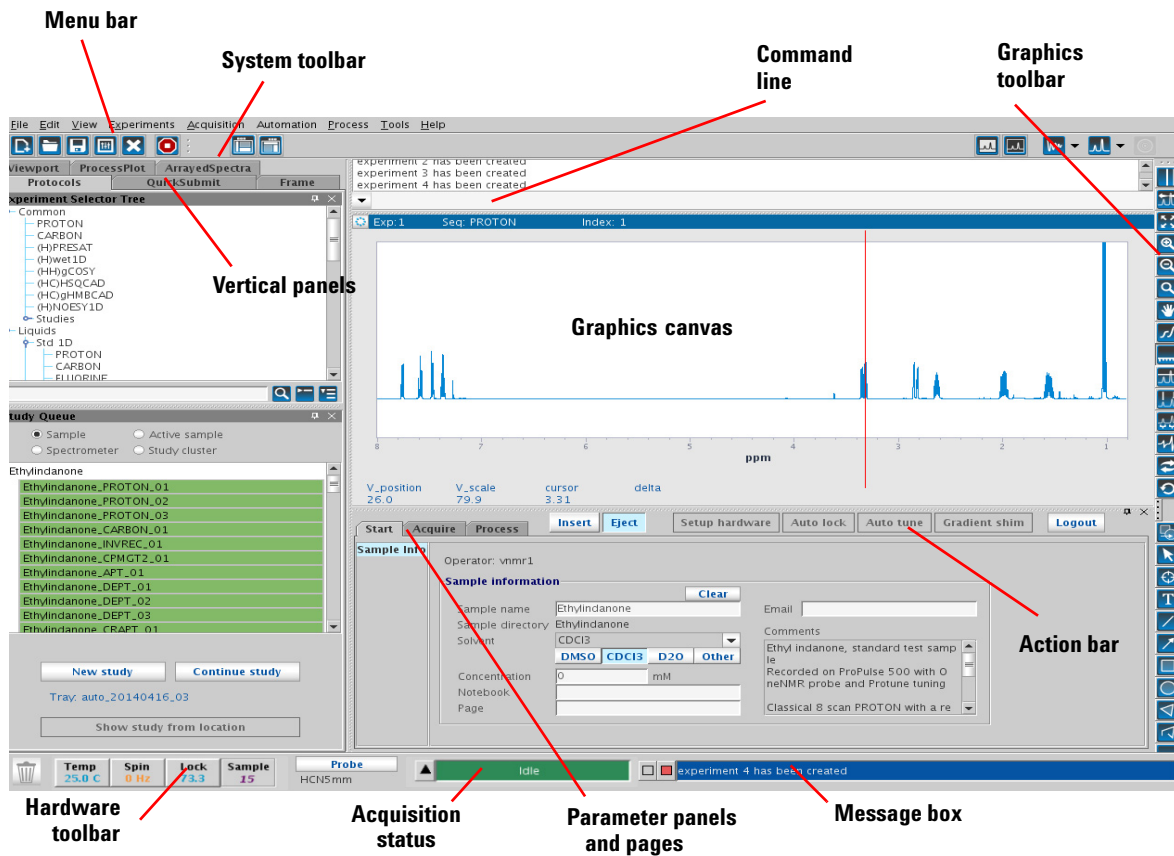


Figure 2 VnmrJ user interface

Toolbars

The VnmrJ toolbars provide quick access to commonly used functions. To view a description of a toolbar icon, move the mouse cursor over the icon until the description appears.

System toolbar



The system toolbar provides access to common system functions. It also lets you show or hide graphics toolbars.

Table 3 System toolbar controls













Icon	Description
	Create a new workspace.
	Opens the File Browser to search for and open a file.
	Opens the File Browser to find a location and save data.
	Opens the Styles and Themes pop-up, where you can select or customize colors or color themes for the VnmrJ user interface.
	Cancel a command.
	Save current screen layout: graphics, parameter panel, locator sizes.
	Save current screen layout: graphics, parameter panel, locator sizes.
	Draw spectral data using a light background. This does not change the User Interface theme.

Table 3 System toolbar controls (continued)

Icon	Description
	Draw spectral data using a dark background. This does not change the User Interface theme.
	Opens the Display FID toolbar controls .
	Opens the 1D display spectrum toolbar controls .
	Opens the nD display toolbar controls .

Hardware toolbar

The Hardware toolbar, located at the bottom of the VnmrJ window, shows a trash can icon and a display area dedicated to real-time hardware information.



Figure 3 Hardware toolbar

Sample temperature, spin rate, lock level, and current sample changer location are displayed to the left of the Hardware toolbar.

Status plots

The status plots provide useful information about sample temperature, spin rate, lock level, and current sample changer location. For more information, see [“Status Charts”](#) on page 95.

Acquisition status

Real time events such as system being idle, locking, shimming, or acquiring data are displayed in the field located to the right of the probe file. If the system is active, each event's remaining time is displayed.

Message box

To the right of the Hardware toolbar is a system message box. Error messages and other important system information are displayed in this area.

Graphics toolbar

The Graphics toolbar is used to control the interactive display in the graphics canvas.

See also

[“Common graphics display toolbar controls”](#) on page 19

[“1D display spectrum toolbar controls”](#) on page 20

[“nD display toolbar controls”](#) on page 21







[“Display FID toolbar controls”](#) on page 23

For more information on using the graphics toolbar, see [“Interacting with the Spectrum Using the Graphical Toolbar”](#) on page 165.

Common graphics display toolbar controls

The following tools are common to 1D, nD, and FID display toolbars.

Table 4 Graphic display toolbar controls

Icon	Description
	Reset to full display.
	Zooms in to a region in graphics canvas defined by cursor placement. (Click mouse once to define first cursor and then again to define second cursor.) To zoom further, click to define cursor positions, and then click the zoom icon again.
	Zooms out.
	Pan, or "rubber band" zoom. Click once to define first cursor, then click again and drag.
	Redraw display.
	Return to previous tool menu.

1D display spectrum toolbar controls


The following table shows the icons that appear when you click the 1D Display icon on the graphics toolbar. 

Table 5 1D display Spectrum toolbar















Icon	Description
	One cursor in use, click to toggle to two cursors.
	Two cursors in use, click to toggle to one cursor.
	Click to expand region.
	Click to expand to full spectral display.

Table 5 1D display Spectrum toolbar (continued)

Icon	Description
	Display integrals menu.
	Display partial integrals.
	Display full integral.
	Hide integrals.
	Define integrals.
	Adjust integral level/tilt.
	Delete integrals.
	Display or hide scale.
	Pan or move spectral region.
	Toggle threshold on or off.
	Phase spectrum.

nD display toolbar controls

The following table describes the icons displayed when you click the 2D or 3d Display icon on the Graphics toolbar.



Table 6 nD display toolbar controls
















Icon	Description
	Display color map and show common nD graphics tool.
	Display contour map and show common nD graphics tool.
	Display stacked spectra and show common nD graphics tool.
	Display image map and show common nD graphics tool.
	One cursor in use, click to toggle to two cursors.
	Two cursors in use, click to toggle to one cursor.
	Expand to full display.
	Pan and stretch.
	Show trace.
	Show projections.
	Shows horizontal maximum projection across the top of the 2D display.
	Shows horizontal sum projection across the top of the 2D display.
	Shows vertical maximum projection across the top of the 2D display.
	Shows vertical sum projection down the left side of the 2D display.
	Rotate axes.

Table 6 nD display toolbar controls (continued)

Icon	Description
	Increase vertical scale 20%.
	Decrease vertical scale 20%.
	Phase spectrum menu.
	First spectrum selection.
	Second spectrum selection.
	Enter peak pick menu.





Display FID toolbar controls

The following table contains descriptions of the commands available from the menu that appears when you click the Display FID icon in the Graphics toolbar.

Table 7 Display FID toolbar controls

Icon	Description
	One cursor in use, click to toggle to two cursor
	Two cursors in use, click to toggle to one cursor
	Click to expand to full FID display
	Pan and stretch.
	Click to show real and imaginary

Table 7 Display FID toolbar controls (continued)

Icon	Description
	Click to show real and zero imaginary
	Click to show real only
	Toggle scale on and off
	Phase FID

Annotation toolbar controls

The following table describes the icons displayed in the graphics toolbar (**View > Toolbars > Graphics Toolbar**) in "ds", "dss", and "dconi" display modes.

Table 8 Annotation toolbar controls













Icon	Description
	Toggle to show or hide annotations in graphics canvas and hard copy plot
	Select annotation for editing. Use this mode to move or delete an annotation, or change properties such as color and line thickness.
	Position – displays the value of the position where it is located, in Hz, PPM, etc. The value is updated automatically as the annotation is moved.
	Text – Text with adjustable font, size, style, color, and transparency.
	Line – Line with a adjustable thickness, color, and transparency.
	Arrow – Arrow with adjustable thickness, color, and transparency.
	Box – Box with adjustable thickness, color, and transparency, with rounded or square corners.

Table 8 Annotation toolbar controls (continued)

Icon	Description
	Oval – Oval with adjustable thickness, color, and transparency.
	Polygon – Polygon with adjustable thickness, color, and transparency.
	Polyline – Connected line segments with adjustable thickness, color, and transparency.
	X-Bar – displays its width in Hz, PPM. The value is updated automatically as the annotation is resized.
	Y-Bar – displays its height in intensity units, or for 2D data, in Hz, PPM, etc. The value is updated automatically as the annotation is resized.

Command Line

One of the most powerful aspects of the VnmrJ software is the ability it provides to users to execute commands and macros directly using the Command Line. For more information on VnmrJ commands and macros, see the *Agilent VnmrJ Command and Parameter Reference Guide* and the *Agilent VnmrJ User Programming Guide*.

Graphics Canvas

The Graphics Canvas is used to display and interact with graphic and text information. For more information, see [“Interacting with the Spectrum Using the Graphical Toolbar”](#) on page 165.

When a spectrum is first displayed on the Graphics Canvas, or the display is refreshed, the bar above the Graphics Canvas displays the functions of the left and right mouse buttons and the scroll wheel:

- Use the left mouse button to set the left cursor,
- Use the right button to set the right cursor
- Use the scroll wheel to adjust the vertical scale of the spectrum

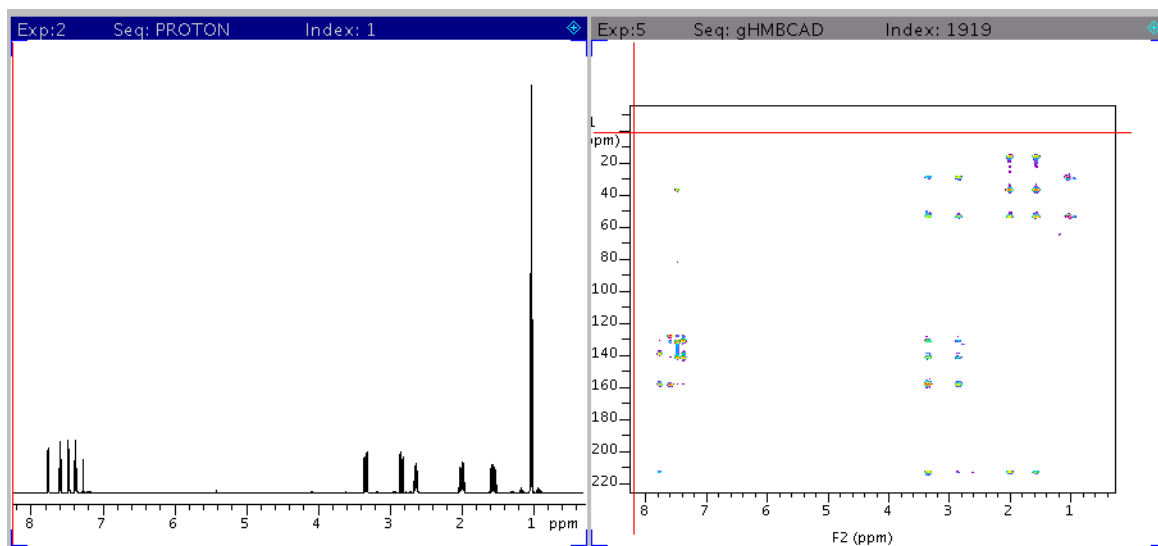


Figure 4 Graphics Canvas

Tray display

If you have a robot sample changer, a graphical display of the changer gives you access to a menu of commands when you right-click the mouse button over the tray display.

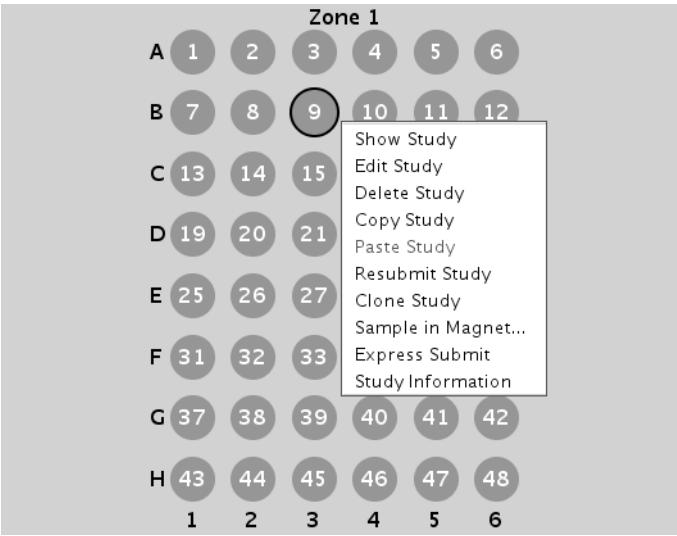


Figure 5 Tray display menu

Table 9 Tray display menu options

Item	Description
Show Study	Displays the queue for the selected location in the Study Queue.
Edit Study	Loads a study into the Study Queue from the tray display in preparation for modification of that study.
Delete Study	Deletes the selected location queue.
Copy Study	Copy Study to clipboard.

Table 9 Tray display menu options (continued)

Item	Description
Paste Study	Paste Study from clipboard.
Resubmit Study	Resubmits the selected location to acquisition.
Clone Study	Clones the selected location queue.
Sample in Magnet	Displays the Sample in Magnet popup, preloaded with that location for a sample change operation.
Express Submit	Utility to submit a sample to a specific location using automation, see Using Express Submit with a sample changer .
Study Information	Displays information about Study.
Swap Queue	Swaps experiments queued in the day to night and vice versa. Only displayed if there is an active Study in the selected location.

Vertical Panels

The vertical panels area of the VnmrJ user interface provides quick access to related functions. Each vertical panel contains one or more functional areas where you perform tasks such as selecting experiments and setting up data display.

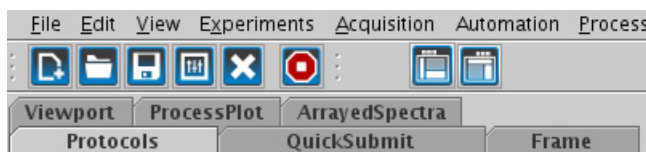


Figure 6 Vertical panel tabs in VnmrJ

For more information, see “[Protocols Vertical Panel](#)” on page 31, “[QuickSubmit vertical panel](#)” on page 39, “[Frame vertical panel](#)” on page 41, “[Viewport vertical panel](#)” on page 45, and “[ProcessPlot vertical panel](#)” on page 49.

Protocols Vertical Panel

The Protocols vertical panel contains the Experiment Selector, the Experiment Selector Tree, and the Study Queue. To show or hide one or more of these panels, click **View** and then select the item you want to show or hide. See also [Experiment Selector](#), [Experiment Selector Tree](#), and [Study Queue](#).

Experiment Selector

The Experiment Selector can be used to load studies into the Study Queue or the current workspace. When an experiment is selected in Experiment Selector, all submissions to automation can be selected by double-clicking and experiment or dragging and dropping it to the Study Queue. You can configure families of experiments and content by using the Experiment Selector. Simple or complex experiments, based on an account or an operator within an account, are accessible as needed.

Use the Experiment Selector Editor to change the order and display of experiments/protocols within the Experiment Selector. For more information, see [“Experiment Selector Editor”](#) on page 90.

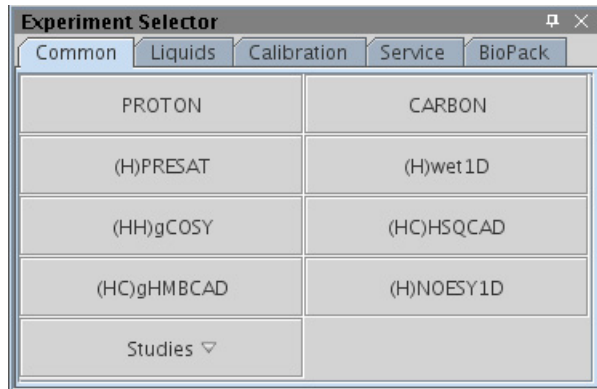


Figure 7 Experiment Selector

Experiment Selector Tree

The Experiment Selector Tree is a convenient way to view the available experiments. Instead of displaying the experiments in tabs (as in the Experiment Selector) experiments are grouped under their respective experiment types. Use the buttons at the bottom of the panel to expand or contract the tree and to search through the tree for an experiment using part or all of the name.

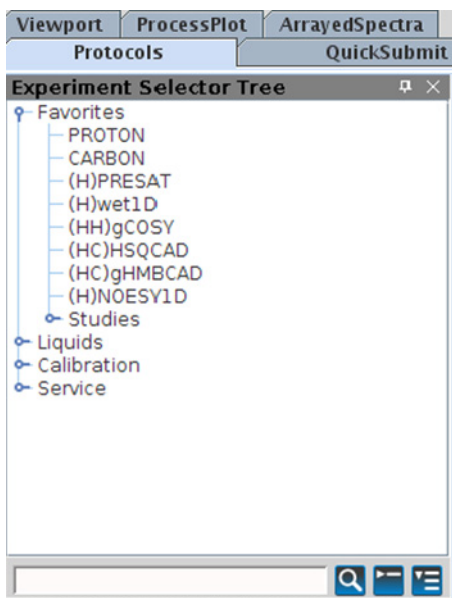





Figure 8 Experiment Selector Tree

Table 10 Experiment Selector buttons

Button	Action
	Type text in this field, and click the search button. The tree view is expanded and selects the first experiment/protocol with a name that contains the text. Click the search button additional times to continue the search further down the tree until the bottom of the tree is reached, at which point the search resumes at the top. Searching for a “Find” text that is not matched anywhere keeps the existing tree view, but does not match a selection.
	Collapses an expanded tree
	Expands the tree view

Study Queue

VnmrJ allows the ability to construct a linked list of experiments as a Study Queue that can be performed on any given sample. The appearance of the Study Queue changes depending on if you have a sample changer installed, or when in Submit mode or Review mode. For more information, see [“Build a Study Queue”](#) on page 151.

A Study Queue is used for both data acquisition and processing. Its many functions are described in more detail in the *Agilent VnmrJ Spectroscopy User Guide*.

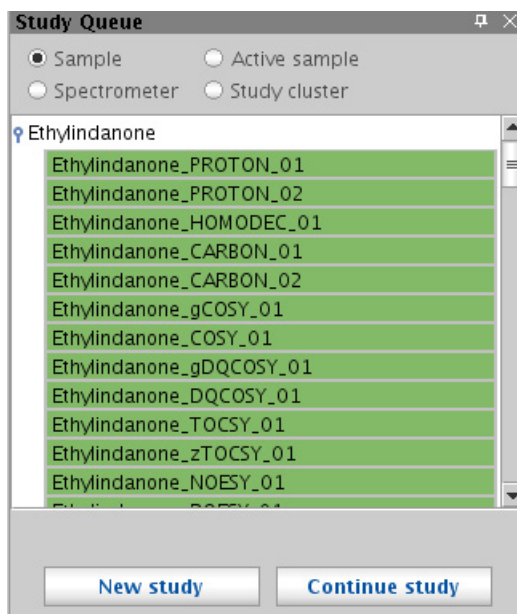


Figure 9 Study Queue—Review mode with no sample changer

Table 11 General Study Queue features

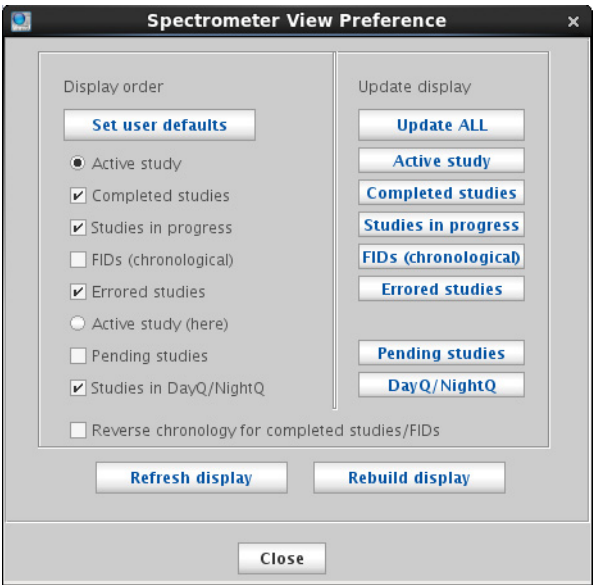
Item	Description
View	<p>Selections that determine what is displayed in the Study Queue:</p> <p>Sample — displays the study linked to the data in the current workspace</p> <p>Spectrometer — displays all studies in the current automation run</p> <p>Active Sample—displays the currently acquiring study</p> <p>Study Cluster—displays study cluster, if one is defined</p>
New Study	Initializes a new study and moves the software to Submit mode
Continue Study	Used to modify the current study displayed in the Study Queue
Show Study from Location (robot changer only)	Loads a study from the tray display

Table 12 Study Queue features — Submit Mode

Item	Description
Cancel	Abandons any changes made to the current study and returns the software to Review mode.
DayQ (robot changer only)	Runs study according to schedule set in DayQ. The schedule is set by the account administrator using the Automation tab of the Edit > Preferences window.
NightQ (robot changer only)	Runs study according to schedule set in NightQ. The schedule is set by the account administrator using the Automation tab of the Edit > Preferences window.
Priority sample (robot changer only)	Allows a sample to be submitted ahead of all other samples in the current automation run. This feature is controlled by the system administrator on an operator-by-operator basis.
Submit	Submits the current study to acquisition, using one of the following choices: Automation—submits the study to the Spectrometer Queue. Foreground exp—submits the study to acquisition in the current workspace. Background—submits the study to a background copy of VnmrJ.
Foreground (shown when not using robot sample changer)	Submits the study to acquisition in the current workspace.
Background (shown when not using robot sample changer)	Submits the study to a background copy of VnmrJ.
Clear Pending Exp from Queue	Deletes all pending experiments from the current Study Queue.

Table 13 Study Queue features — Review Mode

Item	Description
Options	Available when in Spectrometer view. Configure and update display settings on the Spectrometer View Preference window.



Right click over a node to access options, shown below.

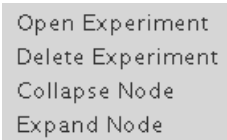


Figure 10 Study Queue node options

Table 14 Node options

Item	Description
Open Experiment	Opens selected experiment and displays parameters in the Parameter Panel.
Delete Experiment	Removes experiment from the Study Queue.
Collapse Node	Collapses selected node so that only experiment name is displayed.
Expand Node	Expands node so that all information is displayed.

QuickSubmit vertical panel

The QuickSubmit panel provides an easy way to quickly submit samples for acquisition.

The screenshot shows the QuickSubmit vertical panel with the following sections:

- Protocols** tab selected, with sub-tabs **QuickSubmit**, **Frame**, and **Viewport**.
- QuickSubmit** section:
 - Next available location: 1
 - Operator: vnmr1
 - Active study: Inactive
 - Buttons: **New study**, **Continue study**
- Parameters** section:
 - Sample name: [text input]
 - Solvent: CDC13 (dropdown menu)
 - Comments: [text input]
- Experiment queue** section:
 - Select experiment: PROTON (dropdown menu)
 - Buttons: **Add to DayQ**, **Add to NightQ**, **Customize**, **Clear queue**
 - New Study: [text input]
 - Checkboxes: ☐ Priority sample, ☐ Next location
 - Buttons: **Submit**, **Logout**
- Messages** section:
 - Ready for a New Study...
 - Next Submission will start at...
 - PriorityQ : 11:32 AM
 - DayQ : 11:32 AM
 - NightQ : 06:00 PM
 - Buttons: **Edit exp list**, **Message history**

Figure 11 QuickSubmit vertical panel

Table 15 QuickSubmit Options

Item	Description
New study	Click to begin a new study.
Continue study	Click to continue the study currently in the Study Queue.
Parameters	Enter descriptive sample parameters and comments.
Experiment queue	From the Select Experiment drop-down list, select an experiment, then click Add to DayQ or Add to NightQ to place the experiment in the Study Queue. Continue to add experiments as desired.
Customize	
Clear queue	Clears the experiments from the queue.
Submit	Submits the current QuickSubmit queue for acquisition.
Logout	Click to log out of the VnmrJ system.
Edit exp list	Edit the existing experiment list.
Message history	Click to see a log of previous messages.

Frame vertical panel

Use the Frame vertical panel to create an inset frame. An inset frame initially shares the same workspace and data as the viewport. However, you can change or remove it. For details on creating and working with insets, see the *Agilent VnmrJ Spectroscopy User Guide*.

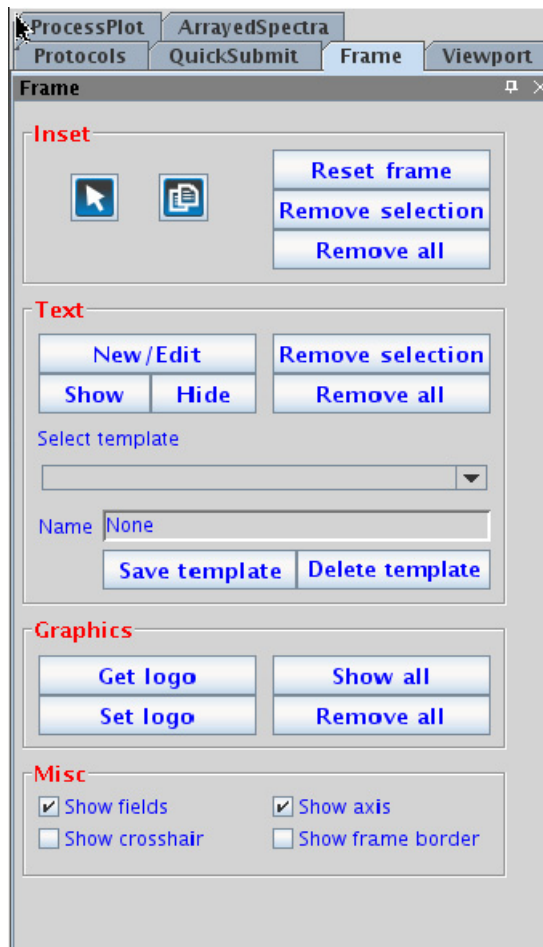


Figure 12 Frame vertical panel

Table 16 Frame vertical panel options



Item	Function
Inset	
	Default mode — left mouse click moves the left cursor and right mouse click moves right mouse cursor.
	Inset mode — left mouse drag a box over a spectrum region creates an inset frame of the region. A viewport can have multiple inset frames. Exit inset mode — release mouse button.
Reset frame	Resets inset frame to default
Remove selection	Removes selected frame or item
Remove all	Removes all inset frames
Text	
New/Edit	Create or change a text inset
Show	Show text inset
Hide	Hide text inset
Remove selection	Remove selected text
Remove all	Removes all text
Select template	Select a saved text inset template
Name	Give a name to the text inset
Save template	Save the text inset as a template
Delete template	Delete the selected template

Table 16 Frame vertical panel options (continued)

Item	Function
Graphics	
Get Logo	Lets you select a logo to show in the frame
Set Logo	
Show All	Shows all graphics in the frame
Remove All	Removes all graphics from the frame
Misc	
Show Fields	Display <code>cr</code> , <code>delta</code> , <code>vp</code> etc... fields at the bottom of the viewport.
Show Crosshair	Display cross hair and chemical shifts of the cursor position when the mouse is moved over the spectrum. This is a useful function when the fields are not shown, not in cursor mode (default mode), or when chemical shift of a peak without moving the left cursor is required while in the cursor mode.
Show Axis	Show scale of the axis.
Show frame border	Check the box to display a box around the frame. Clear the box to display the four corners of the selected frame as <i>hot spots</i> for resizing. No border or corner will be displayed if a frame is not selected. An empty frame is not visible until it is selected.

Inset frame buttons

The buttons delete one or all inset frames and restore the default frame to full size.

Button	Function
Delete Inset	Delete the selected inset.
Delete all	Delete all inset frames.
Full size	Restore the default frame to its full size.

Display check boxes

The check boxes control optional display features.

Check box	Function
Cross hair	Display cross hair and chemical shifts of the cursor position when the mouse is moved over the spectrum. A useful function when the fields are not shown, not in cursor mode (default mode), or when chemical shift of a peak without moving the left cursor is required while in the cursor mode.
Fields	Display <code>cr</code> , <code>delta</code> , <code>vp</code> etc... fields at the bottom of the viewport.
Axis	Show scale of the axis.
Show frame border	Check the box to display a box around the frame. Un-check the box to display the four corners of the selected frame as <i>hot spots</i> for resizing. No border or corner will be displayed if a frame is not selected. An empty frame is not visible until it is selected.

Viewport vertical panel

The Viewport vertical panel is used to set up and customize the display of viewports.

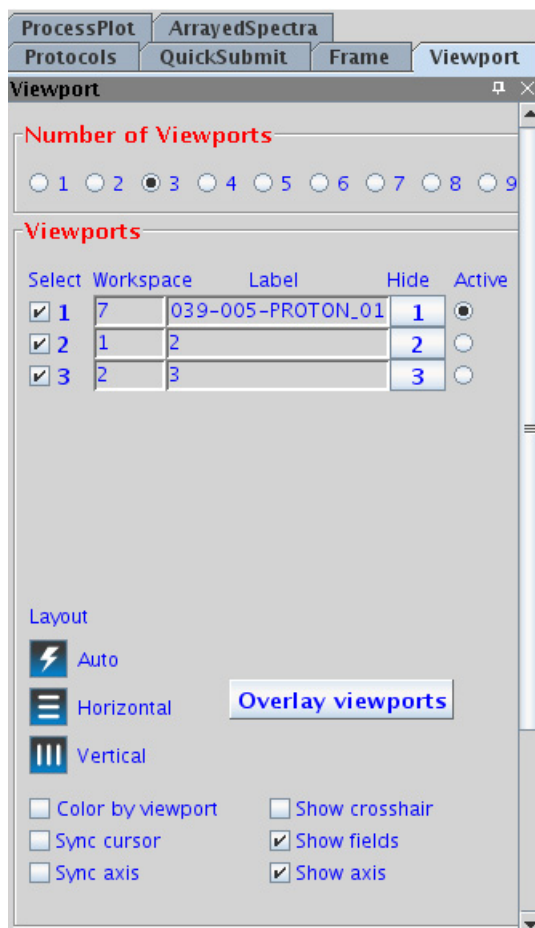


Figure 13 Viewport vertical panel

Table 17 Viewport vertical panel options




Item	Description
Number of viewports	Used to select the number of viewports to display. Available viewports is set in Viewports settings window.
Viewports	<p>Select — When selected, displays that viewport</p> <p>Color — Select color to display data in viewport</p> <p>Workspace — Workspace number (also experiment number shown on upper left corner of viewport)</p> <p>Label — File name or user-defined label</p> <p>Hide — Hide selected viewport</p> <p>Active — Select to make viewport active</p>
Viewport layout	<p> — Auto mode, let VnmrJ arrange the viewports in an optimized row-by-column matrix</p> <p> — Stack viewports horizontally</p> <p> — Arrange viewports vertically</p>
Overlay Viewports	<p>Overlays viewports</p> <p>Stack spectra — show spectra with an offset</p> <p>X — offset in X axis for stacked spectra</p> <p>Y — offset in Y axis for stacked spectra</p>
Color by viewport	Turns on the color option so you can select to display viewports in different colors.
Sync Cursor	Links and synchronizes the cursors and crosshairs in multiple viewports.
Sync Axis	Links and synchronizes axes in multiple viewports. Axis is synchronized to the current active viewport.
Show crosshair	Displays cross hair and chemical shifts of the cursor position when mouse is moved over the spectrum. This is a useful function when the fields are not shown, not in cursor mode (default mode), or chemical shift of a peak without moving the left cursor is required while in the cursor mode.

Table 17 Viewport vertical panel options

Item	Description
Show fields	Shows information fields at the bottom of the active viewport:
<div> <div>vs</div> <div>111.7</div> </div> <div> <div>sp(ppm)</div> <div>35.78</div> </div> <div> <div>wp(ppm)</div> <div>65.00</div> </div> <div> <div>first</div> <div>1</div> </div> <div> <div>last</div> <div>4</div> </div> <div> <div>step</div> <div>1</div> </div>	
Show axis	Displays axes in the viewports

Contour

The contour sub-panel, [Figure 14](#), appears exclusively for the active viewport with 2D data loaded and displayed in contour mode (dpcon).

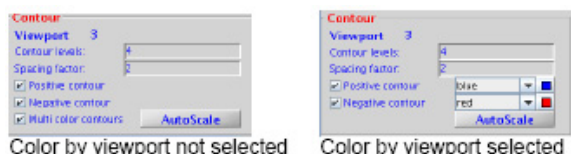


Figure 14 Contour controls

Table 18 Contour panel

Control	Description
Contour levels	Enter a number of contours between 4 and 32 in the text field.
Spacing factor	Enter a number in the text field to specify the spacing between contours. A number between 1.1 and 2 is recommended.
Positive contour	Select this check box to show positive contours using the default color red.
Negative contour	Select this check box to show negative contours using the default color blue.
Color dropdown	Select a color from the menu to use a color other than the default color. Each contour has a color dropdown menu.

Table 18 Contour panel (continued)

Control	Description
Multi color contours	Select this option to use the colors defined in Display Option. If you select the Color by Viewport box, options are not displayed.
AutoScale	Automatically scale the spectrum by clicking.

ProcessPlot vertical panel

The ProcessPlot vertical panel provides quick access to commonly-used options for processing, on-screen display, and plotting. Options in the panel vary depending on current data. Buttons within the panel enable you to open parameter panels that contain more options.

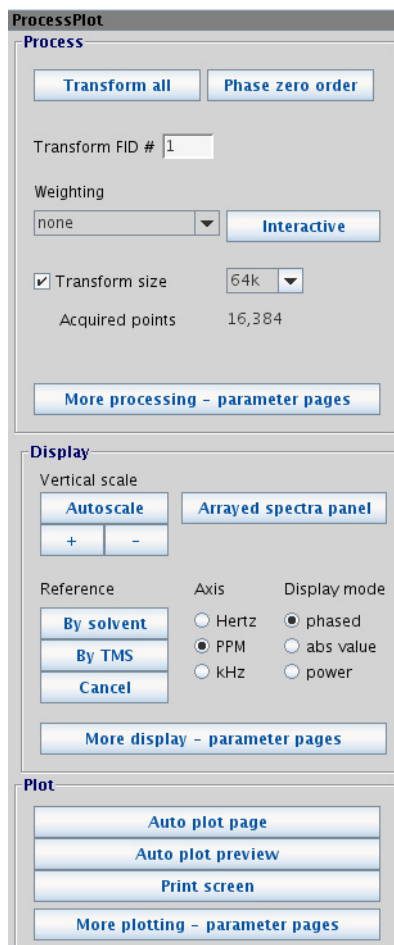


Figure 15 ProcessPlot vertical panel for 1D

Table 19 Options in the ProcessPlot vertical panel

Option	Description
Process	
Transform all	Performs a Fourier transform for all the displayed data.
Phase zero order	Performs a zero order phase correction.
Transform FID #	Select FID # to transform.
Weighting	Select the weighting to be applied to the transform.
Interactive	Select to enter interactive weighting mode.
Transform size	Opens a menu where you can select the number of points to be Fourier transformed (fn).
More processing - parameter pages	Opens the Default page in the Process parameter panel tab for more processing options
Display	
Autoscale	Automatically scales the display vertically.
+/-	Click + or - to increase or decrease the vertical scale.
Arrayed spectra panel	Opens the ArrayedSpectra panel where you can set up display of spectra and FID arrays. See "ArrayedSpectra vertical panel" on page 52.
Reference	By solvent - Reference the spectrum for selected solvent. By TMS - Reference the spectrum to a TMS line. Cancel - Clears the reference line by removing any spectral referencing present, and turns off referencing.
Axis	Select the desired y-axis units: Hertz, ppm, kHz
Display mode	Select the desired display mode: phased, absolute value, or power
More display - parameter pages	Opens the Display page in the Process parameter panel tab for more display options
Plot	
Auto plot page	Executes the <code>plot</code> macro; then the <code>resetplotter</code> macro.

Table 19 Options in the ProcessPlot vertical panel

Option	Description
Auto plot preview	Opens the Plot View popup and displays the plot in Adobe Reader. Use the Plot View popup to save the plot to a file, send to a plotter, or send to an e-mail address.
Print screen	Opens the Print Screen dialog box, where you can set up and print the VnmrJ window or viewport. See “Saving and Printing a Graphics File” on page 182.
More plotting - parameter pages	Opens the Plot page in the Process parameter panel tab for more display options.

ArrayedSpectra vertical panel

The ArrayedSpectra vertical tab contains parameters for displaying and plotting spectra and FID arrays. This procedure applies equally to the display and plotting of both spectra and FID arrays.

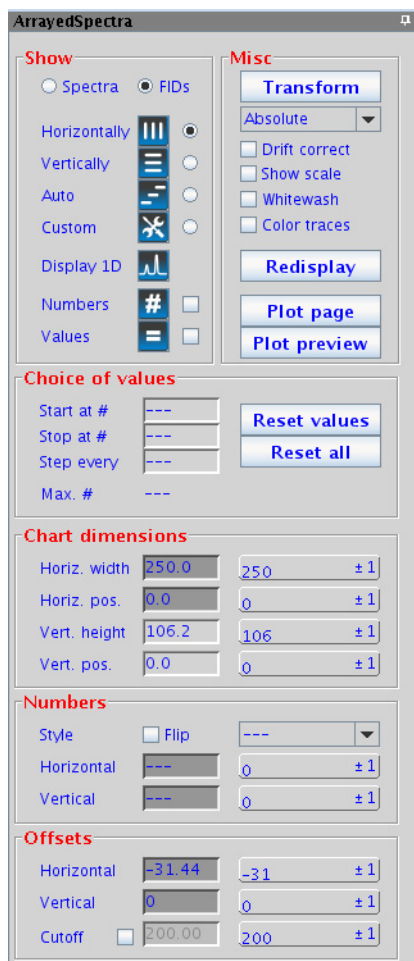


Figure 16 ArrayedSpectra vertical panel

Table 20 Options in the ArrayedSpectra vertical panel

Option	Description
Show	
Spectra or FIDs	Selects to show either spectra or FIDs
Horizontally	Shows the spectra side-by-side
Vertically	Aligns the spectra one above another
Auto	Depends on the previously chosen display mode: <ul style="list-style-type: none"> • If the previous mode showed the spectra full screen (vertical mode or showing only a single 1D) spectra are aligned vertically, and the vertical offset is chosen such that all spectra together cover the entire vertical space. • If the previous mode was horizontal, a vertical offset is added to show the spectra along a diagonal.
Custom	Takes over the display properties of either horizontal, vertical, or auto modes but allows the choice of horizontal and vertical offsets.
Display 1D	Click the icon to show a single spectrum/FID and use the toolbox to manipulate and zoom.
Numbers	When selected, turns on numbering of the array elements displayed.
Values	When selected, turns on display of values for the array elements displayed.
Misc	
Transform	Fourier transform the current FID data
Drift correct	Apply drift correction (corresponds to "dc" command) to all subspectra of the array.
Show scale	Switch on or off a scale below the first spectrum or FID of the array.

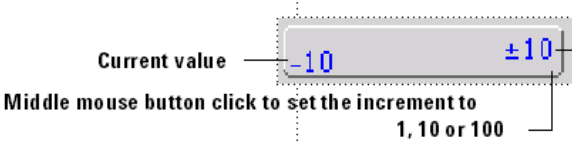
Table 20 Options in the ArrayedSpectra vertical panel (continued)

Option	Description
Whitewash	Aligns the spectra one above another as in the vertical mode, but this mode shows spectra behind each other, avoiding overprinting. Horizontal and vertical offsets can be adjusted.
Color traces	
Redisplay	Refresh the screen.
Plot Page	Send the current array display to the current plotter. Settings on the Plot parameter panel for parameter printing are used. Plotting from the ArrayedSpectra vertical panel does not plot integrals, integral values, and peak frequencies.
Plot Preview	Plot the array to a PDF file and open Acrobat reader with the PDF of the current array. Settings on the Plot parameter panel for parameter printing are used. Plotting from the ArrayedSpectra vertical panel does not plot integrals, integral values, and peak frequencies.
Choice of values	
Start at #	The first element of the array to display
Stop at #	The last element of the array to display
Step every	The element between the beginning and end of the array to display.
Max #	Maximum number of elements to display.
Reset values	Resets just the values to default.
Reset all	Resets all to defaults.
Chart dimensions	Enter the desired values, then adjust the positions as needed using the buttons next to each field. Right mouse click on the button to increase the value or left mouse click on the button to decrease the value the increment shown on the left side of the button.
Horiz. width	Enter the horizontal width for the chart.

Table 20 Options in the ArrayedSpectra vertical panel (continued)

Option	Description
Horiz. pos.	Enter the horizontal position for the chart.
Vert. height	Enter the vertical height for the chart.
Vert. pos.	Enter the vertical position for the chart.
Numbers	
Style	<p>Flip — when selected, rotates the numbers 90 degrees counter-clockwise</p> <p>Drop-down menu — Used to select the position of the numbers. Select Custom to specify a horizontal and vertical positioning of the number with respect to the spectrum. Use the horizontal and vertical fields to type the custom positions.</p>
Horizontal	When using Custom style, lets you enter custom horizontal position.
Vertical	When using Custom style, lets you enter custom vertical position.
Offsets	
Horizontal	<p>Enabled only for vertical, whitewash, or custom array mode.</p> <p>Enter horizontal offset. (Note: the horizontal width must be smaller than the screen width in order to apply any horizontal offset.) Use the button to the right of the field to adjust the position. Right mouse click on the button to increase the value or left mouse click on the button to decrease the value the increment shown on the left side of the button.</p>
Vertical	<p>Enter vertical offset. Use the button to the right of the field to adjust the position. Right mouse click on the button to increase the value or left mouse click on the button to decrease the value the increment shown on the left side of the button.</p>

Table 20 Options in the ArrayedSpectra vertical panel (continued)

Option	Description
 <p>Current value</p> <p>Middle mouse button click to set the increment to 1, 10 or 100</p>	<p>Increment applied to the current setting value.</p> <p>Left click to increase or right click to decrease.</p>
Cutoff	Used to avoid overlapping large lines that may reach into the spectra above.

Parameter Panel

The Parameter Panel shows the pulse sequence, context-specific information, menus, and text entry. The panels under the Acquire and Process tabs change depending on the current pulse-sequence. Parameter Panel information is displayed for the current experiment.

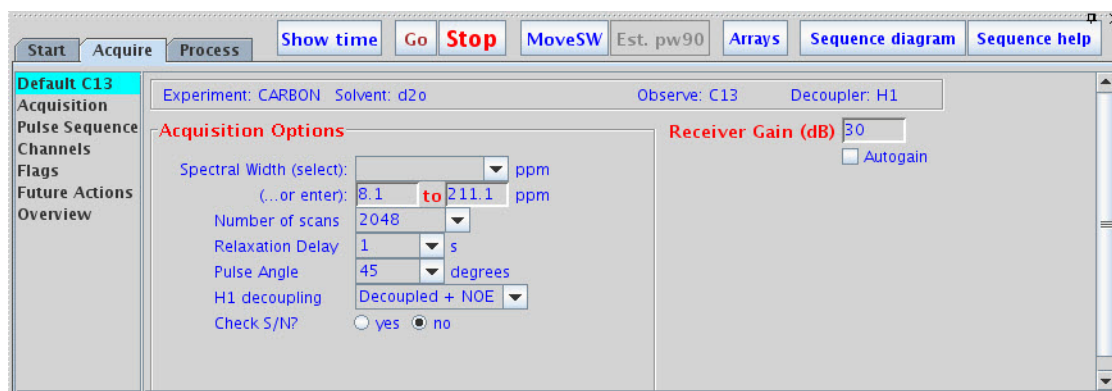


Figure 17 Parameter Panel

Each pane can be resized, reduced to a tab, or closed. Setup, acquire, or process NMR data using the point and click feature of the interface. There are three tabs labeled Start, Acquire, and Process below the graphics window. The top page on each tab displays commonly used functions. Lower pages display detailed functions. Buttons (Action Bar) to the right of the tabs represent tab-specific actions and differ with each tab. The interface can also be accessed using the command line above the Graphics Canvas.

VnmrJ Menus

VnmrJ has an integrated set of tools designed to acquire a series of one and two-dimensional data sets from a library of pulse sequences for any given sample. Access the sophisticated experiments for routine use in a fully automated environment.

Standard menu items are displayed at the top of the VnmrJ window. This section lists menus in alphabetical order rather than in the order they appear in the menu.

Acquisition Menu

The Acquisition menu provides a convenient non-command line access to a number of core VnmrJ commands such as `go`, `ga`, or `au`. The menu option, Parameter Arrays, facilitates the creation of parameter arrays.



Figure 18 Acquisition menu

Table 21 Acquisition menu

Item	Description
Parameter Arrays...	Opens the Array Parameter window, in which you can create and edit parameter arrays.
findZ0	Find Z0 for locking.
Do Gradient Shimming	Opens the gradient shimming menu where you select the gradient map and execute gradient shimming.

Automation Menu

The Automation Menu provides access to automation start, stop, restart and reset controls not generally used during ordinary sample submission and not accessible from the Study Queue. It also provides access to archive queues and automation logs.

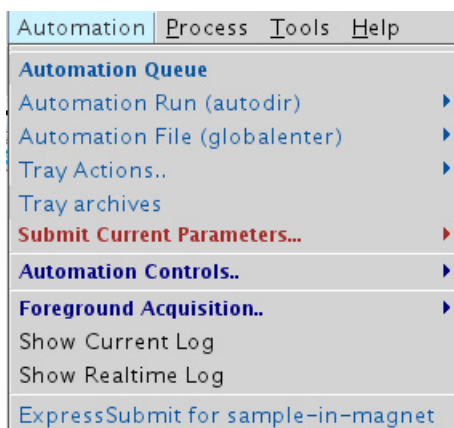


Figure 19 Automation menu

Table 22 Automation menu

Item	Description
Automation Queue	Select to display Automation Queue.
Automation Run (autodir)	<div> New Study Continue Study New Automation Run </div> <p>Start a new study or continue an existing study from an existing automation run. Initiate a new automation run (often done at the start of the day).</p>
Automation File (globalenter)	<div> New Study New Automation File Show AutoFile Tray Submit to Acquisition </div> <p>Edit, create, display and submit to acquisition the "globalenter" version of a Study Queue.</p>
Tray Actions...	<div> Show Tray Hide Tray Show All Studies Automation Run Status Show Study from a Location Recall and resubmit Study from a Location Recall and edit Study from a Location Delete pending Study from a Location </div> <p>Provides functionality that is also available on the Study Queue controls or the right-click menu on the tray locations.</p>
Tray Archives...	Tray archives allows the user to browse completed automation runs or automation files from previous dates.
Submit Current Parameters...	<div> Automation Run as a study to DayQ Automation Run as a study to NightQ Automation File as a study to DayQ Automation File as a study to NightQ </div> <p>Use to manually build any desired experiment in the current workspace and to submit to an automation queue, both day and night.</p>

Table 22 Automation menu (continued)

Item	Description	
Foreground Acquisition...	Allows user to pause after current acquisition, pause immediately, or resume paused study.	
Automation Controls (visible when autosampler is configured)	Pause after current Study	To pause the automation run, manually run an emergency sample, resume the automation run, or to pause a run to fill the magnet with cryogens.
	Pause after current Acquisition	
	Stop-Save-Resume	Stops the running experiment, process or plot, or save to move on to the next experiment in the chain or queue. For example, if a 4 hour experiment were running in automation and after 30 minutes it was processed and nearly complete, then this action allows you to choose a rational action at that time.
	Stop-Discard-Resume	Stops the running experiment and move on to the next item on the list.
	Stop-Save and Stop-Discard	Functions exactly as the submenus described above except that the queue is not resumed until you select Resume Automation from this submenu.
	Pause NOW	Allows you to pause the experiment immediately.
	Pause at scheduled time	Allows the administrator of the account to define in advance an exact time for the automation run to be paused along with a time for automation to resume.
	Resume Automation	Resumes any paused automation run.
	Abort Automation	Stops automation run. During the time of pausing you can use the interface to submit more samples and to acquire NMR data manually. You can also allow time for cryogen fills and the magnet time to recover. The automation run can resume automatically.

Table 22 Automation menu (continued)

Item	Description
Background Acquisition... (visible when no autosampler is configured)	New background run Submits a new acquisition run to background.
	Show all studies Shows status of studies in the Study Queue.
	Pause after current Study Pause after current Acquisition To pause the automation run, manually run an emergency sample, resume the automation run, or to pause a run to fill the magnet with cryogens.
	Stop-Save-Resume Stops the running experiment, process or plot, or save to move on to the next experiment in the chain or queue. For example, if a 4 hour experiment were running and after 30 minutes it was processed and nearly complete, then this action allows you to choose a rational action at that time.
	Stop-Discard-Resume Stops the running experiment and move on to the next item on the list.
	Stop-Save and Stop-Discard Functions exactly as the submenus described above except that the queue is not resumed until you select Resume Automation from this submenu.
	Pause NOW Allows you to pause the experiment immediately.
	Pause at scheduled time Allows the administrator of the account to define in advance an exact time for the acquisition run to be paused along with a time for acquisition to resume.
	Resume Acquisition Resumes any paused acquisition run.
	Abort Acquisition Stops acquisition run.
Show Current Log	Displays the current compact acquisition log in a text editor window.
Show Realtime Log	Displays a compact realtime acquisition log in a popup window.
ExpressSubmit for sample-in-magnet	Submits default experiment (defined in preferences) to the sample in the magnet.

Edit Menu

The available commands and options depend on the rights assigned by the VnmrJ Administrator.

Each of the following menu options opens a dialogue that prompts the user to enter the source and destination workspaces of the items. The command and parameter reference refers to these tools as mp, mf, mt, md, and mz. For more information on commands, parameters, and macros, see the *VnmrJ Command and Parameter Reference Guide*.



Figure 20 Edit menu

Table 23 Edit menu

Item	Description
Move Parameters...	Opens the Move Parameters window that allows parameters to be moved from one experiment number to another.
Move FID...	Opens the Move FID window to move an FID from one experiment number to another.
Move Text...	Opens the Move Text window to move text from one experiment number / workspace to another.
Move Display parameters...	Opens the Move Display Parameters window to move display parameters from one experiment number to another.
Move Integral Resets...	Opens the Move Integral Resets window to move integral resets from one experiment number to another.
New Pulse Shapes (Pbox)...	Opens the powerful Pbox tool for the creation of pulses and decoupling shapes.
View Pulse Shapes...	Opens the Pulse tool, a Bloch simulator for viewing the effects of any shaped pulse.
New/Edit Macro...	Opens a macro directly in a text editor.
Toolbar...	Enables the addition of a button to the top bar of the user interface with a user-specific function.
Display Options	Opens a graphical interface from which you can modify and save/recall the colors used in every tool used in VnmrJ.
Edit Config Profile...	Allows modification of what experiments are shown in the Experiment Selector tool. The starting point is based on the profile assigned to them by the VnmrJ administrator
Edit Experiment Selector	Enables you to change the way a protocol is displayed in the Experiment Selector. You can add or edit folders, change the order of display, or change displayed names This information is saved separately for each operator.
Parameter Pages	Enables you to build/modify Parameter pages.

Table 23 Edit menu (continued)

Item	Description
ToolPanel Tabs	Opens the Tool Panel Editor, where you can configure what vertical panels are available to view, and move their position in the vertical panel pane. You can also save the configuration in a file.
Viewports...	Enables you to toggle viewports. It is a tool to view multiple workspaces simultaneously, on or off.
Applications...	Enables you to define an account with collections of Applications Directories. An Applications Directory is a specific directory path that could contain macros, parameter, templates, and so on. For example, the AutoTest facility can be toggled on or off with this menu item.
Operator Preferences...	Enables the account administrator to allow the individual operators to manage their own preferences for the interface to automatically preset items as email address, preferred solvent, plotter, or notebook. In this example Operator Preferences is not active because in the Preferences menu, User Remembrance is not enabled. The list of choices for this list is completely general and is defined by the account administrator.
Preferences...	Enables the account administrator to define items such as data saving template and default behaviors for plotting, automatic creation of pdf plot files, and a number of operator privileges. This menu item is discussed in detail in “VnmrJ Preferences” on page 103.
System Settings...	Opens a graphics tool with which system options are defined.

Experiments Menu

Unlike the Experiments Selector which can be configured by both the administrators and individual operators in terms of content, the Experiments menu shows the full selection of experiments accessible for the account. Use the Experiment Selector tool to perform automation submissions.

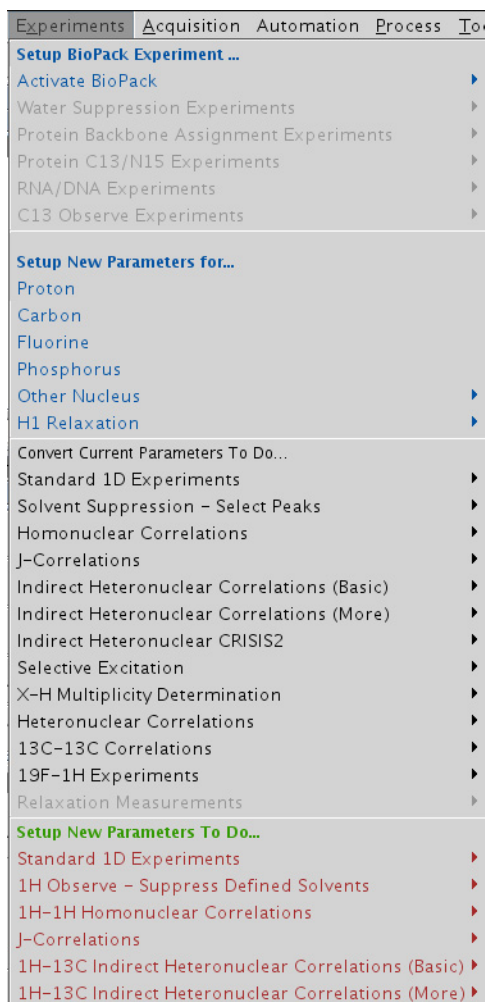


Figure 21 Experiments menu

Table 24 Experiments menu

Item	Description
Setup BioPack Experiment	(Only available when BioPack option is enabled.) For information on using BioPack, see the <i>Agilent VnmrJ 4 BioPack Users Guide</i> .
Activate BioPack	(Only available when BioPack option is enabled.) For information on using BioPack, see the <i>Agilent VnmrJ 4 BioPack Users Guide</i> .
Setup New Parameters for...	Executes a simple retrieval of standard parameters for the selected experiment and also completely clears all sample tags (parameters used to define a sample's identity).This is a clean slate.
Convert Current Parameters To Do...	<p>Sets up the selected requested experiments but retains all sample tags.</p> <p>If you choose to use the Experiment selector without first requesting New Study by the Study Queue controls the result is the same as this conversion.</p> <p>The conversion of parameters with retention of sample identification parameters is the modality of "more on this sample." The acquired data is auto saved and added to the pre-existing data acquired the current study</p>
Setup New Parameters To Do...	Allows a simple retrieval of default parameters for all 2D and a few 1D experiments, according to your need.
Hadamard Experiments	Provides access to all of the Hadamard Fast methods 2D experiments.
Solid-State Experiments	Allows access to all routine Solids NMR experiments

File Menu



Figure 22 File menu

Table 25 File menu

Item	Description
New Workspace	Creates a new workspace for use. Workspaces are called exp1, exp2, and so on, up to exp9999. A workspace is a directory where data is acquired or processed.
Join a NEW Workspace	Creates a new workspace and then actively joins the workspace in the interface. Following is an example of the command line equivalent: cexp (7) j exp7 will create a new workspace and join exp7.
Open...	Accesses the Open window (also called the Experiment Selector Editor) where you can browse for and open files.
Save As...	Opens the File Browser window where you can specify the location and name for saving the data in the current workspace.

Table 25 File menu (continued)

Item	Description
Auto Save	Saves the data that has been acquired in an experiment workspace using the template set up in User Preferences. See Templates Tab. The location and file name are automatically set based upon the values defined in the Preferences Templates tool.
Printers...	Allows you to select a valid printer and/or plotter for output.
Print Screen...	Allows you to print the current screen.
Auto Plot	Calls the appropriate automatic plotting routine for any type of data in the current workspace.
Create a Plot Design...	Opens Plot Designer to create plot designs or output.
Review PDF Plots...	Allows you to review the PDF plot in Adobe Acrobat. You can set the user preference to create a pdf plot automatically for data that has been acquired of a given sample.
Switch Operators...	Allows the current operator of the system to logout during automation thereby freeing the system for use by another operator.
Exit VnmrJ	Executes an exit of the VnmrJ program. It is equivalent to typing <code>exit</code> in the command line.

Help Menu

The Help menu provides links to help and reference information.

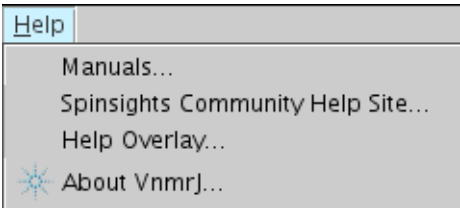


Figure 23 Help menu

Table 26 Help menu

Item	Description
Manuals...	Opens online help where you can view manuals in html or PDF format.
Spinsights Community Help Site...	Opens the Agilent Spinsights home page, where you can find resources such as community forums, downloads, and news.
Help Overlay...	Opens the Help Overlay, which gives you a visual overview of the VnmrJ user interface.
About VnmrJ ...	Opens information about the VnmrJ 4 software.

Process Menu

The Process menu provides tools for common tasks as an alternative to the command line

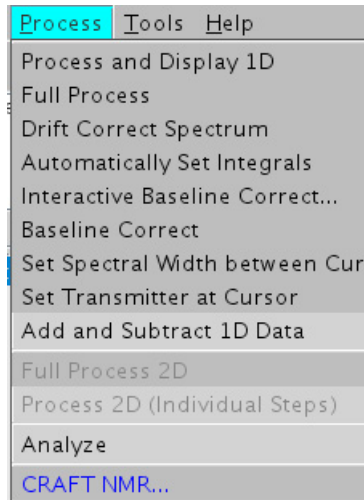


Figure 24 Process menu

Table 27 Process menu

Item	Description
Process and Display 1D	Process and display 1D data.
Full Process	Process and display 1D data using the processing associated with the protocol.
Drift Correct Spectrum	Apply drift correction along both axes of a 2D data set.
Automatically Set Integrals	Automatically find and set integral regions.
Baseline Correct	Apply baseline correction.
Set Spectral Width between Cursors	Mark new spectral width on the graphics screen using the left and right cursors and set the new spectral width.
Set Transmitter at Cursor	Mark new transmitter location on the graphics screen and set the transmitter.
Add and Subtract 1D Data	Results are shown displayed in current when second spectrum is selected.
Full Process 2D	Process and display 2D data using the processing and display parameters associated with the protocol.

Table 27 Process menu (continued)

Item	Description
Process 2D (Individual Steps)	Step by step processing of 2D data.
Analyze	Use to analyze COSY correlations, spin simulation, deconvolution, and regression.
CRAFT NMR	Opens the CRAFT application, (Complete Reduction to Amplitude Frequency Table). CRAFT lets you convert an FID or a collection of FIDs into the component NMR signals in the form of a chemicalshift (frequency) / amplitude / linewidth table. For more information, see the <i>VnmrJ CRAFT User Guide</i> .

Tools Menu

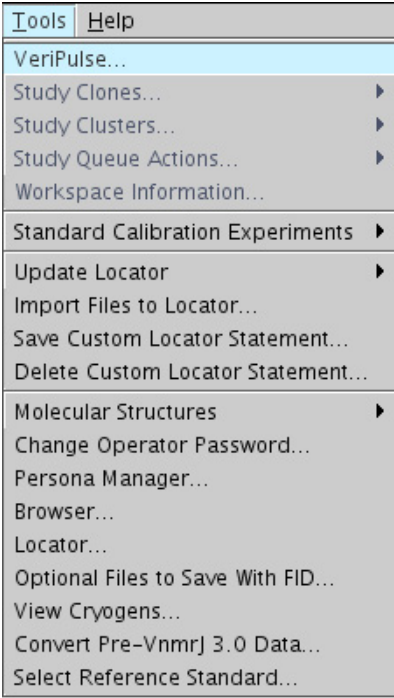


Figure 25 Tools menu

Table 28 Tools menu

Item	Description
VeriPulse...	If VeriPulse is enabled, opens the VeriPulse window that enables you to perform automated testing and calibration. See the <i>Agilent NMR System Calibrations User Guide</i> for information.
Study Clones...	See “Study Clones submenu” on page 77

Table 28 Tools menu (continued)

Item	Description
Study Clusters...	Opens menus with commands that let you create study clusters. A study cluster lets you treat a set of FIDs (from different studies) as a single group. For details, see the <i>VnmrJ Spectroscopy User Guide</i> .
Study Queue Actions...	Displays two menu options: Refresh Study Queue—Updates the study information in the Study Queue window. Clear Study Queue—Clears the Study Queue window.
Workspace Information...	Opens a window that displays the status of all workspace ongoing processes.
Probe Tuning	May display two menu options: Auto Tune Probe...—Opens the ProTune auto-tuning dialog window. Appears only if ProTune accessory is installed and configured. Manually Tune Probe...—Opens the “mtune” panel.
Enforce ProbelD	For installed probes with ProbelD, once ProbelD is configured in System Configuration, this selection enables the ProbelD function. ProbelD prepopulates calibration target values appropriate for the current probe and ensures that the probe file selected in the Probe popup matches the probe that is currently attached to the system.
Disable ProbelD	Disables ProbelD functions.
Standard Calibration Experiments	See “Standard Calibration Experiments submenu” on page 82.
Update Locator	Opens a submenu that provides choices for updating the different parts of the Locator database.

Table 28 Tools menu (continued)

Item	Description
Import Files to Locator...	Opens a window for importing files to the Locator database.
Save Custom Locator Statement...	Opens a window to save custom Locator statements.
Delete Custom Locator Statement...	Opens a window for deleting custom Locator statements.
Molecular Structures	<p>Display all—Display all molecular structures.</p> <p>Plot all—Plot all molecular structures.</p> <p>JChempaint...—Opens the open source application JChempaint (molecular drawing program) in a separate window. Select the Help menu for an online manual.</p> <p>Jmol—Opens the open source application Jmol (3D molecular viewing program) in a separate window. Select the Help menu for an online manual.</p>
Change Operator Password...	Opens a window for changing operator passwords. If the operator has an operating system login account, the password will also be changed.
Persona Manager...	Opens the Persona Manager, where the administrator adds and configures system operators and personas.
Browser...	Opens a file browser window.
Locator...	Opens a Locator window.
Optional Files to Save With FID	Choose additional files that can be automatically saved in the .fid directory.
Sample in Magnet...	Tool to command the sample changer to change samples and to assign the sample position currently in the magnet.

Table 28 Tools menu (continued)

Item	Description
View Cryogens	Opens the CryoMonitor pop-up where you can read the current levels of cryogens and see the level history. This option requires that a cryogen monitor is installed and configured in system configuration.
Convert pre-VnmrJ 3.0 data...	Tool to convert pre-VnmrJ 3 data for use in VnmrJ 3.x.
Select Reference Standard...	Choose the reference compounds used for chemical shift referencing.

Study Clones submenu

The Study Clones submenu is a group of convenience utilities.

A Study Clone is a set of experiments built in the Study Queue with any desired parameter customizations. This set of experiments is automatically represented as a new button in the Experiment Selector. After creation, the user can repeat the exact set of experiments simply by selecting that button either in or out of automation and with or without a sample changer. One use of a Study Clone is to set the desired parameters for a very fast PROTON spectrum, such as `nt=1 ss=0 gain=4` and Clone current Exp with a button label `Fast_H`. It might also be useful to set parameters for a slow quantitative PROTON experiment such as `d1=60 pw(90) ss=2 nt=8` and Clone current Exp with a label as `Quant_H`. The user can use the Study clones to recall complex sets of multidimensional experiments or to simply establish a convenience button for commonly done tasks. Clones from other clones can also be created by adding more experiments in any desired fashion.

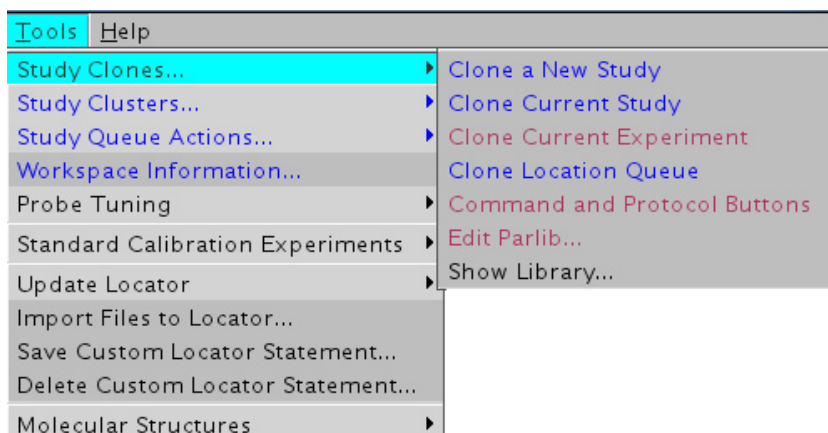


Figure 26 Study Clones submenu

Table 29 Study Clones submenu

Item	Description
Clone New Study	Enters the Study Queue mode to create a new Study that shall be saved as a new clone. Presents a "Save Study" button on the Study Queue window to save the new Study.
Clone Current Study	Use to assign a name for the button associated with a study of a list of experiments assembled in the Study Queue.
Clone Current Experiment	Allows user to assign a name for the button option associated with the currently loaded or displayed experiment. For example, use this option if an FID from a previous study is retrieved in the current workspace, and the user wants to create a clone of the single experiment that had been acquired on that sample.
Clone Location Queue	Allows creation of a button associated with the study completed for the selected tray sample location.

Table 29 Study Clones submenu (continued)

Item	Description
Command and Protocol Buttons	Use the Protocols menu option to devise a method to create a button to run a macro to setup an experiment.

In the Type field, select either **Experiment** or **Command**.

If **Command** is selected, a button option is created to call a macro that, for example, analyzes the lineshape. Based on the result, the user can decide to call a group of non-spin shim routines. A Study Clone could then be composed of a PROTON experiment customized appropriately for running 1H lineshape, followed by the command protocol to analyze lineshape in the Study Clone. This Study clone can be submitted at any time and automates the task of refining shims.

Table 29 Study Clones submenu (continued)

Item	Description
Edit Parlib...	Enables users to view the details of any valid VnmrJ protocol. Edit/Make parlib can also create new protocols based on the contents of the parameter set in the current workspace.

Use the Study Clones tool to create a modified version of an already existing protocol, such as PROTON or CARBON experiments with specific parameters for a quick or long experiment. Do not use Edit/Make Parlib. In order to properly utilize the tool, the user must have a basic understanding of the concept of modules and locked parameters (Plock).

The following is an example view of Edit/Make parlib for a band-selected 2D experiment.

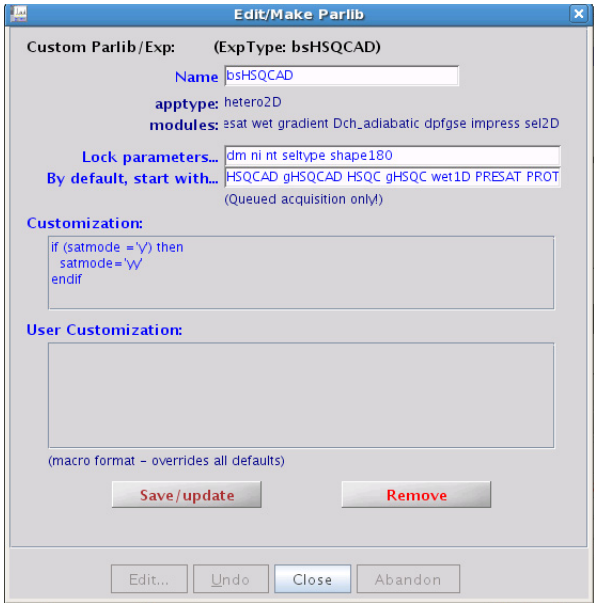


Table 29 Study Clones submenu (continued)

Item	Description
	<p>Both the apptype and the list of modules are different from the PROTON protocol. The user can query the values and modules for the apptype parameter by using the <code>apptype?</code> and <code>modules?</code> commands before creating a new protocol from a pulse sequence and parameter set that originated from outside VnmrJ, such as the user library. Assign the value for modules to the Lock parameters list and add any parameter needed to be kept at setup time.</p> <p>In the above PROTON experiment, basic protocols include the following key concepts:</p> <ul style="list-style-type: none"> • Name—Displays the name of the set parameter and the protocol's button. • apptype—Displays a generic helper macro name for setting up a group of protocols. In VnmrJ apptype is optional. Common apptypes are std1D, homo2D, hetero2D, and lstd1Dmodules. (The names for the little min-parameter sets that are combined to create the existing parameter set.) • Module—Displays a module and all of the values for the parameters in that module, type on the command line module ('popup','modulename'), for example module ('popup','presat'). • Default Starting Experiment—Define a default experiment as a starting point to morph the default experiment into the desired experiment. Type <code>REQexp?</code> from the command line to query the value for the default starting experiment. • Customization—Shows the contents of the macro name_setup or in the case of this example PROTON_setup. Not editable in this tool but it can be edited in a text editor. • User Customization—Creates a macro from the text typed in this field. userprotocolname or for this example userPROTON.
Show Library	Opens a graphical tool to backup then remove old buttons and to review the executable actions of buttons.
Show Library	Opens a graphical tool to backup then remove old buttons and to review the executable actions of buttons.

Standard Calibration Experiments submenu

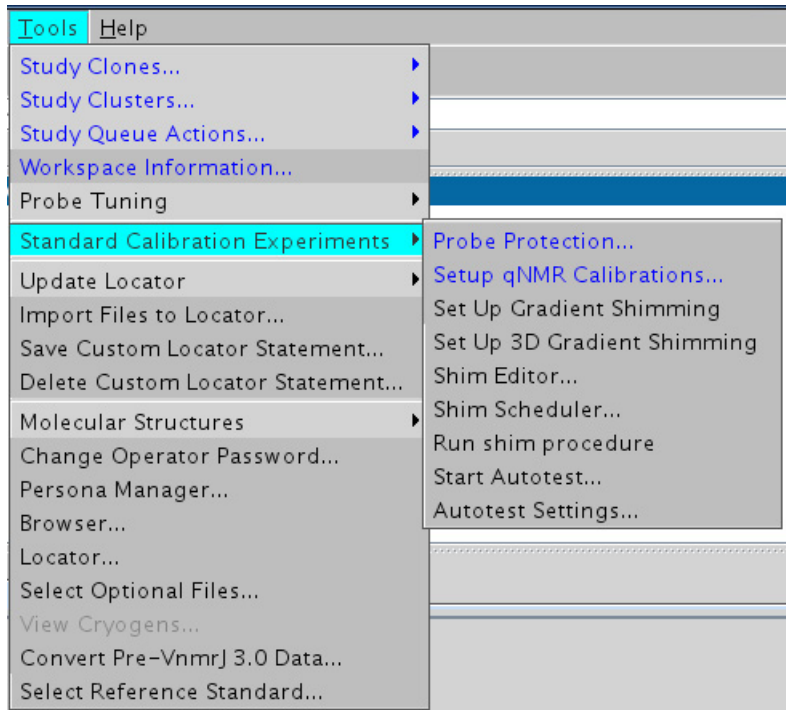


Figure 27 Standard Calibration Experiments submenu

Table 30 Standard Calibration Experiments submenu

Item	Description
Probe Protection...	Opens a window to set up maximum allowed power values for probe protection. See below.
Setup qNMR Calibrations...	Setup qNMR Calibrations-Opens a window to calibrate and set up quantitation tools.
Setup Gradient Shimming	Setup Gradient Shimming-Loads the pulse sequence and panels for making a shim map for gradient shimming.
Shim Editor	Opens the Shim Menu Editor, where you set up or change shim menus.

Table 30 Standard Calibration Experiments submenu

Item	Description
Shim Scheduler	Opens the Shim Maintenance Scheduler, where you set up and schedule shim service.
Run Shim Procedure	Executes the shim procedure.
Start Autotest	Opens the Auto Test window, where you can configure and run an autotest. For more information, see the <i>Agilent AutoTest User Guide</i> .
Autotest Settings	Opens the Autotest settings window, where you select the parameters for AutoTest. For more information, see the <i>Agilent AutoTest User Guide</i> .
Setup 3D Gradient Shimming	Selection appears only if this option is installed. Loads the pulse sequence and panels for making a 3D shim map for gradient shimming.

Probe Protection

Probe Protection settings are global parameters used for sample protection. Recommended settings are to leave power protection on and using default settings.

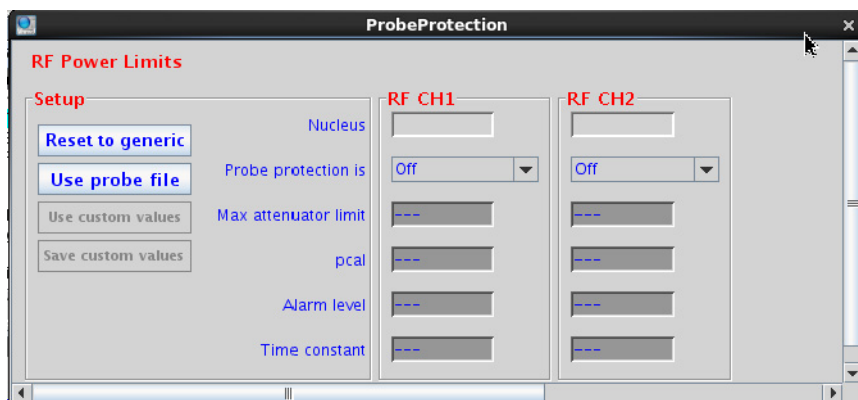


Table 31 Probe Protection

Item	Description
Use probe file	Updates pcal, alarm level, and time constant values using values from the probe.
Use custom values	Use for advanced sample protection.
Save custom values	Saves advanced sample protection values from the interface.
Nucleus	Displays channels with associated nuclei.
Probe protection is:	<ul style="list-style-type: none"> • On—Default state terminates experiments that are predicted to exceed the alarm level. When this happens, PSG prints the relevant rf channel on line 3 and the error window. • Verbose—Same settings as "on" and prints diagnostic messages in process>text. • Warn verbose—Alerts when 90% of alarm is reached and terminates when alarm is reached. • Warn only (off)—Alerts when 90% of alarm is reached and will not terminate. • Off—Will not terminate when the alarm level is reached.
Max. attenuator limit	Displays associated maxattench value for each channel.
pcal	Tpwr level associated with 2 watts of power at the probe.
Alarm level	An energy (power * time) value at which the probe may be damaged or the sample may be overheated.
Time constant	The length of time, typically 5 seconds, for the probe cooling processes.


View Menu

Use this menu to access parts of the interface and restore the interface elements. Close the window by clicking the **Close** button in the upper right hand corner of each window. Access the hardware or graphics toolbars using the Toolbars submenu.



Figure 28 View menu

Changing Display Colors

Using the Styles and Themes window, you can change the colors used for the display and printing of various items in VnmrJ. To open the Styles and Themes window, click the Display Options icon. 

There are four user interface styles available (Default, Classic, Dark and Light), and several look and feel (LAF) choices to customize the interface.

You can also set default colors for drawing 1D and 2D spectral graphics and labels (Display). 1D and 2D spectral drawing have adjustable line thickness for better visibility and report generation. And, when zooming 1D and 2D data, VnmrJ can automatically switch to thicker lines.

The Plot functions let you set up defaults for printing and plotting.

To change the look and feel of the VnmrJ user interface

- 1 On the System Toolbar, click the Display Options icon. 

The Styles and Themes window opens.

- 2 Select the **UI** button.

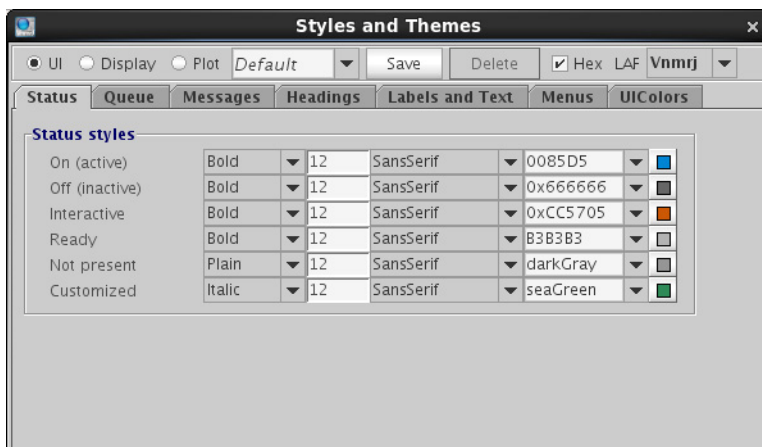


Figure 29 Styles and Themes window with UI selected

- From the drop-down menu, select **Default**, **Classic**, **Light** or **Dark** to change the interface color theme. You can also customize further using the tabs and selections available.

To change color options for spectral drawing

- On the System Toolbar, click the Display Options icon.



The Styles and Themes window opens.

- Select the **Display** button.

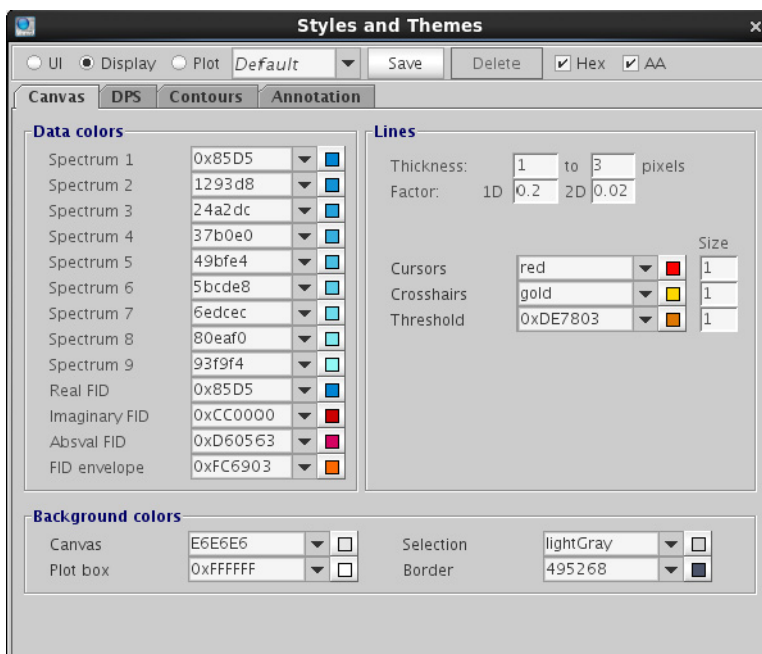


Figure 30 Styles and Themes with Display selected

- Set the widths of lines used to draw 1D and 2D spectral graphics. There are two Line Thickness settings, used depending on whether the number of points in the spectral region is much smaller than the number of pixels in the spectral drawing canvas. The corresponding

2 VnmrJ Interface

To change color options for plotting

Factor parameter controls when the thicker lines will be used. For example, if the Factor parameter is set to 0.2, the thicker lines will be used whenever the ratio of points to pixels is 0.2 or less. If desired, you can set both Thickness values to the same number, so that line drawing will not change when spectra are zoomed.

- 4 Use the other tabs and selections to customize the display of pulse sequence (DPS), Contours, and Annotations.

To change color options for plotting

- 1 On the System Toolbar, click the Display Options icon.



The Styles and Themes window opens.

- 2 Select the **Plot** button.

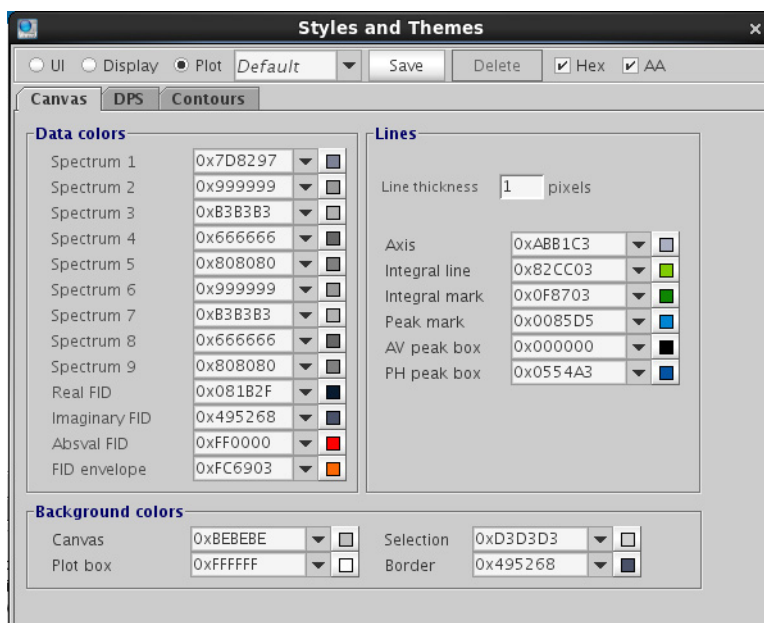


Figure 31 Styles and Themes with Plot selected

- 3** Set the widths of lines and colors to be used to plot 1D and 2D spectral graphics.

The Line thickness sets the same line thickness to all lines associated with the spectral drawing.

- 4** Use the other tabs and selections to customize the plotting of pulse sequence (DPS), Contours, and Annotation.

Experiment Selector Editor

The Experiment Selector Editor lets you customize how protocols are displayed in the Experiment Selector in the Protocols vertical panel. To open the Experiment Selector Editor, on the main menu click **Edit > Edit Experiment Selector**.

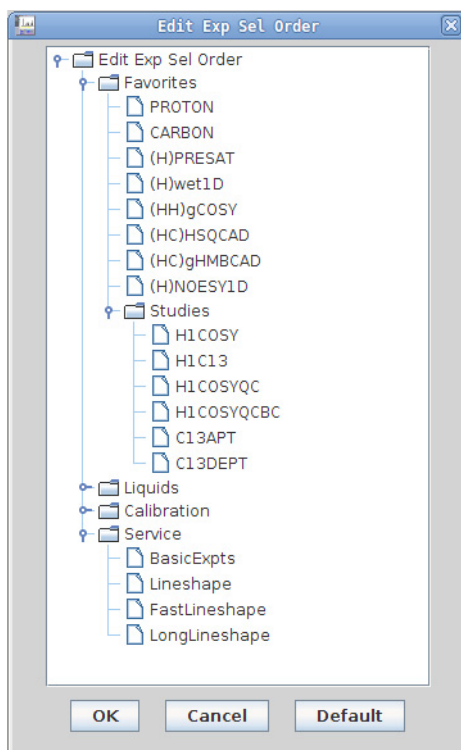


Figure 32 Experiment Selector Editor

The Experiment Selector has the following capabilities.

Table 32 Features of the Experiment Selector Editor

Feature	Action
Drag a protocol or folder to a new location.	Changes the order in which folders and protocols are displayed
OK	Closes the panel and updates the Experiment Selector
Cancel	Discards any changes and keeps the Experiment Selector unchanged
Default	Reverts to the Agilent default order and names
Click 3rd mouse button on an item	Opens a menu
Copy	Copies selected item to clipboard
Paste	Pastes the “copied” item to the position above the location selected
Delete Entry	Deletes selected entry
New Folder	Creates a new Folder (or Node) in the tree. Type in the desired name and then hit the “Enter” key. Failing to hit the enter key causes the folder name to revert to “New Folder”. In that case, click on the name and edit the name, then press Enter.
Add to Favorites	Copies the selected item to the Favorites node. Note: The menu item will show the name of the first Folder/Node displayed in the Experiment Selector. Normally, the first Node is called “Favorites”, however, this is not required. If the first Node is called “Common”, then the menu will display “Add To Common” and the selected item will be copied to the “Common” Folder/Node.
Change the name for an item	Click on the name and type a new name, then press Enter. Failing to press the Enter key will cause the name to revert to its original name.

NOTE

Duplicate names are not allowed in the same folder/node. If you try to paste or rename an item in a folder/node where an identical name exists, an error appears and the action is aborted.

NOTE

Experiment Selector information contained in a protocol .xml file is always active and cannot be overridden by this Editor. Therefore, if (for example) a user has a protocol in his vnmrsys directory area which puts that protocol into a tab named ABC, that tab (or first level in the tree) cannot be permanently renamed, moved, or deleted. Nor can the protocol entry be permanently deleted or removed from its defined location. A warning will appear if one of these actions is attempted for information defined in the protocol.xml file.

File Browser

The file browser opens when you select **File > Open** from the menu bar.

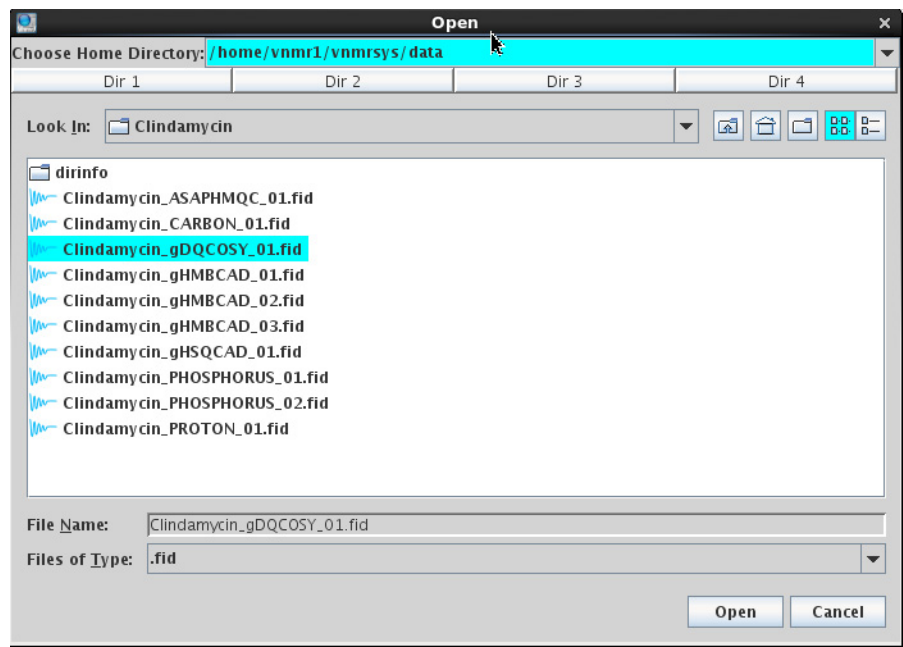


Figure 33 File browser — Open

Table 33 Icons and buttons in the file browser










Button	Description
	Go up one level in the directory tree.
	Go to user's home directory.
	Make a new folder in the current directory.

Table 33 Icons and buttons in the file browser

Button	Description
	Show a list of files and directories at the current directory level.
	Show the details of files and directories at the current directory level.
	Open selected file. Load into current experiment if it is a VnmrJ 3 data file, sequence, or parameter set.
	Save file with the name shown in the File Name: field using the extension shown in the Files of Type: field.
	Cancel selection and close the file browser.
	Click and hold to assign current directory to this button.

Status Charts

The Status charts are accessed from the Hardware Toolbar at the bottom of the VnmrJ main window.



Figure 34 Hardware toolbar in VnmrJ

When you click one of the status plot buttons, a window appears that enables you to log and examine status data.

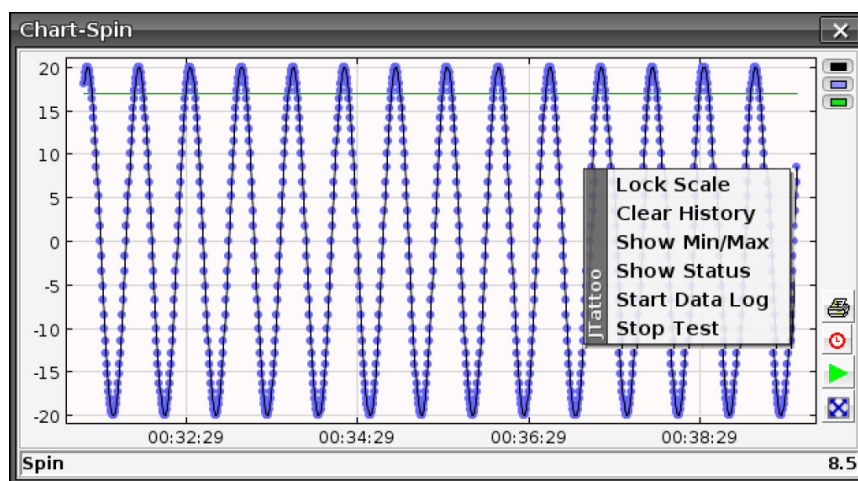


Figure 35 Example of Chart-Spin window with right-click menu

Features of the status chart window

Table 34 Features of the Status Plot windows






Item	Description
	Select color to show/hide the associated element in the chart.
	Opens the Plot Format window. Here, you can <ul style="list-style-type: none">Choose the orientation mode (Portrait, Landscape) The plot will be scaled to fit a full page either vertically or horizontally depending on the chosen orientation mode.Click print in the plot format window to cause the currently displayed data to be printed at the selected printer.
	Toggles the x-axis units between “time-of-day” and “elapsed-time” formats. Initial format is “time-of-day”.
	Click to zoom to display only the most recent data (“Tail” mode). <ul style="list-style-type: none">The amount of data displayed will be set to the x-range selected from most recent mouse “zoom-in” operation (or last 10 seconds if zoom-in action has not yet occurred).After clicking, any new plot data will be automatically added to the end of the visible region.
	Zoom out to fit data in the display (“Fill” mode).
Right-click menu items	
Lock scale	Lock the scale to the current y axis scale. (Click tail or fill button to remove scale lock.)
Clear history	Removes all collected plot data.
Show Min/Max	Displays the maximum and minimum values observed since the start of data collection. (Does not appear in the printer output.)

Table 34 Features of the Status Plot windows

Item	Description
Show Status	Shows the current status of the relevant status variable. (Does not appear in the printer output.)
Start Data Log	Opens a Save dialog box where you can specify a file into which the plot data will be saved.

Using the status chart

This section describes the components of the status chart window, and what you can do with them.

Data Area

Data area where data traces and the grid are drawn.

Toolbar

A set of buttons in a right-side vertical panel (for descriptions, see [Table 34.](#))

Popup menu

Used to select various options (right-click in data area to open the menu)

Axis Area

Values and tick marks shown to the left and below the data area, along with an x-y grid. The Horizontal axis units are displayed in either “Time-of-day” or “Elapsed-time” format. Initial format is “Time-of-day”.




