アカデミックプログラム［B講演］ | 17. 生体機能関連化学・バイオテクノロジー：口頭B講演

2025年3月29日(土) 13:00 ~ 15:30 [A]A301(第3学舎 1号館 [3階] A301)

[[A]A301-4pm] 17. 生体機能関連化学・バイオテクノロジー

座長：三木 卓幸、真島 剛史

日本語
13:00 ~ 13:20
[[A]A301-4pm-01]
巨大ウイルスが持つ新奇ウイルスロドプシンの分光学的特性と機能
○永田 崇[1]、寶本 俊輝[1]、Andrey Rozenberg[2]、Oded Béjà[2]、井上 圭一[1] (1. 東京大学、2. Technion – Israel Inst. of Technology)

英語
13:20 ~ 13:40
[[A]A301-4pm-02]
カロテノイド結合型ロドプシンにおけるカロテノイド分子の新たな役割
○井上 圭一[1,2]、María del Carmen Marín Pérez[3]、藤原 敬允[2,4]、保坂 俊彰[5]、白水 美香子[5]、吉澤 晋[2,4]、Oded Béjà[3] (1. 東大・物性研、2. 東大・新領域、3. イスラエル工科大、4. 東大・大気海洋研、5. 理研)

英語
13:40 ~ 14:00
[[A]A301-4pm-03]
結合サイト改変による生体分子機械GroELへのGTP応答性の付与とその向上
○戸田 諒太朗[1]、Kiyoshi Morishita[1]、三木 卓幸[1]、田口 英樹[2]、相田 卓三[1] (1. 東京大学、2. 東京科学大学)

日本語
14:00 ~ 14:20
[[A]A301-4pm-04]
アデニル酸キナーゼに結合するモノボディの結合様式の検証
中村 伊武輝[1]、長尾 聡[2]、雨坂 心人[3]、折戸 尚樹[1]、廣田 俊[1]、田中 俊一[3]、○松尾 貴史[1] (1. 奈良先端大・物質創成、2. 高輝度光科学研究センター、3. 京都府大・生命環境)

14:20 ~ 14:30
休憩

英語
14:30 ~ 14:50
[[A]A301-4pm-05]
Cytokine-Induced Cancer Cell-Derived Migrasomes Proteomic Analysis
○Ananda Bagus Richky Digdaya Putra[1], Shogo Saito[1], Masayoshi Tanaka[1], Mina Okochi[1] (1. Institute of Science Tokyo)

英語
14:50 ~ 15:10
[[A]A301-4pm-06]
キチナーゼ結合ナノファイバータンパク質の創製と抗真菌活性の評価

○長谷 彩沙[1]、吉本 将悟[2]、平良 東紀[3]、堀 克敏[2]、神谷 典穂[1,4] (1. 九州大学大学院、2. 名古屋大学大学院、3. 琉球大学農学部、4. 九州大学未来化学創造センター)

英語

15:10 ~ 15:30

[[A]A301-4pm-07]

タンパク質混在系で不均一鎖とデコーダーの結合ペアを光捕捉するユビキチン鎖プローブ

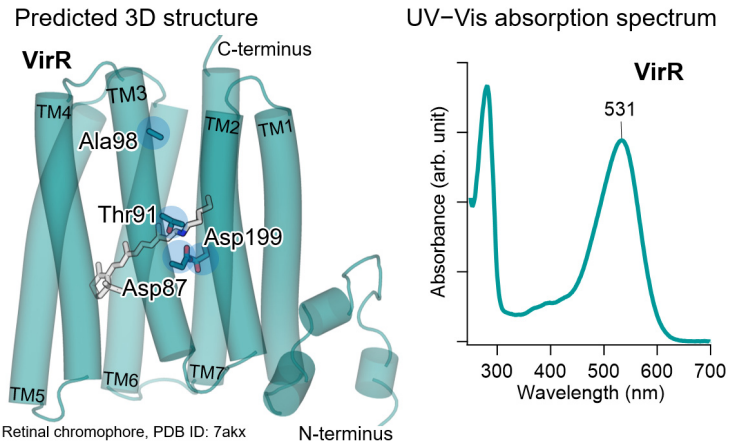
○宇野 大輝[1]、豊田 宇咲乃[1]、古畑 隆史[1]、冨田 拓哉[2]、佐藤 裕介[3,4]、尾勝 圭[5]、深井 周也[5]、佐伯 泰[2]、岡本 晃充[1] (1. 東大院工、2. 東大医科研、3. 鳥大院工、4. 鳥大GSCセンター、5. 京大院理)

