

Academic Program [Oral B] | 17. Biofunctional Chemistry, Biotechnology : Oral B

📅 Tue. Mar 19, 2024 3:55 PM - 4:55 PM JST | Tue. Mar 19, 2024 6:55 AM - 7:55 AM UTC 🏢 H931(931, Bldg. 9 [3F])

[H931-2vn] 17. Biofunctional Chemistry, Biotechnology

Chair: Eiji Nakata, Yusuke Nasu

🇯🇵 Japanese

3:55 PM - 4:15 PM JST | 6:55 AM - 7:15 AM UTC

[H931-2vn-01]

Genetically encoded biosensors for cellular metabolism

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🇯🇵 Japanese

4:15 PM - 4:35 PM JST | 7:15 AM - 7:35 AM UTC

[H931-2vn-02]

Thioester-based coupled fluorogenic assays in microdevice for the detection of single-molecule enzyme activities of esterases with specified substrate recognition

Tatsuya Ukegawa¹, ○Toru Komatsu¹, Mayano Minoda¹, Yasuteru Urano¹ (1. The University of Tokyo)

🇬🇧 English

4:35 PM - 4:55 PM JST | 7:35 AM - 7:55 AM UTC

[H931-2vn-03]

Construction of DNA-based artificial compartments for enzyme cascade reactions

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細胞内代謝を可視化するバイオセンサーの開発

(東大院理¹, JST さきがけ²) ○那須 雄介^{1,2}・上條由貴¹・Robert E. Campbell¹

Genetically encoded biosensors for cellular metabolism

(¹*School of Science, The University of Tokyo*, ²*PRESTO, Japan Science and Technology Agency*) ○Yusuke Nasu,^{1,2} Yuki Kamijo,¹ Robert E. Campbell¹

Fluorescent proteins (FPs) have been proven to be versatile scaffolds for development of biosensors¹. Specifically, GCaMP, a calcium ion (Ca²⁺) biosensor, has been widely employed to monitor neural activities in live model animals. In addition to GCaMP, various FP-based biosensors for non-Ca²⁺ target have been developed. However, few sensors have sensitivity as high as GCaMP, hampering their wide application *in vivo*.

Herein, we present that directed protein evolution and extensive biosensor expression optimization can enable the engineering of FP-based biosensors for a versatile metabolite L-lactate with high sensitivity, specificity, and spatiotemporal resolution in living cultured cells and *in vivo*. L-Lactate, traditionally considered a metabolic waste product, is increasingly recognized as an important intra- and intercellular energy fuel and signaling molecule. This study provides a powerful new optical toolbox, LACCO series, for investigating the emerging roles of extracellular and intracellular L-lactate in live model animals²⁾⁻⁵⁾.

Keywords : *L-Lactate, Fluorescent protein, Genetically encoded biosensor*

蛍光タンパク質は、標的分子依存的な蛍光バイオセンサーの足場としてよく用いられている¹⁾。特にカルシウムイオン (Ca²⁺) センサーである GCaMP は、生きたモデル動物 (*in vivo*) の神経活動をモニターするために広く使用されている。GCaMP 以外にも様々な標的のバイオセンサーが開発されているが、GCaMP ほど高い感度を持つセンサーはほとんどなく、バイオセンサーの *in vivo* での広範な利用は容易ではなかった。そこで本研究では、directed evolution をはじめとするタンパク質工学手法により、高感度、高特異性、および *in vivo* での高時空間分解能を有する乳酸バイオセンサーの開発を目的とした。これまで代謝副産物と考えられてきた乳酸は、細胞内外のエネルギー分子およびシグナル分子として近年注目されている。本研究は、生きたモデル動物における細胞内外の乳酸の新たな役割を解明するための強力なツール (LACCO シリーズ) を提供する²⁾⁻⁵⁾。

