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

Thu. Mar 27, 2025 3:55 PM - 5:15 PM JST | Thu. Mar 27, 2025 6:55 AM - 8:15 AM UTC
[A]A303(A303, Bldg. 1, Area 3 [3F])

[[A]A303-2vn] 17. Biofunctional Chemistry, Biotechnology

Chair: Yuichiro Aiba, Takashi Matsuo

Japanese
3:55 PM - 4:15 PM JST | 6:55 AM - 7:15 AM UTC
[[A]A303-2vn-01]
Unique amino acid metabolism in hyperthermophilic archaea
Yuta Michimori[1], Yuusuke Yokooji[1], Yu Su[1], ○Haruyuki Atomi[1] (1. Kyoto University)

English
4:15 PM - 4:35 PM JST | 7:15 AM - 7:35 AM UTC
[[A]A303-2vn-02]
Enzyme Cascade Immobilization with Porous Coordination Cages: Application to Paper-based Cyanide Detection
○Benjamin Le Ouay[1], Yuri Kanzaki[1], Masaaki Ohba[1] (1. Kyushu University)

English
4:35 PM - 4:55 PM JST | 7:35 AM - 7:55 AM UTC
[[A]A303-2vn-03]
Artificial metal-free peroxidase designed using protein cage
○JIAXIN TIAN[1], Basudev Maity[1], Tiezheng Pan[1], Satoshi Abe[2], Tadaomi Furuta[1], Takafumi Ueno[1] (1. Institute of Science Tokyo, 2. Kyoto Prefectural University)

Japanese
4:55 PM - 5:15 PM JST | 7:55 AM - 8:15 AM UTC
[[A]A303-2vn-04]
Plasmonic structure dependence of the enhanced luminescence of D-Luciferin with the plasmonic chip modified with Luciferase
○K Tawa[1], Hitomi Yamanaka[1], Ayumu Kibata[1], Tomohiro Fukushima[2], Masaki Itatani[2], Yasunori Nawa[1], Kei Murakoshi[2] (1. Kwansei Gakuin University, 2. Hokkaido University)

日本化学会 第105春季年会 (2025)

Unique amino acid metabolism in hyperthermophilic archaea

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Keywords: Archaea; metabolism; genome; amino acid

Archaea represent the third domain of life and exhibit a wealth of unique biological functions and mechanisms, providing valuable insight into the diversity of mechanisms in biology. Understanding the common and distinct properties among the three domains of life is necessary to envision the origin of life and how it evolved. Additionally, the variety of novel biological systems identified in the Archaea, such as metabolic and regulatory mechanisms, provide a vast resource of biomolecules that can be applied in various fields of biotechnology.

Here we will present our recent research on the metabolism of the hyperthermophilic archaeon, *Thermococcus kodakarensis*. The organism is an obligate heterotroph and anaerobe, and grows on a variety of organic compounds including peptides/amino acids, oligo- and poly-saccharides, and organic acids such as pyruvate. The genome consists of 2,088,737 bp with 2,306 predicted coding regions. Among the 2,306 genes, function based on primary structure can only be predicted on approximately half of the genes. Consequently, our understanding on many of the metabolic pathways in *T. kodakarensis* remains incomplete. The presentation here will focus on enzymes and pathways related to amino acids including proline, ornithine, arginine and aspartic acid. The biosynthesis of proline in *T. kodakarensis* utilizes ornithine as a precursor[1]. In search of the source of ornithine, we focused on the arginine deiminase pathway. Although two downstream gene homologs were found on the genome, we were unable to identify a gene corresponding to arginine deiminase. Bioinformatic analyses led to the identification of a novel enzyme, which we designated arginine synthetase[2]. In terms of aspartic acid, *T. kodakarensis* possesses an incomplete tricarboxylic cycle, and the biosynthesis pathway of aspartic acid is unknown. Here, four Class I aminotransferases[3,4] from *T. kodakarensis* were examined to identify the enzyme responsible for the conversion of oxaloacetate to aspartate.

1) R. Zheng, *J. Biol. Chem.* **2018**, *293*, 3625. 2) Y. Michimori, *Proc. Natl. Acad. Sci. USA*. **2024**, *121*, e2401313121. 3) Y. Su, *Front. Microbiol.* **2023**, *14*, 1126218. 4) Y. Michimori, *Proc. Natl. Acad. Sci. USA*. **2024**, *121*, e2311390121.

