シンポジウム | アジア国際シンポジウム：アジア国際シンポジウム―天然物化学・生命科学ディビジョン／生体機能関連化学・バイオテクノロジーディビジョン／医農薬化学ディビジョン―

2025年3月27日(木) 9:00 ~ 11:40 [A]D302(第3学舎 4号館 [3階] D302)

[[A]D302-2am] アジア国際シンポジウム―天然物化学・生命科学ディビジョン／生体機能関連化学・バイオテクノロジーディビジョン／医農薬化学ディビジョン―

座長、シンポジウム関係者：玉村 啓和、清中 茂樹、築地 真也、平山 祐、蓑島 維文、有本 博一

9:00 ~ 9:05

開会挨拶

英語 Invited Lecture

9:05 ~ 9:25

[[A]D302-2am-01]

複数酵素活性の*ex vivo*イメージングに資するactivatable型ラマンプローブの開発

○藤岡 礼任[1]、小関 泰之[2]、神谷 真子[1,3] (1. 東京科学大、2. 東大、3. 自律システム材料学研究センター)

英語 Keynote Lecture

9:25 ~ 9:55

[[A]D302-2am-02]

Gentle dyes for 4D fluorescence imaging

○Zhixing Chen[1] (1. Peking University)

英語 Invited Lecture

9:55 ~ 10:15

[[A]D302-2am-03]

MRIガイド下治療に資する常磁性錯体の設計と応用

○岡田 智[1] (1. 科学大)

10:15 ~ 10:20

休憩

英語 Asia Special Lecture

10:20 ~ 10:50

[[A]D302-2am-04]

Second Near-Infrared Window Fluorescent Probes for in vivo Dynamic Multiplexed Bioimaging

○Fan Zhang[1] (1. Fudan University)

英語 Invited Lecture

10:50 ~ 11:10

[[A]D302-2am-05]

チロシンナーゼを用いた近傍ラベリング法の開発

○朱 浩[1]、呉 在訓[2]、松田 侑奈[1]、美野 丈晴[1]、野中 洋[1,2]、浜地 格[1,2] (1. 京都大学、2. JST ERATO)

英語 Asia Special Lecture

11:10 ~ 11:40

[[A]D302-2am-06]

Chemical and Computational Proteomics for Functional Target Discovery

○Chu Wang[1] (1. Peking University)

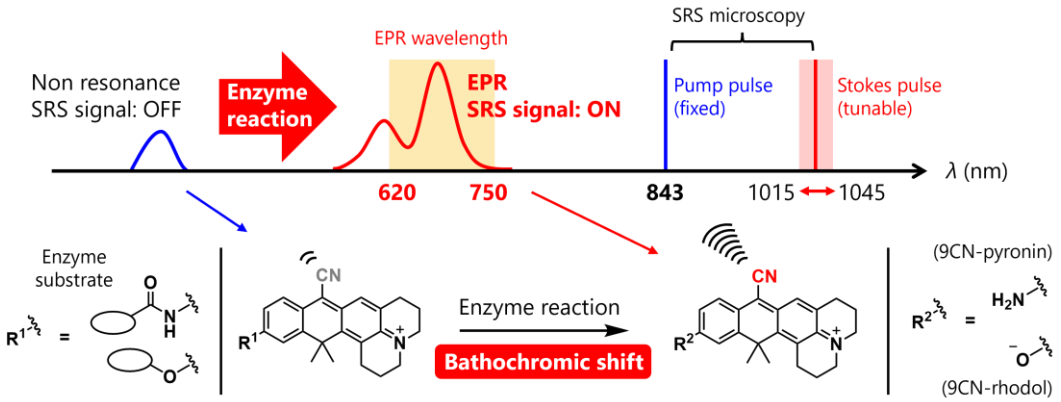
Development of activatable Raman probes for *ex vivo* imaging of plural enzyme activities

([1]*Department of Life Science and Technology, Institute of Science Tokyo*, [2]*Reseatch Center for Advanced Science and Technology, The University of Tokyo,* [3]*Research Center for Autonomous Systems Materialogy, Institute of Science Tokyo*) ○Hiroyoshi Fujioka,[1] Yasuyuki Ozeki,[2] Mako Kamiya,[1,3]

Keywords: *Raman probe*; activatable; enzyme activity; simultaneous detection; aggregation

Label-free imaging has been the prevailing strategy in Raman imaging, but recent development of vibrational tags such as small alkynes or multiplexed Raman probes enabled us to visualize a variety of biomolecules with high specificity. However, in general, existing Raman tags show constant Raman shift and signal intensity, thus its usage has been limited as labeling agents.

