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 ~ 15:20 [A]A304(第3学舎 1号館 [3階] A304)

[[A]A304-1pm] 17. 生体機能関連化学・バイオテクノロジー

座長：古畑 隆史、山口 哲志

日本語
13:00 ~ 13:20
[[A]A304-1pm-01]
ハイブリッドプローブによる小胞体エンドマンノシダーゼ活性複合体の機能解析
○平 啓人[1]、栗原 大輝[2]、廣瀬 光了[1]、戸谷 希一郎[1] (1. 成蹊大学、2. 大阪国際がんセンター)

英語
13:20 ~ 13:40
[[A]A304-1pm-02]
固相合成法を用いた脂肪酸代謝物17,18-EpETEの構造活性相関研究と機能性類縁体の開発
○秋田 真悠子[1]、齋藤 雄太朗[1]、雑賀 あずさ[2]、佐野 友亮[1]、堀田 将志[2]、森本 淳平[1]、上水 明治[3]、青木 淳賢[3]、長竹 貴広[2]、國澤 純[2]、山東 信介[1] (1. 東大院工、2. 医薬健栄研、3. 東大院薬)

日本語
13:40 ~ 14:00
[[A]A304-1pm-03]
発光寿命測定による細胞内ATP・酸素濃度同時イメージング
○尾台 俊亮[1]、藤原 優也、伊藤 栄紘[1]、蒲池 利章[1] (1. 東京科学大学)

英語
14:00 ~ 14:20
[[A]A304-1pm-04]
Nanoparticle-Delivered Bipedal DNA Walker Ratiometric Fluorescence Sensor for miRNA-21 Detection in Cells
○Wenting Wei[1], Han Lin[2], Kuniharu Ijiro[2], Hideyuki Mitomo[2] (1. Grad. Sch. Life Sci., Hokkaido Univ., 2. RIES, Hokkaido Univ.)

英語
14:20 ~ 14:40
[[A]A304-1pm-05]
AktとErkの予測的光制御による解糖系制御機構の解析
○河村 玄気[1]、小澤 岳昌[1] (1. 東京大学大学院理学系研究科)

英語
14:40 ~ 15:00
[[A]A304-1pm-06]
光制御と数理モデルを用いたAktアイソフォーム活性化の時間特性に基づく選択的な細胞内シグナル伝達制御の定量的解明
○関根 由佳[1]、河村 玄気[1]、日下 暁人[2]、小澤 岳昌[1] (1. 東大院理・化学、2. 東大院理・物理)

英語
15:00 ~ 15:20
[[A]A304-1pm-07]
光応答性細胞付着表面を用いた時系列細胞画像と遺伝子の統合1細胞解析技術の開発

○李 雪陽[1]、水野 滉基[3]、佐藤 桜子[5]、小林 海聖[4]、山平 真也[2]、田中 健二郎[3]、加藤 竜司[3]、吉野 知子[4]、岡本 晃充[1]、山口 哲志[2] (1. 東大院工、2. 阪大産研、3. 名大院創薬、4. 農工大院工、5. 農工大)

日本化学会 第105春季年会 (2025)

ハイブリッドプローブによる小胞体エンドマンノシダーゼ活性複合体の機能解析

（成蹊大理工[1]・大阪国際がんセンター[2]）○平 啓人[1]・栗原 大輝[2]・廣瀬 光了[1]・戸谷 希一郎[1]

Functional analysis of ER endomannosidase-active complex using hybrid probes.

([1]*Department of Science and Technology, Seikei University,* [2]*Osaka International Cancer Institute*) ○Akito Taira,[1] Taiki Kuribara,[2] Mitsuaki Hirose,[1] Kiichiro Totani[1]

We discovered endoplasmic reticulum endomannosidase (ER-EM) activity which removes Glcα1-3Man from $Glc_1Man_9GlcNAc_2$ (G1M9) misfolded glycoproteins for promoting them into the degradation pathway in the ER glycoprotein quality control system. And we found the involvement of carboxylesterase 1d (Ces1d) as a component of ER-EM active complex. As a substrate recognition specificity, ER-EM is a multi-point-recognition enzyme, which recognizes the glycans and the hydrophobic aglycone. Therefore, we developed an ER-EM targeting hybrid probe having a Ces1d inhibitor JW972 and G1M9 glycan. The evaluation of ER-EM active complex using the probe showed that Ces1d is involved in hydrophobic aglycon recognition. However, the catalytic site involved in glycohydrolysis is still unclear.

