

Design of Protein Functions Using Computational and Experimental Approaches

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Our research is to design a variety of protein functions using computational and experimental approaches. We try to (1) design enzymes from scratch and reveal the origin of the enzymatic activity, (2) control concerted functions by rationally engineering protein complexes and understand their mechanisms and (3) uncover roles of protein complexes in cells and control cellular functions by creating several customized proteins or protein complexes.

1. Design of Allosteric Sites into a Rotary Molecular Motor by Restoring Lost Function of Pseudo-Active Sites

We have succeeded in designing artificial allosteric sites

(where by binding an effector molecule, activity at the distal active site is regulated) into a rotary molecular motor, *Enterococcus hirae* V₁-ATPase.¹⁾ The allosteric sites were created by restoring lost functions of pseudo-active sites in a pseudo enzyme, of which function is predicted to have been lost during the evolution. Single-molecule experiments together with X-ray crystallography analyses revealed that the rotational rate of the designed V₁-ATPase, which was restored the lost ATP binding ability at the pseudo-active sites, is allosterically accelerated. In principle, our strategy enables us to create allosteric sites into various kinds of protein complexes and to artificially control the concerted functions.

Reference

1) T. Kosugi, T. Iida, M. Tanabe, R. Iino and N. Koga, *Nat. Chem.* **15**, 1591–1598 (2023).