Biofunctional Chemistry Research Section

T. Morii, Professor
E. Nakata, Associate Professor
S. Nakano, Assistant Professor

1. Introduction

A transition to renewable energy technologies requires new chemistry to learn from nature. Nature has developed fantastic solutions to convert the solar energy to the chemical energy and to utilize them in the 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 the biological molecular recognition and chemical reactions. Proteins and protein/nucleic acids assemblies are explored to realize biomimetic function of biological systems, such as visualization of cellular signals by fluorescent biosensors, directed self-assembly of peptides and proteins to build up nanobiomaterials, 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 nanoarchitectures. Followings are main research achievements in fiscal year 2021.

2. Dynamic shape transformation of a DNA scaffold applied for an enzyme nanocarrier

In this study, a three-dimensional DNA scaffold was designed to enable a dynamic shape transition from an open plate-like structure to its closed state of a hexagonal prism structure. A dimeric enzyme xylitol dehydrogenase (XDH) was assembled on the DNA scaffold in its open state in a high loading yield. The enzyme loaded scaffold was subsequently transformed to its closed state by the addition of short DNA closing keys. The enzyme encapsulated in the closed state displayed comparable activity to that in the open state, ensuring that the catalytic activity of enzyme was well maintained in the DNA nanocarrier (Fig. 1).

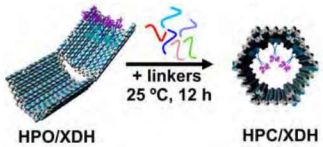

Fig. 1 Schemes representing transformation of XDH-loaded 3D DNA scaffold.

3. Conditional dependence of enzyme cascade reaction efficiency on the inter-enzyme distance

Cascade enzymes in cellular metabolic reactions often suffer from unfavorable kinetics of upstream and downstream enzymes. The kinetic parameters of such sequential enzymes are suggested to be critical in considering the inter-enzyme distance dependence of the cascade efficiencies. In this work, this issue is addressed by evaluating the reaction kinetics of imbalanced cascade enzymes.

An enzyme cascade of XDH and xylulose kinase (XK), derived from the xylose metabolic pathway, was constructed on a 3D DNA scaffold with a dynamic shape transition ability as described above. Evaluation of the cascade reaction efficiencies in the open and closed states revealed little or no inter-enzyme distance dependence, presumably due to the far larger catalytic constant of the downstream enzyme (Fig. 2). The inter-enzyme distance was not the dominant factor for cascade efficiency when the kinetic parameters of the cascade enzymes were imbalanced with the highly efficient downstream enzyme.

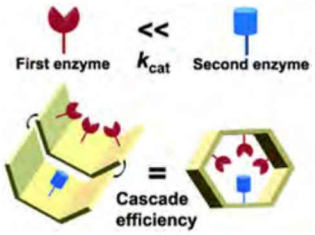

Fig. 2 Schematic representations of the cascade reaction of XDH and XK from a part of xylose metabolic pathway was loaded on 3D DNA scaffold.

4. Tuning the reactivity of a substrate for SNAP-Tag expands its application for recognition driven DNA-protein conjugation

Recognition-driven modification has been emerging as a novel approach to modifying biomolecular targets of interest site-specifically and efficiently. Protein modular adaptors (MAs) are the ideal reaction model for recognition-driven modification of DNA as they consist of both a sequence-specific DNA-binding domain and a self-ligating protein-tag. Coupling a DNA recognition by DNA-binding domain and a chemoselective reaction of protein tag could provide a

highly efficient sequence-specific reaction. However, a MA consisting of a reactive protein-tag and its substrate, for example, SNAP-tag and benzyl guanine, revealed rather nonselective reaction with DNA. Therefore, new substrates of SNAP-tag have been designed to realize sequence-selective rapid crosslinking reactions of MA with SNAP-tag (Fig. 3). The reactions of substrates with SNAP-tag were verified by kinetic analyses to enable the sequence selective crosslinking reaction of MA.

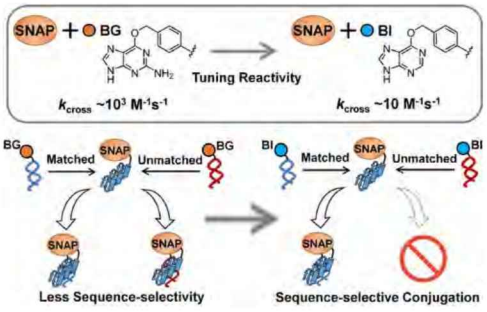

Fig. 3 An illustration of newly designed substrate of SNAP-tag to realize sequence-selective rapid crosslinking by MAs with SNAP-tag.