Accordingly, as a second-generation probe, we had the idea of developing a photocross-linkable hybrid probe that introduced a photocross-linker into the ER-EM target glycan moiety. The photo-crosslinkable probe was examined to be synthesized by a chemoenzymatic synthesis method using UGGT1, which is a glycosyltransferase that converts M9-type glycoprotein to GlM9-type glycoprotein.

This presentation reports on the functional analysis of the ER-EM active complex using first- and second-generation hybrid probes.

Keywords : ER-endomannosidase, Ces1d, Chemoenzymatic synthesis

我々は、小胞体糖タンパク質品質管理機構において $Glc_1Man_9GlcNAc_2$ (G1M9)型不良糖タンパク質から Glcα1-3Man を加水分解し分解経路へと促す小胞体エンドマンノシダーゼ (ER-EM)活性を発見した。また、ER-EM 活性複合体へのカルボキシルエステラーゼ 1d (Ces1d)の関与を見出した。さらに、その基質認識特性として、糖鎖と疎水性アグリコンの二点を認識する多点認識型酵素であることが判明した。

そこで、我々は ER-EM 活性の標的糖鎖である G1M9 型糖鎖と Ces1d 阻害剤 JW972 を構成要素としたハイブリッドプローブを開発した。そして、本プローブによる ER-EM 活性評価の結果、Ces1d は疎水性アグリコンの認識に関与することを見出した。しかし、糖加水分解に関与する触媒サイトは不明である。そこで、第二世代プローブとして、ER-EM 標的糖鎖部位に光架橋剤を導入した光架橋型ハイブリッドプローブの開発に至った。光架橋型プローブは UGGT1 という M9 型糖鎖を G1M9 型糖鎖に変換する糖転移酵素を利用し化学酵素的合成法を利用した合成を検討した。

本発表では、第 1 世代および第 2 世代ハイブリッドプローブを用いた ER-EM 活性複合体の機能解析について報告する。

固相合成法を用いた脂肪酸代謝物 17,18-EpETE の構造活性相関研究と機能性類縁体の開発

（東大院工[1]・医薬健栄研[2]・東大院薬[3]）○秋田真悠子[1]・齋藤雄太朗[1]・雑賀あずさ[2]・佐野友亮[1]・堀田将志[2]・森本淳平[1]・上水明治[3]・青木淳賢[3]・長竹貴広[2]・國澤純[2]・山東信介[1]

Structure-Activity Relationship Study on Fatty Acid Metabolite 17,18-EpETE Using Solid-Phase Synthesis and the Development of a Functional Analog ([1]*Graduate School of Engineering, The University of Tokyo*, [2]*National Institutes of Biomedical Innovation, Health and Nutrition,* [3]*Graduated School of Pharmaceutical Sciences, The University of Tokyo*) ○ Mayuko Akita,[1] Yutaro Saito,[1] Azusa Saika,[2] Yusuke Sano,[1] Masashi Hotta,[2] Jumpei Morimoto,[1] Akiharu Uwamizu,[3] Junken Aoki,[3] Takahiro Nagatake,[2] Jun Kunisawa,[2] Shinsuke Sando[1]

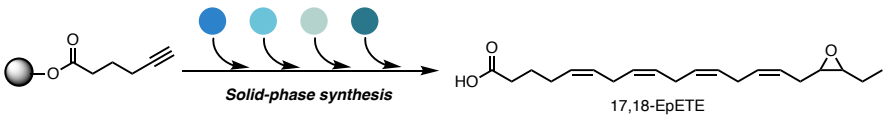
Polyunsaturated fatty acids (PUFAs) and their metabolites are key biomolecules that regulate inflammation and metabolism. While many functional metabolites have been identified, their chemical syntheses usually suffer from complicated, labor-intensive, and time-consuming processes. To solve this, we have developed a solid-phase synthesis for PUFA metabolites.[1] The developed method enables the rapid synthesis of PUFA metabolites by facile procedures.

