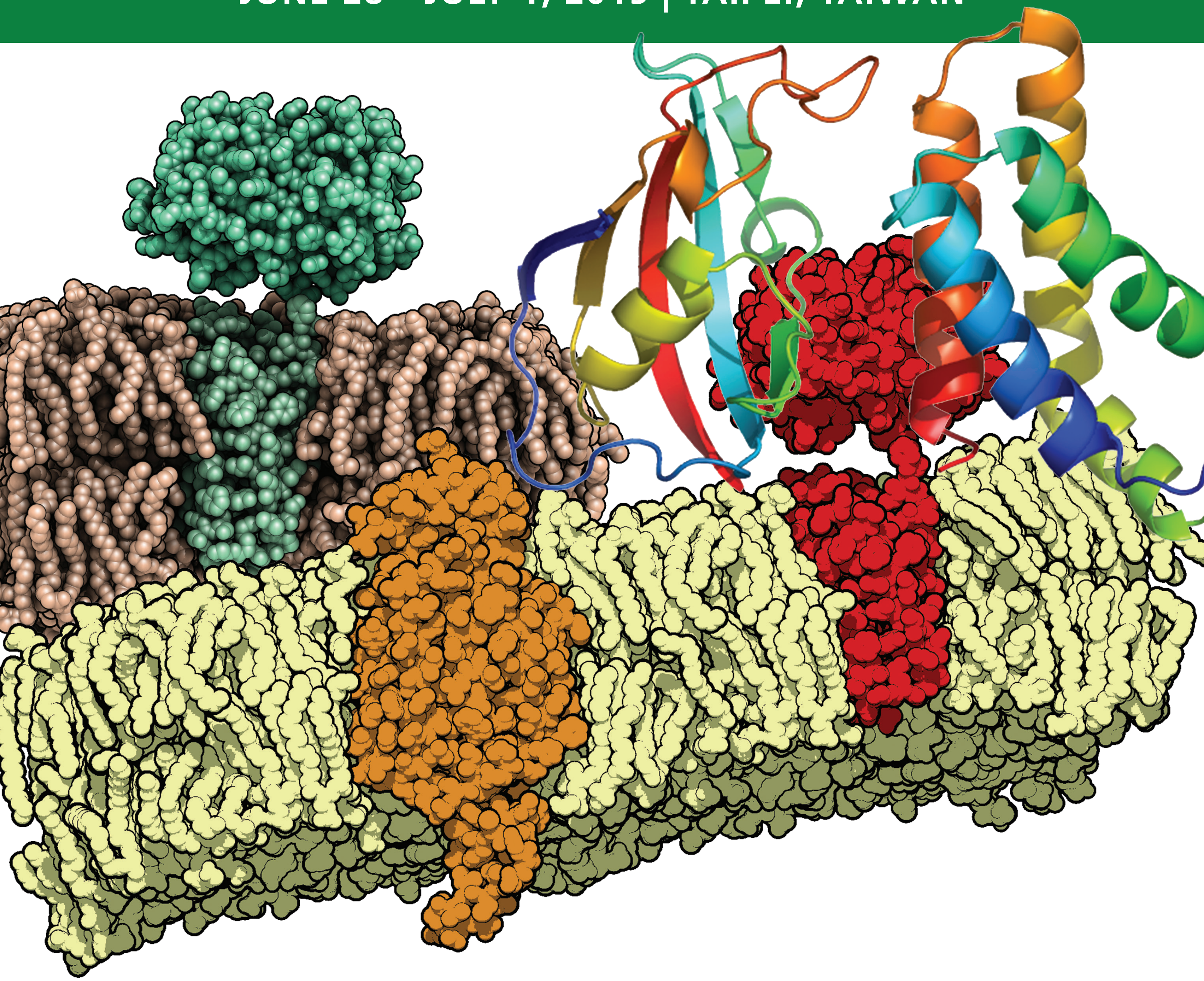
Biophysical Society Thematic Meeting | PROGRAM AND ABSTRACTS

New Biological Frontiers Illuminated by Molecular Sensors and Actuators

JUNE 28 – JULY 1, 2015 | TAIPEI, TAIWAN



Biophysical Society

科技部 Ministry of Science and Technology

中央研究院 ACADEMIA SINICA 1928

國立臺灣大學 敦品勵學 愛國愛人

Organizing Committee

Takanari Inoue, Johns Hopkins University, USA

Robert E. Campbell, University of Alberta, Canada

Chia-Fu Chou, Institute of Physics, Academia Sinica, Taiwan

Jin-Der Wen, National Taiwan University, Taiwan

June 2015

Dear Colleagues,

We would like to welcome you to the BPS Thematic Meeting on ***New Biological Frontiers Illuminated by Molecular Sensors and Actuators***. This landmark international meeting brings together 25 of the leading minds in the areas of biological fluorescence imaging, biological tool development, and implementation of optogenetics, all of which are used to resolve fundamental biological questions that otherwise cannot be addressed by conventional techniques alone. We have more than 96 participants from all over the world. The goal of this meeting is to provide the attendees with a map of the new, and largely unexplored, landscape of powerful optical and chemical tools that enable researchers at the frontier to peer into and harness biological systems with a level of control that was previously unimaginable.

More specifically, the recent emergence of biosensors based on fluorescent proteins, along with optogenetic and chemical dimerization techniques, have created the ability to both visualize and perturb biochemistry in live cells. In coming years we can expect complementary technologies based on magnetic- and force-based stimulation to join optical and chemical techniques as indispensable research tools for gaining novel insights into cellular biology. Due to the multidisciplinary applications of these tools, this meeting is attracting an extremely diversified audience from the fields of biotechnology, method development, chemical biology, material science, cell biology, general chemistry and engineering, computational biology, and synthetic biology. This meeting is thus expected to catalyze the exchange of ideas among this unusually diverse community, thus offering lively, inspiring opportunities for unconventional research collaborations.

We also encourage you to take part in social and cultural activities, because Taipei has a lot to offer. Thank you all for joining our meeting, and we look forward to having a very enjoyable four-day event together!

Sincerely,

Takanari Inoue Jin-Der Wen Robert E. Campbell Chia-Fu Chou

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GENERAL INFORMATION

Registration Hours

The registration desk is located in the lobby area near the Socrates Room at the GIS Convention Center at National Taiwan University (NTU). Registration hours are as follows:

Sunday, June 28	2:00 PM – 6:00 PM
Monday, June 29	8:00 AM – 6:00 PM
Tuesday, June 30	8:00 AM – 6:00 PM
Wednesday, July 1	8:00 AM – 12:00 PM

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in Socrates Room. Speakers are required to bring their laptops. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) All poster sessions will held in Plato Room.

2) A display board measuring 85cm (2.8 feet) wide by 145 cm (4.8 feet) high will be provided for each poster. Poster boards are numbered according to the same numbering scheme as in the abstract book.

3) All posters will be presented on both Monday, June 29 and Tuesday, June 30, from 1:00-2:00 PM. Posters should be set up on the morning of June 29 and removed by 6:00 PM on June 30.

4) During the poster session, presenters are requested to remain in front of their poster boards to meet with attendees.

5) All posters left uncollected at the end of the meeting will be disposed of.

Coffee Breaks

Coffee breaks will be held outside of Socrates Room where tea, coffee, and snacks will be provided.

Internet

Wi-Fi access is available in the Plato Room.

Smoking

Please be advised that smoking is not permitted inside the GIS Convention Center.

Meals

Welcome reception (June 28), luncheons (June 29 and June 30), and the banquet are included in the registration fee.

Name Badges

Name badges are required to enter all scientific sessions and poster sessions. Please wear your badge throughout the conference.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from June 28-July 1 during registration hours.

In case of an emergency, you may contact the following organizers/staff:

Jin-Der Wen
Office: +886-2-33662486
Cell: +886-987-239796

New Biological Frontiers Illuminated by Molecular Sensors and Actuators

Taipei, Taiwan
June 28-July 1, 2015

PROGRAM

Scientific sessions will be held in the Socrates Room and the Poster Sessions in the Plato Room in the GIS Convention Center at NTU unless otherwise noted.

Sunday, June 28, 2015

Welcome/Opening Session

6:00 – 7:00 PM	**Welcome Reception**	**Plato**
7:30 – 7:45 PM	Introduction of the Meeting Organizers	
	Welcome/Opening Remarks by Pan-Chyr Yang, President of the National Taiwan University	
	Introduction of Keynote Speaker by Robert E. Campbell, University of Alberta, Canada	
7:45 – 8:45 PM	Keynote Speaker: Atsushi Miyawaki, RIKEN, Japan *Cruising Inside Cells*	

Monday, June 29, 2015

Session 1	**Harnessing and Manipulating Cellular Processes (I)** Discussion Leader: Robert E. Campbell, University of Alberta, Canada
8:30 – 9:00 AM	Wei-Yuan Yang, Academia Sinica, Taiwan *Optogenetic Investigation of Organelle Quality Control*
9:00 – 9:30 AM	Adam Cohen, Harvard University, USA *All-Optical Electrophysiology with Microbial Rhodopsins*

9:30 – 9:45 AM	Khalid Salaita, Emory University, USA***** *Optomechanical Actuators for Controlling Mechanotransduction in Living Cells*	
9:45 – 10:00 AM	Toru Komatsu, The University of Tokyo, Japan ***** *Synthetically Rerouting Phagocytosis by Rapidly Turning Inert Cells into "Eat You" Mode*	
10:00 – 10:30 AM	**Coffee Break**	**Socrates Lobby**
Session 2	**Harnessing and Manipulating Cellular Processes (II)** Discussion Leader: Wei-Yuan Yang, Academia Sinica, Taiwan	
10:30 – 11:00 AM	Hsiao-Chun Huang, National Taiwan University, Taiwan *Organization of Intracellular Reactions with Heterologous Protein Scaffold*	
11:00 – 11:30 AM	Takeharu Nagai, Osaka University, Japan *Revolutionary Bioimaging with Bright Luminescent Proteins — Comparing Pros and Cons of Fluorescence and Luminescence*	
11:30 AM – 12:00 PM	Michael Lin, Stanford University, USA *Photodissociable Photoswitchable Dimeric Fluorescent Proteins Enable Optical Control of Kinase Activity*	
12:00 – 1:00 PM	**Lunch**	**Chopstix Restaurant**
1:00 – 2:30 PM	**Poster Session I**	**Plato**
Session 3	**Seeing the Unseen in vivo (I)** Discussion Leader: Chia-Fu Chou, Academia Sinica, Taiwan	
2:30 – 3:00 PM	Etsuko Kiyokawa, Kanazawa University, Japan *FRET Imaging in Organoids and Mice*	
3:00 – 3:30 PM	Ian Liau, National Chiao Tung University, Taiwan *Multidisciplinary Optical Approach to Zebrafish Targeting Cardiovascular Research*	
3:30 – 3:45 PM	Francois St-Pierre, Stanford University, USA ***** *Imaging Subcellular Voltage Dynamics in vivo with Improved Genetically Encoded Indicators*	

**Short talks selected from among submitted abstracts*

3:45 – 4:00 PM	Yu-Fen Chang, University of Oxford, United Kingdom *Genetically Encoded Tools to Manipulate and Observe Cellular Dynamics in Cardiac Disease Modelling and Drug Screening*
4:00 – 4:30 PM	**Coffee Break** **Socrates Lobby**
Session 4	**Seeing the Unseen in vivo (II)** Discussion Leader: Ian Liau, National Chiao Tung University, Taiwan
4:30 – 5:00 PM	Zhihong Zhang, Wuhan National Laboratory for Optoelectronics, China *Intravital Microscopy of Fluorescent Protein Model Antigen-elicited Specific Immune Response*
5:00 – 5:30 PM	Oliver Griesbeck, Max Planck Institute of Neurobiology, Germany *Ratiometric in vivo Imaging with "Twitch" Calcium Sensors*
5:30 – 6:00 PM	Ann-Shyn Chiang, National Tsing Hua University, Taiwan *Mapping Memory Circuits in the Drosophila Brain*
6:00 – 7:30 PM	**Dinner on own**
Session 5	**Short Talk Session** Discussion Leader: Jin-Der Wen, National Taiwan University, Taiwan
7:30 – 7:45 PM	Yu Chun Lin, National Tsing Hua University, Taiwan ***** *Rapidly Rewriting Tubulin Codes inside Primary Cilia*
7:45 – 8:00 PM	Ahmed S. Abdelfattah, University of Alberta, Canada ***** *Expanding the Toolbox of Genetically Encoded Voltage Indicators*
8:00 – 8:15 PM	Tomoki Matsuda, Osaka University, Japan ***** *Photo-Manipulation of Intracellular Ca^{2+} by Genetically Encoded Caged Ca^{2+}*
8:15 – 8:30 PM	Shixin Liu, University of California, Berkeley, USA ***** *Complete Kinetic Dissection Reveals the Rate-limiting Mechanism of Transcription Elongation by RNA Polymerase II*
8:30 – 8:45 PM	Chia-Fen Hsieh, Academia Sinica, Taiwan ***** *Probing the Dynamics of Raft Lipids Induced by Receptor-mediated Signaling in Living Cells*

**Short talks selected from among submitted abstracts*

8:45 – 9:00 PM	James Johnson, University of British Columbia, Canada * *Insulin Receptor and IGF1 Receptor Biosensors Employing Between-Domain Fluorescence Tags*