In this work, we aimed at developing activatable Raman imaging probe whose Raman signal is activated upon reaction with target enzyme. Specifically, we utilized a phenomenon that the signal intensity of stimulated Raman scattering (SRS) increases remarkably under electronic pre-resonance (EPR) conditions, in which a molecule is excited at 100–200 nm longer wavelength than its molecular absorption. First, we focused on pyronin derivatives with nitrile at the 9th position (9CN-pyronin) as a scaffold dye. After evaluating photophysical properties, we found 9CN-JCP as a promising scaffold and developed multicolor activatable Raman imaging probes which can detect plural enzyme activities simultaneously in living cells[1]. However, hydrolysis products of these probes tend to leak out from target cells, making it difficult to distinguish cells or region which express target enzyme in tissues. To overcome this problem, we focused on rhodol derivatives with nitrile at the 9th position (9CN-rhodol), which tend to form aggregate in aqueous solution than 9CN-pyronins, and we expected that we would be able to reduce leakage of activated probes if we can induce aggregate formation in target cells. After evaluating stability, brightness and aggregate forming ability, we found 9-CN-JCR-Bn-βGal as a promising probe which enabled us to perform *ex vivo* imaging of enzyme-expressing cells or region in *Drosophila* tissues[2].



1) H. Fujioka, J. Shou, R. Kojima, Y. Urano, Y. Ozeki, M. Kamiya, *J. Am. Chem. Soc*. **2020**, *142*, 20701–20707. 2) H. Fujioka, M. Kawatani, S. Spratt, A. Komazawa, Y. Misawa, J. Shou, T. Mizuguchi, H. Kosakamoto, R. Kojima, Y. Urano, F. Obata, Y. Ozeki, M. Kamiya, *J. Am. Chem. Soc*. **2023**, *145*, 8871–8881.

Gentle dyes for 4D fluorescence imaging

Zhixing Chen[1*]

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Phototoxicity has become a prevailing issue in the super-resolution era when boosted illumination is applied, compromising the physiological relevance of the recorded data. We advocate leveraging chemical approaches to tackle phototoxicity. By exploiting chemical motifs such as triplet state quenchers and biocompatible auxiliaries, we systematically upgrade the commonly used fluorescent markers toward alleviated phototoxicity. These gentle dyes can be directed to various cellular targets spanning mitochondria, DNA, cytoskeleton, insulin granule, and specific proteins, enabling time-lapse super-resolution imaging with minimal photodamage. For example, PK Mito Orange probe is a mitochondrial inner membrane stain that enables 30 frames of STED recording and multi-color imaging of mitochondrial components. PK Zinc dyes enable multiplexed imaging of insulin secretion in isolated islets. These biocompatible probes, with high specificity and gentle behavior under excitation light, promise to offer reliable spatial-temporal information in the era of 4D multiplexed nanoscopy.

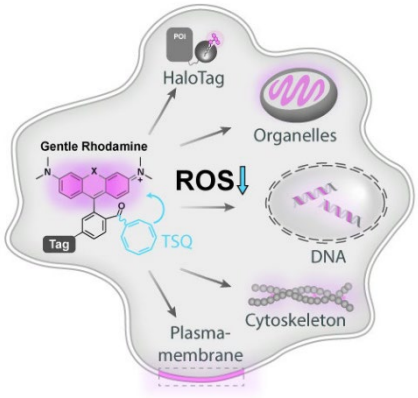


Fig. 1 Gentle rhodamines for general live cell imaging

Reference:

[1] Liu, T.#; Kompa, J.#; Ling, J.#; et al Gentle rhodamines for live-cell fluorescence microscopy. ACS Central Science 2024

[2] Liu, T.[#]; Stephan, T.[#]; Chen, P.; Keller-Findeisen, J.; Chen, J.; Riedel, D.; Yang, Z.; Jakobs, S.; Chen, Z.* Multi-color live-cell STED nanoscopy of mitochondria with a gentle inner membrane stain. PNAS 2022, *119*, e2215799119 [Cover]

