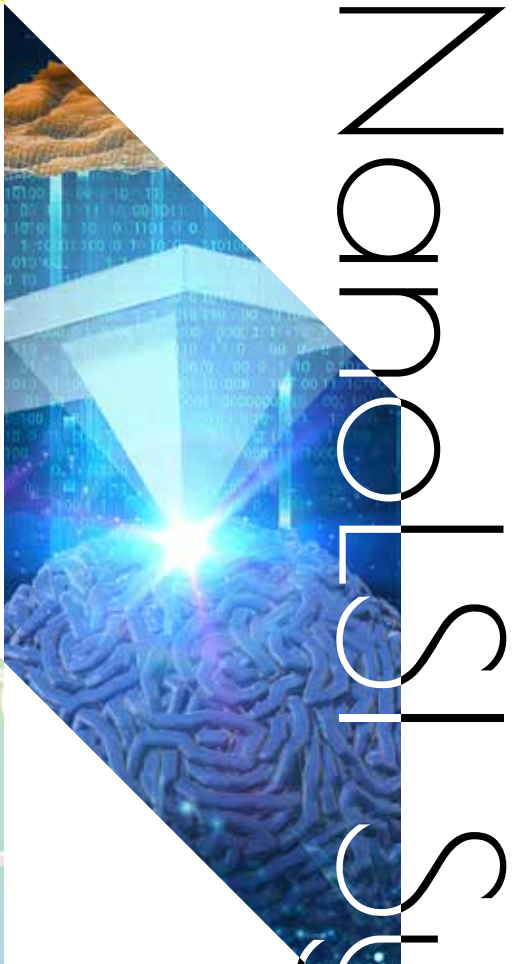
5th NanoLSI Symposium



NanoLSI
WPI KANAZAWA UNIVERSITY

Understanding Nanoscale Biological Processes in the Cells

March
1-2,2022

Institute for Frontier Science Initiative

Cancer Research Institute
Kanazawa University

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KANAZAWA UNIVERSITY

PROGRAM

Understanding Nanoscale Biological Processes in the Cells

	Day	JST	SGT	CET	GMT	Speaker	Title	Page
		3:00 PM	2:00 PM	7:00 AM	6:00 AM	Takeshi FUKUMA Director, WPI-NanoLSI	Opening remarks	

Session1

Nanometrology
Chair: Noriyuki KODERA Professor, WPI-NanoLSI

	Day	JST	SGT	CET	GMT	Speaker	Title	Page
1	MAR 1 (Tue)	3:15 PM	2:15 PM	7:15 AM	6:15 AM	Takeshi FUKUMA Professor WPI-NanoLSI	Visualizing Intracellular Nanostructures of Living Cells by Nanoendoscopy-AFM	P3
2		3:45 PM	2:45 PM	7:45 AM	6:45 AM	Yanjun ZHANG Associate Professor WPI-NanoLSI	Scanning Ion Conductance Microscopy-Based Nanoprobe for Biosensing at Single-Cell and Single-Molecule Level	P4
3		4:15 PM	3:15 PM	8:15 AM	7:15 AM	Lorena REDONDO-MORATA Senior Researcher INSERM / Institut Pasteur de Lille	Non-Equilibrium Dynamics and Nanomechanics of Lipid Membranes	P5
4		4:45 PM	3:45 PM	8:45 AM	7:45 AM	George R. HEATH University Academic Fellow University of Leeds	Localization Atomic Force Microscopy	P6
		5:15 PM	4:15 PM	9:15 AM	8:15 AM	Break (15 min.)		

Session2

Computational Science I
Chair: Alexander MIKHAILOV Professor, WPI-NanoLSI

	Day	JST	SGT	CET	GMT	Speaker	Title	Page
1	MAR 1 (Tue)	5:30 PM	4:30 PM	9:30 AM	8:30 AM	Carsten BETA Professor University of Potsdam	Insights into Amoeboid Motility, Combining Experiments, Data Analysis, and Modeling	P7
2		6:00 PM	5:00 PM	10:00 AM	9:00 AM	Takashi SUMIKAMA Assistant Professor WPI-NanoLSI	Movies of Binding and Unbinding of Biomolecules and Analyses on Them	P8
3		6:30 PM	5:30 PM	10:30 AM	9:30 AM	Holger FLECHSIG Assistant Professor WPI-NanoLSI	Simulation Atomic Force Microscopy	P9

Session3

Life Science
Chair: Rikinari HANAYAMA Professor, WPI-NanoLSI

	Day	JST	SGT	CET	GMT	Speaker	Title	Page
1	MAR 2 (Wed)	3:00 PM	2:00 PM	7:00 AM	6:00 AM	Hiroshi KAWASAKI Professor Kanazawa University	Mechanisms Underlying the Development and Evolution of the Mammalian Cerebral Cortex	P10
2		3:30 PM	2:30 PM	7:30 AM	6:30 AM	Makoto SATO Professor Kanazawa University	The Wave of Differentiation Orchestrates Neurogenesis and Column Formation in the Drosophila Brain	P11
3		4:00 PM	3:00 PM	8:00 AM	7:00 AM	Takeshi IMAI Professor Kyushu University	Automatic Reconstruction of Neuronal Circuits with Super-Multicolor Labeling	P12
4		4:30 PM	3:30 PM	8:30 AM	7:30 AM	Hiroki R. UEDA Professor University of Tokyo	Systems Biology of Mammalian Sleep/Wake Cycles: Phosphorylation Hypothesis of Sleep	P13
		5:00 PM	4:00 PM	9:00 AM	8:00 AM	Break (15 min.)		

Session4

Computational Science II
Chair: Satoru OKUDA Associate Professor, WPI-NanoLSI

	Day	JST	SGT	CET	GMT	Speaker	Title	Page
1	MAR 2 (Wed)	5:15 PM	4:15 PM	9:15 AM	8:15 AM	Damien HALL Assistant Professor WPI-NanoLSI	Computational Modeling at the Nano Life Science Interface	P14
2		5:45 PM	4:45 PM	9:45 AM	8:45 AM	Rakesh DAS Research Fellow National University of Singapore	Enzymatic Activity on Chromatin Organization – A Polymer Model Study	P15
3		6:15 PM	5:15 PM	10:15 AM	9:15 AM	Masashi TACHIKAWA Associate Professor Kyoto University	Physical Model Simulations for Understanding the Structures of Organelle Shapes	P16
		6:45 PM	5:45 PM	10:45 AM	9:45 AM	Alexander MIKHAILOV Professor, WPI-NanoLSI	Closing remarks	

CV & Abstracts

Takeshi FUKUMA

Director/Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan

Contact : fukuma [at sign] staff.kanazawa-u.ac.jp
Please replace [at sign] with @.

Research Interests

Atomic Force Microscopy, Nanoscale Measurement Technologies, Electrical Engineering, Interfacial Sciences, Life Sciences, Electrochemistry

Academic Background

2003		Doctor of Engineering, Department of Electronic Science and Engineering, Kyoto University

Professional Careers

2017 - present		Director/Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University
2017 - present		Professor, Faculty of Frontier Engineering, Kanazawa University
2012 - 2017		Professor, Faculty of Electronic Eng. and Computer Sci., Kanazawa University
2007 - 2012		Associate Professor, Frontier Science Organization, Kanazawa University
2005 - 2007		Senior Scientist, Physics Department, Trinity College Dublin (Ireland)
2001 - 2005		Research Fellow, Kyoto University

Scientific Activities

2003 - present		Instrumentation and applications of atomic-resolution liquid-environment AFM.
1999 - 2003		Instrumentation and applications of atomic-resolution ultrahigh vacuum AFM.

