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

Wed. Mar 26, 2025 9:00 AM - 11:30 AM JST | Wed. Mar 26, 2025 12:00 AM - 2:30 AM UTC
[A]D501(D501, Bldg. 4, Area 3 [5F])

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

Chair: Shin Mizukami, Hiroshi Nonaka

Japanese
9:00 AM - 9:20 AM JST | 12:00 AM - 12:20 AM UTC
[[A]D501-1am-01]

Development of subtype-selective labeling reagents for endogenous dopamine receptors in the live brain

○Hiroshi Nonaka[1,2], Takuma Takeshi[1], Seiji Sakamoto[1,2], Itaru Hamachi[1,2] (1. Graduate School of Engineering, Kyoto University, 2. ERATO, JST)

Japanese
9:20 AM - 9:40 AM JST | 12:20 AM - 12:40 AM UTC
[[A]D501-1am-02]

Creation of receptor-based fluorescent sensor and mapping of protease activity in live mouse brain.

○Seiji Sakamoto[1,2], Mengchu Wang[1], Kazuki Shiaiwa[1], Hiroshi Nonaka[1,2], Itaru Hamachi[1,2] (1. Graduate School of Engineering, Kyoto University, 2. JST ERATO)

Japanese
9:40 AM - 10:00 AM JST | 12:40 AM - 1:00 AM UTC
[[A]D501-1am-03]

In vivo protein labeling by ligand-directed ArNASA chemistry

○Tomonori Tamura[1], Masaharu Kawano[1], Dai Sugihara[1], Itaru Hamachi[1] (1. Kyoto University)

English
10:00 AM - 10:20 AM JST | 1:00 AM - 1:20 AM UTC
[[A]D501-1am-04]

Detection of dipeptidyl peptidase-4 activity *in vivo* with a rationally designed hyperpolarized MRI molecular probe

○Akihito Goto[1], Hiroyuki Yatabe[1], Abdelazim Elsayed Elhelaly[3], Takayasu Sugiyma[1], Keisuke Saito[1], Norikazu Koyasu[2], Fuminori Hyodo[3], Masayuki Matsuo[3], Keita Saito[4], Yoichi Takakusagi[4,5], Hiroshi Ishikita[1], Kazutoshi Yamamoto[2], Murali Cherukuri Krishna[2], Yutaro Saito[1], Shinsuke Sando[1] (1. The University of Tokyo, 2. National Institutes of Health, 3. Gifu University, 4. National Institutes for Quantum Science and Technology, 5. Chiba University)

10:20 AM - 10:30 AM JST | 1:20 AM - 1:30 AM UTC

Break

English
10:30 AM - 10:50 AM JST | 1:30 AM - 1:50 AM UTC
[[A]D501-1am-05]

Live-cell super-resolution imaging of mitochondrial inner membranes using a fast-blinking fluorophore

○Bochao Li[1], Toshiyuki Kowada[1], Takahiro Fujiwara[2], Shin Mizukami[1] (1. Tohoku University, 2. Kyoto University)

English

10:50 AM - 11:10 AM JST | 1:50 AM - 2:10 AM UTC

[[A]D501-1am-06]

Development of optochemogenetic technology for regulating intracellular droplet formation

○Muhammad Wildan Saifudin[1], Toshiyuki Kowada[1,2], Hayashi Yamamoto[3], Shin Mizukami[1,2] (1. Graduate School of Life Sciences, Tohoku University, 2. Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 3. Department of Molecular Oncology, Institute for Advanced Medical Sciences, Nippon Medical School)

English

11:10 AM - 11:30 AM JST | 2:10 AM - 2:30 AM UTC

[[A]D501-1am-07]

Degradation of lipid droplets by in-cell self-assembly of autophagy-tethering chimeras

○Yi DING[1], Ira NOVIANTI[1,2], Toshiyuki KOWADA[1,2], Shin MIZUKAMI[1,2] (1. Graduate School of Life Sciences, Tohoku University, 2. Institute of Multidisciplinary Research for Advanced Materials, Tohoku University)

日本化学会 第105春季年会 (2025)

