# Versatile Protein Encapsulation in Crystals of Spherical Coordination Cages

([1]*Grad. School of Engineering, The Univ. of Tokyo*, [2]*UTIAS, The Univ. of Tokyo,* [3]*Institute for Molecular Science*) ○Hongkun Liu,[1] Takahiro Nakama,[1] Makoto Fujita[2,3]

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Protein immobilization in solid supports is a promising approach to improve the practical performance of enzymes as heterogeneous catalysts. Despite successes with various matrices, they often suffer from limited protein scope, low loading capacity, and ambiguous environments around the protein. Previously, we reported protein encapsulation in $M_{12}L_{24}$ spherical coordination cages, leading to significant stabilization of the caged protein.[1,2,3]

Here, we demonstrate a versatile method to immobilize proteins in porous single crystals by encapsulation in the $M_{12}L_{24}$ coordination cages (**Fig. 1**). Each protein was encapsulated in a well-defined cavity of $M_{12}L_{24}$ cages prior to crystallization by the conjugation with bis(pyridine) ligands (**L**) and subsequent self-assembly with Pd(II) ions (**M**). Crystals of the caged proteins were then obtained by vapor diffusion (**Fig. 1A,B**). This pre-encapsulation strategy allowed us to immobilize more than 10 different proteins in the crystal of $M_{12}L_{24}$ cages without any significant changes in conditions. Fourier-transform infrared (FTIR) and X-ray absorption fine structure (XAFS) spectroscopy provided structural information on immobilized proteins. The superoxide dismutase 1 (SOD1) immobilized in the cage crystal catalyzed superoxide ($O2^{\bullet-}$) disproportionation with an 82% residual activity. Remarkably, the co-immobilization of two enzymes, SOD1 and cytochrome c (cyt c), in a single crystal has enabled a cascade reaction with a proximity-induced 4.2-fold increase in catalytic performance compared to the enzymes immobilized in different cage crystals (**Fig. 1C**).

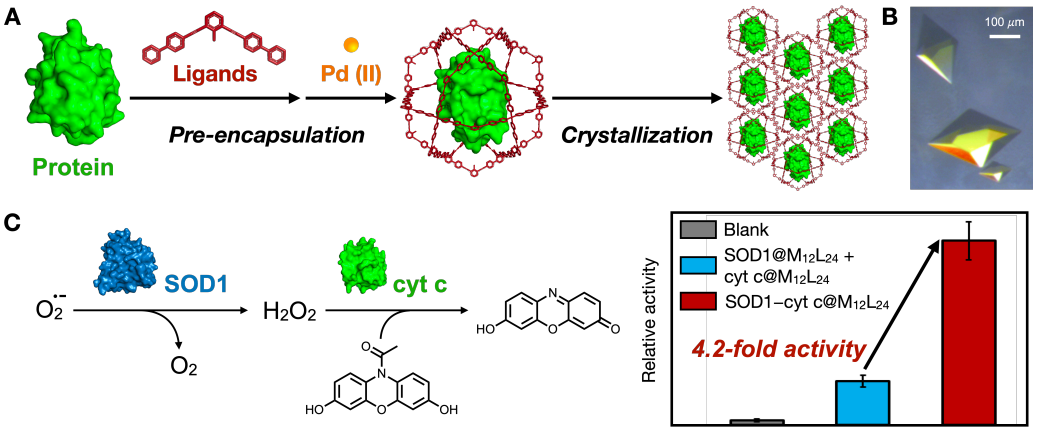


**Fig. 1** (**A**) Schematic diagram of protein immobilization in crystals of $M_{12}L_{24}$ cages. (**B**) Crystals of caged SOD1. (**C**) Cascade reaction by two enzymes immobilized in the $M_{12}L_{24}$ cage crystal.

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