November 27, 2024

Exploring Uncharted
Nanoscale Frontiers
in Life Sciences

OIST

NanoLSI
WPI KANAZAWA UNIVERSITY

8th NanoLSI Symposium

at OIST

8th NanoLSI Symposium

Exploring Uncharted Nanoscale Frontiers
in Life Sciences

NOVEMBER 27, 2024

VENUE

OKINAWA INSTITUTE OF SCIENCE AND TECHNOLOGY GRADUATE UNIVERSITY
1919-1 TANCHA, ONNA-SON, KUNIGAMI-GUN, OKINAWA, 904-0495, JAPAN

Welcome Message from the Organizers of the 8th NanoLSI Symposium Jointly held with OIST

Human prosperity has advanced alongside the development of science and technology, opening numerous uncharted frontiers. While many mysteries have been solved, numerous unknowns remain, particularly in the life sciences. At the nanoscale level, fundamental questions persist, such as the dynamics of proteins and nucleic acids on the surfaces and within cells, the essential building blocks of living organisms.

As a WPI research institute pioneering cutting-edge microscopy techniques for visualizing nanoscale life phenomena, we are excited to co-organize an international symposium with OIST on "Exploring Uncharted Nanoscale Frontiers in Life Sciences."

This symposium aims to foster transdisciplinary research and catalyze new collaborations among researchers from diverse fields. With this opportunity, we greatly look forward to the active discussions of the participants.

Satoshi Mitarai

Satoshi Mitarai, Dean of Research and Professor

Organizer, OIST

A. Kusumi

Akihiro Kusumi, Professor

Symposium Scientific Organizer, OIST

Rikinari Hanayama, Professor

Symposium Scientific Organizer, WPI-NanoLSI

The 8th NanoLSI Symposium at OIST

Exploring Uncharted Nanoscale Frontiers in Life Sciences

November 27, 2024 / Day 1 **Sydney Brenner Lecture Theater (Seminar Room B250)**

8:30 AM	Registration and arrival tea & coffee
9:00 AM	Opening remarks by Takeshi Fukuma, Professor and Director of NanoLSI
	Session #1 9:10 AM - 11:10 AM
	Chair: Akihiro Kusumi & Rikinari Hanayama
9:10 AM	Takeshi Fukuma (NanoLSI)
9:40 AM	Amy Shen (OIST)
10:10 AM	Shinji Watanabe (NanoLSI)
10:40 AM	Matthias Wolf (OIST)
11:10 AM	Lunch
	Session #2 12:10 PM - 2:10 PM
	Chair: Shinji Watanabe & Matthias Wolf
12:10 PM	Yukiko Goda (OIST)
12:40 PM	Marco Terenzio (OIST)
1:10 PM	Richard W. Wong (NanoLSI)
1:40 PM	Miki Nakajima (NanoLSI)
2:10 PM	Tea & coffee break
	Session #3 2:40 PM - 4:40 PM
	Chair: Miki Nakajima & Marco Terenzio
2:40 PM	Clemens M. Franz (NanoLSI)
3:10 PM	Tomomi Kiyomitsu (OIST)
3:40 PM	Hanae Sato (NanoLSI)
4:10 PM	Akihiro Kusumi (OIST)
4:40 PM	Closing remarks by Akihiro Kusumi, Professor of OIST
	Networking dinner/Poster session 5:30 PM - 7:30 PM Lab 5 Atrium
5:30 PM	Poster session
	Flash talks in the first 30 minutes

November 28, 2024 / Day 2 **9:00 AM - 12:00 PM** **Lab Tour**

ORAL SESSIONS

Visualizing Nanoscale Dynamics and Mechanics in Living Cells by Nanoendoscopy AFM

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Takeshi Fukuma is the Director and a Professor of Nano Life Science Institute (WPI-NanoLSI), Kanazawa University. He received his Ph.D. in Engineering at Kyoto University in 2003. After that, he worked as a postdoctoral researcher at Kyoto University, a senior scientist at Trinity College Dublin and an associate professor at Kanazawa University. Since 2012, he has been a professor at Kanazawa University. Since 2017, he has also been the Director of WPI-NanoLSI at Kanazawa University. He has been engaged in the development of in-liquid atomic force microscopy techniques and their applications to various nanoscience research in academic and industrial fields. So far, he has received the MEXT Young Scientists Award in 2011, the JSPS Prize in 2018, and the MEXT Science and Technology Award in 2023.

Presentation Abstract:

Atomic force microscopy (AFM) has been a powerful tool that allows to directly visualize nanoscale dynamics of proteins and DNAs in liquid without labeling. However, such high-resolution AFM imaging typically requires fixation of biological samples onto a solid substrate. Therefore, it has been often questioned if the observed structures or phenomena truly represent those inside living cells. To overcome this problem, we recently developed nanoendoscopy AFM (NE-AFM), where a needle probe is inserted into a living cell to perform intracellular AFM observations[1]. So far, we have demonstrated 3D imaging of the whole cell structure and actin fibers, and 2D imaging of dynamic structural changes in the actin cortical fibers on the inner surface of the bottom cell membrane. In addition, we demonstrated that these measurements do not cause serious damage to a cell despite the repeated insertion of the probe into the cell.

NE-AFM has two distinctive advantages over other imaging techniques. One of them is the capability of nanoscale imaging at intra-cellular interfaces. To take advantage of this, we are investigating dynamics of focal adhesions (FAs). FAs are the intracellular structure connecting between actin fibers and extracellular matrix and play critical roles in cell adhesion and motility. By combining NE-AFM and confocal fluorescent microscope, we simultaneously observed time-lapse changes of the 3D FA structures and paxillin distributions during their growth. From the confocal image, we can identify the position of FAs and perform their 3D-AFM imaging. The 3D-AFM images reveal that the FAs become thicker during their growth. Besides, the actin fiber associated with the FA was initially in contact with the upper cell membrane but detached as the FA grows. This indicates that actin molecules are provided from the cortical actin network during the fiber growth. These detailed nanoscale structural changes are directly captured by NE-AFM in living cells.

Another strength of NE-AFM is the capability to measure the nanomechanical properties in living cells. With this capability, we are investigating nuclear envelope (NE) elasticity. NE is supported by lipids, lamins and lamin associated domains (LADs) of chromatins. Among them, LADs are much thicker than others and hence considered to determine the NE elasticity. Meanwhile, LAD organization is considered to be related to the gene expression and related diseases known as nuclear envelopathies. Therefore, there have been great interests in NE elasticity measurements. In addition, nuclear elasticity has attracted attention in cancer research area due to its correlation with cell resistance to the external pressure during its migration and invasion. To address these issues, we use NE-AFM to measure NE elasticity by directly indenting the NE surface with a needle probe. So far, we found that the NE elasticity increases when the serum was depleted and decreases when the EMT was induced by applying TGFβ. This is reasonable as the gene expression activity should decrease when the cells are arrested at the G0 phase and increase when the cells become more invasive due to the EMT. These results confirm strong correlation between the NE elasticity and gene expression activity. With this basic understanding, we are now exploring various possibilities of NE-AFM studies on NE elasticity.

Recent 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.

Advancing Population Genetics and Disease Detection through Microfluidics and Lab-on-a-Chip Technologies

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Amy Shen is the Provost and a Professor at OIST in Japan, where she has led the Micro/Bio/Nanofluidics Unit since 2014. Before joining OIST, she was a faculty member in Mechanical Engineering at the University of Washington, USA. Her research focuses on microfluidics and lab-on-a-chip technologies at the bio/nano interface, with broad applications in biotechnology. Amy is a Fellow of the American Physical Society, the Royal Society of Chemistry, and the Society of Rheology. She has received numerous accolades, including the NSF CAREER Award, the Ralph E. Powe Junior Faculty Enhancement Award, and was a Fulbright Scholar in 2013. She serves as an associate editor for Soft Matter and is on the editorial advisory boards of ACS Sensors, Journal of Rheology, and Physics of Fluids. Notably, she delivered the 2019 Bergveld Lecture at the University of Twente, Netherlands.

Presentation Abstract:
Microfluidics and lab-on-a-chip devices have become powerful platforms for manipulating fluids at small scales, significantly advancing biophysics and biotechnology research. In this talk, I will present two examples of using microfluidics in microbial population genetics and disease diagnosis. The first example involves a microfluidic device with a controlled microenvironment designed to study population genetics, where microbial populations proliferate in small channels. In these environments, reproducing cells organize into parallel lanes, and as they shift, they can potentially expel other cells from the channel. By combining theoretical models and experiments, we found that genetic diversity is rapidly lost along these lanes. Specifically, our experiments demonstrated that a population of proliferating *Escherichia coli* in a microchannel organizes into lanes of genetically identical cells within just a few generations. The second example highlights the development of advanced microfluidic sensing platforms for rapid and sensitive detection of biomarkers. One such platform employs an opto-microfluidic approach, utilizing localized surface plasmon resonance (LSPR) with gold nanospikes fabricated by electrodeposition within a microfluidic device, coupled with an optical probe, to detect antibodies against the SARS-CoV-2 spike protein in diluted human plasma with a detection limit of approximately 0.5 pM (0.08 ng/mL) within 30 minutes. Additionally, recent work by Mazzaracchio et al. (2023) demonstrates the potential of a duplex electrochemical microfluidic sensor in distinguishing between natural and vaccine-induced humoral responses. Moreover, the versatility of microfluidic platforms extends beyond infectious disease diagnostics, as shown by Funari et al. (2024), who developed a multiplexed opto-microfluidic biosensing platform for the detection of prostate cancer biomarkers. These examples collectively underscore the broad applicability and efficacy of microfluidic technologies in advancing diagnostics across various fields.

Fig. 1: Competition between two E. coli strains in microchannels with 2 open ends.

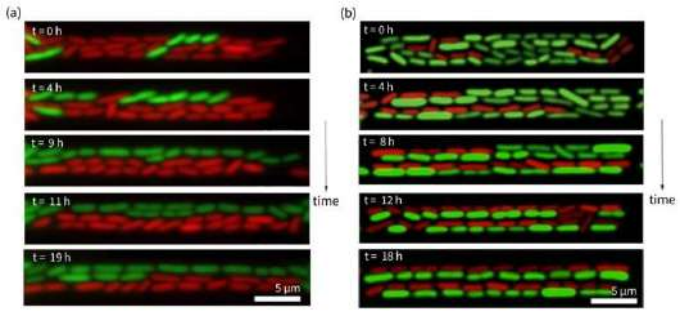
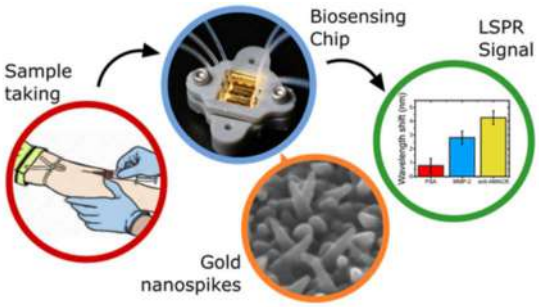


Fig. 2: Microfluidic sensors for biomarker detection.



Selected publications:

1. Riccardo Funari, Kang-Yu Chu, Amy Q Shen, Multiplexed opto-microfluidic biosensing: advanced platform for prostate cancer detection, *ACS Sensors*, 9, 2596-2604, (2024).
2. Tamara M. Iakimova, Benjamin Heidt, Amy Q. Shen, Surface-imprinted polymers based on 3D printing resin for selective bacteria detection, *Cell Reports Physical Science*, 101853, (2024).
3. Tianlong Zhang, Dino Di Carlo, Chwee Teck Lim, Tianyuan Zhou, Guizhong Tian, Tao Tang, Amy Q. Shen, Weihua Li, Ming Li, Yang Yang, Keisuke Goda, Ruopeng Yan, Cheng Lei, Yoichiroh Hosokawa, Yaxiaer Yalikun, Passive microfluidic devices for cell separation, *Biotechnology Advances*, 71, 108317, (2024).
4. Vincenzo Mazzaracchio, Mauricio Rios Maciel, Tatiana Porto Santos, Kazumi Toda-Peters, Amy Q. Shen, Duplex electrochemical microfluidic sensor for COVID-19 antibody detection: natural versus vaccine-induced humoral response, *Small*, 19, 2207731, (2023).
5. Anzhelika Koldaeva, Hsieh-Fu Tsai, Amy Q. Shen, Simone Pigolotti, Population genetics in microchannels, *PNAS*, 110, e2120821110, (2022).
6. Riccardo Funari, Hidehiro Fukuyama, Amy Q. Shen, Nanoplasmonic multiplex biosensing for COVID-19 vaccines, *Biosensors and Bioelectronics*, 208, 114193, (2022).
7. Simon J. Haward, Cameron C. Hopkins, and Amy Q. Shen, Stagnation points control chaotic fluctuations in viscoelastic porous media flow, *PNAS*, 118 (38), e211165118, (2021).
8. Cameron C. Hopkins, Simon J. Haward and Amy Q. Shen, Tristability in viscoelastic flow past side-by-side microcylinders, *Physical Review Letters*, 126 (5), (2021).
9. San To Chan, Frank P. A. van Berlo, Hammad A. Faizi, Atsushi Matsumoto, Simon J. Haward, Patrick D. Anderson, and Amy Q. Shen, Torsional fracture of viscoelastic liquid bridges, *PNAS*, 118 (24), e2104790118, (2021).
10. Shivani Sathish and Amy Q. Shen, Toward the development of rapid, specific, and sensitive microfluidic sensors: a comprehensive device blueprint, *JACS Au*, 1, 11, 1815–1833, (2021).
11. Riccardo Funari, Kang-Yu Chu, Amy Q. Shen, Detection of antibodies against SARS-CoV-2 spike protein by gold nanospikes in an opto-microfluidic chip, *Biosensors and Bioelectronics*, 169, 112578, (2020).
12. Hsieh-Fu Tsai, Joanna Gajdac, Tyler Sloan, Andrei Rarese, Amy Q. Shen, Usiigaci: Label-free instance-aware cell tracking under phase contrast microscopy using machine learning, *SoftwareX*, 9, 230-237, (2019).
13. Johanna Roether, Kang-Yu Chu, Norbert Willenbacher, Amy Q. Shen, and Nikhil Bhalla, Real-time monitoring of DNA immobilization and detection of DNA polymerase activity by a microfluidic nanoplasmonic platform, *Biosensors and Bioelectronics*, 142, 111528, (2019).
14. Cameron Hopkins, Simon J. Haward, and Amy Q. Shen, Purely elastic fluid-structure interactions in microfluidics: implications for mucociliary flows, *Small*, 1903872, (2019).

