アカデミックプログラム［A 講演］ | 16. 天然物化学・ケミカルバイオロジー：口頭 A 講演

2025年3月28日(金) 9:00 ~ 11:10 [A]A404(第3学舎 1号館 [4階] A404)

[[A]A404-3am] 16. 天然物化学・ケミカルバイオロジー

座長：藤岡 礼任、蓑島 維文

日本語
9:00 ~ 9:10
[[A]A404-3am-01]
代謝型グルタミン酸受容体 (mGlu1) の*in vivo*化学遺伝学的制御におけるアロステリックリガンドの改良

○森川 桂伍[1]、堂浦 智裕[2]、近藤 匠[2]、山崎 友照[3]、藤永 雅之[3]、張 明栄[3]、掛川 渉[4]、清中 茂樹[2,5] (1. 名古屋大学工学部、2. 名古屋大学大学院工学研究科、3. 量子科学技術研究開発機構、4. 慶応義塾大学医学部、5. 名古屋大学未来社会創造機構量子化学イノベーション研究所)

日本語
9:10 ~ 9:20
[[A]A404-3am-02]
アデノシンA_{2A}受容体に対する化学遺伝学を指向した変異体-阻害剤ペアの親和性向上戦略

○大木 伸一[1]、松岡 佑真[2]、堂浦 智裕[2]、清中 茂樹[3] (1. 名古屋大学工学部、2. 名古屋大学大学院工学研究科、3. 名古屋大学未来社会創造機構量子化学イノベーション研究所)

英語
9:20 ~ 9:30
[[A]A404-3am-03]
Investigation of the binding mode of stylissatin A on lysosomal PPCA and development of its analogs for anti-obesity agents

○Dewi Luthfiana[1], Yiting Sun[1], Ayumi Dakiiwa[1], Takahiro Shibata[1], Masaki Kita[1] (1. Nagoya University)

英語
9:30 ~ 9:40
[[A]A404-3am-04]
Small Molecule-Mediated Paralytic Effect on Movement Regulation in Symbiotic Dinoflagellates

○Carlos Augusto Loayza-Cassano[1], Hiroshi Yamashita [2], Yuta Tsunematsu[1], Masaki Kita[1] (1. Graduate School of Bioagricultural Sciences, Nagoya University, 2. Fisheries Technology Institute, Japan Fisheries Research and Education Agency)

英語
9:40 ~ 9:50
[[A]A404-3am-05]
Synthesis of Nanographenes with Ca^{2+} Triggered “Turn-On” Blinking Properties toward Super-Resolution Biosensing

○Zhiqiang Gao[1], Hao Zhao[1], Akimitsu Narita[1] (1. Okinawa Institute of Science and Technology)

英語
9:50 ~ 10:00
[[A]A404-3am-06]

Self-Blinking Nanographene with Cationic Side Chains for Super-Resolution Bioimaging of Live-Cell Membrane

○Hao Zhao[1], Xingfu Zhu[2], Xiaomin Liu[2], Mischa Bonn[2], Akimitsu Narita[1,2] (1. Okinawa Institute of Science and Technology Graduate University, 2. Max Planck Institute for Polymer Research)

10:00 ~ 10:10

休憩

日本語

10:10 ~ 10:20

[[A]A404-3am-07]

細胞膜のリーフレット選択的な極性イメージングを目指したテトラジン含有発蛍光性プローブの開発

○大塚 悠生[1]、山本 智也[1,2]、菊地 和也[1,2] (1. 大阪大学大学院工学研究科、2. 大阪大学免疫学フロンティア研究センター)

英語

10:20 ~ 10:30

[[A]A404-3am-08]

Development of Fluorogenic Probes for Monitoring Protein-protein Interactions with Reversible Labeling Strategy

○Jiatong Li[1], Masafumi Minoshima[1], Kazuya Kikuchi[1,2] (1. Graduate School of Engineering, Osaka University, 2. Immunology Frontier Research Center, Osaka University)

日本語

10:30 ~ 10:40

[[A]A404-3am-09]

赤色蛍光を示す可逆的タンパク質ラベル化プローブの開発

○大谷 奈々[1]、蓑島 維文[1]、菊地 和也[1,2] (1. 大阪大学大学院工学研究科、2. 大阪大学大学院免疫フロンティア研究センター)

日本語

10:40 ~ 10:50

[[A]A404-3am-10]

新規グルタチオン定量型ラマンプローブの開発

○村尾 侑大[1]、藤岡 礼任[1]、河谷 稔[1]、Spencer Spratt[2]、車 一宏[2]、小関 泰之[2]、神谷 真子[1,3] (1. 東京科学大生命理工、2. 東大先端研、3. 自律システム材料学研究センター)

日本語

10:50 ~ 11:00

[[A]A404-3am-11]

同時多重検出可能な光スイッチング型ラマンプローブの開発

○松本 叡[1]、藤岡 礼任[1]、河谷 稔[1]、赤星 光[2]、Spencer Spratt[2]、車 一宏[2]、小関 泰之[2]、神谷 真子[1,3] (1. 東京科学大学生命理工学院、2. 東大先端研、3. 自律システム材料学研究センター)

日本語

11:00 ~ 11:10

[[A]A404-3am-12]

細胞保護活性を有する脂肪酸グリセリド誘導体の合成と機能解明

○宇津木 優季[1]、権 来悟[1]、坂田 優希[1]、松野 研司[2]、大野 修[1] (1. 工学院大、2. 安田女子大)

