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 RNA and a peptide or a protein, and reconstitution of the functional assemblies of receptors and enzymes on the nanoar-chitectures. The following are the major research achievements in the fiscal year 2023.

2. An Artificial Liposome Compartment with Size Exclusion Molecular Transport

The cellular compartment plays an essential role in organizing the complex and diverse biochemical reactions within the cell. By mimicking the function of such cellular compartments, the challenge of constructing artificial compartments has been taken up to develop new biochemical tools for efficient material production and diagnostics. The important features required for the artificial compartment are that it isolates the interior from the external environment and is further functionalized to control the transport of target chemicals to regulate the interior concentration of both substrate and reaction products. In this study, an artificial compartment (lipo-WS) with size-selective molecular transport function was constructed by using a DNA origami-guided liposome prepared by modifying the method reported by Perrault *et al.* (*ACS Nano* 2014, **8**, 5132). This completely isolates the liposome interior (lipo), including the DNA origami skeleton (WS), from the external environment and allows the assembly of a defined number of molecules of interest inside and/or outside the compartment. By incorporating a bacterial membrane protein, OmpF, into the liposome, the resulting artificial compartment (lipo-OmpF-WS) was shown to transport only the molecule of interest with a molecular weight below 600 Da from the external environment into the interior of the compartment.

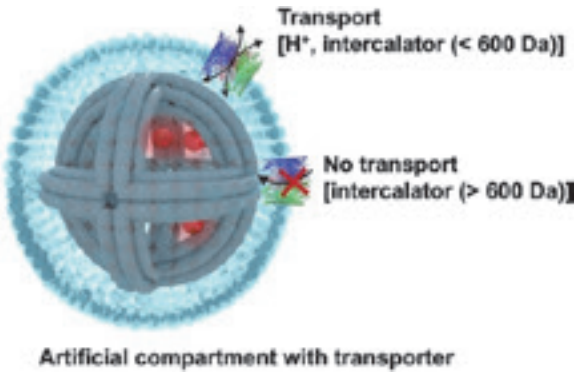

Fig. 1 Illustration of the insertion of OmpF into the membrane of lipo-WS (lipo-OmpF-WS) to construct an artificial compartment with a transporter.

3. Near Quantitative Ligation Results in Resistance of DNA Origami Against Nuclease and Cell Lysate

There have been limited efforts to ligate the staple nicks in DNA origami which is crucial for its stability against thermal and mechanical treatments, and chemical and biological environments. Here, two near-quantitative ligation methods for the native backbone linkage at the nicks in origami are demonstrated: i) a cosolvent dimethyl sulfoxide (DMSO)-assisted enzymatic ligation and ii) enzyme-free chemical ligation using CNBr. Both methods achieved over 90% ligation in 2D origami, only the CNBr method resulted in ≈80% ligation in 3D origami, while the enzyme alone yielded 31–55% (2D) or 22–36% (3D) ligation. Only the CNBr method was efficient for 3D origami. The CNBr-mediated reaction was completed within 5 min, while DMSO method took overnight. Ligation by these methods improved the structural stability up to

30 °C, stability during the electrophoresis and subsequent extraction, and stability against nuclease and cell lysate. These methods are simple, non tedious, and superior in terms of cost, reaction time, and efficiency.

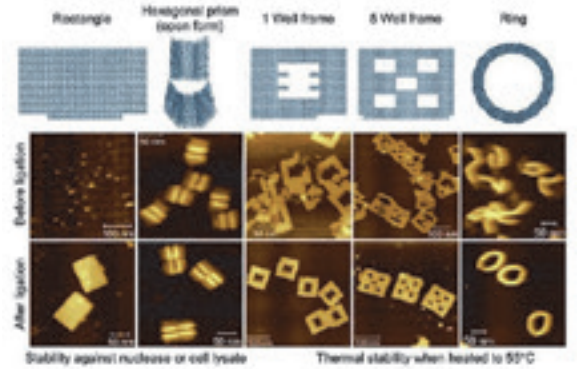

Fig. 2 Various DNA Origami shapes and atomic force microscopy images before and after ligation.

