Published in final edited form as:

Nat Chem Biol. 2013 March ; 9(3): 143–144. doi:10.1038/nchembio.1182.

Cage redesign explains assembly

Elizabeth C Theil and

The Children's Hospital Oakland Research Institute, Oakland, California, USA, and the Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, North Carolina, USA

Paola Turano

The Magnetic Resonance Center (CERM), University of Florence, Florence, Italy, and the Department of Chemistry, University of Florence, Florence, Italy

Elizabeth C Theil: etheil@chori.org; Paola Turano: turano@cerm.unifi.it

Abstract

Control of protein self-assembly and disassembly, which is central to metabolism and engineering applications, remains challenging. Here, a perspicacious redesign of interfaces in the multisubunit ferritin protein cage provides single, modifiable subunits that assemble with Cu^{2+} templating and give insights into the cage assembly code.

Supramolecular protein assemblies occur throughout cell biology and have been engineered for delivering drugs, sensors and imaging agents as well as for synthesizing nanomaterials and polymers. Yet several features of these assemblies are poorly understood: often, disassembly conditions inhibit reassembly. Isolated subunits from such assemblies can be unstable. Additionally, the codes that drive self-assembly are not always obvious. Viral capsids¹, bacterial carboxysomes² and ferritin protein cages are archetypical self-assembled proteins. The ferritin family, composed of Fe^{2+} -O catalytic protein nanocages and caged iron biominerals, is ubiquitous, mainly intracellular and, in animals, required for life³. To gain insight into and control over these systems, and ferritin in particular, Huard *et al.*⁴ now report a new protein engineering approach that allows them to isolate stable monomers of human H ferritin subunits and control reassembly of natural or modified multisubunit complexes.

Ferritins are antioxidants: conversion of Fe^{2+} to caged minerals using O_2 or H_2O_2 both consumes oxy radical-generating species and conserves iron for recovery from stress. Ferritin minerals are also concentrated forms of iron that can be used for metabolism. These protein cages, with multiple sites for catalysis, mineral nucleation and mineral growth, are used in applications of natural proteins to nanotechnology^{5,6}. Each ferritin cage, composed of 12 or 24 identical or similar polypeptide subunits folded into four α -helix bundles, is pierced by ion channels⁷ around three-fold symmetry axes; the channels control Fe^{2+} entry (via catalysis) and exit (via mineral dissolution). Although the secondary and quaternary structures of ferritin protein cages are highly conserved, the location, iron ligands, catalytic mechanisms and sequences vary; sequence conservation can be as low as 20%. Indeed, ferritin cages synthesized in animal tissues have different numbers of catalytic sites and nucleation channels or sites of conversion from catalytic products to mineral nuclei during transit to the mineral growth cavity⁸; these differences in protein cage structure similarly translate into differences in iron mineral crystallinity within the mineral cavity. The wide

range of variability among ferritin family members has slowed identification of the cage self-assembly code and target residues that could be manipulated to yield reversible disassembly and assembly.

Excitingly, the new design strategy of Huard *et al.*⁴ yields large amounts of single, ferritin polypeptide subunits, making them accessible for study, modification and controlled reassembly. The overall process, named reverse metal-templated interface redesign (rMeTIR; Fig. 1), includes inserting metal-coordinating residues at protein-protein interfaces while removing native contacts, yielding complexes that are dependent on the desired metal ion for assembly. To apply the strategy to ferritin, the authors first introduced two bis-histidine motifs on separate subunits to bind added Cu^{2+} , allowing control of cage assembly. The dimer (or C_2) ferritin cage symmetry axes, with the largest interfacial surfaces within the protein cage, were selected for modification. By judiciously replacing conserved C_2 interfacial residues having intersubunit hydrophobic and hydrogen bonding interactions (Leu56, Arg63 and Glu67) with histidines and two nearby hydrogen-bonding residues with carboxylates (Y39E, N74E) to introduce some electrostatic repulsion, single subunits were obtained in good yields. Interestingly, the Cu^{2+} -triggered cage assembly was not structurally perturbed by Cu^{2+} removal by EDTA⁴. Ferritin cage function was also retained in the engineered construct: caged iron minerals with more than 300 iron atoms formed when Fe^{2+} was added, as shown by transmission electron microscopy. The isolated subunits, which could be derivatized with one of two fluorophores (including IAEDANS), were dependent on the newly introduced metal-binding residues for assembly, as upon reassembly no Cu^{2+} was found at the natural Cu^{2+} sites, which occur on C_3 and C_4 symmetry axes in ferritin protein crystals⁹.