Honors

2018		15th JSPS Prize, Japan Society for the Promotion of Science
2017		Hokkoku Bunka Award, Hokkoku Shinbun
2011		The Young Scientists' Prize, The Commendation for Science and Technology by the MEXT

Publications

1. Penedo, M.; Miyazawa, K.; Okano, N.; Furusho, H.; Ichikawa, T.; Alam Mohammad, S.; Miyata, K.; Nakamura, C.; Fukuma, T., Visualizing intracellular nanostructures of living cells by nanoendoscopy-AFM. ***Sci. Adv***. 2021, 7 (52), eabj4990.

2. Fukuma, T.; Garcia, R., Atomic- and Molecular-Resolution Mapping of Solid-Liquid Interfaces by 3D Atomic Force Microscopy. ***ACS Nano*** 2018, 12 (12), 11785-11797.

Visualizing Intracellular Nanostructures of Living Cells by Nanoendoscopy-AFM

Takeshi FUKUMA

Director/Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan

Atomic force microscopy (AFM) is the only method that allows label-free imaging of nanoscale biomolecular dynamics and hence plays a critical role in solving biological questions that cannot be addressed only by the major bio-imaging tools (e.g., fluorescence and electron microscopy). However, such imaging is possible only for the systems either extracted from a cell or reconstructed on a solid substrate, and thus nanodynamics inside living cells largely remain inaccessible with the current nano-imaging techniques. Here we overcome this limitation by nanoendoscopy-AFM, where a specially designed long nanoprobe is inserted into a living cell and scanned in two- or three-dimension (2D/3D). We present direct imaging of the whole cell structure, 3D configurations of the actin fibers, and 2D nano-dynamics of the inner scaffold of the bottom plasma membrane. Importantly, our fluorometric assay reveals that such imaging does not give detectable changes in the cell viability. Unlike previous AFM techniques using ultrasonic waves or elastic responses, this method allows an AFM probe to directly access the target intra-cellular components so that we can exploit full range of AFM capabilities such as high-resolution imaging, nanomechanical mapping, and molecular recognition. These features should greatly expand the range of intra-cellular structures and properties observable in a live cell.

References

[1] Penedo, M.; Miyazawa, K.; Okano, N.; Furusho, H.; Ichikawa, T.; Alam Mohammad, S.; Miyata, K.; Nakamura, C.; Fukuma, T., Visualizing intracellular nanostructures of living cells by nanoendoscopy-AFM. *Science Advances* **2021,** *7* (52), eabj4990.

[2] Yurtsever, A.; Yoshida, T.; Badami Behjat, A.; Araki, Y.; Hanayama, R.; Fukuma, T., Structural and mechanical characteristics of exosomes from osteosarcoma cells explored by 3D-atomic force microscopy. *Nanoscale* **2021,** *13* (13), 6661-6677.

[3] Penedo, M.; Shirokawa, T.; Alam, M. S.; Miyazawa, K.; Ichikawa, T.; Okano, N.; Furusho, H.; Nakamura, C.; Fukuma, T., Cell penetration efficiency analysis of different atomic force microscopy nanoneedles into living cells. *Sci. Rep.* **2021,** *11* (1), 7756.

[4] Fukuma, T.; Garcia, R., Atomic- and Molecular-Resolution Mapping of Solid-Liquid Interfaces by 3D Atomic Force Microscopy. *ACS Nano* **2018,** *12* (12), 11785-11797.

[5] Fukuma, T.; Ueda, Y.; Yoshioka, S.; Asakawa, H., Atomic-scale distribution of water molecules at the mica-water interface visualized by three-dimensional scanning force microscopy. *Phys. Rev. Lett.* **2010,** *104* (1), 016101.

Yanjun ZHANG

Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan

Contact : yanjunzhang [at sign] staff.kanazawa-u.ac.jp
Please replace [at sign] with @.

Scanning Ion Conductance Microscopy-Based Nanoprobe for Biosensing at Single-Cell and Single-Molecule Level

Yanjun ZHANG

Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan

Research Interests

Single Cellular Biology, Biosensing, 3D imaging, Single Molecular Detecting

Academic Background

2002 - 2005 | PhD, Imperial College London, UK

Professional Careers

2020 - present Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2014 - 2020 Senior Research Associate, Imperial College London, UK
2007 - 2014 Professor/PI, Tianjin Medical University General Hospital, China
2005 - 2007 Postdoctoral Research Associate, Imperial College London, UK
2002 - 2005 Research Assistant, Imperial College London, UK

Studies of our group focus on development of SICM and its applications to single-cell imaging. We have developed a non-contact single-cell mechanical probing method with hopping SICM nanoprobe. Moreover, we have developed a range of functionalized SICM-based nanoprobes for single cell and single molecule biosensing. Importantly, these functional nanoprobes can be integrated with SICM scanning and enable us to perform 3D mapping of extracellular and intracellular chemicals with higher spatial and temporal resolution. These new-developed SICM-based nanoprobes have been successfully used for pH and ROS biosensing of individual living cancer cells. We have also developed a new class of SICM-based nanoprobe FET sensors dubbed nexFET (nanopore extended Field Effect Transistor) that combines the advantages of non-contact SICM scanning, nanopore single molecule sensing, FET, and recognition chemistry. These SICM-based nexFETs enable label-free and real-time biosensing with heightened sensitivity and selectivity, and down to the single-molecule level.

Publications

1. Yanjun Zhang*, et al. High-resolution Label-free 3D Mapping of Extracellular pH of Single Living Cells. Nature Communications. 2019, 10:5610.
2. Ren Ren, Yanjun Zhang*, et al. Nanopore Extended Field-effect Transistor for Selective Single-molecule Biosensing. Nature Communications. 2017, 8:586.
3. Yanjun Zhang, et al. Spearhead Nanometric Field-Effect Transistor Sensors for Single-Cell Analysis. ACS Nano. 2016, 10 (3):3214–3221.

References

[1] Yanjun Zhang*, et al. High-resolution Label-free 3D Mapping of Extracellular pH of Single Living Cells. Nature Communications. 2019, 10:5610.

[2] Ren Ren, Yanjun Zhang*, et al. Nanopore Extended Field-effect Transistor for Selective Single-molecule Biosensing. Nature Communications. 2017, 8:586.

[3] Yanjun Zhang, et al. Spearhead Nanometric Field-Effect Transistor Sensors for Single-Cell Analysis. ACS Nano. 2016, 10 (3):3214–3221.

Lorena REDONDO-MORATA

Senior Researcher, Inserm / Institut Pasteur de Lille, France

Contact : lorena.redondo-morata [at sign] inserm.fr
Please replace [at sign] with @.

Research Interests

In vitro membrane reconstituted systems, lipid protein interactions, lipid biophysics, atomic force microscopy, (nano)mechanics.

Academic Background

2008 - 2012	PhD in Biotechnology, University of Barcelona (Spain)
2007 - 2008	Master in Molecular Biotechnology, University of Barcelona (Spain)
2001 - 2007	Degree in Pharmacy, University of Barcelona (Spain)

Professional Careers

2018 - **present**	Senior Researcher Inserm, Institut Pasteur de Lille (Lille, France)
2013 - 2017	Postdoctoral Fellow, Inserm, Simon Scheuring's lab (Marseille, France)
2011 - 2013	Assistant professor, Physical Chemistry Department, University of Barcelona (Spain)
2007 - 2011	Research grant of the Institute for Bioengineering of Catalonia (IBEC, Spain)

Scientific Activities

2021 - **present**	Council member of the French Biophysical Society

Honors

2017	Distinguished Referee, European Physical Journal
2016	SBE-33 award (Spanish Biophysical Society award for young Biophysicist with age under 33)
2013	Journal of Molecular Recognition award
2012	Cum laude, University of Barcelona

Publications

1. Perissinotto, F.; Janel, S.; Lopez-Alonso, J.; Dupres, V.; Bechinger, B.; Lafont, F.; Redondo-Morata, L Biophys. J., 120 (3), 191A, 2021.
2. Redondo-Morata, L; Losada-Perez, P.; Giannotti, M.I.; ISBN: 9780128210215, Elsevier, 2020.
3. Mierzwa, B.*; Chiaruttini, N.*; Redondo-Morata, L.*; Nature Cell Biology, 19(7):787-798, 2017.

