Biofunctional Chemistry Research Section

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1. Introduction

A transition to renewable energy technologies requires new chemistry to learn from nature. Nature has developed fantastic solutions to convert solar energy to chemical energy and to utilize them in exceptionally efficient manners for almost 3 billion years. It is our challenge to understand the efficient bioenergetic processes of nature and to construct bio-inspired energy utilization systems. The research interests in 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 acids 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 artificial receptors and enzymes based on the complex of RNA and a peptide or a protein, and reconstitution of the functional assemblies of receptors and enzymes on the nanoar-chitectures. The followings are the main research achievements in the fiscal year 2022.

2. Controlled assembly of fluorophores inside a nanoliposome

Cellular compartmentalization plays an essential role in organizing the complex and multiple biochemical reactions in the cell. An artificial compartment would provide powerful strategies to develop new biochemical tools for material production and diagnosis, but it is still a great challenge to synthesize the compartments that encapsulate materials of interest while controlling their accurate locations, numbers, and stoichiometry. Chemical characteristics of a liposome-encapsulated compartment, which has great potential to locate various materials of interest with precise control of their locations and numbers in the compartment, were evaluated. A nanoliposome was constructed inside a ring-shaped DNA origami skeleton and further equipped with a double-stranded DNA platform to assemble molecules of interest in the nanoliposome (Fig. 1). Upon formation of the nanoliposome, a pH-sensitive fluorophore on the bridged platform showed little or no response to the pH change of the outer buffer, ensuring that the molecules assembled on the platform are effectively shielded from the outer environment. The ring-shaped DNA skeleton equipped with a double-stranded DNA platform allows spatial assembly of several functional molecules inside the nanoliposome to isolate them from the outer environment.

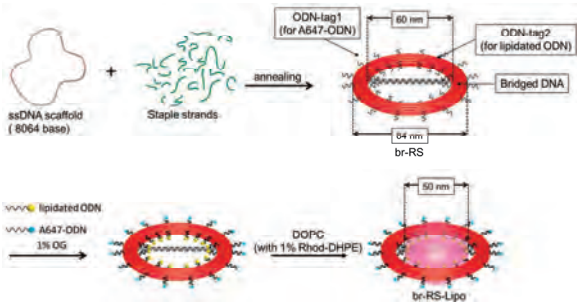

Fig. 1 Design of a bridged ring-shaped DNA origami skeleton that guides formation of a nanoliposome.

3.Dynamic assembly of cascade enzymes by the shape transformation of a DNA scaffold

Within cells, the close spatial arrangement of cascade enzymes facilitates the channeling of intermediates and enhances cascade reaction efficiency. Reconfigurable DNA nanostructures, owing to their structural controllability and precise spatial addressability, are promising tools for mimicking such processes. In this study, a 3D DNA origami scaffold, with a dynamic shape transformation from its open boat form to a closed hexagonal prism induced by toehold-mediated strand displacement, is designed to investigate the enzyme cascade reaction of xylose reductase and xylitol dehydrogenase from D-xylose metabolic pathway. Enzymes are assembled on the DNA scaffold in its open state, which is subsequently closed by the assistance of DNA sequence-specific closing keys. The enzyme cascade efficiency is much higher in the static encapsulated closed state than in the open state due not only to the enzyme proximity but also the environmental factors of 3D DNA structure (Fig. 2 and 3). These results provide novel insights into controlling enzyme

cascade reactions by inducing the shape transformation of DNA nanostructures and how environmental factors affect the action of multi-enzyme complexes in the cell.

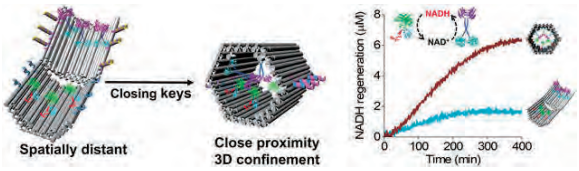

Fig. 2 Schematic representations of the cascade reaction of XR and XDH from a part of xylose metabolic pathway was loaded on 3D DNA scaffold with the shape transformation.

