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

Tue. Mar 19, 2024 9:00 AM - 11:00 AM JST | Tue. Mar 19, 2024 12:00 AM - 2:00 AM UTC H936(936, Bldg. 9 [3F])

[H936-2am] 17. Biofunctional Chemistry, Biotechnology

Chair: Hiroki Akiba, Takahiro Muraoka

Japanese
9:00 AM - 9:20 AM JST | 12:00 AM - 12:20 AM UTC
[H936-2am-01]

Development of antigen-templated biepitopic chemical reactions (BATER) based on structural information

○Hiroki Akiba[1,2], Kentaro Nishiyama[1], Satoshi Nagata[2], Kouhei Tsumoto[2,3], Haruhiko Kamada[1,2], Hiroaki Ohno[1,2] (1. Grad. Sch. Pharm. Sci., Kyoto Univ., 2. NIBIOHN, 3. Sch. Eng., Univ. of Tokyo)

Japanese
9:20 AM - 9:40 AM JST | 12:20 AM - 12:40 AM UTC
[H936-2am-02]

Artificial antibodies against a photo-switchable small molecule for optical cell manipulation

○Tomoki Miyazaki[1], Tomoshige Fujino[2], Tatsuyuki Yoshii[1], Mamoru Funane[2], Naoya Murata[2], Chung Nguyen Kim[2], Kai Tahara[1], Masaru Yoshikawa[1], Natsumi Fukaya[1], Satoru Nagatoishi[4], Kouhei Tsumoto[4], Gosuke Hayashi[2], Hiroshi Murakami[2,3], Shinya Tsukiji[1] (1. Graduate school of engineering, Nagoya Institute of Technology, 2. Graduate School of Engineering, Nagoya University, 3. Institute of Nano-Life-Systems, Institute of Innovation for Future Society, Nagoya University, 4. Graduate School of Engineering, The University of Tokyo)

English
9:40 AM - 10:00 AM JST | 12:40 AM - 1:00 AM UTC
[H936-2am-03]

Oxidative Protein Folding Driven by Disulfide Compounds Containing Cyclic Polyamine Ligands

○Keita Mori[1], Takahiro Muraoka[1,2] (1. Tokyo University of Agriculture and Technology, 2. KISTEC)

Japanese
10:00 AM - 10:20 AM JST | 1:00 AM - 1:20 AM UTC
[H936-2am-04]

Synthesis, inhibitory activity evaluation, and utilization of the fluorescence characteristics of PAI-1 inhibitors that have anthranilic acid scaffold

○Yuna Hamada[1], Shin-ichi Kawaguchi[1], Akiya Ogawa[2], Toshio Miyata[3] (1. The Univ. of Saga, 2. The Univ. of Osaka Metropolitan, 3. The Univ. of Tohoku)

Japanese
10:20 AM - 10:40 AM JST | 1:20 AM - 1:40 AM UTC
[H936-2am-05]

Mechanism of glycerol-induced toxicity in *Methylosinus trichosporium* OB3b cultured with lanthanide ion

○Wataru Shiina[1], Hidehiro Ito[1], Toshiaki Kamachi[1] (1. Tokyo Institute of Technology)

English

10:40 AM - 11:00 AM JST | 1:40 AM - 2:00 AM UTC

[H936-2am-06]

Dramatic enhancement of cytochrome c catalytic activity associated with a Rh coordination cage

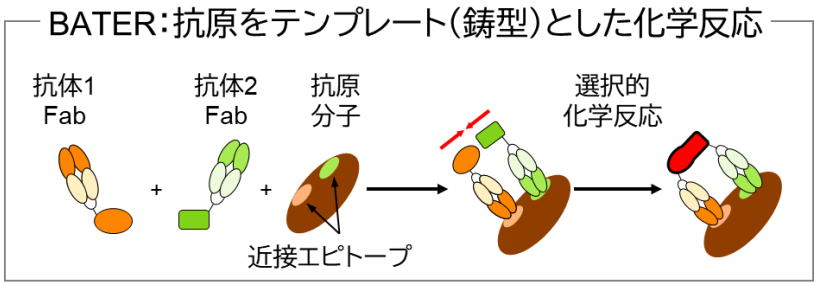
○Benjamin Le Ouay[1], Yuri Kanzaki[1], Purna Kanta Boruah[1], Masaaki Ohba[1] (1. Kyushu University)

