



NOVENBER A. SI

# NanoLSI Symposium



Nanoprobe Technology for Understanding Molecular Systems

NOVEMBER

14-15, 2022

VENUE

HYBRID-FLEXIBLE FORMAT

ANA CROWNE PLAZA HOTEL KANAZAWA, JAPAN

## 6th NanoLSI Symposium

I	November 14, 2022 DAY 1					Nove	
8:30 am	Reception ope	ening and arrival tea			8:30	AM	Reception opening and arrival tea
9:00 am	Opening remo	arks: Prof. Takeshi Fukuma, Director a	f NanoLSI		9:00	AM	Session #4 9:00AM -11:00AM
9:10 ам	Session #1 9 Chairperson: Session theme:	<ul> <li>Prof. Takeshi Fukuma &amp; Prof. Yasufumi Tak Probing Inside of Living Cells by SPM</li> <li>Prof. Ricardo Garcia (Instituto deCiencia de Advances in Single Cell Nanomechanics:</li> <li>Prof. Hiroshi Uji-i (Hokkaido University) Remote Excitation Single Cell Endoscopy</li> <li>Assoc. Prof. Paolo Actis (University of Leeds) Longitudinal Single-Cell Profiling with a N</li> <li>Prof. Takeshi Fukuma (NanoLSI, Kanazawa U Intracellular Structures and Mechanics of</li> </ul>	<b>ahashi</b> Materiales de Madrid, CSIC) Subsurface Imaging and Nanorheolog anobiopsy Platform Jniversity) .iving Cells Investigated by Nanoendos	у сору-АҒМ			Chairperson: Session theme:Prof. Richard W. Wong Recent Advances in Nuclear BiologProf. Robert Goldman (Northwei Intermediate Filament Networ Prof. Ohad Medalia (University The Remarkable Structure and Assoc. Prof. Takeshi Shimi (Tokyo The Role of A-Type Lamins in I Assoc. Prof. Hanae Sato (Nanol Real-Time Imaging of Transcription)
11:10 am	Poster session	1	Lunch		11:00	AM	Poster session
1:00 pm	Session #2 1	1:00PM -3:00PM			1.30	PM	Session #5 1:30PM -3:00PM
	Session theme:	<ul> <li>Molecular Assemblies and Reactions in Various</li> <li>Prof. Steven De Feyter (KU Leuven)</li> <li>Formation and Functionalization of 2D Ma</li> <li>Assoc. Prof. Satoshi Arai (NanoLSI, Kanazav</li> <li>Subcellular Thermometry and Nanoheatin</li> <li>Prof. Shiki Yagai (Chiba University)</li> <li>Synthetic Supramolecular Polymers with D</li> <li>Assoc. Prof. Hitoshi Asakawa (NanoLSI / Na</li> <li>Molecular Pockets in 2D Supramolecular</li> </ul>	Dimensional Spaces Aterials: A Molecular Approach va University) g Using Functional Dyes averse Topological Features anoMaRi, Kanazawa University) Assemblies Investigated by 3D-AFM				Chairperson: Session theme:Prof. Mikihiro Shibata & Prof. Nor Nanoprobe Technology for UnderstaDr. Atsushi Miyawaki (RIKEN Cente Cruising in the CellProf. Mikihiro Shibata (NanoLSI, Ka Correlation between Function an Prof. Yasufumi Takahashi (NanoLSI, Development of Organelle College)
3:00 pm	Tea break				3:00	PM	Closing remarks: Overall Organizer Prof. Mikih
3:20 рм	Session #3 3 Chairperson: Session theme:	<ul> <li>Asst. Prof. Holger Flechsig</li> <li>Theory and Modelling of Intracellular Dynamics</li> <li>Dist. Prof. Tsvi Tlusty (Ulsan National Institute of So The Collective Dynamics of Enzymatic Nano-1</li> <li>Asst. Prof. Chiho Watanabe (Hiroshima University Cell-Size Space Effect on Molecular Diffusion</li> <li>Dr. Tatsuo Shibata (RIKEN Center for Biosystems Emergence of Cell Chirality from the Spatial C</li> <li>Prof. Ralf Metzler (University of Potsdam) Beyond Brownian Motion: From Data to Mod</li> </ul>	cience and Technology) Machines 1) and Liquid-Liquid Phase Separation Dynamics Research) rganization of Actin and Myosin Cytoskel els	ełon			Photo session
5:40 рм	Banauet 5:4	OPM -7:10PM		☆ pre-registration required		1	Pre-event
	Banquet toast:	Prof. Yoshihiro Fukumori, Vice	Director of NanoLSI				November 13, 2022 4:00 PM - 5:00 PM

#### · 15, 2022 DAY 2

- University Feinberg School of Medicine) onnect the Nucleus to the Cell Cortex
- rich)
- chanics of Nuclear Lamin Filaments
- tute of Technology)
- ir of Nuclear Envelope Ruptures
- anazawa University)
- al Feedback in Nonsense-Mediated mRNA Decay



- bility of Protein Complex Revealed by HS-AFM
- azawa University / Nagoya University)
- Technology Using Nanopipette

#### nibata

ıl lab tour @ NanoLSI Building, Kakuma Campus, Kanazawa University

## ABSTRACTS

P 08	Ricardo Garcia / Instituto de Cier
P 10	Hiroshi Uji-i / Hokkaido Universit
P 12	Paolo Actis / University of Leeds
P 14	Takeshi Fukuma 🖊 NanoLSI, Kanc
P 16	Steven De Feyter / KU Leuven
P 18	Satoshi Arai / NanoLSI, Kanazav
P 20	Shiki Yagai / Chiba University
P 22	Hitoshi Asakawa 🖊 NanoLSI / Na
P 24	Tsvi Tlusty / Ulsan National Institu
P 26	Chiho Watanabe / Hiroshima Uni
P 28	Tatsuo Shibata / RIKEN Center for
P 30	Ralf Metzler / University of Potsdo
P 32	Robert Goldman / Northwestern
P 34	Ohad Medalia / University of Zu
P 36	Takeshi Shimi / Tokyo Institute of
P 38	Hanae Sato / NanoLSI, Kanazav
P 40	Atsushi Miyawaki / RIKEN Cente
P 42	Mikihiro Shibata 🖊 NanoLSI, Kan
P 44	Yasufumi Takahashi 🖊 NanoLSI, K

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Kanazawa University / Nagoya University



## Ricardo Garcia



Professor of Nanoscience and Nanotechnology Instituto de Ciencia de Materiales de Madrid, CSIC, Spain

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#### Research Interests

Atomic force microscopy, Nanomechanics, Biophysics, Solid-liquid interfaces, Scanning probe lithography

### Education

1990

PhD in Physics, Universidad Autonoma de Madrid, Madrid, Spain

#### **I** Professional Experience

2014 - present	Professor of Nanoscience, Instituto de Ciencia de Materiales de Madrid/CSIC/Spair
2004 - 2013	Professor, Instituto de Microelectrónica de Madrid/CSIC/Spain
2002 - 2004	Research Scientist, Instituto de Microelectrónica de Madrid/CSIC/Spain
1994 - 2001	Tenured Scientist, Instituto de Microelectrónica de Madrid/CSIC/Spain
1991 - 1993	Post-Doctoral Associate, Institute of Molecular Biology/University of Oregon/USA
1990 - 1991	Post-Doctoral Fellow/ Dept. Chemistry/University of New Mexico/USA

#### Honors

2019	Beller Lectureship Award, American Physical Society/USA
2016	Nanotechnology Recognition Award, American Vacuum Society/USA
2013	ERC Advanced Grant, European Research Council
2011	Achievement Recognition Award, CSIC/Spain
2010	Nanotechnology Prize, Regional Government of Madrid/Spain
2007	Fellow, American Physical Society/USA

#### Publications

- Gisbert, V.G.; Benaglia, S.; Yhlig, M.R.; Proksch, R.; Garcia, R. High-speed nanomechanical mapping of the early stages of collagen growth by bimodal force microscopy. ACS Nano 2021, 15, 1850-1857.
- 2. Garcia, P.D.; Guerrero, P.D.; Garcia, R. Nanorheology of living cells measured by AFM-based force-distance curves. Nanoscale 2020, 12, 9133-9143.
- Garcia, R. Nanomechanical mapping of soft materials with the atomic force microscope: methods, theory and applications. Chemical 3. Society Reviews 2020, 49, 5850-5884.
- 4. Uhlig, M.R.; Martin-Jimenez, D.; Garcia, R. Atomic-scale mapping of hydrophobic layers on graphene and few-layer MoS2 and WSe2 in water. Nature Communications 2019, 10, 2606.
- 5. Fukuma, T. & Garcia, R. Atomic-and molecular-resolution mapping of solid-liquid interfaces by 3D atomic force microscopy. ACS Nano 2018, 12, 11785-11797.

## Advances in Single Cell Nanomechanics: Subsurface Imaging and Nanorheology

Ricardo Garcia

#### Instituto de Ciencia de Materiales de Madrid, CSIC, Madrid, Spain

The development of high-resolution, label-free, quantitative and subsurface microscopy methods of living cells remains a formidable problem. Force microscopy-based measurements are contributing to our understanding of single cell properties. However, current AFM-methods have several limitations. First, the spatial resolution is in the 100 nm range. Second, the description of a cell is restricted to its elastic properties which implies that the measurements are semi-quantitative. Third, AFM does not provide images of intracellular structures (sub-surface).

This presentation introduces an integrated approach to overcome those limitations. We present a method to determine the complete nanomechanical response of a cell from a force-distance curve. The method incorporates the cell's finite-thickness, a power-law nanorheology model and the deformation history of the cell. It transforms the experimental data into viscoelastic parameters of the cell as a function of the indentation frequency. The method enables to generate three-dimensional maps of eukaryotic cells.

