Biofunctional Chemistry Research Section

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1. Introduction

A transition to renewable energy technologies requires new chemistry to learn from nature. For almost 3 billion years, nature has developed fantastic solutions to convert solar energy into chemical energy and to use it in exceptionally efficient way. Our challenge is to understand nature's efficient bioenergetic processes and to design bio-inspired energy utilization systems. The research interests of our group focus on the design of biomacromolecules and their assemblies for molecular recognition, catalysis, and signal transduction in water, the solvent of life. We take synthetic, organic chemical, biochemical and biophysical approaches to understand biological molecular recognition and chemical reactions. Proteins and protein/nucleic acid assemblies are explored to realize the biomimetic function of biological systems, such as visualization of cellular signals by fluorescent biosensors, directed self-assembly of peptides and proteins to build up nano-bio materials, tailoring of artificial receptors and enzymes based on the complex of biofunctional molecules such as nucleic acid, peptides and/or a proteins, and reconstitution of the functional assemblies of receptors and enzymes on the nanoarchitectures. The following are the major research achievements in the fiscal year 2024.

2. Self-Assembled Fluorophore-Based Probe for Efficient Detection of Endogenous Nitroreductase Activity in *Escherichia Coli*

Fluorescent probes are functional molecules whose fluorescent properties are transformed as a response to specific stimuli. Understanding the mechanisms of these transformations is essential for the design of these stimuli-responsive fluorescent probes. A rational design strategy has been developed to construct stimuli-responsive supramolecular cluster fluorescent probes. They operate by a new mechanism called self-assembly induced lactone formation (SAILac) to control the fluorescence properties of SNARF, an asymmetric xanthene fluorophore. Here, to expand SAILac applicability, the structure-activity relationship of the fluorophore scaffold is studied. SNARF-OBn(pNO$_2$), designed as nitroreductase-reactive fluorescent probe based on the SAILac mechanism, is selected as the initial structure. As the result of the structure-activity relationship studies, a new nitroreductase-reactive fluorescent probe, Rhodol-OBn(pNO$_2$), is created, having a superior signal-to-noise (S/N) ratio with higher reactivity toward nitroreductase than the original probe. By using Rhodol-OBn (pNO$_2$), the activity of endogenous nitroreductase in *Escherichia coli* is successfully detected.

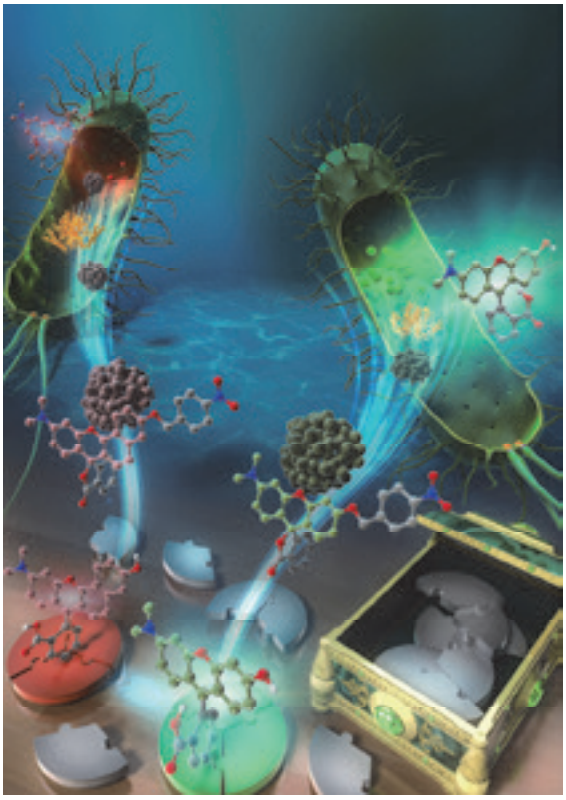

Fig. 1 An illustration of the optimization of the self-assembled fluorophore based probe for efficient detection of endogeneous nitroreductase activity in *E. coli*.

