NanoLSI
WPI KANAZAWA UNIVERSITY

From Molecules to Cells and Tissue
– Bridging Scales
with Nanoprobe Technology

7th NanoLSI Symposium

in Berlin

NOVEMBER 2-3, 2023

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From Molecules to Cells and Tissue
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VENUE

HARNACK HOUSE THE CONFERENCE VENUE OF THE MAX PLANCK SOCIETY
IHNESTR. 16-20 – 14195 BERLIN, GERMANY

Welcome Message from the Organizer and Co-Organizer of the 7th NanoLSI Symposium

It is a great pleasure to welcome you to the 7th NanoLSI Symposium in Berlin. The Nano Life Science Institute (WPI-NanoLSI) at Kanazawa University aims at a fundamental understanding of a wide range of life-related phenomena through transdisciplinary research in the fields of Nanometrology, Life Science, Supramolecular Chemistry, and Computational Science. At this international symposium, distinguished researchers from Japan, Canada, Switzerland, and Germany will gather for two days to promote interdisciplinary research and new collaborations among scientists from diverse backgrounds, and to discuss cutting-edge nanoprobe technologies and their impact across a wide range of scales: "From Molecules to Cells and Tissue – Bridging Scales with Nanoprobe Technology". We are delighted to welcome all participants to our historic venue at Harnack House and we are looking forward to interesting and stimulating presentations and discussions.

Carsten Beta
Organizer

Clemens M. Franz
Co-Organizer

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Carsten Beta

Professor of Biological Physics at the University of Potsdam, Germany, and NanoLSI Overseas PI at Kanazawa University, Japan — works at the interface of physics and biology. His research concentrates on cell motility and chemotaxis of both bacterial swimmers and amoeboid cells and is based on experimental studies using live-cell imaging and microfluidics, as well as theoretical modeling inspired by concepts of active matter physics, nonlinear dynamics, and pattern formation.

Clemens M. Franz

Associate Professor at NanoLSI, Kanazawa University, Japan – applies atomic force microscopy (AFM) and other advanced microscopy techniques to study cell adhesion and mechanotransduction mechanisms. His research focuses on quantitating receptor-mediated adhesion forces, visualizing dynamic receptor-ligand complexes by high-speed AFM, as well as intracellular exploration by large-scale scanning probe microscopy.

7th NanoLSI Symposium

November 2, 2023 / Day 1 8:50 AM – 6:30 PM (CET) (Open venue: 8:00 AM)

8:00 AM **Registration and arrival tea & coffee**

8:50 AM **Opening remarks: Takeshi Fukuma, Director of Nano Life Science Institute, Kanazawa University**

9:00 AM **Session #1 9:00 AM – 11:00 AM**

Chairpersons: Clemens Franz & Noriyuki Kodera
Session theme: Proteins in operation

1. Noriyuki Kodera (NanoLSI, Kanazawa University)
   Video imaging of dynamic behaviors of biomolecules by high-speed atomic force microscopy
2. Ulrich Schwarz (Heidelberg University)
   Adsorption and self-assembly of SAS-6 rings on a surface studied with high-speed AFM and computer simulations
3. Clemens Franz (NanoLSI, Kanazawa University)
   Investigating actomyosin contractility-driven adhesion modulation by complementary high-speed AFM and fluorescence microscopy
4. Erik Schäffer (University of Tübingen)
   Ultraresolution picotensiometry of kinesin motors

11:00 AM **Poster session @Meitner Hall**

1:00 PM **Lunch**

2:00 PM **Session #2 2:00 PM – 4:00 PM**

Chairperson: Takeshi Fukuma
Session theme: From cells to tissue

1. Masanobu Oshima (NanoLSI/CRI, Kanazawa University)
   Identification of genotype-linked nano-scale physical properties of intestinal tumor cells
2. Anna Taubenberger (Technische Universität Dresden)
   Quantifying tumor spheroid mechanical properties in confining 3D microenvironments
3. Satoru Okuda (NanoLSI, Kanazawa University)
   Versatile computational modeling of 3D multicellular dynamics and application to collective cell migration
4. Martin Falcke (Max Delbrück Center for Molecular Medicine)
   On multistability and constitutive relations of cell motion on fibronectin lanes

4:00 PM **Tea & coffee break**

4:30 PM **Session #3 4:30 PM – 6:30 PM**

Chairpersons: Mark MacLachlan & Satoru Okuda
Session theme: Material properties and control

1. Mark MacLachlan (The University of British Columbia/NanoLSI, Kanazawa University)
   Supramolecular chemistry using bionanomaterials
2. Martin Bastmeyer (Karlsruhe Institute of Technology)
   3D cellular microenvironments to study cell mechanics
3. Svetlana Santer (University of Potsdam)
   From molecular actuation to microscopic motion
4. Shinji Watanabe (NanoLSI, Kanazawa University)
   Time-resolved imaging of nanomechanics and nanostructures of living cells with scanning ion conductance microscopy

6:30 PM **Photo session**

7:00 PM **Dinner buffet (by invivation only) 7:00 PM – 9:30 PM**

November 3, 2023 / Day 2 9:00 AM – 1:30 PM (CET) (Open venue: 8:30 AM)

8:30 AM **Registration and arrival tea & coffee**

9:00 AM **Poster session @Meitner Hall**

10:00 AM **Session #4 10:00 AM – 12:00 PM**

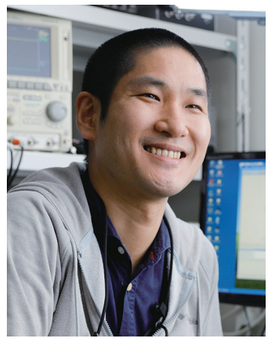
Chairperson: Masanobu Oshima
Session theme: Single cell dynamics

1. Takeshi Fukuma (NanoLSI, Kanazawa University)
   Visualizing nanoscale dynamics and mechanics in living cells by nanoendoscopy AFM
2. Salvatore Chiantia (University of Potsdam)
   Investigating virus-host interactions at a single cell level using quantitative fluorescence microscopy
3. Daniel Müller (ETH Zürich)
   Mechanically quantifying and guiding biological processes
4. Tomoyoshi Yamano (Graduate School of Medical Sciences / NanoLSI, Kanazawa University)
   Development of engineered exosome for immune regulation

12:00 PM **Closing remarks Carsten Beta, Organizer of the 7th NanoLSI Symposium, University of Potsdam/NanoLSI**

12:30 PM **Lunch 12:30 PM – 1:30 PM**

ORAL SESSIONS



Noriyuki Kodera

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Research Interests

Biophysics, molecular motors, cytoskeletons, atomic force microscopy

Education

2005	Ph.D., Div. of Basic Sci., Grad. Sch. Nat. Sci. & Tech., Kanazawa Univ.
2003	M. Sci., Div. of Math. & Phys. Sci., Grad. Sch. Nat. Sci. & Tech., Kanazawa Univ.
2001	B. Sci., Dept. of Phys, Fac. of Sci., Kanazawa Univ.

Professional Experience

2018 – present	Prof., WPI-NanoLSI, Kanazawa Univ.
2013 – 2017	PRESTO Researcher, JST
2010 – 2018	Asst. Prof. & Assoc. Prof., Bio-AFM Frontier Research Center, Inst. Sci. & Eng., Kanazawa Univ.
2005 – 2010	Research Fellow (DC2 & PD), JSPS and Postdoctoral Fellow, CREST, JST

Honors

2017	JSPS Prize
2013	Award for Science and Technology (Development Category), The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
2012	Young Researcher’ s Nanoprobe Technology Prize (JSPS Nanoprobe Technology 167)

Publications

1. Yoshimi K, Takeshita K, Kodera N, ..., Mashimo T, “Dynamic mechanisms of CRISPR interference by Escherichia coli CRISPR-Cas3” . Nature Communications 13, 4917 (2022).
2. Kodera N, Noshiro D, Dora SK, ..., Longhi S, Ando T, "Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy". Nature Nanotechnology 16, 181-189 (2021).
3. Imai H, Uchiumi T, Kodera N, "Direct visualization of translational GTPase factor pool formed around the archaeal ribosomal P-stalk by high-speed AFM". PNAS 117, 32386-32394 (2020).

Video Imaging of Dynamic Behaviors of Biomolecules by High-Speed Atomic Force Microscopy

Noriyuki Kodera

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

High-speed atomic force microscopy (HS-AFM) is a unique microcopy that allows direct real-time visualization of biological macromolecules in action under near-physiological conditions, without any chemical labeling. Typically, the temporal resolution is sub-100 ms, and the spatial resolution is 2–3 nm in the lateral direction and ~0.1 nm in the vertical direction [1]. A wide range of biomolecular systems and their dynamic processes have been studied by HS-AFM, providing deep mechanistic insights into how biomolecules function. Notably, HS-AFM is particularly powerful in visualizing large and flexible biomolecular systems, which conventional structural biology methods are unable to visualize. These unique features of HS-AFM are well demonstrated in recent research results that visualize the structural dynamics of intrinsically disordered regions, single polypeptide chains with height of ~0.5 nm in a protein [2], the gathering of translation factors around the ribosomal stalk complex [3], and the long-range DNA degradation of CRISPR-Cas3 driven by its helicase and nuclease activities [4].

In the presentation, after overviewing the principles and performance of HS-AFM, AFM images showing dynamic behaviors of biomolecules will be shown. In addition, our recent efforts to improve the speed performance of HS-AFM will be discussed [5].

References

1. Ando, T.; Uchihashi, T.; Kodera, N., High-speed AFM and applications to biomolecular systems. Anuu. Rev. Biophys. 2013, 42, 393-414.
2. Kodera, N.; Noshiro, D., Dora, S. K.; ...; Longhi, S.; Ando T., Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy. Nat. Nanotechnol. 2021, 16, 181-189.
3. Imai, H.; Uchiumi, T.; Kodera, N., Direct visualization of translational GTPase factor pool formed around the archaeal ribosomal P-stalk by high-speed AFM. PNAS 2020, 117, 32386-32394.
4. Yoshimi, K.; Takeshita, K.; Kodera, N.; ... ; Mashimo, T., Dynamic mechanisms of CRISPR interference by Escherichia coli CRISPR-Cas3. Nat. Commun. 2022, 13, 4917.
5. Umeda, K.; McArthur, S. J.; Kodera, N., Spatiotemporal resolution in high-speed atomic force microscopy for studying biological macromolecules in action. Microscopy 2023, 72, 151-161.

Ulrich S. Schwarz

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Institute for Theoretical Physics and BioQuant, Heidelberg University, Germany

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Research Interests

Theoretical biophysics, cell mechanics and adhesion, cell organization, self-assembly

Education

1998	PhD in theoretical physics MPI and U Potsdam
1994	Physics diploma LMU Munich

Professional Experience

2009 – present	Full professor for Theoretical Physics at Heidelberg University
2008 – 2009	Full professor for Theoretical Biophysics at Karlsruhe Institute of Technology (KIT)
2001 – 2007	Emmy Noether Junior Research Group Leader at MPI Potsdam and U Heidelberg
1998 – 2000	Postdoc Weizmann Institute, Israel

Honors

2021 – 2022	Fellow of the Marsilius Kolleg Heidelberg
2020 – present	Member of the biophysics panel of the German Research Foundation
2019 – present	Fellow of the Max Planck School Matter to Life
2017	Outstanding teaching award of the Department of Physics and Astronomy
2013	Outstanding Referee of the American Physical Society
2011 – 2013	Speaker of the division Biological Physics of the German Physical Society

Publications

1. Anna Zelená, Johannes Blumberg, Dimitri Probst, Rūta Gerasimaitė, Gražvydas Lukinavičius, Ulrich S. Schwarz, and Sarah Köster. Force generation in human blood platelets by filamentous actomyosin structures. Biophysical Journal, 122(16):3340–3353, 2023.
2. Oliver M Drozdowski, Falko Ziebert, and Ulrich S Schwarz. Optogenetic control of migration of contractile cells predicted by an active gel model. Communications Physics, 6(1):158, 2023.
3. Gonen Golani and Ulrich S Schwarz. High curvature promotes fusion of lipid membranes: Predictions from continuum elastic theory. Biophysical Journal, 122(10):1868–1882, 2023.
4. Santiago Gomez Melo, Dennis Wörthmüller, Pierre Gönczy, Niccolo Banterle, and Ulrich Sebastian Schwarz. Grand canonical Brownian dynamics simulations of adsorption and self-assembly of SAS-6 rings on a surface. The Journal of Chemical Physics, 158(8), 2023.
5. Markus Mund, Aline Tschanz, Yu-Le Wu, Felix Frey, Johanna L Mehl, Marko Kaksonen, Ori Avinoam, Ulrich S Schwarz, and Jonas Ries. Clathrin coats partially preassemble and subsequently bend during endocytosis. Journal of Cell Biology, 222(3):e202206038, 2023.

