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]A304(第3学舎 1号館 [3階] A304)

## [[A]A304-4pm] 17. 生体機能関連化学・バイオテクノロジー

座長：前之園 信也、仲本 正彦

日本語
13:00 ~ 13:20
[[A]A304-4pm-01]
細胞膜との相互作用に基づく脂質ナノ粒子の設計
○岡本 行広[1]、Xuehui Rui[1]、渡邊 望美[1] (1. 大阪大学 大学院基礎工学研究科)

英語
13:20 ~ 13:40
[[A]A304-4pm-02]
磁性-プラズモンハイブリッドナノ粒子を用いたライソゾーム病モデル細胞からのライソゾームの磁気分離
○高橋 麻里[1]、The Son Le[1]、平塚 祐一[1]、松村 和明[1]、前之園 信也[1] (1. 北陸先端大)

英語
13:40 ~ 14:00
[[A]A304-4pm-03]
アップコンバージョンナノ粒子を用いた非視覚オプシン発現細胞の近赤外光操作
○小手川 福笑[1]、高橋 麻里[1]、平塚 祐一[1]、松村 和明[1]、小島 大輔[2]、前之園 信也[1] (1. 北陸先端科学技術大学院大学、2. 東京大学)

英語
14:00 ~ 14:20
[[A]A304-4pm-04]
塩橋を介して多数のリポソームで構成された管状人工組織の創製と特性評価
○小島 知也[1]、朝倉 浩一[1]、伴野 太祐[1] (1. 慶應義塾大学)

14:20 ~ 14:30
休憩

日本語
14:30 ~ 14:50
[[A]A304-4pm-05]
自己集合体間*in situ*クリック反応によるCAIX多価リガンドの発現およびメカニズム解析
○坂本 蓮太郎[1]、木場 勇希[1]、仲本 正彦[1]、松崎 典弥[1] (1. 阪大院工)

日本語
14:50 ~ 15:10
[[A]A304-4pm-06]
蛋白質液滴のオートファジーにおける初期過程の蛍光可視化
○高嶋 一平[1]、馮 逸凡[1]、山本 林[2]、水上 進[1] (1. 東北大学、2. 日本医科大学)

日本語
15:10 ~ 15:30
[[A]A304-4pm-07]

# 遷移金属触媒反応を介した人工細胞膜ダイナミクスの制御

○濵口 怜[1]、Damian Graf[2]、Thomas Ward[2]、金原 数[1] (1. 科学大、2. バーゼル大)

日本化学会 第105春季年会 (2025)

# 細胞膜との相互作用に基づく脂質ナノ粒子の設計

（阪大院基礎工）○岡本 行広・Xuehui Rui・渡邊 望美

Construction of lipid nanoparticles based on the interaction between cell membrane

(Graduated School of Engineering Science, Osaka University)
○**Yukihiro Okamoto**, Xuehui Rui, Nozomi Watanabe

In the development of drugs or drug carriers, the analysis of the interaction between the plasma membrane and drug or drug delivery carrier is significantly important. Thus, a detailed study about interaction should be conducted as well as the study of plasma membranes and drug delivery carriers. In this paper, I will introduce our results based on the analysis of the plasma membrane and the interaction between drug or drug delivery carriers, and then would like to propose the methodology for the drug delivery carrier development and the evaluation method for membrane lipid therapy.

*Keywords : lipid nanoparticle, cell membrane, lipid membrane analysis, membrane lipid therapy*

一部の薬物は，細胞膜に作用してシグナル伝達の変化，そしてがん細胞にアポトーシスを誘起する(membrane lipid therapy)．一方，薬物キャリア(DDS carrier)は，免疫系細胞による認識を回避し，目的とする疾患部位へ到達することが求められる．これらのことを考えると，薬物ならびに DDS carrier と細胞膜との相互作用，そして相互作用の結果生じる細胞膜の物性変化の解析は，最適な membrane lipid therapy 剤や DDS carrier 設計において重要であると考えられる．そこで，本発表では，membrane lipid therapy 剤と DDS carrier の設計に関して，ミクロレベルでは細胞膜の特性解析，マクロレベルでは細胞への取り込みという観点で，細胞膜との相互作用に焦点をあてた研究を実施したので報告する．

