

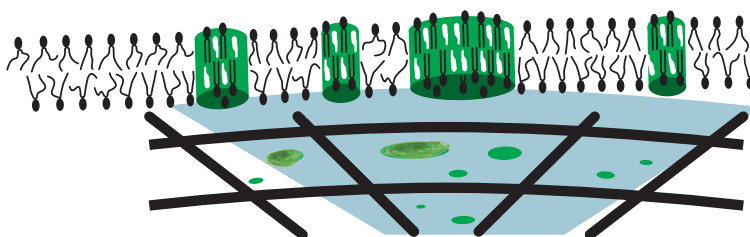
Program

Nano-Meso membrane mechanisms

27–29
January, 2010
Hotel Fujita Kyoto

The Sixth iCeMS
International Symposium

The Thirteenth
Membrane Research Forum



Sponsored by:

Membrane Mechanisms Project, International Cooperative Research Project (ICORP)

Japan Science and Technology Agency (JST)

Sokabe Cell-Mechanosensing Project (ICORP/SORST), JST

The Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University

Center for Meso-Bio Single-Molecule Imaging (CeMI) of iCeMS

National Centre for Biological Sciences (NCBS), Bangalore, India
(Partner Institution of iCeMS)

Purdue University Center for Basic and Applied Membrane Sciences (PUBAMS)
(Partner Institution of iCeMS)



The 13th International Membrane Research Forum
The 6th iCeMS International Symposium

Featuring
Nano-Meso Membrane Mechanisms

January 27 - 29, 2010

Hotel Fujita Kyoto

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Message from Director of the iCeMS

January 27, 2010

On behalf of all my colleagues at the iCeMS, Institute for Integrated Cell-Material Sciences at Kyoto University, I welcome you to the Sixth iCeMS International Symposium.

iCeMS is founded as a response to the Japanese government initiative, called World Premier International Research Centers (WPI program). The initiative is for establishing globally visible research centers here in Japan, which will attract top-level researchers from around the globe, particularly talented young scientists who will become world leaders in the future. The proposal to establish iCeMS was funded as one of the five such centers throughout Japan. iCeMS places a strong emphasis on international collaborations, and the iCeMS international symposium, to be held in a series, is one of our major means to develop our ties with international scientific communities.

We at the iCeMS strive to develop the fundamental understanding and control of molecular complexes in the meso-scale of 5-100 nm (meso-control), as the cell appeared to develop them through evolution. We consider these efforts critical for creating the science and technology of the next generation, and we intend to do these, with a strong focus on pluripotent stem (ES and iPS) cells. We are holding this Sixth iCeMS Symposium on "NANO-MESO MEMBRANE MECHANISMS", as an important part of our effort for understanding meso-scale events. It is also held as 13th International Membrane Research Forum. Two of the iCeMS partner institutions, National Centre for Biological Science (India) and Purdue University Center for Basic and Applied Membrane Sciences (PUBAMS), are co-sponsoring this symposium. I hope that the subject matters discussed here will provide good examples for interesting and useful meso-scale molecular interactions that control cellular functions.

Thank you very much again for joining us at this symposium. I hope you will enjoy this meeting.

Norio Nakatsuji, D.Sc.
Director and Professor
Institute for Integrated Cell-Material Sciences (iCeMS)
Kyoto University

Welcome to Sixth iCeMS International Symposium Thirteenth International Membrane Research Forum

January 27, 2010

We would like to welcome everybody who is participating in this symposium, particularly the speakers from abroad. It is our great pleasure to report that many scientists expressed considerable interest in this forum, and volunteer to present their research results.

This symposium is an attempt to bring together scientists from different research backgrounds, who are working on meso-scale complexes and interactions, particularly those in/on biological membranes. Here, the meso-scale means the space scale between 5 and 100 nm, where key molecular complexes and interactions take place in the cell. These meso-structures, including protein complexes and domains in the meso-scale in/on biological membranes, are important for membrane functions, such as transport of ions and molecules across the membrane and the signal transduction. Meanwhile, findings made in meso-scale biological complexes would greatly contribute to advancing meso-space sciences and technologies.

With this forum, we are trying to provide a common platform for scientists working on various aspects of meso-scale structures and interactions in biological membranes. The aim is "synthesis" rather than specialization. We believe that the time is ripe for combining our forces together to elucidate meso-scale interactions in/on the membrane.

