

Biofunctional Chemistry Research Section

T. Morii, Professor
 E. Nakata, Associate Professor
 P. Lin, Assistant Professor

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 an 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 nanoarchitectures. 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.

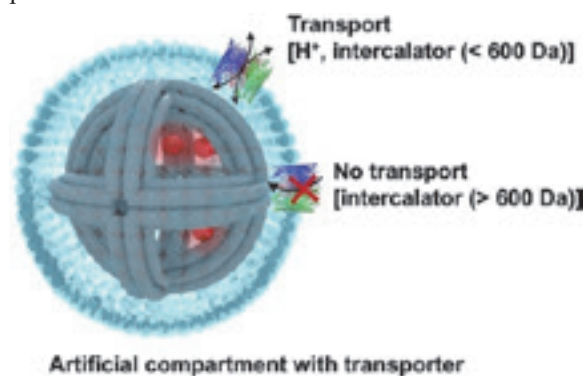


Fig. 1 Illustration of the insertion of OmpF into the membrane of lipo-WS (lipo-OmpF-WS) to construct an artificial compartment with a transporter.

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 $\approx 80\%$ 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.

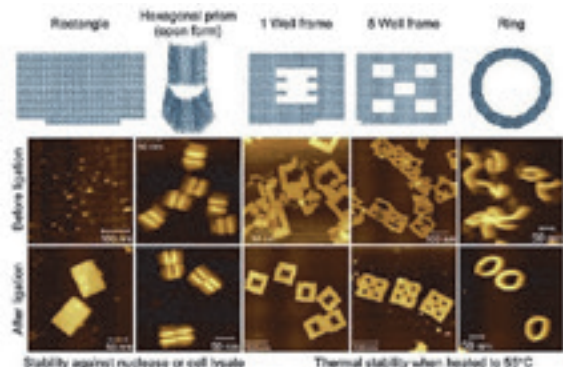


Fig. 2 Various DNA Origami shapes and atomic force microscopy images before and after ligation.

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.

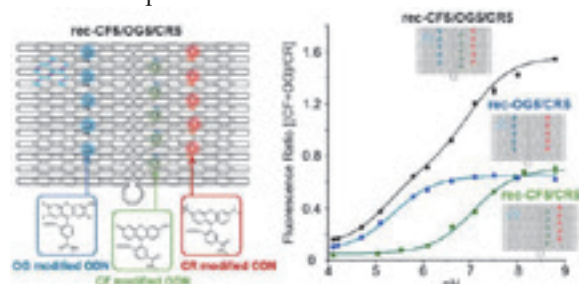


Fig. 3 An illustration of a DNA origami scaffold assembled with three types of fluorophores and their response.

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.

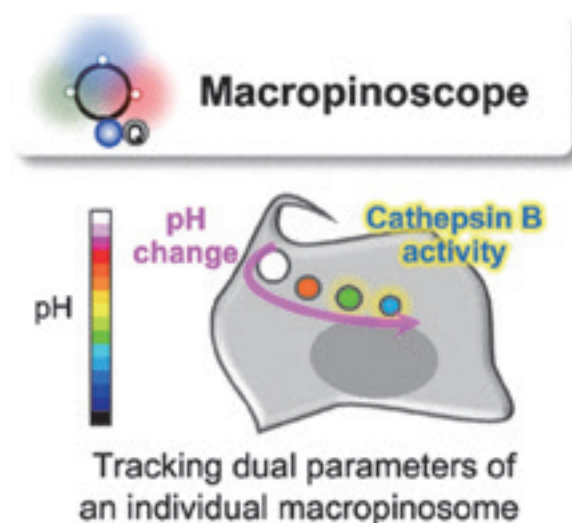


Fig. 4 Illustration of the analysis of simultaneous sensing of pH and cathepsin B activity using CF/OG/CR/CTSBsub-Dex in live cells.

The presented works were supported in part by the Grants-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan to T.M. (No. 23H02083, and 23K17977) and E.N. (No.20H02860, 22K19110 and 22H05418). And these works were also supported by JST CREST Grant Number JPMJCR18H5 to T.M.

