Re-design of an artificial protein nanocage TIP60: structural analysis of a mutant assembled by responding to metal ions

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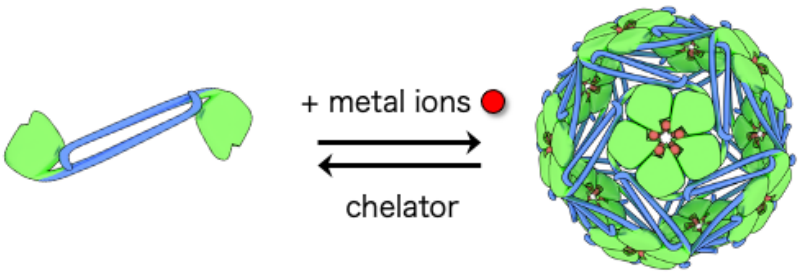
Protein cages composed of multiple protein molecules hold promise of nanomaterials for cargo delivery. The most widely accepted approach to introduce cargo molecules in the inner space is the dissociation and association of protein cage in the presence of the cargo. However, dissociation process of protein cages requires harsh treatment such as acidification that reduces the yield of the cargo encapsulated cages. Thus, developing a protein cage that can reversibly associate/dissociate under mild conditions is beneficial for applications.

We have previously produced an artificial protein nanocage TIP60 composed of 60-mer designed fusion proteins[1,2]. However, TIP60 could not dissociate without harsh treatments. In this study, TIP60 was re-designed for conferring reversibly associate/dissociate mechanisms. For this purpose, we initially introduced mutations at the interface of subunits and found a mutant, K67E, that does not spontaneously assemble into 60-mer. We assumed that the typical metal ions that favored to interact with the side chain of glutamate would recover subunit interactions. As a result, the mutant was reassembled to 60-mer in response to typical metal ions, such as Ba ions. Removal of metal ions by EDTA dissociated the 60-mer again. Cryo-EM structure of the metal-induced 60-mer (mTIP60) showed the potential map corresponding to the Ba ions at the interface region of the subunit near the mutation position, indicating that the metal ions bridge the subunits. We believe that this mild association/dissociation system of mTIP60 would be broadly used for cargo encapsulations.



1) N. Kawakami *et al., Angew. Chem. Int. Ed.*, **57**, 12400–12404 (2018).
2) J. Obata *et al., Chem. Commun.*, **57**, 10226–10229 (2021).