脳内の内在性ドパミン受容体を標的としたサブタイプ選択的ラベル化剤の開発

（京大院工[1]、JST ERATO[2]）○野中 洋[1,2]、武士 拓磨[1]、坂本 清志[1,2]、浜地 格[1,2]

Development of subtype-selective labeling reagents for endogenous dopamine receptors in the live brain ([1]*Graduate School of Engineering, Kyoto University*, [2]*ERATO, JST*)
○Hiroshi Nonaka[1,2], Takuma Takeshi[1], Seiji Sakamoto[1,2], Itaru Hamachi[1,]

The dopamine system is associated with brain functions such as motor control, motivation, learning, and reward systems. Dopamine receptors are responsible for signal transduction in the dopamine system. Because the downstream signaling and expression sites differ for each dopamine receptor subtype, subtype-selective functional analysis is keenly required. In this study, we have developed labeling reagents for subtype-selective chemical modification of endogenous dopamine receptors.
Keywords : Ligand directed chemistry; Dopamine receptor; *GPCR; Brain*

ドパミン神経系は、運動調節・意欲学習・報酬系などの脳機能と関連している。ドパミン神経系において、ドパミン受容体はシグナル伝達を担っている。ドパミン受容体のサブタイプごとに下流のシグナル伝達や発現部位が異なることから、サブタイプ選択的な機能解析が求められている。

我々はこれまでにタンパク質の選択的化学修飾法であるリガンド指向性化学の開発を行ってきた[1]。アシルイミダゾール基を反応基とするリガンド指向性化学（LDAI 化学）を用いることで、AMPA 型グルタミン酸受容体（AMPAR）など神経伝達物質受容体の生きたマウス脳内でのラベル化や、それを用いた AMPAR の未知動態の解明[2]、受容体近傍プロテオーム解析[3]、受容体近傍の環境センシング[4]に成功している。今回我々は、内在性ドパミン受容体の機能解析を指向し、ドパミン受容体をサブタイプ選択的に化学標識可能な標識試薬の開発を行なった。ドパミン受容体とサブタイプ選択的なリガンドとの共結晶構造情報をもとに、標識試薬（ラベル化剤）を設計・合成した。HEK293T 細胞に強制発現させたドパミン受容体に新規ラベル化剤を反応させ、ブロッティングや CLSM 解析したところ、ドパミン受容体へのサブタイプ選択的なラベル化が観測された。開発したラベル化剤は、生きているマウスの脳内でも標的受容体への標識が示唆され、今後ドパミン神経系を解析する上での有望な足がかりを得ることに成功した。

1) S. Kiyonaka, I. Hamachi *et al., Nat. Commun.,* **8**, 14850 (2017).
2) H. Nonaka, I. Hamachi *et al., Proc. Natl. Acad. Sci. USA.,* **121**, e2313887121 (2024).
3) T. Tamura, I. Hamachi *et al., Nat. Chem. Biol.*, **21**, 109–119 (2025).
4) S. Sakamoto, H. Nonaka, I. Hamachi *et al., bioRxiv* (2024).
https://doi.org/10.1101/2024.05.23.594618

受容体を基体とする蛍光センサーの構築と生きたマウス脳内でのプロテアーゼ活性検出

（京大院工[1]・JST ERATO[2]）○坂本清志[1,2]・王萌初[1]・白岩和樹[1]・野中洋[1,2]・浜地格
Creation of Receptor-Based Fluorescent Sensor and Mapping of Protease Activity in Live Mouse Brain. ([1] *Graduate School of Engineering, Kyoto University*, [2]*JST ERATO*) ○Seiji Sakamoto,[1,2] Mengchu Wang,[1] Kazuki Shiraiwa,[1] Hiroshi Nonaka,[1,2] Itaru Hamachi[1,2]

