Academic Program [Oral B] | 17. Biofunctional Chemistry, Biotechnology：Oral B

Wed. Mar 18, 2026 1:00 PM - 3:40 PM JST | Wed. Mar 18, 2026 4:00 AM - 6:40 AM UTC | H937 (937, Bldg. 9 [3F])

[H937-2pm] Oral B

Chair: Mina Okochi, Hiroyuki Shinchi

English

1:00 PM - 1:20 PM JST | 4:00 AM - 4:20 AM UTC

[H937-2pm-01] Surface Modification of an Algal Cell with Elongated DNA strands for Coating with Functional Materials to Expand Cellular Function

○YINGQI MU[1], Yusuke Yonamine[2] (1. Hokkaido university, 2. Kyushu University)

Japanese

1:20 PM - 1:40 PM JST | 4:20 AM - 4:40 AM UTC

[H937-2pm-02] Development of fluorescent pyruvate biosensors toward metabolism imaging

○Shosei Imai[1], Saaya Hario[1], Kazuhiro Sawada[1], Cong Vu[2], Takuya Terai[1], Kei Takahashi-Yamashiro[1], Satoshi Arai[2], Osamu Nureki[1], Robert E. Campbell[1] (1. The University of Tokyo, 2. Kanazawa University)

English

1:40 PM - 2:00 PM JST | 4:40 AM - 5:00 AM UTC

[H937-2pm-03] Single-cell Raman spectroscopic analysis of bacteroid in soybean nodules and evaluation of the symbiotic nitrogen fixation activity

○Shunnosuke Suwa[1], Masahiro Ando[2], Takuma Kyotani[1], Kento Hasegawa[1], Safiullah Habibi[3], Masako Kifushi[1], Yohei Nishikawa[2,4], Toyoaki Anai[6], Naoko Ohtsu[5], Haruko Takeyama[1,2,7] (1. Grad. Sch. Adv. Sci. Eng., Waseda Univ., 2. Res. Org. Nano Life Innov., Waseda Univ.Res. Org. Nano Life Innov., Waseda Univ., 3. Fac. Agric. Tokyo Univ. Agri. Tech., 4. Biomanufacturing Proc. Res. Center, AIST., 5. Inst. Agric. Tokyo Univ. Agri. Tech., 6. Fac. Agric., Kyushu Univ., 7. Inst. Adv. Res. Biosyst. Dyn., Waseda Res. Inst. Sci. Eng., Waseda Univ.)

English

2:00 PM - 2:20 PM JST | 5:00 AM - 5:20 AM UTC

[H937-2pm-04] Major Metabolic Alterations of a Bacterium in Gaseous Environment

○Shori Inoue[1], Taisei Naobayashi[2], Kanako Tokiyoshi[2], Shogo Yoshimoto[1], Maiko Hattori[1], Teppei Niide[3], Hiroshi Shimizu[3], Yoshihiro Toya[3], Hiroshi Tsugawa[2], Katsutoshi Hori[1] (1. Nagoya University, 2. Tokyo University of Agriculture and Technology, 3. The University of Osaka)

2:20 PM - 2:40 PM JST | 5:20 AM - 5:40 AM UTC

Break

English

2:40 PM - 3:00 PM JST | 5:40 AM - 6:00 AM UTC

[H937-2pm-05] Development of a lectin-pull down method using sugar chain-functionalized gold nanoparticles and magnetic beads

○Koki Murata[1], Kentaro Kato[2], Yuri Kurogi[1], Masahiro Wakao[1], Yasuo Suda[1], Hiroyuki Shinchi[1] (1. Grad. Sch. Sci. and Eng., Kagoshima Univ., 2. NEKKEN, Nagasaki Univ.)

English

3:00 PM - 3:20 PM JST | 6:00 AM - 6:20 AM UTC

[H937-2pm-06] Effects of ionization conditions on the ion mobility of glycans

○Hao Feng[1], Takumi Yamaguchi[1,2,3] (1. JAIST, 2. Nagoya City Univ, 3. ExCELLS)

English

3:20 PM - 3:40 PM JST | 6:20 AM - 6:40 AM UTC

[H937-2pm-07] a microwell-based system for analyzing cytotoxicity and cell-sorting at the single-cell level

○Ryotaro Yamamoto[1], Shinya Yamahira[2], Shouichi Sakakihara[2], Akimitsu Okamoto[1], Satoshi Yamaguchi[2]
(1. The Univ. of Tokyo, 2. The Univ. of Osaka)