巨大ウイルスが持つ新奇ウイルスロドプシンの分光学的特性と機能

（東大物性研[1]・Technion – Israel Institute of Technology[2]）○永田 崇[1]・寶本 俊輝[1]・Andrey Rozenberg[2]・Oded Béjà[2]・井上 圭一[1]
Spectroscopic properties and functions of a novel rhodopsin from a giant virus ([1]*The Institute for Solid State Physics, The University of Tokyo*, [2]*Technion – Israel Instute of Technology*)
○Takashi Nagata,[1] Shunki Takaramoto,[1] Andrey Rozenberg,[2] Oded Béjà,[2] Keiichi Inoue[1]

Microbial rhodopsins, found in many microorganisms, are photo-sensitive membrane proteins with diverse functions such as light-dependent ion transport. Viral rhodopsins of giant viruses have the unique ability to localize to the endoplasmic reticulum (ER) and trigger light-dependent Ca^{2+} release from the ER when expressed in animal cells[1,2]. Recently, novel microbial rhodopsins were discovered from giant virus genomes through metagenomic analysis. These included new viral rhodopsins (VirRs) that are closely related to known viral rhodopsin groups but represent a separate evolutionary branch. To characterize these VirRs, we expressed them in mammalian cultured cells and conducted spectroscopic and functional analyses. In this presentation, we will present the characteristics of VirRs and discuss their functions.
Keywords : Photo-sensitive protein, rhodopsin, giant virus

微生物等が持つロドプシンは、光依存的なイオン輸送などの機能を持つ光受容膜タンパク質である。巨大ウイルスから同定されたウイルスロドプシンは、動物細胞に発現させると小胞体に局在し、小胞体からの光依存的な Ca^{2+} 放出を引き起こすというユニークな機能を持つ[1,2]。最近、メタゲノム解析によって巨大ウイルスゲノムから新たな微生物ロドプシンが発見された。これらの新奇ウイルスロドプシン（VirRs）は、分子系統的に既知のウイルスロドプシンのグループには属さないが、近縁である。VirRs の特徴を明らかにするため、我々は VirRs を哺乳類培養細胞で発現させ、分光学的および機能解析を行った。本発表では、VirRs の特性について示し、その機能について議論する。



1) Viral rhodopsins 1 are an unique family of light-gated cation channels. D. Zabelskii, A. Alekseev, K. Kovalev et al., *Nat. Commun.* **2020,** *11* (1), 5707.
2) Hijacking of internal calcium dynamics by intracellularly residing viral rhodopsins. A.-S. Eria-Oliveira, M. Folacci, A. A. Chassot, et al., *Nat. Commun.* **2024,** *15* (1), 65.

カロテノイド結合型ロドプシンにおけるカロテノイド分子の新たな役割

（東大物性研[1]・東大新領域[2]・イスラエル工科大[3]・東大大気海洋研[4]・理研[5]）○井上 圭一[1,2]、María del Carmen Marín Pérez[3]、藤原 敬允[2,4]、保坂 俊彰[5]、白水 美香子[5]、吉澤 晋[2,4]、Oded Béjà[3]

The novel role of carotenoid molecules in carotenoid-binding rhodopsins (ISSP, *The University of Tokyo*[1], *Graduate School of Frontier Sciences, The University of Tokyo*[2], *Faculty of Biology, Technion – Israel Institute of Technology*[3], *AORI, The University of Tokyo*[4], *RIKEN*[5]) ○Keiichi Inoue[1,2], María del Carmen Marín Pérez[3], Takayoshi Fujiwara[2,4], Toshiaki Hosaka[5], Mikako Shirouzu[5], Susumu Yoshizawa[2,4], Oded Béjà[3]