- 1. Garcia, P.D.; Guerrero, C.R.; Garcia, R. Subsurface Imaging of Cell Organelles by Force Microscopy. ACS Nano 2019, 13, 9629-9637
- 2. Garcia, R. Intracellular forces from stiffness. Nat. Mater. 2019, 18, 1037-1038
- 3. Garcia, P.D.; Guerrero, P.D.; Garcia, R. Nanorheology of living cells measured by AFM-based force-distance curves. Nanoscale 2020, 12, 9133-9143
- 4. Sanchez, J.G.; Espinosa, F.M.; Minguez, R.; Garcia, R. The viscoelasticity of adherent cells follows a single power-law with distinct local variations within a single cell and across cell lines. Nanoscale 2021, 13, 16339-16348.
- 5. Garcia, R. Nanomechanical mapping of soft materials with the atomic force microscope: methods, theory and applications. Chemical Society Reviews 2020, 49, 5850-5884



## Hiroshi Uji-i

Professor RIES, Hokkaido University, Japan Department of Chemistry, KU Leuven

E-mail: hiroshi.ujii@es.hokudai.ac.jp, hiroshi.ujii@kuleuven.be Website: https://www.es.hokudai.ac.jp/labo/lnn/Top\_English.html https://sites.google.com/site/hiroshisgroup/

#### Research Interests

Nanoscopy, Plasmonics, Single molecule spectroscopy

#### Education

2002	PhD, Department of Chemistry, Tohoku University, Japan
1999	Ms, Department of Applied Physics, Osaka University, Japan

#### Professional Experience

2015 - present	Full Professor, Research Institute for Electronic Science (RIES), Hokkaido University, Japan
2011- present	Research Professor ('hoofddocent'), Katholieke Universiteit Leuven (KULeuven) Belgium
2004 - 2011	Senior Post-Doctoral Researcher for Center of Excellence Katholieke Universiteit Leuven (KULeuven) Belgium
2002 - 2004	Post-Doctoral Research Fellow, Katholieke Universiteit Leuven (KULeuven) Belgium

#### **Honors**

ERC starting grant "Plasmonics-based Energy Harvesting for Catalysis: PLASMHACAT" 2015 PRESTO/JST 2009

### Publications

His research activity has been published in over 150 peer-reviewed international journals and three book chapters (h-index of 43 (web of science)).5 publications related to Endoscopy

- Nanoscale, 2022. 14, 5439 5446 1.
- ACS Applied Nano Materials 2021, 4, 9, 9886-9894. 2.
- Analytical Chemistry, 2021, 93, 12, 5037-5045 3.
- Nature Communications, 2015, 6, 6287. 4
- 5. Nano Letters, 2009, 9, 995 1001



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## Remote Excitation Single Cell Endoscopy

Hiroshi Uji-i

RIES, Hokkaido University, Sapporo, Japan KU Leuven, Leuven, Belgium iCeMS, Kyoto University, Kyoto, Japan

Silver nanowires (AgNWs) serve as plasmonic waveguides for propagating surface plasmon polaritons (SPPs), allowing the spatial confinement and transfer light energy over micrometer distance through the structures below sub-diffraction limited diameter. In addition to this, surface plasmon allows us to concentrate light energy in nanometer regions, such as at the nanowire end, leading to a massive enhancement of electromagnetic filed that can be used for surface enhanced Raman scattering (SERS) or fluorescence (SEF) spectroscopy/microscopy. In this contribution, we will discuss noble nanoscopic techniques using a combination of SERS/SEF detection and sub-diffraction limit SPPs waveguiding for spectroscopic/microscopic. Specifically, we demonstrate that SPPs launched along an AgNW can remotely excite SERS [1] and SEF [2] in the vicinity of the nanowire surface due to the SPPs wave-guiding effect. The ability to transfer SERS/SEF excitation over several microns will be discussed with respect to single-cell endoscopy, understanding of the interaction between anti-cancer drug molecules and cellular components such as DNA for drug delivery systems [3], and surface characterization using tip-enhanced Raman/fluorescence microscopy [4].

- 1. H. Uji-i et al., Analytical Chemistry, 93, 12, 5037–5045 (2021). Advanced Materials, 26, 5124-5128 (2014). Nano Lett., 9, 995 - 1001 (2009).
- 2. H. Uji-i et al, Nature Commun. 6, 6287 (2015).
- 3. H. Uji-i et al, Sci. Rep., 9, 2666 (2019).
- 4. H. Uji-i et al, Nanoscale, 14, 5439 5446 (2022).



## Paolo Actis



Associate Professor of Bionanotechnology University of Leeds, UK

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#### Research Interests

Single molecule analysis with nanopores, Single cell analysis, Nanoelectrochemistry

#### Education

2008	PhD in Electrochemistry, Grenoble Institute of Technology, FR
2005	Msc Materials Science, University of Turin, IT

#### Professional Experience

2021 - present	Associate Professor, University of Leeds, UK
2016 - 2021	University Academic Fellow, University of Leeds, UK
2014 - 2016	Consultant and Project Manager, Bio Nano Consulting, UK
2012 - 2014	Postdoctoral Scholar, Imperial College London, UK
2008 - 2012	Postdoctoral Scholar, University of California, Santa Cruz, US

#### **Publications**

- 1. Nanopore Fingerprinting of Supramolecular DNA Nanostructures, S Confederat, I Sandei, G Mohanan, C Wälti, P Actis, 2022, **Biophysical Journal**
- 2. A subcellular cookie cutter for spatial genomics in human tissue, AG Bury, A Pyle, F Marcuccio, DM Turnbull, AE Vincent, G Hudson, P Actis, 2022, Analytical and Bioanalytical Chemistry, 1-10
- Rational design of DNA nanostructures for single molecule biosensing, M Raveendran, AJ Lee, R Sharma, C Wälti, P Actis, 2020, Nature 3. Communications 11 (1), 1-9
- 4. Macromolecular crowding enhances the detection of DNA and proteins by a solid-state nanopore, CC Chau, SE Radford, EW Hewitt, P Actis, 2020, Nano Letters 20 (7), 5553-5561
- 5. Ribosome fingerprinting with a solid-state nanopore, M Raveendran, AR Leach, T Hopes, JL Aspden, P Actis, 2020, ACS Sensors 5 (11), 3533-3539

## Longitudinal Single-Cell Profiling with a Nanobiopsy Platform

Paolo Actis

#### School of Electronic and Electrical Engineering, University of Leeds, UK

Physiological and pathological processes within the human body are controlled by complex cell-cell interactions within the context of a dynamic microenvironment. The ability to dynamically measure phenotypes (i.e. gene expression, protein activities, ion fluctuations, signalling) at the single cell level is key to understanding cellular behaviour in a complex environment. I will present the integration of nanopipettes with scanning probe microscopy techniques to enable the extraction of genetic material from living cells. I will discuss the longitudinal profiling of brain cancer cells to characterize the transcriptional reprogramming at the single cell level after chemotherapy and radiotherapy.

#### References

- 1. Sampling from single cells, P Actis, Small Methods, 2018 2 (3), 1700300 28
- Seger, N Pourmand, ACS nano, 2014, 8 (1), 546-553
- 3. Evaluation of mRNA localization using double barrel scanning ion conductance microscopy. Y Nashimoto, Y Takahashi, Y Zhou, H Ito, H Ida, K Ino, T Matsue, H Shiku, ACS nano, 2016, 10 (7), 6915-6922

2. Compartmental genomics in living cells revealed by single-cell nanobiopsy, P Actis, MM Maalouf, HJ Kim, A Lohith, B Vilozny, RA



## Takeshi Fukuma



## Intracellular Structures and Mechanics of Living Cells Investigated by Nanoendoscopy-AFM

Takeshi Fukuma

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

Atomic force microscopy (AFM) has been a powerful tool for directly visualizing nanoscale structures, dynamics and mechanics of biomolecular systems. However, it has been a great challenge to directly access intracellular components of living cells since AFM was originally developed as a 2D surface imaging tool. To overcome this limitation, we have recently developed nanoendoscopy AFM, where we insert a needle-like probe into a living cell to perform a 2D/3D AFM measurement. With the developed technique, we have demonstrated imaging of the whole cell structures, actin fibers, focal adhesions without giving a serious damage to the living cell. In addition, the method also allowed us to map the elasticity distribution on a nucleus to reveal its dependence on the cell cycle and cancer progression. Owing to the capability to directly access to the intracellular component with a probe, most of the functionality of the AFM should, in principle, be available even in a living cell. We also combined this method with confocal or TIRF microscope. The correlative imaging by fluorescence microscopy and AFM allows simultaneous imaging of dynamic transports of specific molecules and structural development of intracellular component. These methods should provide new insights into the nanodynamics and nanomechanics inside living cells.

#### References

- 1. Penedo, M.; Miyazawa, K.; Okano, N.; Furusho, H.; Ichikawa, T.; Alam Mohammad, S.; Miyata, K.; Nakamura, C.; Fukuma, T., Visualizing intracellular nanostructures of living cells by nanoendoscopy-AFM. Sci. Adv. 2021, 7 (52), eabj4990.
- 2. Penedo, M.; Shirokawa, T.; Alam, M. S.; Miyazawa, K.; Ichikawa, T.; Okano, N.; Furusho, H.; Nakamura, C.; Fukuma, T., Cell penetration efficiency analysis of different atomic force microscopy nanoneedles into living cells. Sci. Rep. 2021, 11 (1), 7756.

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

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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, Japan

#### **I** Professional Experience

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

#### Honors

2018	15 <sup>th</sup> JSPS Prize, Japan Society for the Promotion of Science
2017	Hokkoku Bunka Award, Hokkoku Shinbun
2011	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technolog The Young Scientists' Prize

#### **Publications**

- 1. Yurtsever, A.; Wang, P. X.; Priante, F.; Morais Jaques, Y.; Miyata, K.; MacLachlan, M. J.; Foster, A. S.; Fukuma, T., Probing the Structural Details of Chitin Nanocrystal-Water Interfaces by Three-Dimensional Atomic Force Microscopy. Small Methods 2022, 2200320.
- 2. Penedo, M.; Miyazawa, K.; Okano, N.; Furusho, H.; Ichikawa, T.; Alam Mohammad, S.; Miyata, K.; Nakamura, C.; Fukuma, T., Visualizing intracellular nanostructures of living cells by nanoendoscopy-AFM. Sci. Adv. 2021, 7 (52), eabj4990.
- 3. Fukuma, T.; Garcia, R., Atomic- and Molecular-Resolution Mapping of Solid-Liquid Interfaces by 3D Atomic Force Microscopy. ACS Nano 2018, 12 (12), 11785-11797.