Engineering Algal Cell Surfaces with Long DNA Networks for Metal Nanoparticle Immobilization and Photofunction Expansion

(*[1]Graduate School of Life Science, Hokkaido University, [2]Graduate School of Engineering, Kyushu University. [3]Research Institute for Electronic Science, Hokkaido University*)
○Yingqi Mu[1], Yusuke Yonamine[2], Hideyuki Mitomo[3], Kuniharu Ijiro[3]

Keywords: Cell Engineering, Polymeric DNA Network, Algae Cell, Metal nanoparticles

[Introduction] Cell engineering has been utilized to alter cellular functions. However, it is still challenging to significantly enhance them by gene manipulation. Conversely, modifying natural cells with artificial materials can strikingly expand cellular functions. DNA is a suitable polymer for cell-surface engineering owing to its biocompatibility, enzymatic extendibility, and negatively charged backbone, which provides an effective scaffold for immobilizing cationic materials. In this study, the unicellular green algae *Chlamydomonas reinhardtii* (CR) was used as a model photosynthetic organism. Using DNA modified on the CR surface as a scaffold, surface modification with cationic Quantum dots (QDs) to expand the light-harvesting capability of CR cells from the visible to the ultraviolet (UV) region without disrupting normal photosynthesis, while Gold nanorods (Au NRs) is expected to generate ROS under NIR irradiation.

[Results and Discussion] A DNA primer (X-motif) was conjugated with an oligopeptide of 4-hydroxyproline (HYP_{10}), which binds strongly to the CR cell wall,[1] enabling stable immobilization on the CR cell surface. The surface-anchored DNA primers were subsequently elongated via a slippage amplification mechanism using DNA polymerase (Klenow fragment exo(–); KF^-) and dNTPs,[2] forming a thick DNA layer on the cell surface (Figure 1). It was confirmed that Au NRs and QDs modified with cationic ligands were successfully immobilized onto the DNA layer based on fluorescence images and microscopic observations (Figure 1). In addition, QD-modified CR cells exhibited photosynthetic activity under UV light by converting it into visible light, indicating an expansion of their light-harvesting capability. Taken together, this DNA-coating-based surface engineering strategy provides a versatile platform for immobilizing functional materials and expanding cellular photofunctionality.

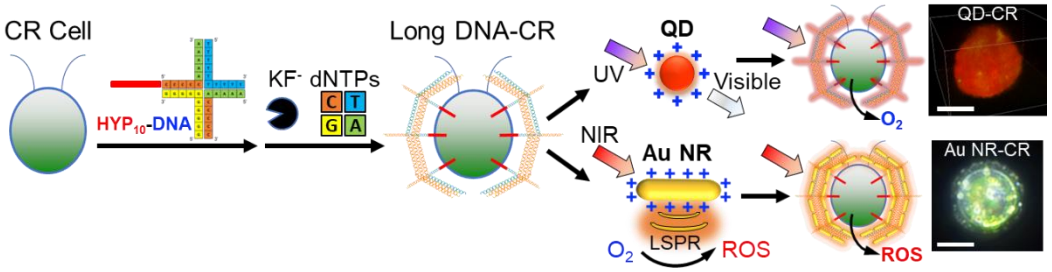


Figure 1. Immobilization of DNA primers (X-motif) on the CR cell surface via a cell wall-binding HYP_{10} peptide, forming a DNA layer by polymerase-mediated elongation (KF^-), followed by coating with cationic Au NRs and QDs. Scale bar: 5 μm.

1) D. B Weibel *et al., PNAS,* **2005,** *102,* 11963. **2)** A. B. Kotlyar *et al., Nucleic Acids Res.,* **2005,** *33,* 525.

蛍光ピルビン酸センサーの開発による代謝経路のイメージング

（東大院理[1]・金沢大ナノ生命科学研究所[2]）○今井 渉世[1]・針尾 紗彩[1]・澤田 和宏[1]・Cong Vu[2]・寺井 琢也[1]・高橋-山城 恵生[1]・新井 敏[2]・濡木 理[1]・Robert E. Campbell[1]
Genetically encoded fluorescent pyruvate biosensors for metabolism imaging ([1]*Graduate School of Science, The University of Tokyo*, [2]*NanoLSI, Kanazawa University*)○Shosei Imai,[1] Saaya Hario,[1] Kazuhiro Sawada,[1] Cong Vu,[1] Takuya Terai,[1] Kei Takahashi-Yamashiro,[1] Satoshi Arai,[1] Osamu Nureki,[1] Robert E. Campbell[1]