立体構造情報に基づいた抗原テンプレート反応の開発

（京大院薬[1]・医薬健栄研[2]）○秋葉宏樹[1,2]・西山健太郎[1]・永田諭志[2]・津本浩平[2]・鎌田春彦[1,2]・大野浩章[1,2]
Development of antigen-templated biepitopic chemical reactions (BATER) based on structural information ([1]*Graduate School of Pharmaceutical Sciences, Kyoto University*, [2]*National Institutes of Biomedical Innovation, Health and Nutrition*) ○ Hiroki Akiba,[1,2] Kentaro Nishiyama,[1] Satoshi Nagata,[2] Kouhei Tsumoto,[2] Haruhiko Kamada,[1,2] Hiroaki Ohno[1,2]

A chemical reaction that proceeds in the presence of a specific template by utilizing the structural complementarity of biomolecules is called a template reaction. We have developed a template reaction called biepitopic antigen-templated chemical reaction (BATER) based on the interaction of two different antibodies binding to different epitopes of an antigen molecule. Using fluorogenic click reaction, we demonstrated that BATER was observed in dependence on the epitopes of a model antigen, TNFR2, recognized by the pair of antibody fragments (Fab). We also demonstrated that the linker length affected the reaction rates. Observation of the reactions in other antigen–antibody pairs suggested that the limitation of BATER was dominated by the potential frequency of collisions of reacting moieties, which can be designed by the information of tertiary structure of the antigen–antibody complexes.
Keywords : Antibody; biorthogonal reactions; template reaction; epitopes; protein-protein interaction

生体高分子などの構造相補性を利用することで、特定の鋳型（テンプレート）存在下で特異的に進行する化学反応をテンプレート反応と呼ぶ。我々は、抗原分子の異なる 2 つのエピトープに結合する抗体の抗原との相互作用をテンプレート反応の鋳型に利用したテンプレート反応（biepitopic antigen-templated chemical reaction: BATER）を開発した。TNFR2 をモデルに、異なるエピトープに結合する抗体に由来する抗原結合フラグメント（Fab）を得て、これに対して発蛍光性のクリック反応を観察するための官能基を Fab の C 末端選択的に導入した。蛍光観察によって、抗原 TNFR2 分子存在下において選択的に、抗体が認識するエピトープペアとリンカー長に依存したクリック反応が観察された[1)]。さらに我々は他の抗原・抗体組み合わせを利用することで、Fab の C 末端に互いに修飾された反応性官能基の分子衝突が可能な距離に 2 つの Fab が結合する設計とすれば BATER が観察されることを示した。



1) Nishiyama *et al*, *Angew. Chem. Int. Ed.* **2023,** *62,* e202306431.

光操作のための小分子光スイッチ結合性人工抗体の創製

(名工大院工[1]・名大院工[2]・名大未来ナノ[3]・東大院工[4]) ○宮崎 友輝[1]・藤野 公茂[2]・吉井 達之[1]・舟根 守[2]・村田 直哉[2]・キムグエン チュン[2]・田原 海[1]・吉川 優[1]・深谷 菜摘[1]・長門石 曉[4]・津本 浩平[4]・林 剛介[2]・村上 裕[2,3]・築地 真也[1]
Artificial antibodies against a photo-switchable small molecule for optical cell manipulation ([1]*Graduate School of Engineering, Nagoya Institute of Technology*, [2]*Graduate School of Engineering,* [3]*Institute of Nano-Life-Systems, Institute of Innovation for Future Society, Nagoya University,* [4]*Graduate School of Engineering, The University of Tokyo*)○Tomoki Miyazaki[1], Tomoshige Fujino[2], Tatsuyuki Yoshii[1], Mamoru Funane[2], Naoya Murata[2], Chung Nguyen Kim[2], Kai Tahara[1], Masaru Yoshikawa[1], Natsumi Fukaya[1], Satoru Nagatoishi[4], Kouhei Tsumoto[4], Gosuke Hayashi[2], Hiroshi Murakami[2,3], Shinya Tsukiji[1]