Enzyme Cascade Immobilization with Porous Coordination Cages: Application to Paper-based Cyanide Detection

([1]*Graduate School of Science, Kyushu University*) ○Benjamin Le Ouay[1]; Yuri Kanzaki[1]; Masaaki Ohba[1]

Keywords: *Enzyme immobilization, Enzymatic cascade, Coordination cage, Metal-organic polyhedra, Paper-based sensors*

Enzyme immobilization is an essential technological development for biocatalysis, as it greatly improves the recyclability and stability of usually hard to isolate and sensitive enzymes for numerous applications. Recently, our research group established water-soluble porous coordination cages (PCCs) as a versatile platform for enzyme bio-immobilization. The PCCs enabled the easy immobilization of enzymes with high retention of catalytic performance in water, by forming charge-driven PCC-enzyme aggregate.[1] Since then, the method has been expanded to the co-immobilization of enzymes with additional filler proteins, enabling systematic and easy tuning of enzyme concentration in the aggregates and greatly reducing limitations related to mass transfer.[2]

In this presentation, we describe the systematic co-assembly of several different enzymes with PCCs to establish cascade reactions (Figure 1). We notably explored the catalytic couple of glucose oxidase (GOx) and horseradish peroxidase (HRP) as a model system, enabling the colorimetric detection of glucose through the oxidation of a chromogenic dye. The development of efficient system is greatly assisted by our reliable and near quantitative immobilization methods, which allows the easy tuning of enzyme ratio for an ideal cascade throughput.

Another benefit of the immobilization method is its bottom-up self-assembly nature, forming micrometer-sized aggregates from the association of nanometer-sized entities. Taking advantage of this, we developed a strategy to reliably immobilize enzymes in the standardized porosity of cellulose filter-paper, with excellent retention of enzyme content. Combining all these, we used the method to develop paper-based sensors for naked-eye detection of analytes. In particular, using the strongly inhibiting effect of cyanides anions on peroxidase activity, a new type of cyanide detection system was successfully developed.

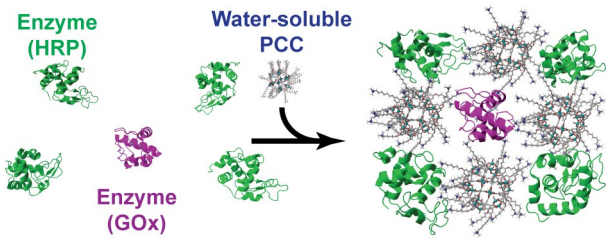

Figure 1. *Immobilization of the GOx-HRP enzyme cascades with water-soluble PPCs.*

1) B. Le Ouay, R. Minami, P.K. Boruah, R. Kunitomo, Y. Ohtsubo, K. Torikai, R. Ohtani, C. Sicard, M. Ohba, *J. Am. Chem. Soc.* **2023**, *145*, 11997. 2) Y. Kanzaki, R. Minami, K. Ota, J. Adachi, Y. Hori, R. Ohtani, B. Le Ouay, M. Ohba, *ACS Appl. Mater. Interfaces* **2024**, *16*, 54423.

Artificial metal-free peroxidase designed using protein cages

([1]*Graduate School of Life Sci. and Technol., Institute of Science Tokyo.* [2]*Graduate School of Life and Environmental Sciences, Kyoto Prefectural University)* ○Jiaxin Tian[1], Basudev Maity[1], Tiezheng Pan[1], Satoshi Abe[2], Tadaomi Furuta[1]and Takafumi Ueno[1]

Keywords: Histidine-cluster, Ferritin cage, peroxidase, X-ray structure analysis

In artificial enzyme design, a common approach is to design simple peptides with functional residue clusters rather than designing entire proteins[1]. But this often limits stability, and precise active site formation, and requires metal molecules. Recently, self-assembled histidine peptides[2] demonstrated metal and cofactor-free peroxidase-like activity, but they lacked a study about the supramolecular structures. Based on this, my study rationally designs histidine clusters on a ferritin protein cage surface to promote catalytic reactions. Previous work on ferritin showed that introducing multiple Phe at the 2-fold symmetric interface improved stability[3]. Therefore, we speculated that introducing multiple histidine at the 2-fold symmetric interface could lead to the formation of Histidine catalytic sites because Histidine has both hydrogen bond donor/acceptor properties in various natural proteins. (**Figure 1a**).

We designed and prepared a series of histidine mutants at the 2-fold symmetric interface of the ferritin cage. We evaluated the catalytic oxidation of TMB by ferritin mutants in the presence of H_2O_2. Among them, **Fr-H6** with six Histidine mutated, showed the highest catalytic activity. The results showed the formation of a blue-colored one-electron oxidized TMB product, which has a specific absorbance band at 652 nm (**Figure. 1b**). The wild-type ferritin did not show any such activity under similar experimental conditions, suggesting the involvement of mutated histidine residues in the reaction for **Fr-H6**. The X-ray crystal structure analysis of **Fr-H6** revealed the presence of mutated histidine residues surrounding the two-fold symmetry interface and formed His cluster.