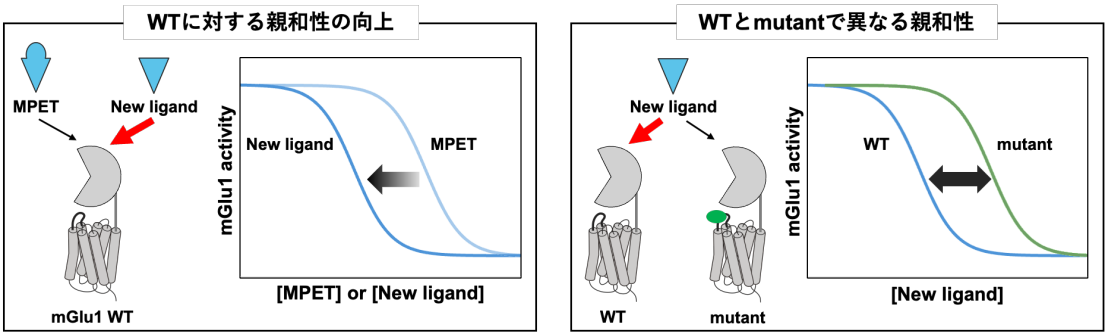
日本化学会 第105春季年会 (2025)

代謝型グルタミン酸受容体（mGlu1）の *in vivo* 化学遺伝学的制御におけるアロステリックリガンドの改良

（名大工[1]・名大院工[2]・QST[3]・慶應大医[4]・名大量子イノベ研[5]）○森川 桂伍[1]・堂浦 智裕[2]・近藤 匠[2]・山崎 友照[3]・藤永 雅之[3]・張 明栄[3]・掛川 渉[4]・清中 茂樹[2,5]
Improving allosteric ligands for in vivo chemogenetic regulation of metabotropic glutamate receptor subtype 1 (mGlu1) ([1]*School of Engineering,* [2]*Graduate school of engineering, Nagoya University,* [3]*National Institutes for Quantum Science and Technology,* [4]*Keio University School of Medicine,* [5]*Research Institute for Quantum and Chemical Innovation, Nagoya University*)
○Keigo Morikawa[1], Tomohiro Doura[2], Takumi Kondo[2], Tomoteru Yamasaki[3], Masayuki Fujinaga[3], Ming-Rong Zhang[3], Wataru Kakegawa[4], Shigeki Kiyonaka[2,5]

Metabotropic glutamate receptor subtype 1 (mGlu1) is a GPCR widely expressed in the central nervous system, and known to play a vital role in memory and learning. Since the physiological functions of mGlu1 differ depending on the cell type in which it is expressed, methods for regulating mGlu1 in specific cell types are required to elucidate the mechanisms of memory and learning. By combining MPET derived from FITM, an allosteric inhibitor of mGlu1, with a mGlu1 mutant that is not inhibited by MPET, we established a cell type-specific method for controlling mGlu1. Using this method, we successfully regulated mGlu1 in a cerebellar Purkinje cell-specific manner *in vivo*. However, positron emission tomography (PET) analysis revealed that MPET is rapidly excreted from the brain. We're searching for new ligands to overcome this issue, and will report the findings obtained during this process.
Keywords : Chemogenetics; Pharmacokinetics; GPCR; mGlu1

代謝型グルタミン酸受容体 1 型 (mGlu1) は、中枢神経系に広く発現する GPCR であり、記憶や学習に重要な役割を果たすことが知られている。一方、mGlu1 の生理機能は発現する細胞種ごとに異なるため、記憶・学習のメカニズムを解明するには特定細胞種における mGlu1 制御法が必要となる。我々は mGlu1 のアロステリック阻害剤である FITM を誘導体化した MPET と、MPET によって阻害されない mGlu1 変異体を組み合わせることで、細胞種選択的な mGlu1 制御系を構築した。本手法を用いることで、マウス小脳のプルキンエ細胞選択的な mGlu1 の制御を実現している。しかし、PET (陽電子断層撮影法) 解析の結果から、MPET は脳内から速やかに排出されてしまうことが示唆されている。現在、下図の 2 つの観点に基づき、この課題を克服する新たなリガンドの探索を行なっており、本発表ではその過程で得られた知見について報告する。



日本化学会 第105春季年会 (2025)

アデノシン A_{2A} 受容体に対する化学遺伝学を指向した変異体-阻害剤ペアの親和性向上戦略

(名大工[1]・名大院工[2]・名大量子イノベ研[3]) ○大木 伸一[1]・松岡 佑真[2]・堂浦 智裕[2]、清中 茂樹[2,3]

Strategy to increase the affinity of inhibitors to engineered adenosine A_2A receptors for chemogenetic control ([1]School of Engineering, Nagoya University, [2]Graduate School of Engineering, Nagoya University, [3]Research Institute for Quantum and Chemical Innovation, Nagoya University) ○Shinichi Oki[1], Yuma Matsuoka[2], Tomohiro Doura[2], Shigeki Kiyonaka[2,3]

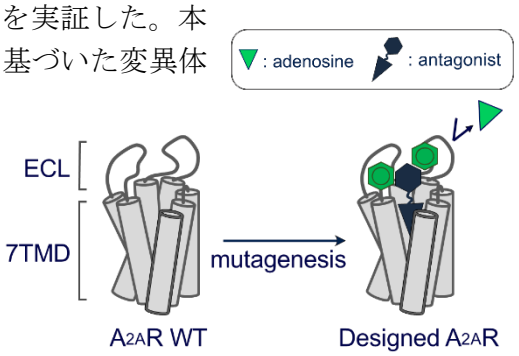
G protein-coupled receptors (GPCRs) form the largest family of membrane proteins. GPCRs are activated by a lot of extracellular signals such as some chemical substances, light and so on. GPCRs trigger signal transductions and regulate a wide range of physiological processes. Because the physiological functions of GPCRs differ depending on the cell types, developing methods to regulate GPCRs in a cell type-specific manner is necessary to understand their physiological functions.

