

Protein Nanocages

The Versatile Molecular Shell

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While scientists and engineers are working hard to build micro- and nano-scale structures, nature has perfected the ability to build highly symmetrical and complex architecture at all scales. *Vanda* 'Miss Joaquim' orchid – the Singapore national flower – and diatom are a couple examples of beautiful macro- and micro-scale structures among many others. Zooming into subcellular structures, ATPase and ribosome are nano-scale molecular machines responsible for the synthesis of ATP – the energy currency of life – and for translating genetic information into protein, respectively. The proteins that are synthesized can self-assemble into nanostructures of various shapes and sizes.

Protein nanocages are such structure. They are composed of multiple protein subunits that self-assemble with excellent precision forming a hollow highly symmetrical and complex caged structure of nanometer size (1/1000 of the average width of a strand of human hair). The smallest repeating unit usually consists of two, three, or five protein subunits. The repeating units subsequently self-assemble to form tetrahedral, dodecahedral, or icosahedral structures – similar to a soccer ball – of 24, 60, or more protein subunits with or without pores on the apices. The beauty of the multiple-subunit nature of the protein nanocage is that a modification on a single subunit will result in multiple modifications on the fully assembled structure. Explorations of the protein nanocages have unveiled their versatility as multifunctional nanoplatforms.^[1]

Among the myriad of natural protein nanocages a few have been extensively studied (Fig. 1): Ferritin (Ftn), E2 protein, heat shock protein (Hsp), DNA-binding protein from starved cells (Dps), viral

capsids, and the vault. Most of these protein nanocages assume spherical shape while the vault assumes an ellipsoidal shape. Their natural functions vary from regulation of iron homeostasis in a physiological system by storing excess iron within its core, catalysis of glycolysis product to the substrate of tricarboxylic acid cycle in glucose metabolism, folding/unfolding of other proteins in response to elevated temperature, protecting DNA from oxidative damage, nucleic acid cargo carrier, to functions that are yet to be elucidated.

By leveraging on their natural catalytic property and ability to store molecules within

the hollow core, scientists have successfully used these nanocages as reaction vessels and templates to synthesize nanoparticles.^[2] This synthesis method results in highly uniform-sized nanoparticles owing to the precise natural control of the template. Beside iron nanoparticles, other metal nanoparticles such as gold, silver, manganese, platinum, as well as semi-conductor nanoparticles have been synthesized.^[3] The hybrid metal core with a protein shell has been shown to be promising in applications as magnetic resonance imaging (MRI) contrast agent and electrocatalyst in the methanol oxidation reaction in a fuel cell.^[4]

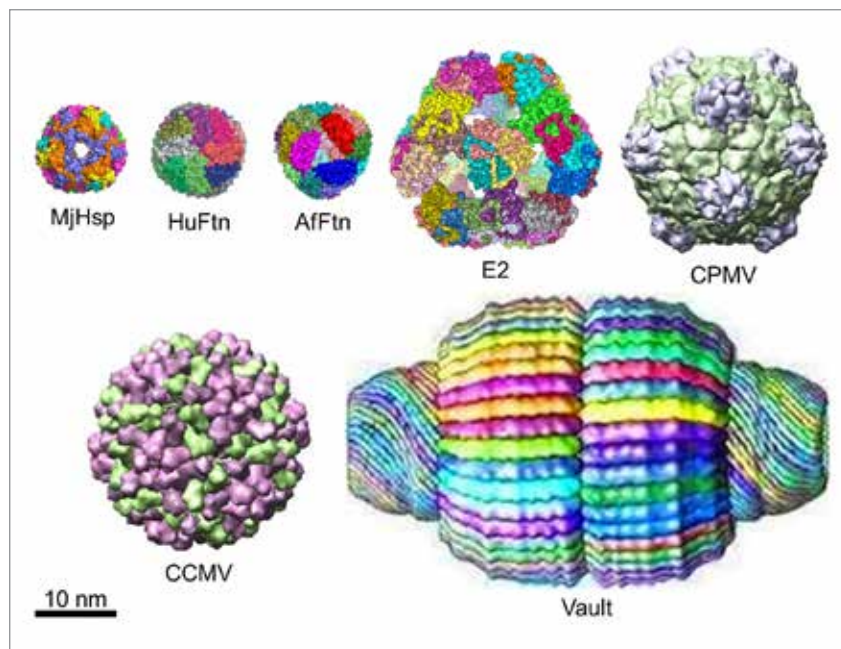


Figure 1. Schematic presentation of some commonly studied protein nanocages and their sizes. **MjHsp**: *Methanococcus jannaschii* heat shock protein (PDB code 1SHS); **HuFtn**: human ferritin (PDB code 2FHA); **AfFtn**: *Archaeoglobus fulgidus* ferritin (PDB code 1S3Q); **E2**: *Geobacillus stearothermophilus* E2 protein of pyruvate dehydrogenase multi-enzyme complex (PDB code 1B5S); **CPMV**: Cowpea mosaic virus (PDB code 1NY7); **CCMV**: Cowpea chlorotic mottle virus (PDB code 1ZA7); **Vault**: major vault protein (PDB code 2ZV5). The colors indicate a single or a cluster of protein subunits. Images are obtained from Protein Data Bank (www.pdb.org) and drawn using PyMOL.^[14]