[1] membrane lipid therapy 剤に関する研究 [1]: membrane lipid therapy 剤として期待される 2OHOA 分子をリポソーム化し，各がん細胞に投与した結果，リポソーム化することでアポトーシス率の向上に成功した．また，二光子顕微鏡解析により，2OHOA リポソーム投与後に，細胞膜の流動性が低下することを明らかとした．

[2] 免疫回避を目指す DDS carrier に関する研究 [2] : macrophage 細胞由来の細胞膜を DDS carrier として期待される cubosome 表層に固定化し，細胞膜固定化 DDS carrier を調製した．そして SAXS 解析や cryo-TEM 解析などより cubic 相を維持していることを明らかとした．cubosome は，血中では，albumin などの分子により崩壊する点が問題視されているが，細胞膜の固定化により，安定性の向上を実現した．さらに，フローサイトメトリーや *in vivo* imaging の結果より，macrophage 細胞による取り込みの軽減ならびに血中滞留時間の向上に成功した．

Membrane lipid therapy 剤や DDS carrier の研究において，細胞膜との相互作用に関する知見を設計にフィードバックすることで，これら薬物や DDS carrier の性能の向上が期待できる．

1) *Scientific Reports*, **2024,** *14*, 15831
2) *J. Mater. Chem. B*, **2024,** *12*, 8702-8715

# Magnetic separation of lysosomes from cells with lysosome dysfunction using superparamagnetic-plasmonic hybrid nanoparticles

(*School of Materials Science, JAIST*) ○Mari Takahashi, The Son Le, Yuichi Hiratsuka, Kazuaki Matsumura, Shinya Maenosono

**Keywords**: Nanoparticles; Magnetic separation; Plasmonic imaging; Lysosomes

Proteomics, which is a comprehensive analysis of proteins extracted from specimens has an important role in understanding the function of proteins. Specifically, comparison of proteins extracted from normal and dysfunctional cells can contribute to drug discovery. In this study, we focus on lysosomes and lysosomal storage diseases. Niemann-Pick type C disease is one such disease and is caused by the mutation of either NPC1 or NPC2 proteins resulting in accumulation of lipids in the lysosome and death.[1)]

In order to separate lysosomes we previously fabricated Ag@FeCo@Ag core@shell@shell magnetic plasmonic hybrid nanoparticles (MPNPs) as a magnetic separation probe (Fig. 1a).[2)] Then the MPNPs were introduced to animal cells and their location traced in the endosomal-lysosomal pathway (Fig. 1b). Then, magnetic separation of lysosomes was performed using a magnetic column (Miltenyi Biotec). Fig. 1c represents western blot (WB) results. Lysosomal marker protein (LAMP2) was detected from the positive selection (PS) which is the magnetically separated fraction while the negative control (GAPDH) was not detected in PS suggesting that magnetic separation of lysosomes was successful.[3)] Although the separation protocol for COS1 cells and HEK293 cells was established, for the sake of proteome analysis, cell types were changed to A549 cells for which an NPC1 knockout (KO) cell line is available. With a modified magnetic separation protocol, lysosomes from wild-type (WT) and NPC1 KO A549 cells were separated.

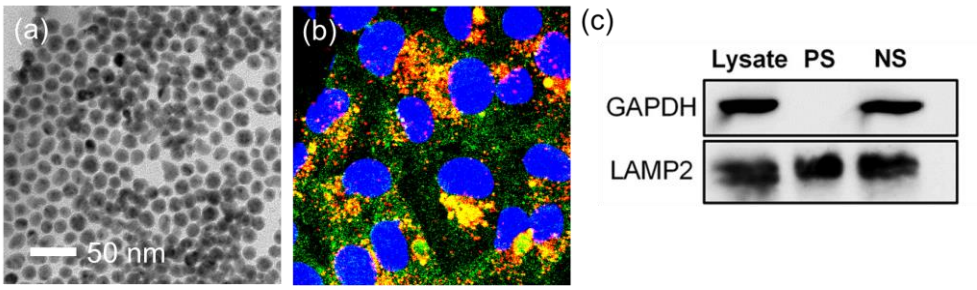


**Figure 1** (a) TEM image of Ag@FeCo@Ag NPs. (b) Confocal laser scanning microscope image of COS-1 cells. Blue: nucleus; green: NPs; red: lysosomes. (c) WB results of whole cell lysate, PS and negative selection (NS). GAPDH was used as a negative control. LAMP2 was a lysosomal protein.