We have previously demonstrated that mutagenesis in the extracellular loops of adenosine A_{2A} receptor ($A_{2A}R$), a class A GPCR, can reduce the binding affinity of $A_{2A}R$ inhibitors while preserving the response to adenosine. In this study, we investigated $A_{2A}R$ mutant-selective enhancement of the binding affinity of $A_{2A}R$ inhibitors by different approaches intended to reduce the dissociation rate of the inhibitors from the $A_{2A}R$ mutant or to increase the association rate of the inhibitors to the $A_{2A}R$ mutant by designing $A_{2A}R$ mutants based on ligand–receptor binding kinetics. Here we report the detailed results.

Key words: G protein-coupled receptor, Adenosine A_{2A} receptor, Chemogenetics

G タンパク質共役型受容体（GPCR）は膜タンパク質の最大ファミリーで、化学物質や光など多様な細胞外シグナルによって活性化され細胞内へ情報を伝達する。GPCR は様々な生理機能を有しているが、同一 GPCR であっても発現する細胞種によって生理機能が異なることが知られている。そのため、GPCR の生理機能を理解するために、GPCR を細胞種特異的に制御する方法の開発が望まれる。

私たちは以前に、class A GPCR の 1 つであるアデノシン A_{2A} 受容体（$A_{2A}R$）の細胞外ループへの変異導入によって、アデノシンへの応答を維持したまま阻害剤との親和性を大きく低下させることが可能であることを実証した。本研究では、リガンド–受容体間の結合速度論に基づいた変異体設計により、阻害剤の受容体からの解離速度の低下、または阻害剤の受容体への結合速度の向上を意図した異なるアプローチによる変異体選択的な阻害剤の親和性向上について検討した。本発表ではその詳細について報告する。



Investigation of the binding mode of stylissatin A on lysosomal PPCA and development of its analogs for anti-obesity agents

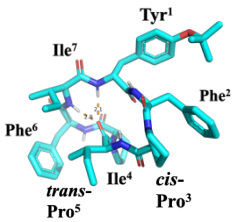
○Dewi Luthfiana,[1] Yiting Sun,[1] Ayumi Dakiiwa,[1] Takahiro Shibata,[1] Masaki Kita[1]

[1]*Graduate School of Bioagricultural Sciences, Nagoya University*

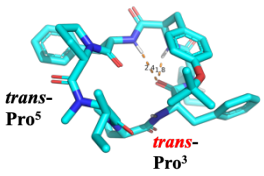
Keywords: stylissatin A, anti-obesity, dmpy-NASA-DACN, PPCA

Stylissatin A (SA) is an anti-inflammatory cyclic heptapeptide of marine sponge *Stylissa massa*.[1] SA inhibited nitric oxide production in LPS-stimulated murine macrophage RAW264.7 cells with low cytotoxicity. SA and its derivatives (SAs), especially D-Tyr[1]-*t*BuSA potently inhibit adipocyte differentiation in the murine 3T3-L1 preadipocyte.[2] SAs also inhibit the interaction between lysosomal protective protein cathepsin A (PPCA) and neuraminidase 1 (Neu1), which causes lipid droplet degradation.[3] Understanding SA-PPCA interaction is essential for the discovery and development of novel anti-obesity drugs. Therefore, to investigate the binding mode of SAs on PPCA, dmpy-NASA-DACN probe was designed. By the Huisgen reaction, dmpy-NASA-ligand conjugates can be easily prepared for in-situ labeling and binding position analysis of unknown targets.

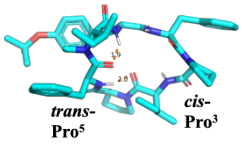
Additionally, conformational study revealed that *N*-methylation of amide moiety on isoleucine residue induced significant conformational change on SAs. While introducing *N*-methyl group can alter the conformation and intramolecular hydrogen-bond potential, it may also impact the target selectivity and biological activity of the peptide. To synthesize these peptides, solid-phase peptide synthesis (SPPS) followed by macrocyclization in solution were employed. This study will elucidate the impact of *N*-methyl group incorporation on target selectivity and bioactivity of SA analogs through in-vitro and in-vivo evaluations.



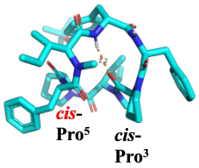
D-Tyr[1]-*t*BuSA



D-Tyr[1]-*t*BuSA-(*N*Me-Ile[4])



D-Tyr[1]-*t*BuSA-(*N*Me-Ile[7])



D-Tyr[1]-*t*BuSA-(*N*Me-Ile[4,7])

1) M. Kita, B. Gise, A. Kawamura, H. Kigoshi, *Tetrahedron Lett*., **2013**, *54*, 6826-6828. 2) M. Zhang, T. Sunaba, Y. Sun, K. Sasaki, H. Isoda, H. Kigoshi, M. Kita, *Chem. Commun*., **2019**, *55*, 5471-5474. 3) Y. Sun, A. Dakiiwa, M. Zhang, T. Shibata, M. Kita, *Chem. Eur. J*., **2024**, e202402049.