This presentation focuses on 17,18-EpETE, a metabolite of eicosapentaenoic acid (EPA), and reports its solid-phase synthesis, biological activity evaluation, and the development of functional analog. 17,18-EpETE exhibits anti-inflammatory activity by activating GPR40, a G protein-coupled receptor on the surface of neutrophils.[2] We identified functional analogs by evaluating the GPR40 activation ability and anti-inflammatory activity of analogs obtained by the solid-phase synthesis.

Keywords :　Polyunsaturated Fatty Acid; Solid-Phase Synthesis

多価不飽和脂肪酸とその代謝物は、炎症や代謝を制御する重要な生体分子群である。近年、多くの機能性代謝物が同定されているが、その化学合成は煩雑で多大な時間と労力を必要とする。この問題を解決するため、当研究室では、固相合成法を用いた多価不飽和脂肪酸代謝物の合成法を開発した[1]。本手法は、簡便な操作による多価不飽和脂肪酸代謝物の迅速な合成を可能にした。

本発表では、eicosapentaenoic acid (EPA) の代謝物の一つである 17,18-EpETE に着目し、その固相合成と生物活性評価および機能性類縁体の開発について報告する。17,18-EpETE は、好中球表面の G タンパク質共役受容体である GPR40 に結合し、抗炎症作用を示す[2]。固相合成法を用いて合成した類縁体の GPR40 活性化能や抗炎症作用の評価をもとに、機能性の類縁体を見出した。



1) Y. Saito *et al.*, *ChemRxiv* **2024**, DOI: 10.26434/chemrxiv-2024-p2tcc.
2) T. Nagatake *et al.*, *J. Aller. Clin. Immunol.* **2018**, *142*, 470–484.

発光寿命測定による細胞内 ATP・酸素濃度同時イメージング

（東京科学大学[1]）○尾台 俊亮[1]・藤原 優也[1]・伊藤 栄紘[1]・蒲池 利章[1]
Simultaneous imaging of intracellular ATP and oxygen concentration by FLIM/PLIM measurement ([1]*Institute of Science Tokyo*) ○Shunsuke Odai,[1] Yuya Fujihara,[1] Hidehiro Ito,[1] Toshiaki Kamachi[1]

Measurement of ATP and oxygen dynamics in living cells facilitates the understanding of cellular energy metabolism. Here, we report a simultaneous imaging system detecting ATP and oxygen in living cells. This system visualizes the intracellular ATP and oxygen concentrations using the fluorescence lifetime of ATP sensor protein, QUEEN-37C, and the phosphorescence lifetime of platinum porphyrin, respectively. In this study, simultaneous imaging of intracellular ATP and oxygen concentrations was achieved from FLIM/PLIM measurements of QUEEN-37C-expressing HeLa cells stained with platinum porphyrin. Furthermore, we observed intracellular ATP and oxygen dynamics after addition of 2-deoxy-D-glucose, a glycolytic inhibitor.
Keywords : FLIM/PLIM; ATP dynamics; Oxygen dynamics; QUEEN-37C

生体のエネルギー物質である ATP は、主にミトコンドリアでの酸化的リン酸化により酸素消費を伴って産生される。一方で、がん細胞など一部の細胞は、好気的条件下においても解糖系により ATP を産生している。このように ATP と酸素は細胞内で複雑に関わっており、細胞のエネルギー産生を理解するためには、ATP と酸素の動態を同時に調べることが重要である。