4. A Ratiometric Fluorescent Probe for pH Measurement over a Wide Range Composed of Three Types of Fluorophores Assembled on a DNA Scaffold

The desirable characteristics of the sophisticated fluorescent pH probe are ratiometric detection characteristics and a wide detection range. In this study, three types of fluorophores with different fluorescence properties were assembled on a DNA origami nanostructure. The DNA nanostructure has the advantage of being a scaffold that can assemble different types of fluorophores with control over their number and position. The defined number of three different fluorophores, i.e., pH-sensitive fluorescein (CF) and Oregon Green (OG), and pH-insensitive tetramethylrhodamine (CR), assembled on the DNA scaffold provided a ratiometric fluorescent pH probe with a wide pH detection range that could cover the variation of intracellular pH.

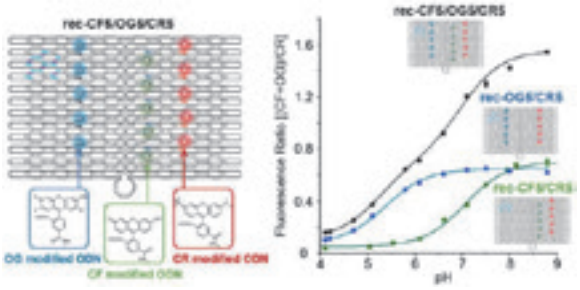

Fig. 3 An illustration of a DNA origami scaffold assembled with three types of fluorophores and their response.

5. Macropinoscope: Real-Time Simultaneous Tracking of pH and Cathepsin B Activity in Individual Macropinosomes

A fluorescent sensor that allows simultaneous analysis of environmental factors in a confined cellular space is useful for understanding precise molecular interactions in living cells and their biological responses. Macropinocytosis is a ubiquitous endocytic pathway for massive uptake of extracellular fluids, resulting in the formation of macropinosomes. Although macropinocytosis can affect intracellular delivery and cancer proliferation, information on the intracellular behavior of macropinosomes is limited. Here, we aimed to develop a macropinoscope, a sensor that simultaneously detects pH and cathepsin B activity in individual macropinosomes. A macropinosome-specific marker, dextran (70 kDa), was used as a platform, onto which fluorescein (CF), Oregon Green (OG), and tetramethylrhodamine (CR) were loaded for ratiometric pH sensing and imaging. A cathepsin B-cleavable peptide sequence carrying sulfo-Cy5 and the quencher BHQ-3 was also loaded; cleavage of the sequence was detected as an increase in sulfo-Cy5 fluorescence. A steep decrease in pH was observed 5–10 min after macropinosome formation, which was accompanied by an immediate increase in cathepsin B activity. Our design concept will lead to the development of other macropinoscopes for the simultaneous detection of other parameters in individual macropinosomes.

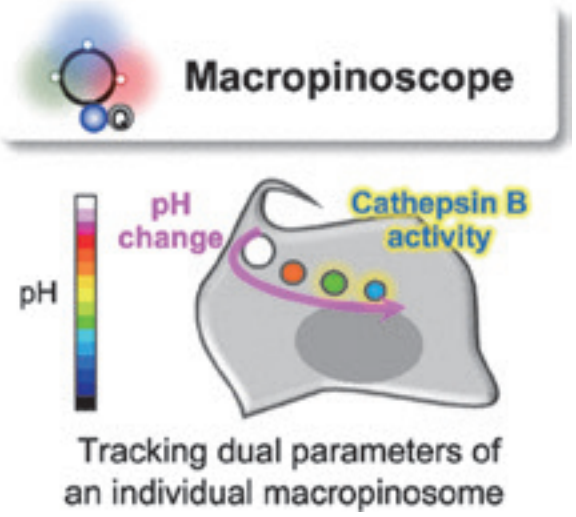

Fig. 4 Illustration of the analysis of simultaneous sensing of pH and cathepsin B activity using CF/OG/CR/CTSBsub-Dex in live cells.

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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（大韓民国）, トポイソメラーゼ反応の 1 分子計測
森井孝, POSTECH（大韓民国）, 分子ライブラリーによる蛍光 RNP センサーの開発

森井孝, POSTECH（大韓民国）, 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（大韓民国）, 細胞内酵素組織体の構築

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