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26日(水) 13:00 ~ 15:30 [A]A301(第3学舎 1号館 [3階] A301)

[[A]A301-1pm] 17. 生体機能関連化学・バイオテクノロジー

座長：安部 聡、稲葉 央

日本語
13:00 ~ 13:20
[[A]A301-1pm-01]
無細胞タンパク質結晶化による天然変性タンパク質の微量迅速構造決定
小島 摩利子[1]、安部 聡[2]、古田 忠臣[3]、○上野 隆史[3] (1. 北大、2. 京府大、3. 科学大)

日本語
13:20 ~ 13:40
[[A]A301-1pm-02]
細胞内タンパク質結晶の分子設計による刺激応答性構造相転移
○菊池 幸祐[1]、永間 美咲[1]、田中 潤子[1]、Haonan Kong[1]、安部 聡[2]、上野 隆史[1] (1. 東京科学大学、2. 京都府立大学)

日本語
13:40 ~ 14:00
[[A]A301-1pm-03]
ペプチドを介したTau由来ペプチドの提示による微小管超構造体の構築
○稲葉 央[1]、景山 大地[1]、渡 宗英[1]、角五 彰[2]、松浦 和則[1] (1. 鳥取大院工、2. 京大院理)

英語
14:00 ~ 14:20
[[A]A301-1pm-04]
自己切断ペプチドのセレクションベースの発見とその機能解析
○西尾 聡一郎[1]、菅 裕明[2]、後藤 佑樹[1] (1. 京都大学大学院、2. 東京大学大学院)

14:20 ~ 14:30
休憩

英語
14:30 ~ 14:50
[[A]A301-1pm-05]
ヘテロキラルオリゴ N メチルアラニンを足場としたタンパク質リガンドの設計
○横峰 真琳[1]、白鳥 陽太[1]、高場 圭章[2]、眞木 さおり[2]、米倉 功治[2,3]、山東 信介[1]、森本 淳平[1] (1. 東大院工、2. 理研、3. 東北大多元研)

英語
14:50 ~ 15:10
[[A]A301-1pm-06]
細胞内浸透圧ストレスに応じたYKペプチド融合sfGFPの人工的な液滴形成
○清水 雅俊[1]、相田 卓三[1]、三木 卓幸[1] (1. 東京大学)

英語
15:10 ~ 15:30
[[A]A301-1pm-07]

Construction of Artificial Lipid-Containing Vesicular Compartments in Living Cells Using Tryptophan-Rich Peptides

◯Qinxuan Yang[1], Takuzo Aida[1], Takayuki Miki[1] (1. The University of Tokyo)

無細胞タンパク質結晶化による天然変性タンパク質の迅速微量構造決定

（北大環境科学[1]・京府大生命[2]・科学大生命[3]・ASMat[4]）小島 摩利子・安部 聡・古田 忠臣・○上野 隆史[3,4]

Rapid Structure Determination of Intrinsically Disordered Proteins Using Cell-free Protein Crystallization ([1] Faculty of Environmental Earth Science, Hokkaido University, [2]Department of Biomolecular Chemistry, Kyoto Prefectural University, [3]*School of Life Science and Technology*, [4]*ASMat, Institute of Science Tokyo*) Mariko Kojima,[1] Satoshi Abe,[2] Tadaomi Furuta,[3] ○ Takafumi Ueno[3, 4]

Protein folding is regulated by interactions with ions, small molecules, and other proteins, allowing functional adaptation to environmental changes. Intrinsically disordered proteins (IDPs) flexibly alter their structures through interactions with other proteins, enabling diverse functions. While their structural regulation is crucial for drug discovery and medicine, IDPs are challenging to analyze due to their flexibility, and the mechanism of their structural stabilization remains unclear. This study utilized cell-free protein crystallization (CFPC) technology to establish a structural analysis screening method for IDPs and investigate the intermolecular interactions. CFPC, based on the wheat germ system with a 100 μL volume, achieved to obtain high-resolution crystal within 24 hours. We have successfully applied CFPC to determine the structure of the IDP targets using polyhedra crystal (PhC) as the fusion tag. systems.