Pyruvate links glycolysis to the TCA cycle, playing central metabolic roles. However, a lack of high-performance pyruvate biosensors has limited our ability to explore physiological roles of pyruvate. Here, we present GreenPy1 and ApplePy1 series, green and red pyruvate biosensors, respectively[1]. They comprise a fluorescent protein fused to a pyruvate binding protein (Fig. A), and their performance was optimized through directed evolution and rational mutagenesis. As a result, we obtained four green and four red variants with different affinities. These biosensors exhibit large fluorescence intensity changes and wide affinity ranges (10s of μM to several mM, Fig. B). Their high performance was confirmed in HeLa cells, where they detected pyruvate concentration changes both in the cytosol and mitochondria. Notably, these biosensors also enable quantitative imaging. GreenPy1 and ApplePy1 can be used for ratiometric and lifetime imaging, respectively, to eliminate the effects of fluorophore concentration and enable quantitative analysis. Finally, we determined cryo-EM structures of the pyruvate biosensor to get insight into the molecular mechanism. This study provides valuable tools to investigate the physiological role of pyruvate for metabolic regulation.

Keywords : Fluorescent biosensor; Pyruvate; Fluorescent lifetime; cryoEM

ピルビン酸は解糖系と TCA 回路をつなぐ代謝の中核分子であるが、高性能な蛍光バイオセンサーが報告されておらず、その生理機能の理解は限定的であった。そこで本研究では、緑色および赤色ピルビン酸センサーGreenPy1 および ApplePy1 シリーズを開発した[1]。センサーはピルビン酸結合タンパク質に蛍光タンパク質を挿入することで作製し(Fig. A)、指向性進化法を用いることで性能の改善を試みた。その結果、大きな蛍光強度変化を実現し、また親和性の異なる 4 種類の変異体をそれぞれで作製することに成功した(数十 μM から数 mM に及ぶ K_d、Fig. B)。GreenPy1 および ApplePy1 はそれぞれレシオおよび蛍光寿命イメージングにより定量解析が可能である。加えて、cryo-EM 構造解析により応答機構を明らかにした。本研究は、代謝制御におけるピルビン酸の生理的役割の解明に有用なツールを提供する。

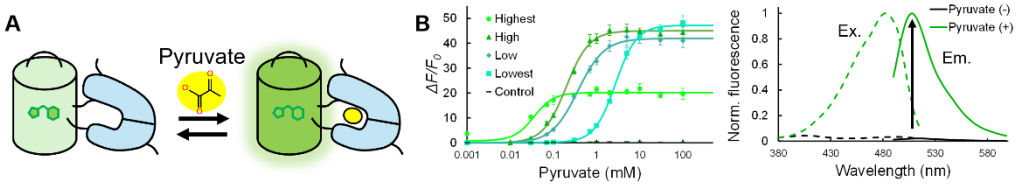


Fig. A) Design of pyruvate biosensors. B) The titration and fluorescence spectra of GreenPy1.

[1] Imai et al., *bioRxiv*, 2025.04.17.649293.

Raman spectroscopy-based evaluation of symbiotic nitrogen fixation activity in soybean nodules

([1]*Grad. Sch. Adv. Sci. Eng., Waseda Univ.*, [2]*Res. Org. Nano Life Innov., Waseda Univ.*, [3]*Fac. Agric. Tokyo Univ. Agri. Tech.*, [4]*Biomanufacturing Proc. Res. Center, AIST.*, [5]*Inst. Agric. Tokyo Univ. Agri. Tech.*, [6]*Fac. Agric., Kyushu Univ.*, [7]*Inst. Adv. Res. Biosyst. Dyn., Waseda Res. Inst. Sci. Eng., Waseda Univ.*) ○Shunnosuke Suwa[1], Masahiro Ando[2], Takuma Kyotani[1], Kento Hasegawa[1], Safiullah Habibi[3], Masako Kifushi[1], Yohei Nishikawa[2,4], Toyoaki Anai[6], Naoko Ohtsu[5], Haruko Takeyama[1,2,7]

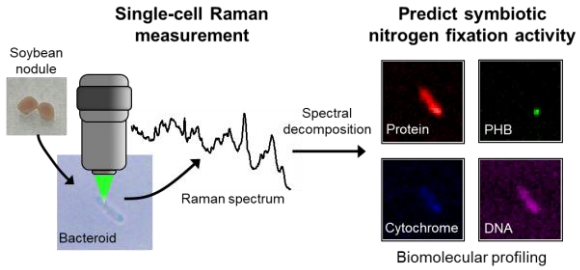
Keywords: *Spectroscopy, Rhizobia, Spectral Deconvolution, Plant, Machine Learning*