Non-Equilibrium Dynamics and Nanomechanics of Lipid Membranes

Lorena REDONDO-MORATA
Senior Researcher, Inserm / Institut Pasteur de Lille, France

In lipid membranes, the ultimate lipid phase coexistence to be fully understood are transient nanodomains, often (confusedly) referred to as lipid rafts [1]. Based on the current knowledge, microdomains in equilibrium are no longer considered suitable models for the biological structure that rafts represent. Multiscale spatiotemporal measurements of the membrane mechanical properties can help to experimentally address different scenarios where membrane micro- and nano-domain formation finds theoretical support. Atomic Force Microscopy (AFM)-based Force Spectroscopy can resolve coexistence of domains at concentrations where height differences at domain boundaries are not detectable [2], providing an ideal approach to investigate the mechanical properties of lipid bilayers at the nanoscale, their elastic constants [3] but also their plastic deformation and rupture [2]. High-Speed AFM imaging, in turn, provides us information about the dynamics of the domain boundaries. Non-equilibrium fluctuations by tuning membrane (local and global) environment actively controlling curvature, leaflet asymmetry, solid support topography and addition of external components would help complete the manifold where domain formation occurs. Here, we will discuss two examples of non-equilibrium membrane fluctuations. First, the in situ conversion of sphingomyelin to ceramide. Ceramide is produced in cells from sphingomyelin by means of the enzymatic activity of endogenous sphingomyelinase, impacting in the physical chemical properties of the membrane, inducing changes in the curvature, phase, segregation, and order. Last, we will discuss about the effect of Mag2 and PGLa, two antimicrobial peptides which, upon their interaction with biomembranes, they have been shown to gradually insert into the lipid bilayer as heterodimer clusters inducing several membrane perturbations as the alteration of lipid packing, pore openings and membrane disintegration.

References

[1] F. M. Goñi, Chemistry and Physics of Lipids, 218 (2019), p. 34.

[2] L. Redondo-Morata et al., Langmuir, 28 (2012), p. 12851.

[3] L. Redondo-Morata, R. L. Sanford, O. S. Andersen et al, Biophysical Journal, 111 (2016), p. 363.

George R. HEATH

University Academic Fellow, School of Physics & Astronomy, University of Leeds, UK

Contact : g.r.heath [at sign] leeds.ac.uk
Please replace [at sign] with @.

Research Interests

High-Speed AFM, membrane proteins, protein dynamics, lipid membranes.

Academic Background

2010 - 2015	PhD - School of Physics and Astronomy, University of Leeds. Supervisors: Prof Stephen Evans and Dr Simon Connell
2006 - 2010	Integrated Masters and BSc Physics - School of Physics and Astronomy, University of Leeds.

Professional Careers

2019 - present	University Academic Fellow, School of Physics & Astronomy, University of Leeds.
2017 - 2019	Postdoctoral Associate - Prof Simon Scheuring. Department of Anaesthesiology, Cornell University, Weill Cornell, US.
2014 - 2017	Postdoctoral Fellow - Prof Lars Jeuken, School of Biomedical Sciences, University of Leeds.

Publications

1. Sanganna Gari RR, Montalvo-Acosta JJ, **Heath G R,** Jiang Y, Gao X, Nimigea CM, Chipot C, Scheuring S. Correlation of membrane protein conformational and functional dynamics. *Nat. Commun.* 12, 4363, (2021).
2. **Heath G R,** Kots E., Robertson J L, Lansky S, Khelashvili G, Weinstein H & Scheuring S. Localization Atomic Force Microscopy. *Nature*, 594, 385–390, (2021).
3. Matin T R*, **Heath G R***, Huysmans, G H, Boudker O & Scheuring S. Millisecond dynamics of an unlabeled amino acid transporter. *Nat. Commun.* 11(1), 1-11, (2020).
4. **Heath G R** & Scheuring S. Advances in high-speed atomic force microscopy (HS-AFM) reveal dynamics of transmembrane channels and transporters. *Curr. Opin. Struct. Biol.* 57, (2019).
5. **Heath, G R** & Scheuring S. High-speed AFM Height Spectroscopy Reveals µs-dynamics of Unlabeled Biomolecules. *Nat. Commun.* 9, 4983, (2018).
6. **Heath G R**, Harrison P L, Strong P N, Evans S D, Miller K. Visualization of diffusion limited antimicrobial peptide attack on supported lipid membranes. *Soft Matter.* 14, 29, (2018).

Localization Atomic Force Microscopy

George R. HEATH

University Academic Fellow, School of Physics & Astronomy, University of Leeds, UK

Understanding structural dynamics of biomolecules at the single-molecule level is vital to advancing our knowledge of molecular mechanisms. Currently, there are few techniques that can capture dynamics at the sub-nanometre scale and in physiologically relevant conditions. Atomic force microscopy (AFM) has the advantage of analysing unlabelled single molecules in physiological buffer and at ambient temperature and pressure, but its resolution can limit the assessment of conformational details of biomolecules. Here I will present localization AFM (LAFM), a technique developed to overcome current resolution limitations. By applying localization image reconstruction algorithms to peak positions in high-speed AFM and conventional AFM data, we increase the resolution beyond the limits set by the tip radius, and resolve single amino acid residues on soft protein surfaces in native and dynamic conditions. LAFM enables the calculation of high-resolution maps from either images of many molecules or many images of a single molecule acquired over time, facilitating single-molecule structural analysis. LAFM is a post-acquisition image reconstruction method that can be applied to any biomolecular AFM dataset.

References

[1] Heath G R, Kots E., Robertson J L, Lansky S, Khelashvili G, Weinstein H & Scheuring S. Localization Atomic Force Microscopy. *Nature*, 594, 385–390, (2021).

Carsten BETA

Professor, Biological Physics, University of Potsdam, Germany

Contact : beta [at sign] uni-potsdam.de
Please replace [at sign] with @.

Research Interests

Cell motility, actin dynamics, bacterial swimming,
live-cell imaging, microfluidics, nonlinear dynamics, pattern formation, reaction-diffusion systems, active particles

Academic Background

2004	Doctorate (Dr. rer. nat.), Physical Chemistry, Fritz-Haber-Institut (MPG) and Freie Universität Berlin (advisor Prof. Dr. Gerhard Ertl)
1996 - 2001	Studies in chemistry, Universität Tübingen, Universität Karlsruhe, Ecole Normale Supérieure Paris, Diploma (advisor Prof. Dr. Marie Farge)

Professional Careers

10/2017 - present	Professor of Biological Physics (full professor), U Potsdam
2009 - 2017	Professor of Biological Physics (associate professor), U Potsdam
2007 - 2009	Juniorprofessor of Biological Physics (assistant professor), U Potsdam
2005 - 2007	Group Leader, Max Planck Institute for Dynamics und Self-Organization, Göttingen
2005	Post-doctoral research fellow, Cornell University and University of California, San Diego, USA

Scientific Activities

10/2021 - present	Member of the council of the German Physical Society
10/2020 - present	Member of the steering committee, Collaborative Research Center SFB 1294 “Data Assimilation”
2012 - 2014	Head of the Institute of Physics and Astronomy, U Potsdam

Honors

2020	Excellence in Teaching Award, Faculty of Science, U Potsdam
1997 - 2001	Fellow of the Studienstiftung des Deutschen Volkes (German Academic Merit Foundation)
2000	Procter & Gamble Förderpreis

Publications

1. Flemming, S., Font, F., Alonso, S., and Beta, C. (2020). How cortical waves drive fission of motile cells. PNAS, 117:6330-6338.