そこで、我々がこれまでに開発したリン光寿命イメージング顕微鏡(PLIM)による細胞内酸素濃度イメージングシステム[1]と、岡田らが開発した ATP センサー蛍光タンパク質 QUEEN-37C[2] を組み合わせることで、酸素と ATP を同時にイメージング可能なシステムを構想した。QUEEN-37C は ATP 非結合と結合状態で構造が変化し、発色団の環境も影響を受けることで最適励起波長や蛍光寿命が変化する。そのため、蛍光寿命イメージング顕微鏡(FLIM)を用いることで、QUEEN-37C による細胞内 ATP 濃度イメージングが可能となる。本研究では、リン光性白金ポルフィリン PtTCPP で染色した QUEEN-37C 発現 HeLa 細胞の FLIM/PLIM 同時測定から、細胞内 ATP・酸素濃度同時イメージングを達成した。さらに、解糖系阻害剤 2-deoxy-D-glucose 添加後の細胞内 ATP・酸素動態を観察した。

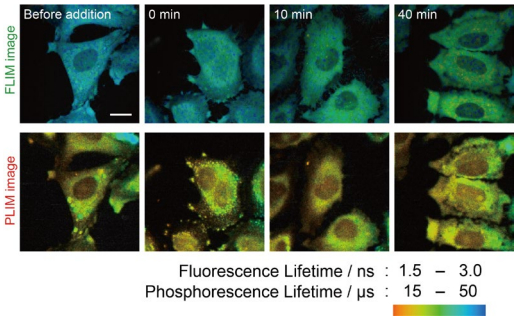


Figure FLIM and PLIM images of QUEEN-37C expressed HeLa cells stained with Pt-porphyrin, PtTCPP, before and after addition of 2-deoxy-D-glucose.

(1) Kurokawa, H.; Ito, H.; Inoue, M.; Tabata, K.; Sato, Y.; Yamagata, K.; Kizaka-Kondoh, S.; Kadonosono, T.; Yano, S.; Inoue, M.; Kamachi, T., *Sci. Rep.* **2015**, *5*, 10657.
(2) Yaginuma, H.; Okada, Y., bioRxiv October 9, **2021**, p 2021.10.08.463131.

Nanoparticle-Delivered Bipedal DNA Walker Ratiometric Fluorescence Sensor for miRNA-21 Detection in Cells

([1]*Graduate School of Life Science, Hokkaido University*, [2]*Research Institute for Electronic Science, Hokkaido University*) ○Wenting Wei,[1] Han Lin,[2] Kuniharu Ijiro,[2] Hideyuki Mitomo[2]

Keywords: Cancer Cell Analyses; miRNA-21; Bipedal DNA Walker Sensor; Ratiometric Detection; Fluorescence Imaging

The DNA walker signal amplification mechanism is a powerful tool for detecting low-abundance microRNA. However, variations in biological environments can influence the amplification efficiency of DNA walkers, leading to inconsistencies and making it challenging to compare results obtained under different conditions. To address this issue, we developed a dual-foot DNA walker sensor based on an inverted hairpin structure. The sensor features identical tracks and cutting mechanisms for both feet, which release fluorescence quenched by AuNPs through cleavage. One generates an amplified internal standard signal triggered by an excess assistant strand (Cy5 signal), and the other produces the detection signal in response to the target molecule (FAM signal) (Fig.1A). By replacing the absolute fluorescence intensity of FAM with the relative fluorescence intensity ratio of FAM to Cy5 (FAM/Cy5), this approach effectively corrects signal fluctuations across different samples and experimental conditions, ensuring calibrated and reliable results for miRNA detection. *In vitro* experiments confirm that the FAM/Cy5 ratio exhibits a strong linear relationship and high stability with the logarithm of miRNA-21 concentration (Fig.1B). Further, ratio-based fluorescence imaging has revealed, with high contrast, the distribution and differential expression levels of miRNA-21 in various cancer cell types (Fig.1C). These results demonstrate that the proposed sensor provides an excellent platform for miRNA detection and underscore its promising potential in cancer diagnostics.