Adsorption and Self-assembly of SAS-6 Rings on a Surface Studied with High-Speed AFM and Computer Simulations

Ulrich S. Schwarz

Institute for Theoretical Physics and BioQuant, Heidelberg University, Germany

Spindle assembly abnormal protein 6 (SAS-6) is an essential protein for centriole duplication and cell division. SAS-6 dimers assemble into a nine-fold ring of diameter 22 nm, from which nine spokes radiate outward and guide the polymerization of the nine microtubule triplets of the centriole. Therefore, SAS-6 rings define the characteristic nine-fold symmetry of centrioles, cilia and flagella. Recently, the assembly of the SAS-6 rings has been reconstituted on flat mica surfaces and monitored in real time with photothermally-actuated off-resonance tapping high-speed AFM (PORT-HS-AFM) [1]. The assembly curves were analyzed with image processing and a kinetic model for self-assembly (the coagulation-fragmentation equations in the reaction-limited approximation). It was found that the presence of the surface shifts the reaction equilibrium up by four orders of magnitude compared to the solution. Using molecular dynamics computer simulations, it was further found that the surface induces a conformational change in the SAS-6 dimers that allows for ring formation and leads to a residual asymmetry in the arrangement of the spokes. The PORT-HS-AFM experiments also revealed that rings can form with other than the nine-fold symmetry. Using Brownian dynamics computer simulations, it was found that the selectivity for the nine-fold symmetry competes with reversibility of the assembly pathway, which is critical to avoid kinetic trapping [2]. Together, these studies demonstrated the intricate interplay between surfaces and protein assembly, and how it can be quantitatively analyzed by combining high-speed AFM with computer simulations.

References

1. Banterle, Niccolò, et al. "Kinetic and structural roles for the surface in guiding SAS-6 self-assembly to direct centriole architecture." Nature Communications 12.1 (2021): 6180.
2. Gomez Melo, Santiago, et al. "Grand canonical Brownian dynamics simulations of adsorption and self-assembly of SAS-6 rings on a surface." The Journal of Chemical Physics 158.8 (2023).

Clemens M. Franz

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Research Interests

Atomic Force Microscopy, Fluorescence Microscopy, Cell Biology, Biophysics, Biomaterials

Education

2003 | Doctor of Philosophy (PhD) in Cell Biology, University College London, UK

Professional Experience

2019 – present	Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2018 – present	Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2016 – 2018	Senior Lecturer, Institute of Zoology, Karlsruhe Institute of Technology, Germany
2007 – 2016	Group Leader Nanobiology, Center for Functional Nanostructures, Karlsruhe Institute of Technology, Germany
2003 – 2007	Postdoctoral Research Fellow, Cellular Machines Group, Max Planck Institute for Molecular Cell Biology and Genetics and Technical University Dresden, Germany

Honors

2014	KIT Research Fellow
2000	Boehringer Ingelheim Fonds Scholar

Publications

1. Eroles, M.; Lopez-Alonso, J.; Ortega, A.; Boudier, T.; Gharzeddine, K.; Lafont, F.; Franz, C. M.; Millet, A.; Valotteau, C.; Rico, F., Coupled mechanical mapping and interference contrast microscopy reveal viscoelastic and adhesion hallmarks of monocyte differentiation into macrophages, Nanoscale 2023, 15 [29] 12255-12269

2. Baumann, H.; Schwingel, M.; Sestu, M.; Burcza, A.; Marg, S.; Ziegler, W.; Taubenberger, A.V.; Muller, D.J.; Bastmeyer, M.; Franz, C. M., Biphasic reinforcement of nascent adhesions by vinculin, Journal of Molecular Recognition 2023, 6 [36], e3012

3. Marchesi, A.; Umeda, K.; Komekawa, T.; Matsubara, T.; Flechsig, H.; Ando, T.; Watanabe, S.; Kodera, N.; Franz, C. M., An ultra-wide scanner for large-area high-speed atomic force microscopy with megapixel resolution, Scientific Reports 2021, 11, 13003

Investigating Actomyosin Contractility-driven Adhesion Modulation by Complementary High-speed AFM and Fluorescence Microscopy

Clemens M. Franz

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

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 able to 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.

References

1. Mohammed, T.O.; Lin, Y.R.,; Weissenbruch, K.; Ngo, K.X.; Zhang, Y.; Kodera, N.; Bastmeyer, M.; Miyanari, Y.; Taoka, A.; Franz, C.M., S100A11 promotes focal adhesion disassembly via myosin II-driven contractility and Piezo1-mediated Ca2+ entry, bioRxiv 2023
2. Baumann, H.; Schwingel, M.; Sestu, M.; Burcza, A.; Marg, S.; Ziegler, W.; Taubenberger, A.V.; Muller, D.J.; Bastmeyer, M.; Franz, C. M., Biphasic reinforcement of nascent adhesions by vinculin, Journal of Molecular Recognition 2023, 6 [36], e3012
3. Marchesi, A.; Umeda, K.; Komekawa, T.; Matsubara, T.; Flechsig, H.; Ando, T.; Watanabe, S.; Kodera, N.; Franz, C. M., An ultra-wide scanner for large-area high-speed atomic force microscopy with megapixel resolution, Scientific Reports 2021, 11, 13003

Erik Schäffer

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Research Interests

Molecular machines, microtubules and associated proteins, DNA-binding proteins, single-molecule biophysics, optical tweezers

Education

2001	Doctoral degree in physics, University of Konstanz, Germany

Professional Experience

2012 – present	Professor of Cellular Nanoscience at the Center for Plant Molecular Biology (ZMBP), University of Tübingen, Germany
2007 – 2012	Research group leader at the Biotechnology Center (BIOTEC), TU Dresden, Germany
2002 – 2006	Post-doctoral fellow, Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany
1999 – 2001	Research assistant at the Polymer Chemistry Department, University of Groningen, The Netherlands
1999	Research assistant at the Polymer Science and Engineering Department, University of Massachusetts, Amherst, USA
1998 – 1999	Research assistant at the Department of Physics, University of Konstanz, Germany
1995 – 1997	Research assistant at the Department of Physics, University of Massachusetts, Amherst, USA

Honors

2016	Prize for Bold Science, State of Baden-Württemberg
2016	ERC Proof of Concept, European Research Council (ERC)
2010	ERC Starting Grant, European Research Council (ERC)
2007	Emmy-Noether fellowship, German Science Foundation (DFG)
2001	DSM Prize for Chemistry and Technology 2001 laureate

Publications

1. Sudhakar, S., Jachowski, T. J., Kittelberger, M., Maqbool, A., Hermsdorf, G. L., Abdosamadi, M. K., and Schäffer, E. Germanium nanospheres for ultraresolution picotensiometry of kinesin motors. Science 371, eabd9944 (2021)

2. Ramaiya, A., Roy, B., Bugiel, M., and Schäffer, E. Kinesin rotates unidirectionally and generates torque while walking on microtubules. Proc. Natl. Acad. Sci. U. S. A. 114, 10894–10899 (2017).

3. Bormuth, V., Varga, V., Howard, J., and Schäffer, E. Protein friction limits diffusive and directed movements of kinesin motors on microtubules. Science 325, 870–873 (2009).

Ultraresolution Picotensiometry of Kinesin Motors

Erik Schäffer

Cellular Nanoscience, ZMBP, University of Tübingen, Germany

Simultaneously measuring the nanoscale motion and forces that molecular machines generate provides insights into how they work mechanically to fulfill their cellular function. To study these machines, we developed germanium nanospheres as probes for optical tweezers. With these high–refractive index nanospheres, we have improved the spatiotemporal resolution of optical tweezers and discovered that the motor kinesin takes 4-nanometer substeps. Further, instead of detaching from their microtubule track under load, motors slid back on it, enabling rapid reengagement in transport. Germanium nanospheres are promising for bioimaging, sensing, optoelectronics, nanophotonics, and energy storage. For optical trapping, the nanospheres open a new temporal window by which to uncover hidden dynamics in molecular machines.

Reference

1. S. Sudhakar, M.K. Abdosamadi, T.J. Jachowski, M. Bugiel, A. Jannasch, E. Schäffer, Germanium nanospheres for ultraresolution picotensiometry of kinesin motors, Science 2021, 371, eabd9944.

Masanobu Oshima

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Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
Cancer Research Institute, Kanazawa University, Japan

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Website: http://genetics.w3.kanazawa-u.ac.jp/english/

Research Interests

Cancer Biology, Mouse Models and Organoid Models for Tumors, Nano-Scale Study for Malignancy

Education

1988	Hokkaido University Graduate School of Veterinary Medicine, Japan

Professional Experience

2017 – present	Professor, WPI-NanoLSI, Kanazawa University, Japan
2005 – present	Professor, Cancer Research Institute, Kanazawa University, Japan
2000 – 2005	Associate Professor, Kyoto University Graduate School of Medicne, Japan
1997 – 1999	Research Associate, Merck Research Laboratories (Merck), USA
1992 – 1997	Staff Scientist, Banyu Pharmaceutical Co., Ltd., Japan
1988 – 1992	Staff Scientist, Chugai Pharmaceutical Co., Ltd., Japan

Honors

2015	The Commendation for Science and Technology by the Ministry of Education, Culture, Sports, Science and Technology (MEXT)
2012	JCA-Mauvernay Award, Japanese Cancer Association (JCA)

Publications

1. Wang D, Sun L, Okuda S, Yamamoto D, Nakayama M, Oshima H, Saito H, Kouyama Y, Mimori K, Ando T, Watanabe S, and Oshima M. Nano-scale physical properties characteristic to metastatic intestinal cancer cells identified by high-speed scanning ion conductance microscope. Biomaterials, 280: 121256, 2022.
2. Kok SY, Oshima H, Takahashi K, Nakayama M, Murakami K, Ueda HR, Miyazono K, and Oshima M. Malignant subclone drives metastasis of genetically and phenotypically heterogenous cell clusters through fibrotic niche generation. Nat Commun, 12 (1): 863, 2021.
3. Takeda H, Kataoka S, Nakayama M, Ali MAE, Oshima H, Yamamoto D, Park JW, Takegami Y, An T, Jenkins NA, Copeland NG, and Oshima M. CRISPR-Cas9 mediated gene knockout in intestinal tumor organoids provides functional validation for colorectal cancer driver genes. Proc Natl Acad Sci USA, 116: 15635-15644, 2019.
4. Oshima H, Kok SY, Nakayama M, Murakami K, Voon DC, Kimura T, and Oshima M. Stat3 is indispensable for damage-induced crypt regeneration but not for Wnt-driven intestinal tumorigenesis. FASEB J, 33: 1873-1886, 2019.