With the rMeTIR-designed monomers in hand, the authors were able to measure, for the first time, unfolding transitions for single subunits by CD spectroscopy: unexpectedly, the monomer lost structure at 40 °C or in 1 M guanidine, whereas the complete cage was stable to >80 °C or 4–6 M guanidine, demonstrating the huge stability gain from the interfacial interactions formed during cage assembly. The critical role of C_2 interfacial geometry in ferritin cage self-assembly was emphasized by the metal selectivity in rMeTIR, where Cu^{2+} , which uses paired, square planar histidine coordination, was substantially more effective in triggering cage assembly than Zn^{2+} or Ni^{2+} , which use tetrahedral (Zn^{2+}) or octahedral (Ni^{2+}) histidine coordination geometry. This metal selectivity in reassembly of the rMeTIR-modified ferritin cage provided an added benefit of identifying the hydrogen bond geometry of the C_2 axes as a critical feature in ferritin's protein-protein self-assembly code.

The very large size of ferritin cages (480 kDa and 240 kDa) and of other protein assemblies has limited the study of reactivity, dynamics and subdomain folding and unfolding in solution by atomic resolution methods such as NMR spectroscopy. Although attempts to study high-molecular-weight assemblies have driven the development of innovative NMR approaches, which, in the case of ferritin, have revealed unpredicted structural and functional features such as protein-based aggregation of the Fe^{3+} -O- Fe^{3+} catalytic products and conversion to Fe_2O_3 - H_2O mineral precursors⁸, the methods are far from standardized. rMeTIR⁴ has the potential to obtain subunits in good yields and specifically labeled subunits in reassembled protein cages from all ferritins. Given the rich complexity and variability of the ferritin protein cage family, the possibilities for additional ferritin cage assembly information, modifications and applications are vast. With such constructs of ferritins and other protein assemblies available, NMR and previously inaccessible methodologies can be used to obtain higher-resolution structural and mechanistic information that unravel the role of subunit cooperativity important in viral pathogenesis and normal cell biology as well as to modulate properties of these natural containers for the delivery of drugs and sensors in nanotechnology.

References

1. Douglas T, Young M. *Science*. 2006; 312:873–875. [PubMed: 16690856]
2. Tanaka S, et al. *Science*. 2008; 319:1083–1086. [PubMed: 18292340]
3. Theil EC. *Curr Opin Chem Biol*. 2011; 15:304–311. [PubMed: 21296609]
4. Huard DJE, Kane KM, Tezcan FA. *Nat Chem Biol*. 2013; 9:169–176. [PubMed: 23340339]
5. Kobayashi M, et al. *Chem Commun (Camb)*. 2011; 47:3475–3477. [PubMed: 21305096]
6. Terashima M, et al. *Biomaterials*. 2011; 32:1430–1437. [PubMed: 21074263]
7. Tosha T, Ng HL, Bhattasali O, Alber T, Theil EC. *J Am Chem Soc*. 2010; 132:14562–14569. [PubMed: 20866049]
8. Turano P, Lalli D, Felli IC, Theil EC, Bertini I. *Proc Natl Acad Sci USA*. 2010; 107:545–550. [PubMed: 20018746]
9. Bertini I, et al. *J Am Chem Soc*. 2012; 134:6169–6176. [PubMed: 22424302]

Figure 1.

Engineering controllable protein assemblies with rMeTIR. **(a)** Modification of self-assembling, 24-subunit ferritin cages was achieved by introducing intersubunit metal-binding sites and eliminating intersubunit hydrogen bonding sites in the C2-C2 interface (shown as two purple bundles of four α-helices) to produce single subunits that self-assemble in the presence of Cu²⁺. **(b)** A single subunit of a ferritin cage, made accessible by rMeTIR in large yields, for study and modification. **(c)** The engineered metal site between two of the 24 subunits, occupied by Cu²⁺. **(d)** Cross-section of an assembled ferritin nanocage after single-subunit modification with the fluorescent dye IAEDANS (green) and subsequent reassembly of tagged subunits by addition of Cu²⁺. Panels **a**, **c** and **d** reprinted with permission from ref. 4.