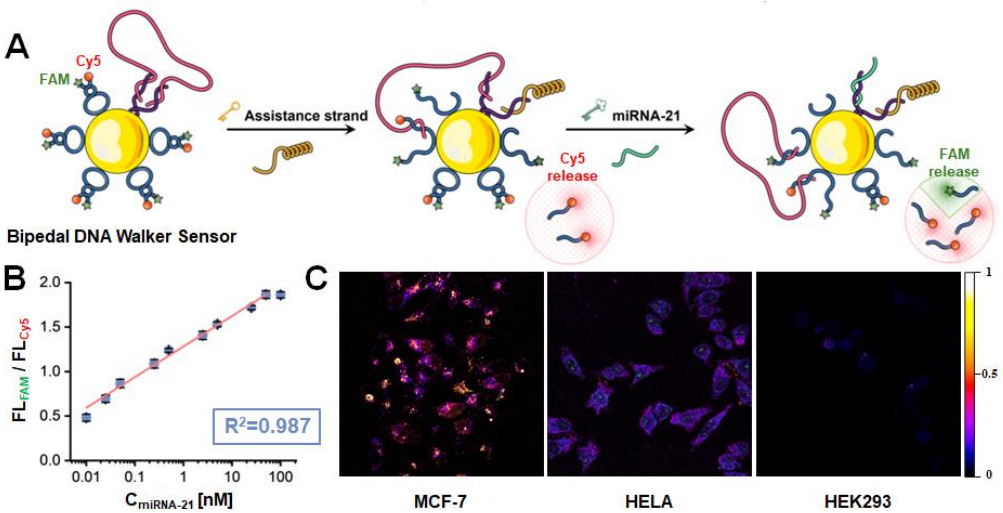


Fig. 1 (A) Principle of the sensor to detect miRNA-21. (B) The linear relationship between FL_{FAM}/FL_{Cy5} and different $\log C_{miRNA-21}$. (C) Ratiometric fluorescence imaging of miRNA-21 in various cells.

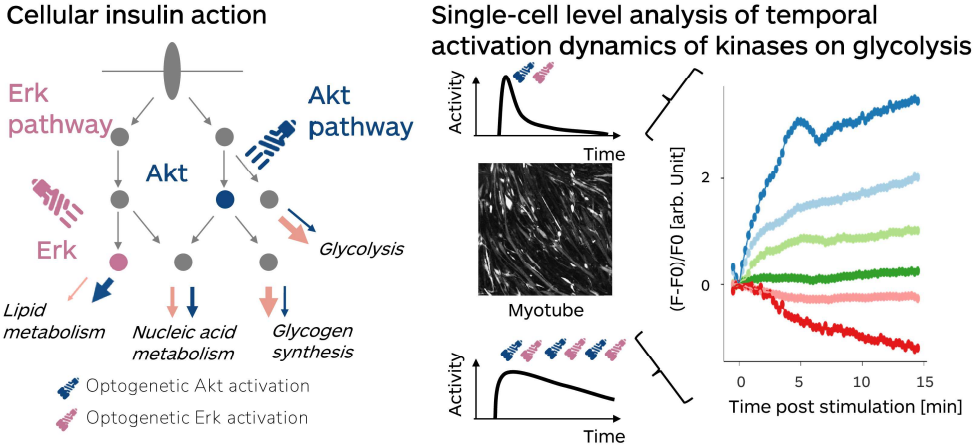
Analysis of glycolysis regulation through predictive optogenetic control of Akt and Erk pathways

([1]*1. Graduate School of Science, The University of Tokyo*)
○Genki Kawamura,[1] Takeaki Ozawa[1]

Keywords: optogenetics, mathematical model, glycolysis, temporal dynamics, kinase

Postprandial metabolism is tightly regulated through the action of hormone insulin and its downstream signaling pathways. It has been suggested that different temporal insulin secretion patterns modulate the activation dynamics of various kinases such as Akt and Erk, controlling appropriate metabolic responses depending on the cellular nutrient availability. While our previous research in C2C12 myotube demonstrated that metabolic signaling network induced by the Akt isoform, Akt2,[1)] partially regulates glycolysis, the precise interplay between temporal kinase activation and glycolytic control remains elusive.