A variety of extracellular proteases in central nervous system (CNS) play significant roles to maintain the highly ordered brain functions. However, methods for analyzing detailed localization and activity of each protease in live mouse brain are still limited. In this study, we attempted to construct an AMPA-type glutamate receptor (AMPAR) based fluorescent protease sensor in live mouse brain by a combination of ligand directed acyl imidazole (LDAI) chemistry and inverse electron demand Diels–Alder (IEDDA) click reaction. For creating AMPAR-based protease sensor, we initially introduced trans-cyclooctene (TCO) click handle and Alexa Fluor 555 (Ax555) fluorophore on AMPARs in live mouse brain using the LDAI chemistry. In the second step, Ax647 and methytetrazine (MeTz) conjugated substrate peptide for a target protease was directly administrated into the mouse brain, which allowed the rapid and selective chemical labeling of target receptors *via* IEDDA reaction.
Keywords : In-Brai Ligand Ddirected Chemistry; Inverse Electron-Demand Diels-Alder (IEDDA) Reaction; Gluramate Receptor; Protein Labeling; Protease Sensor

脳内には様々な細胞外プロテアーゼが存在し、細胞外マトリックスやシナプス構成因子の切断を介して、神経突起伸長、シナプス形成、シナプス可塑性などに関与し、高次脳機能を支える重要な役割を果たしている。しかしながら、生きた動物の脳内で、どのプロテアーゼが、どこで、どのようなタイミングで活性化され機能しているかについての詳細は未だ不明な点が多い。本研究では、当研究室で開発した脳内リガンド指向性アシルイミダゾール (in-brain LDAI) 化学と逆電子要請型 Diels-Alder (IEDDA) クリック反応の組み合わせによって、生きたマウス脳内で、AMPAR 型グルタミン酸受容体を基体とするプロテアーゼセンサーの構築を試みた [1,2]。具体的には、LDAI 化学を用いて、生きたマウス脳内のグルタミン酸受容体に、trans-cyclooctene (TCO) 基と Alexa Fluor 555 (Ax555) を特異的に導入した。次の段階では、Alexa Fluor 647 (Ax647) と methytetrazine (MeTz) 基を付加した標的プロテアーゼ特異的な基質配列ペプチドを投与し、IEDDA クリック反応を介した迅速かつ選択的なラベル化を行なった。ラベル化された受容体に活性型プロテアーゼが近接した場合、基質ペプチドの切断を介して Ax555/Ax647 蛍光強度のレシオ値もしくは蛍光色素間の FRET 効率が変化するセンサー設計である。今回の報告では、脳内での発現が知られる Matrix Metalloproteinase 9 (MMP9) を標的として、生きたマウス脳内での MMP9 活性のマッピングに成功したのでその詳細について報告する。

1. H. Nonaka and I. Hamachi *et al. Proc. Natl. Acad. Sci. USA.*, **121**, 6:2313887121 (2024).
2. S. Sakamoto, H. Nonaka, I. Hamachi *et al.* doi: biorxiv.org/content/10.1101/2024.05.23.594618v1.

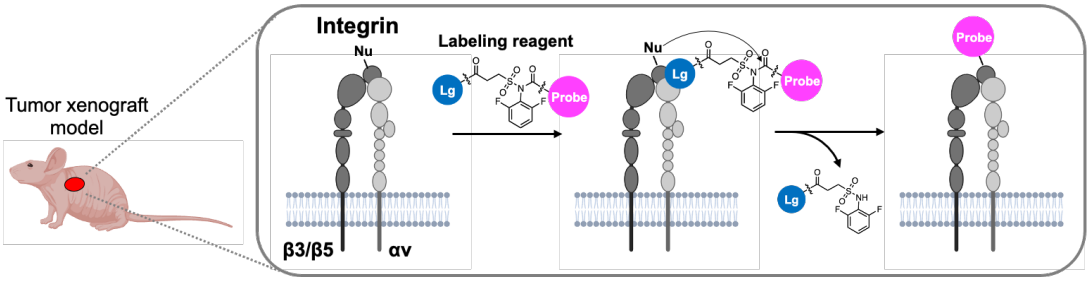
日本化学会 第105春季年会 (2025)

