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

2024年3月18日(月) 13:00 ~ 15:30 H934(9号館 [3階] 934)

[H934-1pm] 17. 生体機能関連化学・バイオテクノロジー

座長：山村 昌平、小澤 岳昌

日本語
13:00 ~ 13:20
[H934-1pm-01]
細胞膜固定化材料の作製と生体材料としての特性解析

○岡本 行広[1,2]、Xuehui Rui[1]、渡邊 望美[1]、馬越 大[1] (1. 大阪大学大学院基礎工学研究科、2. 大阪大学大学院基礎工学研究科未来ラボ)

日本語
13:20 ~ 13:40
[H934-1pm-02]
脂質複合化 DNA ブラシに吸着した金ナノ粒子の溶媒による集合変化

○与那嶺 雄介[1]、石 雅麗[2]、三友 秀之[1]、居城 邦治[1] (1. 北大電子研、2. 北大院生命)

英語
13:40 ~ 14:00
[H934-1pm-03]
ペプチドナノファイバーを基盤とした光応答性人工細胞骨格の創製

○梁 応冰[1]、稲葉 央[1]、松浦 和則[1] (1. 鳥取大学大学院)

英語
14:00 ~ 14:20
[H934-1pm-04]
Surface coating of an algal cell with elongated DNA strands to control the loading and releasing of cationic materials.

○YINGQI MU[1], Yuseke Yonamine[2], Hideyuki Mitomo[2], Kuniharu Ijiro[2] (1. Hokkaido University, 2. Research Institute for Electronic Science, Hokkaido University (RIES, Hokkaido Univ.))

14:20 ~ 14:30
休憩

日本語
14:30 ~ 14:50
[H934-1pm-05]
遺伝子変異がん細胞の検出のためのペプチド核酸プローブと1細胞マイクロアレイチップ技術の開発

○重藤 元[1]、北松 瑞生[2]、大槻 高史[3]、飯塚 明[4]、秋山 靖人[4]、山村 昌平[1] (1. 産総研、2. 近畿大、3. 岡山大、4. 静岡がんセンター)

英語
14:50 ~ 15:10
[H934-1pm-06]
概日時計同調時におけるNADPHオキシダーゼの役割を解明するための発光プローブの開発

○河村 玄気[1]、田丸 輝也[2]、小澤 岳昌[1] (1. 東京大学、2. 東邦大学)

英語

15:10 ~ 15:30

[H934-1pm-07]

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的ダイナミクスと下流シグナル伝達の定量的解析

○関根 由佳[1]、河村 玄気[1]、小澤 岳昌[1] (1. 東大院理・化学)

細胞膜固定化材料の作製とその同定

(阪大院基礎工[1]・阪大院基礎工未来ラボ[2])
○岡本 行広[1,2]・Xuehui Rui[1]・渡邊 望美[1]・馬越 大[1]

Preparation and Characterization of Cell Membrane Coated Materials

([1]*Graduated School of Engineering Science, Osaka University,* [2]*Graduated School of Engineering Science, the Multidisciplinary Research Laboratory System at Osaka University*)
○**Yukihiro Okamoto**[1,2], Xuehui Rui[1], Nozomi Watanabe[1], Hiroshi Umakosh[1]

We can find various and tremendous cell species in our body and in nature, and these cells possess specific molecules and show superior functions. Thus, cell membrane coated materials can be expected as superior materials mimicking the cell ability. In drug delivery system (DDS), the escape from immune system is one of the most required matters, and DDS carriers must be equipped with this ability. In this paper, we attempted to prepare cell membrane coated DDS carriers to attain this demand. At first, the confirmation of cell membrane coated lipid nanoparticles was conducted by cryo-TEM, and subsequently the property analysis of the nanoparticles was performed by DLS, zeta potential, SDS PAGE *etc*. Finally, the performance as DDS carriers was evaluated by toxicity assay, drug release assay, uptake ratio by tumor cells and macrophage cells *etc*. These data successfully demonstrated that cell membrane coating is effective approach for superior DDS carriers.
Keywords :cell membrane coating; membrane property analysis; drug delivery system material

我々の体内，自然界には多種多様な細胞が存在しており，優れた機能を呈している．この優れた機能を模倣する手法の一つとして，細胞膜の利用が考えられる．DDS において免疫系からの回避は重要な事項の一つであり，自己の体内に存在する細胞から細胞膜を回収し，DDS キャリアに固定化することで，従来の DDS キャリア材料に免疫系からの回避という特性を付与できると考えられる．

