Synthesis of hollow spherical cages with polyhedral structures and application to the encapsulation of a whole protein in the cage

In nature, huge capsular structures are constructed by the self-assembly of simple repeating units through many weak interactions like hydrogen bonds, affording well-determined and functionalized cavities at the molecular level. Such capsular structures show biological functions for controlling vital activities through the encapsulation of specific substances in the cavities: for example, virus capsids and the relatively small protein, ferritin, store and release DNA/RNA and iron ions, respectively, and the relatively large protein chaperonin repair three-dimensional structures of trapped proteins.

Chemists have been inspired by the efficient synthesis of capsular structures in natural systems, and a variety of hollow molecules have been synthesized through the self-assembly of artificial molecules connected with weak interactions. The use of coordination bonds between an organic ligand molecule and a metal ion is one of the most successful examples of constructing well-defined, hollow structures owing to the appropriate strength of the bond and the inherent nature of metal ions with defined bond numbers and bond directions around the metal center. The structures of these metal-organic cages are limited to simple Platonic or Archimedean polyhedra and to small molecular sizes, typically approximately 1 to 2 nm, because nature entropically favors a higher symmetry and a smaller number of components.

Here, we report our recent achievements in the synthesis of a self-assembled metal-organic complex with the first stellated polyhedral framework [1] and in the encapsulating of a whole protein within the huge, well-defined cavity [2,3]. We have developed a method for the self-assembly of metal-organic cages from organic ligands (L) bearing two pyridyl groups as coordination sites and transition metal ions (M) with compositions of $M_{12}L_{24}$, where the functionalization of the ligand promises a reliable chemical functionalization of the product complex.

Stellated polyhedron is a mathematically defined polyhedral family with concave surfaces, constructed by extending the faces of a polyhedron until they intersect (Fig. 1(a)). A stellated polyhedral complex is difficult to synthesize owing to its more complicated structure than the reported Platonic or Archimedean polyhedral ones. Therefore, we employed the strategy of firstly constructing an Archimedean polyhedral complex with a cuboctahedral framework, i.e., an $M_{12}L_{24}$ sphere, with the coordination bonds of the pyridyl group **A** shown in Fig. 1(b) in blue and Pd(II) ions, followed by stellation by the 2nd coordination between the pyridyl group **B** shown in red and additional Pd(II) ions. Although the pyridyl groups **A** and **B** have the same coordination properties, the molecular design to use the rigidly fixed pyridyl group **A** and the flexibly tethered pyridyl group **B** enabled us to efficiently prepare the stellated complex based on the robust framework of the $M_{12}L_{24}$ sphere. The product structures of the first $M_{12}L_{24}$ cuboctahedron bearing the free pyridyl group **B** and the final $M_{18}L_{24}$ stellated cuboctahedron were clearly revealed by nuclear magnetic resonance (NMR), mass spectrometry (MS), and finally synchrotron X-ray diffraction studies carried out at beamline **BL38B1** in SPring-8 and at NE3A beamline in the Photon Factory (PF) (Fig. 1(c)).

On the basis of the robust $M_{12}L_{24}$ sphere

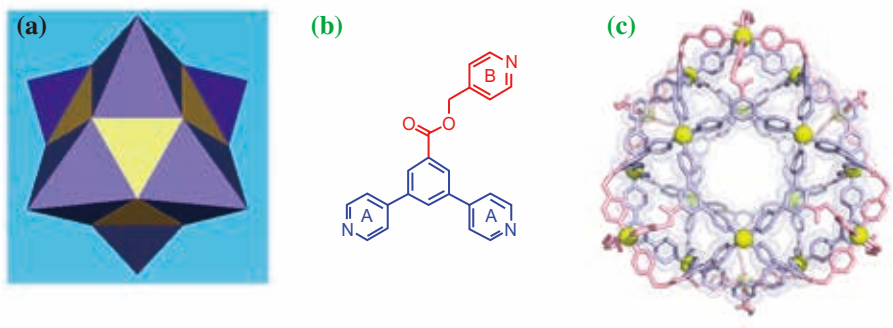

Fig. 1. **(a)** Mathematically defined stellated cuboctahedron. **(b)** Structure of the ligand for the synthesis of metal-organic complex with stellated polyhedral structure. **(c)** X-ray crystal structure of the stellated cuboctahedral complex.