リガンド指向性 ArNASA 化学による in vivo タンパク質ラベリング

（京大院工 [1]）○田村 朋則 [1]・河野 正晴 [1]・杉原 大 [1]・浜地 格 [1]
In vivo protein labeling by ligand-directed ArNASA chemistry ([1]*Graduate School of Engineering, Kyoto University*) ○Tomonori Tamura,[1] Masaharu Kawano,[1] Dai Sugihara,[1] Itaru Hamachi[1]

Ligand-directed chemistry has been recognized as a powerful strategy for selective labeling of endogenous proteins with synthetic reagents in living systems. Although there are several successful examples, the in vivo application of this technique often faced challenges because of the sluggish labeling rate and low in vivo stability of reagents. Here we report our recent efforts on protein labeling in vivo using the latest reactive group, *N*-acyl-*N*-aryl-sulfonamide (ArNASA). The ArNASA-appended labeling reagents selectively labeled their target protein, integrin $\alpha_v\beta3/5$, in cultured cells and in tumor-bearing mice.
Keywords : ArNASA; Ligand-directed chemistry; Integrin; Protein labeling

我々のグループは、生きた細胞や動物個体内の狙った内在性タンパク質を標識（ラベリング）・機能化するための手法として『リガンド指向性化学』を長年開発してきた。[1] この手法では標的タンパク質に対するリガンドと機能性プローブを Cleavable な反応基を介して連結したラベル化剤を用いる。これまでに、反応基としてトシル基やアシルイミダゾール基を有するラベル化剤を用いて、それぞれ赤血球内炭酸脱水酵素やマウス脳内神経伝達物質受容体の選択的ラベリングに成功している。[2,3] 一方、従来の反応基はラベル化速度（効率）や血中安定性に課題があり、脳以外の臓器やがん組織への適用は依然として困難であった。こうした背景の中、ごく最近我々は *N*-acyl-*N*-aryl-sulfonamide (ArNASA)反応基が血清存在下でも効率的に標的タンパク質を修飾可能であり、in vivo での適用に有望であることを見出した。[4] そこで本研究では、がん細胞に発現するインテグリン$\alpha_v\beta3/5$ を標的に複数の ArNASA 型ラベル化剤を合成し、培養細胞および担がんマウス体内におけるラベル化能を評価した。本発表ではこれらラベル化剤の構造活性相関についてその詳細を報告する。



1) T. Tamura and I. Hamachi, *J. Am. Chem. Soc.*, **2019**, *141*, 2782–2799.
2) S. Tsukiji, *et al.*, *Nat. Chem. Biol.*, **2009**, 5, 341–343.
3) H. Nonaka, *et al.*, *Proc. Natl. Acad. Sci.*, **2024**, *121*, e2313887121.
4) M. Kawano, *et al.*, *J. Am. Chem. Soc.*, **2023**, *145*, 26202–26212.

合理的に設計された超偏極 MRI 分子プローブによる生体内ジペプチジルペプチダーゼ-4 活性の検出

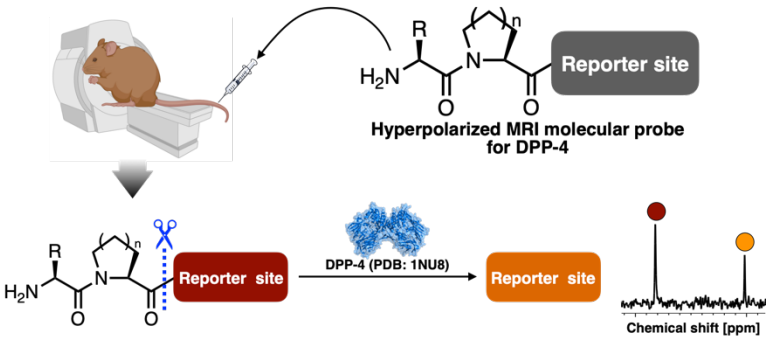
（東大院工[1]・米国国立衛生研究所[2]・岐阜大医[3]・量子科学技術研究開発機構[4]・千葉大院融合理工[5]） ○後藤　彰仁[1]・谷田部　浩行[1]・Abdelazim Elhelaly[2]・杉山　高康[1]・斉藤　圭亮[1]・子安　憲一[2]・兵藤　文紀[3]・松尾　政之[3]・齋藤　圭太[4]・高草木洋一[4,5]・石北　央[1]・山本　和俊[2]・Murali Krishna[2]・齋藤　雄太朗[1]・山東信介[1]