Small Molecule-Mediated Paralytic Effects on Movement Regulation in Symbiotic Dinoflagellates

○Carlos Augusto Loayza-Cassano[1], Hiroshi Yamashita[2], Yuta Tsunematsu[1], Masaki Kita[1]

[1]*Graduate School of Bioagricultural Sciences, Nagoya University*

[2]*Fisheries Technology Institute, Japan Fisheries Research and Education Agency*

Keywords: symbiotic dinoflagellate; host regulation mechanism; paralytic effect; inter-strain effect

Symbiotic dinoflagellates form essential relationships with diverse marine invertebrates, including corals, giant clams, and jellyfish. These dinoflagellates exhibit two distinct forms—motile and coccoid—with the latter being predominant within host animals[1]. However, the mechanisms by which host organisms regulate these forms and the movement of dinoflagellates remain poorly understood, as do the interactions between different dinoflagellate strains.

In this study, methanolic extracts from different strains of dinoflagellates were tested to examine potential inter-strain effects. Notably, the extract of one strain induced a reversible paralytic effect, temporarily inhibiting movement in other dinoflagellates. This effect subsided within 24 hours of exposure and removal of the extract, indicating a non-toxic response. Fractionation revealed that the dichloromethane layer retained these paralytic properties, and further separation via open column chromatography identified specific fractions responsible for the effect. Interestingly, some alkaloids of plant origin were also found to exhibit similar reversible paralytic effects on dinoflagellates, suggesting the potential role of small molecules in influencing dinoflagellate behavior.

These findings highlight a non-lethal mechanism through which certain compounds might influence dinoflagellate behavior, potentially shedding light on inter-strain interactions and regulatory processes in symbiotic relationships.

1) Fujise, L., Yamashita, H., & Koike, K. (**2014**). Application of calcofluor staining to identify motile and coccoid stages of Symbiodinium (Dinophyceae). *Fisheries science*, *80*, 363-368.

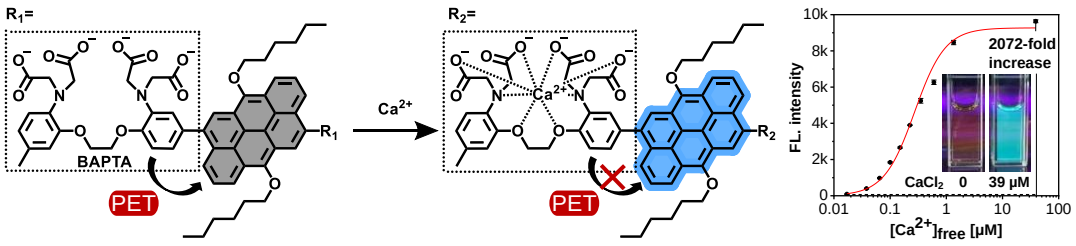
Synthesis of Nanographenes with Ca^{2+} Triggered "Turn-On" Blinking Properties toward Super-Resolution Biosensing

(Organic and Carbon Nanomaterials Unit, Okinawa Institute of Science and Technology Graduate University) ○ Zhiqiang Gao, Hao Zhao, Akimitsu Narita

Keywords: Nanographenes, Turn-On Fluorophores, Super-Resolution Imaging, Biosensing

Optical super-resolution imaging by single-molecule localization microscopy (SMLM) has emerged as a powerful tool to localize and visualize the intracellular distribution of target analytes (*e.g.*, metal ions, proteins), contributing to the understanding of life-activities.[1] Rhodamines, BODIPYs and cyanines are currently used for SMLM biosensing. However, they typically require blinking buffers to achieve effective blinking, which are toxic to live cells, prohibiting the *in-situ* biosensing applicability in life-science. Dibenzo[*hi*,*st*]ovalene (DBOV), a red-emissive and highly photostable nanographene, has demonstrated self-blinking properties and its diaza analog had additionally exhibited proton-/metal ion-induced quenching of emission and blinking, highlighting great potential for SMLM biosensing.[2] Nevertheless, their "always-on" fluorescence and blinking properties often leads to low signal-to-background ratios and thus makes it difficult to acquire high quality images. It is crucial to develop analyte-sensitive nanographenes, especially with "Turn-On" type emission and blinking properties.

We report the synthesis of a water-soluble anthanthrene derivative functionalized with 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid (ATR-BAPTA). ATR-BAPTA exhibited the longest-wavelength absorption peak at 458 nm and weak emission with a maximum at 468 nm in water. BAPTA groups can quench the fluorescence of the parent fluorophore through the photoinduced electron transfer (PET) process, which is prohibited by coordinating with Ca^{2+} ions to allow the emission.[3] Fluorescence spectroscopy revealed that the emission of ATR-BAPTA can be activated by Ca^{2+} ions, realizing >2000-fold of "Turn-On" effect, which is among the highest values in the literature. We achieved the synthesis of a water-soluble nanographene with Ca^{2+} triggered "Turn-On" emission, opening a new venture for long-term and real-time super-resolution biosensing in cell biology.



1) S. Pujals, N. Feiner-Gracia, P. Delcanale, I. Voets, L. Albertazzi, *Nat. Rev. Chem.* **2019**, 3, 68. 2) E. Jin, Q.Yang, C-W. Ju, Q. Chen, K. Landfester, M. Bonn, K. Müllen, X. Liu, A. Narita, *J. Am. Chem. Soc.* **2021,** 143, 10403. 3) C. Deo, S.-H. Sheu, J. Seo, D. E. Clapham, Luke. D. Lavis, *J. Am. Chem. Soc.* **2019,** 141, 13734.