We hope that you will enjoy this meeting and mingle with other scientists having very different backgrounds.

Organizers

iCeMS and the CeMI (Center for Meso-Bio Single-Molecule Imaging) of iCeMS

Jiro Usukura, Masahiro Sokabe, and Aki Kusumi

Executive Board for the Membrane Research Forum

Satyajit Mayor

National Centre for Biological Sciences (NCBS, iCeMS Partner Institution)

Ken Ritchie

Department of Physics, Purdue University and
Purdue Univ. Center for Basic and Applied Membrane Sciences (PUBAMS)

January 27 8:45 - 18:00

Opening
Aki Kusumi

8:45 - 8:50

Membrane Mechanisms Project, ICORP-JST
iCeMS and Institute for Frontier Medical Sciences
Kyoto University

Welcome Address
Norio Nakatsuji

8:50 - 9:00

iCeMS (Director) and Institute for Frontier Medical Sciences
Kyoto University

Welcome to the Sixth iCeMS International Symposium

Keynote Lecture 1

9:00 - 9:40

Chair: Norio Nakatsuji

Ira Mellman

Genentech, Inc.

Generation of signals and signaling domains

Coffee Break

9:40 - 10:00

Keynote Lecture 2

10:00 - 10:40

Chair: Robert G. Parton

Martin Alexander Schwartz

Cardiovascular Research Center

Dept. of Microbiology and Biomedical Engineering

Mellon Prostate Cancer Research Institute, University of Virginia

Integrin regulation of membrane domain trafficking

Seminar 1:

10:40 - 11:40

Chair: Ira Mellman

Haruhiko Bito

Dept. of Neurochemistry

University of Tokyo Graduate School of Medicine

CaM kinase signaling in neuronal microdomains

Makoto Kinoshita

Dept. of Molecular Biology, Division of Biological Science

Nagoya University Graduate School of Science

Assembly of the septin cytoskeleton in vitro and in vivo

Kazumitsu Ueda

iCeMS, Kyoto University

Kyoto University Graduate School of Agriculture

Physiological consequence of membrane lipid transport by ABC proteins

Lunch 11:40 - 13:00

Keynote Lecture 3 13:00 - 13:40 Chair: Jiro Usukura

Robert G. Parton Institute for Molecular Bioscience
University of Queensland
New Insights into the Formation and Function of Caveolae

Coffee Break 13:40 - 14:00

Keynote Lecture 4 14:00 - 14:40 Chair: Tadashi Yokosuka

Jay T. Groves Dept. of Chemistry, University of California, Berkeley
Signaling clusters in cell membranes

Seminar 2: 14:40 - 15:40 Chair: Jay T. Groves

Tadashi Yokosuka, Takashi Saito
Laboratory for Cell Signaling
RIKEN Research Center for Allergy and Immunology
Dynamic regulation of T-cell costimulation by TCR microclusters and the c-SMAC

Yuichi Takakuwa, Ichiro Koshino
Tokyo Women's Medical University
Regulation of erythrocyte membrane stability and malaria parasite invasion by lipid raft-mediated signal transduction

Jiro Usukura EcoTopia Science Institute, Nagoya University
Spatial structure and specificity of the actin cytoskeleton from membrane to cytoplasm

Coffee Break 15:40 - 16:00

16:00 - 18:00

**Progress Report Symposium
Cell Mechanosensing Project (2005-2010)**

**"Clarification of mechanotransduction
mediated by supramolecular complex"
ICORP/SORST, JST**

Keynote Lecture 5 16:00 - 16:40 Chair: Kishio Furuya

Masahiro Sokabe Nagoya University Graduate School of Medicine
ICORP/SORST Cell Mechanosensing Project, JST, Nagoya Japan
**Overview of the Mechanosensing Project:
Comparative biophysics of cell mechanosensing**

Seminar 3: 16:40 - 17:20 Chair: Kishio Furuya

Kimihide Hayakawa ICORP/SORST Cell Mechanosensing Project, JST, Nagoya Japan
A single actin filament works as a mechanosensor

Hiroaki Hirata ICORP/SORST Cell Mechanosensing Project, JST, Nagoya Japan
Force-dependent regulation of actin polymerization at focal adhesion

Keynote Lecture 6 17:20 - 18:00 Chair: Masahiro Sokabe

Boris Martinac Victor Chang Cardiac Research Institute, Sydney, Australia

**Structure and function of bacterial mechanosensitive channels:
An improved open channel model of MscL determined from FRET,
confocal microscopy, and simulation**

18:15 Bus Transportation to Yasaka-michi

Please get together in the lobby area after the last talk by Dr. Boris Martinac.