今回採用した脂質ナノ粒子は，粒径を 50 nm～400 nm 前後に調製可能であり，疎水性/親水性薬物を含有できるため，DDS 材料として期待されている．これをコア材料とし，この表層を細胞膜で修飾したナノ粒子を作製した．その後，DDS キャリアとしての特性を評価した．

Cryo-TEM や SAXS 解析から細胞膜を固定化した脂質ナノ粒子の形成を確認した．続いて，粒子径，ゼータ電位，細胞膜固定化後の膜タンパク質の評価を DLS, zeta 電位測定，SDS-PAGE により実施し，膜タンパク質分子を損なうことなく，200 nm 前後のナノ粒子の形成を確認した．最後に，DDS キャリアとしての性能評価を実施した．調製したナノ粒子の取り込み量をマクロファージと腫瘍細胞で比較した結果，マクロファージ細胞での取り込む量の低下が確認された．このため，調製したナノ粒子は免疫系を回避し，多くのナノ粒子が腫瘍細胞に取り込まれることが期待できる．

以上の結果より，細胞膜を固定化した脂質ナノ粒子は DDS キャリアとして有望な材料であると考えられる．

脂質複合化 DNA ブラシに吸着した金ナノ粒子の溶媒による集合変化

（北大電子研[1]・北大院生命[2]）○与那嶺 雄介[1]・石 雅麗[2]・三友 秀之[1]・居城 邦治[1]
Assembly of Gold Nanoparticles Absorbed into a DNA-Lipid Complex Brush Responding to Solvent Environments ([1]*Research Institute for Electronic Science, Hokkaido University*, [2]*Graduate School of Life Science, Hokkaido University*) ○Yusuke Yonamine,[1] Yali Shi,[2] Hideyuki Mitomo,[1] Kuniharu Ijiro[2]

A complex of positively charged lipid membranes and nucleic acids forms dynamic and diverse structures due to the fluidity of the long alkyl chains of the lipids. Previously, we fabricated a DNA brush of a defined length on a substrate at high density.[1] In this study, we developed a DNA -lipid complex brush via modifying cationic lipids with ionic bonds, adsorbed gold nanoparticles (AuNP) through hydrophobic interactions, and investigated the behavior when the solvent environment was changed (Fig. 1). As a result, hydrophobic AuNPs bound to a double-stranded DNA-lipid complex brush showed peak shift of the extinction spectrum to longer wavelengths when the solution was changed from organic solvent to water. This result suggested that long alkyl groups of the DNA-lipid complex aggregated with each other in a polar solvent and the distance of AuNPs became closer, causing plasmon coupling.
Keywords : DNA-Lipid complex; Gold nanoparticle; Polymer brushes

正電荷を持つ脂質膜と核酸は静電的相互作用によって結合し、その複合体は脂質の長鎖アルキル基の流動性によって動的で多様な構造を形成する。我々はこれまでに、規定した長さの DNA を基板上に高密度に固定した DNA ブラシを作製してきた[1]。本研究では、これにイオン結合でカチオン性脂質を修飾した、脂質複合化 DNA ブラシを作製し、金ナノ粒子（AuNP）を疎水性相互作用により吸着させ、溶媒環境を変化させた際の挙動を調査した（Fig. 1）。その結果、疎水性 AuNP は二本鎖の脂質複合化 DNA ブラシに結合し、溶液を有機溶媒から水に変えたところ、消光スペクトルのピーク位置が長波長側にシフトした。極性溶媒下ではアルキル基同士が凝集し、それに伴いAuNPが近接し、プラズモンカップリングが起こったと考えられる。この変化は可逆的であった。

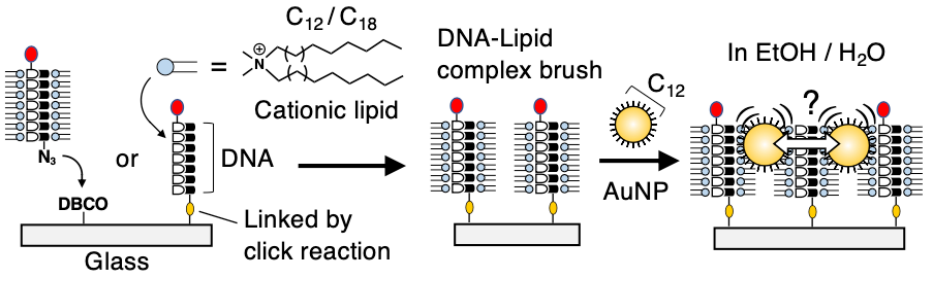


Figure 1. A DNA-Lipid complex brush was formed on a glass substrate and gold nanoparticles were absorbed into the brush through hydrophobic interaction. Assemble behavior was investigated when solvent environment was changed.

