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]A303(第3学舎 1号館 [3階] A303)

[[A]A303-4pm] 17. 生体機能関連化学・バイオテクノロジー

座長：加藤 俊介、森田 能次

日本語
13:00 ~ 13:20
[[A]A303-4pm-01]
非天然金属ポルフィリノイド補因子を大腸菌内で生産する新規生合成経路の構築
○小西 俊輔[1]、加藤 俊介[1]、林 高史[1] (1. 大阪大学)

日本語
13:20 ~ 13:40
[[A]A303-4pm-02]
アセト乳酸合成酵素を用いたα-ブロモエステルのラジカル的アシル化反応
○藤沢 修斗[1]、加藤 俊介[1]、林 高史[1] (1. 大阪大学大学院工学研究科)

英語
13:40 ~ 14:00
[[A]A303-4pm-03]
光増感色素とタンパク質を組み合わせた人工フォトレドックス酵素による光誘起イミン還元反応
○加納 龍成[1]、大洞 光司[1]、林 高史[1] (1. 阪大)

日本語
14:00 ~ 14:20
[[A]A303-4pm-04]
シトクロムP450BM3による有機小分子の酸化を誘起するジカルボン酸含有新規デコイ分子の開発
○石上 恵[1]、有安 真也[1]、笠井 千枝[1]、愛場 雄一郎[1]、杉本 宏[2]、荘司 長三[1] (1. 名古屋大学、2. 理研 SPring-8)

14:20 ~ 14:30
休憩

日本語
14:30 ~ 14:50
[[A]A303-4pm-05]
リモート金属結合部位を最適化した立体選択的マイケル付加反応を触媒する人工金属酵素の創製
○森川 才翔[1]、松本 隆聖[1]、森田 能次[1]、藤枝 伸宇[1] (1. 大阪公大院農)

英語
14:50 ~ 15:10
[[A]A303-4pm-06]
立体選択的ヘテロDiels-Alder反応を指向した人工非ヘム金属酵素の構築
○松本 隆聖[1]、吉岡 紗穂[2]、森田 能次[1]、藤枝 伸宇[1,2] (1. 大阪公大院農、2. 大阪府大院生命)

日本語
15:10 ~ 15:30

[[A]A303-4pm-07]

メタンモノオキシゲナーゼと光化学系II再構成リポソームを用いた高効率光駆動メタン/メタノール変換

○伊藤 栄紘[1]、能戸 湧太[1]、蒲池 利章[1] (1. 東京科学大学)

非天然金属ポルフィリノイド補因子を大腸菌内で生産する新規生合成経路の構築

（阪大院工）○小西 俊輔・加藤 俊介・林 高史

Construction of a New Biosynthetic Pathway in *E. coli* for the Bioproduction of Abiotic Metalloporphyrins (*Graduate School of Engineering, Osaka University*) ○Shunsuke Konishi, Shunsuke Kato, Takashi Hayashi

Biocatalysts have recently attracted increasing attention as a sustainable way for the production of fine chemicals. To expand the repertoires of the biocatalysts, we here developed a new biosynthetic pathway to produce abiotic metallocofactors through the genetic engineering of *E. coli* cells. As shown in Figure 1, a new biosynthetic pathway involving three heterogeneous enzymes (UROD, CPO and CpfC) was designed to prepare abiotic metalloporphyrinoids. Coproporphyrin III was found to be accumulated in *E. coli* cell by heterogeneously expressing two enzymes (UROD and CPO). Furthermore, the formation of the abiotic metallocoproporphyrin III cofactors (M = Co, Mn, Ni, Cu, Zn) were confirmed after adding metal salt into the cell culture. Finally, the construction of artificial metalloenzyme using metallocoproporphyrin III cofactors was succeeded.

Keywords : Biosynthesis; Heme; Metalloporphyrin; Artificial Metalloenzyme

酵素や微生物を用いたバイオプロセスは、持続可能な物質変換技術として近年注目を集めている。本研究では、これらバイオプロセスの有用性を非天然の物質変換反応へと拡張するべく、非天然の酵素補因子を微生物内で生産する新規生合成経路の開発に取り組んだ。具体的には、大腸菌が持つ既存のヘム生合成経路を改変し、鉄以外の金属（Co, Mn, Ni, Cu, Zn 等）が挿入された coproporphyrin III 錯体を生産する新規生合成経路を開発したので報告する。まずはじめに、下図に示すような 2 種類の酵素（UROD, CPO）を大腸菌に異種発現させることで、大腸菌本来のヘム生合成経路から分岐した coproporphyrin III の新規生合成経路を構築した。つづけて、培養終了後に金属塩を外部から添加することで、各種金属が挿入された coproporphyrin III 錯体の生成を確認した。この反応は金属挿入酵素である CpfC の異種発現でさらに効率的に進行することを明らかにした。さらに本発表では、この coproporphyrin III 錯体を活性中心とする人工金属酵素の構築についても報告する。