2. Alirezaeizanjani, Z., Großmann, R., Pfeifer, V., Hintsche, M., Beta, C. (2020). Chemotaxis strategies of bacteria with multiple run modes. Sci. Adv., 6:eaaz6153.

3. Westendorf, C., Negrete, J., Bae, J.A., Sandmann, R., Bodenschatz, E., and Beta, C. (2013). Actin cytoskeleton of chemotactic amoebae operates close to the onset of oscillations. PNAS, 110:3853-3858.

Insights into Amoeboid Motility, Combining Experiments, Data Analysis, and Modeling

Carsten BETA

Professor, Biological Physics, University of Potsdam, Germany

The actin-driven motility of eukaryotic cells plays a central role in many biological functions such as wound healing, embryonic morphogenesis, and cancer metastasis. It is driven by coherent patterns of activity in the actin cytoskeleton. Here, we present experimental results demonstrating the rich diversity of wave patterns in the actin cortex of motile amoeboid cells. We show that actin waves mediate switches between different modes of motility, an amoeboid and a more persistent, keratocyte-like migratory mode, and may also trigger cell cycle-independent cytofission events. We analyzed our data using a Python-based toolbox that we have developed to quantify the shape dynamics of motile cells. Our experimental findings are rationalized in the framework of a noisy bistable reaction-diffusion model that mimics the evolution of cortical patterns and couples to a dynamic phase field to take the cell shape dynamics into account.

References

[1] S. Flemming , F. Font, S. Alonso, and C. Beta, How cortical waves drive fission of motile cells, PNAS, 6337, Vol 117, No. 12 (2020).

[2] S. Alonso, M. Stange, C. Beta, Modeling random crawling, membrane deformation and intracellular polarity of motile amoeboid cells, PLoS ONE 13(8), e0201977 (2018).

[3] D Schindler, T Moldenhawer, M Stange, V Lepro, C Beta, M Holschneider, and W. Huisinga, Analysis of protrusion dynamics in amoeboid cell motility by means of regularized contour flows, PLoS Computational Biology 17 (8), e1009268 (2021).

[4] E Moreno, S Flemming, F Font, M Holschneider, C Beta, S Alonso, Modeling cell crawling strategies with a bistable model: From amoeboid to fan-shaped cell motion, Physica D: Nonlinear Phenomena 412, 132591 (2020).

Takashi SUMIKAMA

Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
PRESTO Researcher, Japan Science and Technology Agency, Japan

Contact : sumi [at sign] staff.kanazawa-u.ac.jp
Please replace [at sign] with @.

Research Interests

Chromosome structure, Polymer simulation, Molecular dynamics simulation, Analyzing molecular motions

Academic Background

2003 - 2008	Graduate School of Science, Nagoya University
1997 - 2003	Department of Chemistry, Nagoya University

Professional Careers

2020 - present	Researcher, PRESTO, Japan Science and Technology Agency
2020 - present	Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University
2018 - 2020	Postdoctoral Fellow, Kanazawa University
2010 - 2018	Assistant Professor, University of Fukui
2008 - 2010	Postdoctoral Fellow, Institute for Molecular Science
2008 - 2008	Postdoctoral Fellow, Nagoya University

Scientific Activities

2018	Advisory committee member of the Biophysical Society of Japan

Honors

2018	Hiroshi and Aya Irisawa Memorial Promotion Award for Young Physiologists from the Physiological Society of Japan

Publications

1. Mita, Sumikama (co-first), Iwamoto, Matsuki, Shigemi, Oiki, *Proc. Natl. Acad. Sci. USA* 118, e2017168118 (2021).
2. Sumino, Sumikama (co-first), Uchihashi, Oiki, *Sci. Adv.* 5, eaax0495 (2019).
3. Ajito, Ueno, Kim, Sumikama (co-first), *J. Am. Chem. Soc.* 140, 13793 (2018).
4. Sumikama, Oiki, *J. Am. Chem. Soc.* 138, 10284 (2016).

Movies of Binding and Unbinding of Biomolecules and Analyses on Them

Takashi SUMIKAMA
Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
PRESTO Researcher, Japan Science and Technology Agency, Japan

Repeated binding and unbinding of biomolecules are the basis of cellular function at the nanoscale. For example, the binding and unbinding of actin-myosin complex is essential for muscle contraction [1], and that of ions and ion channels is the molecular basis of electrical conduction in the nervous system [2]. Accordingly, one of the most effective ways to fundamentally understand biological functions is to capture movies filming such dynamics of molecules and analyze their trajectories. My first topic would be selective ion transport through the K^+ channel, in which the movies of repetitive ion binding and unbinding were taken by the molecular dynamics (MD) simulation. The selectivity simulated was found to be more than two orders of magnitude smaller than expected but was comparable with the electrophysiological experiments [3]. The second topic will be repetitive binding and unbinding of a toxin from a scorpion to the K^+ channel filmed by the high-speed atomic force microscopy (HS-AFM). A theoretical analysis using the kinetic model concluded that the toxin binds to the channel almost solely via the induced-fit mechanism [4]. The last topic would be a collaboration between the MD simulation and HS-AFM experiment to reveal the molecular mechanism of lipid digestion by phospholipase, in which the MD simulation reproduced the structure of phospholipase bound to the membrane observed in the HS-AFM.

References

[1] Kodera, Yamamoto, Ishikawa, Ando, Video imaging of walking myosin V by high-speed atomic force microscopy. *Nature* 468, 72 (2010).

[2] Sumikama, Oiki, Digitalized K^+ Occupancy in the Nanocavity Holds and Releases Queues of K^+ in a Channel. *J. Am. Chem. Soc.* 138, 10284 (2016).

[3] Mita, Sumikama (co-first), Iwamoto, Matsuki, Shigemi, Oiki, Conductance selectivity of Na^+ across the K^+ channel via Na^+ trapped in a tortuous trajectory. *Proc. Natl. Acad. Sci. USA* 118, e2017168118 (2021).

[4] Sumino, Sumikama (co-first), Uchihashi, Oiki, High-speed AFM reveals accelerated binding of agitoxin-2 to a K^+ channel by induced fit. *Sci. Adv.* 5, eaax0495 (2019).

Holger FLECHSIG

Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan

Contact : flechsig [at sign] staff.kanazawa-u.ac.jp
Please replace [at sign] with @.

Research Interests

Biophysics, Computational biology, Protein dynamics, Allostery, Simulation AFM, 3D reconstruction

Academic Background

2011 | Doctoral degree, Dr. rer. nat. | *Physics* at Fritz Haber Institute of the Max Planck Society Berlin, Technical University Berlin

2007 | Diploma degree | *Physics, Theoretical physics* at Free University Berlin

Professional Careers

2018 - present | Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University

2017 - 2018 | Researcher, Department of Mathematical and Life Science, Hiroshima University, and Department of Information Sciences, Ochanomizu University

2015 - 2017 | Assistant Professor, Department of Mathematical and Life Science, Hiroshima University

2011 - 2015 | PostDoc, Department of Physical Chemistry, Fritz Haber Institute of the Max Planck Society, and Department of Mathematical and Life Science, Hiroshima University

Publications

1. R. Amyot, A. Marchesi, C.M. Franz, I. Casuso, H. Flechsig. *Simulation atomic force microscopy for atomic reconstruction of biomolecular structures from resolution-limited experimental images.* **PLoS Comput Biol**, 2022

2. R. Amyot, H. Flechsig. *BioAFMviewer: an interactive interface for simulated AFM scanning of biomolecular structures and dynamics.* **PLoS Comput Biol** 16, e1008444, 2020