1) T.-Y. Chang, *et al*., *J. Biol. Chem.* **2005**, *280*, 20917. 2) M. Takahashi *et al*., *Langmuir* **2015**, *31*, 3) T. S. Le, *et al*., *ACS Nano* **2022**, *16*, 885.

# Near-infrared light manipulation of non-visual opsin-expressing cells using upconversion nanoparticles

([1]*School of Materials Science, JAIST*, [2]*Department of Biological Sciences, School of Science, The University of Tokyo*) ○Fukue Kotegawa,[1] Mari Takahashi,[1] Yuichi Hiratsuka,[1] Kazuaki Matsumura,[1] Daisuke Kojima,[2] Shinya Maenosono[1]

**Keywords**: Upconversion Nanoparticles; Optogenetics; Photoreceptor; Calcium Imaging

Although the demand for UV light-induced photochemical reactions *in vivo* has been increasing, UV light has been limited due to its low tissue penetration from outside the organisms and high cytotoxicity. To address this, a technique is desired which uses upconversion nanoparticles (UCNPs), which when irradiated by near-infrared (NIR) light (with high tissue penetration from outside the organisms) convert it to UV light *in vivo*. In the field of optogenetics, visual opsins that respond to visible light have been studied. Recently, UV light-responsive non-visual opsin, OPN5[1] has much attraction, which is expressed in the deep brain and is involved in metabolic functions.[2]

In this study, we synthesized NIR→UV conversion-capable UCNPs and combined them with OPN5-expressing HEK293T cells. As UCNPs, $NaYF_4$:Yb,Tm@$NaLuF_4$ core@shell NPs with enhanced UV light emission were synthesized (**Fig. 1**).[3] For OPN5-expressing HEK293T cells, an OPN5 expression plasmid was synthesized in which the FLAG tag was expressed in the extracellular domain, and transfected into HEK293T cells. Then, UCNPs were bound to the cells using the FLAG tag. In this presentation, we will explain how we demonstrated that it is possible to manipulate OPN5 by binding UCNPs to OPN5-expressing HEK293T cells, and then generating UV light in the vicinity of OPN5 through NIR light irradiation, as evidenced by intracellular $Ca^{2+}$ imaging.

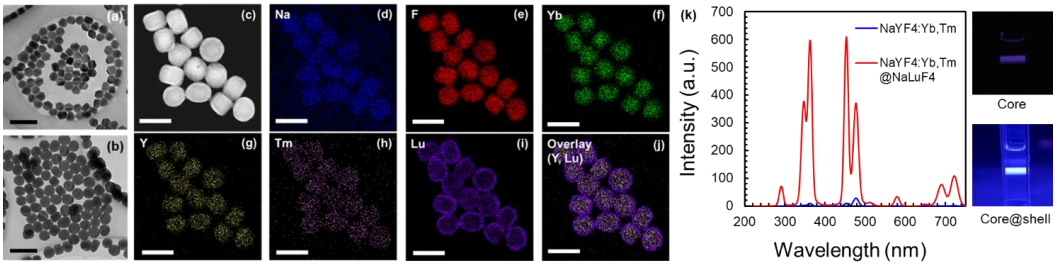


**Fig. 1.** TEM images of (a) $NaYF_4$:Yb,Tm core and (b) $NaYF_4$:Yb,Tm@$NaLuF_4$ core@shell UCNPs (scale bar = 200 nm). (c) HAADF-STEM image, and (d–j) EDS elemental maps of core@shell UCNPs (scale bar = 100 nm). (k) Emission spectra and photos of emission when irradiating NIR light (980 nm) to as-synthesized core (blue curve) and core@shell UCNPs (red curve) dispersed in hexane.

1) E. E. Tarttelin *et al*., *FEBS Lett*. **2003**, *554*, 410; 2) K. X. Zhang *et al*., *Nature* **2020**, *585*, 420; 3) N. T. Nguyen *et al*., *ACS Nano* **2023**, *17*, 382.

