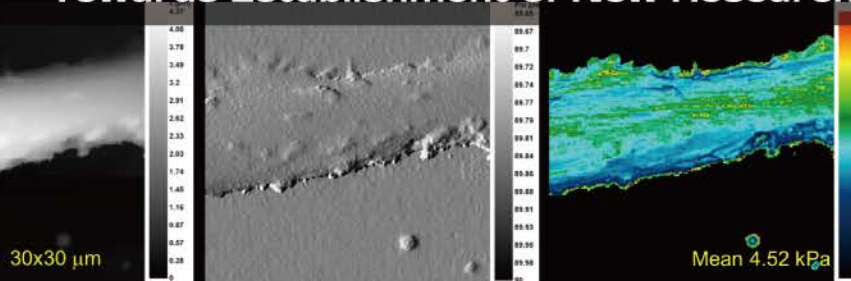


# THE 2ND NANOLSI SYMPOSIUM IN LONDON

— Towards Establishment of New Research Field: Nanoprobe Life Science —



**DATE: MONDAY, NOVEMBER 19, 2018**  
**VENUE: THE CUMBERLAND HOTEL**

## Timetable

- 9:00 am Opening remarks
- 9:10 am Session 1
- 10:20 am Tea break, Poster viewing
- 10:50 am Session 2
- 12:00 pm Lunch, Poster viewing
- 1:30 pm Session 3
- 3:10 pm Tea break, Poster viewing
- 3:40 pm Session 4
- 5:20 pm Closing remarks
- 5:50 pm Banquet

## Invited Speakers (Alphabetical)

- Prof. Joshua Edel from Imperial College London
- Prof. Julia Gorelik from Imperial College London
- Prof. Bart W. Hoogenboom from University College London
- Prof. David Klenerman from University of Cambridge
- Prof. Patrick Unwin from University of Warwick

## Symposium Website

<https://nanolsi.kanazawa-u.ac.jp/2nd-sympo/>

## Full Abstract Book

[https://nanolsi.kanazawa-u.ac.jp/wp-content/uploads/2018/11/NanoLSI-2nd\\_abstract.pdf](https://nanolsi.kanazawa-u.ac.jp/wp-content/uploads/2018/11/NanoLSI-2nd_abstract.pdf)



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## NanoLSI Website

<https://nanolsi.kanazawa-u.ac.jp/>

- Organized by** Nano Life Science Institute, Kanazawa University
- Co-Organized by** Cancer Research Institute of Kanazawa University  
Institute for Frontier Science Initiative, Kanazawa University
- Supported by** Japan Society for the Promotion of Science (JSPS) London



金沢大学  
KANAZAWA  
UNIVERSITY

# The 2nd NanoLSI Symposium in London

Towards Establishment of New Research Field:  
Nanoprobe Life Science

– Date –

Mon. November 19<sup>th</sup>, 2018      9:00-17:50

– Venue –

The Cumberland Hotel – London, UK

Sessions and Posters: Tyburn II

Banquet: Ocean Room



# The 2nd NanoLSI Symposium in London

–Towards Establishment of New Research Field: Nanoprobe Life Science

## PROGRAMME

MC: Kenji Nakashima, Head of NanoLSI Administrative Office, Kanazawa University

### OPENING REMARKS

- 9:00-9:05 Yoshihiro Fukumori  
Vice President, Kanazawa University,  
Deputy Administrative Director, NanoLSI
- 9:05-9:10 Takeshi Fukuma  
Director, NanoLSI

### SESSION 1 – Chair: Yasufumi Takahashi

- 9:10-9:40 Nanopipette biosensors for single-cell analysis  
*Prof Yuri Korchev (Imperial College London, NanoLSI)*
- 9:40-10:00 High-speed atomic force microscopy for nano-scale imaging of biological samples  
*Assoc. Prof Mikihiro Shibata (NanoLSI)*
- 10:00-10:20 Development of High-Speed Ion Conductance Microscope  
*Assist. Prof Shinji Watanabe (NanoLSI)*
- 10:20-10:50 **Tea Break, Poster Viewing**

### SESSION 2 – Chair: Rikinari Hanayama

- 10:50-11:20 Nanoscale SICM/FRET insights into beta-adrenergic receptor distribution and cAMP compartmentation in cardiac myocytes  
*Prof Julia Gorelik (Imperial College London)*
- 11:20-11:40 Three-Dimensional Atomic Force Microscopy for Visualizing Distribution of Water and Molecular Chains at Solid-Liquid Interfaces  
*Prof Takeshi Fukuma (NanoLSI)*
- 11:40-12:00 Probing molecular processes at solid-liquid interfaces  
*Prof Adam S. Foster (Aalto University, NanoLSI)*
- 12:00-12:10 **Photo Session**
- 12:10-13:30 **Lunch, Poster Viewing**

### **SESSION 3 – Chair: Shigehisa Akine**

- 13:30-14:00      Novel strategies in molecular sensing  
*Prof Joshua Edel (Imperial College London)*
- 14:00-14:30      Multifunctional Nanoscale Imaging with Nanopipettes  
*Prof Patrick Unwin (University of Warwick)*
- 14:30-14:50      Metabolic control of cancer stemness  
*Prof Atsushi Hirao (NanoLSI)*
- 14:50-15:10      Cellulose Nanocrystals: Materials for Photonic Structures  
*Prof Mark MacLachlan (University of British Columbia, NanoLSI)*
- 15:10-15:40      **Tea Break, Poster Viewing**

### **SESSION 4 – Chair: Masanobu Oshima**

- 15:40-16:10      New fluorescence based methods for imaging single molecules  
*Prof David Klenerman (University of Cambridge)*
- 16:10-16:40      Assembly and membrane perforation by immune proteins  
*Prof Bart Hoogenboom (University College London)*
- 16:40-17:00      The nuclear pore complex: nanomachine as gatekeeper of molecular transport across nuclear membranes  
*Prof Richard Wong (NanoLSI)*
- 17:00-17:20      Macrocyclic Peptide Technology Targeting HGF and MET receptor  
*Prof Kunio Matsumoto (NanoLSI)*

### **17:20            Closing Remarks**

Prof Yuri Korchev  
Imperial College London, NanoLSI

### **17:50-19:50    Banquet**

Toast:    Seizo Morita (Administrative Director, NanoLSI)  
Closing: Takeshi Fukuma (Director, NanoLSI)

## ORAL PRESENTATION

Yuri Korchev (Imperial College London, NanoLSI)	1
Mikihiro Shibata (NanoLSI)	3
Shinji Watanabe (NanoLSI)	5
Julia Gorelik (Imperial College London)	7
Takeshi Fukuma (NanoLSI)	10
Adam S. Foster (Aalto University, NanoLSI)	12
Joshua Edel (Imperial College London)	14
Patrick Unwin (University of Warwick)	16
Atsushi Hirao (NanoLSI)	19
Mark MacLachlan (University of British Columbia, NanoLSI)	21
David Klenerman (University of Cambridge)	23
Bart Hoogenboom (University College London)	25
Richard Wong (NanoLSI)	27
Kunio Matsumoto (NanoLSI)	29





## Yuri Korchev

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

### Contact:

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### Education:

1983 B.Sc. in Biology & Physiology, University of St Petersburg  
1984 M.Sc. in Physiology, University of St Petersburg  
1989 Ph.D. in Biophysics and Cytology, Russian Academy of Sciences

### Professional Career:

1984 - 1991 Research Scientist, Russian Academy of Sciences, St Petersburg, Russia  
1991 - 1992 Wellcome Trust Research Fellow, St George's Hospital Medical School, UK  
1992 - 1995 Post-Doctoral Research Fellow, St George's Hospital Medical School, London, UK  
1995 - 1997 Post-Doctoral Research Fellow, Charing Cross & Westminster Medical School, UK  
1997 - 2001 Lecturer, Faculty of Medicine, Imperial College London, UK  
2001 - 2003 Senior Lecturer, Faculty of Medicine, Imperial College London, UK  
2003 - 2005 Reader, Faculty of Medicine, Imperial College London, UK  
2005 - present Professor, Faculty of Medicine, Imperial College London, UK

**Research Interests:** Development and applications of scanning ion conductance microscopy and nano-FET and nanopore sensors for biomedical research.