[3] Zhang, Y.;Ling, J.; Liu, T.; Chen, Z.* Lumos maxima – How robust fluorophores resist photobleaching? Curr. Opin. Chem. Biol. 2024, 79, 102439 [invited review]

Design and application of paramagnetic complexes for MRI-guided therapy

(*Institute of Integrated Research, Institute of Science Tokyo*) ○Satoshi Okada
Keywords: MRI; gadolinium complexes; amyloid β; boron neutron capture therapy

Neurodegenerative diseases like Alzheimer's disease (AD) and cancers demand advanced therapeutic strategies. The development of imaging agents capable of guiding and monitoring treatment processes plays a crucial role in enhancing therapeutic outcomes. Here, I introduce design and application of paramagnetic complexes to facilitate MRI-guided therapeutic applications in AD diagnostics and boron neutron capture therapy (BNCT).

Amyloid β (Aβ) peptide fibrils have been considered as the primary toxic species causing AD, although recent studies indicate that oligomers and protofibrils formed during the pre-fibrillation stage exhibit higher toxicity. Thus, visualizing Aβ aggregation in the brain is critical for both diagnostic and mechanistic studies of AD. In our previous studies, we identified a curcumin derivative "Comp.B" which showed 100-fold higher Aβ aggregation inhibitory activities than curcumin by using a structure-activity relationship (SAR) matrix.[1] On the basis of this approach, we synthesized a Gd-DO3A-Comp.B probe by conjugating Comp.B to a gadolinium complex (Gd-DO3A).[2] Fluorescence assay and transmission electron microscopy showed Gd-DO3A-Comp.B effectively inhibited Aβ aggregation. T_1 relaxation times decreased in a dependent manner of pre-incubation time of the Aβ monomer, demonstrating the probe ability to monitor Aβ fibrillation via T_1 assay.

BNCT is a promising cancer therapy based on the nuclear reaction between boron-10 (^{10}B) and thermal neutrons to selectively destroy tumor cells, although the non-invasive estimation of ^{10}B biodistribution remains challenging. To enable non-invasive tracking, we developed Gd-MID-BSA by conjugating a boron cluster (MID) and a gadolinium complex (Gd-DO3A-Mal) to bovine serum albumin (BSA).[3] Biodistribution studies in a tumor mice model demonstrated a tumor T_1 relaxation time reduction from 2.4 s at pre-injection to 1.2 s at 24 hours post-injection, confirming sustained tumor accumulation of Gd-MID-BSA. The B/Gd ratio in organs were consistent with the B/Gd ratio in Gd-MID-BSA, demonstrating that boron concentrations can be predicted from MRI signals. Neutron irradiation at 24 hours after Gd-MID-BSA injection showed superior tumor suppression compared with MID-BSA, indicating the additional contribution of ^{157}Gd neutron capture reactions.

These results demonstrate the potential of the paramagnetic complexes for MRI-guided diagnostics and therapies. The ability to visualize and quantify molecular dynamics in vivo could improve the precision and efficacy of treatments in neurodegenerative diseases and cancer.

1) R. Y. Utomo et al., *Bioorg. Med. Chem.* **2021**, *46*, 116357. 2) R. Y. Utomo et al., *RSC Adv.* **2022**, *12*, 5027. 3) S. Okada et al., *Mol. Pharmaceutics* **2023**, *20*, 6311.

Second Near-Infrared Window Fluorescent Probes for in vivo Dynamic Multiplexed Bioimaging

([1]*Department of Chemistry, Fudan University, China*) ○Fan Zhang[1]
Keywords: *Rare earth luminescent nanomaterials; NIR; Biomedical analysis*

Fluorescent imaging and sensing with high spatio-temporal resolution and sensitivity allow the direct visualization of dynamic biological interests at different levels of components from the molecules, cells in vitro to the tissues, organs in vivo. Disastrous light attenuation and background autofluorescence in tissue at conventional imaging window of 400-900 nm have limited this technique for in vivo analysis, but they both decrease at progressively longer wavelength. Over the past decade, advances in the development of functional fluorophores operating in the second near-infrared window (NIR-II; 1000–1700 nm) have allowed the investigations of deep anatomical features in vivo with high resolution and sensitivity. However, inhomogeneous signal attenuation due to biological matter hampers the application of multiple-wavelengths NIR-II probes to multiplexed imaging. Here we present lanthanide-doped NIR-II nanoparticles with engineered luminescence lifetimes for in vivo quantitative imaging using time-domain multiplexing. To achieve this, we devise a systematic approach based on controlled energy relay that creates a tunable lifetime range spanning 3 orders-of-magnitude upon a single emission band. We consistently resolve selected lifetimes from the NIR-II nanoparticle probes at depths up to 8 mm in biological tissues, where signal-to-noise ratio derived from intensity measurements drops below 1.5. We demonstrate that robust lifetime coding is independent of tissue penetration depth, and we apply in vivo multiplexing to identify tumour subtypes in living mice. Our results correlate well with standard ex vivo immunohistochemistry assays, suggesting that luminescence lifetime imaging could be used as a minimally invasive approach for disease diagnosis.