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

Financial Support

1. Grant-in-Aid for Scientific Research

森井孝, 基盤研究(B), 代謝経路を内在する人工小器官の創製と機能発現原理の確立

森井孝, 挑戦的研究(萌芽), 光合成による二酸化炭素固定化鍵酵素の基質を拡張した分子コンビナートの構築

中田栄司, 学術変革領域研究(A) (公募研究), DNA を構造ビルディングブロックとした酵素の集積状態の構築

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

中田栄司, 挑戦的研究(萌芽), DNA-タンパク質間相互作用を介したタンパク質ポリマーのプログラム合成

2. Others

森井孝, (株) ジャパンインターナショナル総合研究所, 新エネルギーのための研究教育学術活動助成

Lin Peng, (公財) 京都大学教育研究振興財団, 細胞内生体分子凝集体をDNAナノ構造体で模倣して酵素反応を調節する

Publications

H. Hirose, E. Nakata, Z. Zhang, Y. Shibano, M. Maekawa, T. Morii, and S. Futaki, Macropinoscope: Real-Time Simultaneous Tracking of pH and Cathepsin B Activity in Individual Macropinosomes, *Analytical Chemistry*, 95, 30, 11410-11419, 2023

E. Nakata, K. Gerelbaatar, M. Asif, H. Konishi, Y. Shibano, P. Lin, T. Morii, A Ratiometric Fluorescent Probe for pH Measurement over a Wide Range Composed of Three Types of Fluorophores Assembled on a DNA Scaffold, *Chemistry*, 5, 3, 1832-1842, 2023

S. Zhang, E. Nakata, P. Lin, T. Morii, An Artificial Liposome Compartment with Size Exclusion Molecular Transport, *Chemistry-A European Journal*, e202302093, 2023

K. Krishnamurthy, Arivazhagan Rajendran, E. Nakata, T. Morii, Near Quantitative Ligation Results in Resistance of DNA Origami Against Nuclease and Cell Lysate, *Small Methods*, 8, 1, 2300999, 2023

N. Yoshikawa, S. Yamazaki, A. Nakaoku, Y. Manabe, N. Tohnai, E. Nakata, H. Takashima, 1,10-Phenanthroline-Based Octahedra Induced by Protonation of a Nitrogen Atom: Structures and Emission Properties, *ChemistrySelect*, 8, e202303561, 2023

E. Nakata, H. Dinh, L. Peng, T. Morii, Enzyme cascade reactions on DNA origami scaffold, *DNA and RNA Origami: Methods and Protocols (Methods in Molecular Biology)*, 2639, 275-300, 2023

Presentations

中田栄司, 森井 孝, DNA の利活用: センシングと分子コンビナート, システムナノ技術に関する特別研究専門委員会 第5期第2回研究会, 情報通信機構(NICT) (東京), 2023.7.4

E. Nakata, T. Morii, Multiple functional molecules assembled DNA nanostructure for bioimaging application, ACBC2023, Jeju, Korea, 2023.8.22

H. Yang, P. Lin, E. Nakata, T. Morii, Construction of an artificial CO₂ compartment using DNA nanostructure as a scaffold, 第14回エネルギー理工学研究所国際シンポジウム, 京都大学宇治キャンパス, 2023.8.30-9.1

M. Asif, E. Nakata, Y. Shibano, K. Gerelbaatar, T. Morii, Investigating macropinocytosis dynamics: development of a multiple-target detectable sensor based on DNA nanostructure, 第 14 回エネルギー理工学研究所国際シンポジウム, 京都大学宇治キャンパス, 2023.8.30-9.1

T. Morii, Toward the construction of artificial organelles, 第 14 回エネルギー理工学研究所国際シンポジウム, 京都大学宇治キャンパス, 2023.9.1

S. Chuaychob, Novel Process for Carbon Fixation Reaction Inspired by Natural Photosynthesis, 第 14 回エネルギー理工学研究所国際シンポジウム, 京都大学宇治キャンパス, 2023.9.1

中田栄司, 広瀬久昭, Asif Mashal, 二木史朗, 森井孝, 細胞内環境多元同時センサー創出, CREST「細胞外微粒子」2023 年度 領域会議 プログラム, 愛知県・ポートメッセなごや, 2023.9.6-7

P. Lin, T. Hayashi, H. Dinh, E. Nakata, M. Kinoshita, T. Morii, DNA ナノ構造体に配置した酵素の反応促進機構, 第 16 回バイオ関連化学シンポジウム, 東京理科大学野田キャンパス, 2023.9.8-10

S. Chuaychob, W. Hou, M. Shimizu, S. Nakano, A. Rajendran, E. Nakata, T. Morii, Mechanism of RNA Protein Aggregate Formation as a Cause of Triplet Repeat Disease, 第 16 回バイオ関連化学シンポジウム, 東京理科大学野田キャンパス, 2023.9.8-10