### Publications:

1. Ren, R et al, (2017). Nanopore extended field-effect transistor for selective single-molecule biosensing. *Nature comm.* 8: 586 <https://doi.org/10.1038/s41467-017-00549-w>.
2. U Vivekananda, et al. (2017) Kv1. 1 channelopathy abolishes presynaptic spike width modulation by subthreshold somatic depolarization. *Proc. Natl. Acad. Sci. U.S.A.*, 114 (9), 2395-2400
3. Y Zhang, et al. (2016). Spearhead Nanometric Field-Effect Transistor Sensors for Single-Cell Analysis. *ACS nano* 10, 3214-3221

# Nanopipette biosensors for single-cell analysis.

Yuri Korchev

*Imperial College London, Division of Medicine, London W12 0NN, UK*

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

Molecular Biology has advanced we know much about the individual molecular components that make up living cells down to the level of the individual atoms. The challenge, however, is to fully understand the functional integration of these components. This requires determining how the molecular machines that make up a living cell are organized and interact together not at the atomic length scale but on a nm scale. To do this we need to develop and applying nanoscale techniques for the visualization and quantification of cell machinery in real-time and on living cells. This will lead to detailed, quantitative models of sub-cellular structures and molecular complexes under different conditions for both normal and diseased cells.

This approach ultimately requires the development of novel biophysical methods. We have recently pioneered the development of an array of new and powerful biophysical tools based on Scanning Ion Conductance Microscopy that allow quantitative measurements and non-invasive functional imaging of single protein molecules in living cells.

Scanning ion conductance microscopy and a battery of associated innovative methods are unique among current imaging techniques, not only in spatial resolution of living and functioning cells, but also in the rich combination of imaging with other functional and dynamical interrogation methods [1]. There are a significant advance to deliver nanotechnological solutions to biosensing that are affordable, integrated, fast, capable of multiplexed detection and monitoring, and crucially to offer high selectivity for the specific detection of trace levels of analyte in biological fluids. Herein, we design a new class of nanometric field-effect-transistor (FET) sensors [2] and dubbed nexFET (nanopore extended Field Effect Transistor) [3] that combine the advantages of nanopore single molecule sensing, FETs and recognition chemistry.

The nexFET biosensors, with controllable gate voltage enable higher molecular throughput, enhanced signal-to-noise and even heightened selectivity via functionalization of the nexFET with an embedded receptor. This is shown for sensitive and selective detection of an anti-insulin antibody in the presence of its IgG isotype as well as within complex mixtures such as blood serum. Self-assembled nanoporous sensors at the tip of nanopipette can be used for simultaneous SICM imaging and chemical imaging and also can be combined with FET sensors.

## References:

[1]. A Leo-Macias et al. Nanoscale visualization of functional adhesion/excitability nodes at the intercalated disc. (2016).

***Nature comm.*** 7:10342. doi: 10.1038/ncomms10342

[2] Y Zhang, et al. Spearhead Nanometric Field-Effect Transistor Sensors for Single-Cell Analysis. (2016). ***ACS nano*** 10, 3214-3221, DOI: 10.1021/acsnano.5b05211

[3] R Ren, et al. Nanopore extended field-effect transistor for selective single-molecule biosensing. (2017) ***Nature Com.*** 8, DOI: 10.1038/s41467-017-00549-w





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### Education:

2003 B.S. Nagoya Institute of Technology, Department of Materials Science and Engineering, Japan  
2005 M.S. Nagoya Institute of Technology, Department of Materials Science and Engineering, Japan  
2007 Ph.D. from Nagoya Institute of Technology, Japan

### Professional Career:

2005-2007 Research Fellow of the Japan Society for the Promotion of Science, DC1, Japan.  
2007-2008 Research Fellow of the Japan Society for the Promotion of Science, PD, Japan  
2008-2011 Research Fellow of the Japan Society for the Promotion of Science, SPD, Japan  
2011-2013 JSPS Postdoctoral Fellow for Research Abroad, Duke University, USA  
2013-2015 Postdoctoral Research Fellow, Max Planck Florida Institute for Neuroscience, USA  
2015-2016 Postdoctoral Research Fellow, Kanazawa University, Japan  
2016-2017 Associate Professor, Kanazawa University, Kanazawa, Japan  
2017-present Associate Professor, Nano Life Science Institute, Kanazawa University, Kanazawa, Japan

### Scientific Activities:

2005-present Member of the Biophysical Society of Japan  
2014-present Member of the Biophysical Society

**Research Interests:** Bio-imaging, High-speed atomic force microscopy, protein dynamics, cell biology

### Honors:

2009 Early Career Award in Biophysics Society of Japan  
2018 The Young Scientists' Prize from The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology

### Publications:

1. **M. Shibata\***, H. Nishimasu\*, N. Kodera, S. Hirano, T. Ando, T. Uchihashi, and O. Nureki\*, *Nat. Commun.*, 8: 1430. (2017).
2. **M. Shibata\***, T. Uchihashi, T. Ando, and R. Yasuda\*, *Sci. Rep.*, 5: 8724. (2015).
3. **M. Shibata**, H. Yamashita, T. Uchihashi, H. Kandori, and T. Ando, *Nat. Nanotech.* 5: 208 - 212 (2010).

# High-speed atomic force microscopy for nano-scale imaging of biological samples

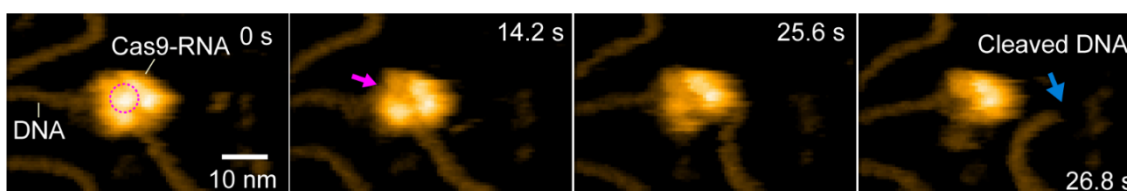
Mikihiro Shibata

*Nano Life Science Institute (WPI-NanoLSI),  
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Structural biology has long been contributing to our understanding of how proteins function by providing their detailed structures at the atomic level. And yet, the revealed structures have been restricted to static information, limiting the level of our understanding. This restriction is now removed by high-speed atomic force microscopy (HS-AFM) that allows direct visualization of individual protein molecules in action at sub-molecular resolution. HS-AFM studies performed in the last few years have provided new mechanistic insight into the functional mechanism of proteins [1]. For example, HS-AFM movies of bacteriorhodopsin (bR), which functions as a light-driven proton pump, clearly showed that, upon illumination, a cytoplasmic portion of bR displaced toward adjacent molecules at  $\sim 0.7$  nm [2]. Therefore, high-resolution visualizations of protein dynamics are powerful approaches for studying elaborate biomolecular processes under realistic conditions.

In this study, we applied HS-AFM to visualize the real-space and real-time dynamics of CRISPR-Cas9, which has been widely used for numerous applications, such as genome editing. The CRISPR-associated endonuclease Cas9 binds to a guide RNA and cleaves double-stranded DNA with a sequence complementary to the RNA guide. Our HS-AFM movies showed that apo-Cas9 adopts unexpected flexible conformations. On the other hand, Cas9–RNA forms a stable bilobed structure and interrogates a target site on the DNA by three-dimensional diffusion. These HS-AFM movies also provided real-time visualization of the Cas9-mediated DNA cleavage reaction. Notably, the Cas9 HNH nuclease domain fluctuates upon DNA binding, and subsequently adopts an active conformation, where the HNH active site is docked at the cleavage site in the target DNA. Collectively, our HS-AFM data extend our understanding of the action mechanism of CRISPR-Cas9 [3].

**Figure 1** A sequential HS-AFM images of Cas9–RNA–DNA during DNA cleavage reaction.



## References:

- [1] T. Ando, T. Uchihashi, and S. Scheuring, *Chem. Rev.* **2014**, 114, 3120-3188.
- [2] M. Shibata, H. Yamashita, T. Uchihashi, H. Kandori, and T. Ando, *Nat. Nanotech.* **2010**, 5, 208-212.
- [3] M. Shibata<sup>\*,\*</sup>, H. Nishimasu<sup>\*,\*</sup>, N. Kodera, S. Hirano, T. Ando, T. Uchihashi, and O. Nureki<sup>\*</sup>, *Nat. Commun.* **2017**, 8, 1430. (+equal contribution, \*co-corresponding author)



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### Education:

2006 Ph.D. Graduate School Science and Technology, Niigata University, Japan, Ph. D. (Science)

### Professional Career:

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

### Scientific Activities:

2012-2013	Committee members of JPS-Division 4 (Semiconductor, Mesoscopic System and Quantum Transport)
2018-present	Committee members of JSAP-M&BE (Molecular Electronics and Bioelectronics)

**Research Interests:** Scanning probe microscopy techniques for visualizing various physical phenomena

### Publications:

1. S. Watanabe, and T. Ando, "High-speed XYZ-nanopositioner for scanning ion conductance microscopy" *Appl. Phys. Lett.* 111, 113106(4 pp), (2017).
2. Satoru Miyamoto, T. Miura, S. Watanabe, K. Nagase, and Y. Hirayama, "Localized NMR Mediated by Electrical-Field-Induced Domain Wall Oscillation in Quantum-Hall-Ferromagnet Nanowire" *Nano Lett.* 16(3), 1596 (6 pp), (2016).
3. T. Hatano, W. Kume, S. Watanabe, K. Akiba, K. Nagase, and Y. Hirayama, "Anisotropic nuclear-spin diffusion in double quantum wells" *Phys. Rev. B* 91, 115318 (7 pp), (2015).