- Elapsed time units are Hours:minutes:seconds measured from the time that VnmrJ is started or from when “Clear Data” is selected from the pull-down menu.
- Time of Day units are “month/day hours:minutes:seconds” in local time.

Status line

Shows variable type and value (drawn below x-axis area).

Zooming

The following modes of zooming are provided:

- Click and drag the left mouse button down and right to zoom in. Click and drag the left mouse button up and left to zoom out.
- Zoom in using the green arrow “tail” button in the toolbar. 
 - Click the green arrow “tail” icon in the toolbar  to display only the most recent data. The amount of data displayed will be set to the x-range selected from most recent mouse “zoom-in” operation (or last 10 seconds if zoom-in action has not yet occurred).
 - After you click the “tail” icon, any new plot data will be automatically added to the end of the visible region.
- Zoom out using the “fill” button 
 - Click the “fill” button in the right-side toolbar. This action sets the plot y and x range to show all current data scaled to fit within the display.

Scale locking/unlocking

- In “zoom-in” mode, the x and y ranges will be “locked” to the boundaries set by the selected “zoom-box”.
- In “Fill” mode, the x and y ranges will autoscale to make sure that all of the data in the plot buffer is displayed (ranges are “unlocked”).
- In “Tail” mode the x range will normally be locked to show only the most recently collected data and the y range will be “unlocked” so that all of the displayed data is shown.
- In any mode scaling can be “locked” to the current y values by selecting **Lock scale** from the chart popup menu.
- Scale-locking is removed by selecting either the fill or tail button in the interface

Data collection

- The amount of data that is buffered and displayed in the status plots is controlled by the “values” attribute in the “statusbutton” sections of HardwareBar.xml (a system resource file).
 - The Current default is 1000 points. For example, values="1000".

- If more points than “values” are collected earlier points are discarded from the plots.
- Setting “values” to -1 will cause all data to be retained.
- All collected Plot data can be removed by selecting “Clear History” from the chart popup menu.

Data display

Status plots can show the following “traces”

- “Lines” a set of connecting lines drawn between successive data points
- “Points” individual “dots” displayed for each collected point
- “Set Value” The “target” or setpoint value for the relevant status variable type

Each display trace can be made visible or hidden by selection the associated color toggle button in the right-side toolbar.

Data Logging

A text file containing status history can be generated as follows:

- Right-click in the chart area, and then select **Start Data Log** from the display popup menu.

This opens a standard system “save” dialog window (see [Figure 36](#)). Browse to an existing file or type the name of a new file to save the log data. Click **Save File Path**. When the file dialog closes, new data will automatically be written to the specified file. Any old data in the file will be discarded (That is, a new file will be created each time).

2 VnmrJ Interface

Using the status chart

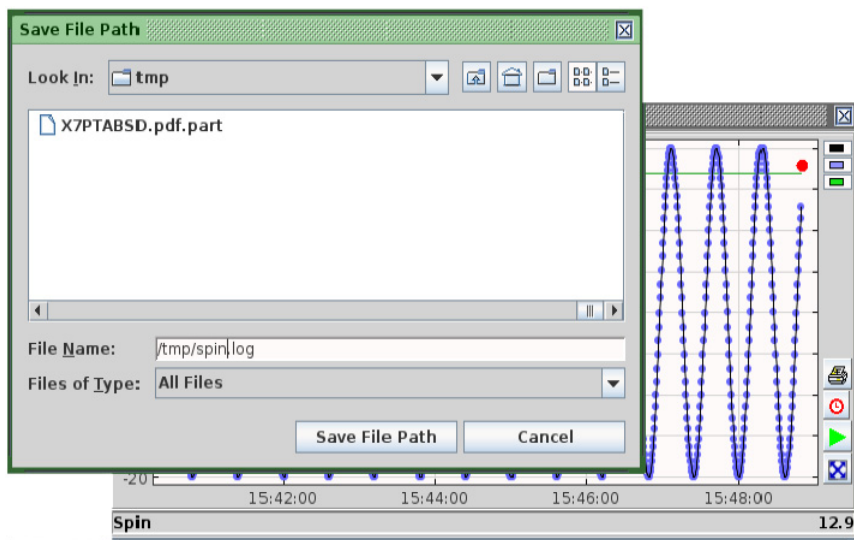


Figure 36 Status chart with save window

- Only new data acquired in the plot after logging starts will be added to the file.
- If logging is active a red “recording dot” will be displayed in the upper right area of the plot (see [Figure 36](#)).

To stop data logging, right-click in the data area to open the chart popup menu and select **Stop Data Log**.

Log file data format is:

“Time-of-day” “elapsed-time” “value”

Example output

DATE TIME VALUE

10/01 11:33:14 000:00:45 -17.3

10/01 11:33:15 000:00:45 -16.4

10/01 11:33:15 000:00:46 - 15.3

Plotting

- 1 Click the “printer” icon in the right-side toolbar to open a system Print dialog box.
- 2 Select the printer, orientation, and appearance in the General, Page Setup, and Appearance tabs.
- 3 Click **Print** in the plot format dialog to cause the currently displayed data to be printed at the selected printer. The plot will be scaled to fit a full page either vertically or horizontally depending on the chosen orientation mode.

Annotation

To include optional text information, right-click in the data area, and select the following options from the popup menu:

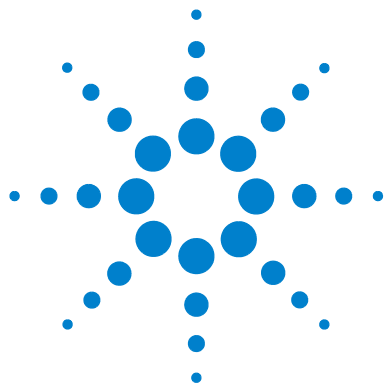
Show Min Max will show the maximum and minimum values observed since the start of data collection.

Show Status will show the current status of the relevant status variable.

Note: Annotation text does not appear in the “printer” output.

2 VnmrJ Interface

Using the status chart



3 VnmrJ Preferences

Templates Tab	104
Automation Tab	112
SQview Tab	119
Queue Tab	120
eOptions Tab	123
Data Mirror Tab	126
SampleTags Tab	128
UserPrefs Tab	130

The default behavior of VnmrJ at the user account level has several customizable options. These options are different from those that can be accessed by the administrator of VnmrJ. The optimal operation of these customization options requires a properly set up probe file for the probe in use.

Select **Preferences** from the **Edit** menu on the main VnmrJ window to access the VnmrJ preferences setup window. Note that some of the options appear only if the **Enable Email Options** check box has been selected. Ensure that an email server has been properly configured on the host computer.



Templates Tab

The **Edit > Preferences > Templates** tab can be used to select where the data is saved and how data is to be named.

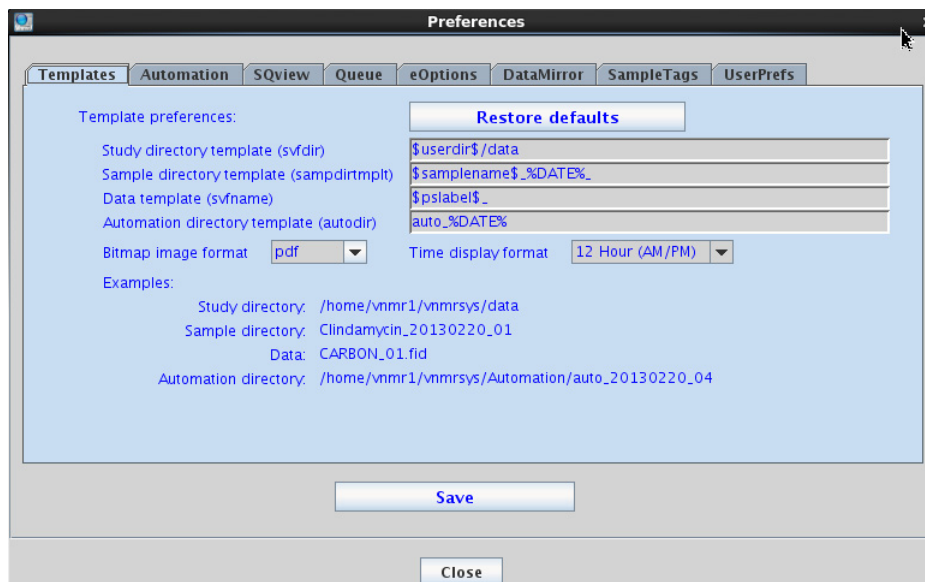


Figure 37 Preferences—Templates tab

The following describes the options for the Templates tab:

Table 35 Options in the Preferences—Templates tab

Item	Description
Study Directory Template	Defines where the data for all the samples is stored. It defines the parameter <code>svfdir</code> . Refer to Format of the Entry Level for the exact format of this entry.
Sample Directory Template	Defines the directory inside the study directory where all the data for any sample are stored. It defines the parameter <code>sampdirtmplt</code> . This is always a subdirectory for the study directory, <code>svfdir</code> . Refer to Format of the Entry Level for the exact format of this entry.
Data Template	Defines the actual name that is given to the individual fid files. It defines the parameter <code>svfname</code> . This file is saved inside <code>sampdirtmplt</code> . Refer to Format of the Entry Level for the exact format of this entry.
Automation Directory Template	Defines location of the automation directory, where the system stores all the information to do the automation run. It defines the parameter <code>autodir</code> . Refer to Format of the Entry Level for the exact format of this entry.

Table 35 Options in the Preferences—Templates tab

Item	Description
Bitmap Image Format	<p>Drop-down menu with the following selections:</p> <p>tif—This creates a TIFF format document, typical for accurate representation of bitmap images. A TIFF document can be read with most common image and word processing programs.</p> <p>pdf—This creates a document according to the Adobe PDF™ document format. A PDF document can be read using Adobe Acrobat Reader. The PDF documents created from VnmrJ require Adobe Reader version 5 or higher.</p> <p>pcx—This creates a document using the PCX protocol, adequate for representations of bitmap images. PCX documents can be read with most common image and word-processing documents.</p> <p>jpg—This creates a JPEG format document accurate for the representation of real-life photos. JPEG documents can be read with most common image and word processing programs.</p>
Time display format	Select the desired format for displaying time from the drop-down menu.

Format of the Entry Level

The format used to enter the directories in the first three fields is the same as was used previously for "autoname". A detailed description of it appears under the description of "autoname" in the *Command and Parameter Reference Manual* for VnmrJ. A brief description is shown here.

There are three types of text that can be entered in these fields. All the options can be mixed together generating very powerful saving options.

- 1 Fixed text, such as `"/home/vnmr1/data."` This is interpreted as is without any special translations. If the text defines an absolute directory path then this path will be used (example `/vnmr/data`). If there is no absolute path then the directory will be created as a subdirectory of the previously defined directory path. So if sample

directory is defined as "mysamples/today" then the subdirectory will be created for the study directory.

- 2 Text enclosed in "\$" signs. This will substitute the enclosed text with the value of the VnmrJ parameter with the same name. For example **\$samplename\$** will be substituted with "mysample" if **samplename='mysample'**. Any VnmrJ parameter can be used for this. The most useful ones will be the ones defining sample parameters (**studyowner**, **samplename**, **solvent**, etc.) and experiment parameters (**plabel** which shows what experiment is run for example). So "**\$samplename\$/\$plabel\$**" will translate into "mysample/PROTON" in the case of a proton spectrum.
- 3 Text enclosed in "%" signs. This will substitute the enclosed text with the entry on the actual enterQ file being used. This can be very cryptic but some useful options are outlined here. For more detailed description the user is referred to the *Command and Parameter Reference Manual*.
 - a %DATE%: This will be substituted with the date that the spectrum is acquired. Other allowed entries are %DAY%, %MO%, %MOC%, %YR%, %YR2%, %HR%, %MIN% and %SEC%.%RX% where X is a number: This will be substituted with a numerical extension in the form "00", "01", "02" etc. The number X defines the number of digits that will be used for the extension, so %R2% will generate "01" while %R3% will generate "001" etc. This ensures that a unique name is generated for every directory created. A %R2% will be appended automatically to the study, sample and automation directory templates if no "%RX%" is explicitly defined. One can suppress the numbering entirely using %R0%. This is, however, not recommended for the Data Template as it may accidentally overwrite data. When using %R0% at the end of the "Sample Directory Template", all data on the same sample but from different Automation Runs (typically from two or more consecutive days) will be saved into the same sample directory, which may be desirable.

The examples at the bottom of the **Templates** tab show what each of the strings entered translates to. Some useful examples are displayed below.

The example shown in [Figure 38](#) on page 108 will direct all study data to *home/chemp/vnmrsys/data/<operator name>*. This is accomplished by the use of the **\$operator_\$** argument. Note that the more proper parameter to use in VnmrJ would be **\$studyowner\$**. The data for each sample will further be saved in a directory named <notebook>_<page>, where notebook and

3 VnmrJ Preferences

Templates Tab

page are the notebook and page numbers that were entered in the Start tab of the parameter panel. The revision number is suppressed here. The actual FIDs will be saved as <experiment>_<samplename> with a three digit revision after it.

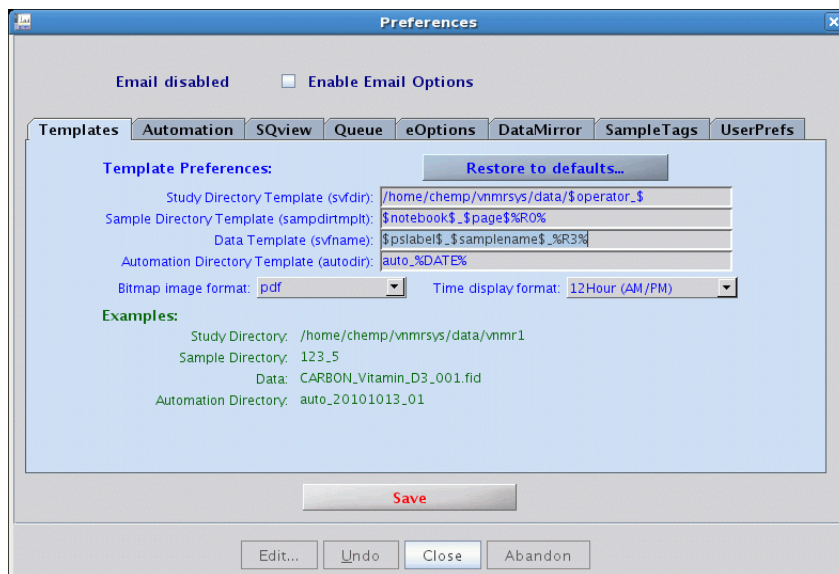


Figure 38 Data saving templates

The example shown in [Figure 39](#) on page 109 illustrates the inclusion of a fixed text in the sample directory name (Book_<notebook>).

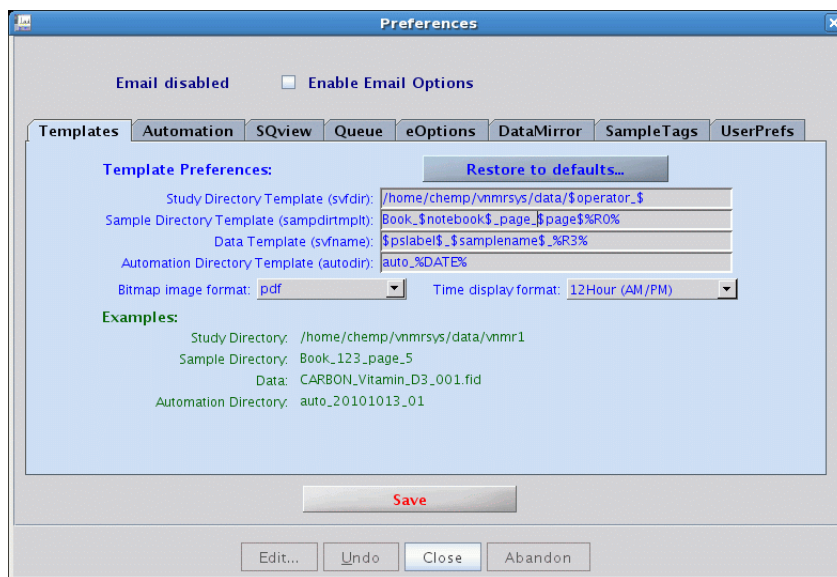


Figure 39 Example—Inclusion of text in Sample Directory Template

The example in [Figure 40](#) on page 110 shows the date is part of the directory name.

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Templates Tab

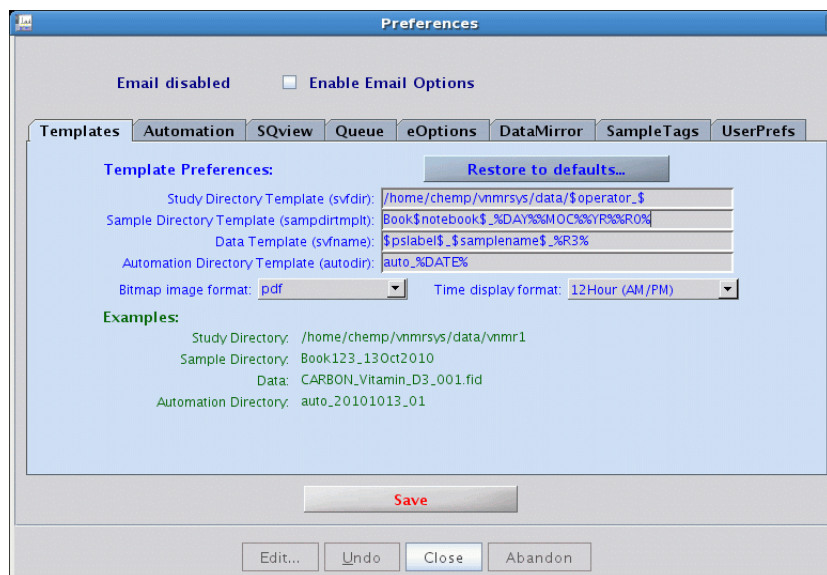


Figure 40 Example—Inclusion of date in Sample Directory Template

Data from one operator can be sorted to different subdirectories according to the date the data was recorded.

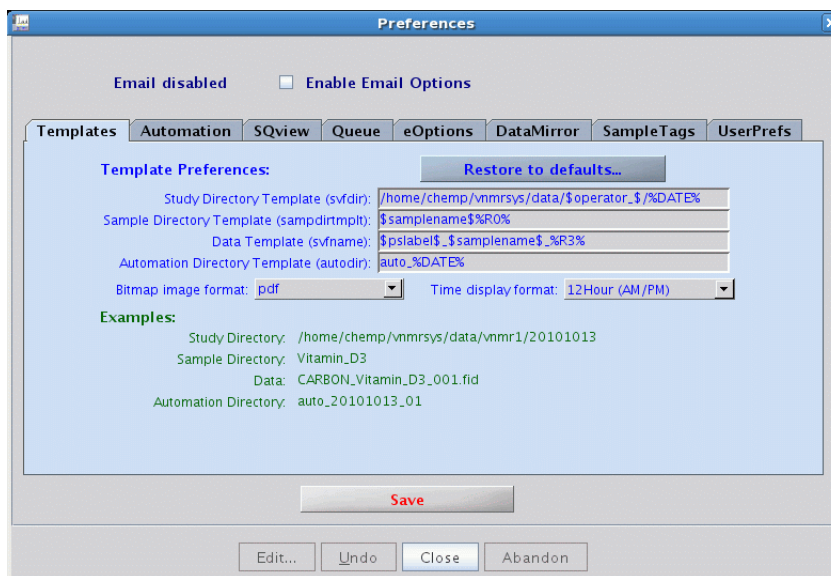


Figure 41 Example—Inclusion of date in Study Directory Template

NOTE

Any changes done on this window should be saved before exiting the window. Otherwise, changes are lost. The changes require the creation of a new automation run to become active.

Automation Tab

Automation preferences are set in the **Edit > Preferences > Automation** tab.

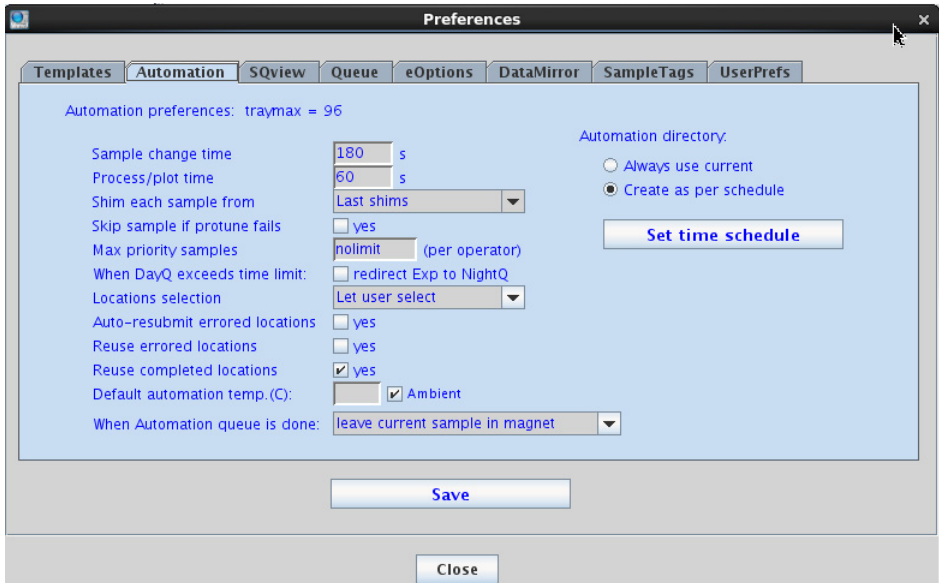


Figure 42 Preferences–Automation tab

The **Automation** tab defines the defaults that will be used for automation experiments. Defaults are described in the following table.

Table 36 Preferences — Automation tab

Item	Description
Traymax	This parameter indicates what type of autosampler is actually used. If this is not set up properly then it needs to be changed in the System Preferences window. Only vnmr1 can make this change.
Sample Change Time	This is the approximate time required for the sample change using the autosampler, the finding of the z0 value for the automatic locking, the automatic tuning (ProTune) and the automatic shimming. The default value is 120 seconds which should be sufficient for most cases. It is generally advised to round the time rounded up, so if the system needs 100 seconds to enter instead 120. This ensures that the time calculations will not fall off because of too short estimates.
Process/Plot Time	This is the time required to process, plot and save the spectrum. The default value of 10 seconds should be sufficient. Use a longer value only if you are using a slower computer.
Shim Each Sample From	<p>This defines the shims started by the system when the shim tries to shim a new sample. There are two options:</p> <ol style="list-style-type: none"> 1 Last shims: Last shims use the shims from the previous sample run 2 Default shims: Default shims load the shim file that is in the probe file. In actual operation with properly prepared samples the outcome should be equivalent. If however that range of samples and tubes used varies widely then the use of Default shims give more consistent results.
Skip Sample if Protune Fails	<p>This check-box appears only if ProTune is installed. If checked then the system skips the sample in the unlikely event that the automatic tuning fails.</p> <p>When the check box is not selected, the data acquisition proceeds.</p>
Maximum Priority Samples	This defines the maximum priority samples an operator can submit in each automation run. The right to have priority samples is granted from the VnmrJ administrative interface.
When DayQ Exceeds Time Limit	By default, with "redirect Exp to NightQ" unselected experiments that are attempted to be added to the DayQ but exceed the DayQ limit result in an error and are not added to the StudyQ. With this option selected, such Experiments are added to the NightQ instead, Select redirect Exp to NightQ if the DayQ has exceeded it's time.
Locations Selection	This drop-down menu defines whether users will be allowed to choose the sample location manually (Let User Select option) or not (Next Available option). The Next Available option will ignore the user input and submit the experiment queue for the next available sample position in the autosampler.
Auto-Resubmit Errored Locations	Select yes to automatically resubmit samples back into the queue that have errored.

Table 36 Preferences — Automation tab (continued)

Item	Description
Reuse Errored Locations	Select yes to automatically reuse samples that have errored.
Reuse Completed Locations	Select yes to automatically reuse samples that have completed.
Default Automation Temp. (C)	Defines the temperature default for all samples run in automation. If at the time New Automation run is started, the temperature control is enabled in the account, a new temperature can be specified at customization time. The software checks if the chosen temperature is safe given the choice of solvent for that sample. In addition, if variable temperature is allowed in sample changer automation, it is important to set the value for the tin (temperature interlock) parameter contained in <code>~/vnmrsys/modules/cpQdefaults</code> be set to "w" so that the sample changer will not insert any sample until the probe has reached the requested temperature.
When Automation Queue is Done	<p>This dropdown menu defines what should happen when the last sample of a StudyQ is done and there are no more sample in the queue:</p> <ul style="list-style-type: none"> • put standby sample (loc=>traymax>) into magnet. This removes the last sample run from the magnet thus allowing its owner to retrieve it from the autosampler tray. The standby sample needs to be placed in the position indicated in the field, position 96 in the case of the figure above. That particular sample location is not available for use. • leave current sample in magnet. This leaves the last sample of the StudyQ in the magnet for further use. • remove current sample from magnet. Only removes the current sample from the magnet without inserting a (standby) sample. This option is only available with 7600AS and 7510AS sample changers.
Automation Directory	<p>Determines when a new automation directory shall be created:</p> <ul style="list-style-type: none"> • Always use current. The current automation directory is used until the user creates a new one by selecting "Automation Run (autodir) -> New Automation Run" from the "Automation" menu. • Create as per schedule. A new automation directory is created on each day on which a previous NightQ ended as defined with the "Set Time Schedule" window.
Set Time Schedule	Opens the Automation Schedule window to set time schedules for automation runs. See " Automation Schedule " on page 114.

Automation Schedule

To open the Automation Schedule tool, click **Edit > Preferences** to open the **Preferences** window. Then select the **Automation** tab and then click **Set Time Schedule**.

	<input type="checkbox"/> New Autodir <input checked="" type="checkbox"/> NightQ	New DayQ begins at	MaxTime per Sample	<input type="checkbox"/> variable	Current NightQ merges at	MaxTime per Sample
Monday:	<input checked="" type="checkbox"/> New Autodir <input checked="" type="checkbox"/> NightQ	8:00AM	30min	<input type="checkbox"/> variable	6:00PM	Unlimited
Tuesday:	<input checked="" type="checkbox"/> New Autodir <input checked="" type="checkbox"/> NightQ	8:00AM	30min	<input type="checkbox"/> variable	6:00PM	Unlimited
Wednesday:	<input checked="" type="checkbox"/> New Autodir <input checked="" type="checkbox"/> NightQ	8:00AM	30min	<input type="checkbox"/> variable	6:00PM	Unlimited
Thursday:	<input checked="" type="checkbox"/> New Autodir <input checked="" type="checkbox"/> NightQ	8:00AM	30min	<input type="checkbox"/> variable	6:00PM	Unlimited
Friday:	<input checked="" type="checkbox"/> New Autodir <input checked="" type="checkbox"/> NightQ	8:00AM	30min	<input type="checkbox"/> variable	6:00PM	Unlimited
Saturday:	<input type="checkbox"/> New Autodir <input checked="" type="checkbox"/> NightQ		30min	<input type="checkbox"/> variable	6:00PM	Unlimited
Sunday:	<input type="checkbox"/> New Autodir <input checked="" type="checkbox"/> NightQ		30min	<input type="checkbox"/> variable	6:00PM	Unlimited

New automation directory will be automatically created at/after (when the first study is initiated):
Mon(8:00) Tue(8:00) Wed(8:00) Thu(8:00) Fri(8:00)
Automation directory will NOT be created on Sat Sun. Previous day automation run continues.

Save this Schedule

Close

Figure 43 Automation Schedule window—Automation Run tab

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Automation Schedule

Automation Time Scheduler

Automation Run Automation File

<-----ShortQ-----> <-----LongQ----->

Total time MaxTime per Sample Total time MaxTime per Sample

Monday: 10 hrs 30min 14 hrs Unlimited

Tuesday: 10 hrs 30min 14 hrs Unlimited

Wednesday: 10 hrs 30min 14 hrs Unlimited

Thursday: 10 hrs 30min 14 hrs Unlimited

Friday: 10 hrs 30min 14 hrs Unlimited

Saturday: 10 hrs 30min 14 hrs Unlimited

Sunday: 10 hrs 30min 14 hrs Unlimited

Save this Schedule

Close

Figure 44 Automation Time Scheduler—Automation File

The **Automation Schedule** tool allows the account administrator to define how time is allocated during the day or night for Automation Runs (AutoRuns, typically on demand sample automation) and Automation Files (AutoFiles, sample queues built for submission at a time chosen by the account administrator). Selecting the All Day check simply turns off time management and is a better choice for a research environment as opposed to an open system being accessed by a large number of chemists. Using the **Automation Schedule** tool, the user can define different queue times for

different times of the day as well as after hours' queues. At sample submission time with the NightQ enabled, a chemist can submit certain experiments to the day and others to the night.

There are definitions for each day of the week. The first two columns define when the day and night starts. They are only active if the "All day" option is not selected. The third column, Max Time per Sample, defines how much experiment time will be allocated per sample during the day. The column with the same name further to the right defines the maximum time per sample during day and night runs.

The definitions for the AutoFiles are similar, with the fields defining how long the total time for short and long queues should be and how long the maximum time per sample allowed for short and long queues should be.

The maximum time per DayQ sample can also be made variable, for example, to allow users longer runs during lunchtime. To do this:

- 1 Select the **variable** check box. A new button **set/view** appears.

The screenshot shows the 'Automation Schedule' section of the VnmrJ Preferences dialog for the day 'Monday'. It is organized into four columns: 'New DayQ begins at', 'MaxTime per Sample', 'Current NightQ merges at', and 'MaxTime per Sample'. Under 'New DayQ begins at', there are checkboxes for 'New Autodir' and 'NightQ', both of which are checked. To the right of these checkboxes are two dropdown menus: the first is set to '8:00AM' and the second is set to '30min'. To the right of the '30min' dropdown is a 'variable' checkbox, which is also checked. Under 'Current NightQ merges at', there is a dropdown menu set to '6:00PM'. Under the final 'MaxTime per Sample' column, there is a dropdown menu set to 'Unlimited'. A 'set/view' button is located to the right of the 'variable' checkbox.

- 2 Select a longer MaxTime per Sample to accommodate the longer allowed "lunch queue".
- 3 Click it to see and/or edit the detailed DayQ time limits for that day.

3 VnmrJ Preferences

Automation Schedule

DayQ time limits for Monday :

DayQ begins: 8:00 ends: 18:00
Maxtime/sample 2:00

#Start	End	Duration
#-----		
8:00	12:00	0:30
12:00	14:00	2:00
14:00	18:00	0:30

New schedule

Start: 2:00PM
End: 6:00PM
Maxtime: 30min

Add

Save Clear Cancel

- 4 Enter the new, "split" DayQ limits by adding pieces of schedule:
 - a Start with first schedule part: Select the **DayQ start** (here 8:00 AM), select the **End** (to the beginning of "lunch", here noon) and select **Maxtime** (here the default 30 min). Click **Add**.
 - b Now enter the start and end times during which longer DayQs are allowed (here 2 h). Click **Add** again.
 - c Finally, enter the second **normal** time period (afternoon). Click **Add**.
 - d Click **Save** to keep this variable schedule. The variable time limits schedule can be erased to start afresh by clicking **Clear**. Click **Cancel** if you want to exit this panel without saving.

A variable schedule may have more than three entries.

Note that any changes done on the automation schedule need to be saved (red **Save** button) before exiting the window, otherwise they will be lost. The changes will usually require the creation of a new automation run in order to become active.

SQview Tab

The **Edit > Preferences > SQview** tab allows the user to change the Study Queue display order or restore the display order to default.

Select the **Show Scout Fids** check box to display all prescan FIDs in the Study Queue.

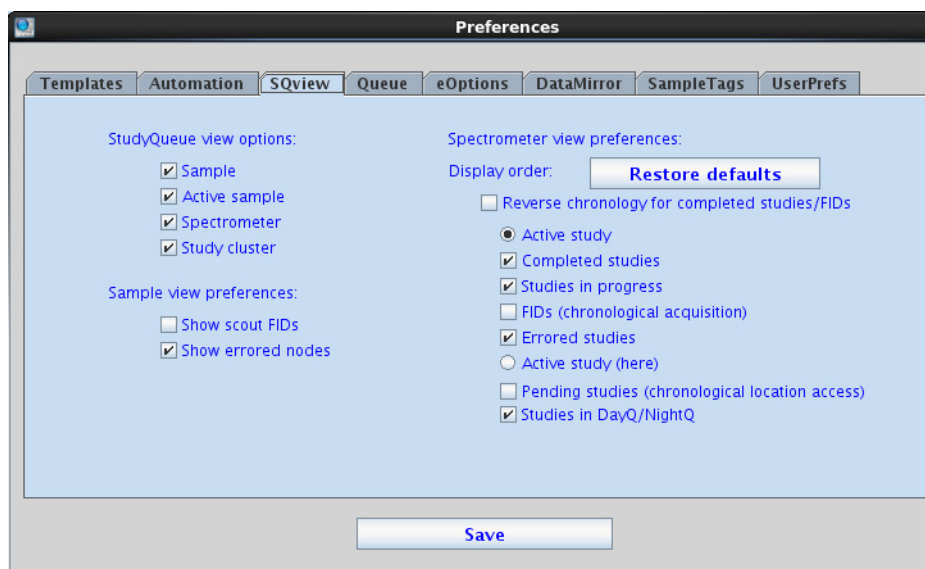


Figure 45 Preferences-SQview tab

Queue Tab

The **Edit > Preferences > Queue** tab offers options for the actual automation queue. All settings at this page are default settings. Each sample submission will start with these default settings. They can be overwritten during the sample submission.

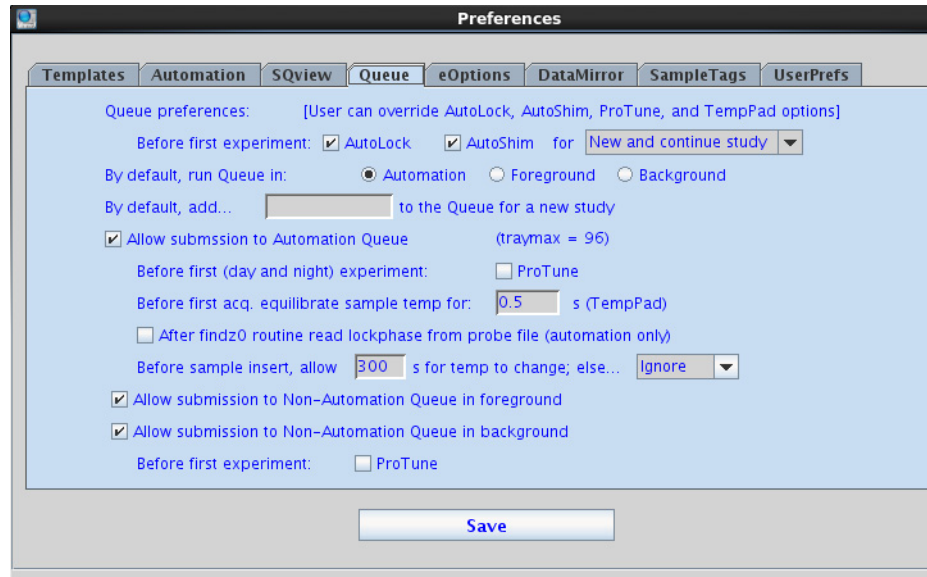


Figure 46 Preferences–Queue tab

Table 37 Preferences — Queue tab

Item	Description
Before First (Day and Night) Experiment	Defines whether the system will automatically lock and shim before the first experiment of a sample queue.
By default run queue in	Select whether to run the queue automatically, in the foreground, or in the background.
By default, add...	This option allows for an experiment to be automatically added by default to each new queue. For example, "PROTON".

Table 37 Preferences — Queue tab (continued)

Item	Description
Allow Submission to Automation Queue	This setting activates the automation queue. If this is not desired then this should be unchecked.
Before First (Day and Night) Experiment	If the system is equipped with a ProTune module, this selection defines whether the system will automatically run ProTune before the first experiment of a sample queue.
Before first acquisition equilibrate sample Temp for ___seconds (TempPad)	This sets the amount of time that the system will wait for the temperature to equilibrate after it inserts the sample into the magnet but before recording any spectra. The default value is 2 seconds which should be sufficient for experiments conducted at or near ambient temperatures. Increasing this value has got the advantage of having the temperature better equilibrated in the sample but the disadvantage of increasing the time required for each sample.
After findz0 routine read lockphase from probe file (automation only)	This is a setting for optimizing the lock system before spectra are recorded. If selected then the lock phase is set to the value in the probe file overwriting any value that has been set manually. The normal value for this box is unchecked. It should only be checked if there is a suspicion that the lock phase is not set properly. In this case the probe file should be updated with the correct value for the lock phase.
Before sample insert, allow ___seconds for Temp to change; Else...	<p>This value selects the amount of time the system waits after retrieving a completed sample but before inserting the next sample for the temperature to equilibrate. This is a useful feature when operators are allowed to run samples in automation at different temperatures. It can be used to avoid the case where a sample was run at a temperature higher than the boiling point or lower than the freezing point of the next sample's solvent. The time defined here is the maximum time that the system will wait for the temperature to equilibrate. If the temperature equilibrates faster, the run continues. The actions in the pull-down menu define what will happen if such a condition is met and there is no waiting time defined. The possible actions are:</p> <p>"Ignore", which causes the system to ignore the condition and proceed with the sample submission, "Abort", which causes the system to abort the automation run and "Alert" which gives a warning to the operator.</p>
Allow submission to Non-automation Queue in Foreground	Select to allow users to run manual Study queues on one sample in the foreground. The foreground option will run all of the StudyQ's experiments in the current VnmrJ experiment.

Table 37 Preferences — Queue tab (continued)

Item	Description
Allow submission to Non-automation Queue in Background	Select to allow users to run manual Study queues on one sample in the background. The background option runs the single Study queue in the same way as a standard automation run; by holding all the information in the autodir directory.
Before First Experiment	If the system is equipped with a ProTune module, the option for automatic tuning before the first experiment appears here. If automatic tuning before the first experiment is not desired then this box should be unchecked.

NOTE

Any changes done on this window need to be saved (red button **Save**) before exiting the window, otherwise they will be lost. The changes will require the creation of a new automation run in order to become active.

eOptions Tab

Electronic options are set in the **Edit > Preferences > eOptions** tab.

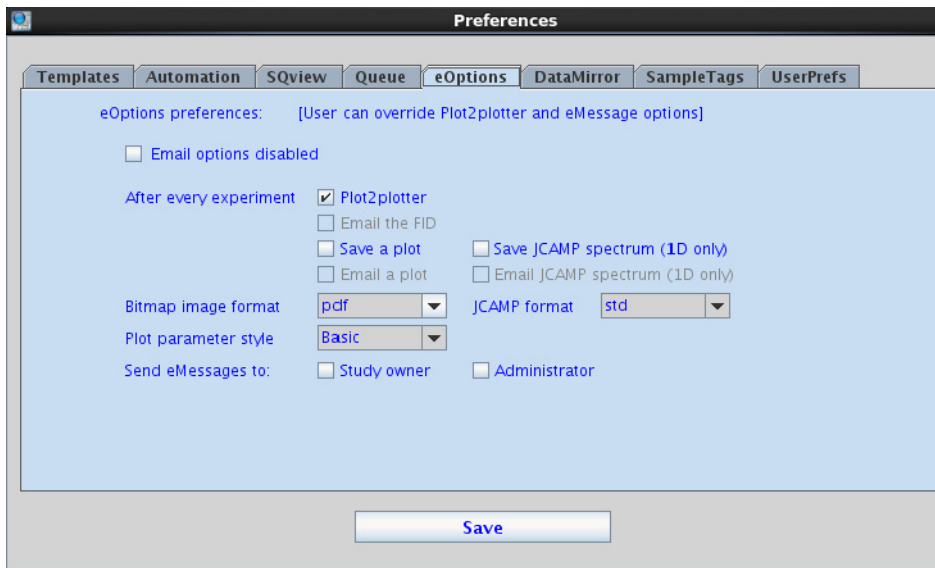


Figure 47 Preferences-eOptions tab

The **eOptions** tab defines actions that will be taken to produce an electronic output of the recorded spectra. The default options set on this tab will be the default option with every new study queue. Each operator has the option to override these in the **Start** parameter panel during the submission of the queue. The available options define what happens after every experiment.

Table 38 eOptions tab

Item	Description
Plot2plotter	Select to produce a hardcopy output to a defined plotter.
Save a plot	Select to save the plot in the format defined in the Bitmap Image Format field.

Table 38 eOptions tab (continued)

Item	Description
Email a plot	This option is enabled only if the "Enable Email Options" is checked at the top of the window. If it is, then the system will send a plot of the recorded spectrum as an e-mail attachment to the e-mail address of the operator who submitted the sample.
Save JCAMP spectrum (1D ONLY)	Select to save a 1D JCAMP Spectrum in the format defined in the JCAMP Format field.
Email JCAMP spectrum (1D ONLY)	Select to email a 1D JCAMP Spectrum as an e-mail attachment to the e-mail address of the operator who submitted the sample. This option is enabled only if the "Enable Email Options" is checked at the top of the window.
Email the fid	This option is also enabled if the "Enable Email Options" is checked at the top of the window. If it is then the system will send the entire fid of the recorded spectrum as an e-mail attachment to the e-mail address of the operator who submitted the sample. This option should be used with care as NMR spectra, especially multidimensional ones, can grow to very large sizes which may not be accommodated by the available capacity and bandwidth of the e-mail servers.
Bitmap image format	<p>This pull-down menu defines the format of the plot that will be saved or e-mailed. Options are:</p> <ul style="list-style-type: none"> • tif. This creates a TIFF format document, typical for accurate representation of bitmap images. A TIFF document can be read with most common image and word processing programs. • pdf. This creates a document according to the Adobe PDF™ document format. A PDF document can be read using Adobe Acrobat Reader. The PDF documents created from VnmrJ require Adobe Reader version 5 or higher. • pcx. This creates a document using the PCX protocol, adequate for representations of bitmap images. PCX documents can be read with most common image and word-processing documents. • jpg. This creates a JPEG format document accurate for the representation of real-life photos but are less recommended for line-art pictures. JPEG documents can be read with most common image and word processing programs.
JCAMP Format	<p>This pull-down menu defines the JCAMP format for 1D that will be saved or e-mailed:</p> <ul style="list-style-type: none"> • std. Contains the processed spectrum in binary encoded text format. • xy. X-Y ASCII text table of the processed spectrum that can be read by spreadsheet programs like Excel or mathematical software.

NOTE

Any changes done on this window need to be saved (red button **Save**) before exiting the window, otherwise they will be lost. The changes will require the creation of a new automation run in order to become active.

Data Mirror Tab

VnmrJ provides the option to automatically store another copy of the data recorded in automation on a local or remote disk. Use the **Edit > Preferences > Data Mirror** tab to set up this feature.

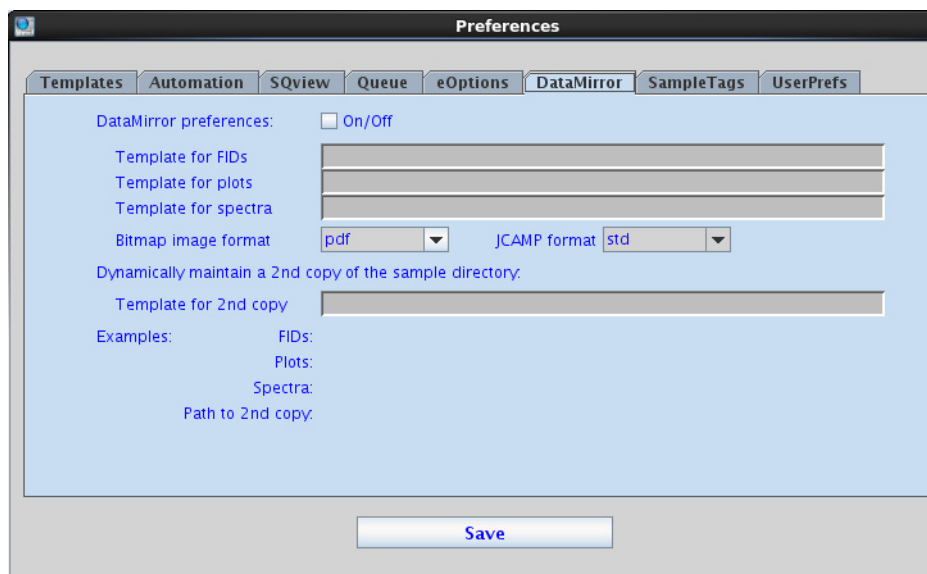


Figure 48 Preferences — DataMirror tab

If the option is turned on (**ON/OFF** check box at the top of the window) the feature is activated and each dataset is saved twice: first at the locations defined in the **Templates** tab, second at the locations defined on this tab. The syntax for the templates is identical to the ones in the [“Templates Tab”](#) on page 104.

There are three types of files that can be mirrored: FIDs, Plots and Spectra. The entire sample directory can be mirrored as well which is defined in the last field on this tab.

Data mirroring can be useful for backup. When used with externally mounted or network drives, users can access data at other systems. This can have several benefits, reducing the load on the spectrometer computer host and reducing the need for extensive network transfers of data.

A complementary tool to this is the UNIX function of rsync. The way to setup rsync is described in the *User Programming Guide*.

NOTE

Any changes done on this window need to be saved (red **Save** button) before exiting the window, otherwise they will be lost. The changes will require the creation of a new automation run in order to become active.

SampleTags Tab

The **Edit > Preferences > SampleTags** tab allows the system administrator to define which parameters required for each sample will be recorded and transferred to all experiments in one sample queue.

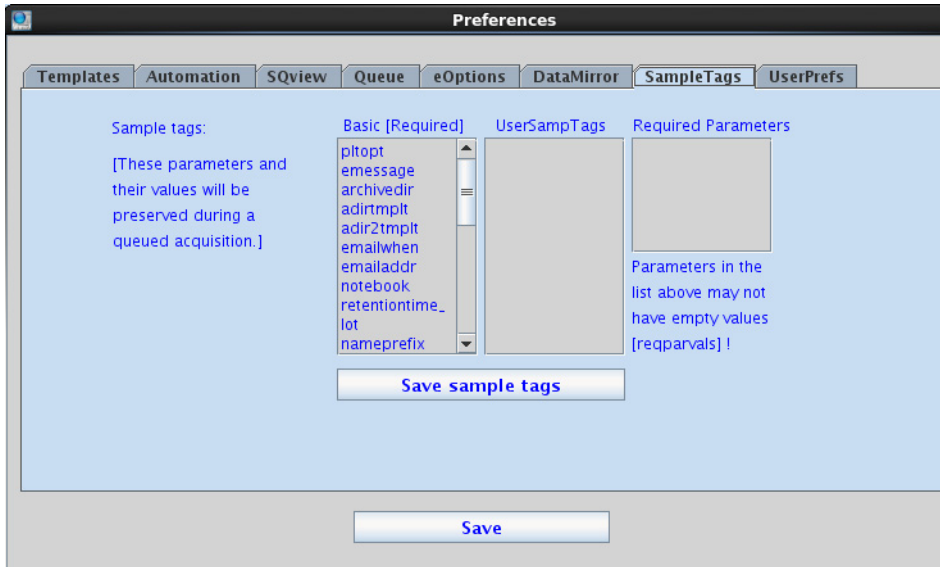


Figure 49 Preferences — SampleTags tab

The window displays three lists of parameters.

Table 39 Preferences — Sample Tags tab

Item	Description
Basic	These are parameters are the standard set captured by default with every parameter set, such as samplename, the data directory where data are saved, etc.

Table 39 Preferences — Sample Tags tab (continued)

UserSamp Tags	Any parameters that the account owner has created and wants to have available for all spectra. For example, a string variable named <code>charge_code</code> could be created and then added to this list. That variable would then be captured with every spectrum and would be available for use in the data save templates.
reqparvals	This column contains the parameters that will be absolutely required to be entered, otherwise the automation run will not be able to proceed. Note that any parameter appearing as data saving parameter in the Templates tab will automatically be required and does not need to be entered again here.

NOTE

Any changes done on this window need to be saved (red **Save Sample Tags** button) before exiting the window, otherwise they will be lost. The changes will require the creation of a new automation run in order to become active.

UserPrefs Tab

The **Edit > Preferences > UserPrefs** tab allows the account administrator to set up operator options.

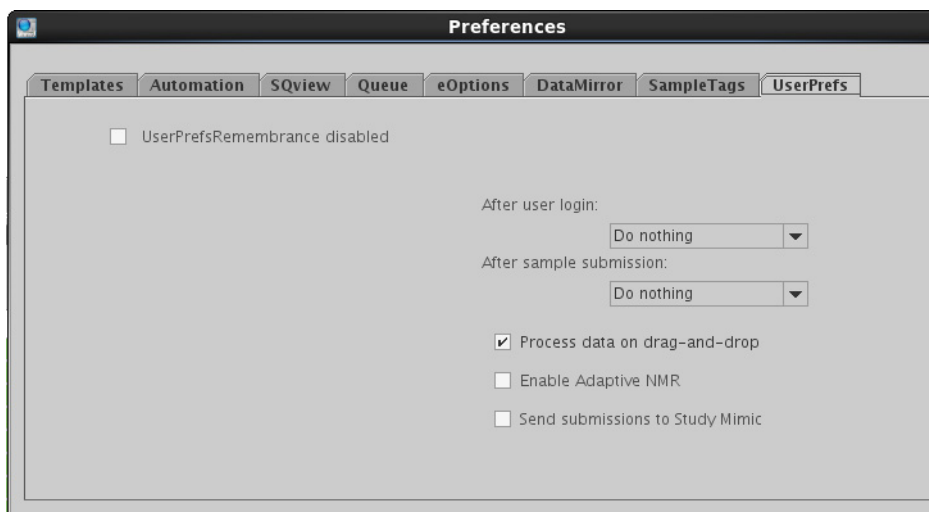


Figure 50 Preferences — UserPrefs tab

The first task the account administrator should perform using the **UserPrefs** tab is to decide the action to be taken after sample submission.

Table 40 Preferences — UserPrefs tab options

Item	Description
Do nothing	Enables the user to leave the list of experiments in the StudyQueue in place for submission in full or in part to another location.
Clear Queue	Clears the experiment list while leaving the interface in submission mode for easy creation of a new queue for other sample locations.
Quit Submit mode	Automatically "clicks" the Done button, exits the sample submission mode and leaves the operator in Data Review mode.

Table 40 Preferences — UserPrefs tab options (continued)

Item	Description
Operator logout	Quits the submit mode and logs that operator out automatically. This may be useful for a high-volume NMR service lab with lots of operators who tend to generally submit one sample at a time.

The account administrator should next define the interface personality at the time of operator login.

The choices are as follows:

Table 41 Options for defining user interface at login

Choice	Description
Do nothing	Displays the manual/sample review mode - the user must click New Study to begin an Automation.
Show Tray	Displays the manual/sample review mode showing the automation tray
Ready for submit	Automatically "clicks" " New Study " button to enable the submission mode of the interface

In the **UserPrefs** tab, the option **UserPrefsRemembrance** enables the user to set values for any desired parameters that can be pre-set upon entry of sample submission mode. The parameters can be unique for each operator, allowing users to easily manage pre-filling of items such as preferred solvent, notebook, email address, and so on.

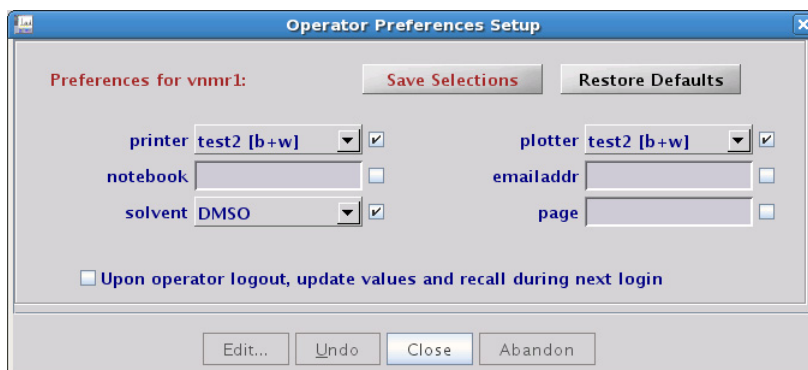
NOTE

Any changes done on this window need to be saved (red button **Save**) before exiting the window, otherwise they will be lost. The changes will require the creation of a new automation run in order to become active.

Once **UserPrefsRemembrance** is enabled and the parameter list for remembrance is defined by the account administrator, individual operators can manage their own preferences for those parameters via the menu **Edit> Operator Preferences**:

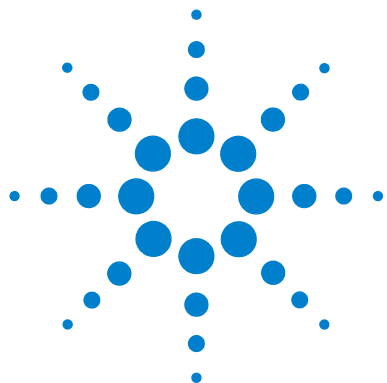
3 VnmrJ Preferences

UserPrefs Tab



In this example, the operator choices for printer, plotter, and solvent have been defined by the account administrator by entering those checks or parameter names into the entry box. After the first time, the account administrator must select the **Setup Default** values button to assign defaults for the user to start modifying values.

The menu for plotter and printer read valid devices known to the NMR spectrometer and each operator can choose the location where the data hard copy is plotted. The userRemembrance tools help prevent keying errors of items such as a laboratory notebook and preferred solvents. In this example the email address is controlled by the operator and not an administrative task for the account administrator. An operator can choose to direct PDF plot emails to any desired email address.



4

Preparing for an Experiment

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Starting VnmrJ

- 1 Log in to the workstation.
- 2 Double-click the VnmrJ icon.

The VnmrJ program window opens. See [Figure 2 on page 16](#).

Preparing for an Experiment

Before you can acquire a spectrum, prepare the sample and set up the experiment using the following steps.

- “[Prepare the sample](#)” on page 136
- “[Load the sample](#)” on page 137
- “[Tune the Probe](#)” on page 139
- “[Optimize the Lock](#)” on page 142
- “[Shim the System](#)” on page 143
- “[Set up the Experiment](#)” on page 147

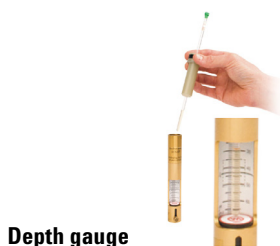
After you complete these steps, go to [Chapter 5](#), “Acquiring Data” to review how to acquire the data.

Prepare the sample

- 1 Prepare the NMR sample by dissolving the analyte in a deuterated solvent.

Use a concentration that will completely dissolve the sample, usually between ~ 1 mg/mL and ~ 50 mg/mL.

- 2 Transfer between 600 μ L and 750 μ L of the solution into a 5-mm NMR tube.
- 3 Positioning the NMR tube:
 - a Insert the NMR tube into a spinner turbine.
 - b Carefully place the spinner turbine into the top of the sample depth gauge.



- c Carefully center the sample on the thick black line in the middle of the depth gauge window
- d Remove the spinner-turbine from the depth gauge.

This step insures that the sample is positioned in the probe coil after insertion into the magnet.

Load the sample

For systems without a robot sample changer: Load the sample into the system.

- 1 Click **Eject** on the VnmrJ **Start > Standard** parameter panel to lift the current sample to the top of the magnet bore.
- 2 Carefully remove the current sample and replace it with the new sample.



- 3 Click **Insert** on the **Start > Standard** parameter panel to lower the sample into the magnet.

For systems with a robot sample changer:

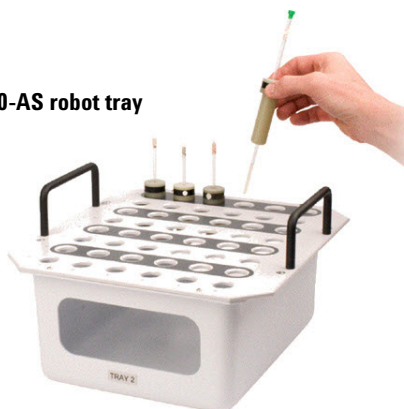
- Load your sample into the robot tray and note the location. Avoid blocking the location of the sample currently in the magnet.
7600/7620-AS: The current location is displayed on the LCD information panel.

4 Preparing for an Experiment

Load the sample

7510-AS: The location directly over the magnet bore is reserved for the sample currently in the magnet.

7600-AS robot tray



7510-AS robot tray



Tune the Probe

Probe tuning is required when there is a significant change in the polarity of the solvent. Changing from a non-polar organic solvent to a more polar organic solvent or aqueous solvent generally requires retuning the probe. Changes in the ionic strength of the solution (for example, low salt to high salt) also require retuning of the probe.


Tuning probes on systems with ProTune

Agilent NMR Systems spectrometers equipped with ProTune provide the features described in [Table 42](#). To open the ProTune window, click **Tools > Probe Tuning > Auto Tune Probe**. For details on tuning a system using ProTune, see the *Agilent VnmrJ Spectroscopy User Guide*.

Table 42 ProTune features and functions

Feature	Button, label, or message	Description
Diagnostic and tuning	Motor Communication OK, Sweep Communication OK	Status of Ethernet communications between the module and the workstation
	Abort Command	Stops current command
	Drop-down list	Probe name and channel number
	Corrected Data/Raw Data	Toggles window to display corrected or raw data
	Tune Probe (MHz)	Tunes frequency
	Threshold (dB)	Criteria for successful tuning
	Refresh (times)	The number of times the plot should update with new data
	Center (MHz)	Sets center value of the sweep range
	Span (MHz)	Sets span value of the sweep range

Table 42 ProTune features and functions (continued)

Feature	Button, label, or message	Description
	Absval Plot/Polar Plot	Toggles between polar plot (imaginary and real reflection) and absolute value plot (reflection v. frequency).
	Cmd	Executes the ProTune command in the field.
Settings	Tune/Match Backlash	Difference between the number of steps traveled from the tune frequency and the number of steps traveled back to the tune frequency.
	Tune/Match KHz/Step Size	Number of KHz the dip moves in 1 step
	Tune/Match Reflection/Step	Minimum dip movement in one step with no regard to frequency. Positive values are inside the circle of origin, and negative values are outside the circle of origin.
	Dip Frequency	Frequency the dip occurs
	Dip Reflection	Reflection value at the dip
	Match at Freq MHz	Reflection at the desired frequency
Controls and output	Tune	Tune motor control
	Match	Match motor control
		Displays the graph in full scale view.

Manual tuning using mtune

If your system does not include ProTune, you can manually tune the system using **Tools > Manual Tune Probe**. The mtune routine runs in the graphics canvas and uses VnmrJ panels. You can also run manual tuning from the command line. For details on manually tuning the system, see the *Agilent VnmrJ Spectroscopy User Guide*.

Optimize the Lock

Under computer control, the lock system maintains a constant field at the sample as the static field generated by the superconducting magnet drifts slowly with time or changes due to external interference. Locking makes the resonance field of the deuterium in the deuterated solvent coincide with the lock frequency.

The lock level can be viewed by clicking on the **Lock** button on the hardware bar.

For information on how to perform lock optimization, see the *Agilent VnmrJ Spectroscopy User Guide*.

Shim the System

There are various ways to shim the system, either manually or using an automated Proshim method. For details on all shimming methods, see the *Agilent VnmrJ Spectroscopy User Guide*.

Shimming on the lock signal manually

Monitor the intensity of the lock signal while adjusting the shim settings. Each shim setting controls the current through shim coils that control magnetic field gradients in different directions. The Z direction must be parallel to the vertical direction of the probe, and it is for this reason that the height of the sample in the NMR tube affects the Z shim settings rather dramatically. For details on how to manually shim the system, see the Agilent VnmrJ Spectroscopy User Guide.

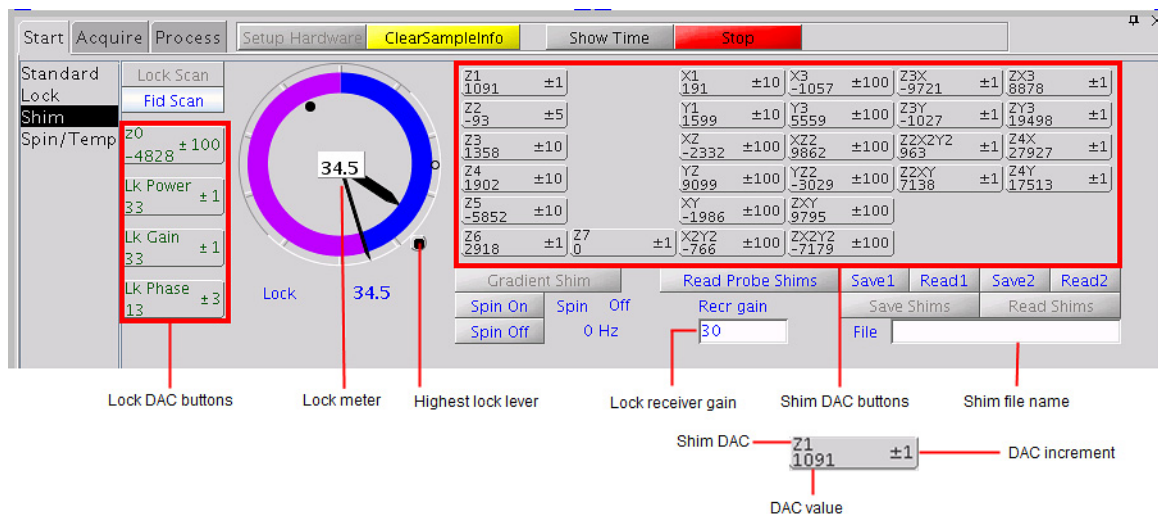


Figure 51 Location of shim buttons and controls

Proshim

With VnmrJ, you can create shim methods that can be saved and used again. The Proshim editor is used to create or modify shim methods.

To open the Proshim window to build or edit Proshim methods, click **Tools > Standard Calibration Experiments > Shim Editor**. For details on using Proshim, see the *VnmrJ Spectroscopy Users Guide*.

Use the Proshim editor ([Figure 52](#)) to:

- Display the method selected in the Current Method group
- Edit the method selected in the Current Method group
- Build a new method
- Save changes to a shim method

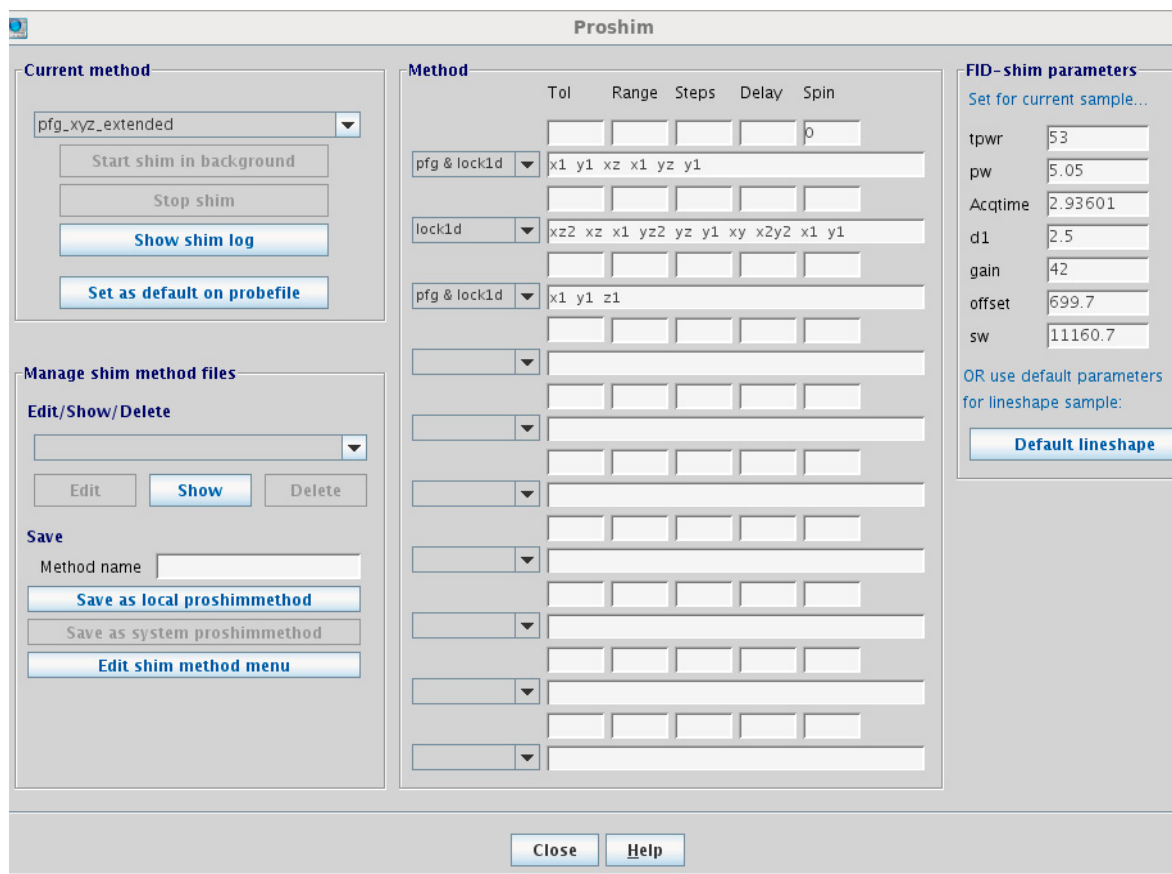


Figure 52 Proshim window

Manage shim method files

After making edits to the shim method and Start shim is run, you can save the edited Shim.

Use Manage shim method files to

- Save the edited shim method as a local or system shim method
- Display a shim method
- Edit a shim method

4 Preparing for an Experiment

Use a Proshim method in automation

- Delete a shim method

Use a Proshim method in automation

Once a Proshim method is created, it can be used to shim a sample as part of an automated study.

- 1 Create a New Study and add the desired experiments to the Study Queue.
- 2 On the Sample Info page, use the Select shim map pull-down to choose a Proshim method.
- 3 The specified Proshim method will now be used when the study is submitted to acquisition.

Set up the Experiment

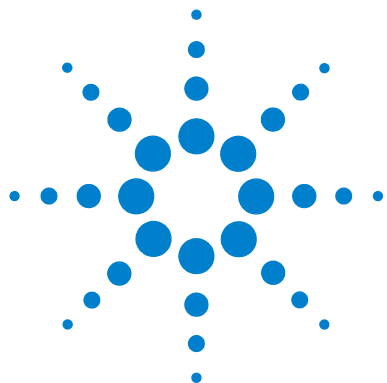
Set up the experiment using the pages in the **Start** tab in the Parameter Panel.

Figure 53 Sample Info tab of the Parameter Panel

- 1 Under the **Start** tab, select the **Sample Info** page.
 - a Fill in the information for the sample, select a **Solvent**, and enter the comments.
 - b Enter a name in the **Sample Name** field to name the sample.
 - c Define the sample, if desired, by filling in the optional **Email**, **Concentration**, **Notebook**, **Page**, and **Comment** fields.
- 2 Insert the sample (if not already loaded).
- 3 Regulate spinning and temperature on the **Spin/Temp** page.
- 4 Find Z0 and adjust the lock using the **Shim** and **Lock** pages.
- 5 Shim the system to adjust the field homogeneity using the controls provided on the **Shim** page.

4 Preparing for an Experiment

Set up the Experiment



5

Acquiring Data

Acquire a Spectrum Manually [150](#)

Build a Study Queue [151](#)

Run a Study Queue [153](#)

Using VnmrJ, spectra are typically acquired using a Study (with or without a robot sample changer). A Study is a collection of one or more experiments that you want to perform on one or more samples. However, you can also acquire a single spectrum manually. This section contains information on how to acquire spectra using VnmrJ.



Agilent Technologies

Acquire a Spectrum Manually

For manual acquisition,

- 1 Use the [Experiment Selector](#) or the [Experiment Selector Tree](#) in the Protocols vertical panel to select an experiment.
- 2 Use the pages in the Start and Acquire tabs of the [Parameter Panel](#) to view and change parameters. See the *Agilent VnmrJ Spectroscopy User Guide* for details on acquisition settings and pages.
- 3 When ready, click the green Acquire button at the top of the Parameter Panel to start acquisition.

Using a Study Queue to Acquire Data

A study is a ordered list of experiments that can be performed on any given sample. When data is collected using the Study Queue, information regarding that sample, including raw data, processed data, and plots, are linked together and automatically collected into a Sample Directory. These links allow customized information from one experiment (for example, calibrated pulse widths, optimized sweep widths, and solvent suppression conditions) to be automatically incorporated into subsequent acquisitions, and the automatic addition of high-resolution reference spectra to 2D plots.

Build a Study Queue

The Study Queue contains an ordered list of experiments you want to perform on one or more samples (with or without a robot sample changer). To create a study and add experiments to the Study Queue, use the following steps.

- 1 **Protocols** vertical panel, under **Study Queue**, click **New Study**. This puts the Study Queue in Submit mode, where you can build a study and submit it to acquisition.
- 2 Double-click to select experiments from the Experiment Selector or the Experiment Selector Tree to add to the Study Queue.

5 Acquiring Data

Build a Study Queue

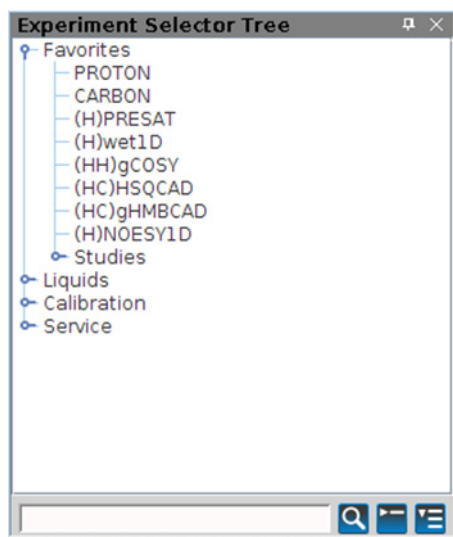


Figure 54 Experiment Selector Tree

Each experiment is added to the Study Queue and displayed as a node.

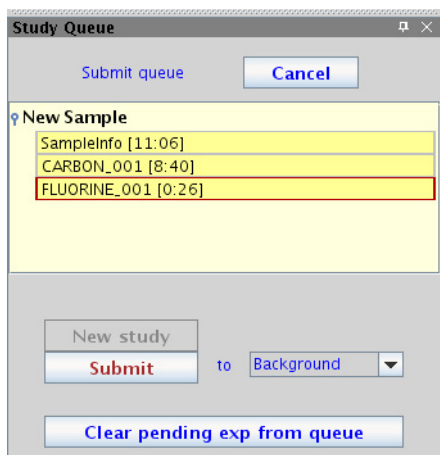


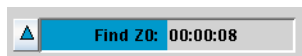
Figure 55 Study Queue

Run a Study Queue

- 1 Click **Submit** to run the Study Queue.

The Acquisition Status display on the hardware toolbar shows:

- the task being performed
- time left for the task to run
- **Idle** when waiting for a process to complete, or **Inactive** when no process is being run.



The Study Queue experiment nodes appear green if completed, blue if active, and yellow if queued.

NOTE

For systems with a robot sample changer:

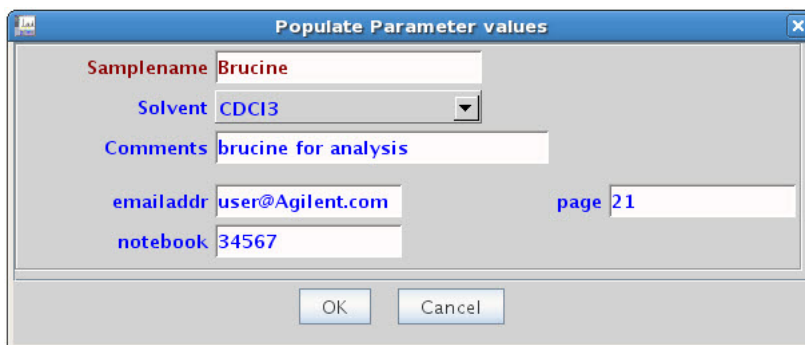
The same Study Queue can be submitted to multiple samples by:

changing the sample information, selecting a sample-tray location, and clicking **Submit**.

Using Express Submit with a sample changer

The Express Submit utility uses automation to quickly submit a sample to a specific tray location. To use Express Submit: a sample changer must be present and selected in **Edit > System Settings > System config** and a default experiment which will be added to each new study must be defined in: **Edit > Preferences > Queue** tab.

- 1 Right-click on the location in the Tray Display that holds the sample and select **Express Submit**.



The screenshot shows a window titled "Populate Parameter values" with a close button in the top right corner. The window contains the following fields and values:

- Samplename** (red label): Brucine
- Solvent** (blue label): CDCI3 (dropdown menu)
- Comments** (blue label): brucine for analysis
- emailaddr** (blue label): user@Agilent.com
- notebook** (blue label): 34567
- page** (blue label): 21

At the bottom of the window are two buttons: "OK" and "Cancel".

Figure 56 Express submit - populate parameter values

- 2 In the Express Submit popup, choose the solvent, fill in the required fields (red labels), and fill in any of the optional fields as desired.
- 3 Click **OK** to submit the default experiment to that location and close the pop-up window.

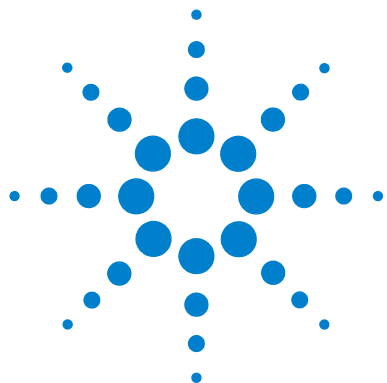
Stopping an Experiment

There are four ways to stop an experiment:

- Click the **Stop** button.
- Click **Acquisition** in the VnmrJ menu bar, and then select **Abort Acquisition**.
- Click the **Stop** button in the bar at the top of the Parameter Panel (when either the **Start** or the **Acquire** panel is selected).
- Enter aa on the command line.

5 Acquiring Data

Stopping an Experiment



6 Processing Data

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Save Current Process or Display Parameters	177

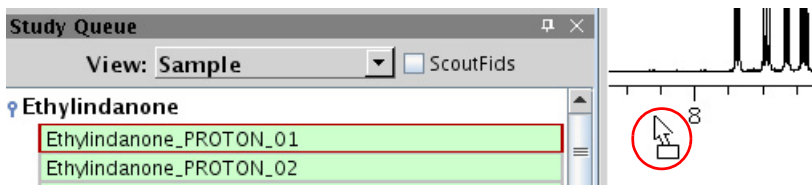
Once a spectrum is acquired, there are a wide variety of ways you can view and process the data. This section describes some of the most commonly used processing tasks. See the *Agilent VnmrJ Spectroscopy User Guide* for more detailed information.



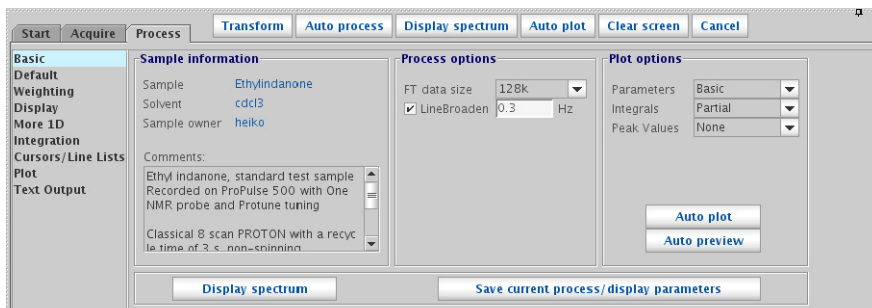
Loading Data from the Study Queue

- 1 Select and drag a completed experiment node to the graphics canvas to automatically process and load the data set.

In the following example, “Ethylindanone_Proton_01” has been selected from the Study Queue and is being dragged to the Graphics Canvas.



- 2 Click on the **Process** tab to display the data manipulation tools.
Basic processing tools are available on the **Process > Basic** parameter panel.



Retrieving a Data Set

Before you can reprocess a data set, the desired data set must be in the current workspace. For information on changing to a different workspace/experiment/viewport, see the *Agilent VnmrJ Spectroscopy User Guide*.

Use the file browser to open a data set

To retrieve the data: **File> Open**.

The resulting pop-up lets you access the desired data directory.

- 1 Select the desired file.
- 2 Click the **Open** button.

The selected data set is loaded into the current workspace.

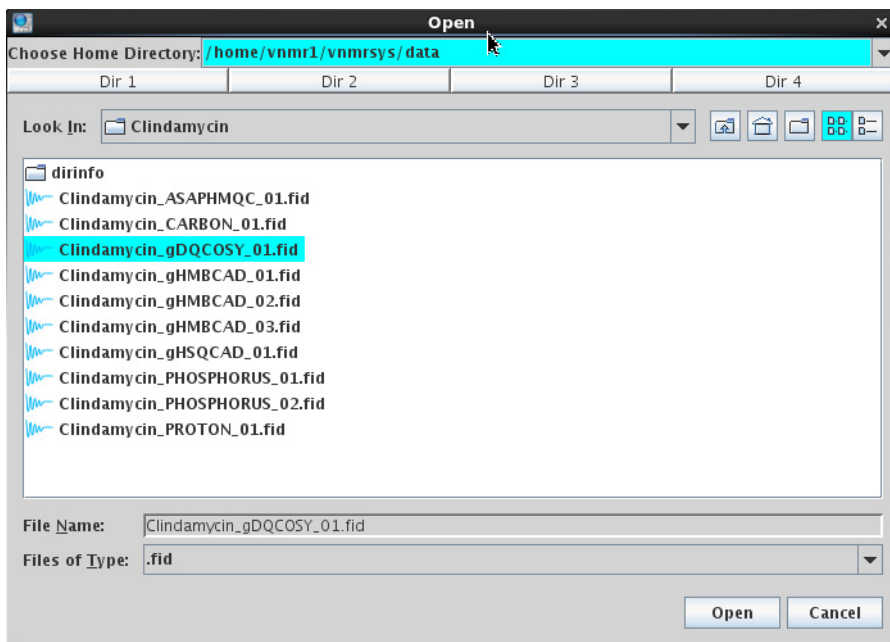
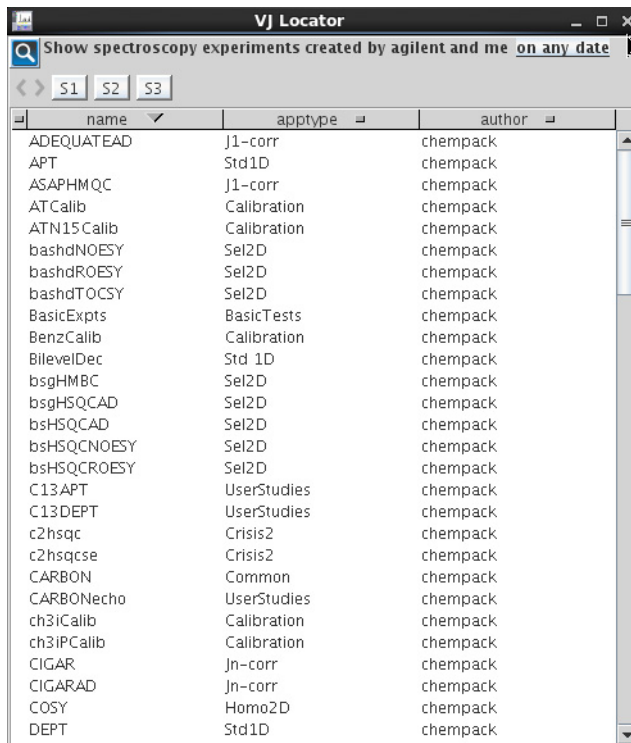


Figure 57 Open dialog box

Use the VJ Locator to search the database

The VJ Locator, [Figure 58](#), is a database browser that provides access to data sets, experiments, shim sets, commands, and so on.



The screenshot shows the VJ Locator window with a search bar at the top containing the text "Show spectroscopy experiments created by agilent and me on any date". Below the search bar are three tabs labeled S1, S2, and S3. The main area displays a table with three columns: name, apptype, and author. The table lists various experiments and their associated types and authors.

name	apptype	author
ADEQUATEAD	J1-corr	chempack
APT	Std1D	chempack
ASAPHMQC	J1-corr	chempack
ATCalib	Calibration	chempack
ATN15Calib	Calibration	chempack
bashdNOESY	Sel2D	chempack
bashdROESY	Sel2D	chempack
bashdTOSY	Sel2D	chempack
BasicExpts	BasicTests	chempack
BenzCalib	Calibration	chempack
BilevelDec	Std 1D	chempack
bsgHMBc	Sel2D	chempack
bsgH5QCAD	Sel2D	chempack
bsH5QCAD	Sel2D	chempack
bsH5QCNOESY	Sel2D	chempack
bsH5QCROESY	Sel2D	chempack
C13APT	UserStudies	chempack
C13DEPT	UserStudies	chempack
c2hsqc	Crisis2	chempack
c2hsqcse	Crisis2	chempack
CARBON	Common	chempack
CARBONecho	UserStudies	chempack
ch3iCalib	Calibration	chempack
ch3iPCalib	Calibration	chempack
CIGAR	Jn-corr	chempack
CIGARAD	Jn-corr	chempack
COSY	Homo2D	chempack
DEPT	Std1D	chempack

Figure 58 VJ Locator

The Locator provides quick access to information on all or part of the disk environment. The administrator determines the scope of the Locator actions.

Locator menu and controls

The magnifying glass and the current Locator statement are at the top of the VJ Locator window, as shown in [Figure 59](#).

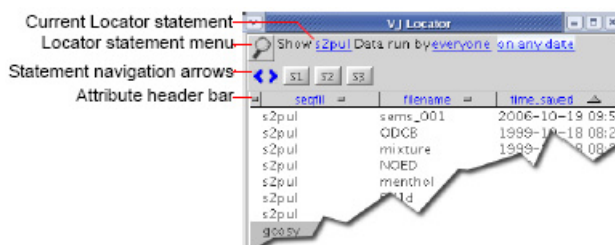


Figure 59 Locator menus and controls

Click the magnifying glass to open a menu of currently available Locator statements. This menu includes both statements provided by Agilent and those customized and saved by the user for searching the database. For more details on using the Locator, see the *Agilent VnmrJ Spectroscopy User Guide*.

Drag and drop items from the locator

To select an item, click the item in the **Locator** list. The selected item can be dragged to the graphics canvas or the Parameter Panel area for an appropriate action. For example, dragging a data set to the graphics canvas retrieves that data set into the current experiment workspace and displays the spectrum. Dragging a workspace to the graphics canvas selects that experiment workspace. Dragging and dropping an item has an action appropriate to the context. Often the same effect can be obtained by double-clicking an object.

Fourier Transform the Data Set

Fourier transform of 1D and 2D data can be performed in a variety of ways. For more information, see [Fourier transform of one-dimensional data](#) and [Fourier transform of two-dimensional data](#). For more information on Fourier transform, see the *Agilent VnmrJ Spectroscopy User Guide*.

Fourier transform of one-dimensional data

The data is typically Fourier transformed into a spectrum before analysis. The data is stored as time-domain data. The FT converts it to frequency-domain data. FT can be done using any of the following buttons/ menu:

- The blue-green **Transform** button in the Action Bar
- The **Transform All** button on the upper left of the Process / Default page (it is also on the Process/Weighting page)
- The **Process** button in the middle bottom of the Process / Basic page
- The **Process** menu, which has options for either **Process** or **Display 1D** and **Full Process**

Fourier transform of two-dimensional data

The FT of a 2D dataset can also be done in several equivalent ways:

- The blue-green **Transform** button in the Action Bar
- The **Full 2D Transform** button on the upper left of the Process / Default page (it is also on the Process/Weighting page and the Process/More 2D page)
- The **Process** button in the middle bottom of the Process / Basic page
- The **Process** menu, which has options for Full Process 2D
- The full processing of a 2D spectrum requires a Fourier Transform along two perpendicular directions, called t1 and t2. These two processing steps can be done one at a time if desired. This allows you to customize the processing parameters in each direction (if desired). For more details, see the corresponding Spectroscopy Guide.

Alter Processing Parameters

More information can be extracted from an NMR spectrum if the processing parameters are optimized specifically for that spectrum (optimized in ways which help answer the questions being asked). Examples include changing the parameters that control processes like zero-filling, weighting functions (and apodizations), linear prediction, referencing, or integral reset points. These parameters are typically controlled using the **Process** tab.

Zero-filling

Zero-filling is controlled by the Transform Size controls, which are located on multiple pages, such as Basic, Default, Weighting, and More 1D pages. If the number of points used is larger than the number of points acquired, zero filling is being performed. This helps to better define shapes of the peaks. It is common and advantageous to transform twice as many points as were acquired. The user can transform fewer acquired data points, which allow the FT calculation to be faster, but at the expense of a less well-defined lineshape.

Weighting and apodization

The detailed parameters controlling the weighting functions and functions that are applicable to the FID before the FT, are defined in the Weighting page in the Process tab. There is also a simplified set of controls on the Default page. Typically, weighting functions are used to increase signal-to-noise, at the expense of broader lines. These functions are used to remove truncation wiggles from the spectrum as needed. The "apodization" process is when the weighting functions are used to remove truncation wiggles

Linear prediction

All of the detailed parameters that control linear prediction are located in the **More 1D** page. There is a simplified set of controls on the Default page. The **Auto** buttons set up a default set of conditions for either forward or backward linear prediction. Many of the protocols in the Experiment

Selector set up appropriate parameters for using linear prediction automatically. For more detailed information about controlling linear prediction, see the *Agilent VnmrJ Spectroscopy User Guide*.

Referencing

Automatic processing uses a routine to provide default referencing for the spectrum. To alter the referencing, use the controls located in either the Default or the Display pages. Referencing By Solvent or By TMS automatically analyzes the spectrum. Referencing the spectrum to a certain cursor position requires the user to place the cursor at the desired location in the spectrum and type in the desired numerical value for that position in the Reference cursor to entry box.

Integration

Integral regions are automatically set up for Proton spectra during automated processing. Further manipulations of the integral can be made by using the controls in the Integration page on the Process tab, or in the graphical toolbar, see Interact with the spectrum using the graphical toolbar.

Phasing

Proper spectral phasing is automatically set up for all spectra during automated processing. Further manipulations of the phasing can be made using the graphical toolbar.

Interacting with the Spectrum Using the Graphical Toolbar

The graphics control bar for the active viewport is to the right of the graphics canvas. Use the buttons in the bar to control the interactive display in the graphics canvas. For details on specific toolbars, see

[“Common graphics display toolbar controls”](#) on page 19

[“1D display spectrum toolbar controls”](#) on page 20

[“nD display toolbar controls”](#) on page 21

[“Display FID toolbar controls”](#) on page 23

[“Annotation toolbar controls”](#) on page 24

Integration and graphics controls

The graphics controls for displaying, and plotting integrals is located on the [“1D display spectrum toolbar controls”](#) on page 20.

Aligning and Stacking Spectra

Spectra can be a mixture of 1D and 2D data sets, all 2D data sets, or all 1D data sets, provided the following requirements are met:

- All selected viewports need to use a common scale.
- Data in the viewports may have different nuclei, different spectral widths, or different spectral regions. The common scale is determined based on data in all selected viewports and determines whether alignment or stacking is possible. Overlaid and stacked spectra are drawn based on the common scales.
- Alignment is enabled if more than one axis in more than one viewport has the same axis (H1, C13 etc).
- Stacking is enabled when data in all viewports have the same axis/axes.

For details on aligning and stacking spectra, see the *Agilent VnmrJ Spectroscopy Users Guide*.

Displaying and Plotting Integrals

The graphical controls for displaying and plotting integrals are located on the Display 1D Spectrum toolbar (Figure 60). For step-by-step instructions, see the *Agilent VnmrJ Spectroscopy User Guide*.

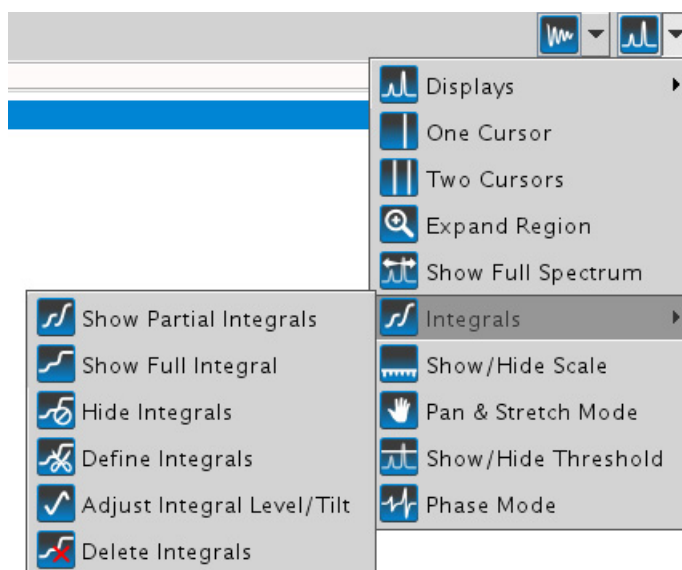


Figure 60 Controls for displaying and plotting integrals

Baseline Correction

Most operations performed on spectra assume a "good" baseline. Line lists, integrations, resolution measurements, 2D volume integrations, and so on measure intensities from "zero" and do not perform any baseline adjustments. Perform a baseline correction operation before performing further data reduction if the baseline in your spectrum is not "good." Two types of baseline corrections are provided, linear and non-linear, and are available using the buttons on the **Display** page of the **Process** tab.

Working with Viewports

All VnmrJ graphics are displayed in frames. The viewport has a default frame that occupies the entire viewport graphics area. An inset frame initially shares the same workspace and data as the original frame and is configured in the same way as the default frame. Insets are configured and modified using the Frame vertical panel. (See “[Frame vertical panel](#)” on page 41.) For more information on creating and changing inset frames, see the *Agilent VnmrJ Spectroscopy User Guide*.

Using Viewports Controls

Use the Viewport vertical panel to control the appearance of data in the viewports. See “[Viewport vertical panel](#)” on page 45. The viewport controls are present if there are two or more viewports.

Show and hide viewports

The selected viewports are arranged on the graphics canvas based on the layout selection. See “[Set viewport layout](#)” on page 171.

- 1 In the Viewport vertical panel, select the check box next to each viewport to show on the graphics canvas.
- 2 Clear the check box next to a view port to hide the viewport.
- 3 Point the cursor to the box next to the viewport label and hold down the left mouse button to temporarily hide a viewport.
- 4 Release the mouse button to show the viewport.

The viewports do not change their layout on the graphics screen. This tool is used when overlay viewports is selected.

Make a viewport active

- 1 In the Viewport vertical panel, click the radio button associated with a viewport to make the viewport active.

The title bar of the active viewport is colored. The inactive viewports have gray title bars.

- 2 Use the horizontal and vertical panel tools to work on the data in active viewport or begin data acquisition using the active panel.

Experiments started from the current active panel are run in the order of submission. Systems running an automated sample changer use only experiment 1 (which is in viewport 1) to submit samples to the automation queue. All other viewports are used for data processing and analysis.