Identification of Genotype-linked Nano-Scale Physical Properties of Intestinal Tumor Cells

Masanobu Oshima

Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
Cancer Research Institute, Kanazawa University, Japan

Accumulation of driver gene mutations is responsible for colon cancer development. However, it has not yet been fully understood, which driver mutations induce each process of malignant progression, such as invasion, dissemination, and metastasis. To examine the malignant progression mechanism, we generated mouse models that carry driver mutations in driver genes, Apc (A), Kras (K), Tgfbr2 (T), Trp53 (P), and Fbxw7 (F) in various combinations, and established tumor-derived organoids from these models. Comprehensive histological analyses of in vivo tumors clarified the link between genotype and phenotypes. Namely, AT and AP double mutations induce submucosal invasion, AKT and AKP triple mutations causes advanced malignancy like EMT and intravasation, and AKTP and AKTPF multiple combinations drive liver metastasis at high frequency. Interestingly, we found that non-metastatic organoids can metastasize when co-migrated with metastatic cells by "polyclonal mechanism" . We next examined physical properties of the membrane surface of theses organoid cells by using high-speed ion conductance microscope (HS-SICM) time-lapse imaging. Notably, highly metastatic AKTP and AKTPF cells showed specific micro-ridges on the cell surface, and these structures were actively and continuously moving. Moreover, the stiffness of the metastatic AKTP and AKTPF cells was significantly lower compared with non-metastatic and benign tumor cells. Such results from organoid studies will be important for understanding the mechanism of malignant progression of cancer.

References

1. Sakai E, Nakayama M, Oshima H, Kouyama Y, Niida A, Fujii S, Ochiai A, Nakayama KI, Mimori K, Suzuki Y, Hong CP, Ock CY, Kim SJ, and Oshima M. Combined mutation of Apc, Kras and Tgfbr2 effectively drives metastasis of intestinal cancer. Cancer Res. 2018, 78 (5), 1334-1346,.
2. Kok SY, Oshima H, Takahashi K, Nakayama M, Murakami K, Ueda HR, Miyazono K, and Oshima M. Malignant subclone drives metastasis of genetically and phenotypically heterogenous cell clusters through fibrotic niche generation. Nat. Commun. 2021, 12 (1), 863.
3. Wang D, Sun L, Okuda S, Yamamoto D, Nakayama M, Oshima H, Saito H, Kouyama Y, Mimori K, Ando T, Watanabe S, and Oshima M. Nano-scale physical properties characteristic to metastatic intestinal cancer cells identified by high-speed scanning ion conductance microscope. Biomaterials 2022, 280, 121256.
4. Wang D, Nguyen HG, Nakayama M, Oshima H, Sun L, Oshima M, and Watanabe S. Mapping nanomechanical properties of basal surfaces in metastatic intestinal 3D living organoids with high-speed scanning ion conductance microscopy. Small 2023, 19 (9), e2206213.

Anna Taubenberger

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Center for Molecular and Cellular Bioengineering (CMCB), TU Dresden, Germany

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Research Interests

Cell and tissue mechanics, tumor microenvironment, bioengineered in vitro models

Education

2009	Doctor of Engineering, Technische Universität Dresden, Germany
2005	Diploma (Dipl.-Ing.) Engineering, Technische Universität Dresden, Germany

Professional Experience

2020 – present	Group leader, MSNZ fellow, CMCB TU Dresden
2013 – 2020	PostDoctoral Research fellow, CMCB TU Dresden
2009 – 2012	PostDoctoral Research fellow, Queensland University of Technology, Brisbane, Australia

Honors

2020	Mildred Scheel Nachwuchszentrum (MSNZ) Medical Scientist
2019	Poster Prize EACR
2011	Early Career Research Fellowship QUT
2010	DFG PostDoc fellowship

Publications

1. Abuhattum S, Kotzbeck P, Schlüßler R, Harger A, Ariza Schellenberger A, Kim K, Escolano JC, Müller T, Braun J, Wabitsch M, Tschöp M, Sack I, Brankatschk M, Guck J, Stemmer K, Taubenberger AV. Adipose cells and tissues soften with lipid accumulation while in diabetes adipose tissue stiffens. Sci Rep 12: 10325 (2022).

2. Mahajan V, Beck T, Gregorczyk P, Ruland A, Alberti S, Guck J, Werner C, Schlüßler R, Taubenberger AV. Mapping tumor spheroid mechanics in dependence of 3D microenvironment stiffness and degradability by Brillouin microscopy. Cancers 13:5549 (2021).

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Quantifying Tumor Spheroid Mechanical Properties in Confining 3D Microenvironments

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Tumor growth and invasion occurs in a mechanically altered tumor microenvironment, which is thought to contribute to cancer progression. Effects of matrix stiffness on tumor cells are well studied in a systematic manner using bioengineered 3D tumor in-vitro models. Previous studies indicate that tumor spheroids change their growth and invasion behavior when grown in stiffened microenvironments. Still, it remains poorly understood how the cells mechanically adapt to confining 3D microenvironments. Here we cultured single breast and pancreatic cancer cells to form tumor spheroids within ECM-mimicking biohybrid hydrogels of defined mechanical properties. Systematic modulation of hydrogel stiffness and/or degradability were associated with changes in cell morphology, gene expression and spheroid growth. Brillouin microscopy and atomic force microscopy revealed that spheroids altered their mechanical properties under confinement at the cellular and multicellular scale and when adopting an invasive phenotype. Drugs interfering with cell-cell junctions and intermediate filaments, but not F-actin filaments or microtubules affected the mechanical phenotype of tumor spheroids measured in situ. Taken together, our study provides insights into how tumor cells adapt their mechanical properties to microenvironment stiffness and confinement and when forming multicellular and invasive structures, which is relevant to tumor formation and progression.

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Research Interests

Biomechanics, Multicellular dynamics, Morphogenesis, Computational mechanics

Education

2013 | Doctor of Engineering, Department of Micro Engineering, Kyoto University, Japan

Professional Experience

2019 – present	Associate Professor, Nano Life Science Institute, Kanazawa University, Japan
2016 – 2021	PRESTO Researcher, Japan Science and Technology Agency, Japan
2016 – 2018	Special Postdoctoral Researcher, RIKEN Center for Developmental Biology, Japan
2013 – 2016	Researcher, RIKEN Center for Developmental Biology, Japan

Honors

2021	Seguchi Award, Japan Society of Mechanical Engineers
2020	Rising Star Award, Japan Science and Technology Agency PRESTO Area
2019	Japan Society of Mechanical Engineers Award (Paper)
2017	Yamaguchi Medal, Asian-Pacific Association for Biomechanics
2017	Incentive Award (Research), Japan Society of Mechanical Engineers

Publications

1. S Okuda*, T Hiraiwa, "Modelling contractile ring formation and division to daughter cells for simulating proliferative multicellular dynamics," The European Physical Journal E 46: 56 (2023)

2. R Matsuzawa, A Matsuo, S Fukamachi, S Shimada, M Takeuchi, T Nishina, P Kollmannsberger, R Sudo, S Okuda*, T Yamashita*, "Multicellular dynamics on structured surfaces: Stress concentration is a key to controlling complex microtissue morphology on engineered scaffolds," Acta Biomaterialia 166: 301-316 (2023)

3. S Okuda*, T Hiraiwa, "Long-term adherent cell dynamics emerging from energetic and frictional interactions at the interface," Physical Review E 107 (3): 034406 (2023)

Versatile Computational Modeling of 3D Multicellular Dynamics and Application to Collective Cell Migration

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In embryogenesis and cancer invasion, cells migrate collectively as a cluster in 3D tissues. Many studies have elucidated the mechanisms of either individual or collective cell migration on 2D substrates; however, it remains unclear how cells migrate collectively as a cluster in 3D tissues. To address such 3D multicellular dynamics, we developed a versatile 3D vertex model and applied it to collective cell migration in 3D space. Numerical simulations revealed that polarized interfacial tension enables cells to migrate collectively as a cluster through a 3D tissue. In this mechanism, the interfacial tension induces unidirectional flow of each cell surface from the front to the rear along the cluster surface. Importantly, multiple migratory modes were induced depending on the strength of polarity, adhesion, and noise, i.e., cells migrated either as single cells, as a cluster, or aligned like beads on a string, as occurs in embryogenesis and cancer invasion. These results suggest that the simple expansion and contraction of cell-cell boundaries enables cells to move directionally and generate the variety of collective migrations observed in living systems.

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Research Interests

Biophysics, Statistical Physics, Non-linear Dynamics

Education

2005	Habilitation: Theoretical Physics, Freie Universität Berlin
1995	Promotion Dr. rer. nat.: Theoretical Physics, Technische Universität Berlin
1987	Diploma Theoretical Physics, Technische Universität Chemnitz

Professional Experience

2014	apl. Professor, Dept. of Physics, Humboldt University Berlin
2008 – present	Research group leader, Max Delbrück Center for Molecular Medicine Berlin
2001 – 2008	Research assistant, Hahn-Meitner-Institute Berlin
1997 – 98 and 2000	Research assistant, Max-Planck-Institute for the Physics of Complex Systems Dresden
1995 – 97, 99	Post doc, Postdoc positions at the Institute for Nonlinear Science and the Physics Department of the University of California San Diego, USA
1995	Post doc, Chemical Engineering Department of the University of Virginia in Charlottesville, USA
1990 – 1995	Research assistant, TU Berlin
1987 – 1990	Engineer for control systems, VEB Chemieanlagenbau Leipzig-Grimma, Direktionsbereich Generallieferant, Karl-Marx-Stadt

Honors

2003	Schrödinger Prize for interdisciplinary research of the Donors' Association for the Promotion of the Sciences and Humanities in Germany 2003, together with J.D. Lechleiter and P. Camacho

Publications

1. Amiri B, Heyn JCJ, Schreiber C, Rädler JO, & Falcke M (2023) On multistability and constitutive relations of cell motion on fibronectin lanes. Biophysical Journal 122(5):753-766.
2. Schreiber C, Amiri B, Heyn JCJ, Rädler JO, & Falcke M (2021) On the adhesion–velocity relation and length adaptation of motile cells on stepped fibronectin lanes. Proceedings of the National Academy of Sciences 118(4):e2009959118.
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On Multistability and Constitutive Relations of Cell Motion on Fibronectin lanes

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Cell motility on flat substrates exhibits coexisting steady and oscillatory morphodynamics, the biphasic adhesion-velocity relation, and the universal correlation between speed and persistence (UCSP) as simultaneous observations common to many cell types. Their universality and concurrency suggest a unifying mechanism causing all three of them. Stick-slip models for cells on 1dimensional lanes suggest multistability to arise from the non-linear friction of retrograde flow. This study suggests a mechanical mechanism controlled by integrin signalling on the basis of a biophysical model and analysis of trajectories of MDA-MB-231 cells on Fibronectin lanes which additionally explains the constitutive relations(1, 2). The experiments exhibit cells with steady or oscillatory morphodynamics and either spread or moving with spontaneous transitions between the dynamic regimes, spread and moving and spontaneous direction reversals. Our biophysical model is based on the force balance at the protrusion edge, the noisy clutch of retrograde flow and a response function of friction and membrane drag to integrin signaling. The theory reproduces the experimentally observed cell states, characteristics of oscillations and state probabilities. Analysis of experiments with the biophysical model establishes a stick-slip oscillation mechanism, explains multistability of cell states and the statistics of state transitions. It suggests protrusion competition to cause direction reversal events, the statistics of which explain the UCSP. The effect of integrin signalling on drag and friction explains the adhesion-velocity relation and cell behavior at Fibronectin density steps. The dynamics of our mechanism are non-linear flow mechanics driven by F-actin polymerization and shaped by the noisy clutch of retrograde flow friction, protrusion competition via membrane tension and drag forces. Integrin signalling controls the parameters of the mechanical system.

