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

Wed. Mar 26, 2025 1:00 PM - 3:40 PM JST | Wed. Mar 26, 2025 4:00 AM - 6:40 AM UTC
[A]D501(D501, Bldg. 4, Area 3 [5F])

[[A]D501-1pm] 17. Biofunctional Chemistry, Biotechnology

Chair: Tomoya Yamamoto, Kenji Yatsuzuka

Japanese
1:00 PM - 1:10 PM JST | 4:00 AM - 4:10 AM UTC
[[A]D501-1pm-01]
Synthesis and Evaluation of Fluorescence Probes for Long-term Membrane Retention

○Ayane Ode[1], Kosei Shioji[1], Hidefumi Iwashita[1] (1. Fukuoka University)

Japanese
1:10 PM - 1:20 PM JST | 4:10 AM - 4:20 AM UTC
[[A]D501-1pm-02]
Plasma Membrane-Specific Fluorescence Probe for Visualizing Lipid Peroxidation and Ferroptosis Detection

○Rina Tokunaga[1], Kosei Shioji[1], Hidefumi Iwashita[1] (1. Fukuoka University)

Japanese
1:20 PM - 1:30 PM JST | 4:20 AM - 4:30 AM UTC
[[A]D501-1pm-03]
Development of Aldehyde Dehydrogenase 1A3-responsive Turn-on Red Fluorescent Probes

○Yuki Nishida[1], Koki Miki[1], Huiying Mu[1], Koji Miki[1], Kouichi Ohe[1] (1. Kyoto University)

Japanese
1:30 PM - 1:40 PM JST | 4:30 AM - 4:40 AM UTC
[[A]D501-1pm-04]
Development of Turn-on Fluorogenic Probes based on *meso*-vinyl-BODIPY Scaffold for Coenzyme A Detection

○Sayoko Yamamoto[1], Huiying Mu[1], Koji Miki[1], Kouichi Ohe[1] (1. Kyoto University)

English
1:40 PM - 1:50 PM JST | 4:40 AM - 4:50 AM UTC
[[A]D501-1pm-05]
Synthesis and Characterization of Unsymmetric Thieno-Phospha-Rhodamine for Near-Infrared Imaging

○Sibo Ma[1], Masayasu Taki[2], Shigehiro Yamaguchi[3,4,1] (1. Graduate School of Science, Nagoya Univ., 2. iGCORE, Gifu Univ., 3. ITbM, Nagoya Univ., 4. IRCCS, Nagoya Univ.)

Japanese
1:50 PM - 2:00 PM JST | 4:50 AM - 5:00 AM UTC
[[A]D501-1pm-06]
Diarylphosphonium-Bridged Fluorescent Dyes for Mitochondrial Imaging

○Ayato Hakamada[1], Masayasu Taki[2], Masahito Murai[1], Yoshimasa Kawaguchi[3], Shiroh Futaki[3], Shigehiro Yamaguchi[1,4,5] (1. Graduate School of Science, Nagoya University, 2. iGCORE, Gifu University, 3. ICR, Kyoto University, 4. ITbM, Nagoya University, 5. IRCCS, Nagoya University)

English
2:00 PM - 2:10 PM JST | 5:00 AM - 5:10 AM UTC

[[A]D501-1pm-07]

Development of a mitochondria-targeting fluorescent probe for visualizing Zn^{2+} flux

○EunHye Sung[1], Toshiyuki Kowada[1,2], Shin Mizukami[1,2] (1. Graduate School of Science, Tohoku University, 2. Institute of Multidisciplinary Research for Advanced Materials, Tohoku University)

2:10 PM - 2:20 PM JST | 5:10 AM - 5:20 AM UTC

Break

Japanese

2:20 PM - 2:30 PM JST | 5:20 AM - 5:30 AM UTC

[[A]D501-1pm-08]

Microglia-targeting two-photon excitation probe for simultaneous multicolor imaging

○Kenji Yatsuzuka[1], Kana Tsujimura[2], Yutaro Saito[3], Atsushi Tsurumune[4], Hiroaki Wake[3], Shigeki Kiyonaka[1,4] (1. Graduate School of Engineering, Nagoya University, 2. School of Engineering, Nagoya University, 3. Nagoya University Graduate School of Medicine, 4. Research Institute for Quantum and Chemical Innovation, Institutes of Innovation for Future Society, Nagoya University)

Japanese

2:30 PM - 2:40 PM JST | 5:30 AM - 5:40 AM UTC

[[A]D501-1pm-09]

In vivo multicolor imaging of mouse microglia

○Kana Tsujimura[1], Kenji Yatsuzuka[2], Yutaro Saito[3], Atsushi Tsurumune[4], Hiroaki Wake[3], Shigeki Kiyonaka[2,4] (1. School of Engineering, Nagoya University, 2. Graduate School of Engineering, Nagoya University, 3. Nagoya University Graduate School of Medicine, 4. Research Institute for Quantum and Chemical Innovation, Institutes of Innovation for Future Society, Nagoya University)

Japanese

2:40 PM - 2:50 PM JST | 5:40 AM - 5:50 AM UTC

[[A]D501-1pm-10]

Investigation for selective detection of APN/LAP with DNP-MRI molecular probe

○Hidetoshi Terada[1], Hiroyuki Yatabe[2], Yutaro Saito[2], Keita Saito[3], Yoichi Takakusagi[3,4], Shinsuke Sando[2] (1. School of Engineering, The University of Tokyo, 2. Graduate School of Engineering, The University of Tokyo, 3. National Institutes for Quantum Science and Technology, 4. Graduate School of Science, Chiba University)

Japanese

2:50 PM - 3:00 PM JST | 5:50 AM - 6:00 AM UTC

[[A]D501-1pm-11]

In vivo long-term observation of the hyperpolarized MRI signal with a glycine-derived probe

○Ruki Ashikaga[1], Yutaro Saito[1], Hiroyuki Yatabe[1], Yohei Kondo[1], Hiroshi Nonaka[1], Abdelazim Elsayed Elhelaly[2], Fuminori Hyodo[2], Matsuo Masayuki[2], Keita Saito[3], Yoichi Takakusagi[3,4], Kazutoshi Yamamoto[5], Murali C. Krishna[5], Shinsuke Sando[1] (1. Guraduate School of Engineering, The University of Tokyo, 2. School of Medicine, Gifu University, 3. National Institutes for Quantum Science and Technology, 4. Graduate School of Science, Chiba Universit, 5. National Institutes of Health, USA)

Japanese

3:00 PM - 3:10 PM JST | 6:00 AM - 6:10 AM UTC

[[A]D501-1pm-12]

Development of nanostructured lipid carrier for ^{19}F MRI probe

○Shiho Sugiura[1], Tomoya Yamamoto[1,2], Kazuya Kikuchi[1,2] (1. Graduate School of Engineering, Osaka Univ., 2. Immunology Frontier Research Center, Osaka Univ.)

English

3:10 PM - 3:20 PM JST | 6:10 AM - 6:20 AM UTC

[[A]D501-1pm-13]

Development of Core-shell Silica Nanoparticles for *in vivo* ^{19}F MRI

○YUE WU[1], Masafumi Minoshima[1,2,3], Kazuya Kikuchi[1,2] (1. Graduate School of Engineering, Osaka Univ., 2. Immunology Frontier Research Center, Osaka Univ., 3. JST PRESTO)

Japanese

3:20 PM - 3:30 PM JST | 6:20 AM - 6:30 AM UTC

[[A]D501-1pm-14]

Development of small ^{19}F MRI probe using lipid nanodisc

○Kodai Fukushima[1], Tomoya Yamamoto[1], Kazuya Kikuchi[1,2] (1. Graduate School of Engineering, Osaka Univ., 2. Immunology Frontier Reaserch Center, Osaka Univ.)