# Fabrication and Characterization of Tubular Artificial Tissues Composed of Multiple Liposomes via Salt Bridges

([1]*Faculty of Science and Technology, Keio University*) ○Tomoya Kojima,[1] Kouichi Asakura,[1] Taisuke Banno[1]

**Keywords**: Liposomes; Vesicles; Artificial Cells; Artificial Tissues; Tubular Structures

Multicellular organisms have hierarchical structures where multiple cells assemble to form tissues, resulting in complex 3D structures. Inspired by them, creation of artificial tissues composed of multiple artificial cells could lead to novel bioinspired materials.[1] We previously found the formation of artificial tissues composed of multiple liposomes by designing salt bridge intermolecular interaction.[2] Next, since controlling shapes is one of the important factors as materials, we have succeeded in developing new techniques to fabricate the artificial tissues into fibrous shapes.[3] As the next step towards more complex structures, tubular structures are focused on because they have holes in which solutions can be penetrated, which has a potential to be used as flow reactors. In this study, we report methods to fabricate liposome-based artificial tissues into tubular shapes and their functionalities.

Liposomes containing amphiphilic amines or carboxylic acids were mixed to obtain assemblies of multiple liposomes via salt bridges. The liposome assemblies were loaded tightly into a pipette tip under centrifugation. A rod representing a core was then inserted into the center of the pipette tip to function as a hole, and the liposome assemblies were pushed with a mechanical pipette to obtain a liposome-based tubular artificial tissue. The tubular structure was confirmed by using image analysis of brightness under high-intensity light irradiation, where gray values decreased at the center of the artificial tissue, indicating the formation of a hole. We will report the precise shape controllability of the tubular structures, such as the size of the holes, and their functionalities as flow reactors.


1) A. Rebasa-Vallverdu, M. Antuch, B. Rosetti, N. Braidotti, P. Gobbo, *ChemSystemsChem* **2024**, *6*, e202400014. 2) T. Kojima, Y. Noguchi, K. Terasaka, K. Asakura, T. Banno, *Small* **2024**, *20*, 2311255. 3) T. Kojima, K. Asakura, P. Gobbo, T. Banno, *Adv. Sci. in press*.

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# Emergence of Multivalent Ligands of CAIX by Interpolymer *in situ* Click reaction and Mechanistic Analysis

(*Grad. Sch. of Eng., Osaka Univ.*[1]) ○Rentaro Sakamoto,[1] Yuki Koba,[1] Masahiko Nakamoto,[1] Michiya Matsusaki[1]

**Keywords**: Cancer; Click reaction; Carbonic anhydrase; Inhibition; Conformational change

Nanomaterials with multivalent ligands have been widely studied in cancer therapy. However, non-selective recognition remains challenges due to tumor heterogeneity. In this context, *in situ* emergence of multivalent ligands for target cells would be effective to realize the recognition with high selectivity, affinity, and adaptivity. We report the emergence of multivalent ligands of Carbonic anhydrase IX (CAIX) by interpolymer *in situ* click reaction.

$N_3$-PEG-U, precursor ligand, was synthesized by introducing CAIX target moiety: U-104 (U) and azidoacetic acid ($N_3$-AcOH) to poly (ethylene glycol) ($M_W$: 10 kDa) (**Fig. 1A**). DBCO-PGA, precursor scaffold, was synthesized by introducing dibenzocyclooctyne-amine (DBCO-amine) to poly (L-glutamic acid) ($M_W$: 120 kDa). Dynamic light scattering revealed that both $N_3$-PEG-U and DBCO-PGA formed nano-assemblies, whereas the size decreased after mixing the two (**Fig. 1B**). These results suggested the conformational change in both nano-assemblies as the consequence of click reaction. Treatment of MDA-MB-231 with either $N_3$-PEG-U or DBCO-PGA had no effect on cell proliferation, while treatment with both polymers inhibited cell proliferation (**Fig. 1C**). In addition, *in situ* ligand synthesized in the presence of cells showed higher efficacy than *ex situ* one synthesized in the absence of cells, indicating the adaptive binding interface provided by *in situ* click reaction (**Fig. 1D and E**).