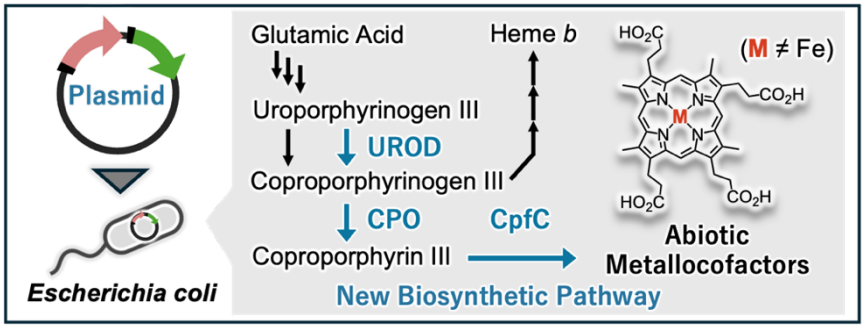


Figure 1. Biosynthetic pathway for the production of abiotic metalloporphyrinoids.

日本化学会 第105春季年会 (2025)

アセト乳酸合成酵素を用いたα-ブロモエステルのラジカル的アシル化反応

（阪大院工）○藤沢 修斗・加藤 俊介・林 高史

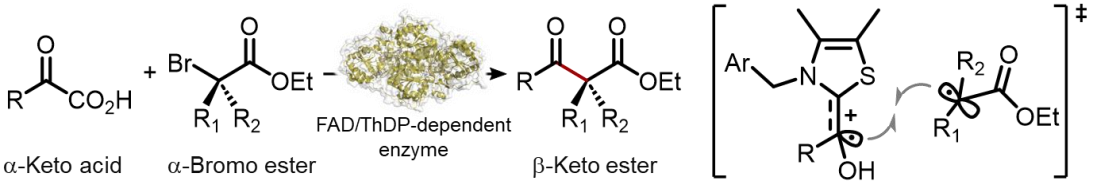
Radical Acylation of α-Bromoesters Catalyzed by Engineered Acetolactate Synthase

(*Graduate School of Engineering, Osaka University*) ○Shuto Fujisawa, Shunsuke Kato, Takashi Hayashi

Biocatalysis has recently emerged as a new sustainable method to produce chemical feedstocks. To expand the catalytic repertoire of enzymes, we have here investigated a biocatalytic radical acylation of α-bromo ester using FAD/ThDP-dependent enzymes. The FAD cofactor in the enzyme active site seems to promote the one-electron oxidation of the Breslow intermediate formed by the reaction of ThDP with α-keto acids, thereby achieving the challenging coupling reaction of acyl radical. Based on this hypothesis, we started to screen various types of FAD/ThDP-dependent enzymes from genome database. As a result, acetolactate synthase from *Thermobispora bispora* (TbALS) was found to catalyze the target acylation of α-bromo ester. Furthermore, a genetic engineering approach based on docking simulation was used to design enzyme variants with enhanced catalytic activity. Moreover, a series of mechanistic studies including EPR measurements supported the radical mechanism for this reaction.

Keywords : Biocatalysis; Acetolactate synthase; Radical reaction; NHC organocatalyst

持続可能社会の実現に向けた新たな物質生産プロセスとして、近年、酵素や微生物等の生体触媒を用いた物質変換に注目が集まっている[1]。本研究では、これら生体触媒の反応適用範囲を、天然の反応のみならず、非天然の化学反応へと拡張するために、FAD/ThDP 依存性酵素を用いたα-ブロモエステルのラジカル的アシル化反応を実施した（下図）。酵素活性部位内で ThDP とα-ケト酸から生成する Breslow 中間体とα-ブロモエステルを、FAD が選択的に一電子酸化・還元することで、二種のラジカル種のカップリング反応が進行することが期待される。この仮説に基づき種々の FAD/ThDP 依存性酵素をスクリーニングした結果、*Thermobispora bispora* 由来のアセト乳酸合成酵素（TbALS）が、本反応において有望な触媒活性を示すことを見出した。さらに、基質と酵素に対するドッキングシミュレーションに基づく遺伝子工学的改変を実施することで、その触媒活性が向上することを明らかにした。また、EPR 測定により、本反応がラジカル的反応機構で進行していることが示唆された。



1) K. Chen, F. Arnold, *Nat. Catal.* **2020,** *3,* 203-213.