Japanese

3:30 PM - 3:40 PM JST | 6:30 AM - 6:40 AM UTC

[[A]D501-1pm-15]

Development of OFF-ON Type Nanoparticle-based ^{19}F MRI Contrast Agent

○Hiroto Fukuda[1], Jeremy Salaam[1], Masafumi Minoshima[1,2,3], Kazuya Kikuchi[1,3] (1. Graduate School of Engineering, Osaka Univ., 2. JST PRESTO, 3. Immunology Frontier Research Center, Osaka Univ.)

日本化学会 第105春季年会 (2025)

高膜滞留性蛍光プローブの合成と機能評価

（福大院理）○大出 綾音・塩路 幸生・岩下 秀文

Synthesis and Evaluation of Fluorescence Probes for Long-term Membrane Retention

(*Graduate School of Science, Fukuoka University*)○Ayane Ode, Kosei Shioji, Hidefumi Iwashita

Plasma membrane consists of bilayer of phospholipids and plays a crucial role in many cellular processes. Fluorescence probes have been used to visualize the plasma membrane morphology that reflects cell status and functions. However, the long-term retention of fluorescence probes in the plasma membrane for tracking has been a challenging problem. In this study, we synthesized fluorescence probes with a naphthalimide scaffold bearing 1) an ionic functional group at the head of the probe and 2) a long-chain alkyl group at the end of the probe to improve membrane retention of the fluorescence probe (Fig.1). To verify the retention ability of the probe in the plasma membrane, the synthesized probes were evaluated by time-lapse imaging of live cells. The results revealed that the ionic functional group and the alkyl chain length effectively contribute to the membrane retention of the fluorescence molecule. Furthermore, we tried to visualize the dynamics of the plasma membrane with a betaine-based fluorescence probe that showed a high affinity to the plasma membrane.

Keywords : Plasma membrane; Retention; Phospholipid; Fluorescence probe

細胞膜は、リン脂質の二重膜から構成されており、多くの細胞プロセスにおいて重要な役割を果たしている。細胞膜の形態は、細胞の状態や機能変化を反映しているため、それらをモニタリングする手法として蛍光プローブが用いられている。しかし、汎用されてきた蛍光プローブの多くは細胞膜を染色後、徐々に細胞質内に移行するため、蛍光プローブの細胞膜滞留性に課題があった[1]。

本研究では蛍光プローブの細胞膜への滞留性を向上させるため、ナフタルイミド蛍光体を母骨格にもち、親水性官能基および疎水性官能基を併せもつ7種類の蛍光プローブを合成した（Fig.1）。プローブの頭頂部には、スルホ基やベタイン基などのイオン性官能基を導入し、末端部にはペンチル基やドデシル基などの長さの異なるアルキル鎖を導入した。合成したプローブ類を生細胞へ添加し、蛍光顕微鏡による経過観察にて細胞膜滞留性を評価した。その結果、イオン性官能基およびアルキル鎖長の違いによって細胞膜への滞留性が異なることを明らかにした。さらに、膜滞留性が最も高いベタイン型の蛍光プローブを用いて、細胞膜の膜動態の可視化を試みた。

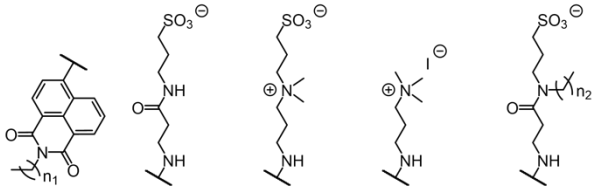


Fig.1 Chemical structure of fluorescence probes

1) Mayeul Collot *et al*., *Bioconjugate Chem*., **2020**, *31*, 875-883.

細胞膜滞留性蛍光プローブによる脂質過酸化の可視化とフェロトーシス検出

（福大院理）〇徳永 里奈・塩路 幸生・岩下 秀文

Plasma Membrane-Specific Fluorescence Probe for Visualizing Lipid Peroxidation and Ferroptosis Detection (*Graduate School of Science, Fukuoka University*) 〇Rina Tokunaga, Kosei Shioji, Hidefumi Iwashita

Phospholipids are the main constituents of biomembrane and are oxidized by the ROS, leading to lipid peroxidation. Although lipid peroxide accumulation through the process is associated with ferroptosis, an iron-dependent form of cell death, the lipid peroxidation-produced biomembranes have not been identified[(1)]. In this study, we synthesized the plasma membrane-specific fluorescence probe, LipoxPM, to visualize lipid peroxidation and evaluated it using ferroptosis-induced cells. Using this probe, we were able to visualize lipid peroxidation in the plasma membrane during ferroptosis, showing that the plasma membrane is one of the biomembranes associated with ferroptosis. In addition, we tried to visualize lipid peroxidation flux within the cell by combinatory use of LipoxPM and C11-BODIPY[(2)], a fluorescence probe to detect intracellular lipid peroxidation (Fig. 1). These probes indicated that ferroptosis-linked lipid peroxidation spreads from intracellular membranes to the plasma membrane.

Keywords : fluorescence probe; lipid peroxidation; plasma membrane; ferroptosis

生体膜の構成要素であるリン脂質は、活性酸素種により連鎖的な酸化反応を受け脂質過酸化を引き起こす。この過程で生じる過酸化脂質の蓄積は、細胞死の一つであるフェロトーシスに関係しているものの、酸化の対象となる生体膜は同定されていない[(1)]。本研究では細胞膜に限定して脂質の酸化を検出する蛍光プローブ（LipoxPM）を合成し、フェロトーシス誘導細胞を用いて評価した。その結果、細胞膜はフェロトーシス過程で脂質の酸化を受ける生体膜の一つであることを明らかにした。さらに、細胞内オルガネラ膜中の脂質過酸化を検出する蛍光プローブ（C11-BODIPY）[(2)]と LipoxPM との時間依存的な脂質過酸化を比較すると、フェロトーシスによる脂質の酸化が細胞内オルガネラ膜から細胞膜へと拡散することを見出した(Fig. 1)。

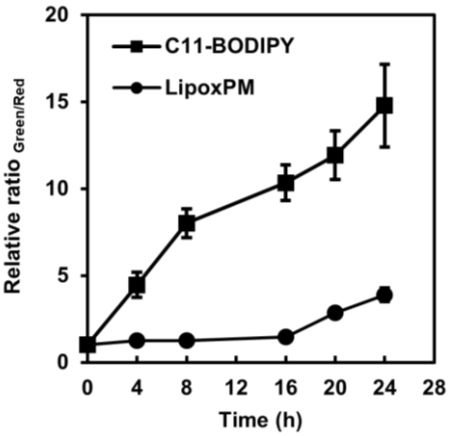


Fig. 1. The relative ratio between fluorescent intensities of two channels with LipoxPM and C11-BODIPY.

(1) R. Tian *et al*., *Nat. Neurosci.*, **2021**.
(2) G. P. C. Drummen *et al*., *Free Radic. Biol. Med.*, **2002**.