# Development of High-Speed Ion Conductance Microscope

Shinji Watanabe

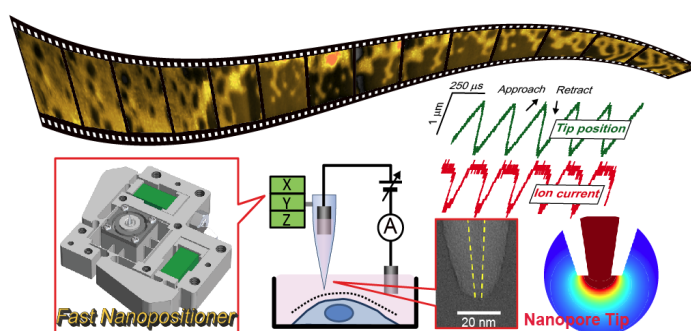
*Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Kanazawa 920-1192, Japan*

In biological studies, scanning probe microscopy (SPM) has been utilized to visualize nanometer-scale surface structures of samples under physiological liquid environments. Atomic force microscopy (AFM) is very popular and powerful SPM due to its excellent spatio-temporal resolution. Nevertheless, the AFM probe makes contact with the sample surface, and the exerted forces provide the deformation to very soft samples such as the plasma membranes of live eukaryotic cells, resulting in low-resolution images and serious damage to the samples [1]. Scanning ion conductance microscopy (SICM)[2] is an alternative imaging way based on an entirely different working principle for visualizing topographic images (Fig. 1). SICM exploits an electrolyte-filled glass pipette (nanopipette) as a probe and an ion current passing through between an electrode inside the nanopipette and another in an external bath electrolyte. This ion current is very sensitive to the tip-sample surface separation; therefore, SICM can capture topographic images without any tip-sample contact. However, the temporal resolution of SICM is much lower than that of AFM. In SICM, thus, it is hard to visualize topographic changes in specimens that occur in under a minute without sacrificing the spatial resolution. This significantly limits the applicability of SICM in biological studies.

We recently succeeded in improving the temporal resolution of SICM without sacrificing the spatial resolution to capture morphological changes in biological samples with nanometer-scale resolution [3]. To improve them, we developed several hardware components of SICM. The tip-scan-type high-speed XYZ-nanopositioner, its undesired vibration damping method, the nanopipette tip exhibiting a high spatial resolution, and the concentration gradient method of the electrolyte salt solution enhancing the signal to noise ratio. These developments allow high-speed SICM images which capture morphological changes in cell membranes in a liquid environment with nanometer-scale resolution. We believe that our studies will strongly contribute to extend the SICM capabilities in biological applications.

## References:

- [1] J. Seifert, J. Rheinlaender, P. Novak, Y. E. Korchev, and T. E. Schäffer, *Langmuir*, 31(24), 6807-6813.
- [2] P. K. Hansma, B. Drake, O. Marti, S. A. C. Gould, and C. B. Prater, *Science* **1989**, 243, 641-643.
- [3] S. Watanabe, and T. Ando, *Appl. Phys. Lett.* **2017**, 111, 113106-1- 113106-4.



**Figure 1** Schematics of high-speed SICM equipment.



## Julia Gorelik

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### Education:

- |      |  |
|------|--|
| 1989 | M.Sc., Biology, St Petersburg Teachers' University, Russia |
| 1996 | Ph.D., Cell Biology, Russian Academy of Sciences           |

### Professional Career:

- |              |   |
|--------------|---|
| 1993-1996    | PhD student, Institute of Cytology St Petersburg, Russia        |
| 1996-1998    | Research Scientist, Institute of Cytology St Petersburg, Russia |
| 1998-2005    | Post-Doctoral Research Fellow, Imperial College London          |
| 2005-2010    | Lecturer, NHLI, Imperial College, London                        |
| 2010-2011    | Senior Lecturer, NHLI, Imperial College, London                 |
| 2011-2014    | Reader in Cellular Biophysics, Imperial College London          |
| 2014-Present | Professor of Cellular Biophysics, Imperial College London       |

**Research Interests:** Interface between physics and cardiovascular research. Scanning Ion Conductance Microscopy (SICM), functional imaging of the cardiovascular system

### Honors:

- Wellcome Trust University Award
- Imperial College Research Excellence Award
- Invited Professorship, Pierre and Marie Curie University, Paris,
- Invited Professorship, Verona University, Italy,

### Publications:

- Sanchez-Alonso JL, Bhargava A, O'Hara T, Glukhov AV, Schobesberger S, Bhogal NK, Sikkel MB, Mansfield C, Korchev YE, Lyon AR, Punjabi PP, Nikolaev VO, Trayanova NA, Gorelik J. () Microdomain-Specific Modulation of L-type Calcium Channels Leads to Triggered Ventricular Arrhythmia in Heart Failure. *Circ Res.* **2016**; *119*(8):944-55
- Glukhov AV, Balycheva M, Sanchez-Alonso JL, Ilkan Z, Alvarez-Laviada A, Bhogal N, Diakonov I, Schobesberger S, Sikkel MB, Bhargava A, Faggian G, Punjabi PP, Houser SR, **Gorelik J.** Direct Evidence for Microdomain-Specific Localization and Remodeling of Functional L-Type Calcium Channels in Rat and Human Atrial Myocytes. *Circulation.* **2015**; *132*(25):2372-84
- Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, **Gorelik J.**  $\beta$ 2-Adrenergic Receptor Redistribution in Heart Failure Changes cAMP Compartmentation. *Science.* **2010**, *327*(5973):1653-7

# Nanoscale SICM/FRET insights into beta-adrenergic receptor distribution and cAMP compartmentation in cardiac myocytes

Julia Gorelik

*Imperial College London*

The  $\beta_1$ - and  $\beta_2$  adrenergic receptors dominate the cardiac response to catecholamines through their coupling to  $G_s$  proteins and to the production of the common second messenger cAMP (Xiang and Kobilka (2003)). Cyclic AMP controls the catecholamine-dependent changes in rate, force and speed of contraction of the heart. However, selective stimulation of these two receptor subtypes leads to clearly distinct physiological and pathophysiological responses. In healthy cardiac myocytes  $\beta_1$ - but not  $\beta_2$ ARs stimulate the cAMP-dependent protein kinase mediated phosphorylation of phospholamban and cardiac contractile proteins (Xiao (2001)). Heart-specific overexpression of  $\beta_1$ - but not of  $\beta_2$ AR in transgenic mice induces progressive cardiac hypertrophy and heart failure (Engelhardt *et al* (1999)). Such subtype specific differences have been attributed to distinct patterns of cAMP compartmentation from the two receptor subtypes observed using electrophysiological and fluorescent biosensors (Nikolaev *et al* (2006)). However, the exact distribution of the  $\beta_1$ - and  $\beta_2$ ARs is still elusive. Unfortunately, methods to detect such localised effects have so far been limited.

Our new functional approach combines scanning ion conductance microscopy (SICM) with measurements of cAMP production by fluorescence resonance energy transfer (FRET) after localized receptor stimulation (Nikolaev *et al* (2010)). SICM is a non-optical method which uses a nanopipette as a scanning probe to image the surface topography of living cells and allows to resolve the structural features of cardiac myocytes such as Z-grooves and transverse (T) tubules with a resolution equal to the pipette's inner diameter (Novak *et al* (2009)), typically ~50 nm. After the acquisition of the cell surface topography we precisely position the pipette onto various membrane regions of defined morphology to locally apply agonists and antagonists of the  $\beta$ ARs. Local stimulation is achieved by applying pressure into the pipette while constantly superfusing the cells with the buffer/antagonist solution from the side (Nikolaev *et al* (2006)). Upon selective stimulation of  $\beta_1$ - and  $\beta_2$ ARs we measure cAMP synthesis in defined areas of the cells by FRET microscopy using a sensitive cAMP sensor Epac2-camps (Nikolaev *et al* (2004)).

To study the localization of receptors and cAMP signals in cardiomyocytes isolated from healthy adult rat hearts, we locally stimulated  $\beta_1$ - and  $\beta_2$ ARs either in the T-tubule or on the crest of the cell. Selective stimulation of  $\beta_1$ ARs in both regions resulted in a robust decrease of FRET, reflecting the stimulation of cAMP synthesis by the receptors localized in different parts of the membrane. In sharp contrast,  $\beta_2$ AR selective stimulation led to cAMP signals only in the T-tubules, but not in the outer regions of the membrane.