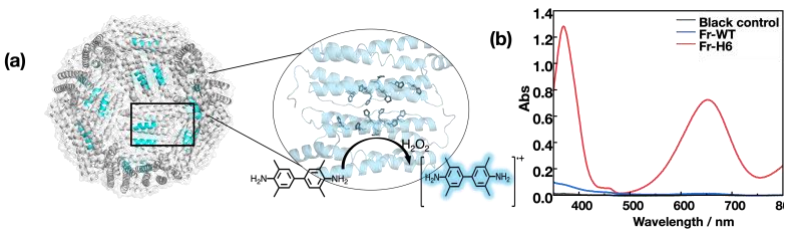

Figure1. Overview of the work. (a) Schematic representation showing the construction of a catalytic histidine-rich site near 2-fold symmetric interface (b) Absorption spectra showing the TMB oxidation by **Fr-H6** in the presence of H_2O_2 and the activity in control FrWT.

Our results showed that the histidine clusters in the ferritin cage contribute to the reduction of H_2O_2 and electron transfer from the substrate. Additionally, ferritin provides a simple method to design the active site in enzyme mimetics precisely.

Reference (1) S. L. Manna, *et al.*, *Int J Mol Sci.,*2021.22,23 (2) Q. Liu *et al.*, *Nat. Mater.*, 2021, 20, 395–402 (3) Y. Hishikawa, *et al.*, *Chem. Eur. J.*, 2023, 29

ルシフェラーゼ固定化プラズモニックチップにおけるD - ルシフェリン増強発光のプラズモニック構造依存性

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Plasmonic structure dependence of the enhanced luminescence of D-Luciferin with the plasmonic chip modified with Luciferase

([1]*Kwansei Gakuin University,* [2]*Hokkaido University*) ○Keiko Tawa,[1] Hitomi Yamanaka,[1] Ayumu Kibata,[1] Tomohiro Fukushima,[2] Masaki Itatani,[2] Yasunori Nawa,[1] Kei Murakoshi[2]

Chemiluminescence by enzymatic reaction of luciferin (substrate) and luciferase (enzyme) has been studied as firefly luminescence. In our lab, fluorescence has been enhanced with a plasmonic chip, which is the silver-coated wavelength-seized grating pattern for application to sensitive biosensor. In this study, enhancement of chemiluminescence was studied using a plasmonic chip. The plasmonic chips with 4 kinds of different pitches (300, 400, 480, 600 nm) and with different patterns (hole-array, line and space, concentric circles) were prepared. Under the microscope, chemiluminescence was observed. The chip with 480 nm-pitch showed the most enhanced luminescence and the reaction rate was more than 6-fold accelerated compared with a flat silver substrate.

Keywords : Plasmon, chemiluminescence, luciferin, luciferase, enzymatic reaction

ルシフェリン（基質）・ルシフェラーゼ（酵素）反応による化学発光機構についてはホタル発光としてこれまでに多くの研究がなされており、化学発光にはアデノシン3リン酸(ATP)を必要とすることから、食品検査にも応用されている。当研究室では蛍光増強チップデバイスとして金属薄膜で覆われた波長サイズの構造を持つプラズモニックチップの開発を行い、プラズモン共鳴による増強蛍光を用いたバイオ検出に関わる研究を進めてきた。本研究ではプラズモニックチップをルシフェリンの化学発光増強に応用することを検討した。

プラズモニックチップは 1)異なるピッチ(Λ)の格子パターンと 2)同ピッチでパターン形状が異なる格子を調製した。1)ではΛ300, 400, 480, 600 nm の 4 種類を、2)はライン＆スペース(LS)、ホールアレイ(HA)、Bull's eye 同心円(BE)パターンのレプリカを光ナノインプリント法で調製し、rf スパッタ法で Ti/Ag/Ti/SiO_2 を成膜した。プラズモニックチップにポリドーパミン膜を形成してルシフェラーゼを表面に吸着させた。そこへ、Mg^{2+}と ATP を含むルシフェリンのトリス-グリシン緩衝液を加え、EM-CCD カメラを搭載した正立顕微鏡を用いて発光を検出した。Fig. 1 に示すようにΛ480 nm で最大の発光強度を検出した。Michaelis Menten 式よりΛ480 nm の HA パターンでは銀で被覆された平坦な基板の 6 倍以上の反応速度定数が得られた。

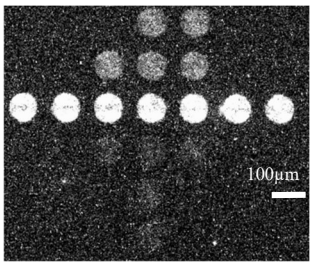

Fig.1 Chemiluminescence image of luciferin under luciferase modified to a plasmonic chip: 1st and 2nd lines (Λ600 nm), 3rd line (Λ480 nm), and 4th-6th lines (Λ400 nm).