accepting designable functionalization, we mimicked natural cages to encapsulate a whole biomolecule in a well-defined synthetic cage, as collaboration works with a group led by Institute for Molecular Science Professor Koichi Kato. Small organic molecules can be artificially encapsulated in a hollow cage, within which the structure or activity of the guest molecule can be controlled. If the encapsulation of a biomolecule in an artificial host is achieved, the structure and activity of the biomolecule could also be controlled at will through the design of the host, which should be of great use in drug development and other industrial applications. However, so far, it has not been possible to enclose larger biomolecules such as proteins because it has not been possible to increase the size of precisely structured artificial molecular capsules.

We noticed that the $M_{12}L_{24}$ sphere constructed from extended ligands and Pd(II) ions has a diameter of 6.3 nm, and that the cavity could encapsulate a whole protein. A ligand tethered to ubiquitin, a relatively small globular protein (76 residues, 8.6 kDa, approximately 3–4 nm in diameter), and a ligand bearing a hydrophilic sugar chain were synthesized; these organic ligands were then mixed with Pd(II) ions in a solvent. The $M_{12}L_{24}$ sphere encapsulating ubiquitin surrounded by a hydrophilic internal surface owing to the 23 attached sugar moieties was automatically formed through self-assembly (Fig. 2).

The precise structure of the product was revealed by NMR, and the determined diffusion coefficient supported the protein-trapping state in the cage. MS studies to determine the molecular weight were not successful unfortunately; however, collaboration works with a group led by Osaka University Associate Professor Susumu Uchiyama succeeded in analyzing of the molecular weight in solution using ultracentrifugation data, showing that the $M_{12}L_{24}$ sphere preserved the protein stably in solution.

We optimized the crystallization conditions and intensively worked on synchrotron X-ray diffraction studies carried out at **BL38B1**, **BL41XU**, **BL26B1**, and **BL26B2** beamlines in SPring-8 and at NE3A and BL17A beamlines in the PF.

Collaboration works with groups led by the Graduate School of Frontier Sciences (concurrently RIKEN SPring-8 center) Professor Takata and JASRI Group Leader Dr. Kumasaka finally determined the crystal structure by the maximum entropy method (MEM). The complex enclosing the whole protein at the center of the artificial capsule was clearly visualized.

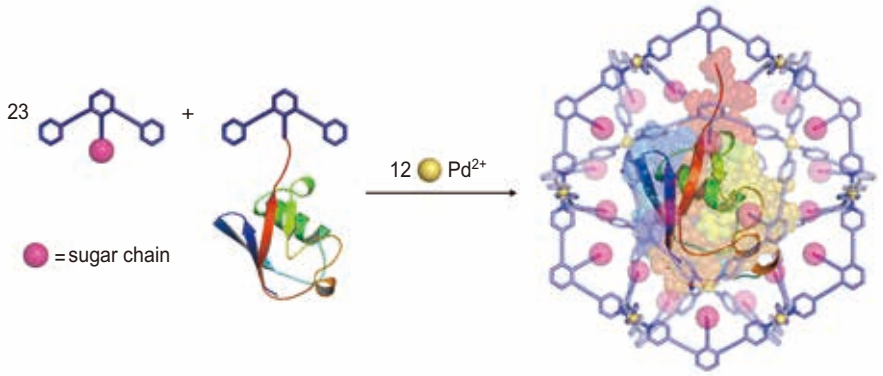

Fig. 2. Synthetic scheme of an $M_{12}L_{24}$ sphere with sugar-decollated inner surface encapsulating a whole ubiquitin molecule in the cavity.

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