In a rat model of chronic heart failure,  $\beta_2$ ARs redistributed from the transverse tubules to the cell crest, leading to diffuse receptor-mediated cAMP signaling. We showed that surface morphology and T-tubular structure are significantly disrupted in chronic heart failure cells (Lyon *et al* (2009)), and that impacts on the redistribution of sarcolemmal  $\beta_2$ AR and localized secondary messenger signaling.

## References:

- (1) Engelhardt S, Hein L, Wiesmann F, Lohse MJ. Progressive hypertrophy and heart failure in beta1-adrenergic receptor transgenic mice. *Proc Natl Acad Sci U S A* **1999**; 96(12):7059-7064.

- (2) Lyon AR, MacLeod KT, Zhang Y, Garcia E, Kanda GK, Lab MJ, Korchev YE, Harding SE, Gorelik J. Loss of T-tubules and other changes to surface topography in ventricular myocytes from failing human and rat heart. *Proc Natl Acad Sci U S A* **2009**; *106*(16):6854-6859.
- (3) Nikolaev VO, Bunemann M, Hein L, Hannawacker A, Lohse MJ. Novel single chain cAMP sensors for receptor-induced signal propagation. *J Biol Chem* **2004**; *279*(36):37215-37218.
- (4) Nikolaev VO, Bunemann M, Schmitteckert E, Lohse MJ, Engelhardt S. Cyclic AMP imaging in adult cardiac myocytes reveals far-reaching beta1-adrenergic but locally confined beta2-adrenergic receptor-mediated signaling. *Circ Res* **2006**; *99*(10):1084-1091.
- (5) Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, Gorelik J.  $\beta$ 2-Adrenergic Receptor Redistribution in Heart Failure Changes cAMP Compartmentation. *Science*. **2010**; *26*; *327*(5973):1653-7.



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### Education:

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

### Professional Career:

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

### Scientific Activities:

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

**Research Interests:** Atomic Force Microscopy, Nanotechnology, Solid-Liquid Interfaces, Nanobio-science

### Honors:

2008	The 4th Incentive Award for Young Scientist, Biophysical Society of Japan
2010	19th MRS-J Academic Symposium Incentive Award, Materials Research Society of Japan
2011	The Young Scientists' Prize, The Commendation for Science and Technology by the MEXT
2017	Hokkoku Bunka Award, Hokkoku Shinbun

### Publications:

1. K. Honbo, S. Ogata, T. Kitagawa, T. Okamoto, N. Kobayashi, I. Sugimoto, S. Shima, A. Fukunaga, C. Takatoh, T. Fukuma, *ACS Nano*, 10 (2016) 2575-2583.
2. K. Miyata, J. Tracey, K. Miyazawa, V. Haapasilta, P. Spijker, Y. Kawagoe, A. S. Foster, K. Tsukamoto, T. Fukuma, *Nano Lett.*, 17 (2017) 4083-4089.



# Three-Dimensional Atomic Force Microscopy for Visualizing Distribution of Water and Molecular Chains at Solid-Liquid Interfaces

Takeshi Fukuma

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

At a solid-liquid interface, water molecules interact with a surface to present non-uniform three-dimensional (3D) distribution referred to as hydration structures. In addition, atoms and molecules constituting the surface are not necessarily static but dynamically fluctuating or diffusing to induce various interfacial phenomena. Consequently, a solid-liquid interface is not two-dimensional but has a three-dimensional extent. However, such 3D fluctuating structures are very difficult to image, which has limited our understanding on various interfacial phenomena in biology, physical chemistry, electrochemistry, tribology, mineralogy and environmental sciences. To overcome this problem, we have been working on the development and applications of 3D atomic force microscopy (3D-AFM) [1]. In this technique, AFM tip is scanned in a 3D interfacial space and the force applied to the tip during the scan is recorded to produce a 3D force image. During the tip scan, the tip interacts with surrounding water and other flexible molecules so that the obtained force map represents their time-averaged distribution. So far, this method is mostly used for visualizing hydration structures on various minerals such as mica, calcite, fluorite and graphite. However, it is also shown that it can be used for visualizing hydration structures of softer materials such as biological molecules (e.g., bRs, GroELs and DNAs). One of these measurements, imaging of a lipid/water interface [2], suggests that the obtained force image reflects not only the hydration structures but also the 3D distribution of flexible molecular chains. This implies that 3D-AFM may allow us to visualize various 3D structures as long as the tip can penetrate into the subsurface area without causing irreversible damages. To explore this possibility, we have been working on visualization of various interfacial structures with different level of complexity and inhomogeneity.

## References:

- [1] T. Fukuma, Y. Ueda, S. Yoshioka, H. Asakawa, "Atomic-Scale Distribution of Water Molecules at the Mica-Water Interface Visualized by Three-Dimensional Scanning Force Microscopy", *Phys. Rev. Lett.*, 104 (2010) 016101.
- [2] H. Asakawa, S. Yoshioka, K. Nishimura, T. Fukuma, "Spatial Distribution of Lipid Headgroups and Water Molecules at Membrane/Water Interfaces Visualized by Three-Dimensional Scanning Force Microscopy", *ACS Nano*, 6 (2012) 9013-9020.

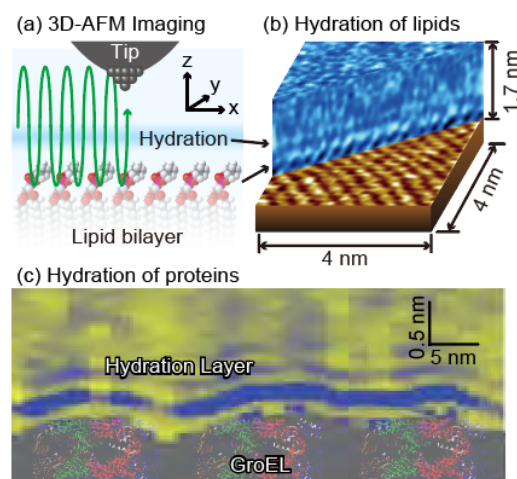


Fig. 1: 3D-AFM imaging of nanobio interfaces.



## Adam S. Foster

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### Education:

1997 Masters in Theoretical Physics, Newcastle University, UK  
2000 PhD in Theoretical Solid State Physics, University College London, UK

### Professional Career:

2000 – 2004 Postdoctoral researcher, Helsinki University of Technology, Finland  
2004 – 2009 Academy of Finland senior research fellow, Helsinki University of Technology, Finland  
2009 – 2012 Professor, Tampere University of Technology, Finland  
2012 – present Professor, Aalto University, Finland  
2015 – present Research professor, Kanazawa University, Japan  
2017 – present Visiting professor, Graduate School Materials Science in Mainz, Germany

### Research Interests:

Surfaces and Interfaces at the Nanoscale group (SIN), internationally competitive simulation group, computational modelling of nanoscale phenomena, understanding and controlling the atomic processes dominating function at the surfaces and interfaces of application critical materials

**Honors:** 2009 – Awarded **Väisälä prize** by Suomalainen Tiedeakatemia

### Publications:

1. Elemental Identification by Combining Atomic Force Microscopy and Kelvin Probe Force Microscopy, *Fabian Schulz, Juha Ritala, Ondrej Krejci, Ari Paavo Seitsonen, Adam S. Foster and Peter Liljeroth*, ACS Nano **12** (2018) 5274
2. Multiple hetero-atom substitution in a graphene nanoribbon, *Shigeki Kawai, Soichiro Nakatsuka, Takuji Hatakeyama, Rémy Pawlak, Tobias Meier, John Tracey, Ernst Meyer and Adam S. Foster*, Sci. Adv. **4** (2018) eaar7181
3. Machine learning hydrogen adsorption on nanoclusters through structural descriptors, *Marc Jäger, Eiaki Morooka, Filippo Federici Canova, and Adam Foster*, npj Computational Materials **4** (2018) 37

# Probing molecular processes at solid-liquid interfaces

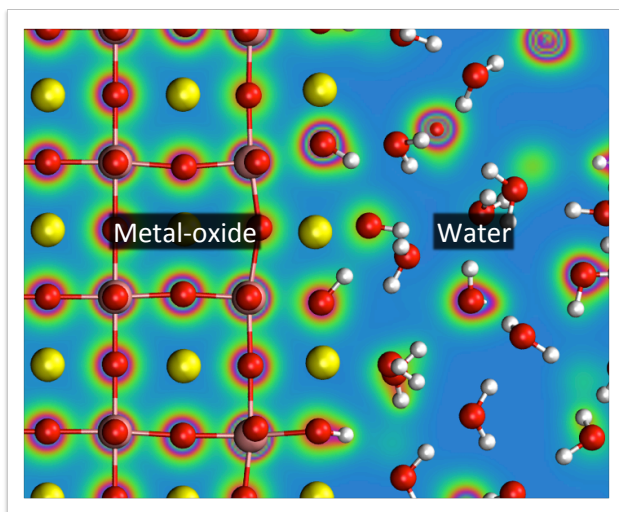
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*Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan*

*Graduate School Materials Science in Mainz, Germany*

The development of high-resolution Atomic Force Microscopy (AFM) in liquids heralded an optimism that reliable atomic and molecular resolution at solid-liquid interfaces would soon be readily available. AFM is already established as an extremely powerful tool for nanoscale imaging of biological systems in solution, but higher resolutions would open the door to atomic scale understanding of the wide spectrum of processes occurring in solution. Despite several outstanding successes, high-resolution AFM in liquids has been plagued by challenges in interpretation and environment control. Many previous studies of the interaction of water with solid surfaces have assumed a simple interface, but the latest advances show that functional properties are sensitive to its detailed atomic/electronic structure and dynamics.