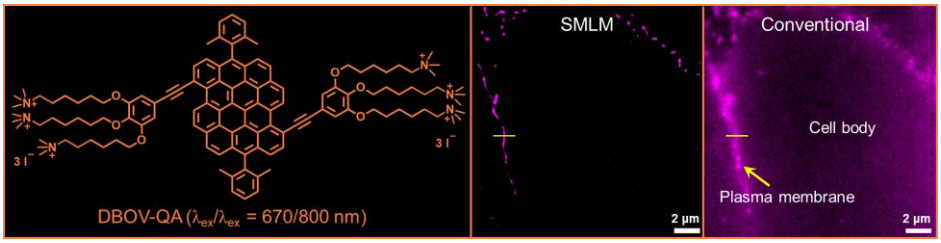
Self-Blinking Nanographene with Cationic Side Chains for Super-Resolution Bioimaging of Live-Cell Membrane

([1]*Organic and Carbon Nanomaterials Unit, Okinawa Institute of Science and Technology Graduate University*, [2]*Max Planck Institute for Polymer Research*) ○Hao Zhao,[1] Xingfu Zhu,[2] Xiaomin Liu,[2] Mischa Bonn,[2] Akimitsu Narita[1,2]

Keywords: Nanographene; Self-Blinking; Membrane Targeting; Super-Resolution; Bioimaging

Single-molecule localization microscopy (SMLM) is a powerful technique to achieve super-resolution imaging beyond the diffraction limit. SMLM protocols usually include small-molecule dyes (*e.g.*, cyanine, rhodamine, and oxazine). Unfortunately, these typically require blinking buffers with external additives, such as glutathione, ascorbic acid, and oxygen-scavenging agent, to realize effective blinking. Such buffers are incompatible with live cells, restricting their applicability in life science.[1] Nanographenes have emerged as self-blinking fluorophores for SMLM imaging. Dibenzo[*hi*,*st*]ovalene (DBOV), a highly stable and red emissive nanographene, has enabled blinking buffer-free SMLM imaging of amyloid fibrils, live-cell lysosomal dynamics, and neural nascent proteins via click chemistry, demonstrating great promise for highly versatile SMLM.[2] Nevertheless, nanographenes with intrinsic subcellular targeting properties have remained unexplored.

We report the synthesis of a water-soluble DBOV derivative bearing cationic side chains with quaternary ammonium terminals (DBOV-QA). DBOV-QA exhibited the longest-wavelength absorption peak at 670 nm and a broad near-infrared emission with a maximum at 800 nm in water. Single-molecule fluorescence studies revealed the self-blinking characteristics. Moreover, cellular distribution analysis demonstrated its high affinity to plasma membrane structures, allowing for the super-resolution SMLM bioimaging of live-cell membranes. We thus achieved a membrane-targeting nanographene with self-blinking properties, paving the way to visualizing and tracking nanoscale membrane structures and dynamics by super-resolution microscopy.



1) G. T. Dempsey, J. C. Vaughan, K. H. Chen, M. Bates, X. Zhuang, *Nat. Methods*, **2011**, *8*, 1027. 2) X. Zhu, Q. Chen, H. Zhao, Goudappagouda, Q. Yang, M. Gelléri, S. Ritz, D. Ng, K. Koynov, S. H. Parekh, V. K. Chetty, B. K. Thakur, C. Cremer, K. Landfester, K. Müllen, M. Terenzio, M. Bonn, A. Narita, X. Liu, *J. Am. Chem. Soc.* **2024**, *146*, 5195.

日本化学会 第105春季年会 (2025)

細胞膜のリーフレット選択的な極性イメージングを目指したテトラジン含有発蛍光性プローブの開発

(阪大院工[1]・阪大免フロ[2]) ○大塚 悠生[1]・山本 智也[1,2]・菊地 和也[1,2]

Tetrazine-based fluorogenic probe toward leaflet-selective polarity imaging of cell membranes ([1]*Graduate School of Engineering, Osaka University*, [2]*Immunology Frontier Research Center, Osaka University*) ○ Yusei Otsuka,[1] Tomoya Yamamoto,[1,2] Kazuya Kikuchi[1,2]

Polarity-responsive fluorescent probes, which shift their emission wavelength according to the polarity of their environment, have been widely applied to visualize the states of various molecular complexes within cells, such as hydrophobic packing in the cell membrane and protein denaturation or aggregation. However, background fluorescence often prevents the selective visualization of polarity in target regions, necessitating washing steps during imaging.

In this study, we focused on the click reaction of tetrazine. Tetrazine functions as a quencher; however, after click reactions with TCO (trans-cyclooctene) or BCN (bicyclo[6.1.0]nonyne), its quenching effect is eliminated, enabling fluorescence activation. Utilizing this mechanism, we hypothesized that it would be possible to selectively visualize the polarity of specific intracellular regions where target molecules are present.

We first synthesized a polarity-responsive fluorescent probe conjugated with tetrazine. The synthesized probe was confirmed to be quenched in various solvents and to exhibit fluorescence activation upon reaction with BCN, showing a broad fluorescence wavelength shift depending on the polarity of the solvent. In this presentation, we will also report on the application of this probe for leaflet-selective visualization of lipid membrane polarity.

Keywords : Fluorescent probes, Cell membrane, Click chemistry

極性応答蛍光プローブは、溶媒等の周囲の極性環境に応じて蛍光波長が変化する特徴を持つ。このような蛍光プローブは、細胞膜中の疎水性パッキング、タンパク質の変性や凝集など、細胞内の様々な分子複合体の状態を可視化するために応用されてきた。しかし、バックグラウンド蛍光により標的領域の極性を選択的に可視化できず、イメージングの際に、洗浄が必要となるといった課題がある。

本研究で我々は、テトラジンのクリック反応に着目した。テトラジンは消光基として機能する一方、TCO（*trans*-cyclooctene）や BCN（Bicyclo[6.1.0]nonyne）とのクリック反応後は消光効果がなくなるため、発蛍光性を付与することができる。この機構を用いることで、標的分子が存在する細胞内の特定領域の極性を選択的に可視化できると考えた。