Photoinduced Imine Reductions Catalyzed by an Artificial Photoredox Enzyme Containing a Photosensitizer with a Protein Matrix

(*Graduate School of Engineering, Osaka University*) ○Ryusei Kano, Koji Oohora, Takashi Hayashi

Keywords: Photoreduction; Artificial enzyme; Photoredox catalyst; Lipocalin; Fluorescein

Imine reduction is an important reaction for generating useful bioactive amines. Various catalysts have been reported to promote this reaction, with photoredox catalysts emerging as promising candidates for sustainable amine synthesis. Developing this reaction using biomolecules as scaffolds is expected to expand its utility.[1] Previously, a unique cyclic reaction network has been constructed to achieve enantioselective imine reductions using a combination of a photoredox catalyst and a natural enzyme.[2] However, the integration of photoredox catalysts with biomolecules has been limited. In this context, we have recently investigated the incorporation of a xanthene dye into a protein matrix as an artificial photoredox enzyme for the photoreduction of dihydroisoquinoline derivatives (Fig. 1).

In this study, fluorescein or eosin Y was employed as an organic photosensitizer, and lipocalin, a protein previously engineered to bind a fluorescein molecule for living cell imaging, was employed.[3] First, computational optimization of protein sequence yielded a variant with a 7-fold increase in expression yield. Second, mutations around the dye-binding site of the protein matrix were performed to improve the catalytic activities for photoinduced imine reductions. Site-directed mutations of potent residues suggested by alanine scanning were carried out. Fluorescein in the H86D mutant exhibited 3.5-fold higher catalytic activity compared to that from the wild-type protein. Fluorescence titration of the substrate revealed that the high binding affinity of the substrate for the H86D mutant incorporating the xanthene dye contributed to the improvement in the catalytic ability.

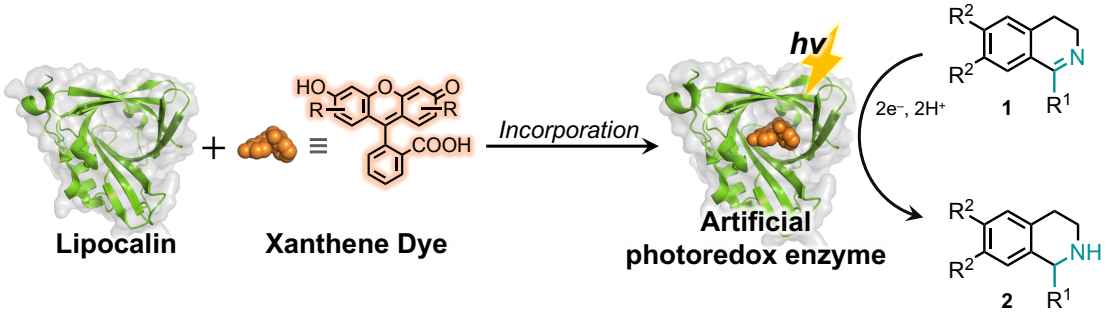


Fig. 1. Photoinduced imine reduction catalyzed by an artificial photoredox enzyme.

1) R. Kano, K. Oohora, T. Hayashi *J. Inorg. Biochem.*, **2024**, *259*, 112657. 2) O. S. Wenger *et al. Chem. Sci.*, **2018**, *9*, 5052. 3) A. Skerra *et al. Proteins*, **2003**, *53*, 121.