ALDH1A3 に応答する turn-on 型赤色蛍光プローブの開発

（京大院工）○西田　有輝・三木　康輝・Huiying Mu・三木　康嗣・大江　浩一
Development of Aldehyde Dehydrogenase 1A3-responsive Turn-on Red Fluorescent Probes (*Graduate School of Engineering, Kyoto University*) ○Yuki Nishida, Koki Miki, Huiying Mu, Koji Miki, Kouichi Ohe

Cancer stem cells (CSCs), which are subpopulation of cancer cells, are involved in tumor initiation, proliferation, and recurrence.[1] Several aldehyde dehydrogenase (ALDH) isoforms are known to be overexpressed in CSCs and used as biomarkers for CSC detection.[2] Among ALDH isoforms, ALDH1A3 is known to be involved in the malignant potential of cancer, [3] and the development of a probe to detect it will lead to the establishment of a simple diagnostic method for malignant potential of cancer. We have developed turn-on red fluorescent probe **NBn-A** to detect ALDH1A3 activity in CSCs.[4] However, the detection of ALDH1A3 activity is still difficult because of the low responsiveness of **NBn-A**. In this study, turn-on red fluorescent probe **NC5-A** with high responsiveness to ALDH1A3 was synthesized.[5] Results of cell experiments using **NC5-A** are described in the presentation.

Keywords: Cancer Stem Cell; Fluorescence Probe; Aldehyde Dehydrogenase; Cyanine Dye

がんに少分布するがん幹細胞 (CSCs) は、がんの発生や自己複製などに重要な役割を持つ[1]。CSCs ではアルデヒド脱水素酵素 (aldehyde dehydrogenase : ALDH) が過剰発現しており[2]、CSCs のバイオマーカーとして注目されている。また、ALDH の isoform のうち ALDH1A3 はがんの悪性度と関与していることが知られている[3]。そのため ALDH1A3 を検出するプローブの開発は、がんの悪性度の簡便な診断方法の確立に繋がると考えた。当研究室では、ALDH1A3 に応答する turn-on 型蛍光プローブ **NBn-A** を開発した(Figure 1a) [4]。しかし、**NBn-A** の ALDH1A3 に対する応答性が低いことが問題であった。本研究では、ALDH1A3 に対する応答性が高い turn-on 型赤色蛍光プローブ **NC5-A** を新たに開発した(Figure 1a) [5]。**NBn-A** に対し ALDH1A3 を作用させたところ、1 時間後に発光量が約 1.5 倍の増大しか示さなかったのに対して、**NC5-A** は約 12 倍の発光量の増大を示した(Figure 1b)。**NC5-A** を用いた細胞実験の結果についても発表する。

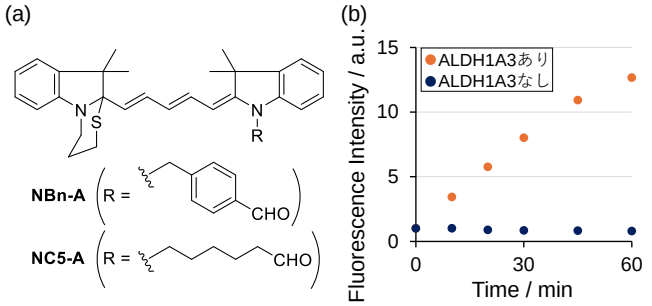


Figure1. (a) ALDH-responsive turn-on fluorescent probes **NBn-A** and **NC5-A**. (b) Time-dependent fluorescence intensity change at 662 nm of **NC5-A** (5 μM) with/without ALDH1A3 (50 nM).

[1] Y. Zhao, Q. Dong, J. Li, K. Zhang, J. Qin, J. Zhao, Q. Sun, Z. Wang, T. Wartmann, K. W. Jauch, P. J. Nelson, L. Qin, C. Bruns, *Semin. Canc. Biol.* **2018**, *53*, 139.
[2] H. Tomita, K. Tanaka, T. Tanaka, A. Hara, *Oncotarget* **2016**, *7*, 11018.
[3] J.-J. Duan, J. Cai, L. Gao, S.-C. Yu, *J. Enzyme Inhib. Med. Chem*. **2023**, *38*, 6689.
[4] K. Miki, D. Yamanaka, H. Mu, K. Miki, K. Ohe, The 103rd CSJ Annual Meeting, D1441-1am-07.
[5] 三木康嗣，三木康輝，西田有輝，麻植雅裕, Huiying Mu，大江浩一，特願 2024-89182.

meso-vinyl-BODIPY 骨格を持つ turn-on 型補酵素 A 応答性蛍光プローブの開発

(京大院工) ○山元　紗代子・Huiying Mu・三木　康嗣・大江　浩一
Development of turn-on fluorogenic probes based on *meso*-vinyl-BODIPY scaffold for coenzyme A detection (*Graduate School of Engineering, Kyoto University*) ○ Sayoko Yamamoto, Huiying Mu, Koji Miki, Kouichi Ohe

Recently, we have developed fluorogenic probes based on *meso*-vinyl-BODIPY (**VB**) dyes for the detection of intracellular thiols [1]. However, these probes lack selectivity and react with all free thiols. Coenzyme A (CoA), an essential cofactor involved in various cellular enzymatic reactions and metabolism, represents a specific target for selective detection. In this study, we present CoA-responsive fluorogenic probes relying on the core structure of **VB** and the chemical properties of CoA. By incorporating a cationic moiety into the side chain of **VB** as a recognition site, the probe exhibited an increased selectivity and reactivity towards CoA (Figure 1). In this presentation, the photophysical properties of the synthesized probes and their applications in cellular imaging will be discussed.

Keywords : BODIPY dye; Coenzyme A; Fluorogenic probe; imaging

当研究室では、細胞内に存在するチオールに反応することで蛍光を発する *meso*-vinyl-BODIPY (**VB**) 色素を開発した[1]。しかし、この分子プローブには特定のチオールに対する選択性がない。補酵素 A (CoA) は細胞内の様々な酵素反応や代謝プロセスに関与している。本研究では、**VB** の分子骨格と CoA の化学構造を利用して CoA 応答性蛍光プローブを開発した (Figure 1) 。**VB** の側鎖に認識部位としてカチオン性官能基を導入することで CoA との反応性と選択性が上がることを見出した。本発表では、合成したプローブの光物性と細胞染色結果について議論する。

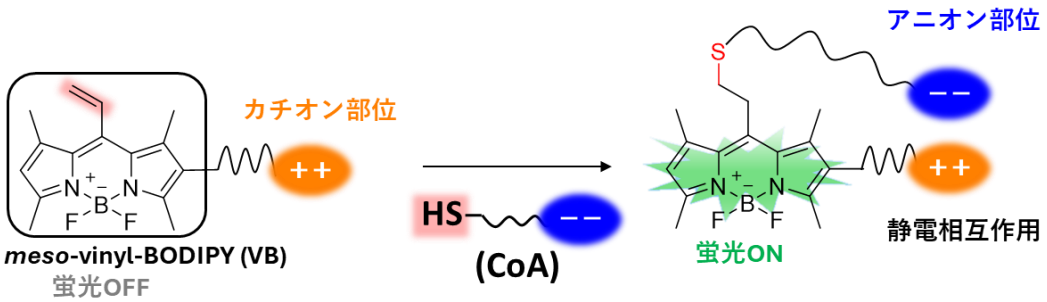


Figure 1. Design strategy of fluorogenic probes for coenzyme A detection based on *meso*-vinyl-BODIPY.

[1] Mu, H.; Miki, K.; Kubo, T.; Otsuka, K.; Ohe, K. *Chem. Commun*. **2021**, *57*, 1818–1821.