Tuesday, June 30, 2015

Session 6	**Illuminating Novel Biology (I)** Discussion Leader: Takanari Inoue, Johns Hopkins University, USA
8:30 – 9:00 AM	Tobias Meyer, Stanford University, USA *Molecular Choreography of Polarity Regulation in Cell Migration*
9:00 – 9:30 AM	Kenzo Hirose, University of Tokyo, Japan *Synaptic Function Illuminated by a Hybrid-Type Fluorescent Glutamate Probe*
9:30 – 9:45 AM	Yuning Hong, University of Melbourne, Australia * *Revealing Proteostasis Capacity in Cells by a Fluorescent Sensor*
9:45 – 10:00 AM	Kenneth Madsen, University of Copenhagen, Denmark * *Investigating Cellular Activity in Dissociated Neuronal Cultures Using Novel pH and PKA Activity Biosensors*
10:00 – 10:30 AM	**Coffee Break** **Socrates Lobby**
Session 7	**Illuminating Novel Biology (II)** Discussion Leader: Tobias Meyer, Stanford University, USA
10:30 – 11:00 AM	Jin Zhang, Johns Hopkins University, USA *Illuminating Biochemical Activity Architecture of the Cell*
11:00 – 11:30 AM	Yulong Li, Peking University, China *Spying Neurotransmitter Release by New Genetically-encoded Indicators*
11:30 AM – 11:45 AM	Junji Suzuki, The University of Tokyo, Japan * *Imaging Intraorganellar Ca^{2+} at Subcellular Resolution Using CEPIA*
11:45 AM – 12:00 PM	Marcus Wilhelmsson, Chalmers University of Technology, Sweden * *Sensing and Structure Investigations of Nucleic Acid Systems Using Fluorescent Base Analogue FRET-Probes*

**Short talks selected from among submitted abstracts*

12:00 – 1:00 PM	**Lunch**	**Lacuz Restaurant**
1:00 – 2:30 PM	**Poster Session II**	**Plato**
Session 8	**Making the Invisibles Visible (I)** Discussion Leader: Lee-Wei Yang, National Tsing Hua University, Taiwan	
2:30 – 3:00 PM	Jung-Chi Liao, Academia Sinica, Taiwan *Actuators within Sensors: The Architecture of Primary Cilia*	
3:00 – 3:30 PM	Stephen Michnick, Université de Montréal, Canada *Imaging Spatiotemporal Dynamics of the Invisible Protein Interactome*	
3:30 – 4:00 PM	Janet Iwasa, University of Utah, USA *Bringing Molecular Mechanisms to Life with 3D Animation*	
4:00 – 4:30 PM	**Coffee Break**	**Socrates Lobby**
Session 9	**Making the Invisibles Visible (II)** Discussion Leader: Stephen Michnick, Université de Montréal, Canada	
4:30 – 5:00 PM	Lee-Wei Yang, National Tsing Hua University, Taiwan *Theories Describing Sensors and Responders Reveal Important Allosteric Sites in Enzymes*	
5:00 – 5:30 PM	Mark Prescott, Monash University, Australia *Genetically Encoded Dark Acceptors for Use in FRET and pcFRET Applications*	
5:30 – 5:45 PM	Wei Zhang, University of Alberta, Canada * *Developing Genetically Encoded Actuators with an Engineered Photocleavable Protein, PhoCle*	
5:45 – 6:00 PM	Yingqi Wang, Tsinghua University, China * *Self-referenced Quantitative FRET with Dual-switchable Donor-Acceptor Pair*	
7:00 – 9:00 PM	**Banquet**	**La Maree Restaurant**

**Short talks selected from among submitted abstracts*

Wednesday, July 1, 2015

Session 10	**Physical Approaches to Illuminate Novel Cellular Properties (I)** Discussion Leader: Sanjay Kumar, University of California, Berkeley, USA
8:30 – 9:00 AM	Maxime Dahan, Institut Curie, France *Magnetic Control of Intracellular Signaling*
9:00 – 9:30 AM	Yves de Koninck, Institut Universitaire en Santé Mentale de Québec, Canada *Novel Fibreoptics Probes for in vivo Optogenetics: From Single Cells to Hard-to-Get-to Areas of the Nervous System*
9:30 – 9:45 AM	Hang Yin, University of Colorado Boulder, USA * *Chemical Biology Sensors for Membrane Curvature and Lipid Composition*
9:45 – 10:00 AM	Yi-Lan Chen, National Taiwan University, Taiwan ** *Observing the Helicase Activity of Ribosome 30S during Translation Initiation by Using Optical Tweezers*
10:00 – 10:30 AM	**Coffee Break** **Socrates Lobby**
Session 11	**Physical Approaches to Illuminate Novel Cellular Properties (II)** Discussion Leader: Maxime Dahan, Institut Curie, France
10:30 – 11:00 AM	Cees Dekker, Delft University of Technology, The Netherlands *Nanofabrication as a Tool to Study the Effects of Cell Shape on Chromosome and Protein Organization in Bacteria*
11:00 – 11:30 AM	Sanjay Kumar, University of California, Berkeley, USA *Cutting the Tension with a Laser: Biophotonic Dissection of Stress Fibers and Contractile Signaling*
11:30 AM – 12:00 PM	Zoher Gueroui, École Normale Supérieure (ENS), Paris, France *Engineering Spatial Gradient of Signaling Proteins Using Magnetic Nanoparticles*
12:00 PM	**Closing Remarks and *Biophysical Journal* Poster Awards**

**Short talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

Cruising Inside Cells

Atsushi Miyawaki.
RIKEN, Wako, Japan.

The behavior of biomolecules 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. There is some concern that a whale fitted with a transmitter may be given the cold shoulder and thus ostracized by other whales. In live cell imaging, a fluorescent probe replaces a transmitter. We label a fluorophore on a specific region of a biomolecule and bring it back into a cell. We then visualize how the biomolecule behaves. 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 serendipitous findings can arise out of such a sportive mind, a frame of mind that prevails when enjoying whale-watching.

Optogenetic Investigation of Organelle Quality Control

Wei Yuan Yang.
Academia Sinica, Taipei, Taiwan.

Autophagy is a trafficking process that enables eukaryotic cells to transport cytoplasmic materials into lysosomes for recycling and degradation, and one of its prominent functions is the selective elimination of dysfunctional organelles. This allows cells to rid themselves of unwanted stress to maintain health and avoid the activation of cell death. One vivid example of this is Parkin-mediated mitophagy: mitochondria defective in protein import results in their selective elimination through the autophagic machinery. The lack of proper mitophagy has been implicated as one cause of Parkinson's disease. Autophagy is therefore a vital strategy for cellular quality control of organelles. Here I will discuss the prospect of utilizing organelle-specific photosensitizers to probe these processes inside living cells.

All-optical Electrophysiology with Microbial Rhodopsins

Adam Cohen.
Harvard, Cambridge, USA.

In the wild, microbial rhodopsin proteins convert sunlight into biochemical signals in their host organisms. Some microbial rhodopsins convert sunlight into changes in membrane voltage. We engineered a microbial rhodopsin to run in reverse: to convert changes in membrane voltage into fluorescence signals that are readily detected in a microscope. Archaerhodopsin-derived voltage-indicating proteins enable optical mapping of bioelectric phenomena with unprecedented speed and sensitivity. We are engineering new molecular logic into microbial rhodopsins by taking advantage of their strong optical nonlinearities. For instance, we engineered a bistable rhodopsin into a light-gated voltage integrator which converts a transient electrical impulse into a stable photochemical product.

Optomechanical Actuators for Controlling Mechanotransduction in Living Cells

Khalid Salaita[1,2].
[1]Emory University, Atlanta, USA, [2]Georgia Institute of Technology & Emory University, Atlanta, GA, USA.

See abstract: Pos-37 Board 37

Synthetically Rerouting Phagocytosis by Rapidly Turning Inert Cells into "Eat You" Mode

Toru Komatsu[1,4], Hiroki Onuma[1], Tetsuo Nagano[2], Yasuteru Urano[1,3].Takanari Inoue[5].
[1]The University of Tokyo, Tokyo, Japan, [3]The University of Tokyo, Tokyo, Japan, [4]JST PRESTO, Tokyo, Japan, [5]Johns Hopkins University, Baltimore, MD, USA.[2]The University of Tokyo, Tokyo, Japan,

See Abstract: Pos-19 Board 19

Organization of Intracellular Reactions with Heterologous Protein Scaffold

Hsiao-Chun Huang.
National Taiwan University, Taipei, Taiwan.

Prokaryotes lack membranous organelles to compartmentalize biochemical reactions. To optimize the efficiency of engineered metabolic pathways in E.coli, artificial organelles based on porous protein shells and synthetic RNA/protein scaffold have been proposed to bring metabolic enzymes to close proximity and thus speed up reactions. In this study, we aim to develop, in E.coli, a unique protein scaffold to spatially assemble pathways of interest, based on heterologous expression of Caulobacter crescentus proteins, PopZ and SpmX. We have cloned and standardized both PopZ and SpmX genes from Caulobacter crescentus, and created fluorescent fusions to validate their localization and capability as molecular scaffold in E.coli. We scanned different expression levels of PopZ and established stable formation of PopZ complex in E.coli. We found that SpmX remained diffused throughout when it was singly expressed in E.coli. When SpmX was co-expressed with PopZ, it then co-localized with PopZ foci, suggesting that there is direct interaction between PopZ and SpmX. We then tested if SpmX can serve as the adaptor for PopZ to bring different proteins of interest to close proximity. As a proof of concept, we performed bimolecular fluorescence complementation. No fluorescence was observed when these two split fluorescent fusion proteins were co-expressed. Fluorescent foci was detected when PopZ is present, strongly suggesting that PopZ complex can serve as a molecular scaffold to spatially assemble pathways via SpmXΔC adaptors. With this scaffold device, we plan to assemble and optimize the efficiency of a foreign metabolic pathway in E.coli. We anticipate this system to be user-friendly in wide range of microbial metabolic engineering applications.

Revolutionary Bioimaging with Bright Luminescent Proteins - Comparing Pros and Cons of Fluorescence and Luminescence

Takeharu Nagai.
Osaka University, Osaka, Japan.

Fluorescent proteins is an indispensable tool for live imaging of cells and cell structures. But the requirement for external illumination definitely precludes its universal application because it can cause problems including photobleaching, photodamage and the unintended activation of other light-responsive proteins. Luminescent proteins such as luciferase is an alternative to fluorescence that does not require an excitation light. However, luminescence imaging has been limited by the dim brightness and lack of color variation of existing luminescent proteins. To overcome this drawback, we conducted random mutagenesis on Renilla reniformis luciferase (Rluc) gene to improve the intensity. Then, the luminescence intensity was further increased by fusion of the improved Rluc to a yellow fluorescent protein Venus with a high BRET efficiency. The chimeric protein showed much brighter luminescence than the original Rluc, enabling not only real-time imaging of intracellular structures in living cells with spatial resolution equivalent to fluorescence but also sensitive tumor detection in freely moving mice which has never been possible before. We also developed color variants of the Nano-lantern by substitution of the Venus with a different wavelength fluorescent protein. Furthermore we applied these Nano-lanterns to design Ca2+, cAMP, and ATP indicators, thereby we succeeded imaging these bioactive molecules in environments where fluorescent indicators have failed. These luminescent proteins will revolutionize conventional bioimaging by allowing visualization of biological phenomena not seen before at the single-cell, organ, and whole-body level, in animals and plants. While luminescence may be a practical alternative in situations where fluorescence is problematic, the signal intensity of the Nano-lanterns is still more than 100 times weaker than fluorescent proteins. In the symposium, I will compare pros and cons of fluorescence and luminescence for bioimaging.

Photodissociable Photoswitchable Dimeric Fluorescent Proteins Enable Optical Control of Kinase Activity

Michael Lin
Stanford University, USA.

About twenty years ago, the cloning of fluorescent proteins catalyzed a revolution in biological research. With ideal characteristics as tags and as components of reporter proteins, fluorescent proteins enabled visualization of biological processes in living cells, with spatial detail and in real time. A second optical revolution is now in the making, with ongoing efforts to use light to control rather than to sense biological activities. While examples exist of adapting natural photoregulatory to regulate biology in mammalian cells, this approach has limitations. We have been exploring the hypothesis that fluorescent proteins can be engineered into ideal photoregulatory proteins as well. We recently engineered the photoswitchable fluorescent protein Dronpa into a photodissociable tetramer, then developed a design for caged proteins in which two copies of Dronpa are fused to a protein of interest so that tetramerization blocks protein activity and photodissociation activates it. We will present recent advances in improving the performance of photodissociable Dronpa domains and in generalizing the caged protein design to an important class of regulatory proteins, the kinases.

FRET Imaging in Organoids and Mice

Etsuko Kiyokawa.
Kanazawa Medical University, Kahoku-gun, Japan.

Based on the principal of fluorescence resonance energy transfer (FRET), we are now able to observe the protein activities in the living cells. Using MDCK organoid and the FRET biosensor for small GTPase Rac1, we found Rac1activity is higher at the lateral than the apical plasma membrane in the mature cyst. Elevating the Rac1 activity at the apical membrane induced the luminal cell filling, indicating that the suppression of the Rac1 is required for maintenance of the epithelial structures. The transgenic mice expressing the FRET biosensors were established and recent development of two photon microscopy enables us to observe individual cells in the living mice. We time-lapse-imaged the activities of extracellular signal-regulated kinase (ERK) and protein kinase A (PKA) in neutrophils in inflamed intestinal tissue. ERK activity in neutrophils rapidly increased during spreading on the endothelial cells and showed positive correlation with the migration velocity on endothelial cells or in interstitial tissue. We are currently trying to observe cancer cell migration in the liver. References1. Mizuno R., KamiokaY., Kabashima K., Imajo M., Sumiyama K., Nakasho E., Ito T., Hamazaki Y., Okuchi Y., Sakai Y., Kiyokawa E., Matsuda M.: In vivo imaging reveals PKA regulation of ERK activity during neutrophil recruitment to inflamed intestines. J Exp Med: 211(6):1123-36, 2014.2. Yagi S., Matsuda M., Kiyokawa E.: Suppression of Rac1 activity at the apical membrane of MDCK cells is essential for cyst structure maintenance. EMBO Rep: 13(3), 237-243, 2012

Multidisciplinary Optical Approach to Zebrafish Targeting Cardiovascular Research

Ian Liau.
National Chiao Tung University, Hsinchu, Taiwan.

