PeptLab

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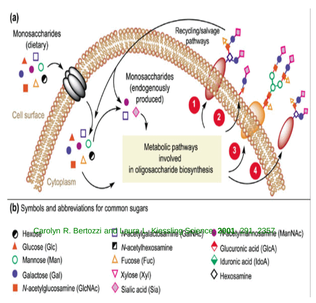
Glycosylation and Neurodegenerative disorders

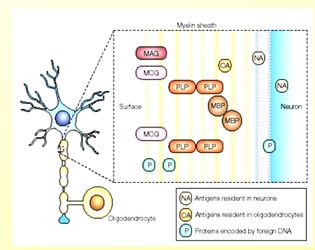
N-Glycosylation is a co-translational phenomenon that modifies the immunogenicity of an antigen, inducing a specific tridimensional conformation in the protein and playing a significant role in immune responce.
Indeed, a loss or change in glycosylation (glycosylation defects) are often associated with a large number of neurodegenerative disorders, as Multiple Sclerosis (MS) and Rett Syndrome. In recent years, novel forms of glycoproteins have been characterized: it has been found that in *Halobacterium halobium*, β-D-glucose is attached on the Asn side chain.
The Asn residue is always located in a specific aminoacidic sequon of the type Asn-Xaa-Ser/Thr (where Xaa is any amino acid except Pro).



Carolyn R. Bertozzi and Laura L. Kiessling Science 2001, 291, 2357

Multiple Sclerosis

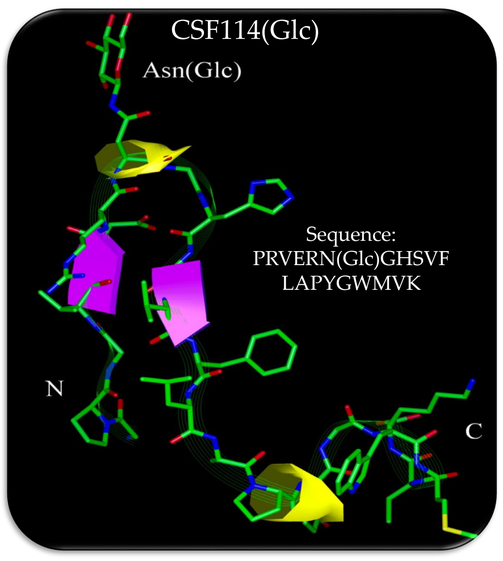
Multiple Sclerosis (MS) is the most known chronic, inflammatory, demyelinating disease of the central nervous system (CNS), characterized by a progressive neurodegeneration, caused by an autoimmune response to self-antigens in genetically susceptible individuals. It can be considered as a group of diseases, whose different mechanisms have not been yet characterized. In the last years, the role of autoantibodies has been reevaluated and they can be used as specific disease biomarkers.



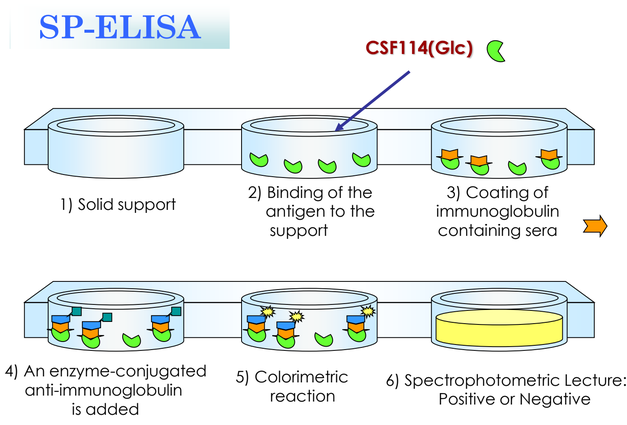
Rett Syndrome

Rett syndrome (RTT) is a relatively rare clinical form of autism affecting almost exclusively females, with a frequency of about 1:10,000 worldwide. RTT is specifically characterized by loss of acquired purposeful hand skills, loss of acquired spoken language, gait dyspraxia and stereotypic hand movements. RTT has recently been proposed as a model of Mutiple Sclerosis (MS), a severe neurological progressive disease. Up to 95% of typical RTT cases are caused by mutations in the methyl-CpG binding protein 2 (MeCP2), a gene located in the Xq28 region of the human genome that encodes a protein whose full list of functions still remains unknown.