To address this, we developed an optogenetic system to precisely control the activities of kinases Akt and Erk using a simulation model that can predict the light-induced kinase activation thereby enabling induction of kinase activation dynamics. Since an optogenetic system for Akt was previously established, we newly developed an orthogonal optogenetic system for Erk and demonstrated that two kinases can be independently controlled using blue and red-light illumination. We then constructed an ordinary differentiation equation-based simulation model by estimating kinetic parameters in the equations based on the experimentally obtained Erk activation pattern with various light illumination patterns. Furthermore, we employed intracellular fluorescent lactate sensor[2)] to monitor the single-cell level regulation of glycolysis. By integrating these experimental and computational approaches, we aim to elucidate how distinct temporal activation patterns of Akt and Erk contribute to the regulation of glycolysis in response to physiological cues.



References: 1) Kawamura, et al., *Sci. Signal.* **16**, eabn0782 (2023)., 2) Nasu et al., *Nat. Commun.* **14**, 6598., (2023).

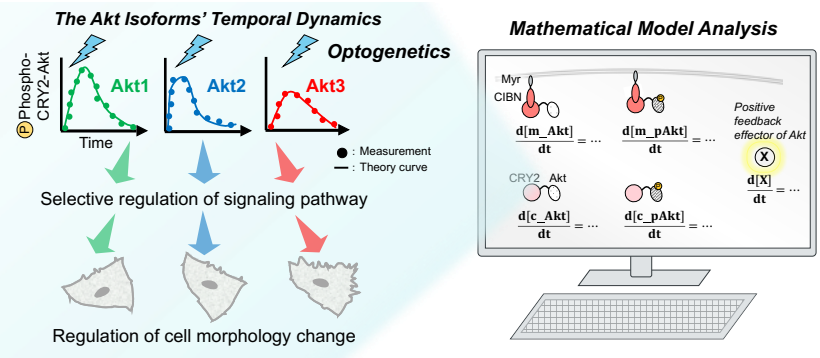
Quantitative Elucidation of Intracellular Signaling Mediated by Isoform-Specific Temporal Dynamics of Akt Using Optogenetics and Mathematical Model

([1]*Department of Chemistry, School of Science, The University of Tokyo,* [2]*Department of Physics, School of Science, The University of Tokyo*) ○Yuka Sekine,[1] Genki Kawamura,[1] Akito Kusaka[2], Takeaki Ozawa[1]

Keywords: Optogenetics, Mathematical Model, Akt Isoform, Temporal Dynamics, Cell Morphology Change

A Ser/Thr kinase Akt plays central roles in cellular signal transduction. Akt exists as three isoforms, Akt1, Akt2, and Akt3. Among several factors that generate the Akt isoforms' specificity to signaling pathway regulation, their temporal activation patterns have drawn attention. In this study, we aim to quantitatively reveal the isoforms' temporal dynamics and their selective regulation of signal transduction and subsequent cellular responses. Because three Akt isoforms have different functions on various diseases, understanding their temporal dynamics will broaden possibilities of new therapeutic methods for these diseases.

To investigate each isoform, we applied a principle of an optogenetics approach named PA-Akt.[1] By using PA-Akt1/2/3, we found that each Akt isoform has its specific temporal properties. Also, we utilized a mathematical model that enables analysis of the measured isoforms' dynamics through the perspective of molecular kinetics associated with Akt activation states. Moreover, we aim to reveal roles of the Akt isoforms' dynamics on cell morphology change. We developed a quantitative single cell level evaluation pipeline for cell morphology and found that photo-activation of Akt3 induced the largest morphology change. Therefore, we set up a hypothesis of the existence of an unknown signaling mechanism selectively regulated by the Akt isoforms. We are testing this hypothesis with an approach that includes an examination of its relationship with the isoforms' temporal dynamics and an analysis of the morphological features of myoblast cells.