## Steven De Feyter

Division of Molecular Imaging and Photonics, Department of Chemistry,



## Formation and Functionalization of 2D Materials: A Molecular Approach

Steven De Feyter

#### Division of Molecular Imaging and Photonics, Department of Chemistry, KU Leuven, Belgium

In this presentation, I will mainly focus on the functionalization of graphite, graphene, and transition metal dichalcogenides using molecules, though the concepts can be applied to other 2D materials too. Nanostructuring is at the heart of all functionalization protocols that we develop because it opens new possibilities for control and functionality. A variety of scanning probe microscopy methods are used for visualization, characterization, and manipulation. The first approach is based on molecular self-assembly at the interface between a liquid or air, and graphite or graphene. A second approach is based on the covalent attachment of molecules on 2D materials via covalent chemistry. It will be demonstrated how in addition to bottom-up strategies that provide control on the density and layer thickness, as well as submicron to nanoscale nanostructuring, also top-down scanning probe microscopy and optical lithography can be used to structure such covalently modified surfaces. A third approach does not focus on the functionalization of the surface, but uses the surface as a support for the in-plane covalent stitching of molecules, leading to the formation of on-surface 2D dynamic covalent polymers.

#### References

- 1. L. Verstraete, S. De Feyter, Chem. Soc. Rev. 50, 5884 (2021).
- 2. L. Daukiya, J. Teyssandier, S. Eyley, S. El Kazzi, M. C. Rodríguez González, B. Pradhan, W. Thielemans, J. Hofkens, S. De Feyter, Nanoscale 13, 2972 (2021)
- 3. K. Tahara, Y. Kubo, S. Hashimoto, T. Ishikawa, H. Kaneko, A. Brown, B. E. Hirsch, S. De Feyter, Y. Tobe, J. Am. Chem. Soc. 16, 7699 (2020)
- 4. M. C. Rodríguez González, A. Leonhardt, H. Stadler, S. Eyley, W. Thielemans, S. De Gendt, K. S. Mali, S. De Feyter, ACS Nano 6, 10618 (2021)
- 5. S. Freddi, M. C. Rodriguez Gonzalez, P. Carro, L. Sangaletti, S. De Feyter, Angew. Chem. Int. Ed. 61, e202200115 (2022)
- 7. G. Zhan, Z. F. Cai, K. Strutyński, L. Yu, N. Herrmann, M. Martinez-Abadia, M. Melle-Franco, A. Mateo-Alonso, S. De Feyter, Nature 603, 835(2022).

## Research Interests

Scanning probe microscopy, Nano(bio)chemistry, Surface functionalization

#### **Education**

1997	PhD in Chemistry (KU Leuven)
1993	Master in Chemistry (KU Leuven

#### Professional Experience

2011-present	Full Professor, Department of Chemistry, KU Leuven (Belgium)
2004 - 2011	Associate Professor, Department of Chemistry, KU Leuven (Belgium)
1999 - 2004	Postdoctoral Researcher, Department of Chemistry, KU Leuven (Belgium)
1998 - 1999	Postdoctoral Researcher, California Institute of Technology (USA)

Professor

KU Leuven, Belgium

E-mail: steven.defeyter@kuleuven.be Website: www.defeytergroup.org

#### Honors

2019	Lavoisier Lectures (Université Paris Diderot)
2018	Elected member of the "European Academy of Sciences"
2016	26th IOCF Yoshida Lectureship
2014	Elected member of the Royal Flemish Academy of Belgium for Science and the Arts: Natural Sciences
2013	European Research Council (ERC) Advanced Grant (NANOGRAPH@LSI)

#### **Publications**

- ] Observing Polymerization in Two-Dimensional Dynamic Covalent Polymers. G. Zhan, Z.-F. Cai, K. Strutyński, L. Yu, N. Herrmann, M. Martínez-Abadía, M. Melle-Franco, A. Mateo-Alonso, S. De Feyter. Nature, 2022, 603, 835–840.
- 2. Detection and Stabilization of a Previously Unknown 2D (Pseudo)polymorph using Lateral Nanoconfinement. A. M. Bragança, A. Minoia, R. Steeno, J. Seibel, B. E. Hirsch, L. Verstraete, O. Ivasenko, K. Müllen, K. S. Mali, R. Lazzaroni, S. De Feyter, J. Am. Chem. Soc., 2021, 143, 11080-11087
- 3. Multicomponent Covalent Chemical Patterning of Graphene M. C. Rodríguez González, A. Leonhardt, H. Stadler, S. Eyley, W. Thielemans, S. De Gendt, K. S. Mali, S. De Feyter. ACS Nano, 2021, 15, 6, 10618-10627

6. G. Zhan, Z.-F. Cai, M. Martínez-Abadía, A. Mateo-Alonso, S. De Feyter, J. Am. Chem. Soc. 13, 5964 (2020)



## Satoshi Arai



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

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#### Research Interests

Chemical Biology, Bioimaging, Cell Engineering, Fluorescence Lifetime Microscopy

#### Education

2007

Doctor of Engineering, Polymer Chemistry, Waseda University

#### Professional Experience

2021 - present	FOREST Researcher (JST)
2019 - present	Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japar
2016 - 2019	Assistant Professor, Res. Inst. for Sci. and Eng., Waseda University, Singapore
2015 - 2019	PRIME Researcher (AMED)
2012 - 2016	Senior Research Fellow, WASEDA Bioscience Research Institute in Singapore, Singapore
2011 - 2012	Research Fellow, National University of Singapore , Singapore
2009 - 2010	Postdoctoral Fellow, Cooperative Bio Sci. Res. Inst., Waseda University, Japan
2007 - 2009	Research Associate, Dept. of Life Sci. and Med. Biosci., Waseda University, Japan

#### Honors

2014	The Award for Encouragement of Research in IUMRS-ICA2014
2014	Tanaka Kikinzoku, MMS Award
2013	The Molecular Biology Society of Japan, Travel Award

#### **Publications**

- 1. Modulation of Local Cellular Activities using a Photothermal Dye-Based Subcellular-Sized Heat Spot, Ferdinandus, M. Suzuki, C. Q. Vu, Y. Harada, S. R. Sarker, S. Ishiwata, T. Kitaguchi, S. Arai\*, ACS Nano, 16, 6, 9004-9018 (2022)
- 2 A palette of site-specific organelle fluorescent thermometers, X. Liu, T. Yamazaki (equal first), H.-Y. Kwon, S. Arai\*, Y.-T. Chang\*, Materials Today Bio, 16, 100405 (2022)
- 3. RGB-Color Intensiometric Indicators to Visualize Spatiotemporal Dynamics of ATP in Single Cells, S. Arai, R. Kriszt, K. Harada, L-S. Looi, S. Matsuda, D. Wongso, S. Suo, S. Ishiura, Y-H. Tseng, M. Raghunath, T. Ito, T. Tsuboi, T. Kitaguchi, Angew. Chem. Int. Ed., 57, 10873-10878 (2018).

## Subcellular Thermometry and Nanoheating Using Functional Dyes

Satoshi Arai

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

Temperature at nano- and microscale is a significant physical element to drive cellular system. To elucidate the system thermodynamically, the development of technology to measure and regulate subcellular temperature has been indispensable, named thermal cell engineering. Previously, using temperature-sensitive fluorescent dyes, we reported organelle targetable fluorescent thermometers that enable the read-out of temperature at organelles as detectable fluorescent signals such as intensity and lifetime. Yet, targeted organelles were only limited to mitochondria and ER as well-known heat sources in thermogenic cells. Very recently, we extended this to several organelles including lipid droplets, golgi, plasma membrane, nucleus, and lysosome which have never been reached by the other sensing methods. Using fluorescent lifetime imaging (FLIM), we further demonstrated these thermometers in quantitative thermometry of heat production in brown adipocytes.

Other than that, we have been developing local heating method at subcellular level. A key thing is a functional photothermal dye which absorbs near-infrared (NIR) light and converts to heat efficiently. Arrangement of the dyes to the target place, followed by NIR laser stimulation, allows to create a tiny heat spot at single cellular level. In this talk, we will share recent progress on thermal engineering in live cell and their cellular applications.

#### References

- 1. Modulation of Local Cellular Activities using a Photothermal Dye-Based Subcellular-Sized Heat Spot, Ferdinandus, M. Suzuki, C. Q. Vu, Y. Harada, S. R. Sarker, S. Ishiwata, T. Kitaguchi, S. Arai\*, ACS Nano, 16, 6, 9004-9018 (2022)
- 2. A palette of site-specific organelle fluorescent thermometers, X. Liu, T. Yamazaki (equal contribution), H.-Y. Kwon, S. Arai\*, Y.-T. Chang<sup>\*</sup>, Materials Today Bio, 16, 100405 (2022)
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3. RGB-Color Intensiometric Indicators to Visualize Spatiotemporal Dynamics of ATP in Single Cells, S. Arai, R. Kriszt, K. Harada,

4. Gold Nanoshell-Mediated Remote Myotube Activation, A. Marino, S. Arai, Y. Hou, A. Degl'Innocenti, V. Cappello, B. Mazzolai, Y-T. Chang, V. Mattoli, M. Suzuki, G. Ciofani, ACS Nano 11, 2494–2508 (2017). Highlighted at Nature Nanotechnology 12.