Detection of dipeptidyl peptidase-4 activity *in vivo* with a rationally designed hyperpolarized MRI molecular probe ([1]*Graduate School of Engineering, The University of Tokyo*, [2]*National Institutes of Health, USA*, [3]*School of Medicine, Gifu University*, [4]*National Institutes for Quantum Science and Technology*, [5]*Graduate School of Science, Chiba University*) ○Akihito Goto[1], Hiroyuki Yatabe[1], Abdelazim Elsayed Elhelaly[3], Takayasu Sugiyama[1], Keisuke Saito[1], Norikazu Koyasu[2], Fuminori Hyodo[3], Masayuki Matsuo[3], Keita Saito[4], Yoichi Takakusagi[4,5], Hiroshi Ishikita[1], Kazutoshi Yamamoto[2], Murali Cherukuri Krishna[2], Yutaro Saito[1], Shinsuke Sando[1]

Dipeptidyl peptidase-4 (DPP-4) is a biologically important enzyme related to various diseases such as type 2 diabetes and cancers. Therefore, the detection of DPP-4 activity *in vivo* is a promising technique for diagnosis. In this study, we developed a molecular probe for detecting DPP-4 activity *in vivo* with hyperpolarized MRI.[1] In the presentation, we will show the design strategy for a hyperpolarized MRI molecular probe targeting DPP-4 and the results of *in vivo* experiments aimed at preclinical applications. Furthermore, on the basis of quantum mechanics/molecular mechanics calculation, we will explain how the optimized molecular structure shows excellent enzymatic reactivity for DPP-4.

Keywords : Molecular imaging; Hyperpolarized MRI; Magnetic resonance imaging; Molecular probe; Dipeptidyl peptidase-4

ジペプチジルペプチダーゼ-4 (DPP-4) は、2 型糖尿病やがんなどの疾患に関与する酵素である。そのため、生体内 DPP-4 活性の検出は、疾患診断の有力な手法として期待される。本研究では、超偏極 MRI を用いて、生体内 DPP-4 活性を検出するための超偏極 MRI 分子プローブの開発を行った。本発表では、DPP-4 を標的とする超偏極 MRI 分子プローブの合理的な設計戦略及び、開発した分子プローブの臨床応用に向けた生体内応用実験の結果を示す。また、最適化された分子構造が DPP-4 に対して優れた酵素反応性を示すメカニズムに関して、量子化学計算により得られた考察を述べる。



[1] K. Golman *et. al.*, *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 11270–11275.

Live-cell super-resolution imaging of mitochondrial inner membranes using a fast-blinking fluorophore

([1]*Graduate School of Life Sciences, Tohoku University,* [2]*Institute of Multidisciplinary Research for Advanced Materials, Tohoku University,* [3]*Institute for Integrated Cell-Material Sciences, Kyoto University*) ○Bochao Li,[1] Toshiyuki Kowada,[1,2] Takahiro Fujiwara,[3] Shin Mizukami[1,2]
Keywords: Fluorescent probe; Single-molecule localization microscopy; Spontaneous blinking; Mitochondria

Single-molecule localization microscopy (SMLM) is a powerful technique for super-resolution imaging of living cells at the nanoscale by switching fluorophores between fluorescent (ON) and non-fluorescent (OFF) states. For this purpose, spontaneously blinking fluorophores have been developed by employing an intramolecular spirocyclization reaction of rhodamines (Fig. a). This approach enables live-cell SMLM imaging under low laser intensity and eliminates the need for exogenous cytotoxic additives. The temporal resolution of SMLM imaging depends on the blinking kinetics (τ_{on} and τ_{off}, representing the lifetimes of the open and closed forms, respectively). A shorter τ_{on}, aligned with the faster recording speed of the camera, is essential for high temporal resolution, while an appropriately short τ_{off} ensures sparse but sufficient fluorescence signals required for SMLM imaging. However, the relatively slow blinking kinetics of a widely used fluorophore HMSiR ($\tau_{on} \approx$ 10–300 ms, $\tau_{off} >$ 1 s) restrict temporal resolution to the minute scale, making it challenging to capture rapid dynamics. Consequently, there is a demand for a general strategy for designing fast-blinking fluorophores capable of resolving second-scale cellular dynamics.