The multivalency concept to develop ligands bearing multiple copies of Asn(Glc) to increase autoantibody affinity



CSF114(Glc) is a simple, reliable, and efficient synthetic glycopeptide, capable of detecting autoantibodies in a statistically significant number of Multiple Sclerosis (MS) patients and Rtt Syndrome. The corresponding native antigen(s) possibly triggering anti-CSF114(Glc) antibodies have not been yet identified and characterized. In this work, autoantibodies purified from MS patients' sera by immunoaffinity against CSF114(Glc) were used to reveal possible antigens in brain tissue.



ELISA is the main technology for screening human sera in many reference laboratories of clinical chemistry. In our laboratory, ELISA was used for the validation processes of the first test, MSPepKit, detecting autoantibodies in MS patient sera. In this case, **CSF114(Glc)** is the Antigenic Probe exploiting the specificity of immunoglobulin recognition.

However, despite being highly specific, sensitive and reliable, ELISAs require multistage time-consuming processes. Moreover, they are difficult to standardize and show large variations between manufacturers and different laboratories.

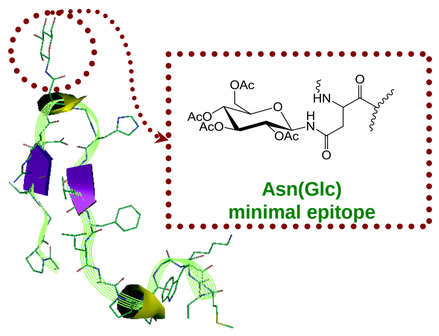
Lolli, F. et al. *Proc. Natl. Acad. Sci.* **2005**, 102, 10273-10278; F. Lolli,et al. *J. Neuroimmunol.* **2005** 167(1-2), 131-137, A.M. Papini *J. Pept. Sci.* ***2009****; 15: 621–628*.

The multivalency concept to develop ligands bearing multiple copies of Asn(Glc) to increase autoantibody affinity

Aberrant modifications of proteins components of organs or tissues target of immune-mediated diseases can contribute to create neo-antigens, producing autoantibodies to the neoepitopes. Autoantibodies can be important biomarkers of disease activity but they are often characterised by a low binding affinity affecting their reliable identification. This is possibly because of the lower molecular weight of the epitope compared to the protein antigen. To explore the potentiality of the multivalent interaction to increase the binding affinity, the poly-Asn(Glc) epitope, **CT35**, was designed and synthesized.
The original N-glucosylated type I' β-turn peptide structure CSF114(Glc) and the new multimeric probe CT35 were tested in SP-ELISA on sera of RTT patients and MS patients compared to normal blood donors (NBD).

Monovalent Interaction

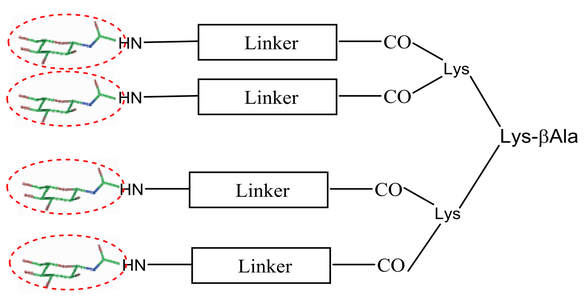
The β-hairpin motif exposing the minimal epitope Asn(Glc) on the tip of a type I' β-turn of the β-hairpin structure.