Rhizobia inhabit in Soybean nodules as bacteroids, where they convert atmospheric nitrogen into ammonia via symbiotic nitrogen fixation—a critical process for plant growth and health. Despite extensive research aimed at enhancing this symbiosis, the specific biomolecular contributors, particularly metabolites, remain poorly understood. This study aims to identify key biomolecules involved in symbiosis using Raman spectroscopy as a label-free and comprehensive molecular detection strategy[1].

Soybean plants were cultivated, and nitrogen fixation activity of their nodules was quantified across several growth stages using an acetylene reduction assay. Subsequently, single-cell Raman spectroscopy was employed to characterize the biomolecules within individual bacteroid. Spectral deconvolution via Multivariate Curve Resolution-Alternating Least Squares[2] (MCR-ALS) and random forest regression were utilized to establish biomolecular profiles and correlate them with recorded nitrogen fixation levels.

As a result, the model predicted the nitrogen fixation activity with high accuracy ($R^2 > 0.71$) and found that the activity was different depending on the soybeans growth stages and nodules sizes, in consistent with the reported study[3]. The Raman spectroscopic study and MCR-ALS successfully illuminated the biomolecules such as proteins, lipid nucleic acid and even polyhydroxybutyrate (PHB), a biopolymer. And the subsequent spectral analysis.

This research was partially supported by the Cabinet Office, Moonshot Research and Development Program on Agriculture, Forestry and Fisheries (JPJ009237, funding agency: Bio-oriented Technology Research Advancement Institution).



1) Suwa, et al, *Plant Biotechnology*, 42(3), 335-343, 2025
2) Ando & Hamaguchi, *Journal of Biomedical Optics*, 19(1), 011016, 2014

気相環境の細菌細胞における代謝ネットワークの再編成

(名大院工[1]・農工大院工[2]・阪大院情報[3]) ○井上翔理[1]・直林大生[2]・時吉花菜子[2]・服部舞子[1]・吉本将悟[1]・二井手哲平[3]・清水浩[3]・戸谷吉博[3]・津川裕司[2]・堀克敏[1]
Major Metabolic Alterations of a Bacterium in Gaseous Environment ([1]*Graduate School of Engineering, Nagoya University*, [2]*Tokyo University of Agriculture and Technology*, [3]*Graduate School of Information Science and Technology, University of Osaka*) ○Shori Inoue[1], Taisei Naobayashi[2], Kanako Tokiyoshi[2], Shogo Yoshimoto[1], Maiko Hattori[1], Teppei Niide[3], Hiroshi Shimizu[3], Yoshihiro Toya[3], Hiroshi Tsugawa[2], Katsutoshi Hori[1]

Gas-phase bioprocesses that immobilize microbial cells on solid carriers enable the efficient conversion of poorly water-soluble gaseous substrates, thereby offering significant potential to advance bioremediation and bioproduction[1)]. However, although the microorganisms in gas-phase are exposed to various environmental stresses, the metabolic adjustment that sustains bacterial cell activity under these water-limited conditions remains poorly understood. In this study, we elucidated the comprehensive metabolic alterations in a highly adhesive bacterium *Acinetobacter* sp. Tol 5 degrading toluene in aqueous and gaseous environments. Integrated omics analysis revealed that intracellular metabolism was significantly reorganized to adapt to water-limited conditions, specifically characterized by enhanced nitrogen source recycling and the utilization of storage lipids. These findings provide insights into the strategies of bacteria adapting to gaseous environments, offering fundamental information for the rational design of robust gas-phase bioprocesses and a deeper understanding of environmental microbiology.
Keywords : Bacteria, Bioproduction, Omics analysis, Metabolism, Toluene