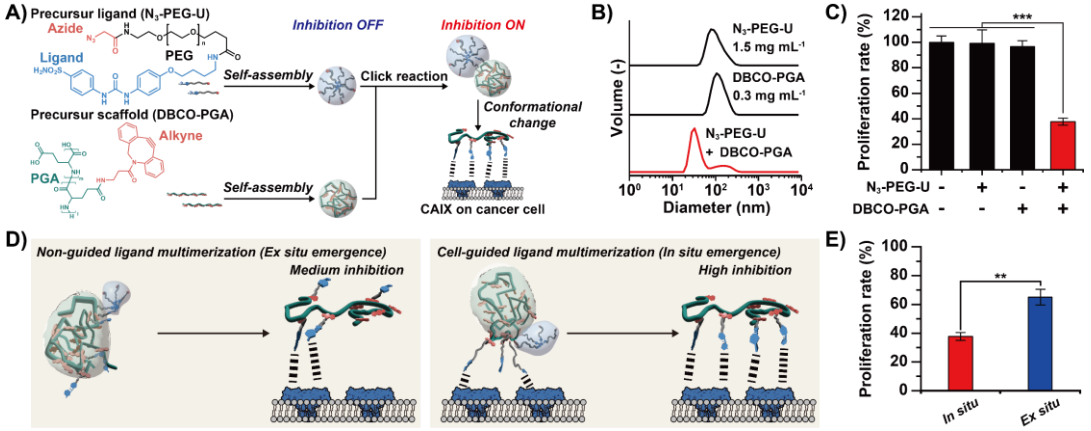


**Fig. 1. (A)** Conceptual illustration of this study. **(B)** Hydrodynamic diameter of both polymers. **(C)** Cell proliferation upon treatment with $N_3$-PEG-U and DBCO-PGA, and the combination. **(D)** Schematic illustration of *in situ* emergence of multivalent ligands. **(E)** Comparison with cell proliferation upon treatment with *in situ*/*ex situ* synthesized multivalent ligands. Note that [$N_3$] and [DBCO] are 1000 and 2000 μM, respectively. Statistical significance was determined using one-way ANOVA followed by Tukey's post-test (n = 3 $^{**}p < 0.01$, $^{***}p < 0.001$).

# 蛋白質液滴のオートファジーにおける初期過程の蛍光可視化

（東北大多元研[1]・東北大院生命[2]・日本医大[3]）○髙嶋　一平[1]・馮　逸凡[2]・山本　林[3]・水上　進[1,2]
Fluorescence imaging of initiation process in autophagy of protein condensates ([1]*Institute of Multidisciplinary Research for Advanced Materials, Tohoku University*, [2]*Graduate School of Life Sciences, Tohoku University*, [3]*Institute for Advanced Medical Sciences, Nippon Medical School*) ○Ippei Takashima,[1] Yifan Feng,[2] Hayashi Yamamoto,[3] Shin Mizukami[1,2]

Liquid-liquid phase separation of protein condensate (hereafter referred to as “droplet”) regulates intracellular protein activities through the autophagic degradation (fluidophagy) which has recently attracted attention as an intracellular event. Here we report a chemical tool to spatiotemporally detect the initial process of fluidophagy via the fluorogenic inverse electron-demand Diels-Alder (IEDDA) reaction. In this method, diene (tetrazine) and dienophile of the IEDDA reaction are introduced into the protein on the droplet and LC3B on the isolation membrane, respectively. When the droplet contacts the isolation membrane during autophagy process, both reactants are placed in close proximity, promoting the fluorogenic IEDDA reaction along the contact interface. In contrast, the reaction does not proceed without autophagy induction due to the controlled reactivity of the designed reactant pair. In this presentation, we will also show the result using this tool to monitor fluidophagy process from contact between the droplet and the isolation membrane to lysosome fusion.