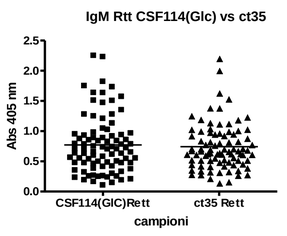
CSF114(Glc)

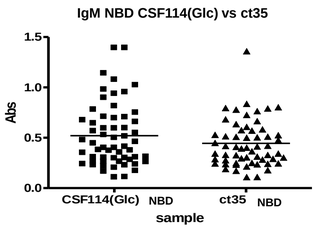
VS

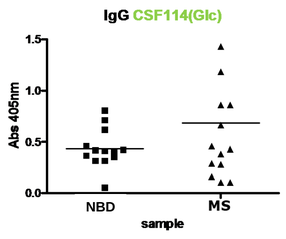
Multivalent Interaction

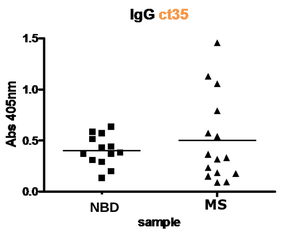


CT35





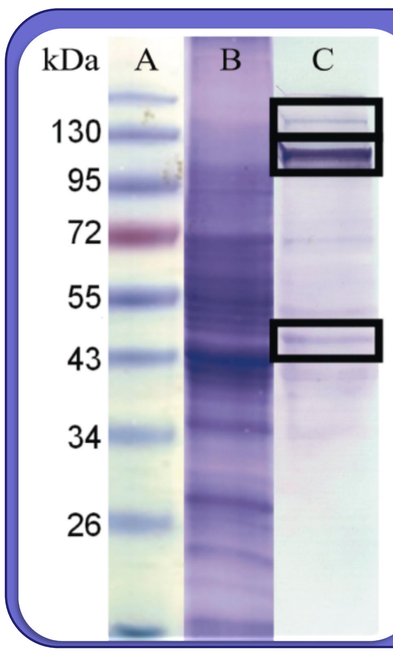




J. Hayek et al. Italian Patent n FI2012A000107.

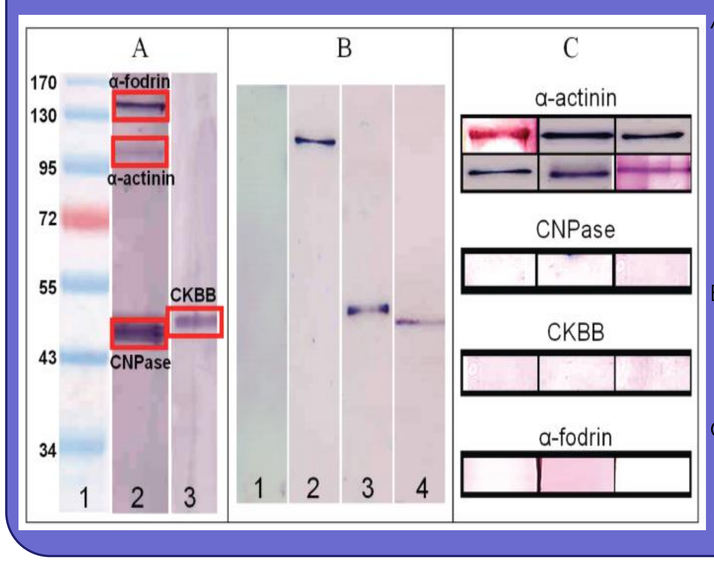
The data showed that mono - and multi-N-glucosylated epitopes recognized IgM antibodies with comparable affinity.

Alpha actinin is specifically recognized by multiple sclerosis autoantibodies isolated using an N-glucosylated peptide epitope



Rat brain proteins are selectively recognized by CSF114(Glc) specific IgGs from Multiple Sclerosis (MuS) patients. SDS-PAGE of rat brain proteins and Western Blot (WB)using anti-CSF114(Glc) purified from individual MuS patients' sera. The circled bands in lane C indicate as the prevailing band at 98 kDa, detected in all patients examined (6/6) whereas the others bands (47 and 130 kDa) were recognized only in three out of six patients' sera. (A) MW markers; (B) brain proteins SDS-PAGE; (C) WB of brain proteins with anti-CSF114(Glc).