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#### Research Interests

Self-assembled molecular materials, Supramolecular polymers

### Education

2002

Doctor of Science, Graduate School of Science and Technology, Ritsumeikan University, Japan

#### Professional Experience

2022 - present	Professor, Institute for Advanced Academic Research (IAAR), Chiba University, Japan
2021 - present	Visiting Professor, The University of Tokyo, Japan
2017 - 2022	Professor, Institute for Global Prominent Research (IGPR), Chiba University, Japan
2010 - 2017	Associate Professor, Graduate School of Engineering, Chiba University, Japan
2006 - 2010	PRESTO Researcher, Japan Science and Technology Agency, Japan
2002 - 2006	Assistant Professor, Faculty of Engineering, Chiba University, Japan

#### Honors

2021	Award for Creative Work for 2021, The Chemical Society of Japan
2020	The 17th JSPS Prize, Japan Society for the Promotion of Science

#### **Publications**

- 1. Takahashi, S.; Yagai, S., Harmonizing Topological Features of Self-Assembled Fibers by Rosette-Mediated Random Supramolecular Copolymerization and Self-Sorting of Monomers by Photo-Cross-Linking, J. Am. Chem. Soc., 2022, 144(29), 13374-13383.
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- 4. Datta, S.; Kato, Y.; Higashiharaguchi, S.; Aratsu, K.; Isobe, A.; Saito, T.; Prabhu, D. D.; Kitamoto, Y.; Hollamby, M. J.; Smith, A. J.; Dalgliesh, R.; Mahmoudi, N.; Pesce, L.; Perego, C.; Pavan, G. M.; Yagai, S., Self-assembled poly-catenanes from supramolecular toroidal building blocks. Nature, 2020, 583, 400-405.
- 5. Kitamoto, Y.; Pan, Z.; Prabhu, D. D.; Isobe, A.; Ohba, T.; Shimizu, N.; Takagi, H., Haruki, R.; Adachi, S.; Yagai, S., One-shot preparation of topologically chimeric nanofibers via a gradient supramolecular copolymerization. Nature Commun., 2019, 10, Article number: 4578.

## Synthetic Supramolecular Polymers with Diverse Topological Features

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One-dimensional molecular aggregates, known as supramolecular polymers, are emerging next-generation polymeric materials owing to their reversible bonding. However, to compete over covalent counterparts, development of supramolecular polymers with well-defined higher-order structures (topologies) are desired. We have addressed this issue based on our unexpected discovery a decade ago on the formation of nanorings from hydrogen-bonded macrocyclic aggregates (rosettes) of barbituric acid-functionalized  $\pi$ -conjugated molecules in alkane solvent (J. Am. Chem. Soc. 2009, 131, 5408; Angew. Chem. Int. Ed. 2012, 51, 9679). The shape persistency of the nanoring along with its uniformity in diameter (ca. 14 nm) allowed us to propose that the generation of "intrinsic curvature" upon the continuous stacking of rosettes. Based on this idea, we synthesized various π-conjugated compounds based on the above molecular design to create curved supramolecular polymers with well-defined topological features. With the self-assembly behaviors of these newly prepared compounds, which have been revealed particularly by direct visualization of their supramolecular polymer chains by AFM, we can now comprehensively illustrate new aspects of our curved supramolecular polymers including complex self-assembly pathways and energy landscapes, dynamic topological change, topological extension by copolymerization, and truly topological structures in molecular assembly.

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## Hitoshi Asakawa



## Molecular Pockets in 2D Supramolecular Assemblies Investigated by 3D-AFM in Liquid

Hitoshi Asakawa

#### Nanomaterials Research Institute (NanoMaRi), Kanazawa University, Japan Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan

Two-dimensional (2D) supramolecular assemblies of various building blocks on surfaces have been studied for constructing molecular architectures. The following important tasks are to establish design methodologies that create functions of the supramolecular assemblies and analysis methods of the functions. We recently proposed building blocks with a three-dimensional shape and the potential to form nanosized "molecular pockets" in the 2D supramolecular assemblies. The molecular pockets are expected to have molecular recognition functions like macrocyclic molecules and are helpful for various applications such as sensing, separation, and chemical storage. The recognition function of molecular pockets fundamentally originates from intermolecular interactions in real space. Thus, the intermolecular interactions around pockets, as well as the molecular-scale structures, should be investigated to understand their recognition functions. However, the direct observation of molecular-scale intermolecular interactions is difficult even with any analytical methods. Three-dimensional AFM (3D-AFM) is a promising technique for analyzing the interactions around molecular pockets in real space. The 3D-AFM has been developed as a unique technique to visualize the distribution of intermolecular interactions with sub-nanometer (sub-nm) spatial resolution and piconewton (pN) force sensitivity. The 3D-AFM images obtained in this study showed that attractive forces are localized in the molecular pockets formed by the self-assembly of the 3D building blocks. The characteristic attractive forces are likely caused by a decrease in the hydrogen bond networks compared with bulk water. Our results demonstrate that the 3D-AFM technique can visualize the distribution of interaction forces related to molecular recognition and should contribute to the design of functional supramolecular assemblies.

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#### Research Interests

Atomic force microscopy, Molecular self-assembly, Intermolecular interactions, Molecular recognition, Interfacial science

### **Education**

2007

Docter of Engineering, Department of Biological Functions Engineering, Kyushu Institute of Technology, Japan

### **Professional Experience**

2018 - present	Group Leader/Associate Professor, Nanomaterials Research Institute (NanoMaRi), Kanazawa University, Japan
2017 - present	Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2016 - present	Associate Professor, Division of Material Chemistry, Kanazawa University, Japan
2010 - 2016	Assistant Professor, Bio-AFM Frontier Research Center, Kanazawa University, Japan
2008 - 2010	Postdoctoral Fellow, Frontier Science Organization, Kanazawa University, Japan

### Honors

2019	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology; The Young Scientists' Prize
2013	Nanoprobe Technology Young Scientist Award from No. 167 Committee of Japan Society for the Promotion of Science (JSPS)

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Chiral Monolayers with Achiral Tetrapod Molecules on Highly Oriented Pyrolytic Graphite. J. Phys. Chem. C 2020, 124,







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#### Research Interests

Physical biology, protein dynamics and evolution, active matter, information engines, quantum concepts in dissipative matter,

### Education

2000 - 2004 Fellow, Center for Physics and Biology, Rockefeller University, New York. Host: Prof. Albert Libchaber 2000 Ph.D. in Physics, Weizmann Institute. Advisor: Prof. Sam Safran

#### Professional Experience

2015 - present	Distinguished Professor, Department of Physics, UNIST, Ulsan
2015 - present	Group Leader, Center for Soft and Living Matter, Institute for Basic Science
2011 - 2015	Long-term Member, Institute of Advanced Study, Princeton.
2005 - 2013	Senior researcher, Physics of Complex Systems, Weizmann Institute.

#### **Publications**

- Piñeros, W.D. & Tlusty, T. (2022) Spontaneous Chiral Symmetry Breaking in a Random Driven Chemical System. Nature Comm. 13, 2244.
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## The Collective Dynamics of **Enzymatic Nano-Machines**

Tsvi Tlusty

Physics Department, Ulsan National Institute for Science and Technology (UNIST), Center for Soft and Living Matter, and Institute of Basic Science (IBS), Republic of Korea

The cellular milieu is teeming with biochemical nano-machines, in particular enzymes - catalysts that speed up the rate of metabolic reactions by orders of magnitude, thereby making life possible. In this talk, we will examine the collective dynamics of these enzymatic nano-machines at multiple spatiotemporal scales of physics and evolution.

We will first discuss the internal dynamics of enzymes. Proteins such as enzymes are a matter of dual nature. As a physical object, an enzyme molecule is a folded chain of amino acids with multifarious biochemistry, but it is also an instantiation along an evolutionary trajectory determined by the enzyme's function. We will describe a physical framework that links the evolution of the gene sequence to the emergence of large-scale dynamical modes in the enzyme, revealing basic features of the genotype-to-phenotype map.

Then, we will discuss how the internal dynamics of enzymes may lead to collective external interactions. In the classical view, catalysis is a localized chemical process where enzymes operate independently and feel the external environment only as thermal agitation that jolts the activated complex past the transition state' s energy barrier. The cell, however, is bustling with activity that generates significant athermal agitation, provoking the hypothesis we will examine that enzymatic catalysis is a collective, many-body process in which enzymes affect each other by inducing longrange hydrodynamic forces. To conclude, we will discuss potential ways to measure the proposed collective effects.

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## Chiho Watanabe



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#### Research Interests

Lipid membrane, Artificial cell, Polymer solution, Water, Cell-size space effect, Neurodegenerative disease

### Education

2015 PhD, Universeity Paris 7, Paris Diderot, France 2012 Master's degree in Engineering, Tokyo University of Agriculture and Technology, Japan

#### Professional Experience

2020 - present	Assistant Professor at Graduate School of Integrated Sciences for Life, Hiroshima University, Japan
2019 - 2020	Specially Appointed Assistant Professor at Komaba Institute for Sciences (KIS), the University of Tokyo, Japan
2016 - 2018	Postdoctoral Researcher at Tokyo University of Agriculture and Technology, Japan

#### Honors

2021	17th Early Career Award in Biophysics, The Biophysical Society of Japan (BSJ)
2021	Young Oral Presentation Award, The 72nd Divisional Meeting of Division of Colloid and Surface Chemistry
2020	The Emerging Science Lecture Prize at the 17th Australia-Japan Colloids Symposium, Australasian Colloids and Interface Society

### **Publications**

- 1. Watanabe, C.; Furuki, T.; Kanakubo, Y.; Kanie, F.; Koyanagi, K.; Takeshita, J.; Yanagisawa, M. Cell-Sized Confinement Initiates Phase Separation of Polymer Blends and Promotes Fractionation upon Competitive Membrane Wetting. ACS Materials Lett. 2022, 1742-1748. https://doi.org/10.1021/acsmaterialslett.2c00404.
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- Watanabe, C.; Yanagisawa, M. Evaporation Patterns of Dextran-Poly(Ethylene Glycol) Droplets with Changes in Wettability and 3. Compatibility. Life 2022, 12 (3), 373. https://doi.org/10.3390/life12030373.
- Harusawa, K.; Watanabe, C.; Kobori, Y.; Tomita, K.; Kitamura, A.; Kinjo, M.; Yanagisawa, M. Membrane Surface Modulates Slow 4. Diffusion in Small Crowded Droplets. Langmuir 2021, 37 (1), 437–444. https://doi.org/10.1021/acs.langmuir.0c03086.
- Watanabe, C.; Kobori, Y.; Yamamoto, J.; Kinjo, M.; Yanagisawa, M. Quantitative Analysis of Membrane Surface and Small 5. Confinement Effects on Molecular Diffusion. J. Phys. Chem. B 2020, 124 (6), 1090-1098. https://doi.org/10.1021/acs.jpcb.9b10558

## Cell-Size Space Effect on Molecular Diffusion and Liquid-Liquid Phase Separation

Chiho Watanabe

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Cells typically have a size of a few to several tens of micrometers and contain a solution with large amounts of biopolymers covered with a cell membrane (lipid membrane). There is interest in how cell-size spatial confinement affects the internal solution behavior that often differs from those in bulk systems. We attempted to decipher the cell-size space effect (CSE) by using polymeric droplets (w/o emulsions) as artificial cells, which can realize macromolecular crowding and cell-size confinement by lipid membranes, which are the characteristics of real cells. Specifically, we studied cell-size confinement effects on 1) molecular diffusion, which is the basis of solution properties, and 2) liquid-liquid phase separation (LLPS), which has attracted attention in recent years from experiments. Molecular diffusion was measured by fluorescence correlation spectroscopy (FCS), and it was found that the diffusion coefficient is slower in cell-size droplets than in larger ones. In addition, we found that phase separation is induced through cell-sized confinement of polymer mixtures, which are in a one-phase state in larger droplets or bulk solutions of microliters or larger. Furthermore, it was indicated that the membrane interfacial properties are important for both phenomena. We would like to introduce these physico-chemical studies on the cell-size space effect.