**Figure 1** Snapshot from an *ab initio* molecular dynamics simulation of a  $\text{TiO}_2$ -water interface showing atomic positions and electron density.

In this work, we briefly review our general approach for simulating high resolution AFM on solid-liquid interfaces [1], but then introduce recent studies that demonstrate how the combination of simulations and experiments can be used to gain insight into dynamic processes, including dissolution [2]. However, the application of these simulation approaches to more complex systems, particularly large organic assemblies, requires new methodologies. Here, we will outline our approach to machine learn the fundamental behaviour of water at interfaces as a function of local chemical environment and develop a predictive tool for AFM simulations that remains rapid for any system.

## References:

- [1] T. Fukuma, B. Reischl, N. Kobayashi, P. Spijker, F. F. Canova, K. Miyazawa, and A. S. Foster, *Phys. Rev. B* **92** (2015) 155412
- [2] K. Miyata, J. Tracey, K. Miyazawa, V. Haapasilta, P. Spijker, Y. Kawagoe, A. S. Foster, K. Tsukamoto, and T. Fukuma, *Nano Lett.*, **17** (2017) 4083



## Joshua Edel

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### Contact:

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### Education:

2004                      PhD in Physical Chemistry, Department of Chemistry, Imperial College London  
2000                      BSc (Hon) in Chemistry, Department of Chemistry, University of British Columbia

### Professional Career:

2016 –                    Professor of Biosensing and Analytical Sciences, Department of Chemistry, Imperial  
2006 – 2016            Lecturer - Reader, Department of Chemistry, Imperial  
2005 – 2006            Research Fellow, Rowland Institute at Harvard, Harvard University  
2004 – 2005            Postdoctoral Research Assistant, School of Applied and Engineering Physics, Cornell University

**Research Interests:** Single molecule biological screening using electrical and optical methods, Early stage diagnostics (cancer, neurodegenerative disorders), single cell screening, electrovariable plasmonics, microfluidics

### Honors:

2017                    ERC Consolidator Investigator Award, EU  
2015                    President's Prize for Excellence, Imperial College London, UK  
2015                    ERC Proof of Concept Award, EU  
2012 – 2016            ERC Starting Investigator Award, EU

### Publications:

1. Paulose et al., *Nanoscale Tweezers for Single Cell Biopsies*, **Nature Nanotechnology** (2018, in press)
2. Cadinu et al., *Double Barrel Nanopores as a New Tool for Controlling Single-Molecule Transport*, **Nano letters** (2018)
3. Barik et al., *Graphene-edge dielectrophoretic tweezers for trapping of biomolecules*, **Nature Communications** (2017).
4. Montelongo et al., *Electrotunable Nanoplasmonic Liquid Mirror*, **Nature Materials** (2017).

# Novel strategies in molecular sensing

Joshua Edel

*Department of Chemistry, Imperial College London*

Analytical Sensors plays a crucial role in today's highly demanding exploration and development of new detection strategies. Whether it be medicine, biochemistry, bioengineering, or analytical chemistry the goals are essentially the same: 1) improve sensitivity, and 2) maximize throughput. In order to address these key challenges, the analytical community has borrowed technologies and design philosophies which has been used by the semiconductor industry over the past 20 years. By doing so, key technological advances have been made which include the miniaturization of sensors and signal processing components which allows for the efficient detection of nanoscale object. One can imagine that by decreasing the dimensions of a sensor to a scale similar to that of a nanoscale object, the ultimate in sensitivity can potentially be achieved – the detection of single molecules. This talk highlights novel strategies for performing single molecule screening.

## References:

1. P. Cadinu, G. Campolo, S. Pud, W. Yang, J.B. Edel, C. Dekker, A.P. Ivanov, *Double Barrel Nanopores as a New Tool for Controlling Single-Molecule Transport*, **Nano letters** (2018)
2. R. Ren, Y. Zhang, B.P. Nadappuram, B. Akpinar, D. Klenerman, A.P. Ivanov, J.B. Edel, Y. Korchev, *Nanopore extended field-effect transistor for selective single-molecule biosensing*, **Nature Communications** (2017).
3. X. Lin, A.P. Ivanov, J.B. Edel, *Selective single molecule nanopore sensing of proteins using DNA aptamer-functionalised gold nanoparticles*, **Chemical Science** (2017).
4. J.Y.Y Sze, A.P. Ivanov, A.E.G. Cass, J.B. Edel, *Single Molecule Multiplexed Nanopore Protein Screening in Human Serum using Aptamer modified DNA Carriers*, **Nature Communications** (2017).
5. P. Cadinu et al. *Single Molecule Trapping and Sensing using Dual Nanopores separated by a Zeptolitre Nanobridge*, **Nano Letters** (2017).
6. K.J. Freedman, L.M. Otto, A.P. Ivanov, A. Barik, S.H. Oh, J.B. Edel, *Nanopore Sensing at Ultra-low Concentrations using Single Molecule Dielectrophoretic Trapping*, **Nature Communications** (2016).



## Patrick Unwin

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### Education:

1985 BSc (Hons) Chemistry, Class I, University of Liverpool, UK

1989 DPhil, Physical Chemistry, University of Oxford, UK

### Professional Career:

1988-90 Junior Research Fellow in Physical Science, Balliol College, University of Oxford

1990-91 SERC/NATO Fellow, University of Texas at Austin, USA

1992-96 Lecturer in Physical Chemistry, University of Warwick

1996-98 Senior Lecturer in Physical Chemistry, University of Warwick

1998-present Professor of Chemistry, University of Warwick

### Scientific Activities:

2010-2015 European Research Council Advanced Grant

2016-present Editorial Committee of *Annual Review of Analytical Chemistry* (*Annual Reviews* series)

2014-present Director EPSRC-Warwick Molecular Analytical Science Centre for Doctoral Training

**Research Interests:** Development of fast, ultrasensitive, multifunctional probe imaging techniques that provide a new way of “seeing” electrochemistry and interfacial processes in action, to reveal active surface sites directly, unambiguously and quantitatively.

**Honors:** Charles N. Reilly Award, Society of Electroanalytical Chemistry, USA (2018); International Society of Electrochemistry-Elsevier Prize for Experimental Electrochemistry (2017); Royal Society-Wolfson Research Merit Award (2017); Tilden Prize, Royal Society of Chemistry (2012)

### Publications:

1. Nanoscale Structure-Dynamics within Electrocatalytic Materials, *J. Am. Chem. Soc.*, 2017, 139, 16813.
2. Surface Charge Visualization at Viable Living Cells, *J. Am. Chem. Soc.*, 2016, 138, 3152.
3. High-Speed Electrochemical Imaging *ACS Nano*, 2015, 9, 8942.