19:00 - 21:00 Mixer at Oblio Restaurant (see the map 2)

Welcome Speech

Jiro Usukura EcoTopia Science Institute, Nagoya University

January 28 8:45 - 19:00

Keynote Lecture 7 8:45 - 9:25 Chair: Madan Rao

Shinya Kuroda Dept. Biophysics and Biochemistry, University of Tokyo
Temporal coding of ERK and Akt signaling pathway

Coffee Break **9:25 - 9:45**

Keynote Lecture 8 9:45 - 10:25 Chair: Satyajit Mayor

Gerard Marriott Dept. of Bioengineering, University of California, Berkeley
Optical switches: New molecular tools for rapid and reversible manipulation of protein interactions and for high contrast fluorescence imaging

Seminar 4: 10:25 - 11:25 Chair: Gerard Marriott

Hiroaki Yokota, Yoshie Harada
iCeMS, Kyoto University
Single-molecule observation of DNA –helicase interactions

Makoto Kiso iCeMS, Kyoto University
Dept. of Applied Bioorganic Chemistry, Gifu University
Synthetic Ganglioside Probes: Versatile Tools for Bio-membrane Research

Eri Hayakawa International Research and Educational Institute for Integrated Medical Sciences (IREIIMS), Tokyo Women's Medical University
A Mycobacterium tuberculosis-Derived Lipoarabinomannan (LAM) Inhibits Membrane Fusion by Modulating Lipid Membrane Domains

Lunch **11:25 - 12:20**

Light lunch will be served on site

Keynote Lecture 9 12:20 - 13:00 Chair: Hideki Koyanaka

Alexander I. Kolesnikov

Oak Ridge National Laboratory

Neutron scattering studies of water confined in nano-channels

Coffee Break 13:00 - 13:20

Keynote Lecture 10 13:20 - 14:00 Chair: Yasushi Hiromi

Roger Morris

School of Biomedical and Health Sciences
King's College London

LRP1-prion protein interactions and the control of the neuronal surface

Seminar 5: 14:00 - 15:20 Chair: Samuel T. Hess

Toshihide Kobayashi RIKEN
Imaging lipid domains

Taroh Kinoshita Immunology Frontier Research Center, Osaka University
Lipid and glycan remodeling in GPI-anchored proteins

Toyoshi Fujimoto Nagoya University Graduate School of Medicine
Quantitative electron microscopy to observe nanoscale distribution of
membrane lipids

Yasushi Hiromi National Institute of Genetics
Generation of "pattern" within the axonal membrane

Coffee Break 15:20 - 15:40

Keynote Lecture 11 15:40 - 16:20 Chair: John Heuser

Ken P. Ritchie Dept. of Physics, Purdue University
Molecular Diffusion in E. coli

Keynote Lecture 12 16:20 - 17:00 Chair: Martin Alexander Schwartz

Samuel T. Hess Dept. of Physics and Astronomy
Institute for Molecular Biophysics, University of Maine
Localization Microscopy Reveals Nanoscale Details of Membrane Organization

17:15 - 18:55 Poster Session and Snack

The program is attached at the end of this booklet.
Beer, sake, soft drinks, and snacks will be served.

17:15 – 18:05 Odd number poster presentation

18:05 – 18:55 Even number poster presentation

19:00 Cab Transportation to Kusumi JST Lab

If you are interested in this tour, please get together in the lobby area.
It takes about 5 min to the lab.

19:15 - 21:00 Tour of Kusumi JST Lab

Demo-experiments will be performed by Koichiro Hirosawa and Takahiro Fujiwara.