Add a label to the viewport

The default label for a viewport is the currently loaded experiment file name.




- 1 In the Viewport vertical panel, click inside a viewport label box (viewport does not have to be active).
- 2 Select the contents of the box and overwrite the text with new text.
- 3 Click outside the text box.

A new label is now associated with this viewport.

Set viewport layout

In the Viewport vertical panel, select a layout icon to arrange the viewports on the graphics canvas. Click the **Overlay Viewports** to stack viewports on top of one another. The overlay layout is useful for placing high resolution 1D spectra on the appropriate 2D axes.

Table 43 Viewport layout

Icon	Description
	Auto layout arranges viewports in an optimized row by column matrix
	Horizontal layout of viewports
	Vertical layout of viewports

Synchronize cursors and axes

Use the following controls in the Viewport vertical panel to synchronize cursors and axes.

Table 44 Cursors and axis

Check box	Description
Sync cursor	Select this check box to link and synchronize the cursors and crosshairs in multiple viewports.
Sync Axis	Select this check box to link and synchronize axes in multiple viewports. Axis is synchronized to the current active viewport.

Set crosshair, fields, and axis display options

Use the following controls in the Viewport vertical panel to turn on or off crosshair, fields, and axes.

Table 45 Crosshair, fields, axis display

Check Box	Description
Show crosshair	Displays cross hair and chemical shift(s) of the cursor position when mouse is moved over the spectrum. This is a useful function when the fields are not shown, not in cursor mode (default mode), or chemical shift of a peak without moving the left cursor is required while in the cursor mode.
Show fields	Select this check box to show information fields at the bottom of the active viewport canvas: <div> <div>vs</div> <div>111.7</div> </div> <div> <div>sp(ppm)</div> <div>35.78</div> </div> <div> <div>wp(ppm)</div> <div>65.00</div> </div> <div> <div>first</div> <div>1</div> </div> <div> <div>last</div> <div>4</div> </div> <div> <div>step</div> <div>1</div> </div>
Show axis	Select this check box to show the axis or remove the check to hide the axis.

Assign colors to spectra by viewport

Use the Viewport vertical panel to assign colors to spectra displayed in viewports.

Table 46 Assigning Colors to Spectra

Check box	Description
Color by viewport	<div><div>Select this check box to display the spectral data using colors assigned by the viewport, see Figure 13.</div><div>Default color assignment:<ul style="list-style-type: none">• Spectra are displayed using a different color for each viewport if the box is checked.• The spectra are displayed using the defaults assigned in the Display options window if this box is not checked.</div><div>Change a color assignment:<ul style="list-style-type: none">• Click on the dropdown color menu for a viewport and select a color for the spectral display in the viewport.</div></div>

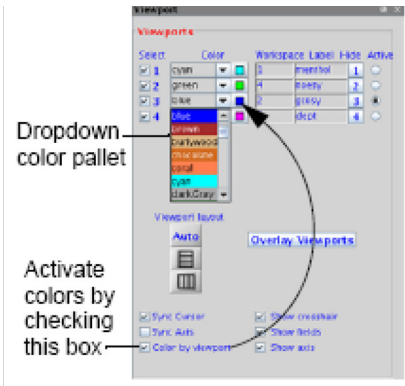


Figure 61 Setting Spectra Colors by Viewport

Using viewports as a spectral interpretation tool

The **Viewports** tool displays and presents data and provides a powerful interface to interpret spectral data. A collection of NMR data sets acquired on a given sample are typically more informative when considered together and simultaneously. Information presented in a 1D data set is often complimentary to that provided by a homonuclear or heteronuclear 2D data set. Many 2D data sets are closely linked from an interpretational point of view. Viewports provide powerful options for interrogating spectral data.

Overlaying homonuclear data sets

When interpreting homonuclear data, concurrently consider COSY or scalar coupling interactions (that is, through-bond) with NOESY or dipolar coupling interactions (that is, through-space).

For example, Figure 62 shows the graphics window with COSY and NOESY data sets in Viewports 1 and 2, respectively, collected on the ethylindanone standard sample. The **Overlay** and **Align Spectra** buttons have been used to provide a composite display. By selecting the **Show Crosshair** check box, a yellow cursor line, or crosshair, is displayed simultaneously in each window. The crosshairs are linked, allowing responses from one data set to be immediately interpreted with respect to the other. This display clearly differentiates those responses observed in the NOESY spectrum that are derived from long-range relaxation from the vicinal and geminal responses.

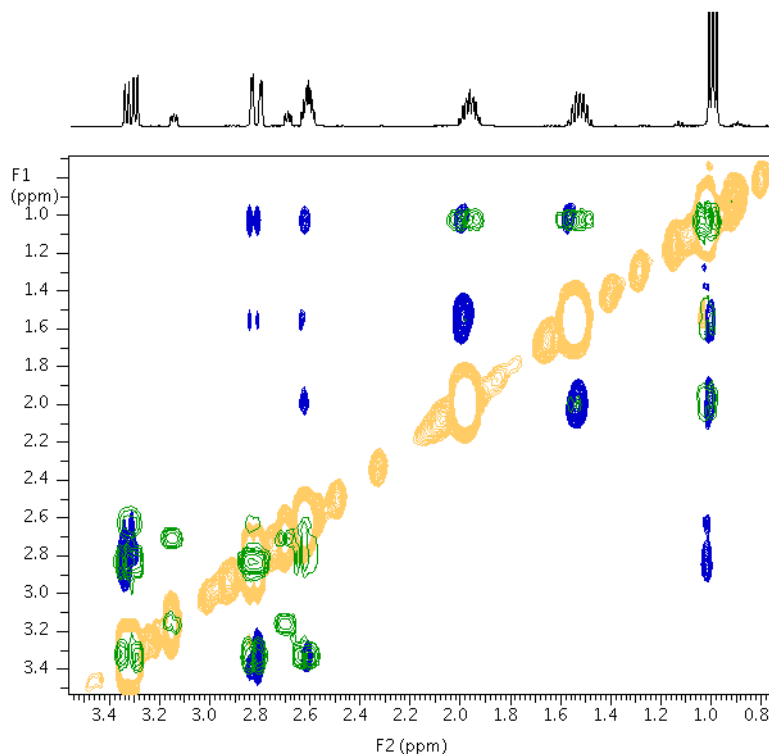


Figure 62 Overlaid COSY and NOESY spectra of ethylindanone

COSY responses are shown in green, while the phase-sensitive NOESY data are displayed in blue and yellow.

Cross referencing heteronuclear data sets

Many heteronuclear data sets provide complimentary insights into molecular structure. As shown in [Figure 63](#) on page 175, a Viewports overlay of HSQC and HMBC data allows visualization of both 1-bond and long-range coupling in a single, easy-to-interpret arrangement. This type of display is very valuable when spectra become congested, and allows unambiguous assignment of long-range responses to 1-bond J_{CH} spin pairs.

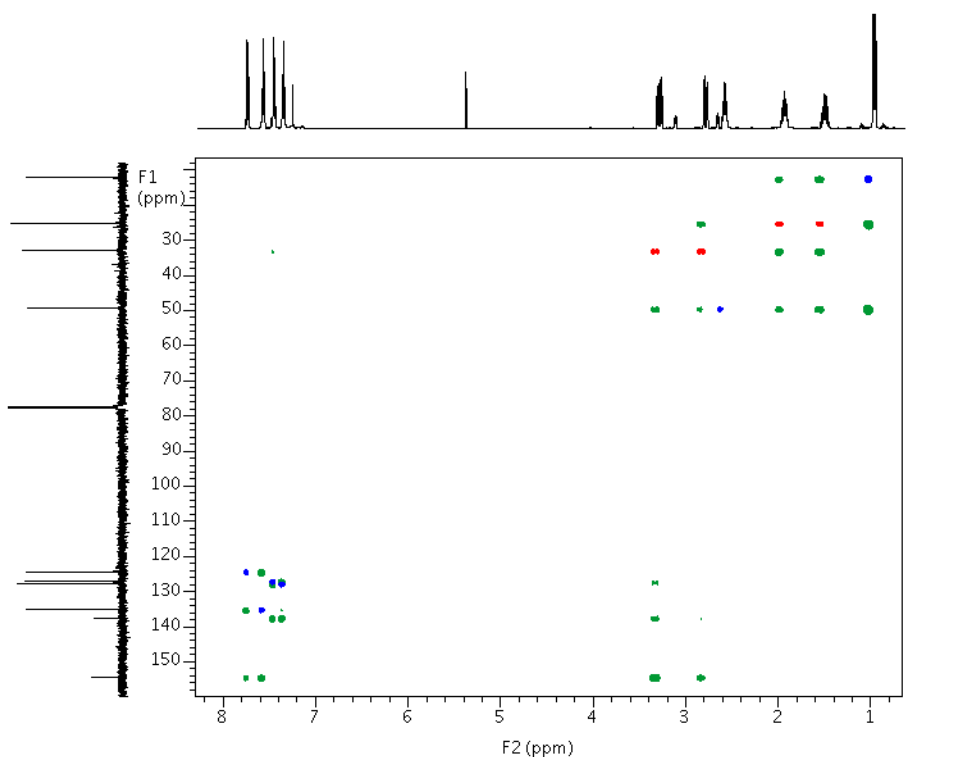


Figure 63 Overlaid HSQC and HMBC spectra of ethylindanone

6 Processing Data

Cross referencing heteronuclear data sets

HMBC responses are shown in green, while the phase-sensitive multiplicity-edited HSQC data are displayed in blue (odd number of protons) and red (even number of protons).

Save Current Process or Display Parameters

In the **Default** page of the **Process** tab, there is a button labeled **Save Current Process/Display Parameters**. Click this button to save a parameter set in the dataset with all the current processing and display information, in a manner that is suitable for GLP requirements. This action stores parameters such as phasing, integral regions (resets points and isadj), and the display information that would be stored by the `s1` and `r1` commands (described above). (It does not store information about referencing, symmetrization, baseline correction, or drift correction.) The next time any **Process** button is clicked, the last-stored set of display parameters will be used to display the resulting spectrum.

6 Processing Data

Save Current Process or Display Parameters



7 Plotting Data

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Agilent Technologies

Plotting Data Saved as a Study

1 In the Parameter Panel, click **Process > Plot** to display the plotting tools.

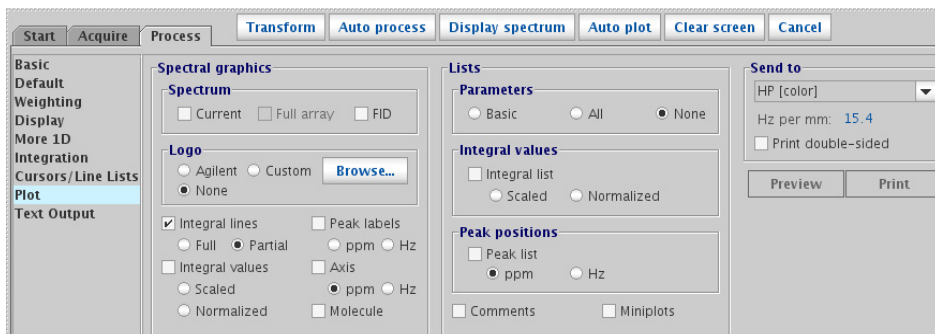


Figure 64 1D plot setup in the Plot page of the Process tab

These preferences, once set, are retained for a given spectrum. By default, most of these options are turned off the first time that the Plot panel is accessed for a given spectrum, so it will usually be necessary to turn several options on, especially the Spectrum **Current** check box, which toggles whether or not the plot includes a graph of the spectrum.

Auto plotting tools are also available on the **Process > Basic** parameter panel.

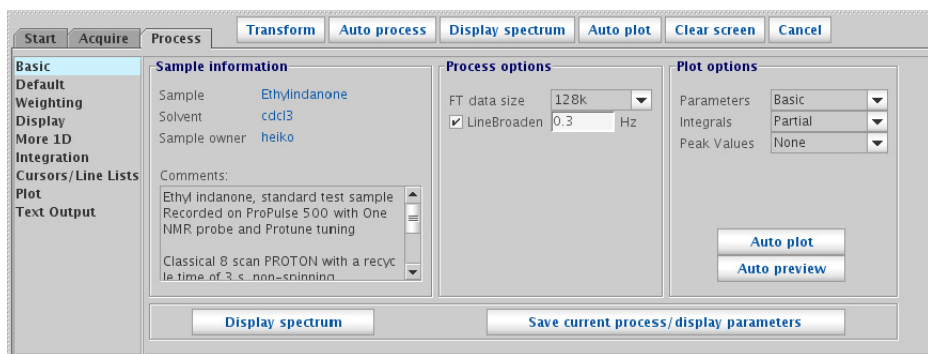
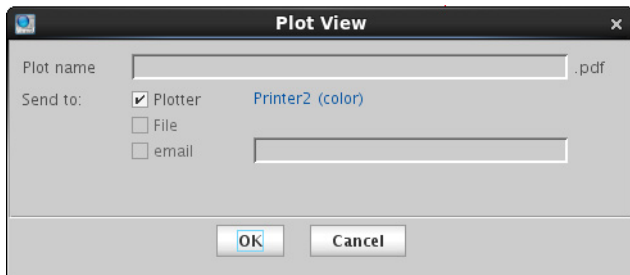


Figure 65 Plot options in the Basic page of the Process tab

- 2 Use the Graphics Toolbar to adjust the displayed spectrum.
Hovering the mouse over the icons displays tool tips.
- 3 Click **Auto Preview** to render a duplicate of the displayed spectrum in Adobe Reader.

The **Plot View** dialog box appears.



- a Review the formatted plot and readjust as needed.
- 4 Select one or more check boxes on the **Plot View** dialog box to print, save as a PDF file, or email the plot.
- 5 Click **OK**.

Saving and Printing a Graphics File

After processing, the 1D or 2D spectrum is displayed in the graphics canvas so that the scale, expansion, and threshold can be adjusted.

To print the spectrum:

- 1 Click **File** from the menu.
- 2 Select **Print Screen**.
The pop-up in [Figure 66](#) appears.
- 3 Select the **name** of the printer to print to it.
- 4 Select the print area, either **Viewports** or **VnmrJ Window**. Viewport will capture the contents of the Viewport, while VnmrJ Window will capture the entire VnmrJ window.
- 5 Choose the **number of copies** to print.
- 6 Click the **Print** or the **Preview** button.

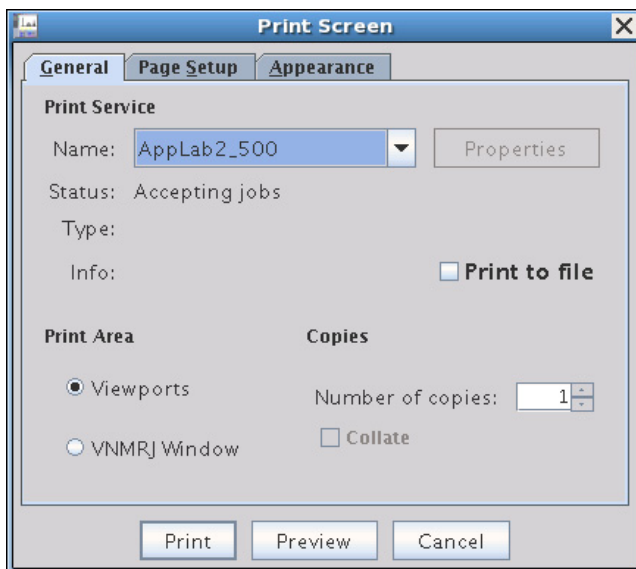


Figure 66 General tab of the Print Screen pop-up

Plotting the Data

Plotting is based around the concept of a plot file. Setting up and submitting a plot can be done from the vertical and horizontal panels and from the command line. The **Plot** parameter page is accessed from the **Process** tab after the spectrum or FID is displayed. Items selected on the **Plot** parameter page (Figure 67 and Figure 68) are added to a temporary plot file. The Preview button opens the plot in Adobe Reader where you can preview it, then save or send it to a printer or email. The **Print** button submits the plot file to the selected printer.

For details on plotting data, see the *Agilent VnmrJ Spectroscopy User Guide*.

Plot options for 1D data

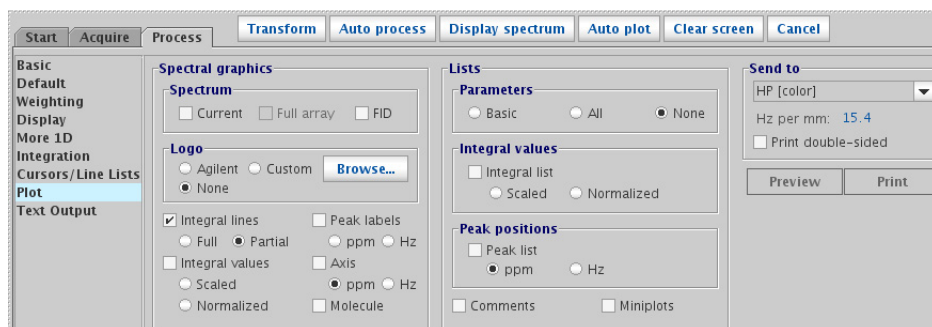


Figure 67 The Plot parameter page for a 1D data set

Table 47 Options in the 1D Plot panel

Item	Description
Spectral graphics	
Spectrum	Select how to display the spectrum on the plot: Current, Region, Full array, FID

Table 47 Options in the 1D Plot panel (continued)

Item	Description
Logo	Select to include a logo on the plot: Agilent, None, or Custom (If Custom, click Browse and then find and select the logo file to use.)
Additional graphics options	
Integral lines	Select to include full or partial lines on plot
Integral values	Select to include scaled or normalized integral values on plot
Axis	Select to display axis on plot in ppm or Hz
Peak labels	Select to display peak labels on plot in ppm or Hz
Molecule	Select if molecule is displayed on graphics canvas to include it in the plot
Lists	
Parameters	Select level of detail to include for parameters list: Basic, All, or None
Integral values	Select Integral list to display integral values, then select Scaled or Normalized
Peak positions	Select Peak list to include list of peak frequencies in lists, then select units of ppm or Hz
Comments	Select to include comments in lists
Miniplots	Select to include miniplots in lists
Send to	In the drop-down menu, select the printer to use.
Print double-sided	If the selected printer has the ability to print double-sided, select this option to enable double-sided printing for the plot.
Preview	Opens the plot in Adobe Reader with currently selected options, along with the Plot View pop-up where you can select to save the file, print it, or email it.
Print	Sends the plot with currently selected options to the selected printer.

Plot options for 2D data

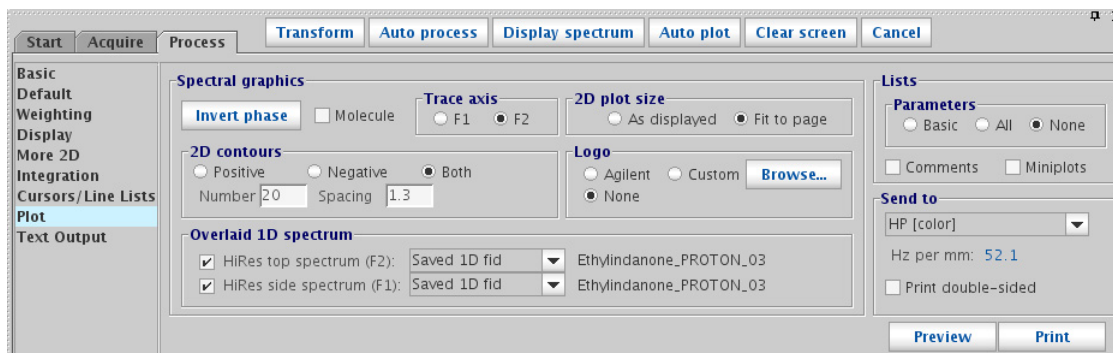


Figure 68 The Plot parameter page for a 2D data set

Table 48 Options in the Plot parameter page for a 2D data set

Item	Description
Spectral graphics	
Invert phase	Click to invert phase
Molecule	Select if molecule is displayed on graphics canvas to include it in the plot
Trace axis	Select F1 or F2
2D plot size	Select As displayed or Fit to page
2D contours	Select to include Positive, Negative, or Both Enter Number of contours to display Enter the Spacing for the contours
Logo	Select to include a logo on the plot: Agilent, None, or Custom (If Custom, click Browse and then find and select the logo file to use.)

Table 48 Options in the Plot parameter page for a 2D data set (continued)

Item	Description
Overlaid 1D spectrum	Select to include 1D spectrum at the top and side of the 2D plot. HiRes top spectrum (F2) HiRes side spectrum (F1) For each, select Saved 1D fid, Projection, or Other workspace.
Lists	
Parameters	Select level of detail to include for parameters list: Basic, All, or None
Comments	Select to include comments in lists
Miniplots	Select to include miniplots in lists
Send to	In the drop-down menu, select the printer to use.
Print double-sided	If the selected printer has the ability to print double-sided, select this option to enable double-sided printing for the plot.
Preview	Opens the plot in Adobe Reader with currently selected options, along with the Plot View pop-up where you can select to save the file, print it, or email it.
Print	Sends the plot with currently selected options to the selected printer.

Changing Color Themes

Printer and Plotter color output is defined using the **Styles and Themes** window, **Plot** option, which provides access to the display colors and the VnmrJ interface colors. You access the **Styles and Themes** window using **Edit > Display Options**. There, you can set colors and save the color “theme” to a file. Once you save a color theme file, you can import and use it at a later time. Setting the styles and themes options are described in detail in the *Agilent VnmrJ Spectroscopy User Guide*.

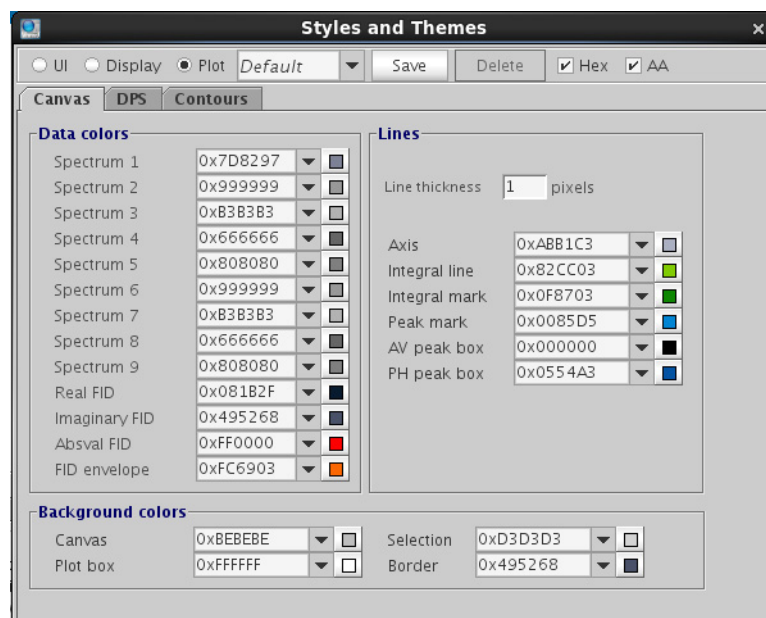


Figure 69 Styles and Themes window

Pasting Text into a Text Editor or Other Application

Text output that appears in the **Integration**, **Cursors/Line Lists/Text Output** parameter pages can be pasted into a text editor to be saved or used elsewhere.

- 1 Highlight the text to be pasted by clicking the left mouse button and dragging the mouse to the end of the desired text.
- 2 Release the mouse button at the end of the desired text. The selected text is highlighted indicating what has been selected.
- 3 Start the text editor or application and place the mouse cursor on the active document.
- 4 Click the middle mouse button to paste the highlighted text into the text editor.

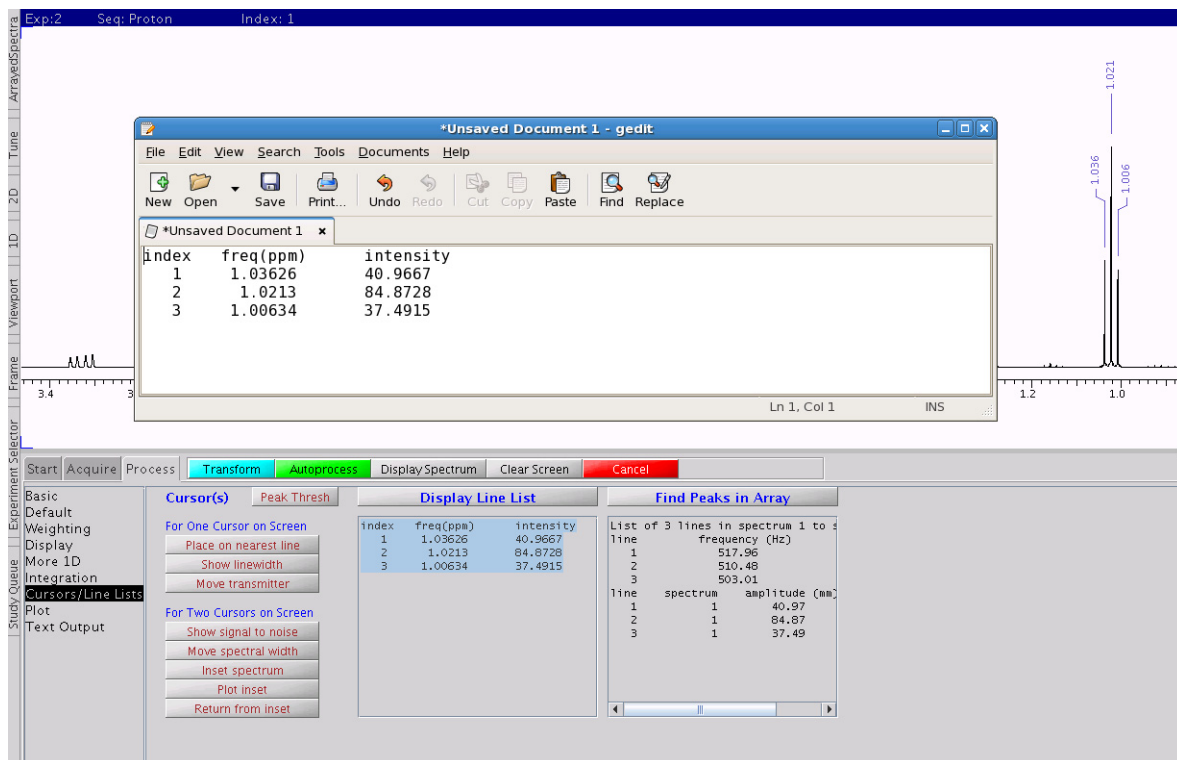
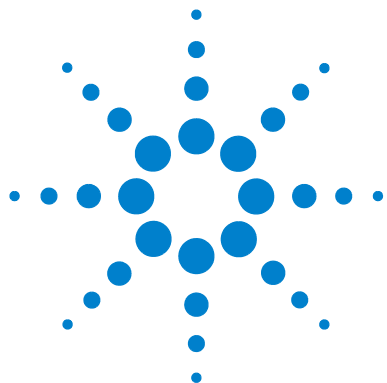


Figure 70 Contents of the Display Line List text box copied into the Linux gedit text editor.



8 Customizing VnmrJ Actions

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VnmrJ has incorporated tools that allow each user account to be customized to suit the individual user tastes. There are 6 tools that can be used to create new buttons in the Experiment Selector, each generating a distinct button. These tools are located in the **Tools > Study Clones** menu. This section describes how to use these tools to customize the Experiment Selector.



Clone a New Study

This tool allows the user to create a new study based on those protocols already present in the Experiment Selector. Studies created using this tool will inherit any subsequent changes made to those protocols. For instance, if a Study Clone is made that includes the CARBON protocol, any subsequent changes made to the behavior of the CARBON protocol will be reflected in the Study Clone, also.

This tool is commonly used to create a composite study of multiple experiments. It is also very useful when a user wants to create a customized copy of an existing protocol without changing the original protocol (that is, a new "**Q_PROTON**" button with a very long relaxation delay, or an "**overnight_CARBON**" button that is parameterized to run all night).

Selecting this tool places the software into Submit Mode and opens an empty study in the Study Queue. The user can then populate this study with any experiment(s) from the Experiment Selector and customize each node in the queue as desired (double click on the node to open it in the current workspace, make any changes, and click the green **Save** button on the parameter panel toolbar). Once the study is configured as desired, click the yellow **Save Study** button on the bottom of the **Study Queue** window. This will open the **Save Study Queue** popup.

In the **Save Study Queue** popup window:

- 1 Select the **Allow Customization** check box if you wish to allow users to customize the nodes in this study on a per-sample basis. Deselecting the check box will lock the study so that users can make no changes.
- 2 Select the **Include Sample Tags** check box to write the current sampltags into the new study clone.
- 3 Choose where the new study will be saved from the **Applications Directory** drop-down menu.
- 4 Choose the tab in the **Experiment Selector** where the new button will appear by selecting an existing tab from the drop-down menu, or select **New Tabname** from the list and fill in the tabname field to create a new tab.
- 5 Fill in the Study Name field with the name of the study. This is the name that will appear on the new button. This name can not be the name of a macro currently in the search tree.

6 Click **Save/Update** to create the new button.

The Experiment Selector will now be updated to reflect the addition of the new study. Clicking this button will build the saved study in the study queue or in the current experiment, as appropriate.

Clone Current Study

This tool is identical to Clone New Study (“[Clone a New Study](#)” on page 190), except that it acts on the study currently present in the Study Queue. This allows the user to clone a completed study or to create a study clone while entering a study to be run on a sample.

Clone Current Experiment

This tool is typically used when the user wishes to clone the experiment in the current workspace. In contrast to Study Clones (“[Clone a New Study](#)” on page 190 and “[Clone Current Study](#)” on page 192), the **Clone Experiment** tool creates a new parameter file that is read in directly when the new button is selected. This truly yields an exact duplicate of the parameter set in curpar that can be used in a study queue. This tool is commonly used when it is desired to collect exactly the same data set on a large number of samples.

Before selecting this tool, configure the experiment in the current workspace as desired. Then select **Tools> Study Clones> Clone Current Experiment**. The **Clone Experiment** popup is opened.

- 1 Select the **Allow Customization** check box if you wish to allow users to customize the parameters in this experiment on a per-sample basis. Deselecting the check box will lock the experiment so that the users can make no changes.
- 2 Select the **Include Sample Tags** check box write the current sampletags into the new experiment clone.
- 3 Choose where the new experiment will be saved from the **Applications Directory** drop-down menu.
- 4 Choose the tab in the **Experiment Selector** where the new button will appear by selecting an existing tab from the drop-down menu, or select **New Tabname** from the list and fill in the tabname field to create a new tab.
- 5 Fill in the **Study Name** field with the name of the study. This is the name that will appear on the new button. This name can not be the name of a macro currently in the search tree.
- 6 Click **Save/Update** to create the new button.

Clone Location Queue

This tool allows the user to create a study clone from a location queue in the sample tray display.

First, select a location in the sample tray (that already has a study queue assigned to it) by clicking on that location. Then select **Tools > Study Clones > Clone Location Queue**. This will open the **Save Study Queue** popup.

In the **Save Study Queue** popup window:

- 1 Select the **Allow Customization** check box if you wish to allow users to customize the nodes in this study on a per-sample basis. Deselecting the check box will lock the study so that the users can make no changes.
- 2 Select the **Include Sample Tags** check box write the current sampletags into the new study clone.
- 3 Choose where the new Study will be saved from the **Applications Directory** drop-down menu.
- 4 Choose the tab in the **Experiment Selector** where the new button will appear by selecting an existing tab from the drop-down menu, or select **New Tabname** from the list and fill in the tabname field to create a new tab.
- 5 Fill in the **Study Name** field with the name of the study. This is the name that will appear on the new button. This name can not be the name of a macro currently in the search tree.
- 6 Click **Save/Update** to create the new button.

Command and Protocol Buttons

This tool serves two purposes. Creating a Command Protocol allows the user to execute a VNMJR command or macro at run time as part of a Study Queue. This function does not collect data but returns to the next item in the study upon completion. The Experiment Protocol function is used to generate an experiment button in the Experiment Selector (that is, it generates the appropriate XML file) to call a pre-existing set-up macro for loading a pulse sequence and parameter file. In other words, this tool can be used to import pre-VJ3 experiments and their associated files into the current system.

Protocol

My Library: ▼

Show Details Delete

Type ☒ Experiment ☐ Command

Name CARBON

Tabname std1D ▼ Select OR

std1D Enter

MenuLevel

Submenu

Label CARBON

Action ☒ Execute.. CARBON

☐ Recall current parameters (rtp)

Req. Exp

ExpTime 8 min, 44 sec

AppDir Home account ▼

☐ AutoPrompt customization

Make protocol

Close Help

To create a Command Protocol:

- 1 Select **Tools > Study Clones > Command and Protocol Buttons** to open the **Protocol** popup.
- 2 Confirm that the **Command** check box is selected.
- 3 Fill in the name to be used for the protocol.
- 4 Choose the tab in the **Experiment Selector** where the new protocol will appear by selecting an existing tab from the drop-down menu, or select **New Tabname** from the list and fill in the tabname field to create a new tab.
- 5 Confirm that the **Execute** check box is selected
- 6 Fill in the field with the name of the macro to be run in the protocol.
- 7 Choose where the new protocol will be saved from the **App Dir** drop-down menu.
- 8 Click **Make Protocol**.
- 9 The new Command Protocol will now appear in the Experiment Selector.

To create an Experiment Protocol:

- 1 First, execute the set-up macro for the desired experiment in the current workspace and parameterize the experiment as desired.
- 2 Select **Tools > Study Clones > Command and Protocol Buttons** to open the **Protocol** popup.
- 3 Confirm that the **Experiment** check box is selected.
- 4 Fill in the name to be used for the protocol.
- 5 Choose the tab in the **Experiment Selector** where the new protocol will appear by selecting an existing tab from the drop-down menu, or select **New Tabname** from the list and fill in the tabname field to create a new tab.
- 6 Choose the **Recall** current parameters check box to use the parameters in curpar as the basis for loading the new experiment, or choose the **Execute** check box and fill in the name of a set-up macro to parameterize the new experiment.

- 7 Fill in the **Req Exp** field with a rational starting experiment(s) as desired.
 - a Leaving this field blank will cause the new protocol to load from the default set-up macro (based on the value of tn) when used in the Study Queue, or it will load on top of the parameters in the current workspace when used in Review mode.
 - b By filling in this field, the new protocol will check for the presence of the specified experiments and operate on the last such parameter set that exists in the sample queue, or it will add the required experiment to the queue if one doesn't already exist. This is the default behavior for the protocols supplied with the system and is the recommended action.
- 8 Choose where the new protocol will be saved from the **App Dir** drop-down menu.
- 9 Click **Make Protocol**.

The new protocol will now appear in the Experiment Selector. Assuming that the **Required Experiments** field was populated appropriately, the protocol will behave as do all the standard system protocols. Adding the new protocol to a Study Queue will preserve the sample tags and locked parameters (that is, *sw*, *pw*, solvent suppression parameters, etc.) from the last valid starting data set in that queue, and the node can be customized as desired.

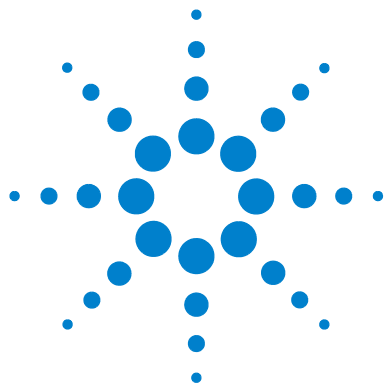
Edit Parlib

This tool is used to create a complete protocol, including a set-up macro, parlib entry, XML file, etc., for inclusion in the Experiment Selector. This tool is intended to be used only when the starting point is a VJ3 system protocol.

The **Edit Parlib** tool is commonly used to add a new protocol for acquisition of data on a nucleus different than those supplied in the standard Experiment Selector interface (i.e., ^{29}Si , ^{17}O , ^{23}Na , etc.).

After configuring the parameters in the current workspace appropriately:

- 1 Select **Tools > Study Clones > Edit Parlib** to open the **Edit Parlib** popup.
- 2 Fill in the name to be used for the protocol.
- 3 Choose the tab in the **Experiment Selector** where the new protocol will appear by selecting an existing tab from the drop-down menu, or select **New Tabname** from the list and fill in the tabname field to create a new tab.
- 4 Populate the **Lock Parameters** field with those parameters whose values should be retained by subsequent experiments.
- 5 Fill in the **By default, start with** field with any protocol that must be executed before the new one being created.
- 6 Fill in the **User Customization** field with any customization that should be performed at set-up time.
- 7 Click **Save/Update**.
- 8 The new protocol will appear in the **Experiment Selector**.



9 Customer Training

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This chapter describes the familiarization training provided by the Agilent installation engineer at time of installation. This training is intended to be a general overview of the instrument, basic maintenance requirements, software features, data acquisition and storage, file maintenance, and other routine tasks. Comprehensive training classes are offered at various Agilent Applications Laboratories around the world. Call your sales representative or contact the Agilent Technologies NMR systems office nearest you for class offerings, schedules and cost.



Training

The installation engineer is providing this training as a service to the customer to use the spectrometer. The engineer may not be able to answer detailed questions beyond the scope of this training. There is no expectation that the installer can provide all of the training needs for the customer. Training is most effective if the end user performs all the tasks.

This training covers only basic operation of a solution-state NMR spectrometer – it does not cover the operation of spectrometers doing bio-NMR (samples in H₂O), solids, or imaging applications. This training is for a maximum duration of one day, and for a maximum attendance of one customer for each training session. To obtain more complete training, or to train a larger group of people at your customer's facility, please suggest that the customers attend one of Agilent's applications lab training classes, arrange an on-site training visit, call the Agilent Technical Assistance Center (TAC) or your local salesperson, or refer to the appropriate manual.

Previous Experience with Agilent NMR Software

If customers are already familiar with certain topics, they may choose to skip the topics. Customers may have no knowledge of how this version of software has changed since their last experience with a previous version of VnmrJ software.

Documentation

Give an overview of the documentation. Explain where to start looking for information and where to get help.

- *QuickStart Guide* – start all new users here.
- *VnmrJ 4 Administration Guide* – start all new system administrators here. This provides quick start information and detailed explanations.
- *VnmrJ 4 Familiarization Guide* - gives an overview of the VnmrJ 4 software and how to use it to acquire data. Also provides descriptions of the User Interface.
- *Spectroscopy User Guide* – a more advanced manual for experienced users, or for those performing more advanced experiments.
- *Command and Parameter Reference Manual (CPR)* – a favorite reference manual, which contains details on every command, macro, and parameter.
- *User Programming Manual* – (Optional, advanced) the primary manual for details about writing new pulse sequences, creating shapes, or modifying parameter characteristics. Any activity that might be called “programming” is documented here.
- Additional manuals (Probe manuals, accessories) – these are general reference material.

Show the customer how to get the documentation.

- Various hard-copy manuals are shipped with the spectrometer.
- Purchase hard copies.
- Visit the Agilent Spinsights site, <http://www.spinsights.net>. A password is required.
- Access the online documentation using VnmrJ 4 Help, using **Help > Manuals**.
- The installation DVD downloads documentation located on the VnmrJ 4 interface.

Sample Requirements

- 1 Review with the end user the contents of the *Quick Start Guide*, including sections on how to prepare and load a sample.
- 2 Point out the depth gauge, show how to use it, and how to document the number to which it is currently set. (The customer should document this number in a logbook.)

Introduction to VnmrJ 4 Operation

Instruct the customer to follow all the steps in the *Quick Start Guide* to acquire a spectrum. This allows customers to acquire the first spectrum (acquire, process, plot, and save – all automatically).

Do this by preferably using the sealed “2-Ethyl Indanone” sample (provided with the system; part number 0190185503).

Overview of the VnmrJ 4 Interface

Give an overview of the layout of the VnmrJ 4 display, using the following list. Point out the location and simple use of:

- The **Main Menu** (for example, shows the location of **File > Open**, **Edit > System Settings**, and **Tools > Probe Tuning**)
- The **Vertical Panels** (show the Protocols, Frame, and Viewport tabs)
- The **Graphics Window** (this is where the spectral data always appears)
- The **Graphics Toolbar** (shows that it can be moved and docked elsewhere)
- The **Parameter panels** (shows how to navigate between the Start, Acquire, and Process tabs, and their various panels)
- The **Action Toolbar** (shows how to use the Show Time button on the Acquire tab, and the Transform button on the Process tab)
- The **Hardware Toolbar** (point out the following: Trash Can, Real-Time Monitors, Probe Selection, Acquisition Status, and Error-Message Display. (Optional) up-arrow acqstatus display)
- The **Command Line** (optional, show how to type `pwd` to print the working directory, or `ls` to list files)

Basic (Automated) VnmrJ 4 Operation

Give the customer more insight into the operation of VnmrJ 4 by doing the following:

- 1 Acquire data “In the Study Queue” – by using the **Submit** button and the **StudyQ**.
 - a Acquire a queue composed of a ^1H 1D (PROTON), a ^{13}C 1D (CARBON), APT, gCOSY, and gHSQCAD (typically on 2-ethylindanone in CDCl_3).
 - b Fill out the **Start > Sample Info page** (specifically Sample Name, Solvent, Comment, tune, autolock, autoshim).
 - c Show the customer how to customize parameters for a node (within the StudyQ, prior to acquisition). Use the **Acquire > Acquisition** page to customize various parameters (that is, nt, dl, gain).

NOTE

The data is saved automatically when using the StudyQ, using the filename templates within Edit / Preferences / Templates.

- d Acquire enough data to be sure to use the **New Study**, **Submit**, and **Continue Study** buttons.
- 2 Acquire data “Without the Study Queue” (in “manual mode”) – by using the **Acquire** button.
 - a Show the customer how to save data in this mode by using **File > AutoSave**.
- 3 Show the customer how to bring old data back into the StudyQ (**File > Open**).
- 4 Show the customer how to reprocess data in the StudyQ (for example, use the **Transform** button in the action bar of the **Process** tab)
- 5 Show the customer how to replot data in the StudyQ (use the **Process > Plot** page, **File > Print Screen**, or optionally the command line)
- 6 Customize the protocols by adding a new button:
 - a Clone a study (using **Tools > Study Clones > Clone Current Study**)

Review where the documentation for these tasks is located within the manuals.

Detailed (Manual) VnmrJ 4 Operation

Teach the end user detailed software operation by doing each of the following:

- 1 Demonstrate the use of the features in the Main Menu (such as, **File > Open, Acquisition > Abort Acquisition, Tools > Probe tuning**, and so forth).
- 2 Demonstrate the use of the icons in the System Toolbar (such as, **Cancel Command, Stop Acquisition**). Point out the “Floating Tool Tips” that tell you what the icon will do, even without pushing it, and the use of context-sensitive help.
- 3 Demonstrate use of **File > Open** and **File > Save As** pop-up windows to open and save data, respectively.

These windows can each be in different locations within the directory structure.

These windows only show the files determined by “Files of Type”. They may not show all the files in each directory unless configured to do so with the “Files of Type” menu.
- 4 Demonstrate the Action bar buttons (that is, **Acquire, Show Time, and Autoprocess**).
- 5 Demonstrate use of the command line, and adjusting the size of the error window.
 - a Type `pwd` and `ls`, then show how the text output goes to the **Process > Text** window.
 - b Adjust the size of the error window – and then hide the command line completely – by dragging the window size.
- 6 (Optional) Teach the use of multiple experiments (`cexp (3)`, `jexp3`, `mf (2,3)`, and so forth).
- 7 (Optional) Teach the end user closing and pinning windows, and how to use the View menu to open closed windows. Demonstrate pinning and closing on the graphical tool bar, the parameter panels page, the hardware toolbar on the bottom, and a vertical panel. Show how to redisplay closed windows by using the View menu.
- 8 Show how to use the depth gauge (and the knurled locking knob).

9 Insert and Eject

- a from the buttons on the panels.
- b from the switch on the upper barrel.
- c from the command line (by typing `eject` and `insert`, or `e` and `i`. This is optional.

NOTE

You cannot eject with one method and then insert using another method. This may confuse the hardware.

10 Teach the end user “semi-manual” locking (push the **Find z0** button).

Teach the end user manual locking (adjust Z0, lock power, and lock gain).

An ideal target lock level is 80%, so the lock gain should be adjusted to achieve this. Point out that users should generally note at which value they set the lock level (to ensure that it is normal for a given lock power, lock gain, and solvent, and to verify after time that the lock level did not decay too much during long-term acquisitions).

11 Teach the end user “semi-manual” shimming (by using the **Gradient Shimming** button on the **Start > Lock** page).

12 Teach the end user manual shimming (show use of the **Start > Shim** page).

13 Adjust lock phase (on the **Start > Lock** and **Start > Shim** pages) while monitoring the lock level.

(Explain that this should be done only when the system is well shimmed, and ideally on a standard sample that has a sharp lock -- like the lineshape sample. The lock phase should be documented in the system logbook. The optimal lock phase value can change by 90 degree steps whenever the console is power cycled.)

14 Save shims:

- a To the local user account (the default, saves the shims in `~/vnmrsys/shims`).
- b (Optional) To the system account (`/vnmr/shims`).

15 Load shims:

- a** From the combination of the probe file and the preferences option.
- b** From the Shim page (Note that this may require the use of a terminal window or the Locator to find the exact filename.)
- c** By dragging and dropping from the **Tools > Browser** window.
- d** (Optional) By dragging and dropping from the **Tools > Locator** window.
- e** By using the **Open** button in the **File > Open** window (you need to select “**shim**” via the display menu to see the files).

16 Select a probe file, and learn how to determine if a given probe file is either a “System” or a “User” probe file. Learn how to determine if a given probe file is a “Probe ID” file or not. Show how ProbeID is turned on-and-off by using the configuration page and the check boxes in the probe pop-up window.

17 Show the customer how to review the contents of a probe file (that is, check the Edit box, review the tabs for different nuclei).

18 Show the customer how to set the Temperature (use the **Spin > Temp** page).

19 Show the customer how to set the spin rate (use the **Spin > Temp** page).

We suggest you also select (mark) the check box for “Control spinner from this panel only”.

20 Tuning:

- a** If ProTune is installed, show how to manually use ProTune (by using **Tools > Probe Tuning > Auto Tune Probe**). Tune a nucleus.
- b** Show the customer how to use mtune to view (monitor) the quality of tuning (by using **Tools > Probe Tuning > Manually Tune Probe**).
- c** Using mtune, demonstrate tuning by using the knobs on the bottom of the probe if they are accessible (no ProTune), or by using the knobs on the ProTune box.
- d** (Optional) Show the customer how to tune using the Tune box on the tether.
[To do so, set the `rf` on channel 1 to H1 and channel 2 to C13, then adjust the controls on the tether box (channels = 1 or 2, gain = 8) and tune to the resulting display].

- 21 Show the customer how to use the graphical toolbar:
- a On a 1D spectrum, demonstrate expanding, contracting, integral on/off, threshold, and phasing.
Ensure that every end user spends time learning how to phase manually. This is a difficult concept to master if you have never used it before, and it is impossible to teach over the phone, so we need at least one user at each site to have mastered how to phase manually. The next most confusing (but not difficult) task is integration. See the optional topic below.
 - b On a 2D spectrum, demonstrate expanding, vertical-scale adjustment, panning, trace, and rotate.
- 22 (Optional) Teach setting up the integral.
For beginning users, this is easier to teach by using the **Process > Integration** page.
This task can also be done using the graphical toolbar.
- 23 Show the customer how to plot “manually” (when not using the StudyQ for automation):
- a By using the buttons on the parameter panels.
 - b (Optional) By entering `pl pscale` page on the command line
- 24 (Optional) Show the customer how to setup gradient shimming and make a new map (that is, explain how to use **Tools > Standard Calibration Experiments > Set up Gradient shimming**). Detailed directions on how to do this are in the *Gradient Shimming* chapter of the *Spectroscopy Guide*. Contrast this with gradient shimming via the **Lock > Gradient Shim** button, which requires no user interaction.
- 25 (Optional) Show the customer how to setup arrays and then clear them (demonstrate a pw array).
- 26 (Optional) Show the customer how to use the Arrayed Spectra vertical panel to process and display arrayed data.
- 27 (Optional) Show the customer how to set up Quantification.

Linux Training

- 1 Provide the end user with written copies of the usernames and passwords for root, vnmr1, and any other user accounts, as were set up by the installer.
- 2 Show the customer how to login a user account.
- 3 Show the customer how to logout of a user account.
Let the users login to each account so they know each password works.
- 4 Show the customer how to start VnmrJ 4 (click on the icon on the desktop, or enter `vnmrj` in a terminal window)
- 5 Show how to exit VnmrJ 4 (**File > Exit VnmrJ 4**).
Explain that this should be done before a user logs out of the user account (if possible). Do this by using **File > Exit** in the Main menu, or entering “exit”.
- 6 Show the customer how to start VnmrJ 4 Adm (which requires vnmr1 permission) (click on the icon on the desktop, or enter `vnmrj adm` in a terminal window)
- 7 Show the customer how to reboot the host computer:
 - a From the Linux menu
 - b (Optional) From a terminal window, enter `su`, enter the root password, enter `reboot`
- 8 Show the customer how to reboot the communication with the console: `su acqproc`, reset the master, `su acqproc`, load shims.
(Discuss that “resetting the master” is optional here.)
(Run `/vnmr/bin/makesuacqproc` if needed.)
- 9 (Optional) Show the customer how to set up a printer in Linux.
- 10 (Optional) Show the customer how to read the manual pages in a terminal window (that is `>man ls`), or call up the Help documentation in Linux.
- 11 (Optional) Show the customer how to use `>ps -ef`, then `>kill (>kill -3 pid)`. (This requires root permission.)
- 12 (Optional) Show the customer how to tar up (data) directories into a single tar file:
`tar cvf filename.tar filename` – this copies filename into filename.tar.

`tar xvf filename.tar` – this extracts the contents of filename.tar.

13 (Optional) Discuss how to obtain and load patches to VnmrJ 4.

VnmrJ 4 Administration

Provide training in VnmrJ 4 Administration:

- 1 Make a new user. (New User, [enter **User Login** and **Interface Type**], Save)
 - a Explain that there are three interface types: Spectroscopy, Imaging, LC-NMR/MS. Use Spectroscopy for liquids and solids NMR.
 - b Explain how making a new user may not allow that user to access existing local files in another user account [probe file, gshimlib, shapelib, and so forth].
- 2 (Optional) Make a new Operator in a User Account.
- 3 (Optional) Show the customer how to “Switch Operators” in VnmrJ 4.
- 4 (Optional) Show the customer how to set the default operator password.
- 5 Delete a user account by highlighting the username, right-click, delete.
- 6 Update a user account (**Configure > Users > Update users**).
- 7 Show the customer how to set up a Printer in VnmrJ 4.
- 8 (Optional) Show the customer how to use VnmrJ 4 Accounting.
- 9 (Optional, if installed) Show the customer how to turn Probe ID on-and-off, and use the probe popup in both modes.

VnmrJ 4 Administration - Quick Start

Review all the topics in the Admin Quick Start chapter of the *VnmrJ 4 Administration Guide*.

- 1 Show the customer how to use **Edit > Preferences** (User Preferences).
- 2 Review the contents of every tab.
 - a **Templates tab:** Show the customer how to modify the automation directory and file-saving parameters.
 - b **Queue tab:** Show the customer how to modify the defaults for autolocking, autoshimming, and protune.
- 3 Show the customer how to use **Edit > System Settings**
 - a Show the customer how to turn on/off “automatic processing upon drag-and-drop”.
 - b Show the customer how to turn on/off WYSIWYG.
Explain what this does, and that it may influence how much of the graphics screen is used to display data.
 - c Show the customer how to turn on the PFG usage for each new user (which is the same as `pfgon='nny'`).
- 4 Show the customer how to select a printer and plotter for each new VnmrJ 4 user (**File > Printers**).
- 5 Show the customer how to use **Edit > System Settings > System Config** pop-up window. (Explain that this is only accessible to the `vnmr1` user.)
- 6 (Optional) Show the customer how to use “appdirs” (**Edit > Applications**; this is needed for Biopack, and so forth).
- 7 Show the customer how to change a password:
 - a In VnmrJ 4 (**Tools > Change Password**)
 - b (Optional) In VnmrJ_Adm
 - c (Optional) In Linux (`>passwd username`)
- 8 Explain how the Experiment Protocol buttons read the calibrations stored in the Probe File. (When you push the **Proton** button, you read in a proton parameter set, and update it to account for the values stored in the probe file. So if the probe file is not set properly, the calibrations – and hence the data – will not be proper). The probe file is now required for proper operation on VnmrJ 4

- 9** Show the customer how to do a probe-file calibration.
 - a** Demonstrate it on an existing probe file (that is, using the Doped D2O sample). Also rerun the methyl iodide calibration if time allows.
 - b** Explain that the “target values” in some of the calibrations come from the probe specification sheets (which may not be readily available to the end user when they want them).
 - c** (Optional) Add and then remove a nucleus from the probe file by using the button on the probe popup.
- 10** (Optional) Show the customer how to change the probe file’s default shimmap (Probelkmap in the probe file). Explain that this exact file is what is used during automated acquisition (even if another map is set in the gradient shimming parameter panels [gmapsys]).
- 11** (Optional) Show the customer how to start and stop the cold probe (if appropriate) using the Cryo vertical panel
- 12** Document the following into a logbook:
 - a** The console serial number (Show the customer where it is)
 - b** Write down lockfreq (in the System Configuration panel, see the section below)
 - c** The air-flow and air-pressure values on both the pneumatics box and on “the wall” (at the regulator at the source of the gas to the room)
 - d** The current value of the lock phase
 - e** The value that the depth gauge screw is set to
- 13** Save a copy of shims into /vnmr/shims
 - a** By using the command line (svs('/vnmr/shims/shimname'))
 - b** By using the VnmrJ 4 GUI on the Start > Lock page (type in the whole path name: /vnmr/shims/shimname)
 - c** By using the terminal window
- 14** Show the customer how to calibrate Protune.

Hardware Overview

- 1 Point out the console, the RF front end, the Pneumatics box, the probe (Optional: ProTune, the robotics, cold-probe units, pfg cable (which moves top-to-bottom sometimes), FTS, Remote Status Unit, Tune Box (on the tether, and so forth).
 - a Show the customer where the console serial number is located (inside the front door).
- 2 Review **Edit > System Settings** (as vnmr1) as needed to explain hardware options.
 - a Write down lockfreq.
 - b Take a screenshot of the config pop-up window and save it in vnmr1's home directory as a backup. (use the Linux menu **Applications > Accessories > Take Screenshot**).
- 3 Show the customer where the extra probes (in their boxes) are located (if appropriate).
- 4 Show the customer how to place a red plastic cap on the probe when it is not in use, especially when it is in the probe case. Explain that this is important to keep debris out of the probe, especially static-prone particles of probe-case foam.
- 5 Show the customer how to power cycle the console after a power interruption.
 - a Point out and explain the use of the blue-green button on VNMR5.
 - b There are also power switches on the RF Front End unit, the Protune unit, the PFG amp, the console's power supplies, and so forth.
- 6 Show the customer where the "master reset button" is located in the console (again).
- 7 (Optional) Demonstrate probe installation and operation:
 - a Show the customer how to install the probe.
 - b Show the customer how to reposition the probe (that is, push the probe up, push the upper barrel down, tighten the clamp).
 - c Show the customer how to manually tune the probe, if automated probe tuning is not present.
- 8 The arrows on the directional couplers point away from the probe and towards the filters and console.

- 9** (Optional) Explain the meanings of the console lights and the lights on the pneumatic box.
- 10** (Optional) Explain how to use the FTS unit.
- 11** Train the customer on magnet safety and cryogen fill techniques:
- Warning signs posted
 - Cryogenics handling and safety
 - Magnet filling
 - Flow meters and Cryogen meters
 - Homogeneity disturbances

Customer Support Information

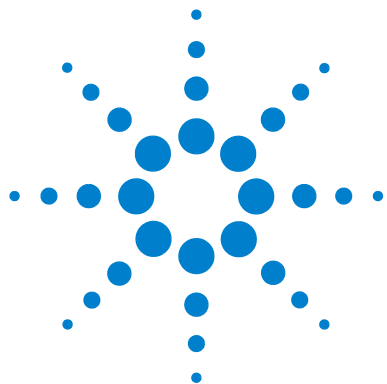
- 1 Provide the contact details to be used when help is needed.
Contact Center: 1-800-227-9770, option 3, option 7.
 - a Show the customer where to find the console serial number (which will be needed when you call TAC).
 - b Show the customer how to use Help > About VnmrJ to find the software version and patch level.
- 2 Show the customer the Agilent Web site (<http://www.chem.agilent.com/en-us/ContactUS/Pages/ContactUs.aspx>), especially the “User Pages”.
 - a The user needs to register to use the “User Pages” (to get the username and password).
 - b (Optional) Register the user right now.
- 3 Show the customer how to get to the Agilent Web site for NMR help.
 - a Through a regular Web browser (www.chem.agilent.com > Instruments & Systems > Nuclear Magnetic Resonance).
 - b Through the online help within the VnmrJ 4 user interface, by selecting **Help > Spinsights Community Help Site**.

Lock Frequency (lockfreq)

Magnets lose a small amount of their current over time. The magnet “drift” means that the frequency needed to lock the deuterium signals will decrease slowly with time. This means that the Z0 value for any given solvent will slowly decrease with time. Eventually the system “lockfreq” will need to be reset to compensate. This needs to be done when the Z0 value runs out of range, making it impossible to establish lock. This will typically be observed first on CDCl₃ and C₆D₆. When the drift happens, which may take six months to two years, contact Agilent service for detailed instructions on how you can “reset your lockfreq due to magnet drift”.

Administrative Chores

- 1** Show the customer where the NMR data from installation is stored (for example, S/N, lineshape, and so forth).
- 2** Obtain sign-off on the installation.



10

Automated Data Collection and Spectra Interpretation

Automated Data Acquisition [222](#)

Building a Study [224](#)

Interpreting the Indanone Spectra [227](#)

This chapter contains a basic walkthrough of collecting and analyzing NMR data using the automated tools in VnmrJ.



Automated Data Acquisition

The automated data acquisition procedures outline several 1D and 2D experiments using the ethylindanone sample.

These experiments demonstrate the capabilities of the spectrometer, the correct calibration of the instrument, and validate the correct functioning of the instrument. If a sample changer is present, submit acquisitions to background.

The following is part of the training session. How to:

- Set up and use the VnmrJ interface
- Use the Experiment Panel to select the application type, Std1D, Hetero 2D, and Homo 2D experiments
- Set up 1D, 2D, gradient (if appropriate hardware is installed) and non gradient protocols. Click the experimental protocols for proton and carbon 1D, homonuclear 2D, and heteronuclear 2D experiments from the list of protocols.
- Create, run, save data, and plot the results obtained from the composite protocol

These experiments can be run using either the Account Owner or Operator interface.

Refer to the *Agilent VnmrJ Administration Guide* for instructions on setting the user interface (both the administrator and operator) and for working with the VnmrJ interface.

Sample for Automated Data Acquisition

Sample	Sample size	Part number
2% 2-ethyl-1-indanone in chloroform- <i>d</i>	5 mm	01-901855-03

Login to VnmrJ

- 1 Log in using the operating system VnmrJ Account Owner.
- 2 Select the account owner from the **Operator** menu, enter the password, and click **OK**.
- 3 Select **Automation > Automation Run > New Automation Run**.
- 4 Make sure that the printer/plotter is set up, pfgon is set properly, and shim map has been copied into /vnmr/shimmaps.

Setting up the study and lock solvent

- 1 Insert the indanone sample (01-901855-03).
- 2 Click **New Study** in the Study Queue.

Operator: vnmr1

Sample information Clear

Sample name: Clindamycin

Sample directory: Clindamycin

Solvent: D2O DMSO CDC13 D2O Other

Concentration: 0 mM

Notebook:

Page:

Email:

Comments: Clindamycin phosphate 10 mg in D2O 400-MR with OneNMR probe

Figure 71 The Sample info page

- 3 Click the **Start** tab, then in the **Sample info** page enter the **Sample name:**
2-ethyl-1-indanone
- 4 Select **chloroform** from the **Solvent** menu.
- 5 Confirm that the following items are selected:
 - **Autoplot**
 - **Shim**
- 6 Confirm that **Lock?** is set to **Yes (alock <>n)**.

Do not click **Email when study complete** or **Email when fid complete**.

Building a Study

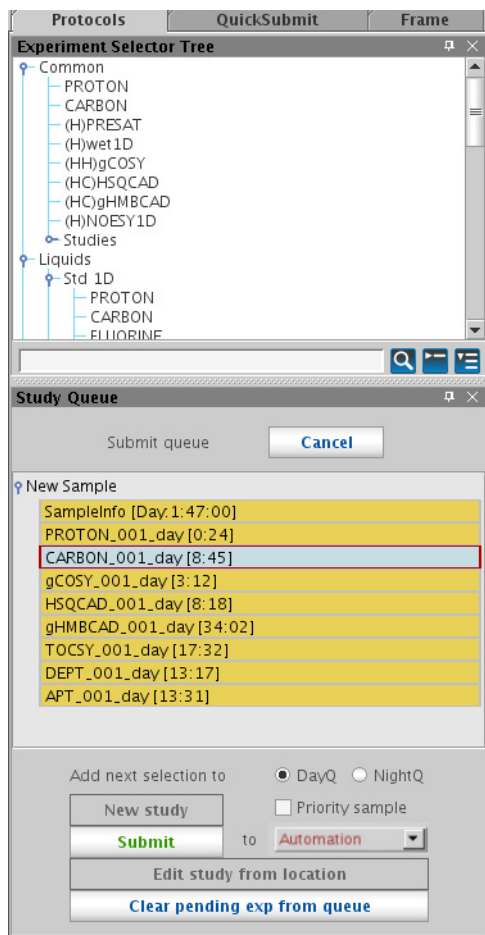


Figure 72 Protocols tab showing Experiment Selector Tree and Study Queue

Common tab

In the Experiment Selector tree, select **Common**, [Figure 72](#), and double-click the following experimental protocols in the following order:

- 1 **PROTON**
- 2 **CARBON**
- 3 **(HH)gCOSY**
- 4 **(HC)HSQCAD**
- 5 **(HC)gHMBCAD**

Under **Liquids**, click **(HH)Homo2D** and select

- 6 **TOCSY**

then click **Std1D**, and select:

- 7 **DEPT**
- 8 **APT**

Customizing the parameters and starting data acquisition

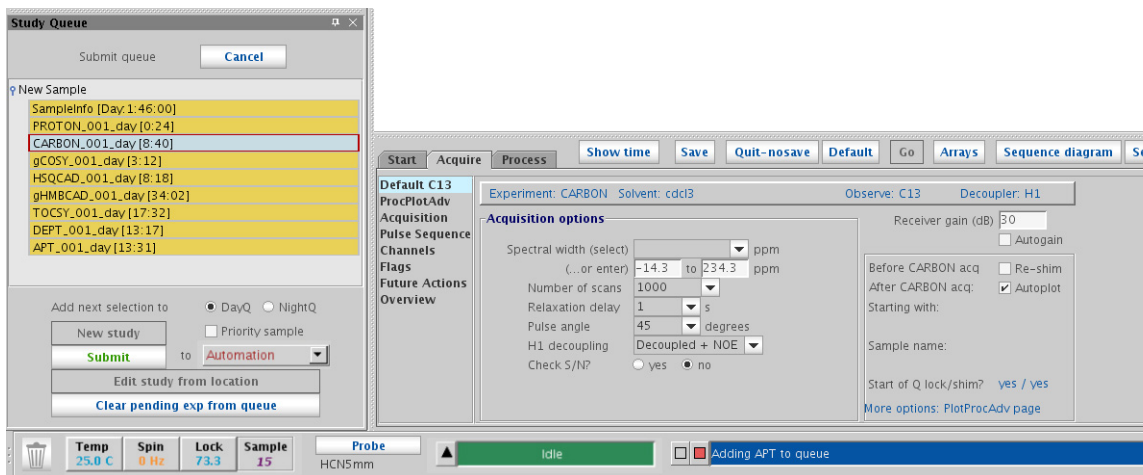


Figure 73 Customized carbon

10 Automated Data Collection and Spectra Interpretation

Starting data acquisition using a study

The **Study Queue** contains the protocols in the order each one was selected and should look similar to the study queue shown in [Figure 73](#).

- 1 Double-click on the CARBON protocol time to retrieve the parameters.
- 2 Click the **Acquire** tab.
- 3 Select the **Defaults C13** page (if more than one page is available).
- 4 Select **1000** from the **Number of Scans** menu.

Starting data acquisition using a study

- 1 Select **Background** from the **Study Queue-Submit to** pulldown menu.
- 2 Click **Submit**.
 - All the protocols are locked automatically.
 - Acquisition starts.

Interpreting the Indanone Spectra

In this section, the data obtained from the automated probe calibration and 2% 2-ethyl-1-indanone sample are interpreted.

- “Calibration – When is it Necessary” on page 227
- “Interpretation of the Calibration Data” on page 227
- “Interpretation of 2-Ethyl-1-Indanone Spectra” on page 235

Calibration – When is it Necessary

The spectrometer must first be calibrated before acquiring spectra of a sample if:

- A new probe is installed.
- The original calibration used a sample in an organic solvent and the new sample has changed from an organic solvent to an aqueous solvent or aqueous solvent with a high salt concentration.
- The current probe calibrations have not been verified recently.

Interpretation of the Calibration Data

The first of the spectra, shown in [Figure 74](#), is a ^1H observe with CDCl_3 as the lock solvent. This data is saved as H1ref. This spectrum contains three $^{13}\text{CH}_3\text{I}$ resonances centered at about 2.2 ppm. The center resonance is from the protons attached to carbon-12 and the two outer resonances are from the protons attached to ^{13}C . The other resonances in the sample are from trimethylphosphite that has reacted with the methyl iodide.

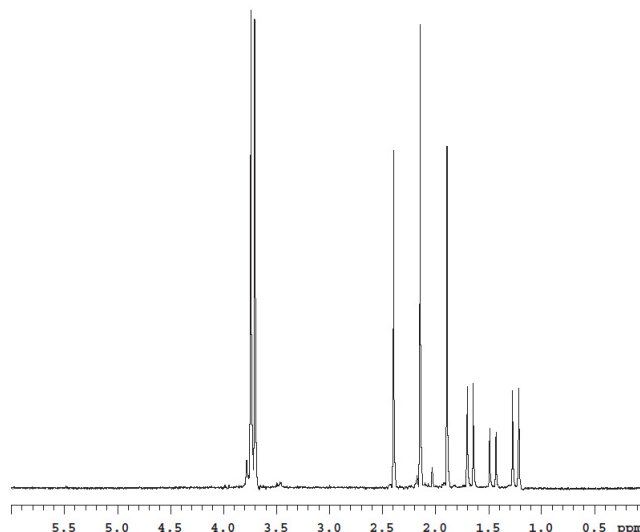


Figure 74 ^1H Spectrum of ^{13}C -Methyl iodide

All trimethylphosphite has reacted to form a phosphonate ester $(\text{CH}_3)_3\text{P}(=\text{O})(\text{OCH}_3)_2$. This phosphonate ester has a doublet at about 1.5 ppm, methyl group attached directly to ^{31}P and a triplet of doublets centered around 4 ppm that arise from ^{13}C (outer pair of doublets) and ^{12}C inner doublet of the methyl ester. The analysis of this sample is fully discussed in an article by Paul Keifer in *Magnetic Moments* (Keifer, P.A., *Magnetic Moments*, 1996, 8 (#2), 18–20). The reaction results in a sample is partially enriched to give approximately 60% abundance of ^{13}C in methyl iodide. The natural abundance of ^{13}C is 1.1% so this level of enrichment is more than adequate for the purposes of calibration.

The spectrum, shown in Figure 75, is an array of increasing ^1H pulse widths based on the ^1H pulse you specified in the Acquire window. If you did not enter a value for the pulse width it is set to the default targets pw90 is set to 15 μs and τ_{pwr} of 51.

The observe transmitter power is set to the value you specified and reduce by 3 for the first test. If the resulting pw90 is shorter then the value you specified (or the default, if you did not specify a pw90 target) the next test is started. If the pw90 is longer then the target, the observe power is increased. Two attempts are made. If the calibration fails to achieve a pw90 that is less than the specified pw90 the AutoCalibration exits. If either attempt yields a pw90 that is less then the specified value the AutoCalibration routine adjusts the observe power the remaining tests are aborted. If the pw90 is less then the specified value, the AutoCalibration then adjusts the power until the measured pw90 is no greater then the specified value but not more then 0.5 μs less than this value. The spectra from a successful calibration are saved as H1pw90.

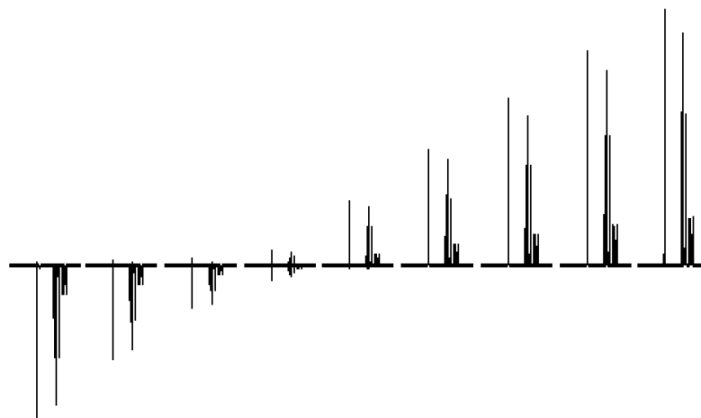


Figure 75 ^1H pw Array

The carbon pwx90 calibration is the next experiment. The pulse sequence changes from s2pul to PWCAL. The specification for the carbon pw90 and tpwr are used as the target values. If no values were specified, the default values of 15 μs at power, in this case pwxlv1, of 51 are used as the target values. Just as with the proton pw90 calibration, the AutoCalibration makes two attempts to achieve the specification and exits

10 Automated Data Collection and Spectra Interpretation

Interpretation of the Calibration Data

the AutoCalibration if the target specification is not reached after the second attempt. The data from the PWXCAL are saved as C13pwx and shown in [Figure 76](#).

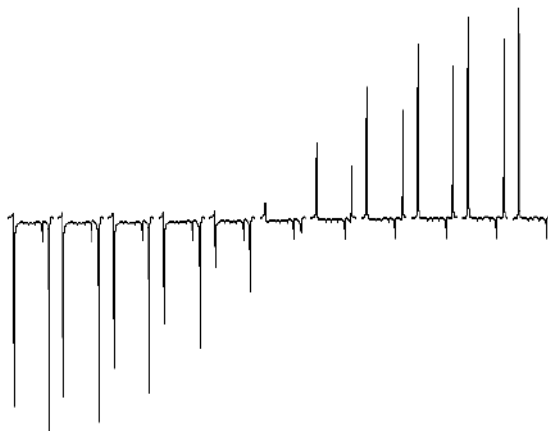


Figure 76 ^{13}C pwx Array

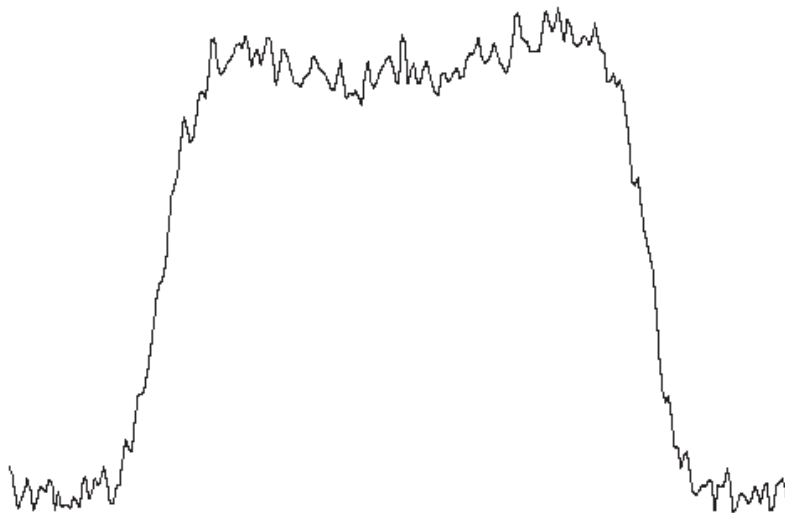


Figure 77 Gradient profile

The next two experiments are run only if you have gradients. The first experiment calibrates the Z-gradient strength, produces the profile shown in [Figure 77](#), and stores this information in the parameter gcal.

The next experiment calculates the ratios of the gradients to be used in various $^1\text{H}\{^{13}\text{C}\}$ indirect detection experiments and stores this information in the parameter Cgrad (for only ^{13}C), [Figure 78](#).

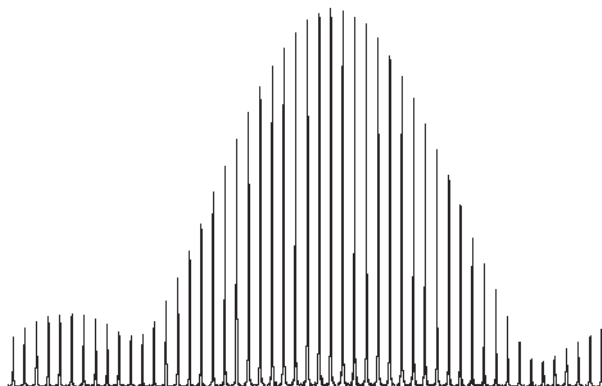


Figure 78 Gradient calibrations

The next calibration is carbon observe pulse width and the pulse sequence is changed to `s2pul` for direct observation of the carbon. The calibration will follow the same pattern as the calibration of the proton `pw90` and the carbon `pw90` using default values for target values if no target specification is given. A reference carbon spectrum is obtained first. The full reference spectrum contains three sets of resonances, at the far right (approximately -22 ppm) is the ^{13}C resonance from methyl iodide, the doublet at 10 ppm is from the ^{13}C resonance from the phosphonate methylester, and the 1:1:1 triplet (far left) at 78 ppm is the ^{13}C resonance of chloroform- d , $^2\text{HCCl}_3$. The carbon `pw90` calibration is analogous to the proton calibration. The reference carbon spectrum, shown in [Figure 79](#), is saved as `C13ref`.

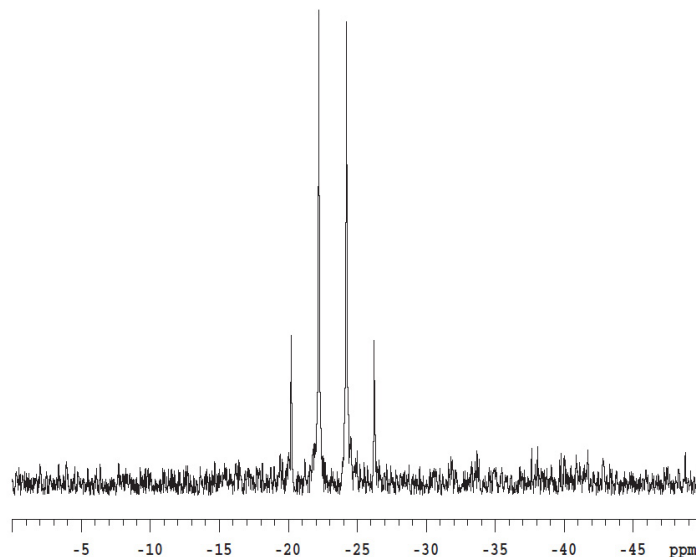


Figure 79 Proton coupled ^{13}C spectrum of ^{13}C -Methyl iodide

The carbon observe `pw90` is determined using a `pw` array, see [Figure 80](#), and saved as `C13pw90`.

The final calibration is of the proton decoupler. The first calibration step determines the value of γH_2 at a decoupler power of 40 (the default value).

This measurement is made using continuous wave, `cw`, decoupling. The pulse sequence is the same as in the previous experiment, carbon observe with proton decoupling, except `pw` is now set to a fixed value, decoupler modulation mode; `dmm` is set to 'c', decoupler mode `dm`; is set to 'yyy', and the decoupler offset; `dof` is arrayed to produce the spectra shown in Figure 16.

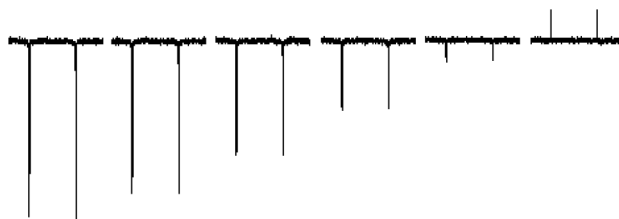


Figure 80 ^{13}C Observe pw array of proton coupled spectra3

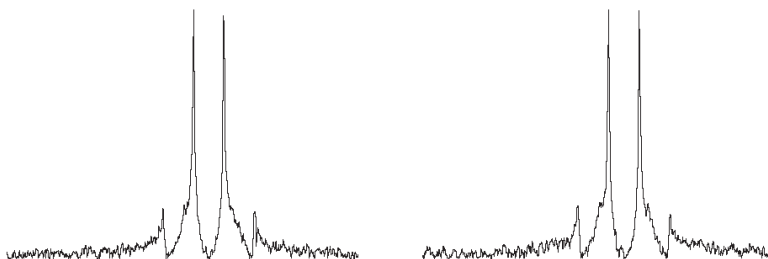


Figure 81 Proton decoupler dof array

The first estimate of the proton decouple pw90 is made from these spectra. Using the relationship between the pulse width and the decoupler field strength, γH_2 , shown in the equation below, the decoupler pw90 is determined.

$$\gamma H_2 = \frac{1}{4(\text{pw90})} \quad \text{pw90} = \frac{1}{4(\gamma H_2)}$$

The sequence is now set to `ppcal` and the proton decoupler 90° pulse, `pp`, is determined. These spectra, shown in Figure 82 are saved as `Hdec_dept`.

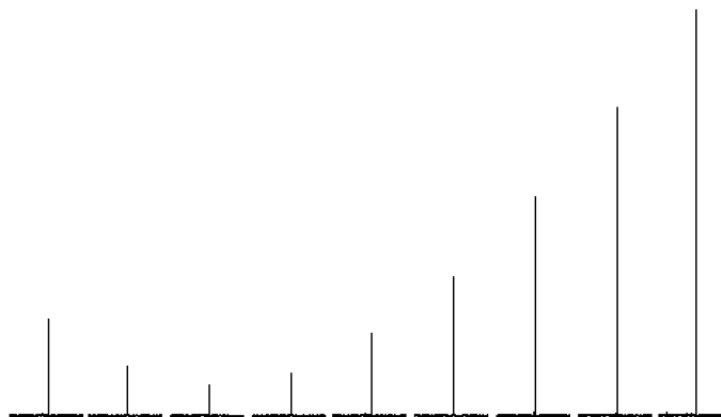


Figure 82 Calibration of the decoupler 90° pulse width

These parameters and calibrations are used to setup WALTZ decoupling. This completes the calibrations. During the calibration procedure, spectra and the array values are plotted to provide a permanent record of the calibrations.

Interpretation of 2-Ethyl-1-Indanone Spectra

The proton NMR shows several distinct features. First, there are some impurities in the sample. These impurities, shown in Figure 83, are at the 2% level and some crosspeaks will show up in the 2D. The very large triplet for the methyl group has ^{13}C satellites at $J=125$ Hz. The singlet at 7.24 ppm is the residual CHCl_3 in the CDCl_3 solvent.

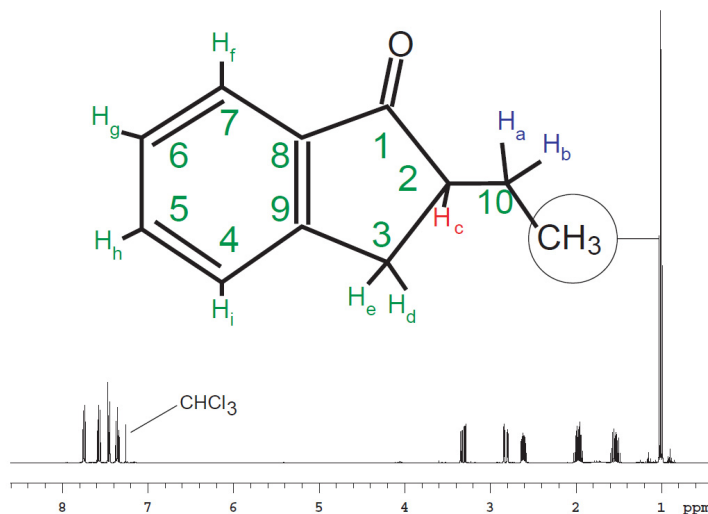


Figure 83 Proton spectrum of 2-Ethyl-1-Indanone

The protons are assigned in the two expansions. The assignments are based upon the 2D data for the compound. The protons of the two CH₂ groups in the molecule, shown in Figure 84, are magnetically nonequivalent and show up as individual multiplets. The signals at 1.9 ppm and 1.55 ppm belong to protons on carbon 10. The two double doublets at 2.8 ppm and 3.26 ppm belong to protons on carbon 3. A complex multiplet at 2.6 ppm is the single proton on carbon 2.

The protons of the aromatic ring, shown in Figure 85, are assigned based on the gHMBC and gCOSY data. Some minor impurities can be seen as well as the residual CHCl₃ signal.

If the sample is shimmed very well there may be some truncation artifact on the CHCl₃ signal.

The gradient COSY shows cross peaks describing the coupling pathways. Some smaller cross peaks are also present in the spectrum which actually arise from the impurities shown in Figure 86. An example of this is the cross peak at 3.5 ppm.

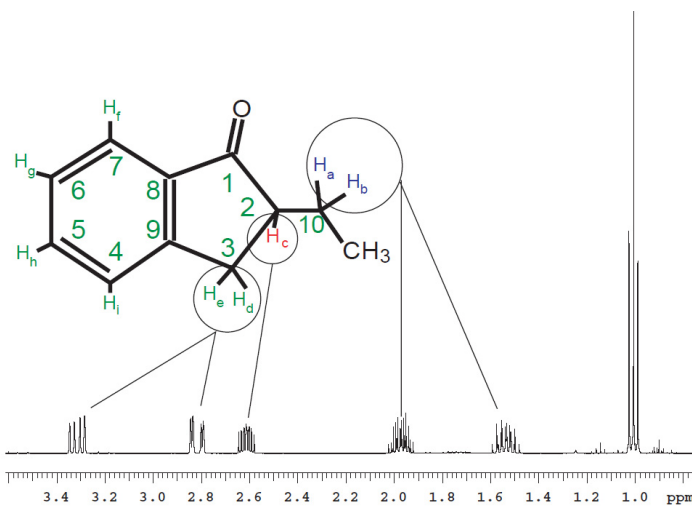


Figure 84 Aliphatic region of the 2-Ethyl-1-Indanone spectrum

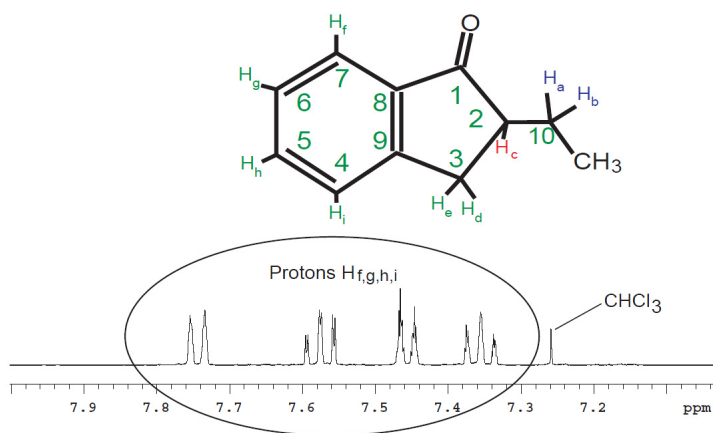


Figure 85 Aromatic region of the 2-Ethyl-1-Indanone spectrum

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Interpretation of 2-Ethyl-1-Indanone Spectra

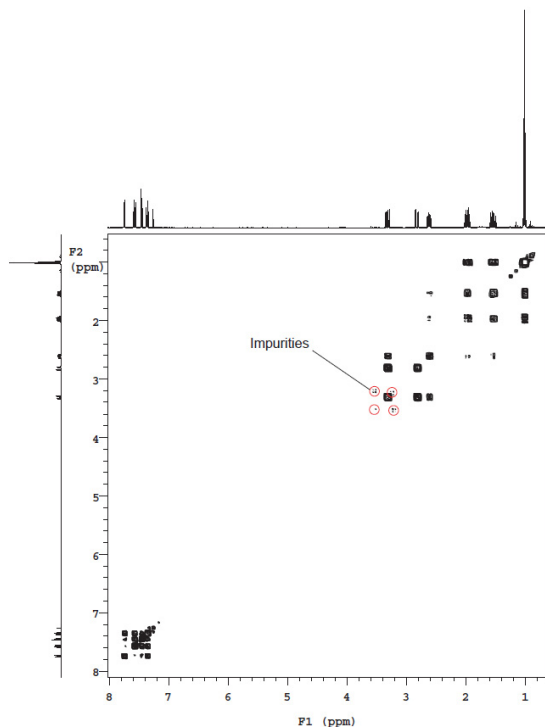


Figure 86 Gradient COSY of 2-Ethyl-1-Indanone

The methyl triplet in [Figure 87](#) shows cross peaks to the H10 protons. The H10 and H2 protons cross peaks multiplicity shows them to be weakly coupled (the J value is small).

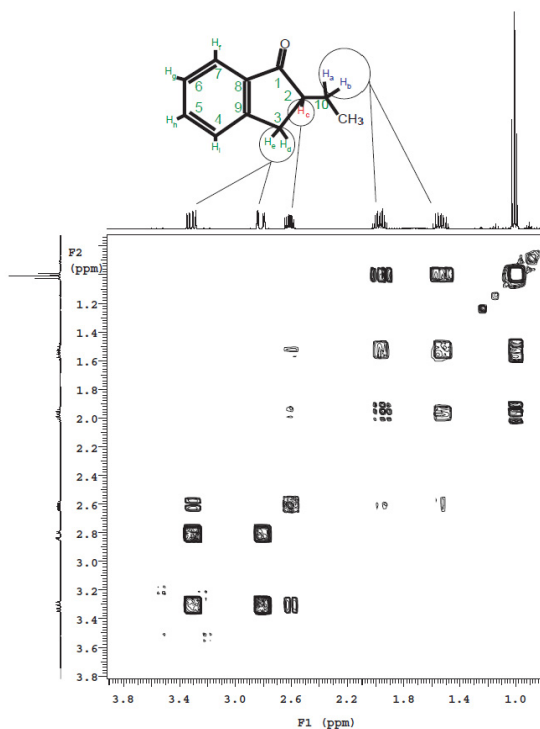


Figure 87 Gradient COSY (gCOSY) of aliphatic region of 2-Ethyl-1-Indanone

Assignment of the aliphatic region, begins with H7, the most deshielded proton, [Figure 88](#). From H7 direct connectivity is apparent to H6. The rest of the assignment is H6 to H5 (the other triplet) and then to H4. The assignment of H7 to the signal at 7.72 ppm is confirmed by the gHMBC data.

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Interpretation of 2-Ethyl-1-Indanone Spectra

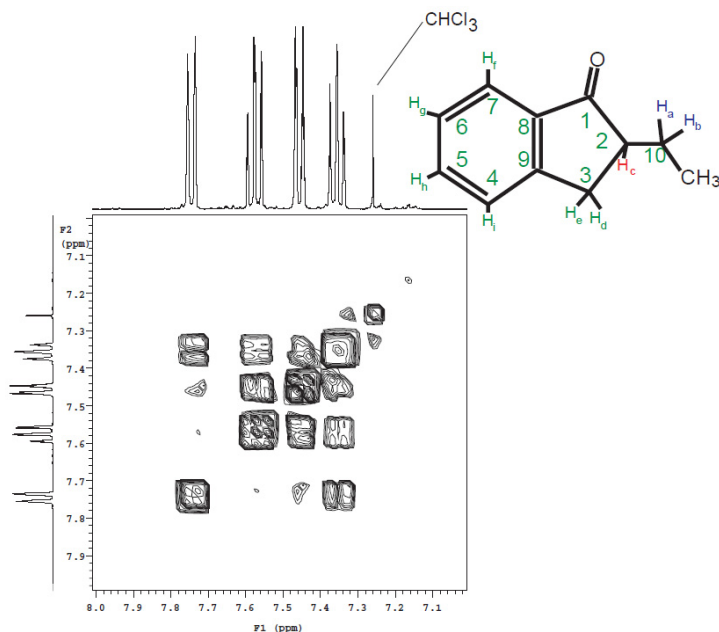


Figure 88 Gradient COSY (gCOSY) of the aromatic region of 2-Ethyl-1-Indanone

TOCSY is a phase sensitive experiment. The cross peaks are narrower than in the COSY giving higher “resolution”. Correlations among all protons in a spin system are observed in the TOCSY spectrum, see [Figure 89](#). The critical parameter is mix. In this case mix is 0.08 seconds which is sufficient to show correlations throughout the entire spin system. Shorter mix times will reveal fewer correlations.

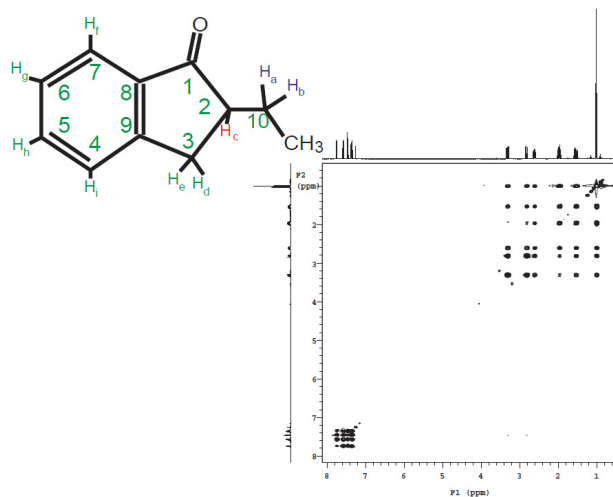


Figure 89 TOCSY of 2-Ethyl-1-Indanone shows correlations among all protons

The expansion shows the completely defined spin system starting with the CH_3 group and ending with protons on C10, [Figure 90](#). A total of 5 crosspeaks are seen in the row.