- 1) **Nasu, Y.**, Shen, Y., Kramer, L. & Campbell, R. E. “Structure- and mechanism-guided design of single fluorescent protein-based biosensors.” *Nat. Chem. Biol.* **17**, 509–518 (2021).
- 2) **Nasu Y.**, et al. “A genetically encoded fluorescent biosensor for extracellular L-lactate”, *Nature Communications*, **12**, 7058 (2021).
- 3) Le and Hario et al. “High performance genetically-encoded green fluorescent biosensors for intracellular L-lactate” *bioRxiv* 2022.10.19.512892 (2022).
- 4) **Nasu Y.**, et al. “A red fluorescent genetically encoded biosensor for extracellular L-lactate”, *bioRxiv* 2022.08.30.505811 (2022).
- 5) **Nasu Y.**, et al. “Lactate biosensors for spectrally and spatially multiplexed fluorescence imaging” *Nature Communications*, **14**, 6598 (2023).

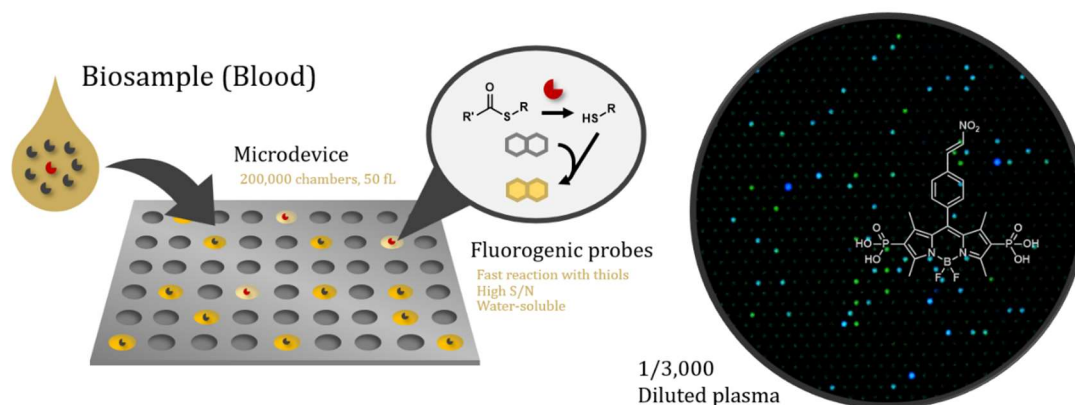
チオールを用いたカップルドアッセイに基づく血液中 1 分子エステラーゼ活性計測技術の開発

(東大院薬¹・東大院医²) 請川 達也¹・○小松 徹¹・箕田 麻弥乃¹・浦野 泰照^{1,2}
 Thioester-Based Coupled Fluorogenic Assays in Microdevice for the Detection of Single-Molecule Enzyme Activities of Esterases with Specified Substrate Recognition (¹*Graduate School of Pharmaceutical Sciences, The University of Tokyo*, ²*Graduate School of Medicine, The University of Tokyo*) Tatsuya Ukegawa,¹ ○Toru Komatsu,¹ Mayano Minoda,¹ Yasuteru Urano^{1,2}

Single-molecule enzyme activity assay is a platform that enables the analysis of enzyme activities at single proteoform level. The limitation of the targetable enzymes is the major drawback of the assay, but the general assay platform is reported to study single-molecule enzyme activities of esterases based on the coupled assay using thioesters as substrate analogues. The coupled assay is realized by developing highly water-soluble thiol-reacting probes based on phosphonate-substituted boron dipyrromethene (BODIPY). The system enables the detection of cholinesterase activities in blood samples at single-molecule level.