Synthesis and Characterization of Unsymmetric Thieno-Phospha-Rhodamine for Bioimaging

([1]*Graduate School of Science, Nagoya University*, [2]*Institute for Glyco-core Research (iGCORE), Gifu University,* [3]*Institute of Transformative Bio-Molecules (ITbM), Nagoya University,* [4]*Integrated Research Consortium on Chemical Sciences (IRCCS), Nagoya University*) ○Sibo Ma,[1] Masayasu Taki,[2] Shigehiro Yamaguchi[1,3,4]

Keywords: Bioimaging; Fluorescent Probe; Rhodamine; Near Infrared Imaging

Near-infrared (NIR) imaging has emerged as a highly promising modality due to its reduced tissue scattering and absorption, along with alleviated interference from autofluorescence in the NIR spectral region. However, conventional NIR dyes, such as cyanine and aza-BODIPY-based dyes, suffer from inherent limitations, including poor water solubility and low photostability. To overcome these challenges, NIR-emissive phospha-rhodamine dyes (PORs), in which an endocyclic oxygen atom of the rhodamine fluorophore is replaced with an electron-withdrawing phosphoryl group, have been developed.[1] We conceived that the substitution of benzene ring in PORs with an electron-donating thiophene ring would result in increasing in the HOMO energy level, leading to a bathochromic shift in the absorption and emission spectra, thereby enhancing deep tissue imaging capabilities.

In this study, we synthesized a series of unsymmetric thieno-phospha-rhodamine (**TPR**) dyes with varied amino substituents, such as pyrrolidyl (**TPR-py**), diethylamino (**TPR-Et**), and dimethylamino (**TPR-Me**) groups, on the thiophene ring (Figure 1a). These dyes exhibited similar photophysical properties, with absorption and emission maxima around 700 nm and 800 nm, respectively, resulting in large Stokes shift over 1600 cm^{-1} in the NIR region. Cell staining experiments with **TPRs** revealed that the electron-donating character of the amino groups significantly influenced the intracellular localization of the **TPR** dyes. Specifically, **TPR-py** primarily stained mitochondria (Figure 1b), while **TPR-Me** was predominantly localized in lysosomes (Figure 1c).

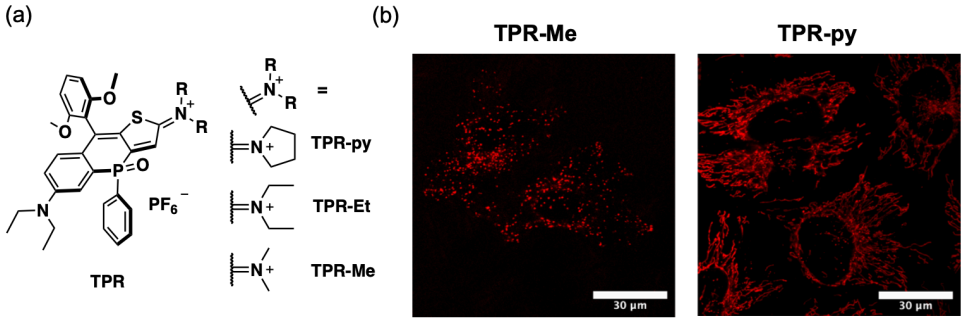


Figure 1. (a) Structure and (b) imaging result of **TPRs**.

1) Grzybowski, M.; Taki, M.; Senda, K.; Sato, Y.; Ariyoshi, T.; Okada, Y.; Kawakami, R.; Imamura, T.; Yamaguchi, *Angew. Chem. Int. Ed.* **2018**, *57*, 10137-10141.

ジアリールホスホニウム架橋型ミトコンドリア蛍光プローブの開発

○袴田 彩仁[1]、多喜 正泰[2]、村井 征史[1]、川口 祥正[3]、二木 史朗[3]、山口 茂弘[1,4,5]
Diarylphosphonium-Bridged Fluorescent Dyes for Mitochondrial Imaging (*[1]Graduate School of Science, Nagoya University, [2]Institute for Glyco-core Research (iGCORE), Gifu University, [3]Institute for Chemical Research (ICR), Kyoto University, [4]Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, [5]Integrated Research Consortium on Chemical Sciences (IRCCS), Nagoya University*) ○ Ayato Hakamada[1], Masayasu Taki[2], Masahito Murai[1], Yoshimasa Kawaguchi[3], Shiroh Futaki[3], Shigehiro Yamaguchi[1,4,5]

We have developed a diphenylphosphonium-bridged fluorophore, PLaS, which exhibits remarkable photophysical properties. In an aqueous solution containing 10% DMSO, PLaS showed a large Stokes shift, with absorption and emission maxima at 460 nm and 575 nm, respectively, and a high fluorescence quantum yield of 0.60. The cationic nature of the PLaS fluorophore facilitated its selective localization to mitochondrial inner membranes, as confirmed by STED imaging. Furthermore, we functionalized PLaS with a propyl sulfonate group, yielding PLaS-C_3SO_3. While PLaS-C_3SO_3 is intrinsically membrane-impermeable, co-incubation with a cell-penetrating peptide enabled selective staining of the mitochondrial outer membrane in living cells.
Keywords : Fluorescence probe, phosphonium, mitochondrial inner membrane, mitochondrial outer membrane, super-resolution imaging

ミトコンドリアは ATP 合成やアポトーシスをはじめとする細胞活動を担い，その機能不全は多くの疾患の原因となる．本研究では，ミトコンドリア動態を可視化する新たな蛍光プローブとして，ジフェニルホスホニウムで架橋したラダー型蛍光色素 PLaS を開発し，蛍光プローブの小型化および多機能化を実現した (Figure 1a)．メトキシ基を有する PLaS-C_1 は，10% DMSO を含む水溶液中で 465 nm および 575 nm に吸収および発光極大を示した．興味深いことに，本色素は 4135 cm^{-1} という大きなストークスシフトを有しながら， 0.60 という高い蛍光量子収率を示した．HeLa 細胞を染色したところ，ミトコンドリアへの特異的な集積が認められ，STED 顕微鏡により明瞭なクリステ構造が観察されたことから，ミトコンドリア内膜プローブとして機能することがわかった (Figure 1b).

さらに，プロピルスルホン酸基を導入した細胞膜非透過性の PLaS-C_3SO_3 を合成した．これを細胞膜透過性ペプチドと共培養したところ，ミトコンドリア外膜が染色される様子が確認された (Figure 1c).

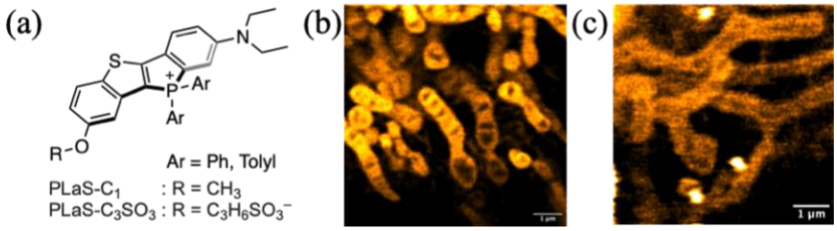


Figure 1. (a) Structures of PLaSs. (b) STED image of mitochondrial inner membrane with PLaS-C_1. (c) STED image of mitochondrial outer membrane with PLaS-C_3SO_3.

Development of a mitochondria-targeting fluorescent probe for visualizing Zn^{2+} flux

([1]*Graduate School of Science, Tohoku University*, [2]*Institute of Multidisciplinary Research for Advanced Materials, Tohoku University*) ○ EunHye Sung,[1] Toshiyuki Kowada,[1,2] Shin Mizukami[1,2]

Keywords: *Small-molecule fluorescent probe, Fluorescence imaging, Zn^{2+}*

Zinc ion (Zn^{2+}), which is one of the most essential elements in mammalian cells, plays critical roles in enzymatic activities, cellular signaling, and transcription. Dyshomeostasis of Zn^{2+}, particularly within mitochondria, can lead to loss of mitochondrial membrane potential, ATP depletion, increased production of reactive oxygen species, and apoptosis.[1] Therefore, tools for visualizing intracellular Zn^{2+} dynamics are essential to understand the physiological roles of Zn^{2+}.