シトクロム P450BM3 による有機小分子の酸化を誘起するジカルボン酸含有デコイ分子の開発

（名大院理[1]・理研 SPring-8[2]）○石上 恵[1]・有安 真也[1]・笠井 千枝[1]・愛場 雄一郎[1]・杉本 宏[2]・荘司 長三[1]

Development of dicarboxylic acid-based decoy molecules which induce the oxidation of organic small molecules by cytochrome P450BM3 ([1]*Graduate School of Science, Nagoya University*, [2]*SPring-8, RIKEN*) ○Megumi Ishigami,[1] Shinya Ariyasu,[1] Chie Kasai,[1] Yuichiro Aiba,[1] Hiroshi Sugimoto,[2] Osami Shoji[1]

Cytochrome P450BM3, which catalyzes the hydroxylation of long-chain fatty acids, can catalyze the oxidation of organic small molecules with structures entirely different from the native substrate. While reported decoy molecules are mainly composed of amino acid derivatives, in this study, we focus on developing decoy molecules based on dicarboxylic acids, readily available in diverse structures. Previously, we discovered that decoy molecules (Cn-Pht) synthesized via condensation of alkyl chain amines (carbon number, n) with phthalic acid effectively promoted styrene epoxidation. To elucidate the structural importance of the phthalic acid moiety, we compared the activity with corresponding C10-Pht analogs and analyzed the crystal structure of P450BM3 bound to C12-Pht. The results reveal that Cn-Pht decoys can mimic the binding mode of the native substrate, suggesting that the bent structure of phthalic acid is promising as a decoy molecule.

Keywords : Cytochrome P450BM3;Decoy Molecule; Dicarboxylic Acid; Epoxidation

長鎖脂肪酸水酸化酵素シトクロム P450BM3 に活性化分子「デコイ分子」を添加することで、天然基質とは構造が大きく異なる有機小分子の酸化反応を進行させることが可能になる[1]。既報のデコイ分子はアミノ酸を主体とする骨格に限定されていたが、本研究では多様な構造が容易に入手可能なジカルボン酸を基盤としたデコイ分子の開発を指向した。これまでに直鎖アルキルアミン（炭素数 n）とフタル酸を縮合させた分子（Cn-Pht）がスチレンエポキシ化を効果的に加速することを見出した。フタル酸部分の有効性を検証するため、C10-Pht 類縁体を系統的に評価し、さらに P450BM3 とフタル酸含有デコイ分子の共結晶構造解析を行った。すると、デコイ分子が天然基質の結合様式を非常に良く模倣していることが明らかになり、フタル酸の持つ屈曲構造がデコイ分子として重要であることが示唆された。

Ref. [1] O. Shoji *et al.*, *Angew. Chem. Int. Ed.* **2017**, *56*,10324.

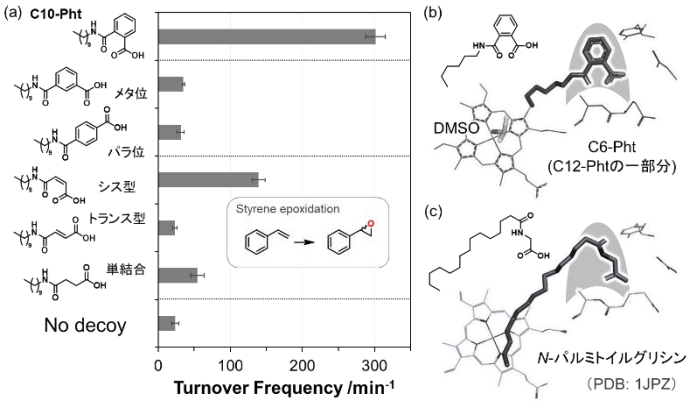


図 1（a）種々のデコイ分子によるスチレンエポキシ化活性値（b）デコイ分子結合型および（c）天然基質結合型 P450BM3

リモート金属結合部位を最適化した立体選択的マイケル付加反応を触媒する人工金属酵素の創製

（阪公大院農 [1]）○森川 才翔 [1]・松本 隆聖 [1]・森田 能次 [1]・藤枝 伸宇 [1]

Development of artificial metalloenzymes catalyzing stereoselective Michael addition reactions with optimized remote metal binding sites ([1] *Graduate School of Agriculture, Osaka Metropolitan Univ.*) ○Saito Morikawa,[1] Ryusei Matsumoto,[1] Yoshitsugu Morita ,[1] Nobutaka Fujieda[1]

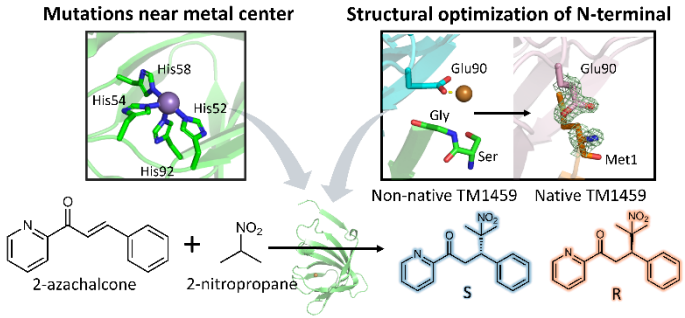
Since chalcones are useful as a building block for pharmaceutical ingredients, a variety of reaction systems are required. One of them is enantioselective Michael addition reaction. In the previous study, 90 % enantiomeric excess of the *S*-enantiomer was achieved in Michael addition reactions using 2-nitropropane and 2-azachalcone as substrates by using inorganic catalysts. In this reaction, however, a large amount of catalyst was required. To reduce the catalyst amount and invert the enantioselectivity, another reaction system is needed.