本研究ではまず、テトラジンと結合した極性応答蛍光プローブを合成した。合成したプローブが様々な溶媒中で消光されていること、BCN との反応で発蛍光性を示し、溶媒の極性に応じて幅広い蛍光波長変化を示すことを確認した。本講演では開発したプローブを脂質膜のリーフレット選択的な極性の可視化に応用した研究結果についても報告する予定である。

Development of Fluorogenic Probes for Monitoring Protein-protein Interactions with Reversible Labeling Strategy

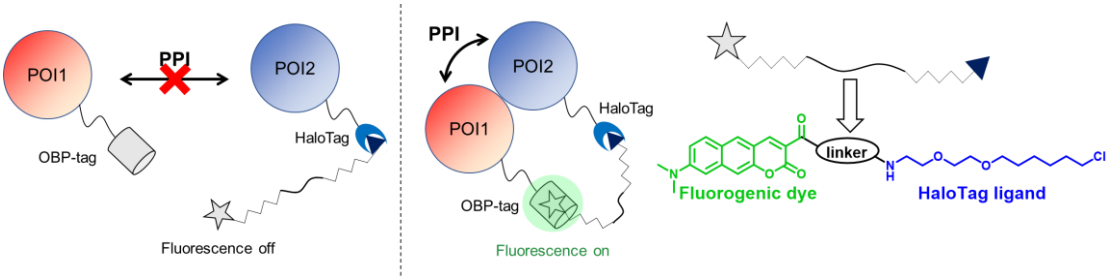
○ Jiatong Li,[1] Masafumi Minoshima,[1] Kazuya Kikuchi.[1,2] (1. Graduate School of Engineering, Osaka University, 2. Immunology Frontier Research Center, Osaka University)

Keywords: *Fluorescent Probes, Protein-protein Interactions, Fluorescence Microscopy, Reversible Interactions, Live-cell imaging*

Protein-protein interactions (PPI) play essential roles in signal transduction and molecular transportation in cells[1]. Understanding PPI reveals the network of gene products and function in cells. Fluorescence imaging is a powerful tool for visualizing biomolecules in living cells, enabling researchers to study biological processes with high spatiotemporal resolution. Fluorescence Resonance Energy Transfer (FRET) [2]-based techniques and Bimolecular Fluorescence Complementation (BiFC)[3] methods have been widely used for investigating PPIs. Nevertheless, interpreting PPIs by these approaches may be confounded by various factors, such as fluorescence crosstalk and fluorescence artifact by spontaneous complementation, respectively.

To address these challenges and advance the detection of PPIs, our research focuses on a reversible labeling strategy utilizing chemical fluorescent probes. This study introduces an innovative imaging approach for visualizing PPIs based on a fluorogenic and reversible protein labeling system. The key of this method is the OBP-tag, a protein tag designed to be fused with the protein of interest (POI), enabling dynamic labeling and visualization with reversibility.

This approach represents a novel method for PPI detection. A series of fluorescent probes were synthesized and characterized, and their irreversible binding to HaloTag and reversible interaction with OBP were successfully validated. Significant fluorescence shifts were observed in response to PPIs. Using N-Cadherin interactions as a model in live-cell imaging experiments, we demonstrated visualization at cell adhesion sites and confirmed the reversible labeling capabilities.



(1)Lu, H. et al. *Sig Transduct Target Ther* **2020**, *5* (1), 213.
(2)Park, S.-H. et al. *J. Am. Chem. Soc.* **2019**, *141* (10), 4273–4281.
(3)Wang, S. et al. *ACS Chem. Biol.* **2018**, *13* (5), 1180–1188.

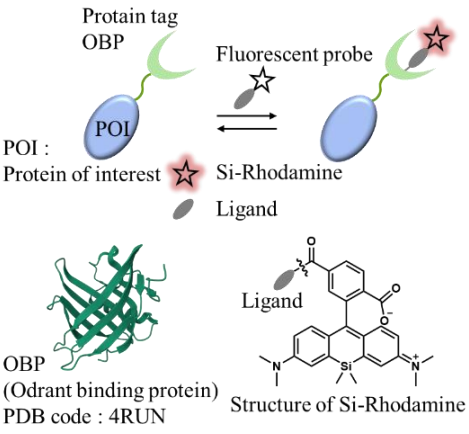
赤色蛍光を示す可逆的タンパク質ラベル化プローブの開発

（阪大院工[1]・阪大免フロ[2]）○大谷奈々[1]・蓑島維文[1]・菊地和也[1,2]
Development of Reversible Protein Labeling Probe with Red fluorescence ([1]*Graduate School of Engineering, Osaka University*, [2] *Immunology Frontier Research Center, Osaka Univ*) ○ Nana Otani,[1] Masafumi Minoshima,[1] Kazuya Kikuchi[1,2]

Fluorescent protein labeling is an important tool for the imaging-based analysis of the dynamics of proteins. During microscopic observation, the fluorescence signals are gradually diminished because of photobleaching by strong excitation light, which limits the observation time. Non-covalent labeling methods using protein tags have recently been re-evaluated because the labeling state is apparently maintained by probe exchange [(1)]. We have developed a non-covalent labeling system using odorant binding protein (OBP) as a protein tag [(2)]. The fluorescent probes were developed by conjugating environmentally responsive coumarin dye derivatives with fatty acids as OBP-tag ligands. However, coumarin dye derivatives are limited to blue to green fluorescence and their photostability is insufficient.

In this study, we focused on Si-Rhodamine derivatives with red fluorescence and high photostability for developing new protein labeling probes via OBP-tag. The design, synthesis, and fluorescent properties of the probes will be presented.