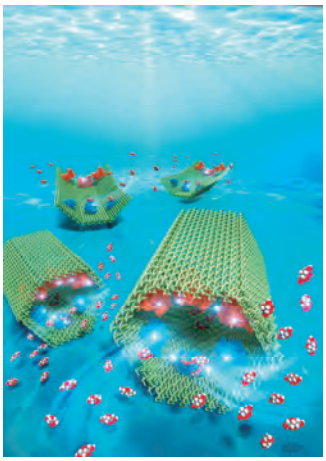

Fig. 3 The image of the cascade reaction of XR and XDH on 3D DNA scaffold with the shape transformation.

4. FRET-based cathepsin probes for simultaneous detection of cathepsin B and D activities

Fluorescent cathepsin probes were prepared by modification of peptidic substrates for cathepsin B (CTSB) and cathepsin D (CTSD) with FRET pairs (Fig. 4). Fluorophores with distinguishable emission characteristics were applied to CTSB and CTSD probes with their appropriate quenchers to simultaneously monitor the activity of CTSB and/or CTSD. Conjugation of both the CTSB and CTSD probes with short single-stranded DNA drastically increased their reactivity to cathepsins over the parent probes possibly by improving their solubility. The activity of CTSB and CTSD were simultaneously detected by using these orthogonal FRET-based cathepsin probes.

Fig. 4 DNA conjugated FRET-based probes for cathepsin B (CTSB) and cathepsin D (CTSD) were designed to simultaneously detect the activity of both cathepsins.

5. A two-step screening to optimize the signal response of an auto-fluorescent protein-based biosensor

Auto-fluorescent protein (AFP)-based biosensors transduce the structural change in their embedded recognition modules induced by recognition/reaction events to fluorescence signal changes of AFP. The lack of detailed structural information on the recognition module often makes it difficult to optimize AFP-based biosensors. To enhance the signal response derived from detecting the putative structural change in the nitric oxide (NO)-sensing segment of transient receptor potential canonical 5 (TRPC5) fused to enhanced green fluorescent protein (EGFP), EGFP-TRPC5, a facile two-step screening strategy, *in silico* first and *in vitro* second, was applied to variants of EGFP-TRPC5 deletion-mutated within the recognition module (Fig. 5). In *in silico* screening, the structural changes of the recognition modules were evaluated as root-mean-square-deviation (RMSD) values, and 10 candidates were efficiently selected from 47 derivatives. Through *in vitro* screening, four mutants were identified that showed a larger change in signal response than the parent EGFP-TRPC5. One mutant in particular, 551-575, showed four times larger change upon reaction with NO and H_2O_2. Furthermore, mutant 551-575 also showed a signal response upon reaction with H_2O_2 in mammalian HEK293 cells, indicating that the mutant has the potential to be applied as a biosensor for cell measurement. Therefore, this two-step screening method effectively allows the selection of AFP-based biosensors with sufficiently enhanced signal responses for application in mammalian cells.

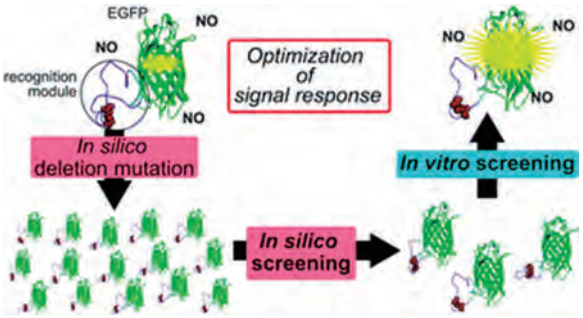

Fig. 5 Schematic representations of a two-step screening to optimize the signal response of AFP based biosensor (NO sensor).

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Collaboration Works

森井孝, Ghent University（ベルギー）, 選択的 DNA 修飾

森井孝, 中田栄司, Rajendran Arivazhagan, Ewha Womans University（韓国）, トポイソメラーゼ反応の１分子計測

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森井孝, 仲野瞬, POSTECH（韓国）, 生理活性物質を高感度で検出するセンサーの開発

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

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2. Others

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