Optical manipulation of tag-fused proteins using photo-functional synthetic molecules is a powerful approach for controlling cell functions. In this study, we developed a novel chemo-optogenetic tool for cell manipulation based on an artificial antibody–photo-switchable small molecule pair. First, we generated artificial antibodies that bind to the cis-form of azobenzene using the TRAP display. The clone #16 showed high affinity and specificity to cis-azobenzene in vitro and in cells. By fusing clone #16 to signaling proteins, we successfully demonstrated photo-reversible control of cell signaling, such as the Raf/ERK pathway, and cell migration.
Keywords: In vitro selection, Artificial antibody, Azobenzene, Tag protein, Intracellular signal transduction

細胞内のシグナル分子を光で操作する技術は、時空間分解能の高い細胞機能制御を可能にする強力なアプローチである。その一つに、HaloTag や SNAP-tag に代表される「タグタンパク質」を用いた手法がある。これらのタグタンパク質は特定の小分子と特異的に結合するため、それら小分子の光機能化（ケージド化やフォトクロミック化など）によって二種類のタグタンパク質間の相互作用を光制御する光化学遺伝学法が報告されている[1,2]。一方、従来のツールは既存の「タグタンパク質ー小分子ペア」に依存しているため、その種類は少ない。また、小分子の光機能化にも構造的な限界がある。そこで我々は、“任意の光機能性小分子に結合する人工抗体タグを進化分子工学により創製する”という新しい方法論に基づいた光操作ツールの開発を目指した。

本研究では、光で結合の可逆的な ON/OFF 制御が可能な人工抗体タグー小分子ペアの確立を目指し、cis 型アゾベンゼンに特異的に結合するアンティカリン型人工抗体の in vitro セレクションを行なった。人工抗体ライブラリの作成後、TRAP 提示法[3]によるセレクションを行なった結果、アゾベンゼンの trans 型には結合を示さず、cis 型に特異的に結合を示す人工抗体クローンの取得に成功した（$K_d \approx 14$ nM）。この人工抗体は、動物細胞内に安定に発現でき、細胞内での光可逆的な結合と解離が可能であった。さらに、人工抗体に種々のシグナル分子を融合することで、脂質・キナーゼのシグナル活性や細胞運動の光可逆的な操作に成功した。

1) E. R. Ballister et al., *Nat. Commun.*, **2014**, 5, 5475. 2) T. Mashita et al., *ChemBioChem*., **2019**, 5, 1382. 3) T. Ishizawa et al., *J. Am. Chem. Soc.*, **2013**, 14, 5433.

Oxidative Protein Folding Driven by Disulfide Compounds Containing Cyclic Polyamine Ligands

([1]*Graduate School of Engineering, Tokyo University of Agriculture and Technology*, [2]*KISTEC*) ○Keita Mori,[1] Takahiro Muraoka[1,2]

Keywords: Protein Folding; Metal Complex; Disulfide Compound; Redox Reaction; Cyclic Polyamine Ligand

Protein folding is driven and precisely controlled by various interactions, resulting in the formation of native conformations. In particular, folding of many functional proteins is accompanied by disulfide (S–S) bonding between cysteine residues, called oxidative folding. Therefore, it has been demanded to artificially promote oxidative folding for production of therapeutic proteins and inhibition of pathological aggregates. Previously, disulfide compounds exhibiting desirable redox activity have been reported as a synthetic folding modulator.[1,2] In this study, we developed disulfide derivatives tethering cyclic polyamine ligands (cpa-SS) for efficient promotion of oxidative folding (Fig. 1a). It was expected that the cyclic polyamines can interact with substrate proteins via hydrogen bonds and metal coordination[3] to accelerate S–S introduction and suppress protein aggregation.