January 29 8:45 - 13:00

8:45 - 13: 00

**Progress Report Symposium
Membrane Mechanisms Project (2005-2010)
ICORP, JST**

Akihiro Kusumi (PI) 15 min
Opening and introduction

Chair: Ken P. Ritchie

Satyajit Mayor (PI) 40 min
National Centre for Biological Sciences (NCBS)
The construction of membrane domains in living cell membranes: a role for cortical
actin activity

Takahiro Fujiwara 20 min
Hop diffusion of membrane lipids and proteins in the plasma membrane as directly
observed by high-speed single fluorescent-molecule tracking

Nobuhiro Morone (Collaborator) 15 min
National Center of Neurology and Psychiatry
National Institute of Neuroscience
Three-dimensional reconstruction of the membrane skeleton at the plasma
membrane interface by freeze-etch electron tomography

Coffee Break 10:15 - 10:35

Chair: Roger Morris

- Madan Rao (Collaborator) 35 min
National Centre for Biological Sciences (NCBS)
Active dynamics of cortical actin and mechanisms of molecular clustering
at the cell surface
- Ziya Kalay (Collaborator) 15 min
iCeMS, Kyoto University
The effects of spatial confinement on the reaction rates of macromolecules
in the plasma membrane
- John Heuser (Advisor) 25 min
iCeMS, Kyoto University
Dept. of Cell Biol. and Physiol., Washington Univ.
Murder and mayhem in brain cells, as caused by mesoscale prion-aggregates
- Rinshi Kasai 15 min
First determination of the dimer dissociation constant of GPCR
in the living cell plasma membrane
- Kenichi Suzuki 20 min
Mechanism for Raft-based signal transduction as studied by single-molecule
tracking
- Kenichi Suzuki 15 min
Lipid-stabilized homo-dimers of GPI-anchored proteins
based on ectodomain protein interactions
- Chieko Nakada 15 min
Recruitment of NMDA receptor to the synapse by the concerted actions of
exocytosis and lateral diffusion in the plasma membrane
- Akihiro Kusumi
Closing of the Progress Report

Closing of the Symposium

Masahiro Sokabe

Nagoya University Graduate School of Medicine
ICORP/SORST Cell Mechanosensing Project, JST, Nagoya Japan

Poster Session Program

January 28

17:15 - 18:55

Presentation Time

Odd Numbers 17:15-18:05

Even Numbers 18:05-18:55

Cabs for the Kusumi Lab Tour will start leaving at 19:00.
If you are interested in participating in the lab tour, please come to the lobby area of the hotel at 19:00.

Name	Affiliation
1. Hiromune Ando	iCeMS, Kyoto University Dept. of Applied Bioorganic Chemistry, Gifu University Synthesis and Functional Evaluation of Fluorescent glycolipid probes for the single molecular tracking of raft markers
2. Rajshri Joshi	National Institute of Genetics, SOKENDAI Dept. of Genetics Molecular addresses: motifs involved in compartment-specific localization of guidance receptors in Drosophila axons
3. Hiroko Yukinaga	Dept. of Pathology and Biology of Diseases Graduate School of Medicine, Kyoto University Cell sorting analysis of glioblastoma cells expressing FRET biosensors for GTPases
4. Michinori Matsuo	Laboratory of Cellular Biochemistry, Div. of Applied Life Science Graduate School of Agriculture, Kyoto University Mechanisms by which glia-derived apoE-containing lipoproteins stimulate axon elongation of CNS neurons
5. Reiko Kato	Laboratory of Cellular Biochemistry, Div. of Applied Life Science Graduate School of Agriculture, Kyoto University Lipid transporter ABCG1 affects lipid raft and alters amyloid processing

6. Masato Ishigami Laboratory of Cellular Biochemistry, Div. of Applied Life Science
Graduate School of Agriculture, Kyoto University
Purification and analysis of ATPase activity of human MDR1 expressed
in FreeStyle293cells

7. Yasuhisa Kimura Laboratory of Cellular Biochemistry, Div. of Applied Life Science
Graduate School of Agriculture, Kyoto University
Large scale production and purification of ATP-sensitive potassium channels
in cultured human cells

8. Kazuhiro Fujita Dept. of Computational Biology
Graduate School of Frontier Sciences, University of Tokyo
Decoupling of receptor and downstream signals in Akt pathway by its low-pass filter
characteristics

9. Yusuke Maeda Dept. of Immunoregulation
Research Institute for Microbial Diseases, Osaka University
GPHR is a novel anion channel critical for acidification and functions of the Golgi apparatus

10. Morihisa Fujita Dept. of Immunoregulation
Research Institute for Microbial Diseases, Osaka University
Regulation of GPI-anchored protein transport from the ER by GPI structural remodeling

11. Yoshiaki Yano and Katsumi Matsuzaki
Graduate School of Pharmaceutical Sciences, Kyoto University
FRET analysis of oligomerization of metabotropic glutamate receptor using novel
coiled-coil tag-probe labeling and spectral imaging