- 1. https://doi.org/10.1021/acs.langmuir.2c01397 (planned to be published before the symposium)
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#### Research Interests

Biophysics of cells and development, Cell and tissue chirality, Collective cell migration

#### Education

Ph.D. Graduate School of Arts and Sciences, The University of Tokyo, Japan 1999 1994 B.S. Faculty of Science, Kyoto University, Japan

#### Professional Experience

2018 - present	Team Leader, Laboratory for Physical Biology, RIKEN Center for Biosystems Dynamics Research, Japan
2015 - 2018	Team Leader, Laboratory for Physical Biology, RIKEN Quantitative Biology Center, Japan
2010 - 2014	Unit Leader, Laboratory for Physical Biology, RIKEN Center for Developmental Biology, Japan
2005 - 2010	Associate Professor, Department of Mathematical and Life Sciences, Hiroshima University, Japan
2002 - 2005	Lecturer, Department of Mathematical and Life Sciences, Hiroshima University, Japan
2001 - 2003	Research Scientist and Humboldt Research Fellowship, Fritz Haber Institute of Max Planck Society, Germany
1999 - 2001	Postdoctoral Research Fellow, Research Institute for Mathematical Sciences, Kyoto University, Japan

#### Honors

The Young Scientists' Prize, the Commendation for Science and Technology, the MEXT 2010

#### **Publications**

- 1 Sato, K., Hiraiwa, T. & Shibata, T. Cell Chirality Induces Collective Cell Migration in Epithelial Sheets. Physical Review Letters 115,188102 (2015).
- 2. Nishikawa, M., Hörning, M., Ueda, M. & Shibata, T. Excitable Signal Transduction Induces Both Spontaneous and Directional Cell Asymmetries in the Phosphatidylinositol Lipid Signaling System for Eukaryotic Chemotaxis. Biophys J 106, 723–734 (2014).
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- 4. Shibata, T. & Fujimoto, K. Noisy signal amplification in ultrasensitive signal transduction. PNAS 102, 331–336 (2005)
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## Emergence of Cell Chirality from the Spatial Organization of Actin and Myosin Cytoskeleton

Tatsuo Shibata

#### Laboratory for Physical Biology, RIKEN Center for Biosystems Dynamics Research, Japan

The chirality or left-right asymmetry of the morphogenesis and arrangement of organ is essential for their function and development. The chirality of organ and tissue is derived from the chirality of the cells that compose of them, and the cellular chirality emerges by organizing the molecular chirality within the cell. However, the principle of how molecular chirality is organized to lead to cellular chirality is still unclear. To address this question, we experimentally study the dynamical chiral behaviors of single epithelial cells and provide a theoretical understanding of how the chiral behaviors at the single-cell level arise from the molecular-level chirality. We first found that the nucleus rotates, and the cytoplasm circulates in the clockwise direction, which is driven by the actin and myosin cytoskeleton. During the rotation, the actin and myosin cytoskeleton organizes in a vortex-like chiral orientational order, which apparently drives the rotation. However, when the formation of stress fibers was repressed by drug treatment, the vortex-like chiral order disappeared and concentric achiral order emerged while the cells still rotate. Hence, the cell can rotate without any macroscopic chiral order of the cytoskeleton. To elucidate the mechanism of these chiral rotation with and without macroscopic chiral orientational order of the cytoskeleton, we analyzed a hydrodynamic model considering the effect of chirality of cytoskeleton. Our experiment and theory suggest that the cell chirality emerges as a collective behavior of chiral molecules even without any macroscopic chiral orientational order of the cytoskeleton.

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4. Hiraiwa, T., Kuranaga, E. & Shibata, T. Wave Propagation of Junctional Remodeling in Collective Cell Movement of Epithelial



## Ralf Metzler



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#### Research Interests

Stochastic processes, nonequilibrium statistical physics, search processes, molecular reactions, soft and biological mat- ter, data science, machine learning, Bayesian analysis

### Education

1996

Doctor of Natural Science, Physics Dept, University of Ulm, Germany

#### **Professional Experience**

2011 - present	Chair Professor for Theoretical Physics, University of Potsdam, Germany
2007 - 2011	Professor ("Extraordinarius") for Complex Bio Materials, Technical University of Munich (TUM), German
2006 - 2007	Associate Professor and Canada Research Chair in Biological Physics, University of Ottawa, Canado
2002 - 2006	Assistant Professor, NORDITA-Nordic Institute for Theoretical Physics, Copenhagen, Denmark

#### Honors

2023	Distinguished Visiting Professor, Department of Chemistry, Indian Institute of Technology Bombay, Mumbai, India
2023	Heilbronn distinguished visiting fellowship (Mathematics of Movement programme at Isaac Newton Institue), Heilbronn Institute for Mathematical Research
2022	APCTP Distinguished Fellow (Senior Advisory Group Scientist), Asia Pacific Center for Theoretical Physics, Pohang, Korea
2020	Higgs Associate, Higgs Centre for Theoretical Physics, University of Edinburgh, Scotland
2017	SigmaPhi Prize 2017 for outstanding achievements in Statistical Physics
2010	Finland Distinguished Professor (FiDiPro) at Tampere University of Technology, Academy of Finland
2006	Canada Research Chair in Biological Physics, Government of Canada
2000	Emmy Noether fellowship, German Science Foundation
1998	Amos de Shalit named fellowship, Minerva German-Israeli binational Foundation
1998	Feodor Lynen fellowship, Alexander von Humboldt Foundation

## Beyond Brownian Motion: From Data to Models

Ralf Metzler

Institute of Physics & Astronomy, University of Potsdam, Germany

After a brief historical introduction to Brownian motion I will address recent experimental and simulations results on diffusive transport in complex systems, ranging from dynamics in single molecules, motion of molecules and tracers in living biological cells, to ants and birds. The main characteristic of these system is the occurrence of non-Gaussian displacement distributions and/or anomalous diffusion of the form  $< r^2(t) > - t^{\alpha}$  with  $\alpha \neq 1$ . Due to the non-universality of such kind of stochastic dynamics, it is important to know the precise underlying physical process [1]. I will discuss several examples. Data-science methods help us in identifying such physical processes encoded in experimental records and to extract physical parameters. I will provide an overview over different approaches and their performance in the context of diffusive motion [2,3].

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- 3. G. Muñoz-Gil, G. Volpe, M. A. Garcia-March, E. Aghion, A. Argun, C. B. Hong, T. Bland, S. Bo, J. A. Conejero, N. Firbas, Ò. Garibo i Orts, A. Gentili, Z. Huang, J.-H. Jeon, H. Kabbech, Y. Kim, P. Kowalek, D. Krapf, H. Loch-Olszewska, M. A. Lomholt, J.-B. Masson, P. G. Meyer, S. Park, B. Requena, I. Smal, T. Song, J. Szwabiński, S. Thapa, H. Verdier, G. Volpe, A. Widera, M. Lewenstein, R. Metzler, and C. Manzo, Nature Comm. 12, 6253 (2021).

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- G. Muñoz-Gil et al., Objective comparison of methods to decode anomalous diffusion, Nature Comm. 12, 6253 (2021)
- 2 E. Yamamoto, T. Akimoto, A. Mitsutake, and R. Metzler, Universal relation between instantaneous diffusivity and radius of gyration of proteins in aqueous solution, Phys. Rev. Lett. 126, 128101 (2021)
- 3. D. Krapf et al, Spectral Content of a Single Non-Brownian Trajectory, Phys. Rev. X 9, 011019 (2019)
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- 5. A. Godec and R. Metzler, Universal proximity effect in target search kinetics in the few encounter limit, Phys. Rev. X 6, 041037 (2016)
- 6. J.-H. Jeon, M. Javanainen, H. Martinez-Seara, R. Metzler, and I. Vattulainen, Protein crowding in lipid bilayers gives rise to non-Gaussian anomalous lateral diffusion of phospholipids and proteins, Phys. Rev. X 6, 021006 (2016)
- 7. J. Shin, A. G. Cherstvy, and R. Metzler, Sensing viruses by mechanical tension of DNA in responsive hydrogels, Phys. Rev. X 4,021002 (2014)
- 8. J. H. P. Schulz, E. Barkai, and R. Metzler, Aging renewal theory and application to random walks, Phys. Rev. X 4, 011028 (2014)

Continue to next page



## Robert D Goldman

Professor of Cell and Developmental Biology The Stephen Walter Ranson Professor Emeritus Feinberg School of Medicine, Northwestern University, USA

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#### Research Interests

Cell Biology: Cytoskeleton, Intermediate Filaments, Nuclear Lamins and Nuclear Architecture