The designed compound cpa-SS was synthesized by conjugating cyclic polyamines with a disulfide-containing alkyl linker. Bovine Pancreatic Trypsin Inhibitor (BPTI) was used as a substrate of oxidative folding. Reverse-phase HPLC analysis revealed that cpa-SS promoted BPTI folding more efficiently than glutathione (GSSG), a typical redox-active biomolecule. The folding ability of cpa-SS became more significant when a reduced form of disulfide compounds (cpa-SH and GSH) was added to promote shuffling of S–S bonds. Furthermore, the folding efficiency was improved in the presence of transition metal ions such as Cu^{II} and Ni^{II}, which can bind to the cyclic polyamine ligands (Fig. 1b). On the other hand, GSSG hardly yielded native BPTI in the presence of Cu^{II} ions due to Cu^{II}-dependent aggregation of BPTI. These results suggested that cpa-SS have a dual effect: (i) promotion of oxidative folding and (ii) inhibition of metal-dependent protein aggregation. We will also include effects of different cyclic polyamine ligands and folding assay of other protein substrates in the presentation.

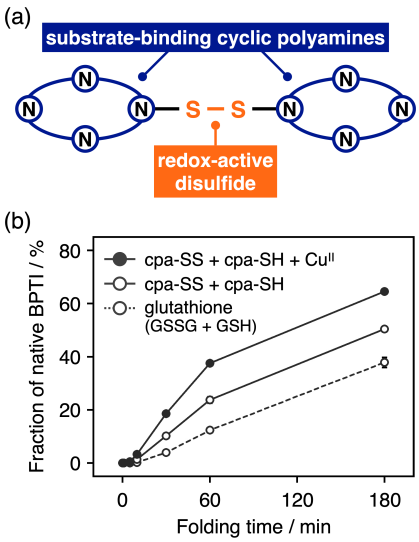


Fig. 1 (a) Disulfide compound with cyclic polyamines (cpa-SS). (b) Time-course analysis of oxidative folding of BPTI by cpa-SS.

1) J. C. Lukesh III, K. A. Andersen, K. K. Wallin, R. T. Raines, *Org. Biomol. Chem.* **2014**, *12*, 8598.
2) S. Okada, Y. Matsumoto, R. Takahashi, K. Arai, S. Kanemura, M. Okumura, T. Muraoka, *Chem. Sci.* **2023**, *14*, 7640.
3) L. O. Gerlach, J. S. Jakobsen, K. P. Jensen, M. R. Rosenkilde, R. T. Skerlj, U. Ryde, G. J. Bridger, T. W. Schwartz, *Biochemistry* **2003**, *42*, 710.

アントラニル酸骨格を有する PAI-1 阻害剤の合成、阻害活性評価と蛍光特性の利用

（佐賀大院先進[1]・佐賀大農[2]・大阪公立大院工[3]・東北大院医[4]）○濱田 悠菜[1]・川口 真一[1,2]・小川 昭弥[3]・宮田 敏男[4]

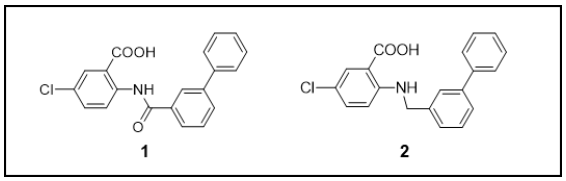
Synthesis, inhibitory activity evaluation, and utilization of the fluorescence characteristics of PAI-1 inhibitors that have anthranilic acid scaffold ([1]*Graduate School of Advanced Health Science, Saga University*, [2]*Faculty of Agriculture, Saga University,* [3]*Graduate School of Engineering, Osaka Metropolitan University,* [4]*Graduate School of Medicine, Tohoku University*)○Yuna Hamada,[1]Shin-ichi Kawaguchi,[1,2] Akiya Ogawa[3], Toshio Miyata[4]

Plasminogen activator inhibitor-1 (PAI-1) is an important protein which inhibits tissue type plasminogen activator (tPA) and prevents thrombolysis. Because PAI-1 level in various diseases is increased, inhibition of PAI-1 is expected. In this research, we synthesized anthranilic acid derivatives which inhibit PAI-1 and evaluated them in vitro. These derivatives also have fluorescence characteristics, so we quantified compound amount in cell with fluorescence detection HPLC.