3. D. Loutchko, H. Flechsig. *Allosteric communication in molecular machines via information exchange: what can be learned from dynamical modeling.* **Biophys Rev** 12, 443-452, 2020

4. H. Flechsig, A.S. Mikhailov. *Simple mechanics of protein machines.* **J Roy Soc Interface** 16, 20190244, 2019

5. H. Flechsig. *Design of elastic networks with evolutionary optimized long-range communication as mechanical models of allosteric proteins.* **Biophys J** 113, 558-571, 2017

6. H. Flechsig, A.S. Mikhailov. *Tracing entire operation cycles of molecular motor hepatitis C virus helicase in structurally resolved dynamical simulations.* **Proc Natl Acad Sci USA** 107, 20875-20880, 2010

Simulation Atomic Force Microscopy

Holger FLECHSIG
Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan

Observations of biomolecular structures and their conformational dynamics by atomic force microscopy (AFM) are restricted to changes of the molecular surface with limited resolution, preventing detailed understanding from experiments alone. We have developed simulation atomic force microscopy (sAFM) which allows to reconstruct 3D atomistic molecular structures from 2D topographic AFM images [1,2,3]. I present applications and demonstrate how the obtained full atomistic information advances the molecular understanding beyond topographic imaging.

References

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[3] H. Flechsig. *Simulation atomic force microscopy to predict correlated conformational dynamics in proteins from topographic imaging.* **bioRxiv** doi:10.1101/2021.10.15.464530 (2021)

Hiroshi KAWASAKI

Professor, Department of Medical Neuroscience, Graduate School of Medical Sciences, Kanazawa University, Japan

Contact : kawasaki [at sign] med.kanazawa-u.ac.jp
Please replace [at sign] with @.

Research Interests

Development and evolution of the brain, Disease model

Academic Background

1994 - 1998	Graduate School of Medicine, Kyoto University
1984 - 1990	School of Medicine, Kyoto University

Professional Careers

2013 - present	Professor, Graduate School of Medical Sciences, Kanazawa University
2004 - 2012	Project Associate Professor, School of Medicine, The University of Tokyo
2002 - 2004	Research Associate, Howard Hughes Medical Institute/Duke University
2001 - 2002	Lecturer, Institute for Fronter Medical Sciences, Kyoto University
1998 - 2001	Assistant Professor, Institute for Fronter Medical Sciences, Kyoto University
1998 - 1998	Research Fellowship for Young Scientists, JSPS
1990 - 1994	Clinical Neurologist, Kyoto Univ Hosp/Shimada Municipal Hosp/Shizuoka General Hosp

Scientific Activities

2020 - present	Associate Editor, Frontiers in Neuroscience
2016 - present	Editorial Board, Scientific Reports

Honors

2006 - 2010	PRESTO Researcher, JST

Publications

1. Neuron, 28, 31-40, 2000
2. Proc Natl Acad Sci U S A., 99, 1580-1585, 2002
3. Developmental Cell, 27, 32-46, 2013
4. eLife, 6, e29285, 2017
5. Cell Reports, 20, 2131-2143, 2017
6. eLife, 9, e54873, 2020

Mechanisms Underlying the Development and Evolution of the Mammalian Cerebral Cortex

Hiroshi KAWASAKI

Professor, Department of Medical Neuroscience, Graduate School of Medical Sciences, Kanazawa University, Japan

The cerebral cortex of the brain has changed significantly during evolution. The cerebral cortex has become larger, and folds appeared on the surface of the cerebral cortex. Although these changes of the cerebral cortex are considered to be important for acquiring higher brain functions during evolution, the mechanisms underlying the expansion and folding of the cerebral cortex remain unclear. This is partially because the cerebral cortex of mice is relatively small and does not have cortical folds. Therefore, we have been utilizing ferrets, which have larger and folded cerebral cortex and established genetic manipulation techniques for the ferret cerebral cortex by combining *in utero* electroporation and the CRISPR/Cas9 system. Using our technique, we investigated the roles of sonic hedgehog (Shh) signaling in the development of the ferret cerebral cortex. We found that activation of Shh signaling resulted in additional folding in the ferret cerebral cortex. Consistently, suppression of Shh signaling by expressing Hhip-DeltaC22 inhibited cortical folding. Interestingly, we found that Shh signaling was more activated in the developing cerebral cortex of ferrets than that of mice, suggesting that Shh signaling is involved in the evolution of the cerebral cortex. Our technique for the ferret cerebral cortex should be useful for investigating the mechanisms underlying the development and evolution of the cerebral cortex.

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[4] eLife, 6, e29285, 2017

Makoto SATO

Professor, Mathematical Neuroscience Unit, Institute for Frontier Science Initiative, Kanazawa University, Japan

Contact : makotos [at sign] staff.kanazawa-u.ac.jp
Please replace [at sign] with @.

Research Interests

Developmental neurobiology, Mathematical modeling, Machine learning

Academic Background

1995 - 2000	Ph. D. Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo
1991 - 1995	B.S. Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo

Professional Careers

2015 - **present**	Professor, Institute for Frontier Science Initiative, Kanazawa University
2012 - 2015	Professor, Brain/Liver Interface Medicine Research Center, Kanazawa University
2010 - 2013	PRESTO, Japan Science and Technology Agency
2008 - 2012	Associate Professor, Frontier Science Organization, Kanazawa University
2002 - 2008	Assistant Professor, Institute of Molecular and Cellular Biosciences, University of Tokyo
2000 - 2002	Postdoctoral Fellow, Department of Biochemistry, University of California, San Francisco

Scientific Activities

2015 - **present**	Mathematical modeling of biological phenomena
2002 - **present**	Developmental neurobiology of Drosophila visual system
2000 - 2002	Roles of FGF signaling in long range cell-cell communication
1994 - 2000	Positional information and pattern formation in the developing peripheral nervous system

Honors

2012	The MEXT Young Scientists Prize

Publications

1. Intracellular trafficking of Notch orchestrates temporal dynamics of Notch activity in the fly brain. Wang, M., Han, X., Liu, C., Takayama, R., Yasugi, T., Ei, S., Nagayama, M., Tanaka, Y. and Sato, M. **Nature Communications** 12, 2083 (2021).

2. DWnt4 and DWnt10 regulate morphogenesis and arrangement of the columnar structures through Fz2/PCP signaling in the *Drosophila* brain. Han, X., Wang, M., Liu, C., Trush, O., Takayama, R., Akiyama, T., Naito, T., Tomomizu, T., Imamura, K. and Sato. M. **Cell Reports** 33, 108305 (2020).

3. Dscam1 establishes the columnar units through lineage-dependent repulsion between sister neurons in the fly brain. Liu, C., Trush, O., Han, X., Wang, M., Takayama, R., Yasugi, T., Hayashi, T., Sato, M. **Nature Communications** 11, 4067 (2020).

4. N-cadherin orchestrates self-organization of neurons within a columnar unit in the *Drosophila* medulla. Trush, O., Liu, C., Han, X., Nakai, Y., Takayama, R., Murakawa, H., Carrillo, J. A., Takechi, H., Hakeda-Suzuki, S., Suzuki T. and Sato, M. **Journal of Neuroscience** 39, 5861-5880 (2019).

5. Notch-mediated lateral inhibition regulates proneural wave propagation when combined with EGF-mediated reaction diffusion. Sato, M., Yasugi, T., Minami, Y., Miura, T. and Nagayama, M. **Proceedings of the National Academy of Sciences** 113, E5153-E5162 (2016).