微生物を触媒とするバイオプロダクションは、環境負荷の低い高付加価値化合物の合成法として注目されている。中でも、高付着性細菌 *Acinetobacter* sp. Tol 5 を担体に固定化し、基質を水に溶解させることなく気相中の分子拡散を利用して供給する気相反応プロセスは、難水溶性基質の効率的な利用法として応用が進められている[1)]。一方で、気相環境下の微生物は、高酸素濃度や乾燥による極端なストレスに曝されるものの、そのような環境下で細胞機能を維持するための代謝調節機構については、十分に解明されていない。本研究では、液相および気相環境下でトルエンを分解する Tol 5 細胞を対象に、メタボロームとトランスクリプトームの統合解析により、代謝変化を網羅的に調査した。その結果、 気相環境下では窒素源の再利用および貯蔵脂質の代謝が変化していることが明らかとなった。これらの知見は、気相環境に適応する細菌の生存戦略に関する洞察を与え、気相バイオプロセスの合理的設計に向けた基盤情報を提供するものである。

1) Gas-phase bioproduction of a high-value-added monoterpenoid (*E*)-geranic acid by metabolically engineered *Acinetobacter* sp. Tol 5. A. Usami, M. Ishikawa, K. Hori, *Green Chem.*, **2020**, 22, 1258-1268.

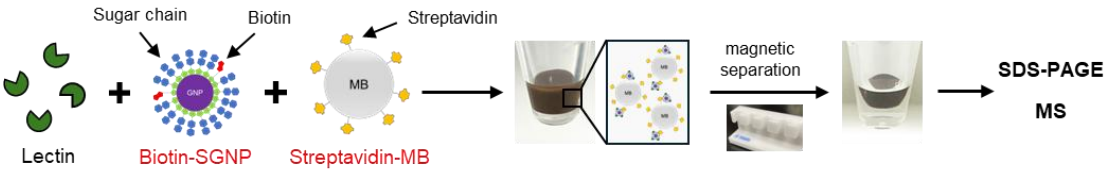
Development of a lectin-pull down method using sugar chain-functionalized gold nanoparticles and magnetic beads

([1]*Graduate School of Engineering, Kagoshima University*, [2]*NEKKEN, Nagasaki University*)
○Koki Murata,[1] Kentaro Kato,[2] Yuri Kurogi,[1] Masahiro Wakao,[1] Yasuo Suda,[1] Hiroyuki Shinchi[1]

Keywords: Sugar chain; Lectin; Gold nanoparticles; Pull-down method

Sugar chains play crucial roles in diverse biological processes, including intercellular recognition, immune response, and pathogen infection. These processes are initiated by the specific recognition of sugar chain structures by sugar chain-binding proteins, known as lectins. Therefore, analysis of sugar chain–lectin interactions is essential for understanding physiological functions and pathological conditions. In our previous studies, we developed sugar chain-functionalized nanoparticles (SNPs) as analytical tools for investigating sugar chain–lectin interactions [1,2]. SNPs aggregate in the presence of specific multivalent lectins, enabling simple visual detection of these interactions. However, analysis of lectins bearing a single sugar chain binding site (monovalent lectins) remains challenging because they do not induce aggregation. To overcome this limitation, we established a lectin pull-down assay that combines sugar chain-functionalized gold nanoparticles (SGNPs) with magnetic beads (MB), allowing interaction analysis independent of lectin valency.

In the lectin pull-down method, lectins bound to biotin-conjugated SGNPs (Biotin-SGNPs) are isolated using streptavidin-conjugated MB (Streptavidin-MB) and analyzed by SDS-PAGE. Biotin-SGNPs immobilized with four different sugar chains (β-galactoside, α-mannoside, sialylgalactoside, or dextran) and three different Biotin-PEGn-TA (n = 3, 11, or 35) were prepared. We found that the spacer length of biotin is a critical determinant for magnetic separation and must be optimized according to the length of immobilized sugar chains. Using four lectins (RCA120, Con A, WGA, and CEL-III), we confirmed that each lectin was selectively pulled down using Biotin(PEG11)-SGNPs. Notably, this method enabled the pull-down of the monovalent lectin CEL-III. Furthermore, Biotin-SGNPs successfully captured CEL-III spiked into serum samples. Taken together, the lectin pull-down assay provides a valuable approach for investigating lectin–sugar chain interactions and offers potential for lectin isolation from biological samples.



[1]Nakamura-Tsuruta S. *et al*., *J. Biochem*. **2008**, *143*, 833-839.
[2] Shinchi H., *et al*., *Chem. Asian J*. **2012**, *7*, 2678-2682.