Relative to other common model animals, the zebrafish (Danio rerio) possesses numerous unique features such as rapid development, genetic tractability, low cost of maintenance and optical transparency at the larval stage. The underlying function and gene of its cardiovascular system is similar to that of human beings, further making it particularly attractive for fundamental study or for drug screen targeting cardiovascular diseases. Toward this end, we have developed multidisciplinary optical approaches to assess the cardiovascular function of zebrafish. With pseudodynamic 3D imaging, we determined precisely the cardiac function of zebrafish. We show that the conventional 2D approach tends to overestimate the cardiac parameters and to produce results of greater variation. As demonstration, we characterized zebrafish subject to pharmacological interventions of varied cardiac activities and evaluated a zebrafish model of cardiomyopathy. We found that the cardiac function of zebrafish exhibits pharmacological responses similar to human beings. With image-guided Raman micro-spectroscopy, we interrogated the pharmacological response of hypercholesterolemia zebrafish subject to two commonly prescribed anti-hyperlipidemic drugs (ezetimibe and atorvastatin) in situ and in vivo. While the treatment of either drug alone decreased the vascular deposition of lipids, only atorvastatin exerted a profound anti-oxidative effect on vascular fatty lesions. Beyond its efficacies in suppressing both the accumulation and oxidation of vascular lipids, atorvastatin expedited the clearance of vascular lipids. The pleotropic therapeutic effect of atorvastatin observed on zebrafish is notably consistent with the known pharmaceutical effects of this drug on human beings. In view of the growing interest of using zebrafish in both fundamental and applied cardiovascular research, we envisage that multidisciplinary optical approach should benefit fields ranging from investigation of the pathophysiology of cardiovascular diseases to a pharmaceutical evaluation of cardiac activity and toxicity.

Imaging Subcellular Voltage Dynamics in Vivo with Improved Genetically Encoded Indicators

Francois St-Pierre, Helen H. Yang, Xiaozhe Ding, Ying Yang, Thomas R. Clandinin, Michael Z. Lin.
Stanford University, Stanford, CA, USA.

See Abstract: Pos-41 Board 41

Genetically Encoded Tools to Manipulate and Observe Cellular Dynamics in Cardiac Disease Modelling and Drug Screening

Yu-Fen Chang, Frances Book, Mark J. Davies, Matthew J. Daniels.
University of Oxford, Oxford, United Kingdom.

See abstract: Pos-5 Board 5

Intravital Microscopy of Fluorescent Protein Model Antigen-Elicited Specific Immune Response

Zhihong Zhang.
Huazhong University of Science and Technology, Wuhan, China.

Intravital optical imaging provided a useful approach for clarifying how, when, and where the immune cells involved in tumor immunity. Model antigens have been widely used to simplify the investigation of complicated anti-tumor immune response. However, the classical model antigens (e.g., OVA) cannot be directly detected without fluorescent labeling. Thus, it's necessary to develop a visualized model antigen system based on fluorescent protein itself for the intravital imaging of tumor immunity. Fluorescent protein KatushkaS158A displayed perfect optical characters and was quite suitable for optical imaging in vivo. The data indicated that it elicited both cellular and humoral immune response in the immunized C57BL/6 mice, resulting in the attenuation of the tumorigenesis of KatushkaS158A-expressing melanoma cells (K-B16) in vivo. To visualize the specific anti-tumor immune response in tumor microenvironment, EGFP-transgenic C57BL/6 mice were immunized twice with IFA-emulsified KatushkaS158A on Day 14 and Day 7 before the implantation of K-B16 cells into the dorsal skin-fold window chambers. The intravital imaging data indicated that strong and specific immune response against K-B16 cells was occurred in the KatushkaS158A-immunized mice at 2 days after the implantation of K-B16 cells, which swarms of EGFP+ immunocytes rushed toward the tumor cells with significantly higher motility and retained in the middle of the tumor area with high density. These responsive immunocytes eliminated K-B16 cells and blocked the growth of melanoma cells in vivo. Thus, KatushkaS158A protein was not only acted as a fluorescent marker for tumor imaging, but also used as a model antigen to elicit specific tumor immune response in C57BL/6 mice.

Ratiometric in vivo Imaging with "Twitch" Calcium Sensors

Oliver Griesbeck.
Max-Planck-Institute of Neurobiology, Martinsried, Germany.

I will describe a collection of FRET-based calcium biosensors with a minimized calcium binding domain and thus a reduced number of calcium binding sites per sensor. They are based on the C-terminal lobe of Troponin C and were characterized by NMR and SAXS. Their FRET responses were optimized by a large scale functional screen in bacterial colonies, refined by a secondary screen in hippocampal neurons. Further improvements in brightness lead to sensors with excellent properties in vivo. When imaging neuronal activity in mouse cortex and olfactory bulb the performance of the most sensitive variants matched that of synthetic calcium dyes. Moreover, improved Twitch sensors allowed for high resolution imaging of calcium fluctuations during tissue migration and activation of T-lymphocytes upon encountering their antigen. The sensitivity, brightness, biocompatibility and linear response properties should make them widely useful for cellular imaging applications.

Mapping Memory Circuits in the Drosophila Brain

Ann-Shyn Chiang.
National Tsing Hua University, Hsinchu, Taiwan.

Long-term memory (LTM) involves gene activation and new protein synthesis that alters synaptic connections between neurons. Knowing where these genes and proteins interact is critical for understanding LTM formation. Recently, we showed that new proteins in the mushroom body (MB) efferent MB-V3 and afferent DAL neurons are necessary for LTM formation in Drosophila. Here, using a temperature-sensitive ribosomal-cleavage toxin to block protein synthesis, we report an ensemble of neurons where new proteins induced after learning are necessary for LTM formation. Monitoring gene activities with a photoconvertible fluorescent protein KAEDE, we showed that different memory neurons use different gene products at different times during memory formation. Our findings begin to reveal a spatiotemporal neural ensemble storing protein-synthesis-dependent LTM in the Drosophila brain.

Rapidly Rewriting Tubulin Codes inside Primary Cilia

Emily Su[1,2], Takanari Inoue[2,1], **Yu Chun Lin**[3,2].
[3]Institute of Molecular Medicine, Naitonal Tsing Hua Univesity, Hsinchu City, Taiwan.[1]Department of Biomedical Engineering, School of Medicine, Johns Hopkins University, Baltimore, MD, USA, [2]Department of Cell Biology, School of Medicine, Johns Hopkins University, Baltimore, MD, USA,

See abstract: Pos-25 Board 25

Expanding the Toolbox of Genetically Encoded Voltage Indicators

Ahmed S. Abdelfattah[1], Samouil L. Farhi[2], Yongxin Zhao[1], Daan Brinks[2], Adam E. Cohen[2], Robert E. Campbell[1].
[1]University of Alberta, Edmonton, AB, Canada, [2]Harvard University, Cambridge, MA, USA.

See abstract: Pos-1 Board 1

Photo-Manipulation of Intracellular Ca^{2+} by Genetically Encoded Caged Ca^{2+}

Tomoki Matsuda, Noritaka Fukuda, Takeharu Nagai.
Osaka University, Ibaraki, Osaka, Japan.

See abstract: Pos-32 Board 32

Complete Kinetic Dissection Reveals the Rate-Limiting Mechanism of Transcription Elongation by RNA Polymerase II

Shixin Liu[1], Manchuta Dangkulwanich[1], Toyotaka Ishibashi[1], Maria Kireeva[2], Lucyna Lubkowska[2], Mikhail Kashlev[2], Carlos Bustamante[1,3].
[1]University of California, Berkeley, Berkeley, CA, USA, [2]National Cancer Institute, Frederick, MD, USA, [3]Howard Hughes Medical Institute, Berkeley, CA, USA.

See abstract: Pos-27 Board 27

Probing the Dynamics of Raft Lipids Induced by Receptor-mediated Signaling in Living Cells

Chia-Fen Hsieh, Yii-Lih Lin[1,2], Chia-Fu Chou[1].
[1]Academia Sinica, Taipei, Taiwan, [2]National Taiwan University, Taipei, Taiwan.

See abstract: Pos-12 Board 12

Insulin Receptor and IGF1 Receptor Biosensors Employing Between-Domain Fluorescence Tags

James D. Johnson, Howard Cen, Søs Skovsø, Tobias Albrecht.
University of British Columbia, Vancouver, Canada.

See abstract: Pos-17 Board 17

Molecular Choreography of Polarity Regulation in Cell Migration

Tobias Meyer.
Stanford University, Stanford, USA.

We are investigating molecular mechanisms of polarization both in neutrophils and endothelial cells using fluorescent reporters and chemical and genetic perturbations. I will be presenting work on the order of events by which neutrophils rapidly transition from an unpolarized state to a polarized migrating state in a series of steps involving the small GTPases Cdc42, RhoA, Rac as well as Ras and PI3K. Once neutrophils are persistently migrating they steer their front towards chemotactic sources using primarily local Cdc42 and RhoA activities. In contrast to neutrophils, endothelial cells show two types of polarization, one operating in leader cells using a combination of small GTPases, Ca2+ and diacylglycerol signals and one operating in follower cells based on small GTPases that are locally directed by curved VE-cadherin-based membrane invaginations that we termed "Cadherin fingers". I will be presenting results on how curved membranes generated by these cadherin fingers help orient follower cells.

Synaptic Function Illuminated by a Hybrid-Type Fluorescent Glutamate Probe

Kenzo Hirose.
UTokyo, Tokyo, Japan.

To facilitate our understanding of the basic features of synaptic transmission, we have been developing a series of fluorescent glutamate probes named EOS. EOS is a hybrid type fluorescent probe consisting of a fluorescent dye and a glutamate binding domain of AMPA-type glutamate receptor. eEOS, our most recent version of EOS, was obtained by a combinatorial screening. eEOS has good photostability and a large dynamic range. Using eEOS, we successfully imaged glutamate release from single presynaptic terminals of cultured hippocampal neurons. The amounts of glutamate release under various conditions were analyzed to evaluate synaptic parameters that govern the quantal nature of neurotransmitter release. Results show that there exist multiple release sites at each synaptic terminal. There are large varieties in the number of the release sites as well as in release probability. Intriguingly the two synaptic parameters were not correlated. Thus each synapse has its own 'personality' characterized by these independent parameters. We then asked how nanoscale architectures formed by presynaptic proteins control neurotransmitter release. We undertook STORM microscopy for nanoscale visualization of presynaptic proteins. Notably, Mun13-1 molecules were found to assemble as small clusters; there were multiple Mun13-1 clusters per active zone. By combining the glutamate imaging technique and STORM microscopy, we directly counted and compared the number of the release sites and the number of Mun13-1 clusters. We found that these numbers were highly correlated. We also found that Muc13-1 clusters recruited syntaxin-1. These data indicate that Munc13-1 clusters are molecular entities for functional release. Reconstitution experiments in non-neuronal cells reveal that the formation of nanoclusters relies on self-organizing properties of Munc13-1 molecules sites. The self-organizing property of presynaptic molecule underlies presynaptic weights for synaptic computation.

Revealing Proteostasis Capacity in Cells by a Fluorescent Sensor

Yuning Hong.
University of Melbourne, Melbourne, Australia.

See abstract: Pos-11 Board 11

Investigating Cellular Activity in Dissociated Neuronal Cultures Using Novel pH and PKA Activity Biosensors

Thorvald F. Andreassen, Sofie E. Pedersen, **Kenneth Madsen**.
University of Copenhagen, Copenhagen, Denmark.

See abstract: Pos-31 Board 31

Illuminating Biochemical Activity Architecture of the Cell

Jin Zhang.
The Johns Hopkins University, Baltimore, USA.

It has become increasingly clear that cellular biochemical activities are compartmentalized in nanoscale domains that define the biochemical architecture of the cell. Despite advances in molecular sensors and optical imaging, direct interrogation of any minute activity domains at the molecular length scale remains a challenge. In this talk, I will focus on cAMP and Ca2+ regulated signaling activities and present studies where we combined genetically encoded fluorescent biosensors, superresolution imaging, targeted biochemical perturbations and mathematic modeling to probe the biochemical activity architecture of the cell.

Spying Neurotransmitter Release by New Genetically-encoded Indicators

Yulong Li.
Peking University, P.R.China, Beijing, China.