1) S. Nakamura *et al., ACS Omega,* **2017,** *2,* 2208.

Creation of Photoresponsive Artificial Cytoskeleton based on Peptide Nanofibers

(*Graduate School of Engineering, Tottori University*) ○ Yingbing Liang, Hiroshi Inaba, Kazunori Matsuura

Keywords: Cytoskeleton; Liposome; Peptide Nanofiber; Assembly/Disassembly; Photoresponse

Polymerization and depolymerization of cytoskeleton structures such as microtubules and actin filaments in eukaryotic cells impact cell deformation and migration. Recently, it has reported that the assembly and disassembly of nanofibers composed of actin inside giant unilamellar vesicles (GUVs) can affect their morphology.[1] However, there are no studies on spatiotemporal control of GUV deformation using cytoskeleton-like self-assembled nanofibers. In this study, we developed photoresponsive spiropyran/merocyanine (SP/MC)-modified peptide nanofibers to create an artificial cytoskeleton that can induce a morphological change in GUVs (Fig. 1A).[2]

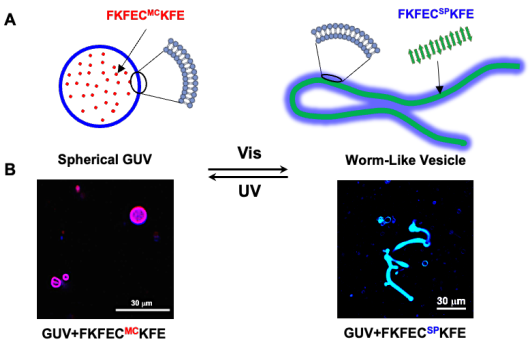


Fig. 1 Schematic diagram (A) and CLSM images (B) of the reversible morphologic changes of GUVs encapsulated FKFEC$^{SP/MC}$KFE .

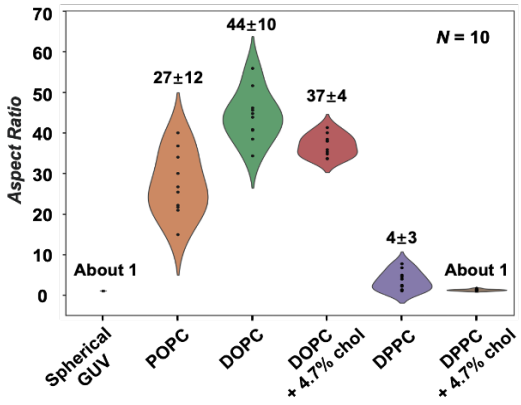


Fig. 2 The effect of membrane fluidity on the aspect ratio of GUVs induced by photoisomerization of SP/MC-peptide.

We synthesized an SP/MC-modified *β*-sheet peptide (FKFEC$^{SP/MC}$KFE) with reversible structural conversion by photo-irradiation. The SP-peptide formed β-sheet nanofibers, whereas the photoisomerization to the MC-peptide almost completely dissociated the nanofibers. Spherical GUVs encapsulating the MC-peptide dramatically and reversibly changed into worm-like vesicles by the photoisomerization to the SP-modified peptide (Fig. 1B). In addition, we investigated how membrane fluidity affects the GUV deformation induced by photoisomerization of SP/MC-peptide (Fig. 2). GUV conprising of flexible DOPC with two double bonds in their structure deformed more drastically than GUV conprising of mono-unsaturated POPC. The addition of cholesterol decreased the membrane fluidity, but the deformation remained drastic. In contrast, GUV conprising of rigid DPPC with a saturated fatty acid chain underwent minimal morphological changes.