#### Education

1967

Ph.D. in Biology, Princeton University, USA

#### Professional Experience

2021 - present	Professor of Cell and Developmental Biology, Stephen Walter Ranson Professor Emeritus, Feinberg School of Medicine, Northwestern University, USA
1981 - 2021	The Stephen Walter Ranson Professor and Chairman Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, USA
1981 - 1991	Program Leader, Cell Biology, Northwestern University Cancer Center, USA
1977 - 1981	Professor of Biological Sciences, Carnegie-Mellon University, USA
1973 - 1974	Associate Professor of Biological Sciences, Carnegie-Mellon University, USA
1969 - 1973	Assistant Professor of Biology, Case Western Reserve University, USA
1967 - 1969	American Cancer Society, Eleanor Roosevelt Postdoctoral Fellow at the Medical Research Council Institute of Virology, Glasgow, Scotland
1967 - 1968	American Cancer Society, Eleanor Roosevelt Postdoctoral Fellow at the Royal Postgraduate Medical School, London, Enaland

#### **Publications**

- 1. Nuclear lamin isoforms differentially contribute to LINC complex-dependent nucleocytoskeletal coupling and whole-cell mechanics. Vahabikashi, A.; Sivagurunathan, S.; Nicdao, FAS.; Han, YL.; Park, CY.; Kittisopikul, M, Wong, X.; Tran, JR.; Gundersen, GG.; Reddy, KL.; Luxton, GWG.; Guo, M.; Fredberg, JJ.; Zheng, Y.; Adam, SA.; Goldman, RD. Proc Natl Acad Sci U S A. 2022 Apr 26;119 (17):e2121816119.
- Computational analyses reveal spatial relationships between nuclear pore complexes and specific lamins. Kittisopikul, M.; Shimi, T.; 2 Tatli, M.; Tran, JR.; Zheng, Y.; Medalia, O.; Jagaman, K.; Adam, SA.; Goldman, RD. J Cell Biol. 2021 Apr 5;220(4):e202007082.
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## Intermediate Filament Networks Connect the Nucleus to the Cell Cortex

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Recent insights into the interactions of the nuclear and cytoplasmic Intermediate Filament (IF) systems demonstrate their functions in integrating the global response of mesenchymal cells to mechanical stress. Within the nucleus, each of the Type V IF proteins, lamins A, C, B1, and B2 assembles into a distinct meshwork. These meshworks are interspersed with nuclear pore complexes to form the nuclear lamina (NL). The lamin complex connects the inner nuclear membrane to chromatin. Evidence will be presented showing that the lamin meshworks are also connected to a juxtanuclear cage of Type III vimentin IFs (VIFs) located at the outer membrane of the nuclear envelope and that this cage connects to an extensive cytoplasmic network of VIFs associated with the cell surface. Our data show that the lamin meshworks are connected to the juxtanuclear cage of VIFs by specific components of the linker of nucleoskeleton and cytoskeleton (LINC) complex containing proteins spanning the inner and outer nuclear membranes. In the cortical region, VIFs are found deep within bundles of F-actin (stress fibers), thereby forming interpenetrating networks (IPNs). Using super-resolution microscopic techniques combined with live cell imaging, cryo-EMT, fluorescence recovery after photobleaching, atomic force microscopy, optical tweezer microrheology, traction force microscopy and particle tracking microrheology, we demonstrate that VIFs function in regulating the stiffness of the cell surface, the cytoplasm, overall cell contractility, adhesion and motility. These results are supported by analyses of reconstituted cytoskeletal networks prepared from purified proteins. Overall, these results alter the commonly held view that the contractile and adhesive properties of mammalian cells are solely regulated by F-actin and its associated proteins. This work involves collaborations with the Laboratories of David Weitz at Harvard University, Ming Guo at MIT, Paul Janmey at the University of Pennsylvania and Ohad Medalia at the University of Zurich. The work is supported by the NIH.

- 1. Computational analyses reveal spatial relationships between nuclear pore complexes and specific lamins. Kittisopikul, M.; Shimi, T.; Tatli, M.; Tran, JR.; Zheng, Y.; Medalia, O.; Jaqaman, K.; Adam, SA.; Goldman, RD. J Cell Biol. 2021 Apr 5;220 (4):e202007082.
- 2. Nuclear lamin isoforms differentially contribute to LINC complex-dependent nucleocytoskeletal coupling and whole-cell mechanics. Vahabikashi, A.; Sivagurunathan, S.; Nicdao, FAS.; Han, YL.; Park, CY.; Kittisopikul, M, Wong, X.; Tran, JR.; Gundersen, GG.; Reddy, KL.; Luxton, GWG.; Guo, M.; Fredberg, JJ.; Zheng, Y.; Adam, SA.; Goldman, RD. Proc Natl Acad Sci U S A. 2022 Apr 26;119(17):e2121816119.



## Ohad Medalia



#### Professor of Biochemistry Department of Biochemistry, University of Zurich, Switzerland

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### Research Interests

The structure and mechanical properties of the nuclear envelope, Nuclear lamins, Structural analysis of intermediate filaments, Cryo-electron tomography

### Education

2001	Ph. D. in Chemistry, Department of Organic Chemistry. The Weizmann Institute of Science, Israel
1994	B. Sc. In Chemistry. Faculty of Chemistry, Tel-Aviv University, Israel

### Professional Experience

2017 - present	Professor, Department of Biochemistry, University of Zurich, Switzerland
2011 - 2017	Associated Professor, Department of Biochemistry, University of Zurich, Switzerland
2011 - 2015	Adjunct Professor, Ben Gurion University, Israel
2005 - 2009	Visiting Scientists, Max-Planck Institute of Biochemistry, Germany
2005 - 2009	Senior Lecturer, Ben Gurion University, Israel

### Honors

2019	ERC Synergy Grant
2014	The Charles University, Medical Faculty Medal of Honor
2010	Toronto Prize
2009	The ARCHES Award
2009	ERC Starting Grant
2008	The FEI European Microscopy Award
2004	The Alon Fellowship
2003	Max-Planck Institute for Biochemistry Junior Research Award
2001	The Elchanan E. Bondi Memorial Prize for Ph. D. students.
2001	The Rothschild Fellowship
2001	The European Commission Individual Fellowship
2000	The Lev Margulis Young Investigators Award of Merit
1999	The Wolf Foundation Fellowship- for Excellent Ph.D students.



Ohad Medalia

#### Department of Biochemistry, University of Zurich, Switzerland

Lamins are nuclear intermediate filaments (IFs) of metazoan cells. They assemble into fibrous structures that are positioned between the inner nuclear membrane and the peripheral chromatin, although a small fraction of lamins is present in the nucleoplasm. Lamins are required to maintain nuclear structure and, together with many interaction partners, are involved in most nuclear activities. Mutations in lamins cause a group of >14 distinct diseases called laminopathies, it is not clear how lamins are organized in vivo and how these mutations affect lamin functions. Understanding how lamins are assembled, and how mutations in lamins and lamin binding proteins affect lamin filament assembly and cellular localization is essential for understanding the their modus operandi. Moreover, lamins are intimately interacting with chromatin. Using variety of microscopy modalities, such as protein design, atomic force microscopy, molecular dynamics (MD) simulations, quotative fluorescence and cryo-electron microscopy, we provided unprecedented understanding of the remarkable structure and mechanical properties of nuclear Lamins. The results of this study indicate the unique mechanical and functional properties of lamins.

#### **Publications**

- 1. Schuller, A., Wojtynek, M., Mankus D., Tatli M., Kronenberg-Tenga R., Regmi S., Dasso M., Weis K., Medalia, O.\*, Schwartz, TU\*. (2021) The cellular environment shapes the nuclear pore complex architecture. Nature, 598 (7882):667-671.
- 2. Turgay Y, Eibauer M, Goldman AE, Shimi T, Khayat M, Ben-Harush K, Dubrovsky-Gaupp A, Sapra KT, Goldman RD, Medalia O. (2017) The molecular architecture of lamins in somatic cells. Nature 543(7644):261-264.
- 3. Patla, I., Volberg, T., Elad, N., Hirschfeld-Warneken, V., Grasshof, C., Fässler, R., Spatz, J., Geiger, B., and Medalia, O., (2010) Dissecting the molecular architecture of integrin-mediated focal adhesion by cryo-eelectron tomography. Nat. Cell.Biol. 12(9):909-15
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- 5. Medalia, O., Weber, I., Frangakis, A.S., Nicastro, D. Gerisch, G., and Baumeister, W. (2002) Macromolecular Architecture in Eukaryotic Cells Visualized by Cryo-electron Tomography. Science. 298, 1209-1213.



## Takeshi Shimi



Specially Appointed Associate Professor/Dr. Institute of Innovative Research, Tokyo Institute of Technology, Japan

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#### Research Interests

Structure, dynamics and functions of the nuclear envelope, particularly lamina/lamins

#### **Education**

2005

Ph.D. in Biology, Osaka University Graduate School of Science, Japan

#### **I** Professional Experience

2018 - present	Specially Appointed Associate Professor, Institute of Innovative Research, Tokyo Institute of Technology, Japan
2016 - 2018	Research Assistant Professor, Northwestern University, USA
2015 - 2016	Staff Scientist, The University of Chicago, USA
2012 - 2015	Research Assistant Professor, Northwestern University, USA
2006 - 2012	Postdoctoral Research Fellow, Northwestern University, USA
2005 - 2006	Postdoctoral Research Fellow, Advanced ICT Research Institute, Japan

#### Publications

- Nucleoplasmic lamin C rapidly accumulates at sites of nuclear envelope rupture with BAF and cGAS. Kono, Y.; Adam, SA.; Reddy KL; 1. Zheng, Y.; Medalia O.; Goldman RD.; Kimura H.; Shimi, T., J Cell Biol. 2022 (In press)
- Computational analyses reveal spatial relationships between nuclear pore complexes and specific lamins. Kittisopikul, M\*.; Shimi, T\*.; 2. Tatli, M.; Tran, JR.; Zheng, Y.; Medalia, O.; Jagaman, K.; Adam, SA.; Goldman, RD., (\*egual contribution). J Cell Biol. 2021 Apr 5;220 (4):e202007082.
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## The Role of A-Type Lamins in Repair of Nuclear Envelope Ruptures

Takeshi Shimi

#### Institute of Innovative Research, Tokyo Institute of Technology, Japan

The nuclear lamina (NL) lines the inner nuclear membrane of the nuclear envelope (NE) to maintain nuclear structure in mammalian cells. The major NL component, the nuclear lamins contribute to the protection against NE rupture induced by mechanical stress. However, the specific role of the lamins in repair of NE ruptures has not been fully determined. Our analyses using immunofluorescence and live-cell imaging revealed that among all the lamin isoforms (LA, LB1, LB2, and LC), only nucleoplasmic LC rapidly accumulated at sites of NE rupture induced by laser microirradiation and single-cell compression in mouse embryonic fibroblasts. The recruitment of LC from the nucleoplasm to the rupture sites required the binding of the immunoglobulin-like fold domain to Barrier-toautointegration factor (BAF). BAF accumulation at the rupture sites and DNA sensing of cytoplasmic cyclic GMP-AMP synthase (cGAS) were partially dependent on LA/C. These results suggest that nucleoplasmic LC, BAF, and cGAS concertedly accumulate for rapid repair of NE ruptures.