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Research Interests

supramolecular chemistry, materials chemistry, biomaterials, photonic materials

Education

1999	PhD, Department of Chemistry, University of Toronto, Canada
1995	BSc, Department of Chemistry, University of British Columbia, Canada

Professional Experience

2017 – present	Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University
2016 – 2022	Associate Dean for Research & Graduate Studies, Faculty of Science, UBC
2014 – 2022	Director, NSERC CREATE Training Program in Nanomaterials (NanoMat)
2011 – present	Professor, UBC
2007 – 2011	Associate Professor, UBC
2001 – 2007	Assistant Professor, UBC
1999 – 2001	NSERC Postdoctoral Fellow, MIT, Cambridge, USA

Honors

2015 – 2029	Tier 1 Canada Research Chair in Supramolecular Materials
2016	Fellow of the Royal Society of Chemistry (UK)
2016	Award for Excellence in Materials Chemistry, Canadian Society for Chemistry
2014	Steacie Prize
2014	Fellow of the Royal Society of Canada
2013	Rutherford Memorial Medal in Chemistry, Royal Society of Canada
2013	JSPS Invitational Fellowship for Research
2012-2014	E.W.R. Steacie Memorial Fellowship

Publications

1. Cho, K.; Andrew, L. J.; MacLachlan, M. J. "Uniform Growth of Nanocrystalline ZIF-8 on Cellulose Nanocrystals: Useful Template for Microporous Organic Polymers" Angew. Chem. Int. Ed. 2023, 62, e202300960.
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Supramolecular Chemistry Using Bionanomaterials

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Bionanomaterials, such as cellulose nanocrystals (CNCs), are attractive for incorporation into new materials as they are extracted from renewable resources, they are biodegradable, and their properties can be changed through facile surface modification. Furthermore, CNCs are known to organize into a chiral nematic liquid crystalline phase in water. Upon drying, their order can be retained in solid films, with the organization resembling a helical stacking of the nanocrystals. These films with chiral nematic order display photonic properties (they appear iridescent) when the helical pitch of the structure is on the order of the wavelength of visible light. By deliberately changing the helical pitch of the structure, one can readily tune the wavelength of light reflected by the films.
In this presentation, I will discuss our group's efforts to construct new photonic materials using CNCs as an active component or a template. In particular, we have been constructing elastomers and shape-memory composite materials whose photonic properties change upon mechanical stimulation.

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Martin Bastmeyer

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Research Interests

Cell mechanics, Stem Cell Biology, Advanced Microscopy, Developmental Neurobiology

Education

1989	PhD in Biology, MPI Tübingen and University Kaiserslautern
1984	Diploma in Biology, University Kaiserslautern

Professional Experience

2021 – present	Director Institute of Biological and Chemical Systems - Biological Information Processing (IBCS-BIP) at KIT
2004 – present	Professor for Cell- and Neurobiology, Karlsruhe Institute of Technology (KIT), Germany
2001 – 2004	Professor for Neurobiology, University Jena, Germany
1998 – 2001	Independent Research Group Leader, University Konstanz, Germany
1993 – 1994	Postdoc, Salk Institute, San Diego, USA
1988 – 1998	Postdoc, MPI Tübingen and University Konstanz, Germany

Honors

2018	BRIDGE Fellowship of the Japanese Society for the Promotion of Sciences (JSPS)
2016	Erwin-Schrödinger-Prize for Interdisciplinary Research
2014	Advanced Researcher Fellowship Japanese Society for the Promotion of Sciences (JSPS)
1998	Heisenberg Fellowship of the DFG

Publications

1. K Weißenbruch, J Grewe, M Hippler, M Fladung, M Tremmel, K Stricker, US Schwarz, M Bastmeyer (2021) Distinct roles of nonmuscle myosin II isoforms for establishing tension and elasticity during cell morphodynamics. eLife 10: doi: 10.7554/eLife.71888.
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3. M Hippler, E Blasco, J. Qu, M Tanaka, C Barner-Kowollik, M Wegener, M Bastmeyer (2019) Controlling the shape of 3D microstructures by temperature and light. Nat Commun. 10: 232. doi: 10.1038/s41467-018-08175-w.
4. B Richter, V Hahn, S Berthels, TK Claus, M Wegener, G Delaittre, C Barner-Kowollik and M Bastmeyer (2017) Guiding cell attachment in three-dimensional microscaffolds selectively functionalized with two distinct adhesion proteins. Adv. Mater. 29: DOI: 10.1002/adma.201604342
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3D Cellular Microenvironments to Study Cell Mechanics

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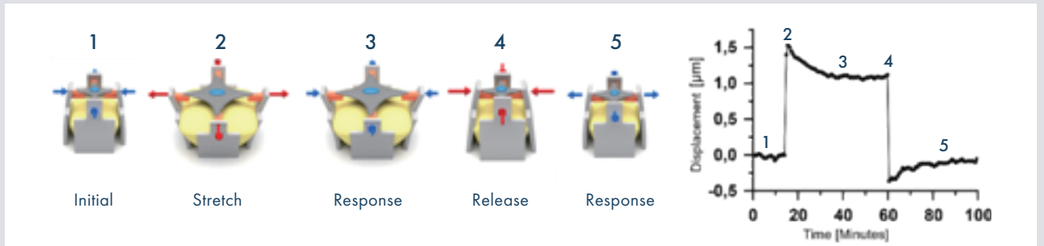
Cell behavior and differentiation are not only influenced by biochemical cues but also by physical properties like adhesive geometry, topography, and stiffness of the 3D extracellular environment. Correspondingly, cells exert, sense, and respond to physical forces by a variety of mechanisms. In this talk I will discuss how 3D laser nanoprinting can be applied to design 3D cellular microenvironments in the µm range with defined geometries and adjustable flexibility [1,2]. By varying the adhesion geometry, we study the interplay between extracellular physical factors and cell function in a systematic approach. Since the elastic modulus of the scaffold material varies between E=140-350 MPa, measurements of cell adhesion forces (typically in the range of 50-100 nN) in relation to adhesion geometry are also feasible. Furthermore, we are developing stimuli-responsive resists for 3D laser nanoprinting [3]. These hydrogels change their mechanical properties upon an external stimulus and thus have great potential for applications to study cellular mechanobiology. We have manufactured a miniaturized device that can be used to mechanically stretch single cells in a well-defined temporal and spatial manner and to simultaneously measure cellular contraction forces during the experiment [4] (Fig. 1). In combination with CRISPR/Cas9-generated knockout cell lines of proteins involved in cellular force production (e.g., non-muscle myosin II isoforms, a-actinin isoforms) this system will allow to study the molecular basis and regulation of force generation by non-muscle cells [5].



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2. C Barner-Kowollik et al. (2017) 3D Laser Micro- and Nano-Printing: Challenges for Chemistry. Angew. Chem. Int. Ed. 56: 15828-15845, doi: 10.1002/anie.201704695
3. CA Spiegel et al. (2020) 4D Printing at the Microscale. Adv Func Mater 30: doi: 10.1002/adfm.201907615
4. M Hippler et al. (2020) Mechanical stimulation of single cells by reversible host-guest interactions in 3D microscaffolds. Sci Adv. 6(39): eabc2648. doi: 10.1126/sciadv.abc2648.
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Svetlana Santer

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Research Interests

Light responsive polymers and nano-objects, physics and chemistry at interfaces, functional thin polymer films, manipulation of nano-objects

Education

1997-2000	PhD (Dr. rer.nat.), at the University of Ulm, Germany
1989-1996	Study of physics at the St.-Petersburg State University, Russia

Professional Experience

2009 – present	Professor for Experimental Physics, University of Potsdam, Germany
2008 – 2009	Junior Fellow at the Freiburg Institute of Advanced Studies, Freiburg, Germany
2007	Habilitation in Microsystem Technology (MST) at IMTEK, Freiburg, Germany
2003 – 2007	Research Assistant at the Institute for Microsystem Technology (IMTEK), Freiburg, Germany
2001 – 2003	Group Leader "Interface Analysis" at the Freiburger Materialforschungszentrum (FMF), Germany
2000 – 2001	PostDoc at IMTEK, Freiburg, Germany

Honors

2007	Heisenberg stipend, DFG
2004	Eliteförderprogramm für Postdoktorandinnen und Postdoktoranden, Landesstiftung Baden-Württemberg
2003	Margarethe von Wrangell Habilitationsstipendium
1995	Soros Preis for young researchers for the work on "Interaction of Molecule DNA with Antitumor Compounds

Publications

1. Polarization controlled fine structure of diffraction spots from an optically induced grating, J. Jelken, C. Henkel, S. Santer*, Appl. Phys. Lett. 2020, 116, 051601.
2. Light induced reversible structuring of photosensitive polymer films, J. Jelken, S. Santer* RSC Adv. 2019, 9, 20295.
3. Remote control of soft nano-objects by light using azobenzene containing surfactants, S. Santer, J. Phys. D Appl. Phys. 2017, 51, 013002.

From Molecular Actuation to Microscopic Motion

Svetlana Santer

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Azobenzene molecules can be considered as molecular actuators that convert optical energy in mechanical work. In this talk I will show several interesting examples where azobenzene can be used in order to actuate matter on large time and length scales. In all these examples the azobenzene plays the role of a transducer that mediates between different states of size, shape, position and interfacial energy of several nano-scale soft materials.
In the first part of my talk I will show how using a home made set-up combining an atomic force microscope (AFM) and two-beam interferometry. It is possible to address two major points concerning the experimental efforts in understanding surface relief grating (SRG) formation in azobenzene containing polymers: (i) how is the orientation of the electric field vector within the interfering electromagnetic fields related to the topographical pattern within the SRG; (ii) how can one measure locally the opto-mechanical forces emerging during topography change. We will discuss three distinct systems: polymer films, polymer brushes, and azobenzene containing polymer nanoparticles. [1-4]
In the second part of my talk I will show how, using azobenzene containing surfactant, [5] one can manipulate microparticles and even induce their self-propulsion when trapped at a solid/liquid interface. The physical origin of this genuine behavior is related to the phenomenon of light driven diffusioosmosis (LDDO). [6,7] We will discuss how to establish light-driven hydrodynamics as a useful and versatile tool for investigating collective motion of self-propelled particles and aggregation. At the very end of my talk I will present further examples of azo-induced actuation of soft matter. Microgel particles made light sensitive with azo-surfactants can change their volume (growing or shrinking) by up to a factor of 8 and LCST point between 32°C and 85°C in response to illumination with two different wavelengths. [8, 9]

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2. Di Florio, G.; Brundermann, E.; Yadavalli, N.S.; Santer, S.A.; Havenith, M. Nano Letters, 14 (2014) 5754.
3. Loebner, S.; Lomadze, N.; Kopyshev, A.; Koch, M.; Guskova, O.; Grenzer Saphiannikova, M.; Santer, S. A. J. Phys. Chem. B, 122 (2018) 2001.
4. Lomadze, N.; Kopyshev, A.; Bargheer, M.; Wollgarten, M.; Santer, S. Scientific Reports, 7 (2017) 8506.
5. Santer, S. J. Phys. D: Applied Physics, 51 (2017) 013002.
6. Feldmann, D.; Maduar S.R.; Santer, M.; Lomadze, N.; Vinogradova O.I.; Santer, S. Scientific Reports, 6 (2016) 36443.
7. Arya, P.; Umlandt, M.; Jelken, J.; Feldmann, D.; Lomadze, N.; Asmolov , E. S.; Vinogradova, O. I.; Santer, S. A. The European Physical Journal E, 44(50) (2021), 1-10.
8. Schimka, S.; Gordievskaya, Y.D.; Lomadze, N.; Lehmann, M.; von Klitzing, R.; Rumyantsev, A.M.; Kramarenko, E.; Santer, S. J. Chem. Phys., 147 (2017) 031101.
9. Jelken J.; Jung S.; Lomadze N.; Gordievskaya Y. D.; Kramarenko E. Y.; Pich A.; Santer S. Adv. Funct. Mater., (2021), 2107946.