Previously, we developed a small molecule–protein hybrid probe to quantify labile Zn^{2+} concentration in various organelles by combining **ZnDA-3H** (Figure 1a) with HaloTag technology.[2] In this study, to extend the versatility of our probes, we developed a new fluorescent probe, **ZnDA-3TPP**, which enables the monitoring of Zn^{2+} dynamics in mitochondria without HaloTag. This mitochondria-targeting probe is particularly advantageous for applications in cells that are difficult to transfect, such as neurons, and also allows HaloTag to be used for another purpose in general cell studies. The substitution of the HaloTag ligand with a triphenyl phosphonium (TPP) moiety facilitates the spontaneous probe retention in mitochondria through electrostatic interaction with negatively charged mitochondrial inner membrane. In vitro fluorescence measurement demonstrated that **ZnDA-3TPP** exhibits Zn^{2+}-dependent fluorescence enhancement with a K_d of 0.28 nM, indicating that **ZnDA-3TPP** possesses sufficient binding affinity to visualize labile Zn^{2+} in mitochondria (60 pM^2). Fluorescence imaging of HeLa cells treated with **ZnDA-3TPP** confirmed that the probe localized predominantly to the mitochondria (Figure 1b,c).

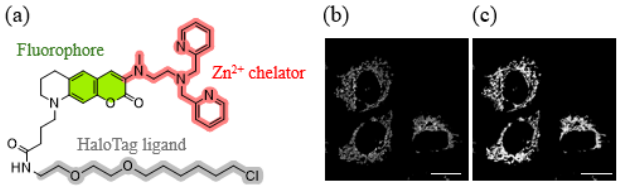


Figure 1. (a) Structure of **ZnDA-3H**. (b,c) Confocal fluorescence microscopic images of **ZnDA-3TPP** (b) and MitoTracker Deep Red FM (c) in HeLa cells. Scale bar: 20 μm.

1) H. Liu, L. Li, and R. Lu, *J. Cell. Physiol.*, **2024**, *239*, e31223. 2) R. Liu, T. Kowada, Y. Du, Y. Amagai, T. Matsui, K. Inaba, and S. Mizukami, *ACS Sens.* **2022**, *7*, 748–757.

多色同時イメージングを志向したミクログリア標的二光子励起プローブの開発

(名大院工[1]・名大工[2]・名大院医[3]・名大量子イノベ研[4]) ○八塚 研治[1,2]・辻村 佳奈[2]・齋藤 祐太朗[3]・鶴旨 篤司[4]・和氣 弘明[3,4]・清中 茂樹[1,2,4]

Microglia-targeting two-photon excitation probe for simultaneous multicolor imaging

([1]*Graduate School of Engineering, Nagoya University*, [2]*School of Engineering, Nagoya University*, [3]*Nagoya University Graduate School of Medicine*, [4]*Research Institute for Quantum and Chemical Innovation, Institutes of Innovation for Future Society, Nagoya University*) ○ Kenji Yatsuzuka[1], Kana Tsujimura[2], Yutaro Saito[3], Atsushi Tsurumune[4], Hiroaki Wake[3,4], Shigeki Kiyonaka[1,4]

Two-photon excitation microscopy (TPM) enables intravital imaging using femtosecond pulsed infrared lasers to excite fluorophores through low-energy two-photon absorption. Unlike visible light, the low absorption and scattering properties of near infrared light allow for greater transparency, making it suitable for deep-tissue imaging. To enhance the rare two-photon absorption, laser light is concentrated near the focal point, increasing spatial photon density and decreasing background. Usually, TPM employs the same or similarly designed probes with single photon microscopy, only with a few specifically designed for two-photon excitation.

In this study, we developed a two-photon excitation probe targeting microglia, immune cells involved in neurological disease pathology in brains. We focused on the differences between two-photon and single-photon excitation to design effective intravital imaging probes for TPM. This presentation will cover the probe design and evaluation process.

Keywords : Multicolor imaging; Two-photon excitation; Microglia; Fluorescent probe

二光子励起顕微鏡法は生体イメージングに利用される手法である。この方法では本来一つの光子で励起される蛍光基をよりエネルギーの低い二つの光子で励起するために、超短パルスの近赤外レーザーを励起光源として用いる。近赤外光は、可視光と比較して生体組織における吸収・散乱が少なく透過性に優れるため、組織深部の観察に適している。また通常ほとんど起きない二光子励起現象を誘起するため、焦点付近にレーザー光を集中させ、空間的に光子密度を高めており、結果的にバックグラウンド蛍光が低減される。このように二光子励起顕微鏡法では単光子励起で多用される共焦点顕微鏡によるイメージングとは異なる環境で実施される。しかし、二光子顕微鏡で用いられる色素やプローブは通常の単光子励起の時と同じものや同じコンセプトに基づいて設計されたものであり、二光子励起専用の設計がなされることは珍しい。

今回我々は脳内の免疫細胞であり、近年神経疾患における病態に関与する細胞として着目されているミクログリアを標的とする二光子励起プローブの開発を行った。この研究では二光子励起と単光子励起の違いや二光子顕微鏡と共焦点顕微鏡の違いに着目し、二光子顕微鏡での生体内イメージングで効果的に利用可能なプローブを開発した。本発表では我々の二光子励起プローブの設計と性能評価について報告する。

日本化学会 第105春季年会 (2025)

ミクログリアを標的としたマウス *in vivo* マルチカラーイメージング

(名大工[1]・名大院工[2]・名大院医[3]・名大量子イノベ研[4]) ○辻村 佳奈[1]・八塚 研治[1,2]・齋藤 祐太朗[3]・鶴旨 篤司[4]・和氣 弘明[3,4]・清中 茂樹[1,2,4]

In vivo multicolor imaging of mouse microglia ([1]*School of Engineering, Nagoya University*, [2]*Graduate School of Engineering, Nagoya University*, [3]*Nagoya University Graduate School of Medicine*, [4]*Research Institute for Quantum and Chemical Innovation, Institutes of Innovation for Future Society, Nagoya University*) ○Kana Tsujimura[1], Kenji Yatsuzuka[1,2], Yutaro Saito[3], Atsushi Tsurumune[4], Hiroaki Wake[3,4], Shigeki Kiyonaka[1,2,4]

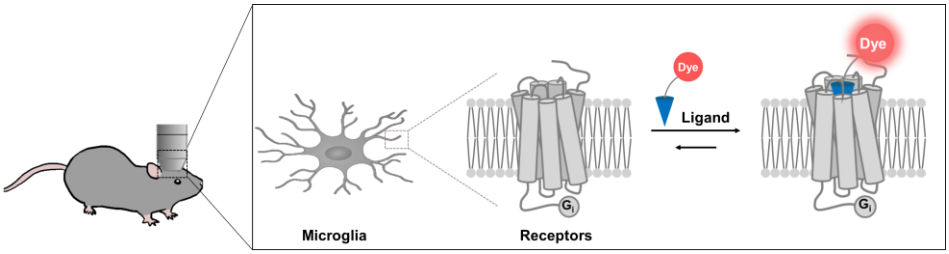
Microglia, the primary immune cells of the central nervous system, have attracted significant attention due to their implication in the onset and progression of neurological diseases through their functional disruption. Two-photon excitation microscopy (TPM) is useful for observing a particular cell type like microglia *in vivo*. However, white-light lasers used in TPM require significant time for wavelength switching, interfering with simultaneous multicolor imaging and limiting the use of *in vivo* imaging to analyze their function.