The nerve communication relies on the vesicular release of various neurotransmitters from presynaptic terminals. The various neurotransmitters are chemical diverse, but universally concentrated into small synaptic vesicles (SVs, diameter 40-50 nm) before their discharge upon the nerve activities. How high the concentration of the transmitters can reached inside the SVs? How to visualize their release, especially in vivo? When and how long would they act on postsynaptic neurons? We have developed various genetically-encoded sensors aiming to answer those questions. First, we have generated voltage sensors that specifically targeted to the SVs. These sensors allow us, for the first time, to measure the membrane potential across the SVs, unapproachable by conventional electrophysiological means. Because the uptake of neurotransmitter are determined by the proton-electrochemical gradients, with the known intravesicular pH and membrane potential, we could estimate the steady state neurotransmitter concentration. Secondly, to aid visualization of transmitter release in vivo, we have generated super pH sensitivity fluorescent protein (pHlamingo) with large apparent stokes shift. pHlamingo's excitation and emission peaks are at 460 nm and 610 nm, respectively. Its pH dependent fluorescent emission (pH7.5/pH5.5) is 20% higher than pHluorin. When targeting to SVs, pHlamingo's convenient excitation peak and superior pH sensitivity allows it to report the release of neurotransmitter release, especially for the in-vivo 2-photon microscopy settings. Finally, we have constructed sensors that can convert the ligand-dependent structural rearrangement of receptors to the folding of a fluorescent protein. When targeting to the neuronal cell surface, these sensors can sense transmitter release such as acetylcholine. We anticipate that the further optimization of the new generation sensors would provide critical help to deepen our understanding the regulation of neurotransmitter release in health and disease.

Imaging Intraorganellar Ca^{2+} at Subcellular Resolution Using CEPIA

Junji Suzuki[1], Kazunori Kanemaru[1], Kuniaki Ishii[2], Masamichi Ohkura[3], Yohei Okubo[1], Masamitsu Iino[1].
[1]The University of Tokyo, Bunkyo-ku, Tokyo, Japan, [2]Yamagata University, Iida-nishi, Yamagata, Japan, [3]Saitama University, Sakura-ku, Saitama, Japan.

See abstract: Pos-43 Board 43

Sensing and Structure Investigations of Nucleic Acid Systems Using Fluorescent Base Analogue FRET-Probes

Marcus Wilhelmsson.
Chalmers University of Technology, Gothenburg, Sweden.

See abstract: Pos-47 Board 47

Actuators within Sensors: The Architecture of Primary Cilia

Jung-Chi Liao, T T. Yang.
Academia Sinica, Taipei, Taiwan.

Primary cilia are cellular sensors associated with important signaling pathways including hedgehog signaling and Wnt signaling. Intraflagellar transport is actively regulated to deliver precursors and other molecules in primary cilia. Despite the importance, our knowledge of the ciliary architecture is limited, hindering a structure-based understanding of ciliary functions. Here we reveal the molecular architecture at the base of primary cilia using superresolution microscopy. We found that there are multiple levels of trafficking rests for intraflagellar transport proteins and transmembrane proteins, suggesting the gating regulation at the ciliary base is performed at different resting sites upon the architecture of primary cilia.

Imaging Spatiotemporal Dynamics of the Invisible Protein Interactome

Stephen Michnick.
Université de Montréal, Montréal, QC, Canada.

The last decade has witnessed great innovation in methods to detect the spatiotemporal dynamics of molecular and particularly protein-protein interactions in living cells and organisms. At the same time, our global understanding of protein interactomes suggests that protein-protein interactions are highly interconnected, perhaps forming irreducibly complex networks. This goes for interactions between post-translational modifying enzymes and their substrates. The seeming complexity of interactomes implies that development of truly specific reporters of any interactions in the cell is impossible; that in fact what these reporters detect are many "invisible" interactions among proteins having nothing to do with what we think we are detecting. In this presentation I will provide examples, comparing proteome wide detection versus specific reporters of signal transduction, which illustrate how complex a signaling interactome can be. I will then present the argument that lack of specificity of reporters does not render them useless. Used wisely reporters provide a window into the richness of biochemical activities in living cells and new ideas about how matter is organized in living processes.

Bringing Molecular Mechanisms to Life with 3D Animation

Janet Iwasa.
University of Utah, Salt Lake City, USA.

In recent years, there has been a rapid growth in the use of 3D animation as a means to communicate complex biological processes to a wide range of audiences. Using animation software from the entertainment industry, it is possible to synthesize data from diverse sources to create a coherent and contextualized view of how molecular and cellular systems operate. These visualizations have served not only to make molecular concepts more accessible to students and the public at large, but have also proven to be extremely useful for researchers seeking to build and refine their hypotheses. In an effort to make animation tools more readily available to researchers, we have embarked on a project to create a novel molecular biology-centric 3d animation application, called Molecular Flipbook, created specifically for cell and molecular biologists. In addition to provide an intuitive means to create molecular animations, Molecular Flipbook also allows users to share and view animations on an online database.

Theories Describing Sensors and Responders Reveal Important Allosteric Sites in Enzymes

Lee-Wei Yang.
National Tsing Hua University, Hsinchu, Taiwan.

Accumulated experimental and theoretical evidences have shown that protein functions as a physiochemically connected network. Allostery, understood in this new context, is a manifestation of residue communicating over remote sites via this network and hence a recently rising interest in identifying communication pathways mediating allosteric controls. In this study, we demonstrate that a new formulation of linear response theory (LRT) can describe a two-stage conformational relaxation- 1. ligand-induced conformational changes at a few tens to a hundred of picoseconds and 2. an early molecular 'twitch' that is faster than conformational relaxation by an order of magnitude. Predictions based on LRT agree with observations from site-specific UV resonance Raman, time-resolved X-ray and sound speed in a condensed medium. With the computational ease of the current implementation, we can easily perturb the protein network by thousands of times where time-resolved atomic trajectories can be tracked following each perturbation. Frequently used 'communication centers' are identified and it is found by experiments that mutations of these centers, many remote from the catalytic site, would greatly impact the hydride transfer rate in DHFR. Mutations on those that do not serve as communication centers impact the catalysis minimally. We also show the signal propagation is directional, highly anisotropic and need not be reciprocal. We favorably consider the method's applicable future in probing functionally sensitive distant mutants by the physical approach herein proposed.

Genetically Encoded Dark Acceptors for Use in FRET and pcFRET Applications

Craig Don Paul[1], Daouda Traore[1], Anne Pettikiriarachichi[2], Matthew Wilce[1], Seth Olsen[4], Csaba Kiss[3], Matthew Perugini[5], Toby Bell[1], Rodney Devenish[1], Andrew Bradbury[3], **Mark Prescott**[1]. [1]Monash University, Melbourne, Australia, [3]Los Alamos National Laboratory, Los Alamos, NM, USA, [5]La Trobe University, Melbourne, Victoria, Australia.[2]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia, [4]University of Queensland, Brisbane, Queensland, Australia,

Fluorescent proteins are available with optical properties that make them suitable for use as FRET donor/acceptor pairs. Many have been used to construct biosensors for monitoring cellular events. The use of a non-fluorescent or dark acceptor for FRET applications has a number of advantages, amongst which is facilitating the use of fluorescent probes to monitor additional parameters, otherwise precluded by the presence of emission from a fluorescent acceptor.

Here we report on the properties and application of two novel genetically encoded dark acceptors useful for FRET experiments. The first protein called Phanta, can be used as a photochromic FRET (pcFRET) acceptor and is best suited to FPs with green emission such as EGFP. Phanta has a very low fluorescence quantum yield ($\Phi F = 0.008$) and can be reversibly, and repeatedly photoswitched between one of two absorbing states (495 nm and 395 nm) on sequential exposure to cyan or violet light. pcFRET can be readily estimated from donor emission images acquired with Phanta in each of the two photoswitched states.

The second protein called Ultramarine is an intensely blue coloured non-fluorescent (ΦF, 0.004) monomeric protein. The broad absorbance spectrum of Ultramarine makes it suitable as an acceptor for a number of different fluorescent donors including those with cyan, green, yellow or orange emissions. Fluorescence lifetime imaging is required to monitor FRET in live cells when using Ultramarine.

Developing Genetically Encoded Actuators with an Engineered Photocleavable Protein, PhoCle

Wei Zhang, Robert E. Campbell.
University of Alberta, Edmonton, Canada.

See abstract: Pos-54 Board 54

Self-referenced Quantitative FRET with Dual-Switchable Donor-Acceptor Pair

Yingqi Wang, Xiaodong Liu.
Tsinghua University, Beijing, China.

See abstract: Pos-45 Board 45

Magnetic Control of Intracellular Signaling

Maxime Dahan[1,2].
[1]Institut Curie, Paris, France, [2]CNRS, Paris, France.

Live cell imaging has shown how the establishment and maintenance of cell polarity relies on complex mechanisms by which signaling cascades become regulated at sub-cellular levels. Yet, how signaling networks are spatio-temporally coordinated into a polarized cell is not elucidated. Here, we present a new tool to activate signaling pathways inside living cells. In our approach, magnetic nanoparticles (MNPs) functionalized with active proteins are inserted in the cytosol of mammalian cells where they behave as signaling nanoplatforms. By exerting magnetic forces, MNPs are then manipulated in the cytosol to position their signaling activity at different subcellular locations. The cellular response to this spatially resolved biochemical perturbation is quantified in term of effector recruitment, signalling activity and cytoskeleton/membrane dynamics. We show that MNPs of different sizes, from 50nm to 500nm in diameter, can be used to generate different spatial perturbation patterns. While large MNPs are trapped in internal structures and require large forces (>10 pN) to be displaced, they allow us to create precise point-like perturbations at the cell periphery. In contrast, the smaller MNPs diffuse fast in the cytosol (~1$\mu m^2/s$) and are used to create gradient of signaling activity. We applied our magneto-genetic technique to the Rho-GTPase signaling network which orchestrates cell polarity and migration. We demonstrate that the pathway linking Rac1 to actin polymerization is spatially restricted to the protrusive areas of the cell by transporting TIAM1-coupled particles at different subcellular locations while monitoring GTPase activation and actin polymerization. Our approach can be extended, in cultured cells or in other models, to a large variety of biomolecules (DNA, mRNA, peptides…) whose precise localization is critical in biological functions. Overall, it should thus contribute to enhance our understanding of how local biochemical information is spatially modulated, processed and integrated at the cell scale.

Novel Fibreoptics Probes for in vivo Optogenetics: From Single Cells to Hard-to-Get-to Areas of the Nervous System

Yves De Koninck.
Laval University, Quebec, Canada.

The future of neuroscience lies in our ability to assess, in a context sensitive manner, how each component of the enormously complex nervous system integrates, processes and transfers neurochemical information. Although colossal advances in understanding cell signalling events have been made using *ex vivo* preparations, the highly reactive and plastic brain imposes *in vivo* studies to assess their relevance to normal function and pathology. Thus, the true enabling discovery technologies will be those that bridge single cell molecular signalling studies with whole animal physiological and behavioural assessments. The recent advent of photoactivatable proteins to generate novel sensors and actuators open new arrays of possibilities on this front. Yet, harnessing their full potential remains limited by properties of light such as diffraction, absorption and scattering which restrict resolution and depth of observation/intervention. Our ability to probe and control cellular and molecular events across the length and time scales relevant to brain functions (from millisecond to hours and days) *in vivo* hinges on the development of novel techniques to deliver light and measure events with extreme sensitivity and precision. I will describe recent developments we have made to enable single cell signalling and electrophysiology studies in deep brain structures with a micro-optrode that not only enables combined electrical and optical Ca^{2+} measurements from single neurons but also probing intrinsic membrane properties optogenetically. I will also describe novel fibreoptics geometries that enable light deliveries and collection in configurations that enable probing structures that would otherwise not be accessible, including approaches such as panoramic confocal imaging and epidural optogenetics.

Chemical Biology Sensors for Membrane Curvature and Lipid Composition

Hang Yin.
University of Colorado Boulder, Boulder, USA.

See abstract: Pos-52 Board 52

Observing the Helicase Activity of Ribosome 30S during Translation Initiation by Using Optical Tweezers

Yi-Lan Chen[1], Jin-Der Wen[2].
[1]Genome and Systems Biology Program, National Taiwan University, Taipei, Taiwan, [2]Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan.

See abstract: Pos-6 Board 6

Nanofabrication as a Tool to Study the Effects of Cell Shape on Chromosome and Protein Organization in Bacteria

Cees Dekker.
Kavli Institute NanoScience Delft, Delft, Netherlands.

The general principles underlying the interplay between intracellular molecular networks and the cell boundary are essential to cell biology, yet largely unexplored. Here, we use nanofabricated chambers to 'sculpt' living bacterial cells into defined shapes that deviate from their natural phenotype, and subsequently we study the organization of proteins and DNA in these shape sculptures.

I will show our ability to shape live E. coli bacteria into novel shapes such as rectangles, squares, triangles and circles. We use these to explore the spatial adaptation of Min proteins that oscillate pole-to-pole in rod-shape Escherichia coli to assist cell division. In a wide geometric parameter space from 2x1x1 to 11x6x1 μm3, Min proteins exhibit versatile oscillation patterns, sustaining rotational, longitudinal, diagonal, stripe, and even transversal modes. These patterns are found to directly capture the symmetry and scale of the cell boundary, and the Min concentration gradients scale in adaptation to the cell size within a characteristic length range of 3–6 μm. Numerical simulations reveal that local microscopic Turing kinetics of Min proteins can yield global symmetry selection and gradient scaling, when and only when facilitated by the three-dimensional confinement of cell boundary. These findings contrast previous geometry-sensing models based on the longest distance, membrane area or curvature, and reveal that spatial boundaries can facilitate simple molecular interactions to result in far more versatile functions than previously understood.

Furthermore I will show preliminary data on the organization of (single) chromosomes in shaped cells that illustrate how DNA is organized in an expanded cytosolic volume. Finally, I will briefly sketch some of our ideas to explore the building of synthetic cells, specifically our first steps to establish synthetic cell division.

