BIOGRAPHICAL SKETCH

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NAME: Takanari Inoue

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POSITION TITLE: Professor of Cell Biology, Cell Dynamics, Pharmacology and Molecular Sciences, Biological Chemistry, and Biomedical Engineering, Deputy Director of Center for Cell Dynamics

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Tokyo (Tokyo, Japan)	B.S.	05/98	Pharmaceutical Science
University of Tokyo (Tokyo, Japan)	Ph.D.	03/03	Chemical & Cell Biology
Stanford University (Palo Alto, CA)	Postdoctoral	03/08	Chemical & Systems Biology

A. Personal Statement

My multidisciplinary scientific journey started 25 years ago when I joined a chemical biology laboratory at the University of Tokyo as a first-generation college student. I was initially trained as an organic chemist, synthesizing designer small molecules, and then continued this work as a graduate student in a collaborator's lab specialized in cell biology/physiology. After setting up my own lab as an Assistant Professor at Johns Hopkins University School of Medicine in 2008, I continued developing molecular sensors and actuators for the study of chemotaxis. My young lab was an important contributor to a key discovery in the field – the identification of a positive feedback loop between PI3K and membrane deformation. During this time, I have incorporated a general engineering concept into our chemical and cell biology research program to establish "*Synthetic Cell Biology*". Under this non-reductionistic theme, I not only deconstruct cellular processes, but also reconstruct minimal molecular components from scratch to achieve seemingly complex dynamic cell behaviors. An unofficial motto in my lab is a quotation from the great physicist Richard Feynman: "What I cannot create, I do not understand." Our studies driven by hypothesis, technology and curiosity have had a strong impact on the cell migration and cytoskeleton communities as indicated by over 800 reagent requests; patent filings; press releases and public interviews; along with invited lectureships. More recently, we have extended these techniques to study phagocytosis, another representative dynamic cellular process. Ultimately, the reconstitution of these dynamic cell behaviors based on our molecular actuators can be used to endow cells with important therapeutic functions with applications in the treatment of cancers. These new biomedical directions will be facilitated by my affiliation with one of the top medical schools in the country. This puts me in a perfect position to translate our basic biological discoveries intto the treatment of human diseases in the near future.

Current supporting funds:

1R01GM136858 (T Inoue)	9/1/20-8/31/24	4.2 calendar mos.
5R01GM123130 (T Inoue)	9/1/18-6/30/23	1.4 calendar mos.
HFSP Research Program Grant (T Inoue)	12/1/20-11/30/23	0.6 calendar mos.
NSF 000819255 (T Inoue)	3/1/22-2/28/26	2.0 calendar mos.

Citations:

1. Nakamura H., Lee A.A., Afshar A.S., Lin Y.C., Tanigawa M., Suarez A., Razavi S., DeRose R., Bobb D., Hong W., Gabelli S.B., Goutsias J., **Inoue T** "Intracellular production of hydrogels and synthetic RNA granules by multivalent interactions" ***Nature Materials*** 17, 79-89 (2018) (PMC5916848) (Supported by GM092930)

2. Wu HD, Kikuchi M, Dagliyan O, Aragaki AK, Nakamura H, Dokholyan NV, Umehara T, **Inoue T** "Rational design and implementation of a chemically inducible hetero-trimerization system" ***Nature Methods*** 17, 928–936 (2020) (PMC in development) (Supported by R01GM123130)
3. Deb Roy A, Gross EG, Pillai GS, Seetharaman S, Etienne-Manneville S, **Inoue T.** "Non-catalytic allostery in α-TAT1 by a phospho-switch drives dynamic microtubule acetylation" ***Journal of Cell Biology*** 221, e1-20 (2022) (PMC in development) (Supported by R01GM123130)
4. Watanabe S, Nihongaki Y, Itoh K, Watanabe S, **Inoue T*** "Defunctionalizing Intracellular Organelles with Genetically-Encoded Molecular Tools Based on Engineered Phospholipase A/Acyltransferases" ***Nature Communications*** 13, 1-15 (2022) (PMC9338259) (Supported by R01GM136858)

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2019 – Director, Center for Cell Dynamics, Johns Hopkins University
2018 – Professor, Cell Biology, Cell Dynamics, Pharmacology and Molecular Sciences, Biological Chemistry, and Biomedical Engineering, Johns Hopkins University
2008 – 2018 Assistant/Associate Professor of Cell Biology and Cell Dynamics, Johns Hopkins University
2003 – 2008 Postdoctoral Fellow, Chemical & Systems Biology, Bio-X Program, Stanford University
1998 – 2003 Graduate Student, Chemical & Cell Biology, University of Tokyo (Tokyo, Japan)
1994 – 1998 Undergraduate Student, Pharmaceutical Sciences, University of Tokyo (Tokyo, Japan)

