

Biomolecular Organization Chemistry Research Section

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1. Introduction

Living organisms accomplish a wide range of material transformations under mild conditions by utilizing enzymes as highly efficient biological catalysts. In contrast, conventional industrial processes often rely on high temperatures, high pressures, or metal catalysts, requiring substantial energy input and resource consumption. Biological systems therefore represent an attractive model of “high efficiency with low energy cost.” In addition, while industrial systems depend heavily on finite resources such as fossil fuels and generate significant waste, living systems achieve sustainable energy utilization by harnessing sunlight, recycling organic and inorganic materials, and operating within closed metabolic cycles. These systems not only minimize environmental burden but also maintain continuous resource circulation. Another distinguishing feature of biological energy utilization is its integration with information processing. Metabolic pathways are dynamically regulated in response to environmental and cellular conditions, enabling adaptive control over growth, differentiation, and stress responses. This level of flexibility and coordination remains difficult to reproduce in artificial systems.

Our laboratory aims to understand and reconstruct these unique features of biological systems—namely, energy-efficient catalysis, sustainable resource utilization, circular processes, and adaptive regulation—through chemical approaches. By doing so, we seek to establish new design principles for next-generation energy and material transformation systems.

The following are the major research achievements in the FY 2025.

2. Enzyme cascade reactions encapsulated in a liposome compartment with size-limited molecular transport

Cellular metabolic pathways face challenges such as slow turnover, loss of intermediates, and undesired side reactions. To overcome these limitations, cells compartmentalize enzymes within organelles, creating specialized microenvironments that enhance cascade efficiency through increased local concentrations, selective permeability, and substrate channelling. Inspired by this strategy, we previously developed a liposomal compartment incorporating a DNA origami

wireframe skeleton and the membrane transporter OmpF, enabling selective molecular transport (*Chem. – Eur. J.*, **2023**, 29, e202302093; *ChemBioChem*, **2025**, 26, e202401041).

In this study, we applied this platform to construct a two-step xylose cascade system consisting of xylose reductase and xylitol dehydrogenase. While small molecules such as substrates and intermediates diffuse through OmpF, essential cofactors remain confined within the compartment. Using this well-defined system, we aim to quantitatively evaluate how compartmentalization and enzyme spatial organization influence cascade reaction efficiency, addressing a key challenge in the design of synthetic metabolic systems.

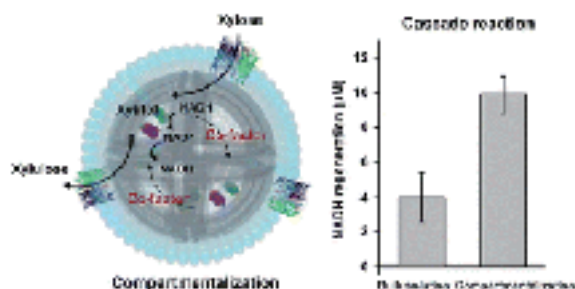


Fig. 1 Liposome-coated DNA origami compartments encapsulating defined cascade enzymes and cofactors enhance reaction efficiency under low cofactor conditions. The benefit diminishes at higher NADH levels, highlighting the roles of intermediate diffusion and enzyme spatial organization in confined environments.

3. Construction of a CO₂-Fixing Compartment Using a Shape-Transforming DNA Scaffold

A shape-transformable 3D DNA nanoscaffold was developed to mimic the confined environment of carboxysomes for CO₂ fixation. The scaffold switches between open and closed states, enabling comparison of identical RuBisCO populations under different spatial conditions. RuBisCO was site-specifically immobilized via a CLIP-GCN4 adaptor, and structural transformation was confirmed by FRET analysis. In the closed state, enzymes are brought into close proximity to mimic natural confinement. Comparative analysis showed similar CO₂-fixation activity in both states.

This system provides a versatile platform to study the effects of enzyme number, proximity, and nanoscale confinement on metabolic efficiency.

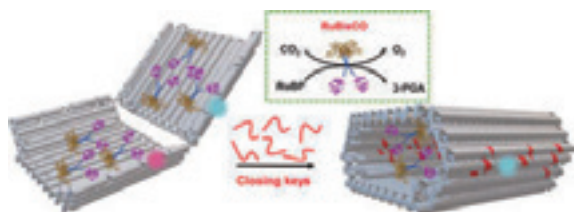


Fig. 2 A shape-transforming DNA scaffold encapsulates RuBisCO within a controllable, self-contacting compartment that mimics the carboxysome.

4. Single-Molecule Kinetic Exploration of Functional Substates in an Evolving Phosphotriesterase

Enzymes achieve catalysis by dynamically sampling diverse conformational states. Beyond this plasticity, individual enzyme molecules occupy metastable substates, forming an ensemble of functional substates within a population. Since shifts in functional substate dynamics drive phenotypic variation, their evolutionary trajectories are central to the emergence of new functions. However, the challenge of measuring functional substates has hindered our understanding of their role in enzyme evolution and the optimization of conformational substates. Here, we address this gap by investigating how functional and conformational substates are modulated during enzyme functional transitions, using single-molecule kinetic assays and molecular dynamics simulations. We analyzed wild-type phosphotriesterase (PTE) and 18 evolved variants that transitioned from the native PTE to promiscuous arylesterase (AE) activity. Our findings reveal that evolutionary transitions reshape functional and conformational substate landscapes: PTE-specialized variants exhibit broader substate distributions, whereas AE-specialized variants display more uniform substates. These results provide the first direct evidence that enzyme evolution is accompanied by coordinated shifts in functional and conformational substate equilibria, optimizing catalytic efficiency. This work highlights the power of single-molecule techniques in uncovering how heterogeneous enzyme populations navigate substate transitions and ultimately shape enzyme evolvability.

In this study, we provided the probes that enable the direct measurement of individual enzymatic functions, thereby facilitating the evaluation of enzyme evolutionary processes and supporting the outcomes of this research.

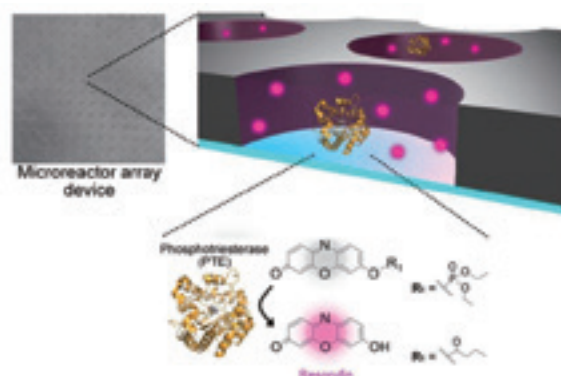


Fig. 2 Schematic illustration of the microreactor array platform for single-molecule enzyme analysis. Individual microreactors confine phosphotriesterase (PTE) molecules together with fluorogenic substrates, enabling compartmentalized reactions. Enzymatic hydrolysis produces the fluorescent product resorufin, allowing direct monitoring of catalytic activity within each microreactor.

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Collaboration Works

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

中田 栄 司, University of British Columbia (カナダ) , Enzyme evolution

Financial Support

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中田 栄 司, 学術変革領域研究(A), DNA-酵素ハイブリッド構造体による酵素集積状態の構築 (公募研究)

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2. Others

(該当なし)

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