In this study, we used artificial metalloenzymes, catalysts that combine a protein with a metal ion. As a result, we achieved to improve enantioselectivity in the Michael addition reaction by introducing mutations near the metal center and optimization strategy for the N-terminal region. In this presentation, we will discuss the details of the results and the mechanism.

Keywords : Artificial metalloenzyme; Macromolecular ligand; X-ray crystallography; SUMO

カルコン骨格は医薬品原料のビルディングブロックとして有用であることが知られている。そのため、様々な反応系が必要とされ、そのひとつがエナンチオ選択的マイケル付加反応である。先行研究では無機触媒を用いることで、2-ニトロプロパンと2-アザカルコンを基質としたマイケル付加反応において、*S* 体のエナンチオマー過剰率 90 %を達成している。[1] しかし、多量の触媒が必要であり、*R* 体の選択性が未報告であることから、より効率的な反応系が必要とされている。

本研究では、タンパク質と金属イオンを組み合わせた触媒である人工金属酵素を触媒として用いた。また、金属中心近傍への変異導入と N 末端領域に対する配列最適化を行うことで、マイケル付加反応におけるエナンチオ選択性の向上を達成した。本発表では、その結果の詳細とメカニズムについて議論する予定である。



1) Yirong Zhou, Qiang Liu and Yuefa Gong, *Org. Biomol. Chem.,* **2012**, *10*, 7618–7627
2) Nobutaka Fujieda and co-workers, *Angew. Chem*., **2020**, *132*, 7791–7794

Stereoselective Inverse Electron Demand Hetero-Diels-Alder Reactions Catalyzed by Non-Heme Metalloenzymes

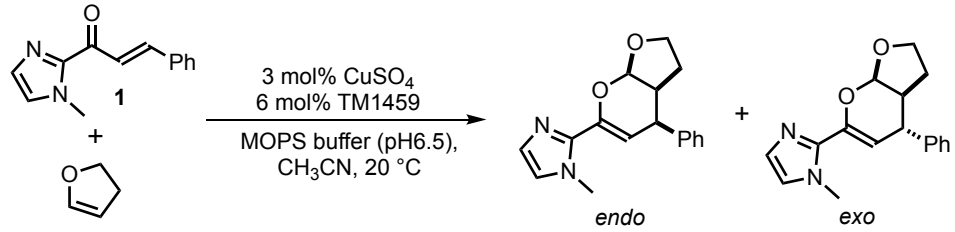
([1]*Graduate School of Agriculture, Osaka Metropolitan University*, [2]*Graduate School of Life and Environmental Sciences, Osaka Prefecture University*) ○Ryusei Matsumoto,[1] Saho Yoshioka,[2] Yoshitsugu Morita,[1] Nobutaka Fujieda[1,2]

Keywords: Artificial Metalloenzymes; Macromolecular Ligands; Stereodivergent reaction

Recently, a variety of approach toward developing artificial metalloenzymes (ArMs) have emerged all over the world. ArMs are defined as highly regio- and/or enantioselective catalysts consisting of a protein matrix and a synthetic metal complex. Therefore, ArMs can harness excellent reactivity derived from the metal complexes as well as enzymatic ability such as exquisite chemical micro-environment to accelerate even difficult and desirable chemical reactions.[1]

Our group developed artificial metalloenzymes by using a cupin-type protein (TM1459) obtained from hyperthermophile, *Thermotoga Maritima*, where well-defined amino acid residues are disposed around the metal center. This metal binding site consists of 4-histidine residues in a same geometry to that of the tris(2-pyridylmethyl)amine (TPA) ligand system. By using this protein as a metal-ligands, we have recently developed the artificial non-heme metalloenzyme with high stereoselectivity by mutating 4-histidine tetrad at the metal binding site for some asymmetric reactions.[2]

In this study, we screened thus obtained mini-library of mutants for the inverse electron-demand hetero Diels-Alder reaction. As a result, Cu-H52A mutant with 3-his triad, showed *endo*-selectivity, but low enantioselectivity and yield as well. Therefore, the pose of substrate docked into cavity by *in silico* simulation suggested that there are some steric repulsion between the substrate and surrounded amino acids. Finally based on this notion, we constructed the Cu-H52G/I108D mutant which showed enhanced selectivity (94 % ee) and yield (92 %). In addition, further substrate scope was investigated and the series of substrate also showed good stereoselectivity.