[1] C. Li, X. Zhang, B. Yang, F. Wei, Y. Ren, W. Mu, X. Han, *Adv. Mater.*, **34**, 2204039 (2022)
[2] Y. Liang, S. Ogawa, H. Inaba, K. Matsuura, *Front. Mol. Biosci.*, **10**, 1137885 (2023)

Surface Coating of an Algal Cell with Elongated DNA Strands to Control the Loading and Releasing of Cationic Materials

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

Keywords: Cell Engineering, DNA Polymerase, Algae Cell, Functional Materials

【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 extend cellular functions. To produce the "functionalized cell", it is supportive to form a polymer layer on the cell surface that mediates adhesion between cells and functional materials in physiological conditions. DNA is a suitable polymer as it is biocompatible and can be extended even under cell culture conditions with enzymatic reactions. Furthermore, negatively charged phosphate groups of DNA can function as a scaffold for loading cationic materials. In this study, as a model cell for functionalization, a unicellular alga, Chlamydomonas reinhardtii (CR), was modified with a DNA primer. The immobilized DNA primers were then elongated with a DNA polymerase to cover the CR cells with long DNA chains. A cationic gold nanoparticle was loaded onto the DNA layer through electrostatic interaction and released via degradation with an endonuclease to demonstrate endowment and deprivation of the function.

【Results and Discussion】

A DNA primer (X-motif) was conjugated with an oligopeptide of 4-hydroxyproline (HYP_{10}) that binds tightly to the CR cell wall[1] and immobilizes onto CR cell surface. By adding a DNA polymerase, Klenow fragment exo (-) (KF^-),[2] long double-stranded DNA chains with repeating sequences were elongated via slippage mechanisum[2] to form a thick DNA layer on the cell surface (Figure 1). A gold nanorod (GNR) coated with a cationic ligand (11-MTAB) was further modified onto the surface of the elongated DNA-coated cells through electrostatic interaction. Moreover, the release of modified GNRs was demonstrated by adding an endonuclease, DNase I, which degrades double- and single-stranded DNA (Figure 2).

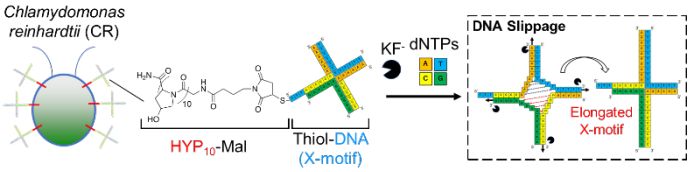


Figure 1. Elongation of DNA primers (X-motif) immobilized on CR cell surface with a DNA polymerase (KF-).

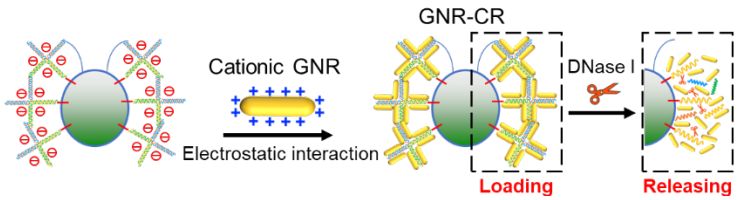


Figure 2. Cationic GNRs were loaded on CR cell surface through electrostatic interaction and released with an endonuclease (DNase I).

1) D. B Weibel *et al.*, *PNAS*, **2005,** *102*, 11963-11967.

2) A. B. Kotlyar *et al.*, *Nucleic Acids Res.*, **2005,** *33*, 525–535.

遺伝子変異がん細胞の検出のためのペプチド核酸プローブと 1 細胞マイクロアレイチップ技術の開発

（産総研・健康医工学 [1]・近畿大・応用化学 [2]・岡大院・統合科学 [3]・静岡がんセンター・免疫治療研究部 [4]） 重藤 元 [1]・北松 瑞生 [2]・大槻 高史 [3]・飯塚 明 [4]・秋山 靖人 [4]・山村 昌平 [1]

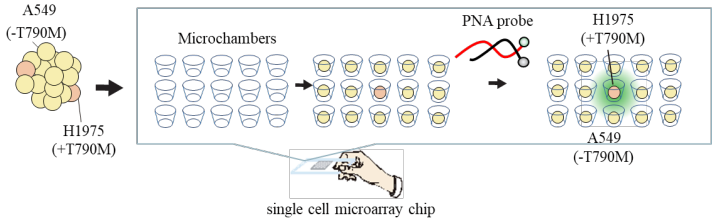
Development of the analyzing method for single nucleotide-mutated single-cancer cells using Peptide Nucleic acid Probes and Single-Cell Microarray

([1] *Health & Medical Res. Inst., AIST*, [2] *Dep. of App. Chem., Kindai Univ.,* [3] *Grad. Sch. of ISEHS, Okayama Univ.*, [4] *Immunotherapy Div., Shizuoka Cancer Cent. Res. Inst.*) ○Hajime Shigeto,[1] Mizuki Kitamatsu,[2] Takashi Ohtsuki,[3] Akira Iizuka, [4] Yasuto Akiyama,[4] Shohei Yamamura[1]

The gene mutations such as T790M in EGFR provides to cell proliferation and acquisition against anticancer drug resistance. In this study, we developed a novel system to detect the cancer cells harboring T790M mutation by combining technology of peptide nucleic acid probes and cell microarray chip. The developed method enables the easily and sensitively detection a small number of target cells from a various number of non-mutated cells.