Development of high-speed scanning ion conductance microscopy for investigating nanostructural dynamics on cellular surfaces

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Shinji Watanabe is an Associate Professor of NanoLSI, Kanazawa University. He studied condensed matter physics at Niigata University (Japan), where he received his Ph.D. in 2006. He worked for one year as a postdoctoral researcher at Niigata University. From 2007 to 2009 and 2009 to 2012, he was an Assistant Professor and a Postdoctoral Researcher at Tohoku University (Japan), respectively. From 2012, he became an Assistant Professor at Kanazawa University (Japan). Since 2020, he is an Associate Professor at Kanazawa University. His current research focuses on the development of scanning probe techniques for wide range applications of nanosciences.

Presentation Abstract:

In my talk, I will introduce recent advancements in scanning ion conductance microscopy (SICM) for live cell imaging. SICM is a type of scanning probe microscopy that uses a glass nanopipette as a probe to achieve nanoscale imaging of sample surfaces in a liquid environment. This technique is particularly advantageous due to its low-invasive measurement principle, allowing for long-term visualization of cellular surfaces. Recent developments in SICM, carried out by several groups including my group [1], have enabled the capture of nanostructural dynamics on the cellular surface at sub-second time scales. Moreover, simultaneous imaging of topography and elasticity has been developed, providing comprehensive understanding of cellular states by combining multiple types of information.

Despite these advancements, there is still a need to improve the imaging rate of SICM for broader applications in nanobiosciences. Enhancing accessibility to complex structures, such as fragile tissues, remains an area of ongoing development. In my presentation, I will review recent SICM advancements and discuss the challenges we face in improving spatiotemporal resolution and accessibility to samples with complex architectures. Additionally, I will present our recent work on genotype-defined cancer cells [2], including three-dimensional organoids [3], where SICM has been used to identify quantitative differences in cellular states through physical parameters such as roughness, membrane fluctuation, and local elasticity.

Recent publications:

1. S. Watanabe, Satoko Kitazawa, Linhao Sun, and Toshio. Ando. Development of High-Speed Ion Conductance Microscopy, Rev. Sci. Instrum. 90(12): 123704 (2019).
2. Dong Wang, Han Gia Nguyen, Mizuho Nakayama, Hiroko Oshima, Linhao Sun, Masanobu Oshima, Shinji Watanabe, Mapping Nanomechanical Properties of Basal Surfaces in Metastatic Intestinal 3D Living Organoids with High-Speed Scanning Ion Conductance Microscopy, Small, 2206213 (2022).
3. Dong Wang, Linhao Sun, Satoru Okuda, Daisuke Yamamoto, Mizuho Nakayama, Hiroko Oshima, Hideyuki Saito, Yuta Kouyama, Koshi Mimori, Toshio Ando, Shinji Watanabe, Masanobu Oshima. Nano-scale physical properties characteristic to metastatic intestinal cancer cells identified by high-speed scanning ion conductance microscope. Biomaterials 280: 121256 (2022).

3D TEM reconstruction of Au nanoparticle at sub-Ångstrom resolution

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Matthias Wolf is a tenured Professor at Okinawa Institute of Science and Technology Graduate University and heads the Molecular Cryo-Electron Microscopy Unit.
His research focuses on structural virology and microbiology using biophysical techniques. His work is most known for high-resolution single particle cryo-EM. More recently, his group has also been extending these techniques to three-dimensional reconstruction of nanoparticles.
Matthias earned a PhD degree in biophysics and structural biology from Brandeis University (USA), and a master's degree in pharmacy from the University of Innsbruck (Austria). He spent 5 years of postdoctoral research time at Harvard Medical School and Children's Hospital Boston. He joined OIST as Principal Investigator in 2011, and was promoted to Assistant Professor in 2012, Associated Professor in 2019, and tenured full Professor in 2021. Also since 2021 he has been appointed a Joint Associate Research Fellow at the Institute of Biological Chemistry at Academia Sinica in Taipei/Taiwan.

Presentation Abstract:
Three-dimensional (3D) structural analysis is crucial to investigate the structural and functional properties of nanoparticles. Transmission electron microscopy (TEM) is a widely used technique to perform such characterization, however, conventional TEM images only provide two-dimensional projections of the 3D object examined. Here we propose a novel core-towards-surface 3D reconstruction strategy based on methods used in single-particle cryo-EM to reconstruct an average 3D model of 18±2 nm gold nanoparticles by starting from a 3 nm core, and expanding the reconstruction stepwise towards the surface of the nanoparticle. Our tailored approach enabled us to reconstruct the entire volume of the nanoparticle at sub-Ångstrom resolution. The excellent agreement between the experimental 3D reconstruction and a theoretical map calculated by quenched molecular dynamics demonstrates that our method is suitable to provide statistically relevant 3D structures of nanoparticles which can be subsequently used to perform ensemble analysis for strain mapping or to determine the lattice parameter of alloyed nanoparticles of different compositions.

Recent publications:

1. Engineered protein subunit COVID19 vaccine is as immunogenic as nanoparticles in mouse and hamster models.
   Melissa M Matthews, Tae Gyun Kim, Keon Young Kim, Fumiko Obata, Satoshi Shibata, Noriko Shibata, Higor Alves Iha, Vladimir Meshcheryakov, Tzung-Yang Hsieh, Mary Collins, Miho Tamai, Daiki Sasaki, Jun Fujii, Hiroki Ishikawa, Matthias Wolf
   Scientific Reports 14, 25528 (2024)
   https://doi.org/10.1038/s41598-024-76377-y
2. Electromagnetic lensing using the Aharonov–Bohm effect
   MT Schreiber, C Cassidy, M Saidani, M Wolf
   New Journal of Physics 26 (4), 043012 (2024)
   https://iopscience.iop.org/article/10.1088/1367-2630/ad3b31/meta
3. Nearly complete structure of bacteriophage DT57C reveals architecture of head-to-tail interface and lateral tail fibers
   Rafael Ayala, Andrey V Moiseenko, Ting-Hua Chen, Eugene E Kulikov, Alla K Golomidova, Philipp S Orekhov, Maya A Street, Olga S Sokolova, Andrey V Letarov, Matthias Wolf
   Nature Communications 14 (1), 8205 6 (2023)
   https://www.nature.com/articles/s41467-023-43824-9
4. Structural insights into RNA bridging between HIV-1 Vif and antiviral factor APOBEC3G
   T Kouno, S Shibata, M Shigematsu, J Hyun, TG Kim, H Matsuo, M Wolf
   Nature Communications 14 (1), 4037 (2023)
   https://www.nature.com/articles/s41467-023-39796-5

A role for synapse-astrocyte connection in shaping the morphological complexity of astrocytes

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Yukiko Goda is Professor of Synapse Biology Unit at OIST. She received BSc from the University of Toronto and PhD from Stanford University. After postdoctoral training at the Salk Institute, she joined the faculty of Biology Division, University of California, San Diego in 1997. She then moved to the UK in 2002 as a Senior Group Leader in the MRC Laboratory for Molecular Cell Biology at University College London, and from 2011 to 2022, she was a team leader at the RIKEN Brain Science Institute (renamed RIKEN Center for Brain Science in 2018). Dr. Goda's research interests focus on synapses and astrocytes. Her laboratory investigates the cellular principles by which synaptic strengths are set and dynamically modified in defined neural circuits that are consequential to supporting particular behaviors.

Presentation Abstract:
The amyloid precursor protein (APP) has been intensely studied for its role in Alzheimer's disease, but its physiological function remains unclear. In neurons, APP and its homologs, the amyloid precursor-like proteins (APLPs) are present at synapses and promote synaptogenesis. Astrocytes also express APP although a role for astrocytic APP has not been fully explored. We have studied the expression and function of APP in rodent astrocytes *in vitro* and *in vivo*. shRNA-mediated knockdown of astrocytic APP compromises astrocyte morphological elaboration in hippocampal cultures and in the intact brain. Our results highlight a role of astrocytic APP and possibly of APLPs in shaping astrocyte morphological complexity. We are currently examining how astrocytic APP affects the dynamics of tripartite synapses.

Recent publications:

1. Chater TE†, Eggl MF†, Goda Y*, Tchumatchenko T*. (2024) Competitive processes shape multi-synapse plasticity along dendritic segments. *Nat Commun 15*, 7572. doi: 10.1038/s41467-024-51919-0.
2. Letellier M, Goda Y. (2023) Astrocyte calcium signaling shifts the polarity of presynaptic plasticity. *Neuroscience 525*, 38-46.
3. Saint-Martin M, Goda Y. (2022) Astrocyte-synapse interactions and cell adhesion molecules. *FEBS J 290*, 3512-3526. doi: 10.1111/febs.16540.
4. Chipman PH, Fung CCA, Fernandez A, Sawant A, Tedoldi A, Kawai A, Gautam SG, Kurosawa M, Abe M, Sakimura K, Fukai T, Goda Y. (2021) Astrocyte GluN2C NMDA receptors control basal synaptic strengths of hippocampal CA1 pyramidal neurons in the *stratum radiatum*. *eLife 10*, e70818. doi: 10.7554/eLife.70818.
5. Tong R, Chater TE, Emptage NJ, Goda Y. (2021) Heterosynaptic crosstalk of pre- and postsynaptic strengths along segments of dendrites. *Cell Rep 34*, 108693. doi: 10.1016.

Reduced mitochondrial activity and impairment in axonal translation during aging in sensory neurons

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Marco Terenzio did his PhD in the laboratory of Prof. Giampietro Schiavo at Cancer Research UK in London, with the aim of identifying regulators of neurotrophin receptors endosomal distribution in motor neurons. After a brief first postdoc at the German Cancer Research Centre (DKFZ) in Heidelberg, Germany, where he studied the mechanisms of mitochondrial clearance, he joined the laboratory of Prof. Mike Fainzilber at the Weizmann Institute in Israel, where he investigated the mechanisms underlying cell length sensing in neurons and the axonal translational response to sciatic nerve injury. He now studies the mechanism underlying mRNA granules transport and translation in axons of sensory neurons and motor neurons. He also studies the mechanisms behind the pathogenesis of ALS.

Abstract Authors:
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2. Complex Fluids and Flows Unit, Okinawa Institute of Science and Technology Graduate University, Kunigami-gun, Okinawa, Japan.

Presentation Abstract:
Axonal translation is an important mechanism which plays a role in maintaining axonal morphology as well as mediating axonal recovery after injury. Mitochondria are trafficked along the axons and provide energy required for several intracellular mechanisms including molecular transport and local translation. Decline in mitochondria activity is one of the hallmarks of aging. However, it is still unclear whether this decline corresponds to a similar reduction in the extent of axonal translation in aging neurons. We utilized microfluidic devices to separate cell body and axons of DRG neurons. Using live imaging, we found a significant decrease in the level of axonal translation as well in the number of translational hotspots in aging neurons. We also showed that attempting to increase mitochondria activity had a positive effect on axonal translation in aging neurons. We think that this research sheds a light on axonal translation in aged neurons and its relationship with energy sources inside the axonal organelles, which might present an opportunity for therapy in the future.

Recent publications:

1. FMRP Long-Range Transport and Degradation Are Mediated by Dynlrb1 in Sensory Neurons. El-Agamy SE, Guillaud L, Kono K, Wu Y, Terenzio M. Mol Cell Proteomics. 2023 Nov;22(11):100653.
2. Biodegradable and Electrically Conductive Melanin-Poly (3-Hydroxybutyrate) 3D Fibrous Scaffolds for Neural Tissue Engineering Applications. Agrawal L, Vimal SK, Barzaghi P, Shiga T, Terenzio M. Macromol Biosci. 2022 Dec;22(12):e2200315.
3. Development of 3D culture scaffolds for directional neuronal growth using 2-photon lithography. Agrawal L, Saidani M, Guillaud L, Terenzio M. Mater Sci Eng C Mater Biol Appl. 2021 Dec;131:112502.
4. DYNLRB1 is essential for dynein mediated transport and neuronal survival. Terenzio M, Di Pizio A, Rishal I, Marvaldi L, Di Matteo P, Kawaguchi R, Coppola G, Schiavo G, Fisher EMC, Fainzilber M. Neurobiol Dis. 2020 Jul;140:104816.

Nanoimaging of SARS-CoV-2 viral invasion toward the nuclear pore territories

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Richard Wong is Professor of WPI-NanoLSI. He is employing several nano-imaging methods to elucidate the nano-meso-structure and functionality of several intracellular transport systems of viral proteins and tiny organelles. Of particular interest are nuclear pores, nucleosomes, microtubules, and extracellular vesicles (EV).
Richard obtained his PhD at the Department of Anatomy & Cell Biology, The University of Tokyo, under the guidance of Prof. Nobutaka Hirokawa. As a postdoctoral researcher with Nobel laureate Günter Blobel at Howard Hughes Medical Institute at Rockefeller University in New York, Richard has investigated the structure and function of the nuclear pore complex.

Presentation Abstract:

I am going to briefly introduce nano imaging of nuclear pore territories in my talk.

