Synthetic Molecular Wireframes Reinforce Protein Stability through Molecular Chaperone-like Structural Refolding Effects

Daishi Fujita,[1,2] *Ryoto Suzuki*,[3] *Makoto Fujita*[3]
[1] *Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, JAPAN*
[2] *PRESTO, Japan Science and Technology Agency, JAPAN*
[3] *Department of Applied Chemistry, The University of Tokyo, JAPAN*
dfujita@icems.kyoto-u.ac.jp

Spatial isolation of molecules is often a powerful strategy for regulating their molecular behavior. Biological systems well-employ such mechanisms, however, scientists have yet to rival nature, particularly for macromolecular substrates. Our team has recently demonstrated that only a "wireframe" molecular scaffold is sufficient to improve the structural and enzymatic properties of a protein encapsulated within (Figure 1). The three-dimensionally confined enzyme (cutinase-like enzyme (CLE): a protein for plastic degradation) did not show any structural melting by DSC experiments up to 130 °C (the T_m of native CLE is around 50 °C). Remarkable stability in a 10:90 aqueous–acetonitrile solution for tens of days was observed, even at room temperature. A kinetic assay of the enzymatic reaction revealed that the key to this stability is the isolated space, which aids protein refolding in a manner reminiscent of molecular chaperones (Figure 2). Although the encapsulated enzyme did partially denature in solutions containing high proportions of organic solvent, it refolded back to the original tertial structure when half-aqueous solvent conditions were restored. Isotope-labeled NMR studies also supported such refolding behavior. The key to protein encapsulation is the self-assembly of giant wireframe hollow metal complexes,[1] some of which are the largest known artificially self-assembled objects that still possess a precise atomic composition.[2, 3] To the best of our knowledge, the M_nL_{2n} family is the first class of such atomically precise synthetic assemblies capable of encapsulating whole protein molecules.[4] Figure 1 shows the encapsulation strategy. Organic bidentate ligands (L), possessing a 2-formyl pyridyl group that interacts with the N-terminus of the protein through reversible covalent bond formation, self-assemble around the protein upon the addition of Pd(II) ions (M) to afford the caged protein. This protein reinforcing methodology has potential applications for the industrial use of enzymes or as a research tool for enzymology.

Figure 1.

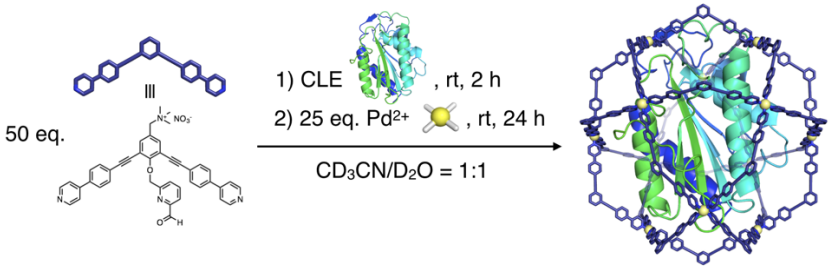
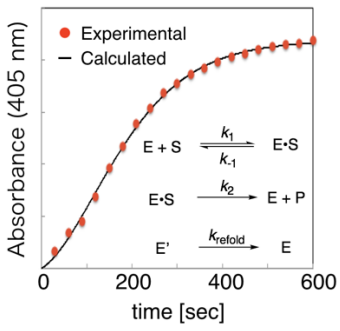


Figure 2.



1. K. Harris, D. Fujita, M. Fujita *Chem. Commun.* **2013**, *49*, 6703-6712.
2. D. Fujita, Y. Ueda, S. Sato, N. Mizuno, T. Kumasaka, M. Fujita *Nature* **2016**, *540*, 563.
3. D. Fujita, Y. Ueda, S. Sato, H. Yokoyama, N. Mizuno, T. Kumasaka, M. Fujita *Chem* **2016**, *1*, 91.
4. D. Fujita *et al. Nat. Commun.* **2012**, *3*, 1093.