#### References

1. Nucleoplasmic lamin C rapidly accumulates at sites of nuclear envelope rupture with BAF and cGAS. Kono, Y.; Adam, SA.; Reddy KL.; Zheng, Y.; Medalia O.; Goldman RD.; Kimura H.; Shimi, T., J Cell Biol. 2022 (In press)



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### Research Interests

Transcription, Translation, mRNA decay

### Education

2006

Ph.D. in Biochemistry University of Tokyo, Department of Biophysics and Biochemistry, Tokyo, Japan

### **I** Professional Experience

2022 - present	Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2022 - 2022	Research Assistant Professor, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY, USA
2021 - 2022	Research Assistant Professor, Department of Anatomy & Structural Biology, Albert Einstein College of Medicine, Bronx, NY, USA
2017 - 2021	Faculty Associate, Department of Anatomy & Structural Biology, Albert Einstein College of Medicine, Bronx, NY, USA
2016 - 2017	Faculty Associate, Department of Cell Biology/Stem Cell Institute and Anatomy & Structural Biology, Albert Einstein College of Medicine, Bronx, NY, USA

#### **Honors**

2019	Aegean Conference Travel Award, 3rd International Conference on the Long and the Short of Non-Coding RNAs, Crete, Greece
2019	Travel Award, RNA 2019 Meeting, Kraków, Poland
2009 - 2011	JSPS Postdoctoral Fellowship for Research Abroad, Japan Society for the Promotion of Science (JSPS), Japan

#### **Publications**

Li W\*, Maekiniemi A\*, Sato H, Osman C, Singer RH (2022) An improved imaging system that corrects MS2-induced RNA destabilization. Nature Methods in press. (\*contributed equally)

- 1. Sato H and Singer RH (2021) Cellular variability of nonsense-mediated mRNA decay. Nature communications 12:7203.
- 2 Sato H\*, Das S\*, Singer RH, Vera M. (2020) Imaging of DNA and RNA in Living Eukaryotic Cells to Reveal Spatiotemporal Dynamics of Gene Expression. Annual Review of Biochemistry 89:159-187 (\*contributed equally)
- 3 Sato H, Wu B, Delahaye F, Singer RH, Greally JM. (2019) Retargeting of macroH2A following mitosis to cytogenetic-scale heterochromatic domains. J Cell Biol. 218:1810-1823.

## Real-Time Imaging of Transcriptional Feedback in Nonsense-Mediated mRNA Decay

Hanae Sato

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Nonsense-mediated mRNA decay (NMD) is a translation-coupled mRNA decay pathway triggered by a premature termination codon (PTC). Although only cytoplasmic ribosomes define the in-frame stop codon, unexpected transcriptional changes at the PTC-containing gene have been reported previously. While this observation implies PTC detection at the transcription site prior to the cytoplasmic translation or crosstalk between cytoplasmic decay and transcription, it has never been addressed with high temporal and spatial resolution. Here we utilize a real-time imaging approach that allows simultaneous detection of transcription sites expressing either wild-type or NMD-targeted β-globin reporter genes in the same cell to capture the transcriptional dynamics at the PTC-containing gene allele. Our study provides a robust link between cytoplasmic decay and transcription in the nucleus.

#### References

- 1. Sato H and Singer RH (2021) Cellular variability of nonsense-mediated mRNA decay. Nature communications 12:7203.
- Dynamics of Gene Expression. Annual Review of Biochemistry 89:159-187 (\*contributed equally)

2. Sato H\*, Das S\*, Singer RH, Vera M. (2020) Imaging of DNA and RNA in Living Eukaryotic Cells to Reveal Spatiotemporal



## Atsushi Miyawaki

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#### Research Interests

Bioimaging

### Education

Ph.D. Osaka University School of Medicine, Japan, field of study: Signal Transduction 1991 1987 M.D. Keio University School of Medicine, Japan, field of study: Medicine

### Professional Experience

2022 - present	Visiting Professor, Institute for Life and Medical Sciences, Kyoto University, Japan
2013 - present	Team Leader, Biotechnological Optics Research Team, RIKEN Center for Advanced Photonics, Japan
2012 - present	Visiting Professor, Graduate School of Nanobioscience, Yokohama City University, Japan
2010 - 2011	Visiting Professor, Faculty of Science, Toho University, Japan
2010 - present	Director, RIKEN CBS-Olympus Collaboration Center, Japan (previously named RIKEN BSI-Olympus Collaboration Center)
2009 - present	Visiting Professor, Keio University School of Medicine, Japan
2008 - 2018	Deputy Director, RIKEN Brain Science Institute, Japan
2007 - 2010	Deputy Director, BSI Olympus Collaboration Center
2007 - present	Visiting Professor, Graduate School of Advanced Science and Engineering, Waseda University, Japan
2006 - 2011	Visiting Professor, National Institute for Basic Biology, The National Institute of Natural Sciences, Japan
2006	Visiting Professor, Tokyo University of Science, Japan
2006 - 2013	Research Director,ERATO MIYAWAKI Life Function Dynamics Project, Japan Science and Technology Agency, Japan
2005 - 2010	Visiting Professor, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan
2004 - 2009	Group Director, Advanced Technology Development Group, RIKEN Brain Science Institute, Japan
1999 - present	Laboratory Head, Lab for Cell Function Dynamics, RIKEN Center for Brain Science, Japan (previously named RIKEN Brain Science Institute)
1997 - 1998	Research Pharmacologist, The University of California, San Diego, USA
1995 - 1997	HFSP, Long-Term Fellowship, The University of California, San Diego, USA
1993 - 1998	Assistant Professor, The Institute of Medical Science, The University of Tokyo, Japan
1991 - 1993	Researcher, The Institute of Medical Science, The University of Tokyo, Japan

## Cruising in the Cell

Atsushi Miyawaki

#### **RIKEN** Center for Brain Science, Japan, **RIKEN** Center for Advanced Photonics, Japan

The behavior of biochemical molecules moving around in cells makes me think of a school of whales wandering in the ocean, captured by the Argus system on the artificial satellite. When bringing a whale back into the sea --- with a transmitter on its dorsal fin, every staff member hopes that it will return safely to a school of its species. A transmitter is now minute in size, but it was not this way before. There used to be some concern that a whale fitted with a transmitter could be given the cold shoulder and thus ostracized by other whales for "wearing something annoying." How is whale' s wandering related to the tide or a shoal of small fish? What kind of interaction is there among different species of whales? We human beings have attempted to fully understand this fellow creature in the sea both during and since the age of whale fishing. In a live cell imaging experiment, a luminescent probe replaces a transmitter. We label a luminescent probe on a specific region of a biological molecule and bring it back into a cell. We can then visualize how the biological molecule behaves in response to external stimulation. Since luminescence is a physical phenomenon, we can extract various kinds of information by making full use of its characteristics.

Cruising inside cells in a supermicro corps, gliding down in a microtubule like a roller coaster, pushing our ways through a jungle of chromatin while hoisting a flag of nuclear localization signal --we are reminded to retain a playful and adventurous perspective at all times. What matters is mobilizing all capabilities of science and giving full play to our imagination. We believe that such serendipitous findings can arise out of such a sportive mind, a frame of mind that prevails when enjoying whale-watching.

#### Honors

2021 Highly Cited Researchers 2021, Clarivate Analytics Recognition of ranking among the top 1% of researchers for most cited documents in Cross-Field, 2021 Japan Academy Prize, 2021 Keio Medical Science Prize (Keio University Medical Science Fund), 2020 Takeda Medicine Prize (Takeda Science Foundation), 2017 Uehara Prize, 2017 Medal with Purple Ribbon, 2015 Shimadzu Prize, 2015 The Thirty-Seventh Annual W. Alden Spencer Award, 2014 Arthur Kornberg Memorial Award, 2013 Fujihara Award, 2012 Inoue Prize for Science, 2008 The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, 2007 Keio University Sanshikai Kitazato Award, 2007 Tsukahara Nakaakira Award, 2006 Japan Society for the Promotion of Science Prize, 2006 Harvard University, Department of Chemistry and Chemical Biology, Woodward Visiting Scholar, 2004 The 4th Yamazaki-Teiichi Prize Winner Biological Science & Technology.

