Iron in Biology

Iron is an essential element for virtually all living organisms:
- the iron content in bacteria is of the order of $10^5$-$10^6$ iron ions per cell;
- virtually all living microorganisms require a minimum effective concentration of $10^{-8}$ M for growth.

Iron ranks fourth in abundance of all elements on the Earth’s crust, but its bioavailability is limited.
Under aerobic conditions iron(III) is the dominant oxidation state and is characterized by an extremely low solubility (of the order of $10^{-18}$ M, at pH of 7.4).
Iron(II) is more soluble, but available to organisms only in anaerobic environments. In the presence of air it becomes toxic due to its redox chemistry which leads to the formation of ferric iron and reactive oxygen species.
As a consequence of these considerations, iron in biological systems is found tightly sequestered by high-affinity iron-binding proteins.

Ferritin

The solution that Nature has evolved to create a reservoir of bioavailable iron is a set of iron storage proteins that shield iron and prevent it from damaging other molecules, yet allow it to be released when needed. Ferritins are the major class of iron storage proteins found in bacterial, plant and animal cells.
Ferritins form hollow spherical particles with external diameter of about 12 nm and a large internal cavity (~ 8 nm) in which 2000 to 4500 iron atoms can be stored as iron(III)-oxo biomineral.

Ferritins self-assemble from 24 subunits with 4-helix bundle structure. Some of them host in their interior a ferric oxidase site, i.e., a reaction center where the uptake of iron(II) is catalytically oxidized to iron(III) and then migrates to the biomineralization cavity. The structure is pierced with several channels that are proposed to play a role in iron transport to and from the interior.
The biomineralization of iron in ferritin is a complex multistep reaction:
$$2\text{Fe}^{2+} + \text{O}_2 + (\text{H}_2\text{O})_x \rightarrow \text{Fe}_2\text{O}_3(\text{H}_2\text{O})_x + 4\text{H}^+ + \text{H}_2\text{O}_2$$
The overall process is governed by iron trafficking processes:
- The ferrous ions enter the protein shell and diffuse to the ferroxidase site, in the middle of four-helix bundle.
- Diferric products of the catalytic coupling with O$_2$ should navigate through the protein cage, as the nanomineral nuclei grow in the large central ferritin cavity.
The process requires a number of weak transient interactions between the protein matrix and the ferrous and ferric species.

Ferritin NMR @ CERM

Why NMR?
NMR might represent a key investigation tool for its unique ability to reveal transient interactions between protein amino acids and other chemical entities including metal ions.
Ferritin (MW 480 kDa) is much larger than proteins generally affordable by NMR.
At CERM we have developed a combined solution/solid state NMR approach that has allowed us to obtain a partial protein assignment.
Interaction studies with iron have provided insight into the mechanism of the reaction at the ferroxidase site and on the pathway of ferric precursors travelling towards the biomineralization cavity.

Complementary X-ray studies
Using soaking of ferritin crystal in iron(II) solutions followed by flash-freezing we have been able to observed transiently bound iron species at the ferroxidase site.

These studies complement lower-resolution NMR information in solution.

The role of the channels
We have prepared a number of mutants to analyze the role of residues in the pores to investigate the role of these channels measuring the kinetic of iron uptake and release.

References
- Lalli D, Turano P. Solution and solid state NMR approaches to draw iron pathways in the ferritin nanocage. Acc Chem Res. 2013, http://dx.doi.org/10.1021/ar4000893