Keywords : Intrinsically disordered protein; Protein microcrystal; Cell-free protein crystallization

タンパク質のフォールディングはイオンや低分子, タンパク質との相互作用により制御され,外部環境に応答して異なる機能を発現する. 天然変性タンパク質(Intrinsically disordered protein; IDP)は, 他のタンパク質との相互作用により柔軟に構造変化させることで多様な機能を発現するタンパク質であり, その構造制御は創薬医学の分野で注目されている. しかし IDP はその柔軟性ゆえに構造解析が困難であり, IDP が他のタンパク質表面で構造を固定する仕組みは未解明のままである. 本研究では,著者らが開発した無細胞タンパク質結晶化技術(Cell-free protein crystallization; CFPC)を用いて,[1] IDP の構造解析スクリーニング法を確立し, IDP の構造決定に必要となる分子間相互作用を解析した.[2] CFPC では 100 μL スケールのコムギ胚芽無細胞タンパク質合成反応を用い, 24 時間以内に高分解能の結晶を取得できる.[2] これまでにも細胞内タンパク質結晶である多角体(polyhedra crystal; PhC)を鋳型とした標的タンパク質の構造決定に成功しており,[3] PhC を融合タグとした様々なタンパク質標的への応用が期待できる.

1) S. Abe, et al., ***Sci. Rep.,*** 12, 16031 (2022).
2) M. Kojima, et. al., ***Proc. Natl. Acad. Sci. USA***, 121, e2322452121 (2024).
3) M. Kojima, et al., ***Biomater. Sci.,*** 11, 1350-1357 (2023).

細胞内タンパク質結晶の分子設計による刺激応答性構造相転移

（東京科学大学[1]・京都府立大学[2]）○菊池 幸祐[1]・永間 美咲[1]・田中 潤子[1]・Haonan Kong[1]・安部 聡[2]・上野 隆史[1]
Design of in-cell protein crystals for the stimuli-responsive structural phase transitions ([1]*School of Life Science and Technology, Institute of Science Tokyo*, [2]*Department of Biomolecular Chemistry, Kyoto Prefectural University*) ○Kosuke Kikuchi,[1] Misaki Nagama,[1] Junko Tanaka,[1] Haonan Kong,[1] Satoshi Abe,[2] Takafumi Ueno[1]

Protein crystals have gained significant attention as biomaterials for catalysts, storage, and sensors. In crystals, protein subunits organize in a highly symmetrical and energetically stable manner. This hinders the design of protein crystals that can undergo crystalline-crystalline structural phase transitions. Previous efforts were centered on re-crystallization after the dissolution of the original crystal, whereas crystalline-crystalline structural phase transitions were less explored owing to their difficulty and complexity. In this work, we engineered in-cell protein crystals to achieve stimuli-responsive structural phase transitions (Figure 1).
Keywords: In-cell Protein Crystals; Protein Engineering; Stimuli-responsiveness; Phase Transition; Small-angle X-ray Scattering

タンパク質結晶は生体材料として注目を集めており，触媒や分子貯蔵への応用研究が進められている．結晶において分子は秩序正しく配置されているが，その秩序構造を変化させることは困難である．したがって，溶解・再結晶を経ることなく三次元結晶の結晶ー結晶相転移を実現するような分子設計はいまだ確立されていない[1,2]．本研究では，細胞内タンパク質結晶のエンジニアリングにより，刺激応答性構造相転移の実現を目指した（Figure 1）．