Microbial rhodopsins are seven-transmembrane photoreceptive membrane proteins that use retinal as their chromophore. Previously, only a few H^+-pumping rhodopsins were known to bind 4-keto carotenoids as antenna pigments, facilitating retinal isomerization and enhancing H^+ transport through excitation energy transfer (EET). In contrast, we recently found that 3-OH carotenoids not only bind to a broader range of rhodopsins as antenna pigments (Fig. 1) but also promote H^+ transport by optimizing protein structure and accelerating photoreactions.

Keywords : Rhodopsin; Retinal; Carotenoid; Proton pump; Chloride pump

微生物ロドプシンはレチナールを発色団とする、7回膜貫通型の光受容型膜タンパク質である。これまで、ごく一部の H^+ポンプ型ロドプシンが 4-keto 型のカロテノイドを光捕集アンテナとして結合し、励起エネルギー移動（EET）によってレチナールの異性化反応を誘起し、H^+輸送量を向上させることが知られていた。これに対し、最近我々は、3-OH 型のカロテノイドがより広範なロドプシンに結合し、アンテナ色素として働くだけではなく（Fig. 1）、タンパク質の構造最適化や光反応の加速など、様々な形で H^+輸送を促進していることを明らかにしたため、それについて報告する[1–3]。

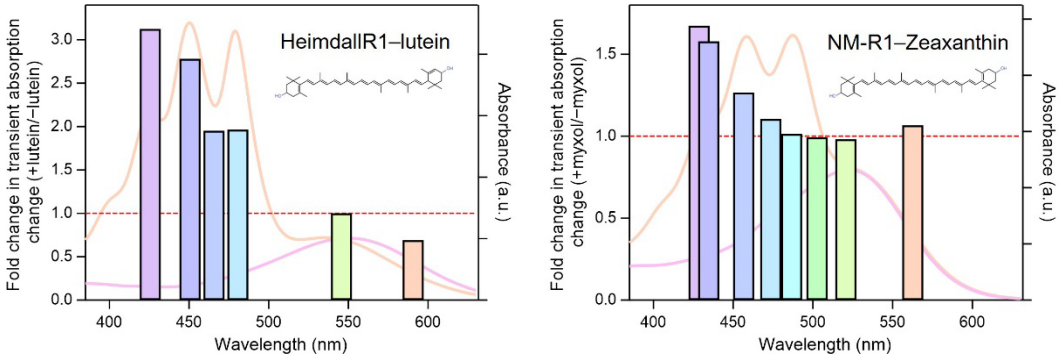


Figure 1. Fold change in transient absorption signals observed in rhodopsins, HeimdallR1 (left) and NM-R1 (right), with and without carotenoid, representing EET between carotenoid and retinal.

1) A. Chazan, et al., *Nature* **2023**, *615*, 535.
2) G. Tzlil, et al., *bioRxiv* **2024**, https://doi.org/10.1101/2024.09.18.613612.
3) T. Fujiwara, et al., *bioRxiv* **2024**, https://doi.org/10.1101/2024.11.08.622755.

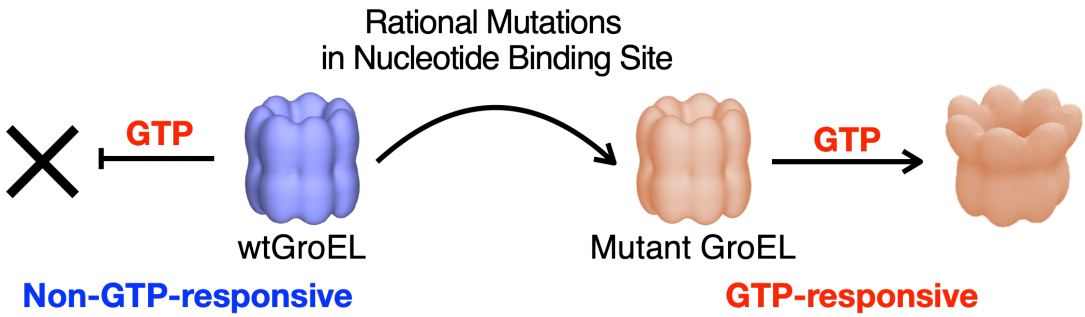
Imparting GTP Responsiveness to Biomolecular Machine GroEL by Binding Pocket Engineering