First, I would like to talk about the different jobs that nuclear pore complexes (NPCs) do. Inside the NPC, an assembly of natively unfolded ("spider cobweb-like") proteins dictates the chemical and size selectivity of transport into and out of the nucleus. NPCs are not only as intracellular supply chain terminals controlling the transport of proteins, NPCs also play critical roles in spindle polarity, the formation of aneuploidies, the growth of colon and brain cancer, and the location of super enhancers at the epigenomic and spatial levels.

Second, I would like to talk about NPCs dynamic structures and how viral proteins move towards to NPCs. NPCs restrict free diffusion to molecules below 5 nm while facilitating the active transport of selected cargoes, sometimes as large as the pore itself. This versatility implies an important pore plasticity. The limitation of traditional optical imaging is due to diffraction, which prevents achieving the required resolution for observing a diverse array of organelles and proteins within cells. Super-resolution techniques have effectively addressed this constraint by enabling the observation of subcellular components on the nanoscale. Nevertheless, it is crucial to acknowledge that these methods often need the use of fixed samples. This also raises the question of how closely a static image represents the real intracellular dynamic system. High-speed atomic force microscopy (HS-AFM) is a unique technique used in the field of dynamic structural biology, enabling the study of individual molecules in motion close to their native states. Subsequently, we promptly utilize HS-AFM real-time imaging and cinematography approaches to record different viral proteins, microtubules, EVs and how they transport towards nuclear pore from purified proteins, cells, organoids, and mouse brain tissues.

Recent publications:

1. Sajidah ES, … and Wong RW. Nanoimaging of SARS-CoV-2 viral invasion toward the nucleus and genome. Cell Rep. Phys. Sci. 5: 102111. (2024)
2. Hazawa M, … and Wong RW. Super enhancer trapping by the nuclear pore via intrinsically disordered regions of proteins in squamous cell carcinoma cells. Cell Chemical Biology 31: 792–804. (2024)
3. Ikliptikawati DK, … and Wong RW. Nuclear transport surveillance of p53 by nuclear pores in glioblastoma. Cell Reports 42:112882. (2023)
4. Lim K, … Hanayama R and Wong RW. Nanoscopic assessment of anti-SARS-CoV-2 spike neutralizing antibody using high-speed AFM. Nano Letters 23: 619-628. (2023)

Targeting ADAR1: understanding its role in drug resistance, developing a sensitive detection biosensor, and analyzing its structural dynamics

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Miki Nakajima is a Professor at the WPI-NanoLSI and the Faculty of Pharmaceutical Sciences at Kanazawa University. Prior to joining Kanazawa University as an Assistant Professor, she was a Research Associate at the School of Pharmaceutical Sciences, Showa University. She earned her Bachelor's and Master's degrees in Pharmaceutical Sciences from Hokkaido University and obtained her PhD from Kanazawa University.

Her primary research interest is in interindividual variability in drug response and toxicity. She investigates the regulatory mechanisms of drug-metabolizing enzyme expression through RNA modification, microRNAs, and lncRNAs, with a particular focus on cancer.

Presentation Abstract:

Human RNA undergoes various modifications, among them, the most prevalent modification is A-to-I RNA editing catalyzed by ADAR (adenosine deaminase acting on RNA). This process involves the conversion of adenosine (A) to inosine (I) in double-stranded RNA regions, which can influence RNA stability, splicing, and protein functions. The aberrant expression of ADAR is associated with various cancers, highlighting its potential as both a prognostic biomarker and a therapeutic target. Our study also revealed that ADAR1 expression was higher in breast cancer tissues compared to normal tissues, suggesting that ADAR1 contributes to cancer proliferation. We found that ADAR1 increased the expression of DHFR (dihydrofolate reductase), a target protein of methotrexate, an anti-cancer drug, by disrupting the binding of miR-25-3p and miR-125-3p, leading to drug resistance. We demonstrated that suppressing ADAR1 expression decreased DHFR level and enhanced the effectiveness of methotrexate, indicating that inhibiting ADAR1 can help overcome resistance to anti-cancer drugs.

We screened DNA aptamers against ADAR1 and identified Apt38, an aptamer with high binding affinity for ADAR1. Although its inhibitory effects on A-to-I RNA editing were weak, we leveraged its binding capability to develop an electrochemical biosensor for the precise detection of ADAR1 in biological samples. The biosensor combines Apt38 with an electrochemical transduction method, utilizing a sandwich assay format with specific antibodies and gold nanoparticles. It detects ADAR1 at concentrations as low as 0.53 nM via differential pulse voltammetry (DPV), offering a highly sensitive, cost-effective, and rapid detection method with significant implications for cancer prognosis and monitoring.

Limited structural information on ADAR1 complexes has hindered the identification of effective inhibitors. To address this challenge, we employed 3D computational modeling and high-speed atomic force microscopy (HS-AFM) to study the dynamics of ADAR1. We identified key interface regions (IFx and IFy) within the deaminase domain that are crucial for ADAR1 dimerization and observed stable dimeric structures in the presence of substrate dsRNA. Our findings also underscore the role of the flexible N-terminal region in maintaining ADAR1 dimer stability and dynamics. These insights are essential for developing targeted inhibitors to modulate ADAR1 activity, paving the way for new therapeutic interventions.

Recent publications:

1. M. Nakano and M. Nakajima. Adenosine-to-inosine RNA editing and N^6-methyladenosine modification modulating expression of drug metabolizing enzymes. Drug Metab. Dispos. 50: 624-633 (2022).
2. M. Biyani, K. Sharma, S. Maeda, H. Akashi, M. Nakano, M. Nakajima, and M. Biyani. A novel aptamer-antibody sandwich electrochemical sensor for detecting ADAR1 in complex biological samples. Biosens. Bioelectron. X 19: 100491 (2024).
3. M. Biyani, Y. Isogai, K. Sharma, S. Maeda, H. Akashi, Y. Sugai, M. Nakano, N. Kodera, M. Biyani, and M. Nakajima. High-speed atomic force microscopy and 3D modeling revealed the structural dynamics of ADAR1 complexes. submitted.

Quantitative methods for investigating focal adhesion nanomechanics

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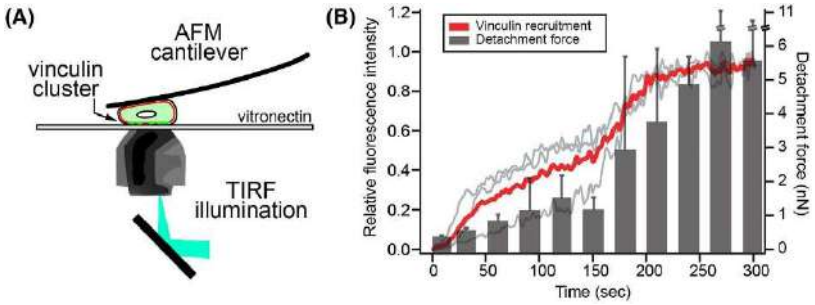
Clemens Franz is Associate Professor at WPI NanoLSI, Kanazawa University, where he is developing AFM applications for cell adhesion, matrix and mechanobiology research.
Clemens obtained his PhD in Cancer Cell Biology from University College London under the supervision of Prof. Anne Ridley. During his postdoc with Prof. Daniel Muller at Max-Planck-Institute for Cell Biology and Genetics Dresden, he applied AFM-based single-cell force spectroscopy to quantitate cell adhesion force from the single-molecule level to the cell scale. After becoming a group leader at Karlsruhe Institute of Technology, he continued integrating optical microscopy and AFM for cell adhesion and extracellular matrix research.

Presentation Abstract:
Focal adhesions are dynamic integrin adhesion sites where intracellular contractile forces generated by actin stress fibers are transmitted onto the extracellular environment, thereby driving processes such as cell migration, tissue invasions, and extracellular matrix (ECM) remodeling. At the same time, many mechano-sensitive focal adhesion components themselves undergo force-induced conformational changes and functional regulation. High-speed atomic force microscopy (HS-AFM) can image such force-induced conformational changes of focal adhesion-associated proteins under physiological conditions and in real-time. Here, we have applied HS-AFM in combination with fluorescence microscopy to investigate actomyosin contractility-dependent adhesion modulation, including the tension-driven opening of Ca^{2+} channels near mechanically stressed focal adhesion sites, leading to intracellular Ca^{2+} influx, recruitment of Ca^{2+}-binding proteins such as S100A11, and subsequent focal adhesion disassembly. Furthermore, we have established methods to image individual integrin receptor-ligand pairs by HS-AFM and show how force-induced conformational changes modulate integrin receptor binding strength to the ECM protein laminin. Lastly, by combining cell deroofing with large-range/high-resolution HS-AFM imaging, we are can image large intracellular protein assemblies and even entire organelles down to molecular resolution, while preserving them in a functional state. In this way, we have generated the first molecular resolution-scale overview images of entire actin stress fibers and analyzed nanostructural and -mechanical changes during myosin II-driven actin stress fiber contraction. Thus, HS-AFM can provide unique nanoscale structural insight into both intra- and extracellular biomechanical processes underlying cell/matrix adhesion regulation.



Recent publications:

1. L. Akter, H. Flechsig, A. Marchesi, and C.M. Franz, Observing dynamic conformational changes within the coiled-coil domain of different laminin isoforms by high-speed atomic force microscopy, Int. J. Mol. Sci. 25, 4 (2024)
2. T.O. Mohammed, Y.-Y. Lin, … and C.M. Franz, S100A11 promotes focal adhesion disassembly via myosin II-driven contractility and Piezo1-mediated Ca2+ entry, J. Cell Sci. 137 (2): jcs261492 (2024)
3. Z. Ye,…C.M. Franz, V. Torre, and Arin Marchesi, Structural heterogeneity of the ion and lipid channel TMEM16F, Nat. Comm. 15, 110 (2024)
4. H. Baumann,…and C.M. Franz, Biphasic reinforcement of nascent adhesions by vinculin, J. Mol. Rec. e3012 (2023)

Unexpected features of early embryonic division in medaka

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Tomomi Kiyomitsu obtained his PhD at Kyoto University (supervised by Prof. Mitsuhiro Yanagida) in 2008. He continued his PhD studies of chromosome segregation as a postdoctoral fellow in Yanagida's laboratory until 2010. From 2010-2013, Tomomi started his spindle positioning study as an HFSP long-term fellow in Prof. Iain M. Cheeseman's laboratory at the Whitehead Institute, MIT, USA. He then moved back to Nagoya University, Japan (to Prof. Gohta Goshima's laboratory) in 2013 and continued his work as an assistant professor, a lecturer, and a JST PRESTO researcher. He started his own unit at OIST in April 2020. He has been a JST FOREST researcher since 2023.

Presentation Abstract:

During early embryogenesis in animals, a large, fertilized egg undergoes repeated cell divisions to create numerous small, differentiated cells. This process comprises a series of dynamic physical and biochemical changes, including cell size reduction, zygotic gene activation, and cell cycle remodeling. Regardless of these drastic cellular changes, unified parental chromosomes must be accurately duplicated and segregated to all blastomeres to maintain and transmit genomic information. Although sizes and cleavage patterns of fertilized eggs vary among species, a microtubule-based bipolar structure, the mitotic spindle, is generally assembled in each blastomere to segregate duplicated chromosomes into daughter cells. Recent studies have shown that embryonic divisions in bovines and humans are error-prone, but mechanisms of spindle assembly and chromosome segregation in vertebrate embryos remain poorly understood, compared to those in somatic cells. Recently, we have established live functional assay systems in medaka (*Oryzias latipes*) embryos by combining high-quality live imaging with CRISPR/Cas9-mediated genome editing and an auxin-inducible degron 2 (AID2)-based protein knockdown system (Kiyomitsu et al., Nature Communications 2024). In this talk, I will present our recent findings showing unique and unexpected features of early embryonic divisions. Especially, I focus on RCC1, a guanine nucleotide exchange factor for Ran, which is non-essential for spindle assembly in small somatic human cells (Tsuchiya et al., *Current Biology* 2021), but becomes essential in large medaka early embryos (Kiyomitsu et a., *Nature Communications* 2024).

Recent publications:

1. Kiyomitsu T. (An Editor of Book): The Mitotic Spindle. ***Methods in Molecular Biology***, Springer Nature. *in press*
2. Kiyomitsu A, Nishimura T, Hwang SJ, Ansai S, Kanemaki MT, Tanaka M, Kiyomitsu T. Ran-GTP assembles a specialized spindle structure for accurate chromosome segregation in medaka early embryos. ***Nature Communications*** (2024) Feb 1;15(1):981. doi: 10.1038/s41467-024-45251-w.
3. van Toorn M, Gooch A, Boerner S, Kiyomitsu T. NuMA deficiency causes micronuclei via checkpoint-insensitive k-fiber minus-end detachment from mitotic spindle poles. ***Current Biology*** (2023) Feb 6;33(3):572-580.e2.
4. Kiyomitsu T. Using Optogenetics to Spatially Control Cortical Dynein Activity in Mitotic Human Cells. ***Methods in Molecular Biology*** (2023) 2623:73-85.s
5. Tsuchiya K, Hayashi H, Nishina M, Okumura M, Sato Y, Kanemaki MT, Goshima G, Kiyomitsu T. Ran-GTP Is Non-essential to Activate NuMA for Mitotic Spindle-Pole Focusing but Dynamically Polarizes HURP Near Chromosomes. ***Current Biology*** (2021) Jan 11;31(1):115-127.e3.

Crosstalk Between Cytoplasmic Decay and Nuclear Transcription?: Insights from Real-Time Imaging of Nonsense-Mediated mRNA Decay

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Hanae Sato is an RNA biologist with extensive research experience in biochemistry and imaging technologies. After completing her Ph.D. at the University of Tokyo, she broadened her expertise in the RNA field at the University of Rochester and Albert Einstein College of Medicine, USA. Her current research focuses on the regulation of mRNA from transcription to decay, with a particular emphasis on understanding the molecular mechanisms underlying nonsense-mediated mRNA decay (NMD). She employs a range of approaches, including advanced single-molecule mRNA fluorescence imaging technologies. Since founding her lab at NanoLSI, Kanazawa University, in 2022, she has also explored RNA therapeutics, aiming to develop treatments for disorders linked to nonsense mutations.