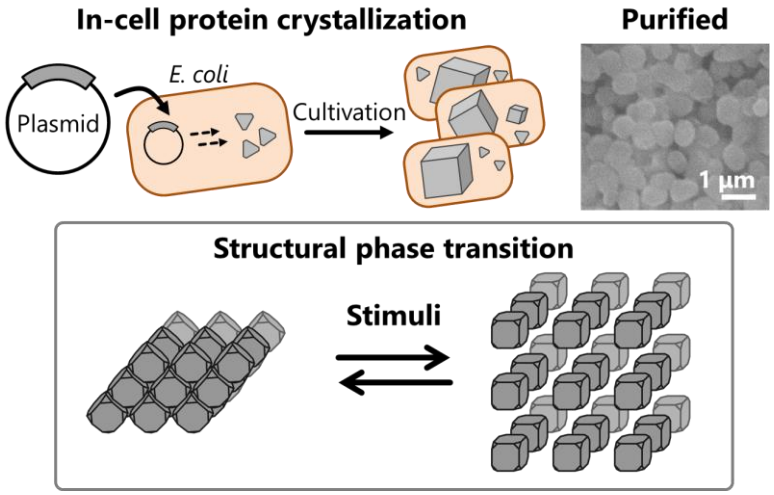


Figure 1. Design of in-cell protein crystals for the stimuli-responsive phase transitions.

1) Du, M. *et al. Nano Lett.* **21**, 1749-1757 (2021).
2) Ramberg, K. O. *et al. J. Am. Chem. Soc.* **143**, 1896-1907 (2021).

ペプチドを介した Tau 由来ペプチドの提示による微小管超構造体の構築

（鳥取大院工[1]・京大院理[2]）○稲葉央[1]・景山大地[1]・渡宗英[1]・角五彰[2]・松浦和則[2]
Peptide-mediated display of Tau-derived peptide for construction of microtubule superstructures ([1]*Graduate School of Engineering, Tottori University*, [2]*Graduate School of Science, Kyoto University*) ○Hiroshi Inaba[1], Daichi Kageyama[1], Soei Watari[1], Akira Kakugo[2], Kazunori Matsuura[1]

Microtubules are one of the cytoskeletons composed of tubulin. In nature, complex microtubule superstructures like doublets and bundles exist to perform unique functions. We have developed Tau-derived peptides (TP) that bind to the inside of microtubules for the encapsulation of nanomaterials. By presenting TP-fused tetrameric proteins on the microtubules, we have achieved the formation of microtubule superstructures by tubulin binding to the exposed TP.[1] In this study, we constructed microtubule superstructures by linking the KA7 peptide (repeated lysine-alanine sequence) which bind to the outer surface of microtubules[2] to TP. Confocal microscopy and transmission electron microscopy showed that KA7-TP enabled construction of microtubule superstructures such as doublets and bundles.
Keywords: Tau-derived peptide; Microtubules; Tubulin; Superstructures

チューブリンからなる細胞骨格である微小管は、一般的に一巻きのチューブ構造を形成する。一方、天然には微小管が横方向に連なったダブレットやバンドルなどの複雑な超構造体が存在し、これらを人工的に構築できればその理解や材料応用に繋がる。我々は、微小管内部に結合する Tau 由来ペプチド（TP）を開発し、微小管内部へのナノ構造体の導入に成功している。さらに、TP 融合四量体タンパク質を微小管外部表面に提示し、露出した TP へのチューブリンの結合により微小管超構造体の構築を達成している[1]。本研究では、微小管外部に結合するリシンとアラニンを 7 回繰り返す KA7 ペプチド[2]を TP に連結した KA7-TP を開発し、その結合を利用した微小管超構造体の構築を試みた（Fig. 1）。異なるリンカーを有する 3 種類の KA7-TP およびその N 末端に赤色蛍光色素 TMR を修飾した TMR-KA7-TP を合成した。共焦点レーザー蛍光顕微鏡（CLSM）により全ての TMR-KA7-TP が微小管に結合することが示された。KA7-TP を TMR 修飾微小管に添加し、緑色蛍光色素 AF488 修飾チューブリンを複合化後に GTP アナログ（GMPCPP）を添加したところ、CLSM により TMR 微小管と AF488 微小管の共局在が見られた（Fig. 1）。透過型電子顕微鏡においても、ダブレットやバンドルなどの微小管超構造体の構築が確認された。

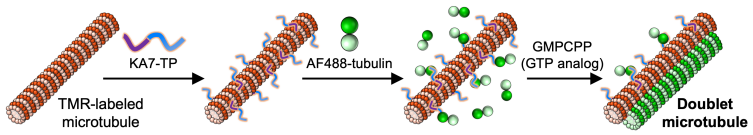


Fig. 1. KA7 連結 TP（KA7-TP）による微小管超構造体の構築

1) H. Inaba[1], Y. Sueki[1], M. Ichikawa[1], A. M. R. Kabir, T. Iwasaki, H. Shigematsu, A. Kakugo, K. Sada, T. Tsukazaki, K. Matsuura, *Sci. Adv.*, **2022**, *8*, eabq3817 ([1]Equal contribution).
2) H. Drechsler, Y. Xu, V. F. Geyer, Y. Zhang, S. Diez, *Mol. Biol. Cell*, **2019**, *30*, 2953.