Other Experience and Professional Memberships

2019 Session Organizer, 92nd Japanese Society of Biochemistry Annual Meeting, Yokohama, Japan
2019 Organizer, ASCB Annual Meeting, Subgroup Session, Washington DC, CA
2016 Organizer, AAA Annual meeting at EB2016, Symposium, San Diego, CA
2016 Organizer, ASCB Annual Meeting, Mini-symposium, San Francisco, CA
2015 Organizer, BPS International Thematic Meeting, Taipei, Taiwan
2015 Organizer, Experimental Biology Meeting Symposium, Boston, MA
2015 Editorial Board Member, *Scientific Reports*
2015 Organizer, ASCB Annual Meeting, Subgroup Session, San Diego, CA
2014 Committee member, R.R. Bensley Award, American Association of Anatomists
2014 Committee member, Young Investigator's Day Award, Johns Hopkins University, MD
2014 Organizer, ASCB Annual Meeting, Subgroup Session, Philadelphia, PA
2013 Organizer, ASCB Annual Meeting, Subgroup Session, New Orleans, LA
2011- Founder and organizer, Japanese Science Seminar in Baltimore (JSSB), Baltimore, MD
2010- Member, Biophysical Society
2008- Ad hoc grant reviewer: National Science Foundation, National Institute of Health (NIGMS, NIDDK), Swiss National Science Foundation, Lowe Trust Foundation, North Carolina Biotechnology Center, National Kidney Foundation of Maryland, Biotechnology and Biological Sciences Research Council, Academia Sinica Grant Review, French National research Agency (ANR), Netherlands Organization for Scientific Research (NWO), European Commission SEP (ERC), Emerson Collective Cancer Foundation, JSPS KAKENHI, Human Frontier Science Program (HFSP)
2005- Member, American Society of Cell Biology

Honors

2022 ASCB Fellow, American Society of Cell Biology
2021 JSPS Prize
2018 JSPS Bridge Award
2017 Hopkins-Allegheny Health Network Cancer Research Award
2017 Hamilton O. Smith Award for Innovative Research
2016 SPIE's Systems Biology Pioneer Award, International Society for Optics and Photonics
2016 Mirowski Discovery Award, Johns Hopkins University
2015 Breakthrough Award in Breast Cancer Research Program, Department of Defense
2015 Catalyst Award, Discovery Award, Johns Hopkins University, Office of the Provost
2014 NIDDK Silvio O. Conte Digestive Disease Research Core Center Investigator
2014 Young Scientists' Prize, Minister of Science and Technology in Japan
2014 R.R. Bensley Award in Cell Biology, American Association of Anatomists

2013 Young Investigator Award from Pharmaceutical Society in Japan
2012-2015 PRESTO investigator, Japanese Science and Technology
2010-2012 NIDDK Polycystic Kidney Center Investigator

Patents

2020 "A versatile method to fabricate giant vesicles that can encapsulate a range of membrane-bound proteins, luminal proteins, and cell lysates" Razavi S., Robinson DN, and **Inoue T.** (U.S. Patent PCT 065237, pending)
2003 "IP_3 Receptor Ligands" U.S. Patent 6,656,927, **Inoue T.**, Kikuchi K. Hirose K., Iino M., and Nagano T.
2000 "IP_3 Receptor Ligands" JPN Patent 4226245, **Inoue T.**, Kikuchi K. Hirose K., Iino M., and Nagano T.

C. Contribution to Science

1. ***Developing advanced molecular sensors and actuators***: We made significant advances in developing technology for studying spatiotemporal signaling, by establishing a set of CID-based techniques for rapidly introducing perturbations into a cell. We were the first to develop a CID technique that was capable of manipulating the activity of signaling molecules within seconds at various subcellular locations in a reversible, photoactivatable, bi-functional and bio-orthogonal manner. Furthermore, we expanded our library of CID targets beyond conventional signaling molecules and have demonstrated its sufficiency in changing biophysical properties such as membrane curvature.

    a. Suarez A., Ueno T., Huebner R., McCaffery J.M., and **Inoue T.*** "Bin/Amphiphysin/Rvs (BAR) family members bend membranes in cells" ***Scientific Reports*** 4 (4693), 1-6 (2014) (PMC1299016)
    b. Miyamoto T., Rho E, Sample V., Akano H., Magari M., Ueno T., Chen M., Tokumitsu H., Zhang J., and **Inoue T.*** "Compartmentalized AMPK Signaling Illuminated by Genetically Encoded Molecular Sensors and Actuators" ***Cell Reports*** 11 (4), 657-670 (2015) (PMC4417068) ***Note***: Press release: "Molecular spies sabotage a protein's activities in specific cellular compartments"
    c. Watanabe S, Nihongaki Y, Itoh K, Watanabe S, **Inoue T*** "Defunctionalizing Intracellular Organelles with Genetically-Encoded Molecular Tools Based on Engineered Phospholipase A/Acyltransferases" ***Nature Communications*** 13, 1-15 (2022) (PMC9338259)
    d. Nakamura H, Rho E, Deng D, Razavi S, Matsubayashi HT, **Inoue T.*** "ActuAtor, a molecular tool for generating force in living cells" ***bioRxiv*** March 3, 2020, https://doi.org/10.1101/2020.03.30.016360