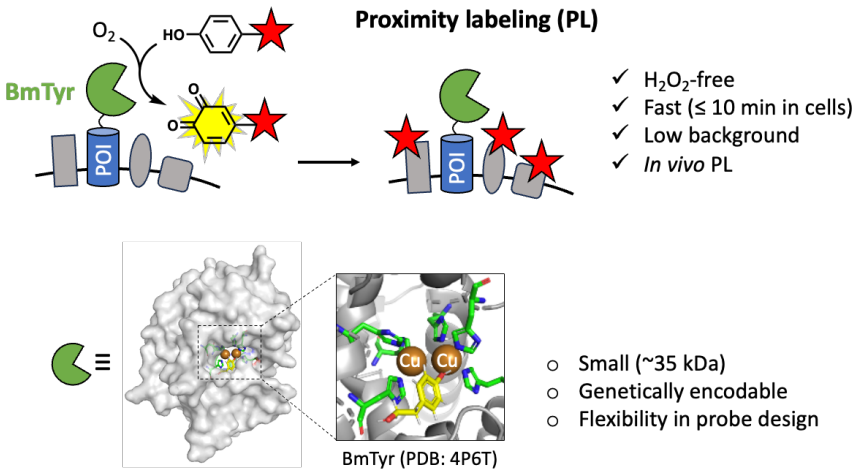
1) J. Ming, Y. Chen, H. Miao, Y. Fan, S. Wang, Z. Chen, Z. Guo, Z. Guo, L. Qi, X. Wang, B. Yun, P. Pei, H. He, H. Zhang, Y. Tang, D. Zhao, G. Wong, J. G. Bünzli, F. Zhang*, *Nat. Photonics*, **2024**, 18, 1254–1262. 2) Y. Chen, Y. Yang, Fan Zhang*, *Nat. Protoc.*, **2024**, 19, 2386-2407. 3) Y. Yang, Y. Chen, P. Pei, Y. Fan*, S. Wang, H. Zhang, D. Zhao, B.i Qian, F. Zhang*, *Nat. Nanotechnol.*, **2023**, 18, 1195-1204. 4) Y. Chen, S. Wang, F. Zhang*, *Nat. Rev. Bioeng.*, **2023**, 1, 60–78. 5) L. Lu, F. Zhang*, *Nat. Nanotechnol.*, **2022**, 17, 566–568. 6)T. Wang+, S. Wang+*, Z. Liu+, Z. He, P. Yu, M. Zhao, J. G. Bünzli, F. Zhang*, *Nat. Mater.*, **2021**, 20, 1571-1578. 7) P. Pei, Y. Chen, C. Sun, Y. Fan*, Y. Yang*, X. Liu, L. Lu, M. Zhao, H. Zhang, D. Zhao, X. Liu, F. Zhang*, *Nat. Nanotechnol.*, **2021**, 16, 1011-1018. 8)Y. Fan, P. Wang, Y. Lu*, R. Wang, L. Zhou, X. Zheng, X. Li, J. A. Piper and F. Zhang*, *Nat. Nanotechnol*., **2018**, 13(10), 941-946.

Development of Tyrosinase-based Proximity Labeling

([1]*Graduate School of Engineering, Kyoto University*, [2]*JST ERATO*) ○Hao Zhu,[1] Jae Hoon Oh,[2] Yuna Matsuda,[1] Takeharu, Mino,[1] Hiroshi Nonaka,[1,2] Itaru Hamachi[1,2]
Keywords: Tyrosinase, Proximity Labeling, Proteomics

Characterizing the protein constituents of a specific organelle and protein neighbors of a protein-of-interest (POI) is essential for understanding the function and state of the organelle and protein networks associated with the POI. Proximity labeling (PL) has emerged as a promising technology for specific and efficient spatial proteomics, which has enabled characterization of a wide range of subcellular structures and protein networks in various cell types and species. Nevertheless, the enzymes most adopted for PL in biological research still have limitations: APEX requires cytotoxic H_2O_2 for activation and thus is poor in biocompatibility for *in vivo* application, BioID shows insufficient labeling kinetics, and TurboID suffers from high background biotinylation. Therefore, new enzymes are keenly desirable for the more flexible and broader application of PL.