Engineering of the protein nanocages has led to the expansion of their functions beyond the natural ones. There are three surfaces that can be engineered: the internal surface, the external surface, and the interface between the subunits.^[5] The internal surface of a protein nanocage does not naturally carry any therapeutic cargo. However, by substituting specific amino acids – the building blocks of protein, it is possible to provide an anchor onto which small molecule or macromolecule therapeutics can be attached to. The anticancer drug doxorubicin has been shown to be successfully encapsulated without jeopardizing the drug efficacy.^[6] Distinct from small molecule encapsulation into the lumen of the other protein nanocages that relies on chemical interactions (e.g. covalent, ionic, and hydrophobic), macromolecule encapsulation into the vault lumen is facilitated by attachment to a protein fragment called INT. The INT binds specifically to the internal surface of the vault through protein-protein interactions and acts as a shuttle to ferry the macromolecule cargos into the vault.^[7]

Towards applications in targeted delivery of the therapeutic cargo, the external surface of the protein nanocages can be decorated with peptides that can aid in binding to the surface of cancer cells.^[8] Besides decorating the external surface with cancer-specific targeting ligands, display of epitopes on the external surface opens a potential of using the protein nanocages as vaccine platforms.^[9] Keeping in mind that the protein nanocages are composed of multiple protein subunits, multiple epitopes and ligands can be simultaneously displayed on the surface and the only limitation is steric hindrance. The interface between subunits is the least explored surface yet the most interesting as it determines the self-assembly feature of the protein nanocages.

The self-assembly process is central to the formation of the caged protein architecture. Modulation of the self-assembly process has implications on controlling molecular therapeutic cargo release from the lumen of the protein nanocage. For example, cancerous tissue environment is slightly more acidic compared to the healthy tissue.^[10] Substituting amino acids located at the interface of subunits

with histidines has been demonstrated to impose pH-responsive disassembly profile on a protein nanocage.^[11] Histidine is an amino acid containing a side chain that is positively charged at acidic pH, while it remains uncharged at neutral and basic pH-s. If clusters of histidines are placed close enough to each other at the interface and are accessible, they will repel each other due to the same charge upon arriving at acidic pH environment. The result is the destabilization of the interaction between the subunits and the disassembly, facilitating molecular release from the lumen of the protein nanocage. Besides applications in controlling release of molecular cargo, the self-assembly process can also be used as a strategy to trap molecules into the lumen. Several studies show that by first disassembling the protein nanocages, one can reassemble them into their original caged architectures. During this reassembly process, molecules such as nanoparticles and other enzymes can be encapsulated.^[12]

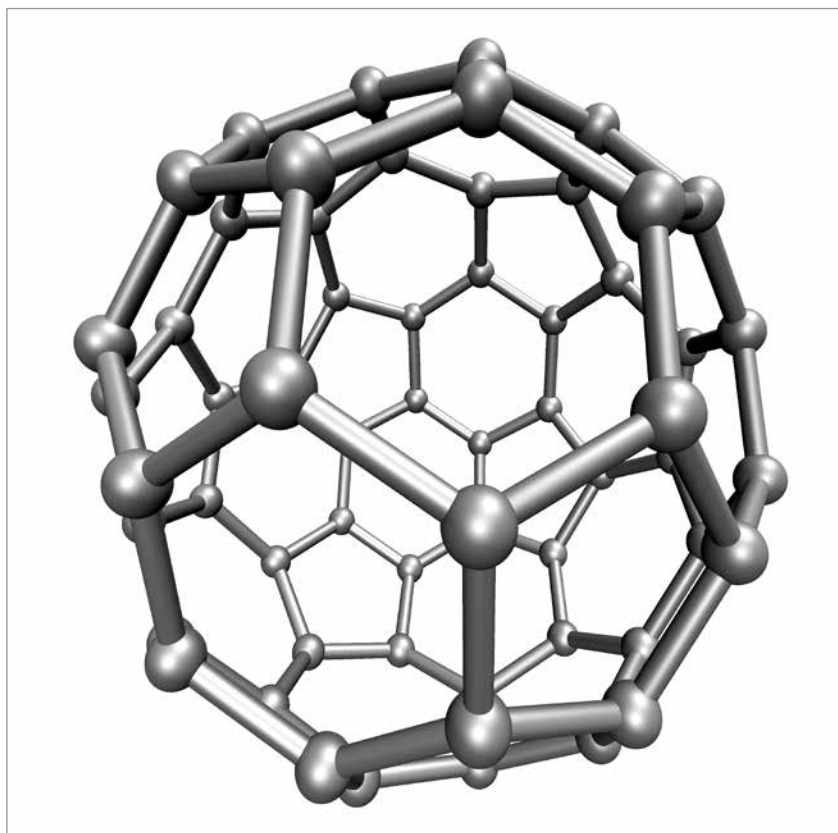
Through understanding the symmetrical nature and self-assembly mechanism of the different protein subunits, scientists are

able to build synthetic protein nanocages from modular building blocks derived from natural proteins. By combining 2 types of basic structural units linked by a short rigid protein structure, Yeates group at UCLA builds synthetic protein nanocages of tetrahedral shape.^[13]

The modularity of the protein nanocage building blocks holds promise to further tailor their properties for specific applications. Collaborative efforts between bioengineers, biochemist, materials scientists, and computer engineers will open new ground on the bioinspired design of hierarchical nanostructures with increasing complexity.

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About the Author



Dr. Sierin Lim is an assistant professor of Bioengineering at the School of Chemical and Biomedical Engineering, Nanyang Technological University. Her research focuses on the design, engineering, and development of hybrid nano/microscale devices from biological parts by utilizing protein engineering as a tool. In particular, she is interested in self-assembling protein-derived nanocages and photosynthetic biological materials. The project scopes range from understanding the self-assembly mechanism of the nanocages, engineering therapeutic/diagnostic (theranostic) agent carriers, to improvement of electron transfer efficiency in a photosynthetic electrochemical cell.

Dr. Lim received the 2012 Asia Pacific Research Networking Fellowship from the International Federation of Medical and Biological Engineering and founded the Biomedical Engineering Society (Singapore) Student Chapter in 2009.