Cutting the Tension with a Laser: Biophotonic Dissection of Stress Fibers and Contractile Signaling

Sanjay Kumar.
University of California, Berkeley, Berkeley, USA.

The ability of the actomyosin cytoskeleton to distribute tensile forces at the cell-matrix interface at specific times and places is now understood to be critical to shape stabilization, motility, tissue assembly, and fate determination. I will discuss efforts my research group has been making to investigate the tensile properties of single stress fibers in living cells using single-cell biophotonic tools. Specifically, we have used femtosecond laser nanosurgery to spatially map the viscoelastic properties of actomyosin stress fibers and found that specific stress fiber compartments exhibit distinct viscoelastic properties that strongly depend on their subcellular location. In an effort to understand how these variations in viscoelastic properties translate into differences in tensile signaling and shape stability contributions, we have combined this method with fluorescence energy resonance transfer (FRET) probes of molecular tension within focal adhesions. These studies reveal that individual stress fibers can distribute tensile loads across a surprisingly large ensemble of adhesions, which is in turn related to the network connectivity of the individual fiber. Finally, we have begun to investigate the relative contributions of myosin activators (MLCK, ROCK) and isoforms (myosin IIA, IIB) to regulating tension within these pools of stress fibers, and we have coupled these measurements with single-cell micropatterning to investigate how viscoelastic properties are related to stress fiber geometry, inter-adhesion distances, and sarcomere/dense body architecture.

Engineering Spatial Gradient of Signaling Proteins Using Magnetic Nanoparticles

Zoher Gueroui.
Ecole Normale Supérieure - CNRS, Paris, France.

Intracellular biochemical reactions are often localized in space and time, inducing gradients of enzymatic activity that may play decisive roles in determining cell' s fate and functions. However, the techniques available to examine such enzymatic gradients of activity remain limited. Here, we propose a new method to engineer a spatial gradient of signaling protein concentration within Xenopus egg extracts using superparamagnetic nanoparticles. We show that, upon the application of a magnetic field, a concentration gradient of nanoparticles with a tunable length extension is established within confined egg extracts. We then conjugate the nanoparticles to RanGTP, a small G-protein controlling microtubule assembly. We found that the generation of an artificial gradient of Ran-nanoparticles modifies the spatial positioning of microtubule assemblies. Furthermore, the spatial control of the level of Ran concentration allows us to correlate the local fold increase in Ran nanoparticle concentration with the spatial positioning of the microtubule-asters. Our assay provides a bottom-up approach to examine the minimum ingredients generating polarization and symmetry breaking within cells. More generally, these results show how magnetic nanoparticles and magnetogenetic tools can be used to control the spatiotemporal dynamics of signaling pathways.

POSTER ABSTRACTS

POSTER SESSION

Monday, June 29, 1:00 PM – 2:30 PM
& Tuesday, June 30, 1:00 PM – 2:30 PM
Plato Room

All posters will be presented on both Monday, June 29 and Tuesday, June 30, from 1:00-2:00 PM. Posters should be set up on the morning of June 29 and removed by 6:00 PM on June 30.

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Settu, Kalpana *(Abstract Withdrawn)*	39-POS	Board 39
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1-POS Board 1

Expanding the Toolbox of Genetically Encoded Voltage Indicators

Ahmed S. Abdelfattah[1], Samouil L. Farhi[2], Yongxin Zhao[1], Daan Brinks[2], Adam E. Cohen[2], Robert E. Campbell[1].
[1]University of Alberta, Edmonton, AB, Canada, [2]Harvard University, Cambridge, MA, USA.

Simultaneous recording of neuronal activity from many locations is key to understanding how brain function emerges from neuronal circuits. Optical imaging using voltage indicators based on green fluorescent proteins (FPs) has emerged as a powerful approach for detecting the activity of many individual neurons with high spatial and temporal resolution. To expand the toolbox of genetically encoded voltage indicators, we engineered two FP-based voltage indicators: A bright red fluorescent voltage indicator (FlicR1) and a green to red highlightable voltage indicator (FlicGR). We used directed protein evolution to screen libraries of thousands of variants to identify clones with sufficient brightness and response amplitude that would report membrane potential changes in mammalian cells.

FlicR1 has voltage sensing properties that are comparable to the best available green indicators. It reports single action potentials in neurons in single trial recordings. Furthermore, FlicR1 can be imaged with wide field fluorescence microscopy using a typical mercury arc lamp, and faithfully reports spontaneous activity in cultured hippocampal neurons and rat brain organotypic slices. We also demonstrate that FlicR1 can be used in conjunction with PsChR, a blue-shifted channelrhodopsin, for all-optical neuronal activation and activity recording, although blue light photoactivation of the FlicR1 chromophore remains a challenge.

FlicGR is the first example of a highlightable voltage indicator. A powerful application of fluorescent proteins is their use as highlighters where they can be converted from one color to another by light. Using blue light, we can photoconvert FlicGR from green to red. We demonstrate that both the green and red forms are sensitive to membrane potential changes in mammalian cells.

2-POS Board 2

Structural Dynamic Model of Programmed Ribosomal Frameshifting

Kai-Chun Chang[1], Emmanuel Salawu[2,3], Lee-Wei Yang[2,3], Jin-Der Wen[1].
[1]Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan, [2]Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu, Taiwan, [3]Bioinformatics Program, Institute of Information Sciences, Academia Sinica, Taipei, Taiwan.

Programmed ribosomal frameshifting (PRF) is promoted by a slippery sequence and a road-blocking downstream RNA secondary structure, such as a hairpin or pseudoknot (PK). Despite extensive biophysical and biochemical studies of −1 PRF for the past few decades, how the ribosome changes its conformation during PRF and how the RNA structures are unwound remain largely unknown. Recent single-molecule Förster resonance energy transfer (smFRET) experiments indicated that the ribosome may engage in a hyper-rotated state upon encountering an RNA structure.
To model the intrinsic dynamics of the ribosome, we build a full-atom ribosome model devoid of any missing subunits and residues, and then apply anisotropic network model (ANM) combined with a novel technique that removes the "tip effect" by preserving bond lengths during large conformational changes. The ANM model with full modes solved (63,768 modes) not only agrees well with experimental B-factor (correlation coefficient r = 0.63), but also captures the ratcheting motion of the ribosome (orientation correlation C = 0.68). The pairwise distance changes between multiple labeling sites from previous smFRET experiments are also examined, and all quantitatively comparable with our predicted values.
The PK bound to the ribosome is modeled by fitting a solution structure of PK to the cryo-EM map and then refined by steered molecular dynamics simulations. The global conformational change of the ribosome in response to the external force exerted by PK is modeled by linear response theory. We found that PK may force the 30S subunit to "roll" in a direction perpendicular to ratcheting, and thereby prolonging the ratcheted state and inducing tRNA distortion that disrupts tRNA-mRNA interaction, as previously observed. We propose that the "rolled" state may contribute to PRF by storing the potential energy for a later power stroke that unwinds the downstream RNA structure.

3-POS Board 3

Observing Translation Initiation of the Ribosome on rpsO Gene Transcript by Using smFRET

Shu-Ya Chang, Jin-Der Wen.
Institute of Molecular and Cellular Biology, National Taiwan Univercity, Taipei, Taiwan.

Many mRNAs fold into secondary structures; however, their codons must be in single-stranded form to be translated. Previous research has revealed that the ribosome itself has helicase activity during the translation process. The *rpsO* gene transcript of *Escherichia coli* regulates its own translation through the 5' untranslated region (5' UTR), which can fold into a pseudoknot or a double-hairpin conformation. The two structures can be interchanged spontaneously, but the ribosome can only bind the pseudoknot to initiate translation. On the pseudoknot, only the Shine-Dalgarno (SD) sequence but not the AUG start codon is exposed, so the ribosome has to unwind part of the secondary structure to complete the initiation. However, it remains unclear in which stage the conformation is opened by the ribosome. In this study, we characterize the conformational change of the *rpsO* 5' UTR in the presence of the 30S ribosomal subunit and initiator tRNA (charged formyl-methionine tRNA) by using single-molecule fluorescence resonance energy transfer (smFRET). Our results show that the population of the pseudoknot form is increased when 30S binds to the RNA. 30S would begin to search for the AUG start codon after binding to the SD sequence of the pseudoknot by partially unwinding the local structures. In the present of the initiator tRNA, 30S could completely unwind a stem of the pseudoknot and form the pre-initiation complex. These results demonstrate that the 30S ribosomal subunit alone can perform its helicase activity during the initiation stage.

4-POS Board 4

Real-Time Observation of Single Macromolecular Rotation Using Gold Nanorods

Wen-Hsuan Chang[1], Edward Yeung[2], Hung-Wen Li[1].
[1]National Taiwan University, Taipei, Taiwan, [2]Iowa State University, Ames, IA, USA.

Gold nanoparticles have high absorption and scattering cross section. The typical issue of single molecule fluorescence experiments is that fluorescence dyes photobleach and limit the observation time. Scattering of gold nanoparticles do not photobleach, making them suitable for long-time observation and fast optical imaging due to large scattering cross section. In particular, scattering light of gold nanorods is highly polarized along the long axis, allowing the anisotropic detection. Using laser dark-field microscopy, we image individual gold nanorods labeled on DNA molecules to observe the rotation of single macromolecules. Sensitive measurement can be made by out-of-focus images of gold nanorods, and analyzing the defocused image using geometric analysis, with the angle precision determined to be about one-degree. We will present the application to study protein-DNA interactions at the single-molecule level.

5-POS Board 5

Genetically Encoded Tools to Manipulate and Observe Cellular Dynamics in Cardiac Disease Modelling and Drug Screening

Yu-Fen Chang, Frances Book, Mark J. Davies, Matthew J. Daniels.
University of Oxford, Oxford, United Kingdom.

Optogenetic technology allows us to control depolarization of electrically excitable cells using ion-channels that open in response to light. Genetically Encoded Calcium Indicators (GECIs) can be utilized to visualize cellular response upon opto-stimulation in real time. Previously, we have successfully demonstrated the use of channelrhodopsin2 (ChR2), a light sensitive cation channel, with a spectrally compatible red shifted calcium indicator (R-GECO) in a transient expressing neuronal model. Here, we presented a viral delivery system for the two component parts (an improved ChR2 and R-GECO) required to target relevant cardiac substrates such as adult guinea pig cardiomyocytes human-embryonic-stem-cell derived cardiomyocytes (hES-CM), and patient derived iPS cardiomyocyte derivatives allowing light stimulation with simultaneous Ca++ imaging. To prove the concept of contactless drug screening in excitable cells, known small molecules were tested on hES-CMs in multi-well plates. Increased Ca++ influx was observed in hES-CMs upon blue light stimulation after treatment of BayK8644, the L-Type Ca++ channel agonist. On the other hand, cytosolic calcium fell upon light activation after treating with BAPTA, a Ca++ chelator. The contactless nature of this technology enables high-throughput drug screening to be targeted to rapid and reversible phenomena like Ca++ handling, providing a new platform for cardiac disease modelling.

6-POS Board 6

Observing the Helicase Activity of Ribosome 30S during Translation Initiation by Using Optical Tweezers

Yi-Lan Chen[1], Jin-Der Wen[2].
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In prokaryotes, recognition of ribosomal binding site (RBS) which includes the Shine-Dalgarno sequence (SD) and AUG start codon is important for translation initiation. Structured 5'-UTR of mRNA might influence the protein expression. On the other hand, it has been proposed that ribosomal proteins S3 and S4 in the 30S are important to the helicase activity of the ribosome. This implies that the 30S itself might open the structured 5'-UTR during translation initiation.

We test this hypothesis by using optical tweezers. When we added the 30S to bind the SD sequence before a hairpin, the unfolding force of the hairpin became lower. This suggests that the 30S itself interacts with and destabilizes the hairpin. Furthermore, the 30S needs the initiator tRNA (fmet-tRNA) to dock properly on RBS. Therefore, we added both fmet-tRNA and the 30S to form pre-initiation complex. We found 30S was correctly initiated at the start codon. This result might explain how translation initiation happens and how 30S interacts with structured 5'-UTR. We propose that the 30S binds to SD first. Then, the 30S searches and partially opens surrounding structures. Finally, it will accommodate at the correct site after fmet-tRNA pairs to the AUG start codon.

With more control experiments in the near future, we wish this study will help us to understand how the 30S and structured 5'-UTR cooperate to achieve the regulation of translation initiation.

7-POS Board 7

Low-Noise, low-Capacitance Solid-State Nanopore Measurements at High Bandwidth for Biomolecule Analysis

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Solid-state nanopores are promising single molecule sensors, yet their applications are still limited by relatively high noise and low bandwidth. We devised an integrated process to produce silicon nitride (SiN) nanopore membranes suspended on glass substrates, thus successfully lowering the chip capacitance. We obtain higher signal-to-noise ratios and better resolution in ionic current signals than previously reported in solid state nanopores at megahertz bandwidth. Specifically, we show measurements of ionic current during translocation of DNA molecules through thin SiN membrane nanopores of small diameters at megahertz bandwidths with enhanced ionic signal-to-noise ratios. We further discuss the potential of these results to pave the way towards identifying intramolecular DNA sequences with solid-state nanopores, and how these low capacitance glass chip devices could be used in other biomolecule sensing applications.