# Multifunctional Nanoscale Imaging with Nanopipettes

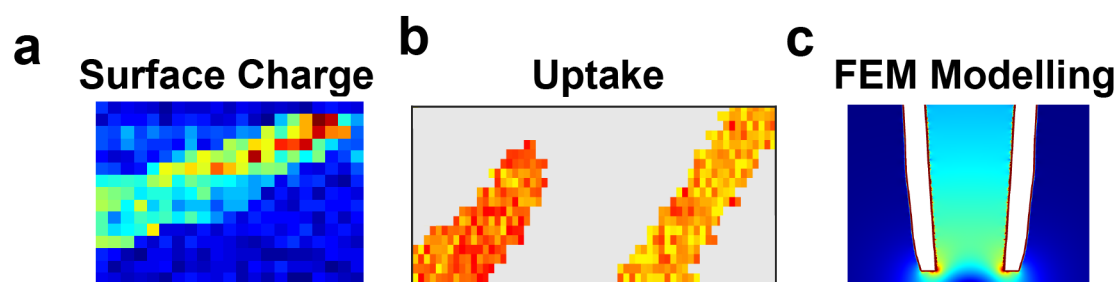
Patrick Unwin

University of Warwick, UK

Advances in microscopy and micro-spectroscopy have revealed the chemistry and physical structure of surfaces and interfaces as never before. Yet, a holistic understanding of interfacial processes, from the functioning of living cells to the operation of electrocatalysts, requires spatially-resolved information on other properties, such as chemical flux and surface charge. We have sought to develop microscopy methods that can reveal *function* and *topography synchronously* and, further, where highly resolved flux data can be combined with information from other microscopy approaches in a *correlative co-location microscopy strategy*, to reveal the relationship between structure and function unambiguously [1,2]. We make use of nanopipettes (with dimensions down to a few 10s nm) containing electrolyte solution and quasi-reference counter electrodes as mobile probes and electrochemical cells that can be moved quickly and intelligently near to surfaces to gather multiple electrochemical flux signals that are processed by finite element method modelling [3]. We shall describe how potential control of the tip, novel feedback and probe scan configurations, multi-channel nanopipette probes, and detailed understanding of mass transport in nanopipettes [4], opens up major new capability of nanopipette imaging probes, with applications ranging from the uptake and delivery of molecules to single cells [5], surface charge at cell membranes [6] and other surfaces [7], surface patterning and printing [8], and identifying key active sites on electrode surfaces and electromaterials [9,10], leading to the rational design of electrocatalysts.

I am grateful to many talented students, postdocs, and research fellows at Warwick, and collaborators elsewhere, for their huge contributions to instrumentation development and applications. I thank the Royal Society for a Wolfson Research Merit Award, and the ERC and EU for funding which enabled many of the developments herein.

**Figure 1.** a) Surface charge map of a root hair cell showing enhanced charge magnitude at the tip. b) Map of heterogeneous uptake rates of a molecule at a root hair cell. c) Electric field profile from FEM simulations at end of a nanopipette near a surface.



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- [1] M. Kang, D. Momotenko, A. Page, D. Perry, and P. R. Unwin, *Langmuir*, **2016**, *32*, 7993–8008.
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- [4] D Perry, A Page, B Chen, BG Frenguelli, PR Unwin, *Anal. Chem.*, **2017**, *89*, 12458–12465.
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- [7] D. Perry, R. Al Botros, D. Momotenko, S. L. Kinnear and P. R. Unwin, *ACS Nano*, **2015**, *9*, 7266–7276.
- [8] [D. Momotenko](#), [A. Page](#), [M. Adobes-Vidal](#) and [P. R. Unwin](#), *ACS Nano*, **2016**, *10*, 8871–8878.
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- [10] [C. L. Bentley](#), [M. Kang](#), and [P. R. Unwin](#), *J. Am. Chem. Soc.*, **2017**, *139*, 16813–16821.





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### Education:

1988 M.D. Jichi Medical University  
1994 Ph.D. University of Tokushima

### Professional Career:

1995 - 1997 Postdoctoral fellow, Japan Society for the Promotion of Science (Kumamoto University)  
1997 - 2001 Postdoctoral fellow, Ontario Cancer Institute, University of Toronto, Canada  
2001 – 2002 Assistant professor, Institute of Molecular Embryology and Genetics, Kumamoto University  
2002 - 2004 Assistant professor, Keio University School of Medicine  
2004 - 2005 Associate professor, Keio University School of Medicine  
2005 – present Professor, Cancer Research Institute, Kanazawa University  
2017- present Director, Cancer Research Institute, Kanazawa University

### Scientific Activities:

2011-present Associate editor of Cancer Science  
2014-present Associate member of Science Council of Japan  
2017-present Executive director of Japanese Society of Hematology

**Research Interests:** Stem cell biology, Metabolism, Cancer therapy

### Honors:

2007 The JSPS (Japan Society for the Promotion of Science) PRIZE  
2010 The Commendation for Science and Technology by the MEXT

### Publications:

1. Tadokoro Y, *et.al.*, *Cell Stem Cell*. 22:713-725, 2018
2. Hoshii T, *et.al.*, *Proc Natl Acad Sci U S A*. 111:3805-10, 2014
3. Naka K, *et.al.*, *Nature*, 463:676-80, 2010
4. Miyamoto K, *et.al.*, *Cell Stem Cell*. 1:101-112, 2007

# Metabolic control of cancer stemness

Atsushi Hirao, MD, PhD

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Cancer Research Institute, Kanazawa University*

Nutrients, such as amino acids and glucose, are critical determinants of cell survival, proliferation and differentiation processes in normal and malignant tissues. Recent studies have revealed critical roles of metabolic control in stem cell properties, so called "stemness", which contribute to malignant progression of cancers. It has been shown that metabolic control is critical for maintenance of hematopoietic stem cell (HSCs) functions. HSCs utilize glycolysis to sustain undifferentiated status, whereas oxidative phosphorylation becomes dominant in progenitors. Unlike HSCs, leukemia stem cells (LSCs) appear to have low glycolytic flow, indicating that there are critical differences in metabolic regulation between these cell types. Therefore, knowledge of the molecular mechanisms of metabolic regulation will provide novel therapeutic approaches for eradication of LSCs. We previously found that fine-tuning of mTOR complex 1 (mTORC1) and forkhead transcription factor FoxO, which function in nutrient sensing signaling pathways, is critical for maintenance of HSC homeostasis. We found that modification of these signals induces apoptosis of LSCs, but not HSCs. Therefore, we believe that investigation of molecular functions in these pathways will lead to the development of successful therapeutics for eradication of LSCs.

In the WPI program, we aim to understand the molecular mechanisms of metabolic regulation for determining stem cell fate in normal and malignant tissues by using Bio-SPM. To do so, we will identify metabolic enzymes that play critical role in cancer malignancy by CRISPR library as therapeutic targets, followed by identification of metabolites as biomarkers. We also develop novel probes that specifically bind to particular metabolites in collaboration with experts of supermolecular chemistry. Finally, we will combine the probe with SPM, such as SICM. By collaborations with experts in nanotechnology, we shall build a path to a new era of cancer biology.

## References:

- [1] Tadokoro Y, *et al.*, Spred1 Safeguards Hematopoietic Homeostasis against Diet-Induced Systemic Stress. *Cell Stem Cell*, **2018**, 22:713-725.
- [2] Hegazy AM, *et al.*, Therapeutic strategy for targeting aggressive malignant gliomas by disrupting their energy balance. *J Biol Chem.*, **2016**, 291:21496-21509.
- [3] Hoshii, T., *et al.*, mTORC1 is essential for leukemia propagation but not stem cell self-renewal. *J. Clin. Invest.*, **2012**, 122: 2114-2129.
- [4] Naka K, *et al.*, TGF $\beta$ -FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature*, **2010**, 463:676-80.
- [5] Miyamoto K, *et al.*, Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell*, 2007, 1:101-12.



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### Education:

1995 B.Sc.(Hons.) Chemistry, University of British Columbia  
1999 Ph.D. Chemistry, University of Toronto

### Professional Career:

1999-2001 NSERC PDF, M.I.T.  
2001-2007 Assistant Professor, UBC  
2007-2011 Associate Professor, UBC  
2011-present Professor, UBC  
2016-present Associate Dean of Research & Graduate Studies, UBC  
2017-present Visiting Research Professor, Kanazawa University

### Scientific Activities:

2014-present Fellow, Royal Society of Canada  
2015-present Director, NSERC CREATE Training Prog. in Nanomaterials Science & Technol. (UBC)  
2016-2018 Advisory Board Member, Materials Chemistry Frontiers, Royal Society of Chemistry

**Research Interests:** Supramolecular chemistry, macrocycles, nanomaterials, cellulose nanocrystals, porous materials.

### Honors:

Strem Award for Pure or Applied Inorganic Chemistry (Canadian Society for Chemistry (CSC), 2013), Rutherford Medal (Royal Society of Canada (RSC), 2013), Invitational Fellowship for Research in Japan (JSPS, 2013), Steacie Prize (2014), Award for Excellence in Materials Chemistry (CSC, 2016)

### Publications:

1. Wang, P. X.; Hamad, W. Y.; MacLachlan, M. J. *Angew. Chem. Int. Ed.* **2018**, *57*, 3360-3365.
2. Wang, P.-X.; Hamad, W. Y.; MacLachlan, M. J. *Nature Comm.* **2016**, *7*, 11515.
3. Shopsowitz, K.E.; Qi, H.; Hamad, W.Y.; MacLachlan, M.J. *Nature* **2010**, *468*, 422-425.