Keywords : Chemical Biology; Enzymes; Liquid Biopsy; Single-molecule Analysis

血液中の 1 分子酵素活性計測によって、疾患と関わる proteoform レベルのタンパク質機能異常を明らかにすることが期待される¹⁾。1 分子酵素活性計測技術は、10 万～100 万個の微小なチャンバーが並列したマイクロデバイスに希釈した生体サンプルをロードし、確率的に 0 または 1 分子の標的酵素が含まれる状態で蛍光基質を用いた活性検出をおこなうことで目的の活性を含む酵素が含まれるチャンバーを「数える」ことによっておこなわれる。本研究では、チオエステルの加水分解反応によって生じるチオールを boron dipyrromethene (BODIPY) を母核とした新規チオール検出プローブを用いてマイクロチャンバー内で検出するカップルドアッセイ系を設計、開発し、これにより血液中の 1 分子エステラーゼ活性の検出をおこなうことを可能とした²⁾。



1) S. Sakamoto et al., *Sci. Adv.* **2020**, 6, eaay0888, 2) T. Ukegawa et al., *Adv. Sci.* **2023**, in press (DOI: 10.1002/advs.202306559)

Construction of DNA-based artificial compartments for enzyme cascade reactions

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Keywords: DNA Scaffold; Artificial Compartments; Enzyme Cascade Reactions; Substrate Channeling; Competitive Enzyme

In cells, enzymes are spatially organized to perform specific sequential reactions within the compartments such as membrane-bound or membraneless organelles.¹ Artificial compartments have been constructed using liposomes, proteins, or polymers, but the applications of these carriers face the challenges of low enzyme loading yields and the difficulty in controlling the location and number of enzymes. With the advantages of precise addressability, DNA scaffolds provide the ideal platforms for enzyme assembly.² In this study, a series of DNA hexagonal prisms with different dimensions were prepared to construct the artificial compartments for enzyme reactions.

A series of scaffold systems were developed for the cascade reactions of xylose reductase (XR) and xylitol dehydrogenase (XDH) from the D-xylose metabolic pathway. The DNA scaffolds of 3D hexagonal prism (HP), medium HP (MHP), and shallow HP (SHP) were prepared by the DNA origami method.³ XR and XDH were specifically located to the scaffold in the open state by the modular adaptor method,⁴ followed by the closing process of the scaffold induced by the closing keys (short DNAs).⁵ XR and XDH were encapsulated in the closed states of HP, MHP, and SHP with an estimated interenzyme distance of ~18 nm, ~10 nm, and ~4 nm, respectively. Alditol oxidase (AldO) was used as a competing enzyme for XDH to evaluate the free diffusion of intermediates (Figure 1).

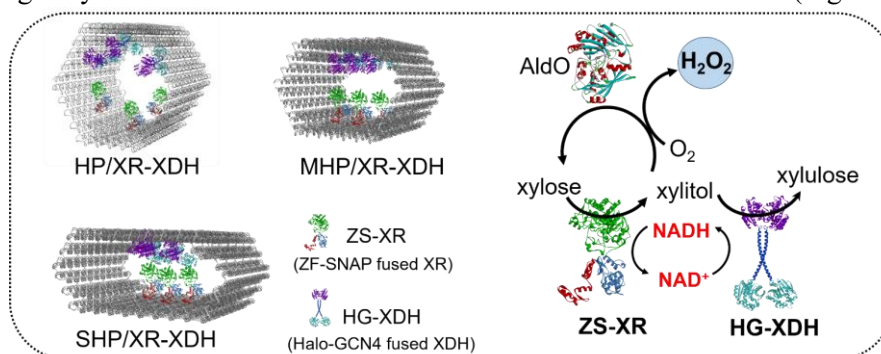


Figure 1. Cascade enzyme reactions of XR and XDH on the DNA scaffolds.

1) R. Wang, X. Liu, B. Lv, W. Sun, C. Li, *ACS Synth. Biol.* **2023**, *12*, 1378. 2) F. Hong, F. Zhang, Y. Liu, H. Yan, *Chem. Rev.* **2017**, *117*, 12584. 3) P. W. K. Rothmund, *Nature* **2006**, *440*, 297. 4) E. Nakata, H. Dinh, T. A. Ngo, M. Saimura, T. Morii, *Chem. Commun.* **2015**, *51*, 1016. 5) P. Lin, H. Dinh, Y. Morita, E. Nakata, T. Morii, *Adv. Funct. Mater.* **2023**, *33*, 2215023.