10 Automated Data Collection and Spectra Interpretation

Interpretation of 2-Ethyl-1-Indanone Spectra

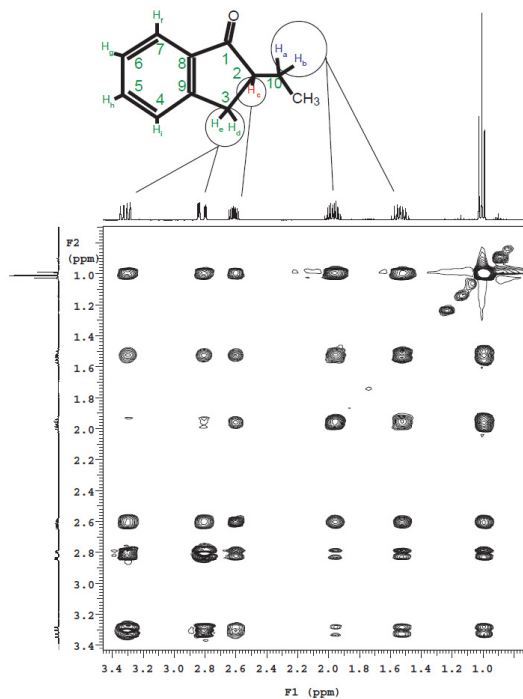


Figure 90 TOCSY of 2-Ethyl-1-Indanone shows correlations among all protons

The indanone sample does not have any significant NOE crosspeaks,
[Figure 91](#)

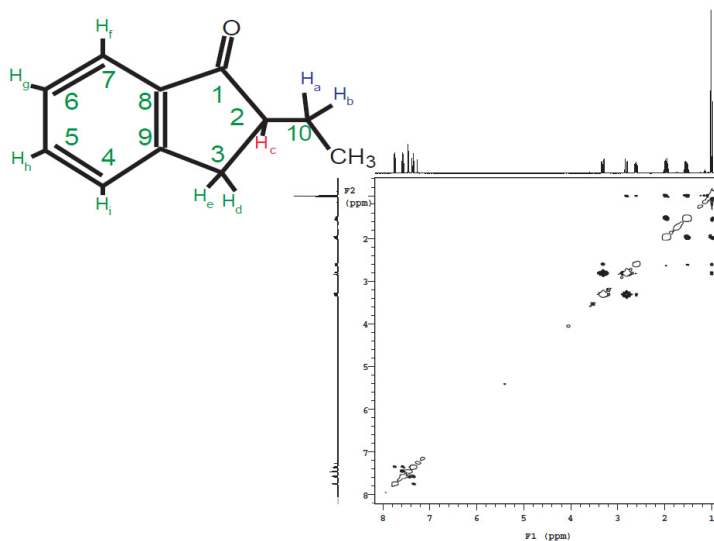


Figure 91 NOESY spectrum of 2-Ethyl-1-Indanone

The main area of interest in this spectrum is to note that the diagonal will be negative and the NOESY crosspeaks will be positive. Crosspeaks which appear to have both positive and negative components are actually not NOE correlations but coupling artifacts.

In the gHSQC (and HSQC) experiment, see [Figure 92](#) the protons correlate with the carbons to which they are attached. The detected nucleus is ^1H and this results in a higher signal-to-noise than the ^{13}C detected heterocor experiment. When compared to the HMQC experiment, the HSQC experiment has the advantage that the $^1\text{H} - ^1\text{H}$ homonuclear coupling does not evolve. As a result the resolution in the 2D plane is higher in the HSQC experiment. The higher resolution has the added advantage of improving the signal-to-noise. The version of the HSQC experiment supplied with NMR spectrometer systems has the added benefit that it will distinguish $-\text{CH}$, $-\text{CH}_2$, and $-\text{CH}_3$ groups. In this case phase is indicated by whether the crosspeak is filled in with multiple contours (above the plane) or is a single contour (below the plane).

10 Automated Data Collection and Spectra Interpretation

Interpretation of 2-Ethyl-1-Indanone Spectra

By contrast to the gHSQC experiment, the gHMBC (and HMBC) experiment shows long range (mostly 2 and 3 bond) ^1H - ^{13}C correlations. This shows connectivity between the non - protonated carbonyl and the protons on C-2.

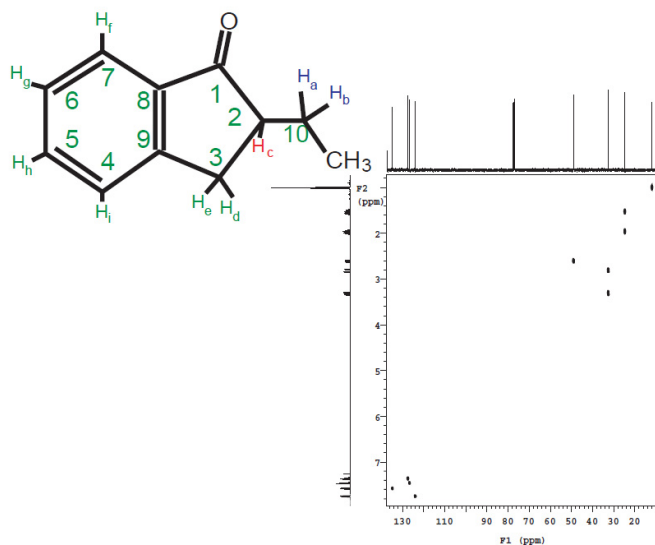


Figure 92 HSQC spectrum of 2-Ethyl-1-Indanone

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G7446-90569



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Agilent VnmrJ 4

Experiment Guide



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Manual Part Number

G7446-90515

Edition

Revision A, March 2013

This guide is valid for 4.0 and later revisions of the Agilent VnmrJ software, until superseded.

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In This Guide...

This guide provides more information about the small-molecule liquids experiments available in the Agilent VnmrJ 4 Experiment Selector.

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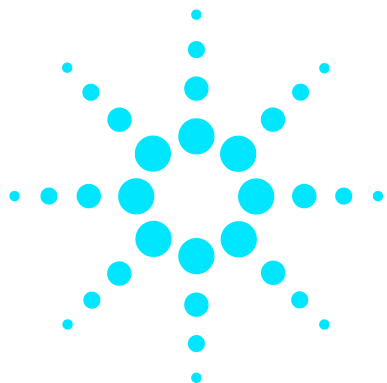
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1 Introduction to Experiments

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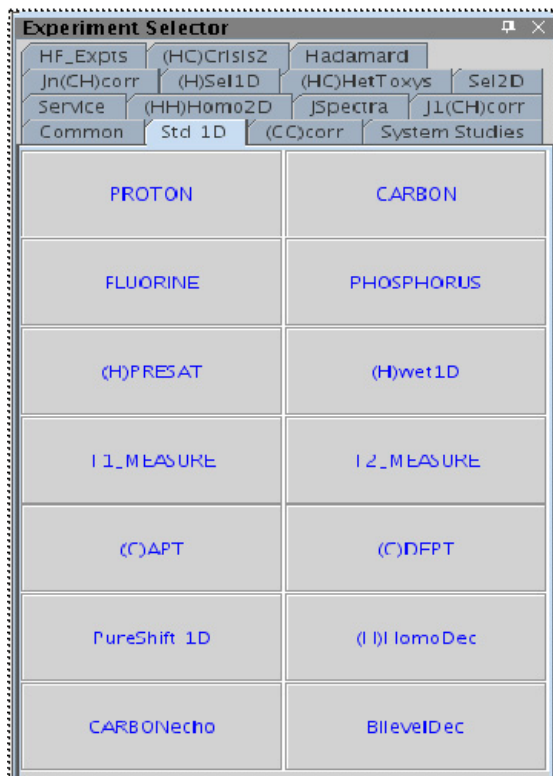
This guide was created for the benefit of organic chemist/novice NMR spectroscopists to intermediate level NMR spectroscopists.

The purpose of this document is to provide more information about the small-molecule liquids experiments available in the VnmrJ 4 Experiment Selector. Included are descriptions of the experiments, their utility, guides to parameter selection, processing, and references for further reading.



Organization

The chapters of this manual are divided into the headings listed on the tabs of the Experiment Selector found in the middle of the left side of the VnmrJ window:



The DOSY2D and DOSY3D experiments are installed with the Dosy package for VnmrJ. If this package was purchased and/or installed, these tabs do appear . A separate DOSY manual is available under the “Help > Online Manuals” menu.

The “Common” tab is a redundant listing of popular routine experiments; similarly, the “System Studies” serve as demonstrations of experiments combined into a single study. Because these experiments are covered

under other tabs, these tabs have not been included. Finally, the “Service” tab is intended for the use of system service personnel, not the users of this manual, and was omitted.

The chapters begin with a general description of the family of experiments and some of the key variants among the experiments. Each experiment in the family is then discussed separately and in depth. The individual experiment descriptions contain a general overview, examples of usage, a pulse sequence diagram, guidance for parameters selection and processing, and literature references. A screenshot of the “Default” panel of the “Acquire” tab, which contains the most pertinent parameters, is shown for each experiment. Pictures of spectra from the experiments are not shown; however, example data sets for most experiments are located in the /vnmr/fidlib folder on your spectrometer.

Modular Experiment Setup

All the sequences in the Experiment Selector were built with a modular design to facilitate parameter setup. By using a “layering” approach where each experiment contributes some property to the final combination, it is possible to configure hundreds of variations without the need for hundreds of individual entries. Each experiment has a customized “Default” panel under the “Acquire” tab that contains the most important and commonly modified parameters for that experiment. Additional panels are added under the “Acquire” tab for more detailed parameters when necessary. When new experiments are created from existing ones, relevant parameters such as the spectral width are retained in a logical fashion.

To illustrate the modular concept, consider a progression of experiments starting with a PROTON and ending with an HSQCADTOXY. A PROTON experiment was acquired with a customized spectral width (sw). If an HSQC experiment is selected from the proton, this sw is maintained in the F2 dimension of the HSQC, as well as other acquisition dominant parameters such as the 90° pulse width (pw90). Set parameters related to the F1 dimension, for example, number of increments (ni) or the ¹³C spectral width. If you decide to acquire an HSQCAD instead, these new F1 parameters are transferred to the HSQCAD and you do not need to reset them. The necessary adiabatic shaped pulses are calculated automatically from parameters in the probe file. Then, to acquire an HSQCADTOXY from the HSQCAD experiment, set only the parameters relating to the TOCSY spinlock (for example, the mixing time). In contrast, if a TOCSY experiment is set up before the HSQCADTOXY, the parameters related to the TOCSY are transferred, and you must set up the HSQC-related parameters instead.

Solvent suppression is also much easier with this modular experiment design. Solvent suppression (“wet”, “presat”, or “purge” type) is available for nearly all the experiments in the selector. Separate experiments such as “presat COSY” or “wet TOCSY” are not necessary, which greatly simplifies the experiment choices. The desired type of solvent suppression is set by choosing the appropriate (H)PRESAT or (H)wet1D experiment and parameters (PURGE suppression is part of (H)PRESAT). Any experiment that is selected later automatically contains the same solvent suppression setup.

The focus of this manual is using the “Experiment Selector” buttons and the “Acquire” tab parameter panels to set up experiments. However, the “Experiments” pull-down menu at the top of the VnmrJ window contains some useful functionality for setting up experiments with nuclei other than the typical ^1H and/or ^{13}C . Briefly, selecting experiments under the “Convert Current Parameters To Do” heading retains the currently set transmitter nucleus (tn) and decoupler nucleus (dn). If the HSQCAD experiment is chosen with tn='F19'/dn='P31', the resulting parameters are for a ^{19}F - ^{31}P HSQCAD experiment. Similarly, if the PRESAT experiment is chosen with tn='F19' or 'P31', the result is a 1D ^{19}F or ^{31}P experiment with presaturation at a selected frequency. This menu allows for easy setup of experiments with “nonstandard” nuclei.

For all the pulse sequences, necessary shaped pulses are calculated automatically from a combination of parameters in the appropriate module and the probe file. Therefore, a probe file containing the relevant calibration information for the experiments is required. See the VnmrJ Installation and Administration manual, Chapter 9, for information on how to set up a probe file.

1 Introduction to Experiments

Modular Experiment Setup



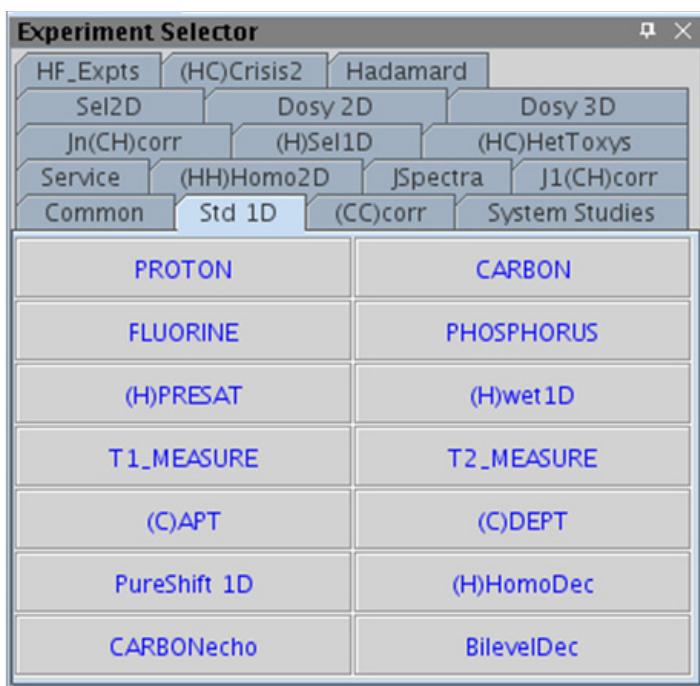
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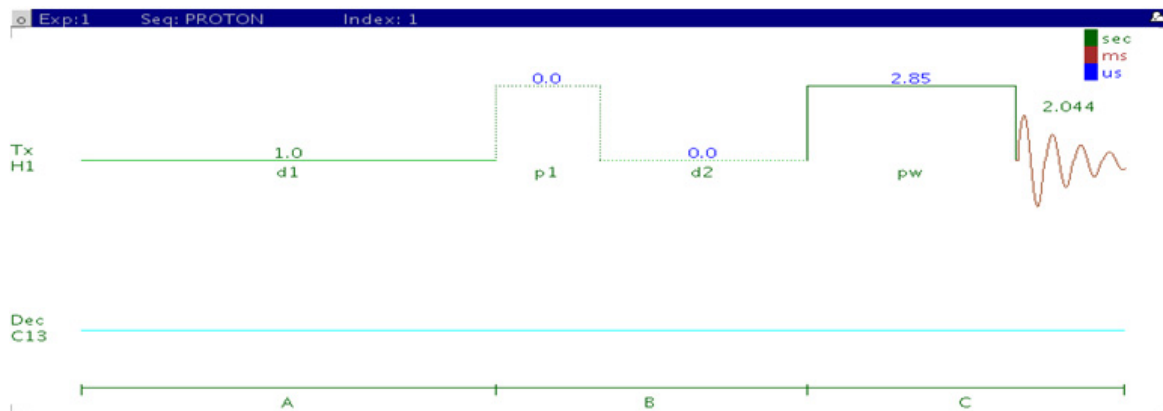


STD 1D Tab

This tab of the Experiment Selector includes the set of standard 1D experiments available, including experiments for proton and other nuclei. The experiments commonly used for proton detection include the standard PROTON 1D experiment, experiments with both presaturation and wet-type solvent suppression, T_1 and T_2 relaxation measurements, plus homonuclear and bilevel decoupling experiments as well as an experiment that produces the equivalent of a broad-band decoupled proton spectrum, called Pureshift 1D. Carbon-detected experiments included are the basic CARBON, (C)APT and (C)DEPT multiplicity selection, and a CARBONecho experiment. Finally, there are tabs for ^{19}F (FLUORINE) and ^{31}P (PHOSPHORUS) 1D experiments, which are also easily adapted for other X-nuclei.



PROTON



General description and usage

Description:

Uses the standard s2pul pulse sequence to acquire a 1D- ^1H dataset.

Usage:

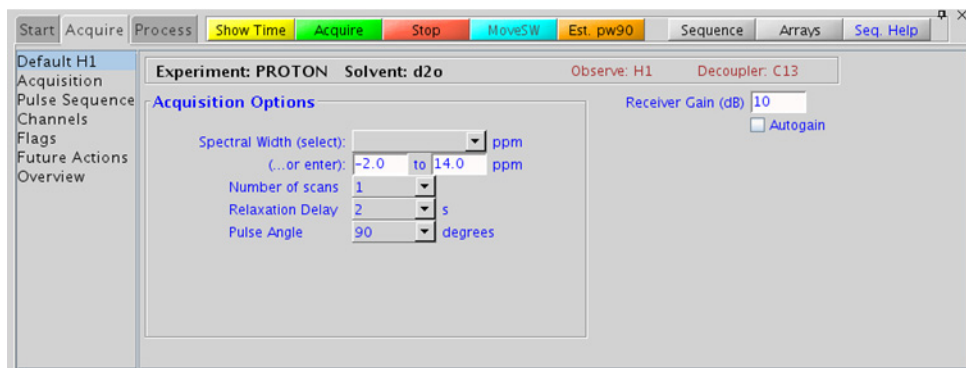
Just as the PROTON experiment is the first step in a typical small molecule structure elucidation, it is also the basic building block for most of the common liquids NMR experiments. When acquiring under automation, most of the 2D experiments require acquisition of a 1D proton first, and it is helpful to begin with this experiment when acquiring manually as well. Important parameters such as solvent and spectral width are transferred to further experiments set up from the proton dataset. All parameters are set from the “Acquire” tab/”Default H1” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from “Start” tab/“Standard” panel
Spectral Width (sw)	Select typical choices from pull down menu or type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle	Select typical choices from pull down menu*-controls the tip angle for the pulse
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the digital resolution (Hz/point, 1/at)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.





Processing

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D proton spectrum is line broadening, with an lb setting from ~ 0.5 - 1 Hz. A suggested optimal value is an lb equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the “Process” tab on the “Weighting” panel. Suggested choices for resolution enhancement (at the expense of S/N) are “pseudo echo” and “res-enhance.” When the weighting function is selected, values for these parameters are calculated based on the acquisition time. They can also be set manually with the “Interactive Weighting” button.

NOTE

Resolution enhancing functions affect the spectral integration- do not use these types of apodization for quantification.

Tips, tricks, and next steps

- The appropriate receiver gain (gain) is most easily set by making sure that the Autogain option is checked on the right side of the “Acquire” tab. The software optimizes the gain setting before data collection.
- To optimize the spectral width (sw) further, place one cursor on each side of the desired region on the spectrum, click the  button located under the spectrum window and reacquire the data.
- To estimate the 90° pulse automatically for subsequent experiments, click the  button located underneath the spectrum window. This calibration is used in lieu of the value in the probe file for future experiments on this sample. Calibration is useful with either salty samples or where perfect tuning is not achievable, both of which can cause longer 90° pulse values.
- The acquisition time (at) which controls the digital resolution (Hz/point, which is equal to $1/at$), is set on the “Acquisition” panel of the “Acquire” tab. While the default digital resolution set from the chosen spectral width is typically much greater than the actual linewidths, check that the acquisition time is sufficient when acquiring large spectral widths or when trying to measure linewidths accurately. For example, in probe lineshape tests.

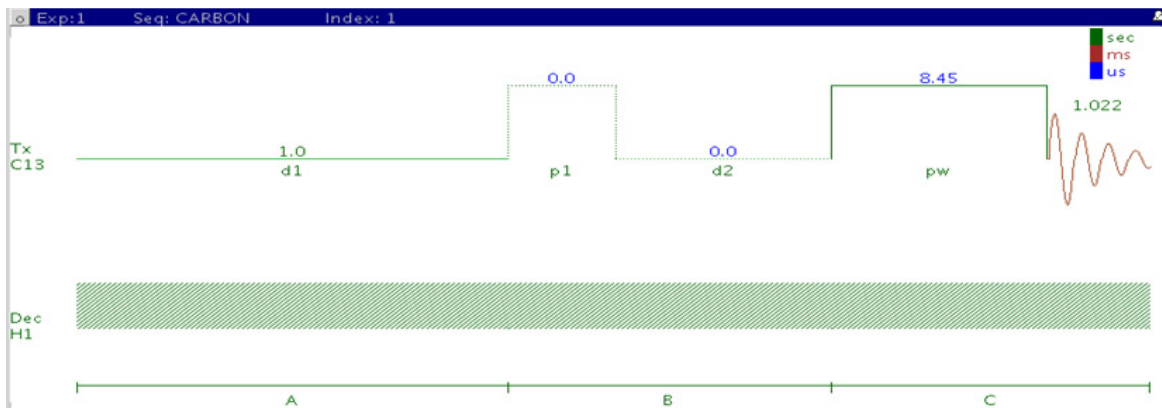
- A pulse tip angle of 45° is common for PROTON experiments. Consider increasing to 90° for quantification. Decrease to smaller values (30° and less) in case of very concentrated samples to avoid receiver/ADC overflow.
- The *total* interscan delay is the sum of the acquisition time and the relaxation delay. When acquiring a multiscan PROTON experiment for quantification, set the interscan delay to at least 5X the longest proton T_1 value in the molecule (see the T1_MEASURE experiment to measure these values). Typical values for an interscan delay range from 3-5s (d1 of 1-3s with an acquisition time of ~ 2 s), which is not appropriate for quantification; consider using ~ 25 s if the T_1 values are not measured. For precise quantification ($<1\%$) a total relaxation delay of $10 \times T_1$ is appropriate.
- The PROTON dataset is used as a starting point for a wide range of experiments. Common next steps include setting up solvent suppression, choosing peaks for selective 1D experiments, and homo- and heteronuclear 2D experiments such as TOCSY and HSQC.

References:

R. J. Abraham, P. Loftus, Proton and Carbon-13 NMR Spectroscopy: An Integrated Approach, Heyden, London, 1978.

D. D. Traficante, Concepts Magn. Reson. 4 (1992) 153-160. (Quantitative Analysis)

CARBON



General description and usage

Description:

Uses the standard s2pul pulse sequence with decoupling to acquire a 1D- ^{13}C dataset.

Usage:

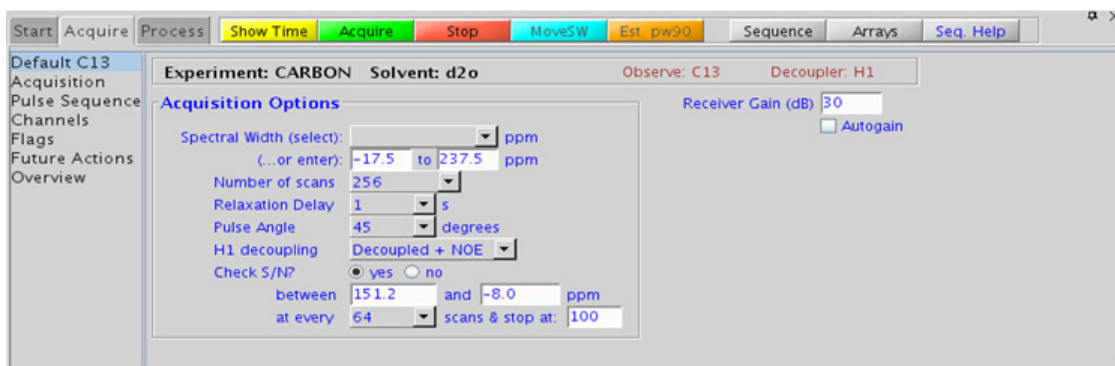
The 1D CARBON experiment is a tool commonly used by organic chemists for structure elucidation and confirmation as the carbon chemical shift is highly sensitive to nearby functional groups. Proton decoupling is traditionally used to simplify Carbon spectra to a series of single lines with little or no coupling constant information available. Carbon chemical shifts are also often reliably calculated from structural input by prediction software. The CARBON experiment, however, requires a relatively large amount of sample (or spectrometer time) as compared to proton-detected experiments due to the lower relative natural abundance of the ^{13}C nuclide and its reduced receptivity when compared to ^1H . All parameters are set from the “Acquire” tab/”Default C13” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from “Start” tab/”Standard” panel
Spectral Width (sw)	Select typical choices from pull down menu or type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle	Select typical choices from pull down menu*-controls the tip angle for the pulse
H1 decoupling (dm)	Select typical choices from pull down menu- turns decoupling and/or NOE enhancement on/off
Check S/N?	Select yes or no- acquires acquisition until S/N value between ppm indicated is > specified value
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the digital resolution (Hz/point, 1/at)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.



Processing

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D carbon spectrum is line broadening, with an lb setting from ~ 0.5 - 1 Hz. Carbon spectra is rarely of high enough S/N for resolution enhancement; however other types of weighting functions are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The acquisition time (at), which controls the digital resolution (Hz/point, which is equal to $1/\text{at}$), is set on the “Acquisition” panel of the “Acquire” tab. Because carbon spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see small coupling; consider lengthening the acquisition time and/or narrowing the spectral width when acquiring coupled carbon spectra.
- The standard proton-decoupled CARBON spectrum is produced by turning on the decoupling during the pulse and acquisition. It is also standard practice to leave the decoupler on during the preacquisition delay (d1), which provides an NOE enhancement to the carbon signals caused by the attached protons. Typically the more protons attached, the larger the enhancement. For example, methyl signals appear larger than quaternary carbon signals. Turn off the NOE enhancement if accurate quantification is desired.
- A common next step for structure elucidation after acquiring the CARBON data is to acquire a series of (C)DEPT experiments to determine the multiplicity of the carbon peaks (for example, methyl, methylene, and methine).
- Carbon T_2 relaxation times can be very long, particularly for quaternary and aromatic carbons, and for degassed samples such as the 10% ethylbenzene standard. Such samples can exhibit the symptom of being difficult or impossible to phase correctly if the spectra are truncated. Increase the acquisition time (at) until the phasing issue is corrected.

- If there is insufficient sample to obtain a CARBON spectrum, consider acquiring proton-detected experiments such as HSQC and HMBC, which give similar information and require much less sample. These experiments can also be configured to give multiplicity information.
- The CARBON dataset can be used as a starting point for carbon-detected correlation experiments such as INADEQUATE or HETCOR spectra. Optimized spectral widths are transferred to the 2D experiment.

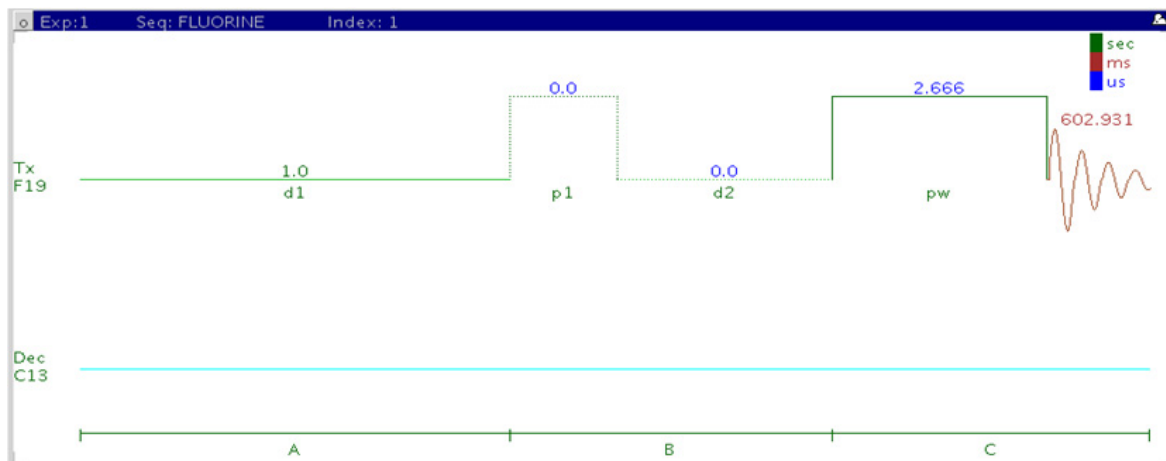
References:

F. W. Wehrli, T. Wirthlin, Interpretation of Carbon-13 NMR Spectra, Heyden, London, 1976.

R. J. Abraham, P. Loftus, Proton and Carbon-13 NMR Spectroscopy: An Integrated Approach, Heyden, London, 1978.

E. Breitmaier, W. Voelter, Carbon-13 NMR Spectroscopy: High-Resolution Methods and Applications in Organic Chemistry and Biochemistry, VCH, 1987.

FLUORINE



General description and usage

Description:

Uses the standard s2pul pulse sequence to acquire a 1D- ^{19}F dataset.

Usage:

The 1D FLUORINE experiment is typically used to confirm the presence of fluorine in a molecule. The large fluorine chemical shift range is highly sensitive to nearby functional groups. The experiment is nearly as sensitive as a 1D PROTON experiment. All parameters are set from the "Acquire" tab/"Default F19" panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from "Start" tab/"Standard" panel
Spectral Width (sw)	Type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle	Select typical choices from pull down menu*-controls the tip angle for the pulse
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.

The screenshot displays the Agilent VnmrJ 4 software interface. At the top, there is a menu bar with options: Start, Acquire, Process, Show Time, Acquire, Stop, MoveSW, Est. pw90, Sequence, Arrays, and Seq. Help. Below the menu bar, the main window is titled "Experiment: FLUORINE Solvent: d2o". On the left side, there is a vertical list of tabs: Default F19, Acquisition, Pulse Sequence, Channels, Flags, Future Actions, and Overview. The "Acquisition" tab is selected. The main area of the window is divided into two sections. The left section, titled "Acquisition Options", contains four rows of controls: "Spectral width - from" with a text box containing "-200.6" and a "to" text box containing "30.6", followed by "ppm"; "Number of scans" with a dropdown menu showing "16"; "Relaxation Delay" with a dropdown menu showing "1" and a unit "s"; and "Pulse Angle" with a dropdown menu showing "30" and a unit "degrees". The right section contains "Receiver Gain (dB)" with a text box containing "30" and a checkbox labeled "Autogain" which is checked. At the top right of the main area, there are two more controls: "Observe: F19" and "Decoupler: C13".

Processing


A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5 - 1 Hz. A suggested optimal value is an lb equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the “Process” tab on the “Weighting” panel. Suggested choices for resolution enhancement (at the expense of S/N) are “pseudoecho” and “res-enhance.” When the weighting function is selected, values for these parameters are calculated based on the acquisition time. They can also be set manually with the “Interactive Weighting” button.

CAUTION

Resolution enhancing functions affect the spectral integration- do not use these types of apodization for quantification.

Many NMR probes contain fluorinated materials, which cause broad signals that can distort the baseline (especially when lying next to or outside the spectral window). The default processing parameters are set to use linear prediction to back predict the first seven points of the spectrum in order to flatten the baseline. These settings can be modified under the “Process” tab on the “More 1D” panel.

Tips, tricks, and next steps

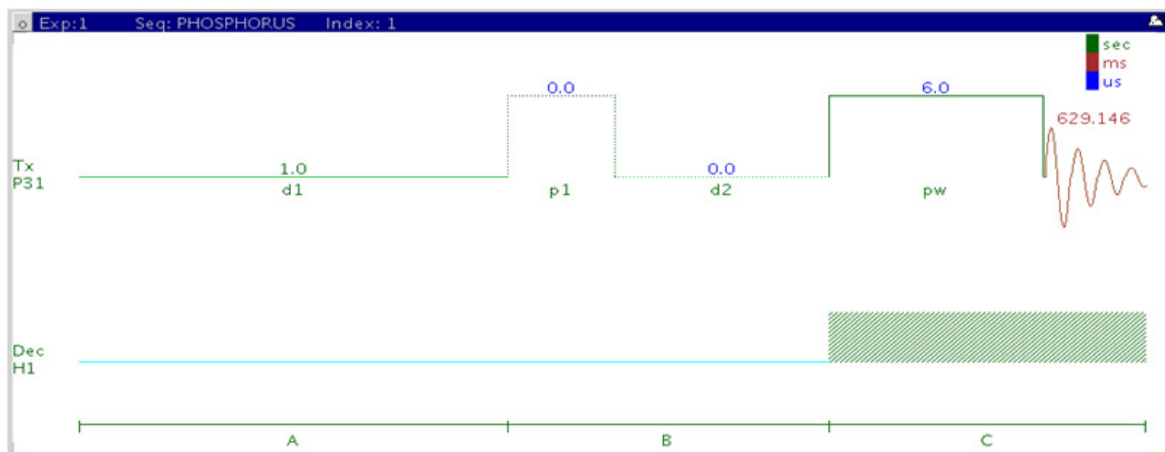
- To optimize the spectral width (sw) further, place one cursor on each side of the desired region on the spectrum, click the  button located under the spectrum window and reacquire the data. The default range is set to cover the typical range of fluorine chemical shifts seen in organic molecules. As this range is large, once the fluorine signals are located, consider narrowing the chemical shift range and reacquiring the spectrum for better resolution.

- The acquisition time (at), which controls the digital resolution (Hz/point, which is equal to $1/at$), is set on the “Acquisition” page of the “Acquire” tab. Because FLUORINE spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see small couplings; consider lengthening the acquisition time and/or narrowing the spectral width.
- The *total* interscan delay is the sum of the acquisition time (at) and the relaxation delay (d1). When acquiring a multiscan FLUORINE experiment for quantification, set the interscan delay to at least 5X the longest fluorine T_1 value of the signals of interest.
- Because of the potentially wide spectral window, determination and use of 90° pulses with FLUORINE NMR experiments can be problematic. Measure the pw90 with the transmitter offset (tof) positioned near an actual ^{19}F resonance.
- This fluorine experiment produces a proton-coupled ^{19}F spectrum. See the HF experiments (Chapter 11) for a proton-decoupled version of the experiment.
- When acquiring impure samples of fluorinated compounds from a biological origin, consider using fluorine spectra in lieu of a more complicated proton spectrum as appropriate.

References:

- E. G. Brame, Anal. Chem. 34 (1962) 591-592. (Spectra Structure Correlations of F19)
- E. F. Mooney, An Introduction to ^{19}F NMR Spectroscopy, Heyden, London, 1970.
- S. Berger, S. Braun, H. O. Kalinowski, NMR Spectroscopy of the Non-Metallic Elements, Wiley, New York, (1997) 398-698.
- J. Battiste, R. A. Newmark, Prog. NMR. Spectrosc. 48 (2006) 1-23. (Multidimensional ^{19}F NMR)

PHOSPHORUS



General description and usage

Description:

Uses the standard s2pul pulse sequence to acquire a 1D- ^{31}P dataset.

Usage:

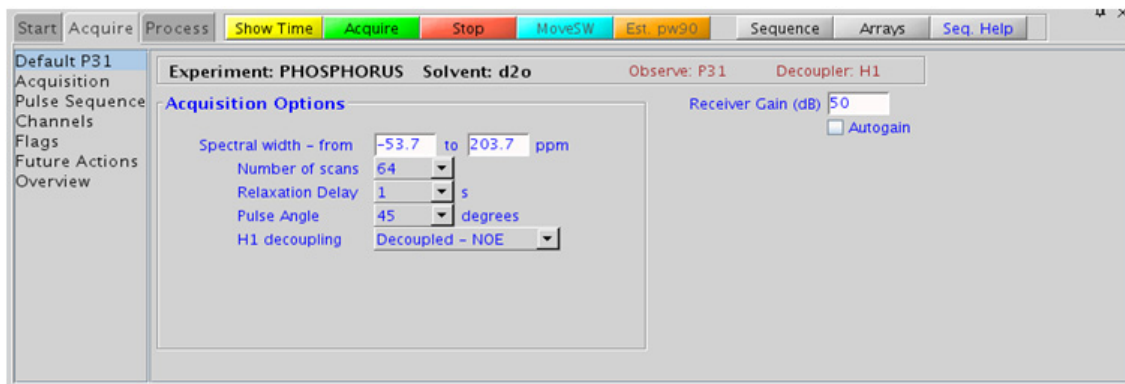
The PHOSPHORUS experiment is typically used to confirm the presence of phosphorus in a molecule. The large phosphorus chemical shift range is highly sensitive to nearby functional groups and the oxidation state of the phosphorus atom. All parameters are set from the "Acquire" tab/"Default P31" panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from "Start" tab/"Standard" panel
Spectral Width (sw)	Type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*- controls the delay between scans
Pulse Angle	Select typical choices from pull down menu*- controls the tip angle for the pulse
H1 decoupling (dm)	Select typical choices from pull down menu- turns decoupling and/or NOE enhancement on/off
Check S/N?	Select yes or no- acquires acquisition until S/N value between ppm indicated is > specified value
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.




Processing

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~ 0.5 - 1 Hz. Other types of weighting functions are available under the “Process” tab on the “Weighting” panel.

PHOSPHORUS spectra often contain broad signals, which distort the baseline. The default processing parameters are set to use linear prediction to back predict the first five points of the spectrum in order to flatten the baseline. These settings can be modified under the “Process” tab on the “More 1D” panel.

Tips, tricks, and next steps

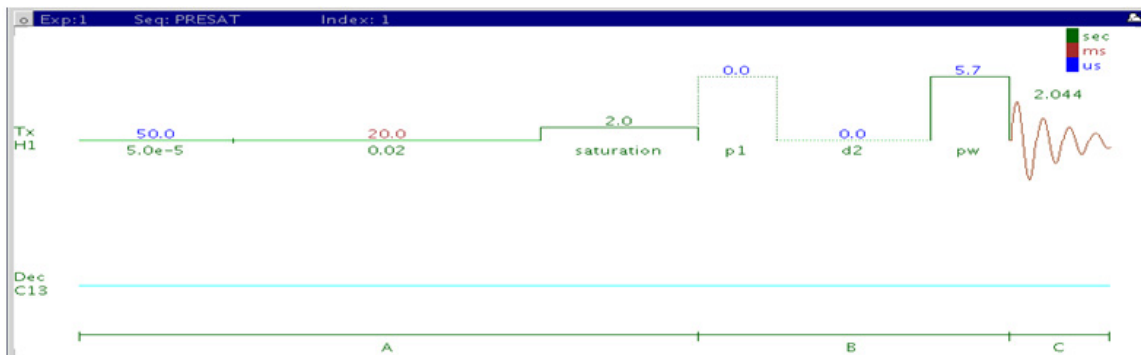
- To optimize the spectral width (sw) further, place one cursor on each side of the desired region on the spectrum, click the  button located under the spectrum window and reacquire the data.
- The appropriate receiver gain (gain) is most easily set by making sure that the Autogain option is checked on the right side of the “Acquire” tab. The software optimizes the gain setting before data collection.
- The acquisition time (at), which controls the digital resolution (Hz/point, which is equal to $1/at$), is set on the “Acquisition” page of the “Acquire” tab. Because PHOSPHORUS spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see coupling; consider lengthening the acquisition time and/or narrowing the spectral width.
- If the sample was purified using reversed-phase HPLC, remember that many mobile phases commonly used in chromatography contain nonvolatile phosphate buffers. Such samples contain large background phosphorus signals from the buffer; consider using a different buffer such as formic acid when anticipating the need of ^{31}P NMR data.

- It is also possible to use presaturation to suppress a large, undesired peak in the phosphorus spectrum. From the PHOSPHORUS parameter set, go to the “Experiments” menu at the top of the VnmrJ window and choose “Convert current parameters to do... Solvent Suppression – Select Peaks => PRESAT submenu”. The “Default” panel now displays the appropriate parameters to configure the frequency/frequencies for suppression.

References:

- O. Khul, Phosphorous-31 NMR Spectroscopy, Springer (2008) 1-131.

(H)PRESAT



General description and usage

Description:

A standard s2pul pulse sequence to acquire a 1D-¹H dataset with a presaturation option to suppress strong signals. Single peaks are suppressed with a hard pulse, multiple peaks with a shaped pulse.

Usage:

Presaturation is a simple method of removing strong signals from a spectrum using irradiation during all or part of the relaxation delay. Common reasons for wanting to suppress signals include: (i) the presence of large solvent signals, (ii) use of nondeuterated solvents, like H₂O, (iii) use of samples with large background impurities or buffers such as urea or acetate, (iv) use of samples of low concentration when the residual proton signal from the deuterated solvent overwhelms the peaks of interest. The most straightforward method is to acquire a standard PROTON spectrum or a scout scan first and then interactively choose the peaks to suppress from this data. The software also allows the peaks to be chosen automatically via a threshold, or via a pull-down menu with several selections for water. These different setup methods are detailed in

the “Tips, tricks, and next steps” section. If additional experiments are set up from the (H)PRESAT data set, all the presaturation parameters are transferred to the new experiment. All parameters are set from the “Acquire” tab/”Default” panel unless otherwise specified.

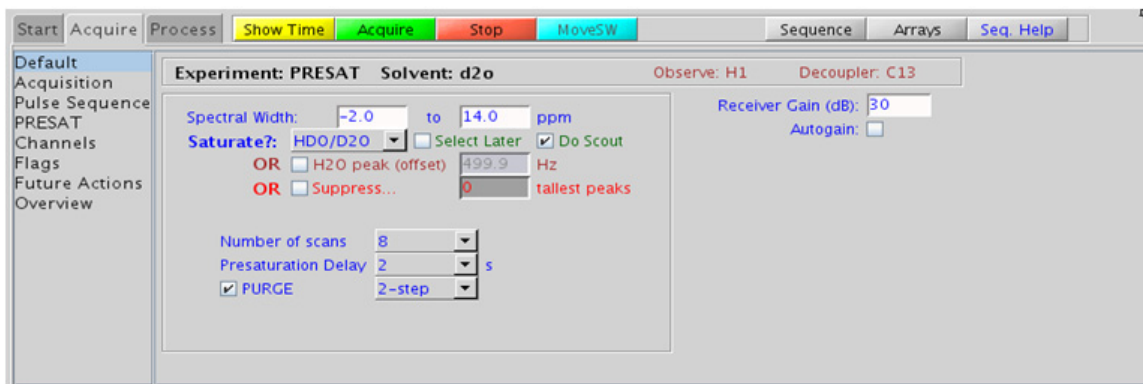
Key parameters

Parameter	Description
Solvent (solvent)	Set from “Start” tab/”Standard” panel
Spectral Width (sw)	Type in specific ppm values (if using previously acquired proton data to select peaks for suppression, do not change)
Saturate? (satmode)	Choices of water type to suppress in pull-down, or check boxes to select later from scout scan, or type in specific water frequency, or suppression of n tallest peaks
Number of scans (nt)	Select typical choices from pull down menu*
Presaturation Delay (satdly)	Select typical choices from pull down menu*- controls the delay between scans
PURGE (prgflg)	Check box adds PURGE pulse to improve saturation, choose 2-step (default) or 4-step

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.

2 Standard 1D - Std 1D Processing

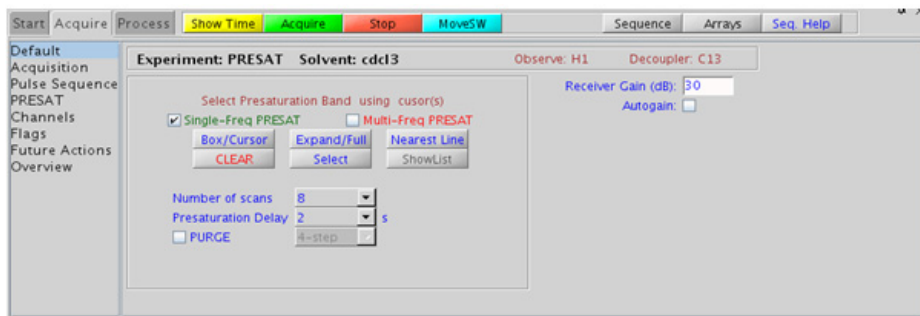


Processing

The processing is typically the same as processing used for standard PROTON spectrum, except manual phasing may be required due to an unphaseable residual signal from the suppressed peaks. A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from $\sim 0.5 - 1$ Hz. A suggested optimal value is line broadening equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- Choosing the (H)PRESAT experiment from a previously acquired PROTON data set allows for interactive selection of the peaks to be suppressed, and the “Default” panel appears differently. Choose single or multifrequency presat with the check boxes, place the cursors on or around the peak to be suppressed and click **Select**. The width of the peaks chosen determine the power used for the presaturation. Repeat for additional peaks.



If a PROTON spectrum has not been previously acquired but the “Select Later” and “Do Scout” options were checked on the “Default” panel shown in the “Key parameters” sections, the scout scan is acquired and the data displayed, now with the “Default” panel shown to facilitate the interactive peak selection.

- The appropriate receiver gain (gain) is most easily set by making sure that the Autogain option is checked on the right side of the “Acquire” tab. The software optimizes the gain setting before data collection. Selecting autogain is a good choice in most solvent suppression experiments acquired in automation.
- When you click the check box to “Suppress... n tallest peaks”, and type a value for n, a scout scan is acquired, and then the software sets up suppression on n peaks as measured from the threshold. This approach works well when acquiring in automation mode.
- The choices for water type in the “Saturate?” pull-down menu control the power of the RF field used for the presaturation. The highest power (50-Hz field) is used for the 90% H₂O, while the other choices (HDO/D₂O, wet DMSO) use lower power. The saturation frequency (satfrq) for the 90% H₂O suppression is automatically optimized for maximum efficiency.
- The power, length, and offset of the presat pulse can be modified manually in the “PRESAT” panel. Remember that presat powers are typically low (typically a small, negative number) and the length usually 2s or less. If additional interscan delay time is needed, the relaxation delay d1 can be set to a larger value than the presat delay.

- Adding the 2-step (or 4-step) purge option can significantly improve the presaturation efficiency, particularly for large water peaks. However the efficiency of the purge option is dependent upon the pw90 and B1 (in)homogeneity of the probe. The 4-step purge produces the best water suppression. Note, however, that the purge phase cycle is *added* to all subsequent experiments, for example, the minimum nt for 4-step PURGE is 4.
- Once the solvent suppression is set up satisfactorily, selecting another experiment type. For example, zTOCSY, NOESY, and so on, automatically retain suppression parameters for the new experiment. To turn off presaturation, unselect the “Presaturation” check box on the “PRESAT” panel.
- (H)PRESAT also suppresses any exchangeable protons. Use wet-type suppression to preserve these signals

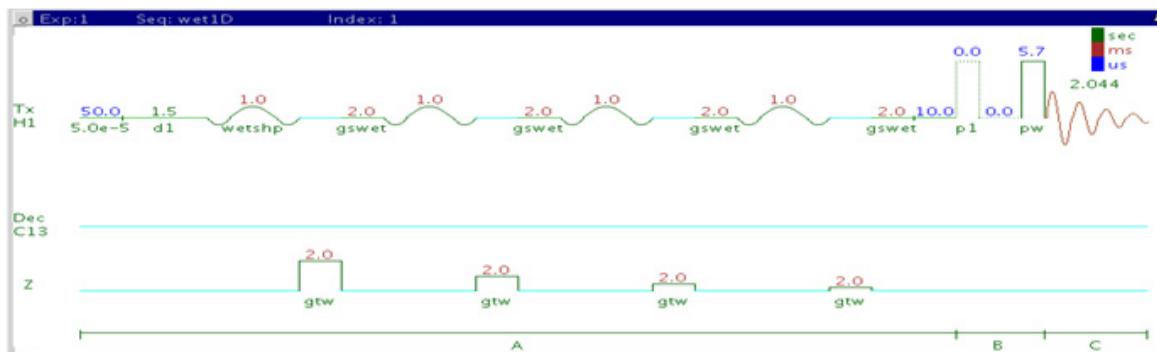
References:

P. J. Hore, Methods Enzymol. 176 (1989) 64-77. (Solvent Suppression)

M. Gueron, P. Plateu, M. Decorps, Prog. NMR Spectrosc. 23 (1991) 135-209. (Solvent Signal Suppression)

R. T. MacKay, Ann. Rep. NMR. Spectrosc. 66 (2009) 33-76. (Advances in Solvent Suppression)

(H)wet1D



General description and usage

Description:

Pulse sequence to acquire a 1D- ^1H dataset with selective excitation pulses to exclude strong signals.

Usage:

WET solvent suppression removes strong signals from a spectrum by creating a shaped pulse that excites only the solvent resonances followed by the use of gradients to suppress the excited resonances. WET suppression has the advantage over other methods (like (H)PRESAT) that the exchangeable protons are often not suppressed as much. It also facilitates the decoupling and hence removal of ^{13}C satellites of organic solvents. However (H)wet1D does not result in the smallest residual H_2O signal, and it can also produce baseline artifacts that interfere somewhat with integration. Common reasons for wanting to suppress signals include: (i) the presence of large solvent signals, (ii) use of nondeuterated solvents, for example H_2O , (iii) use of samples with large background impurities or buffers, such as urea or acetate, or (iv) use of samples of low concentration when the residual proton signal from the deuterated solvent

overwhelms the peaks of interest. The most straightforward method is to acquire a standard PROTON spectrum or a scout scan first and from this data, interactively choose the peaks to be suppressed. The software also allows the peaks to be chosen automatically via a threshold or a number of common solvent selections. These different setup methods are detailed in the “Tips, tricks, and next steps” section. If additional experiments are set up from the (H)wet1D data set, all the presaturation parameters are transferred to the new experiment. All parameters are set from the “Acquire” tab/”Default” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	LSet from “Start” tab/”Standard” panel
Spectral Width (sw)	Type in specific ppm values (if using previously acquired proton data to select peaks for suppression, do not change)
WET peaks	Choices of define now (shows list of solvents when checked) or check boxes to select later from scout scan, or suppression of n tallest peaks, or type in specific water frequency
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*- controls the delay between scans
C13 decoupling during WET?	Turns decoupling on to suppress solvent ^{13}C satellites, for example, from CH_3CN
Optimize CF	Optimizes the compression factor for the transmitter power used for the wet shape

NOTE


You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.



Processing

The processing is typically the same processing used for standard PROTON spectrum, except manual phasing may be required due to an unphaseable residual signal from the suppressed peaks. A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5 - 1 Hz. A suggested optimal value is line broadening equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- Choosing the (H)wet1D experiment from a previously acquired PROTON data set allows for interactive selection of the peaks to be suppressed, and the “Default” panel appears differently (see the following figure). Choose single or multifrequency presat with the check boxes. Place the cursors on or around the peak to suppress and click . Repeat for additional peaks. A threshold can also be set to select peaks automatically.



If a PROTON spectrum has not been previously acquired but the “Select Later” and “Do Scout” options were checked on the “Default” panel shown in the “Key parameters” sections, the scout scan is acquired and the data displayed, now with the “Default” panel shown above to facilitate the interactive peak selection.

- The appropriate receiver gain (gain) is most easily set by making sure that the Autogain option is checked on the right side of the “Acquire” tab. The software optimizes the gain setting before data collection. Selecting autogain is a good choice in most solvent suppression experiments acquired in automation.
- When you click the check box to “Suppress... n tallest peaks”, and type a value for n, a scout scan is acquired, and then the software sets up suppression on n peaks as measured from the threshold. This approach works well when acquiring in automation mode.
- When selecting peaks for suppression via the solvent check boxes, note that the most common choices are displayed, while clicking the “more” button shows additional, less typical choices.
- Try to keep the widths of the selected peak >60 Hz, as peaks that are too narrow create long, shaped pulse durations.
- If the pw90 is reasonably accurate, the suppression quality using the default parameters is adequate for most samples. Click the “Optimize CF” check box to optimize suppression. This arrays and attempts to optimize a scaling factor for the shaped pulses tpwr_cf, looking for the value that produces the best suppression. If the desired number of scans is large (>32), this optimization takes a fair amount of time. Consider lowering nt for the optimization and then increasing after the parameters are set. This optimization can also be done manually from the “WET” panel.

2 Standard 1D - Std 1D

Tips, tricks, and next steps

- Once the solvent suppression is set up satisfactorily, selecting another experiment type. For example, zTOCSY, NOESY, and so on, automatically retain suppression parameters for the new experiment. To turn off WET, unselect the “WET” check box on the “WET” panel.

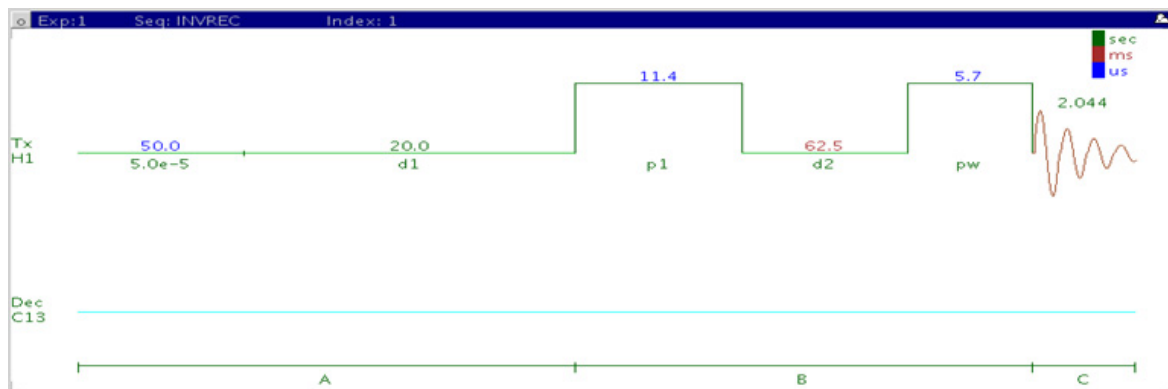
References:

R. Ogg, P. Kingsley, J. Taylor, J. Magn. Reson. B104 (1994) 1-10. (WET)

S. Smallcombe, S. L. Patt, P. Keifer, J. Magn. Reson. A117 (1995) 295-303. (WET – Applications to LCNMR)

S. L. Patt, J. Magn. Reson. 96 (1992) 94-102. (Shifted Laminar Pulses)

T1_MEASURE



General description and usage

Description:

Uses an inversion-recovery pulse sequence (180° -d2- 90°) to measure spin-lattice (T_1 or longitudinal) relaxation rates.

Usage:

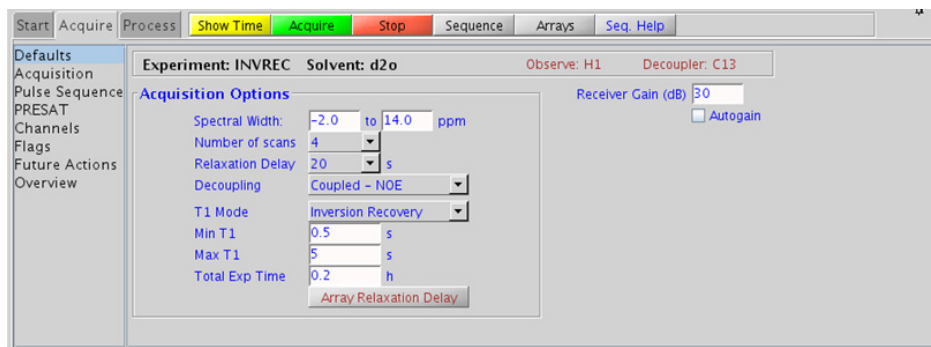
Knowledge of the T_1 relaxation rates for the sample is important for multiscan quantification experiments. Accurate quantification requires an interpulse delay of at least 5x the longest T_1 of interest in the molecule. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters







Parameter	Description
Solvent (solvent)	Set from "Start" tab/"Standard" panel
Spectral Width (sw)	Type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*- controls the delay between scans; set longer for accurate T_1 measurements
Decoupling (dm)	Select choice from pull down menu- for X nuclei, choose coupled/uncoupled; +/- NOE
T1 Mode	Select choice from pull down menu- choose inversion recovery or progressive saturation
Min/Max T1; Total Exp Time	Use to set the array of values for the T_1 d2 delay- choose a min/max value, select desired total exp time (controls # of steps) and click Array Relaxation Delay

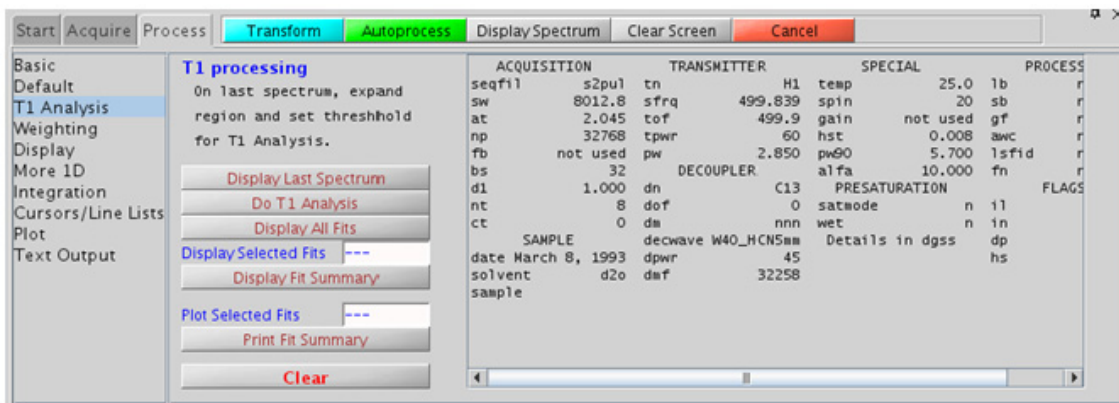
NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.




Processing

The T1_MEASURE experiment, with the inversion recovery option selected, produces an array of 1D spectra, where the peaks heights slowly progress through zero and then become the opposite phase as the value of d2 increases. To process the entire array, use , or type “wft”. To display a single spectrum in the array, use the   buttons to choose a spectrum. Alternatively type ds(#), where # is the number of the spectrum in the array you wish to display. Clicking  on the vertical “ArrayedSpectra” panel displays all the spectra in a horizontal array. On the “Process” tab, there is a special panel labeled “T1 Analysis”. This panel contains tools to facilitate the T₁ analysis. Click , expand the spectral region for the T₁ analysis and set the threshold just below the shortest analysis peak. Now click  to fit the data to T₁ curves for each peak. Use the display and print buttons to view the results.



Tips, tricks, and next steps

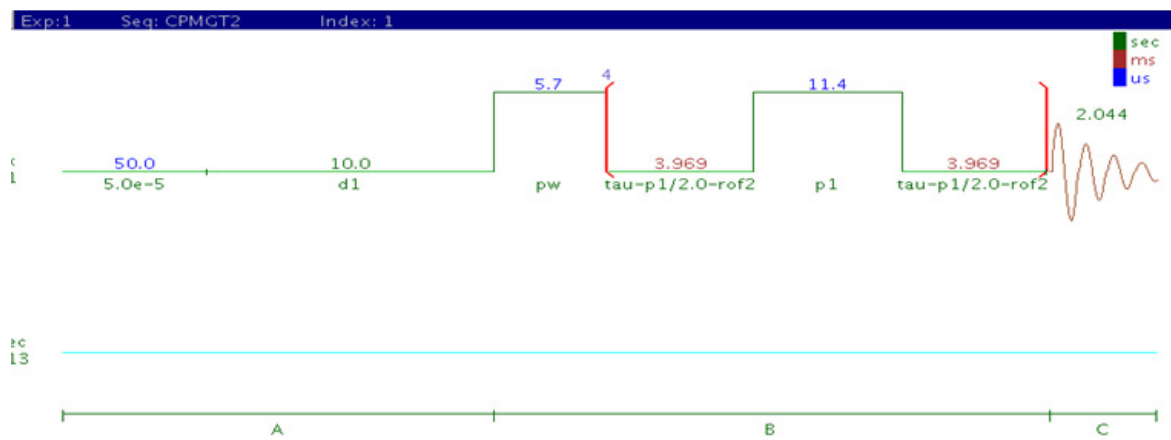
- For the most accurate results, ensure that the relaxation delay is 5x the longest expected T₁- the default value is 20s, which assumes the longest T₁ is < 4s. If you find that your T₁ values of interest are longer, lengthen this delay appropriately and reacquire the data.

- The appropriate receiver gain (gain) is set from a PROTON spectrum with a 90° pulse angle. The “Autogain” option is not available for arrayed spectra.
- An accurate 90° pulse measurement is important for this experiment. For best results, measure the 90° pulse for your sample with a standard proton experiment before the T₁ analysis.
- To save time, you can perform the analysis initially with a small array containing a broad range of d2 values. Once you know the approximate value of d2 that produces the null, you can perform a second analysis with smaller array steps to target the exact value.
- To view the values in the d2 array, click  displayed underneath the spectrum when the “Acquire” tab is selected.
- If you are estimating the T₁ value visually from the null spectrum, remember that T₁ = d2*1.44, at the null point, as T₁ relaxation is a first-order rate process.

References:

A. Derome, Modern NMR Techniques for Chemistry Research, Pergamon Press, New York, 1987.

T2_MEASURE



General description and usage

Description:

Uses a spin-echo pulse sequence to measure spin-spin (T_2 or transverse) relaxation rates.

Usage:

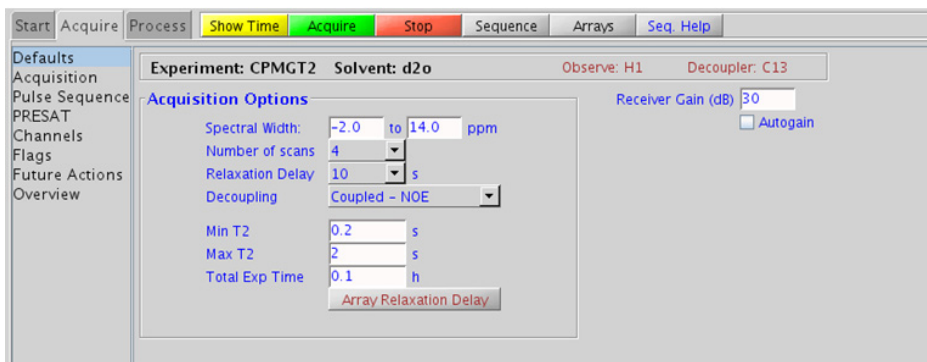
This experiment allows one to measure the transverse relaxation (T_2) in the absence of magnetic field inhomogeneity, rather than the rate constant for the decay of the FID (T_2^*), which is reflected in the 1D linewidths. T_2 relaxation rates are correlated with molecular size, so their measurement can provide insight for small molecules regarding aggregation, binding to large molecules (for example, proteins), or other noncovalent interactions. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters


Parameter	Description
Solvent (solvent)	Set from "Start" tab/"Standard" panel
Spectral Width (sw)	Type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*- controls the delay between scans; set longer for accurate T_2 measurements
Decoupling (dm)	Select choice from pull down menu- for X nuclei, choose coupled/uncoupled; +/- NOE
Min/Max T2; Total Exp Time	Use to set the array of values for the T_2 delay- choose a min/max value, select desired total exp time (controls # of steps) and click "Array Relaxation Delay" button






NOTE

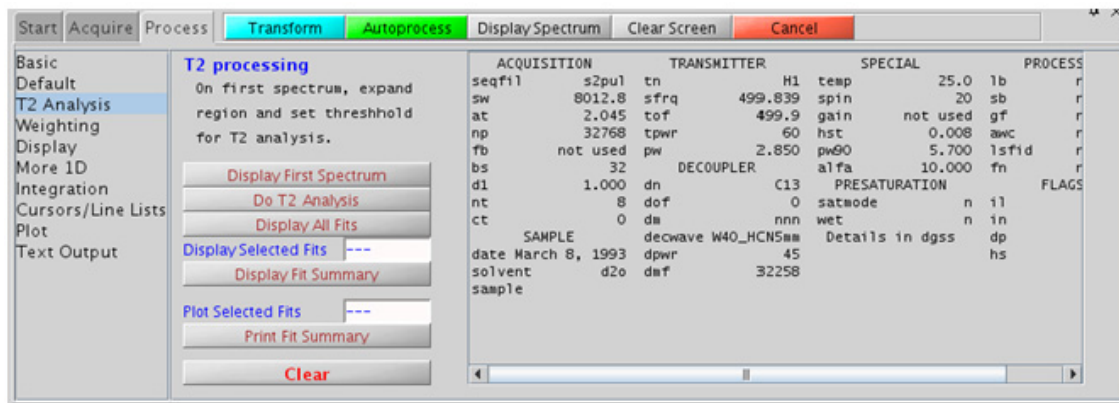
You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.



Processing


The T2_MEASURE experiment produces an array of 1D spectra, where the peaks heights slowly progress to zero as the value of the delay approaches the value for full T₂ relaxation. To process the entire array, use 

or type “wft”. To display a single spectrum in the array, use the   buttons to choose a spectrum. Alternatively type ds(#), where # is the number of the spectrum in the array you wish to display. Clicking  on the vertical “ArrayedSpectra” panel or typing “dssh” displays all the spectra in a horizontal array. On the “Process” tab, there is a special panel labeled “T₂ Analysis”. This panel contains tools to facilitate the T₂ analysis. Click , expand the spectral region for the T₂ analysis and set the threshold just below the shortest analysis peak. Now click  to fit the data to T₂ curves for each peak. Use the display and print buttons to view the results.



Tips, tricks, and next steps

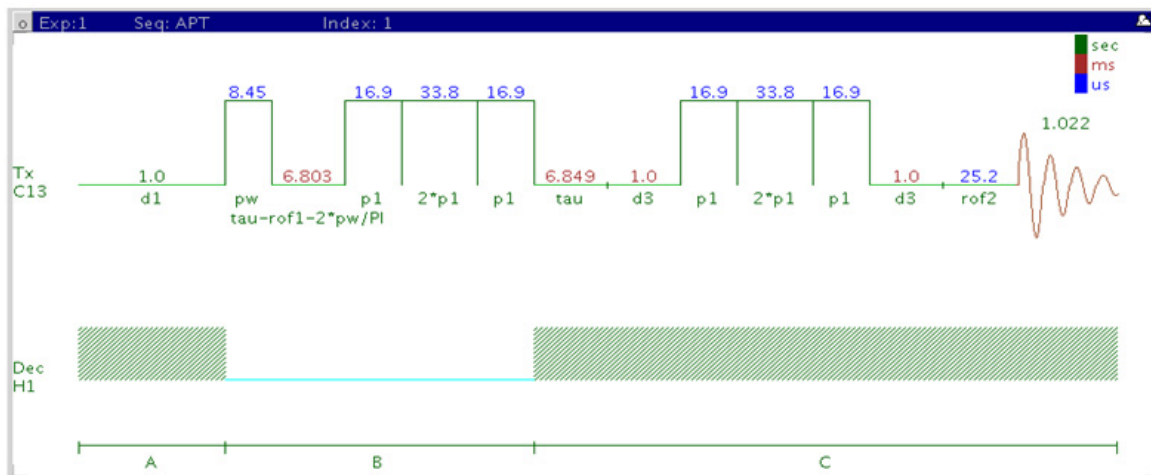
- An accurate 90° pulse measurement is important for this experiment. For best results, measure the 90° pulse for your sample with a standard proton experiment before the T₂ analysis.

- The appropriate receiver gain (gain) is set from a PROTON spectrum with a 90° pulse angle. The “Autogain” option is not available for arrayed spectra.
- To save time, the analysis can be performed initially with a small array containing a broad range of T₂ values. Once the approximate value of T₂ that produces the null is determined, a second analysis can be performed with smaller array steps to target the exact value.
- To view the values in the d2 array, click  displayed underneath the spectrum when the “Acquire” tab is selected.
- Because broad peaks have short T₂ values, this sequence is sometimes used to remove large, broad, background peaks from spectra and flatten the baseline (“T2 filter”). Select a single, short value (0.1-0.2s) for the T₂ value (bigtau) and acquire the data.
- Strictly speaking, the T2_MEASURE experiment only works well on singlets. For example, it is good for ¹³C spectra. For coupled systems, multiplet distortion occurs during the echo, the degree of which depends on the value of T₂ delay.

References:

- H. Y. Carr, E. M. Purcell, Phys. Rev. 94 (1954) 630-638. (T2)
- S. Meiboom, D. Gill, Rev. Sci. Instrum. 29 (1958) 688-691. (T2)
- A. Derome, Modern NMR Techniques for Chemistry Research, Pergamon Press, New York, 1987.

(C)APT



General description and usage

Description:

Attached proton test experiment to determine carbon multiplicity.

Usage:

The (C)APT experiment is used as an adjunct or a replacement for a 1D CARBON spectrum. The default parameters result in a spectrum with methyl and methine-type carbon signals having opposite phase with respect to that of methylenes and quaternary-type carbons. Quaternary carbon lines typically are smaller than methylene signals, and can often be distinguished in this manner. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from "Start" tab/"Standard" panel
Spectral Width (sw)	Select typical choices from the pull down menu or type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu* - controls the delay between scans
Check S/N?	Select yes or no- acquires data until S/N value between ppm indicated is > specified value

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.

Start Acquire Process Show Time Acquire Stop Sequence Arrays Seq. Help

Defaults Acquisition Pulse Sequence Channels Flags Future Actions Overview

Experiment: APT Solvent: d2o Observe: C13 Decoupler: H1

Acquisition Options

Spectral Width (select) [pull-down] ppm
 (...or enter): -17.5 to 237.5 ppm

Number of scans 256

Relaxation Delay 1 s

Check S/N? ☒ yes ☐ no

between 151.2 and -8.0 ppm
 at every 64 scans & Stop at 100

Receiver Gain (dB) 50
☐ Autogain

Processing

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~ 0.5 - 1 Hz. Other types of weighting functions are available under the “Process” tab on the “Weighting” panel. Manual phasing may be necessary as the spectra often contain both positive and negative peaks. Proper phasing is typically with the solvent resonance “up” which results in quaternary and CH₂ carbons also pointing up and CH/CH₃ carbons pointing down.

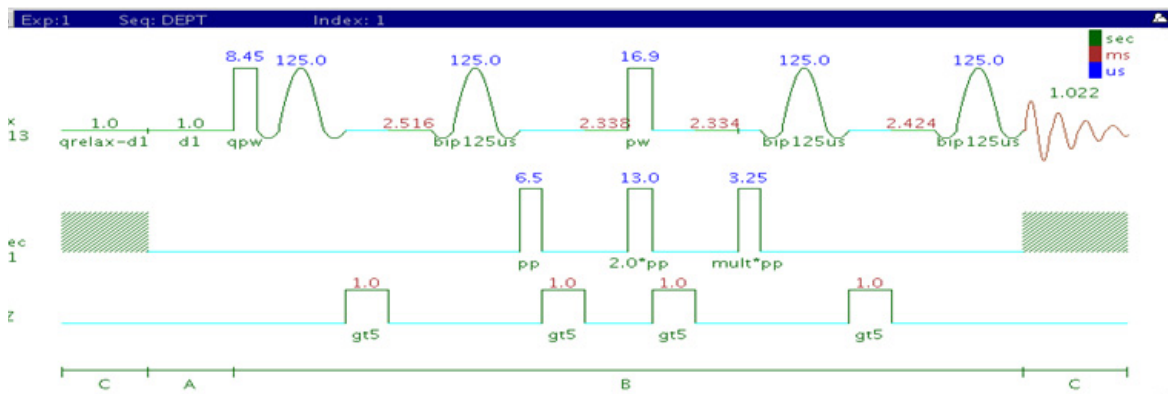
Tips, tricks, and next steps

- Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is highly concentrated or ¹³C-labelled.
- The (C)DEPT experiment can produce edited carbon subspectra containing a single type of carbon multiplicity for easy interpretation. However the (C)APT is a single experiment, compared to multiple experiments for the full (C)DEPT series. Protonated carbons are also enhanced in DEPT by magnetization transfer from the protons as opposed to APT.
- If there is insufficient sample to obtain an (C)APT spectrum, consider acquiring proton-detected experiments such as gHSQC, which gives similar information and requires much less sample. The gHSQC experiment can also be acquired with multiplicity editing to give the same type of information as the (C)APT experiment.

References:

S. L. Patt, J.N. Shoolery, J. Magn. Reson. 46 (1982) 535-539. (APT)

(C)DEPT



General description and usage

Description:

Experiment to determine carbon multiplicity.

Usage:

(C)DEPT experiments are used to produce edited subspectra of 1D carbon data that contain only carbons of a certain multiplicity type. Choices include (1) methines and methyls peaks positive, methylenes negative, (2) methines only, or (3) methyls, methylenes, and methines (no quaternary carbons). Mathematical combinations of the three choices can be used to produce spectra that contain only a specific type of carbon, for example, methylenes are only produced by the linear combination of choices 1 - 3. Additionally, quaternary carbons can be selected (DEPTQ) to be in-phase or anti-phase with respect to the methyls or not to be present at all (traditional DEPT). With full DEPTQ editing (eight spectra), quaternary carbons can also be separated out. Individual subspectra for each carbon are generated with the "Full Edit" choice of the "XH Multiplicity editing" pull down. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

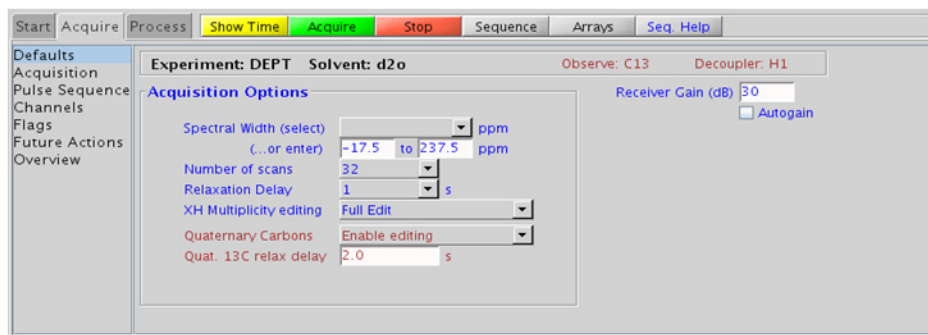
Key parameters

Parameter	Description
Solvent	Set from "Start" tab/"Standard" panel
Spectral Width (sw)	Select typical choices from the pull down menu or type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*
XH Multiplicity editing (mult)	Select choice of (C)DEPT experiment from pull down menu
Quaternary Carbons (qphase)	Select choice of DEPTQ- allows for the addition of antiphase or in-phase quat. carbons
Quat. 13C relax delay (qrelax)	Allows for an additional delay to be included for subspectra containing quat. carbons in the sequence

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.

2 Standard 1D - Std 1D Processing



Processing

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D proton spectrum is line broadening, with an lb setting from ~ 0.5 - 1 Hz. Other types of weighting functions are available under the “Process” tab on the “Weighting” panel. Manual phasing may be necessary as the spectra can contain both positive and negative peaks.

Tips, tricks, and next steps

- Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is highly concentrated or ^{13}C -labelled.
- The (C)DEPT experiment can produce edited carbon subspectra containing a single type of carbon multiplicity for easy interpretation. Protonated carbons are also enhanced in DEPT by magnetization transfer from the protons as opposed to APT.
- A single DEPT experiment with the “XH/XH3 up & XH2 down” option along with the Quaternary carbon “Antiphase” option is a good alternative to APT, as it provides the same information, but with enhanced sensitivity for protonated carbons.

- If there is insufficient sample to obtain a (C)DEPT spectrum, consider acquiring proton-detected experiments such as gHSQC, which gives similar information and requires much less sample. The gHSQC experiment can also be acquired with multiplicity editing to give the same type of information as the (C)DEPT experiment.

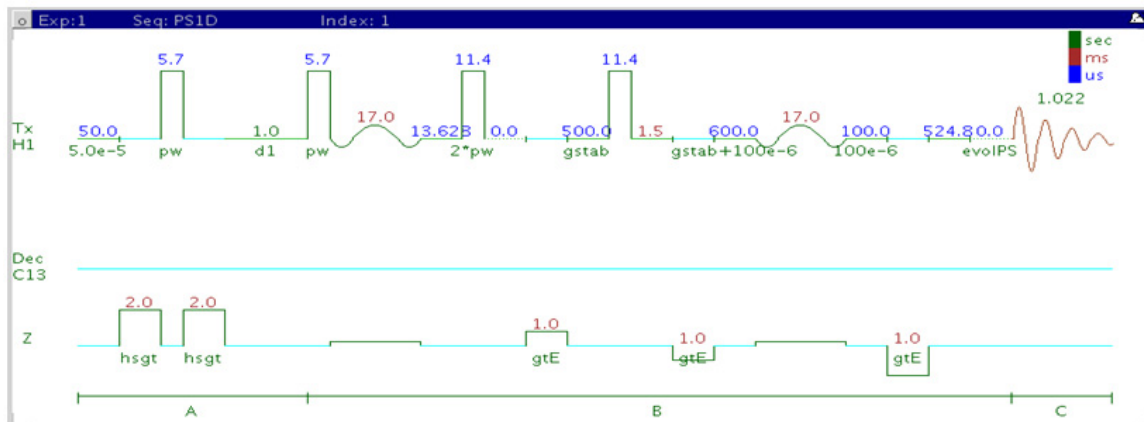
References:

D. M. Doddrell D. T. Pegg M. R. Bendall, J. Magn. Reson. 48 (1982) 323-327. (DEPT)

M. A. Smith, H. Hu, A. J. Shaka, J. Magn. Reson. 151 (2001) 269-283. (BIP Pulses)

P. Bigler, R. Kummerle, W. Bermel, Magn. Reson. Chem. 45 (2007) 469-472. (DEPTq)

PureShift 1D



General description and usage

Description:

Uses a double spin-echo type experiment to acquire an effectively homonuclear-decoupled proton spectrum.

Usage:

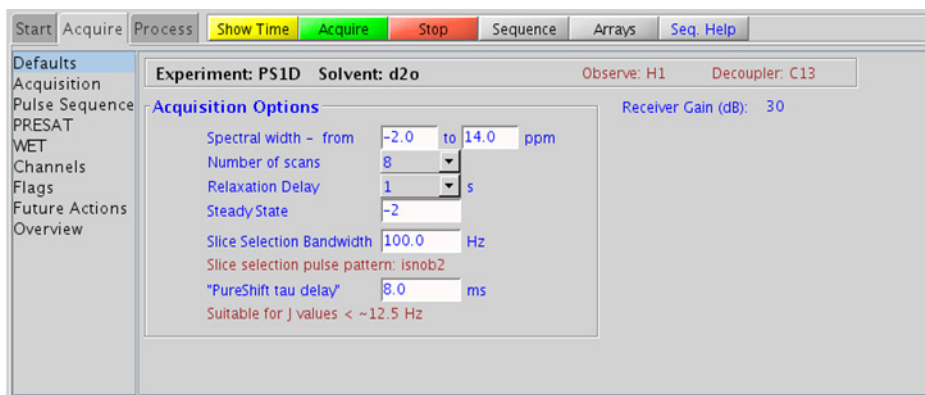
The PureShift 1D experiment is useful to simplify complicated proton spectra dominated by overlapping multiplets. The result is a “proton-decoupled” spectrum of singlets at the true chemical shift of each proton multiplet. The sensitivity is much less than the standard proton experiment. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent(solvent)	Set from “Start” tab/”Standard” panel
Spectral Width(sw)	Type in specific ppm values
Number of scans(nt)	Select typical choices from pull down menu*
Relaxation Delay(d1)	Select typical choices from pull down menu*- controls the delay between scans
Slice Selection Bandwidth	Bandwidth for selective pulse- 100-Hz standard
”PureShift tau delay”	Delay for effective homonuclear decoupling

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.



Processing

As the sensitivity of the PureShift 1D experiment is lacking compared to a standard PROTON, a good processing weighting choice is one which provides S/N enhancement, for example, line broadening (lb ~0.5 - 1 Hz).

Tips, tricks, and next steps

- Smaller bandwidths for the selective pulse result in greater selectivity (for example, decoupling of closely spaced coupling partners), but longer experiment times and lower sensitivity. 100 Hz is a good compromise starting point. Spectral width (sw) can also be decreased to include only the region of interest.
- The “PureShift tau delay” is inversely related to the maximum coupling constant that can be effectively decoupled. Decrease this delay for larger couplings- refer to the display in the panel for the effective maximum coupling constant.

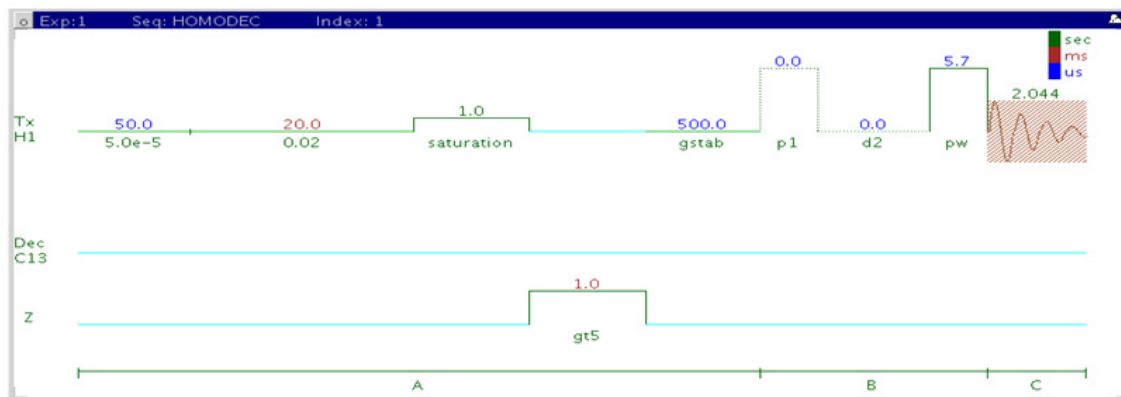
References:

P. Xu, X. Wu, R. Freeman, J. Magn. Reson. 95 (1991) 132-148.
(Homonuclear Decoupling)

K. Zangger, H. Sterk, J. Magn. Reson. 124 (1997) 486-489. (Homonuclear Broadband Decoupling)

J. A. Aguilar, S. Faulkner, M. Nilsson, G. A. Morris, Angew. Chem. Int. Ed., 49 (2010) 3901-3903. (PureShift 1D)

(H)HomoDec



General description and usage

Description:

Uses basic irradiation to decouple a 1D- ^1H dataset at a single frequency (hard pulse) or multiple frequencies (shaped pulse).

Usage:

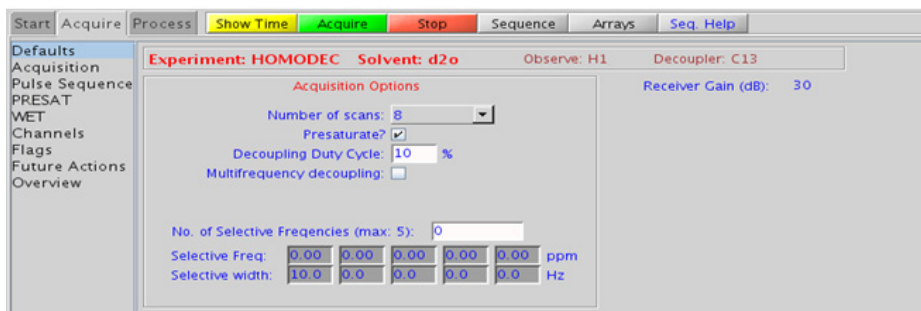
Before widespread use of the 2D COSY and TOCSY experiments, this experiment was widely used to define homonuclear coupling partners. Irradiation at a given frequency for a multiplet results in a collapse of coupled multiplets. Where the TOCSY and/or COSY can be difficult to interpret due to overlap, this experiment may still be a good choice for simplifying highly coupled systems. In addition to the homonuclear decoupling (“homo”), which can be used in any pulse sequence, this sequence simultaneously saturates the signal which is decoupled to remove artifacts. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and widths for the decoupling are known, and can be chosen interactively with the cursor. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from "Start" tab/"Standard" panel
Spectral Width(sw)	Set from previously acquired PROTON*
Number of scans(nt)	Select typical choices from pull down menu*
Presaturate	Check box to presaturate the selected resonances to suppress decoupling artifacts – highly recommended
Decoupling Duty Cycle	Use to control % of time the decoupling is on- longer time improves decoupling at the expense of S/N (10% recommended)
Multifrequency Decoupling	Check box for multiple frequencies
No., ppm, width of selective frequencies	Use top box to set the number of selective frequencies. Boxes underneath are used to set the desired ppm and width in Hz of each frequency

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.

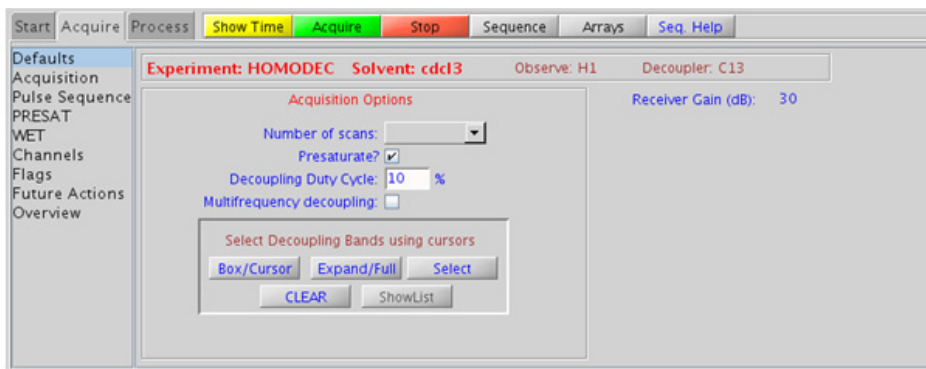


Processing

The processing is typically the same as the process used for standard PROTON spectrum, except manual phasing may be required due to antiphase/negative peaks at the decoupling frequency. A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D proton spectrum is line broadening, with an lb setting from $\sim 0.5 - 1$ Hz. A suggested optimal value is line broadening equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- If the HOMODEC experiment is selected in an experiment already containing PROTON data, the decoupling band selection can be chosen interactively. Place the cursors around the multiplet to decouple and click “Select”. Repeat for multiple decoupling bands; click “CLEAR” to erase previous choices.



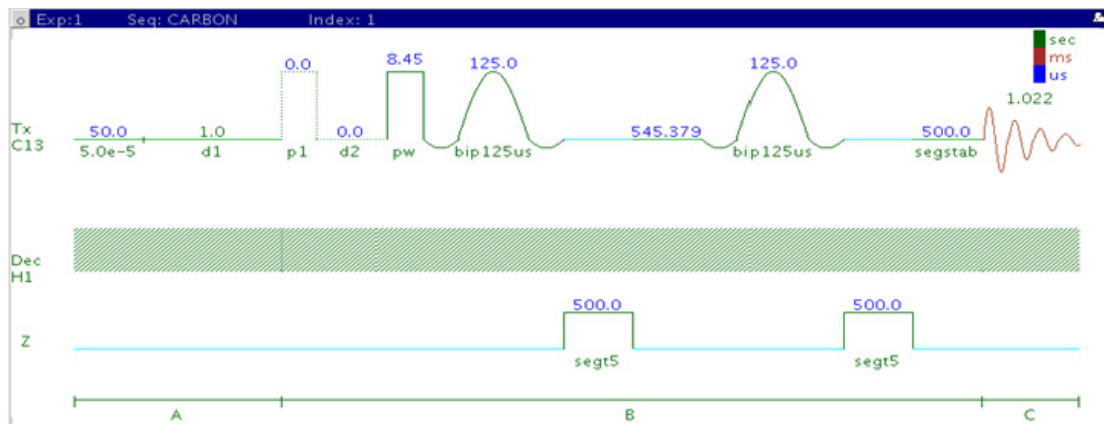
- Acquiring several spectra with single frequency decoupling usually give cleaner spectra and more easily interpretable results than one spectrum with multiple simultaneous decouplings.

- A 2D gCOSY/gDQCOSY spectrum may be a better choice for a standard structure elucidation/assignment problem as it can provide the same information for the entire molecule with a single experiment that can be obtained in <5 min. with a reasonable sample amount.

References:

J. K. M. Sanders, J. D. Merish, Prog. NMR Spectrosc. 15 (1982) 353-400.
(Difference Spectroscopy)

CARBONecho



General description and usage

Description:

Acquires a 1D- ^{13}C dataset with a double echo from bip pulses to allow delayed data acquisition without the need for linear back prediction.

Usage:

This experiment is highly specialized to acquire 1D carbon data on a high Q probe, such as a ^{13}C cold probe, which may have long pulse ringdown times. All parameters are set from the “Acquire” tab/“Default C13” panel unless otherwise specified.

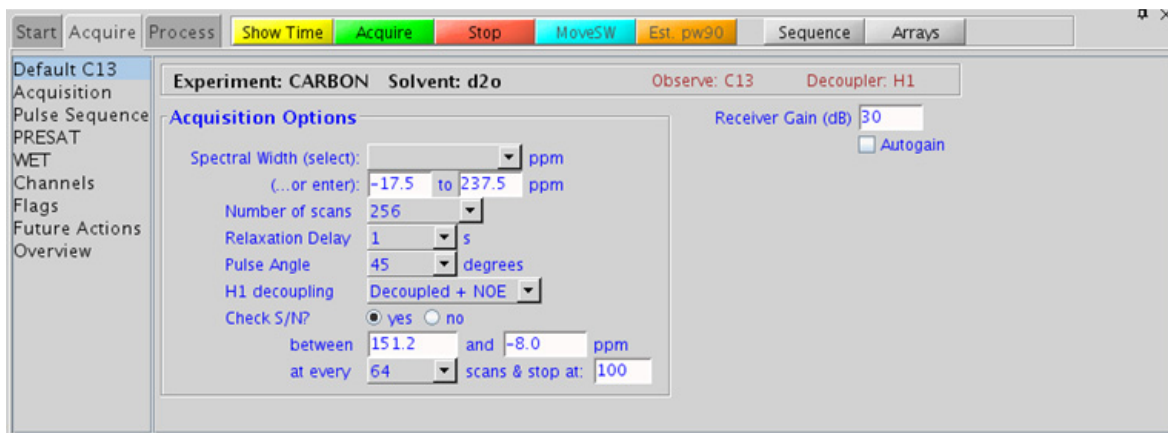
Key parameters

Parameter	Description
Solvent (solvent)	Set from "Start" tab/"Standard" panel
Spectral Width (sw)	Select typical choices from pull down menu or type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle	Select typical choices from pull down menu*-controls the tip angle for the pulse
H1 decoupling (dm)	Select typical choices from pull down menu- turns decoupling and/or NOE enhancement on/off
Check S/N?	Select yes or no- acquires data until S/N value between ppm indicated is > specified value
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.

2 Standard 1D - Std 1D Processing



Processing

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D carbon spectrum is line broadening, with an lb setting from ~ 0.5 - 1 Hz.

Tips, tricks, and next steps

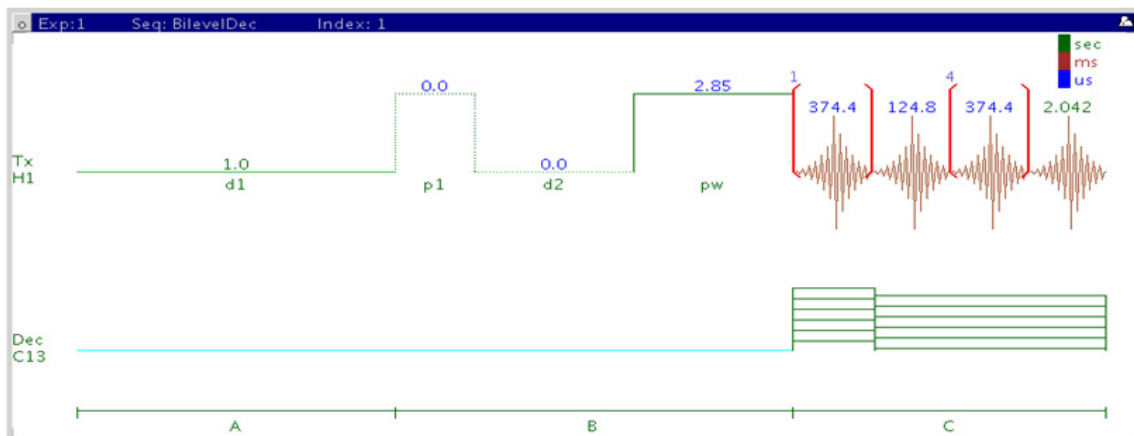
- The acquisition time, which controls the digital resolution (Hz/point, which is equal to $1/at$), is set on the "Acquisition" panel of the "Acquire" tab. Because carbon spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see small coupling; consider lengthening the acquisition time and/or narrowing the spectral width when acquiring coupled carbon spectra.
- Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is highly concentrated or ^{13}C -labelled.

- The standard proton-decoupled CARBONecho spectrum is produced by turning on the decoupling during the pulse and acquisition. It is also standard practice to leave the decoupler on during the preacquisition delay (d1), which provides an NOE enhancement to the carbon signals from attached protons. When more protons are attached to a carbon, the enhancement is larger. For example, methyl signals appear larger than quaternary carbon signals. Turn off the NOE enhancement if integration is desired.