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Research Interests

Scanning probe microscopy, Nanoscience, Nanoscale measurement technologies.

Education

2006	Doctor of Science, Graduate School of Science and Technology, Niigata University, Japan

Professional Experience

2020 – present	Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2017 – 2020	Assistant Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2012 – 2017	Assistant Professor, Bio-AFM Frontier Research Center, Kanazawa University, Japan
2009 – 2012	JST-ERATO Postdoctoral Fellow, Tohoku University, Japan
2007 – 2009	Assistant Professor, Center for the Advancement of Higher Education, Tohoku University, Japan
2007	Assistant Professor, Department of Physics, Tohoku University, Japan
2006 – 2007	JST- SORST Postdoctoral Fellow, Niigata University, Japan
2006	JST-CREST Postdoctoral Fellow, Niigata University, Japan

Publications

1. Wang, D.; Gia Han., N.; Nakayama, M.; Oshima, H.; Sun, L.; Oshima M.; Watanabe, S.; Mapping Nanomechanical Properties of Basal Surfaces in Metastatic Intestinal 3D Living Organoids with High-Speed Scanning Ion Conductance Microscopy, Small, 2022, 2206213.
2. Wang, D.; Sun, L.; Okuda, S.; Yamamoto, D.; Nakayama, M.; Oshima. H; Saito, H.; Kouyama, Y.; Mimori, K.; Ando, T.; Watanabe, S.; Oshima, M., Nano-scale physical properties characteristic to metastatic intestinal cancer cells identified by high-speed scanning ion conductance microscope. Biomaterials 2022, 280, 121256
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Time-Resolved Imaging of Nanomechanics and Nanostructures of Living Cells with Scanning Ion Conductance Microscopy

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Scanning ion conductance microscopy (SICM) is a kind of scanning probe microscopy technique, which uses a glass nanopipette as a probe to detect a tiny ion current through the nanopipette tip opening for nanoscale imaging. SICM has been utilized to observe nanostructures of living cell surfaces and their dynamics with low invasiveness in measurements. Recently, SICM has been advanced to examine the nanomechanical properties of living cell surfaces, allowing us to provide elasticity maps with nanometer-scale resolution. However, there is still a need to improve image rate and spatial resolution for diverse applications of nanobiosciences. In addition, improvement in accessing complicated structures, such as fragile tissues, has also been desired in SICM. We have recently developed a high-speed-scan type of SICM (HS-SICM). With the developed technique, we have simultaneously visualized nanostructures and elasticity distributions of living cancer cells with different malignancies. In addition, dynamic information of SICM images allows us to suggest quantitative differences in cellular states as the difference in physical parameters, such as roughness, mobility, and elasticity. We also applied HS-SICM to investigate nanostructures and nanomechanical properties of three-dimensional living organoids. By developing a simple method to embed organoids in collagen gels, we successfully visualized multiple nano and microstructures and elasticity distributions and their dynamics.

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Research Interests

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

Education

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

Professional Experience

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

Honors

2023	Science and Technology Award (Research), The Commendation for Science and Technology by MEXT
2018	15th JSPS Prize, Japan Society for the Promotion of Science
2017	Hokkoku Bunka Award, Hokkoku Shinbun
2011	Young Scientists' Prize, The Commendation for Science and Technology by MEXT

Publications

1. Yurtsever, A.; Wang, P. X.; Priante, F.; Morais Jaques, Y.; Miyazawa, K.; MacLachlan, M. J.; Foster, A. S.; Fukuma, T., Molecular insights on the crystalline cellulose-water interfaces via three-dimensional atomic force microscopy. Sci. Adv. 2022, 8 (41), eabq0160.
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Visualizing Nanoscale Dynamics and Mechanics in Living Cells by Nanoendoscopy AFM

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Atomic force microscopy (AFM) is the only method that allows direct imaging of nanoscale dynamics of biomolecules in liquid without labeling. However, such high-resolution imaging is possible only for biological systems reconstructed on a solid substrate. To overcome this limitation, we have developed nanoendoscopy AFM, where we insert a needle-like probe into a living cell and perform AFM measurements of the intra-cellular nanoscale dynamics and mechanics. This technique has two distinctive advantages over existing bio-imaging tools. First, it allows high-resolution imaging at an interface, such as the inner surface of the bottom cell membrane or the upper surface of the nucleus. To take advantage of this feature, we are investigating the structure and dynamics of focal adhesions (FAs). FAs connect actin fibers and extra-cellular structures and play important roles in cell motility and adhesion. Combining nanoendoscopy AFM with a confocal fluorescence microscope, we succeeded in direct imaging of the growth of a FA and the actin stress fiber associated with it. Such imaging allows correlating molecular transport visualized by fluorescence microscopy and nanoscale structural changes observed by AFM, helping us to understand the molecular mechanisms of various intra-cellular phenomena. Another advantage is the capability of nanomechanical property measurements. Nanoendoscopy AFM allows direct indentation of an intra-cellular component with a needle probe and quantitative estimation of local elasticity. With this technique, we are investigating nuclear elasticity changes induced by metastatic cancer progression. While the cell membranes become softer with the cancer progression, the nuclear membranes become stiffer. Our preliminary biochemical analyses suggest that the stiffening is due to increased chromatin compaction levels. Such a stiffening may imply its biological role in protecting the DNA from the risk of nuclear rapture during their invasive migration, where high pressure will be applied to the nucleus in passing through a small channel.

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Research Interests

Fluorescence microscopy, fluctuation analysis, virus assembly, membrane biophysics

Education

2008	Doctor of Physics, University of Dresden, Germany

Professional Experience

2021 – present	Professor of Physical Biochemistry, University of Potsdam, Germany
2015 – 2021	Junior Professor of Physical Cell Biochemistry, University of Potsdam, Germany
2014 – 2015	Principal Investigator, Dept. of Biology, Humboldt University, Berlin, Germany
2012 – 2014	Postdoc, Dept. of Biology, Humboldt University, Berlin, Germany
2009 – 2012	Postdoc, Dept. of Biochemistry and Cell Biology, State University of New York, USA
2008 – 2009	Postdoc, Dept. of Physics, Technical University of Dresden, Germany

Honors

2009	LSRF Fellowship, sponsor Howard Hughes Medical Institute (HHMI)
2008	Feodor Lynen Fellowship, Alexander von Humboldt Foundation
2007	Student Research Achievement Award, Biophysical Society

Publications

1. Dunsing V, Petrich A, Chiantia S. Multicolor fluorescence fluctuation spectroscopy in living cells via spectral detection- eLife 2021, doi: 10.7554/eLife.69687
2. Petrich A, Dunsing V, Bobone S, Chiantia S. Influenza A M2 recruits M1 to the plasma membrane: A fluorescence fluctuation microscopy study. Biophysical Journal 2021, doi: 10.1016/j.bpj.2021.11.023
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Investigating Virus-host Interactions at a Single Cell Level Using Quantitative Fluorescence Microscopy

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The pathogenesis of influenza A viruses (IAVs) is influenced by several factors, including IAV strain origin and reassortment, tissue tropism and host type. While such factors were mostly investigated in the context of virus entry, fusion and replication, little is known about the viral-induced changes to the host lipid membranes during assembly. In this work, we applied several biophysical fluorescence microscope techniques (i.e., membrane charge Förster energy resonance transfer sensing, generalized polarization imaging and scanning fluorescence correlation spectroscopy (sFCS)) to quantify the effect of infection by two IAV strains of different origin (avian: FPV; human: WSN) in avian and human cell lines (DF1 and HEK293T). We found that IAV infection affects the membrane charge of the inner leaflet of the plasma membrane. Moreover, we showed that IAV infection impacts lipid-lipid interactions by decreasing membrane fluidity and increasing lipid packing. Because of such alterations, diffusive dynamics of membrane-associated proteins were hindered. Taken together, our results indicate that the infection of avian and human cell lines with IAV strains of different origins had similar effects on the biophysical properties of the plasma membrane at the assembly side.

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Research Interests

Biophysics, Bionanotechnology, Membrane Proteins, Atomic Force Microscopy, Cellular Systems

Education

1997	PhD in Biophysics, Biozentrum, University of Basel, Switzerland

Professional Experience

2021 – present	Head of the Department of Biosystems Science and Engineering, ETH Zürich, Switzerland.
2020 – present	Foreign Scientific Member of the Max-Planck-Institute of Medical Research, Heidelberg, Germany.
2019 – 2021	Co-Head of the Department of Biosystems Science and Engineering, ETH Zürich, Switzerland.
2014 – present	Co-Director of the NCCR Molecular Systems Engineering, Switzerland.
2010 – present	Chair of Biophysics, Department of Biosystems Science and Engineering, ETH Zürich, Switzerland.
2003 – 2005	Director of the Biotechnology Center, Dresden, Germany.
2002 – 2010	Full professor for Cellular Machines (C4-position) at the Biotechnology Center, University of Technology Dresden, Germany
2000 – 2002	Groupleader (C3-position) at the Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany..

Honors

2023	Honorary Professor of the University of Heidelberg, Germany
2022	International Research Award of Germany, Humboldt Foundation, Ministry of Science and Education (BMBF), Germany
2020	Scientific Member of Max-Planck Society, Germany
2019	Marsilius Medal of the University of Heidelberg, Germany
2016	Elected EMBO Member
2011	Fellow of the Center of Advanced Studies of the Ludwig-Maximillian University, Munich, Germany

Publications

1. In mitosis integrins reduce adhesion to extracellular matrix and strengthen adhesion to adjacent cells
M. Huber, J.C. Casares-Arias, R. Fässler, D.J. Müller & N. Strohmeyer. Nature Communications (2023) 14, 2143
2. Gasdermin-A3 pore formation propagates along variable pathways
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M. Krieg, et al. Nature Reviews Physics (2019) 1, 41-57.

Mechanically Quantifying and Guiding Biological Processes

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Mechanobiology emerges at the crossroads of medicine, biology, biophysics and engineering and describes how the response of proteins, cells, tissues and organs to mechanical cues contribute to development, differentiation, physiology and disease. The grand challenge in mechanobiology is to quantify how biological systems sense, transduce, respond and apply mechanical signals. Over three decades, atomic force microscopy (AFM) has emerged as a key platform enabling the simultaneous morphological and mechanical characterization of living biological systems. Here, we will introduce the use of AFM-based nanoscopic assays to mechanically characterize, stimulate and animal cells and cellular systems. For example, we will exemplify how to use AFM-based assays to characterize viruses binding to mammalian cells and demonstrate how to use these insights to direct virus infection in vitro and in vivo for controlling cellular function and to restore vision. In another example we will use AFM to characterize how adherent cells sense the extracellular matrix (ECM) and responds to its biochemical composition and mechanical properties. Finally, we will employ AFM to functionally stimulate neuronal networks while following their electrophysiological response and to understand how individual neurons sense and respond to mechanical stimuli.