F. Komatsubara, P. Lin, E. Nakata, H. Konishi, T. Morii, Enzymatic metabolic reactions compartmentalized in liposome with a skeletal DNA nanostructure, 第 16 回バイオ関連化学シンポジウム, 東京理科大学野田キャンパス, 2023.9.8-10

M. Asif, E. Nakata, K. Gerelbaatar, Y. Shibano, T. Morii, Development of a multiple-target detectable sensor based on DNA nanostructure, 第 16 回バイオ関連化学シンポジウム, 東京理科大学野田キャンパス, 2023.9.8-10

中田栄司, 森井孝, DNA ナノ構造体を足場とした機能性分子の配置, 発動・機働変形ジョイント会議, 秋田県・協働大町ビル, 2023.9.20-21

中田栄司, 森井孝, DNA ナノ構造体を足場とした機能性分子の配置—基盤技術紹介—, 発動・機働変形ジョイント会議, 秋田県・協働大町ビル, 2023.9.20-21

中田栄司, 森井孝, ナノ空間内の環境を同時に検出するセンサー, 学術変革領域研究 (A)「生物を凌駕する無細胞分子システムのボトムアップ構築学」2023 年度 領域会議, 和歌山県・高野山大学, 2023.9.21-23

中田栄司, 森井孝, DNA を構造ビルディングブロックとした酵素の集積状態の構築, 学術変革領域研究 (A)「生物を凌駕する無細胞分子システムのボトムアップ構築学」2023 年度 領域会議, 和歌山県・高野山大学, 2023.9.21-23

中田栄司, 森井孝, DNA ナノ構造体にタンパク質を 1 分子ずつ制御して配置する, 第 72 回高分子討論会, 香川大学, 2023.9.26-28

森井孝, 生物エネルギーシステムの実現に向けた課題, The 6th Asian Chemical Biology Conference (ACBC2023), 京都大学宇治キャンパス本館会議室, 2023.10.12

S. Zhang, E. Nakata, P. Lin, T. Morii, Artificial Liposome Compartment with DNA Origami Scaffold for Size Exclusion Molecular Transport, 第 50 回国際核酸化学シンポジウム (2023 ISNAC), Miyazaki Civic Plaza, 2023.11.1

H. Yang, P. Lin, E. Nakata, T. Morii, "Construction of an artificial CO₂ fixation compartment using DNA nanostructure as a scaffold", 第 50 回国際核酸化学シンポジウム (2023 ISNAC), Miyazaki Civic Plaza, 2023.11.1

S. Chuaychob, W. Hou, M. Shimizu, S. Nakano, A. Rajendran, E. Nakata, T. Morii, In Vitro Study of Aggregate Formation between CUG Repeat RNA and MBNL1 Protein, 第 50 回国際核酸化学シンポジウム (2023 ISNAC), Miyazaki Civic Plaza, 2023.11.1

M. Asif, E. Nakata, Y. Shibano, K. Gerelbaatar, T. Morii, Development of a multiple-target detectable sensor based on DNA nanostructure, 第 50 回国際核酸化学シンポジウム (2023 ISNAC), Miyazaki Civic Plaza, 2023.11.2

P. Lin, H. Yang, E. Nakata, T. Morii, Three-dimensional DNA hexagonal prisms for cascaded enzyme reactions, 第 50 回国際核酸化学シンポジウム (2023 ISNAC), Miyazaki Civic Plaza, 2023.11.2

T. Morii, Molecular recognition and functionalization of nucleic acid and protein assemblies, 第 50 回国際核酸化学シンポジウム (2023 ISNAC), Miyazaki Civic Plaza, 2023.11.2

P. Lin, E. Nakata, T. Morii, Enhanced catalytic activity of an enzyme assembled on a DNA nanostructure, Asia 3 Roundtable of Nucleic Acids (A3RONA 2023), Chengdu, China, 2023.11.11

中田栄司, 森井孝, ナノ構造体上に配置した分子プローブで挑む分子夾雑環境, 分子夾雑に挑む化学@わかやま, 和歌山市民図書館, 2023.11.30

A. Mashal, Insights into Macropinocytosis: A DNA Nanostructure Sensor Approach, 第3回京都大学エネルギー理工学研究所学生研究発表会, 京都大学宇治キャンパス, 2023.12.8