### Publications

- 1 Masahiko Hirano, Ryoko Ando, Satoshi Shimozono, Mayu Sugiyama, Noriyo Takeda, Hiroshi Kurokawa, Ryusaku Deguchi, protein" Nat Biotechnol.2022 Apr 25. doi: 10.1038/s41587-022-01278-2.
- Michikawa T, Yoshida T, Kuroki S, Ishikawa T, Kakei S, Kimizuka R, Saito A, Yokota H, Shimizu A, Itohara S, Miyawaki 2. (6):109966. doi: 10.1016/j.celrep.2021.109966.
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Continue to next page

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A." Distributed sensory coding by cerebellar complex spikes in units of cortical segments." Cell Rep., 2021 Nov 9;37

KatayamaH., HamaH., NagasawaK., KurokawaH., SugiyamaM., AndoR., FunataM., YoshidaN., HommaM., NishimuraT., TakahashiM., IshidaY., HiokiH., TsujihataY., Miyawaki A.(2020)Visualizing and modulating mitophagy for therapeutic studies of

AndoR., Sakaue-Sawano A., Shoda K., Miyawaki A. (2020) Two new coral fluorescent proteins of distinct colors for sharp



## Mikihiro Shibata



#### Professor

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#### Research Interests

Life Sciences, Neurobiology, High-Speed Atomic Force Microscopy, Membrane Proteins

### Education

2007

Doctor of Engineering, Department of Materials Science and Engineering, Nagoya Institute of Technology

#### Professional Experience

2021 - present	Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2017 - 2021	Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2016 - 2017	Associate Professor, Institute for Frontier Science Initiative, Kanazawa University, Japan
2015 - 2016	Postdoctoral Fellow, Department of Physics, Kanazawa University, Japan
2013 - 2015	Postdoctoral Research Fellow, Max Planck Florida Institute for Neuroscience, USA
2011 - 2013	JSPS Postdoctoral Fellow for Research Abroad, Duke University Medical School, USA
2008 - 2011	Research Fellow of JSPS (SPD), Kanazawa University, Japan

#### Honors

The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology; 2018 The Young Scientists' Prize Young Researchers' Prize of the Biophysical Society of Japan 2009

#### **Publications**

- 1. L. Puppulin\*, D. Kanayama, N. Terasaka, K. Sakai, N. Kodera, K. Umeda, A. Sumino, A. Marchesi, W. Weilin, H. Tanaka, T. Fukuma, H. Suga, K. Matsumoto, & M. Shibata\*. Macrocyclic peptide-conjugated tip for fast and selective molecular recognition imaging by high-speed atomic force microscopy. ACS Applied Materials & Interfaces, 13, 46, 54817-54829 (2021).
- 2. M. Shibata, K. Inoue, K. Ikeda, M. Konno, M. Singh, C. Kataoka, R. Abe-Yoshizumi, H. Kandori, & T. Uchihashi. Oligomeric states of microbial rhodopsins determined by high-speed atomic force microscopy and circular dichroic spectroscopy. Sci. Rep. 8(1) 8262 (11 pages) (2018).
- 3. M. Shibata\*, H. Nishimasu\*, N. Kodera, S. Hirano, T. Ando, T. Uchihashi, & O. Nureki\*. Real-space and real-time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy. Nat. Commun. 8(1) 1430 (9 pages) (2017).

## **Correlation Between Function and Mobility** of Protein Complex Revealed by HS-AFM

Mikihiro Shibata

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

Structural biology has long contributed to our understanding of how proteins work by providing detailed structures. However, the reported structures have been restricted to static information. High-speed atomic force microscopy (HS-AFM) allows direct visualization of individual proteins in action at sub-nanometer resolution under near physiological conditions. Our HS-AFM studies performed in past few years have provided new mechanistic insight into the functional mechanism of proteins. For example, HS-AFM movies of bacteriorhodopsin (bR), functions as a light-driven proton pump, clearly showed that a cytoplasmic portion of bR displaced toward adjacent bR molecules at ~ 0.7 nm by light [1]. In addition, oligomeric structures of various microbial rhodopsins were directly visualized and the stoichiometry of membrane proteins were determined in a lipid environment [2-4]. HS-AFM movies of CRISPR-Cas9, which has been widely used for genome editing tools, visualized the real-space and real-time DNA cleavage by Cas9 [5]. Moreover, HS-AFM movies of hepatocyte growth factor (HGF) with a macrocyclic peptide HiP-8 showed that binding of HiP-8 to HGF restricted the flexibility of domains of HGF into static closed conformations, resulting in allosteric inhibition [6]. Thus, HS-AFM is a powerful approach to directly visualize protein dynamics in liquid environment. In this symposium, I would like to introduce our recent HS-AFM results of calcium/calmodulin-dependent protein kinase II.

- 1. M. Shibata, H. Yamashita, T. Uchihashi, H. Kandori, and T. Ando. High-speed Atomic Force Microscopy Shows Dynamic Molecular Processes in Photoactivated Bacteriorhodopsin. Nat. Nanotechnol., 5, 208-212 (2010).
- 2. M. Shibata+, K. Inoue+, K. Ikeda, M. Konno, M. Singh, C. Kataoka, R. Abe-Yoshizumi, H. Kandori, and T. Uchihashi. Oligomeric states of microbial rhodopsins determined by high-speed atomic force microscopy and circular dichroic spectroscopy. Sci. Rep., 8, 8262 (2018). (+equal contribution).
- 3. W. Shihoya, K. Inoue, M. Singh, M. Konno, S. Hososhima, K. Yamashita, K. Ikeda, A. Higuchi, T. Izume, S. Okazaki, M. Hashimoto, R. Mizutori, S. Tomida, Y. Yamauchi, R. Abe-Yoshizumi, K. Katayama, S.P. Tsunoda, M. Shibata, Y. Furutani, A. Pushkarev, O. Béjà, T. Uchihashi, H. Kandori, and O. Nureki. Crystal structure of Heliorhodopsin. Nature, 574, 132-136 (2019).
- 4. K.E. Kishi, Y.S. Kim, M. Fukuda, M. Inoue, T. Kusakizako, P.Y. Wang, C. Ramakrishnan, E. F.X. Byrne, E. Thadhani, J. M. Paggi, T. E. Matsui, K. Yamashita, T. Nagata, M. Konno, S. Quirin, M. Lo, T. Benster, T. Uemura, K. Liu, M. Shibata, N. Nomura, S. Iwata, O. Nureki, R.O. Dror, K. Inoue, K. Deisseroth, and H. E. Kato. Structural basis for channel conduction in the pump-like channelrhodopsin ChRmine. Cell, 185, 4, 672-689 (2022).
- 5. M. Shibata\*+, H. Nishimasu\*+, N. Kodera, S. Hirano, T. Ando, T. Uchihashi, and O. Nureki\*. Real-space and real-time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy. Nat. Commun., 8, 1430 (2017). (+equal contribution, \*Co-corresponding author)
- 6. K. Sakai, T. Passioura, H. Sato, K. Ito, H. Furuhashi, M. Umitsu, J. Takagi, Y. Kato, H. Mukai, S. Warashina, M. Zouda, Y. Watanabe, S. Yano, M. Shibata, H. Suga, and K. Matsumoto. Macrocyclic peptide-based inhibition and imaging of hepatocyte growth factor. Nat. Chem. Biol., 15, 598-606 (2019).



## Yasufumi Takahashi



## **Development of Organelle Collection Technology** Using Nanopipette

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Super-resolution microscopy visualized nanoscale dynamics of proteins within the live cell. However, it is difficult to distinguish more than seven fluoresecent proteins due to color restrictions, and a comprehensive evaluation technique for proteins and lipids is required. Scanning ion conductance microscopy (SICM) uses a nanopipette for detecting ion current and is an effective tool for live cell topography imaging. The topography information of SICM is also effective to navigate the nanopipette on the sample surface for detecting the chemical and ejecting/collecting the chemical at a local region on a cell with nanoscale accuracy. Previously, we fabricated nanopipette-based electrochemical and nanopore sensors to achieve oxygen, catecholamine, H<sub>2</sub>O<sub>2</sub>, and pH imaging.<sup>1,3,4,5</sup> However, it is difficult to detect proteins and lipids using electrochemical sensors. The local cytosol collection is also an important application for SICM to reveal the localization of mRNAs at a single-cell level. Since the fine structure of the nanopipette is difficult to control with conventional pressure, we have established a cytosol collection technique that utilizes changes in interfacial tension by potential.<sup>2</sup> This system successfully detected local differences in Actb mRNA expression levels within a single mouse fibroblast cell. However, proteins and lipids analysis remains challenging due to collection volume issues. Machine learning and artificial intelligence have made it possible to perform general human visual recognition and comprehensive judgment and have produced significant results in the structural analysis of membrane proteins. We have developed a machine learning-based single cell recognition and collection system. This auto-collection system not only improves throughput but also has the potential to discover proteins that are expressed in a position-specific manner and molecules related to protein-protein interactions by using AI technology to perform identification equivalent to humans. The collected cytosol will be analyzed by NanoESI.

#### References

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- Zhang, Y. et al. Acs Nano 2016, 10 (3), 3214-21.
- Takahashi, Y.; et al. Proc. Natl. Acad. Sci. U. S. A. 2012, 109 (29), 11540-11545.
- Takahashi, Y.; et al. Angew. Chem. Int. Ed. 2011, 50 (41), 9638-9642.

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#### Research Interests

Scanning ion conductance microscopy, Single cell analysis, Live cell imaging

### Education

2009

Doctor of Philosophy, Graduate School of Environmental Studies, Tohoku University

### **I** Professional Experience

2020 - present	Professor, Department of Electronics, Graduate School of Engineering,Nagoya University, Japan
2020 - present	Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2017 - 2020	Associate Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan
2015 - 2017	Associate Professor, Division of Electrical Engineering and Computer Science, Kanazawa University, Japan
2011 - 2013	Assistance Professor, Advanced Institute for Materials Research (WPI-AIMR), Tohoku University, Japan
2010 - 2011	JSPS Postdoctral Fellowship for Research Abroad, Division of Medicine, Imperial College London, UK

#### Honors

2022	Horiba Masao Award
2020	Nakatani Foundation Incentive Award
2016	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology; The Young Scientists' Prize

#### **Publications**

- Zhang, Y.; Takahashi, Y. et al., Nat. Commun., 2019, 10, 5610. 1.
- Nashimoto, Y.; Takahashi, Y. et al. Acs Nano 2016, 10 (7), 6915-22. 2.
- Zhang, Y. et al. Acs Nano 2016, 10 (3), 3214-21. 3.
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- Takahashi, Y.; et al. Angew. Chem. Int. Ed. 2011, 50 (41), 9638-9642. 5.




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