Here, we introduce a bacterial tyrosinase (BmTyr) as a new PL enzyme suitable for H_2O_2-free, fast (≤ 10 min in living cells), and low-background protein tagging.[1] BmTyr is genetically encodable in mammalian cells and enables subcellular-resolved PL in the nucleus, mitochondrion, and endoplasmic reticulum. We further designed a strategy of ligand-tethered BmTyr for *in vivo* PL, in which BmTyr was conjugated with a small-molecule ligand that can anchor BmTyr to an endogenous POI. Proteomics after the *in vivo* PL identified the neighboring proteins of a neurotransmitter receptor (Grm1 and Drd2) at its resident synapse in a live mouse brain.



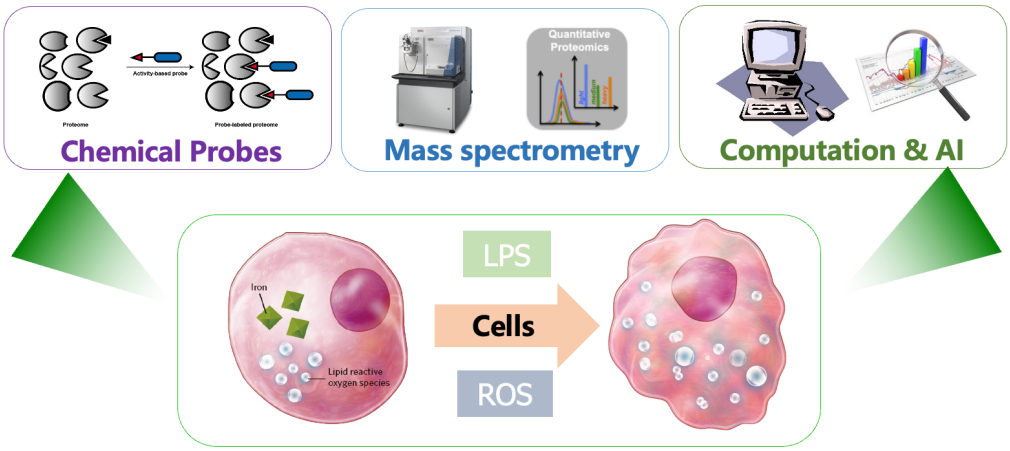
1) H. Zhu *et al.*, *J. Am. Chem. Soc.* **2024**, *146*, 11, 7515.

Chemical and Computational Proteomics for Functional Target Discovery

([1]*College of Chemistry and Molecular Engineering, Peking University*, [2]*Academy for Advanced Interdisciplinary Studies, Peking University*) ○ Chu, Wang[1,2]

Keywords: Activity-based protein profiling; Chemical proteomics; Metal-binding proteins; Itaconate; Cysteine

Genome sequencing projects have revolutionized our view of the complexity of prokaryotic and eukaryotic proteomes; however, we are also left with a daunting challenge of functionally annotating these large number of predicted proteins. Chemical proteomic methods, such as activity-based protein profiling (ABPP), have been developed aiming at systematically discovering new functional targets directly from native proteomes. In this talk, I will present recent progresses from my laboratory which combine ABPP-based chemical proteomic, biochemical and computational strategies to uncover the functional targets of ligand/cofactor binding and post-translational modifications in proteomes, which includes development of chemoproteomic methods[1] for profiling functional cysteines modified by immunoregulatory metabolite itaconate[2] as well as chelated by metals[3].



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3) Yao Cheng, Haobo Wang, Hua Xu, Yuan Liu, Bin Ma, Xuemin Chen, Xin Zeng, Xianghe Wang, Bo Wang, Carina Shiau, Sergey Ovchinnikov, Xiao-Dong Su, Chu Wang. Co-evolution-based prediction of metal-binding sites in proteomes by machine learning. *Nat. Chem. Biol.* **2023**, *19* (5), 548-555.