References:

M. A. Smith, H. Hu, A. J. Shaka, J. Magn. Reson. 151 (2001) 269-283. (BIP Pulses)

BilevelDec



General description and usage

Description:

Used to acquire a carbon-decoupled 1D- ^1H dataset.

Usage:

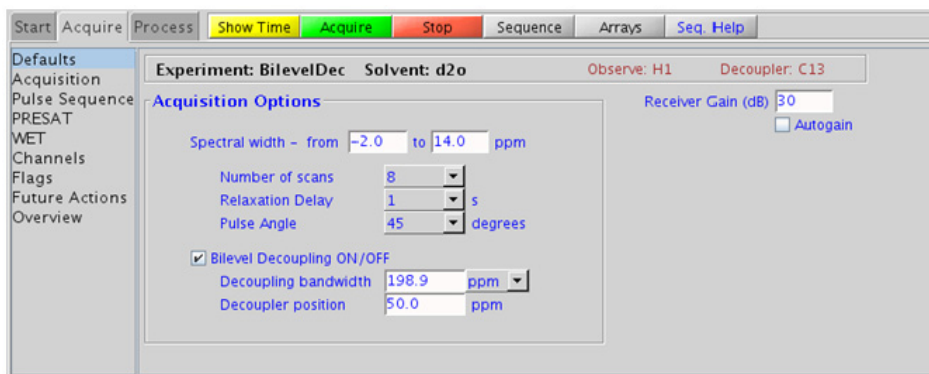
This experiment is designed to acquire a 1D proton spectrum with heteronuclear decoupling. The bilevel style decoupling helps remove cyclic decoupling sidebands associated with large ^1H -X couplings by varying the length of the higher power decoupling at the beginning of the acquisition. These sidebands are often a problem when decoupling a nucleus of high abundance like ^{31}P . This experiment is also used for uniformly ^{13}C -labelled samples, which can have complex, difficult to interpret 1D PROTON spectra from ^{13}C - ^1H coupling.

Key parameters

Parameter	Description
Solvent (solvent)	Set from "Start" tab/"Standard" panel
Spectral Width(sw)	Type in specific ppm values
Number of scans(nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle	Select typical choices from pull down menu*-controls the tip angle for the pulse
Bilevel Decoupling ON/OFF	Check box to turn decoupling on/off
Decoupling bandwidth	Use to the desired bandwidth for decoupling- larger bandwidth means more power
Decoupler position	Use to set carbon decoupling frequency in ppm (50 ppm typically the center)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.



Processing

The processing is typically the same as the processing used for standard PROTON spectrum, except manual phasing may be required due to antiphase/negative peaks at the decoupling frequency. A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D proton spectrum is line broadening, with an lb setting from ~0.5 - 1 Hz. A suggested optimal value is line broadening equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- The appropriate receiver gain (gain) is most easily set by making sure that the Autogain option is checked on the right side of the “Acquire” tab. The software optimizes the gain setting before data collection.
- If the ^{13}C -labelling is localized to a portion of the molecule, for example an aromatic ring, setting the decoupling position to the center of the labeled carbon region and narrowing the decoupling bandwidth can minimize the decoupling power.
- With probes of good ^{13}C efficiency (shorter 90° pulse lengths), BiLevelDEC can be used to acquire ^{13}C decoupled PROTON spectra with ^1H acquisition times of normal length and full band ^{13}C decoupling, provided a sufficiently long d1 relaxation delay is set, such as 10-20s. This experiment can be useful for quantitative NMR or trace analysis where it is desirable to move the ^{13}C satellites under the main ^1H peak resonance.

References:

E. Kupce, R. Freeman, G. Wider and K. Wütrich, J. Magn. Reson, Ser A. 122 (1996) 81-84. (Bilevel Decoupling)

2 Standard 1D - Std 1D

Tips, tricks, and next steps



3 Homonuclear 2D - (HH)Homo2D

(HH)Homo2D Tab 90

gCOSY 92

gDQCOSY 100

TOCSY 108

zTOCSY 112

ROESY 116

ROESYAD 120

NOESY 124

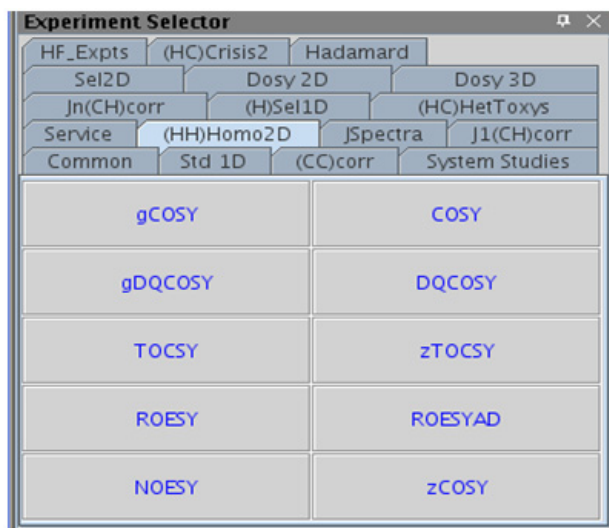
zCOSY 128



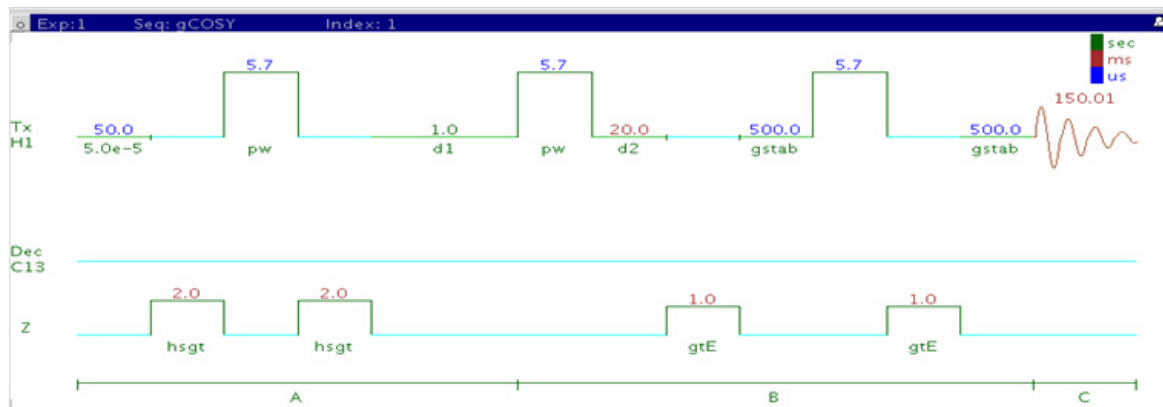
(HH)Homo2D Tab

This tab of the Experiment Selector includes the set of 2D experiments available for proton homonuclear correlations, including both J-coupling and through-space correlation experiments. The J-coupling experiments fall into two families, the COSY family that primarily displays connectivities from two- and three-bond couplings and the TOCSY family, which can show correlations among an entire spin system. The z-filtered versions of the experiments are also available as well as gradient enhanced coherence selection for the COSY experiments. The gradient enhanced version of the experiment experiences better artifact suppression at the expense of some loss of sensitivity (factor of ~ 1.4). In experiments involving z-magnetization (zTOCSY, zCOSY, NOESY) zero-quantum suppression is implemented and switched on by default. The through-space correlation experiments consist of both NOESY and ROESY with an additional option of an adiabatic spinlock for the ROESY. The adiabatic spinlock is preferable as the power is lower, permitting longer mixing times for the ROESYAD.

These experiments are considered essential data for small molecule structure elucidation following the 1D PROTON experiment. With reasonable amounts of material, the J-coupling experiments can often be acquired in 5 minutes or less. Because these experiments are proton-proton correlations, they are the most sensitive and have the best chance of providing additional structural data beyond the PROTON spectrum if the sample is limited.



gCOSY



General description and usage

Description:

Two-Dimensional J-correlation spectroscopy with gradient coherence selection.

Usage:

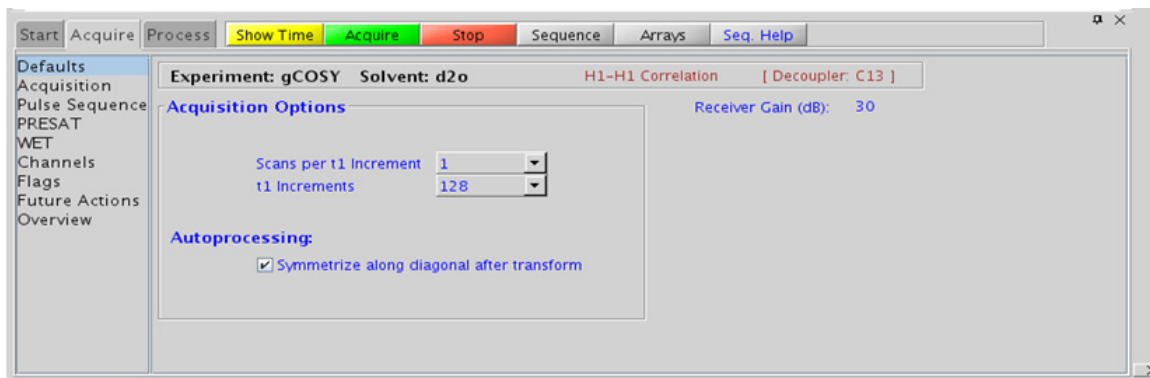
This experiment produces a 2-dimensional, absolute-value, spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON spectrum and crosspeaks between protons with nonzero couplings, typically 2 - 4 chemical bonds apart. This experiment is useful for assignment, particularly when some of the peak identities are known- the others can then be deduced from the crosspeaks observed. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. The gCOSY is a highly robust experiment and, along with TOCSY/zTOCSY, is among the most sensitive of the 2Ds, making it a popular choice for the organic chemistry NMR lab. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu* no required minimum for the gCOSY
t1 Increments (ni)	Select typical choices from pull down menu*-controls the resolution in F1 (minimum ~64)
COSY-beta	Set from "Pulse Sequence" panel pull-down, choose COSY-45, 60, 90, or 135
Symmetrize	Check box for processing option to symmetrize along the diagonal
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down menu to control the extent of linear prediction (default is 2*ni). Menus to change the weighting functions quickly in F2/F1 are also present (defaults are squared sinebells). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

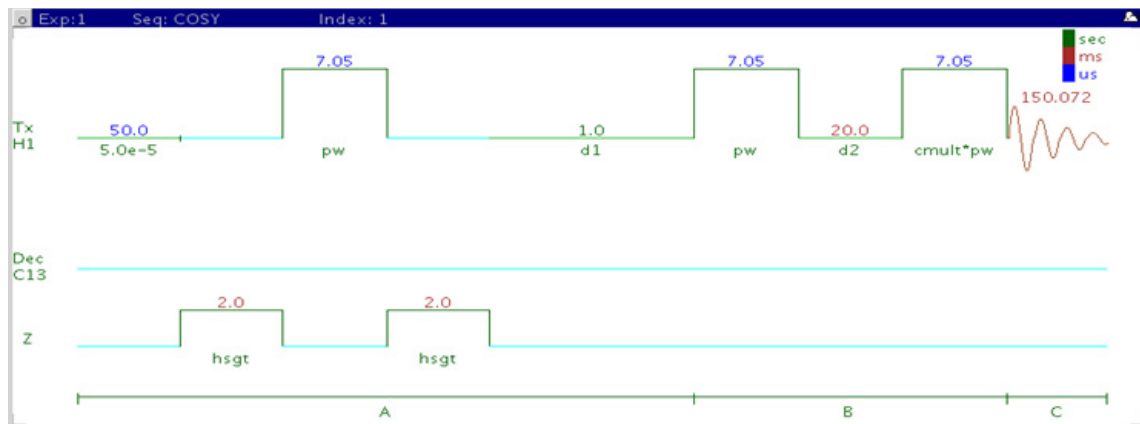
- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the gCOSY is set up from this experiment.
- The gCOSY is a high sensitivity experiment and data can often be collected using one scan per increment with reasonably concentrated samples. Consider increasing the number of increments (ni) and turning off linear prediction in f1 to increase the quality of the data for such samples.
- The COSY experiment has a sensitivity advantage (factor of ~1.4) over the gCOSY, but can produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than gCOSY. The COSY also requires a multiple of four scans per increment, as opposed to the gCOSY, which can be run with one scan per increment.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.

- The default value for the tip angle of the second pulse is set to 90°, producing a gCOSY-90. To acquire a gCOSY-45, go to the “Pulse Sequence” dialog box under the “Acquire” tab and select 45 degrees from the pull-down box for COSY-beta. The gCOSY-45 has less intense diagonal peaks than the gCOSY-90, making close chemical shift correlations easier to see at a loss of sensitivity. A 60° pulse can provide a compromise between the two.
- The d2 delay (default value 20msec) can be increased via the Pulse Sequence panel to emphasize crosspeaks originating from small couplings.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- Spectral symmetrization can appear to provide an easy way of cleaning up artifacts such as t1 noise (noise along the F1 axis often observed at the chemical shift of sharp, intense peaks). However, it can produce spurious crosspeaks that appear real. When in doubt about the validity of a cross peak, examine the unsymmetrized spectrum as well.
- If the concentration is not very low, a gDQCOSY can be considered instead of the gCOSY. It typically leads to cleaner spectra (due to suppression of singlet diagonal peaks, such as t-butyl resonances) and yields higher resolution spectra.
- Complex molecules with many overlapping spin systems can be difficult to assign from a gCOSY. Consider using a zTOCSY in lieu of, or in conjunction with the gCOSY data as the zTOCSY experiment is better at identifying the entire spin system of protons.

References:

- W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

COSY



General description and usage

Description:

Two-Dimensional J-correlation spectroscopy.

Usage:

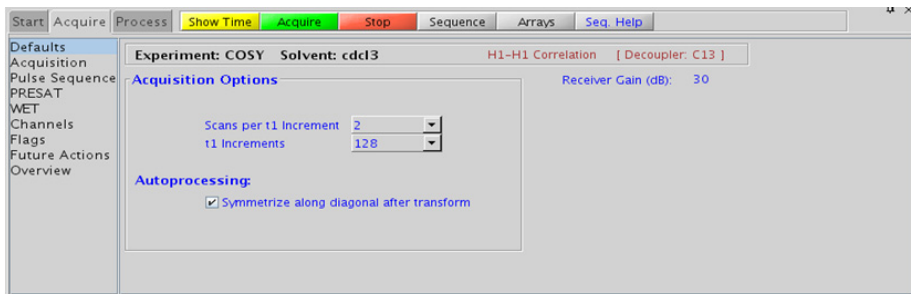
This experiment produces a 2-dimensional, absolute-value, spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons with nonzero couplings, typically 2 - 4 chemical bonds apart. This experiment is useful for assignment, particularly when some of the peak identities are known- the others can then be deduced from the crosspeaks observed. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. The COSY is a highly robust experiment and, along with TOCSY, is among the most sensitive of the 2Ds, making it a popular choice for the organic chemistry NMR lab. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
COSY-beta	Set from “Pulse Sequence” panel pull-down, choose COSY-45, 60, 90, or 135
Symmetrize	Processing option to symmetrize along the diagonal
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are squared sinebells). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

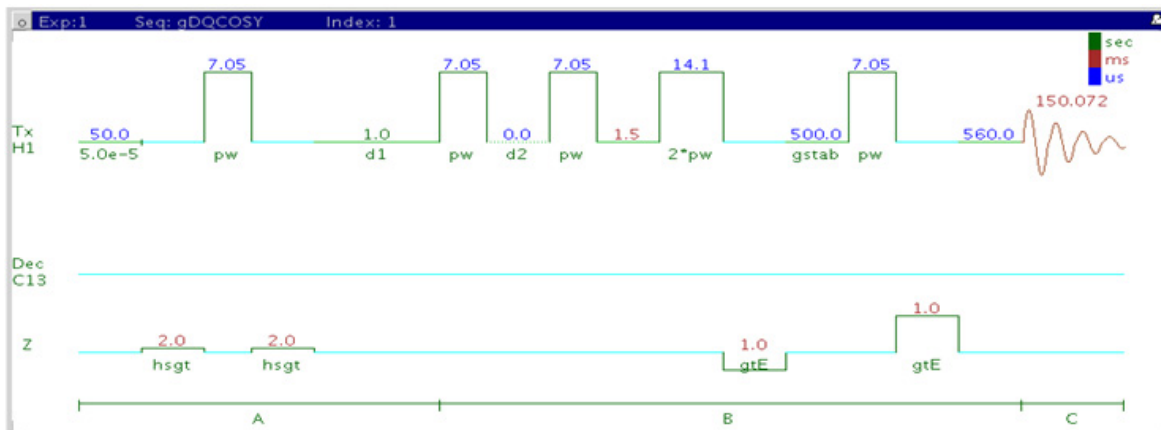
- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the COSY is set up from this experiment.
- The COSY experiment has a sensitivity advantage (factor of ~1.4) over the gCOSY but can produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than gCOSY. The COSY also requires a multiple of four scans per increment, as opposed to the gCOSY, which can be run with one scan per increment.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default value for the tip angle of the second pulse is set to 90°, producing a COSY-90. To acquire a COSY-45, go to the “Pulse Sequence” dialog box under the “Acquire” tab and select 45 degrees from the pull-down box for COSY-beta. The COSY-45 has less intense diagonal peaks than the COSY-90, making close chemical shift correlations easier to see at a loss of sensitivity. A 60° pulse can provide a compromise between the two.
- The d2 delay (default value 20msec) can be increased via the Pulse Sequence panel to emphasize crosspeaks originating from small couplings.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times, and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- Spectral symmetrization can appear to provide an easy way of cleaning up artifacts such as t1 noise (noise along the F1 axis often observed at the chemical shift of sharp, intense peaks). However it can produce spurious crosspeaks that appear real. When in doubt about the validity of a cross peak, examine the unsymmetrized spectrum as well.
- Complex molecules with many overlapping spin systems can be difficult to assign from a COSY- consider using a zTOCSY instead of, or in conjunction with the COSY data as the zTOCSY experiment is better at identifying the entire spin system of protons together.

References:

- J. Jeener, Ampere International Summer School, Basko Polje, Yugoslavia 1971. (Introduction to 2D)
- W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)
- D. L. Turner, Prog. NMR. Spectrosc. 17 (1985) 281-358. (2D NMR)

gDQCOSY



General description and usage

Description:

Two-Dimensional double-quantum filtered J-correlation spectroscopy with gradient coherence selection.

Usage:

This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons with nonzero couplings, typically 2 - 4 chemical bonds apart. This experiment is useful for assignment, particularly when some of the peak identities are known- the others can then be deduced from the crosspeaks observed. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. The advantage of the gDQCOSY over the gCOSY is that peaks with no double-quantum transitions (for example, singlets) are suppressed, producing cleaner spectra. The experiment is also phase sensitive, resulting in better peak

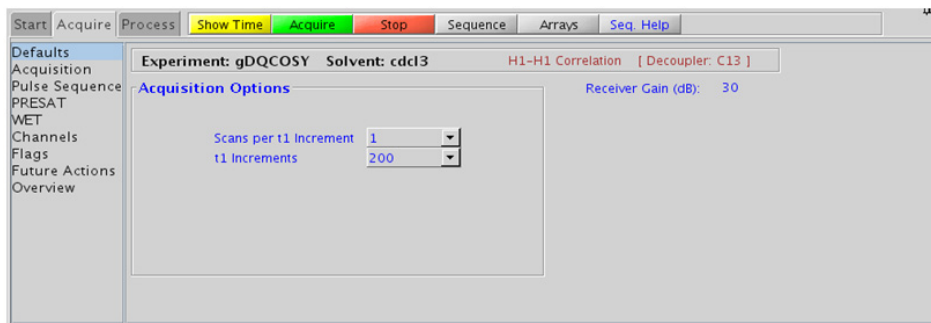
shapes. Calibrate the 90° pulse width accurately for this experiment. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu*- no required minimum for the gDQCOSY
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are shifted sinebells). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

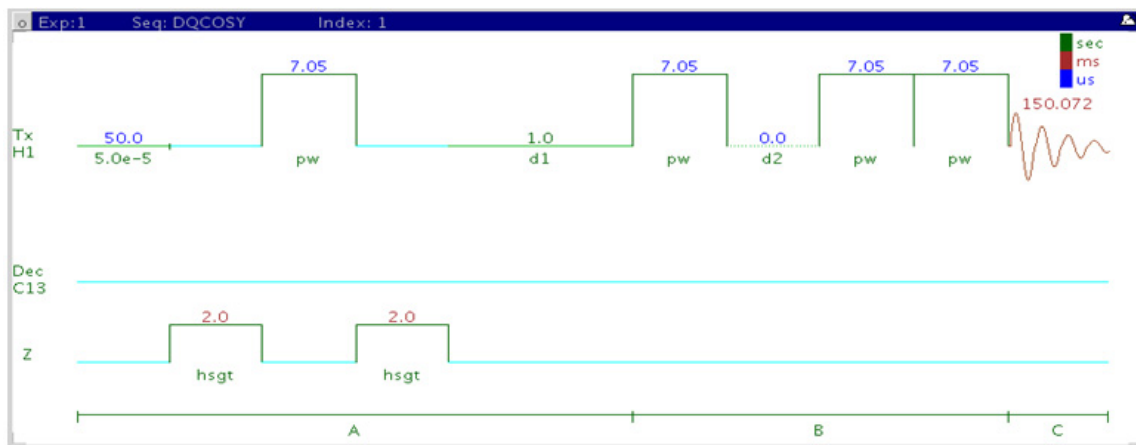
- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the gDQCOSY is set up from this experiment.
- The main advantage of the gDQCOSY versus the gCOSY is better resolution (phase sensitive versus absolute value experiment) and the absence of singlets that display no couplings. For instance, t-butyl signals, which can be a large source of t1 noise because of their intensity, are suppressed in a gDQCOSY.
- The DQCOSY experiment has a sensitivity advantage (factor of ~1.4) over the gDQCOSY but can produce potentially less clean spectra (due to t1 noise from strong signals) than gDQCOSY. The DQCOSY also requires a multiple of four scans per increment, as opposed to the gDQCOSY, which can be run with one scan per increment.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The 90° pulse width is typically calibrated reasonably well for best results from the gDQCOSY experiment. See the PROTON experiment in Chapter 2 to calibrate the pulse.

- Because the J-couplings evolve as an echo in gDQCOSY, the crosspeaks arising from small couplings may not appear until the number of completed increments is sufficient. In general, it is useful to set n_1 to a larger value than is the case in an experiment such as gCOSY.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times, and d_1 relaxation delays (defaults are 0.15s and 1s, respectively).
- The gDQCOSY experiment can be used to measure coupling constants of overlapped multiplets as the crosspeaks contain the full multiplet structure. If used for coupling constant measurement, run this experiment with many increments (n_1 typically to 400 or higher). The zCOSY is a better alternative for coupling constant measurements than the gDQCOSY.
- Complex molecules with many overlapping spin systems can be difficult to assign from a gDQCOSY- consider using a zTOCSY instead of, or in conjunction with the gDQCOSY data as the zTOCSY experiment is better at identifying the entire spin system of protons.

References:

- W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)
- U. Piantini, O. W. Sorensen, R. R. Ernst, J. Am. Chem. Soc. 104 (1982) 6800-6801. (Double Quantum Filter)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

DQCOSY



General description and usage

Description:

Two-Dimensional double-quantum filtered J-correlation spectroscopy.

Usage:

This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons with 2- (geminal) or 3- (vicinal)-bond couplings. This experiment is useful for assignment, particularly when some of the peak identities are known- the others can then be deduced from the crosspeaks observed. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. The advantage of the DQCOSY over the COSY is that peaks with no double-quantum transitions (for example, singlets) are suppressed, producing cleaner spectra. The experiment is also phase sensitive, resulting in better peak shapes. The

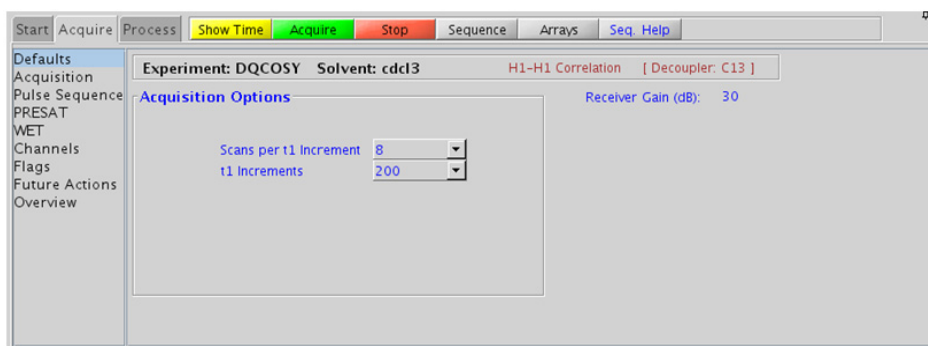
90° pulse width is typically calibrated accurately for this experiment. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are shifted sinebells). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

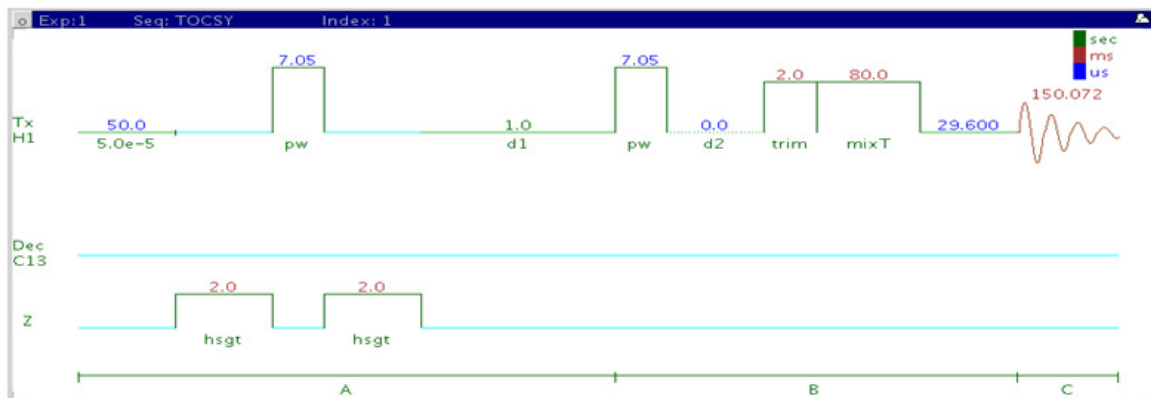
- Spectral width (sw) and receiver gain (gain)-settings transfer from a PROTON if the DQCOSY is set up from this experiment.
- The main advantage of the DQCOSY versus the COSY is better resolution (phase sensitive versus absolute value experiment) and the absence of singlets that display no couplings. For instance, t-butyl signals, which can be a large source of t1 noise because of their intensity, are suppressed in a DQCOSY.
- The DQCOSY experiment has a sensitivity advantage (factor of ~1.4) over the gDQCOSY but can produce potentially less clean spectra (due to t1 noise from strong signals) than gDQCOSY. The DQCOSY also requires a multiple of four scans per increment, as opposed to the gDQCOSY, which can be run with one scan per increment.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- Calibrate the 90° pulse width accurately for best results from the DQCOSY experiment. See the PROTON experiment in Chapter 2 to calibrate the pulse.

- Because the J-couplings evolve as an echo in DQCOSY, the crosspeaks arising from small couplings may not appear until the number of completed increments is sufficient. In general, it is useful to set ni to a larger value than is the case in an experiment such as gCOSY.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times, and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- The DQCOSY experiment can be used to measure coupling constants of overlapped multiplets as the crosspeaks contain the full multiplet structure. If used for coupling constant measurement, run this experiment with many increments (ni typically to 400 or higher).
- Complex molecules with many overlapping spin systems can be difficult to assign from a DQCOSY- consider using a zTOCSY instead of, or in conjunction with the DQCOSY data as the zTOCSY experiment is better at identifying the entire spin system of protons.

References:

- W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227.
 (COSY)
- U. Piantini, O. W. Sorensen, R. R. Ernst, J. Am. Chem. Soc. 104 (1982)
 6800-6801. (Double Quantum Filter)

TOCSY



General description and usage

Description:

Two dimensional total J-correlation spectroscopy for scalar coupled spin systems.

Usage:

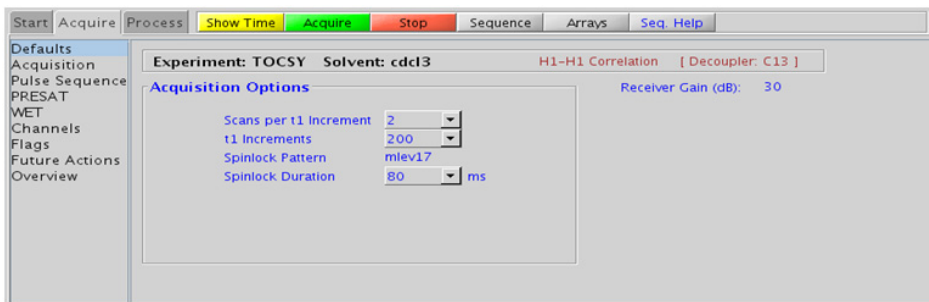
This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks potentially between all protons in a scalar coupled spin system. This experiment is useful for assignment, particularly for complex molecules with multiple overlapping spin systems, for example, peptides. For structure elucidation, the molecular fragments containing individual spin systems can be mapped out from the correlations. The TOCSY is a highly robust experiment and, along with gCOSY, is among the most sensitive of the 2Ds, making it a popular choice for the organic chemistry NMR lab. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Spinlock Pattern	Pull-down menu on the “Pulse Sequence” panel- MLEV17 or DIPSI2 work well for small molecules; clean MLEV17 or DIPSI2 are good choices for large molecules
Spinlock Duration (mixT)	Select from pull down menu- 80ms work best for large spin systems*
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are Gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the TOCSY is set up from this experiment.
- The TOCSY is a high sensitivity experiment and data can often be collected using one scan per increment with reasonably concentrated samples. Consider increasing the number of increments (ni) to increase the quality of the data for such samples.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings (such as for a COSY), select a shorter spinlock time, for example, 30ms or less. Such short spinlock TOCSY is a good alternative to gCOSY as TOCSY gives pure absorptive lineshape and potentially improved resolution.

- The MLEV17 and DIPSI2 spinlock patterns are good choices for small molecules, and the DIPSI3 and clean MLEV are typically used for larger molecules.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times, and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- The zTOCSY experiment contains a zero-quantum filter and usually results in cleaner spectra than the TOCSY experiment. Only zTOCSY experiment can be combined with zero-quantum filters.

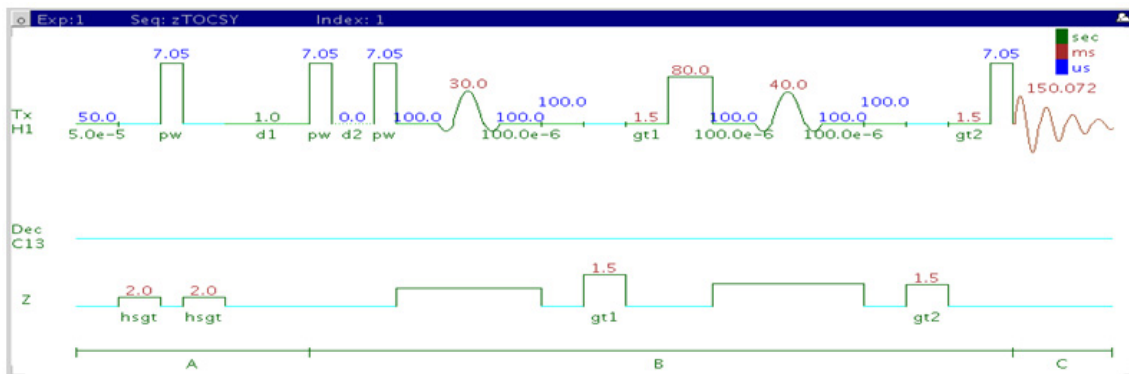
References:

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)

C. Griesinger, G. Otting, K. Wuethrich, R. R. Ernst, J. Am. Chem. Soc. 110 (1988) 7870-7872. (TOCSY with clean MLEV17)

J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)

zTOCSY



General description and usage

Description:

Two Dimensional total J-correlation spectroscopy for scalar coupled spin systems with a zero-quantum filter for artifact suppression.

Usage:

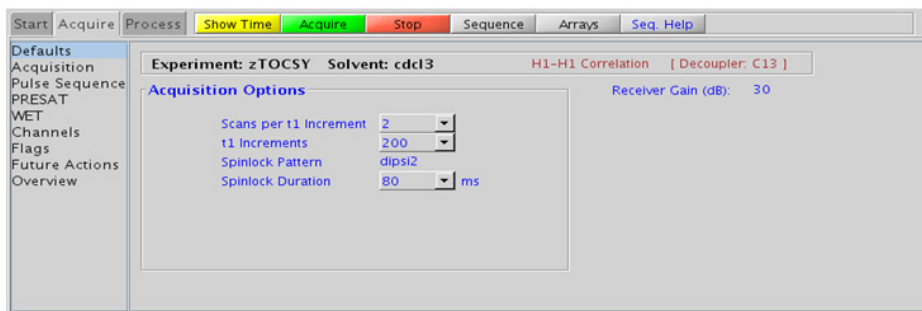
This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks potentially between all protons in a scalar coupled spin system. This experiment is useful for assignment, particularly for complex molecules with multiple overlapping spin systems, for example, peptides. For structure elucidation, the molecular fragments containing individual spin systems can be mapped out from the correlations. The zTOCSY is a highly robust experiment and, along with gCOSY, is among the most sensitive of the 2Ds, making it a popular choice for the organic chemistry NMR lab. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Spinlock Pattern	Pull-down menu on the “Pulse Sequence” panel- DIPSI2 works well for small molecules, DIPSI3 is a good choice for large molecules
Spinlock Duration (mixT)	Select from pull down menu- 80ms work best for large spin systems*
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are Gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the zTOCSY is set up from this experiment.
- The TOCSY is a high sensitivity experiment and data can often be collected using one scan per increment with reasonably concentrated samples. Consider increasing the number of increments (ni) to increase the quality of the data for such samples
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. To show primarily vicinal and geminal couplings (such as for a COSY), select a shorter spinlock time, for example, 30ms or less. Such short spinlock zTOCSY is a good alternative to gCOSY as zTOCSY gives pure absorptive lineshape and potentially improved resolution.
- The DIPSI2 spinlock pattern is a good choice for small molecules, and the DIPSI3 is typically used for larger molecules

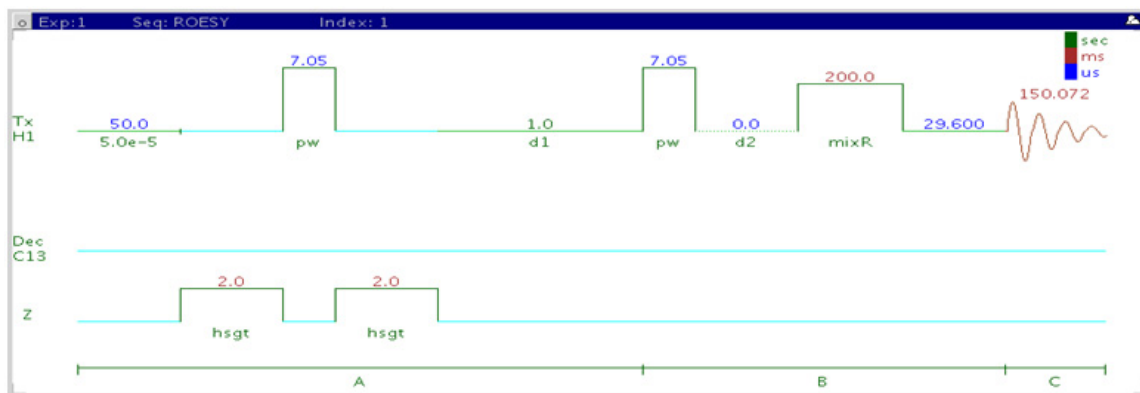
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times, and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- The zTOCSY experiment contains a zero-quantum filter and usually results in cleaner spectra than the TOCSY experiment.

References:

J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

ROESY



General description and usage

Description:

Two-Dimensional through-space correlation spectroscopy in the rotating frame.

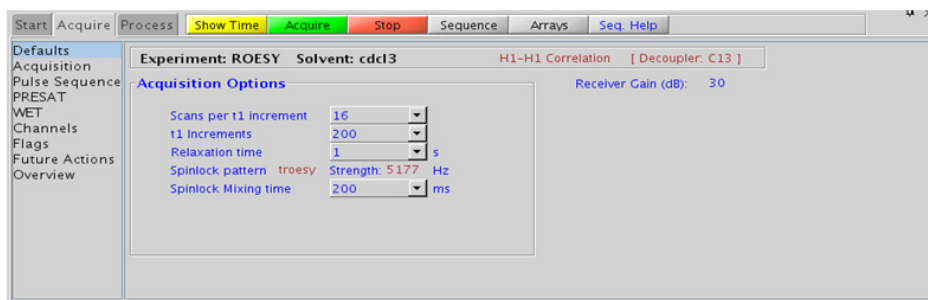
Usage: This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically $<4 \text{ \AA}$). As such the ROESY experiment is often used to help determine stereochemistry for rigid ring systems or to confirm attachments between proximal, but noncoupled portions of a molecule. The ROESY is the experiment of choice for intermediate-sized molecules (typically between 800-1000 MW) where the NOE can be zero. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Relaxation time (d1)	Select typical choices from pull down menu*-controls the delay between scans
Spinlock Pattern	Pull-down menu on “Pulse Sequence” panel- choose troesy (recommended) or cw
Spinlock Mixing time	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment, except for minor manual phasing changes that can be required as the ROE peaks are negative. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the F₂ data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F₂/F₁ are also present (defaults are Gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

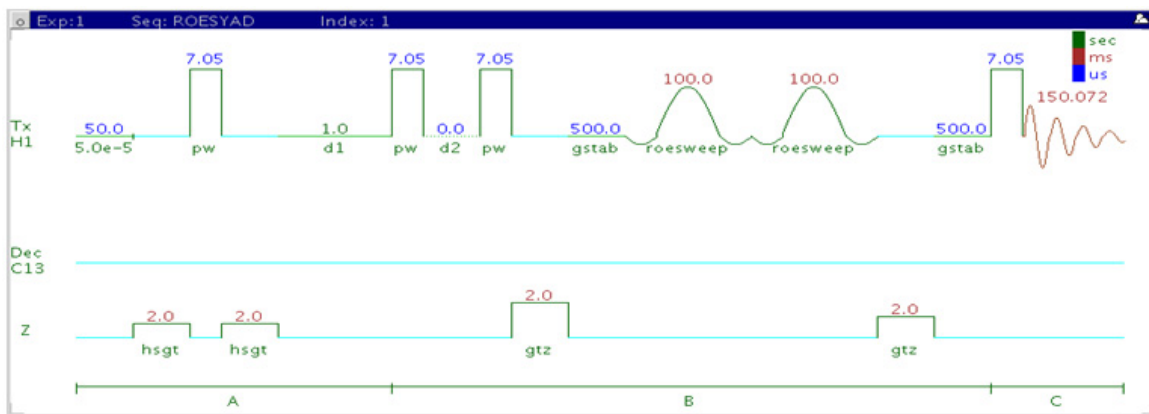
- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the ROESY is set up from this experiment.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times, and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- A mixing time of 200ms is a good starting place for most small molecules. Weak ROE interactions can require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks can result in sample heating. It is a good idea not to exceed a 500ms spinlock.

- Because the mixing time for the ROESY is limited, weaker correlations may not be observable. ROESY is the experiment of choice over NOESY for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOEs. Consider running NOESY experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d_6 -DMSO or d_6 -DMSO with ~10 - 20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- Crosspeaks in the ROESY spectrum that have the same phase as the diagonal can arise from chemical exchange during the ROE mixing time or TOCSY-type correlations (couplings). The exchange crosspeaks provide information about interconverting molecular conformations and can be used to recognize such molecular dynamic processes.
- The ROESYAD experiment uses adiabatic pulses for the spinlock, which may be preferred over the ROESY.

References:

- A. A. Bothner-By, R. L. Stephens, J. Lee, C. D. Warren, J. W. Jeanloz, J. Am. Chem. Soc. 106 (1984) 811-813. (CAMELSPIN)
- A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)
- T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc 114 (1992) 3157-3159. (EASY-ROESY)

ROESYAD



General description and usage

Description:

Two-Dimensional through-space correlation spectroscopy in the rotating frame with adiabatic pulses for the spinlock.

Usage:

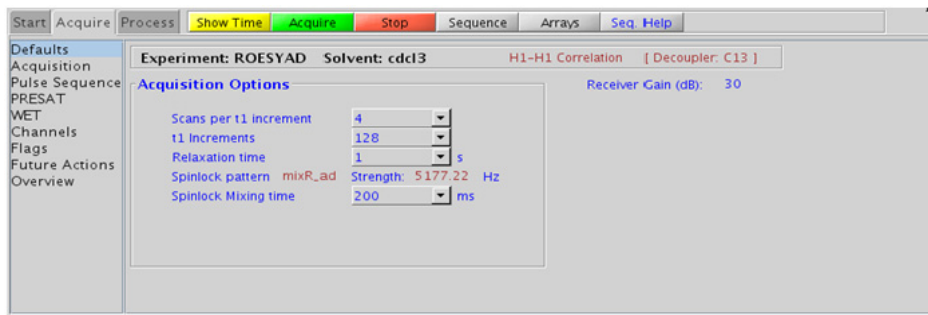
This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically $<4 \text{ \AA}$). As such the ROESYAD experiment is often used to help determine stereochemistry for rigid ring systems or to confirm attachments between proximal, but noncoupled portions of a molecule. The ROESYAD is the experiment of choice for intermediate-sized molecules (typically between 800-1000 MW) where the NOE can be zero. Because of its lower-power adiabatic spinlock the ROESYAD is usually superior to the traditional ROESY experiment. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Relaxation time (d1)	Select typical choices from pull down menu*-controls the delay between scans
Spinlock Mixing time	Select from pull down menu- 200ms works well for most small molecules*
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment, except for minor manual phasing changes that can be required, as the ROE peaks are negative. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the F₂ data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2**n*_i). Menus to change the weighting functions in F₂/F₁ are also present (defaults are Gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

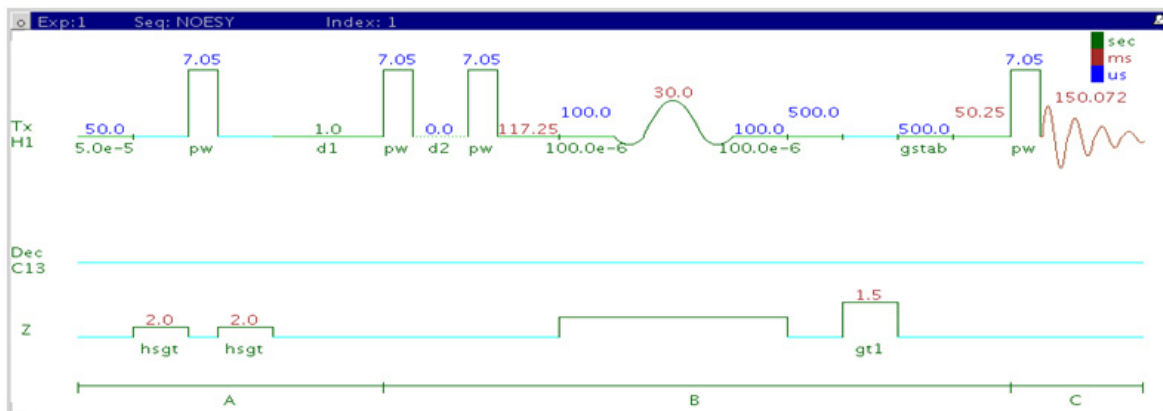
- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the ROESYAD is set up from this experiment.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times, and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- A mixing time of 200ms is a good starting place for most small molecules. Weak ROE interactions can require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks can result in sample heating. It is a good idea not to exceed a 500ms spinlock.

- Because the mixing time for the ROESYAD is limited, weaker correlations may not be observable. ROESYAD is the experiment of choice over NOESY for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOEs. Consider running NOESY experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d_6 -DMSO or d_6 -DMSO with ~10 - 20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- Crosspeaks in the ROESYAD spectrum that have the same phase as the diagonal can arise from chemical exchange during the ROE mixing time or TOCSY-type correlations (couplings). The exchange crosspeaks provide information about interconverting molecular conformations and can be used to recognize such molecular dynamic processes.
- The ROESYAD experiment uses adiabatic pulses for the spinlock, which may be preferred over the ROESY.

References:

- A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)
- T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc 114 (1992) 3157-3159. (EASY-ROESY)
- D. F. Hansen, L. E. Kay, J. Biomol. NMR, 37 (2007) 245-255. (Adiabatic Spinlock)

NOESY



General description and usage

Description:

Two-dimensional through-space correlation spectroscopy.

Usage:

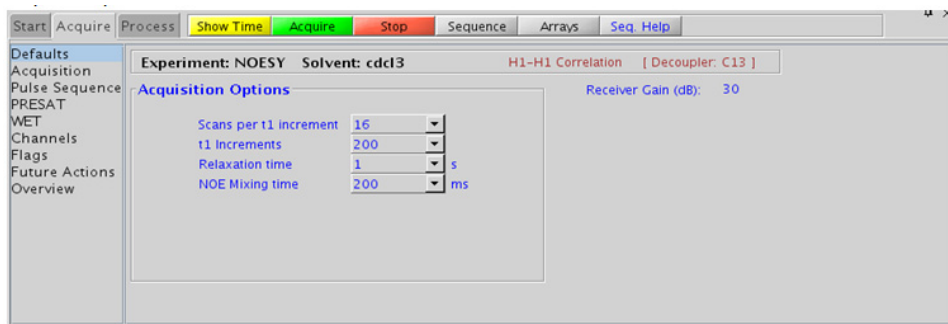
This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically $<5 \text{ \AA}$). As such the NOESY experiment is often used to help determine stereochemistry for rigid ring systems or to confirm attachments between proximal, but noncoupled portions of a molecule. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Relaxation time (d1)	Select typical choices from pull down menu*-controls the delay between scans
NOE Mixing time (mixT)	Select from pull down menu- 500ms works well for most small molecules*
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
ZQ filter	Check box on the “Pulse Sequence” panel- zero-quantum filter for artifact suppression. Strongly recommended- default is on.

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment, except for minor manual phasing changes that can be required, as the NOE peaks are negative for smaller molecules. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to change the weighting functions in F2/F1 are also present (defaults are Gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

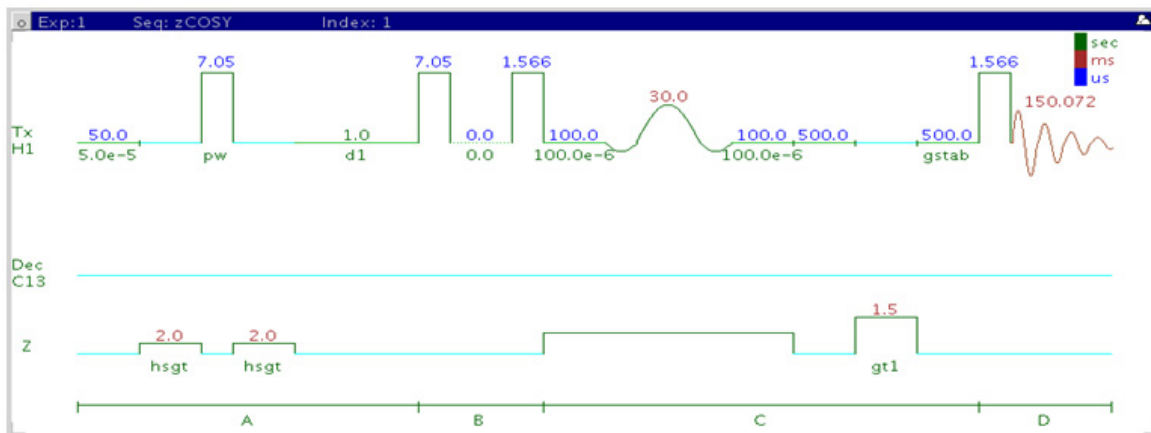
- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the NOESY is set up from this experiment.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times, and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions can require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase, and levels off. This time is the optimal mixing time.

- Medium-sized molecules (MW ~500 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOEs. Consider acquiring a ROESYAD for these molecules. Alternatively consider, (i) choosing a more viscous solvent such as d₆-DMSO or d₆-DMSO with ~10 - 20% D₂O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- Small molecules can have crosspeaks arising from chemical exchange during the NOE mixing time and have the same phase as the diagonal. The exchange crosspeaks provide information about interconverting molecular conformations and can be used to recognize such molecular dynamic processes.

References:

- S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)
- M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

zCOSY



General description and usage

Description:

Two-dimensional J-correlation spectroscopy.

Usage:

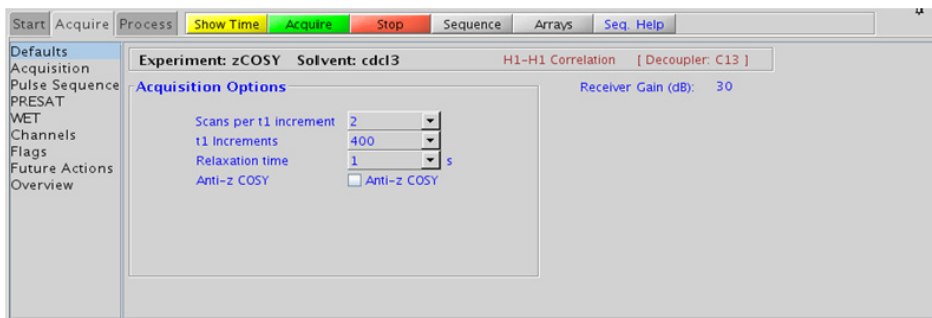
This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons with nonzero couplings, typically 2 - 4 chemical bonds apart. This experiment is mostly used to determine homonuclear coupling constants, especially smaller long-range couplings. These are typically obstructed by the complex coupling pattern in a traditional COSY but are accessible in zCOSY because of its E.COSY (Exclusive COSY) pattern. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Relaxation time (d1)	Select typical choices from pull down menu*-controls the delay between scans
Anti-z COSY	Check box to acquire an anti-z COSY (broadband proton decoupled)
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to change the weighting functions in F2/F1 are also present (defaults are sinebells). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the zCOSY is set up from this experiment.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup unrelaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times, and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- The zCOSY is an attractive alternative to the traditional E.COSY (Exclusive COSY) experiment. zCOSY gives the same crosspeak pattern as E.COSY but can be run with single scan per increment.

References:

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

3 **Homonuclear 2D - (HH)Homo2D**

Tips, tricks, and next steps



4 Heteronuclear 2D 1-bond - J1(CH)corr

J1(CH)corr Tab 134

HSQCAD 136

gHSQCAD 140

HSQC 146

gHSQC 150

HMQC 154

gHMQC 158

HETCOR 162

gHETCOR 166

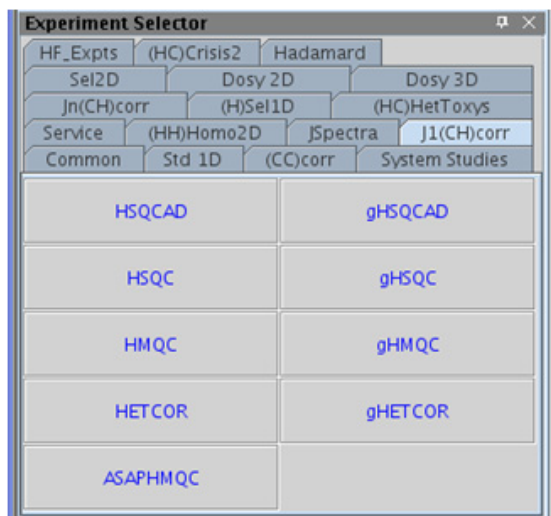
ASAPHMQC 170



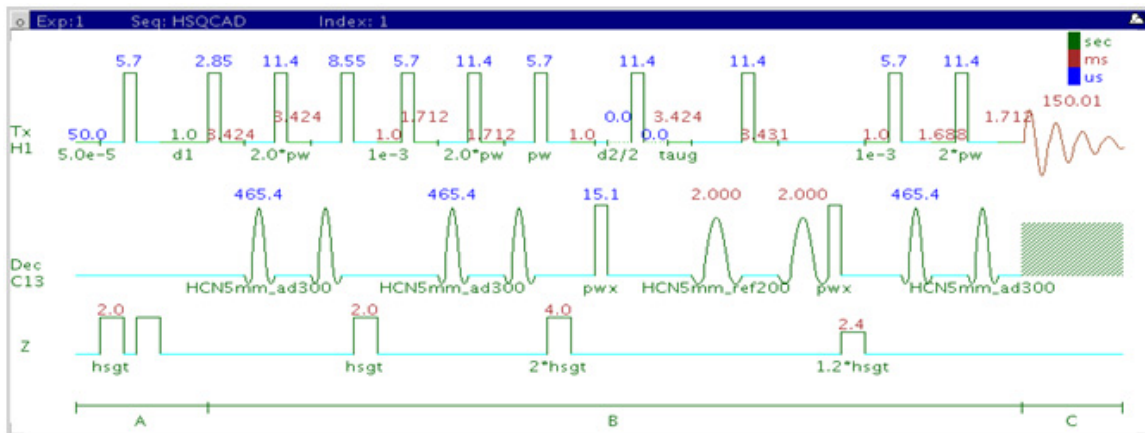
J1(CH)corr Tab

This tab of the experiment selector contains 2D experiments for correlating directly attached protons to carbons. HSQC and its variants are the most commonly used family, as they typically provide the best sensitivity and peak shape. These experiments are available both with and without gradient coherence selection and adiabatic 180° carbon pulses. The gradient coherence selected versions of the experiments experience better artifact suppression at the expense of losses of sensitivity (factor of ~1.4). The adiabatic versions, HSQCAD and gHSQCAD are highly recommended, as they provide more uniform inversion profiles for a wide ¹³C spectral width, and, at the same time, compensate for any ¹³C pwx imperfections in the 180° pulses. Gradient and non-gradient HMQC experiments are also available, as well as a “fast” experiment called ASAPHMQC, which can provide a complete HX correlation spectrum with reasonable resolution in about a minute or less for samples of ~40mM concentration. Finally, there are both gradient and non-gradient versions of the ¹³C-detected HETCOR experiment. The HSQC and HETCOR experiment (and their variants) also support multiplicity-editing, where signals from the methylene group appear with opposite sign from the signals arising from methyl and methine groups. Note that although the default parameters for these experiments are setup for ¹H-¹³C correlations, other X-nuclei may be used instead of carbon provided the appropriate calibrations for these nuclei are in the probe file.

The 1-bond proton-carbon correlation is extremely useful for structure confirmation and elucidation. Peak assignments are easily made from this data; if a particular carbon assignment is known, its attached proton assignment can be derived, and vice versa. Knowledge gained from carbon chemical shift prediction can also be applied to the proton assignments with this type of data. The proton-detected experiments, HSQC and HMQC, are also considerably more sensitive than a 1D CARBON and thus permit access to carbon data for much less concentrated samples.



HSQCAD



General description and usage

Description:

Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses.

Usage:

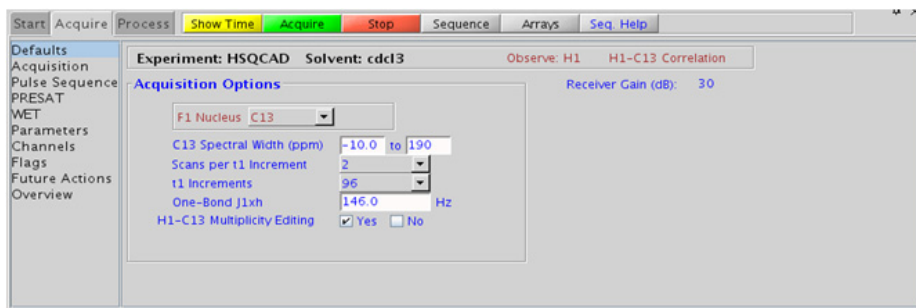
The HSQCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ - 146 Hz works well for many carbons
H1-C13 Multiplicity Editing	Check box to turn on/off multiplicity editing
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the HSQCAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm=’nnn’ on the command line.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This results in cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.
- The multiplicity-editing feature uses the CRISIS technique that relies on a hypothetical relationship between one-bond coupling constant and carbon chemical shift. Carbons with coupling constants that deviate significantly from this relationship (for example cyclopropanes or ethylene oxides) result in imperfect editing.
- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution.
- The HSQCAD experiment has a sensitivity advantage (factor of ~ 1.4) over the gHSQCAD, but due to t_1 noise from strong signals such as t -butyl, may produce potentially less clean spectra than its gradient counterpart.
- The HSQCAD uses adiabatic 180° carbon pulses and often gives better results than the HSQC, due to much improved uniform inversion over a wide ^{13}C spectral width.
- Datasets with moderate S/N are better processed with 2^*ni linear prediction instead of the default (4^*ni). Remember to reset the window function along F1 axis if linear prediction parameters are changed.

References:

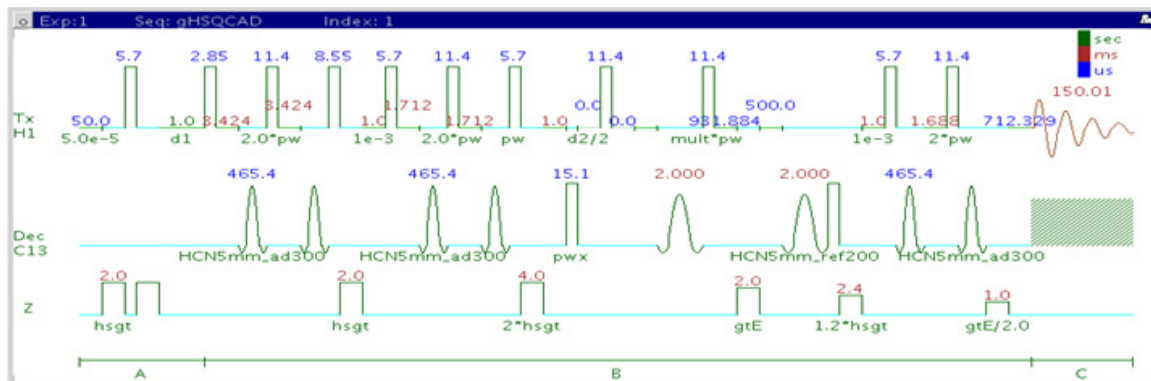
G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189.
(HSQC)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256.
(Adiabatic Pulses)

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003)
253-259 (HSQCAD / CRISIS-HSQC)

4 Heteronuclear 2D 1-bond - J1(CH)corr gHSQCAD

gHSQCAD



General description and usage

Description:

Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses and gradient coherence selection.

Usage:

The gHSQCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

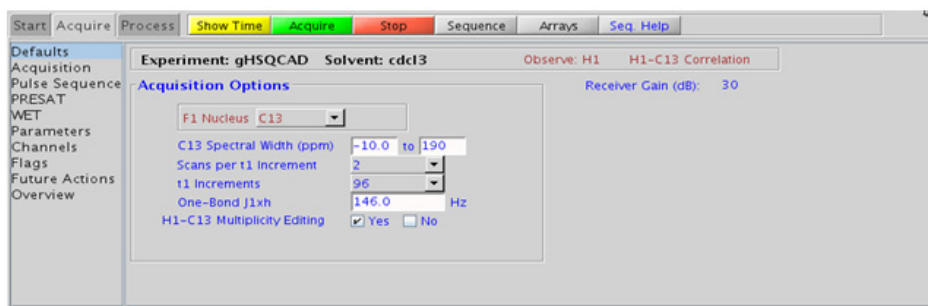
Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
H1-C13 Multiplicity Editing	Check box to turn on/off multiplicity editing
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

4 Heteronuclear 2D 1-bond - J1(CH)corr Processing



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gHSQCAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing `dm='nnn'` on the command line.

- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This results in cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- Using the multiplicity editing feature result in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.
- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution.
- The HSQCAD experiment has a sensitivity advantage (factor of ~ 1.4) over the gHSQCAD, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.
- The gHSQCAD uses adiabatic 180° carbon pulses and often gives better results than the gHSQC, due to much improved uniform inversion over a wide ^{13}C spectral width.
- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). Remember to reset the window function along F1 axis if linear prediction parameters are changed.

References:

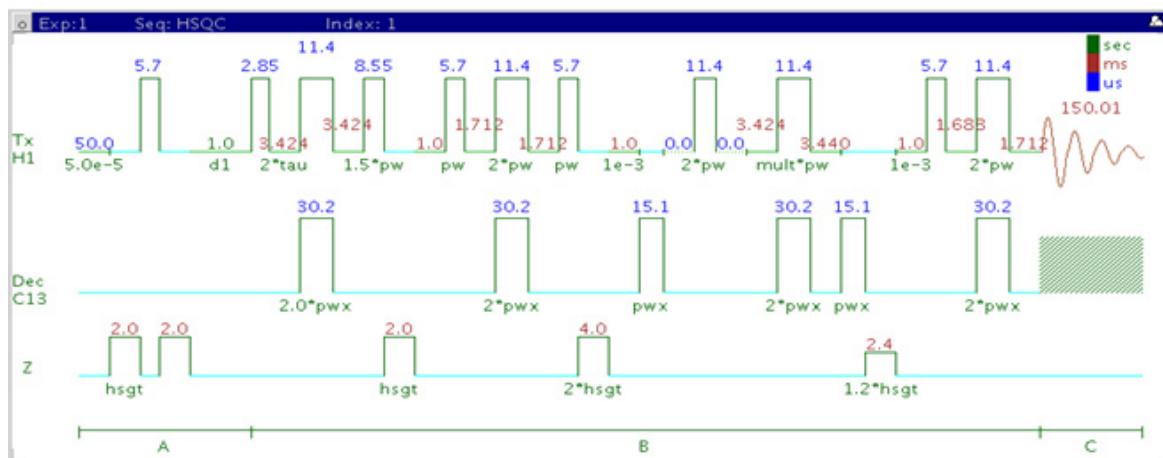
- G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)
- E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

4 Heteronuclear 2D 1-bond - J1(CH)corr

Tips, tricks, and next steps

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003)
253-259 (gHSQCAD / CRISIS-gHSQC)

HSQC



General description and usage

Description:

Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy.

Usage:

The HSQC experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

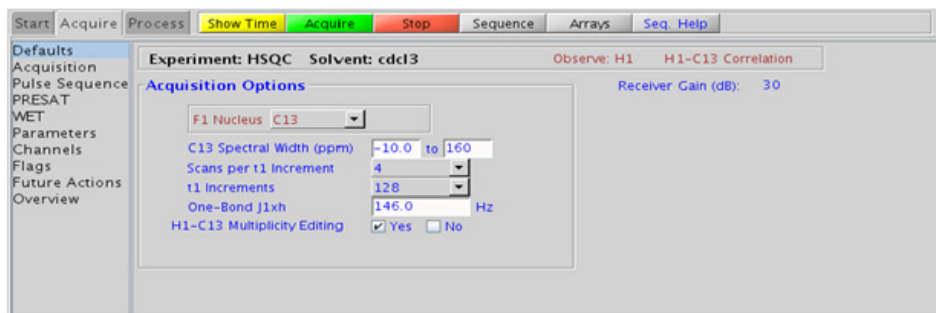
Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ - 146 Hz works well for many carbons
H1-C13 Multiplicity Editing	Check box to turn on/off multiplicity editing
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

4 Heteronuclear 2D 1-bond - J1(CH)corr Processing



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the HSQC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing `dm='nnn'` on the command line.

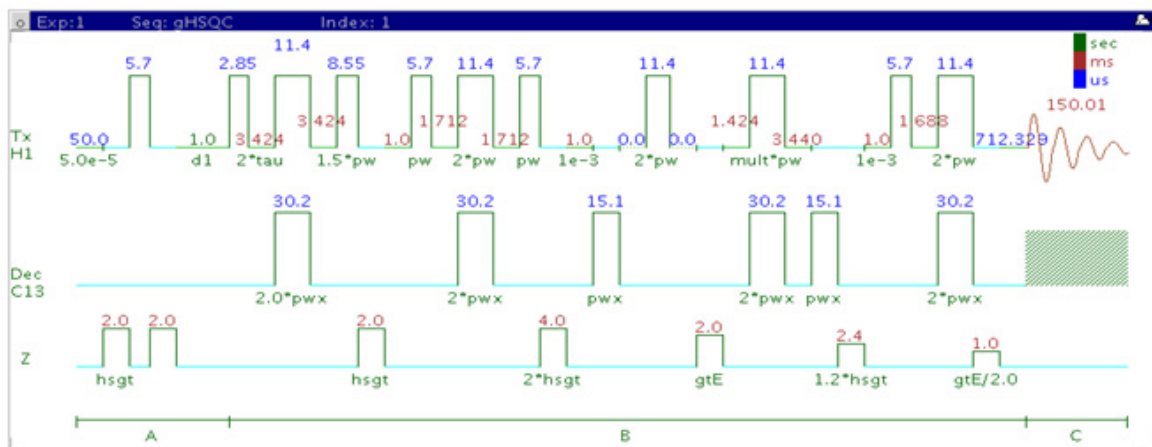
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- Using the multiplicity editing feature results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. There is a sensitivity loss with this feature due to unmatched coupling constants and echo time, but the extra information about multiplicity is usually worth the loss in sensitivity. Hence this feature is on by default. Alternately, consider using HSQCAD to minimize this sensitivity loss.
- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution.
- The HSQC experiment has a sensitivity advantage (factor of ~ 1.4) over the gHSQC, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.
- The HSQCAD and gHSQCAD use adiabatic 180° carbon pulses and invariably gives better results than the HSQC.
- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). Remember to reset the window function along F1 axis if linear prediction parameters are changed.

References:

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

A. G. Palmer III, J. Cavanagh, P. E. Wright, M. Rance, J. Magn. Reson. 93 (1991) 151-170. (HSQC)

gHSQC



General description and usage

Description:

Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with gradient coherence selection.

Usage:

The gHSQC experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

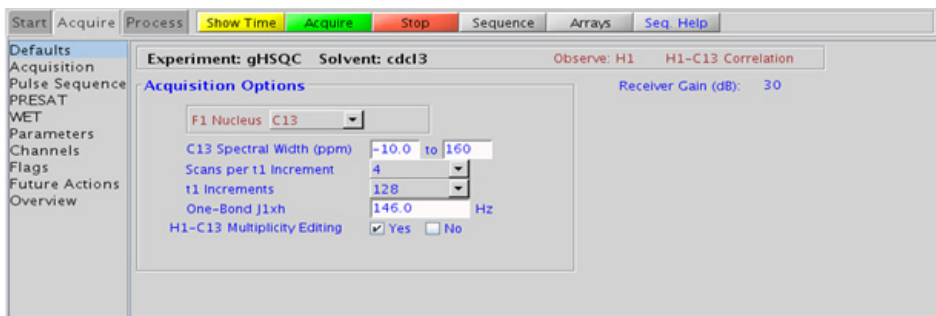
Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimensionScans per t1 increment (nt)
Select typical choices from pull down menu*	
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
H1-C13 Multiplicity Editing	Check box to turn on/off multiplicity editing
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

4 Heteronuclear 2D 1-bond - J1(CH)corr Processing



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gHSQC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing `dm='nnn'` on the command line.

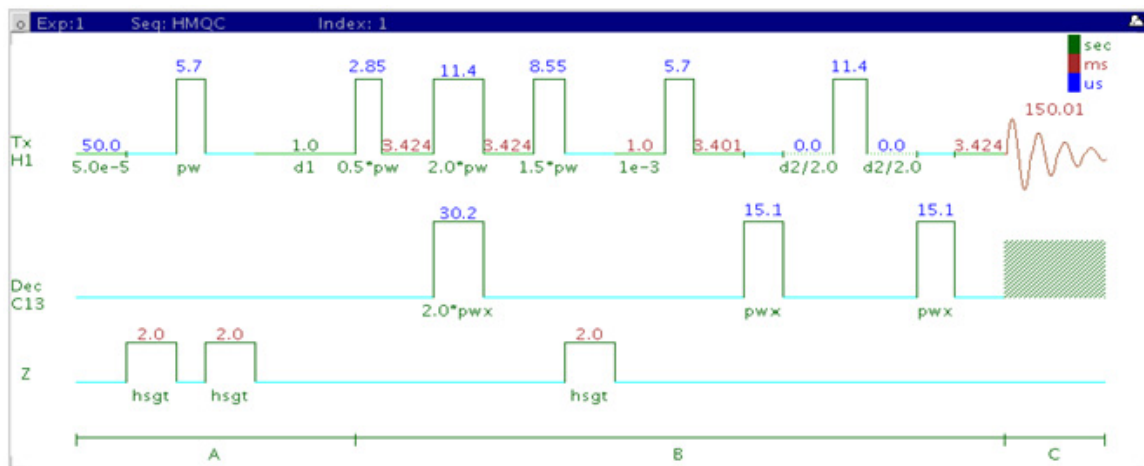
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- Using the multiplicity editing feature results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. There is a sensitivity loss with this feature due to unmatched coupling constants and echo time, but the extra information about multiplicity is usually worth the loss in sensitivity. Hence this feature is on by default. Alternately, consider using gHSCAD to minimize this sensitivity loss.
- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution.
- The HSQC experiment has a sensitivity advantage (factor of ~ 1.4) over the gHSQC, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.
- The HSQCAD and gHSQCAD use adiabatic 180° carbon pulses and invariably give better results than the gHSQC.
- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). Remember to reset the window function along F1 axis if linear prediction parameters are changed.

References:

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

HMQC



General description and usage

Description:

Two dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy.

Usage:

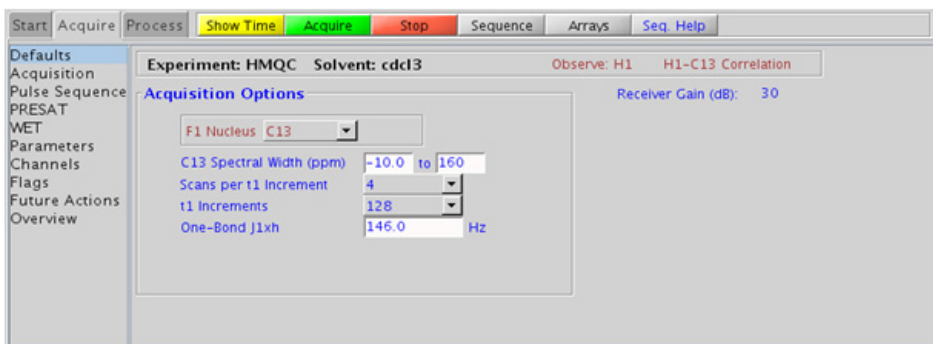
The HMQC experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ - 146 Hz works well for many carbons
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from “Pulse Sequence” panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the HMQC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm=’nnn’ on the command line.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The HMQC experiment has a sensitivity advantage (factor of ~ 1.4) over the gHMQC, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.

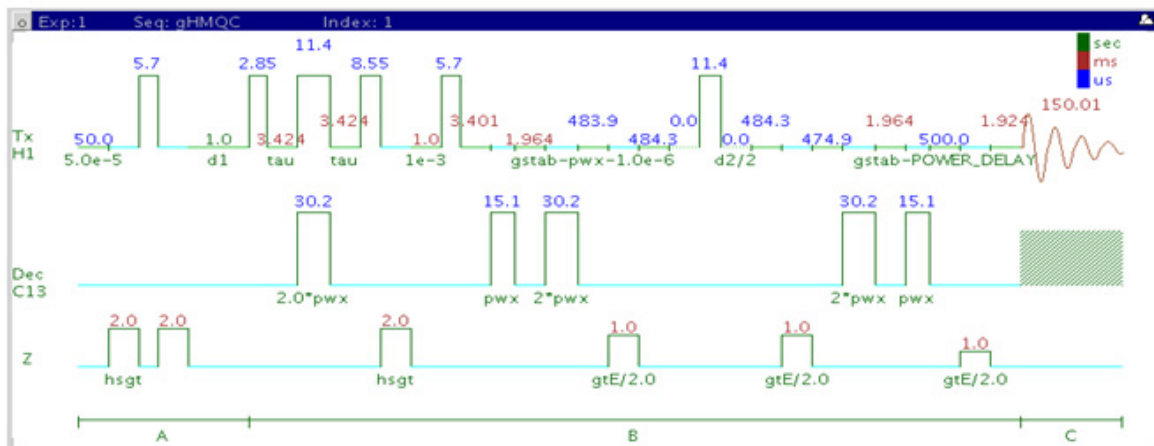
- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution. In addition, the HSQC experiments have the added ability to do multiplicity- editing.

References:

L. Muller, J. Am. Chem. Soc. 101 (1979) 4481-4484. (HMQC)

A. Bax, R. H. Griffey, B. L. Hawkins, J. Magn. Reson. 55 (1983) 301-315. (HMQC)

gHMQC



General description and usage

Description:

Two dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy with gradient coherence selection.

Usage:

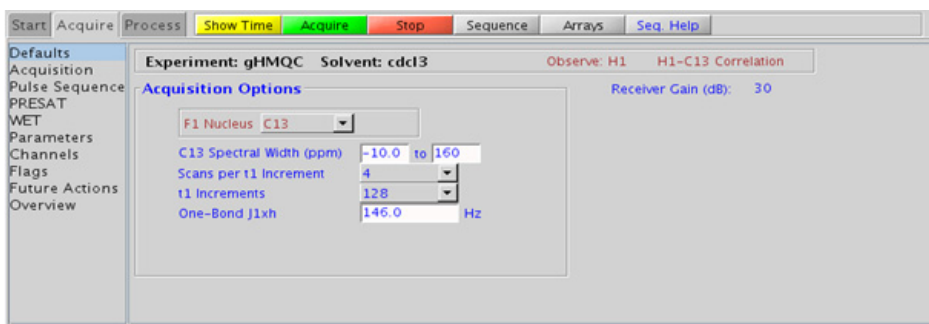
The gHMQC experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ - 146 Hz works well for many carbons
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from “Pulse Sequence” panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

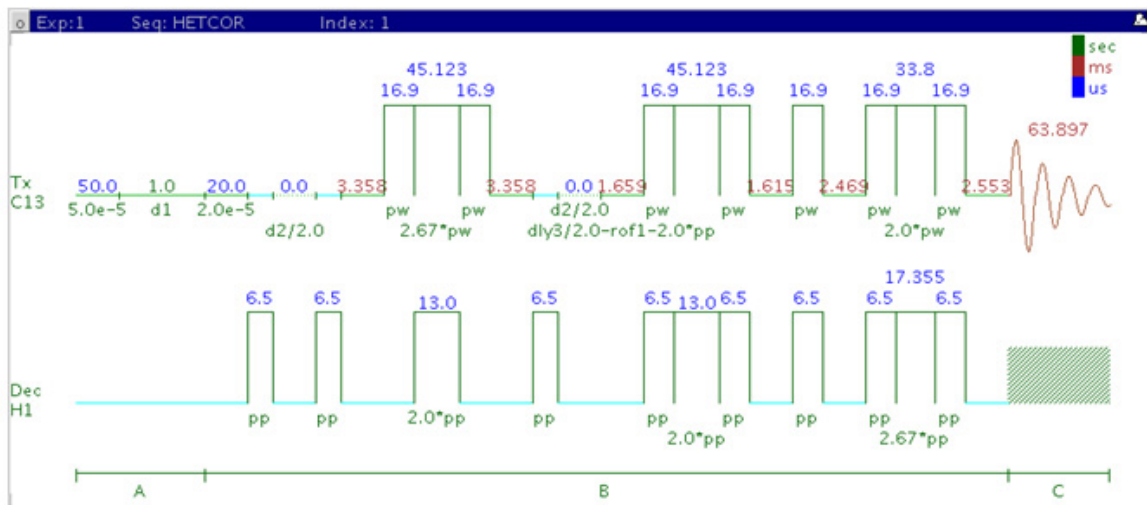
- Spectral width (sw) setting transfers from a PROTON if the gHMQC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm=’nnn’ on the command line.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The HMQC experiment has a sensitivity advantage (factor of ~ 1.4) over the gHMQC, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution. In addition, the HSQC experiments have the added ability to do multiplicity editing.

References:

- L. Muller, J. Am. Chem. Soc. 101 (1979) 4481-4484. (HMQC)
- A. Bax, R. H. Griffey, B. L. Hawkins, J. Magn. Reson. 55 (1983) 301-315. (HMQC)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

HETCOR



General description and usage

Description:

Two dimensional heteronuclear 1-bond shift correlation with carbon observe.

Usage:

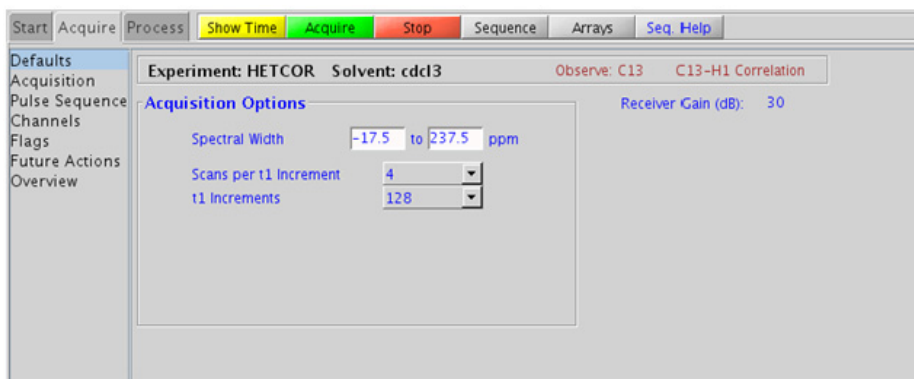
The HETCOR experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. However unlike the HSQC/HMQC experiments HETCOR is carbon-detected, meaning the F1 dimension is ^1H and the F2 dimension is ^{13}C . Because the HETCOR is significantly less sensitive than the proton-detected HSQC/HMQC, use it only under special circumstances where higher resolution is desired in the carbon dimension. The proton decoupling power (during acquisition) used is also usually less. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired CARBON or type in desired values
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.064s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond Coupling	Set value for the average $^1J_{X,H}$ on "Pulse Sequence" panel- 146 Hz works well for many carbons
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

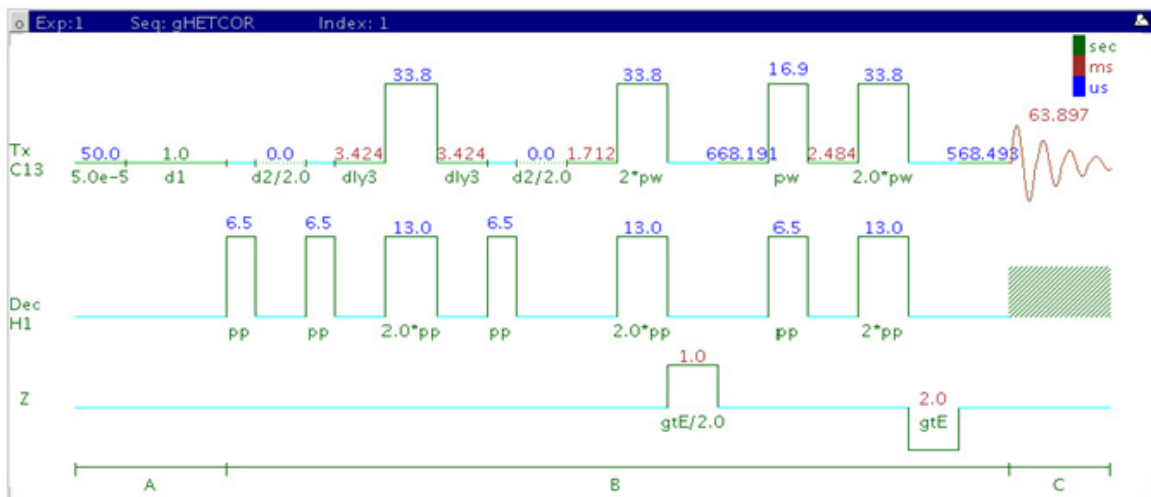
- Spectral width (sw) setting transfers from a CARBON if the HETCOR is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. The default setting for this parameter is off.
- The embedded multiplicity- editing results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons.
- The HETCOR also generates an effective ^1H broadband decoupling along the F1 (H1) dimension.
- The HETCOR experiment has a sensitivity advantage (factor of ~ 1.4) over the gHETCOR, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.

- In general, the HSQC-type experiments are far more sensitive than the HETCOR. The advantages to the HETCOR are lower decoupling power and potentially higher resolution for carbon. The band-selective HSQC experiment, (HC)bsHSQCAD (see Chapter 7), however, is a much more sensitive experiment and may provide a more convenient way to achieve higher ^{13}C resolution.

References:

- A. Bax, G. A. Morris, J. Magn. Reson. 42 (1981), 501-505. (HETCOR)
- D. L. Turner, Prog. NMR. Spectrosc. 17 (1985) 281-358. (2D NMR)

gHETCOR



General description and usage

Description:

Two dimensional heteronuclear 1-bond shift correlation with carbon observe and gradient coherence selection.

Usage:

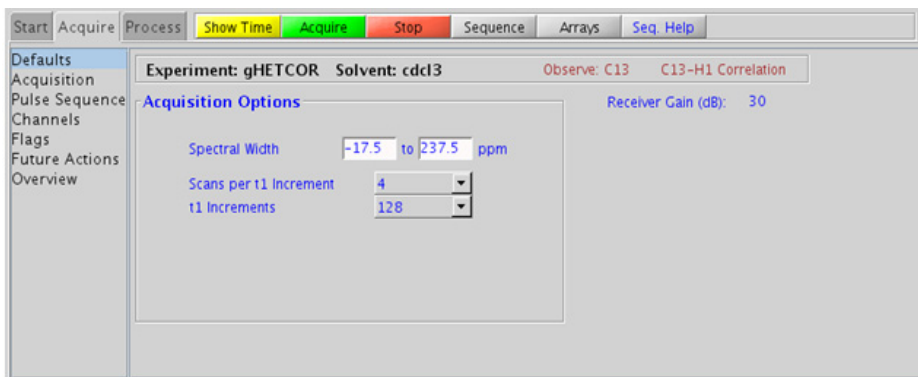
The gHETCOR experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. However unlike the gHSQC/gHMQC experiments gHETCOR is carbon-detected, meaning the F1 dimension is ^1H and the F2 dimension is ^{13}C . Because the gHETCOR is significantly less sensitive than the proton-detected gHSQC/gHMQC, and use it only under special circumstances where higher resolution is desired in the carbon dimension. The proton decoupling power (during acquisition) used is also usually less. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired CARBON or type in desired values
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.064s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond Coupling	Set value for the average $^1J_{X,H}$ on "Pulse Sequence" panel- 146 Hz works well for many carbons
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a CARBON if the gHETCOR is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. The default setting for this parameter is off.
- The embedded multiplicity editing results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons.
- The gHETCOR also generates an effective ^1H broadband decoupling along the F1 (H1) dimension.
- The HETCOR experiment has a sensitivity advantage (factor of ~ 1.4) over the gHETCOR, but due to t1 noise from strong signals from t-butyl, may produce potentially less clean spectra than its gradient counterpart.

- In general, the gHSQC-type experiments are far more sensitive than the gHETCOR. The advantages to the gHETCOR are lower decoupling power and potentially higher resolution for carbon. The band-selective HSQC experiment, (HC)bsgHSQCAD (see Chapter 7), however, is a much more sensitive experiment and may provide a more convenient way to achieve higher ^{13}C resolution.

References:

- A. Bax, G. A. Morris, J. Magn. Reson. 42 (1981), 501-505. (HETCOR)
- D. L. Turner, Prog. NMR. Spectrosc. 17 (1985) 281-358. (2D NMR)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

ASAPHMQC



General description and usage

Description:

Two dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses and an “ASAP” feature for rapid recycle times.

Usage:

This experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is the most useful of the HMQC series as it contains a feature that allows for very fast recycle time. With a reasonably concentrated sample, it is possible to obtain an ASAPHMQC spectrum with adequate resolution in about 1 minute. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

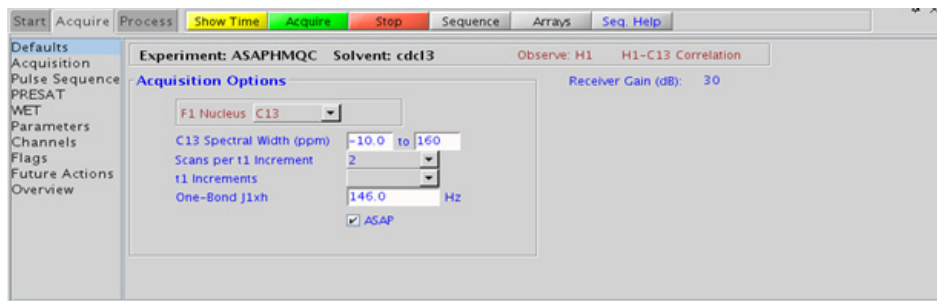
Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.064s for ASAP
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ 146 Hz works well for many carbons
ASAP	Check box to turn on/off ASAP feature (off gives an HMQC with adiabatic 180° X-nuclei pulses)
Relaxation time (d1)	Set from "Acquisition" panel- controls the delay between scans (default is 0.064s)
Mixing time (mix)	Set from "Pulse Sequence" panel- mixing time for adiabatic pulses (default is 25ms)
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

4 Heteronuclear 2D 1-bond - J1(CH)corr Processing



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the ASAPMQC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- ASAPMQC as a fast method gives significantly better sensitivity than other members of the heteronuclear one-bond correlation experiments for a given spectrometer time. This alone overweighs other advantages of traditional experiments.

- During the short relaxation delay (d1), the ASAPHMQC employs a TOCSY-type magnetization transfer from ^{12}C -attached protons (which are kept along z during the pulse sequence) to the observed ^{13}C -attached protons. Note that because of this transfer, the ASAPHMQC works best if one or several protons are in the neighborhood (i.e., spin system) of the observed ^{13}C - ^1H pair. Lone ^{13}C - ^1H pairs without neighboring protons may therefore show up at a much lower intensity. In such cases it may help to increase d1 from the default 60ms.
- The adiabatic pulses and rapid recycle time make the ASAPHMQC experiment a good choice for obtaining carbon information in automation. This experiment is also a good way to rapidly obtain “survey” carbon information for a subsequent band-selective experiment (see Chapter 7).

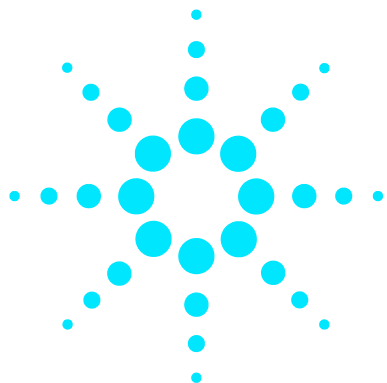
References:

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256.
 (Adiabatic Pulses)

E. Kupce, R. Freeman, Magn. Reson. Chem. 45 (2007) 2-4. (ASAPHMQC)

4 Heteronuclear 2D 1-bond - J1(CH)corr

Tips, tricks, and next steps



5 Heteronuclear 2D multiple-bond - Jn(CH)corr

Jn(CH)corr Tab 176

gHMBCAD 178

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gH2BCAD 186

gH2BC 190

CIGAR 194

CIGARAD 198

HMBC 202

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gHMBCRELAY 212



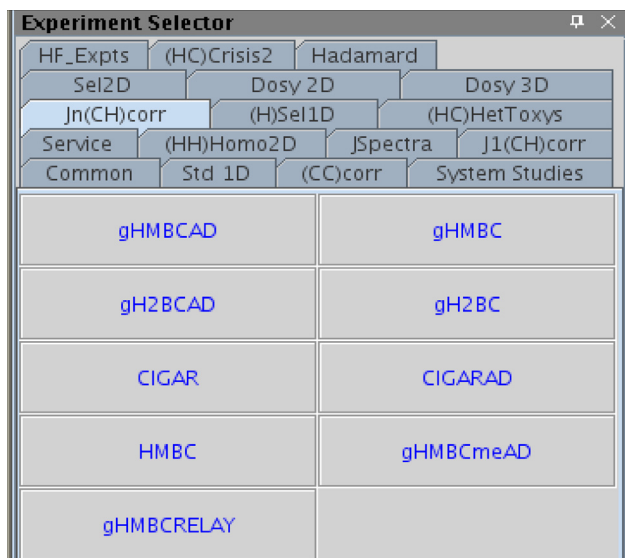
Jn(CH)corr Tab

This tab of the experiment selector contains 2D experiments showing long-range couplings from protons to carbon nuclei. The most commonly used experiment type, the HMBC, is available both with and without adiabatic 180° carbon pulses, and also with and without gradient coherence selection. The gradient enhanced version of the experiment has better artifact suppression at the expense of a loss of sensitivity (factor of ~ 1.4). The adiabatic version, gHMBCAD is very highly recommended, as it provides more uniform inversion profiles for a wide ^{13}C spectral width, and at the same time compensates for any ^{13}C pwx imperfections in the 180° pulses. There is a multiplicity- edited version of this experiment, gHMBCmeAD, and an experiment that provides similar information to differentiate between 2-bond and >2-bond couplings, the gHMBCRELAY. The H2BC experiment (adiabatic and non-adiabatic) is used to show specifically 2-bond proton-carbon correlations involving non-quaternary carbons. This experiment is, in principle, an HMQC-COSY equivalent. Finally, there are the adiabatic and non-adiabatic CIGAR experiments. While the HMBC experiments are typically optimized for a single Jn(XH) coupling constant, the CIGAR experiments attempt to optimize a range for this coupling constant. This experiment is particularly effective for ^{15}N , where the Jn(NH) coupling constants can vary widely.

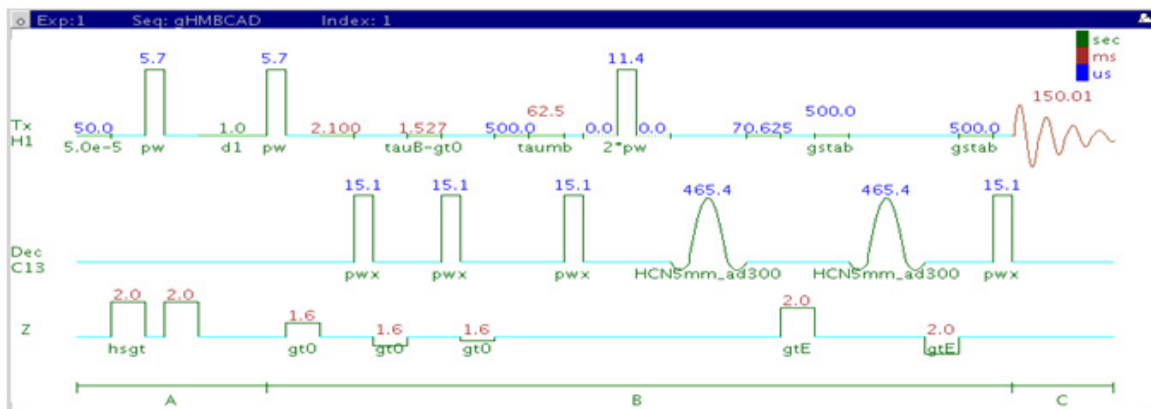
Long-range proton-carbon correlations are often used in structure elucidation to provide key connectivity data between separated spin systems and to quaternary carbons. The HMBC type experiments are generally more sensitive than 1D CARBON spectra for detecting quaternary carbons (with the exception of carbons which are 3 or 4 bonds away from any proton). The sensitivity of these experiments does vary widely from correlation to correlation. When the HMBC type experiments are used with less concentrated samples, while it may not show all correlations, even a partial long range correlation data set is highly valuable for structure elucidation.