Keywords : PNA probe; Single Cell Microarray Chip; SNP; EGFR; Lung cancer

がん細胞は発生過程において多様な遺伝子変異を獲得する。Epidermal Growth Factor Receptor (EGFR)における exon19 E746-A750 の欠損や L858R、T790M の非同義置換変異を獲得することはがん細胞の増殖促進、抗がん剤耐性能の獲得をもたらす。我々はこれまで抗がん剤耐性細胞の新たな診断方法の開発を目的とし EGFR mRNA 中の 3 つの遺伝子変異をそれぞれ検出するためのペプチド核酸 (Peptide Nucleic Acid, PNA) を使用した PNA プローブを開発してきた。また数百万個～数千万個の細胞から 1 個の標的を検出する集積型の細胞マイクロアレイチップの開発に成功している。本研究では非変異がん細胞中から、T790M に変異を有する EGFR mRNA を発現する単一がん細胞を検出・分離するシステムを開発した。T790M に変異を有する肺がん細胞株 NCI-H1975 と変異を持たない肺がん細胞株 A549 を混合したサンプルを、1 細胞マイクロアレイチップにより単一細胞に分離した。その後 T790M 変異を特異的に検出する PNA プローブを用いてチップ上で染色を行った。NCI-H1975 細胞を A549 にスパイクし解析を行ったところ 10%以下の NCI-H1975 細胞を定量検出することが可能であった。これらの結果から我々の開発するシステムは多様な細胞が含まれるがん組織中の少数の抗がん剤耐性細胞を簡易、高感度に検出可能であることが示された。



(1) Yamamura., et al., *sensors*, 17, 2410 (2017)
(2) Shigeto H., et al., *Analyst*, 144, 4613-4621 (2019)
(3) Shigeto H., et. al., *Micromachines*, 11, 628 (2020)

Development of bioluminescent probes to analyze the role of NADPH oxidase 1 in circadian clock synchronization

(*[1]Graduate School of Science, The University of Tokyo, [2]School of Medicine, Toho University*) ○Genki Kawamura,[1] Teruya Tamaru,[2] Takeaki Ozawa[1]

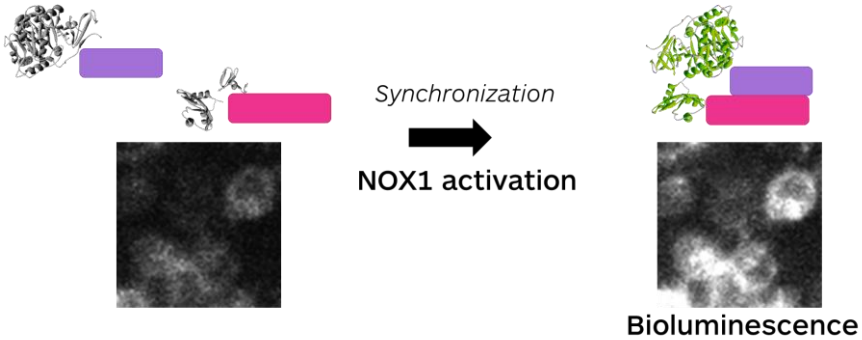
Keywords: Split-luciferase, Bioluminescence imaging, Circadian clock, NADPH oxidase

Cellular circadian clocks adjust their circadian phases to external cues through a process called synchronization. We have previously demonstrated that clocks synchronize to cellular stressors such as reactive oxygen species (ROS) and ultraviolet radiation (UV).[1,2] However, the role of physiological ROS signaling in this synchronization process remains elusive.

In this study, we investigated the role of NADPH oxidase (NOX), a primary source of intracellular ROS, in clock synchronization. NOX is an enzyme family that catalyzes the production of cellular $O_2^{\cdot-}$ and H_2O_2, believed to play a unique role in cellular ROS signaling.[3] We observed that the administration of low levels of H_2O_2 enhances clock oscillation. Moreover, we found that genetical knock-down of a NOX family member, NOX1, disrupted clock synchronization. We found that synchronization stimulation increased cellular H_2O_2 levels, while NOX1 suppression decreased them, suggesting that ROS produced by NOX1 mediates cellular synchronization.