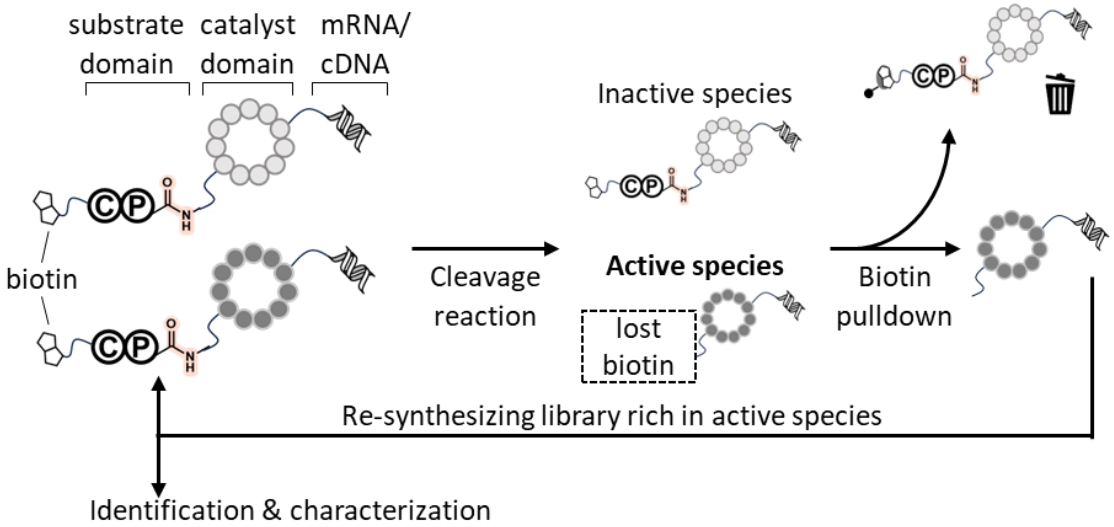
Selection-Based Discovery and Mechanistic Analysis of Self-Cleaving Peptides

([1]*Graduate School of Science, Kyoto University*, [2]*Graduate School of Science, The University of Tokyo*) ○Soichiro Nishio,[1] Hiroaki Suga,[2] Yuki Goto[1]

Keywords: Peptide; mRNA display; Amide bond cleavage; Selection

Amide bonds are remarkably stable, which presents a major challenge for their cleavage despite its critical role in protein degradation and numerous biotechnological processes. In nature, proteases overcome this challenge by adopting highly intricate three-dimensional structures that enable them to catalyze amide bond cleavage. However, short peptides generally lack the structural complexity required to form such catalytic active sites. While significant progress has been made in developing peptide-based catalysts for a variety of chemical reactions, no peptide catalysts have yet been demonstrated to cleave amide bonds effectively.

In this study, we present a novel approach to identify peptides with amide bond cleaving activity using an mRNA display-based selection strategy. We synthesized a molecular library composed of biotin, a Cys-Pro-amide bond as the substrate domain, a random peptide as the catalytic domain, and mRNA/cDNA encoding the peptide sequence. Following the cleavage reaction, active species that lost biotin were separated from inactive ones and selectively amplified to reconstruct the library. Remarkably, this selection campaign serendipitously led to peptides with self-cleaving activity. We will discuss the characterization and catalytic properties of these active peptides, which provide new insights into peptide-based catalysis and suggest promising directions for future research.