Most of these experiments (except gHMBCRELAY, gH2BC and gH2BCAD) rely on ^1H - ^{13}C correlations via long-range coupling constants. In general 3-bond coupling constants (~ 8 Hz) are larger than 2-bond coupling constants (~ 2 -3 Hz) and typically tend to give stronger crosspeaks when the experiment parameters are optimized for 8 Hz. However, care must be exercised in interpreting these crosspeaks from their intensity as arising

from 2 or 3 bond correlations, as they can only be definitively identified as crosspeaks due to smaller coupling constants (and may be 2, 3, or 4 bonds away). The 2-bond correlation experiments gH2BC and gH2BCAD, although less sensitive than the HMBC, are used to distinguish between 2- and 3-bond correlations.



gHMBCAD



General description and usage

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses and gradient coherence selection.

Usage:

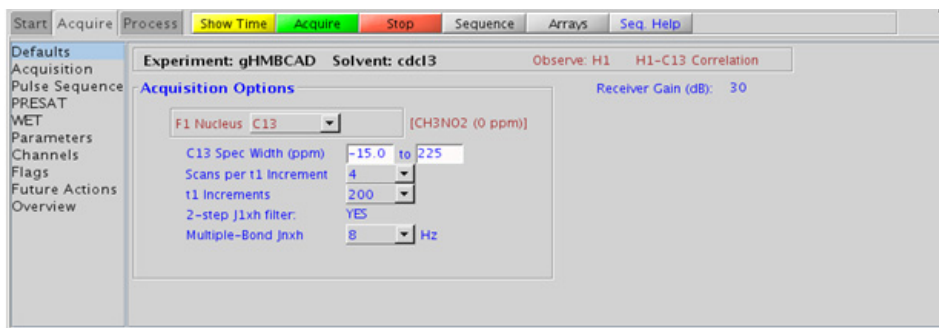
The gHMBCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons with carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. The gHMBCAD experiment is also one of the most sensitive ways to obtain information about quaternary carbon chemical shifts and connectivities. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jn _{xh} (jnxh)	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$ - 8 Hz works well for most molecules
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gHMBCAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- In general, the HMBC-type experiments have the best sensitivity of the Jn(CH)corr experiments- the other experiments provide specialized data for specific information types.

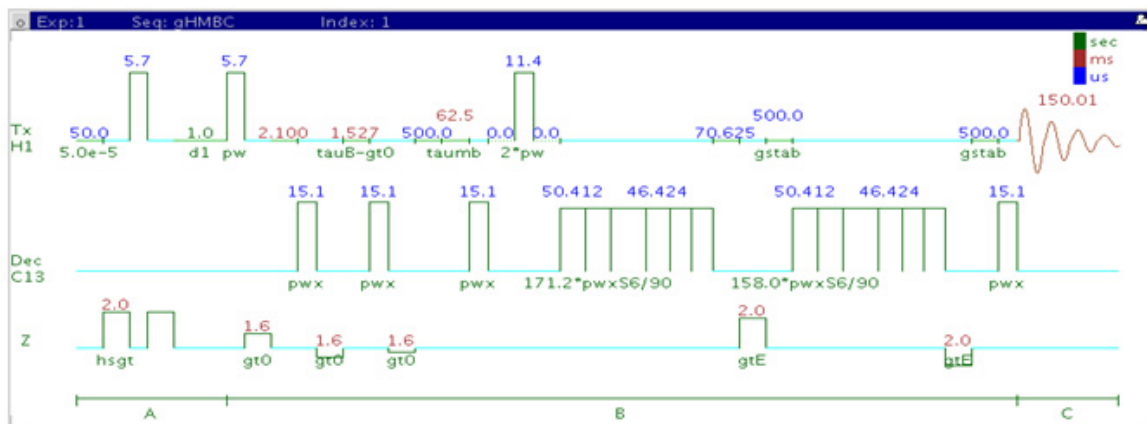
- The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity loss. The CIGARAD experiment is better suited on a reasonably concentrated sample for detecting longer range correlations from a range of coupling constants, but at the expense of sensitivity.
- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the “Pulse Sequence” panel around which one-bond couplings are eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.
- The gHMBCAD uses adiabatic 180° carbon pulses and invariably gives better results than the gHMBC.

References:

- A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)
- C. E. Hadden, Magn. Reson. Chem. 43 (2005) 330-333. (Adiabatic Pulses)

5 Heteronuclear 2D multiple-bond - Jn(CH)corr gHMBC

gHMBC



General description and usage

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with gradient coherence selection.

Usage:

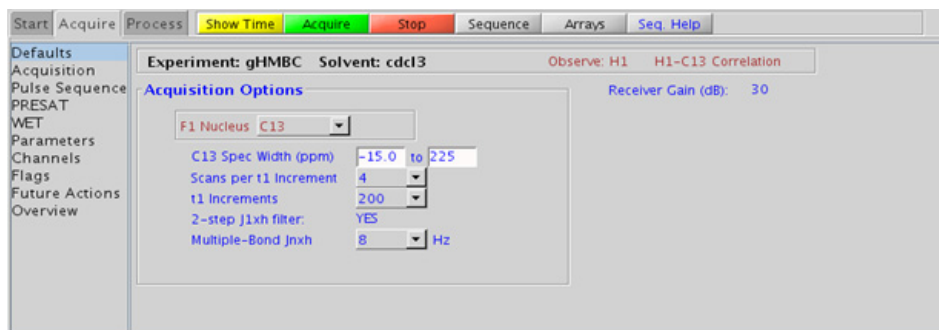
The gHMBC experiment is used to acquire a 2-dimensional spectrum to correlate protons with carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. The gHMBC experiment is also one of the most sensitive ways to obtain information about quaternary carbon chemical shifts and connectivities. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
Multiple-Bond Jn _{xh} (jnxh)	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$ - 8 Hz works well for most molecules
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

Tips, tricks, and next steps

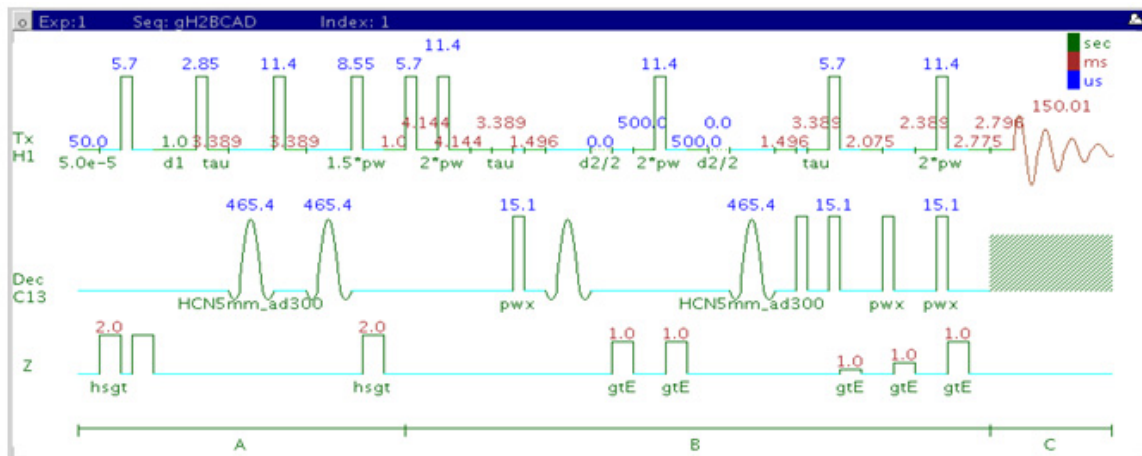
- Spectral width (sw) setting transfers from a PROTON if the gHMBC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The HMBC experiment has a sensitivity advantage (factor of ~ 1.4) over the gHMBC, but due to t_1 noise from strong signals such as *t*-butyl may produce potentially less clean spectra than its gradient counterpart.
- In general, the HMBC-type experiments have the best sensitivity of the Jn(CH)corr experiments- the other experiments provide specialized data for specific information types.

- The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity loss. The CIGAR experiment is better suited on a reasonably concentrated sample for detecting longer-range correlations from a range of coupling constants, but at the expense of sensitivity.
- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one bond couplings. Select two coupling constants on the “Pulse Sequence” panel around which one-bond couplings are eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.
- The gHMBC uses the Shaka6 (S6) pulse to achieve broadband inversion. Parameters relating to this pulse is found on the “Pulse Sequence” tab.
- The gHMBCAD uses adiabatic 180° carbon pulses and invariably gives better results than the gHMBC.

References:

- A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

gH2BCAD



General description and usage

Description:

Two dimensional heteronuclear 2-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses and gradient coherence selection.

Usage:

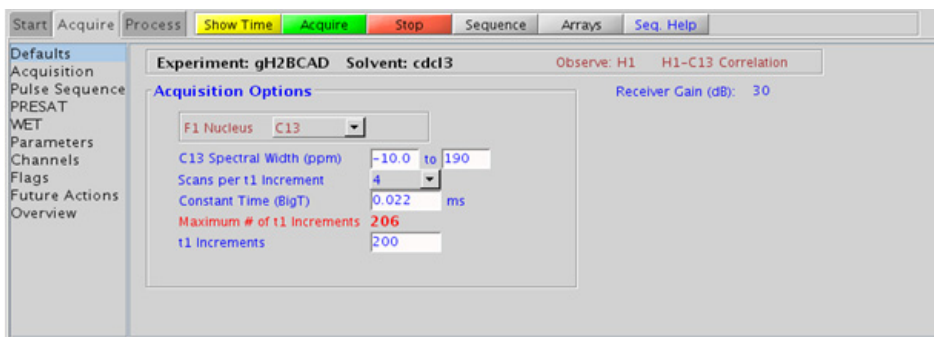
The gH2BCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons with carbons through ^1H - ^1H vicinal and ^1H - ^{13}C one bond coupling connectivity. Although less sensitive than the gHMBCAD, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations. Note that the experiment is constant time in F1, so the constant time delay (BigT) determines the maximum number of increments (ni). All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
Constant Time (BigT)	Type in value for constant time delay- 22ms (default) works for most samples
t1 Increments (ni)	Type in value- controls the resolution in F1 (note maximum allowed from the constant timedelay (BigT)
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- turn on/off TANGO-gradient suppression- default is on

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (256k x 256k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also available (defaults are gaussian in both F1 and F2 dimensions). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gH2BCAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The gH2BCAD is in principle a gHMQC-COSY type experiment. By this very nature, it can only show 2-bond correlations to protonated carbons but not for quaternary carbons. A 22ms bigT delay is an optimal value for COSY magnetization transfer.
- The gH2BCAD experiment is specialized to show only 2-bond correlations, but it is less sensitive than the gHMBCAD experiment. As such, the gH2BCAD is best used as supplemental information to the gHMBCAD data to distinguish between 2- and 3-bond correlations.

- The gH2BCAD uses adiabatic 180° carbon pulses and invariably gives better results than the gH2BC.

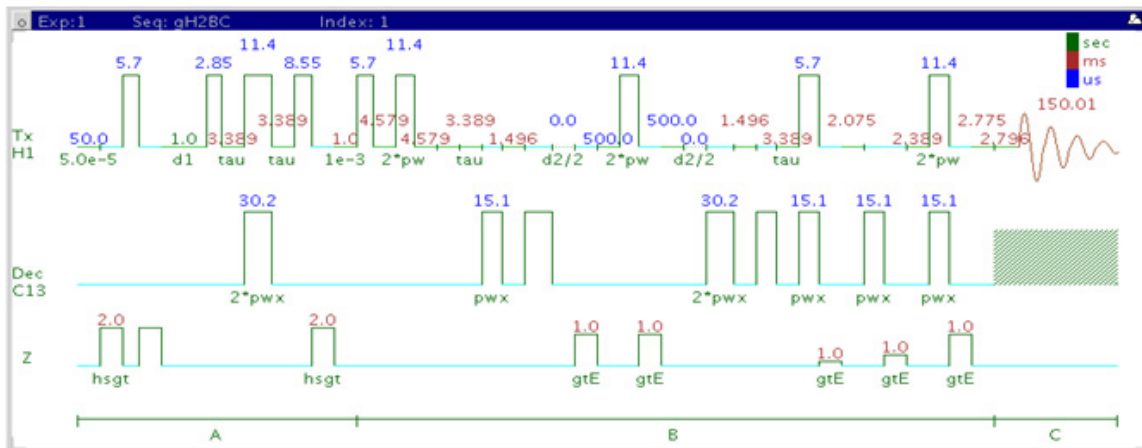
References:

N. T. Nyberg, J. O. Duus, O. W. Sorensen, J. Am. Chem. Soc. 127 (2007) 6154-6155. (H2BC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

gH2BC



General description and usage

Description:

Two dimensional heteronuclear 2-bond J-correlation spectroscopy with gradient coherence selection.

Usage:

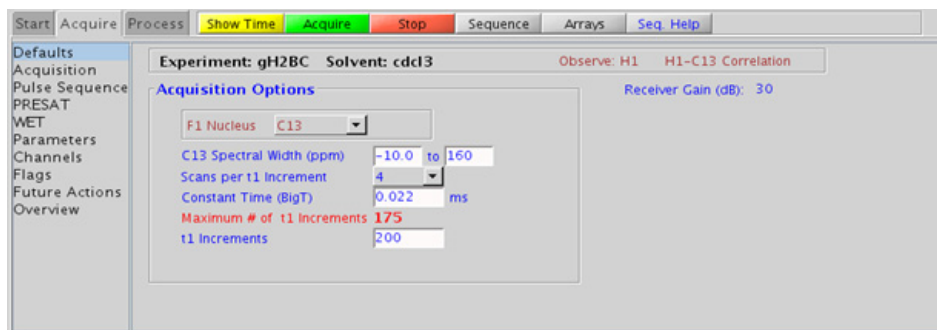
The gH2BC experiment is used to acquire a 2dimensional spectrum to correlate protons with carbons through ^1H - ^1H vicinal and ^1H - ^{13}C one-bond coupling connectivity. Although less sensitive than the gHMBC, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations. Note that the experiment is constant time in F1, so the constant time delay (BigT) determines the maximum number of increments (ni). All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
Constant Time (BigT)	Type in value for constant time delay- 22ms (default) works for most samples
t1 Increments (ni)	Type in value- controls the resolution in F1 (note maximum allowed from the constant timedelay (BigT)
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- turn on/off TANGO-gradient suppression- default is on

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (256k x 256k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also available (defaults are gaussian in both F1 and F2 dimensions). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

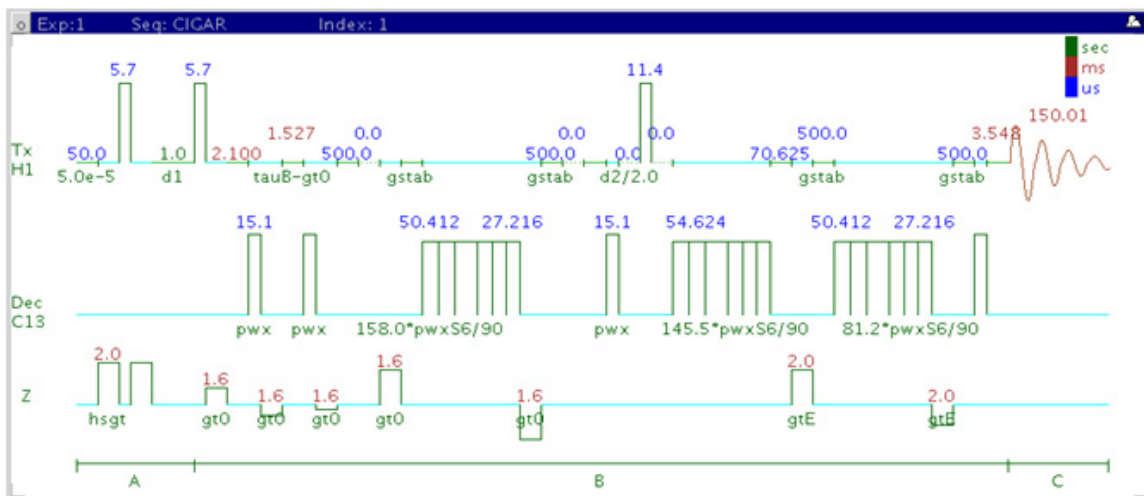
- Spectral width (sw) setting transfers from a PROTON if the gH2BC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The gH2BC is in principle a gHMQC-COSY type experiment. By this very nature, it can only show 2-bond correlations to protonated carbons but not for quaternary carbons. A 22ms bigT delay is an optimal value for COSY magnetization transfer.
- The gH2BC experiment is specialized to show only 2-bond correlations, however it is less sensitive than the gHMBC experiment. As such, the H2BC is best used as supplemental information to the gHMBC data to distinguish between 2- and 3-bond correlations.

References:

N. T. Nyberg, J. O. Duus, O. W. Sorensen, J. Am. Chem. Soc. 127 (2007) 6154-6155. (H2BC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

CIGAR



General description and usage

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy.

Usage:

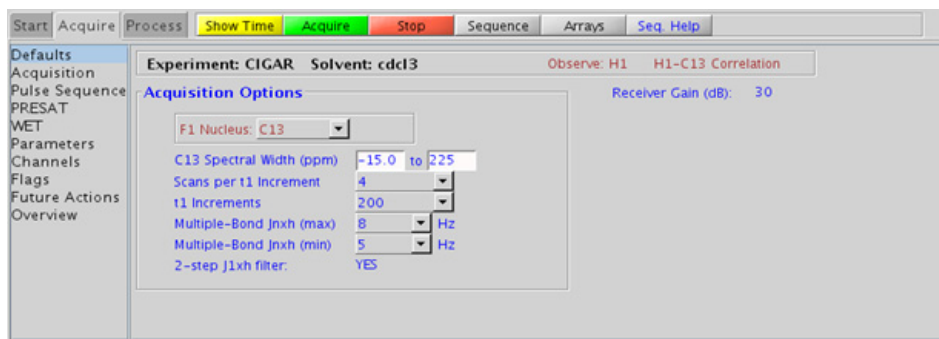
The CIGAR experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over multiple bonds. Although less sensitive than its gHMBC counterpart, this experiment is useful when there is a need to see longer range correlations (>3 bonds and/or with small coupling constants), or when there is a large range of $^nJ_{X,H}$ coupling constants, such as with proton-nitrogen. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
Multiple-Bond jnxh (max/min)	Set values for the maximum/minimum $^n\text{J}_{\text{X,H}}$
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlations-default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

Tips, tricks, and next steps

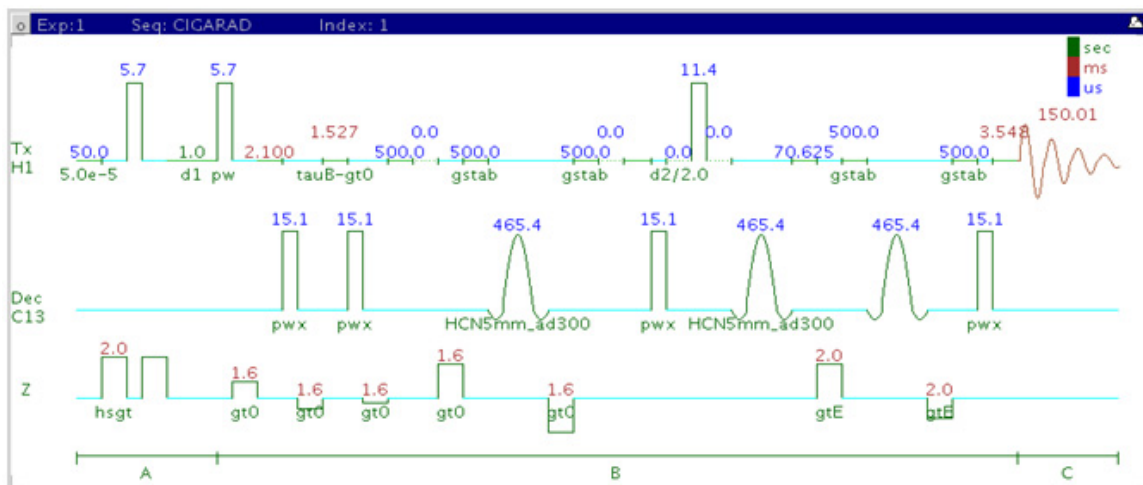
- Spectral width (sw) setting transfers from a PROTON if the CIGAR is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The parameter jscaleU and jscaleD (accessed in the “Pulse Sequence” panel) are typically set to 0 for best sensitivity, which are the default values. However, you can set these to non-zero values to achieve homonuclear J dependent peak “skewing” for either improved peak identification in a crowded region (jscaleU) or to differentiate J^2 vs. J^3 crosspeaks (jscaleD).

- The CIGAR experiment is specialized to allow input of a range for $^nJ_{X,H}$. However, it is less sensitive than the gHMBC experiment (at least by a factor of 2). As such, the CIGAR is best used under circumstances where there is a large range for the $^nJ_{X,H}$ coupling constants, such as for proton-nitrogen. The CIGAR experiment may also be used when the sample is reasonably concentrated and there is a need to see longer range correlations (for example, >3-bond).
- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the “Pulse Sequence” panel around which one-bond couplings are eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.
- The CIGARAD uses adiabatic 180° carbon pulses and invariably gives better results than the CIGAR.

References:

- C. E. Hadden, G. E. Martin, V. V. Krishnamurthy, Magn. Reson. Chem. 38 (2000) 143-147. (CIGAR)
- M. Kline, S. Cheatham, Magn. Reson. Chem. 41 (2003) 307-314. (^{15}N -optimized CIGAR)

CIGARAD



General description and usage

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses.

Usage:

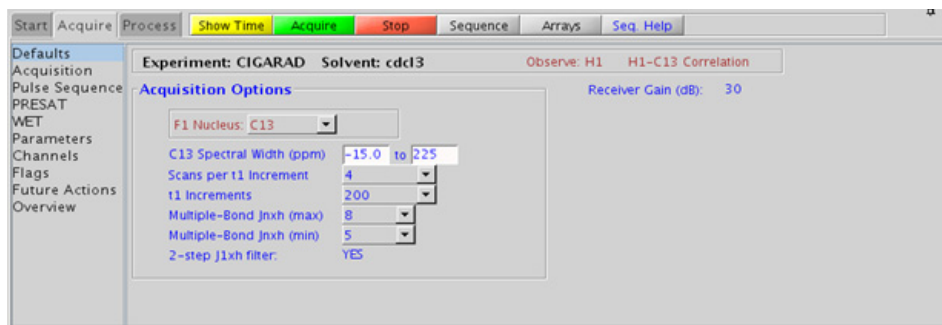
The CIGARAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over multiple bonds. Although less sensitive than its gHMBC counterpart, this experiment is useful when there is a need to see longer range correlations (>3 bonds and/or with small coupling constants), or when there is a large range of $^nJ_{X,H}$ coupling constants, such as with proton-nitrogen. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
Multiple-Bond jnxx (max/min)	Set values for the maximum/minimum $^n\text{J}_{\text{X,H}}$
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlations-default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

Tips, tricks, and next steps

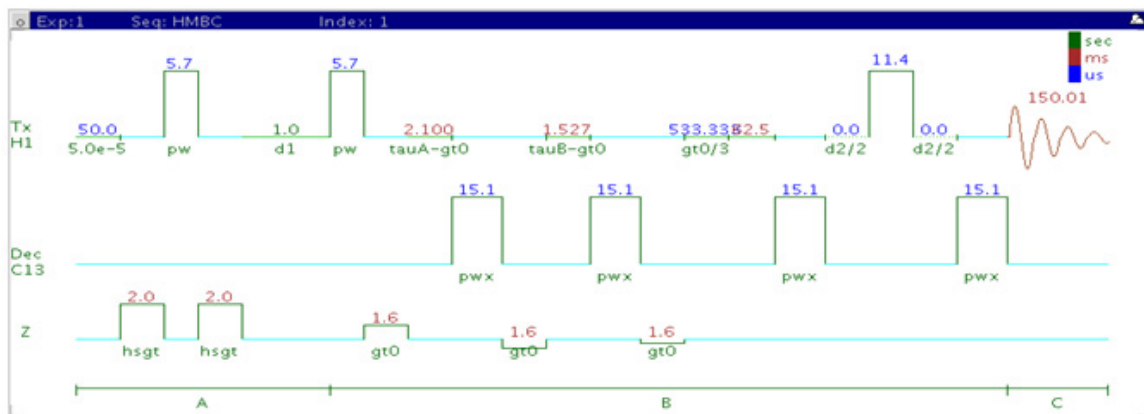
- Spectral width (sw) setting transfers from a PROTON if the CIGARAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The parameter jscaleU and jscaleD (accessed in the “Pulse Sequence” panel) are typically set to 0 for best sensitivity, which are the default values. However, you can set these to non-zero values to achieve homonuclear J dependent peak “skewing” for either improved peak identification in a crowded region (jscaleU) or to differentiate J^2 vs. J^3 crosspeaks (jscaleD).

- The CIGARAD experiment is specialized to allow input of a range for $^nJ_{X,H}$, however it is less sensitive than the gHMBC experiment (at least by a factor of 2). As such, the CIGARAD is best used under circumstances where there is a large range for the $^nJ_{X,H}$ coupling constants, such as for proton-nitrogen. The CIGARAD experiment may also be used when the sample is reasonably concentrated and there is a desire to see longer range correlations (for example, >3-bond).
- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the “Pulse Sequence” panel around which one-bond couplings are eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.
- The CIGARAD uses adiabatic 180° carbon pulses and invariably gives better results than the CIGAR.

References:

- C. E. Hadden, G. E. Martin, V. V. Krishnamurthy, Magn. Reson. Chem. 38 (2000) 143-147. (CIGAR)
- C. E. Hadden, Magn. Reson. Chem. 43 (2005) 330-333. (Adiabatic Pulses)

HMBC



General description and usage

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with gradient coherence selection.

Usage:

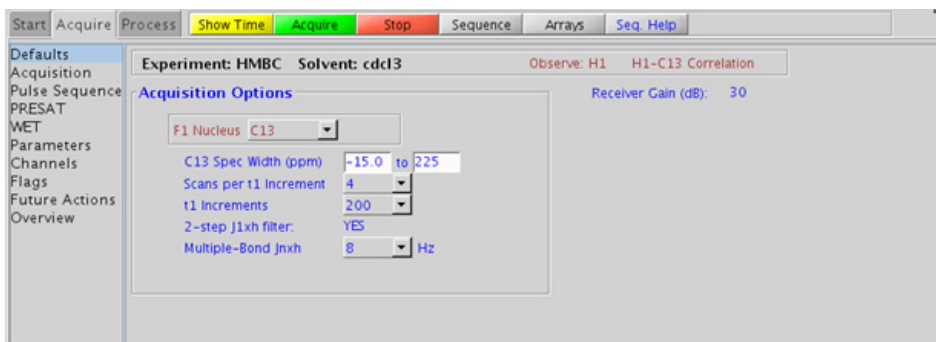
The HMBC experiment is used to acquire a 2-dimensional spectrum to correlate protons with carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. The HMBC experiment is also one of the most sensitive ways to obtain information about quaternary carbons chemical shifts and connectivities. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
Multiple-Bond Jn _{hx} (jn _{hx})	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$ - 8 Hz works well for most molecules
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlation on/off- default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

Tips, tricks, and next steps

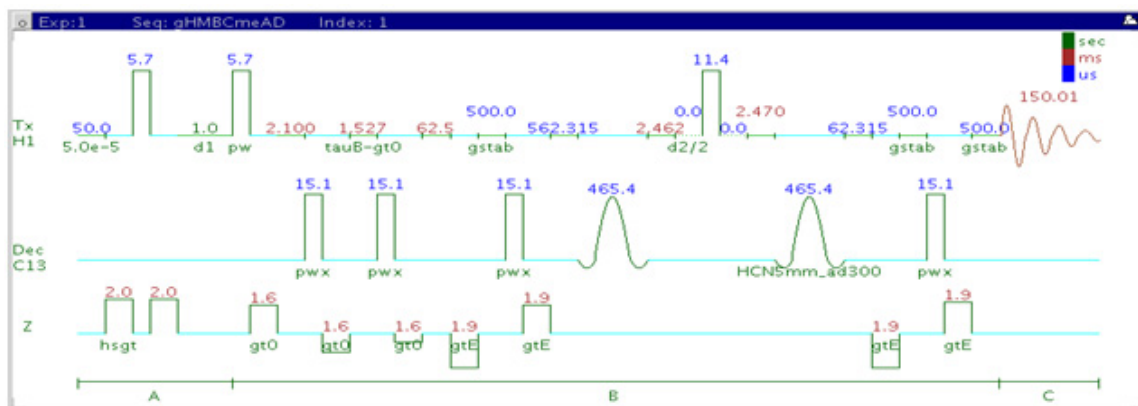
- Spectral width (sw) setting transfers from a PROTON if the HMBC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The HMBC experiment has a sensitivity advantage (factor of ~ 1.4) over the gHMBC, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart. Invariably the gradient coherence selected variants (such as gHMBC or gHMBCAD) are preferred over HMBC.

- The `jnxh` sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity loss.
- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the “Pulse Sequence” panel around which one-bond couplings are eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

References:

A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)

gHMBCmeAD



General description and usage

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with multiplicity-editing (adiabatic 180° X-nuclei pulses and gradient coherence selection).

Usage:

The gHMBCmeAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The experiment is highly useful for structure elucidation as the longer range allows connectivities between isolated proton spin systems to be confirmed. The gHMBCmeAD experiment is a multiplicity edited version of gHMBCAD. This experiment is acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive

to local functional groups and can be reliably predicted with software. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

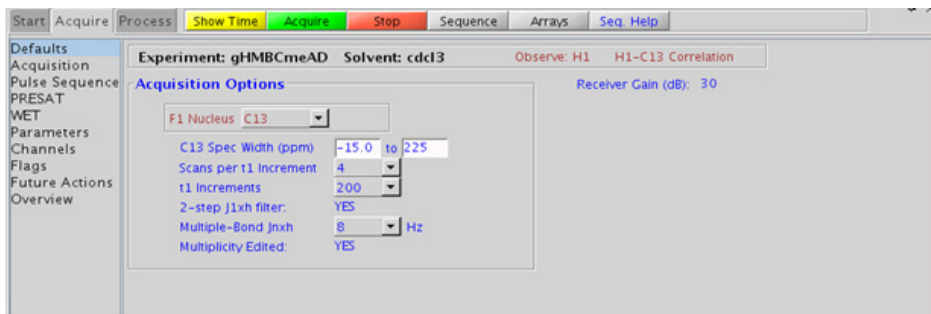
Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxb (jnxh)	Pull-down menu to set value for the average $^n\text{J}_{\text{CH}}$ - 8 Hz works well for most molecules
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.

5 Heteronuclear 2D multiple-bond - Jn(CH)corr Processing



Processing

The multiplicity- editing feature requires running an array of two spectra (default setup). The “Process” tab/“Default” panel has three buttons under the “Transform” heading to select the desired subspectrum. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gHMBCmeAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.

- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The multiplicity-editing feature uses the CRISIS technique that relies on a hypothetical relationship between one-bond coupling constant and carbon chemical shift. Carbons with coupling constants that deviate significantly from this relationship result in imperfect editing.
- As the gHMBCmeAD is acquired as an array of two spectra, it takes twice as long to acquire and there is a small sensitivity loss with the multiplicity-editing.
- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the “Pulse Sequence” panel around which one-bond couplings are eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

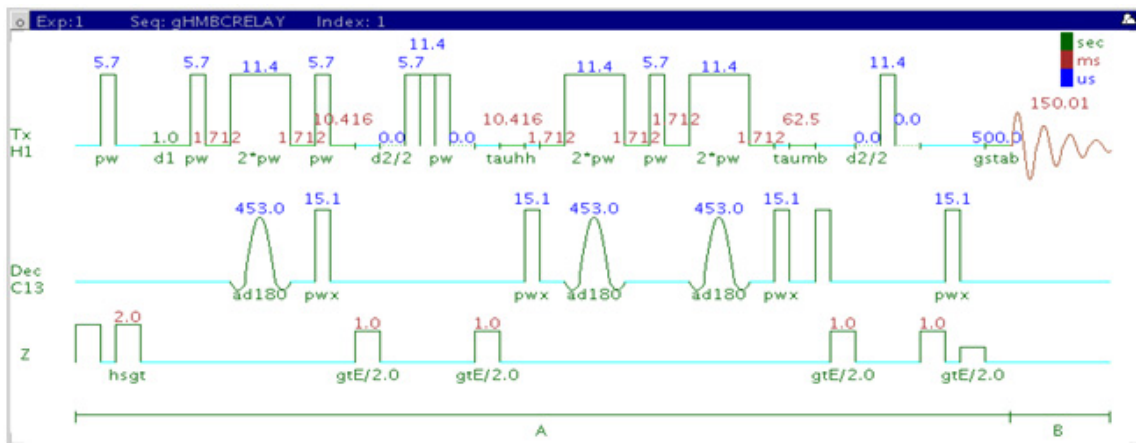
References:

- A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)
- A. J. Benie, O. W. Sorensen, Magn. Reson. Chem. 44 (2006) 739-743. (gHMBCme)
- R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (CRISIS)

5 Heteronuclear 2D multiple-bond - Jn(CH)corr

Tips, tricks, and next steps

gHMBCRELAY



General description and usage

Description:

Two dimensional heteronuclear spectroscopy for the simultaneous detection of $^2J_{C,H}$ and $^nJ_{C,H}$ with adiabatic 180° X-nuclei pulses and gradient coherence selection.

Usage:

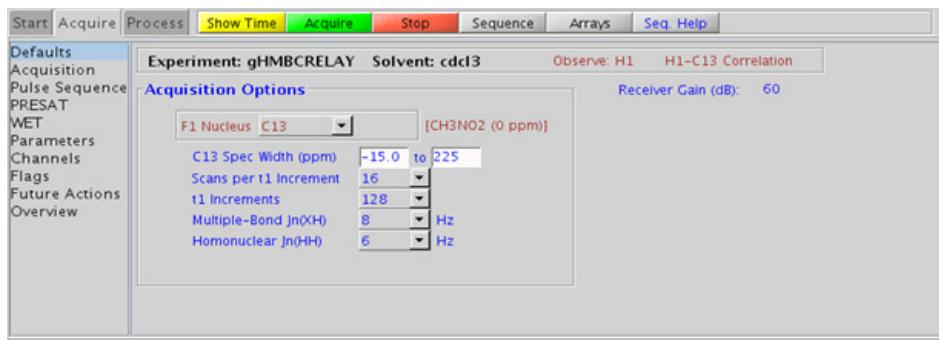
The gHMBCRELAY experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over multiple bonds. Although less sensitive than the gHMBCAD, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations, as the 2-bond correlations are created through a separate pathway and may be separated into a subspectrum. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
Multiple-Bond Jn(xh)	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$ - 8 Hz works well for most molecules
Homonuclear Jn(HH)	Pull-down menu to set value for the optimized $^n\text{J}_{\text{H,H}}$
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The “Process” tab/“Default” panel has three buttons under the “Transform” heading to select the desired subspectrum. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are squared sinebells). More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gHMBCRELAY is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The gHMBCRELAY experiment is specialized to be able to show subspectra containing only 2-bond H-C correlations, or >2-bond correlations, however it is less sensitive than the gHMBCAD experiment. As such, the gHMBCRELAY is best used only when it is important to be able to distinguish between 2- and 3-bond correlations.

References:

T. Sprang, P. Bigler, Magn. Reson. Chem. 42 (2004) 55-60. (HMBCRELAY)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

M. R. Bendall, M. Garwood, K. Ugurbill, D. T. Pegg, Magn. Reson. Med. 4 (1987) 493-499. (Adiabatic Pulses)

5 Heteronuclear 2D multiple-bond - Jn(CH)corr

Tips, tricks, and next steps



6 Selective 1D - (H)Sel1D

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selexcit 240

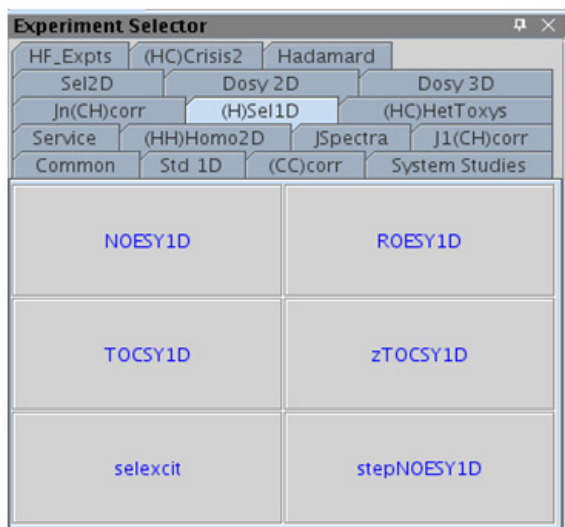
stepNOESY1D 244



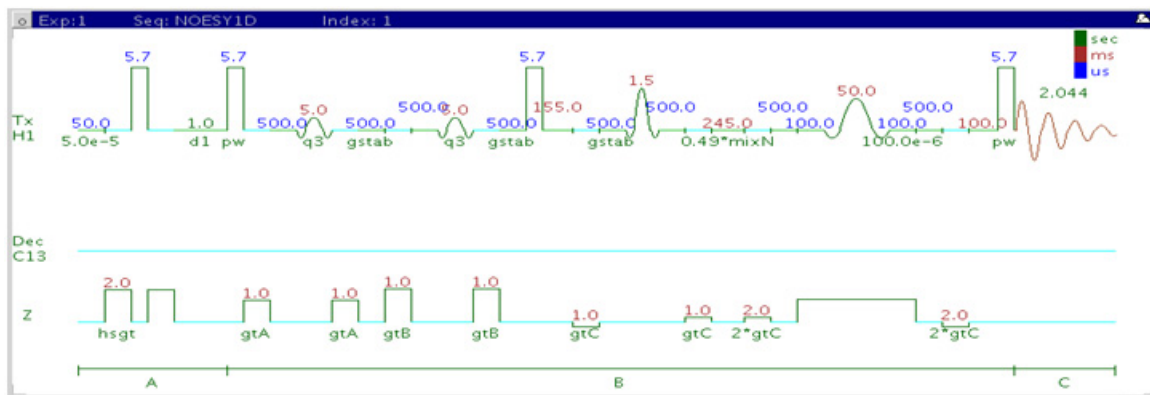
(H)Sel1D Tab

This tab of the experiment selector includes the set of 1D experiments performed on small, user-defined bands or resonances of the proton spectrum using selective pulses. They are useful to obtain targeted information about single resonances or small areas of a complicated proton spectrum. Included are J-coupled experiments (TOCSY1D and zTOCSY1D), through-space experiments (NOESY1D, ROESY1D), as well as a simple selective excitation experiment (selexcit). The zTOCSY1D contains a z-filter to suppress zero-quantum artifacts and, in general, give better results than TOCSY1D. The stepNOESY1D is a specialized experiment that first performs a TOCSY 1D and then a second selective NOESY1D excitation off one of the TOCSY relayed peaks.

The most direct way to set up these experiments is to first acquire a proton spectrum and then choose the appropriate selective 1D experiment. The proton spectrum can then be used as a reference to select the desired peaks/regions for the experiment. Alternatively, if the regions are known *a priori*, the selective 1D experiment can be chosen initially and the regions entered manually.



NOESY1D



General description and usage

Description:

A pulse sequence to acquire a 1D NOESY dataset with a selective pulse to obtain NOE information from a specific frequency.

Usage:

The NOESY1D experiment is an important tool for structure elucidation as it does not require through-bond coupling connectivity for correlations, rather the NOE (Nuclear Overhauser Effect) interaction is seen between protons that are close in space (typically $<5 \text{ \AA}$). As such the NOESY1D experiment is often used to help determine stereochemistry for rigid ring systems, or to confirm attachments between proximal (but non-coupled) portions of a molecule. The selective NOESY1D experiment has an advantage over the 2D NOESY version for simplifying complicated spectra with a lot of overlapping peaks. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for NOE analysis may be chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

Key parameters

Parameters	Description
Solvent (solvent)	Set from “Start” tab/“Standard” panel
Spectral Width(sw)	Set from previously acquired PROTON*
Number of scans(nt)	Select typical choices from pull down menu*
NOE Mixing time (mixT)	Select from pull down menu- 500ms works well for most small molecules*
Run a mix=0 spectrum	Check box to acquire a control spectrum with a mixing time of 0ms- creates an array for mixT
No., ppm, width of selective frequencies	Select number of frequencies from the drop down menu. Enter frequency position(s) and bandwidth(s) in the entry boxes.
ZQ filter	Check box on the “Pulse Sequence” panel- zero quantum filter for artifact suppression. Strongly recommended - default is on.

NOTE


You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.

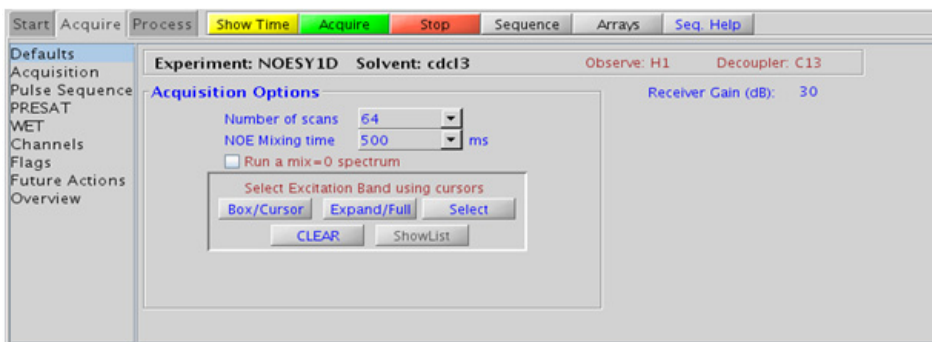
The screenshot displays the 'Acquisition Options' panel within the Agilent VnmrJ 4 software. The top of the panel shows the experiment name 'NOESY1D' and the solvent 'd2o'. Below this, the 'Acquisition Options' section contains several parameters: 'Number of scans' is set to 64, 'NOE Mixing time' is 500 ms, and there is an unchecked checkbox for 'Run a mix=0 spectrum'. A section for selective frequencies includes a dropdown for 'No. of Selective Frequencies (max: 5)' set to 0, and input boxes for 'Selective Freq' (0.00 ppm) and 'Selective width' (10.0 Hz). An 'OR' checkbox for 'Autoestimate from spectrum' is also visible. The right side of the panel shows 'Observe: H1' and 'Decoupler: C13'.


Processing

Same processing as for standard PROTON spectrum, except that manual phasing may be required as the NOE peaks may be negative for smaller molecules. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~ 0.5 -1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- Choosing the NOESY1D experiment from a previously acquired PROTON data set allows for interactive selection of the peaks for NOE analysis, and the “Default” panel appears differently (see below). Place the cursor(s) on or around the peak and click . Repeat for additional peaks. Results are best if isolated peaks are chosen for correlation analysis. It is advisable not to make the widths of the selected peak too narrow as this creates shaped pulses with long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center avoids interference from nearby resonances.



- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation is confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time. In addition, the slope of such a build up curve can be used to determine bond distances between selected spin pairs (using methods such as PANIC).
- Molecules with multiple ring systems or a ring system combined with a long carbon chain may require different optimal mixing times for different parts of the molecule. In general, more flexible parts of the molecule require longer mixing times and more rigid, shorter.
- The NOESY1D contains zero quantum suppression element to suppress artifacts (typically antiphase in nature and interfering with spectral analysis), which is switched on by default.
- The sensitivity of the NOESY1D experiment is *not* equivalent to a PROTON, nor is an 8-scan NOESY1D equivalent to an 8-scan, 256-increment NOESY. Do not hesitate to acquire this experiment with a larger number of scans, particularly for weak/long-distance NOEs.
- When acquiring the NOESY1D with a larger number of scans, it is advisable to acquire a mixN=0 experiment with the *same number of scans* (using , to see peaks that appear from any potential pulse imperfections, so as not to mistake them for NOE correlations. The mixN=0 spectrum can also be used to confirm the selective excitation occurred as expected, verifying a result from the mixN0 spectrum with no observed NOE.
- Medium-sized molecules (MW ~700-1000 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOEs. Consider acquiring a ROESY1D for these molecules. Alternatively one may consider (i) choosing a more viscous solvent such as d₆-DMSO or d₆-DMSO with ~10-20% D₂O (this results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

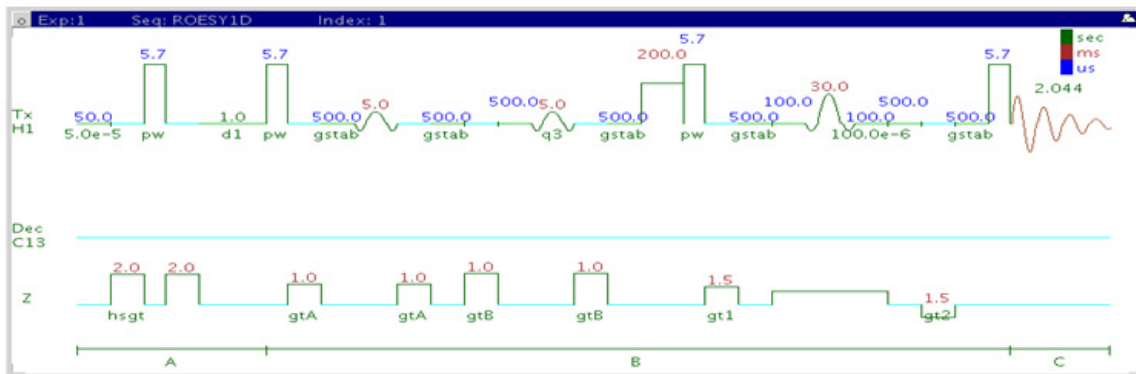
References:

S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

ROESY1D



General description and usage

Description:

A pulse sequence to acquire a 1D ROESY dataset with a selective pulse to obtain ROE information from a specific frequency.

Usage:

The ROESY1D experiment is an important tool for structure elucidation as it does not require through-bond coupling connectivity for correlations, rather the ROE (rotating frame Overhauser effect) interaction is seen between protons that are close in space (typically $<4 \text{ \AA}$). As such, the ROESY1D experiment is often used to help determine stereochemistry for rigid ring systems, or to confirm attachments between proximal but non-coupled portions of a molecule. The selective ROESY1D experiment has an advantage over the 2D ROESY version for simplifying complicated spectra with a lot of overlapping peaks. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for ROE analysis may be chosen interactively with the cursor. All parameters are set from the “Acquire” tab/“Default” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from “Start” tab/“Standard” panel
Spectral Width(sw)	Set from previously acquired PROTON*
Number of scans(nt)	Select typical choices from pull down menu*
SpinLock time	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms
Run a mix=0 spectrum	Check box to acquire a control spectrum with a mixing time of 0ms- creates an array
No., ppm, width of selective frequencies	Select number of frequencies from the drop down menu. Enter frequency position(s) and bandwidth(s) in the entry boxes
ZQ filter	Check box on the “Pulse Sequence” panel- zero quantum filter for artifact suppression. Strongly recommended- default is on.

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.


The screenshot displays the 'Acquisition Options' panel within the Agilent VnmrJ 4 software. The top menu bar includes 'Start', 'Acquire', 'Process', 'Show Time', 'Acquire', 'Stop', 'Sequence', 'Arrays', and 'Seq. Help'. The left sidebar lists various panels: 'Defaults', 'Acquisition', 'Pulse Sequence', 'PRESAT', 'WET', 'Channels', 'Flags', 'Future Actions', and 'Overview'. The main panel shows the following settings:

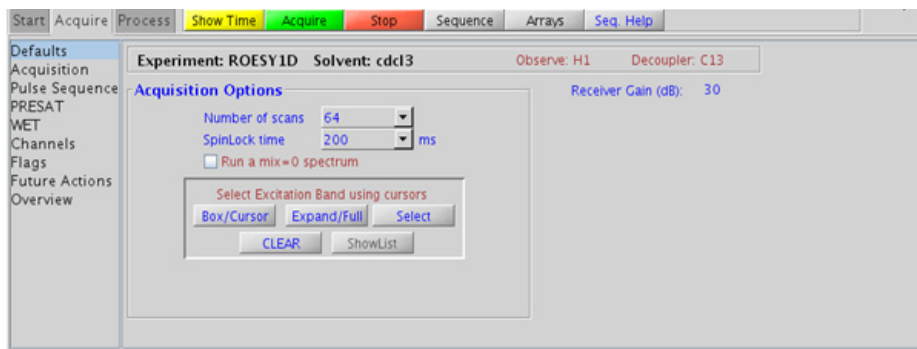
- Experiment:** ROESY1D
- Solvent:** d2o
- Observe:** H1
- Decoupler:** C13
- Receiver Gain (dB):** 30
- Acquisition Options:**
 - Number of scans:** 64 (dropdown)
 - SpinLock time:** 200 ms (dropdown)
 - ☐ Run a mix=0 spectrum
 - No. of Selective Frequencies (max: 5):** 0 (dropdown)
 - Selective Freq:** 0.00, 0.00, 0.00, 0.00, 0.00 ppm
 - Selective width:** 10.0, 0.0, 0.0, 0.0, 0.0 Hz
 - OR** ☐ Autoestimate from spectrum


Processing

Same processing as for standard PROTON spectrum, except that manual phasing may be required, as the ROE peaks are negative. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~ 0.5 -1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- Choosing the ROESY1D experiment from a previously acquired PROTON data set allows for interactive selection of the peaks for ROE analysis, and the “Default” panel appears differently (see below). Place the cursor(s) on or around the peak and click . Repeat for additional peaks. Results are best if the isolated peaks are chosen for correlation analysis. It is advisable not to make the widths of the selected peak too narrow as this creates shaped pulses with long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center avoids interference from nearby resonances.



- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.
- Because the mixing time for the ROESY1D is limited, weaker correlations may not be observable. The ROESY1D is the experiment of choice over NOESY1D for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOEs. One may consider running NOESY1D experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d₆-DMSO or d₆-DMSO with ~10-20% D₂O (this results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- The ROESY1D contains zero quantum suppression element to suppress artifacts (typically antiphase in nature and interfering with spectral analysis), which is switched on by default.
- The sensitivity of the ROESY1D experiment is *not* equivalent to a PROTON, nor is an 8-scan ROESY1D equivalent to an 8-scan, 256-increment ROESY. Do not hesitate to acquire this experiment with a larger number of scans, particular for weak/long-distance ROEs.
- When acquiring the ROESY1D with a larger number of scans, it is advisable to acquire a mixR=0 experiment with the same number of scans (using ) , to see peaks that appear from any potential pulse imperfections so as not to mistake them for ROE correlations. The mixR=0 spectrum can also be used to confirm the selective excitation occurred as expected, verifying a result from the mixR0 spectrum with no observed ROE.
- Sensitivity permitting, the slope of a ROESY1D buildup curve (by an array of mixing time) can be equally used as a NOESY1D buildup curve to determine bond distances between selected spin pairs. (using methods such as PANIC)

References:

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)

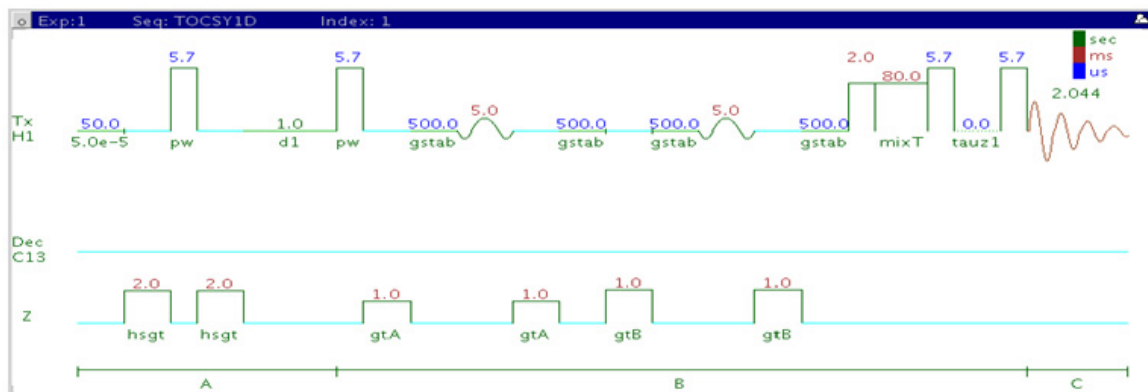
6 Selective 1D - (H)Sel1D

Tips, tricks, and next steps

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

TOCSY1D



General description and usage

Description:

A pulse sequence to acquire a 1D TOCSY dataset with a selective pulse to obtain coupling information from a specific frequency.

Usage:

The TOCSY1D experiment is an important tool for structure elucidation as it can show all protons in a chosen spin system. The selective TOCSY1D experiment has an advantage over the 2D TOCSY version for simplifying complicated spectra with invariably shorter total experiment time. If one proton in a given spin system is resolved, selecting this proton for TOCSY1D analysis produces an edited 1D spectrum containing only the resonances in this spin system. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for TOCSY1D analysis may be chosen interactively with the cursor. All parameters are set from the “Acquire” tab/”Default” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from “Start” tab/“Standard” panel
Spectral Width(sw)	Set from previously acquired PROTON*
Number of scans(nt)	Select typical choices from pull down menu*
SpinLock time	Select from pull down menu- 80ms works best for large spin systems*
Run a mix=0 spectrum	Check box to acquire a control spectrum with a mixing time of 0ms- creates an array
No., ppm, width of selective frequencies	Select number of frequencies from the drop down menu. Enter frequency positions bandwidth(s) in the entry boxes
SpinLock Pattern	Pull-down menu on the “Pulse Sequence” panel- MLEV17 or DIPSI2 works well for small molecules, clean MLEV17 is a good choice for large molecules

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.

Start Acquire Process Show Time Acquire Stop Sequence Arrays Seq. Help

Experiment: TOCSY1D Solvent: d2o Observe: H1 Decoupler: C13

Receiver Gain (dB): 30

Acquisition Options

Number of scans: 16

Spinlock time: 80 ms

☒ Run a mix=0 spectrum 8 scans

No. of Selective Frequencies (max: 5): 0

Selective Freq: 0.00 0.00 0.00 0.00 0.00 ppm

Selective width: 10.0 0.0 0.0 0.0 0.0 Hz

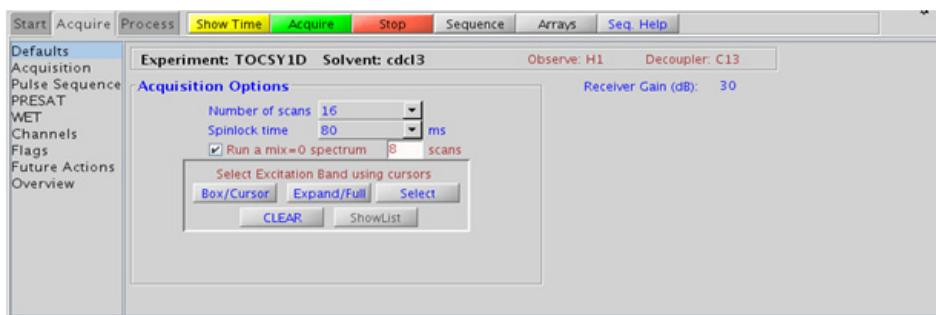
OR ☐ Autoestimate from spectrum

Processing


Same processing as for standard PROTON spectrum. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- Choosing the TOCSY1D experiment from a previously acquired PROTON data set allows for interactive selection of the peaks for analysis, and the “Default” panel appears differently (see below). Place the cursor(s) on or around the peak and click [Select](#). Repeat for additional peaks. Results are best if the more isolated peaks are chosen for correlation analysis. It is advisable not to make the widths of the selected peak too narrow as this creates shaped pulses with long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center prevents interference from nearby resonances.



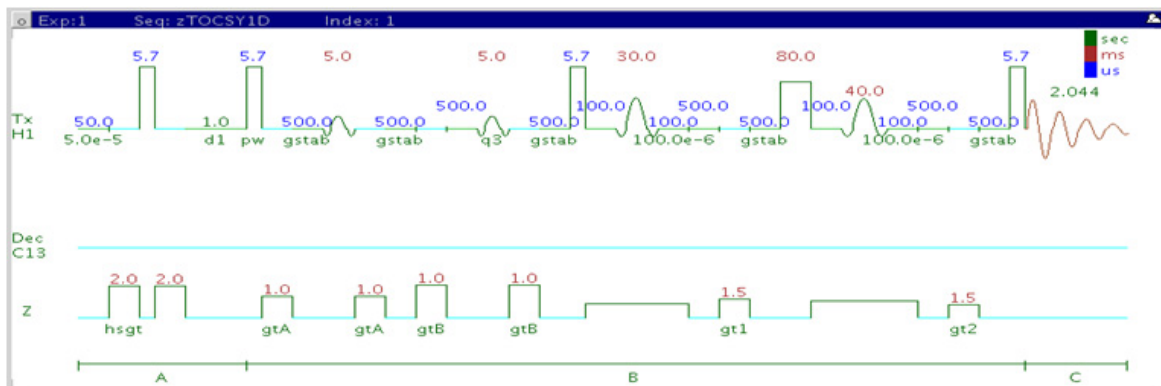
- The zTOCSY1D experiment contains a zero-quantum filter to suppress artifacts and often gives better results than the TOCSY1D.

- The spinlock time ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings (such as for a COSY), select a shorter spinlock time, for example, 30ms or less.
- The MLEV17 and DIPSI2 spinlock patterns are good choices for small molecules and the DIPSI3 and clean MLEV for larger molecules.
- The sensitivity of the TOCSY1D experiment is not equivalent to a PROTON, nor is an 8-scan TOCSY1D equivalent to an 8-scan, 256-increment TOCSY. Do not hesitate to acquire this experiment with a larger number of scans, particular for weak couplings or extended spin systems.
- When acquiring the TOCSY1D with a larger number of scans, it is advisable to acquire a mixT=0 experiment, to see peaks that appear from any potential pulse imperfections, so as not to mistake them for TOCSY correlations. The mixT=0 spectrum can also be used to confirm the selective excitation occurred as expected, verifying a result from the mixT0 spectrum with no observed TOCSY correlation.
- It is also advisable to run a mixT=0 “reference” spectrum (using , when TOCSY1D is acquired with longer spinlock mixing time. Unlike typical NOESY1D or ROESY1D, the selected resonance (i.e., the originally selected peak) may have intensity similar to the TOCSY peak and hence may not be obviously discernable.

References:

- A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)
- J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)
- K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

zTOCSY1D



General description and usage

Description:

A pulse sequence to acquire a 1D zTOCSY dataset with a selective pulse to obtain coupling information from a specific frequency.

Usage:

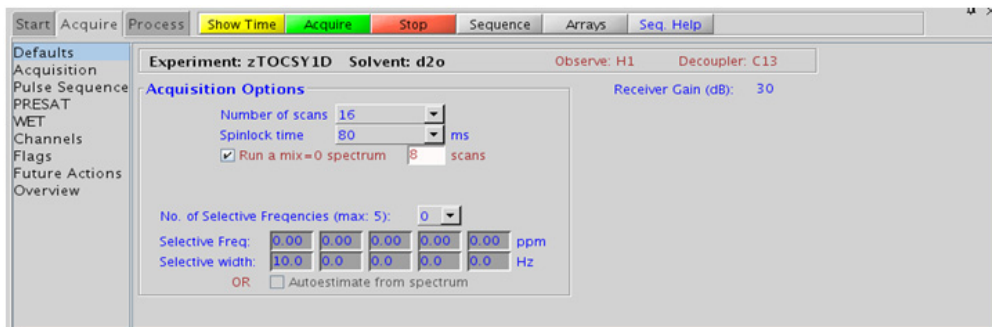
The zTOCSY1D experiment is an important tool for structure elucidation as it can show all protons in a chosen spin system. The selective zTOCSY1D experiment has an advantage over the 2D zTOCSY version for simplifying complicated spectra with a lot of overlapping peaks with invariably shorter total experiment time. If one proton in a given spin system is resolved, selecting this proton for zTOCSY1D analysis produces an edited 1D spectrum containing only the resonances in this spin system. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for zTOCSY1D analysis may be chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from "Start" tab/"Standard" panel
Spectral Width(sw)	Set from previously acquired PROTON*
Number of scans(nt)	Select typical choices from pull down menu*
SpinLock time	Select from pull down menu- 80ms works best for large spin systems*
Run a mix=0 spectrum	Check box to acquire a control spectrum with a mixing time of 0ms- creates an array
No., ppm, width of selective frequencies	Select number of frequencies from the drop down menu. Enter frequency position(s) and bandwidth(s) in the entry boxes
SpinLock Pattern	Pull-down menu on the "Pulse Sequence" panel- DIPS12 works well for small molecules, DIPS13 is a good choice for large molecules

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

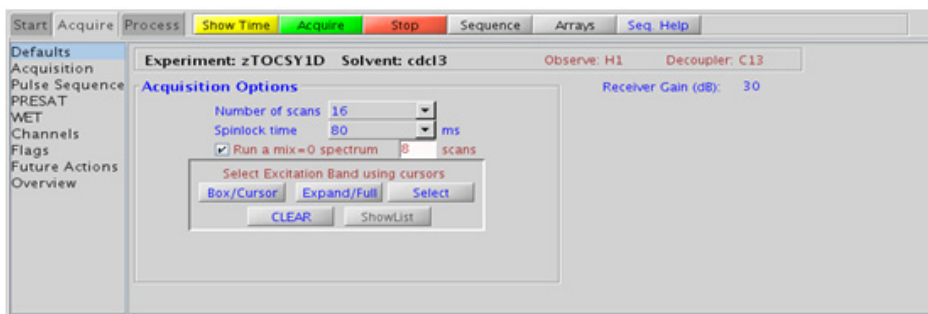


Processing


Same processing as for standard PROTON spectrum. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- Choosing the zTOCSY1D experiment from a previously acquired PROTON data set allows for interactive selection of the peaks for analysis, and the “Default” panel appears differently (see below). Place the cursor(s) on or around the peak and click **Select**. Repeat for additional peaks. Results are best if the more isolated peaks are chosen for correlation analysis. It is advisable not to keep the widths of the selected peak too narrow as it creates shaped pulses with long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center prevents interference from nearby resonances.



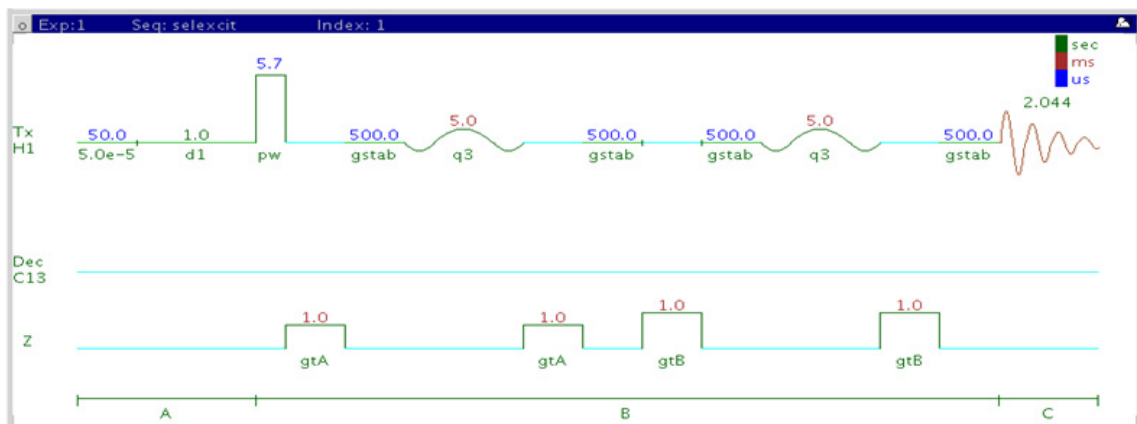
- The zTOCSY1D experiment contains a zero-quantum filter to suppress artifacts and often gives better results than TOCSY1D.

- The spinlock time ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings (such as for a COSY), select a shorter spinlock time, for example, 30ms or less.
- The DIPSI2 spinlock pattern is a good choice for small molecules and the DIPSI3 for larger molecules.
- The sensitivity of the zTOCSY1D experiment is not equivalent to a PROTON, nor is an 8-scan zTOCSY1D equivalent to an 8-scan, 256-increment zTOCSY. Do not hesitate to acquire this experiment with a larger number of scans, particularly for weak couplings or extended spin systems.
- When acquiring the zTOCSY1D with a larger number of scans, it is advisable to acquire a mixT=0 experiment, to see peaks that appear from any potential pulse imperfections, so as not to mistake them for TOCSY correlations. The mixT=0 spectrum can also be used to confirm the selective excitation occurred as expected, verifying a result from the mixT0 spectrum with no observed TOCSY correlation.
- It is also advisable to run a mixT=0 “reference” spectrum (using , when TOCSY1D is acquired with longer spinlock mixing time. Unlike typical NOESY1D or ROESY1D, the selected resonance (i.e., the originally selected peak) may have intensity similar to the TOCSY peak and hence may not be obviously discernable.

References:

- A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)
- J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)
- K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)
- M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

selexcit



General description and usage

Description:

A pulse sequence to acquire a 1D ^1H dataset with a selective pulse to excite only a portion of the spectrum.

Usage:

The selexcit experiment is most commonly used to set up band-selective 2D experiments (see Chapter 7). It can be used, however, to edit the proton spectrum and improve the dynamic range, when there may be large amounts of impurities and/or solvents signals. An example may be a sample from a biological matrix such as bile, which may have large signals in the aliphatic region and it may be desirable to selectively excite the aromatic region of the spectrum only. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for selective excitation are known, and may be chosen interactively with the cursor. All parameters are set from the “Acquire” tab/“Default” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent	Set from “Start” tab/“Standard” panel
Spectral Width (sw)	Set from previously acquired proton*
Number of scans	Select typical choices from pull down menu*
No., ppm, width of selective frequencies	Select number of frequencies from the drop down menu. Enter frequency position(s) and bandwidth(s) in the entry boxes

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.


The screenshot shows the 'Acquisition Options' panel in the Agilent VnmrJ 4 software. The top bar includes tabs for Start, Acquire, Process, Show Time, Acquire, Stop, Sequence, Arrays, and Seq. Help. The main panel displays the following settings:

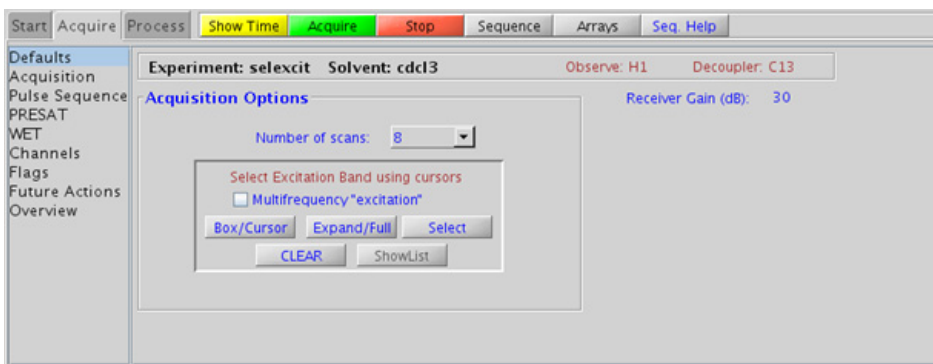
- Experiment: selexcit Solvent: d2o
- Observe: H1 Decoupler: C13
- Receiver Gain (dB): 30
- Number of scans: 8 (dropdown menu)
- No. of Selective Frequencies (max: 5): 0 (dropdown menu)
- Selective Freq: 0.00 0.00 0.00 0.00 0.00 ppm
- Selective width: 10.0 0.0 0.0 0.0 0.0 Hz
- OR ☐ Autoestimate from spectrum

Processing

Same processing as for standard PROTON spectrum. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- Choosing the selexcit experiment from a previously acquired PROTON data set allows for interactive selection of the peaks for analysis, and the “Default” panel appears differently (see below). Place the cursor(s) on or around the peak and click . Repeat for additional peaks. Results are best if the more isolated peaks are chosen for correlation analysis. It is advisable not to make the widths of the selected peak too narrow as this creates shaped pulses of long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center prevents interference from nearby resonances from being selected.

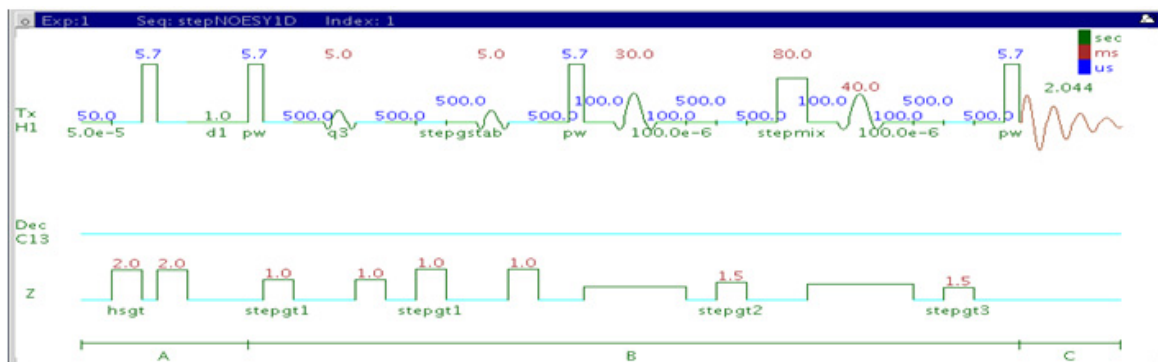


- The selexcit experiment has a check option to do multifrequency excitation. Choosing multiple bands (interactively from the PROTON spectrum) with this check box “ON” results in a single spectrum wherein all of the selected bands are included. However, selection of multiple bands with this check box “OFF” results in multiple spectra each with a single selected band.
- The sensitivity of the selexcit experiment is usually not equivalent to a PROTON, due to some potential loss from the selective pulse.
- Setting up the desired regions from a selexcit experiment is the easiest way to configure a band-selective 2D experiment (see Chapter 7).

References:

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

stepNOESY1D



General description and usage

Description:

A pulse sequence to acquire a 1D TOCSYNOESY dataset with a selective pulse to obtain TOCSYNOESY information from a specific frequency. It is effectively a 1D equivalent of a 3D experiment.

Usage:

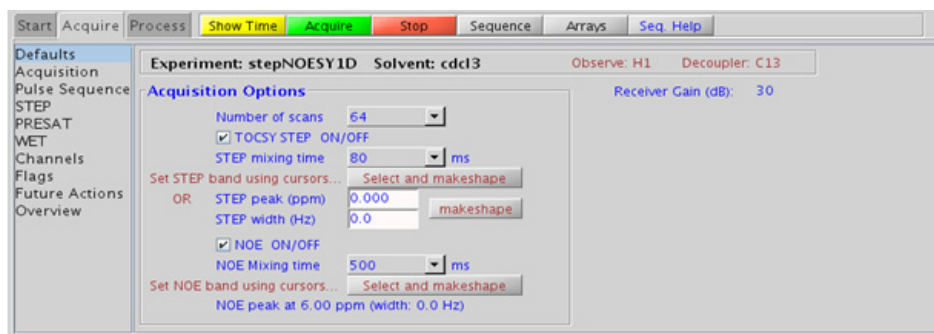
The stepNOESY1D experiment first acquires a TOCSY1D on a selected peak from a PROTON spectrum, and then performs a NOESY1D on one of the TOCSY correlations. This experiment, while somewhat insensitive, can be helpful to simplify and obtain subspectra of impure samples and/or complex spectra when there is one resolved peak from the desired molecule of interest. This is a unique method to obtain NOE information from completely obscured protons in a complex molecule. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies for the TOCSY and subsequent NOE analysis are chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from “Start” tab/“Standard” panel
Spectral Width(sw)	Set from previously acquired PROTON*
Number of scans(nt)	Select typical choices from pull down menu*
TOCSY STEP ON/OFF	Check box to turn off TOCSY to set up STEP band (turn off NOE also); turn on for analysis
STEP mixing time	Select from pull down menu- 80ms works best for large spin systems*
Set STEP band, STEP peak (ppm), width (Hz)	Select desired TOCSY correlation with cursor type in ppm/width, click “Select and makeshape” button
NOE ON/OFF	Check box to turn off NOE to set up NOE band; turn on for analysis
NOE mixing time	Select from pull down menu- 500ms works best well for most small molecules*
Set NOE band	Select desired TOCSY correlation with cursors, click “Select and makeshape” button

NOTE



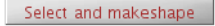
You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Same processing as for standard PROTON spectrum, except that manual phasing may be required as the NOE peaks may be negative for smaller molecules. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the “Process” tab on the “Weighting” panel.

Tips, tricks, and next steps

- The STEP band for the TOCSY may be selected from a previously acquired PROTON dataset, or a quick stepNOESY1D experiment with the TOCSY STEP and NOE off can be used to acquire a quick sample spectrum. Place the cursor(s) on or around the peak to be selected and click . Alternatively, the frequency (ppm) and width (Hz) for the STEP peak may be entered in the boxes provided and  clicked. The NOE band may also be selected from a previously acquired TOCSY1D or zTOCSY1D spectrum. Alternately, a quick stepNOESY1D experiment with the TOCSY STEP on and NOE off can be acquired and used for NOE band selection. Place the cursor(s) on or around the peak to be selected and click . It is advisable to not keep the widths of the selected peaks too narrow, as it creates shaped pulses with long durations, which can, in turn, result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center prevents interference from nearby resonances.
- Use spinlock (STEP mixing time) durations exceeding 150ms with caution as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. A STEP mixing time of 30-80ms is typical for most small molecules, depending on the length of the coupling network and the size of the intervening coupling constants. An 80ms STEP mixing time is a good choice for molecules with extended spin systems.

- The TOCSY spinlock pattern can be selected from the “STEP” tab. The DIPSI2 spinlock pattern is a good choice for small molecules and the DIPSI3 is typically used for larger molecules.
- A mixing time for the NOE of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. As in NOESY1D, the NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time. In addition, the slope of such a build up curve can be used to determine bond distances between selected spin pairs (using methods such as PANIC).
- The sensitivity of the stepNOESY1D experiment is quite low, as it is essentially a 1D equivalent of a 3D experiment, which typically takes a long time to acquire. Do not hesitate to acquire this experiment with a larger number of scans. It may also be helpful to optimize the STEP and NOE mixing times with TOCSY1D and NOESY1D experiments before acquiring the stepNOESY1D.

References:

H. Hu, S. Bradley, K. Krishnamurthy, J. Magn. Reson., 171 (2004), 201 (STEPNOESY1D)

S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

6 Selective 1D - (H)Sel1D

Tips, tricks, and next steps



7 Selective 2D - Sel2D

Sel2D Tab	250
(HC)bsHSQCAD	252
(HC)bsgHSQCAD	256
(HC)bsgHMBC	260
(HC)EXSIDE	264
(HC)bsHSQCNOESY	268
(HC)bsHSQCROESY	274
(HH)bsROESY	280
(HH)bsTOCSY	284
(HH)bsNOESY	288



Sel2D Tab

The experiments in the “Sel2D tab” are F1 band-selected 2D experiments. Band-selection in the F1 dimension saves time and improves resolution in any 2D experiment by reducing the number of increments needed in that dimension, while allowing the operator to focus on the correlation region of interest. In the heteronuclear experiments this can be especially useful for resolving nearly-degenerate ^1H - ^{13}C resonances into unambiguous correlations. With homonuclear spectra it can shed light, for example, into a crowded aromatic region of a NOESY. Such high resolution in the traditional (broadband version) experiment can only be obtained at the cost a very high number of increments (and hence spectrometer time).

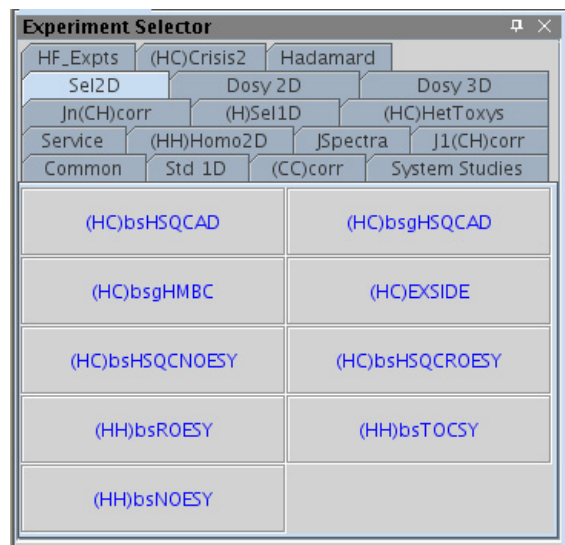
The homonuclear experiments include (HH)bsROESY, (HH)bsTOCSY, and (HH)bsNOESY. All of these experiments are setup either from a PROTON spectrum, a selexcit experiment (selective 1D), or by simple entry of the desired F1 region in the setup panels. All necessary selective pulses are created automatically and the parameters set appropriately. The homonuclear experiments support the option for broadband homonuclear decoupling in the F1 dimension, thereby further improving resolution.

The heteronuclear experiments are proton-carbon by default, with ^{13}C as the band-selected dimension. If the selective 2D experiment is setup from a traditional 2D data set of the same type, for example, HSQC or HMBC, the operator can choose the band-selected frequencies with cursors from the existing spectra. Alternatively, the desired F1 window can be chosen by entering the desired region into the parameter entries. All required shaped pulses are created automatically.

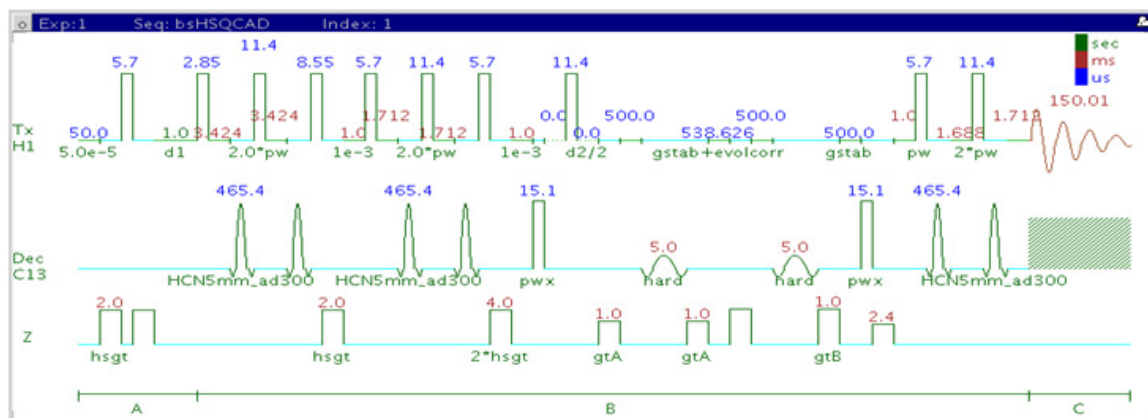
There are two experiments that only appear in the Sel2D tab, (HC)bsHSQCNOESY and (HC)bsHSQCROESY. Because the F1 window can be customized, the HSQCNOESY/ROESY is available for a wide range of structural problems. The information conveyed is similar to the HetToxy family of experiments, for example, “proton A has NOE to a proton B, whose carbon is at that chemical shift”, but with the potential of added resolution for the carbon shifts.

The (HC)EXSIDE is an experiment that provides a method to measure long-range H/C coupling constants. Typically the (HC)EXSIDE is setup from a selexcit experiment, where the multi-band option is used to select

all desired protons, taking care to exclude pairs from the same spin system. The coupling constants are seen as in-phase pairs in the F1 dimension, scaled up by a J-scaling parameter.



(HC)bsHSQCAD



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses and band-selection in F1.

Usage:

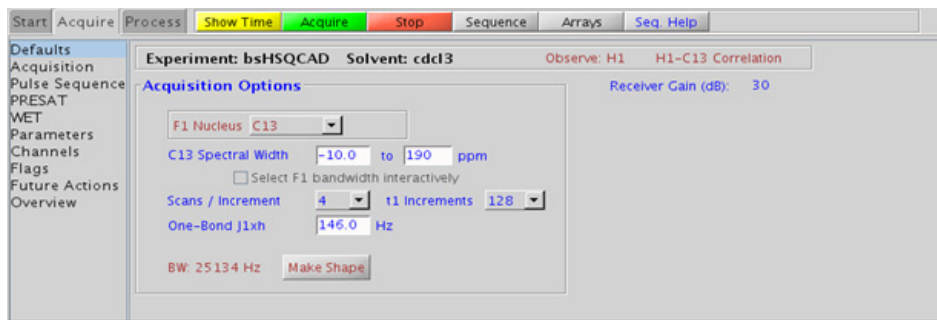
The (HC)bsHSQCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to directly attached carbons in a band-selected region along F1. Using band-selection is an efficient way to increase the resolution in the F1 carbon dimension without adding large numbers of increments and, hence, experiment time. The selected band can either be typed in or chosen interactively from a previously acquired HSQC-type experiment. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired HSQC-type experiment
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ - 146 Hz works well for many carbons
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- check box to turn on/off TANGO-Gradient ^{12}C suppression (default is on- highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

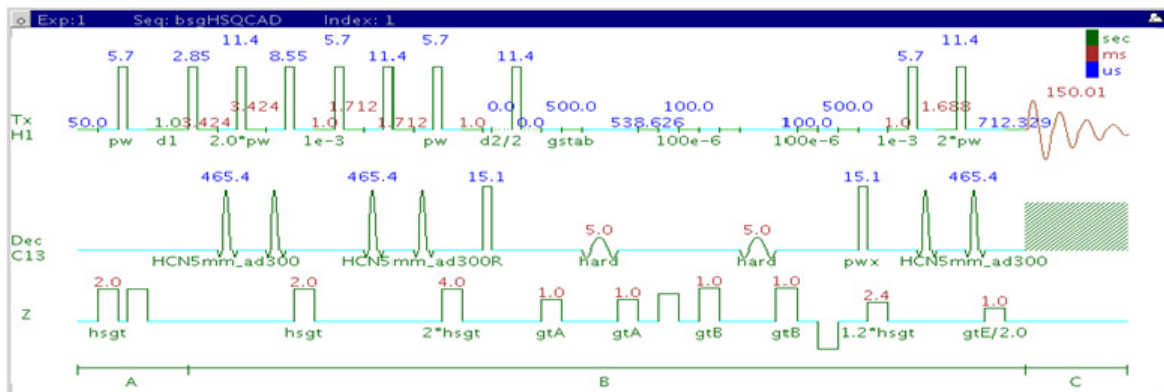
- Spectral width (sw) setting transfers from a PROTON if the (HC)bsHSQCAD is set up from this experiment. All HSQC-related parameters transfer if the experiment is set up from a previously acquired HSQC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.

- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing `dm='nnn'` on the command line.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The (HC)bsHSQCAD experiment has a sensitivity advantage (factor of ~ 1.4) over the (HC)bsgHSQCAD, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart.

References:

- G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189.
(HSQC)
- E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256.
(Adiabatic Pulses)
- C. Gaillet, C. Liqueur, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139 (1999) 454-459. (Band Selective HSQC/HMBC)

(HC)bsgHSQCAD



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses, gradient coherence selection, and band-selection in F1.

Usage:

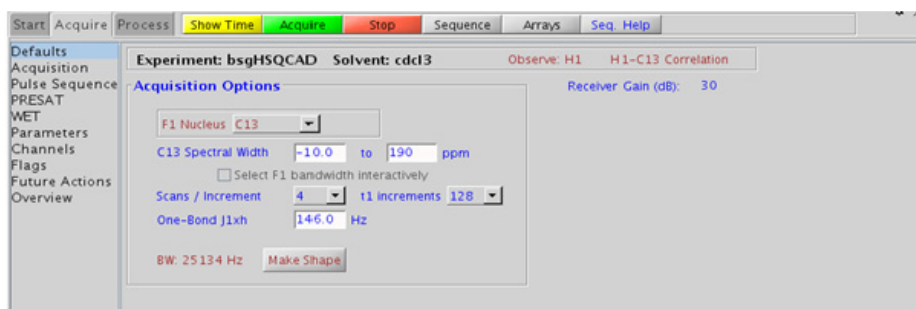
The (HC)bsgHSQCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to directly attached carbons in a band-selected region along F1. Using band-selection is an efficient way to increase the resolution in the F1 carbon dimension without adding large numbers of increments and, hence, experiment time. The selected band can either be typed in or chosen interactively from a previously acquired HSQC-type experiment. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired gHSQCAD
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ - 146 Hz works well for many carbons
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from “Pulse Sequence” panel- check box to turn on/off TANGO-Gradient ^{12}C suppression (default is on- highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the (HC)bsgHSQCAD is set up from this experiment. All HSQC-related parameters transfer if the experiment is set up from a previously acquired HSQC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing `dm='nnn'` on the command line.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The (HC)bsHSQCAD experiment has a sensitivity advantage (factor of ~ 1.4) over the (HC)bsgHSQCAD, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart.

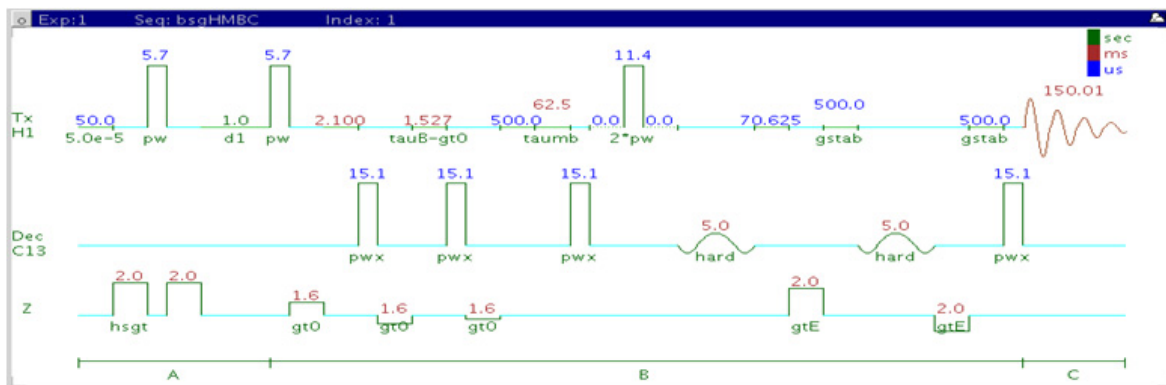
References:

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189.
(HSQC)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256.
(Adiabatic Pulses)

C. Gaillet, C. Liqueur, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139
(1999) 454-459. (Band Selective HSQC/HMBC)

(HC)bsgHMBC



General description and usage

Description:

Two Dimensional heteronuclear multiple-bond J-correlation spectroscopy with gradient coherence selection and band-selection in F1.

Usage:

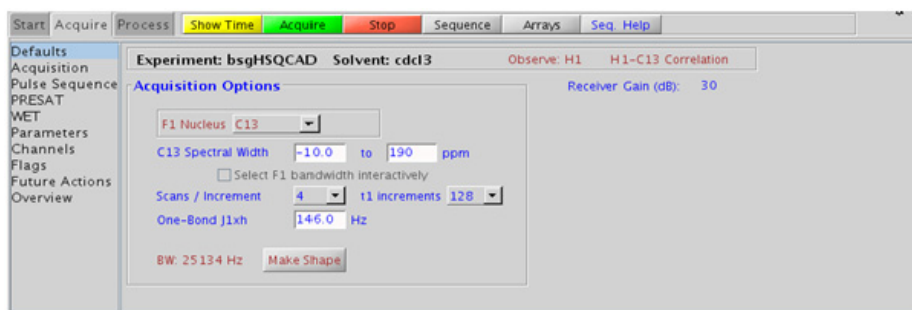
The (HC)bsgHMBC experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons in a band-selected region along F1, usually over 2-3 bonds with the 3-bond correlations typically being stronger. Using band-selection is an efficient way to increase the resolution in the F1 carbon dimension without adding large numbers of increments and, hence, experiment time. The selected band can either be typed in or chosen interactively from a previously acquired HSQC- or HMBC-type data set. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired (CH) correlation experiment
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jn _{xh} (jnxh)	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$ - 8 Hz works well for most molecules
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. This arrangement allows for the best resolution and sensitivity combination.

Tips, tricks, and next steps

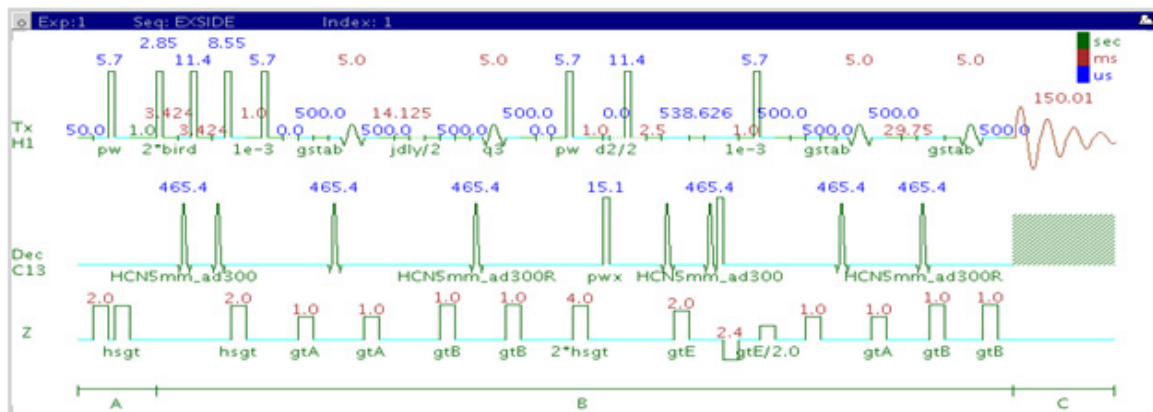
- Spectral width (sw) setting transfers from a PROTON if the (HC)bsgHMBC is set up from this experiment. All HMBC-related parameters transfer if the experiment is set up from a previously acquired HMBC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the “Pulse Sequence” panel around which one-bond couplings are eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.
- The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity.

References:

- A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)
- E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)
- C. Gaillet, C. Liqueur, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139 (1999) 454-459. (Band Selective HSQC/HMBC)

(HC)EXSIDE



General description and usage

Description:

Two Dimensional band-selective HSQC-type experiment for measuring long-range ^1H - ^{13}C coupling constants.