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Research Interests

Immunology, Exosome Engineering, Synthetic biology

Education

2011	Division of Immunobiology, Institute for Biological Sciences, Tokyo University of Science, Japan.

Professional Experience

2023 – Present	JST FOREST Researcher
2022 – Present	Associate professor in Graduate School of Medical Sciences, Kanazawa University, Japan
2019 – 2023	JST PRESTO Researcher
2017 – Present	Associate professor in Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2017 – 2022	Assistant professor in Department of Immunology, Kanazawa University, Japan
2011 – 2017	Postdoctoral researcher in Institute for Immunology, Ludwig-Maximilians University, Germany
2011 – 2011	Visiting researcher in Department of Dermatology, University Hospital Zurich, Switzerland

Publications

1. Keesiang Lim, Goro Nishide, Elma Sakinatus Sajidah, Tomoyoshi Yamano, Yujia Qiu, Takeshi Yoshida, Akiko Kobayashi, Masaharu Hazawa, Toshio Ando, Rikinari Hanayama, Richard W Wong., Nanoscopic Assessment of Anti-SARS-CoV-2 Spike Neutralizing Antibody Using High-Speed AFM. Nano letters. 2023, 23(2) 619-628.
2. Xiabing Lyu, Shota Imai, Tomoyoshi Yamano, Rikinari Hanayama., Preventing SARS-CoV-2 Infection Using Anti-spike Nanobody-IFN-β Conjugated Exosomes. Pharmaceutical research, 2022, 40(4) 927-935
3. Yamano T, Dobeš J, Vobořil M, Steinert M, Brabec T, Ziętara N, Dobešová M, Ohnmacht C, Laan M, Peterson P, Benes V, Sedláček R, Hanayama R, Kolář M, Klein L, Filipp D., Aire-expressing ILC3-like cells in the lymph node display potent APC features. The Journal of experimental medicine. 2019, 216(5) 1027-1037

Development of Engineered Exosome for Immune Regulation

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Exosomes play pivotal roles in cell-cell communication. It has been demonstrated that any protein of interest can be expressed on exosomes through conjugation with tetraspanins, exosome-specific surface proteins. Leveraging this method, we have developed engineered exosomes that simultaneously express several immune modulators. A distinctive feature of this approach is that target cells can concurrently receive all signals from these immune modulators. Using our method, we have developed several engineered antigen-presenting exosomes capable of either positively or negatively regulating immune responses. For example, Antigen Presenting Exosomes (AP-Exo-CTL) are engineered to express a peptide-major histocompatibility class I (pMHCI) complex, a costimulatory CD80 molecule, and IL-2 on their surface, thus enabling the simultaneous presentation of multiple immune modulators to antigen-specific CD8 T cells. This leads to the clonal expansion and differentiation of antigen-specific CTL, inducing potent anti-cancer effects. Conversely, AP-Exo-Treg exosomes are engineered to express the antigen-MHCII complex, IL-2, and TGF-β on their surface, promoting the induction of antigen-specific regulatory T cells. We will discuss the potential and efficacy of these engineered exosomes in modulating immune responses.

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1. Keesiang Lim, Goro Nishide, Elma Sakinatus Sajidah, Tomoyoshi Yamano, Yujia Qiu, Takeshi Yoshida, Akiko Kobayashi, Masaharu Hazawa, Toshio Ando, Rikinari Hanayama, Richard W Wong., Nanoscopic Assessment of Anti-SARS-CoV-2 Spike Neutralizing Antibody Using High-Speed AFM. Nano letters. 2023, 23(2) 619-628.
2. Xiabing Lyu, Shota Imai, Tomoyoshi Yamano, Rikinari Hanayama., Preventing SARS-CoV-2 Infection Using Anti-spike Nanobody-IFN-β Conjugated Exosomes. Pharmaceutical research, 2022, 40(4) 927-935
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POSTER SESSIONS

The 7th NanoLSI Symposium in Berlin; From Molecules to Cells and Tissue – Bridging Scales with Nanoprobe Technology

Nanoscale visualization of Aptamer-CYP24 complex binding dynamics in the development of aptamer therapeutics in cancer

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Abstract

Overexpression of a mitochondrial enzyme CYP24 in cancer cells decreases the level of vitamin D3, which has anti-cancer activity [1]. To enhance vitamin D3 functionality in cancer cells, we identified a CYP24-inhibiting DNA aptamer (Apt-7). In this study, we used high-speed atomic force microscopy (HS-AFM) [2] and molecular docking methods to provide a single-molecule explanation of the aptamer-based CYP24 inhibition mechanism [3]. The HS-AFM revealed the dynamic interactions of Apt-7 and CYP24 at the nanoscale level and the spatio-temporal analysis of captured images enabled us to characterize the binding interaction of Apt-7 and CYP24. The molecular docking results further revealed that Apt-7 combined steadily at active or allosteric binding sites of CYP24 by strong hydrogen bonds and electrostatic interaction. Interestingly, images from HS-AFM showed remarkably similar conformation with molecular docking results. In summary, the integrated approach of HS-AFM and molecular docking provided a bridge to the development of a lead molecule for promising anticancer therapy.

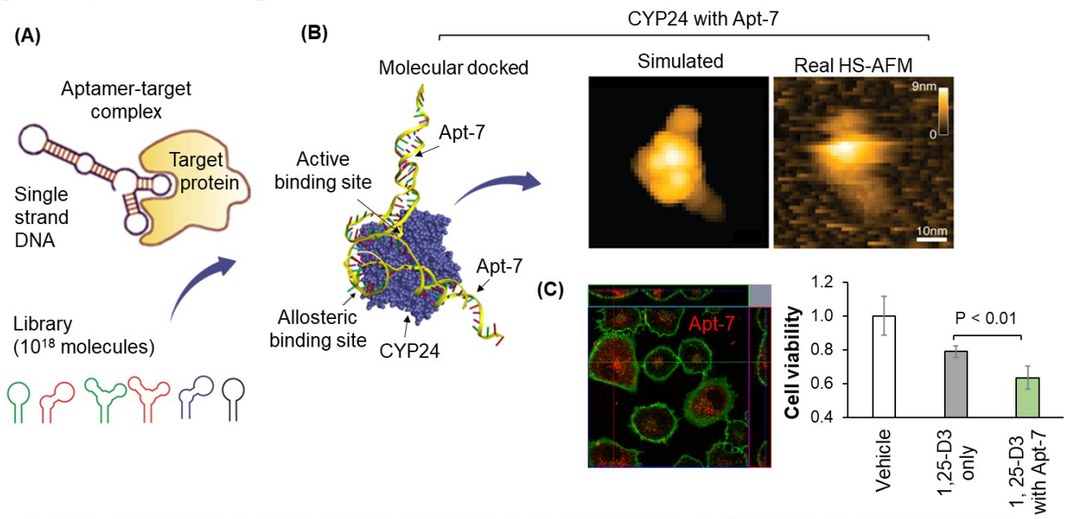

Figure 1. Development of CYP24-inhibiting aptamer for novel cancer therapy. (A) Schematic representation of DNA aptamer binding to target protein and in vitro selection of aptamers using a diverse ssDNA library; (B) simulation (molecular docking and BioAFMViewer) and experimental (HS-AFM) co-analysis of the binding interaction between Apt-7 and CYP24; (C) Apt-7 internalization into cancer cells and effect on cancer cells viability.

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3. **Madhu Biyani**, Kaori Yasuda, Yasuhiro Isogai, Yuki Okamoto, Wei Weilin, Noriyuki Kodera, Holger Flechsig, Toshiyuki Sakaki, Miki Nakajima, and Manish Biyani**, ACS Applied Materials & Interfaces 2022**, 14, 16, 18064-18078.

Light-induced manipulation of colloidal particles in a photosensitive polymer-dye system

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We present a multi-dimensional investigation into the optical and physical characteristics of a complex photosensitive surfactant system. This system comprises mesoporous silica particles in an aqueous solution of a fluorescent dye and an azobenzene-containing surfactant, capable of binding into a complex together. The azobenzene-containing surfactant undergoes reversible photo-isomerization from its *trans*- to *cis*-configuration upon exposure to UV/blue light, with this isomerization process occurring on a picosecond timescale. This unique property enables precise control over the dynamic aggregation or dispersion of mesoporous colloidal particles upon illumination. The formation of the dye/surfactant complex introduces an additional dimension to the investigation of the described system.

The driving force behind the possibility of remotely controlling the colloids is the phenomenon of light-driven diffusioosmosis (LDDO). The porous structure of mesoporous silica colloids allows them to act as a source or sink of the photosensitive surfactant, driving the “local” LDDO process and leading to mutual interactions (repulsion/attraction) between particles, which may be used to impose a fine structure on a 2D particle grid. Depending on the isomerization state, the surfactant has different adsorption affinities to both the particles and the fluorescent dye. The *trans*-isomer readily enters and accumulates in the negatively charged pores of the particles, while also forming a complex with the negatively charged organic dye. However, the *cis*-isomer is expulsed out of the pores and remains in the solution outside the particles; it also doesn’t bind to the dye. Thus, upon irradiation, the azobenzene-containing surfactant isomerizes to its *cis*-state within the colloids’ pores, the dye-surfactant complex gets destroyed and the polymer readily diffuses out, generating a decrease in luminescence and an excess concentration near the colloid’s outer surface, which leads to the creation of a diffusioosmotic flow and the displacement of the particles. This process is wavelength-and intensity-dependent, allowing for the control over these structures. The dye provides an additional dimension to the system, adding to its complexity due to its influence on the surfactant isomerization and the LDDO process.

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2. Feldmann, D.; Maduar S.R.; Santer, M.; Lomadze, N.; Vinogradova O.I.; Santer, S. “Manipulation of small particles at solid liquid interface: light driven diffusioosmosis”. *Scientific Reports*, (2016).
3. Arya P.; Feldmann, D.; Kopyshev, A.; Lomadze N.; Santer, S. Light driven guided and self-organized motion of mesoporous colloidal particles. *Soft Matter*, (2020).
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Nanoscopic Evaluation of Anti-SARS-CoV-2 Spike Neutralizing Antibody Using High-Speed AFM

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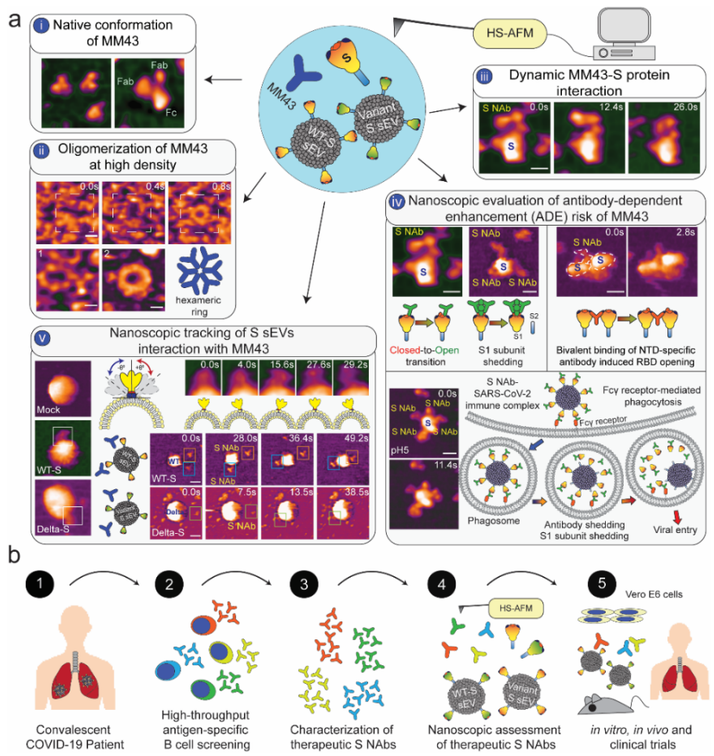
Abstract: SARS-CoV-2, the newly emerging β-coronavirus, has caused the unprecedented COVID-19 pandemic since 2019. Delayed onset of COVID-19 plus highly infectious variants have accelerated the infection rate to worldwide. Although several mRNA vaccines against viral spike (S) protein have been developed to prevent the disease, the protection wanes fast and override by new variants. Anti-S protein neutralizing antibodies (S NAbs) provide an effective alternative to prevent and treat COVID-19, in which people can constantly receive rapid passive immunity against SARS-CoV-2. Direct visualization of the dynamic S NAb and S protein interaction at nanoscopic level using current imaging tools, cryo-EM and confocal imaging for example, remains difficult. We here perform high-speed atomic force microscopy (HS-AFM) imaging to elucidate this interaction, in which it is essential to study the binding pattern of S NAbs and the risk of antigen-dependent enhancement (ADE). We first observed the innate structural properties of an S NAb (MM43; IgG1) such as the Y conformation of IgG antibody, and formation of spontaneous hexameric rings at high density. The S NAb neither induce RBD opening nor S1 subunit shedding after it bound to S protein. On the other hand, we noticed that the S NAb-S protein interaction was stable at acidic pH (pH5). Collectively, these findings suggested that the S NAb has low risk of ADE. Besides protein-protein interaction, we also developed small extracellular vesicles with surface S protein (S sEV) to mimic the S protein of SARS-CoV-2. Dynamic interaction between S NAb and S sEV together with the binding tendency of S NAb could be evaluated through HS-AFM scanning. In summary, we demonstrate a nanoscopic platform that is feasible to study the binding properties of numerous S NAbs either isolated from convalescent COVID-19 patients or via antibody design.