ヘテロキラルオリゴ N メチルアラニンを足場としたタンパク質リガンドの設計

（東大院工[1]・理研[2]・東北大多元研[3]）○横峰 真琳[1]・白鳥 陽太[1]・高場圭章[2]・眞木さおり[2]・米倉 功治[2,3]・山東 信介[1]・森本 淳平[1]

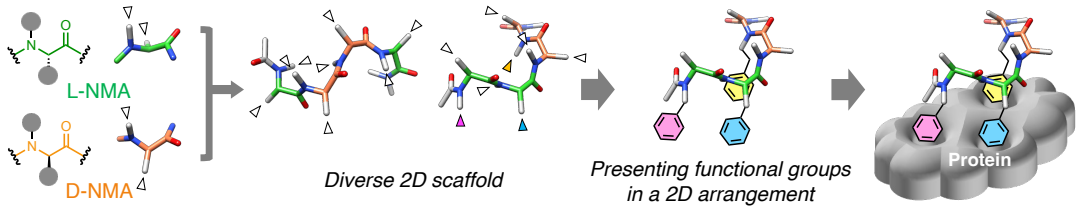
Design of protein ligands based on heterochiral oligo(*N*-methylalanine) as a scaffold

([1]*Graduate School of Engineering, The University of Tokyo*, [2]*RIKEN*, [3]*IMRAM, Tohoku University*)○Marin Yokomine,[1] Yota Shiratori,[1] Kiyofumi Takaba,[2] Saori Maki-Yonekura,[2] Koji Yonekura,[2,3] Shinsuke Sando,[1] Jumpei Morimoto[1]

Biomacromolecules play important roles via molecular recognition, which requires precise spatial arrangements of interaction sites at the nanometer level. Therefore, synthetic molecules that adopt well-defined nanometer-sized shapes in water and allow for the introduction of functional groups are useful as molecular scaffolds for mimicking the functions of biomacromolecules. Peptide-based molecular scaffolds, in particular, are attracting attention due to their high water solubility and ease of synthesis. However, most reported peptide-based molecular scaffolds are linear shapes, as they typically consist of simple repetitive structures, with only limited examples capable of forming two-dimensional shapes. In this presentation, we propose the design of diverse and two-dimensionally extended scaffold shapes by combining L- and D- *N*-methylalanines (NMAs). We also present the results of conformational analysis[1)] and the subsequent design of ligands targeting anti-apoptotic protein MCL-1 based on these findings.

Keywords : Protein–protein interaction; Conformational analysis; Peptide; Molecular design; MCL-1.

生体高分子は分子認識により重要な役割を果たしており、そのためには相互作用部位がナノメートルレベルで精密に空間配置されていることが必要である。よって、水中でナノメートルサイズの規定された形状を取り、官能基を導入可能な合成分子は、生体高分子の機能を模倣する足場分子として有用である。特にペプチド性の足場分子は高い水溶性と合成の容易さから注目されているが、単純な繰り返し構造からなる直線的な足場がほとんどであり、二次元的な足場構造を形成できる例は少ない。本発表では、L 体と D 体の N メチルアラニン（NMA）を組み合わせた多様な二次元的に広がる足場構造の設計を提案し、その構造解析[1)]およびそれに基づく抗アポトーシスタンパク質 MCL-1 のリガンド設計について報告する。



1) J. Morimoto, Y. Shiratori, M. Yokomine, T. Ueda, T. Nakamuro, K. Takaba, S. Maki-Yonekura, K. Umezawa, K. Miyanishi, Y. Fukuda, T. Watanabe, W. Mizukami, K. Takeuchi, K. Yonekura, E. Nakamura, S. Sando, *ChemRxiv* **2023**, 10.26434/chemrxiv-2023-c4mw6.

Artificial Droplet Formation of YK-Peptide-Tagged sfGFP Under Osmotic Stress in Living Cells

(*Graduate School of Engineering, The University of Tokyo*) ○Masatoshi Shimizu, Takuzo Aida, Takayuki Miki

Keywords: Liquid-liquid Phase Separation; Self-assembling peptide; Osmotic pressure; Intracellular supramolecular

Inside cells, proteins and nucleic acids undergo phase separation, leading to the formation of liquid droplets. These droplets play significant roles in various physiological processes, including gene expression, metabolic reactions, and immune responses. To better understand the mechanisms of droplet formation and explore their potential applications in cellular engineering, we have been developing methods to artificially induce droplets within cells using designed peptides. We identified a 13-residue peptide comprising alternating tyrosine (Y) and lysine (K) residues (YK13), which facilitates droplet formation[1]. Specifically, when the peptide NES (nuclear export signal)-YK13 was fused with superfolder Green Fluorescent Protein (NES-YK13-sfGFP) and expressed in COS-7 cells, it was observed to form droplets within the cells. To broaden the application of artificial droplets in cell engineering, it is essential to achieve controllable droplet formation in response to environmental stimuli.