([1]*Graduate School of Engineering, The University of Tokyo*, [2]*Institute of Innovative Research, Institute of Science Tokyo,* [3] *Center for Emergent Matter Science, RIKEN*)
○Ryotaro Toda,[1] Kiyoshi Morishita,[1] Takayuki Miki,[1] Hideki Taguchi,[2] Takuzo Aida[1,3]
Keywords: GroEL, Biomolecular Machines, Nanomaterials, GTP

Biomolecular machines play critical roles in biological processes such as cell division, ion transport, and signal transduction. by converting energy from the selective binding and hydrolysis of nucleotides into mechanical motions.

Biomolecular machine GroEL exhibits large domain opening motions upon ATP binding and hydrolysis, empowering its essential chaperone function. Given this feature, GroEL can serve as a carrier for drug delivery systems (DDS).[1] However, the abundance of ATP in both cancerous and healthy cells poses an inherent challenge to ATP-responsive DDS.

We previously reported that a single mutation in the ATP-binding site confers GTP responsiveness to GroEL. Here, we conducted bioinformatics analysis to investigate which functional groups are frequently used for recognition of the guanine base part of GTP, and several functional groups were found to be commonly used. We further report GroEL mutants with enhanced GTP responsiveness and selectivity over ATP by investigating the key binding site residues. Unlike ATP, GTP is abundant in cancer cells and not in healthy cells, and thus, GTP-driven GroEL mutants are expected to lead to improved drug carriers.



(1) S. Biswas et al., *Nat. Chem.* **2013**, *5*, 613.

アデニル酸キナーゼに結合するモノボディの結合様式の検証

（奈良先端大物質創成[1]・高輝度光科学研究センター[2]・京都府大生命環境[3]） 中村 伊武輝[1]・長尾 聡[2]・雨坂 心人[3]・折戸 尚樹[1]・廣田 俊[1]・田中 俊一[3]・◯松尾 貴史[1]
Binding Mechanism of Monobodies Binding to Adenylate Kinase ([1]*Division of Materials Science, Nara Institute of Science and Technology*, [2] *SPring-8/JASRI,* [3]*Graduate School of life and Environmental Sciences, Kyoto Prefectural University*) Ibuki Nakamura,[1] Satoshi Nagao,[2] Hiroshi Amesaka,[3] Naoki Orito,[1] Shun Hirota,[1] Shun-ichi Tanaka,[3] ◯Takashi Matsuo[1]

We have reported that monobody OP-4, a synthetic binding protein for the OPEN-form adenylate kinase, inhibits the kinase activity by binding to the outside of the kinase active site. Accordingly, we have investigated the binding mechanism of OP-4 using ITC and 2D-NMR. As a result, ITC study indicated the entropy-driven binding mechanism for OP-4. 1H-15N HSQC revealed that the binding of OP-4 induces a slight conformational change of adenylate kinase retaining the structural characteristics of the OPEN-form.
Keywords : monobody; adenylate kinase; protein-protein interactions ;ITC; NMR

アデニル酸キナーゼ（Adk）は、OPEN/CLOSED の２つの構造変換を伴ってリン酸基転移反応（2 ADP ⇄ ATP＋AMP）を触媒する酵素である。我々は、これまでに、人工結合タンパク質の１つであるモノボディ（Mb）を基盤として、大腸菌由来 Adk の OPEN/CLOSED それぞれの構造を認識するモノボディを４種類（CL-1: CLOSED 構造特異的、OP-2, -3, -4: OPEN 構造特異的）得ている[2)]。そこで、本研究では、OPEN 構造を認識する Mb のうち、Adk の酵素活性を阻害する OP-4 と、CLOSED 構造を認識する CL-1 について、結合様式を検証した。