12. Shin-ichiro M. Nomura PRESTO-JST / iCeMS, Kyoto University
Artificial cell-models based on giant vesicles with gene expression system

13. Miho Yanagisawa Graduate School of Science, Kyoto university
Entropy-induced microphase separation on the cell-size liposome containing
PEG(polyethylene glycol)-cholesterol

14. Nobunari Sasaki iCeMS, Kyoto University
Sonic hedgehog regulates dendritic spine formation through a non-canonical pathway

15. Asami Makino Lipid Biology Laboratory, RIKEN Advanced Science Institute
Endoplasmic reticulum cholesterol controls both ApoB secretion and the formation of lipid droplets in cultured hepatocytes
16. Mitsuhiro Abe Lipid Biology Laboratory, RIKEN Advanced Science Institute
Role of Sphingomyelin in cytokinesis
17. Motohide Murate Lipid Biology Laboratory, RIKEN Advanced Science Institute
Nanoscale Analysis of Lateral and Transmembrane Distribution of Membrane Lipids
18. Nobuhiro Hayashi Dept. of Life Science
Graduate School of Bioscience and Biotechnology
Tokyo Institute of Technology
Cross-talk of signaling pathways regulated by myristoylation, a multifunctional posttranslational protein modification
19. Tsuyoshi Endo, Nobuhiro Hayashi
Dept. of Life Science
Graduate School of Bioscience and Biotechnology
Tokyo Institute of Technology
Development of new technique for research of lipid rafts; high- throughput proteomics and site-selective fluorescence labeling of proteins
20. Satoshi Hosoya, Nobuhiro Hayashi
Dept. of Life Science, Tokyo Institute of Technology
Intervention in membrane signaling system of immunocyte by HIV Nef through the myristoylation
21. Eriko Sumiya Institute for Chemical Research, Kyoto University
Bisbromoamide, a marine natural product, induces morphological changes in cultured cells
22. Kishio Furuya Cell Mechanosensing Project, ICORP/SORST-JST
Multi pathways of ATP-release in mammary epithelial cells revealed by ATP real-time imaging
23. Takeshi Nomura Cell Mechanosensing Project, ICORP/SORST-JST
Activation mechanism of bacterial mechanosensitive channel MscL by chlorpromazine

24. Yasuyuki Sawada Dept. of Physiology
Nagoya University Graduate School of Medicine
Analysis of Opening Process of the E-coli Mechanosensitive Channel MscL Using Molecular Dynamics Simulations
25. Azumi Yoshimura¹, Jiro Usukura²
¹iCeMS, Kyoto University
²EcoTopia Science Institute, Nagoya University
Interactions between microtubule and actin filaments beneath the plasma membrane
26. Yoshitaka Kimori, Nobuhiro Morone
National Center of Neurology and Psychiatry
National Institute of Neuroscience
Structural analysis for cell membrane domain by electron microscopic image processing
27. Tomoki Kato, Mikio Furuse, Kazunori Kawasaki
AIST/Kobe University
Tight junction-like structures by claudin-3 protein expressed on plasma membranes of L cells: as studied by quick-freezing-replica and HADDF-STEM
28. Kazunori Kawasaki, Emiko Kobayashi, Tomoki Kato, Yoshikazu Tahara Yoshikazu, Yutaro Hayashi, Kazuhiro Aoyama
AIST/Kyorin University/FEI Japan
Three-dimensional images of intramembrane particles of Na⁺/K⁺-ATPase by using ultra-high vacuum freeze-replica and HADDF-STEM tomography
29. Satomi Mitsuhashi¹, Hideyuki Hatakeyama², Minako Karahashi³, Tomoko Koumura³, Satoru Noguchi¹, Yukiko K Hayashi¹, Ikuya Nonaka¹, Yasuhito Nakagawa³, Yu-ichi Goto², Ichizo Nishino¹.
¹Dept. of Neuromuscular Res., NIN, NCNP
²Dept. of Mental Retardation and Birth Defect Res., NIN, NCNP
³School of Pharmaceutical Sciences, Kitasato University
Choline kinase defect causes muscular dystrophy and mitochondrial dysfunction
30. Masahiro Nakajima Center for Micro-Nano Mechatronics
Graduate School of Engineering
Nagoya University
Quantitative electric microinjection into phospholipid-coated micro-droplet using nano-pipettes