Presentation Abstract:

Eukaryotic cells are compartmentalized into the nucleus and cytoplasm, with gene expression processes occurring in these distinct compartments—transcription in the nucleus and translation in the cytoplasm. Nonsense-mediated mRNA decay (NMD) is a translation-coupled mRNA decay pathway triggered by premature termination codons (PTCs). Although the recognition of in-frame PTCs occurs exclusively in cytoplasmic ribosomes, unexpected transcriptional alterations in genes with PTCs have been observed. This suggests crosstalk between the nucleus and cytoplasm during gene expression regulation. Addressing cellular events in these separate compartments simultaneously remains challenging. In this presentation, I will discuss a study that employs a real-time imaging technique to simultaneously monitor the transcriptional activity of both wild-type and NMD-targeted reporter genes in individual cells. Our findings reveal that NMD, which operates in the cytoplasm, induces significant transcriptional changes in a PTC-specific manner, providing compelling evidence for a robust connection between cytoplasmic decay and nuclear transcription. Additionally, I will present collaborative research conducted at NanoLSI, highlighting the diverse technologies used in our investigations.

Recent publications:

1. Dong-Woo Hwang, Anna Maekiniemi, Robert H. Singer, Hanae Sato. Real-time single-molecule imaging of transcriptional regulatory networks in living cells. Nature Reviews Genetics 25: 272-285 (2024)
2. An improved imaging system that corrects MS2-induced RNA destabilization. Weihan Li, Anna Maekiniemi, Hanae Sato, Christof Osman, Robert H. Singer. Nature Methods 19: 1558-1562 (2022)
3. Cellular variability of nonsense-mediated mRNA decay. Hanae Sato, Robert H. Singer. Nature Communications 12: 7203-7203 (2021)

Development of ultrafast super-resolution single-molecule imaging and discovery of a nano-liquid signal transduction platform (iTRVZ)

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Akihiro Kusumi (Aki) is Professor of OIST, and Professor Emeritus of Kyoto University and Nagoya University. He is developing single-molecule imaging and manipulation methods and instruments, and applying them for revealing the nano-meso-structure and function of the cellular plasma membrane.

Aki obtained his PhD at the Department of Biophysics, Kyoto University, under the guidance of Prof. Shun-ichi Ohnishi. Aki's first postdoc advisor, Prof. James S. Hyde of the Medical College of Wisconsin is responsible for his physics, and his second postdoc advisor, Prof. Malcolm S. Steinberg of Princeton University is responsible for his cell biology.

Presentation Abstract:
I am going to briefly cover two topics in my talk.

First, I would like to talk about development of an ultrafast camera system that enables the highest time resolutions in single fluorescent-molecule imaging to date, which were photon-limited by fluorophore photophysics: 33 and 100 μs with single-molecule localization precisions of 34 and 20 nm, respectively, for Cy3, the optimal fluorophore we identified. Using theoretical frameworks developed for the analysis of single-molecule trajectories in the plasma membrane (PM), this camera successfully detected fast hop diffusion of membrane molecules in the PM, previously detectable only in the apical PM by using less preferable 40-nm gold probes, thus helping to elucidate the principles governing the PM organization and molecular dynamics. Furthermore, as described in the companion paper, this camera allows simultaneous data acquisitions for PALM/dSTORM at as fast as 1 kHz, with 29/19 nm localization precisions in the 640x640 pixel view-field.

Using our newly-developed ultrafast camera, we reduced the data acquisition periods required for photoactivation/photoconversion localization microscopy (PALM, using mEos3.2) and direct stochastic reconstruction microscopy (dSTORM, using HMSiR) by a factor of ≈30 compared with standard methods, for much greater view-fields, with localization precisions of 29 and 19 nm, respectively, thus opening up previously inaccessible spatiotemporal scales to cell biology research.

Second, I will talk about the liquid nano-platform for signal integration on the PM, called iTRVZ. Crosstalk of cellular signaling pathways is essential for integrating them for inducing coordinated final cell responses. However, how signaling molecules are assembled to induce signal integration remains largely unknown. Here, using advanced single-molecule imaging, we found a nanometer-scale liquid-like platform for integrating the signals downstream from GPI-anchored receptors and receptor-type tyrosine kinases. The platform employs some of the focal adhesion proteins, including integrin, talin, RIAM, VASP, and zyxin, but is distinct from focal adhesions, and is thus termed iTRVZ. The iTRVZ formation is driven by the protein liquid-liquid phase separation and the interactions with the raft domains in the plasma membrane and cortical actin. iTRVZ non-linearly integrates the two distinctly different receptor signals, and thus works as an AND gate and noise filter. Using an in-vivo mouse model, we found that iTRVZ greatly enhances tumor growth.

Recent publications:

1. T. K. Fujiwara, … and A. Kusumi. Ultrafast single-molecule imaging reveals focal adhesion nano-architecture and molecular dynamics. J. Cell Biol. 222: e202110162 (2023).
2. T. K. Fujiwara, … and A. Kusumi. Development of ultrafast camera-based single fluorescent-molecule imaging for cell biology. J. Cell Biol. 222: e202110160. (2023).

POSTER SESSIONS

P-01	**Linhao Sun** **Assistant Professor, WPI-NanoLSI** **Kanazawa U**	High Spatiotemporal Resolution Scanning Ion Conductance Microscopy for Exploring the Surface Characteristics of Living Cells
P-02	**Kohei Amada** **PhD Student, JAIST**	Optimizing Conductivity and Stretchability in Ultra-High Molecular Weight Polyethylene through Controlled Filler Distribution
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P-05	**Yanjun Zhang** **Associate Professor, WPI-NanoLSI** **Kanazawa U**	Scanning Ion Conductance Microscopy and its based nanoprobes for single cell biosensing
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P-07	**Lim Kee Siang** **Assistant Professor, WPI-NanoLSI** **Kanazawa U**	Journey to the Nucleus
P-08	**Kohgaku Eguchi** **Senior Staff Scientist, OIST**	Nanoscale analysis of phosphoinositide distribution on cell membranes of mouse cerebellar neurons using SDS-digested freeze-fracture replica labeling
P-09	**Mahmud SM Neaz** **PhD Student, WPI-NanoLSI** **Kanazawa U**	Nanoscopic investigation of RNA-mediated LLPS formation
P-10	**Poulami Mukherjee** **Postdoctoral Fellow, JAIST**	Strategic Design of Prussian Blue Analogs for Electrochemical CO_2 Reduction
P-11	**Ayhan Yurtsever** **Assistant Professor, WPI-NanoLSI** **Kanazawa U**	Revealing Submolecular Structures with 3D-AFM: Applications in Polysaccharide Nanocrystals and Peptide Assemblies
P-12	**Yoshiki Ochiai** **PhD Student, OIST**	SUPREM: an engineered non-site-specific m^6A RNA methyltransferase with highly improved efficiency
P-13	**Lucky Akter** **Postdoctoral Fellow, WPI-NanoLSI** **Kanazawa U**	Observing dynamic conformational changes within the coiled-coil domain of different laminin isoforms using high-speed atomic force microscopy

P-14	**Taiko Oshida** **PhD Student, JAIST**	Phase structure analysis of polymer blends combining unsupervised machine learning and AFM images
P-15	**Djamel Eddine Chafai** **Assistant Professor, WPI-NanoLSI Kanazawa U**	Nanoscale investigation of CRMP2 isoforms' role in microtubule organization using high-speed atomic force microscopy (HS-AFM)
P-16	**Viet Giang Truong** **Senior Staff Scientist, OIST**	Hybrid Metamaterial Plasmonic Tweezers for Direct Trapping and Measuring Conformational Changes of Single Urease Proteins
P-17	**You-Rong Lin** **PhD Student, WPI-NanoLSI Kanazawa U**	Investigating the role of non-muscle myosin II isoforms during stress fiber contraction by atomic force microscopy
P-18	**Nunnarpas Yongvongsoontorn** **Research Assistant Professor, JAIST**	Carrier-Enhanced Efficacy of Molecular Targeted Drug-Loaded Nanoparticles for Cancer Therapy
P-19	**Xiabing Lyu** **Assistant Professor, WPI-NanoLSI Kanazawa U**	Surface-engineered extracellular vesicles to modulate antigen-specific T cell expansion for cancer immunotherapy
P-20	**Esteban Fregoso** **PhD Student, OIST**	A microfluidic-based model for molecular characterization of axonal injury
P-21	**Tamoghna Das** **Assistant Professor, WPI-NanoLSI Kanazawa U**	On relating the structure, dynamics and mechanics of aggregation
P-22	**Joo Eun Chung** **Research Professor, JAIST**	Universal Nanoenhancer For Drug Delivery
P-23	**Saahil Acharya** **Postdoctoral Scholar, OIST**	SynGAP LLPS Condensates as the Basic Platform for Recruiting PSD95 and Receptor Oligomers for Generating Excitatory Synapses
P-24	**Mahima Kumar** **PhD Student, WPI-iCeMS, Kyoto U**	Biomass-derived carbon dots as inflammation theranostics
P-25	**Ming Yang** **PhD Student, OIST**	Spatiotemporal Regulations of RanGTP-dependent Mitotic Spindle Assembly in Medaka Embryos
P-26	**Hannah Connor** **Research Intern of OIST, U Bath**	Improving the Bioavailability of azole antifungals within Composite Hydrogels through Host-Guest Interactions with β-Cyclodextrin

P-01

High Spatiotemporal Resolution Scanning Ion Conductance Microscopy for Exploring the Surface Characteristics of Living Cells

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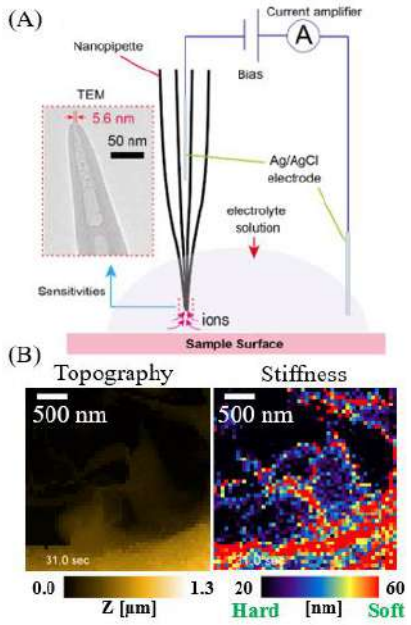
Abstract

Scanning ion conductance microscopy (SICM) is one type of novel nanoprobe technique among atomic force microscopy, scanning tunneling microscopy and others. SICM utilizes ion current changes between two electrodes as feedback signal to outline the three-dimensional profiles of detected samples. The whole imaging process is conducted in a liquid environment (Figure 1A). The most advantage of SICM is its non-contact imaging features, allowing to maintain the structural integrities of flexible bio-samples such as cells.



Recent improvements of SICM in time/space resolutions have sped up its broad applications for imaging the dynamical surface characteristics (morphologies, charge and stiffness) of biological samples including living cells (Figure 1B), neurons, bacteria and many others. These surface characteristics have been found to be of significance as label-free biomarker for cancer diagnosis or as key factor for mediating intercellular communications.

Recent publications:

1. D Wang; L Sun; S Watanabe; M Oshima et al., Nanoscale physical properties characteristic to metastatic intestinal cancer cells identified by high-speed scanning ion conductance microscope, ***Biomaterials***, 2022, 280: 121256

2. Kenry; L Sun; S Watanabe; B Liu et al., In Situ Visualization of Dynamic Cellular Effects of Phospholipid Nanoparticles via High-Speed Scanning Ion Conductance Microscopy, ***Small***, 2022, 18(37): 2203285-2203285

3. D Wang; L Sun; M Oshima; S Watanabe; Mapping Nanomechanical Properties of Basal Surfaces in Metastatic Intestinal 3D Living Organoids with High-Speed Scanning Ion Conductance Microscopy, ***Small***, 2022, 19(7): 2206213.

4. W Phairuang; L Sun; Characterizing Chemical, Environmental and Stimulated Subcellular Physical Characteristics of Size-Fractionated PMs Down to $PM_{0.1}$, ***Environ. Sci. Tech.***, 2024,58 (28), 12368-12378

P-02

Optimizing Conductivity and Stretchability in Ultra-High Molecular Weight Polyethylene through Controlled Filler Distribution

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Abstract

Ultra-high molecular weight polyethylene (UHMWPE) exhibits outstanding mechanical properties and wear resistance. However, it shows high insulation and hence problems related to static charge accumulation. In recent years, there have been some attempts to add a conductive filler, such as graphene nanoplatelets (GP), to UHMWPE with the aim of enhancing its electrical properties and antistatic performance.[1,2] Typically, melt-mixing is used to disperse the filler, but as UHMWPE has a high melt viscosity, it is processed using compression moulding; thus, it is difficult to control the dispersion of fillers. In this study, we propose a strategy to control the distribution of nanofillers. Generally, UHMWPE has a large particle size (~200 μm), and fillers tend to segregate in the interparticle spaces (Figure 1 a), which can hinder particle fusion even with a small amount of fillers, leading to a significant deterioration of mechanical properties.[3] To overcome this, we used a technology[4] developed in our laboratory that disperses Ziegler-Natta catalysts at the nanoscale to produce UHMWPE particles with a size of less than 2 μm, which were then used as a second matrix to create a composite with GP (Figure 1b). As a result, we successfully produced a composite that exhibited electrical conductivity while retaining its mechanical properties. This study demonstrates that filling the voids among coarse with fine particles is essential for achieving both of conductive pathways and the entanglements of polymer chains.