Local direction of opto-mechanical stress in azobenzene containing polymers during surface relief grating formation

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On this poster we present how the photo-induced deformation of azobenzene containing polymers relates to the local direction of opto-mechanical stresses generated during irradiation with interference patterns (IPs). We can substantiate the modeling approach proposed in Ref. 1, which describes the directional photo-deformations in glassy side-chain azobenzene polymers, and prove that these deformations arise from the re-orientation of rigid backbone segments along the light polarization direction. In experiments we inscribe surface relief grating in pre-elongated photosensitive colloids of few micrometers length using different IPs such as SS, PP, +45, SP, RL and LR. The deformation of the colloidal particles is studied in-situ, whereby the local variation of the polymer topography is assigned to the local distribution of electrical field vector for all IPs. Orientation approach correctly predicts local variations of the main axis of light-induced stress in each interference pattern for both initially isotropic and highly oriented materials. With this work, we suggest that the orientation approach implements a self-sufficient and convincing mechanism to describe photo-induced deformation in azobenzene containing polymer films that in principle does not require auxiliary assumptions.

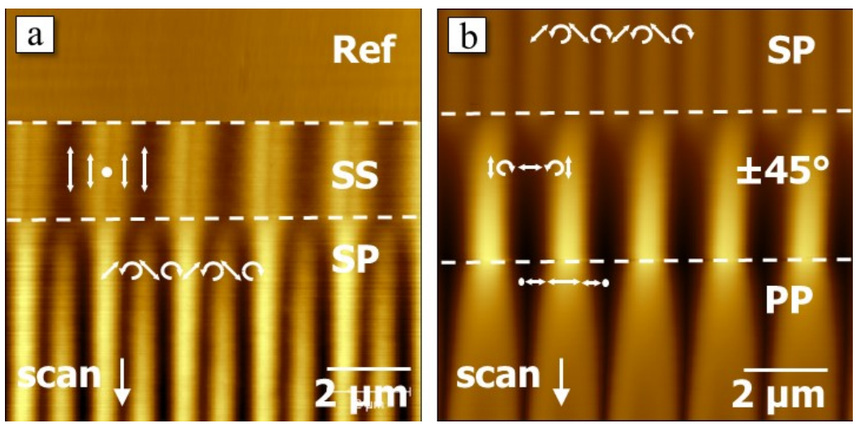

Figure 1. AFM micrographs showing SRG inscribed in the azobenzene containing polymer film using different interference pattern. White dashed horizontal lines indicate positions at the micrographs where the IP is switched. Additionally, the distribution of the electrical field vectors (white arrows) are assigned to the topography variation.

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Deciphering actin structure-dependent preferential cooperative binding of cofilin

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In our earlier study, we observed that the shortened helical pitch in cofilactin, where a cofilin cluster is bound to an actin filament, affected the neighboring bare zone to shorten a half helical pitch (HHP) on the pointed-end (PE) side of the cluster, while the pitch on the barbed-end (BE) side remained similar to the control. The unidirectional growth of cofilin clusters toward the PE of the filament suggested that cofilin favors actin structures with a shorter HHP. However, the exact mechanism underlying the cooperative binding of cofilin to actin filaments based on their favorable actin structure has remained unclear. To delve into this mechanism, we conducted a principal component analysis on actin structures derived from 46 PDB structures. This analysis classified the structural differences in actin associated with different nucleotides and actin binding proteins. In particular, we found a notable contrast in the structure of ADP-actin between F-actin and cofilactin, providing a structural basis for suggesting that the influence of ADP has a minimal effect on actin structure that is conducive to the preferential binding of cofilin. Furthermore, through the use of high-speed atomic force microscopy, we made a novel discovery: the bare half helix with shortened HHP on the PE side of the cluster comprised fewer actin protomers compared to the normal helices. The mean axial distance between two adjacent actin protomers along the same long-pitch strand, referred to as MAD, in the shortened bare HHP exhibited elongation and thermal fluctuation in a higher range (5.0 – 6.3 nm) than the MAD in the normal HHP (4.3 – 5.6 nm). The impediment of cooperative structural changes in helical twisting, achieved through stronger attachment to the lipid membrane, had a more pronounced hindrance on cofilin binding and the expansion of larger clusters than the impact of inorganic phosphate. Collectively, we hypothesize that the shortening of half helices, resulting in elongation in MAD, assumes a pivotal role in facilitating the preferential cooperative binding of cofilin to actin protomers.

Light-Triggered Manipulations of Droplets All in One: Reversible Wetting, Transport, Splitting, and Merging

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Here we report on light-triggered droplet manipulation such as reversible wetting, splitting, merging, and transport [1]. The unique feature of our approach is that the changes in the wetting properties of microscopic droplets of anisotropic (liquid crystalline) liquids adsorbed on photo-switchable films can be triggered just by application of soft optical stimuli, which lead to dynamical, reversible changes in the local morphology of the structure surfaces. The adaptive films consist of an azobenzene-containing surfactant ionically attached to oppositely charged polymer chains. Under exposure to irradiation with light, the azobenzene photo-isomerizes between two states, nonpolar *trans*- and polar *cis*-isomers, resulting in corresponding changes in surface energy and orientation of the surfactant tails at the interface [2]. Additionally, the local increase in the surface temperature due to absorption of light by the azobenzene groups [3] enables diverse processes of manipulation of the adsorbed small droplets, such as the reversible increase of the droplet basal area up to 5 times, anisotropic wetting during irradiation with modulated light, and precise partition of the droplet into many small pieces. Moreover, using a moving focused light spot, we demonstrate the locomotion of the droplet over macroscopic distances with a velocity of up to 150$\mu m \cdot s^{-1}$. Our findings could lead to the ultimate application of a programmable workbench for manipulating and operating an ensemble of droplets, just using simple and gentle optical stimuli.

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Real-time visualization of Structural Maintenance of Chromosomes complexes by high-speed atomic force microscopy

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Many of chromosomal functions are linked to the folding structure of genome DNA, which is meters in length but packed in a micrometer-order nucleus. The structural maintenance of chromosomes (SMC) complexes, including cohesin, condensin, and Smc5/6, play central roles in such three-dimensional genome organization. SMC complexes are ring-shaped ATPase assemblies that topologically encircle DNA strand inside of their ring to form physical links between distantly separated DNA segments. Resent biochemical and single-molecule studies have revealed dynamic behaviors of SMC complexes, however, it remains elusive how the proteinous rings bind and manipulate DNA at the sub-molecular level. In this study, we applied high-speed AFM to directly visualize SMC complexes that bound to DNA. This visualized the structural changes of single SMC molecule with their various behavers on DNA, like one-dimensional diffusion, at 100 millisecond-order resolution. Furthermore, our results obtained with the ATPase mutant suggested that SMC complex initially associates with DNA via ATPase domain, which follows to the late DNA-binding state. Based on these results, we discuss the molecular mechanism by which SMC complexes bind to DNA.

Characterization and optimization of SERS substrates using adenine as benchmark molecule and automation of data evaluation

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Abstract

Providing insight into biomolecular interactions at the nanoscale, Surface-Enhanced Raman Spectroscopy (SERS) is an important tool in biophysics and structural chemistry [1]. Factors such as morphology, shape, and type of nanostructured metal, laser wavelength, and analyte choice affect the performance of SERS substrates. Consequently, efforts invested in characterizing and improving these substrates to enhance their sensitivity, reproducibility, uniformity, and affordability, are paramount.

While the conventional use of fluorescent dyes for benchmarking of the SERS substrates introduces complexities due to resonance Raman scattering effects [2], we focus on adenine as an optimal reporting molecule for characterization of the SERS substrates. Adenine exhibits several advantageous properties making it an ideal choice, particularly for silver substrates. Importantly, adenine is non-resonant at visible wavelengths, but possesses strong Raman cross-section. Furthermore, in the 1 µM − 10 µM concentration range a unique surface complex between silver and adenine forms, providing additional Raman enhancement and presenting opportunities for biophysical investigations [3]. At higher adenine concentrations, we evidenced a layer of oriented adenine molecules on the surface of silver SERS substrates by atomically-resolved 3D AFM.

The large-area SERS substrates characterization and optimization generally represent a demanding task, that requires evaluation of vast Raman datasets. Therefore, we present a novel approach enabling a precise automated SERS background removal and peak identification based on morphology operators [4].

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Exploring the mechanics of cortical dendrite formation through AFM measurements

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Abstract

Dendrites are important structures that receive signals from other neurons. Previous studies have shown that dendrites elongate and branch in response to the signals. Recently, mechanical properties have attracted much attention as a new factor in regulating cell behavior, and it is thought that they also affect dendrite elongation and branching. However, the mechanical properties of dendrites and the surrounding environment are not well understood, and the effect of the mechanical properties on dendrites is unknown. Therefore, to investigate the influence of mechanical properties on dendrites, we isolated and cultured neurons from mouse cortex and measured the properties by atomic force microscopy (AFM). In this presentation, we will report the results obtained so far and discuss future analyses and the influence of mechanical properties on dendrites. We believe that our work will allow us to understand how the mechanical properties of the brain and the nerves themselves influence the formation of neural networks.

High-speed atomic force microscopy revealed alternative interactions between CaMKII holoenzymes depend on their phosphorylation

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Long-term potentiation (LTP) and long-term depression (LTD) are considered fundamental cellular-level mechanisms in memory formation and forgetting, respectively (*1*), and alter synaptic signal efficiency and dendritic spine volume (*2*). At the molecular level, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) plays a pivotal role in the functional and structural plasticity of the brain(*3*). CaMKII is highly abundant within the post-synaptic density (PSD), comparable to the levels of cytoskeletal proteins (*4*). However, its contribution to the mechanism underlying structural plasticity remains elusive. Here, we employed high-speed atomic force microscopy (HS-AFM) (*5*), (*6*), (*7*) to observe the structural dynamics of CaMKII at high concentrations similar to those found within the PSD. Furthermore, we observed the dynamics of the phosphorylation states of CaMKII related to LTP and LTD and analyzed the intermolecular distance between CaMKII holoenzymes. As a result, the interaction among neighboring CaMKII was dependent on their phosphorylated states. These findings suggest that the structural dynamics of CaMKII may contribute in part to the structural plasticity in neurons.