Usage:

The (HC)EXSIDE experiment is used primarily to obtain information about long-range ^1H - ^{13}C coupling constants. Setup is from a previously acquired selexcit experiment. The data appears as a 2D 1-bond ^1H - ^{13}C correlation with proton chemical shifts in F2 and carbon in F1. The long-range ^1H - ^{13}C coupling constants are measured from in-phase pairs in the F1 dimension scaled up by the j-scaling factor. The specific advantage of (HC)EXSIDE in measuring long-range coupling constants is that (i) they are measured along F1 as doublets with the peak separation scaled by a user definable scaling factor and (ii) only the heteronuclear active coupling is observed without any interference from ^1H - ^1H homonuclear couplings. Proton-carbon coupling constants are often used to help define

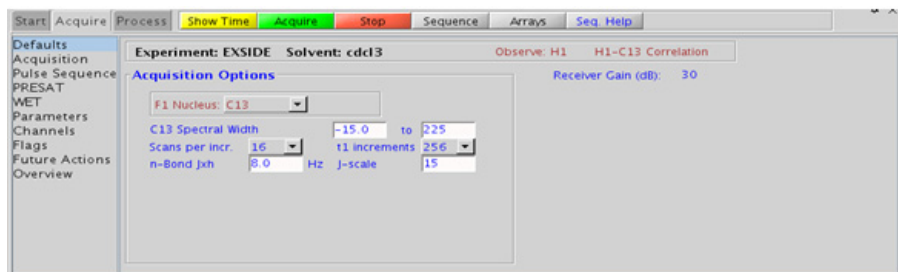
e/z stereochemistry of double bonds or molecular conformations, for example, the torsion angles of peptides. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired selexcit
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
n-Bond jxh (jnxh)	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$ - 8 Hz works well for most molecules
J-scale (jscale)	Scaling factor for the coupling constants in F1
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

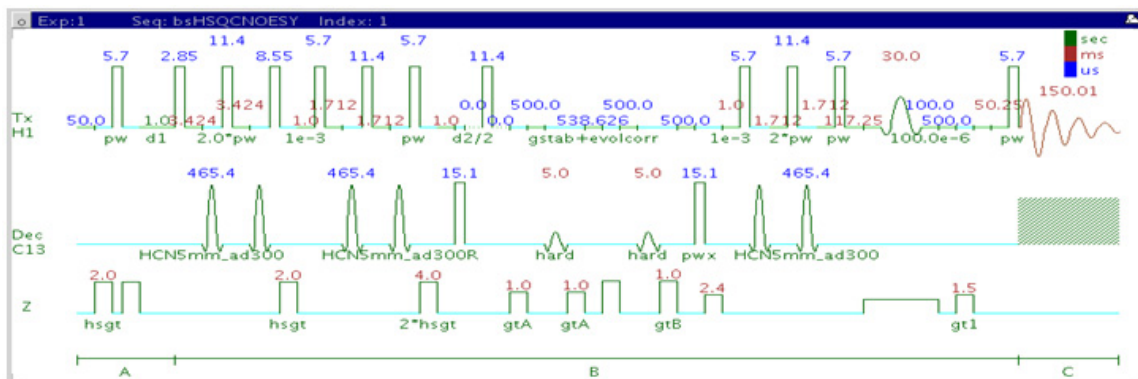
- This experiment is setup from a previously acquired selexcit experiment. Select the multi-band option and choose all desired protons, taking care to select only protons which do not share a mutual homonuclear coupling. Ideally, a COSY-type experiment is acquired and used to guide selection of resonances (not coupled to each other) for the selexcit. If heteronuclear coupling constants need to be determined from two mutually coupled protons, two EXSIDE experiments must be acquired. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.

- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The jnxh parameter sets the optimal value of the proton-carbon coupling constant for detection.
- Proton-carbon long range coupling constants are often used to help define *e/z* stereochemistry of double bonds or molecular conformations, for example, the torsion angles of peptides.

References:

V. V. Krishnamurthy, J. Magn. Reson. Ser. A121 (1996) 33-41. (EXSIDE)

(HC)bsHSQCNOESY



General description and usage

Description:

Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with the addition of a noesy mixing time. Contains adiabatic 180° X-nuclei pulses and band-selection in F1.

Usage:

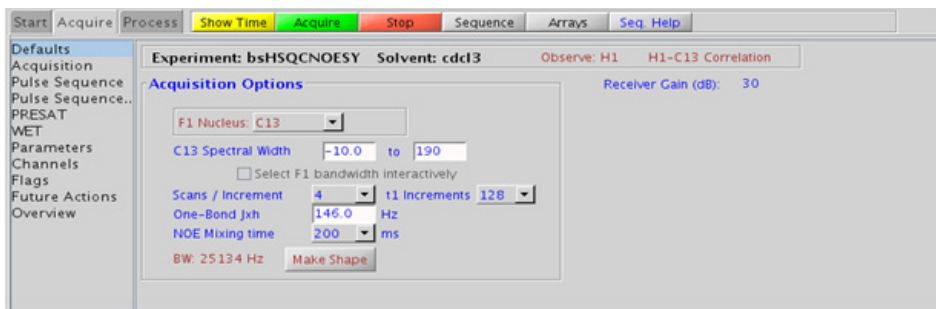
The (HC)bsHSQCNOESY experiment is used to acquire a 2-dimensional spectrum to collect information correlating NOE and carbon chemical shift information, for example, Proton A has an NOE to proton B at carbon chemical shift X. The band-selection along the F1 dimension gives the (HC)bsHSQCNOESY an advantage of better resolution than a non-selective HSQCNOESY. The selected band can either be typed in or chosen interactively from a previously acquired HSQC-type spectrum. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired gHSQCAD
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
NOE Mixing time (mixN)	Select from pull down menu- 500ms works well as a starting point*
Steady State (ss)	Set from second "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the (HC)bsHSQCNOESY is set up from this experiment. All HSQC-related parameters transfer if the experiment is set up from a previously acquired HSQC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.

- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time.
- Medium-sized molecules (MW ~500 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOEs. Consider acquiring a (HC)bsHSQCROESY for these molecules. Alternatively one may consider, (i) choosing a more viscous solvent such as d₆-DMSO or d₆-DMSO with ~10-20% D₂O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- Small molecules may have crosspeaks arising from chemical exchange during the NOE mixing time and have the same phase as the diagonal. The exchange crosspeaks provide information about inter-converting molecular conformations and can be used to recognize such molecular dynamic processes.
- (HC)bsHSQCNOESY spectra may sometimes contain signals with partial antiphase character. Zero quantum suppression is embedded (and switched on by default) into the (HC)bsHSQCNOESY pulse sequence to suppress such artifacts.

References:

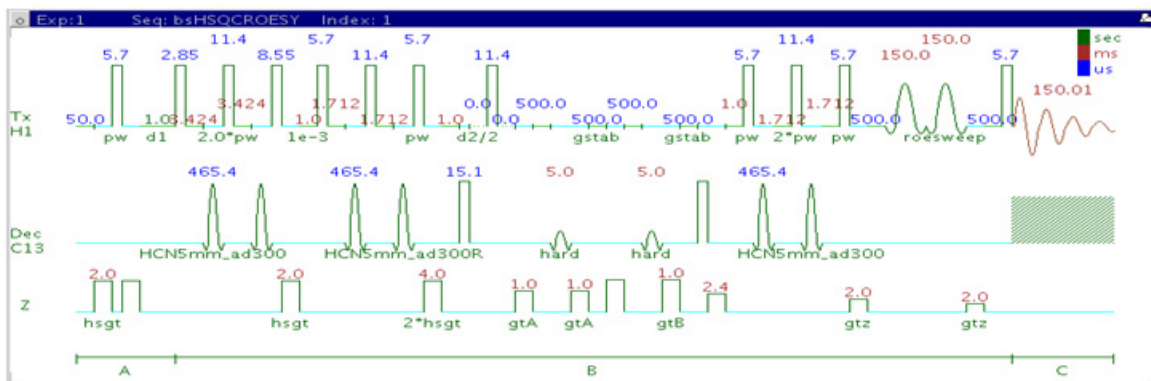
S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

C. Gaillet, C. Liqueur, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139 (1999) 454-459. (Band Selective HSQC/HMBC)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

(HC)bsHSQCROESY



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with the addition of a roesy mixing time. Contains adiabatic 180° X-nuclei pulses and band-selection in F1.

Usage:

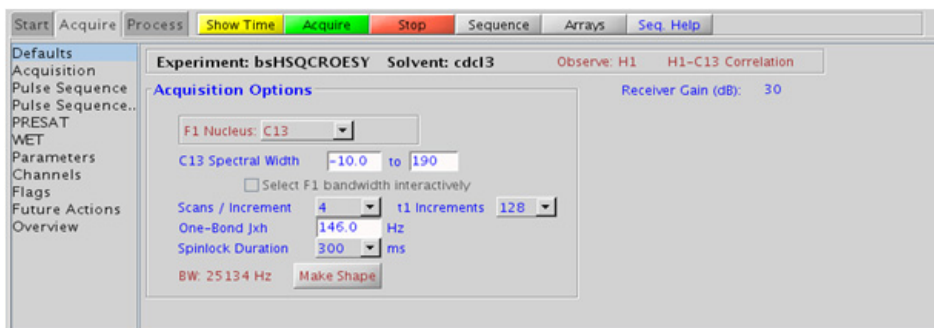
The (HC)bsHSQCROESY experiment is used to acquire a 2-dimensional spectrum to collect information correlating NOE and carbon chemical shift information, for example, Proton A has an ROE to proton B at carbon chemical shift X. The band-selection along F1 dimension gives the (HC)bsHSQCROESY an advantage of a better resolution than a non-selective HSQCROESY. The selected band can either be typed in or chosen interactively from a previously acquired HSQC-type spectrum. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired gHSQCAD
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ - 146 Hz works well for many carbons
Spinlock Duration	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms
Steady State (ss)	Set from second "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the (HC)bsHSQCROESY is set up from this experiment. All HSQC-related parameters transfer if the experiment is set up from a previously acquired HSQC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.

- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.
- Because the mixing time for the (HC)bsHSQCROESY is limited, weaker correlations may not be observable. (HC)bsHSQCROESY is the experiment of choice over (HC)bsHSQCNOESY for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOEs. One may consider running (HC)bsHSQCNOESY experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d₆-DMSO or d₆-DMSO with ~10-20% D₂O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- Crosspeaks in the (HC)bsHSQCROESY spectrum that have the same phase as the diagonal may arise from chemical exchange during the ROE mixing time, or due to TOCSY-type correlations (couplings). The exchange crosspeaks provide information about inter-converting molecular conformations and can be used to recognize such molecular dynamic processes.

References:

- A. Bax, D. G. Davies, J. Magn. Reson. 63 (1985) 207-213. (ROESY)
- E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

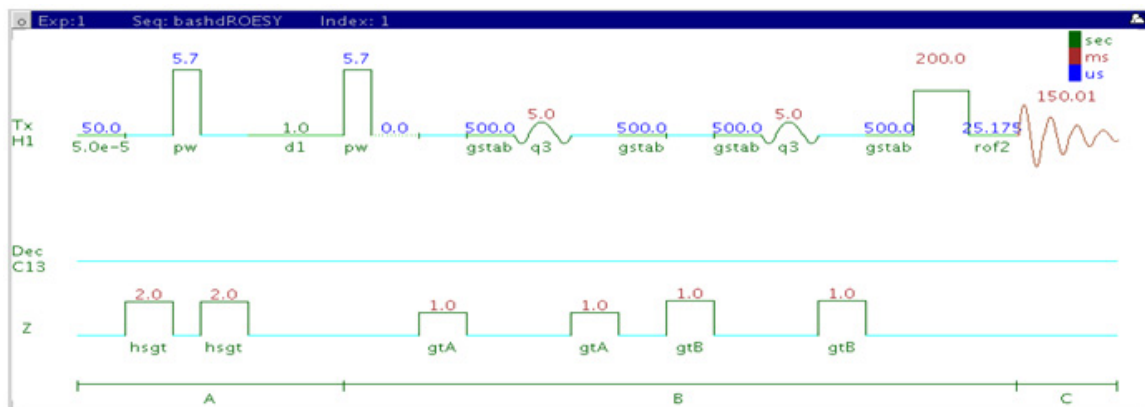
7 Selective 2D - Sel2D

Tips, tricks, and next steps

C. Gaillet, C. Lique, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139 (1999) 454-459. (Band Selective HSQC/HMBC)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

(HH)bsROESY



General description and usage

Description:

Two Dimensional through-space correlation spectroscopy in the rotating frame with band-selection in F1.

Usage:

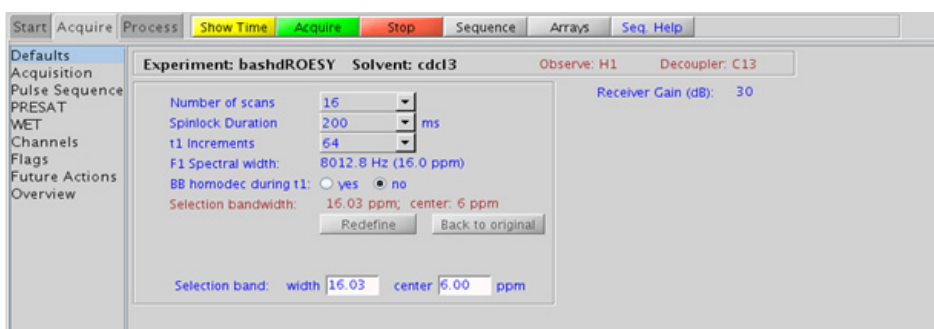
This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically $<4 \text{ \AA}$). This experiment has an added feature above the ROESY of band-selection in F1, which is an efficient way to increase the resolution in the F1 dimension without adding a large number of increments and, hence, experiment time. There is an added experimental option for homonuclear decoupling in F1 to further improve the resolution. The (HH)bsROESY can be very useful to obtain high-resolution through-space correlations to an overlapped area of the spectrum, for example the α -proton region of peptides. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Relaxation time (d1)	Set from “Acquisition” panel, controls the delay between scans
Number of scans (nt)	Select typical choices from pull down menu*
Spinlock Duration	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
BB homodec during t1 (homodec)	Select yes/no for homonuclear decoupling in F1
Selection bandwidth	Set from previously acquired selexcit or type in specific values
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment, except for minor manual phasing changes that may be required, as the ROE peaks are negative. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the F₂ data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F₂/F₁ are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

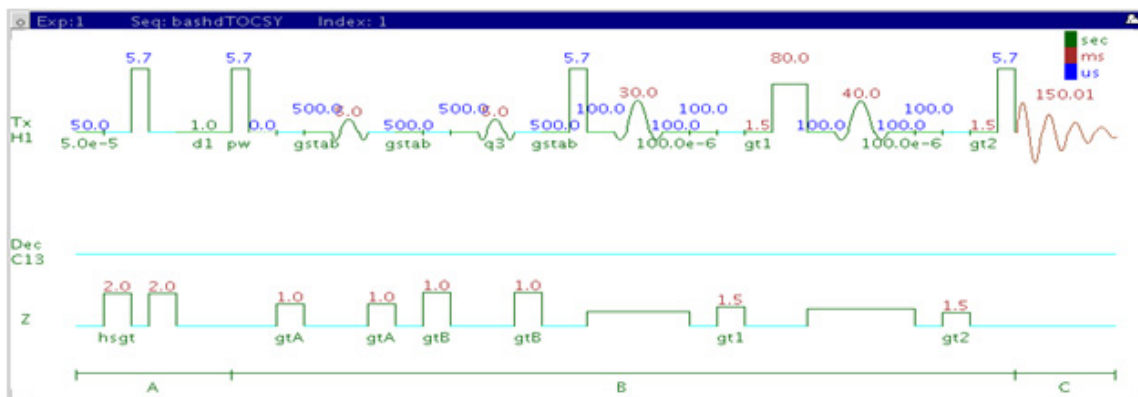
- Spectral width (sw) setting transfers from a PROTON if the (HH)bsROESY is set up from this experiment. The F₁ band is then selected interactively. Optimized selected bandwidth, sw, and receiver gain settings transfer from a selexcit if the (HH)bsROESY is set up from this experiment.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- The homonuclear decoupling option (homodec) along F₁ necessitates that the selected resonances do not have mutual homonuclear coupling among themselves.

- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.
- Because the mixing time for the (HH)bsROESY is limited, weaker correlations may not be observable. (HH)bsROESY is the experiment of choice over (HH)bsNOESY for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOEs. One may consider running (HH)bsNOESY experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d₆-DMSO or d₆-DMSO with ~10-20% D₂O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- Crosspeaks in the (HH)bsROESY spectrum that have the same phase as the diagonal may arise from chemical exchange during the ROE mixing time, or due to TOCSY-type correlations (couplings). The exchange crosspeaks provide information about inter-converting molecular conformations and can be used to recognize such molecular dynamic processes.

References:

- A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)
- T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc 114 (1992) 3157-3159. (EASY-ROESY)
- A. Kaerner, D. L. Rabenstein, Magn. Reson. Chem. 36 (1998) 601-607. (Band Selective ROESY)

(HH)bsTOCSY



General description and usage

Description:

Two Dimensional total J-correlation spectroscopy for scalar coupled spin systems with a zero-quantum filter for artifact suppression and band-selection in F1.

Usage:

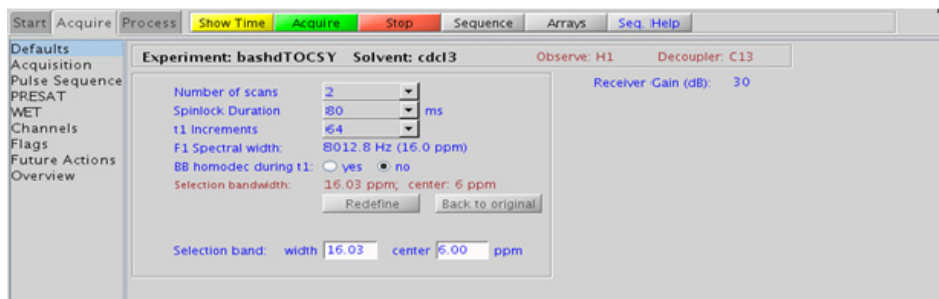
This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between several protons in a scalar coupled spin system. This experiment has an added feature above the zTOCSY of band-selection in F1, which is an efficient way to increase the resolution in the F1 dimension without adding a large numbers of increments and, hence, experiment time. There is an added experimental option for homonuclear decoupling in F1 to further improve the resolution. The (HH)bsTOCSY can be very useful to obtain high-resolution through-bond correlations to a overlapped area of the spectrum, for example the α -proton region of peptides. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Relaxation time (d1)	Set from "Acquisition" panel, controls the delay between scans
Number of scans (nt)	Select typical choices from pull down menu*
Spinlock Duration (mixT)	Select from pull down menu- 80ms works best for large spin systems*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
BB homodec during t1 (homodec)	Select yes/no for homonuclear decoupling in F1
Selection bandwidth	Set from previously acquired selexcit or type in specific values
Spinlock Pattern	Pull-down menu on the "Pulse Sequence" panel- dipsi2 or dipsi3
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

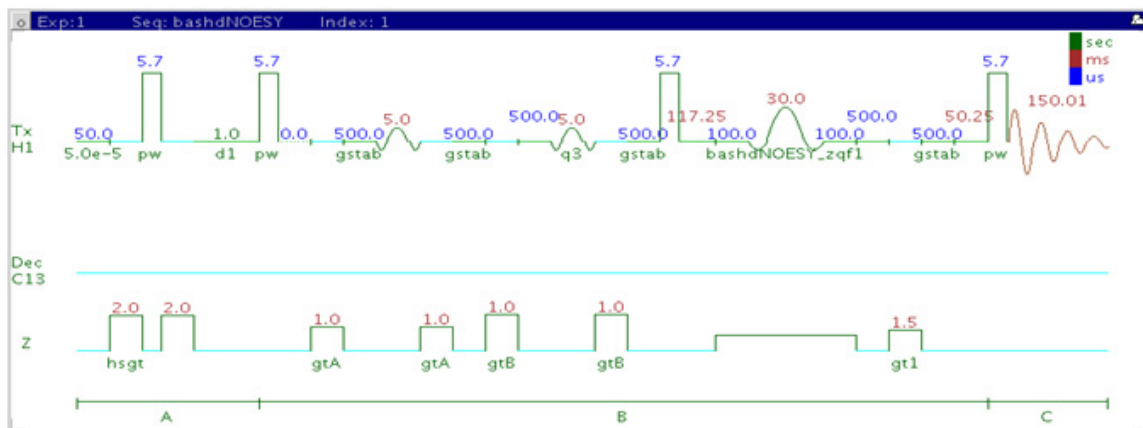
- Spectral width (sw) setting transfers from a PROTON if the (HH)bsTOCSY is set up from this experiment. The F1 band is then selected interactively. Optimized selected bandwidth, sw, and receiver gain settings transfer from a selexcit if the (HH)bsTOCSY is set up from this experiment.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.

- The homonuclear decoupling option (homodec) along F1 necessitates that the selected resonances do not have mutual homonuclear coupling among themselves.
- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, for example, 30ms or less.
- The DIPSI2 spinlock pattern is a good choice for small molecules and the DIPSI3 is typically used for larger molecules
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).
- The (HH)bsTOCSY experiment contains a zero-quantum filter, and usually results in cleaner spectra. The zero-quantum suppression filter is switched on by default.

References:

- A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)
- J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)
- V. V. Krishnamurthy, Magn. Reson. Chem. 35 (1997) 9-12. (Band Selective TOCSY)
- M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

(HH)bsNOESY



General description and usage

Description:

Two Dimensional through-space correlation spectroscopy with band-selection in F1.

Usage:

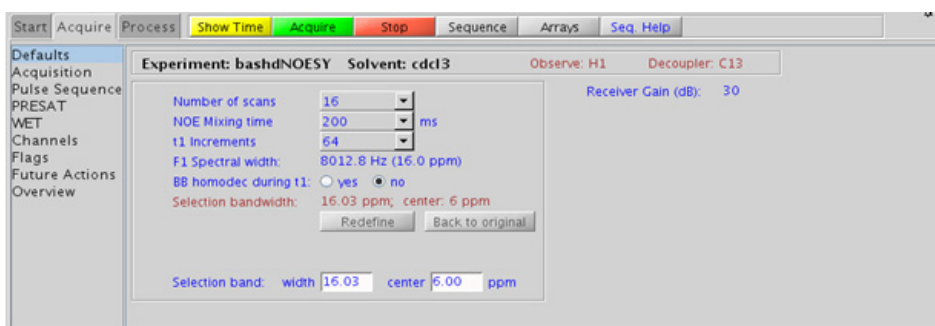
This experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically $<4 \text{ \AA}$). This experiment has an added feature above the NOESY of band-selection in F1, which is an efficient way to increase the resolution in the F1 dimension without adding a large number of increments and, hence, experiment time. There is an added experimental option for homonuclear decoupling in F1 to further improve the resolution. The (HH)bsNOESY can be very useful to obtain high-resolution through-space correlations to a overlapped area of the spectrum, for example the α -proton region of peptides. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
Relaxation time (d1)	Set from “Acquisition” panel, controls the delay between scans
Number of scans (nt)	Select typical choices from pull down menu*
NOE Mixing time (mixN)	Select from pull down menu- 500ms works well as a starting point*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
BB homodec during t1 (homodec)	Select yes/no for homonuclear decoupling in F1
Selection bandwidth	Set from previously acquired selexcit or type in specific values
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment, except for minor manual phasing changes that may be required, as the NOE peaks are negative for smaller molecules. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the (HH)bsNOESY is set up from this experiment. The F1 band is then selected interactively. Optimized selected bandwidth, sw, and receiver gain settings transfer from a selexcit if the (HH)bsNOESY is set up from this experiment.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The homonuclear decoupling option (homodec) along F1 necessitates that the selected resonances do not have mutual homonuclear coupling among themselves.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time.
- Medium-sized molecules (MW ~500 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOEs. Consider acquiring a (HH)bsROESY for these molecules. Alternatively one may consider, (i) choosing a more viscous solvent such as d₆-DMSO or d₆-DMSO with ~10-20% D₂O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- Small molecules may have crosspeaks arising from chemical exchange during the NOE mixing time and have the same phase as the diagonal. The exchange crosspeaks provide information about inter-converting molecular conformations and can be used to recognize such molecular dynamic processes.
- (HH)bsNOESY spectra may sometimes contain signals with partial antiphase character. Zero quantum suppression is embedded (and switched on by default) into the bsNOESY pulse sequence to suppress such artifacts.

References:

S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

7 Selective 2D - Sel2D

Tips, tricks, and next steps

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941.
(Zero Quantum Suppression)



8 Heteronuclear CRISIS2- (HC)Crisis2

(HC)Crisis2 Tab	294
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gc2h2bcme	304
gc2h2bc	308
gc2hmbcme	312
gc2hmbc	318
gc2hsqcse	322
gc2hsqc	326

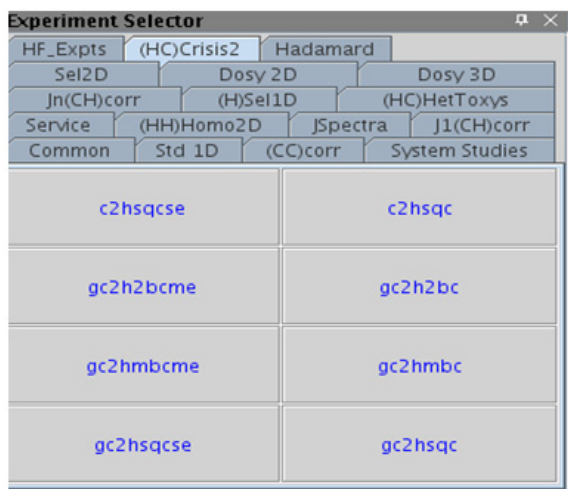


(HC)Crisis2 Tab

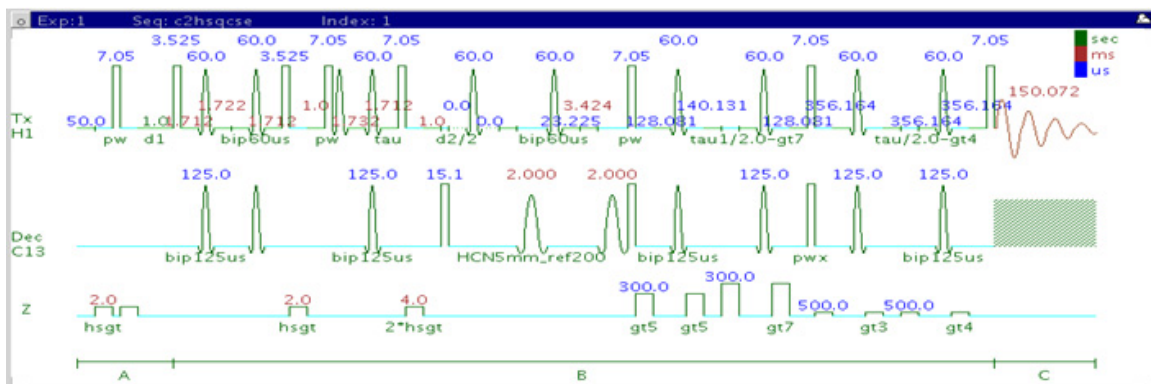
The (HC)Crisis2 experiments are a suite of commonly-used heteronuclear 2D experiments that differ by having bip (broadband inversion pulse) pulses or adiabatic pulses in both the ^{13}C and ^1H channels. For all the experiments in this tab, you can use either bip or adiabatic inversion pulses in one or both channels by the appropriate selection of the bipflg parameter.

The bipflg parameter value typically has two characters. The first character represents channel 1 (^1H) and the 2nd character represents channel 2 (^{13}C). A value of “y” uses bip inversion pulse while a value of “n” uses adiabatic inversion pulses. The advantage of adiabatic pulses is that they are tunable to any specific bandwidth using Pbox utility while the bip pulse shapes are supplied for a given bandwidth and duration, Setup and use of all of these experiments is exactly the same as the corresponding experiments found in the J1(CH)corr (Chapter 4) and the Jn(CH)corr (Chapter 5) tabs.

These experiments can be particularly useful when acquiring a collection of samples in automation that may adversely and variably affect the probe tuning/calibrations because of variations in salt concentrations, different solvents, etc. The bip pulses can compensate for imperfect calibrations, resulting in good data quality under these difficult conditions. Crisis2 experiments, because of their broadband inversion pulses on both channels, are also the best choice for ^{19}F -X correlation.



c2hsqcse



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses and sensitivity enhancement.

Usage:

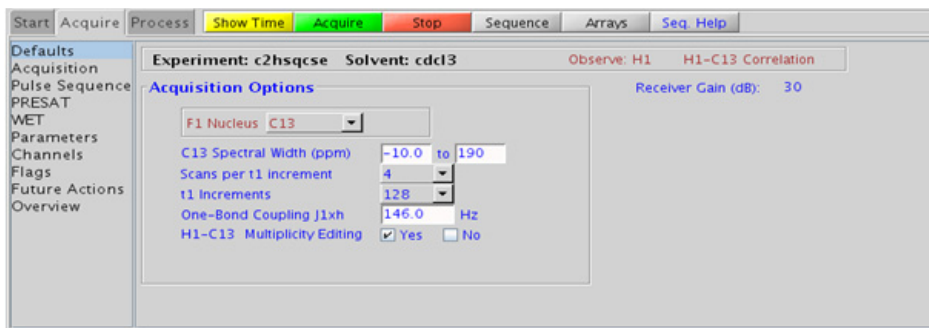
The c2hsqcse experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and vice versa. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
H1-C13 Multiplicity Editing	Check box to turn on/off multiplicity editing
Steady State (ss)	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

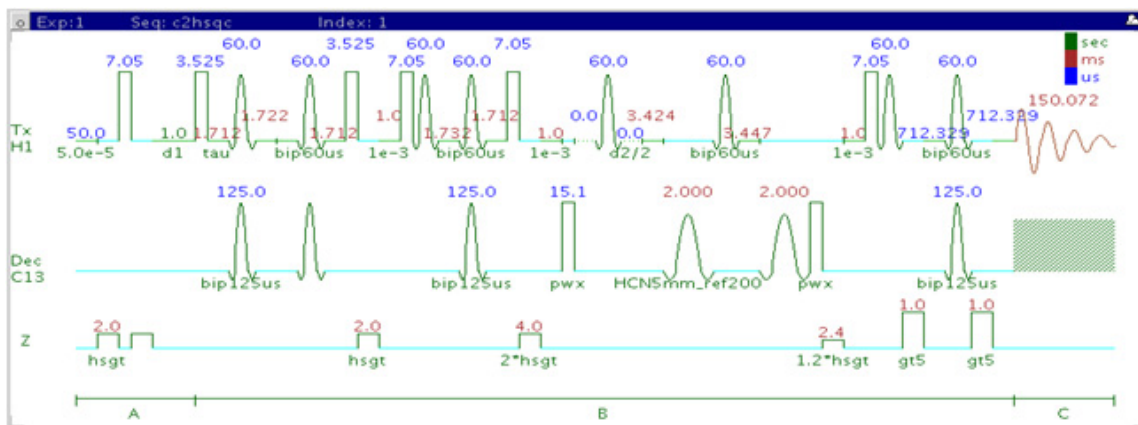
- Spectral width (sw) setting transfers from a PROTON if the c2hsqcse is set up from this experiment. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ¹³C-labelled.
- To measure ¹H-¹³C (or ¹H-X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm=’nnn’ on the command line.

- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- Using the multiplicity editing feature results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.
- The c2hsqcse experiment has comparable sensitivity to the gc2hsqcse, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.
- The c2hsqcse uses adiabatic or bip 180o carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.
- The c2hsqcse has a sensitivity enhancement feature which, in theory, can result in an increase of approximately a factor of 1-1.4 in sensitivity over c2hsqc.
- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). Remember to reset the window function along F1 axis if linear prediction parameters are changed.

References:

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (gHSQCAD / CRISIS-gHSQC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)



General description and usage

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses.

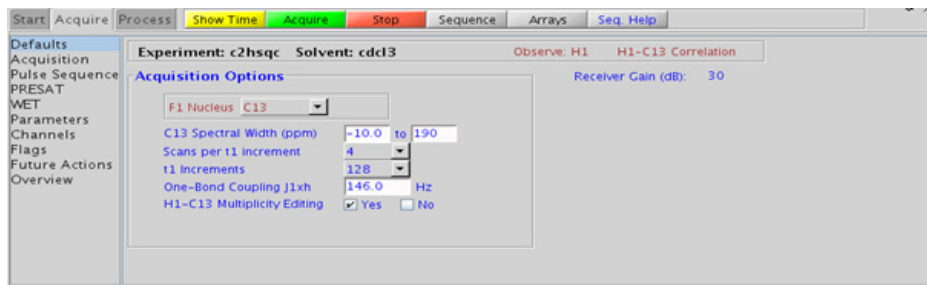
The c2hsqc experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and vice versa. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ - 146 Hz works well for many carbons
H1-C13 Multiplicity Editing	Check box to turn on/off multiplicity editing
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from “Pulse Sequence” panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the c2hsqc is set up from this experiment. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ¹³C-labelled.
- To measure ¹H-¹³C (or ¹H-X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm='nnn' on the command line.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

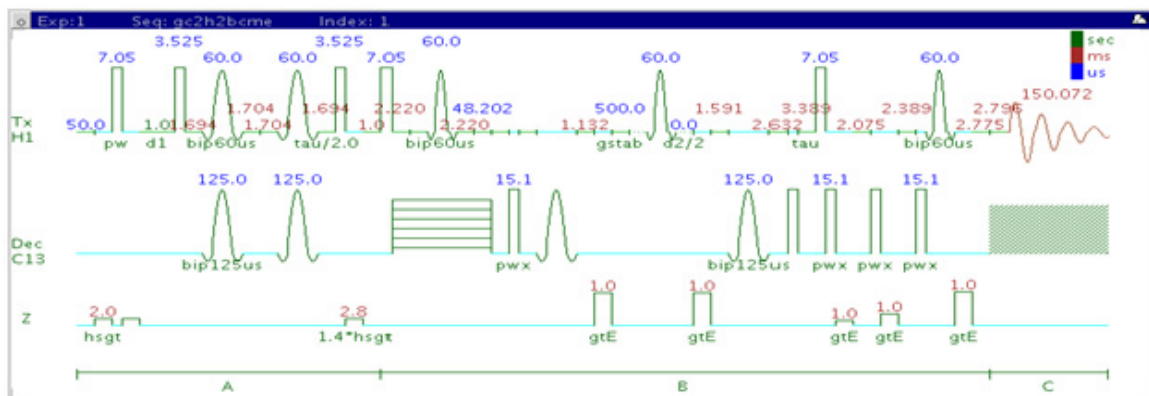
- Using the multiplicity editing feature results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.
- The c2hsqc experiment has a sensitivity advantage (factor of ~ 1.4) over the gc2hsqc, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.
- The c2hsqc uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.
- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). Remember to reset the window function along F1 axis if linear prediction parameters are changed.

References:

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (gHSQCAD / CRISIS-gHSQC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

gc2h2bcme



General description and usage

Description:

Two Dimensional heteronuclear 2-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses, gradient coherence selection, and multiplicity editing.

Usage:

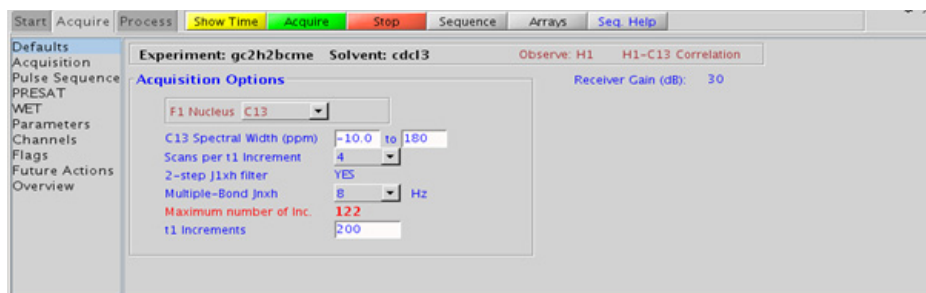
The gc2h2bcme experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over 2 bonds. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. Although the gc2h2bcme is less sensitive than the gc2hmbcme, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations. This is an edited experiment acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. Note that the experiment is constant time in F1, so the constant time delay (BigT) determines the maximum number of increments (ni). All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Type in value- controls the resolution in F1 (note maximum allowed from the constant time delay (BigT))
Multiple-Bond Jn _{xh} (jnxh)	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$ - 8 Hz works well for most molecules
Constant Time (BigT)	Type in value on the “Pulse Sequence” panel for constant time delay- 0.022ms (default) works for most samples
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from “Pulse Sequence” panel- turn on/off TANGO-gradient suppression- default is on

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

This is an edited experiment acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. The “Process” tab/“Default” panel has three buttons under the “Transform” heading to select the desired subspectrum. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present; the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gc2h2bcme is set up from this experiment. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ¹³C-labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The gc2h2bcme is in principle a gHMQC-COSY type experiment. By this very nature, it can only show 2-bond correlations to protonated carbons but not for quaternary carbons. A 22ms bigT delay is an optimal value for COSY magnetization transfer.
- The gc2h2bcme experiment is specialized to show only 2-bond correlations, but it is less sensitive than the gc2hmbcme experiment. As such, the gc2h2bcme is best used as supplemental information to the gc2hmbcme data to distinguish between 2- and 3-bond correlations.

- The multiplicity-editing feature uses the CRISIS technique that relies on a hypothetical relationship between one-bond coupling constants and carbon chemical shifts. Carbons with coupling constants that deviate significantly from this relationship result in imperfect editing.
- As the gc2h2bcme is acquired as an array of two spectra, it takes twice as long to acquire and there is a small sensitivity loss with the multiplicity-editing.
- The gc2h2bcme uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection

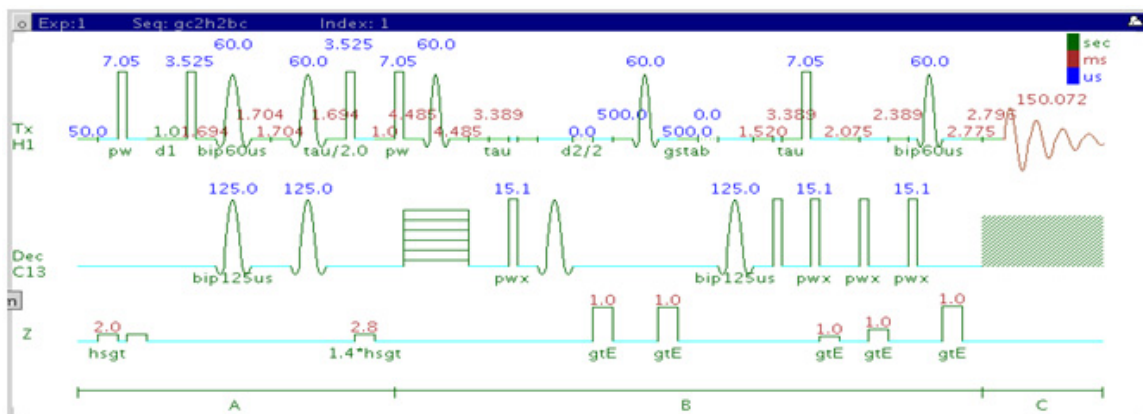
References:

A. J. Benie, O. W. Sorensen, Magn. Reson. Chem. 44 (2006) 739-743.
(Multiplicity Edited HMBC)

N. T. Nyberg, J. O. Duus, O. W. Sorensen, J. Am. Chem. Soc. 127 (2007)
6154-6155. (H2BC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2
with Multiplicity Editing)

gc2h2bc



General description and usage

Description:

Two Dimensional heteronuclear 2-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses and gradient coherence selection.

Usage:

The gH2BCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over 2 bonds. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. Although the gc2h2bcme is less sensitive than the gc2hmbcme, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations. Note that the experiment is constant time in F1, so the constant time delay (BigT) determines the maximum number of increments (ni). All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Type in value- controls the resolution in F1 (note maximum allowed from the constant time delay (BigT))
Multiple-Bond Jnxx (jnxh)	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$ - 8 Hz works well for most molecules
Constant Time (BigT)	Type in value on the “Pulse Sequence” panel for constant time delay- 0.022ms (default) works for most samples
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from “Pulse Sequence” panel- turn on/off TANGO-gradient suppression- default is on

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present; the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

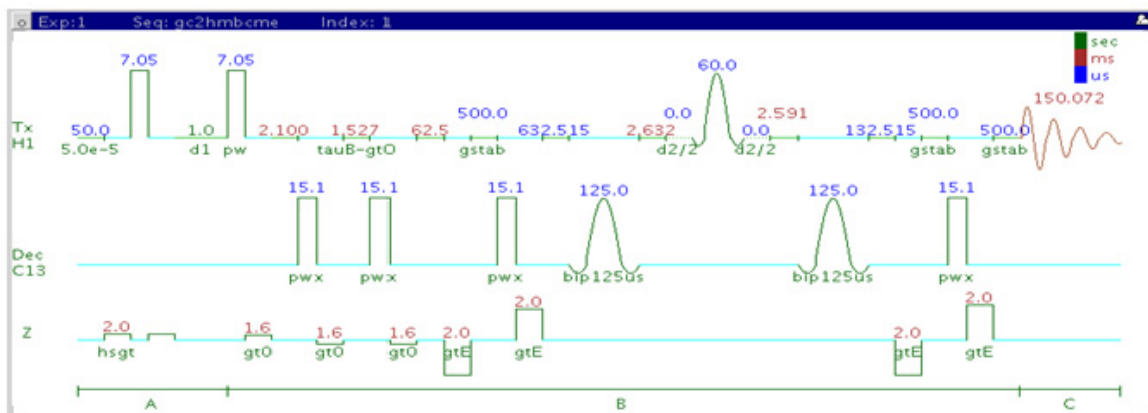
- Spectral width (sw) setting transfers from a PROTON if the gc2h2bc is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The gc2h2bc is in principle a gHMQC-COSY type experiment. By this very nature, it can only show 2-bond correlations to protonated carbons but not for quaternary carbons. A 22ms bigT delay is an optimal value for COSY magnetization transfer.
- The gc2h2bc experiment is specialized to show only 2-bond correlations, but it is less sensitive than the gc2hmhc experiment. As such, the gc2h2bc is best used as supplemental information to the gc2hmhc data to distinguish between 2- and 3-bond correlations.

- The gc2h2bc uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

References:

- N. T. Nyberg, J. O. Duus, O. W. Sorensen, J. Am. Chem. Soc. 127 (2007) 6154-6155. (H2BC)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)
- E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)
- H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

gc2hmbcme



General description and usage

Description:

Two Dimensional heteronuclear multiple-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses, gradient coherence selection, and multiplicity editing.

Usage:

The gc2hmbcme experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. This is an edited experiment acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. For structure elucidation, carbon chemical shifts are

particularly useful as they are highly sensitive to local functional groups and can be reliably predicted with software. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

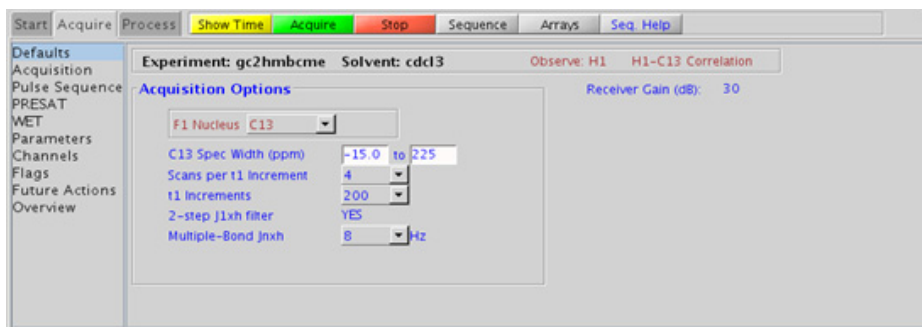
Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxx (jnxh)	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$. 8 Hz works well for most molecules
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.

8 Heteronuclear CRISIS2- (HC)Crisis2 Processing



Processing

This is an edited experiment acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. The “Process” tab/“Default” panel has three buttons under the “Transform” heading to select the desired subspectrum. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. This arrangement allows for the best resolution and sensitivity combination.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gc2hmbcme is set up from this experiment. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ^{13}C -labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The multiplicity-editing feature uses the CRISIS technique that relies on a hypothetical relationship between one-bond coupling constants and carbon chemical shifts. Carbons with coupling constants that deviate significantly from this relationship result in imperfect editing.
- As the gHMBcmeAD is acquired as an array of two spectra, it takes twice as long to acquire and there is a small sensitivity loss with the multiplicity-editing.
- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the “Pulse Sequence” panel around which one-bond couplings are eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.
- The gc2hmbcme uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

References:

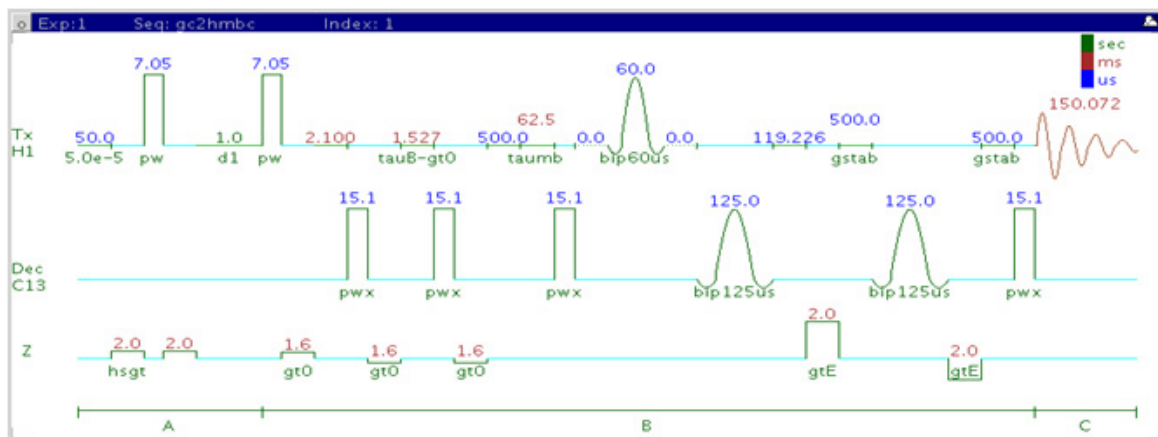
A. J. Benie, O. W. Sorensen, Magn. Reson. Chem. 44 (2006) 739-743.
(Multiplicity Edited HMBC)

8 Heteronuclear CRISIS2- (HC)Crisis2

Tips, tricks, and next steps

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

gc2hmbc



General description and usage

Description:

Two Dimensional heteronuclear multiple-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses and gradient coherence selection.

Usage:

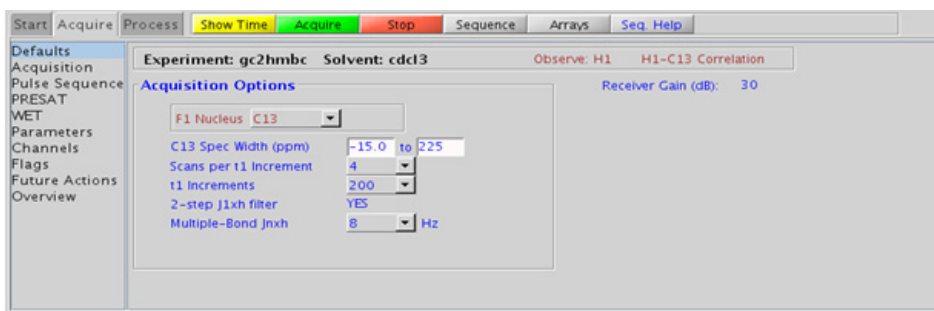
The gc2hmbc experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be reliably predicted with software. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxx (jnxh)	Pull-down menu to set value for the average $^n\text{J}_{\text{X,H}}$ - 8 Hz works well for most molecules
One-Bond suppression	Set from “Pulse Sequence” panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. This arrangement allows for the best resolution and sensitivity combination.

Tips, tricks, and next steps

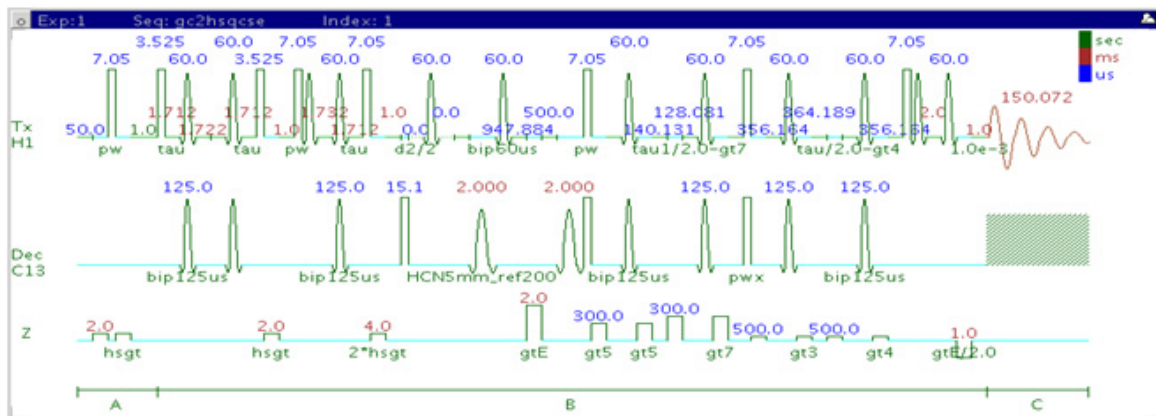
- Spectral width (sw) setting transfers from a PROTON if the gc2hmhc is set up from this experiment. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ¹³C-labelled.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- In general, the HMBC-type experiments have the best sensitivity of the Jn(CH)corr experiments- the other experiments provide specialized data for specific information types.

- The `jnxh` sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity loss. The CIGARAD experiment is better suited on a reasonably concentrated sample for detecting longer range correlations from a range of coupling constants, but at the expense of sensitivity.
- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the “Pulse Sequence” panel around which one-bond couplings are eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.
- The `gc2hmbcme` uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

References:

- A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1996) 2093-2094 (HMBC)
- H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

gc2hsqcse



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses, sensitivity enhancement, and gradient coherence selection.

Usage:

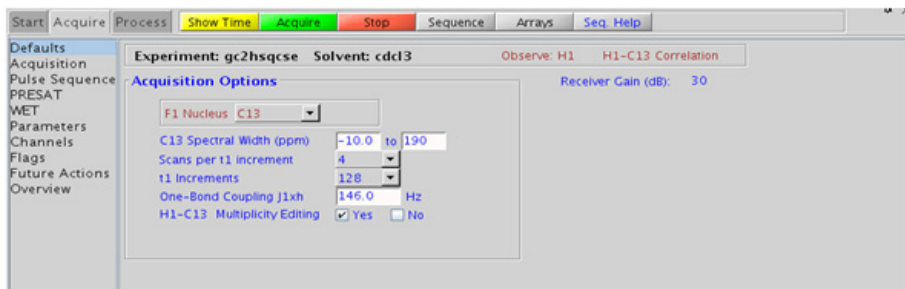
The gc2hsqcse experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and vice versa. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
H1-C13 Multiplicity Editing	Check box to turn on/off multiplicity editing
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from “Pulse Sequence” panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

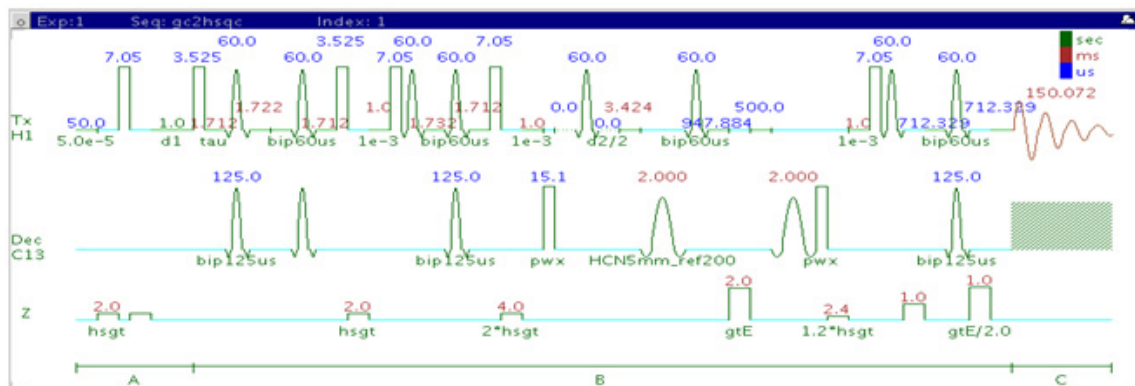
- Spectral width (sw) setting transfers from a PROTON if the gc2hsqcse is set up from this experiment. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ¹³C-labelled.
- To measure ¹H-¹³C (or ¹H-X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm=’nnn’ on the command line.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.
- The c2hsqcse experiment has a comparable sensitivity to the gc2hsqcse, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.
- The gc2hsqcse uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.
- The gc2hsqcse has a sensitivity enhancement feature which, in theory, can result in an increase of approximately a factor of 0.7-1.4 over c2hsqc and a factor of 1 to 2 over gc2hsqc.
- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). Remember to reset the window function along F1 axis if linear prediction parameters are changed.

References:

- G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)
- A. G. Palmer III, J. Cavanagh, P. E. Wright, M. Rance, J. Magn. Reson. 93 (1991) 151-170. (HSQC)
- H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

gc2hsqc



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses and gradient coherence selection.

Usage:

The gc2hsqc experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and vice versa. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1J_{\text{X,H}}$ - 146 Hz works well for many carbons
H1-C13 Multiplicity Editing	Check box to turn on/off multiplicity editing
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
C12-H1 suppression	Set from “Pulse Sequence” panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gc2hsqc is set up from this experiment. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ¹³C-labelled.
- To measure ¹H-¹³C (or ¹H-X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm='nnn' on the command line.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.
- The c2hsqc experiment has a sensitivity advantage (factor of ~ 1.4) over the gc2hsqc, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.
- The gc2hsqc uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.
- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). Remember to reset the window function along F1 axis if linear prediction parameters are changed.

References:

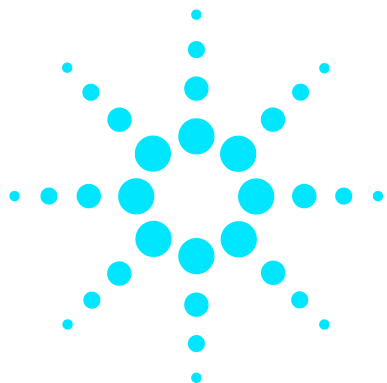
G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

A. G. Palmer III, J. Cavanagh, P. E. Wright, M. Rance, J. Magn. Reson. 93 (1991) 151-170. (HSQC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

8 Heteronuclear CRISIS2- (HC)Crisis2

Tips, tricks, and next steps



9 Hetero-TOCSY - (HC)HetToxys

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HMQCTOXY	358
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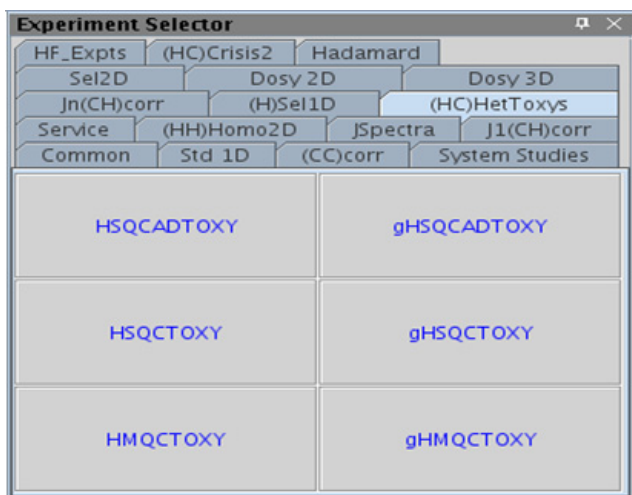


(HC)HetToxys Tab

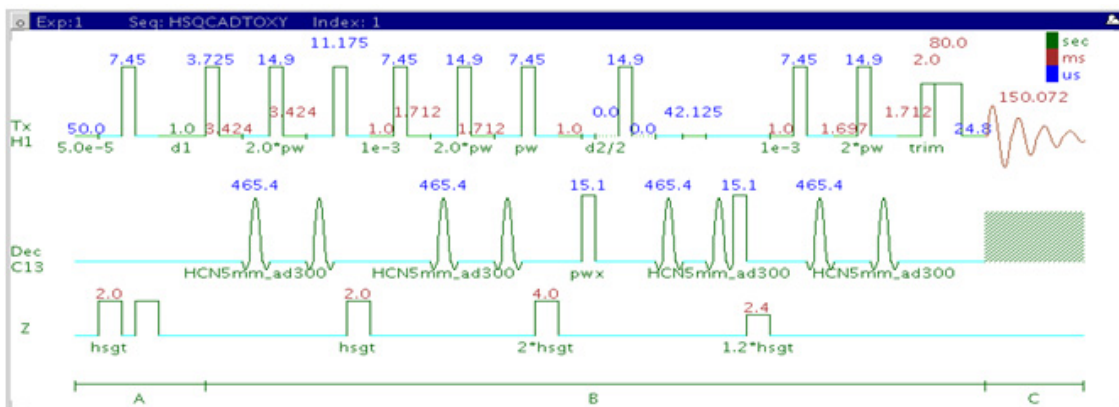
The (HC)HetToxys family of experiments can be used to resolve ambiguous assignments and/or overlap issues that are not resolved after the analysis of a more common set of 2D experiments such as a zTOCSY and a gHSQCAD. These experiments provide two pieces of information; first, typically a correlation is generated that is the same as in the gHSQCAD, for example, “This proton is attached to that carbon”. Second, a series of correlations are generated that are redundant in both F2, the proton dimension, and in F1, the carbon dimension. The information conveyed is “The proton at this chemical shift is coupled to a proton whose carbon is at that chemical shift”.

The most sensitive experiment in the family is the HSQCADTOXY, which, along with the gHSQCADTOXY, has adiabatic 180° carbon pulses. The gradient enhanced version of the experiment does experience better artifact suppression, but at the expense of a loss of sensitivity (factor of ~ 1.4). The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, consider using the non-gradient coherence selection version, with the HSQCADTOXY as the recommended choice. The non-adiabatic experiments in the HetToxy family, however, provide an option to invert or suppress only the responses directly attributed to the H-C pair (HSQC peaks), which can be advantageous for cases of severe overlap in the ^1H dimension.

The (HC)HetToxy family of experiments are very powerful for helping to solve structural problems with many classes of chemical substances wherever there is resonance overlap in the proton spectrum. In compounds such as peptides it is often useful to acquire HSQCADTOXY data for both H-C and H-N. Provided the ^{15}N chemical shifts of each amide are resolved, it is possible to effectively separate the complete ^1H NMR spectrum for each amino acid in the F1 (^{15}N) dimension. Subsequent consultation of the H-C HSQCADTOXY spectrum can make it straightforward to verify all assignments for the carbons as well.



HSQCADTOXY



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock and adiabatic 180° X-nuclei pulses.

Usage:

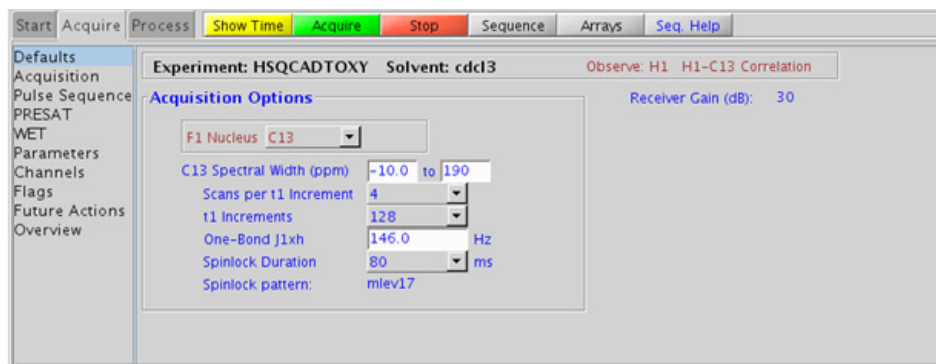
The HSQCADTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^1H - ^1H TOCSY and ^{13}C chemical shift information, for example, Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^1H - ^1H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ 146 Hz works well for many carbons
Spinlock Duration (mixT)	Select from pull down menu- 80ms works best for large spin systems*
C12-H1 suppression	Check box to turn on/off on the “Pulse Sequence” panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the HSQCADTOXY is set up from this experiment. Setup from a TOCSY-type or HSQC-type experiment transfers TOCSY- or HSQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ^{13}C -labelled.
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm=’nnn’ on the command line.
- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, for example, 30ms or less.
- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the “Pulse Sequence” tab).

- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The HSQCADTOXY experiment, like all other heteronuclear TOCSYs, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^1H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.
- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.
- The HSQCADTOXY experiment has a sensitivity advantage (factor of ~ 1.4) over the gHSQCADTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, consider using the non-gradient coherence selection version.
- The HSQCADTOXY uses adiabatic 180° carbon pulses and often gives better results than the HSQCCTOXY, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

References:

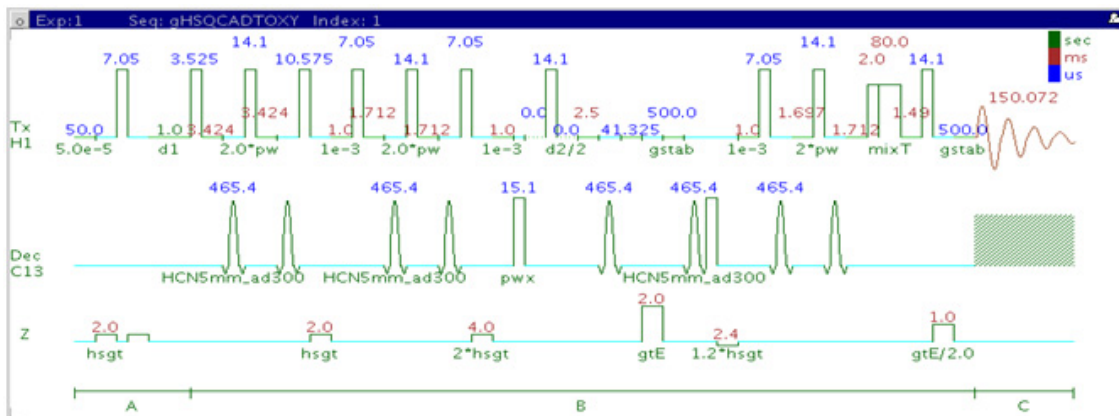
E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256.
(Adiabatic Pulses)

9 Hetero-TOCSY - (HC)HetToxys

Tips, tricks, and next steps

K. E. Kover, O. Prakash, V. J. Hruby, J. Magn. Reson. A103 (1993) 92-96.
(z-filtered HSQC-TOCSY)

gHSQCADTOXY



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock, adiabatic 180° X-nuclei pulses, and gradient coherence selection.

Usage:

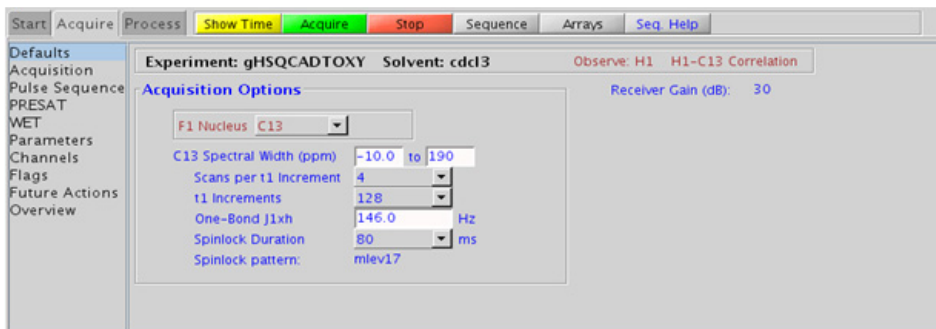
The gHSQCADTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^1H - ^1H TOCSY and ^{13}C chemical shift information, for example, Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^1H - ^1H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
Spinlock Duration (mixT)	Select from pull down menu- 80ms works best for large spin systems*
C12-H1 suppression	Check box to turn on/off on the “Pulse Sequence” panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gHSQCADTOXY is set up from this experiment. Setup from a TOCSY-type or HSQC-type experiment transfers TOCSY- or HSQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm=’nnn’ on the command line.
- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, for example, 30ms or less.
- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the “Pulse Sequence” tab).

- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- The gHSQCADTOXY experiment, like all other heteronuclear TOCSYs, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^1H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.
- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.
- The HSQCADTOXY experiment has a sensitivity advantage (factor of ~ 1.4) over the gHSQCADTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, consider using the non-gradient coherence selection version.
- The gHSQCADTOXY uses adiabatic 180° carbon pulses and often gives better results than the gHSQCCTOXY, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

References:

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256.
(Adiabatic Pulses)

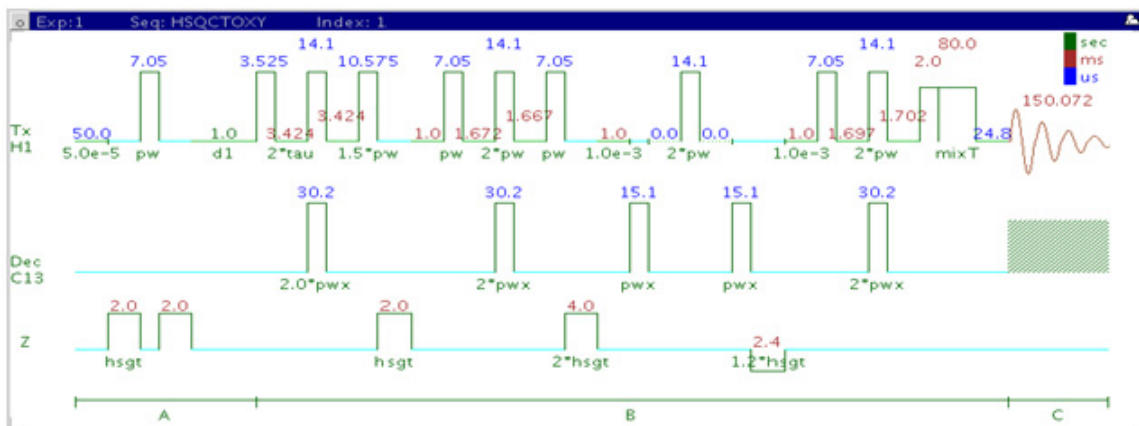
9 Hetero-TOCSY - (HC)HetToxys

Tips, tricks, and next steps

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

K. E. Kover, O. Prakash, V, J. Hruby, J. Magn. Reson. A103 (1993) 92-96. (z-filtered HSQC-TOCSY)

HSQCTOXY



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock and adiabatic 180° X-nuclei pulses.

Usage:

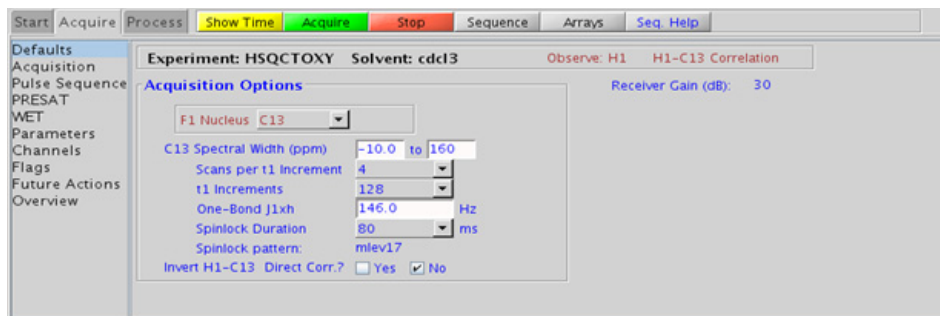
The HSQCTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^1H - ^1H TOCSY and ^{13}C chemical shift information, for example, Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^1H - ^1H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the “Acquire” tab/“Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
Spinlock Duration (mixT)	Select from pull down menu- 80ms works best for large spin systems*
Invert H1-C13 Direct Corr.?	Check box to invert direct proton-carbon correlation
C12-H1 suppression	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the HSQCTOXY is set up from this experiment. Setup from a TOCSY-type or HSQC-type experiment transfers TOCSY- or HSQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.

- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing `dm='nnn'` on the command line.
- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, for example, 30ms or less.
- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the “Pulse Sequence” tab).
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- On the “Pulse Sequence” tab there is a pull-down menu to select in-phase, inverted, or suppressed XH direct correlations. There is a minor sensitivity loss with this feature, however the extra information is obtained with no additional cost in experiment time. When direct correlations suppression is chosen the ^{13}C decoupling can be turned off to place any residual direct correlations away from the true ^1H chemical shifts. This can allow the observation of ^1H - ^1H J-connectivity between protons with very close overlap.
- The HSQCTOXY experiment, like all other heteronuclear TOCSYs, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^1H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.
- The HSQCTOXY experiment has a sensitivity advantage (factor of ~ 1.4) over the gHSQCTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, consider using the non-gradient coherence selection version.
- The HSCADTOXY and gHSCADTOXY use adiabatic 180° carbon pulses and often give better results than the HSQC, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

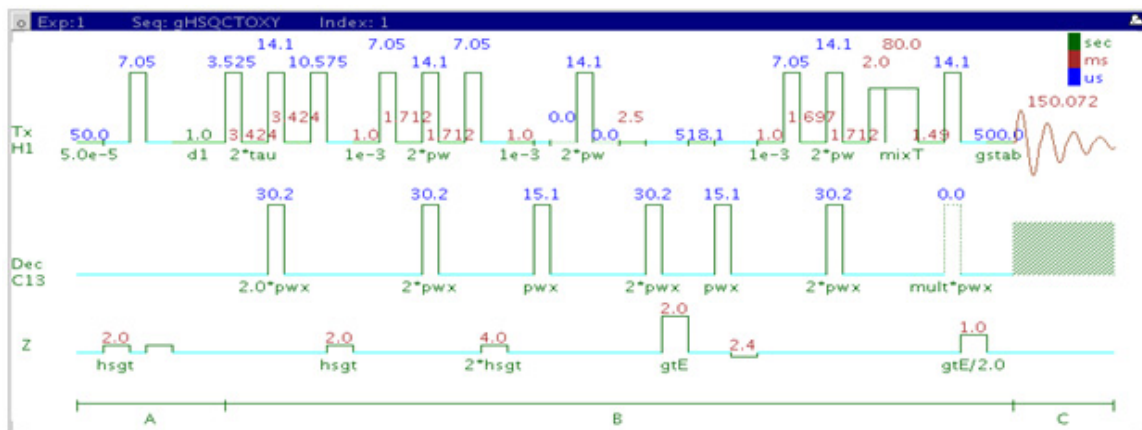
References:

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256.
(Adiabatic Pulses)

K. E. Kover, O. Prakash, V. J. Hruby, J. Magn. Reson. A103 (1993) 92-96.
(z-filtered HSQC-TOCSY)

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003)
253-259 (Multiplicity Editing)

gHSQCTOXY



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock, adiabatic 180° X-nuclei pulses, and gradient coherence selection.

Usage:

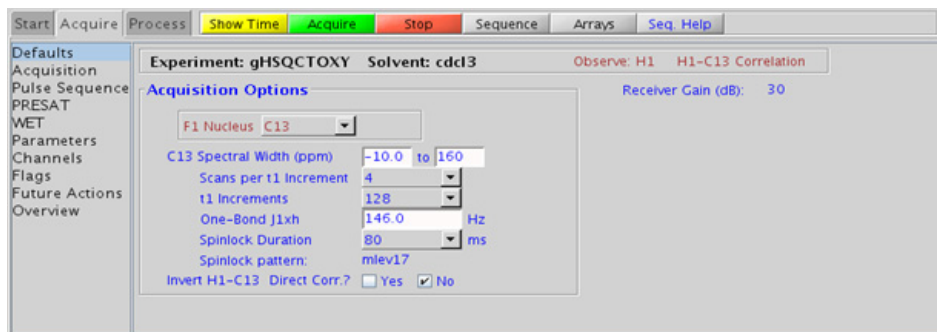
The gHSQCTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^1H - ^1H TOCSY and ^{13}C chemical shift information, for example, Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^1H - ^1H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
Spinlock Duration (mixT)	Select from pull down menu- 80ms works best for large spin systems*
Invert H1-C13 Direct Corr.?	Check box to invert direct proton-carbon correlation
C12-H1 suppression	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gHSQCTOXY is set up from this experiment. Setup from a TOCSY-type or HSQC-type experiment transfers TOCSY- or HSQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.

- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing `dm='nnn'` on the command line.
- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, for example, 30ms or less.
- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the “Pulse Sequence” tab).
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- On the “Pulse Sequence” tab there is a pull-down menu to select in-phase, inverted, or suppressed XH direct correlations. There is a minor sensitivity loss with this feature, however the extra information is obtained with no additional cost in experiment time. When direct correlations suppression is chosen the ^{13}C decoupling can be turned off to place any residual direct correlations away from the true ^1H chemical shifts. This can allow the observation of ^1H - ^1H J-connectivity between protons with very close overlap.
- The gHSQCTOXY experiment, like all other heteronuclear TOCSYs, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^1H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.
- The HSQCTOXY experiment has a sensitivity advantage (factor of ~ 1.4) over the gHSQCTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, consider using the non-gradient coherence selection version.
- The HSQCADTOXY and gHSQCADTOXY use adiabatic 180° carbon pulses and often give better results than the HSQC, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

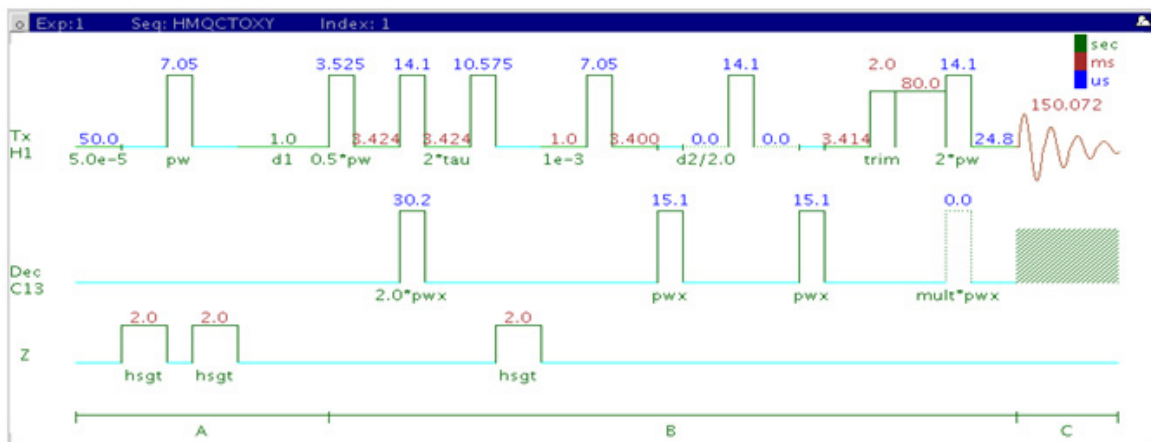
References:

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256.
(Adiabatic Pulses)

K. E. Kover, O. Prakash, V. J. Hruby, J. Magn. Reson. A103 (1993) 92-96.
(z-filtered HSQC-TOCSY)

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003)
253-259 (Multiplicity Editing)

HMQCTOXY



General description and usage

Description:

Two Dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock.

Usage:

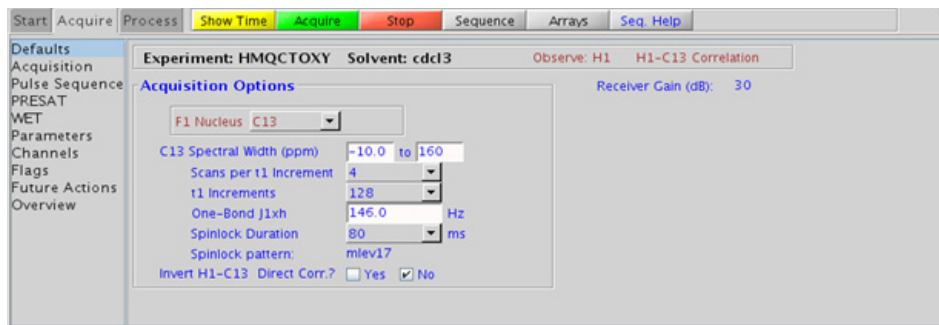
The HMQCTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^1H - ^1H TOCSY and ^{13}C chemical shift information, for example, Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^1H - ^1H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
Spinlock Duration (mixT)	Select from pull down menu- 80ms works best for large spin systems*
Invert H1-C13 Direct Corr.?	Check box to invert direct proton-carbon correlation
C12-H1 suppression	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the HMQCTOXY is set up from this experiment. Setup from a TOCSY-type or HMQC-type experiment transfers TOCSY- or HMQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ¹³C-labelled.

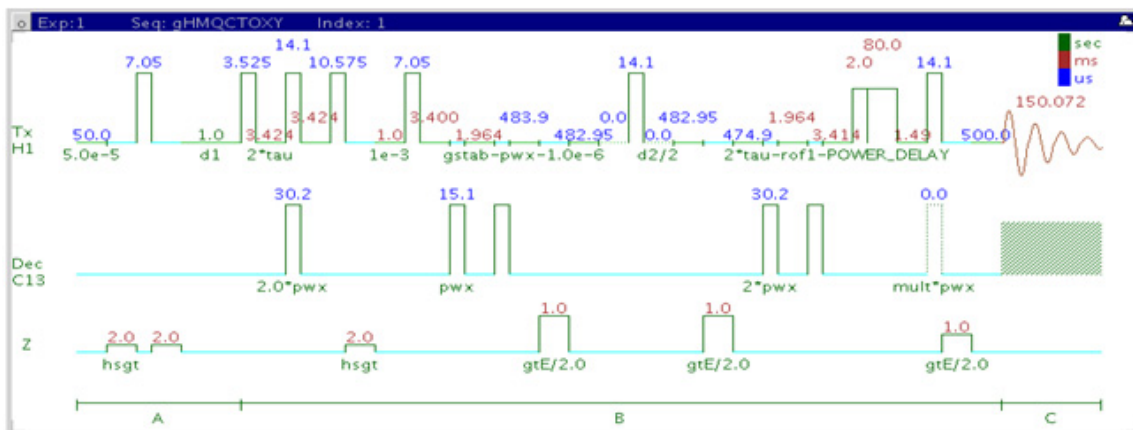
- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing dm=’nnn’ on the command line.
- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, for example, 30ms or less.
- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the “Pulse Sequence” tab.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- On the “Pulse Sequence” tab there is a pull-down menu to select in-phase, inverted, or suppressed XH direct correlations. There is a minor sensitivity loss with this feature, however the extra information is obtained with no additional cost in experiment time. When direct correlations suppression is chosen the ^{13}C decoupling can be turned off to place any residual direct correlations away from the true ^1H chemical shifts. This can allow the observation of ^1H - ^1H J-connectivity between protons with very close overlap.
- The HMQCTOXY experiment, like all other heteronuclear TOCSYs, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HMQC stage is then distributed along the ^1H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.
- The HMQCTOXY experiment has a sensitivity advantage (factor of ~ 1.4) over the gHMQCTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, consider using the non-gradient coherence selection version.

References:

- L. Lerner, A. Bax, J. Magn. Reson. 69 (1986) 375-380. (HMQC-TOCSY)
- R. C. Crouch, T. D. Spitzer, G. E. Martin, Magn. Reson. Chem. 30 (1992) S71-S73. (Multiplicity Edited HMQC-TOCSY)

gHMQCTOXY



General description and usage

Description:

Two Dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock and gradient coherence selection.

Usage:

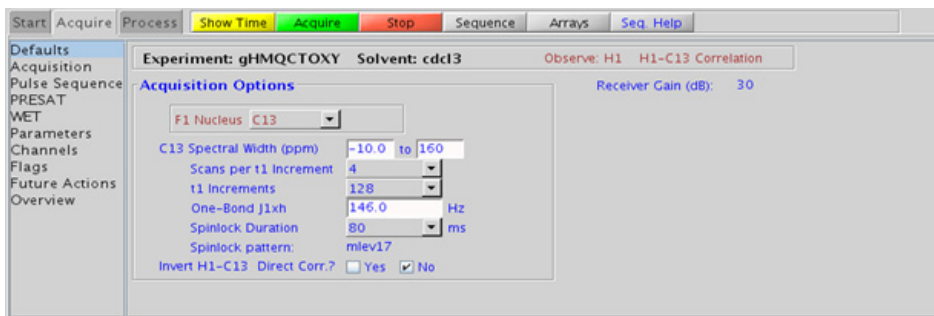
The gHMQCTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^1H - ^1H TOCSY and ^{13}C chemical shift information, for example, Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^1H - ^1H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Set value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for many carbons
Spinlock Duration (mixT)	Select from pull down menu- 80ms works best for large spin systems*
Invert H1-C13 Direct Corr.?	Check box to invert direct proton-carbon correlation
C12-H1 suppression	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the gHMQCTOXY is set up from this experiment. Setup from a TOCSY-type or HMQC-type experiment transfers TOCSY- or HMQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value (?30), unless the sample is extremely concentrated or ^{13}C -labelled.

- To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling can be turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing `dm='nnn'` on the command line.
- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations extend. Use spinlock durations exceeding 150ms with caution, as this can result in sample heating. The efficiency of the TOCSY transfer is directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, for example, 30ms or less.
- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the “Pulse Sequence” tab).
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. However, higher power carbon decoupling may cause some perturbations that may necessitate more ss scans to reach equilibrium. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- On the “Pulse Sequence” tab there is a pull-down menu to select in-phase, inverted, or suppressed XH direct correlations. There is a minor sensitivity loss with this feature, however the extra information is obtained with no additional cost in experiment time. When direct correlations suppression is chosen the ^{13}C decoupling can be turned off to place any residual direct correlations away from the true ^1H chemical shifts. This can allow the observation of ^1H - ^1H J-connectivity between protons with very close overlap.
- The gHMQCTOXY experiment, like all other heteronuclear TOCSYs, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^1H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H - ^1H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.
- The HMQCTOXY experiment has a sensitivity advantage (factor of ~ 1.4) over the gHMQCTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, consider using the non-gradient coherence selection version.

References:

- L. Lerner, A. Bax, J. Magn. Reson. 69 (1986) 375-380. (HMQC-TOCSY)
- R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)
- R. C. Crouch, T. D. Spitzer, G. E. Martin, Magn. Reson. Chem. 30 (1992) S71-S73. (Multiplicity Edited HMQC-TOCSY)



10 Carbon-carbon correlation - (CC)corr

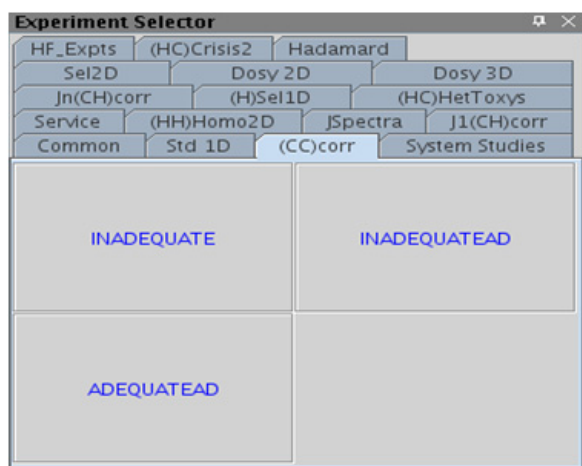
(CC)corr Tab 370
INADEQUATE 372
INADEQUATEAD 376
ADEQUATEAD 380



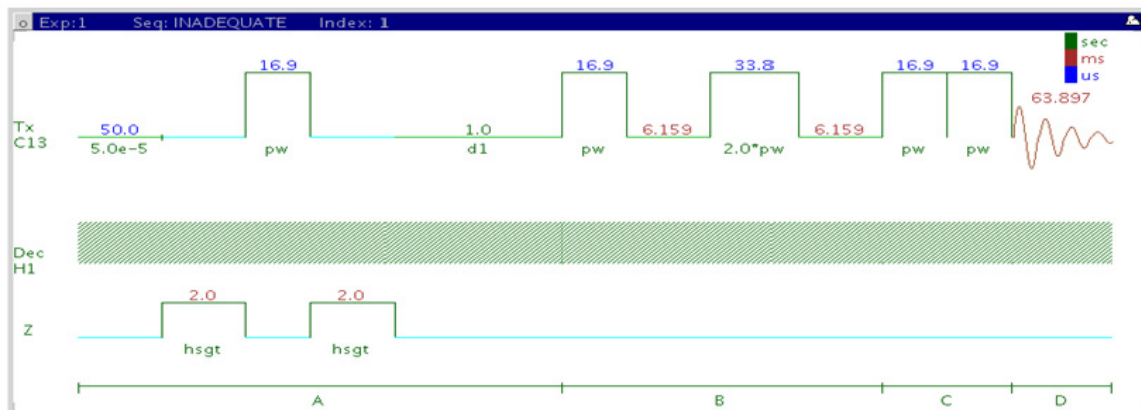
(CC)corr Tab

This tab contains three experiments to map direct correlations from carbon to carbon. Two experiments are ^{13}C -detected (INADEQUATE and INADEQUATEAD) and the third is ^1H -detected (ADEQUATEAD). To unambiguously elucidate the structure of small-molecules, the INADEQUATE and INADEQUATE(AD) experiments have no peer except perhaps X-ray crystallography. They are, however, highly insensitive and thus infrequently used.

A good rule of thumb to determine if the sample is concentrated enough for this class of experiment to be successful within a reasonable time (overnight acquisition) is that the S/N for the CARBON spectrum is ~25:1 after a single scan. The proton-detected ADEQUATEAD experiment is more sensitive. However it has the requirement that a proton must be nearby for the carbon to be detected. The “AD” experiments include adiabatic carbon 180° pulses and are highly recommended, as they provide more uniform inversion profiles for a wide ^{13}C spectral width.



INADEQUATE



General description and usage

Description:

Two Dimensional homonuclear multiple-quantum J-correlation spectroscopy.

Usage:

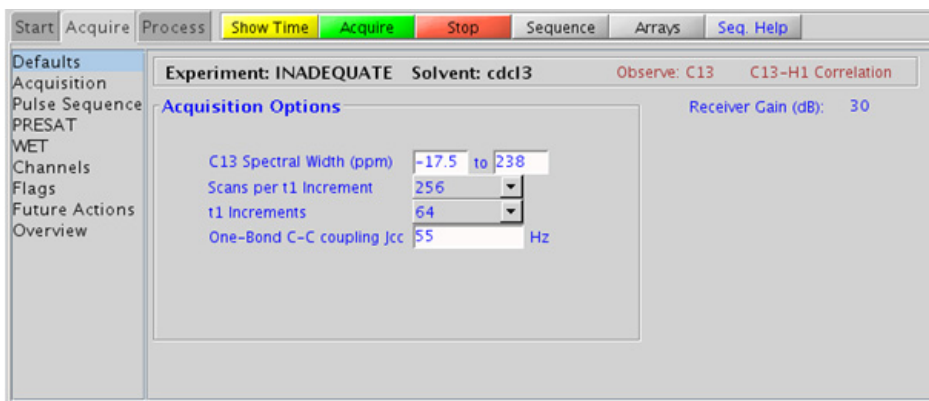
The INADEQUATE experiment shows correlations between carbons that are directly coupled to one another. The data have great utility for structure elucidation as the carbon connectivities are established directly from the correlations. These connectivities can then be used to map the molecular framework, similar to x-ray crystallography. The drawback to the experiment is that as the sensitivity is very low; it should only be attempted with samples of higher concentrations. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
C13 Spectral Width (sw)	Type in desired values in ppm
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.064s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1
One-Bond C-C coupling Jcc (jcc)	Type in value for the average J^1_{C-C} 55 Hz works well for most molecules
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

You can type specific values for these parameters manually in the appropriate boxes on the “Acquisition” panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 2k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a CARBON if the INADEQUATE is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30).
- Spectral width in the F1 dimension (sw1) is set, by default, to $sw*2$. However, a value of $sw1=sw$ (while generating aliased peaks along F1) can be used with no ambiguities in connectivity interpretations.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- Observing quaternary carbons is uniquely challenging. Use of some relaxation agent may be helpful while running the INADEQUATE experiment.
- A good rule of thumb to determine if the sample is concentrated enough for an INADEQUATE experiment is that the S/N for the CARBON spectrum is $\sim 25:1$ after a single scan.

- The INADEQUATEAD uses adiabatic 180° carbon pulses and often gives better results than the INADEQUATE, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ¹³C spectral width.

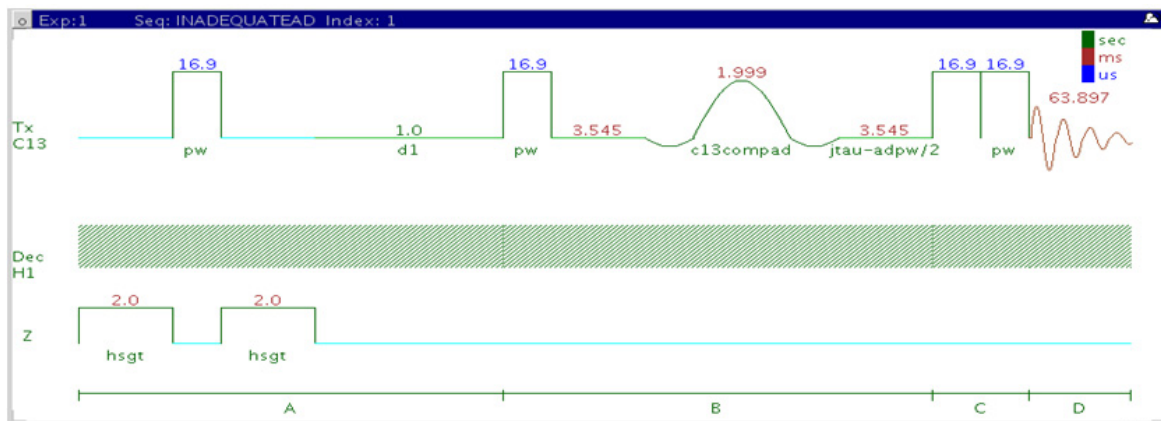
References:

A. Bax, R. Freeman, S. P. Kempsell, J. Am. Chem. Soc. 102 (1980) 4849-4851 (INADEQUATE)

J. Buddrus, J. Lambert, Magn. Reson. Chem. 40 (2002) 3-23.
(INADEQUATE – Recent Developments)

A. D. Bain D. W. Hughes, C. K. Anand, Z. Nie, V. J. Robertson, Magn. Reson. Chem. 48 (2010) 630-641 (INADEQUATE – Practical Aspects)

INADEQUATEAD



General description and usage

Description:

Two Dimensional homonuclear multiple-quantum J-correlation spectroscopy with adiabatic 180° X-nuclei pulses.