Keywords : Protein; Fluorescent probe; Imaging



タンパク質の蛍光ラベル化は、イメージングによる細胞内動態の解析のための重要なツールである。しかし、観察中に顕微鏡の強い励起光により蛍光色素が光褪色を起こすと、観察時間が制限されてしまう。タグタンパク質を用いたラベル化のうち、非共有結合によるラベル化では、光褪色してもプローブの入れ替わりにより、ラベル化状態を維持することが可能であるため、近年再評価されている[(1)]。当研究室では、非共有結合によるラベル化に着目し、Odorant binding protein (OBP) を用いたラベル化システムを開発してきた[(2)]。これまでに、環境応答性のクマリン色素誘導体と脂肪酸からなる蛍光プローブを OBP のラベル化に用いてきた。しかし、クマリン色素誘導体では、蛍光波長が青から緑色に限られてしまい、光安定性も十分でないという課題がある。

本研究では長波長の蛍光波長を持ち、光安定性の高い色素である Si-Rhodamine とその誘導体に着目し、OBP に結合する新たなプローブの開発を目的とした。Si-Rhodamine を色素としたプローブを設計、合成し、OBP との結合による発蛍光性について報告する。

1) J. Kompa *et al*., *J. Am. Chem Soc*. **2023**, *145*, 3075.
2) 蓑島 維文 他, 特願 2023-34762

日本化学会 第105春季年会 (2025)

新規グルタチオン定量型ラマンプローブの開発

(東京科学大生命理工[1]、東大先端研[2]、東京科学大自律システム材料学研究センター[3])
○村尾 侑大[1]・藤岡 礼任[1]・河谷 稔[1]・Spencer Spratt[2]・車 一宏[2]・小関 泰之[2]・神谷 真子[1,3]
Development of a novel Raman probe for glutathione ([1]*Department of Life Science and Technology, Institute of Science Tokyo*, [2]*Research Center for Advanced Science and Technology, The University of Tokyo,* [3]*Research Center for Autonomous Systems Materialogy (ASMat), Institute of Science Tokyo*) ○Yuta Murao,[1] Hiroyoshi Fujioka,[1] Minoru Kawatani,[1] Spencer John Spratt,[2] Kazuhiro Kuruma,[2] Yasuyuki Ozeki,[2] Mako Kamiya[1,3]

Glutathione (GSH) is one of the important antioxidants in cells and plays a pivotal role in maintaining intracellular redox levels, thus various fluorescent probes for detecting GSH have been developed so far. We have focused on the fact that Raman imaging, which detects molecular vibration, is superior capability for multiplexed detection to fluorescence imaging, and aimed to develop a novel Raman probe for detecting GSH. At the previous annual meeting, we reported on a novel GSH-sensitive Raman probe using rhodamine (Rho) scaffold based on our recently established molecular design that Raman signals can be controlled by the absorption wavelength of the molecule. However, the developed probe did not respond reversibly toward GSH in cells.

Then, we focused on malachite green (MG) scaffolds and prepared a series of MG derivatives to develop a new Raman probe toward GSH. As a result, we confirmed that the developed MG derivatives showed different Raman shifts depending on the chemical structure and Raman signal intensity changed upon reaction with GSH. Furthermore, we prepared several probes by conjugating GSH-reactive MG dyes and GSH-unreactive Rho dyes. Currently, we are evaluating which derivatives can function as Raman probes for quantifying GSH concentration in cells based on live-cell imaging. We plan to present these results at this meeting.
Keywords : GSH, Raman probe, rhodamine, malachite green, Raman imaging

グルタチオン（GSH）は細胞内の主たる還元種として酸化還元レベルの維持に寄与しており、これまでに様々な GSH 検出蛍光プローブが開発されてきた。我々は、分子振動を検出するラマン顕微法が蛍光法と比べて多重検出能に秀でていることに着目し、ラマンイメージングに利用可能な GSH 定量型ラマンプローブの開発に取り組んできた。前々回の年会では、分子の吸収波長によりラマン信号強度を制御する原理に基づき、ローダミン色素（Rho）を母核とした GSH プローブについて報告したが、細胞内における GSH 応答可逆性に課題があった。

そこで今回、マラカイトグリーン色素（MG）を母核として誘導体展開を行った結果、色素構造に応じて異なるラマンシフト値を示すこと、GSH と反応することでラマン信号強度が変化することを確認した。さらに、GSH 応答性 MG 色素と非応答性 Rho 色素をリンカーで結合したプローブを複数開発した。現在、ライブセルイメージング評価に基づいて GSH 濃度定量に資するラマンプローブの選定を行っており、本会ではこの成果について発表する予定である。

同時多重検出可能な光スイッチング型ラマンプローブの開発

（東京科学大生命理工[1]・東大先端研[2]・東京科学大自律システム材料学研究センター[3]）○松本 叡[1]・藤岡 礼任[1]・河谷 稔[1]・赤星 光[2]・Spencer John Spratt[2]・車 一宏[2]・小関 泰之[2]・神谷 真子[1,3]

Development of photo-switchable Raman probe with simultaneous detection ([1]*Department of Life Science and Technology, Institute of Science Tokyo*, [2]*Research Center for Advanced Science and Technology, The University of Tokyo*, [3]*Research Center for Autonomous Systems Materialogy (ASMat), Institute of Science Tokyo*) ○Satoshi Matsumoto,[1] Hiroyoshi Fujioka,[1] Minoru Kawatani,[1] Hikaru Akaboshi,[2] Spencer John Spratt,[2] Kazuhiro Kuruma,[2] Yasuyuki Ozeki,[2] Mako Kamiya[1,3]

We have developed a diarylethene-based photo-switchable Raman probe whose Raman signal changes upon light-irradiation, with which we performed super-resolution Raman imaging[1]. Since Raman microscopy is known to exhibit superior potential for multiplexed imaging than fluorescence microscopy, we set out expand the repertoire of photo-switchable Raman probes for multiplexed super-resolution vibrational imaging.