1) Ward. T. R. *et al.*, *Nat. Rev. Methods Primers*, **2024**, *4*, 79. 2) a) N. Fujieda; S. Itoh, *et al.*, *J. Am. Chem. Soc.*, **2017**, *139*, 5149. b) N. Fujieda; S. Itoh, *et al.*, *Angew. Chem. Int. Ed.*, **2020**, *59*, 7717. c) R. Matsumoto; N. Fujieda, *et al.*, *Chem. Sci.*, **2023**, *14*, 3932. d) N. Fujieda; S. Itoh, *et al.*, *Chem. Euro. J.*, **2024**, e202402803

メタンモノオキシゲナーゼと光化学系 II 再構成リポソームを用いた高効率光駆動メタン/メタノール変換

（東京科学大学[1]）○伊藤 栄紘[1]・能戸 湧太[1]・蒲池 利章[1]
Highly efficient photoinduced methane-to-methanol conversion using liposomes containing methane monooxygenase and photosystem II ([1]*Institute of Science Tokyo*) ○Hidehiro Ito [1], Yuta Noto [1], Toshiaki Kamachi[1]

Methanotrophs have a particulate methane monooxygenase (pMMO) which catalyzes the direct methane to methanol. In our previous study, a photoinduced methane oxidation system (PSII-pMMO membrane system) was constructed by reconstitution of purified photosystem II (PSII) from thermophilic cyanobacteria into the membrane fraction containing pMMO. Light irradiation on this PSII reconstituted membrane, electrons obtained from water oxidation by PSII are transferred to pMMO via the quinone pool, and photoinduced methane oxidation was achieved. However, to improve the system, membrane fraction from bacteria does not appropriate because it contains various lipids and proteins etc, which may decrease the efficiency of the photoinduced methane oxidation catalyzed by pMMO. In this study, we tried to achieve a highly efficient photoinduced methane to methanol conversion using pMMO and PSII reconstituted in liposome (PSII-pMMO liposome system). When 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) liposome containing decylplastoquinone was used, efficiency of the methanol production was higher than that of PSII-pMMO membrane system previously.
Keywords : methane monooxygenase, photosystem II, methane oxidation, light, liposome

メタン資化細菌が持つ膜結合型メタンモノオキシゲナーゼ (pMMO)は温和な条件で選択的にメタンをメタノールに酸化する反応を触媒する。先行研究において、pMMO を含む菌体膜画分にシアノバクテリア由来の光化学系 II (PSII)を再構成した光駆動メタン酸化反応系(PSII-pMMO 膜画分系)を構築した[1)]。この反応系に光照射すると、PSII の触媒作用で、水を分解して得た電子がキノンを介して pMMO へと伝達され、メタン酸化反応が進行する。しかし、PSII-pMMO 膜画分系では菌体膜画分を反応場として利用しているため、夾雑タンパク質によるメタン酸化への影響や脂質組成の改良が困難といった問題があった。

本研究では、界面活性剤で可溶化した pMMO、PSII、電子伝達体のキノンを人工脂質二分子膜リポソームに再構成した反応系(PSII-pMMO リポソーム系, Figure 1)を構築した。脂質分子に 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)とデシルプラストキノンを使用した PSII-pMMO リポソーム系では、光駆動メタン酸化反応におけるメタノール生成量が PSII-pMMO 膜画分系よりも増加した。

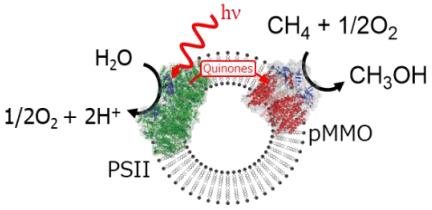


Figure 1 Schematic representation of photoinduced methane-to-methanol conversion using liposomes containing methane monooxygenase and photosystem II.

1) H. Ito; R. Kondo; K. Yoshimori; T. Kamachi, *ChemBioChem* **2018**, *19* (20), 2152–2155.