3. A Practical Approach for Polarity and Quantity Controlled Assembly of Membrane Proteins into Nanoliposomes

Biological membranes achieve selectivity and permeability through protein transporters and channels. The design of artificial compartments with permeable membranes is essential to facilitate substrate

and product transfer in enzymatic reactions. In this study, an E. coli outer membrane protein OmpF fused to a modular adaptor was integrated onto a DNA origami skeleton to control the number and polarity of the OmpF trimer. DNA origami skeleton-guided nanoliposomes reconstituted with functional OmpF exhibit pH-responsiveness and size-selective permeability. This approach highlights the potential to construct artificial compartments that incorporate membrane proteins of defined number and polarity, allowing tunable substrate fluxes.

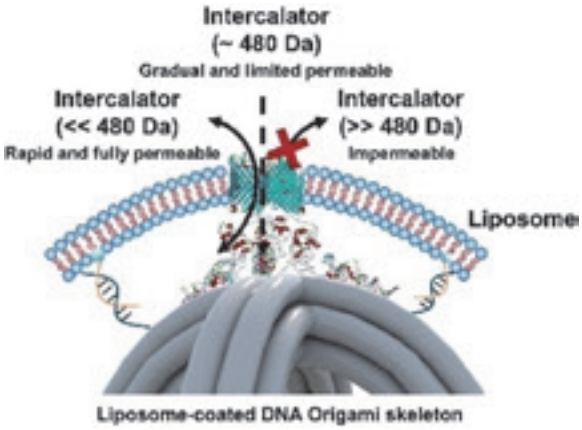

Fig. 2 An image of liposome-coated DNA skeleton with modular adaptor-fused transporter (ZF-OmpF), which incorporated in the liposome.

4. The roles of high-density water layer on the DNA scaffold surface in the modulation of enzyme reactions

It is known experimentally that enzymatic reactions are often accelerated when the enzymes are assembled on the scaffold of DNA nanostructures. However, the exact mechanism by which this acceleration occurs remains unclear. Here, we study the reactions of enzymes with different catalytic mechanisms assembled on a DNA scaffold with various substrates. Analysis of the hydration properties of the substrates using our accurate statistical mechanics theory classifies the substrates into two groups that behave as hydrophilic and hydrophobic solutes, respectively. The reaction of the enzyme on the DNA scaffold is accelerated with a hydrophilic substrate but decelerated with a hydrophobic substrate. We propose a mechanism of acceleration or deceleration in which, due to the formation of a high-density layer of water near the DNA surface with high negative charge density, the concentration of a substrate with high energetic affinity for water within the layer becomes higher than that near a free enzyme, whereas that of a substrate with low energetic affinity becomes lower within the layer. This study provides chemical and physical insights into a general case of biocatalysts, where the rates of chemical reactions occurring at the interface of biomolecules in aqueous environments can differ substantially from those in the bulk solution due to variations in the local concentration of a given ligand.

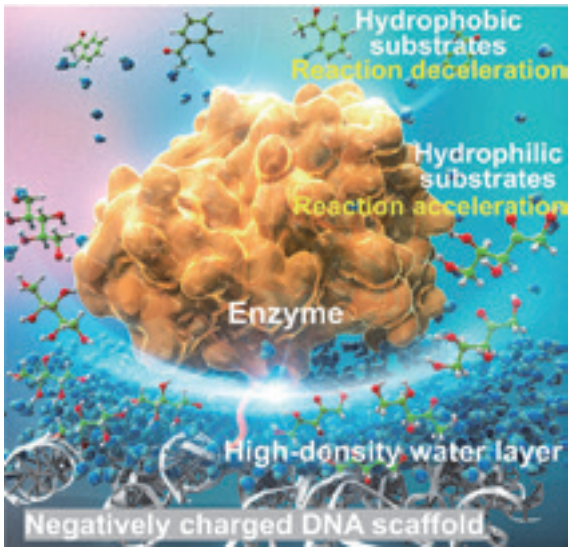

Fig. 3 An illustration of the roles of high-density water layer on the DNA scaffold surface in the modulation of enzyme reactions with hydrophilic substrates or hydrophobic substrates.

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中田栄司, Seoul National University（韓国）, 細胞内酵素組織体の構築

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中田栄司, 東京科学大学, 学術変革領域研究 A・超越分子システム

中田栄司, 名古屋工業大学, 学術変革領域研究 A・超越分子システム

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