*Keywords : fluidophagy; membrane contact site; Fluorescence detection*

タンパク質凝縮の液液相分離（以下、液滴）は細胞内タンパク質の活性制御に関わり、その制御機構の一つとして液滴オートファジー (fluidophagy) が近年注目されている。本発表では、この液滴オートファジーの初期過程として、液滴と隔離膜間の接触を発蛍光型の逆電子需要ディールズアルダー（IEDDA）反応で時空間的に検出する手法を紹介する。本手法では、反応剤ペアであるジエン（テトラジン）とジエノフィルをそれぞれ液滴側のタンパク質と隔離膜上に局在する LC3B へ導入する。オートファジーの誘導により液滴が隔離膜に認識され、両反応剤が近接することで発蛍光型 IEDDA 反応が生じる。また、これら反応剤ペアの構造最適化により、オートファジーが誘導されない場合には反応が進行しないように調整した。さらに本ツールを用いて液滴と隔離膜間での接触からリソソーム融合に至る液滴オートファジー過程のモニタリングを行ったので合わせて報告する。

日本化学会 第105春季年会 (2025)

# 遷移金属触媒反応を介した人工細胞膜ダイナミクスの制御

（科学大院生命[1]・バーゼル大[2]・科学大 ASMat[3]）○濵口 怜[1]・Damian Alexander Graf[2]・Thomas R. Ward[2]・金原 数[1,3]

Control of the dynamics of an artificial cell-membrane via a transition metal-catalyzed reaction

([1]*School of Life Science and Technology, Institute of Science Tokyo*, [2]*Department of Chemistry, University of Basel,* [3]*Research Center for Autonomous Systems Materialogy, Institute of Science Tokyo*) ○Rei Hamaguchi,[1] Damian Alexander Graf,[2] Thomas R. Ward,[2] Kazushi Kinbara[1,3]

Biological membranes contain phase-separated domain structures called rafts, which are abundant in saturated lipids and cholesterol. The rafts are functional domains with dynamic properties, including transient changes in phase-separation states and macroscopic changes in membrane morphology, which are important for maintaining homeostasis in living organisms.[1)] We expected that manipulating such membrane dynamics by bioorthogonal reactions would make it possible to control membrane-mediated biological phenomena artificially. With this goal in mind, we developed a membrane platform that enables a transition metal-catalyzed reaction in the vicinity of the membrane. To construct this platform, we engineered the vesicle surface with an artificial metalloenzyme (ArM) capable of carrying out a bioorthogonal reaction in aqueous media. In addition, by designing a substrate that releases a fatty acid via the catalytic reaction, we found the potential to control the membrane dynamics, such as raft elimination and membrane budding, upon substrate addition (Fig.).

*Keywords : Lipid Bilayer Membrane; Lateral Phase Separation; Artificial Metalloenzyme; Artificial Cell; Molecular Dynamics Simulation*

生体膜は、飽和脂質やコレステロールが豊富なラフトと呼ばれる相分離ドメイン構造を形成している。このラフトは、相分離状態の過渡的変化や、膜の巨視的形態変化といったダイナミクスを有する機能性領域であり、生体の恒常性維持において重要である[1)]。これら膜ダイナミクスを生体直交反応により制御することができれば、膜に媒介された生命現象の人為的操作が可能となる。そこで本研究では、水中で様々な生体直交反応を触媒可能な人工金属酵素に着目し、これらをモデル細胞膜表面に固定化することで、膜近傍における遷移金属触媒反応を実現する膜プラットフォームを構築した。さらに触媒反応により脂肪酸を放出する基質を設計することで、基質の添加に伴うラフトドメインの消失や膜の出芽といった膜ダイナミクスの制御可能性を見出した(Fig.)。

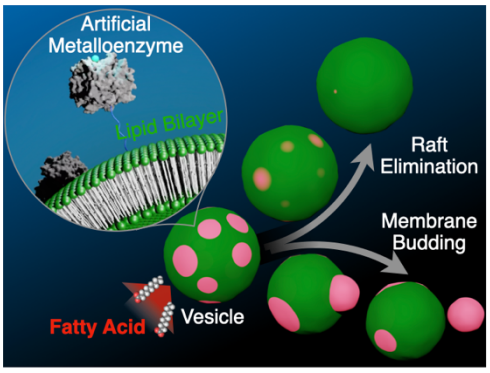


Fig. Membrane dynamics changes in vesicles modified with the ArM.

1) The mystery of membrane organization: composition, regulation and roles of lipid rafts. E. Sezgin, I. Levental, S. Mayor, *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 361–374.