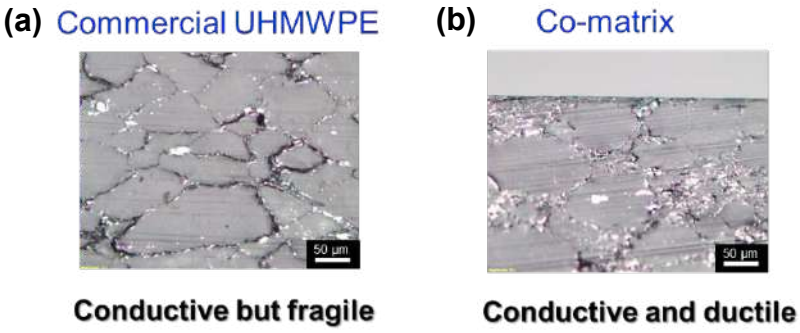

Figure 1. Cross-sectional optical microscope images of UHMWPE/GP composite films. (a) Compression moulding was performed using UHMWPE particles of around 70 μm; (b) UHMWPE particles of around 0.46 μm were added as a second matrix in compression moulding.

References:

1) Alam, F. et al., *Mater. Sci. Eng. B* **2019**, 241, 82-91., 2) Stankovich, S. et al., *Nature* **2006**, 442, 282-286., 3)Yan, X. et al., *Ind. Eng. Chem. Res.* **2023**, 62, 7950-7961., 4) Chammingkwan, P. et al., *Front. Chem.* **2018**, 6, 524.

P-03

Nanoscale analysis of microbial cell wall structures by AFM

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Microorganisms and plants have cell walls that encircle their cells and execute diverse functions by generating turger pressure inside the cell. For instance, in *Colletotrichum Orbiculare*, which is one of a type of plant-pathogenic fungus, spores differentiate into appressoriums on the plant surface and make a turger pressure to punch a hole in the plant cell wall for mycelium to invade. The cell wall structure of the appressorium that supports such strong turger pressure attracted extensive interest from numerous research fields. In this study, we visualized the nanoscale structures of appresorium cell walls using atomic force microscopy (AFM). We used appressoria of *Colletotrichum Orbicular* with both wild-type and melanin mutant (where the melanin gene was knocked out) as our model sample. Melanin mutant cells are known to have lower turger pressure in the appressorium than wild-type cells, and we expected differences in the cell wall structures. The appressoria were grown on a plastic dish and immersed in ultrapure water. We performed AFM measurements using a NanoWizard4 (JPK, Bruker) and 240AC (OPUS) cantilever. High resolution AFM images of the cell wall surface of both wild-type and melanin mutant appressoria in Fig. 1c-d, revealing the detailed fibrous structures of the cell wall with a resolution of less than 10 nm. Our analysis found that the diameter of the fibrous structures was larger in the wild-type cell wall than in the melanin mutant and that globular structures with a size of several tens of nanometers were present in the wild-type cell wall, forming a robust membrane structure that creates strong turger pressure inside the appressorium. This method provides new insights into the mechanism by which cell wall can form such high turger pressures and contribute to the field of biology and materials science.

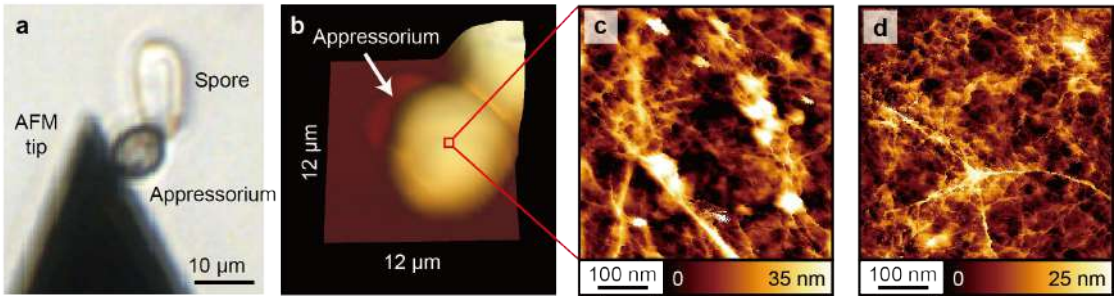

Fig. 1 AFM imaging of cell wall of the appresorium of *Colletotrichum Orbiculare*.
(a) Bright field optical microscopy image and (b-d) AFM images of appressorium of Colletotrichum orbiculare of (b-c) wild type and (d) melanin mutant, respectively.

P-04

Base Editor Screens Uncover Functional Domains in Mitotic Stopwatch Genes

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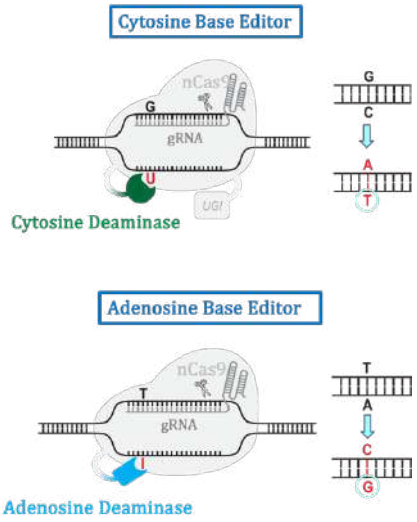
Abstract

Cells duplicate their genome and segregate them equally to each daughter cell during mitosis. This process must be carried out accurately, as any error can lead to defects in the genome and subsequent pathologies, such as cancer. The Mitotic Stopwatch Pathway is a surveillance mechanism that detects error-prone prolonged mitosis by measuring the duration of mitosis and stops the proliferation of potentially defective cells by forming complexes that stabilize the transcription factor p53, which subsequently induces cell arrest or death.

To identify and study functional domains of genes involved in the formation and function of the Mitotic Stopwatch Complex – 53BP1, USP28, p53, and p21 – we performed Base Editor Screens utilizing recently established CRISPR Base Editors that can specifically mutate cytosine to thymine (C-to-T) or adenine to guanine (A-to-G), leading to targeted mutation of single amino acids.

Immortalized Retinal Pigment Epithelial cells (hTERT RPE-1) expressing base editors were infected with a custom-made gRNA library to mutate thousands of codons in the target genes. The infected cells were treated with Polo-Like Kinase 4 inhibitor to prolong mitosis. Only cells that carry a loss-of-function mutation continue to proliferate due to the failure to stabilize p53 or express functional p21 following prolonged mitosis. We also treated cells with Doxorubicin to compare the effect of prolonged mitosis to DNA damage response. Next Generation Sequencing (NGS) of the samples identified multiple sites in 53BP1, USP28, and p53 that are vital and specific for the Mitotic Stopwatch function. Further experiments using single-cell tracking, immunofluorescence staining, and immunoprecipitation assays revealed how specific domains of these genes interact with each other in the context of the Mitotic Stopwatch Complex.



Notably, the screen also identified essential phosphorylation sites. We are currently investigating how these phosphorylation sites contribute to the time-measuring mechanism of the mitotic stopwatch.

Publications

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2. Belal, H., Ng, E.F.Y., Meitinger, F. (2024). 53BP1-mediated activation of the tumor suppressor p53. **Curr Opin Cell Biol** 2024, 91:102424.
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P-05

Scanning Ion Conductance Microscopy and its based nanoprobes for single cell biosensing

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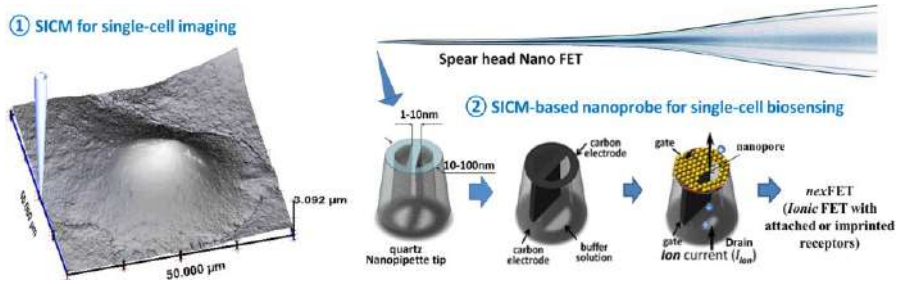
Abstract



Our research focuses on advancing Scanning Ion Conductance Microscopy (SICM) for single-cell imaging. We developed an SICM-based mechanical probing method for cellular stiffness mapping and functionalized nanoprobes for single-cell biosensing. These nanoprobes have been successfully applied to pH and ROS biosensing in individual living cancer cells. Additionally, we've created SICM-based nexFET sensors that combine non-contact SICM, nanopore single-molecule sensing, FET, and recognition chemistry, enabling label-free, real-time biosensing of single cells and single molecules.

Recent publications:

1. Dong Wang, Emily Woodcock, Xi Yang, Hiromi Nishikawa, Elena V Sviderskaya, Masanobu Oshima, Christopher Edwards, **Yanjun Zhang***, Yuri Korchev*. Exploration of individual colorectal cancer cell responses to H2O2 eustress using hopping probe scanning ion conductance microscopy. *Science Bulletin*, 2024, 69: 1909-1919.

2. Fengjie Liu*, Martha Gledhill, Qiao-Guo Tan, Kechen Zhu, Qiong Zhang, Pascal Salaun, Alessandro Tagliabue, **Yanjun Zhang*,** Dominik Weiss1*, Eric P Achterberg, Yuri Korchev*. Phycosphere pH of unicellular nano- and micro- phytoplankton cells and consequences for iron speciation. *The ISME Journal*, 2022, 16: 2329–2336.

3. **Yanjun Zhang***, Yasufumi Takahashi, Sung Pil Hong, Fengjie Liu, Joanna Bednarska, Philip S Goff, Pavel Novak, Andrew Shevchuk, Sahana Gopal, Iros Barozzi, Luca Magnani, Hideki Sakai, Yoshimoto Suguru, Takuto Fujii, Alexander Erofeev, Peter Gorelkin, Alexander Majouga, Dominik J Weiss, Christopher Edwards, Aleksandar P Ivanov, David Klenerman, Elena V Sviderskaya*, Joshua B Edel*, Yuri Korchev*. High-resolution label-free 3D mapping of extracellular pH of single living cells. *Nature Communications*. 2019, 10: 5610.

P-06

The Role of Platinum Positioning in MOF-Derived Pt/C Electrocatalysts for Oxygen Reduction Reaction

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Abstract

Fuel cells are one of the most promising and green alternative technologies for decentralized energy conversion. However, the sluggish kinetics in the cathodic part, oxygen reduction reaction (ORR), remains a key challenge. Platinum (Pt), a commonly used material for ORR, has limitations beyond high cost and scarcity, such as durability issues and poisoning susceptibility. To overcome these issues, Pt is loaded on high-surface-area substrates like carbon black or carbon nanotubes to improve its performance and meet the energy demand.[1,2] The global challenge of Pt/C catalysts arises from precisely controlling the spatial distribution of Pt in a mesoporous carbon carrier, which hinders the understanding of the structural performance relationship. Metal-organic frameworks (MOFs) are ideal platforms for controlling the structural properties of derived carbon materials and are suitable for loading Pt in catalytic applications. Our research focuses on spatial control over Pt nanoparticles (Pt NPs) in UiO-66 MOF—within the MOF framework or on its surface—to investigate how Pt positioning influences performance. Following carbonization and HF etching, the formed Pt/C catalysts were evaluated, revealing that Pt active sites remain intact without aggregation, with the MOF retaining its structural stability, even at high-temperature and rigorous etching conditions, highlighting the structural integrity of derived Pt/C catalysts. The embedded Pt NPs could restrict ionomer interaction and poisoning compared to Pt positioned on the surface, which is advantageous in ORR. The outcomes from this work not only emphasize MOF-based catalysts but also understand the structure-performance relationship, thereby creating a new pathway toward designing highly efficient next-generation sustainable energy materials.

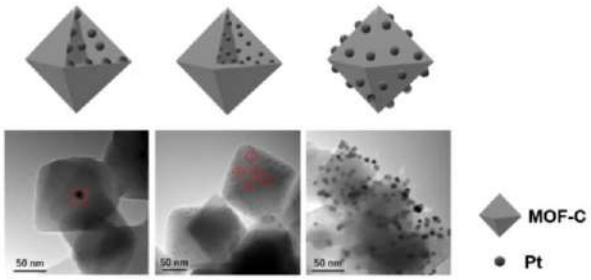

Figure 1. TEM images showing the Pt positioning at different locations of MOF-derived Pt/C catalysts.

Acknowledgment

This work is supported by Mitsui Mining & Smelting Co., Ltd.

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1. Liu, J. *et al.* Carbon‐Supported Divacancy‐Anchored Platinum Single‐Atom Electrocatalysts with Superhigh Pt Utilization for the Oxygen Reduction Reaction. *Angewandte Chemie* 131, 1175–1179 (2019).
2. Zhao, J. *et al.* Manipulating the oxygen reduction reaction pathway on Pt-coordinated motifs. *Nat Commun* 13, 685 (2022).

P-07

Journey to the Nucleus

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Abstract

Nucleus is the command center of the cell, mediating intracellular homeostasis, cellular processes, and more. Nucleocytoplasmic signaling is precisely regulated by nuclear pore complexes (NPCs). These NPCs, which act as selective permeability barrier, are megadalton-sized nanogates composed of about 30 distinct nucleoporins (Nups). NPCs exhibit complex structural organization, conformational dynamics, and functions. In addition to nucleocytoplasmic transport, NPCs play a crucial role in regulating gene expression. Due to their biological significance, NPCs are frequently exploited in pathological conditions including viral infections and cancers. Elucidating the complex properties of NPCs in nuclear transport and gene regulation is essential to decipher their fundamental roles, offering valuable insights for targeting NPCs in the treatment of various diseases. NPC structure has been extensively investigated using a combination of cryo-electron microscopy and AlphaFold. Current super-resolution microscopy techniques provide remarkable resolution for studying NPC dynamics by tagging them with fluorophores. Despite these advancements, direct visualization of NPC properties (native structure, structural dynamics, and nuclear transport) in real-time remains challenging. HS-AFM is a powerful, label-free scanning probe microscope that provides direct observation of biomolecules and organelles at the nanoscopic level with a high spatiotemporal resolution in real-time. Here, we investigate the key questions related to NPC structure and functions during the journey to nucleus using HS-AFM and life science approaches. We first summarize our previous studies, which include the conformational dynamics of fusion proteins during viral entry, disruption of nuclear transport by viral accessory proteins, and the native NPC structure of nuclei isolated from mouse brain tissue. We then report our current progress regarding the molecular properties of SARS-CoV-2 N protein and condensins, as well as the effect of LLPS inhibitor on NPC structure. Lastly, we propose developing biomimetic NPCs as a nanoscopic platform for studying nuclear transport of viral capsid using HS-AFM.