WB of authentic proteins with CSF114(Glc) specific IgGs from MuS patients or commercially available mAbs.



A. WB of rat brain proteins detected with commercial monoclonal IgGs. 1: MW Markers, 2: Rat brain proteins detected by: antialpha fodrin (1:400), anti-CNPase (1:500), anti-alpha actinin (1:100); 3: Rat brain proteins incubated with: anti-CKBB (1:400).
B. WB of authentic proteins incubated with commercial monoclonal IgGs. 1: alpha fodrin; 2: alpha actinin; 3: CKBB; 4: CNPase.
C. WB of authentic proteins detected by anti-CSF114(Glc) IgGs purified from individual MuS patients' sera.

Experimantal stages:

1. purification of autoAbs from MuS sera,
2. detection of Ags in the rat brain displaying immunoreactivity toward the MuS autoAbs,
3. MALDI identification of putative Ags,
4. assessment of immunoreactivity of putative Ags and their standard protein counterparts against either specific mAbs or the CSF114(Glc) Abs.

The CSF114(Glc) Abs detected three distinct protein bands in the rat brain, which upon MALDI and MS/MS analyses led to the identification of four proteins: **alpha fodrin, alpha actinin 1, CNPase and creatine kinase**. However, with the exception of **alpha actinin 1**, CNPase, alpha fodrin and creatin kinase, in their authentic form, failed to be recognized by CSF114(Glc) Abs.

S. Pandey, et al. (2013). MOLECULAR CELLULAR PROTEOMICS, vol. 12(2), p. 277-282

The *Haemophilus influenzae* HMW1 adhesin is glycosylated by the bacterial transferase HMW1C and could be a target of Multiple Sclerosis' autoantibodies

The *Haemophilus influenzae* HMW1 Adhesin Is a Glycoprotein with an Unusual *N*-Linked Carbohydrate Modification[*][S]

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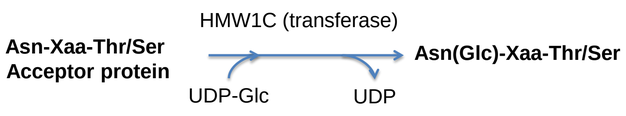
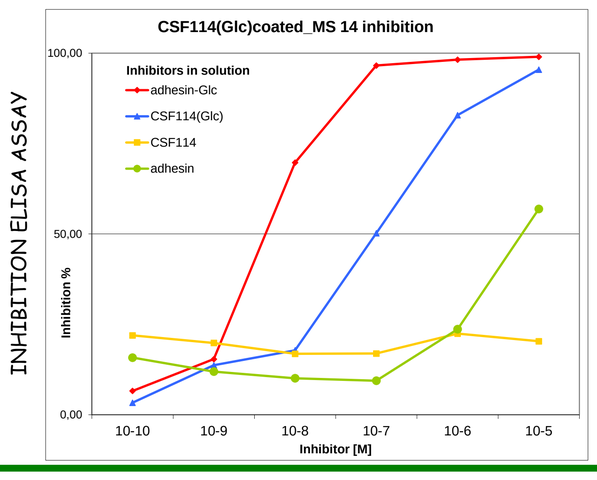
Julia Gross[‡], Susan Grass[§], Alan E. Davis[‡], Petra Gilmore-Erdmann[‡], R. Reid Townsend[‡¶], and Joseph W. St. Geme III[§1]



Structure of mature HMW1 adhesin-(Glc) residues 441- 1536 (36kDa)

Purple: extracellular adhesin known fragment, residues 1205-1536 with 12 potential Asn-Xaa-Thr/Ser glycosites: yellow and red



Anti CSF114(Glc) IgGs have a higher affinity for adhesin-Glc ($IC_{50}=10^{-8.5}$M) than for CSF114(Glc) ($IC_{50}=10^{-7}$M); Non glucosylated protein/peptide have no activity