2. ***Uncovering unconventional roles of membrane phospholipids:*** Phosphoinositides are lipids contained in the membranes, essential for many signaling events. The chemical similarity of the different phosphoinositides have made the study of their roles challenging, a fact further complicated by compensatory mechanisms that exist for its generation in genetic knockdown and knockout studies. With a new generation of molecular actuators that allows for the specific manipulation of phosphoinositide levels in a rapidly inducible manner, we elucidated for the first time unconventional roles that phosphoinositide (4,5)-bisphosphate, or PIP_2, takes on in various biological contexts such as in regulation of ion channel gating, targeting mechanism of small GTPases, and actin reorganization.

    a. Suh BC[#], **Inoue T**[#], Meyer T, and Hille B. "Rapid chemically-induced changes of PtdIns(4,5)P_2 gate KCNQ ion channels" ***Science*** 314, 1454-1457 (2006) ***Note***: "*Perspectives*" (*Science* 314, 1402-1403 (2006)), *"Editor's Choice"* (*Science STKE* 364, tw410 (2006)), "*Spotlight*" (*ACS Chem. Biol.* 1, 608 (2006)), "*Research Highlights*" (*Nature Methods* 4, 7 (2007))
    b. Heo WD, **Inoue T**, Park WS, Kim ML, Park BO, Wandless TJ, and Meyer T. "PI(3,4,5)P_3 and PI(4,5)P_2 lipids target Ras, Rho, Arf and Rab GTPases to the plasma membrane" ***Science*** 314, 1458-1461 (2006)
    c. Ueno T., Falkenburger B.H., Pohlmeyer C., and **Inoue T*** "Triggering Actin Comets Versus Membrane Ruffles: Distinctive Effects of Phosphoinositides on Actin Reorganization" ***Science Signaling*** 4(203), ra87 (2011) ***Note***: "Perspective", (*Science Signaling*, 5, pe7 (2012), "How Actin Gets the PIP"), "*Editorial Guides*" (*Science Signaling*, 5, eg3 (2012), "A Sense of Direction"), Press release: "Rearranging the Cell's Skeleton" (*Biochemist e-VOLUTION*" at Biochemical Society), Featured in F1000, Selected for *Cover*
    d. Stilling S, Kalliakoudas T, Benninghoven-Frey H, **Inoue T**, Falkenburger B. "PIP2 determines length and stability of primary cilia by balancing membrane turnovers" ***Communications Biology*** 5, 1-15 (2022) (PMC8789910)

3. ***New biological findings in chemotaxis:*** Focusing on feedback mechanisms during cell polarization, we discovered that the initial chemotactic event can be deconstructed into two temporally distinct steps: 1) signaling polarization and 2) morphological polarization. In particular, by combining CID-mediated Rac activation with microfluidic spatial control, we demonstrated that gradient steepness, not the mean value, of Rac activity inside cells determines the first step (signaling asymmetry), while the mean value of active Rac critically regulates the second step (morphological asymmetry). Furthermore, we have both experimentally and computationally demonstrated that a linear gradient of Rac is sufficient to trigger efficient cell polarization as well as directed cell migration. We also identified a small GTPase, H-Ras, as one of the missing links in the positive feedback loop that drives cell polarization. To explore the importance of physical cues in chemotaxis, we used a microfluidic device to reveal the molecular mechanisms mediating crosstalk between chemotaxis and physical cell-to-cell contact. This study uncovered that physical contacts significantly perturb chemoattractant-induced PI3K signaling through an ephrin B receptor, demonstrating the important role of physical cellular properties (in contrast to biochemical reactions) in chemotaxis.