Effects of ionization conditions on the ion mobility of glycans

([1]*School of Materials Science, Japan Advanced Institute of Science and Technology*, [2]*Graduate School of Pharmaceutical Sciences, Nagoya City University*, [3]*Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences*)○Hao Feng[1], Takumi Yamaguchi[1,2,3]

Keywords: *Glycans, Ion mobility–mass spectrometry, Electrospray ionization, Conformation*

The structural analysis of glycans remains challenging due to their isomeric complexity and conformational flexibility. Ion mobility–mass spectrometry (IM–MS) separates ionized molecules based on their mobility, which depends not only on charge but also on size and shape. Therefore, IM–MS is considered a useful method for distinguishing glycan isomers that differ in residue sequence and linkage type. While IM–MS also holds potential for the conformational analysis of glycans, the optimal conditions for the mobility analysis of glycan ions remain to be explored. In this study, we systematically examined how ionization parameters affect glycan ion mobility to develop IM–MS structural analyses of glycans.

Mass spectrometric analysis of glycans commonly relies on protonated ions or sodium ion adducts. However, the ambiguity of protonation or sodium-binding sites often complicates mobility analysis. To facilitate the efficient generation of site-defined monoprotonated ions, we attached a tag containing a specific protonation site to the reducing end of glycans. We synthesized glycolipid glycans, such as Galα1-4Galβ1-4Glcβ, with this tag (**Figure 1**) and analyzed them using an IM–MS system. The arrival time distributions of the monoprotonated ions indicated the coexistence of multiple conformations of the glycans; in contrast, interaction with sodium ions perturbed these conformational dynamics. We also examined the effects of ionization conditions—specifically, temperature and solvent environment—on the results of mobility analysis of the glycan ions. High Ionization temperatures and the use of organic solvents increased the conformational dynamics of the glycan ions and broadened their distributions. These findings highlight the utility of this protocol for analyzing glycan conformations.[1)]

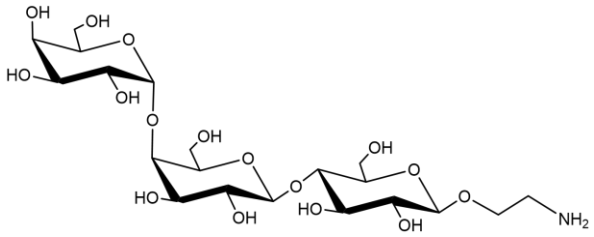


Figure 1. Structure of the glycan labeled with an ethylamine tag for site-specific protonation.

1) H. Feng and T. Yamaguchi, *Molecules*, **30**, 2177 (2025).

A microwell-based system for analyzing cytotoxicity and cell-sorting at the single-cell level

([1]*The University of Tokyo*, [2] *The University of Osaka*) ○Ryotaro Yamamoto,[1] Shinya Yamahira,[2] Shouichi Sakakihara,[2] Akimitsu Okamoto,[1] Satoshi Yamaguchi,[2]
Keywords: cytotoxicity; single-cell analysis; microwell; immune cell;

Cancer immunotherapy is a promising strategy for treating refractory cancers. For example, adoptive transfer of genetically modified T cells, such as CAR-T therapy, has shown remarkable efficacy, particularly in hematological malignancies. [1] However, in solid tumors, effective CAR-T cells are often extremely rare, highlighting the need to evaluate the cytotoxic activity of immune cells at the single-cell level. Although microwell-based single-cell assays have been reported, the small-sized wells lead to confined microenvironments for cells, which restricts cellular motility and morphological dynamics [2] and makes it difficult to accurately assess cytotoxicity against adherent cancer cells.

In this study, a new technology is developed which enables single-cell and large-scale, analysis of immune cell cytotoxicity towards cancer cells under a condition that allows adherent cancer cells to maintain their physiological morphology and activity. Furthermore, individual immune cells can be collected based on their cytotoxic behaviors and subject to gene expression analysis to identify novel biomarkers useful for the preparation and quality control of highly cytotoxic therapeutic T cells.

To this end, a substrate containing large-sized microwells that do not restrict cellular movement or morphological changes was prepared and coated with a photoactivatable cell-adhesive PEG lipid (PA-BAM) developed by our research group. Single-cell-sized light spots were irradiated onto the surface, followed by cell seeding and washing to allow attachment of only a single cell per spot. HeLa cancer cells were then cultured to allow cell spreading, after which KHYG-1 cells were seeded, enabling observation of HeLa-KHYG-1 cell-cell interactions within individual microwells. Using this system, the cytotoxic activity of KHYG-1 cells was successfully evaluated at the single-cell level.

1) S. L. Maude et al., *N. Engl. J. Med*. **2014**, 371, 1507. 2) Y. Zhou et al., *Cell Rep.* **2020**, 31, 107574.