To overcome this limitation, we developed new imaging probes for visualization of microglia in the brain through two-photon excitation. While the initial probe could be utilized for cultured cells, it was unsuitable for *in vivo* imaging. By investigating its physicochemical properties, we successfully developed probes suitable for *in vivo* multicolor imaging by TPM, which allowed us to analyze dynamics of microglia.

Keywords : in vivo imaging; Microglia; Two-photon excitation microscopy; Multicolor imaging

中枢神経系の主要な免疫細胞であるミクログリアは、近年その機能の破綻が神経疾患の発症および進行に深く関与していることが明らかとなり、研究対象として注目されている。ミクログリアのような特定の細胞種を生体で観察するには二光子励起顕微鏡法が有効とされているが、この手法で一般的に使用される白色レーザーは波長変更に時間を要するため、複数の励起波長を利用したマルチカラー同時イメージングは困難であり、動物モデルを用いた細胞機能の詳細な解析には限界があった。

この課題を克服するため、我々は脳内のミクログリアを選択的に標識し、*in vivo* で二光子励起による観察を可能にするイメージングプローブを開発した。当初のプローブは細胞レベルでの観察には適していたが、*in vivo* 環境下での使用には問題があった。そこで、プローブの物性を詳細に検討・改良した結果、*in vivo* マルチカラーイメージングに適したプローブの開発に成功し、生体内でのミクログリア動態解析が可能となった。本発表では、これらの成果について詳細に報告する。



日本化学会 第105春季年会 (2025)

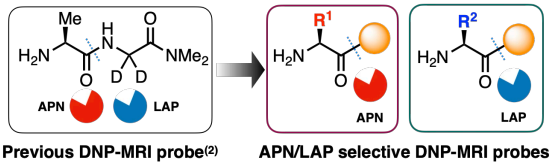
超偏極分子プローブを用いた APN/LAP の選択的活性検出に向けた検討

○寺田　英俊[1]・谷田部　浩行[2]・齋藤　雄太朗[2]・齋藤　圭太[3]・高草木　洋一[3,4]・山東　信介[2]
(東大工[1]・東大院工[2]・量子科学技術研究開発機構[3]・千葉大院融合理工[4])
○ Hidetoshi Terada[1], Hiroyuki Yatabe[2], Yutaro Saito[2], Keita Saito[3], Yoichi Takakusagi[3,4], Shinsuke Sando[2]
([1]*School of Engineering, The University of Tokyo*, [2]*Graduate School of Engineering, The University of Tokyo*, [3]*National Institutes for Quantum Science and Technology*, [4]*Graduate School of Science, Chiba University*)

Dynamic nuclear polarization (DNP) is a quantum technology that significantly enhances the detection sensitivity of molecular probes in magnetic resonance imaging (MRI), enabling in vivo imaging of enzymatic activities[1]. Aminopeptidases (APs) are a group of enzymes that recognize and cleave N-terminal amino acid residue of peptides. Among them, aminopeptidase N (APN) and leucine aminopeptidase (LAP) are recognized as important disease biomarkers. However, the selective detection of APN and LAP activity utilizing molecular probes remains challenging because of their overlapping substrate recognition. Although DNP-MRI molecular probes detecting APN activity in vivo have been reported[2], selective in vivo imaging of AP activity has yet to be achieved. In this study, we present the design, synthesis, and evaluation of DNP-MRI molecular probe candidates capable of selectively detecting APN and LAP activity.
Keywords: Hyperpolarization, Nuclear magnetic resonance, Aminopeptidase, Molecular probe

動的核偏極 (DNP) 法は核磁気共鳴イメージング (MRI) の検出感度を大幅に向上させることができる量子技術である。DNP 法で高感度化した分子プローブを利用する DNP-MRI は、生体内酵素活性イメージング手法として有用である[1]。アミノペプチダーゼ (AP) は、ペプチドの N 末端アミノ酸残基を認識して切断する酵素群であり、中でもアミノペプチダーゼ N（APN）とロイシンアミノペプチダーゼ (LAP) は重要な疾患バイオマーカーである。しかし、一般に APN と LAP は基質認識範囲が重複するため、分子プローブを用いた APN/LAP 選択的な活性検出は困難であった。これまでに、生体内 APN 活性を検出可能な DNP-MRI 分子プローブが報告されているが[2]、選択的な生体内 AP 活性イメージングには至っていない。そこで、本研究では APN/LAP 活性の選択的検出を可能にする DNP-MRI 分子プローブ候補の設計・合成と評価について報告する。

1) Ardenkjær-Larsen, J. H. *et al. Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 10158–10163.
2) Saito Y. *et al. Sci. Adv.* **2022**, *8*, eabj2667.



Previous DNP-MRI probe(2)　APN/LAP selective DNP-MRI probes

日本化学会 第105春季年会 (2025)

グリシン誘導体型分子プローブを用いた生体内での超偏極 MRI シグナルの長時間観測

（東大院工[1]・岐阜大医[2]・量子科学技術研究開発機構[3]・千葉大院融合理工[4]・米国国立衛生研究所[5]）○足利 留樹[1]・齋藤 雄太朗[1]・谷田部 浩行[1]・近藤 洋平[1]・野中 洋[1]・Elhelaly Abdelazim Elsayed[2]・兵藤 文紀[2]・松尾 政之[2]・齋藤 圭太[3]・高草木 洋一[3,4]・山本 和俊[5]・Murali C. Krishna[5]・山東 信介[1]

In vivo long-term observation of the hyperpolarized MRI signal with a glycine-derived probe

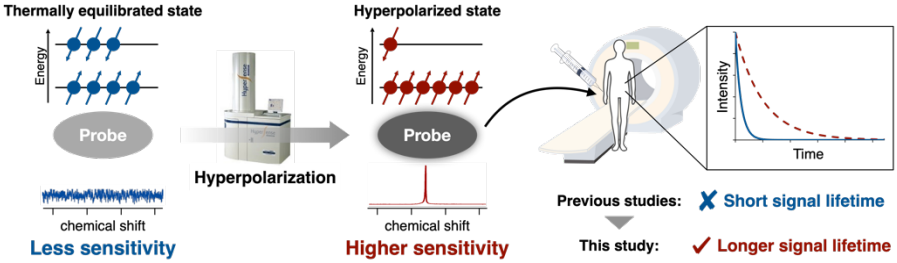
([1]*Graduate School of Engineering, The University of Tokyo*, [2]*School of Medicine, Gifu University*, [3]*National Institutes for Quantum Science and Technology*, [4]*Graduate School of Science, Chiba University*, [5]*National Institutes of Health, USA*) ○Ruki Ashikaga[1], Yutaro Saito[1], Hiroyuki Yatabe[1], Yohei Kondo[1], Hiroshi Nonaka[1], Abdelazim Elsayed Elhelaly[2], Fuminori Hyodo[2], Matsuo Masayuki[2], Keita Saito[3], Yoichi Takakusagi[3,4], Kazutoshi Yamamoto[5], Murali C. Krishna[5], Shinsuke Sando[1]

Nuclear magnetic resonance imaging (MRI) is a prevailing technique that allows noninvasive observation at the depths of the body. However, typical MRI has the disadvantage of low sensitivity, making it difficult to observe the signal of molecular probes administered *in vivo*. Dynamic nuclear polarization (DNP) improves MRI sensitivity by tens of thousands of times.[1] By observing the MR signals enhanced by DNP, it is possible to monitor the dynamic changes of the injected molecular probes and thereby analyze their metabolism *in vivo*. However, existing DNP-MRI molecular probes have a problem of short signal lifetime *in vivo*, limiting the application of DNP-MRI. In this study, we designed a glycine-derived probe based on precise insights into the relaxation mechanism. We performed *in vivo* long-term observation of the hyperpolarized MRI signal with the synthesized molecular probe.