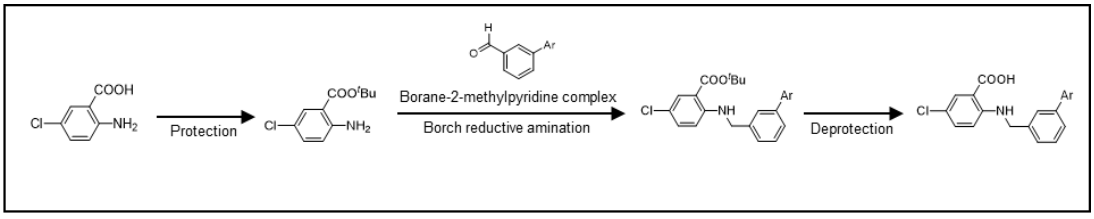
Keywords : Inhibitors; Anthranilic acid; Fluorescence molecules; Fibrinolysis system; Cells

Plasminogen activator inhibitor-1(PAI-1)は線溶系に関与するタンパク質で、tissue type plasminogen activator(tPA)を阻害して血栓溶解を妨げる。血栓症だけではなく様々な疾患で PAI-1 値が上昇することが報告されており、低分子 PAI-1 阻害剤による病状改善が期待されている。これまでにいくつかの PAI-1 阻害剤が報告されているが、いずれも上市していない。

当研究グループでは、PAI-1 阻害活性を示すジフェニルアミド誘導体（**1**）を見出し[1)]、**1** は臨床試験に進んでいる。また、**1** のアミド部分を還元したアントラニル酸誘導体（**2**）においても PAI-1 阻害活性を示すことが明らかになった。**2** はブラックライトの照射により蛍光を示すので、蛍光特性の利用が期待できる。



本研究では、3 段階の反応でアントラニル酸誘導体を合成した。また、PAI-1 阻害アッセイや Cell viability assay で評価した。さらに、細胞への取り込みを蛍光検出 HPLC で確認した。



1) Yamaoka, N. et al., *Bioorg. Med. Chem. Lett.*, **2018**, *28*, 809-813

ランタノイドイオン存在下における *Methylosinus trichosporium* OB3b のグリセロールによる毒性メカニズム

（東京工業大学 [1]）○椎名 渉 [1]、伊藤 栄紘 [1]、蒲池 利章 [1]
Mechanism of glycerol-induced toxicity in *Methylosinus trichosporium* OB3b cultured with lanthanide ion ([1]*Tokyo Institute of Technology*) ○Wataru Shiina[1], Hidehiro Ito[1], Toshiaki Kamachi[1]

Methanotrophs, which can utilize methane as a sole carbon source, were known to exhibit growth inhibition by glycerol [1]. In our previous study [2], we revealed that glycerol causes a toxic effect on *Methylosinus trichosporium* OB3b (OB3b strain) cultured with lanthanide ions.

In this study, we revealed that the glycerol-dependent toxicity in OB3b strain was caused by the oxidation of glycerol by lanthanide-dependent methanol dehydrogenase, XoxF1. The Maillard reaction, protein modification by reactive carbonyl species, occurred in OB3b cells cultured with glycerol and lanthanide ions. We demonstrated from the Maillard reaction that XoxF1 and aldehyde were involved in glycerol-dependent toxicity. We purified XoxF1 from OB3b strain and characterized its methanol and glycerol oxidation activity. Finally, we isolated and characterized an OB3b mutant that could avoid glycerol-dependent toxicity.
Keywords : Methanotroph; Lanthanide; Methanol dehydrogenase; Glycerol

メタンを単一の炭素源として生育することができるメタン資化細菌は、グリセロールによって増殖が阻害される [1]。以前の研究において、メタン資化細菌の一種である *Methylosinus trichosporium* OB3b (OB3b 株)をランタノイドイオン存在下で培養することで、グリセロール毒性が強くなることが明らかとなっている [2]。

本研究では、OB3b 株におけるグリセロール毒性が、ランタノイド依存性メタノールデヒドロゲナーゼである XoxF1 のグリセロール酸化に起因することを明らかにした(Figure)。グリセロール存在下で培養した OB3b 株菌体内で、活性カルボニル種により誘導される Maillard 反応が起きていることを蛍光分析および SDS-PAGE により明らかにした。この Maillard 反応を手掛かりとして、グリセロール毒性に XoxF1 およびアルデヒドが関連していることを示した。さらに、OB3b 株より精製した XoxF1 のメタノールおよびグリセロール酸化活性を評価した。最後に、実験室進化によってグリセロール毒性を回避した OB3b 変異株を単離し、変異遺伝子を解析した。