The Wave of Differentiation Orchestrates Neurogenesis and Column Formation in the *Drosophila* Brain

Makoto SATO

Professor, Mathematical Neuroscience Unit, Institute for Frontier Science Initiative, Kanazawa University, Japan

The wave of differentiation, 'proneural wave', sequentially generates neural stem cells on the surface of the *Drosophila* brain [5]. Combining mathematical modeling and molecular genetic analysis, we demonstrated that intracellular trafficking of Notch causes the non-linear dynamics of Notch signaling activity. As a result, Notch is activated twice along the proneural wave front. The first Notch activity negatively regulates the proneural wave propagation [5], while the second peak controls the temporal transition of the neural stem cells, which ultimately specifies the fate of neurons [1].

Additionally, we showed that the proneural wave controls the formation of columnar structure, the morphological and functional unit of the brain [2,4]. According to the radial unit hypothesis, neurons of the same lineage form a radial unit that contributes to column formation. However, the molecular mechanisms that provide a link between the neuronal lineage and column formation remain elusive. We demonstrated that Down syndrome cell adhesion molecule (Dscam) is temporally upregulated in new born neural stem cells, and is inherited to their daughter neurons [3]. The transient transcription of *Dscam* at the proneural wave front enables the expression of the same Dscam splice isoform within the same neuronal lineage, causing lineage-dependent axonal repulsion, and controls column formation. Thus, the proneural wave orchestrates neurogenesis through temporal Notch dynamics and column formation through temporal Dscam expression [1,3].

References

[1] Intracellular trafficking of Notch orchestrates temporal dynamics of Notch activity in the fly brain. Wang, M., Han, X., Liu, C., Takayama, R., Yasugi, T., Ei, S., Nagayama, M., Tanaka, Y. and Sato, M. **Nature Communications** 12, 2083 (2021).

[2] DWnt4 and DWnt10 regulate morphogenesis and arrangement of the columnar structures through Fz2/PCP signaling in the *Drosophila* brain. Han, X., Wang, M., Liu, C., Trush, O., Takayama, R., Akiyama, T., Naito, T., Tomomizu, T., Imamura, K. and Sato. M. **Cell Reports** 33, 108305 (2020).

[3] Dscam1 establishes the columnar units through lineage-dependent repulsion between sister neurons in the fly brain. Liu, C., Trush, O., Han, X., Wang, M., Takayama, R., Yasugi, T., Hayashi, T., Sato, M. **Nature Communications** 11, 4067 (2020).

[4] N-cadherin orchestrates self-organization of neurons within a columnar unit in the *Drosophila* medulla. Trush, O., Liu, C., Han, X., Nakai, Y., Takayama, R., Murakawa, H., Carrillo, J. A., Takechi, H., Hakeda-Suzuki, S., Suzuki T. and Sato, M. **Journal of Neuroscience** 39, 5861-5880 (2019).

[5] Notch-mediated lateral inhibition regulates proneural wave propagation when combined with EGF-mediated reaction diffusion. Sato, M., Yasugi, T., Minami, Y., Miura, T. and Nagayama, M. **Proceedings of the National Academy of Sciences** 113, E5153-E5162 (2016).

Takeshi IMAI

Professor, Department of Developmental Neurophysiology Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Contact : imai.takeshi.457 [at sign] m.kyushu-u.ac.jp
Please replace [at sign] with @.

Research Interests

Sensory systems, Neural development, Connectomics, Tissue clearing, Fluorescence imaging

Academic Background

2006 Ph.D., Graduate school of Science, The University of Tokyo (Supervisor: Prof. Hitoshi Sakano)

2001 B.S., Department of Biophysics and Biochemistry, Graduate school of Science, The University of Tokyo

Professional Careers

2017 - present Professor, Graduate School of Medical Sciences, Kyushu University

2010 - 2018 Team Leader, RIKEN Center for Developmental Biology (CDB)

2009 - 2015 PRESTO, Japan Science and Technology Agency (JST)

Honors

2015 The Young Scientists' Prize, MEXT

2007 GE Healthcare & Science Prize for Young Life Scientists, Regional Prize Winner

Publications

1. Ke M-T, Fujimoto S, Imai T. SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. *Nat Neurosci.* 16:1154-1161. (2013)
2. Ke MT, et al. Super-Resolution Mapping of Neuronal Circuitry With an Index-Optimized Clearing Agent. *Cell Rep.* 14(11):2718-32.1 (2016)
3. Iwata R, Kiyonari H, Imai T. Mechanosensory-Based Phase Coding of Odor Identity in the Olfactory Bulb. *Neuron* 96 (5), 1139-1152. e7 (2017)
4. Sakaguchi R, Leiwe MN, Imai T. Bright multicolor labeling of neuronal circuits with fluorescent proteins and chemical tags. *eLife* e40350 (2018)
5. Inagaki S, Iwata R, Iwamoto M, Imai T. Widespread inhibition, antagonism, and synergy in mouse olfactory sensory neurons in vivo. *Cell Rep.* 31(13):107814 (2020)
6. Aihara S, Fujimoto S, Sakaguchi R, Imai T. BMPR-2 gates activity-dependent stabilization of dendrites during mitral cell remodeling. *Cell Rep.* 35:109276 (2021)

Automatic Reconstruction of Neuronal Circuits with Super-Multicolor Labeling

Takeshi IMAI

Professor, Department of Developmental Neurophysiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

All the brain functions originate from the neuronal circuits. It is, therefore, important to understand how neurons are interconnected in the brain. Stochastic multicolor labeling (e.g., Brainbow) is a powerful strategy to dissect and reconstruct densely labeled neuronal circuits. We previously developed a stochastic multicolor labeling method with enhanced expression levels that uses a tetracycline-operator system (Tetbow) (Sakaguchi et al., eLife, 2018). In Tetbow, three fluorescent protein (XFP) genes were expressed stochastically in neurons to generate various color hues. We optimized Tetbow for either plasmid or virus vector-mediated multicolor labeling. When combined with the tissue clearing method, SeeDB2, Tetbow was useful to visualize the three-dimensional architecture of individual neurons. For example, we were able to visualize the axonal projection patterns of individual mitral and tufted cells along several millimeters in the mouse olfactory system. However, the number of color hues generated by the combination of three XFPs was limited. Therefore, we still had to trace the multicolor-labeled neurites manually, which was the rate-limiting step in large-scale circuit reconstructions. Here we developed the "super-multicolor labeling" method with seven different XFPs, in which the number of possible color combinations was massively expanded. As trichromatic human eyes can only recognize the combination of red, green, and blue, we also developed a fully automated pipeline for the quantitative analysis of the color combinations generated by the super-multicolor labeling. Using this strategy, we have successfully reconstructed neuronal circuits in 3D "without manual neurite tracing". Our strategy should facilitate fully automated light microscopy-based connectomics.

References

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[2] Ke MT, Nakai Y, Fujimoto S, Takayama R, Yoshida S, Kitajima TS, Sato M, Imai T. Super-Resolution Mapping of Neuronal Circuitry With an Index-Optimized Clearing Agent. *Cell Rep.* 14(11):2718-32.1 (2016)

[3] Sakaguchi R, Leiwe MN, Imai T. Bright multicolor labeling of neuronal circuits with fluorescent proteins and chemical tags. *eLife* e40350 (2018)

Hiroki R. UEDA

Professor, Systems Pharmacology, Graduate School of Medicine, University of Tokyo, Japan
Team Leader, Laboratory for Synthetic Biology, Center for Biosystems Dynamics Research, RIKEN, Japan

Contact : uedah-tky [at sign] umin.ac.jp
Please replace [at sign] with @.

Research Interests

Systems Biology, Synthetic Biology, Tissue clearing, 3D imaging

Academic Background

2004	The University of Tokyo, Graduate School of Medicine, Department of Pharmacology, Ph. D.
2000	The University of Tokyo, Faculty of Medicine, M. D.