Keywords: Nuclear magnetic resonance imaging; Hyperpolarization; Molecular probes

核磁気共鳴イメージング（MRI）は、生体深部を非侵襲的に観測できる技術として、広く普及している。しかし、通常の MRI には感度が低いという欠点があり、生体内に投与した分子プローブのシグナル観測は難しい。動的核偏極法（DNP）は MRI 感度を数万倍向上させることができる量子技術である[1)]。DNP によって高感度化した分子プローブのシグナルを MRI により観測することで、分子プローブの生体内における動的変化を追跡することができ、生体内代謝解析などが可能になる。一方で、既存の DNP-MRI 分子プローブには生体内でのシグナル観測時間が短いという問題がある。そこで、本研究では核スピン緩和機構の理解を基に、グリシン誘導体型分子プローブを設計・合成し、生体内で長時間のシグナル観測を行った。

1) Ardenkjær-Larsen, J. H. *et al. Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 10158–10163.



日本化学会 第105春季年会 (2025)

ナノ構造脂質キャリア型 ^{19}F MRI 造影剤の開発

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

Development of nanostructured lipid carrier for ^{19}F MRI probe ([1]*Graduate School of Engineering, Osaka University,* [2]*Immunology Frontier Research Center, Osaka University*) ○ Shiho Sugiura[1], Tomoya Yamamoto[1,2], Kazuya Kikuchi[1,2]

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique. ^{19}F MRI can trace the localization of ^{19}F MRI contrast agents with high contrast due to 100% natural isotopic abundance and the absence of biological background signals. To enhance signal intensity by increasing the local concentration of ^{19}F, nanoparticle-based ^{19}F MRI contrast agents encapsulating fluorine compounds have been developed. However, nanoparticles tend to accumulate in the liver, and their delivery efficiency to tumors via the enhanced permeability and retention (EPR) effect is generally low.

In this study, we aim to apply nanostructured lipid carriers (NLCs) for ^{19}F MRI contrast agents that are designed to reduce liver accumulation. NLC is a lipid nanoparticle composed of a solid and liquid lipid core. Solid lipids enhance the size stability of nanoparticles, while the MR signal of liquid lipids can be detected due to their mobility. We synthesized fluorinated triglyceride as the liquid lipid. The MR signals of NLC encapsulating fluorinated triglyceride showed high intensity. Size remained stable for at least one week. In this presentation, we will report on the fluorinated compounds in NLCs, MR signal intensity, T_2 relaxation time, and size stability.

Keywords : ^{19}F MRI; lipid nanoparticle; nanostructured lipid carrier

磁気共鳴画像法（MRI）は核磁気共鳴現象を利用した、非侵襲的なイメージング手法である。生体内には ^{19}F がほとんど存在しないため、^{19}F を観測核種とした ^{19}F MRI は、^{19}F 核を有する造影剤の局在を高コントラストで画像化することができる。^{19}F 核の局所濃度を上げてシグナル強度を高めるために有機フッ素化合物を内包したナノ粒子型の ^{19}F MRI 造影剤が開発されてきた。しかし、一般にナノ粒子は肝臓に蓄積しやすく、EPR 効果を用いた腫瘍への送達効率は低い。

本研究では、ナノ構造脂質キャリア（NLC）を用いて肝臓への蓄積を抑えた新規 ^{19}F MRI 造影剤の開発を目指した。NLC は固体脂質と液体脂質のコアから成る脂質ナノ粒子である。固体脂質はナノ粒子のサイズ安定を高め、液体脂質は運動性を有するため ^{19}F 核を導入すると ^{19}F NMR シグナルを生じることが期待できる。液体脂質として、トリグリセリドの末端をフッ素化したフッ化トリグリセリドを合成した。合成したフッ化トリグリセリドを内包した NLC から ^{19}F NMR シグナルが観測され、1 週間以上にわたりサイズ安定性が確認された。本発表では、NLC に内包する有機フッ素化合物の検討と ^{19}F NMR シグナル強度、T_2 緩和時間、サイズ安定性の評価について報告する予定である。

Development of Core-shell Silica Nanoparticles for *in vivo* ^{19}F MRI

(*[1]Graduate School of Engineering, Osaka University, [2]JST PRESTO, [3]Immunology Frontier Research Center, Osaka University*)
○Yue Wu[1], Masafumi Minoshima[1,2], Kazuya Kikuchi[1,3]

Keywords: ^{19}F MRI; Silica Nanoparticle; MRI

Magnetic resonance imaging (MRI) is one of the most well-established clinical imaging techniques, known for its high sensitivity, deep tissue penetration, and non-invasive, radiation-free nature. ^{19}F MRI, in particular, is a powerful tool for in vivo imaging, allowing for the tracking of specific cells and enzyme activities in deep tissues, all in a background-free modality.

Our laboratory has developed FLAME (Fluorine-Accumulated Silica Nanoparticle for ^{19}F MRI Enhancement), a novel ^{19}F MRI nanoprobe enabling *in vivo* ^{19}F MRI[1,2](Figure 1a, b). FLAME features a core-shell structure, in which liquid perfluorocarbon is encapsulated within a silica shell. This design provides the probe with high ^{19}F MR sensitivity and enhanced surface modifiability. However, despite these advantageous properties, the delivery efficiency of FLAME to cancer tissues remains suboptimal. Instead, FLAME demonstrates long-term accumulation in the liver and spleen following administration in mice. This inefficiency is believed to be influenced by its size, as modified FLAME nanoparticles can reach up to 200 nm. Consequently, developing smaller-sized nanoprobes is deemed necessary to improve delivery efficiency.

Herein, we introduce the development of ultrasmall FLAME (u-FLAME) nanoparticles. u-FLAME was synthesized via a one-pot method that prevents an increase in nano-emulsion size. Surface modification with carboxyl groups was achieved through a co-condensation approach to produce u-FLAME-COOH (Figure 1c). *In vivo* experiments demonstrated the rapid clearance of u-FLAME-COOH from the liver (Figure 1d).

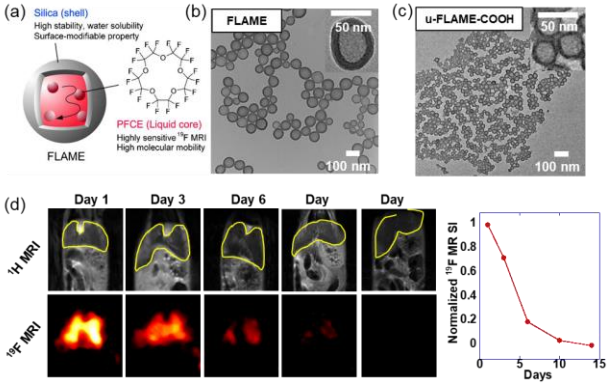


Figure 1. (a) (b) Schematic and TEM image of FLAME; (c) TEM image of u-FLAME-COOH; (d) ^{19}F MRI of u-FLAME-COOH.

1. H. Matsushita *et al.*, *Angew. Chem. Int. Ed.*, **2014**, 53, 1008-1011.
2. T. Nakamura *et al.*, *Angew. Chem. Int. Ed.*, **2015**, 54, 1007-1010.