In this study, we developed a series of fast-blinking fluorophores by optimizing the intramolecular nucleophile, substituting the hydroxy group in HMSiR with an amino group to accelerate the spirocyclization kinetics. By fine-tuning the steric effects at the adjacent position of the ring-closing nucleophile (Fig. b), one of the newly developed fluorophores exhibited sufficiently fast blinking kinetics (τ_{on} = 1.6 ms, τ_{off} = 0.24 s). Leveraging these superior properties, we tracked the fast dynamics of the mitochondrial inner membrane in living cells using SMLM, achieving a 3-s temporal resolution over 20-s duration.

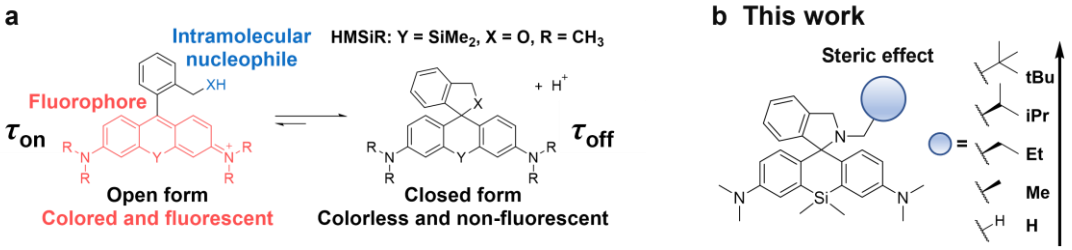


Figure (a) Intramolecular spirocyclization of rhodamines. (b) Design strategy of this work.

Development of Optochemogenetic Technology for Regulating Intracellular Droplet Formation

([1]*Graduate School of Life Sciences, Tohoku University*, [2]*Institute of Multidisciplinary Research for Advanced Material, Tohoku University,* [3]*Department of Molecular Oncology, Institute for Advanced Medical Sciences, Nippon Medical School*) ○ Muhammad W. Saifudin,[1] Toshiyuki Kowada,[1,2] Hayashi Yamamoto,[3] Shin Mizukami[1,2]

Keywords: Autophagy; Photochromism; Droplet formation; Fluorescence imaging

Biomolecular condensates are intracellular membraneless compartments composed of proteins and/or other biomolecules, formed through multivalent weak interactions to exhibit liquid-liquid phase separation. Because biomolecular condensates are associated with various diseases, cells regulate their quantity through a selective autophagy process called fluidophagy. Autophagosome formation in fluidophagy involves the recognition and sequestration of droplets by the isolation membrane. Notably, only droplets with small size and low surface tension can be fully sequestered.[1] However, the detailed mechanism of autophagosome formation in fluidophagy remains unclear, highlighting the need for a method to manipulate droplet formation and properties, such as droplet size and fluidity, in living cells.

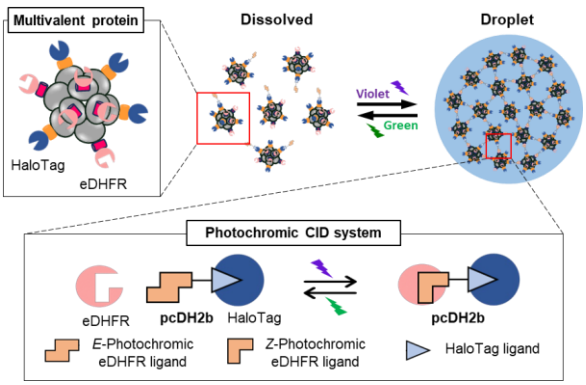


Fig. 1. Strategy for regulating droplet formation.