8-POS Board 8

Investigating the Coordination of Two ATPases in RecBCD Helicase Complex Using Single-Molecule Fluorescence

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RecBCD is an essential trimeric enzyme complex that initiates homologous recombinational repair in E. coli. RecBCD complex contains single-stranded DNA nuclease and two DNA helicases with single-stranded DNA translocase activities with opposite polarities. Previously, we showed that RecB is a 3’-to-5’ single-stranded DNA translocase and RecD is a 5’-to-3’ one. Here, we use total internal reflection fluorescence microscopy to observe and analyze individual cy3-labeled ATP binding and turnover events during RecBCD unwinding at the single-molecule level. This allows us to characterize both ATPase activities of RecB and RecD as well as the coordination between both subunits.

9-POS Board 9

Globin Nitrito Heme Fe-O-N=O/ 2-Nitrovinyl Species: Impications for Myoglobin Helices Dynamics

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Nitrite acts as a 'pool' for the NO signaling molecule under hypoxic and anoxic conditions, when NO synthase activity is impaired. This is supported by the growing number of metallo-proteins that are reported to be able to reduce nitrite to nitric oxide in mammals. Nitrates and nitrites have been viewed as storage pools supporting NO signaling during metabolic stress and their bio activation, involves both enzymatic and non-enzymatic reactions in tissues and blood. Nitrites react with heme proteins leading to several heme Fe adducts and the catalyzed reaction pathways play a vital role for deoxy-hemoglobin and deoxy-myoglobin (Mb) dependent nitrite reduction. Structural information based on X-ray crystallography of the unusual Mb-nitrite interactions has been reported, and mechanisms for nitrite conversion to NO have been proposed. A detailed characterization of the structures and bonding of the bound nitrite and its interaction with the protein environment in its natural environment rather than in crystals, however, is lacking. The description of biological activity in heme proteins responsible for activating small molecules requires identification of ligand movement into the metal and non-metal binding sites at the atomistic level. Mechanisms of nitrite reductase activity in globins are difficult to verify without the dynamical aspects of the ligand binding. Computational Modeling for the NO_2^- adducts of heme proteins is becoming increasingly important. The conditions under which the nitrito heme Fe-O-N=O/ 2-nitrovinyl species is generated in Mb and interacts with the protein matrix are identified at the atomistic level employing Molecular Dynamics Simulations and Density Functional Theory Calculations. Helices movement and flexibility in Mb are strongly affected upon the nitrite binding, altering also the Mb spectroscopic characteristics. We correlate these theoretical findings with results from resonance Raman Spectroscopy.

10-POS Board 10

Fabrication of sub-20 nm Nanopore Arrays in Membranes with Embedded Metal Electrodes at Wafer Scales

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We introduce a method to fabricate solid-state nanopores with sub-20 nm diameter in membranes with embedded metal electrodes across a 200 mm wafer using CMOS compatible semiconductor processes.Multi-layer (metal–dielectric) structures embedded in membranes were demonstrated to have high uniformity (1±0.5 nm) across the wafer. Arrays of nanopores were fabricated with an average size of 18±2 nm in diameter using a Reactive Ion Etching (RIE) method in lieu of TEM drilling. Shorts between the membrane-embedded metals were occasionally created after pore formation, but the RIE based pores had a much better yield (99%) of unshorted electrodes compared to TEM drilled pores (<10%). A doublestranded DNA of length 1 kbp was translocated through the multi-layer structure RIE-based nanopore demonstrating that the pores were open. The ionic current through the pore can be modulated with a gain of 3 using embedded electrodes functioning as a gate in 0.1 mM KCl aqueous solution. This fabrication approach can potentially pave the way to manufacturable nanopore arrays with the ability to electrically control the movement of single or double-stranded DNA inside the pore with embedded electrodes.

11-POS Board 11

Revealing Proteostasis Capacity in Cells by a Fluorescent Sensor

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Maintaining proteostasis is an essential housekeeping function for cell survival. Proteostasis is in principle affected in any disease that involves misfolded or mutant proteins that do not fold with normal efficiencies; and hence overdraw on the finite proteostasis resources of the cell. Tracking the proteostasis capacity of cells has the generic potential to track neurodegenerative diseases of diverse specific molecular origins. Building new approaches to identify the efficiency of proteostasis is thus highly desired in order to track the risk of cells succumbing to damage from protein misfolding and aggregation. Here we describe our approach to revealing the proteostasis states in Huntington's disease cell culture models by a cysteine-reactive aggregation-induced emission (AIE) fluorogen. Stress conditions, such as protein misfolding and environmental stimuli, will lead to an elevated level of unfolded proteins. The fluorogen, TPE-MI, can react with the unfolded proteins with free cysteine residue exposed and turns on its fluorescence. Because of the unique AIE characteristic, reacting with small biothiols, such as glutathione, would not change the fluorescence of TPE-MI. The signal from TPE-MI can thus be correlated to the amount of unfolded proteins and reflect the proteostasis capacity in cells. We then apply this method to investigate the proteostasis capacity in the subpathogenic and pathogenic Httexon 1 cells by using flow cytometry. Our results show distinct fluorescence signals between the pathogenic and subpathogenic cells. By incorporating pulse shape analysis, we are able to differentiate the signals from the non-inclusion and inclusion cell population within the same sample. Our results suggest that the collapse of proteostasis is one of the features for Huntington's diseases and such effect emerges in cells prior to the formation of visible aggregates.

12-POS Board 12

Probing the Dynamics of Raft Lipids Induced by Receptor-Mediated Signaling in Living Cells

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Lipid rafts, the relatively ordered and tightly packed membrane microdomains with enriched cholesterol and glycosphingolipids, play an important role in compartmentalizing cellular processes through assembling of signaling molecules and regulating membrane protein trafficking. However, the direct dynamics of lipid rafts involving signaling pathways remains unclear.
In this study, we directly investigated the dynamics of lipid rafts with the lipid molecules conjugated with single fluorescent dyes. Sphingomyelin (SM) was used as the lipid raft marker while phosphoglycerolipid (DPPE) was used as the non-lipid raft marker. The whole process of signaling was slowed down from few minutes to hours by controlling the ligand-binding area within tens of nanometers. The movements of receptors were also recorded with nano-sized particles.
We believe this is the first direct observation of the dynamics of lipid raft following the ligand-binding receptor. The raft lipids are not just concentrated in the ligand-receptor binding area, but also moved along with the receptor on cell membrane. During the floating process of lipid raft with receptor, the concentration of raft lipids increases 2.8-fold in average in an area of 1 μm^2. Increment in the concentration of raft lipids in a fixed area correlates to a slower movement of the receptors. The diffusion coefficient of receptors can vary from 0.004 to 0.5 $\mu m^2/s$. In this study, we observed the early events of lipid raft aggregation induced by receptor-mediated signaling in living cells.

13-POS Board 13

Radiation-Induced Micronucleus in a Multi-Scale Tissue Modeling Framework

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The surface of skin is lined with several thin layers of epithelial cells that are maintained throughout life time by a small population of stem cells. Ionizing radiation exposures could injure the underlying proliferative cells and induce genomic instability that is correlated with cancer risk. In this work a multi-scale computational model for skin epidermal dynamics that links phenomena occurring at the subcellular, cellular, and tissue levels of organization is applied to simulate the experimental data of cytokinesis-block micronucleus (CBMN) assay in 2 types of 3D in vitro human epidermal tissues. This multi-scale model has been used to simulate the population kinetics and proliferation indexes observed in irradiated swine epidermis, which highlights the importance of considering proliferation kinetics as well as the spatial organization of tissues when conducting investigations of radiation responses. At the subcellular level, several recently published Wnt signaling controlled cell-cycle models are applied, allowing testing of validity of some basic biological rules at the cellular and sub-cellular levels by qualitatively comparing simulation results with published data. We will present results of simulation of skin responses of CBMN to conventional photon techniques as well as highly charged and energetic (HZE) particles used in new Hadron therapy. Combining with the radiation track structure simulated with software RITRACK, this approach could illuminate the roles of various physical, chemical, and biological interactions in determining the relative biological effects of the HZE particles to induce genomic instability. This integrated model will provide a versatile platform to investigate various radiation altered physical, chemical, and biological interactions in different time and spatial scales, which could enhance our understanding of the pathophysiological effects of ionizing radiation on the skin for patients undergoing radiotherapy.

14-POS Board 14

Effect of L and T - Type Calcium Channels in Retinal Ganglion Cells Stimulation

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The retina comprises of atleast 50 distinct types of cell that widely differ in shape and size. It covers the visual field and conveys a complete processed visual image. The Visual system, a marvelous anatomical complex neural circuit composes of image-forming pathways with retinal ganglion cells(RGCs) depending on photoreceptors, rods and cones whereas the non-image-forming pathways involves intrinsically photosensitive RGCs to express the photopigment melanopsin. The retina and the brain communicate through action potentials to realize the time-dependent visual image as projected by the optics of eye.
The paper attempts to develop an all active Fohlmeister–Coleman–Miller(FCM) model with five nonlinear ion channels for RGC, with an intracellular resistance(Ra), a membrane mechanism in parallel with a membrane capacitance(C) and a gap junction conductance(G) between cells. The process augurs the functions of sodium, calcium, delayed rectifier potassium, inactivating potassium, non inactivating calcium activated potassium, L- and T-type calcium conductances in the model.
The simulation shows the repetitive firing of RGCs without application of the Istim due to the depolarization of the RGCs. In ipRGCs, the depolarization of membrane potential results in the activation of VGNCs further depolarizes, leading to activation of L-type VGCCs. The spontaneous electrical activity spreads as rhythmic bursts of action potential between adjacent cells to produce transient elevations in internal calcium concentration.
Such RGC stimulation benefitted severe age-related macular degeneration(AMD), retinitis pigmentosa(RP) patients due to the presence of actively functioning ipRGCs though they are blind. By electrically preferentially stimulating the surviving RGCs with a retinal prosthetic, vision could be partially restored.

15-POS Board 15

Neural Influence on Synchronization of Cardiac Oscillators - A Computational Study

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Biological rhythms, like the cardiac rhythm are often generated by large populations of mutually interacting cellular oscillators. Typically individual oscillators in such populations have intrinsic frequencies with a significant dispersion. The ability of such a population to generate a stable, regulated rhythm depends critically on the nature of interactions among the oscillators. Although the autorhythmic cells in the heart at a wide range of frequencies in culture, they beat at a common frequency set by the sinus rhythm. Two nonlinear oscillators operating at different frequencies can synchronize only under special conditions, which are expected to be more stringent for a large network of oscillators with a range of intrinsic frequencies. Since the heart is a large network of oscillators beating at a wide range of frequencies, synchronization is a serious issue. The problem of synchronization in a grid model of cardiac cells with a considerable gradient in intrinsic frequencies is investigated. Simulations involve the Demir model, which is one among the detailed biophysical cardiac cell models. In the grid models there is a monotonic variation in intrinsic frequency of cells along one of the diagonals, i.e. from the left-top corner to the right-bottom corner. When the frequency range is of the order of the physiological range the entire grid failed to synchronize to a common frequency even after prolonged simulation. It points out the need for an external influence that can coax the cardiac oscillators to beat at a single frequency. In order to achieve synchronization, a simple neural feedback system is proposed in which neurons monitor the activities of cardiac cells and activate an entire “slower” stretch of cells. For properly tuned feedback gain it is shown that synchronization can be achieved within a short period.

16-POS Board 16

Development of New Fluorescent Proteins & Biosensors with Microfluidic Selection Systems

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Creation of new molecular sensors and actuators relies on methods for identifying complex photophysical phenotypes and subsequently performing separations on cell populations. We have developed microfluidic flow cytometry approaches tailored to interrogating the performance of genetically-encoded fluorophores and FRET sensors, and present results of studies employing these technologies.

One of the systems rapidly screens cell-based FP libraries on the basis of multiple photophysical parameters relevant to imaging, including brightness, photostability, and excited-state lifetime (i.e. a proxy for fluorescence quantum yield). Molecular dynamics-guided design was used to create a library of mCherry mutants that was screened with this system, and resulted in identification of a variant with a higher stability β-barrel and improved photostability but reduced brightness due to reduction in the fluorescence quantum yield. To improve brightness, additional rounds of selection were performed by adding excited-state lifetime as a sorting criterion. The multiparameter sort identified multiple clones with improved photostability and up to double the excited-state lifetime of the parent mCherry FP. Some of the mutated positions were previously identified in molecular dynamics simulations as exhibiting large structural fluctuations. Subsequent rounds of screening were used to improve FP folding and maturation.

A second instrument in our lab is tailored to the screening and development of FRET-sensors for quantifying cellular concentrations of specific analytes. It sorts cells at rates of dozens per second, based on the magnitude and kinetics of ligand-induced sensor response on timescales from milliseconds to 15 seconds. We used it to investigate several genetically-encoded FRET sensors, which show unexpected heterogeneity of response. We demonstrate identification and purification of responsive sub-populations of Zn^{2+} sensors in HeLa cells.