脂質ナノディスクを用いた微小 ^{19}F MRI 造影剤の開発

（阪大院工[1]・阪大免フロ[2]）○福島 広大[1]・山本 智也[1]・菊地 和也[1,2]
Development of small ^{19}F MRI probe using lipid nanodsc([1]*Graduate School of Engineering, Osaka University*, [2] *Immunology Frontier Research Center, Osaka University*) ○Kodai Fukushima,[1] Tomoya Yamamoto,[1] Kazuya Kikuchi[1,2]

^{19}F Magnetic Resonance Imaging (^{19}F MRI) is an imaging technique that detects the location of contrast agents containing ^{19}F nuclei. ^{19}F MRI has low intrinsic background signals and enables imaging of the location of ^{19}F nuclei *in vivo* with high sensitivity. To detect ^{19}F nuclei *in vivo*, ^{19}F MRI probes such as silica- or polymer-coated nanoparticles encapsulating fluorinated molecules have been developed[1,2]. These nanoparticles enable highly sensitive detection by increasing the local concentration of ^{19}F nuclei *in vivo*. However, long-term accumulation in the liver and particle size control remain a problem.

To address these problems, we focused on lipid nanodiscs. Lipid nanodiscs are small nanoparticles, approximately 10~20 nm in size, consisting of a phospholipid bilayer and belt-like amphiphilic molecules. The size of lipid nanodiscs can be easily controlled by adjusting the properties of the amphiphilic molecules. In addition, we expected that lipid nanodiscs would suppress the long-term accumulation in the liver due to their small particle size.

In this research, we developed small ^{19}F MRI probes using lipid nanodiscs approximately 10 nm in size by incorporating synthesized ^{19}F-labeled phospholipids. In ^{19}F NMR, ^{19}F-labeled phospholipids in nanodiscs exhibit a T_2 relaxation time of approximately 200 ms. We also performed ^{19}F MRI measurements of ^{19}F-labeled nanodiscs using a phantom. This presentation will also include the results of an *in vivo* experiment with ^{19}F-labeled nanodiscs.

Keywords : ^{19}F MRI; Nanodisc; Phospholipid; Imaging

^{19}F Magnetic Resonance Imaging (^{19}F MRI) は、^{19}F 核を含有する造影剤の生体内での所在を検出するイメージング手法である。^{19}F MRI 造影剤は、内因性バックグラウンドシグナルの影響を受けず、造影剤の位置を感度良く検出できるため、生体イメージングに適している。^{19}F MRI 造影剤として、^{19}F 核含有化合物をシリカやポリマーで内包したナノ粒子型造影剤が開発されてきた[1,2]。ナノ粒子型造影剤は、生体内で ^{19}F 核の局所濃度を高め、感度よく ^{19}F 核の位置を可視化できる。一方、肝臓への長期的な蓄積や粒子サイズ制御に課題があった。

この課題を克服するため、脂質ナノディスクに着目した。脂質ナノディスクは、リン脂質と両親媒性分子から構成される 10~20 nm のナノ粒子である。脂質ナノディスクは両親媒性分子の特性により、粒子サイズ制御を容易に行うことができる。また、微小な粒子径を持つため、肝臓への長期的な蓄積を抑制可能であると予想される。

本研究では、化学合成した ^{19}F 標識化リン脂質を用いて脂質ナノディスクを調製することで、微小 ^{19}F MRI 造影剤を開発した。開発した造影剤は、10 nm 程度の粒子径を持つことが確認された。また、造影剤内の ^{19}F 標識化リン脂質は、^{19}F NMR で約 200 ms の T_2 緩和時間を示し、MRI 測定に適した高い運動性を持つことが示された。さらに、ファントムを用いた ^{19}F MRI 測定によって、造影剤の位置を可視化できた。本発表では、^{19}F 標識化ナノディスクの *In vivo* での評価も報告する予定である。

[1] H. Matsushita, S. Mizukami, F. Sugihara, Y. Nakanishi, Y. Yoshioka, K. Kikuchi, *Angew. Chem. Int. Ed*. **2014**, *53*, 1008–1011.
[2] Y. Konishi, M. Minoshima, K. Fujihara, T. Uchihashi, K. Kikuchi, *Angew. Chem. Int. Ed.* **2023**, *62*, e202308565.

日本化学会 第105春季年会 (2025)

ナノ粒子を用いた OFF-ON 型 ^{19}F MRI 造影剤の開発

（阪大院工[1]・JST さきがけ[2]・阪大免フロ[3]）○福田寛人[1]・Jeremy Salaam[1]・蓑島維文[1,2,3]・菊地和也[1,3]

Development of ^{19}F MRI Probe labeling β-lactamase (*[1]Graduate School of Engineering, Osaka Univ.*, *[2]JST PRESTO*, *[3]Immunology Frontier Research Center, Osaka Univ.*) ○Hiroto Fukuda,[1] Jeremy Salaam,[1] Masafumi Minoshima,[1,2,3] Kazuya Kikuchi[1,3]

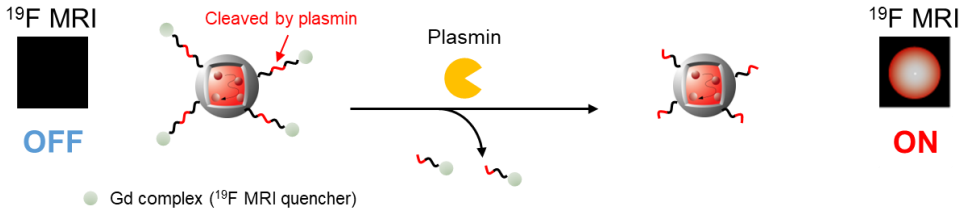
Plasmin is a serine protease that is produced by plasminogen activation. In addition to its role in blood clotting, recent studies have demonstrated that plasmin is involved in the early stages of inflammation response in the liver. Thus, there is a need to detect plasmin activity *in vivo*.[1] ^{19}F magnetic resonance imaging (MRI) is a useful method to non-invasively visualize the probe signals in deep tissue without endogenous background signals. We have developed a nanoparticle comprised of perfluorocarbon core and a silica shell as a ^{19}F MRI nanoprobe. Furthermore, the modification of the nanoparticle with paramagnetic quenchers via an enzyme-specific cleavage linker allowed the detection of the enzyme activity.[2]

In this study, an OFF-ON type ^{19}F MRI nanoprobe was designed for the detection of plasmin activity in the liver. We prepared the nanoprobe modifying the surface with a gadolinium complex via a substrate peptide for plasmin. ^{19}F MRI signal was increased when the gadolinium complex was cleaved off by plasmin.

Keywords : ^{19}F MRI; nanoparticle

プラスミンは前駆体であるプラスミノーゲンから産生されるセリンプロテアーゼ酵素の一種であり、血栓の溶解などさまざまな役割を果たしている。プラスミンは、肝臓における炎症応答に関与していることが近年の研究で明らかになっており、生体内でのプラスミン活性を検出することが求められる[1]。生体内で酵素活性を検出する手法として ^{19}F MRI に注目した。^{19}F MRI は生体深部を非侵襲的に画像化する手法であり、バックグラウンドシグナルなく造影剤の局在をイメージングできる。我々は以前にパーフルオロカーボンをコアに有するシリカナノ粒子を開発している。このナノ粒子は、常磁性ガドリニウム錯体を酵素特異的に切断されるリンカーを介して修飾したものであり、酵素活性の検出に成功している[2]。

本研究では、肝臓におけるプラスミンの活性検出を目指した OFF-ON 型の ^{19}F MRI プローブを設計した。粒子表面に、プラスミンにより切断されるペプチドを介してガドリニウム錯体を修飾した造影剤を作製した。プラスミンとの反応により、ガドリニウム錯体が切り離されることで、^{19}F MRI シグナルが上昇した。



1) S. K. Baker, S. Strickland, *J. Exp. Med*. **2020**, *217*, e20191865.

2) K. Akazawa, *et al., Bioconjug. Chem.* **2018**, *29*, 1720.