OP-4 は、Adk の活性部位の外側のヒンジ部分を含む領域に結合することが X 線小角散乱測定（SAXS）によってわかっている。そこで、結合の駆動力を等温滴定カロリメトリー (ITC) で評価したところ、エントロピー駆動型の結合であることがわかった。また、^{1}H-^{15}N HSQC 測定によって複合体中の Adk の構造状態を検証したところ、OP-4 は、複合体形成時に Adk の主鎖構造に摂動を与えることが示唆された。さらに基質 ATP を用いた ^{31}P-NMR 測定では、OP-4 の存在下でシグナルの広幅化が観測された。このことは、OP-4 の結合によって Adk の主鎖構造に変化が起こるものの、OPEN 構造の特徴は維持されており、基質が酵素内部に接触できることを示している。

一方、CL-1 は、エンタルピー駆動型で結合し、X 線構造解析により Adk の CORE ドメインの外側で多点水素結合、カチオン−π 相互作用を介して結合していることがわかった。また、CL-1 の存在下で Adk の遷移状態アナログである Ap_5A の解離定数が減少した。よって、CL-1 は Adk の CLOSED 状態を安定化していると考えられる。

以上より、Mb は、標的タンパク質の構造状態によって多様な結合様式を示しうることが明らかとなった。

1) I. Nakamura, H. Amesaka, H. Kamikubo, S. Tanaka, T. Matsuo *et al. Protein Sci.* **2023**, *32*, e4813 (2023). 2) I. Nakamura, S. Nagao, H. Amesaka, S. Negi. S. Tanaka, T. Matsuo, *ChemRxiv.* 10.26434/chemrxiv-2024-7591m-v2

Cytokine-Induced Cancer Cell-Derived Migrasomes Proteomic Analysis

([1]*Department of Chemical Science and Engineering, School of Material and Chemical Technology, Institute of Science Tokyo*) ○Ananda Bagus Richky Digdaya Putra,[1] Shogo Saito,[1] Masayoshi Tanaka,[1] Mina Okochi[1]

Keywords: Migrasomes, Extracellular Vesicles, Proteomic Analysis, Cancer Cells

Migrasomes are extracellular vesicles released from migrating cells, playing critical roles in intercellular communication[1]. Cytokines, such as tumor necrosis factor α (TNF-α) and IL-6, play an important role in the body's response to infection, inflammation, or injury[2]. IL-6 amplifier is a phenomenon of excessive secretion of IL-6 by immune cells and non-immune cells. IL-6 amplifier starts when the immune cells receive massive inflammatory signals[3]. Our results showed that the peptide interface increased migrasomes formation and retained the migrasomes on the peptide substrates after cell detachment[4]. Applying this method, migrasomes derived from cancer cells that stimulated with IL-6 increased the IL-6 expression in the cells cultured afterward.

However, cytokine stimulation's impact on the IL-6 amplifier condition in cancer-derived migrasomes remains underexplored. This study aims to investigate the proteomic changes in migrasomes derived from MDA-MB-231 cells, a triple-negative cancer cell line under an IL-6 amplifier condition. Cells were cultured under standard conditions with serum-containing medium, starvation conditions by replacing to serum-free medium, and subjected to IL-6 stimulation. Migrasomes released by cells were isolated using differential centrifugation. Gene expression was analyzed via quantitative PCR, and proteomic profiling was conducted using mass spectrometry.