5. A facile combinatorial approach to construct a ratiometric fluorescent sensor: application for the real-time sensing of cellular pH changes

Realtime monitoring of the cellular environment, such as the intracellular pH, in a defined cellular space provides a comprehensive understanding of the dynamics processes in a living cell. Considering the limitation of spatial resolution in conventional microscopy measurements, multiple types of fluorophores assembled within that space would behave as a single fluorescent probe molecule. Such a character of microscopic measurements enables a much more flexible combinatorial design strategy in developing fluorescent probes for given targets. Nanomaterials with sizes smaller than the microscopy spatial resolution provide a scaffold to assemble several types of fluorophores with a variety of optical characteristics, therefore enablinging a convenient strategy for designing fluorescent pH sensors. In this study, fluorescein (CF) and tetramethylrhodamine (CR) were assembled on a DNA nanostructure with controlling the number of each type of fluorophore. By taking advantage of the different responses of CF and CR emissions to the pH

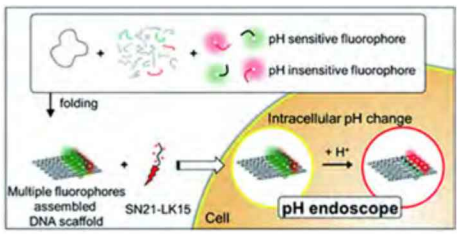

Fig. 4 a DNA origami scaffold assembled with multiple fluorophores to study intracellular pH change.

environment, an appropriate assembly of both CF and CR on DNA origami enabled a controlled intensity of fluorescence emission and ratiometric pH monitoring within the space defined by DNA origami. The CF and CR-assembled DNA origami was successfully applied for monitoring the intracellular pH changes (Fig. 4).

6. Stabilization and structural changes of 2D DNA origami by enzymatic ligation

The low thermal stability of DNA nanostructures is the major drawback in their practical applications. Detailed analyses of the conditions for the enzymatic ligation of the staple strands in 2D square lattice DNA origami provided optimized conditions to enhance the thermal stability of DNA nanostructures (Fig. 5).

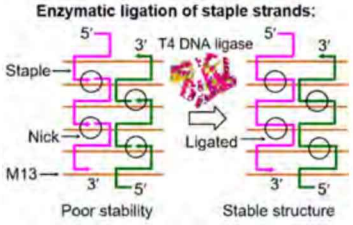

Fig. 5 Enzymatic ligation of staple strands in DNA origami scaffold.

7. Topologically-Interlocked Minicircles as Probes of DNA Topology and DNA-Protein Interactions

The topologically-interlocked minicircle rotaxane and catenane inside a frame-shaped DNA origami were synthesized. To probe the DNA-protein interactions, restriction reactions were carried out on the prepared interlocked structures and other DNAs with different topologies (Fig. 6).

This collaboration work with Prof. Y. Kwon (Ewha Womans University, Korea) was started when she was appointed as a visiting professor of IAE (FY 2013).

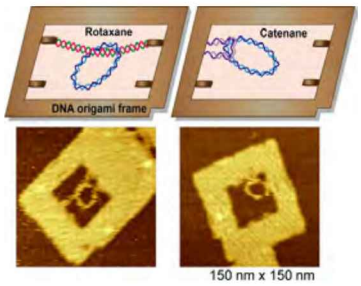

Fig. 6 An illustration of topologically-interlocked minicircles in DNA origami scaffold.

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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

森井孝，仲野瞬，POSTECH（韓国），分子ライブラリーによる蛍光 RNP センサーの開発

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

森井孝，Rajendran Arivazhagan，Vanderbilt University School of Medicine（アメリカ），トポイソメラーゼ作用の分子機構

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

大垣英明，森井孝，片平正人，野平俊之，モンゴル国立大学，インドネシア大学，フィリピン大学ディリマン校，ベトナム国家大学ハノイ校，ラオス国立大学，王立プノンペン大学，研究拠点形成事業 B. アジア・アフリカ学術基盤形成型

Financial Support

1. Grant-in-Aid for Scientific Research

中田栄司，基盤研究(B)，DNA ナノ構造体の階層的自己組織化による高効率な酵素連続反応場の構築

2. Others

森井孝，科学技術振興機構，細胞内環境測定多元同時センサーの開発

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