    a. Thevathasan, J.V., Tan E., Hui Z., Lin Y.C., Li Y., **Inoue T.*** and Fivaz M.***** "The small GTPase HRas shapes local PI3K signals through positive feedback and regulates persistent membrane extension in migrating fibroblasts" ***Molecular Biology of the Cell*** 24, 2228-2237 (2013) (PMC3708728)
    b. Lin B., Yin T., Wu Y.I., **Inoue T.*** and Levchenko A.***** "Interplay between chemotaxis and contact inhibition of locomotion determines exploratory cell migration" ***Nature Communications*** 6, 6619 (2015) (PMC4391292)
    c. Deb Roy A, Gross EG, Pillai GS, Seetharaman S, Etienne-Manneville S, **Inoue T.*** "Non-catalytic allostery in α-TAT1 by a phospho-switch drives dynamic microtubule acetylation" ***Journal of Cell Biology*** 221, e1-20 (PMC in development)
    d. Matsubayashi HT, Mountain J, Yao T, Peterson AF, Deb Roy A, **Inoue T.*** "Non-catalytic role of phosphoinositide 3-kinase in mesenchymal cell migration through non-canonical induction of p85β/AP-2-mediated endocytosis" ***bioRxiv***, December 31, 2022, https://doi.org/10.1101/2022.12.31.522383

4. ***Reconstructing cell functions by synthetic cell biology***: To understand biological systems in a bottom-up fashion, we have taken a synthetic biology approach in reconstituting several biological functions as well as elementary signaling circuit functionalities such as Boolean logic gates. The biological functions include phagocytosis and actin polymerization, while we achieved almost all of the two-input Boolean gates. Momentum is building in the field of synthetic biology to seek interchangeable parts from natural biology that can be assembled into systems that function in artificial settings. These fundamental works provides leverage in generating a biological computer using biomolecules in the near future.

    a. Onuma H., Komatsu T., Arita M., Hanaoka K., Ueno T., Terai T., Nagano T., and **Inoue T.*** "Rapidly rendering cells phagocytic by cell-surface display technique with concurrent Rac activation" ***Science Signaling***, 7, rs4, 1-7 (2014) (PMC4136641)
    b. Kim AK, DeRose R, Ueno T, Lin B, Komatsu T, Nakamura H, **Inoue T.*** "Toward total synthesis of cell function: Reconstituting cell dynamics with synthetic biology" ***Science Signaling*** 9, re1, 1-7 (2016) (PMC in development)
    c. Nakamura H., Lee A.A., Afshar A.S., Lin Y.C., Tanigawa M., Suarez A., Razavi S., DeRose R., Bobb D., Hong W., Gabelli S.B., Goutsias J., **Inoue T*** "Intracellular production of hydrogels and synthetic RNA granules by multivalent interactions" ***Nature Materials*** 2018;17:79-89 (PMC5916848)
    d. Nihongaki Y, Matsubayashi HT, **Inoue T*** "A molecular trap inside microtubules probes luminal access by soluble proteins" ***Nature Chemical Biology*** 17, 888-895 (2021) (PMC8319117)

5. ***Deconstructing spatiotemporally dynamic signaling inside primary cilia***: Despite its significant involvement in physiology and pathophysiology, studies of primary cilia have been hampered primarily by its small size, making it challenging to distinguish signaling events that occur in the primary cilium. We thus recently engineered proteins that can rapidly trap cytoplasmic proteins in the primary cilia upon addition of a synthesized small compound. This novel tool allowed us to measure the trafficking kinetics of proteins in living kidney cells and revealed the ciliary trafficking mechanisms. We also developed a series of genetically encoded calcium indicators targeted to primary cilium and illuminated ciliary calcium signaling. These findings have made a strong impact on the communities as indicated by over 400 reagent requests, several patents, press releases, interviews and invited lectures.

a. Lin Y.C., Niewiadomski P., Lin B., Nakamura H., Phua S.C., Jiao J., Levchenko A., Inoue T., Rohatgi T., and **Inoue T.*** “Chemically-inducible diffusion trap reveals molecular sieve-like barrier at primary cilia“ ***Nature Chemical Biology*** 9, 437-443 (2013) (PMC3870470)
b. Su S., Phua S.C., DeRose R., Kalugin P.N., Katada T., Kontani K., and **Inoue T.*** “Genetically Encoded Calcium Indicator illuminates Ca^{2+} Dynamics in Cilia” ***Nature Methods*** 10, 1105 (2013) (PMC3860264)
c. Francesc R. Garcia-Gonzalo, Siew C. Phua, Elle C. Roberson, Galo Garcia III, Monika Abedin, Stéphane Schurmans, **Inoue T.*,** Jeremy F. Reiter* “Ciliary Phosphoinositides Modulate Hedgehog Signaling” ***Developmental Cell***, 34, 400-409 (2015) (PMC4557815)
d. Phua SC, Chiba S, Suzuki M, Su E, Roberson EC, Pusapati GV, Setou M, Rohatgi R, Reiter JF, Ikegami K, **Inoue T.*** “Dynamic Remodeling of Membrane Composition Drives Cell Cycle through Primary Cilia Excision” ***Cell*** 2017;168:264-279 (PMC in process) ***Note***: “Study Shows How and Why Hairlike Structures on Cells are Lost” PKD News Blog

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/takanari.inoue.1/bibliography/public/