# Cellulose Nanocrystals: Materials for Photonic Structures

Mark MacLachlan

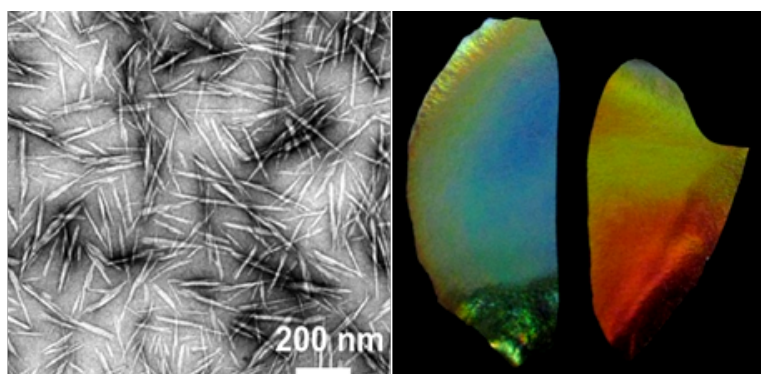
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Cellulose nanocrystals (CNCs) can be extracted from biomass, including cotton and wood pulp. When prepared through sulfuric acid-catalyzed hydrolysis of biomass, CNCs are obtained with sulfate ester groups on their surface. The resulting CNCs (Fig. 1) form stable colloidal suspensions in water, but they also organize into a chiral nematic liquid crystal when concentrated above ~4 wt% in water. This chiral nematic organization is retained in dried films of CNCs, giving films with bright, iridescent colors.[1]

Our group has been active in using CNCs as a template to construct novel chiral nematic mesoporous materials with photonic properties.[2] We have succeeded in preparing silica,[3] organosilica,[4] and polymeric materials[5] through self-assembly or through hard-templating methods.

In this presentation, I will introduce nanoscale biomaterials, illustrate our concept for creating new materials with tunable photonic properties (such as the organosilica glass shown in Fig. 1), and highlight some of our recent work in this area.[6-8]



**Figure 1** TEM image of CNCs (left) and photograph of chiral nematic, mesoporous organosilica (right).

## References:

- [1] J.-F. Revol, L. Godbout and D. G. Gray, *J. Pulp Pap. Sci.*, **1998**, *24*, 146.
- [2] M. Giese, L.K. Blusch, M. K. Khan and M. J. MacLachlan, *Angew. Chem. Int. Ed.*, **2015**, *54*, 2888.
- [3] K. E. Shopsowitz, H. Qi, W. Y. Hamad and M. J. MacLachlan, *Nature*, **2010**, *468*, 422.
- [4] K. E. Shopsowitz, W. Y. Hamad and M. J. MacLachlan, *J. Am. Chem. Soc.*, **2012**, *134*, 867.
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- [6] A. Tran, W. Y. Hamad and M. J. MacLachlan, *ACS Appl. Nano Materials*, **2018**, *1*, 3098.
- [7] T. Hiratani, O. Kose, W. Y. Hamad and M. J. MacLachlan, *Mater. Horizons*, **2018**, in press.
- [8] Y. Cao, W. Y. Hamad and M. J. MacLachlan, *Adv. Opt. Mater.*, **2018**, *6*, 1800412.



## David Klenerman

### Affiliation:

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### Education:

- 1979-1982 BA Chemistry, University of Cambridge
- 1982-1985 PhD Chemistry, University of Cambridge, Department of Chemistry  
(Supervisor: Professor Ian Smith FRS)
- 1985-1987 Fulbright Scholar, Stanford University, California (Supervisor: Professor Richard N. Zare)

### Professional Career:

- 1987-1994 BP Research Centre, Sunbury – Research Scientist, Laser Spectroscopy Group
- 1994-1999 Assistant Director of Research, University of Cambridge
- 1999-2004 Lecturer, Department of Chemistry, University of Cambridge
- 2002-2007 Reader in Biophysical Chemistry, University of Cambridge
- 2007-Present Professor of Biophysical Chemistry, University of Cambridge

**Research Interests:** Single molecule fluorescence, scanning ion conductance microscopy, molecular basis of neurodegenerative disease, molecular basis of T-cell triggering.

### Honors:

- 2012 Elected Fellow of the Royal Society (FRS)
- 2015 Elected Fellow of the Academy of Medical Sciences (FMedSci)
- 2016 Royal Society Glaxo Wellcome Professor of Molecular Medicine

### Publications:

1. Initiation of T cell signaling by CD45 segregation at 'close contacts'. *Nature Immunology* **2016**, 17, 574.
2. Direct Observation of the Interconversion of Normal and Toxic Forms of alpha-Synuclein. *Cell* **2012**, 149, 1048.
3. Inhibiting the Ca<sup>2+</sup> Influx Induced by Human CSF. *Cell Rep* **2017** 21, 3310.

## **New fluorescence based methods for imaging single molecules**

**David Klenerman**

*University of Cambridge*

A major advance in physical chemistry in the last two decades has been the development of quantitative methods to directly observe individual molecules in solution, attached to surfaces, in the membrane of live cells or more recently inside live cells. These advances allow the study of how the individual cellular components interact to form a living cell and how this can go awry in disease. My group has focused on developing these methods and then, with our biological and clinical collaborators, applying them to biological and biomedical problems which have been difficult or impossible to solve using more conventional bulk methods. I will show how we have developed single molecule fluorescence to detect the interaction of molecules in the test-tube and on cells and combined this with pipette based delivery. I will then show how these methods can be used to gain new molecular insights by describing our recent work on the role of proteins aggregates in neurodegenerative disease and the molecular basis of the immune response.



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### Education:

- |      |  |
|------|--|
| 1998 | MSc in Physics, University of Groningen (NL)               |
| 2002 | PhD in Condensed Matter Physics, University of Geneva (CH) |

### Professional Career:

- |              |  |
|--------------|--|
| 2002-2004    | Postdoc at the Department of Physics, University of Basel (CH)   |
| 2005-2007    | Postdoc at the Biozentrum, University of Basel (CH)              |
| 2007-2013    | Lecturer (UK eq. Assistant Professor), University College London |
| 2013-2016    | Reader (UK eq. Associate Professor), University College London   |
| 2016-Present | Professor of Biophysics, University College London               |

### Scientific Activities:

- |              |   |
|--------------|---|
| 1998-2002    | Correlated electron systems, superconductivity, STM/STS |
| 2005-Present | Nanoscale biophysics                                    |

### Research Interests:

In my lab, we are interested in the broad question of how biological molecules interact with each other and (self-)organise to collectively carry out tasks that are important for health and disease. Examples of such tasks are the repair of DNA damage, the perforation of cellular membranes and the regulation of transport into and out of the cell nucleus. While we use a range of biophysical techniques, our favourite tool for this is atomic force microscopy (AFM).

### Honors:

Medal for Scanning Probe Microscopy 2017, of the Royal Microscopical Society

### Publications:

1. C. Leung, A. W. Hodel, A. J. Brennan, N. Lukyanova, S. Tran, C. House, S. C. Kondos, J. C. Whisstock, M. A. Dunstone, J. A. Trapani, I. Voskoboinik, H. R. Saibil, and B. W. Hoogenboom, Real-time visualization of perforin nanopore assembly, *Nature Nanotechnology* 12 (2017): 467–473.
2. A. Bestembayeva, A. Kramer, A. A. Labokha, D. Osmanovic, I. Liashkovich, E. V. Orlova, I. J. Ford, G. Charras, A. Fassati, and B. W. Hoogenboom, Nanoscale stiffness topography reveals structure and mechanics of the transport barrier in intact nuclear pore complexes, *Nature Nanotechnology* 10 (2015): 60–64.

# Assembly and membrane perforation by immune proteins

Bart Hoogenboom

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Pore-forming proteins are crucial armaments in the continuous battle between organisms and their pathogens. In the course of their function, they assemble into oligomeric rings that puncture holes through target membranes. Bacterial toxins are pore-forming proteins [1,2] that penetrate host cell membranes to release nutrients for the bacteria, thus invading or killing the host cells, for example in bacterial pneumonia or meningitis. On the other hand, the immune system of vertebrates secretes pore-forming proteins to kill infected or cancerous cells or invading pathogens. To perform these tasks, pore-forming proteins convert from water-soluble monomers into membrane-bound, oligomeric pores. I will report on experiments in which we disentangle the different steps of this intricate process, largely by real-time and nanometer-scale visualisation, focusing on proteins that play key roles in our immune system [3-5].