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Measurement of temperature gradients in microscopic volumes using thermosensitive microgels

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Microgels are recognized for their remarkable responsiveness to changes in environmental conditions [1]. In this work, we demonstrated a novel method for local temperature determination in aqueous solutions by observing the volume-inhomogeneous collapse of microgels positioned near the substrate. The substrate consists of a glass cover, a thin titanium oxide layer (<1nm), and a variable-thickness gold layer (5-30nm). The localized laser irradiation of the substrate results in the absorption of light by the gold layer, converting it into heat. We studied how the laser wavelength and power levels, as well as distance from the irradiation spot influence the size and shape of the thermosensitive PNIPAM microgel. The obtained dependences allowed us to construct a comprehensive temperature distribution profile relative to the irradiation point, both along the surface and perpendicular to it.
Moreover, experimental data posed an intriguing question about the underlying mechanisms of heat transfer in liquids. What are the roles of thermophoresis, thermo-osmosis, and convection in this process?

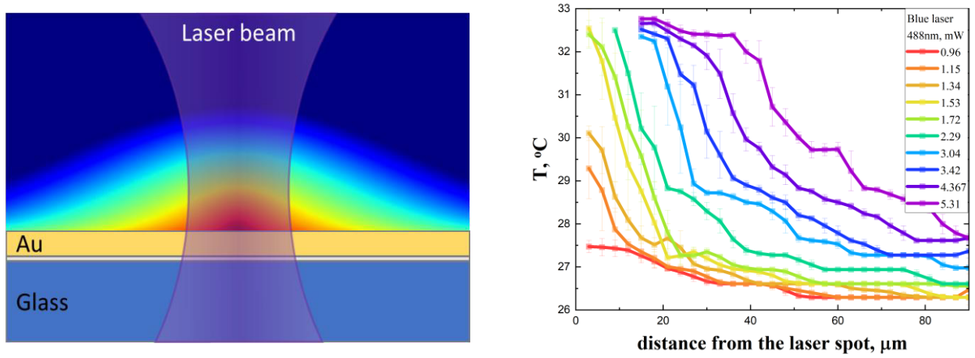

Fig. A scheme that describes a method for local heating (left). Temperature profile of an aqueous solution under irradiation with a blue laser in the case of a 30 nm gold layer (right).

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Separation of small extracellular vesicles using an anion exchange column and revealing their mechanical properties by AFM

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Abstract

Extracellular vesicles (EVs) are nano-sized vesicles with lipid-bilayer membranes, and secreted by almost all cell types. Small extracellular vesicles (sEVs) are typically ranging in size from 30 to 150 nanometers in diameter, and they play important roles in intercellular communication, transporting various bioactive molecules between cells. According to the different biogenesis mechanisms, EVs are commonly classified into three subtypes including exosome (EXOs), microvesicles (MVs) and apoptotic bodies. However, there is currently no method to isolate specific sEVs from a sample containing different types of sEVs, also their mechanical properties have not been characterized.

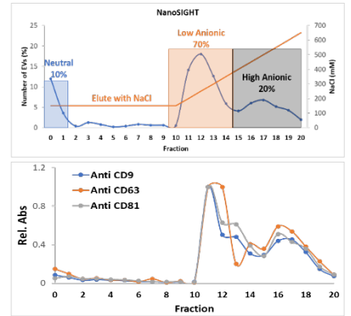


Fig 1. Separation of EVs based on anionic charges and confirmation by EV markers.

In this research, we used an anion exchange column to separate heterogenous sEVs and revealed the detailed structure and nanomechanical properties by Atomic Force Microscopy (AFM). Through the anion-exchange method, sEVs in the culture supernatant can be eluted into different fraction based on surface charge, also analysis of sEV markers indicated that different anionic fractions may correspond to different sEV subtypes. (Fig. 1). Then we isolated the MVs from cells whose plasma membranes were labeled with a membrane-impermeable biotin reagent and detected with streptavidin, found that the anion exchange column can elute MVs into fraction 15 and intraluminal vesicles (ILVs), which are origin of EXOs, into fraction 0 and 12 (Fig. 2). Based on these findings, we separated heterogenous EVs into MVs and EXOs, then AFM analysis revealed that EXOs have higher elasticity and rigidity than MVs. Consequently, we developed a method to clearly separate sEV subtypes derived from different membranes, which overcomes issues of the current sEV isolation method, also elucidate the structural, mechanical characteristics of heterogenous sEVs.

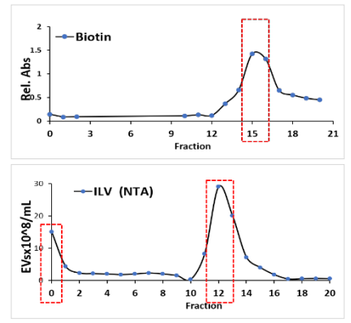
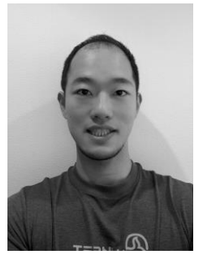


Fig 2. Anionic difference of heterogenous EVs.

Reconstruction of Multicellular Patterns with Synthetic Morphogen System



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During morphogenesis, cell-cell communication through a secreted factor called morphogen plays important roles for regulating precise cell position and arrangement. However, the mechanisms through which diffusing morphogens define precise spatial patterns amidst biological fluctuations remain unclear. Several theories are reported such as gene regulatory network and endocytic pathway of morphogen, but it is difficult to separately analyze each factor in model organisms due to the complexity.

Therefore, we designed and developed a SYnthetic Morphogen system for Pattern Logic Exploration using 3D spheroids (SYMPLE3D). This system allowed us to study how cellular responses to concentration gradients of secreted molecules could generate tissue domains with sharp boundaries. In this system, we utilized an artificial cell-cell communication through secretion and reception of green fluorescent protein (GFP), using synthetic receptor called synthetic Notch Receptor (synNotch). With this receptor, we can program which proteins the cells recognize and which genes the cells turn on in response to that recognition.

We first established GFP-secretor and GFP-receiver cells expressing synNotch. Then, we evaluated artificial morphogen signaling between these cells after spheroid formation. Here, we explored how the culture conditions and the expression of responsive genes can influence multicellular patterns in SYMPLE3D. Interestingly, we found that coupling morphogen signal and cell sorting can sufficiently convert a gradient signal into distinct tissue domains with a sharp boundary. I will introduce and discuss our findings on patterning in this symposium.

Keywords: Synthetic biology, Morphogen, Cell adhesion

The promises of *Dictyostelium discoideum*: Charting the Path for Crawling Cell-Based Biohybrid Designs.

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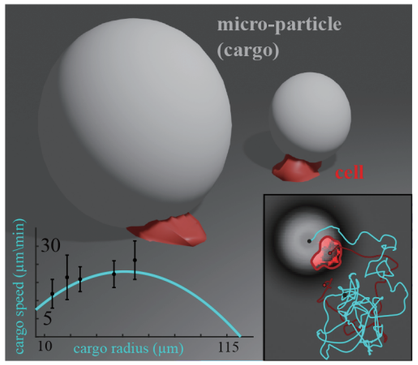

Incorporating motile cells into biohybrid microrobots offers distinct advantages such as sensitive responsiveness to external stimuli, robustness, and a built-in energy source. Drawing inspiration from the motility of leukocytes, we integrated amoeboid *Dictyostelium discoideum* cells to design a cell-cargo biohybrid system. In this design, the cell acts as the exclusive active element, adaptlively maneuvering spherical micro-cargoes when they are encountered [1–3]. Intriguingly, the dynamics of this cell-cargo partnership is governed by the size of the cargo [2]. A singular cell can move cargoes as large as red blood cells, spanning up to approximately 240 microns in diameter, all while applying sub-piconewton range forces. Notably, these cell-generated forces amplify when persistent drag acts on the cargo, underscoring the system's adaptive mechanoresponsiveness in intricate settings [1]. Leveraging their innate chemotactic behavior, the directionality of cargo movement can be harnessed to achieve precision in delivery [1–3]. As amoeboid motility is a hallmark of numerous mammalian cell types, our findings pave the way for the realization of crawling cell-centric biohybrid systems.

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Bacterial swimming under flow conditions: fluorescence microscopy, light-driven micromanipulations and nano-mechanobiology

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By changing the configuration of the flagella bundle, bacterial swimmers adjust their direction and speed of locomotion. The soil bacterium *Pseudomonas putida*, by counterclockwise (CCW) rotation of their flagella, push themselves forward, while by clockwise (CW) rotation they pull themselves in the opposite direction. Additionally, *P. putida* can wrap their bundle of flagella around the cell body.[1,2] However, many reasons and benefits of having different motility patterns still remain unclear. Here, by using different techniques, we study how the swimming pattern of cells changes under flow conditions.

First, using fluorescence microscopy and microfluidics, we show how the contribution of each swimming mode changes under laminar shear flow conditions. Compared to a fluid at rest, we found that in a shear flow, swimmers prefer the pull configuration of flagella over the wrapped one (both emerging under CW flagellar rotation). Moreover, swimmers tend to align in parallel to the flow direction, in contrast to a non-motile mutant (control with the same geometry), which does not show a preferred orientation under the same shear flow conditions. As the next step, to test an instant response of bacterial swimmers to flow, we developed biocompatible light-induced flow techniques to locally advect or trap stained cells.[3] Finally, with the help of AFM, we aim at specifying the nano-mechanical properties of flagella and cell body to develop a mechanistic understanding of flagellar dynamics under flow conditions in the future.

Our research demonstrates how bacteria adapt their swimming pattern to different environmental conditions on the single-cell level. Understanding bacterial behavior and adaptation in such conditions can be crucial for clarifying the processes of spreading and infection in capillaries as well as biofilm formation in a natural environment.

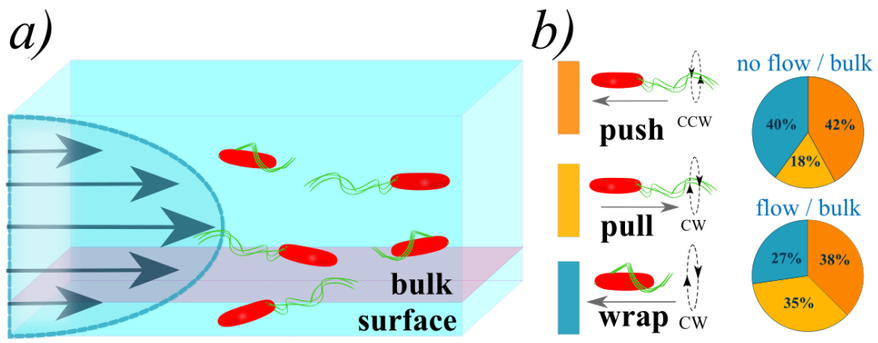

Figure 1. (a) Schematic of bacterial swimming in a laminar shear flow. (b) Distribution of swimming modes under flow and no-flow conditions

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Abstract: See page 13

Open Facility Program

Researchers at Nano Life Science Institute (NanoLSI) are pioneers in the development of original Biological Scanning Probe Microscopy (Bio-SPM) technologies, such as atomic-resolution Atomic Force Microscopy (AFM) (FM-AFM/3D-AFM), high-speed AFM, scanning ion conductance microscopy (SICM), AFM for cell measurement, and in applying them to the life sciences.
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Financial support

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Applicants can be accompanied by up to two others such as fellow researchers, postdocs from their own lab.

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