1) Katsura Y et al. *Sci. Rep.* **2015**, 5, 14589.

Integrated technology of time-series imaging and single-cell gene analysis on photoresponsive gel layers

(*[1]Grad. Sch. Eng., The Univ. of Tokyo, [2]Grad. Sch. Pharm. Sci., Nagoya Univ., [3]Tokyo Univ. Agric. Technol, [4]Grad. Sch. Eng., Tokyo Univ. Agric. Technol, [5]SANKEN, Osaka Univ.*)
○Xueyang Li[1], Koki Mizuno[2], Sakurako Sato[3], Kaisei Kobayashi[4], Shinya Yamahira[5], Kenjiro Tanaka[2], Ryuji Kato[2], Tomoko Yoshino[4], Akimitsu Okamoto[1], Satoshi Yamaguchi[5]
Keywords: Hydrogel, Image analysis, Single cell analysis

Recent advances in single-cell analysis have revealed that the heterogeneity and functional diversity among individual cells are critical in diseases and therapy. Therefore, single-cell imaging technology for comprehensively visualizing various cell behaviors is being actively developed as a tool to identify the cells of interest among a huge number of cells. However, conventional methods based on MEMS devices[1] and droplets[2] do not allow for phenotypic analysis of adherent cells because they cannot take original expanded form in these devices and droplets[1,2]. In this study, we developed a technology that enables integrated time-series cellular imaging of adherent cells combined with single-cell level gene analysis.

A dual-responsive surface consisting of a thin photo-degradable gel layer modified with a photoactivatable PEG-lipid[3] as a photoactivatable cell trapping reagent was used in this research. Cells were incubated on the surface, and the phenotypes of individual cells were exhaustively monitored using fluorescence microscopy. After identifying the target cells based on their phenotypes, light spots with a single-cell size were irradiated to the targeted cells to induce gel degradation, leading to selective single-cell release (Figure 1). The released cells were collected individually using a cell picker for a further single-cell gene expression analysis. We successfully confirmed the expression of genes associated with the observed phenotype using the present image-based single-cell photo-recovery system.

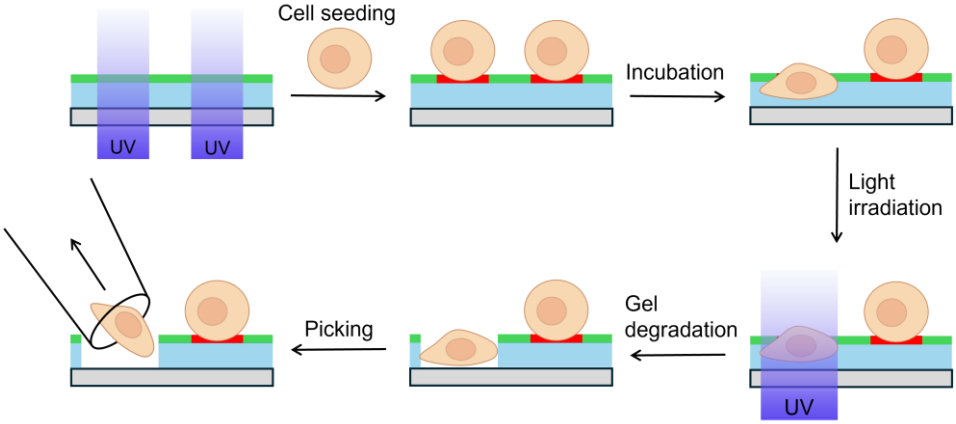


Figure 1. Illustration of the substrate surface enabling both light-induced cell patterning and release

1) Dura *et al.*, *Proc. Natl. Acad. Sci. USA* 2016, **113**, E3599; 2) Madrigal *et al.*, *Proc. Natl. Acad. Sci. USA* 2022, **119**, e2110867119; 3) Yamahira *et al.*, *J. Am. Chem. Soc.* 2022, **144**, 13154.