31. Takahiro K. Fujiwara, Shinji Takeuchi, Yosuke Nagai, Kazuhide Hanaka, Kokoro Iwasawa, Kenichi G.N. Suzuki, and Akihiro Kusumi

ICORP-JST/CeMI, iCeMS, Kyoto University

Direct observation of hop diffusion of single lipid and protein molecules in the plasma membrane by high-speed single fluorescent-molecule tracking

32. Kokoro Iwasawa, Rinshi S. Kasai, Koichiro M. Hirose, Kenichi G. N. Suzuki, Yoshihiro Miwa¹, and Akihiro Kusumi

ICORP-JST/Kyoto University

¹Graduate School of Comprehensive Human Sciences,
University of Tsukuba

Recruitment and mobility of MARCKS on the inner surface of the plasma membrane: a single-molecule tracking study

33. Kenichi G. N. Suzuki, Takahiro K. Fujiwara, Yuki M. Shirai, and Akihiro Kusumi

ICORP-JST/SAKIGAKE-JST/Kyoto University

Both GPI-anchored proteins and non-raft lipids undergo similar rapid hop diffusion in the plasma membrane

34. Ikuko Koyama-Honda, Yuri L. Nemoto, Min Xie, Koji Tsukamoto, Yukiko Shimada¹, Yoshiko Ohno-Iwashita², and Akihiro Kusumi

ICORP-JST/Kyoto university/JSPS,

¹Tokyo Metropolitan Institute of Gerontology

²Iwaki Meisei University

Dynamic co-localization and co-diffusion of cholesterol and cholesterol-enriched domains in the plasma membrane revealed by single-molecule tracking

35. Ikuko Koyama-Honda, Takahiro K. Fujiwara, Rinshi S. Kasai, Kenichi G. N. Suzuki, Miyako Yahara, Eriko Kajikawa, Akihiko Yoshimura¹, and Akihiro Kusumi

ICORP-JST/Kyoto university/JSPS

¹School of Medicine, Keio University / CREST-JST

Facilitation of the signal transfer across the plasma membrane bilayer by the raft-based lipid interactions: a dual-color single-molecule tracking study

36. Kenji A. K. Tanaka, Takahiro K. Fujiwara, Kenichi G. N. Suzuki, Yukihiro S. Kudo, Yuki M. Shirai, Satyajit Mayor, and Akihiro Kusumi

ICORP-JST/Kyoto University

Reexamining lipid microdomains and compartments in muscle cell membranes by single phospholipid molecule tracking

37. Kenji A. K. Tanaka, Kenichi G. N. Suzuki, Shusaku T. Shibutani, Manami S. H. Miyahara, Hisae Tsuboi, Miyako Yahara, Akihiko Yoshimura, Satyajit Mayor, Takahiro K. Fujiwara, and Akihiro Kusumi
ICORP-JST/Kyoto University
Membrane molecules remain mobile even after chemical fixation: a single-molecule tracking study
38. Rinshi S. Kasai, Kenichi G. N. Suzuki, Eric R. Prossnitz¹, Ikuko Koyama-Honda, Takahiro K. Fujiwara, and Akihiro Kusumi
ICORP-JST/Kyoto University
¹University of New Mexico Health Science Center, USA
Dynamic GPCR monomer-dimer equilibrium: a single-molecule tracking study
39. Chieko Nakada, Hiroko Hijikata, Yuri L. Nemoto, Hiroto Yoshida, Shigeo Okabe¹, Takahiro Fujiwara, and Akihiro Kusumi
ICORP-JST/Kyoto University
¹The University of Tokyo School of Medicine
Recruitment of NMDA receptor to the neuronal synapse: cooperative actions of exocytosis and lateral diffusion in the cell membrane
40. Koichiro M. Hirosawa, Kenta J. Yoshida, Kenichi G. N. Suzuki, Takahiro K. Fujiwara, Miyako Yahara, and Akihiro Kusumi
ICORP-JST/Kyoto University
Mechanism of Lyn recruitment to the IgE receptor cluster: dual color single-molecule tracking study



Kusumi JST Lab

Kusumi Lab @Kyoto Univ.
075-751-4112

Hotel I/27-29
Fujita Kyoto

会場から最寄りのコンビニ
nearest convenience store

Media cafe Popeye
next to Kyoto Royal Hotel
located underground (B1)
cafeteria seat
280 Yen / hour
Internet
cafe

500 m



500 m