As a result, 1,339 proteins commonly expressed across conditions, with Ferroptosis and Proteasome related protein groups, were upregulated after starvation and cytokines stimulation based on the KEGG Pathway analysis results. The JAK-STAT pathway's role in inflammation, cell survival, and immune regulation directly intersects with ferroptosis and proteasome. Therefore, we tried to identify the expression of genes involved in the JAK-STAT pathway in migrasomes after stimulation by IL-6, namely JAK1, STAT1, and STAT3, respectively, also the suppressors of cytokine signaling (SOCS) protein group, namely SOCS1 and SOCS3. Following starvation, the expression of IL-6, JAK-1, SOCS-1, and SOCS-3 in migrasomes was upregulated, but subsequent cytokine stimulation led to downregulation. Conversely, STAT-1 (pro-inflammatory) was increased during starvation and reduced after stimulation, while STAT-3 (anti-inflammatory) exhibited the opposite results. This suggests that migrasomes are involved in signaling modulation under stress conditions under starvation and IL-6 stimulation.

1) Ma, L., et al., Cell Res. **2015**, 25 (1), 24–37. 2) Yuan, S., et al., Front. Immunol. **2021**, 12, 659419. 3) Murakami, Y. et al. Sci. Rep. **2017,** 7, 1, 44042 4) Saito, S., et al., Mater. Sci. Eng., C **2021**, 124, 112495.

キチナーゼ結合ナノファイバータンパク質の創製と抗真菌活性の評価

（九大院工[1]・名工大院工[2]・琉球大農[3]・九大未来化セ[4]）○長谷 彩沙[1]・吉本 将悟[2]・平良 東紀[3]・堀 克敏[2]・神谷 典穂[1,4]

Creation and antifungal activity evaluation of a nanofiber protein conjugated with chitinase ([1]*Graduate School of Engineering, Kyushu University*, [2]*Graduate School of Engineering, Nagoya University*, [3]*Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus*, [4]*Division of Biotechnology, Center for Future Chemistry, Kyushu University*) ○Ayasa Nagatani,[1] Shogo Yoshimoto,[2] Toki Taira,[3] Katsutoshi Hori,[2] Noriho Kamiya,[1,4]

Globally, the threat of an alarming increase in fungal infection in various sectors of human health, livestock, and agriculture has exhibited a scary condition. In this study, we propose a novel way to utilize fibrous proteins, showing selective partitioning to polysaccharide-rich phase in an aqueous two-phase system (ATPS), as a new carrier for antifungal reagents. Regarding fibrous proteins, we focused on unique characteristics of Cstalk, which is located at the C-terminus of AtaA, showing the selective partitioning to Dextran (Dex)-rich phase in PEG/Dex ATPS. To validate our concept, antifungal chitinase (Chi), an enzyme specifically targeting the chitin components of fungal cell wall, was site-specifically conjugated with Cstalk via SpyTag/SpyCatcher chemistry to form a Cstalk-Chi complex. The antifungal activity of the complex was much higher than chitinase alone, suggesting that Cstalk facilitated the delivery of Chi to polysaccharide-rich fungal cell wall, thereby enhancing antifungal efficacy.

Keywords : Antifungal, bioconjugation, chitinase, fibrous protein, fungal infection

医療、家畜および農業分野における真菌感染症の増加は、喫緊に対処すべき優先課題となっている。本研究では、水性二相系において多糖類相へ優先的に分配する性質を示す繊維状タンパク質を、抗真菌活性を示す酵素の輸送担体とする新規抗真菌薬を提案する。我々は、巨大な繊維状タンパク質である AtaA の C 末端を構成する Cstalk が、多糖類相に分配性を示すことを見出した。そこで、真菌細胞壁を構成するキチン層を分解する抗真菌酵素であるキチナーゼ (Chi) と Cstalk を複合化し、抗真菌活性評価を行った結果、Chi 単体の場合と比較して、複合体はより高い抗真菌活性を示した。この結果より、Cstalk は真菌細胞壁へ Chi を効率的に輸送するキャリアとして機能し、抗真菌活性の増強に影響を及ぼしている可能性が示唆された。