X. Shuhui, タンパク質を配置した DNA ナノ構造体の集積化, 第3回京都大学エネルギー理工学研究所学生研究発表会, 京都大学宇治キャンパス, 2023.12.8

S. Zhang, Artificial Liposome Compartment with DNA Origami Scaffold for Size Exclusion Molecular Transport, 第3回京都大学エネルギー理工学研究所学生研究発表会, 京都大学宇治キャンパス, 2023.12.8

小西宏明, Construction and analysis of CO₂ fixing enzymes assemblies on 3DDNA nanostructures, 第3回京都大学エネルギー理工学研究所学生研究発表会, 京都大学宇治キャンパス, 2023.12.8

E. Nakata, T. Morii, Bioinspired Metabolic System Construction for Efficient Material Conversion, “Sustainable Technologies for a Better Planet: Challenges & Preparedness for 2050” (STBP2024), JC Bose University, 2024.1.6

中田栄司, 森井孝, DNA ナノ構造体を利用したセンシングデバイス, 先端 ICT デバイスラボ・コラボレーションミーティング, 情報通信機構(NICT) (東京), 2024.1.19

P. Lin, H. Yang, E. Nakata, T. Morii, Construction of artificial carboxysomes for the efficient carbon fixation, The 8th Zhejiang-Kyoto-Ajou Joint Symposium on Energy Science, Hangzhou, China, Hangzhou, China, 2024.1.22

S. Chuaychob, W. Hou, M. Shimizu, S. Nakano, A. Rajendran, P. Lin, E. Nakata, T. Morii, In Vitro Model of a Triplet-Repeat RNA Disease in Myotonic Dystrophy Type 1 Utilizing DNA Nanostructures, The 49th International Congress on Science, Technology and Technology-based Innovation (STT 49), Songkhla, Thailand, 2024.1.24

中田栄司, 森井孝, 細胞内環境多元同時センサー創出, CREST 最終報告会および先端医用材料勉強会, 山鹿市民交流センター, 2024.1.25

中田栄司, Zhang Shiwei, Lin Peng, 今井友也, 森井孝, DNA ナノ構造体を足場とした人工コンパートメントの創製, 第515回生存圏シンポジウム, 京都大学宇治キャンパス, 2024.2.28

P. Lin, E. Nakata, T. Morii, Design of Artificial Metabolic Pathways on DNA Nanoscaffolds, University of Bordeaux - Kyoto University Strategic Partnership Symposium 2024, Bordeaux, France, 2024.3.4

中田栄司, 森井孝, DNA ナノ構造体を足場とした機能性分子の精密配置, 第7回分子ロボティクス年次大会, 東京大学駒場キャンパス, 2024.3.14

A. Rajendran, K. Krishnamurthy, E. Nakata, T. Morii, Chemical ligation of staple nicks in DNA origami, 日本化学会 第104春季年会, 日本大学理工学部, 2024.3.18

F. Komatsubara, E. Nakata, P. Lin, H. Konishi, T. Morii, Enzymatic metabolic reactions compartmentalized in a liposome with a skeletal DNA nanostructure, 日本化学会 第104春季年会, 日本大学理工学部, 2024.3.19

P. Lin, H. Yang, S. Zhang, E. Nakata, T. Morii, Construction of DNA-based artificial compartments for enzyme cascade reactions, 日本化学会 第104春季年会, 日本大学理工学部, 2024.3.19

S. Zhang, E. Nakata, P. Lin, T. Morii, Artificial liposome compartment with DNA origami scaffold for size exclusion molecular transport, 日本化学会 第104春季年会, 日本大学理工学部, 2024.3.20

S. Chuaychob, W. Hou, M. Shimizu, S. Nakano, A. Rajendran, E. Nakata, T. Morii, In vitro model of RNA-Protein aggregates in myotonic dystrophy type 1, 日本化学会 第104春季年会, 日本大学理工学部, 2024.3.20

M. Asif, E. Nakata, Y. Shibano, P. Lin, T. Morii, Development of a method for simultaneous measurement of multiple factors in the intracellular local environment, 日本化学会 第104春季年会, 日本大学理工学部, 2024.3.20

H. Yang, P. Lin, E. Nakata, T. Morii, Construction of an artificial CO₂ fixation compartment using DNA nanostructure as a scaffold, 日本化学会 第104春季年会, 日本大学理工学部, 2024.3.20

S. Xian, E. Nakata, P. Lin, T. Morii, Assembly of the functional proteins located on DNA nanostructures, 日本化学会 第 104 春季年会, 日本大学理工学部, 2024.3.20