Usage:

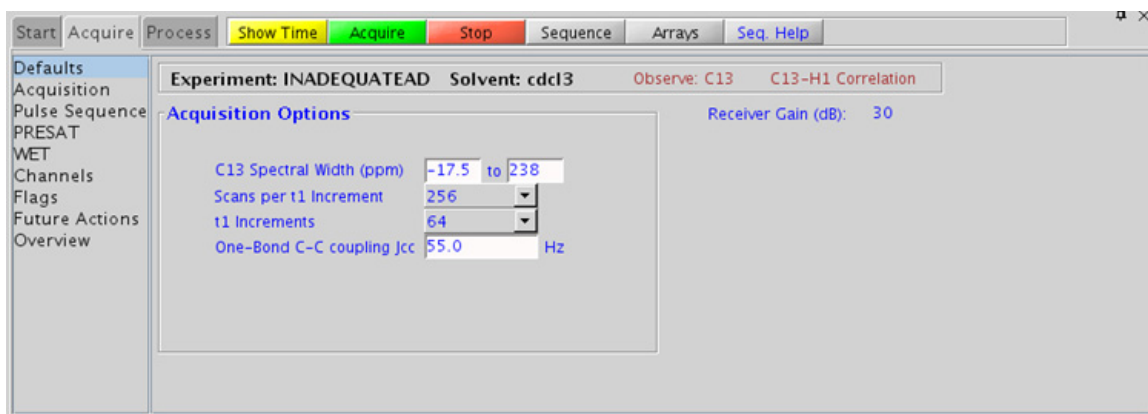
The INADEQUATE experiment shows correlations between carbons that are directly coupled to one another. The data have great utility for structure elucidation, as the carbon connectivities are established directly from the correlations. These connectivities can then be used to map the molecular framework, similar to x-ray crystallography. The drawback to the experiment is that as the sensitivity is very low; it should only be attempted with samples of higher concentrations. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
C13 Spectral Width (sw)	Type in desired values in ppm
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.064s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1
One-Bond C-C coupling Jcc (jcc)	Type in value for the average J_{C-C}^1 55 Hz works well for most molecules
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 2k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a CARBON if the INADEQUATEAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30).
- Spectral width in the F1 dimension (sw1) is set, by default, to $sw*2$. However, a value of $sw1=sw$ (while generating aliased peaks along F1) can be used with no ambiguities in connectivity interpretations.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- Observing quaternary carbons is uniquely challenging. Use of some relaxation agent may be helpful while running the INADEQUATEAD experiment.

- A good rule of thumb to determine if the sample is concentrated enough for an INADEQUATEAD experiment to be successful within a reasonable time (overnight acquisition) is that the S/N for the CARBON spectrum is ~25:1 after a single scan.
- The INADEQUATEAD uses adiabatic 180° carbon pulses and often gives better results than the INADEQUATE, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ¹³C spectral width.

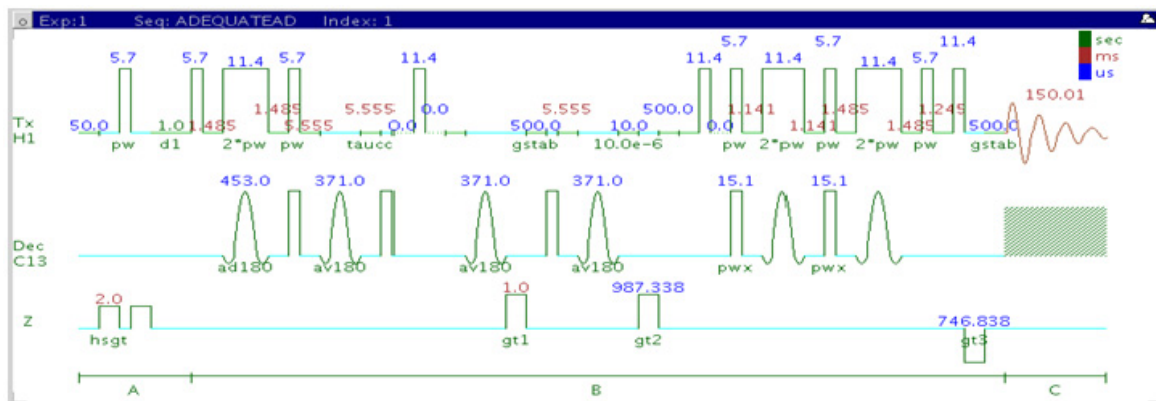
References:

A. Bax, R. Freeman, S. P. Kempsell, J. Am. Chem. Soc. 102 (1980) 4849-4851 (INADEQUATE)

L. Jin, D. Uhrin, Magn. Reson. Chem. 45 (2007) 628-633 (IPAP INADEQUATE with Adiabatic Pulses)

D. Uhrin, Ann. Rep. NMR. Spectrosc. 70 (2010) 1-34 (INADEQUATE – Review)

ADEQUATEAD



General description and usage

Description:

Two Dimensional heteronuclear single or double-quantum J-correlation spectroscopy with adiabatic 180° X-nuclei pulses.

Usage:

The ADEQUATEAD experiment can be acquired either as a single quantum experiment or a double quantum experiment. The single quantum experiment selectively transfers coherences from two adjacent carbons to protons directly attached to one of the carbons. The double quantum experiment can connect carbons that are separated by multiple bonds. The data have great utility for structure elucidation, as the carbon connectivities established indirectly from the correlations can then be used to map the molecular framework. The experiment is more sensitive (theoretically a factor of 32) than the INADEQUATEAD. However, the ADEQUATEAD experiment relies on at least one nearby attached proton,

so correlations between quaternary carbons are not observed. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

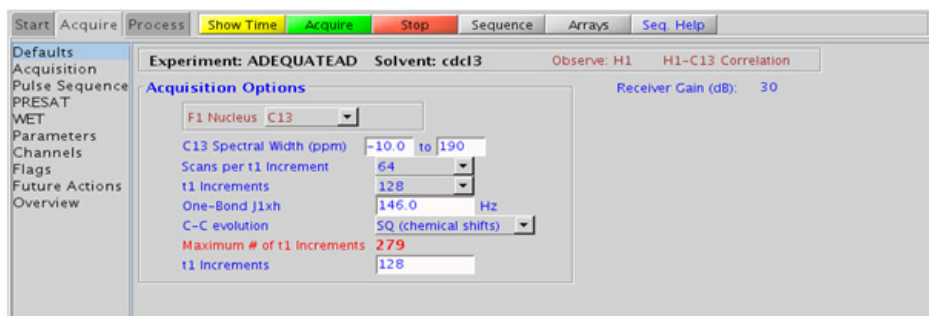
Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn)	Pull- down menu to set F1 nucleus to ^{13}C , ^{15}N , or other*
C13 Spectral Width (ppm)	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh)	Type in value for the average $^1\text{J}_{\text{X,H}}$ - 146 Hz works well for most molecules
C-C evolution	Set to single quantum (SQ) or double quantum (DQ)
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.

10 Carbon-carbon correlation - (CC)corr Processing



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a PROTON if the ADEQUATEAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30).

- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

References:

B. Reif, M. Kock, R. Kerssebaum, H. Kang, W. Fenical, C. Griesinger, J. Magn. Reson. A118 (1996) 282-285 (ADEQUATE)

M. Kock, R. Kerssebaum, W. Bermel, Magn. Reson. Chem. 41 (2003) 65-69. (ADEQUATE with Adiabatic Pulses)

T. Parella, F. S. Ferrando, J. Magn. Reson, 166 (2004) 123-128. (ADEQUATE with Multiplicity Editing)

10 Carbon-carbon correlation - (CC)corr

Tips, tricks, and next steps



11 Proton-fluorine - HF_Expts

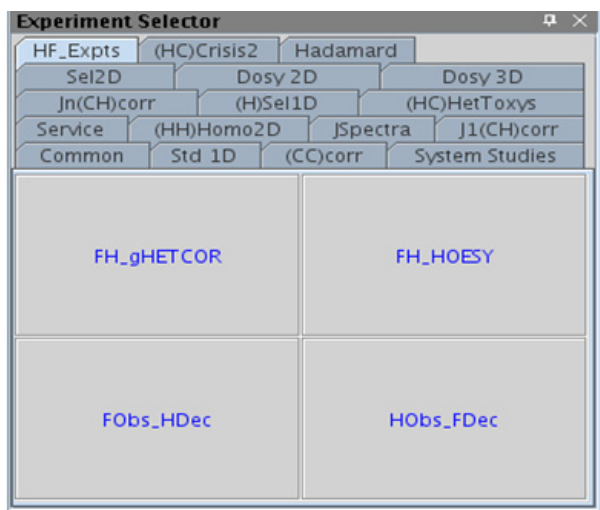
HF_Expts Tab	386
FH_gHETCOR	388
FH_HOESY	392
FObs_HDec	396
HObs_FDec	400



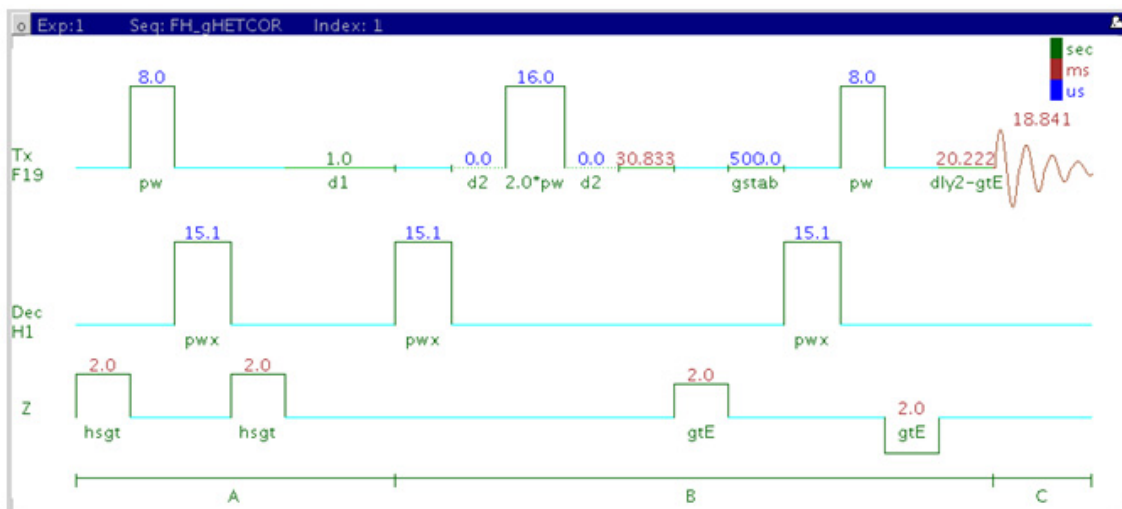
HF_Expts Tab

This tab of the experiment selector includes both 1D and 2D experiments involving ^1H and ^{19}F . All the experiments are designed such that the data are acquired without the need for a second high-band channel in the spectrometer. The 1D experiments are with fluorine observe, proton decouple (FObs_HDec), and *vice versa* (HObs_FDec). The 2D experiments available include both J-coupling (FH_gHETCOR) and through-space (FH_HOESY) correlation experiments.

These experiments can provide a unique handle for structure elucidation and peak assignment of fluorinated compounds. When acquiring impure samples of fluorinated compounds from a biological origin, these experiments can be used to see resonances from the molecule of interest, while excluding background signals. These experiments require a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file. There are unique calibration routines in the channel sharing mode. See to the VnmrJ Installation and Administration manual, Chapter 9, for information on calibration.



FH_gHETCOR



General description and usage

Description:

Two Dimensional heteronuclear ^1H - ^{19}F shift correlation with fluorine observe and gradient coherence selection.

Usage:

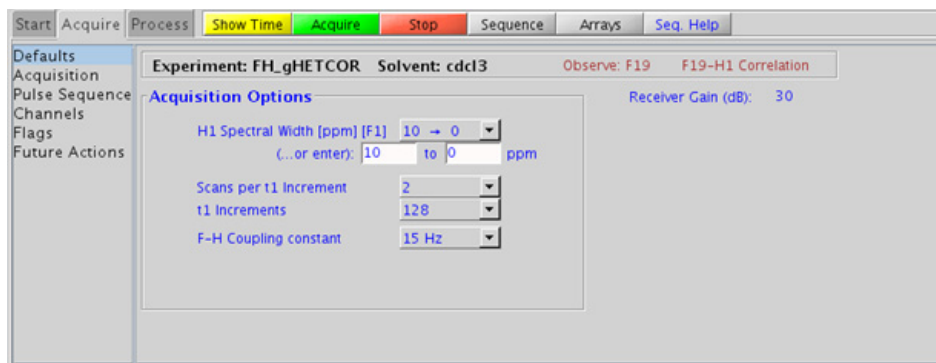
The FH_gHETCOR experiment is used to acquire a 2-dimensional spectrum to correlate protons coupled to fluorines with fluorine detection, meaning the F1 dimension is ^1H and the F2 dimension is ^{19}F . This scheme allows for higher resolution in the fluorine dimension as the chemical shift range is much larger. This experiment is useful to confirm the attachment location(s) for fluorine atom(s) in a molecule. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameters	Description
Spectral Width (sw)	Set from previously acquired FLUORINE or Fobs_HDec*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.019 s
H1 Spectral Width [ppm] [F1]	Select typical choices from pull down menu or type in values
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1
F-H Coupling Constant (jFH)	Set value for the average $^nJ_{F,H}$
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

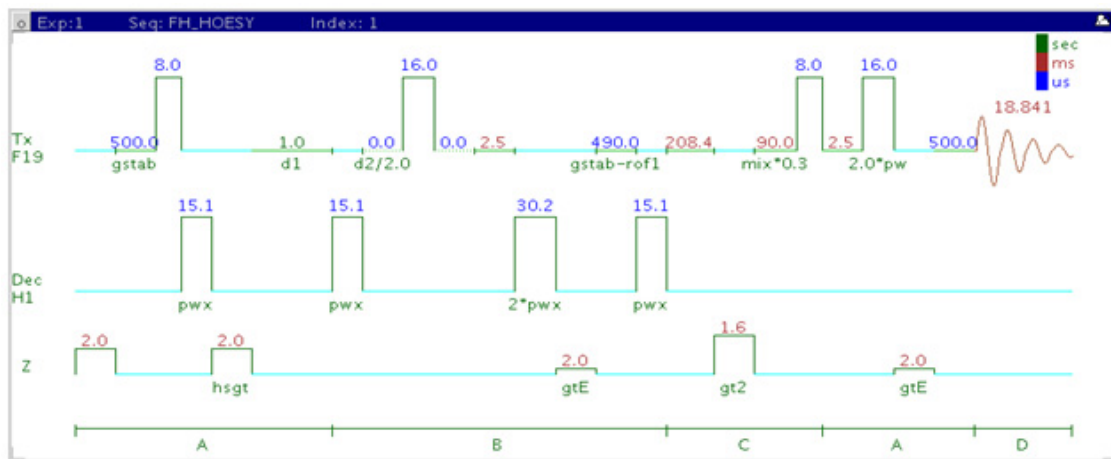
Tips, tricks, and next steps

- Note: this experiment requires a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file.
- Spectral width (sw) and receiver gain (gain) settings transfer from a FLUORINE or FObs_HDec data set if the FH_gHETCOR is set up from these experiments. As ^{19}F has a large chemical shift range, it is highly recommended that the sw is optimized before acquiring this experiment. Using the Fobs_HDec experiment for setup preserves the decoupling parameters. However, decoupling may result in sensitivity losses and unintended signal cancellations. Decoupling can be turned off by setting the parameter dm to the value “nnn”.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. The default setting for this parameter is off.

References:

J. Battiste, R. A. Newmark, Prog. NMR. Spectrosc. 48 (2006) 1-23.
(Multidimensional ^{19}F NMR)

FH_HOESY



General description and usage

Description:

Two Dimensional heteronuclear ^1H - ^{19}F through-space correlation spectroscopy with fluorine observe.

Usage:

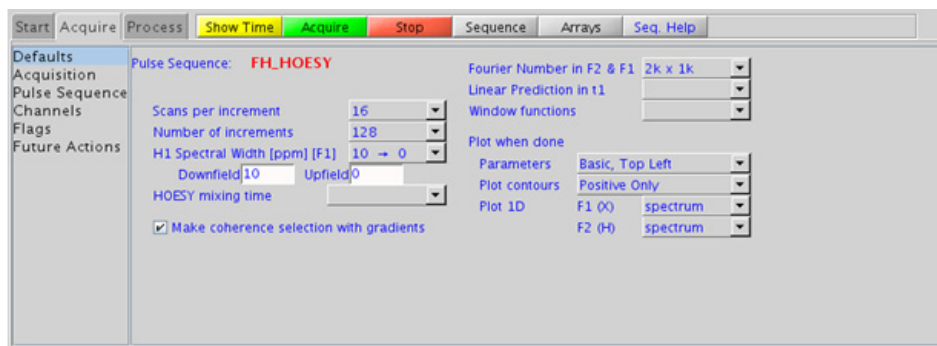
The FH_HOESY experiment produces a 2-dimensional spectrum with crosspeaks between fluorines and protons that are close in space (typically $<5 \text{ \AA}$). The experiment is configured with fluorine detection, meaning the F1 dimension is ^1H and the F2 dimension is ^{19}F . This scheme allows for higher resolution in the fluorine dimension as the chemical shift range is much larger. The FH_HOESY experiment is often used to help determine stereochemistry for rigid ring systems or to confirm attachment locations for fluorines on unsaturated carbons (for example, aromatic) of a molecule. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired FLUORINE or Fobs_HDec*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.019 s
Scans per increment (nt)	Select typical choices from pull down menu*
Number of increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1 (minimum ~64)
H1 Spectral Width [ppm] [F1]	Select typical choices from pull down menu or type in values
HOESY mixing time (mix)	Select from pull down menu*
Make coherence selection with gradient	Check box to turn gradient coherence selection on/off
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans
Relaxation time (d1)	Set from “Acquisition” panel controls the delay between scans

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” or “Pulse Sequence” panels.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment, except for minor manual phasing changes that may be required, as the NOE peaks are negative for smaller molecules. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

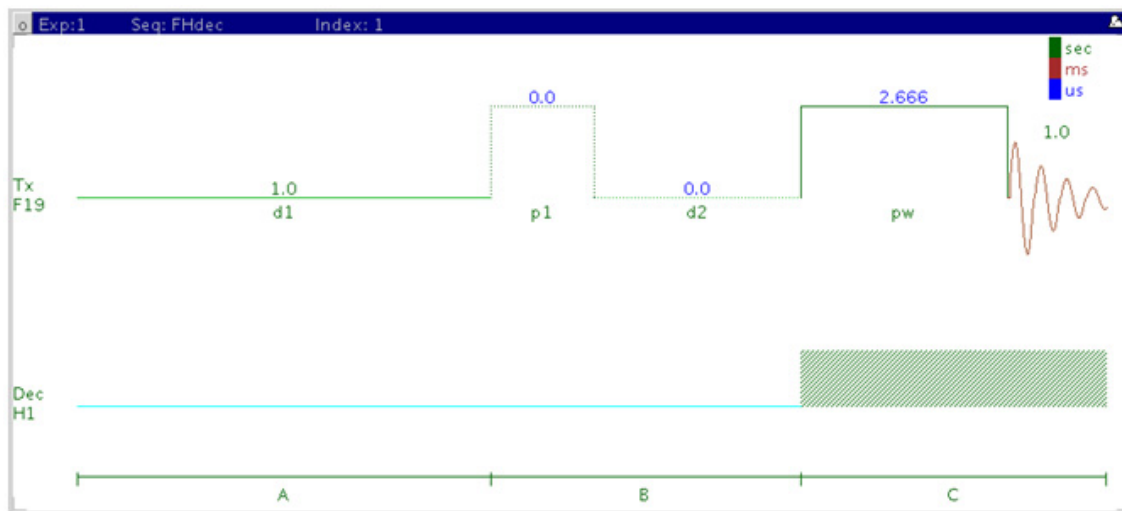
Tips, tricks, and next steps

- Note: this experiment requires a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file.
- Spectral width (sw) and receiver gain (gain) settings transfer from a FLUORINE or FObs_HDec data set if the FH_gHETCOR is set up from these experiments. As ^{19}F has a large chemical shift range, it is highly recommended that the sw is optimized before acquiring this experiment. Using the Fobs_HDec experiment for setup preserves the decoupling parameters. However, decoupling may result in sensitivity losses. Decoupling can be turned off by setting the parameter dm to the value “nnn”.
- The default number of “steady state” or “dummy scans” that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

References:

- J. Battiste, R. A. Newmark, Prog. NMR. Spectrosc. 48 (2006) 1-23.
(Multidimensional ^{19}F NMR)
- W. Bauer, Magn. Reson. Chem. 34 (1996) 532-537. (Gradient Inverse
HOESY)

FObs_HDec



General description and usage

Description:

Uses the standard s2pul pulse sequence to acquire a 1D- ^{19}F dataset with proton decoupling.

Usage:

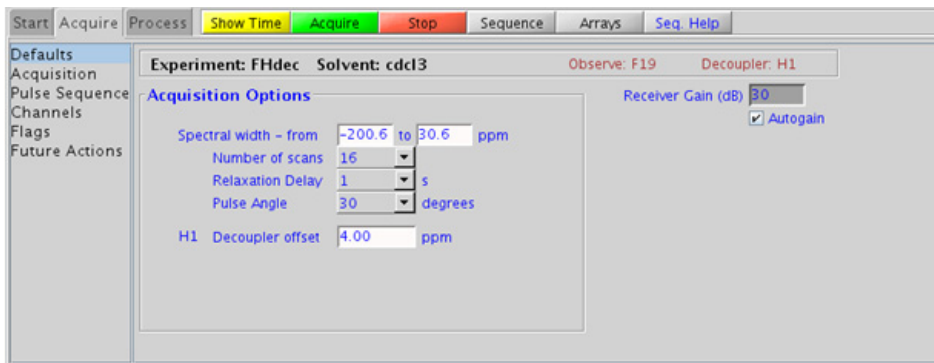
The FObs_HDec experiment is typically used to confirm the presence of fluorine in a molecule. The large fluorine chemical shift range is highly sensitive to nearby functional groups. The experiment is nearly as sensitive as a 1D PROTON experiment, and the decoupling increases the sensitivity by collapsing the ^{19}F peaks into singlets. All parameters are set from the "Acquire" tab/"Default F19" panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from the “Start” tab/“Standard” panel
Spectral Width (sw)	Type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle	Select typical choices from pull down menu*-controls the tip angle for the pulse
H1 Decoupler offset (dof)	Type in value for the center of the proton chemical shift range to optimize decoupling
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the digital resolution (Hz/point, 1/at)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” panel.



Processing

A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is an lb equal to the peak linewidth at half-height. Other types of apodization are available under the “Process” tab on the “Weighting” panel. Suggested choices for resolution enhancement (at the expense of S/N) are “pseudo echo” and “res-enhance.” When the weighting function is selected, values for these parameters are calculated based on the acquisition time. They may also be set manually with the “Interactive Weighting” button.


CAUTION

Resolution enhancing functions affect the spectral integration- do not use these types of apodization for quantification.

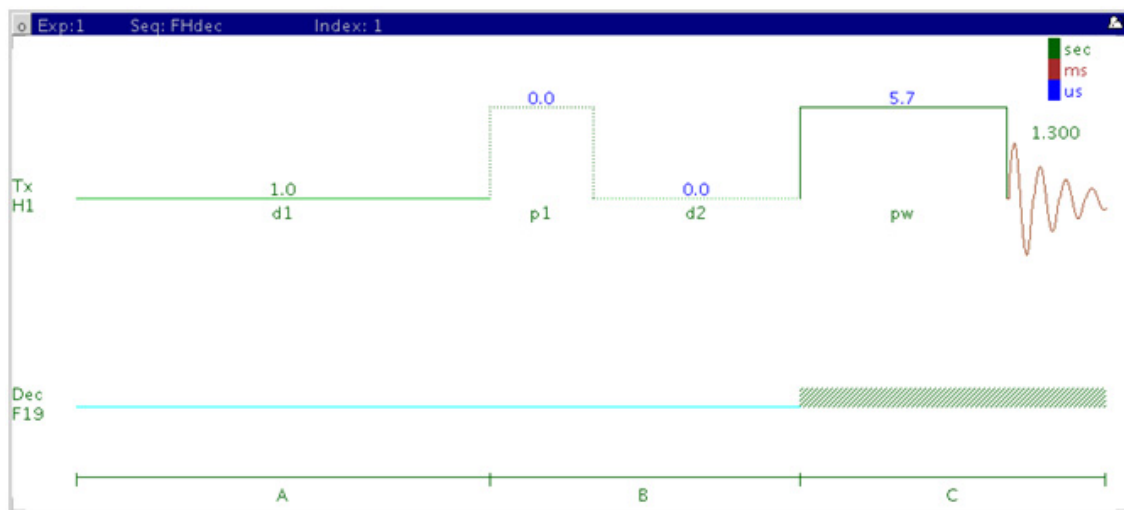
Many NMR probes contain fluorinated materials, which cause broad signals that can distort the baseline (especially when lying next to or outside the spectral window). The default processing parameters are set to use linear prediction to back predict the first seven points of the spectrum in order to flatten the baseline. These settings may be modified under the “Process” tab on the “More 1D” panel.

Tips, tricks, and next steps

- Note: this experiment requires a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file.
- This fluorine experiment produces a proton-decoupled ^{19}F spectrum. See the FLUORINE experiment (Chapter 2) for a proton-decoupled version of the experiment, or set the parameter dm to the value ‘nnn’. Parameters to optimize the decoupling are found on the “Pulse Sequence” tab. However, as the proton chemical shift range is quite small, the default parameters should usually work well (default shape covers an 8000 Hz range).

- To further optimize the spectral width (sw), place one cursor on each side of the desired region on the spectrum, click the  button located under the spectrum window and reacquire the data. The default range is set to cover the typical range of fluorine chemical shifts seen in organic molecules. As this range is very large, once the fluorine signals are located, consider narrowing the chemical shift range and reacquiring the spectrum for better resolution.
- The acquisition time, which controls the digital resolution (Hz/point, which is equal to $1/at$), is set on the “Acquisition” page of the “Acquire” tab. Because FLUORINE spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see small couplings; consider lengthening the acquisition time and/or narrowing the spectral width.
- It must be noted that the total interscan delay is the sum of the acquisition time (at) and the relaxation delay (d1). When acquiring a multi-scan FObs_HDec experiment for quantification, set the interscan delay to at least 5X the longest fluorine T1 value in the molecule.
- When acquiring impure samples of fluorinated compounds from a biological origin, consider using fluorine spectra in lieu of a more complicated proton spectrum as appropriate.

HObs_FDec



General description and usage

Description:

Uses the standard s2pul pulse sequence to acquire a 1D- ^1H dataset with ^{19}F decoupling.

Usage:

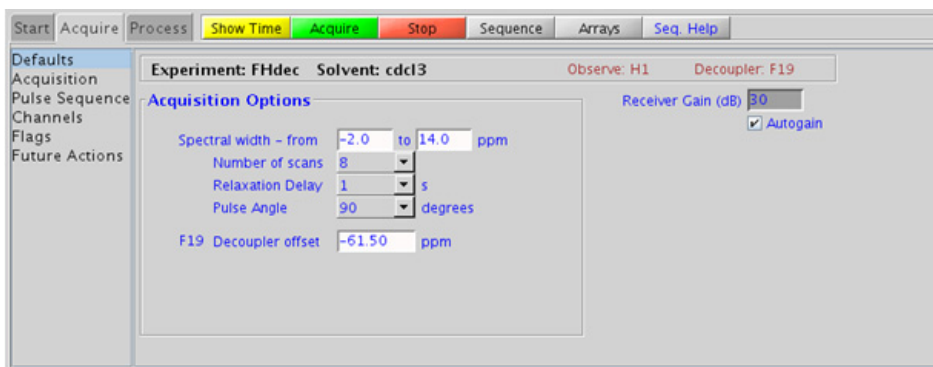
The HObs_FDec experiment can be used to simplify proton spectra with complicated fluorine coupling and to establish which protons are coupled to fluorine. All parameters are set from the “Acquire” tab/”Default F19” panel unless otherwise specified.

Key parameters

Parameter	Description
Solvent (solvent)	Set from the “Start” tab/“Standard” panel
Spectral Width (sw)	Type in specific ppm values
Number of scans (nt)	Select typical choices from pull down menu*
Relaxation Delay (d1)	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle	Select typical choices from pull down menu*-controls the tip angle for the pulse
F19 Decoupler offset (dof)	Type in value for the center of the fluorine chemical shift range to optimize decoupling
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the digital resolution (Hz/point, 1/at)

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” panel.



Processing


A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is an lb equal to the peak linewidth at half-height. Other types of apodization are available under the “Process” tab on the “Weighting” panel. Suggested choices for resolution enhancement (at the expense of S/N) are “pseudo echo” and “res-enhance.” When the weighting function is selected, values for these parameters are calculated based on the acquisition time. They may also be set manually with the “Interactive Weighting” button.

CAUTION

Resolution enhancing functions affect the spectral integration- do not use these types of apodization for quantification.

Tips, tricks, and next steps

- Note: this experiment requires a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file.
- The HObs_FDec experiment produces a fluorine-decoupled ^1H spectrum. See the PROTON experiment (Chapter 2) for a proton-decoupled version of the experiment, or set the parameter dm to the value ‘nnn’. Parameters to optimize the decoupling are found on the “Pulse Sequence” tab. As the fluorine chemical shift range is quite large, it is important to locate the chemical shift(s) of the fluorine(s) for decoupling and set the decoupler offset properly. If there are multiple fluorines in the molecule with large chemical shift differences, consider decoupling a single fluorine at a time. Alternatively, an adiabatic decoupling shape may be created. See the VnmrJ manual for instructions on using Pbox.

- To further optimize the spectral width (sw), place one cursor on each side of the desired region on the spectrum, click the  button located under the spectrum window and reacquire the data. The default range is set to cover the typical range of fluorine chemical shifts seen in organic molecules. As this range is very large, once the fluorine signals are located, consider narrowing the chemical shift range and reacquiring the spectrum for better resolution.
- ^{19}F shaped decoupling (due to large chemical shift range of ^{19}F) typically necessitates higher powers to be used. However decoupling powers above 46 dB are not allowed in the shared mode (thus providing an instrument safety margin). Using the default parameters, the sensitivity of these decoupled experiments is approximately 60% that of the non-decoupled experiment. In case you want to change the decoupling bandwidths (for wider ^{19}F decoupling width) and/or duty cycle (to gain sensitivity and/or lower decoupling power) the decoupling shape must be recreated via the Pulse sequence panel.

11 Proton-fluorine - HF_Expts

Tips, tricks, and next steps



12 JSpectra

JSpectra Tab 406

HETER02DJ 408

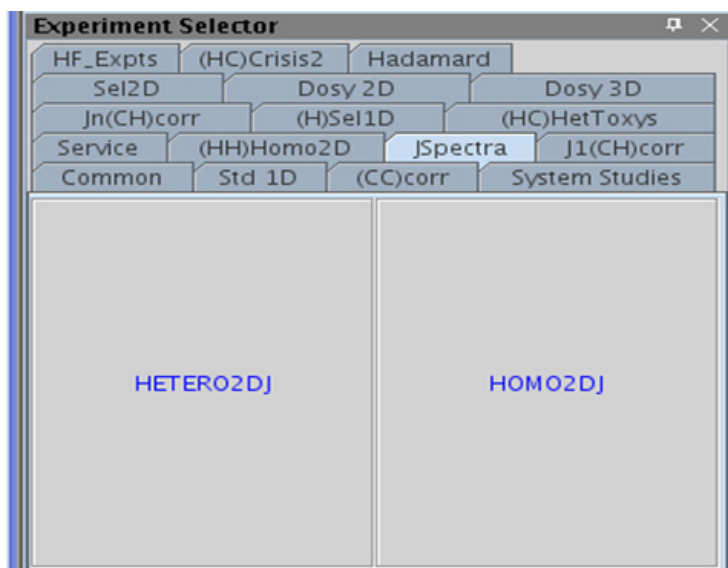
HOM02DJ 412



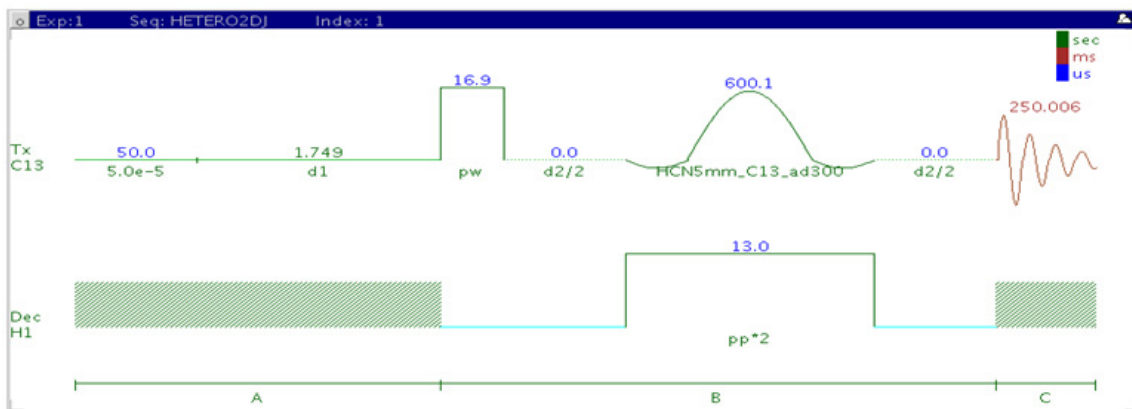
JSpectra Tab

This tab of the experiment selector includes two experiments that provide information regarding coupling constants (J's) in the F1 dimension. The HOMO2DJ experiment has proton chemical shifts in F2 and proton coupling constants in F1, while HETERO2DJ version has carbon chemical shifts in F2 and proton coupling constants in F1. As HETERO2DJ is carbon-detected, it is a very low sensitivity experiment.

These experiments are usually used to resolve complex, overlapping multiplets. The HOMO2DJ is also used to help resolve complicated, multi-component samples, such as from a biological matrix. In addition, with appropriate post-acquisition processing, the F2 projection in HOMO2DJ can be interpreted as a broadband homonuclear decoupled ^1H spectrum.



HETERO2DJ



General description and usage

Description:

Two Dimensional heteronuclear J-resolved spectroscopy.

Usage:

The HETERO2DJ experiment is used to acquire a 2-dimensional spectrum with the ^{13}C chemical shifts in the F2 dimension and the ^1H couplings in F1. The experiment is fairly insensitive as it is carbon-detected.

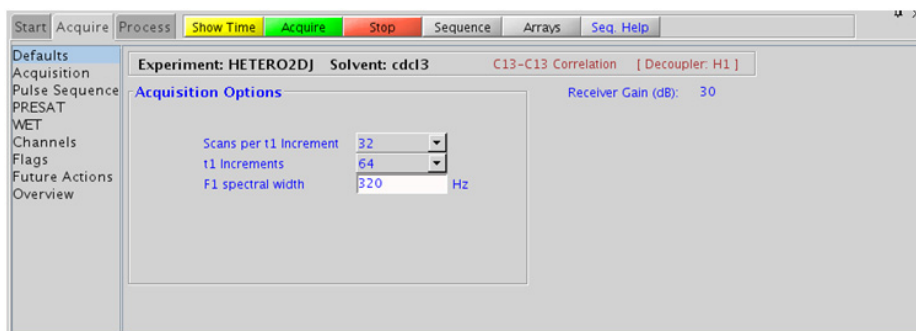
Proton-carbon coupling constants are often used to help define *e/z* stereochemistry of double bonds or molecular conformations, for example, the torsion angles of peptides. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired CARBON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.25s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1
F1 spectral width	Set value for the average range of $^nJ_{C,H}$
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (8k x 128 is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is off). Menus to quickly change the weighting functions in F2/F1 are also present; the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. This arrangement allows for the best resolution and sensitivity combination. As such, the optimal weighting functions are the defaults of a squared shifted sinebell for F2 and gaussian for F1. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) setting transfers from a CARBON if the HETERO2DJ is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C -labelled.
- A typical value for sw1 of at least 3 times the largest expected one-bond coupling constant can avoid any potential folding of methyl quartets.
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. The default setting for this parameter is off.

- The (HC)EXSIDE experiment (see Chapter 7) can also be used to measure selective long-range H-C coupling constants and may provide better sensitivity, particularly when there are only one or two specific couplings of interest.

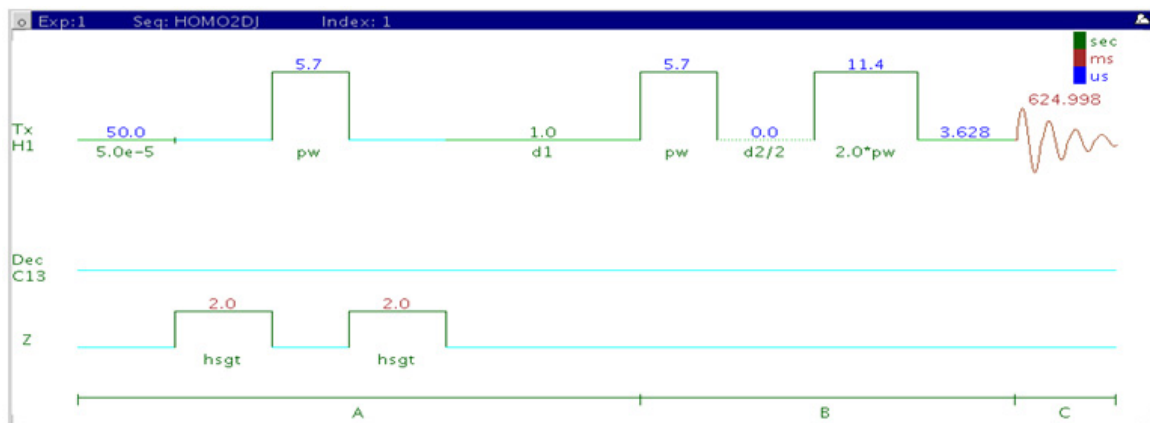
References:

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227.
(COSY)

L. Muller, A. Kumar, R. R. Ernst, J. Magn. Reson. 25 (1977) 383-390. (2DJ)

G. Bodenhausen, G. A. Morris, R. Freeman, D. L. Turner, J. Magn. Reson. 28 (1977) 17-28. (2DJ)

HOM02DJ



General description and usage

Description:

Two Dimensional homonuclear J-resolved spectroscopy.

Usage:

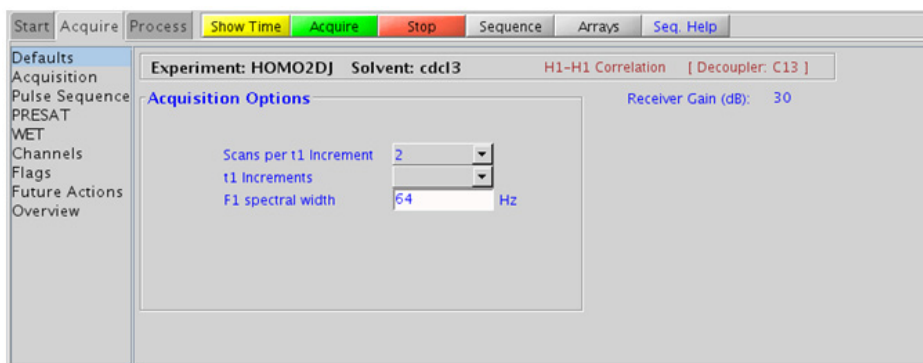
The HOM02DJ experiment is used to acquire a 2-dimensional spectrum with the ^1H chemical shifts in the F2 dimension and the ^1H couplings in F1. This experiment can be used to obtain accurate measurements of couplings when there are complex multiplets and/or overlap in the 1D PROTON spectrum. This experiment can also be used to help resolve overlap in complex multi-component spectra, such as spectra of biofluids. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Spectral Width (sw)	Set from previously acquired PROTON*
Acquisition time (at)	Set from “Pulse Sequence” panel, controls the resolution in F2, default 0.625s
Scans per t1 increment (nt)	Select typical choices from pull down menu*
t1 Increments (ni)	Select typical choices from pull down menu* - controls the resolution in F1
F1 spectral width	Set value for the average range of $^nJ_{H,H}$ – don’t forget couplings constants are both positive and negative
Steady State (ss)	Set from “Pulse Sequence” panel, turn on/off gradient cleanup between scans

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” panel.



Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. The “Process” tab/“Basic” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (8k x 128 is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is off). Menus to quickly change the weighting functions in F2/F1 are also present; the parameters for the weighting functions (defaults are squared sinebells) are calculated automatically from the acquisition time and number of increments. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. More processing details are found under some of the other “Process” panels, such as the “Default”, “Weighting”, and “More 2D” panels.

Tips, tricks, and next steps

- Spectral width (sw) and receiver gain (gain) settings transfer from a PROTON if the HOMO2DJ is set up from this experiment.
- It is highly recommended to adjust sw1 and ni such that ni/sw1 is equal to acquisition time (at) to accommodate the “45° rotate” that is typically applied during processing. Such equal resolution in both F1 and F2 dimension results in much better F2 projection (which mimics a proton decoupled proton spectrum).
- The default number of steady state scans (ss) that are acquired before data acquisition to establish sample equilibrium is 32, which works for most samples. This value is changed on the “Acquisition” panel of the “Acquire” tab if necessary.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.625s and 1s respectively).

References:

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227.
(COSY)

L. Muller, A. Kumar, R. R. Ernst, J. Magn. Reson. 25 (1977) 383-390. (2DJ)

G. Bodenhausen, G. A. Morris, R. Freeman, D. L. Turner, J. Magn. Reson.
28 (1977) 17-28. (2DJ)

12 JSpectra

Tips, tricks, and next steps



13 Hadamard Encoded - Hadamard

Hadamard Tab 418
dqcosyHT 420
gcosyHT 424
hetcorHT 428
hmbcHT 432
hsqcHT 436
hsqctocsyHT 440
noesyHT 444
roesyHT 450
tocsyHT 454

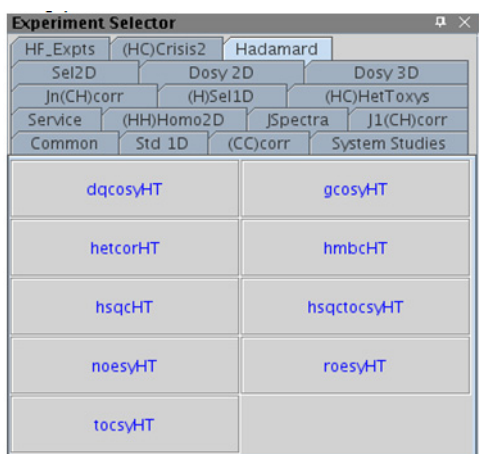


Hadamard Tab

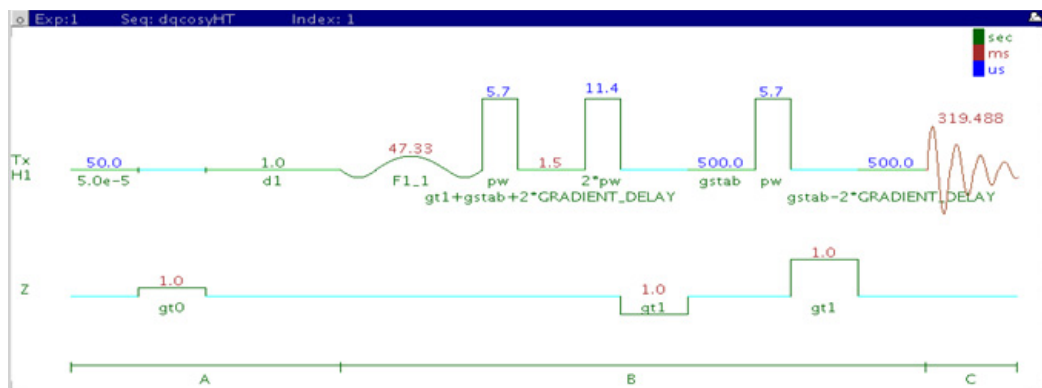
This tab of the Experiment Selector contains a suite of 2D experiments that employ Hadamard encoding. The concept behind Hadamard encoding is to excite the interesting chemical shifts

with an array of frequencies. The Hadamard method of acquiring 2D spectra does offer a 2D spectrum recorded at a fraction of the time of the similar traditional 2D experiment. The sensitivity of a Hadamard experiment is comparable to that of a traditional experiment per unit of time. Consequently the advantage of the Hadamard experiment is realized only when there is enough sensitivity (i.e., sample concentration) to record a fast 2D spectrum.

The specific frequencies for the Hadamard matrix are obtained by selection from a PROTON or a CARBON spectrum as appropriate. The experiments include a full set of standard two-dimensional pulse sequences including homonuclear experiments (dqcosyHT, gcosyHT, tocsyHT, noesyHT, and roesyHT), heteronuclear experiments (hsqcHT, hmbcHT, and hetcorHT), as well as an hsqctocsyHT experiment. All the experiments are processed using the Hadamard transformation, which is set by default in the processing parameters.



dqcosyHT



General description and usage

Description:

Two Dimensional double-quantum filtered J-correlation spectroscopy with Hadamard encoding.

Usage:

The dqcosyHT produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON spectrum and crosspeaks between j-coupled protons. The Hadamard matrix is created from a previously acquired PROTON data set. The advantage of the dqcosyHT over the gcosyHT is that peaks with no double-quantum transitions (for example, singlets) are suppressed, producing cleaner spectra. The experiment is also phase sensitive, resulting in narrower peak shapes. The 90° pulse width is typically calibrated accurately for this experiment. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Hadamard Frequency List	Set from previously acquired PROTON- see Tips, tricks, and next steps for setup description
H1 pulse width (pw)	Type in calibrated 90° pulse value
Relaxation delay (d1)	Type in value- controls the delay between scans
Scans per increment (nt)	Select typical choices from pull down menu*
Steady state scans (ss)	Select typical choices from pull down menu*
Number of increments (ni)	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that are excited)
Number of points in F2 (np)	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2	Select typical choices from pull down menu- usually 2*np

NOTE


Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

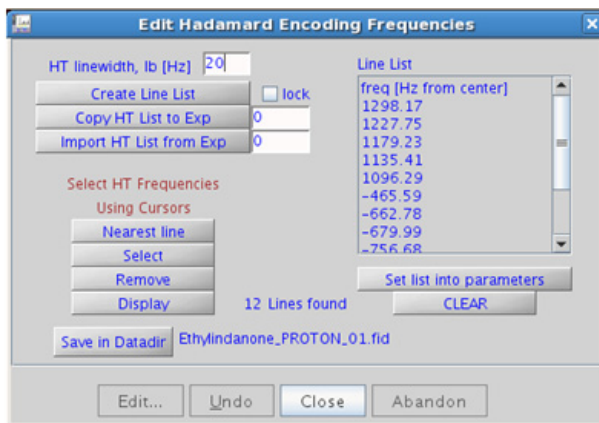
The screenshot shows the 'Hadamard Frequency List Options' panel in the Agilent VnmrJ 4 software. The panel has a title bar with 'Start', 'Acquire', 'Process', 'Show Time', 'Acquire', 'Stop', 'Sequence', 'Arrays', and 'Seq. Help'. The main area is divided into two sections. The left section, titled 'Hadamard Frequency List Options', contains a 'Use' button, a text box with '20 Hz line broadening to', a 'Make HT frequency list' button, and an 'or' button. Below this is a text box with 'Import HT frequencies from exp' and a value of '0'. A status line indicates '12 lines found'. At the bottom are 'Local HT frequencies' and 'Edit HT frequencies' buttons. The right section contains a list of parameters with their values: 'H1 pulse width' (5.70 us), 'Relaxation delay' (1 sec), 'Scans per increment' (1), 'Steady state scans' (4), 'Number of increments' (16), 'Number of points in F2' (4k), and 'Fourier Number in F2' (8k).






Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. Note that a Hadamard- transform is used, such that the parameter “procl” is set to “ht”, which is the default. The “Process” tab/“Default” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details are found under some of the other “Process” panels, such as the “Weighting” and “More 2D” panels.

Tips, tricks, and next steps

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select **Hadamard Experiments > Set Hadamard Encoding** from the Experiments menu to bring up the dialog box below. Click  to automatically create the frequency list:



- Clear  to edit the line list. Place the cursor on the peak to be added or removed and click  or  as appropriate. Click , save the Hadamard frequency list in the Data directory using , and close the dialog box. This frequency list is used for all subsequent Hadamard experiments with PROTON indirect dimension.
- The main advantage of the dqcosyHT versus the gcosyHT is better resolution (phase sensitive versus absolute value experiment) and the absence of singlets that display no couplings. For instance, t-butyl signals, which can be a large source of t1 noise because of their intensity, are suppressed in a dqcosyHT.
- The 90° pulse width needs to be calibrated accurately for best results from the dqcosyHT experiment. See the PROTON experiment in Chapter 2 to calibrate the pulse.

References:

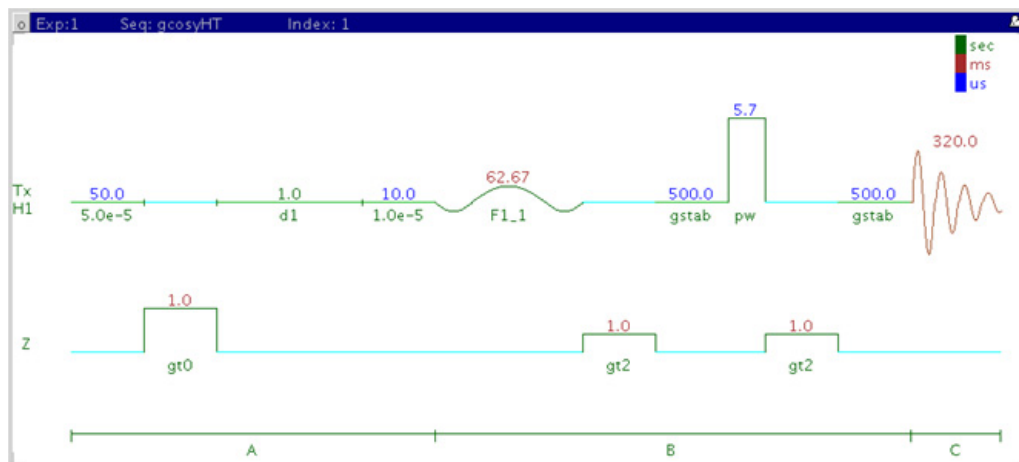
W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

U. Piantini, O. W. Sorensen, R. R. Ernst, J. Am. Chem. Soc. 104 (1982) 6800-6801. (Double Quantum Filter)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

gcosyHT



General description and usage

Description:

Two Dimensional J-correlation spectroscopy with Hadamard encoding and gradient coherence selection.

Usage:

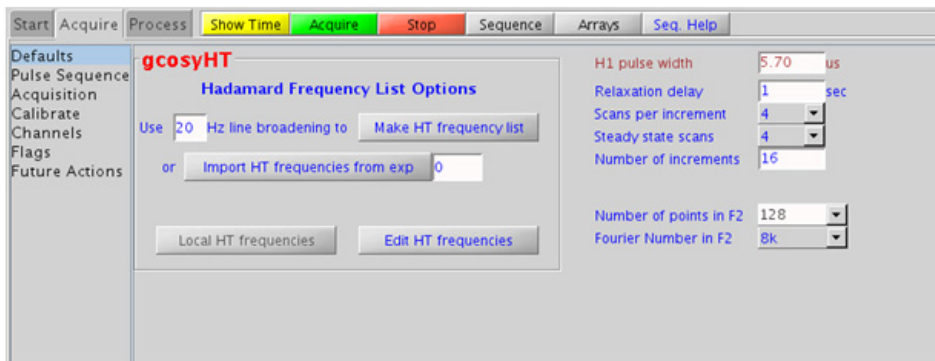
The gcosyHT experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON spectrum and crosspeaks between j-coupled protons. The Hadamard matrix is created from a previously acquired PROTON data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Hadamard Frequency List	Set from previously acquired PROTON- see Tips, tricks, and next steps for setup description
H1 pulse width (pw)	Type in calibrated 90° pulse value
Relaxation delay (d1)	Type in value- controls the delay between scans
Scans per increment (nt)	Select typical choices from pull down menu*
Steady state scans (ss)	Select typical choices from pull down menu*
Number of increments (ni)	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that are excited)
Number of points in F2 (np)	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2	Select typical choices from pull down menu- usually 2*np

NOTE

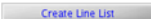
Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

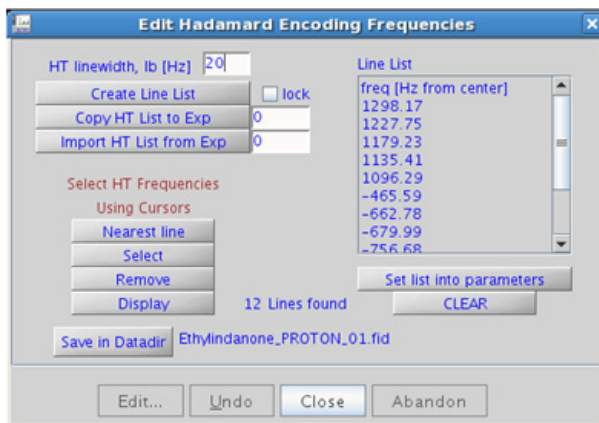





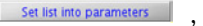

Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter “procl” is set to “ht”, which is the default. The “Process” tab/“Default” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details are found under some of the other “Process” panels, such as the “Weighting” and “More 2D” panels.

Tips, tricks, and next steps

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select **Hadamard Experiments > Set Hadamard Encoding** from the Experiments menu to bring up the dialog box below. Click  to automatically create the frequency list:



- Clear  to edit the line list. Place the cursor on the peak to be added or removed and click  or  as appropriate. Click , save the Hadamard frequency list in the Data directory using , and close the dialog box. This frequency list is used for all subsequent Hadamard experiments with PROTON indirect dimension.

References:

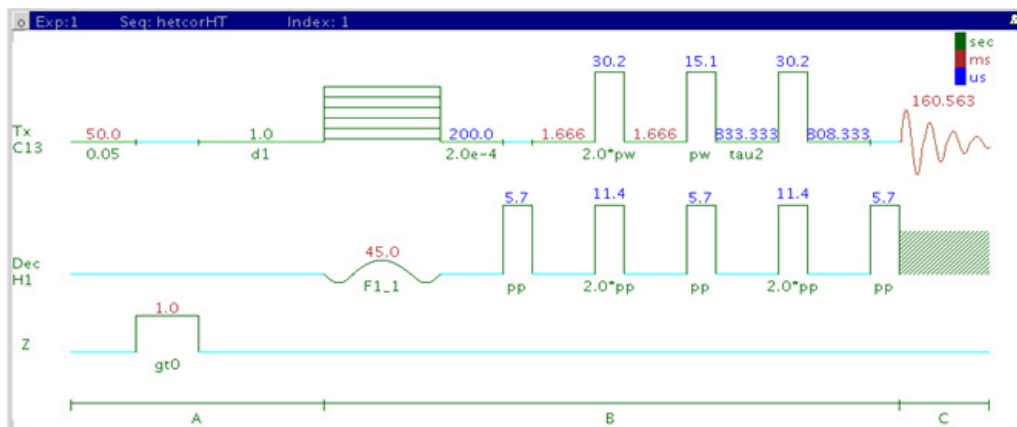
W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

hetcorHT



General description and usage

Description:

Two Dimensional heteronuclear 1-bond correlation with Hadamard encoding (carbon observe).

Usage:

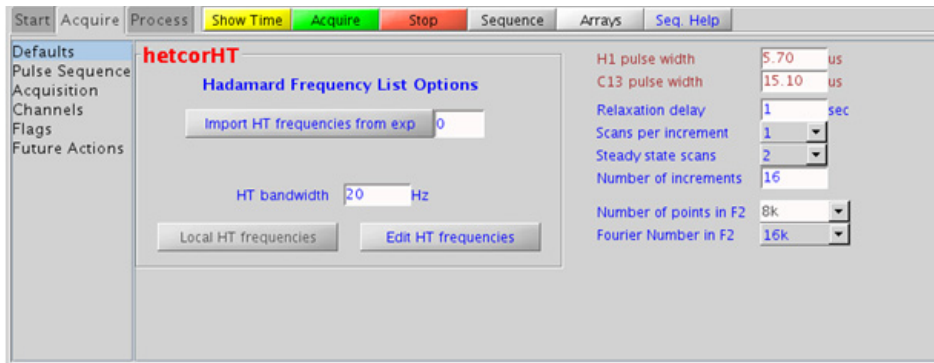
The hetcorHT experiment is used to acquire a 2-dimensional spectrum to correlate protons to directly attached carbons, however unlike the HSQC/HMQC experiments it is carbon-detected, meaning the F1 dimension is ^1H and the F2 dimension is ^{13}C . The Hadamard matrix is created from a previously acquired PROTON data set. The experiment is much less sensitive than the proton-detected hsqcHT, and should only be used under special circumstances where higher resolution is desired in the carbon dimension. All parameters are set from the “Acquire” tab/”Defaults” panel unless otherwise specified.

Key parameters

Parameter	Description
Hadamard Frequency List	Set from previously acquired PROTON- see Tips, tricks, and next steps for setup description
H1/C13 pulse widths (pp/pw)	Type in calibrated 90° pulse values
Relaxation delay (d1)	Type in value- controls the delay between scans
Scans per increment (nt)	Select typical choices from pull down menu*
Steady state scans (ss)	Select typical choices from pull down menu*
Number of increments (ni)	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that are excited)
Number of points in F2 (np)	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2	Select typical choices from pull down menu- usually 2*np

NOTE


Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

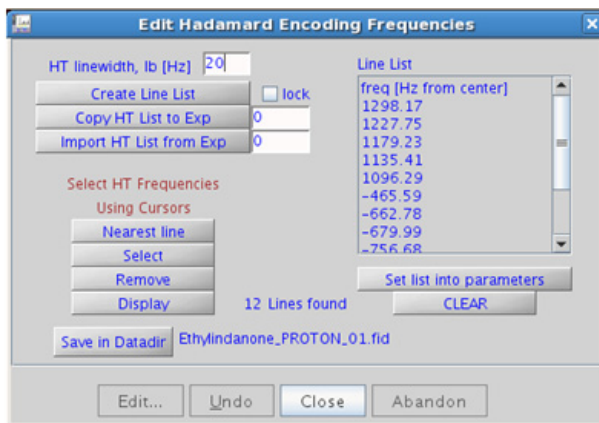


Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter “procl” is set to “ht”, which is the default. The “Process” tab/“Default” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details are found under some of the other “Process” panels, such as the “Weighting” and “More 2D” panels.

Tips, tricks, and next steps

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select **Hadamard Experiments > Set Hadamard Encoding** from the Experiments menu to bring up the dialog box below. Click  to automatically create the frequency list:

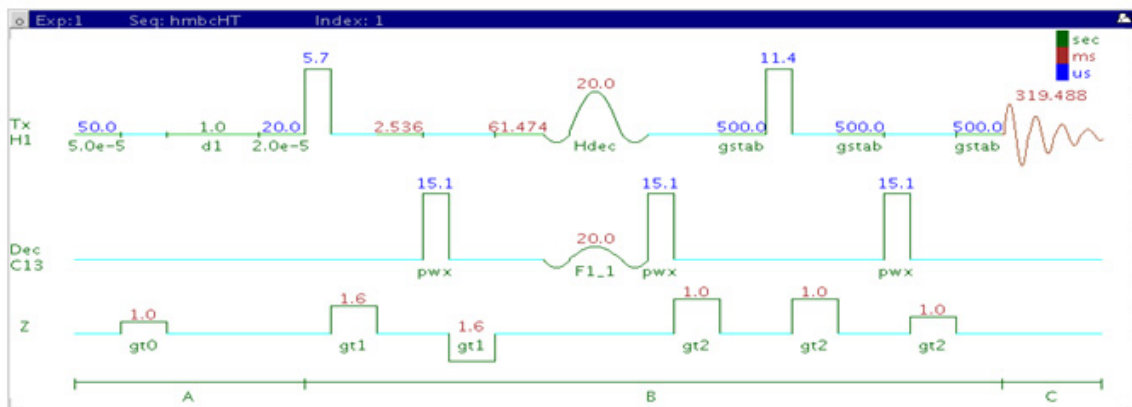


- Clear ☐ lock to edit the line list. Place the cursor on the peak to be added or removed and click or as appropriate. Click , save the Hadamard frequency list in the Data directory using , and close the dialog box. This frequency list is used for all subsequent Hadamard experiments with PROTON indirect dimension.
- The 90° pulse widths for ^1H and ^{13}C need to be calibrated accurately for best results from the hetcorHT experiment.
- In general, the hsqcHT experiment is more sensitive than the hetcorHT. The advantage of the hetcorHT, though, is that a much higher resolution is achieved for the carbon, the F2, dimension. The resulting proton spectrum along the F1 dimensions is effectively a ^1H decoupled proton spectrum.
- Parameters to adjust the type and power for ^{13}C and/or ^1H decoupling are found on the "Pulse Sequence" tab.

References:

- A. Bax, G. A. Morris, J. Magn. Reson. 42 (1981) 501-505. (HETCOR)
- E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)
- E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

hmbcHT



General description and usage

Description:

Two Dimensional heteronuclear multiple-bond J-correlation spectroscopy with Hadamard encoding.

Usage:

The hmbcHT experiment is used to acquire a two-dimensional spectrum to correlate protons to carbons over long range couplings, usually over 2-3 bonds. The Hadamard matrix is created from a previously acquired CARBON data set before acquisition. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Hadamard Frequency List	Set from previously acquired CARBON- see Tips, tricks, and next steps for setup description
H1/C13 pulse widths (pw/pwx)	Type in calibrated 90° pulse values
Relaxation delay (d1)	Type in value- controls the delay between scans
Scans per increment (nt)	Select typical choices from pull down menu*
Steady state scans (ss)	Select typical choices from pull down menu*
Number of increments (ni)	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that are excited)
Number of points in F2 (np)	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2	Select typical choices from pull down menu- usually 2*np

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Start Acquire Process Show Time Acquire Stop Sequence Arrays Seq. Help

Defaults Pulse Sequence Acquisition Calibrate Channels Flags Future Actions

hmbcHT

Hadamard Frequency List Options

Import HT frequencies from exp 0

HT bandwidth 45 Hz


Local HT frequencies Edit HT frequencies

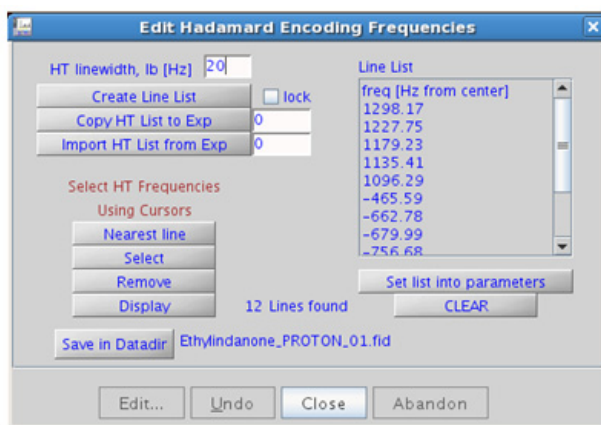
H1 pulse width 5.70 us
C13 pulse width 15.10 us
Relaxation delay 1 sec
Scans per increment 4
Steady state scans 4
Number of increments 16
Number of points in F2 4k
Fourier Number in F2 8k




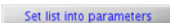

Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter “procl” is set to “ht”, which is the default. The “Process” tab/“Default” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details are found under some of the other “Process” panels, such as the “Weighting” and “More 2D” panels.

Tips, tricks, and next steps

- To setup the Hadamard encoding frequencies, record, process, and autosave a CARBON spectrum. With the PROTON spectrum in current workspace select Hadamard Experiments > Set Hadamard Encoding from the Experiments menu to bring up the dialog box below. Click  to automatically create the frequency list:

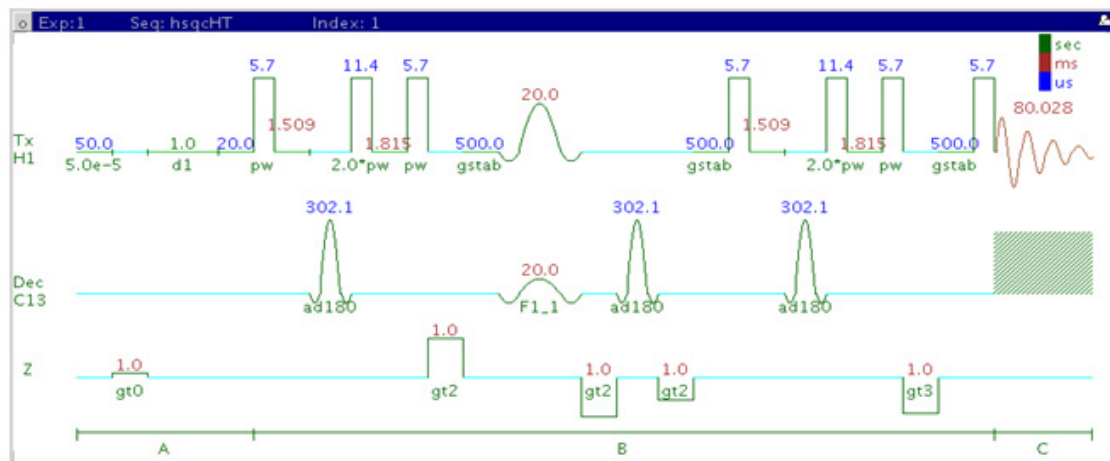


- Clear  to edit the line list. Place the cursor on the peak to be added or removed and click  or  as appropriate. Click , save the Hadamard frequency list in the Data directory using , and close the dialog box. This frequency list is used for all subsequent Hadamard experiments with CARBON indirect dimension.
- While editing the CARBON frequency list, it is important to remove solvent resonances from the list.
- Parameters to adjust the J-couplings $^1J_{C,H}$ and $^nJ_{C,H}$ (defaults are 140 Hz and 8 Hz) are found on the “Pulse Sequence” tab. The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity.

References:

- A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)
- E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)
- E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

hsqcHT



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with Hadamard encoding.

Usage:

The hsqcHT experiment is used to acquire a 2-dimensional spectrum to correlate protons to directly attached carbons. The Hadamard matrix is created from a previously acquired CARBON data set before acquisition. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Hadamard Frequency List	Set from previously acquired PROTON- see Tips, tricks, and next steps for setup description
H1/C13 pulse widths (pw/pwx)	Type in calibrated 90° pulse values
Relaxation delay (d1)	Type in value- controls the delay between scans
Scans per increment (nt)	Select typical choices from pull down menu*
Steady state scans (ss)	Select typical choices from pull down menu*
Number of increments (ni)	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that are excited)
Number of points in F2 (np)	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2	Select typical choices from pull down menu- usually 2*np

NOTE


Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

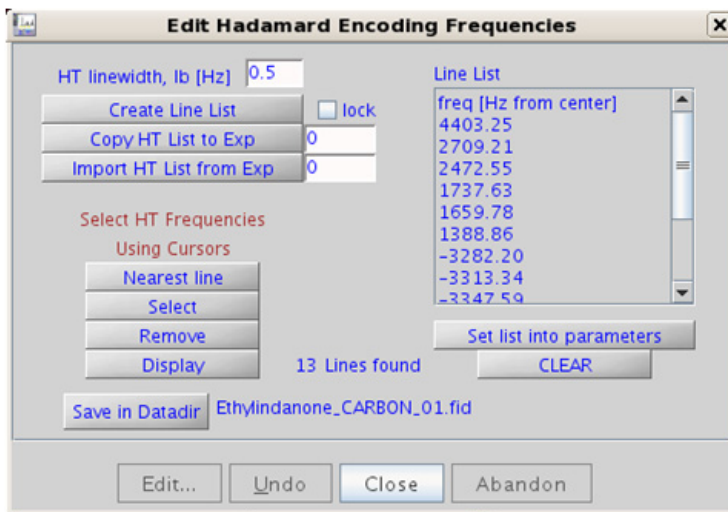
The screenshot displays the 'hsqcHT' acquisition setup window. On the left, a sidebar lists navigation options: Defaults, Pulse Sequence, Acquisition, Calibrate, Channels, Flags, and Future Actions. The main panel is titled 'hsqcHT' and 'Hadamard Frequency List Options'. It features a button 'Import HT frequencies from exp 0' and a field for 'HT bandwidth' set to 45 Hz, with 'Local HT frequencies' and 'Edit HT frequencies' buttons below it. On the right, a list of parameters is shown with their current values and units: H1 pulse width (5.70 us), C13 pulse width (15.10 us), Relaxation delay (1 sec), Scans per increment (4), Steady state scans (4), Number of increments (16), Number of points in F2 (1026), and Fourier Number in F2 (8k).

Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter “procl” is set to “ht”, which is the default. The “Process” tab/“Default” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details are found under some of the other “Process” panels, such as the “Weighting” and “More 2D” panels.

Tips, tricks, and next steps

- To setup the Hadamard encoding frequencies, record, process, and autosave a CARBON spectrum. With the PROTON spectrum in current workspace select Experiments menu ® Hadamard Experiments ® Set Hadamard Encoding from the menu to bring up the dialog box below. Click  to automatically create the frequency list:



- Clear ☐ lock to edit the line list. Place the cursor on the peak to be added or removed and click **Select** or **Remove** as appropriate. Click **Set list into parameters**, save the Hadamard frequency list in the Data directory using **Save in Datadir**, and close the dialog box. This frequency list is used for all subsequent Hadamard experiments with CARBON indirect dimension.
- While editing the CARBON frequency list, it is important to remove solvent resonances from the list.
- Parameters to adjust the type and power for ^{13}C decoupling are found on the “Pulse Sequence” tab. To measure ^1H - ^{13}C (or ^1H -X) coupling constants, the carbon decoupling is turned off by entering “nnn” in the Channel 2 “Dec On/Off” entry on the “Channels” panel or alternatively by typing `dm='nnn'` on the command line.

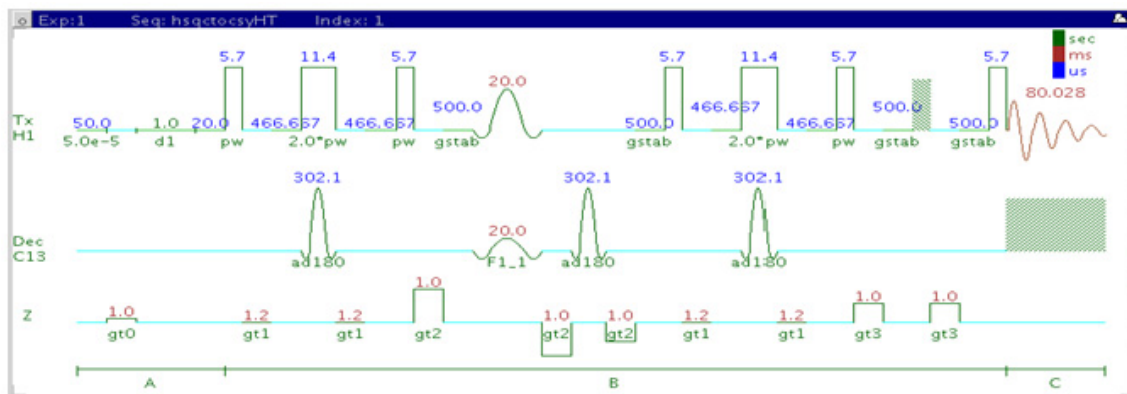
References:

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189.
(HSQC)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122.
(Hadamard – Review)

hsqctocsyHT



General description and usage

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock and Hadamard encoding.

Usage:

The hsqctocsyHT experiment is used to acquire a 2-dimensional spectrum to connect ^1H TOCSY correlations with ^{13}C nuclei chemical shift information. For example, Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^1H TOCSY by spreading them out over the carbon (or other X-nuclei) chemical shift range. The Hadamard matrix is created from a previously acquired CARBON data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

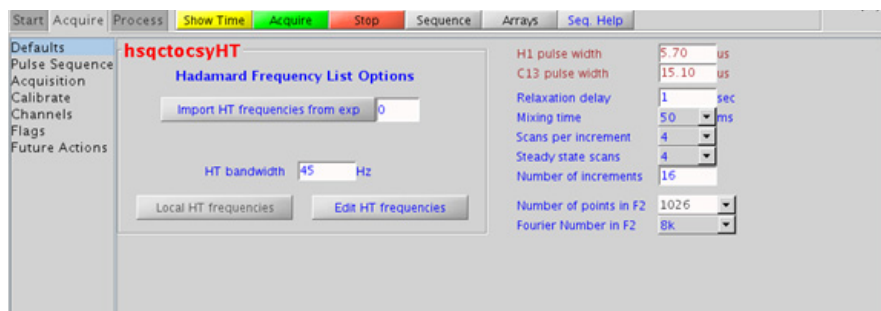
Key parameters

Parameter	Description
Hadamard Frequency List	Set from previously acquired CARBON- see Tips, tricks, and next steps for setup description
H1/C13 pulse widths (pw/pwx)	Type in calibrated 90° pulse values
Relaxation delay (d1)	Type in value- controls the delay between scans
Scans per increment (nt)	Select typical choices from pull down menu*
Steady state scans (ss)	Select typical choices from pull down menu*
Number of increments (ni)	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that are excited)
Mixing time (mix)	Select from pull down menu- 80ms works best for large spin systems*
Number of points in F2 (np)	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2	Select typical choices from pull down menu- usually 2*np
Hadamard Frequency List	Set from previously acquired CARBON- see Tips, tricks, and next steps for setup description
H1/C13 pulse widths (pw/pwx)	Type in calibrated 90° pulse values

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.


13 Hadamard Encoded - Hadamard Processing

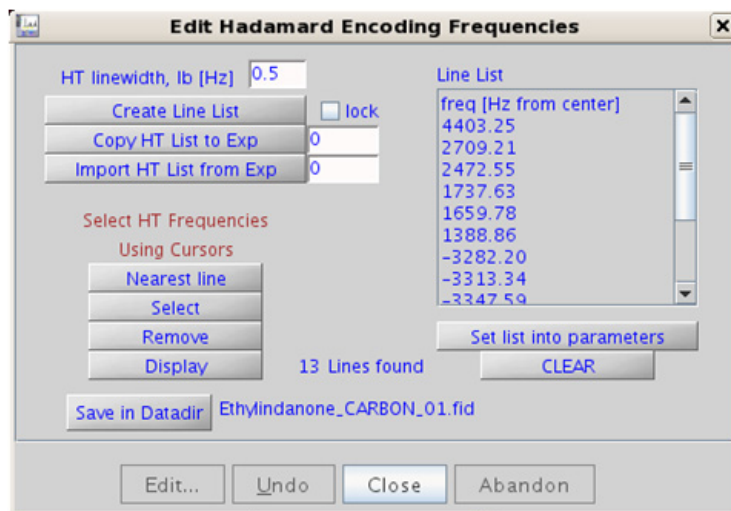


Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter “proc1” is set to “ht”, which is the default. The “Process” tab/“Default” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details are found under some of the other “Process” panels, such as the “Weighting” and “More 2D” panels.

Tips, tricks, and next steps

- To setup the Hadamard encoding frequencies, record, process, and autosave a CARBON spectrum. With the PROTON spectrum in current workspace select **Hadamard Experiments > Set Hadamard Encoding** from the Experiments menu to bring up the dialog box below. Click  to automatically create the frequency list:

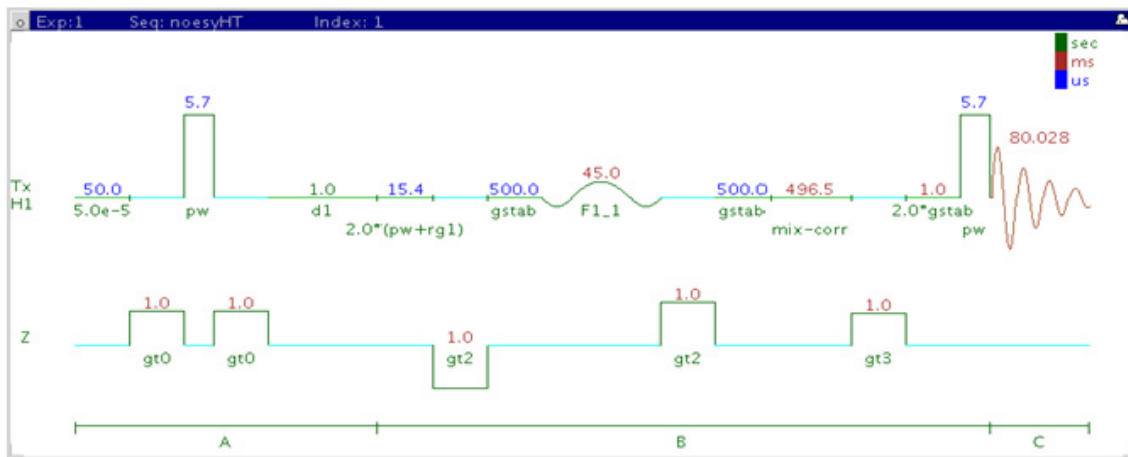


- Clear ☐ lock to edit the line list. Place the cursor on the peak to be added or removed and click **Select** or **Remove** as appropriate. Click **Set list into parameters**, save the Hadamard frequency list in the Data directory using **Save in Datadir**, and close the dialog box. This frequency list is used for all subsequent Hadamard experiments with CARBON indirect dimension.
- While editing the CARBON frequency list, it is important to remove solvent resonances from the list.

References:

- K. E. Kover, O. Prakash, V. J. Hruby, J. Magn. Reson. A103 (1993) 92-96. (z-filtered HSQC-TOCSY)
- E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)
- E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

noesyHT



General description and usage

Description:

Two Dimensional through-space correlation spectroscopy with Hadamard encoding.

Usage:

The noesyHT experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically $<5 \text{ \AA}$). The Hadamard matrix is created from a previously acquired PROTON data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameter	Description
Hadamard Frequency List	Set from previously acquired PROTON- see Tips, tricks, and next steps for setup description
H1 pulse width (pw)	Type in calibrated 90° pulse value
Relaxation delay (d1)	Type in value- controls the delay between scans
Mixing time (mix)	Select from pull down menu- 500ms works well as a starting point*
Scans per increment (nt)	Select typical choices from pull down menu*
Steady state scans (ss)	Select typical choices from pull down menu*
Number of increments (ni)	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that are excited)
Number of points in F2 (np)	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2	Select typical choices from pull down menu- usually 2*np

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” panel.

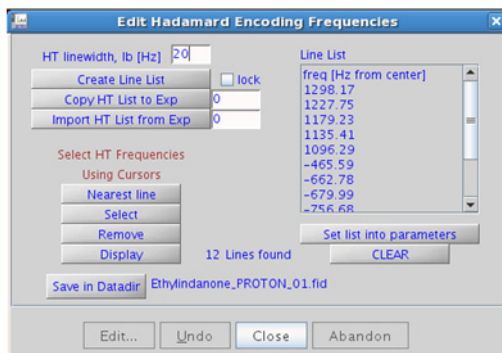
The screenshot shows the 'noesyHT' acquisition panel in the Agilent VnmrJ 4 software. The panel is divided into several sections. On the left, there is a sidebar with options: Defaults, Pulse Sequence, Acquisition, Calibrate, Channels, Flags, and Future Actions. The main area is titled 'noesyHT' and contains a 'Hadamard Frequency List Options' section. This section includes a 'Use' dropdown set to '20', a 'Hz line broadening to' input field, and a 'Make HT frequency list' button. Below this, there is an 'or' option, an 'Import HT frequencies from exp' button, and a '0' input field. At the bottom of this section are 'Local HT frequencies' and 'Edit HT frequencies' buttons. On the right side of the panel, there are several parameters with their values and units: 'H1 pulse width' is 5.70 us, 'Relaxation delay' is 1 sec, 'Mixing time' is 500 ms, 'Scans per increment' is 4, 'Steady state scans' is 4, 'Number of increments' is 16, 'Number of points in F2' is 1026, and 'Fourier Number in F2' is 8k.




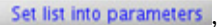

Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter “procl” is set to “ht”, which is the default. The “Process” tab/“Default” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details are found under some of the other “Process” panels, such as the “Weighting” and “More 2D” panels.

Tips, tricks, and next steps

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select Hadamard Experiments > Set Hadamard Encoding from the Experiments menu to bring up the dialog box below. Click **Create Line List** to automatically create the frequency list:



- Clear  to edit the line list. Place the cursor on the peak to be added or removed and click  or  as appropriate. Click , save the Hadamard frequency list in the Data directory using , and close the dialog box. This frequency list is used for all subsequent Hadamard experiments with PROTON indirect dimension.
- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).
- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation is confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time.
- Medium-sized molecules (MW ~500 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOEs. Consider acquiring a roesyHT for these molecules. Alternatively one may consider, (i) choosing a more viscous solvent such as d₆-DMSO or d₆-DMSO with ~10-20% D₂O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- Small molecules may have crosspeaks arising from chemical exchange during the NOE mixing time and have the same phase as the diagonal. The exchange crosspeaks provide information about inter-converting molecular conformations and can be used to recognize such molecular dynamic processes.

References:

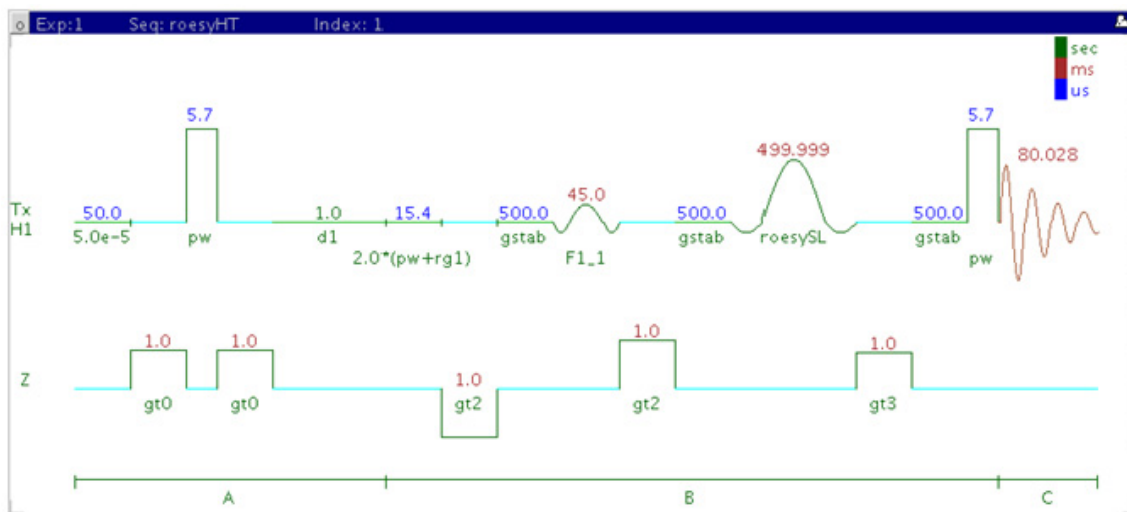
S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

13 Hadamard Encoded - Hadamard

Tips, tricks, and next steps

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122.
(Hadamard – Review)

roesyHT



General description and usage

Description:

Two Dimensional through-space correlation spectroscopy in the rotating frame with Hadamard encoding.

Usage:

The roesyHT experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically $<4 \text{ \AA}$). The Hadamard matrix is created from a previously acquired PROTON data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Hadamard Frequency List	Set from previously acquired PROTON- see Tips, tricks, and next steps for setup description
H1 pulse width (pw)	Type in calibrated 90° pulse value
Relaxation delay (d1)	Type in value- controls the delay between scans
Mixing time (mix)	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms*
Scans per increment (nt)	Select typical choices from pull down menu*
Steady state scans (ss)	Select typical choices from pull down menu*
Number of increments (ni)	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that are excited)
Number of points in F2 (np)	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2	Select typical choices from pull down menu- usually 2*np

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

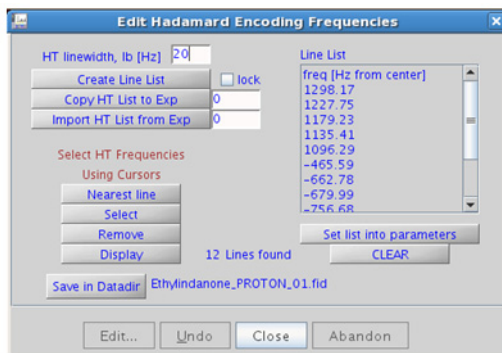
The screenshot shows the 'roesyHT' software interface. The top menu bar includes 'Start', 'Acquire', 'Process', 'Show Time', 'Acquire', 'Stop', 'Sequence', 'Arrays', and 'Seq. Help'. The left sidebar lists navigation options: 'Defaults', 'Pulse Sequence', 'Acquisition', 'Calibrate', 'Channels', 'Flags', and 'Future Actions'. The main window is titled 'roesyHT' and 'Hadamard Frequency List Options'. It contains several input fields and buttons. The 'Use' field is set to '20 Hz line broadening to'. Below it, there is a button 'Make HT frequency list' and a text input 'Import HT frequencies from exp' with a value of '0'. At the bottom, there are two buttons: 'Local HT frequencies' and 'Edit HT frequencies'. On the right side, there are several parameters with their values and units: 'H1 pulse width' (5.70 us), 'Relaxation delay' (1 sec), 'Mixing time' (500 ms), 'Scans per increment' (4), 'Steady state scans' (4), 'Number of increments' (16), 'Number of points in F2' (1026), and 'Fourier Number in F2' (8k).




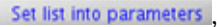

Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter “procl” is set to “ht”, which is the default. The “Process” tab/“Default” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details are found under some of the other “Process” panels, such as the “Weighting” and “More 2D” panels.

Tips, tricks, and next steps

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select Hadamard Experiments > Set Hadamard Encoding from the Experiments menu to bring up the dialog box below. Click **Create Line List** to automatically create the frequency list:

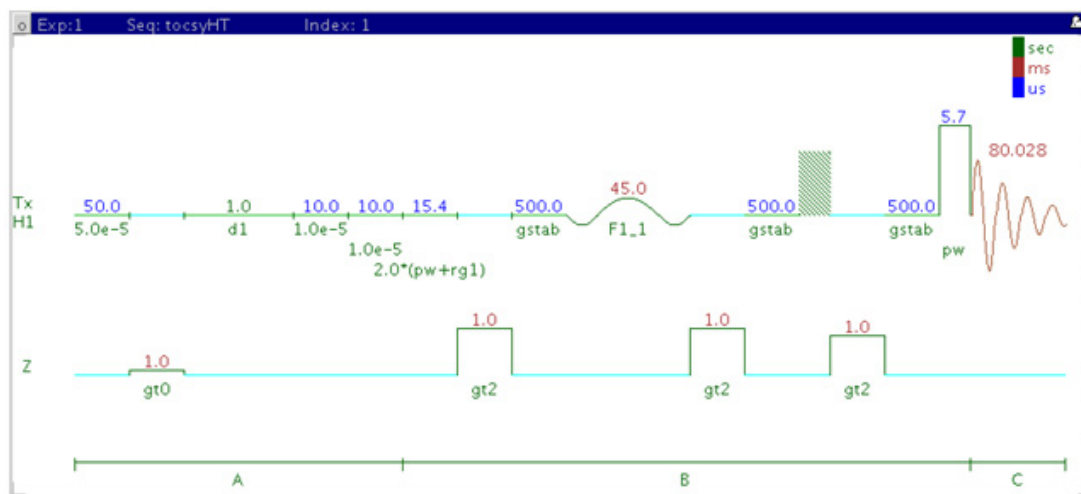


- Clear  to edit the line list. Place the cursor on the peak to be added or removed and click  or  as appropriate. Click , save the Hadamard frequency list in the Data directory using , and close the dialog box. This frequency list is used for all subsequent Hadamard experiments with PROTON indirect dimension.
- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.
- Because the mixing time for the roesyHT is limited, weaker correlations may not be observable. roesyHT is the experiment of choice over noesyHT for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOEs. One may consider running noesyHT experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d₆-DMSO or d₆-DMSO with ~10-20% D₂O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).
- Crosspeaks in the roesyHT spectrum that have the same phase as the diagonal may arise from chemical exchange during the ROE mixing time, or due to TOCSY-type correlations (couplings). The exchange crosspeaks provide information about inter-converting molecular conformations and can be used to recognize such molecular dynamic processes.

References:

- A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)
- E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)
- E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

tocsyHT



General description and usage

Description:

Two Dimensional total J-correlation spectroscopy for scalar coupled spin systems with Hadamard encoding.

Usage:

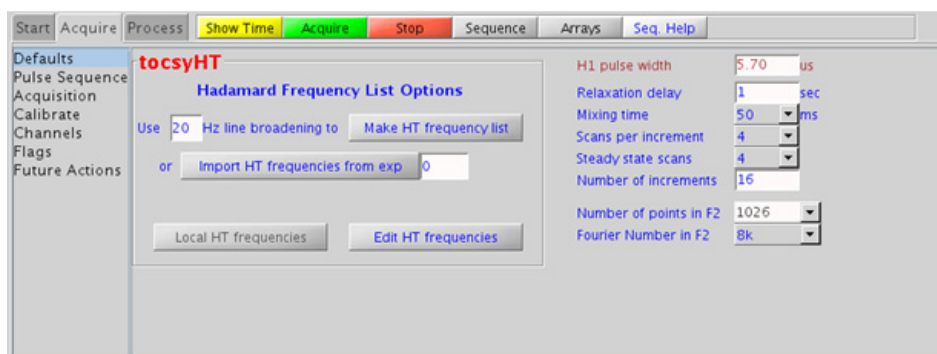
The tocsyHT experiment produces a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between all protons in a scalar coupled spin system. The Hadamard matrix is created from a previously acquired PROTON data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Key parameters

Parameters	Description
Hadamard Frequency List	Set from previously acquired PROTON- see Tips, tricks, and next steps for setup description
H1 pulse width (pw)	Type in calibrated 90° pulse value
Relaxation delay (d1)	Type in value- controls the delay between scans
Mixing time (mix)	Select from pull down menu- 80ms works best for large spin systems*
Scans per increment (nt)	Select typical choices from pull down menu*
Steady state scans (ss)	Select typical choices from pull down menu*
Number of increments (ni)	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that are excited)
Number of points in F2 (np)	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2	Select typical choices from pull down menu- usually 2*np

NOTE

Specific values for these parameters may be typed manually in the appropriate boxes on the “Acquisition” panel.

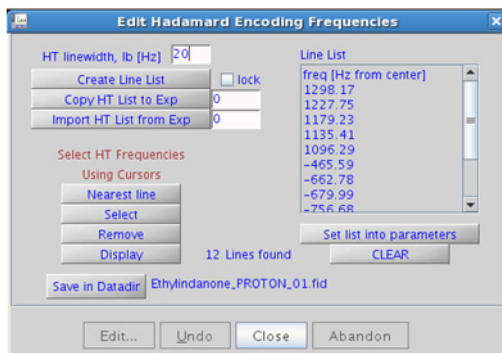





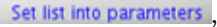

Processing

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter “procl” is set to “ht”, which is the default. The “Process” tab/“Default” panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details are found under some of the other “Process” panels, such as the “Weighting” and “More 2D” panels.

Tips, tricks, and next steps

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select Hadamard Experiments > Set Hadamard Encoding from the Experiments menu to bring up the dialog box below. Click **Create Line List** to automatically create the frequency list:



- Clear  to edit the line list. Place the cursor on the peak to be added or removed and click  or  as appropriate. Click , save the Hadamard frequency list in the Data directory using , and close the dialog box. This frequency list is used for all subsequent Hadamard experiments with PROTON indirect dimension.
- The spinlock time ultimately controls how far along the spin system the correlations extend. A value of 50 or 80ms is typically a good choice.
- A pull-down menu to change the waveform for the tocsyHT spinlock is found on the “Pulse Sequence”. The DIPSI2 spinlock pattern is a good choice for small molecules and FLOPSY8 is typically used for larger molecules.

References:

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

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Revision A, March 2013



G7446-90515



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