In this study, we found that YK11-fused sfGFP responded to osmotic pressure, a stress factor involved in numerous biological events, to induce droplet formation. Initially, NES-YK11-sfGFP dispersed uniformly under normal conditions. When osmotic stress was applied, NES-YK11-sfGFP formed droplets within one minute (Fig. 1). Moreover, we confirmed that the YK11 droplets independently formed with other representative droplets formed in osmotic stress. The droplet formation of NES-YK11-sfGFP was successfully reconstructed in vitro.

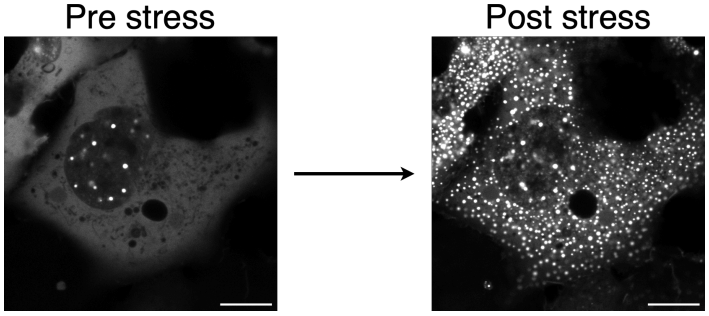


Fig. 1: COS-7 cells expressing NES-YK11-sfGFP, before and after osmotic stress, observed by confocal microscopy (CLSM). Scale bars: 10 μm.

1) Miki, T., Hashimoto, M., Takahashi, H., Shimizu, M., Nakayama, S., Furuta, T., Mihara, H., *Nature Communications* **15**, 8503 (2024)

Construction of Artificial Lipid-Containing Vesicular Compartments in Living Cells Using Tryptophan-Rich Peptides

([1]*Graduate School of Engineering, The University of Tokyo*) ○Qinxuan Yang[1], Takuzo Aida[1], Takayuki Miki[1]

Keywords: Natural peptides, Intracellular assembly, Intracellular compartments, Supramolecular chemistry

Lipid membrane-bound compartments, i.e. organelles, are important in the cell since they provide a closed environment with selective membrane permeability for specific biological reactions. Up to the state, scientists have been trying to manipulate the intracellular membranes by changing their locations and compositions[1]. However, there is no report in constructing new membrane-bound structures as controlling the genesis and assembly of lipids is thought to be challenging. Herein, we introduce the first successful construction of artificial phospholipid-containing compartments in living cells using genetically encoded peptides. We demonstrate that peptide sequence with tryptophan repeats (W) spontaneously forms vesicular structures, and Raman imaging confirms the presence of lipids within these assemblies.

Tryptophan is well known for interaction with phospholipids through hydrogen bonding and hydrophobic interaction, thus we designed a peptide containing 9 tryptophan repeats (W9) fused with super-folder green fluorescent protein (sfGFP) for imaging. Using an approach we previously reported[2], plasmid DNA encoding W9-sfGFP was prepared and delivered to COS-7 cells by lipofection. We hypothesize that W9-sfGFP bind to intracellular membranes first, then formation of W9-domains caused membrane disturbance and bending, which eventually caused independent vesicle formation. Our investigations showed that these vesicles contain lipids and are independent from intracellular organelles. The mechanism of vesicle formation was also studied in in vitro models. This work opens new avenues for constructing and engineering lipid-containing compartments in cellular environments.

1) I. Takanari, *Nat. methods* **2020,** *17* (9), 928-936. 2) T. Miki, *Nat. Commun.* **2021**, *12*, 3412.