Recent publications:

1. **Lim, K** et al., *Cell Host Microbe*, 2024
2. Sajidah, E.S., **Lim, K** et al., *Cell Rep Phys Sci*., 2024
3. Dunajova, Z., Mateu, B.P., Radler, P., **Lim, K** et al., *Nat Phys.*, 2023
4. Nishide, G., **Lim, K**., *J Phys Chem Lett.*, 2023
5. Lostao, A., **Lim, K** et al., *Int J Biol Macromol*., 2023
6. **Lim, K** et al., *Nano Lett.*, 2023

P-08

Nanoscale analysis of phosphoinositide distribution on cell membranes of mouse cerebellar neurons using SDS-digested freeze-fracture replica labeling

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Abstract

Phosphoinositides (PI), a group of membrane-constitutive phospholipids, play an essential role in many aspects of neuronal function, but their distribution pattern on the neuronal membrane has remained unclear. In this study, we used SDS-digested freeze-fracture replica labeling method (SDS-FRL) to observe the distribution of phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$), a stereoisomer of PI, on neuronal membranes of mouse cerebellum under a transmission electron microscope (Fig.1A & B). We found that $PI(4,5)P_2$-labeling gold particles were distributed as nanoclusters on the cytoplasmic side of neuronal cell membranes. $PI(4,5)P_2$ was accumulated in the active zone and associated with P/Q-type voltage-gated calcium channels ($Ca_V2.1$) at the presynaptic boutons of parallel fibers (Fig.1C), indicating that $PI(4,5)P_2$ regulates neurotransmitter release. $PI(4,5)P_2$ was associated with the metabotropic glutamate receptor mGluR1α in the dendritic spine membranes of PCs. The placement of $PI(4,5)P_2$ clusters near mGluR1α possibly contributes to the efficient production of second messengers by activating mGluR1α and subsequent hydrolysis of $PI(4,5)P_2$ by PLCβ. $PI(4,5)P_2$ is also co-localized with GIRK3, a G protein-gated inwardly rectifying potassium channel subunit, indicating the regulation of the PC excitability by $PI(4,5)P_2$. These results demonstrate that visualization of PIs at the nanoscale level by SDS-FRL and spatial analysis of their association with neuronal proteins provide important information to reveal the role of PIs in synaptic transmission.

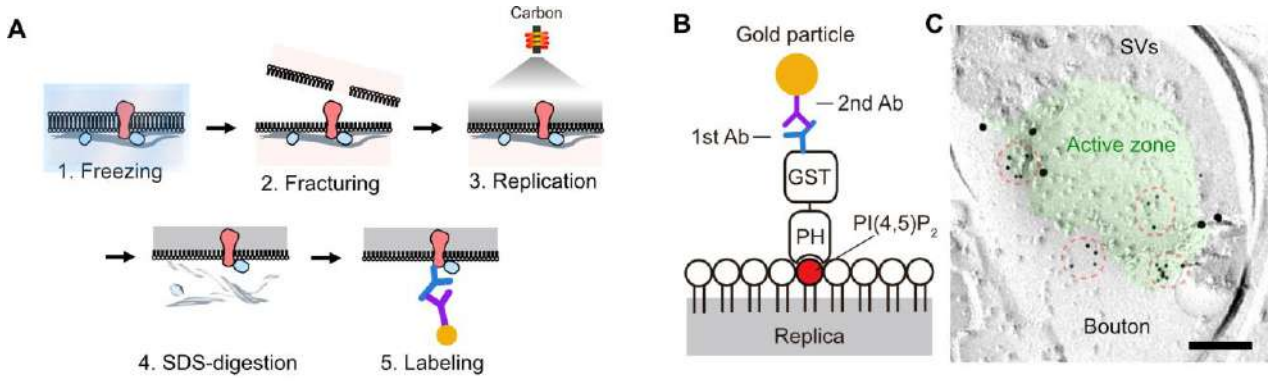

Figure 1. SDS-FRL for $PI(4,5)P_2$ labeling. (A) A schema of SDS-FRL. (B) $PI(4,5)P_2$ labeling using a specific probe (PLCδ1-PH domain). (C) Distribution of $PI(4,5)P_2$ (5 nm, clusters are indicated by red dashed lines) and $Ca_V2.1$ (15 nm) on PF bouton. Green indicates the active zone. Scale bar = 100 nm.

Recent publications:

1. Eguchi K, Le Monnier E, Shigemoto R (2023). *J Neurosci.* 43(23):4197-4216.

P-09

Nanoscopic investigation of RNA-mediated LLPS formation

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Abstract

Biomolecular condensate formed through liquid liquid phase separation (LLPS) involve the accumulation of proteins and RNAs. Recent research highlights the significant role of RNA self-assembly in the formation of these condensates. RNA self-assembly is predominantly driven by robust and redundant RNA-RNA interactions. Naturally two RNAs have the potential to interact with each other at high concentration and it is true for RNAs that do not form strong self-structure. This phenomenon could drive the formation of LLPS through the presence of divalent or organic cations or proteins. Both base pairing and tertiary interaction may form by RNA interactions. Although, RNA is the main component of biomolecular condensates, little is known about role of RNA structure and sequence composition in the formation of LLPS. In this study, we investigate on the intrinsic thermodynamic, kinetic and structural properties of RNAs to elucidate the formation of stable monomer, dimer and higher order structure of RNAs during LLPS formation. We visualize the molecular transitions of RNA structures on the formation condensates using High speed atomic force microscopy. Our data demonstrate that RNA-mediated LLPS formation is a multistep process. In the first step, RNA tends to form secondary and tertiary structures by base-pairing interaction. In second step, tertiary interaction solely influences the formation of several small condensates of RNAs which finally compact together and form large condensate. These findings suggest the critical involvement of RNA structure and sequence composition on the formation of RNA-mediated LLPS.

P-10

Strategic Design of Prussian Blue Analogs for Electrochemical CO_2 Reduction

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Abstract

Excessive CO_2 emissions are one of the most significant drivers of global warming, contributing approximately 76%, according to the Intergovernmental Panel on Climate Change (IPCC). Hence, converting CO_2 into value-added chemicals is an important area of research concerning mitigating environmental impacts. In electrochemical CO_2 reduction, sufficient potential has been shown by metal-organic frameworks (MOFs)-in particular Prussian blue analogs (PBA) family, due to the synergistic interaction of the bimetallic centers in enhancing catalytic activity and selectivity.[1,2] In this work, Co-Fe PBA nanocubes are strategically modified in a two-step process: mild etching and then decoration with MoS_2 nanoflowers. Initial etching results in hollow nanocage structures that greatly increase the surface area and number of active sites. This hollow architecture improves mass and electron transport by maximizing exposure to catalytic sites for the effective adsorption and conversion of CO_2. The subsequent decoration with MoS_2, well known for its catalytic activities in electrochemical reduction, further improves the activity of the modified PBAs due to the introduction of abundant edge sites for interaction with absorbed CO_2. Figure 1 shows the morphological evolution of the designed catalyst. The overall improvement in performance comes from the combined roles of the etching and MoS_2 decoration, contributing to the structural and electronic modifications working together to make the catalyst more effective at facilitating the complex reactions needed to reduce CO_2 efficiently. This work underlines the potential in the design of hybrid PBA-based materials, enabling the overcoming of some critical challenges in technologies for CO_2 conversion.

Figure 1: Schematic representation of designing etched-PBA-MoS_2 for CO_2 reduction reaction.

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2. Mukherjee, P., Sathiyan, K., Vishwanath, R. S. & Zidki, T. Anchoring MoS_2 on an ethanol-etched Prussian blue analog for enhanced electrocatalytic efficiency for the oxygen evolution reaction. *Mater Chem Front* 6, 1770–1778, **2022**.

P-11

Revealing Submolecular Structures with 3D-AFM: Applications in Polysaccharide Nanocrystals and Peptide Assemblies

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https://researchmap.jp/patriot

Three-dimensional atomic force microscopy (3D-AFM), developed by Fukuma et al., has emerged as a standout technique, providing unique structural information for various surfaces, interfaces, and nanostructures with unprecedented detail, reaching molecular and atomic scales [1]. In my research, 3D-AFM was used for two different applications: revealing the submolecular hydration structures around polysaccharide-based nanocrystals (e.g., chitin and cellulose) in water [2,3], and visualizing the molecular organization of water on 2D self-assembled peptide nanocrystals on graphite [4,5,6]. We found that the structured water layer extends approximately 15 Å from the peptide surface, exhibiting a transition from crystalline to semicrystalline and glassy states before merging into bulk water. Notably, this hydration shell dynamically adapts to the physical topology and chemical domains of the peptide surface. These results demonstrate that 3D-AFM is a valuable technique for understanding solvation and hydration in bionanomaterials, highlighting the significant role of water in protein structure and function, and paving the way for future advancements in peptide design for specific applications [7].

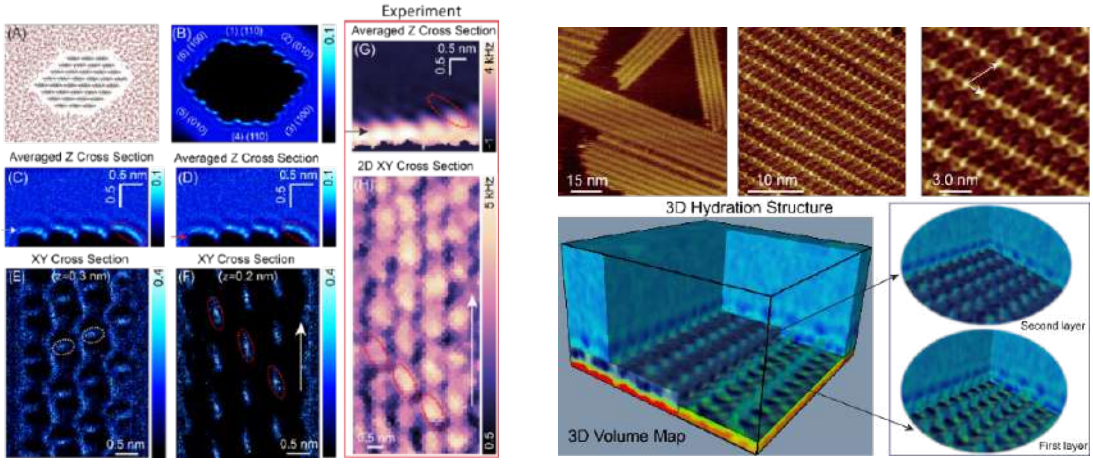

Figure 1: 3D-AFM images at polysaccharide NCs-water and peptide-water interfaces.

Recent publications:

1. *ACS Nano* 2018 *12* (12), 11785-11797

2. Science Advances.8, eabq0160(2022). DOI:10.1126/sciadv.abq0160

3. Small 2023, 19, 2302276

***4. ACS Nano* 2023 *17* (8), 7311-7325**

5. Small 2024, 20, 2400653

6. *ACS Applied Materials & Interfaces* 2023, 15, 23, 27789-27800

7. *In preparation.*

P-12

SUPREM: an engineered non-site-specific m^{6}A RNA methyltransferase with highly improved efficiency.

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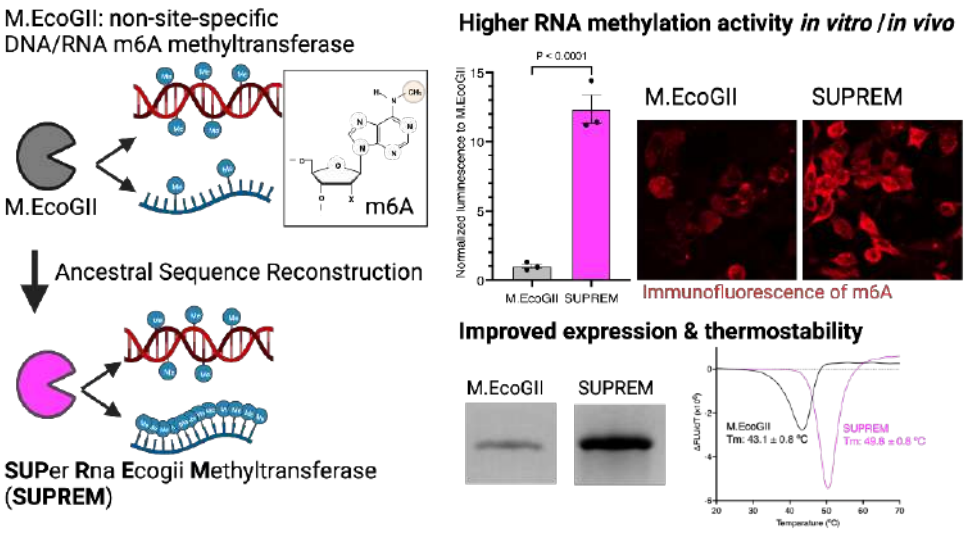
RNA methyltransferases (RNA MTases) play a pivotal role in various physiological and pathological processes through RNA methylation. Harnessing engineered RNA MTases holds promise for advancing synthetic biology tools, enabling precise manipulation of RNA methylation and facilitating RNA labeling within living cells. However, the development of such tools is challenging due to limited understanding of structure-function relationships in RNA MTases. Herein, using ancestral sequence reconstruction we explore the sequence space of the bacterial DNA methyltransferase EcoGII (M.EcoGII), a promising target for protein engineering due to its lack of sequence specificity and its residual activity on RNA. We thereby created an efficient non-specific RNA MTase termed SUPREM, which exhibits 8-fold higher expression levels, 7 ºC higher thermostability, and 12-fold greater m^{6}A RNA methylation activity compared with M.EcoGII. Immunofluorescent staining and quantitative LC/MS-MS analysis confirmed SUPREM's higher RNA methylation activity compared with M.EcoGII in mammalian cells. Our findings indicate that SUPREM holds promise as a versatile tool for *in vivo* RNA methylation and labeling.