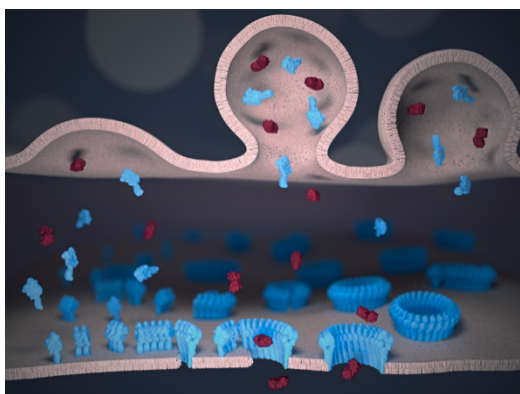


Fig. 1. Schematic of perforin nanopore assembly in the immunological synapse between a killer lymphocyte and a transformed or virus-infected cell [3]. Courtesy of Adrian Hodel.

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### Education:

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2004 Ph.D. (Anatomy & Cell Biology) The University of Tokyo

### Professional Career:

2004-2005 JSPS Postdoctoral fellow, Department of Anatomy & Cell Biology, The University of Tokyo  
2005-2007 HHMI Research Associate, Laboratory of Cell Biology, The Rockefeller University (USA)  
2008-2012 Associate Professor (Tenure-Track), Frontier Science Organization, Kanazawa University  
2012-Present Professor, Biology Course, Faculty of Natural System, Kanazawa University  
2015-2018 Professor & Head of Division in Molecule & Cell Research, Bio-AFM Frontier Research Center, Kanazawa University  
2015-Present Professor & unit leader in Institute for Frontier Science Initiative, Kanazawa University  
2017-Present Professor & Principle investigator in Nano Life Science Institute(WPI-NanoLSI), Kanazawa University

### Scientific Activities:

2015- Referee for Fonds de la Recherche Scientifique, Belgium.  
2015- Referee for Fondation pour la Recherche Médicale, France.  
2008- 2018 Visiting Professorship, Laboratory of Cell Biology, The Rockefeller University, USA.  
2013- Visiting Professorship, Department of Hematology, West China Hospital, Sichuan University

### Research Interests:

To elucidate the structure and function of Nuclear Pore Complex (NPC) in cells

### Honors:

2017- Fellow, The Royal Society of Medicine, United Kingdom.  
2017- Ambassador, American Society of Cell Biology, USA.

### Publications:

1. Lim KS and Wong RW *Cell Chem. Biol.* 2018 25(9):1056-1058.

# The nuclear pore complex: nanomachine as gatekeeper of molecular transport across nuclear membranes

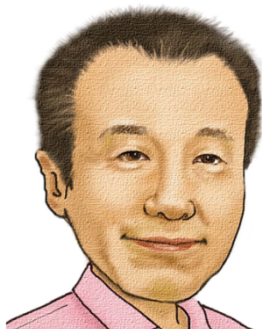
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A cell is like a country. The central government is the nucleus. On the surface of the nucleus, there are thousands of tiny turnstiles and gates (nuclear pores) that are embedded into nuclear membranes. These gates are multiprotein nuclear pore complexes (NPCs). NPCs are the macromolecular turnstiles between the cytoplasm and the nucleus that control the trafficking of proteins, RNAs and viruses. The giant NPC structures are extremely complex [1]. NPCs modulate nuclear shape and organization and have implicated in various cellular activities, such as transcription, autophagy [2], mitosis [3] and cell fate determination [4]. Small molecules can pass freely through NPCs; in contrast, larger molecules (>40 kDa) can pass through the pore smoothly only if they are bound to specific transporter proteins that interact with FG (phenylalanine-glycine)-nucleoporins (FG-Nups), which form a soft and flexible lining composed of approximately 200 intrinsically disordered polypeptide chains inside the turnstile. Several FG-Nup trafficking models have been proposed, such as forest, virtual gating, polymer brush, and selective phase/hydrogel models. Unfortunately, there has been a lack of techniques that can visualize and investigate FG-Nup behavior inside the NPCs of cancer cells. Recently, we show that high-speed atomic force microscopy (HS-AFM) can be used to visualize dynamic changes in nuclear pores in cancer cells [5]. We showed nanoscopic changes of the symmetric pores by morphometric analysis in colon cancer cell. To determine FG-Nup behavior, we concentrated on the central turnstile enclosed by the cytoplasmic filaments. We visualized that FG-Nups are short, stiff, hair-like, twisted ropes having a brush-like manner or forming a broken cobweb shape from their tethering point. We propose a “spiders-spidermen working inside the sticky cobweb” model for the process of central channel shuttling or gene gating by the nuclear pore [5].

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### Education:

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1987-1990	Assistant Professor, Department of Dermatology, Osaka University
1990-1993	Assistant Professor, Department of Biology, Kyushu University
1993-1994	Assistant Professor, Biomedical Research Center, Osaka University
1994-2007	Associate Professor, Graduate School of Medicine, Osaka University
2007-present	Professor, Cancer Research Institute, Kanazawa University
2017-present	Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University

### Honors:

1992	Academic Incitement Award of Japanese Industrial Hygiene Dermatology Association
1996	JB Award of the Japanese Biochemical Society
1997	Incitement Award of the Japanese Cancer Association The 3rd Bio Business Competition JAPAN, The Special Award Nature Medicine-AnGes MG BioMedical Award (Main Award)
2014	The Commendation for Science and Technology by MEXT, Prizes for Science and Technology

### Publications:

1. MiaoW, Sakai K, Imamura R, Ito K, Suga H, Sakuma T, Yamamoto T, Matsumoto K. MET activation by a macrocyclic peptide agonist couples to biological responses differently from HGF in a context-dependent manner. *Int J Mol Sci*, 19: 3141, 2018.
2. Imamura R, Matsumoto K. Hepatocyte growth factor in physiology and infectious diseases. *Cytokine*, 98: 97-106, 2017.
3. Adachi E, Sakai K, Nishiuchi T, Imamura R, Sato H, Matsumoto K. Cell-autonomous changes in Met receptor expression regulate the growth and metastatic characteristics in malignant melanoma. *Oncotarget*, 7: 70779-70793, 2016.
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# Macrocyclic Peptide Technology Targeting HGF and MET receptor

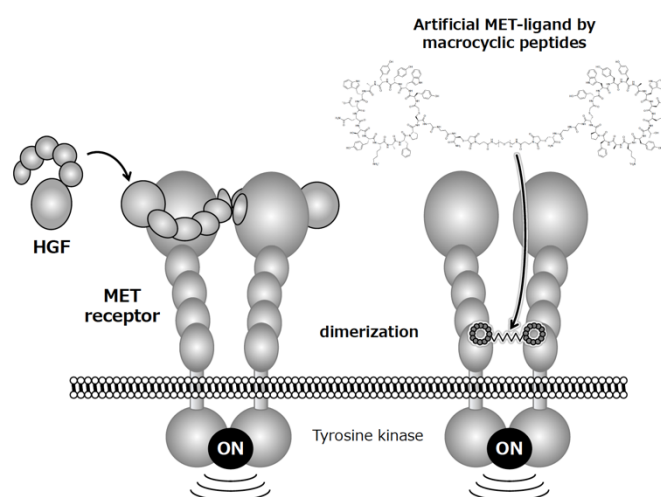
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Hepatocyte growth factor (HGF) is bioactive protein composed of 697 amino acids. HGF binds to and activates the MET transmembrane receptor. HGF-induced activation of the MET receptor drives mitogenesis (cell proliferation), motogenesis (cell migration), morphogenesis (3-D tubulogenesis), and cell survival, thereby participates in regeneration and protection of tissues, including the liver and nervous tissue. Clinical trials using recombinant HGF protein are ongoing for treatment of spinal cord injury and amyotrophic lateral sclerosis. In cancer tissues, aberrant activation of MET receptor by amplification of MET gene and/or overexpression of HGF participate in malignant progression of cancer such as drug resistance and invasion-metastasis.

Using RaPID (Random Peptide Integrated Discovery) system, an innovative drug discovery platform, we obtained macrocyclic peptides that specifically bind to HGF or MET with 1 - 10 nM Kd values. The cross-linking of MET-binding macrocyclic peptides, thereby capable of bivalent display of MET-binding peptides, conferred them an ability to selectively activate MET [1, 2]. These artificial MET-ligands induced dimerization and activation of MET receptor, and exhibited mitogenic, motogenic, and morphogenic activities that are comparable to native ligand HGF. Our approach for generating bivalent macrocyclic peptides as non-protein ligands for cell surface receptors may be useful for developing surrogate ligands with a broad range of potential applications.

HGF is secreted extracellularly as biologically inactive single-chain HGF (scHGF). Protease-mediated processing into two-chain HGF (tcHGF) is critical step for activation of HGF. In normal tissues, HGF exists as inactive scHGF, while tcHGF is generated in restricted tissues such as cancer tissues. The selective detection and inhibition of tcHGF are important for cancer diagnosis and therapeutics.



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