Professional Careers

2013 - present	Professor, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo
2011 - present	Laboratory Head, Laboratory for Synthetic Biology, RIKEN
2017 - 2019	Principal Investigator, International Research Center for Neurointelligence, The University of Tokyo
2016 - present	Affiliate Professor, Graduate School of Information Science and Technology, The University of Tokyo
2011 - present	Invited Professor, Graduate School of Frontier Biosciences, Osaka University
2005 - present	Visiting Professor, Tokushima University

Scientific Activities

2011-Co-founder of Japanese Society of Cell Synthesis Research (2005-), Scientific Editors for EMBO Journal, and Genes to Cells, 2011-Research Supervisor, PREST Control and Design of Cellular Functions, Organizer of 10 symposiums over 5 years. 2013-Director, Japanese Society for Chronobiology, 2020-Vice President, Japanese Society for Chronobiology, 2013-Director, The Molecular Biology Society of Japan, 2014-Associate Editor for IEEE Life Sciences Letters, 2014-Associate Editor for NPJ Systems Biology and Applications, 2014-Member of Science Council of Japan (SCJ), 2014-2018 The representative of Young Academy of Japan, 2016-International Advisory Board for Advanced Biosystems, 2018-iScience, Scientific Advisory Board, 2019-Journal of Biological Rhythms, Editorial Board, 2021-USERN Advisory Board, 2021-Molecular Systems Biology, Advisory Editorial Board

Honors

2018-The Ichimura Prize, 2017-Innovator of the Year, 2015-Yamazaki-Teiichi Prize, 2012-Tsukahara Nakaakira Memorial Award, 2011-Nagase Award, 2011-Changemaker of the year 2011, 2010-JSPS Award, 2009-IBM Science Award, 2006-Young Scientist Award (MEXT), 2005-Tokyo Techno-Forum 21 Gold medal , 2004-Japan Innovator Award.

Publications

1. Tatsuki et al. Neuron, 90(1): 70–85 (2016).
2. Sunagawa et al, Cell Reports, 14(3):662-77 (2016).
3. Susaki et al. Cell, 157(3): 726–39, (2014).
4. Niwa et al, Cell report, 24, 2231-2247. e7 (2018).
5. Ode and Ueda, Front. Psychol. 11, 575328 (2020)

Systems Biology of Mammalian Sleep/Wake Cycles: Phosphorylation Hypothesis of Sleep

Hiroki R. UEDA
Professor, Systems Pharmacology, Graduate School of Medicine, University of Tokyo, Japan
Team Leader, Laboratory for Synthetic Biology, Center for Biosystems Dynamics Research, RIKEN, Japan

The detailed molecular and cellular mechanisms underlying NREM sleep (slow-wave sleep) and REM sleep (paradoxical sleep) in mammals are still elusive. To address these challenges, we first constructed a mathematical model, Averaged Neuron Model (AN Model), which recapitulates the electrophysiological characteristics of the slow-wave sleep. Comprehensive bifurcation analysis predicted that a Ca^{2+}-dependent hyperpolarization pathway may play a role in slow-wave sleep. To experimentally validate this prediction, we generate and analyze 26 KO mice, and found that impaired Ca^{2+}-dependent K^{+} channels (Kcnn2 and Kcnn3), voltage-gated Ca^{2+} channels (Cacna1g and Cacna1h), or Ca^{2+}/calmodulin-dependent kinases (Camk2a and Camk2b) decrease sleep duration, while impaired plasma membrane Ca^{2+} ATPase (Atp2b3) increases sleep duration. Genetical (Nr3a) and pharmacological intervention (PCP, MK-801 for Nr1/Nr2b) and whole-brain imaging validated that impaired NMDA receptors reduce sleep duration and directly increase the excitability of cells. Based on these results, we propose phoshporylation hypothesis of sleep that phosphorylation-dependent regulation of Ca^{2+}-dependent hyperpolarization pathway underlies the regulation of sleep duration in mammals. We also recently developed a simplified mathematical model, Simplified Averaged Neuron Model (SAN Model), which uncover the important role of K^{+} leak channels in NREM sleep. In this talk, I will also describe how we identify essential genes (Chrm1 and Chrm3) in REM sleep regulation, and propose a plausible molecular definition of a paradoxical state of REM sleep.

References

[1] Tatsuki et al. Neuron, 90(1): 70–85 (2016).
[2] Sunagawa et al, Cell Reports, 14(3):662-77 (2016).
[3] Susaki et al. Cell, 157(3): 726–39, (2014).
[4] Niwa et al, Cell report, 24, 2231-2247. e7 (2018).
[5] Ode and Ueda, Front. Psychol. 11, 575328 (2020)

Damien HALL

Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
Department of Applied Physics, Aalto University, Finland.

Contact : hall.damien [at sign] staff.kanazawa-u.ac.jp
Please replace [at sign] with @.

Research Interests

Physical biochemistry of disease: Amyloidosis, virus infection and cancer.

Academic Background

1996 - 1999	PhD in Biochemistry, University of Queensland
1991 - 1995	Bachelor of Science with First Class Honors (Chemistry), University of Queensland

Professional Careers

2021 -	Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2020 - 2021	Visiting Scientist, Nagoya Institute for Technology, Japan
2019 - 2020	Department of Energy ORISE Established Scientist, National Institutes of Health, U.S.A.
2013 - 2018	Senior Research Fellow, Australian National University, AUS
2008 - 2013	Wakate Fellow, University of Tsukuba, Japan
2003 - 2007	Human Frontiers Science Program Long Term Fellow, University of Cambridge, U.K.
1999 - 2003	John E. Fogarty Fellow, National Institutes of Health, U.S.A.

Scientific Activities

2011 - present	Editorial Board of Biophysical Reviews
2008 - 2013	Wakate Fellow Committee

Honors

2019 - 2022	ORISE Established Research Scientist (NIH) U.S.A.
2013 - 2018	ANU Senior Research Fellowship (ANU) AUS.

Publications

1. Hall D. (2020) **FEBS letters**. 594, pp.43-66
2. Hall D. (2020) **Biophysics and Physicobiology.** 17, 30-35.
3. Hall D. et al. (2018). **Analytical biochemistry**. 542, 40-57.

Computational Modeling at the Nano Life Science Interface

Damien HALL
Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
Department of Applied Physics, Aalto University, Finland.

In this talk I describe three different projects which simulate various aspects of cell behavior potentially realizable through atomic force microscopy (AFM) measurement. The first work describes a multiscale model of cell growth, division and epigenetic transfer of a polymer element able to replicate within the cytosol. The multiscale model pairs a stochastic particle model (to represent the cell body) and a large set of coupled ordinary differential equations (to describe the growth and partition of the polymer element). The second piece of work describes probabilistic methods for optimizing AFM-based feature assignment within live cell membranes. A diffusion-based description of the cell membrane components is coupled with an analytical model of the AFM tip raster scanning process in order to assess the effects of relative scanning rate and feature density on the assignment process. The third project is concerned with simulating force curve profiles associated with long AFM tip experiments that 'penetrate' into the cell interior. In this work the cell is modeled as a viscoelastic fluid with internal components assigned a set structure and spacing and modeled as breakable springs. This last piece of work (simulation of a force curve based on known cell structure) is analogous to solving the complementary forward aspect of a more difficult inverse problem (the assessment of cell structure based on analysis of a cell penetration force curve).

References

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[2] Hall, D., 2020. On the nature of the optimal form of the holdase-type chaperone stress response. FEBS letters, 594(1), pp.43-66.

[3] Wakayama, R., Uchiyama, S. and Hall, D., 2019. Ionic liquids and protein folding—old tricks for new solvents. Biophysical reviews, 11(2), pp.209-225.

Rakesh DAS

Research Fellow, Mechanobiology Institute, National University of Singapore, Singapore

Contact : mbird [at sign] nus.edu.sg
Please replace [at sign] with @.