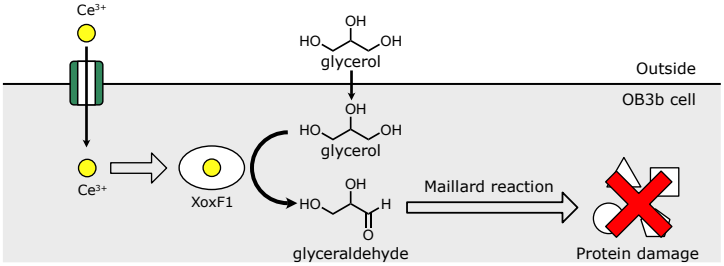


Figure Proposed mechanism of glycerol-dependent toxicity in OB3b strain.

1) Hoefman, S. *et al. PLOS ONE* **2012**, *7* (4), e34196.
2) Shiina, W. *et al. Appl. Environ. Microbiol.* **2023**, *89* (1), e01413-22.

Dramatic Enhancement of Cytochrome c Catalytic Activity Associated with a Rh Coordination Cage

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

Keywords: Cytochrome c; Peroxidase; Metal-organic Polyhedra, Coordination cage, Allosteric effect

The control of enzymes' interactions with nanosystems is a fascinating approach to regulate their catalytic performances. Recently, our group has been investigating the association of enzymes with coordination cages (also known as metal-organic polyhedra, MOPs). MOPs combine several unique features such as a nanometer-sized perfectly defined structure, a high symmetry and the coexistence of several different micro-domains on their surface, that make them very interesting materials to associate with proteins. Thanks to their intrinsic porosity and to their tunable surface properties, MOPs can act as efficient nanosized spacers for the versatile immobilization of enzymes in very mild conditions.[1] However, the influence of the local-scale structure of MOPs on their interactions with enzymes remains to be fully understood.

Here, we describe the systematic study of the influence of a rhodium-based MOP, **Rh-SO$_3$** (Figure 1A), on the catalytic activity of cytochrome c (**CytC**) acting as a peroxidase. Simple mixing of **Rh-SO$_3$** with **CytC** resulted in a dramatic enhancement of catalytic activity, up to a factor 2000 compared to that of free **CytC** at the same concentration (Figure 1B). By comparison, free ligand and Rh acetate had only a minimal influence on the activity of **CytC**, highlighting a MOP-specific effect and the importance of associating Rh metal centers and ligand functional groups in close proximity to activate the enzyme. Analysis of the **Rh-SO$_3$/CytC** dose-response plot suggested the formation of a 1:1 MOP-enzyme assembly as the active specie, with a high stability ($K_{MOP\text{-}Enzyme} > 10^{10}$ M^{-1}). The structural reasons explaining this dramatic activity enhancement are currently under investigation.

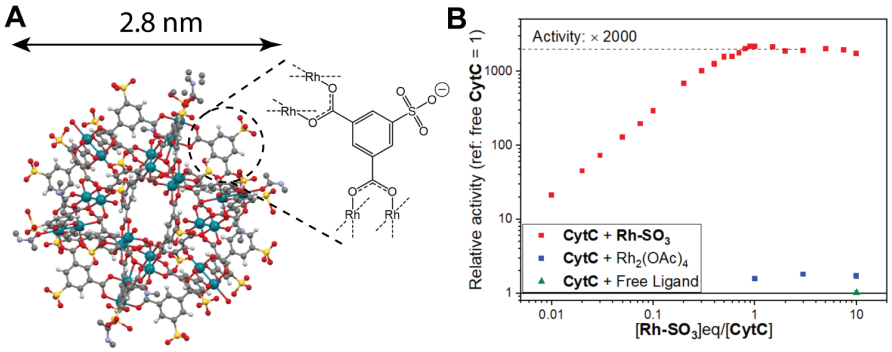


Figure 1. A: Structure of **Rh-SO$_3$** and of its constitutive ligand. **B**: Activity comparison for **CytC** in presence of **Rh-SO$_3$** or its constituents. **CytC** concentration was 1.5 μM.

[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.