17-POS Board 17

Insulin Receptor and IGF1 Receptor Biosensors Employing Between-Domain Fluorescence Tags

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The insulin receptor (InsR) is one of the most studied proteins, but fundamental aspects of its biology remain unclear, including the kinetics of insulin receptor activation and internalization from the plasma membrane. To monitor insulin receptor and insulin-like growth factor 1 receptor (IGF1R) dynamics in real time, we employed a novel tagging strategy wherein fluorescent proteins (TagRFP, TagRFP-T, eGFP, eYFP, TagBFP, tagGFP2) were placed at the extracellular region in between two functional domains. Unlike published insulin receptor fusions that have fluorescent proteins attached to their C-termini, our fusion proteins primarily localized to vesicle-like cytoplasmic structures mirroring the pattern we observed for the endogenous insulin receptors in vivo and in vitro. InsRA and InsRB splice variants had overlapping distributions, in contrasting with the conclusions of previous studies with C-terminal tagged receptors. Using insulin secreting pancreatic beta-cells as a model system, we mapped the steady state internalization and trafficking of between-domain-tagged InsRs and endogenous InsRs to organelles labeled with caveolin1 (Cav1), flotillin1 and Lamp1, which bypass compartments positive for clathrin, Rab5a, Rab7, Rab11a, or Rab4a. TIRF imaging of InsRA-eGFP revealed that the phospho-mimetic Cav1-Y14D mutant significantly shortened the lifetime of InsRA domains at the plasma membrane prior internalization. Ratiometric pH sensor fluorescent proteins and Fluorogen Activating Peptides were also developed to track the dynamics of insulin receptor internalization of insulin receptor clusters in living cells in real time using spinning disk confocal microscopy and high-conetnt imaging systems. We expect that these novel approaches to live-cell imaging of tyrosine kinase receptor dynamics will be important for elucidation of insulin and IGF1 signaling and development of high-content screening for molecules that modify insulin receptor trafficking and activity.

18-POS Board 18

Fluorescent Nucleic Acid Systems: Design, Construction and Biosensing

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Sequence specific hybridization probes composed of fluorescent synthetic oligonucleotides have been developed for genetic analysis in the post-genomic era. Currently such fluorescent oligonucleotides play a decisive role in analysis of the genetic information and DNA sensing such as SNP typing.
Fluorescent nucleic acid systems are widely applied in various fields, from fundamental biological probes to nano-construction, Nucleic acids are used as a scaffold for arranging aromatic fluorophore assemblies, either by insertion into the DNA base pairs or by stacking via the duplex. Moreover, chemical modifications of nucleic acids are accessible by the modified DNA phosphoramidites or postsynthetic approach, and provide with new and interesting fluorescent nucleic acids systems. Fluorescent nucleic acid systems represent an extensive and exciting research area in chemistry as well as in biotechnology and photophysics. We have synthesized and investigated new fluorescent nucleic acid systems for probing single nucleotide polymorphisms (SNPs), structural changes of DNA4 and ligand interaction with RNA bulge. We have developed the new type of molecular beacon, quencher-free molecular beacon (QF-MB), that exhibits several advantageous features, including a high level discrimination between the target and its single-mismatched congeners and an economical device set-up due to the absence of the quencher. We have also designed and synthesized the probing system for quadruplex structures of DNA (G-quadruplex and i-motif) and B-Z transition.
Strong π–π stacking interactions in nucleic acids can be used to generate novel secondary structures. We have investigated the fluorescent phenomena and structures of pyrene modified oligodeoxyadenylate and oligodeoxyguanylate. The covalently linked pyrenes induced the formation of a self-assembled olgiodeoxyadenylate duplex5 and various secondary structures with interesting fluorescence phenomena. Various results using fluorescent nucleic acid systems will be discussed.

19-POS Board 19

Synthetically Rerouting Phagocytosis by Rapidly Turning Inert Cells into "Eat You" Mode

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Cells communicate with outside environment through surface molecules. In order to engineer the surface property of intact living cells on a rapid timescale, we developed a technique with which a protein of interest can be displayed at the cell surface within 15 minutes by adding a chemical dimerizer. In particular, we rapidly displayed a C2 domain of MFG-E8 protein, endowing cells with an ability to selectively bind to apoptotic cells through externalized phosphatidylserine. While this was not sufficient for engulfment of apoptotic cells, additional presence of active Rac in the surface engineered cells triggered engulfment. The surface engineering technique provides a powerful way to immediately alter cellular property, facilitate understanding of cellular communication skills, and may also be employed for cell-based therapy to eliminate targeted cells in living animals.
<B>Reference: <I>Sci. Signaling, <B>7, <I>334, rs4 (2014)

20-POS Board 20

The N-Terminus Fine-Tunes Multiple Ligand-Binding of the Pain Sensing TRPV1 Channel to Adjust Its Response to Capsaicin

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Transient receptor potential vanilloid type 1 (TRPV1) assembles into tetrameric non-selective cation channels to serve as a polymodal sensor for noxious stimuli including capsaicin, acidic pH and painful heat. We exploited tandem repeat tetrameric TRPV1 with all or some of the capsaicin binding pockets replaced by the capsaicin insensitive S512F mutation, to elucidate the mechanism with which TRPV1 differentiates the severity of noxious stimuli through graded ligand occupancy. We created all 16 different wild type-mutant binding permutations to assess the efficacy of capsaicin as an agonist. Binding of one capsaicin molecule is sufficient to open TRPV1, but only partially. Further inclusion of wild type subunits in tandem repeat tetramers promotes ligand induced channel opening. The gating effect of capsaicin is asymmetric, far more pronounced when the only ligand-binding site was introduced into the first repeat of the tandem TRPV1 possessing a single wild-type subunit. A flexible N-terminal domain can more effectively propagate the binding event to the rest of tetrameric complex to open the channel pore. Oppositely, adding a lipid-anchoring motif at the N terminus of the S-F-F-F tandem tetramer retards channel response to capsaicin. We applied ribosomal skipping strategy by introducing the 2A peptide between the first and second subunits, to release the N terminus at the second position of the F_{2A}S-F-F tandem receptor. This drastically enhances the responsiveness of the F_{2A}S-F-F to capsaicin when compared to F-S-F-F. In fact, the channel formed by F_{2A}S-F-F is indistinguishable from the S-F-F-F tandem tetramer. These results highlighted the crucial role of an N-terminus with a full range of mobility for binding the first ligand in the holo-receptor and reveal the design used by this pain sensing receptor to avoid transmission of spurious noxious information.

21-POS Board 21

Optogenetic Toolkit for Regulation of Receptor Tyrosine Kinases Signaling

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Receptor tyrosine kinases (RTKs) are a family of cell-surface receptors which mediate cell migration, proliferation, differentiation and angiogenesis in various cells and tissues.
In Arabidopsis thaliana, cryptochrome is blue light photoreceptor that includes a N-terminal photolyase homology region (PHR) that mediates light-responsive dimerization through binding to chromophores and C-terminal extension domain that involved in signaling transduction by interacting other partner. Homo-oligomerization of cryptochrome 2 is mediated through the PHR domain. PHR of Cryptochrome 2 (CRY2PHR) fused controlling system to receptor tyrosine kinases using CRY2PHR oligomerization promises a powerful tool to study cell signaling because of its fast responsiveness with subsecond time resolution, spatial resolution, reversibility and no need for exogenous cofactors.
Here, we engineered optically controlled RTKs (optoRTKs) by exploiting cryptochrome 2, which homointeracts upon blue light irradiation. After screening, we found out that fibroblast growth factor receptor (FGFR) and tropomyosin receptor kinase (Trk) can be activated by blue light-induced homointeraction of CRY2PHR. The major downstream signaling pathways of RTKs, canonical signals can activated by light stimulation with precise, rapid and reversible manner. The genetically modified light-inducible RTKs, optoRTKs, shed us a potential on the study of receptor tyrosine kinases signaling in various biological systems.

22-POS Board 22

Femtosecond Laser Pulses Induced Thermal Lensing Effect in H_2O via One-Photon Absorption

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Water is essential to the living systems, and the biological importance of water has been recognized for its anomalous chemical and physical properties. Research of photothermoacoustic (PTA) wave in water has attracted much attention in recent years. Also, the field of photoacoustic imaging, cancerous lesion detection, drug delivery, and cell permeabilization has developed rapidly. However, theoretical explanations of the PTA wave generation and propagation mechanism vary widely.
Using the *Z*-scan technique with 820 nm 18 fs-laser pulse trains with train-width and train-to-train separation longer than its thermal diffusivity constant, we investigated PTA wave induced in water. We observed the thermal lensing effect and explained the heat generation by relaxation of the excited molecular motions. The molecular motions, such as vibration and libration, can be excited either by an ultrashort laser pulse via single- or multi-photon process. By comparing the peak and valley separation in the *Z*-scan results, we realized that water molecules gain excess energy from an 820 nm 18 fs-laser pulse via one-photon excitation associated with the 3^{rd}-order overtone of molecular vibration.
This work gives us a relatively simple and sensitive method for investigating nonlinear optical and thermal properties of water and water-rich samples. Therefore, it has great potential to be applied to research in wide range of biological systems.

23-POS Board 23

Some Like It Hot: Biomolecule Analytics Using MicroScale Thermophoresis

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The analysis of biomolecular interactions and their quantification in the early stages of the drug discovery allows faster development of therapeutics and diagnostic techniques. MicroScale Thermophoresis (MST) is a novel and exciting technology to quantify biomolecular interactions and to enhance the discovery process in pharmaceutical industry. The technique is based on thermophoresis, the directed motion of molecules in temperature gradients. This thermophoretic movement is affected by the entropy of the hydration shell around molecules and is highly sensitive to binding and oligomerization reactions, which affect the size, charge, conformation or hydration shell. Furthermore, MST assays are highly adaptable to fit to the diverse requirements of different biomolecules, e.g. membrane proteins to be stabilized in solution. The type of buffer and additives can be chosen freely. Measuring is even possible in complex bioliquids like cell lysate and thus under close to in vivo conditions and without sample purification.

Since this technology is not limited by any changes in size upon binding, even very small compounds and fragments can be investigated for binding to their target molecule. The MST technology allows for the determination of binding affinities and other biophysical parameters such as stoichiometry directly in solution without surface immobilization. Exploiting the intrinsic tryptophan fluorescence of proteins this method provides a truly label-free experimental setup. This combination of high sensitivity towards small molecules and close to native conditions in an immobilization-free and label-free experimental design makes MST a powerful method for drug discovery. A combination of different biophysical techniques should be employed to shed light on different aspects of their mode of action, thus improving the quality of therapeutics and speed up the development process.

24-POS Board 24

Multi-Frequency Modulated LED for Detecting Fluorescence Lifetimes in Complex Composition

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Frequency domain fluorescence lifetime instrument (FD-FLI) is a rapid and accurate method to study biomolecular interactions and dynamics. While fluorescence lifetime represents molecular structure and molecular local environment, complex molecular composition results in multiple fluorescence lifetimes in FLI data. It is usually difficult to identify multiple fluorescence lifetime components from just one FLI measurement. In order to further apply FLI to study multiple molecular components, and to capture fast and dynamic events involving multiple fluorescence lifetimes in one measurement, we developed a novel FLI technique based on light-emitting diode (LED). We applied multi-frequency modulation to the LED, and used a lock-in detection algorithm to acquire all the lifetime components information. We were able to solve multi-lifetime cases with our FLI system. The advantages to use LED as excitation light source of FLI include: small size, high power, and low cost. Especially, LED was modulated at radio frequency directly, therefore provided nanosecond temporal resolution for fluorescence lifetime signals. Since conventional fluorescence lifetime techniques are costly due to use of laser, our LED-based FD-FLI has great potential for wider applications in complex biomolecular studies.
To our knowledge, we are the first group to demonstrate applications of multi-frequency modulated LED in a FD-FLI system. We also applied this technique to Förster resonance energy transfer experiments, and resolved fluorescence lifetimes when complex donor and acceptor composition was present in the sample. With our novel FD-FLI system, we successful resolved all fluorescence lifetimes of donor alone (without acceptor), donor bound to acceptor molecules, and acceptors in the sample in one multi-frequency measurement.

25-POS Board 25

Rapidly Rewriting Tubulin Codes inside Primary Cilia

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Primary cilium is a microtubule-based protrusion on the apical surface of almost every cell type in our body, while functions as a sensory organelle that transduces extracellular cues to specific intracellular functions. Dysfunctions of primary cilia consequently lead to a variety of human diseases known as ciliopathies. Primary cilium is composed of a microtubule buddle called axoneme that gives support to the structure and provides a track for cilia motors-dependent movement. Axonemal tubulins undergo various post-translational modifications (PTMs) which are tightly regulated by specific modifying enzymes. Although axonemal PTMs has been known for several decades, deciphering how they regulate the structure and function of primary cilium is still largely unchartered mainly due to a lack of techniques to locally manipulate axonemal PTMs without affecting microtubule PTMs in the cell body. To overcome this long-standing challenge, we have recently developed a “chemically inducible diffusion trap” (CIDT) technique that allows us to translocate protein of interests (POIs) into primary cilia for manipulation of ciliary composition or signaling locally on a second timescale. With this technique, we translocated PTMs modifying enzymes specifically to axoneme for local perturbation of tubulin PTMs in cilia. Accumulation of one deglutamylase, cytoplasmic carboxypeptidase 5 (CCP5), to axoneme by our CIDT system can efficiently deglutamylate axonemal PTMs in cilia. This novel approach enables us to evaluate the specific roles of axonemal PTMs in the structural integrity and function of primary cilia and to understand how cilium gets organized and regulates itself by axonemal PTMs.