Recently, we developed a photochromic CID (chemically induced dimerization) system capable of photoreversibly controlling the dimerization of two tag proteins, *E. coli* dihydrofolate reductase (eDHFR) and HaloTag, using a photochromic dimerizer, **pcDH**.[2] We also improved this photochromic CID system by combining a newly developed photochromic dimerizer, **pcDH2b**, with a tag protein mutant, thereby reducing background association. Based on this improved photochromic CID system with a multivalent protein (Fig. 1), we developed a novel technology for optically controlling intracellular droplet formation. In this presentation, we will discuss the results regarding optical control of droplet formation and dissolution, including optical control at the sub-cellular region and droplet size regulation.

Reference:
1) J. Agudo-Canalejo *et al.*, *Nature* **591**, 142 (2021). 2) T. Mashita *et al.*, *Nat. Chem. Biol.* **20**, 1461 (2024).

Degradation of lipid droplets by in-cell self-assembly of autophagy-tethering chimeras

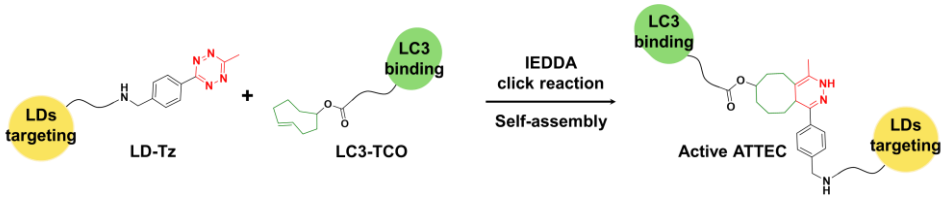
([1]*Graduate School of Life Sciences, Tohoku University*, [2]*Institute of Multidisciplinary Research for Advanced Materials, Tohoku University*) ○ Yi Ding,[1] Ira Novianti,[1,2] Toshiyuki Kowada,[1,2] Shin Mizukami[1,2]

Keywords: Autophagic degradation; Lipid droplet degradation; Autophagy-tethering chimeras; Click reaction

Lipid droplets (LDs) are dynamic organelles characterized by a unique phospholipid monolayer structure and a hydrophobic core composed of neutral lipids. By interacting with other organelles, LDs play critical roles in cellular lipid synthesis, metabolism, and transport processes, as well as in regulating cellular stress. Cancer, the leading cause of global mortality, is characterized by metabolic reprogramming, one of its primary hallmarks. Alterations in LD metabolism have emerged as a key feature of this reprogramming, with abnormal accumulation of LDs observed in various cancers, particularly under hypoxic conditions.[1] Therefore, promoting LD degradation could be a promising approach to mitigate cancer-related metabolic disorders, though research in this area remains limited.

Proximity-inducing bifunctional chimeras can exploit endogenous cellular machinery to modulate various physiological processes. Among these, autophagy-tethering chimeras (ATTECs) utilize the native autophagy-lysosomal pathway to degrade specific cargo, including mitochondria and LDs, by tethering them to phagophores via LC3.[2] However, this strategy has several limitations, such as poor cellular permeability and solubility due to their high molecular weights (> 800 Da), as well as potential on-target toxicity to normal cells.

In this study, we aimed to develop an activatable ATTEC capable of intracellular self-assembly from two smaller, more cell-permeable precursors via a click reaction for the targeted degradation of LDs in cancer cells. The click reaction between tetrazine and *trans*-cyclooctene (TCO) is well-suited for this purpose, as it features rapid kinetics, high yields, and compatibility with physiological conditions without requiring catalysts.[3] Herein, we designed and synthesized a tetrazine-conjugated LD-targeting moiety (**LD-Tz**), which can assemble with a TCO-tagged LC3 ligand (**LC3-TCO**). The resulting click-formed active ATTEC is expected to tether LDs to LC3-II-positive isolation membranes, facilitating their degradation through the selective autophagy.



1) Y. Miyagi *et al.*, *Int. J. Mol. Sci.* **2016**, *17*, 1430. 2) W. Wei *et al.*, *J. Biol. Chem.* **2023**, *299*, 104572. 3) C. Zagni *et al.*, *Bioorg. Chem.* **2024**, *150*, 107573.