Research Interests

Physical, theoretical, and computational biology

Academic Background

2018 - 2019	Extended Senior Research Fellow at SNBNCBS, Kolkata, India
2013 - 2018	Ph.D. student at SNBNCBS, Kolkata, India
2011 - 2013	M.Sc. in Physics from Visva Bharati University, Santiniketan, India
2008 - 2011	B.Sc. in Physics from Visva Bharati University, Santiniketan, India

Professional Careers

2019 - **present**	Postdoctoral researcher at Mechanobiology Institute, National University of Singapore

Scientific Activities

2018	Co-organized “Emergent Phenomena in Classical and Quantum Systems” at SNBNCBS, Kolkata
2018	Took tutorial on MD and parallel computation (OpenMP API and MPI) at SNBNCBS, Kolkata

Honors

2015	Best poster award in a national conference ‘Nanodays 2015’ organized in India
2008 - 2013	INSPIRE scholarship from Dept. of Science and Technology, India

Publications

1. **RD**, Takahiro Sakaue, G. V. Shivashankar, Jacques Prost, and Tetsuya Hiraiwa, *How enzymatic activity is* involved in chromatin organization, arXiv:2112.10460 [physics.bio-ph].
2. **RD**, Manoranjan Kumar and Shradha Mishra, Nonquenched rotators ease flocking and memorize it, Phys. Rev. E 101, 012607 (2020).
3. Sudipta Pattanayak, **RD**, Manoranjan Kumar and Shradha Mishra, Enhanced dynamics of active Brownian particle in a periodic and confined channel, Euro. Phys. J. E 42, 62 (2019).
4. **RD**, Manoranjan Kumar and Shradha Mishra, Polar flock in the presence of random quenched rotators, Phys. Rev. E 98, 060602(R) (2018).
5. **RD**, Shradha Mishra and Sanjay Puri, Ordering dynamics of self-propelled particles in an inhomogeneous medium, Europhys. Lett. 121, 37002 (2018).
6. **RD**, Manoranjan Kumar and Shradha Mishra, Order-disorder transition in active nematic: A lattice model study, Sci. Rep. 7, 7080 (2017).

Enzymatic Activity on Chromatin Organization - A Polymer Model Study

Rakesh DAS

Research Fellow, Mechanobiology Institute, National University of Singapore, Singapore

Spatial organization of chromatin plays a critical role in genome regulation [1]. Various types of affinity mediators and enzymes have been attributed to regulate spatial organization of chromatin from a thermodynamics perspective [2, 3]. However, at the mechanistic level, enzymes act in their unique ways. Here, we construct a polymer physics model following the mechanistic scheme of Topoisomerase-II [4], an enzyme resolving topological constraints of chromatin, and investigate its role on interphase chromatin organization. Our GPU-aided high-performance computer simulations demonstrate Topoisomerase-II's ability to phase separate chromatin into eu- and heterochromatic regions with a characteristic wall-like organization of the euchromatic regions. Exploiting a mean-field framework, we argue that the ability of the euchromatic regions crossing each other due to enzymatic activity of Topoisomerase-II induces this phase separation. Motivated from a recent experimental observation on different structural states of the eu- and the heterochromatic units, we further extend our model to a bidisperse setting and show that the characteristic features of the enzymatic activity driven phase separation survives there. The existence of these characteristic features, even under the non-localized action of the enzyme, highlights the critical role of enzymatic activity in chromatin organization, and points out the importance of further experiments along this line, which may provide insights for the mechanisms of chromatin architecture change upon cell state modifications.

References

[1] G. V. Shivashankar, *Mechanical regulation of genome architecture and cell-fate decisions*, Curr. Opin. Cell Biol. **56**, 115 (2019).

[2] E. M. Hildebrand and J. Dekker, *Mechanisms and functions of chromosome organization*, Trends in Biochem. Sci. **45**, 385 (2020).

[3] A. Agrawal, N. Ganai, S. Sengupta, and G. I. Menon, *Nonequilibrium biophysical processes influence the large-scale architecture of the cell nucleus*, Biophys. J. **118**, 1 (2020).

[4] J. Roca, *Topoisomerase II: a fitted mechanism for the chromatin landscape*, Nucleic Acids Res. **37**, 721 (2009).

Masashi TACHIKAWA

Associate Professor, Institute for Frontier Life and Medical Sciences, Kyoto University, Japan

Contact : match [at sign] infront.kyoto-u.ac.jp
Please replace [at sign] with @.

Research Interests

Cell biology, Organelle, Morphology, Physical model simulation

Academic Background

2019 - present | Depertment of Biophysics, Graduate School of Science, Kyoto University

Professional Careers

2019 - present Associate Professor at Institute for Frontier Life and Medical Sciences, Kyoto University
2010 - 2019 Researcher at Theoretical Biology Laboratory, Riken
2005 - 2010 Researcher at ERATO Complex Biology Project, JST
2001 - 2007 Researcher at The University of Tokyo

Publications

1. Modeling Membrane Morphological Change during Autophagosome Formation. Sakai Y, Koyama-Honda I, Tachikawa M, Knorr RL, Mizushima N, *iScience* **23**(9) 101466, 2020.
2. Modeling the functions of condensin in chromosome shaping and segregation. Sakai Y, Mochizuki A, Kinoshita K, Hirano T, Tachikawa M, *PLoS Comp Biol.* **14**(6) e1006152, 2018.
3. Measurement of caveolin-1 densities in the cell membrane for quantification of caveolar deformation after exposure to hypotonic membrane tension. Tachikawa M, Morone N, Senju Y, Sugiura T, Hanawa-Suetsugu K, Mochizuki A, Suetsugu S, *Scientific Reports* **7**(1) 7794, 2017.
4. Golgi apparatus self-organizes into the characteristic shape via postmitotic reassembly dynamics. Tachikawa M, Mochizuki A, *PNAS* **114**(20) 5177-5182, 2017.
5. Controlling segregation speed of entangled polymers by the shapes: A simple model for eukaryotic chromosome segregation. Sakai Y, Tachikawa M, Mochizuki A, *Physical Review. E* **94**(4) 042403, 2016.
6. Nonlinearity in cytoplasm viscosity can generate an essential symmetry breaking in cellular behaviors. Tachikawa M, Mochizuki A, *Journal of Theoretical Biology* **364** 260-265, 2015.

Physical Model Simulations for Understanding the Structures of Organelle Shapes

Masashi TACHIKAWA

Associate Professor, Institute for Frontier Life and Medical Sciences, Kyoto University, Japan

Eukaryotic cells have various functional units made of lipid-bilayer membrane, called organelles. Although their characteristic morphologies are thought to be closely related to their functions, the mechanisms to produce and maintain them remain largely unknown. We develop computational approaches to understand the mechanisms that govern the organelle structures. The size of organelles is up to 1 μm much larger than the molecular size. Continuum models are suitable for describing the organelle shapes. We employ a dynamically triangulated surface method to describe the tree dimensional shape of membrane.

Two difficult points to understand from molecular biology study are the formation process of a complex organelle shape and the force balance to keep the shape steadily. Here we present two our recent studies on the formation of Golgi apparatus and the energy model for entire shape of inner and outer membranes of mitochondria. In the study of the Golgi apparatus, we mimic the Golgi apparatus formation process in mammalian cells and show the assembly of small membrane particles can self-organize into the characteristic Golgi shapes in a controlled physical condition. Latter study examine the detailed force balance on mitochondria membranes and discuss the relation between shape and these forces.

References

[1] Membrane Simulation Models from Nanometer to Micrometer Scale. Noguchi H, *J. Phys. Soc. Jpn.*, **78**(4) 041007, 2009.

[2] Golgi apparatus self-organizes into the characteristic shape via postmitotic reassembly dynamics. Tachikawa M, Mochizuki A, *PNAS* **114**(20) 5177-5182, 2017.

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