Recent publications:

1. Yoshiki Ochiai, Ben E. Clifton, Madeleine Le Coz, Marco Terenzio, Paola Laurino. SUPREM: an engineered non-site-specific m^{6}A RNA methyltransferase with highly improved efficiency. *Nucleic Acids Research,* gkae887, https://doi.org/10.1093/nar/gkae887

P-13

Observing dynamic conformational changes within the coiled-coil domain of different laminin isoforms using high-speed atomic force microscopy

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Laminins are trimeric glycoproteins with important roles in cell-matrix adhesion and tissue organization. The laminin α, ß, and γ-chains have short N-terminal arms, while their C-termini are connected via a triple coiled-coil domain, giving the laminin molecule a well-characterized cross-shaped morphology as a result. The C-terminus of laminin alpha chains contains additional globular laminin G-like (LG) domains with important roles in mediating cell adhesion. Dynamic conformational changes of different laminin domains have been implicated in regulating laminin function, but so far have not been analyzed at the single-molecule level. High-speed atomic force microscopy (HS-AFM) is a unique tool for visualizing such dynamic conformational changes under physiological conditions at sub-second temporal resolution. After optimizing surface immobilization and imaging conditions, we characterized the ultrastructure of laminin-111 and laminin-332 using HS-AFM timelapse imaging. While laminin-111 features a stable S-shaped coiled-coil domain displaying little conformational rearrangement, laminin-332 coiled-coil domains undergo rapid switching between straight and bent conformations around a defined central molecular hinge. Complementing the experimental AFM data with AlphaFold-based coiled-coil structure prediction enabled us to pinpoint the position of the hinge region, as well as to identify potential molecular rearrangement processes permitting hinge flexibility. Coarse-grained molecular dynamics simulations provide further support for a spatially defined kinking mechanism in the laminin-332 coiled-coil domain. Finally, we observed the dynamic rearrangement of the C-terminal LG domains of laminin-111 and laminin-332, switching them between compact and open conformations. Thus, HS-AFM can directly visualize molecular rearrangement processes within different laminin isoforms and provide dynamic structural insight not available from other microscopy techniques.

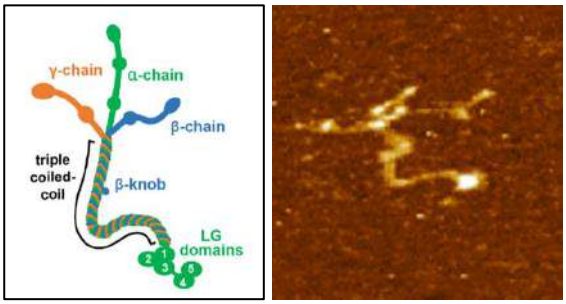

Fig.: Investigating laminin ultrastructure by HS-AFM. Adapted from Akter et al. IJMS 2024.

Recent publications:

1. **Lucky Akter**, Holger Flechsig, Arin Marchesi, and Clemens M. Franz. "Observing Dynamic Conformational Changes within the Coiled-Coil Domain of Different Laminin Isoforms Using High-Speed Atomic Force Microscopy." International Journal of Molecular Sciences 25, no. 4, p.1951, Feb 2024.

2. Tareg Omer Mohammed, You-Rong Lin, **Lucky Akter**, Kai Weissenbruch, Kien Ngo KX, Zhang Y, Kodera N, Bastmeyer M, Miyanari Y, Taoka A, Franz CM. S100A11 promotes focal adhesion disassembly via myosin II-driven contractility and Piezo1-mediated Ca2+ entry. Journal of Cell Science. 137(2):jcs261492 Jan 2024.

P-14

Phase Structure Analysis of Polymer Blends Combining Unsupervised Machine Learning and AFM Images

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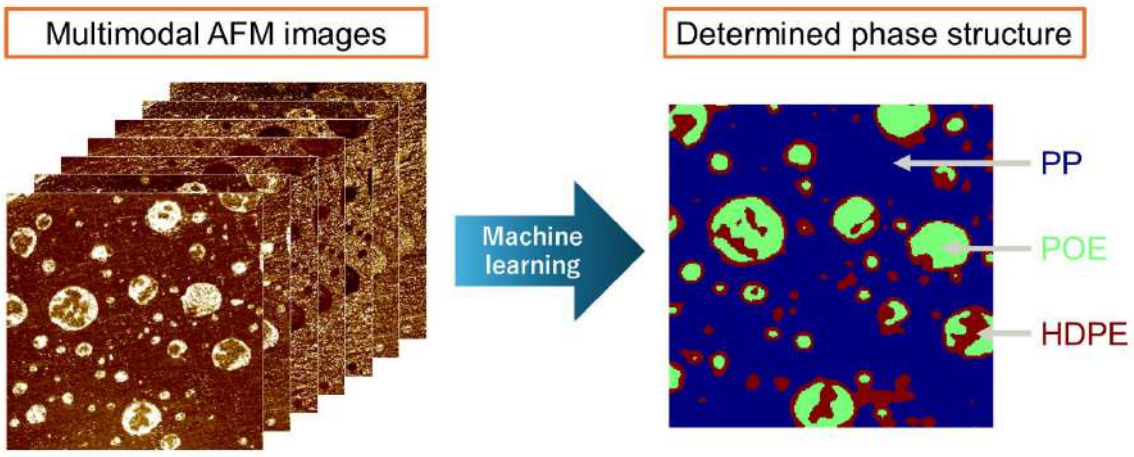
Abstract

Polymer blends, consisting of two or more polymers, can achieve varieties of properties unattainable with homopolymers, where it is critical to determine the phase structure as it has dictated the overall material performance. The transmission electron microscopy has limitations in distinguishing between polymers composed of light elements, making analysis challenging. In this study, we propose using atomic force microscopy (AFM) for phase structure analysis. AFM enables to measure not only the surface roughness but also the nanomechanical, thermal, and electrical properties, etc.[1-3] Machine learning (ML) is an effective tool for batch processing these multimodal images efficiently and accurately. We present a method that combines ML with AFM multimodal mapping images to specify phase structures. We applied this approach to a ternary polymer blend of polypropylene (PP), high-density polyethylene (HDPE), and polyolefin elastomer (POE). The phase structure of the ternary blend was successfully identified using ML clustering techniques applied to appropriately processed multimodal images.



Acknowledgement: The work of T.O. was supported by JST SPRING (JPMJSP2102).

Reference:

1) Gomès, S. et al., *Phys. Status. Solidi. A* **2015**, 212, 477-494., 2) Barth, C. et al., *New J. Phys.* **2010**, 12, 093024., 3) Wu, S. et al., *Friction* **2024**, 12, 2627-2656.

P-15

Nanoscale investigation of CRMP2 isoforms' role in microtubule organization using high-speed atomic force microscopy (HS-AFM)

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Abstract

Collapsin Response Mediator Protein 2 (CRMP2) plays a crucial role in microtubule dynamics and is involved in many cellular processes in neurodevelopment and cancer. While two CRMP2 isoforms exist (CRMP2A and CRMP2B), their specific functions in microtubule organization remain incompletely understood. This study employs high-speed atomic force microscopy (HS-AFM) to investigate the nanoscale interactions between CRMP2 isoforms and microtubules.

We utilized HS-AFM to directly visualize and investigate the dynamic interactions between CRMP2 isoforms and microtubules. Our results reveal that CRMP2A exhibits a distinct capacity to crosslink microtubules, a feature not observed in the other isoform CRMP2B. This crosslinking ability of CRMP2A was found to significantly impact microtubule organization and stability.

This study demonstrates the power of HS-AFM in elucidating the nanoscale mechanisms of protein-microtubule interactions and highlights the distinct functional roles of CRMP2 isoforms in microtubule organization. These insights contribute to our understanding of cellular processes and may have implications for disorders associated with cytoskeletal abnormalities.

Keywords:

CRMP2 proteins; microtubule-associated proteins; High-speed AFM

Acknowledgments

The presenter acknowledges the contribution of Saho Kitagawa, Syeda Rubaiya Nasrin, and Akira Kakugo to this work.

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P-16

Hybrid Metamaterial Plasmonic Tweezers for Direct Trapping and Measuring Conformational Changes of Single Urease Proteins

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Abstract: Direct manipulation of biomolecules without the need for fluorescent labels or tethers is a topic of enduring interest. This study provides an alternative approach for trapping and directly measuring the conformational energy changes of single unmodified proteins using metamaterial plasmonic tweezers, while paving the way for label-free characterization of biomolecules at single-entity levels.

Summary: Although plasmonic nanotweezers can achieve manipulation on a scale smaller than the diffraction limit of light microscopy, direct trapping of sub-10 nm bioparticles in an aqueous environment without modifications remains challenging.[1] Here, we introduce a hybrid Fano-resonant metamaterial plasmonic tweezers (MPT) to exhibit stable nano-trapping.[2-3] We present the heat generation mechanisms, from light-to-heat conversion, over the MPT's surface and review how to effectively transfer this photothermal heating to load and trap single enzymes in real-time.[4] We find that a steep temperature gradient, generated near the hot-spot region of the MPT, can provide additional degrees of freedom in particle/protein trapping and manipulation. By analyzing the spatial probability distribution of trapped particles, we observe that we can distinguish dielectric particles from biomolecules. Many trapping sites are activated simultaneously which determines the intrinsic potential energy landscape of trapped proteins, and can potentially help us to quantify changes in the conformation dynamics of the urease molecules. We believe this photothermal-assisted trapping approach will open new opportunities for developing ultrasensitive, label-free detection and provide a transformative tool for direct protein characterization at the single-molecule level.

Keywords: Conformational changes, Metamaterials, Plasmonic tweezers, Single nanoparticle and Enzyme trapping.

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P-17

Investigating the role of non-muscle myosin II isoforms during stress fiber contraction by atomic force microscopy

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Abstract:
In eukaryotic cells, the actin cytoskeleton is a remarkable multifunctional system. The morphology of the cells is strongly determined by the contractile actomyosin cytoskeleton, which includes actin filaments and motor proteins of the non-muscle myosin II (NM II) class.

NM II is an actin-binding protein that crosslinks actin fibers and provides contractile properties. The three mammalian NM II isoforms—NMIIA, NMIIB, and NMIIC—have both overlapping and unique properties in cells [1]. Knocking out (KO) the NMIIA protein disrupts the organization of stress fibers (SF) and the elongation of focal adhesions (FA). The main function of NMIIA is to dynamically generate tension in cells. Additionally, NMIIB contributes to elastic stability, while NMIIC mediates tensional homeostasis. Optical microscopy shows that NMIIA KO cells and NMIIB KO cells exhibit smaller FAs. Moreover, NMIIA KO cells display a lower density of FAs [2]. However, the ultrastructure of FAs and SFs in these KO cells remains unknown. Studying the ultrastructure can provide more insights into the organization of SFs in NMIIA, NMIIB, and NMIIC KO cells.

Atomic force microscopy (AFM) can be used to explore the ultrastructure and topography, helping us understand intracellular structures. To study stress fibers inside cells with AFM, the first step is to remove the cell membrane without causing significant damage. A common technique for membrane removal is de-roofing, which involves introducing ultrasonic bursts to the cells [3]. We use this technique to remove the cell membrane, allowing us to characterize the topography and mechanical properties of intracellular structures such as SFs. In this study, we use the NMII KO system to investigate the molecular mechanisms of NMII-driven contraction in stress fibers. To better understand the ultrastructure of SFs, we use micropattern to normalize cellular phenotypes. In conclusion, understanding the role of different NMII isoforms will enhance our insight into cell migration and cytoskeleton remodeling.

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Keywords: Atomic force microscope, Non-muscle myosin II, intracellular imaging

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P-18

Carrier-Enhanced Efficacy of Molecular Targeted Drug-Loaded Nanoparticles for Cancer Therapy

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Enhancing the efficacy of anticancer drugs while minimizing adverse effects (AEs) remains a significant challenge in cancer therapy. To confront this challenge, we have pioneered a novel strategy employing micellar nanocomplexes (MNCs) comprising derivatives of epigallocatechin-3-*O*-gallate (EGCG), a key component of green tea which is well known for various therapeutic effects. The green tea-based MNCs provide synergistic therapeutic effects with the drugs carried besides the site-specific accumulation, representing unique and effective drug delivery systems which take advantage of the intrinsic anticancer properties of EGCG-based carrier.[1]

Sunitinib (SU, an anti-angiogenic tyrosine kinase inhibitor)-loaded MNC (SU-MNC) was formed using poly(ethylene glycol)-conjugated EGCG (PEG-EGCG) as such a carrier.[2] On human renal cell carcinoma (HRCC)-xenografted mice, intravenous (i.v.) administration of SU-MNC significantly augmented SU efficacy, allowing for a remarkable 1/50 and 1/28 dose reduction as compared to SU administered i.v. and orally, respectively, rendering it non-toxic while maintaining therapeutic effect. In comparison, a conventional nanocarrier, SU-loaded polymeric micelle (SU-PM) comprised of PEG-*b*-poly(lactic acid) copolymer (PEG-PLA), only reduced toxicity with no elevated efficacy, despite comparable drug-loading and tumor-targeting efficiency to SU-MNC. These results indicate that the improved efficacy of SU-MNC was ascribed to the carrier–drug synergies with the high-performance carrier of PEG-EGCG besides tumor-targeted delivery.