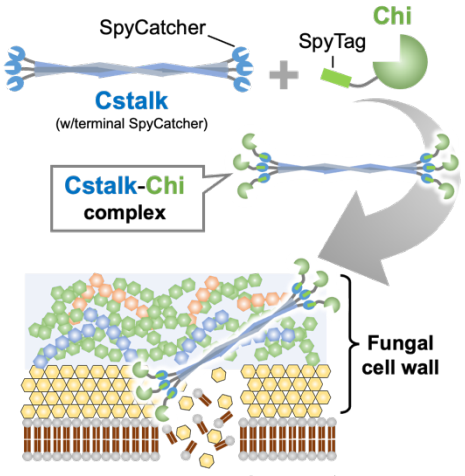


Fig. 1　本研究の概念図

【謝辞】本研究は JSPS 科研費 JP23H00247 の助成を受けたものです。

Ubiquitin chain probes for photo-induced capture of heterotypic chain-decoder binding pairs from a mixture of proteins

([1]*Graduate School of Engineering, The University of Tokyo*, [2]*The institute of Medical Science, The University of Tokyo*, [3]*Graduate School of Engineering, Tottori University*, [4]*GSC center, Tottori University*, [5] *Graduate School of Science, Kyoto University*) ○Taiki Uno[1], Usano Toyoda[1], Takafumi Furuhata[1], Takuya Tomita[2], Yusuke Sato[3, 4], Kei Okatsu[5], Yasushi Saeki[2], Akimitsu Okamoto[1]

Keywords: Heterotypic ubiquitin chain; Photo-crosslinker; Shuttle factor; Protein-Protein interaction

Recently, the heterotypic ubiquitin chain (heterotypic chain), a chain of ubiquitin units multimerized *via* multiple lysine (K) and methionine residues, has attracted much attention as a posttranslational modification that is highly diverse in terms of its structures and functions [1]. In particular, K63/K48 chains, which are the most abundant chain species (20% of all K63 chains in the cell)[2], play important roles in functional regulation of proteins. In NF-κB signaling, it is reported that the formation of K48 branches on K63 chains stabilizes K63 chain-mediated signals by protecting the K63 chains from enzymatic cleavage[3-4]. On the other hand, it is also known that K48-linked branch on K63 chains induces proteasomal degradation to shut down functions of substrate proteins in other cases[2-3]. However, chemical mechanisms that underly two opposing functions remain to be elucidated. To fully understand the structure-function relationships of the K63/K48 chain, a molecular probe that enables comprehensive analysis of protein-K63/K48 chain interaction in a complex environment such as cell extracts containing multiple proteins is highly demanded.

In this study, we developed a heterotypic chain probe functionalized by a photo-crosslinker towards comprehensive analysis of protein-heterotypic chains interaction in a mixture of proteins (Fig 1). Using ubiquitin functionalized with diazirine, we enzymatically synthesized diazirine-functionalized K63/K48 tri-ubiquitin chains that have a K48 branch on the different positions of K63 chains. The photo-crosslinking of diazirine-functionalized K63/K48 tri-ubiquitin with ubiquitin recognition proteins (URPs) was performed in test-tube, and crosslinking efficiencies were varied depending on the branched structure. URPs were also captured by photo-crosslinking even in a mixture of proteins represented by cell extracts. The diazirine-functionalized heterotypic ubiquitin chain would be applicable to comprehensive analysis of the heterotypic chain-URP interaction.

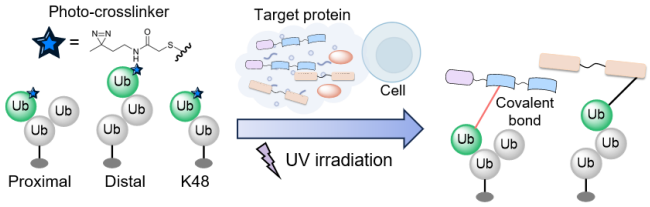


Fig 1. Scheme of photo-induced capture of heterotypic chain-URP pairs in a complex environment.

[1] *Mol.Cell Biol.*, **2023**, *24*, 273-287.; [2] *Proc. Natl. Acad. Sci. USA*, **2018**, *115*, 1401-1408.; [3] *Molecules*, **2020**, *25*, 5200.; [4] *Mol.Cell*, **2016**, *64*, 251-266.