To track NOX1 activity in real-time, we developed a split-luciferase complementation probe that detects temporal changes in NOX1 activity. We found that NOX1 activity increased upon synchronization stimulation concomitantly with cellular ROS level increase. Moreover, we discovered that NOX1 activity oscillates in a circadian manner, suggesting that the circadian clock system regulates NOX1 activity.

In conclusion, we propose a model where the circadian clock controls NOX1 activity, which generates ROS signals that, in turn, maintain the circadian clock synchronization.



References: 1) Kawamura, G. et al, *Commun. Biol.* **2018**, 1 (1), 204. 2) Tamaru, T. et al, *PLOS ONE* **2013**, 8 (12), 1–16. 3) Sies, H.; Jones, D. *Nat Rev Mol Cell Biol* **2020**, 21 (7), 363–383.

Quantitative analysis of Akt isoforms' temporal dynamics and downstream signaling with optogenetics and a mathematical model

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

Keywords: Optogenetics, mathematical model, Akt isoform, temporal dynamics, cell morphology change

Ser/Thr kinase Akt plays pivotal roles in cellular signal transduction pathways. There are three isoforms of Akt; Akt1, Akt2, and Akt3, that shows several different functions. It is suggested that each Akt isoform has its specific temporal dynamics and selectively regulates its downstream signaling and subsequent cellular responses.

To individually examine each Akt isoform's phosphorylation dynamics, we utilized optogenetics approach. We applied a principle of photoactivatable Akt (PA-Akt)[1], which activates Akt by using CRY2/CIBN photo-dimerization, to all the Akt isoforms. We measured the CRY2-Akt isoforms' temporal phosphorylation patterns of light illuminated PA-Akt isoform expressing cells. As a result, we found that each CRY2-Akt isoform has its specific temporal properties. To numerically analyze the measured Akt isoforms' temporal dynamics, we employed mathematical models to estimate isoform-specific kinetic parameters. Moreover, we examined roles of each Akt isoform's dynamics on downstream signaling regulation and found that cell protrusion and retraction occur upon each Akt isoform's photo-activation. From these results, we aim to quantitatively clarify the Akt isoforms' temporal activation kinetics and how each isoform regulates cell morphology change.

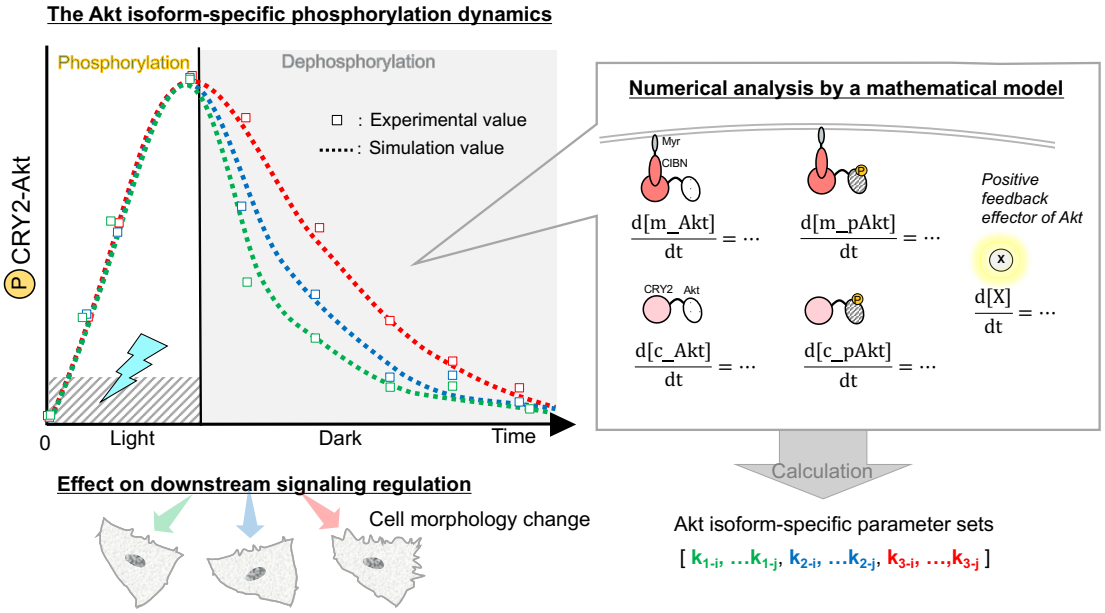


Figure 1 Quantitative analysis of the Akt isoform-specific signaling

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