The green tea based-nanoparticle substantially reduces adverse effects, as systemic toxicity is reduced by enabling dose-sparing through amplified anticancer efficacy and drug targeting to tumors. The high-performance drug carrier of the green tea based-nanoparticle is considered to enable a more effective and safer strategy for cancer therapy, suggesting scope for improved nanomedicine formulation.

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P-19

Surface-engineered extracellular vesicles to modulate antigen-specific T cell expansion for cancer immunotherapy

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Abstract

Extracellular vesicles (EVs) are emerging as mediators of cell–cell communication, including immune regulation. However, despite the recent development of several EV-based cancer immunotherapies, their clinical efficacy remains limited. In this study, we created antigen-presenting extracellular vesicles (AP-EVs) using EV engineering techniques. Specifically, we employed fusion with either tetraspanin or MFG-E8 to replicate the functional characteristics of professional antigen-presenting cells. AP-EVs were also equipped with surface-bound IL-2, which facilitated selective delivery of IL-2 to antigen-specific $CD8^+$ T cells. AP-EVs were engineered to express a peptide-major histocompatibility class I complex, a costimulatory molecule, and IL-2, allowing the simultaneous presentation of multiple immune modulators to antigen-specific $CD8^+$ T cells. This promoted the clonal expansion and differentiation of antigen-specific cytotoxic T lymphocytes, leading to potent anticancer immune responses. Combination therapy with AP-EVs and anti-PD-1 demonstrated enhanced anticancer immunity against established tumors compared with anti-PD-1 monotherapy. Furthermore, we successfully engineered humanized AP-EVs, which selectively stimulated tumor antigen-specific $CD8^+$ T cells. Our engineered EVs represent an effective strategy for cancer immunotherapy.

Recent publications:

1. Preventing SARS-CoV-2 Infection Using Anti-spike Nanobody-IFN-β Conjugated Exosomes
2. Surface-engineered extracellular vesicles to modulate antigen-specific T cell expansion for cancer immunotherapy

P-20

Title: A microfluidic-based model for molecular characterization of axonal injury.

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Abstract

Peripheral nerve injury (PNI) is a common cause of disability and sensorimotor impairment usually associated with trauma, where tensile forces and shear stress along nerves lead to focal demyelination or structural damage, followed by denervation of target tissues, axonal degeneration and cell death (Navarro, Vivó and Valero-Cabré, 2007). After PNI, surviving neurons switch to a growing state where changes in gene expression promote cell survival and axonal regeneration (Sulaiman and Gordon, 2013; Hussain et al., 2020) however, studies showed that variations in location, duration, and mechanism, among other injury components, can evoke distinct cellular responses, which ultimately impact regeneration (Menorca, Fussell and Elfar, 2013). Acute stretch injury of the axons, can trigger strain-compensatory mechanisms and degeneration followed by functional impairments if the tension threshold is exceeded; in contrast, continuous uniaxial stretch at low rates can promote transcriptional changes related to long-term axonal growth (Loverde et al., 2020; Varier et al., 2022); raising the question of whether chronic and acute versions of the same type of injury can evoke differential gene expression patterns that may expose new avenues to enhance axonal regeneration. To address this question, we developed a microfluidic-based system designed to model a stretch axonal injury with spatiotemporal control that can be observed with live imaging setups. We characterized the cellular response to stretch by measuring peaks in calcium activity as an indicator of injury and confirmed the presence of previously known regeneration-associated genes at different timepoints. Sequencing at matching timepoints showed differential expression patterns that will be further analyzed through functional profiling for selection and validation of molecules with potential to modulate axonal regeneration after injury.

P-21

On relating the structure, dynamics and mechanics of aggregation

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Abstract

Aggregation of objects is a natural phenomenon observed at the astronomical scale as the pasta phase in neutron stars to the sub-micron scale as protein aggregates within the cells. The aggregates are dynamic structures of finite length-scale and finite lifetime. This is a special phase of matter that shares the flow property of liquids and the rigidity of a solid to some extent. Due to the diversity and amenability, such materials have attracted much attention into various industrial applications. Using computer simulation, we study the statistical mechanics of a representative system of particulate aggregates. We chart out the possible structures in our model system. We identify the relevant dynamical mechanisms for such structural diversity. We also quantify the local mechanical fluctuations in these systems. We find that the local fractal geometry holds the key to relate the structure, dynamics and mechanics of aggregate forming systems.

P-22

Universal Nanoenhancer For Drug Delivery

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Abstract

Although a few nanomedicines have been approved for clinical use, acknowledging the improved safety given by their propensity of site-specific accumulation, their improved efficacy over conventional drugs has remained marginal. One of typical drawbacks of nanocarriers is low drug-loading capacity that leads to insufficient efficacy, and requires an increase in dosage and/or frequency of administration, which in turn increases carrier toxicity. In contrast, elevating drug-loading would cause the risk of nanocarrier instability resulting in low efficacy and off-target toxicity. This dilemmatic issue has imposed constraints on the design and development of nanocarriers.

We have pioneered the green tea catechin-based nanocarriers comprised of synthetic derivatives of epigallocatechin-3-O-gallate (EGCG), a key ingredient of green tea for treatment of intractable diseases including cancer. The nanocarriers encapsulated various types of drugs (protein, peptide, small molecule drug, and nucleotide) facilitating high drug loading via hydrophobic interaction, hydrogen bonding and π-π stacking, etc., and synergized with the loaded drugs due to the intrinsic therapeutic activities derived from EGCG, producing amplified therapeutic efficacy. By this approach, superior therapeutic outcomes and/or dosage reduction was attained, besides site-specific accumulation. Our study showed that EGCG-based nanocarriers would provide a remedial approach to address various critical limitations of current therapies and nanomedicines such as modest efficacy, adverse effects and carrier toxicity as a universal platform.

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P-23

SynGAP LLPS Condensates as the Basic Platform for Recruiting PSD95 and Receptor Oligomers for Generating Excitatory Synapses

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Abstract

The neuronal post-synapse comprises of nano-scale domains that are precisely opposed to the pre-synaptic neurotransmitter release sites. This nano-scale assembly of post-synaptic receptors and scaffold proteins has been found to be essential for proper synaptic function. However, the biophysical mechanisms that retain receptors and scaffold proteins at the post-synapse are not well understood. Specifically, a mechanism that can induce clusters of PSD95, a key scaffold protein, at low concentrations, such as those expected before the formation of functional post-synapses, and which can induce long-term retention of post-synaptic receptors, remains to be described.

Here, we demonstrate that SynGAP forms liquid-liquid phase-separated condensates through homophilic interactions mediated by its intrinsically disordered region as well as its C-terminal coiled-coil domain, both in vitro as well as in cultured cells. SynGAP recruits PSD95 into these condensates by way of its PDZ-binding motif at its C-terminus, and the recruited PSD95 in turn allows recruitment and immobilization of receptors such as Neuroligin and AMPA receptors (via TARPs). Interestingly, the monomeric mutant of Neuroligin exhibits impaired recruitment to the post-synapse. Further investigations then reveal that oligomerization of Neuroligin, and TARP-AMPA receptor complex, enhances the anchorage of these molecules in the SynGAP condensates containing PSD95, compared to monomeric Neuroligin and TARP. We also reveal that phosphorylation of SynGAP by CaMKII, primarily at serine residues in SynGAP's intrinsically disordered region, suppresses condensate formation.

Taken together, these discoveries show how liquid-like assemblies of SynGAP can recruit PSD95 molecules and retain trans-membrane receptors like Neuroligin and AMPA receptors, depending on their oligomerization state, which could be essential for generating neuronal excitatory synapses. These SynGAP condensates are regulated by phosphorylation induced by the kinase activity of CaMKII.

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P-24

Biomass-derived carbon dots as inflammation theranostics

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Related website: https://scholar.google.com/citations?user=-STDcrsAAAAJ&hl=en

Quantum dots, Nobel Prize winner in Chemistry, have recently gained significant attention in precision medicine due to their unique properties, like high photostability, efficient light absorption, and vibrant luminescence. Currently, a shift towards discovering quantum dots from sustainable sources, aiming to harness their potential as stimuli-responsive biosensors, bioimaging probes, and drug delivery agents. Biomass-waste-derived carbon quantum dots (CQDs) are an attractive alternative to conventional QDs, which often require expensive and toxic precursors, as they offer several merits in eco-friendly synthesis, using renewable sources, and cost-effective production.[1] In this study, we evaluated biomass CQD for their potential application as non-toxic bioimaging agents in in-vitro and *in-vivo* model.[1] Our recent findings suggested they can be advanced as theranostic agents to visualize and modulate inflammation.[2] The future work aims to engineer programmable SMART transcription factors (SMART-TFs) based on CQD technology and explore their nano-bio interaction to expand their utility in diverse biological applications.[3]

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P-25

Spatiotemporal Regulations of RanGTP-dependent Mitotic Spindle Assembly in Medaka Embryos

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Mitotic spindle assembly is a critical process ensuring accurate chromosome segregation during cell division, yet the regulatory mechanisms governing this phenomenon in large vertebrate embryos remain elusive. Building on our previous discoveries in medaka (*Oryzias latipes*) embryos, which revealed the specialized and indispensable role of chromosome-derived RanGTP signals in early embryonic spindle assembly (Kiyomitsu et al., 2024), this study aims to elucidate the mechanisms by which RanGTP is particularly critical during early embryonic division through three specific aims. First, the dynamics of mitotic spindle assembly and the behaviors of microtubule-associated spindle assembly factors (SAFs) will be monitored during early embryogenesis in medaka. Second, the functional domains of chromatin-bound RCC1, a generator of RanGTP, will be pinpointed using auxin-inducible degron 2 (AID2)-mediated protein knockdown-rescue experiments to elucidate the precise molecular elements orchestrating RanGTP signals during early cleavage stages. Finally, the spatiotemporal regulations of downstream targets of RanGTP, particularly microtubule-associated SAFs, will be analyzed to uncover the molecular basis of RanGTP-dependent spindle assembly mechanisms and how these mechanisms change during embryogenesis. This continuous investigation will shed light on the intricate interplay between RCC1 and its effectors, providing a comprehensive understanding of the molecular framework governing RanGTP-based specialized spindle assembly in large, rapidly dividing vertebrate embryos. Such insights hold potential for advancing embryonic development research and may offer guidance for targeted interventions in medical applications or fertility treatments.

P-26

Improving the Bioavailability of azole antifungals within Composite Hydrogels through Host-Guest Interactions with β-Cyclodextrin

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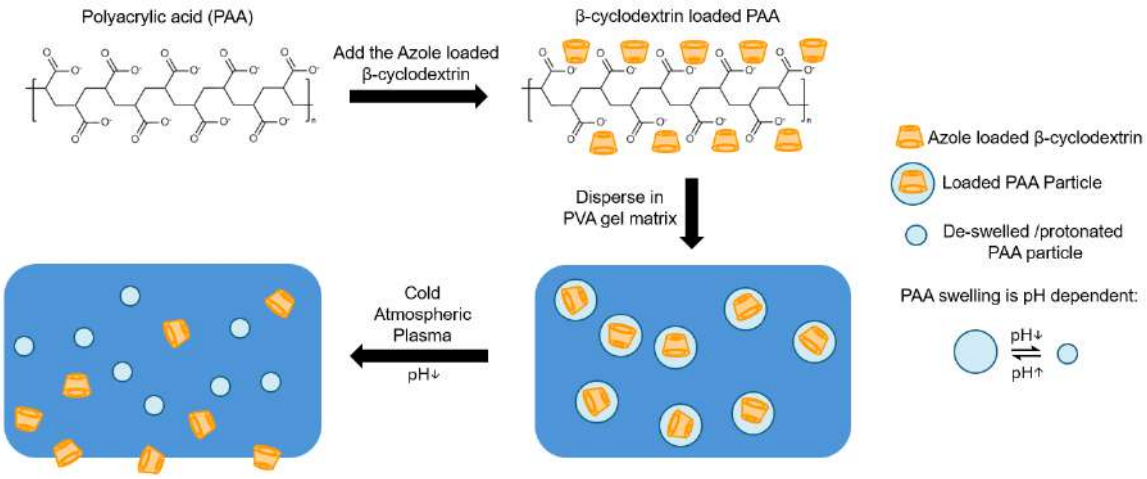
Fungal infections are becoming increasingly prevalent, affecting more than a billion people a year, with high morbidity and mortality rates. Fungal infections disproportionately affect the immunocompromised, elderly and critically ill, severely impacting the quality of life of these patients. Only recently have fungal infections been recognised as a similar barrier to healing comparative to their bacterial counterparts. Hydrogels have been favoured for wound treatment as they promote healing, and their properties can be easily engineered for specific functionality. Treatment of fungal infections are hampered by the insolubility of antifungal drugs, particularly prevalent among the azole class. Cyclodextrin moieties are commonly used to increase the solubility of a range of compounds, such as pharmaceuticals, flavorings, and dyes.



Cyclodextrin can be complexed to the PAA particles within the composite hydrogel, treatment with Cold Atmospheric Plasma (CAP) changes the properties of the hydrogel, enabling triggered release of the aminated β-cyclodextrin. To quantitively study the release characteristics of aminated cyclodextrin loaded hydrogels, ferrocene carboxylic acid was used as a dye moiety, due to its well-defined oxidative potential. This successful proof of principle led to the β-cyclodextrin – azole loaded hydrogels being tested against C. albicans strains. The clotrimazole loaded hydrogel showed clear controlled release and potential synergistic effects with CAP. This system has strong potential for the targeted drug delivery of other insoluble compounds.

Credit: OIST （CC BY 4.0）

9:00AM – 5:00PM
Sydney Brenner Lecture Theater
(Seminar Room B250) — OIST

Poster session at Lab 5 Atrium from 5:30PM

Organizers

Satoshi Mitarai (OIST)
Akihiro Kusumi (OIST)
Rikinari Hanayama (WPI-NanoLSI)

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