First, we predicted the Raman spectra of diarylethene derivatives with various structures based on quantum chemical calculation, which suggested that they showed various patterns of vibrational features. Next, we synthesized more than 30 derivatives, and it turned out that they actually showed various Raman spectra and signal intensities. Among them, we found 3 candidate derivatives that can be detected simultaneously. Moreover, we confirmed photo-switching properties of these candidates in living cells. We are preparing probes with organelle-targeting ligands, and we will also report these results.

Keywords: Raman probe, photo-switching, diarylethene, simultaneous detection, imaging

我々はジアリールエテンを母核として、光照射によってラマン信号が変化する光スイッチング型ラマンプローブを開発し、これを用いた超解像ラマンイメージングを報告してきた[1]。ここで、ラマン顕微法は蛍光法と比べて多重検出能が高い手法として知られているため、同時検出可能な光スイッチング型ラマンプローブの数を拡充できれば多重超解像イメージングが可能になると考え、新規プローブの開発を行った。

はじめに量子化学計算に基づいて様々な構造のジアリールエテン誘導体のラマンスペクトルを予測したところ、その構造ごとに異なるラマンスペクトルを示すことが示唆された。実際に 30 種類以上の誘導体の合成・評価を行ったところ、様々なラマンスペクトル・信号強度を有する誘導体が得られ、この中から同時検出可能なスペクトルを示す 3 種類の候補誘導体を見出した。さらに、これら誘導体は細胞内でもラマン信号を光スイッチング可能であることを確認した。現在、開発した誘導体にオルガネラ局在性リガンドを導入したプローブの開発・評価を行っているところであり、その結果についても報告する予定である。

1) *Sci Adv.* **2023**, *9*, eade9118.

日本化学会 第105春季年会 (2025)

細胞保護活性を有する脂肪酸グリセリド誘導体の合成と機能解明

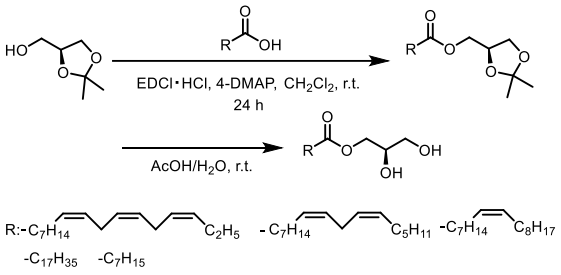
（工学院大先進工[1]・安田女子大薬[2]）○宇津木 優季[1]・権 来悟[1]・坂田 優希[1]・松野 研司[2]・大野 修[1]

Synthesis and functional elucidation of fatty acid glyceride derivatives with cytoprotective activity ([1]*School of Advanced Engineering, Kogakuin University,* [2] *Faculty of Pharmacy, Yasuda Woman's University*) ○Yuki Utsugi,[1] Leo Kwon,[1] Yuki Sakata,[1] Kenji Matsuno,[2] Osamu Ohno [1]

Stress in the endoplasmic reticulum (ER) caused by the accumulation of abnormal proteins induces cell death[1),2)]. ER stress-induced cell death is involved in diseases such as diabetes and stroke when it occurs excessively in certain cells. In this study, we searched for a compound that shows cytoprotective activity towards human cervical cancer HeLa cells treated with thapsigargin, an ER stress inducer. Then, we found 1-linolenoyl-(2*R*)-glycerol, isolated from the MeOH extract of giant hornet *Vespa mandarinia*, as an active compound. To obtain compounds with further potent activity, derivatives with various fatty acid moieties were synthesized and were evaluated for their cytoprotective activity.

Keywords : ER stress; Fatty acid; Cytoprotective activity; Derivative

小胞体ストレスは、重度になると周囲の細胞に影響を及ぼさないよう細胞死を誘導する[1) ,2)]。小胞体ストレス誘導性細胞死は特定の細胞で過剰に起こると、糖尿病や脳卒中などの疾患に関与すると考えられている。本研究では、ヒト子宮頸がん由来 HeLa 細胞に小胞体ストレス誘導剤である thapsigargin を添加することで引き起こされる細胞死を阻害する活性（細胞保護活性）を持つ化合物を天然資源より探索し、オオスズメバチ MeOH 抽出物より 1-linolenoyl-(2*R*)-glycerol を単離した（Fig. 1）。さらなる強活性誘導体を獲得するため、脂肪酸部位に多様性を持たせた誘導体を合成した（Scheme 1）。また、それらの活性を評価することで構造活性相関を解析したところ、不飽和結合と水酸基の存在が活性に寄与していることが示唆された。



Scheme 1. 脂肪酸グリセリド誘導体の合成

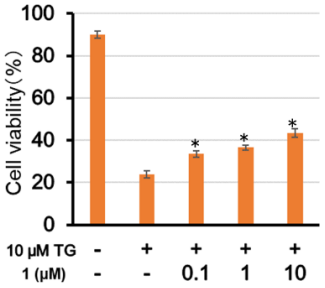


Fig. 1 1-Linolenoyl-(2*R*)-glycerol (**1**) による細胞保護活性（HeLa, trypan blue assay, 24 h）（*$p < 0.01$vs TG group）

1）V. I. Rasheva, P. M. Domingos, *Apoptosis.* **2009**, *14*, 996.
2）S. Kanemoto, K. Imaizumi, *J. Jpn. Biochem. Soc.* **2018**, *90*, 51.