# Dual-surface functionalization of an artificial protein nanoparticle TIP60 using molecular-filtration effect of surface pores

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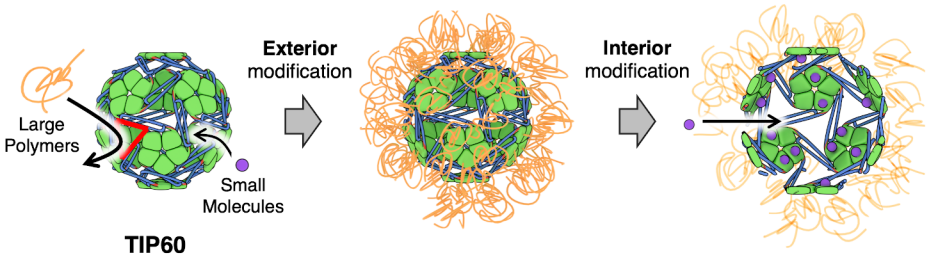
Hollow protein nanocages, which can incorporate cargo molecules in their inner cavity, are attractive materials for various applications such as drug-delivery systems and catalysis[1]. We produced an artificial protein nanoparticle designated as TIP60 by designing fusion proteins composed of a pentamer protein and dimer protein[2]. The TIP60 is a hollow nanoparticle with 20 pores on its surface. The serine residues on the interior and exterior surface of TIP60 can be replaced with cysteine residues as functional groups for site-specific chemical modification. We performed modification experiments using mutants of TIP60 introduced cysteine residues on the interior surface of TIP60 and size-different polymer molecules with maleimide groups specifically reacting with thiols. The polymers smaller than the size of the pores on the TIP60 modified cysteine residues on the interior surface, while the larger polymers could not modify them. This result suggested that the pores on the surface of TIP60 functioned as size-dependent molecular filters, which allowed only molecules smaller than the pore size to penetrate into the inner cavity of TIP60.

Motivated by the finding of size-dependent molecular discriminations by the pores on TIP60, we performed dual-surface modifications of TIP60 by size-different molecules. A double mutant of TIP60 with cysteine residues on both interior and exterior surfaces was first modified by polymer molecules larger than the pore size. These large molecules could modify cysteine residues on the exterior surface. The remained interior cysteine residues were modified by thiol-containing small molecules. We also demonstrated that the redox-responsive release of thiol-containing small molecules from the dual-surface modified TIP60.




1) Y. Zhang, M. S. Ardejani and B. P. Orner, *Chem. Asian J.*, 2016, **11**, 2814. 2) N. Kawakami, H. Kondo, Y. Matsuzawa, K. Hayasaka, E. Nasu, K. Sasahara, R. Arai and K. Miyamoto, *Angew. Chem. Int. Ed.*, 2018, **57**, 12400.