26-POS Board 26

Three-Dimensional Fibroblast Morphology on Compliant Substrates of Controlled Negative Curvature

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Raditionally, cell biological investigations have mostly employed cells growing on flat, two-dimensional, hard substrates, which are of questionable utility in mimicking microenvironments in vivo. We engineered a novel scaffold to achieve cell culture in the third dimension (3D), where fibroblasts lose the strong dorsal–ventral asymmetry in the distribution of cytoskeletal and adhesion components that is induced by growth on flat sub- strates. The design principle of our new 3D substrate was inspired by recent advances in engineering cellular microenvironments in which rigidity and the patterning of adhesion ligands were tuned on two-dimensional substrates; the engineered substrates enable independent control over biochemical and mechanical factors to elucidate how mechanical cues affect cellular behaviours. The 3D substrates consisted of polyacrylamide scaf- folds of highly ordered, uniform pores coated with extracellular matrix proteins. We characterized important parameters for fabrication and the mechanical properties of polyacrylamide scaffolds. We then grew individual fibroblasts in the identical pores of the polyacrylamide scaffolds, examining cellular morphological, actin cytos- keletal, and adhesion properties. We found that fibroblasts sense the local rigidity of the scaffold, and exhibit a 3D distribution of actin cytoskeleton and adhesions that became more pronounced as the pore size was reduced. In small pores, we observed that elongated adhesions can exist without attachment to any solid sup- port. Taken together, our results show that the use of negatively curved surfaces is a simple method to induce cell adhesions in 3D, opening up new degrees of freedom to explore cellular behaviours.

27-POS Board 27

Complete Kinetic Dissection Reveals the Rate-Limiting Mechanism of Transcription Elongation by RNA Polymerase II

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RNA polymerase is a molecular motor that converts chemical energy from ribonucleotide incorporation into mechanical translocation along the DNA template. It is generally accepted that such mechanochemical coupling occurs by a "Brownian ratchet" instead of a "power stroke" mechanism. However, the rate-limiting steps within the Brownian ratchet framework remained controversial. By using single-molecule manipulation and challenging individual yeast RNA polymerase II with a nucleosomal barrier, we separately measured the forward and reverse translocation rates. Surprisingly, we found that the forward translocation rate is comparable to the subsequent catalysis rate, in contradiction to the prevalent assumption that the pre- and post-translocated states rapidly reach equilibrium before catalysis. This finding reveals a linear Brownian ratchet mechanism in which translocation represents one of the rate-limiting steps. This mechanism unifies structural, biochemical, and single-molecule data and suggests that the recently proposed branched ratchet model, which necessitates a putative secondary nucleotide binding site on the enzyme, is not required to explain the observed force-velocity dependence of the polymerase. We further determined the other major on- and off-pathway kinetic parameters in the elongation cycle. The resulting energy landscape shows that the off-pathway states are favored thermodynamically, but not kinetically, over the on-pathway states, conferring the polymerase its propensity to pause and providing a physical basis for transcriptional regulation.

28-POS Board 28

Vertical Nanopillar for in Situ Probe of Nuclear Mechanotransduction

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As the control center of a cell, the cell nucleus contains the cell's genetic materials and regulates gene expression. The stability and deformability of the cell nucleus are important to many biological processes like migration, proliferation, polarization, and the differentiation of stem cells. When cells are exposed to mechanical force, the force will be transmitted via cytoskeleton to the nucleus, induce shape deformation of the nuclear envelopes, and even change the configurations of nucleoskeletons, which give the clue that cell nucleus itself may be able to sense and respond to mechanical signals. However, current techniques for studying nuclear mechanics are limited for inducing subcellular force perturbation in live cells. Here we developed a novel assay of using vertical nanopillar arrays to study the mechanical coupling between cell nucleus and cytoskeleton in live cells. Our results showed that nanopillars can induce deformation of nuclear envelope, and the deformation is controlled by the geometry of the nanopillars, and the stiffness of the nucleus. Also, cytoskeletons such actin and intermediate filaments were showed to play important roles in inducing nuclear deformation. Furthermore, we demonstrate that mechanical perturbation of the nuclear envelope can cause the reorganization of nuclear lamina. Overall, vertical nanopillars provide a non-invasive force to create a subcellular perturbation and can be used as a tool for studying nuclear mechanotransduction in live cells.

29-POS Board 29

Functional Conformational Changes of Blue-light Sensing Cryptochrome

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Cryptochromes, a kind of blue-light sensing proteins, are best known to regulate the entrainment of circadian rhythms responses in diverse organisms. More intriguingly, they are found to involve in the sensing of magnetic fields for insects and small animals. Algae, plant and animal cryptochromes possess vastly different size of carboxy-terminal (C-terminal) extensions, while the C-terminal domain is found to be significant in the functional response of plant cryptochromes. Here, we investigate the interaction partner, functional mechanism, and the possible C-terminal conformational changes of an alga cryptochrome (aCry). Protein pull-down assay is used to screen possible interaction partners of aCry, and a component of the circadian rhythm was found to directly interact with aCry in a blue-light dependent manner. We use single molecule Förster Resonance Energy Transfer (smFRET) to study the conformational changes of aCry. In the dark, aCry stays in the close conformation as monomer. Exposure to blue-light causes aCry to endure a conformational release of C-terminal domain from the PHR domain and partial homodimerization. Chemical reduction of the cryptochrome cofactor, FAD, induces further conformational extension in the presence and absence of blue-light.

30-POS Board 30

Tuning the Temperature of Single Cells: a New Tool to Study Temperature Sensing

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Temperature fluctuation is a common environmental cue that can affect many essential cellular activities including metabolism, proliferation, and apoptosis. Recent study shows that single cells can directly sense the environmental temperature change and respond through metabolic adjustment such as thermogenesis. Yet the mechanism of temperature sensing is largely unknown and remains an intriguing problem. The effort is stymied by the lack of appropriate tools that can induce a wide range of temperature perturbations in live cells with adequate time resolution. To address this problem we have developed a novel platform combining infrared laser induced temperature-jump (T-jump) and fluorescence live-cell imaging, whereupon we can tune the temperature of single cells or cell populations up to 60 degrees Celsius with millisecond resolved time resolution.

31-POS Board 31

Investigating Cellular Activity in Dissociated Neuronal Cultures Using Novel pH and PKA Activity Biosensors.

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A fundamental ambition within the field of neuroscience is to understand and describe neuronal signaling at the single neuron level as well as within complex neuronal circuits. Consequently, new insights into neuronal activity are a requirement in order to uncover the processes governing brain physiology. Traditionally the investigation of neuronal function on the single cell level has been limited to electrophysiological measurements providing great temporal resolution but poor spatial information and flexibility. However, the advents of novel biosensors have provided the tools to circumvent these caveats by allowing us to probe the function of neurons using less invasive techniques with high spatial resolution and increasing flexibility. Here we describe our work in applying biosensors in different neuronal culture systems, firstly to investigate hippocampal acidification using pHluorin as pH sensor and secondly to monitor PKA activity in striatal neurons.
Our group has recently described the use of the pH sensitive pHluorin-GFP variant (1) as a tool to monitor intracellular acidification in hippocampal neurons (2). We found that chemical LTD protocols decrease the intracellular pH in dissociated hippocampal neurons through a currently unknown calcium dependent mechanism. We have now implemented the use of simultaneous measurement of calcium and pH through the use of pHluorin and the calcium sensor R-GECO (3).
We also describe the use of the PKA activity sensor AKAR (4) to investigate discrete dopamine mediated changes in postsynaptic striatal neuron PKA activity. The sensitivity of this assay allows us to monitor changes of PKA activity in response physiological concentrations of agonist providing a unique method to investigate striatal dopamine physiology.

32-POS Board 32

Photo-Manipulation of Intracellular Ca^{2+} by Genetically Encoded Caged Ca^{2+}

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In living organism, Ca^{2+} is one of the most versatile second messenger to control biological processes such as muscle contraction, hormonal secretion and apoptosis induction. Its spatial and temporal dynamics has key role to regulate these physiological phenomena. To reveal such dynamics, variety of Ca^{2+} indicators had been developed. They enabled visualization of Ca^{2+} dynamics in open and clear manner. Nowadays, live cell imaging is providing meaningful Information for research in wide range of biological field. However, for deeper understanding of relationship between Ca^{2+} concentration changing and following response, development of useful tools to manipulate intracellular Ca^{2+} level have been desired.
In current methods, Ca^{2+} concentration is controlled by light through Ca^{2+} binding compounds with photocleavable moieties. However, they require irradiation of toxic ultraviolet wavelength light and/or cell loading associated with disruption of the cell membrane. These properties which have possibility to impair cells become big problem especially in the case of in vivo measurement. In addition to this, Ca^{2+} release from such compounds is irreversible.
In response to this, we developed genetically-encoded photoactivatable calcium ion-releaser PACR (PhotoActivatable Ca^{2+} Releaser). That is composed of Ca^{2+} binding protein and light-sensitive protein. Affinity of PACR for Ca^{2+} was decreased during irradiation of blue light. Thus reversible and repeatable increasing of Ca^{2+} concentration in cell is possible without damage to living specimens. By using PACR, we succeeded nucleus specific temporal Ca^{2+} concentration change in the HeLa cell and excitation of specific neuron in freely moving *C. elegans* by blue light irradiation. This useful tool is expected to contribute on researches to reveal the role of Ca^{2+} dynamics in complex biological phenomena.

33-POS Board 33

Localized Fluorescence Enhancement of Fluorescently Labelled DNA Strands Using Gold Nanovoids.

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Nanoscale spherical voids embedded inside a gold film exhibit strong localised Mie plasmon modes within the nanovoid volume.(1) The energy and electric field distribution of the localised Mie modes can be tuned with void dimension. The fabrication of spherical nanovoids involves a template method, in which the self-assembly of colloidal polymer nanospheres into a close-packed hexagonal array is followed by electrochemical growth of gold around the spheres.(2) The subsequent removal of the spheres leaves behind spherical gold nanaovoids. The use of gold shifts the localised plasmon resonances to the visible part of the electro-magnetic spectrum, crucial for fluorescence applications. The ability of surface plasmon polariton supporting, nanostructured metal substrates to enhance the fluorescence intensity of nearby fluorescence emitters is well-documented.(3, 4) Here, we present experimental results on the localised enhanced fluorescence intensity of fluorescently labelled DNA strands stretched over a gold nanovoid array, and interpret those results in light of the theoretically modelled localised Mie plasmon modes supported by voids of the same dimensionality. These studies have been extended to provide 3D structured gold nanovoids with pores through which the DNA strand is to be transported. Free-standing 3D gold microcavities with a nanopore milled through the base were designed for application using theoretical approaches so that the plasmon enhancement is greatest at the pore.These new structures have been successfully fabricated and the results will be presented.

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34-POS Board 34

Tissue Cell Morphology and Organization in Three-Dimensional Scaffolds of Uniform and Ordered Pores

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In this work, we fabricate novel gelatin scaffolds with uniform pores for 3D cell culture based on a rapid and economical microfluidic method. We cultured three different cell types namely MDCK, C2C12 and 3T3 with distinct epithelial, muscular and connective tissue origin respectively in the novel gelatin scaffolds and found those cells exhibiting morphological and physiological properties very relevant to their *in vivo* counterparts. Epithelial cells (MDCK) organized into characteristic cyst-like structures with apico-basal polarity confirmed by GP-135 and E-cadherin sub-cellular localization. Myoblasts (C2C12) exhibited marked aster-like linear pattern initially and on addition of differentiation medium formed thick myotubes with multiple nuclei. Fibroblasts (3T3) showed a vast range of morphologies which encouraged us to further investigate its properties when grown on scaffolds with various pore sizes. The cell shape appeared more elongated and exhibited higher number of focal adhesions in larger pore than the smaller pore. Also the cell volume increased with the increase in pore size. Further in smaller pore the actin appeared to be diffused whereas prominent stress fibers were visualized in the larger pore. The nuclear morphology was more spherical in the smaller pore which may affect cell proliferation rate. These findings highlight 3D cell organization and fibroblast behavior with respect to varying pore size promoting the systematic study of mechanical properties in tissue engineering and quantitative cellular mechanobiology.

35-POS Board 35

Ionic Mechanism of Propagation in Human Purkinje Fiber Cells: Role of Calcium Ions and Calcium Channels - A Simulation Study

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An important ailment that requires treatment by the physician is cardiac failure ("Heart Failure (HF)"), which usually decreases the contractility of the myocardium. The Purkinje Fiber Cells (PFC) augurs to be an important biological network of the human cardiac ventricular conduction system. It assuages to be tertiary pacemaker of the heart which synchronizes the ventricular conduction.
The paper analyzes the effects of calcium ionic mechanism of propagation using the Zhang model. The strength of contraction of cardiac muscle depends to a great extent on the concentration of calcium ions in the extracellular fluids. The calcium ions play a vital role in excitation, contraction coupling and cardiac muscle relaxation. The model attempts to increase the influx of calcium concentration (0.0004 mM) from the normal Ca2+ (0.0002 mM) value. The results show that the internal rise of calcium concentration increases the excitability of PFC. Similarly, the high calcium level in extracellular and low calcium level in intracellular decreases the excitability of PFC. The calcium channels appear to play a major role in sustaining conduction and propagation when low calcium level in intracellular fluids. The abnormal calcium concentration may affect the PFC excitability and Conduction Velocity, which in turn can lead to cardiac ventricular arrhythmias.

36-POS Board 36

Potassium-Mediated Tumor Invasion: A Mathematical Model

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The cancer cell invasion of tissue appears to be a complex biological process during which cell migration occurs through the extracellular matrix. It engages the potassium channels to regulate the behavior of cancer cell such as proliferation and migration through both canonical and non-canonical ion permeation functions. The pharmacological strategies, in view of their cell surface localization and well- known pharmacology target potassium channel and prove to be a promising therapeutics for cancer.
The paper proposes a hybrid discrete-continuum multiscale model to study the early growth of solid tumors and their ability to degrade and migrate into the surrounding extracellular matrix. It models the cancer cells as discrete individual entities to interact with each other sing a potential function. The theory involves partial differential equations to model the spatio-temporal dynamics of the other variables that includes extracellular matrix, matrix degrading enzymes and potassium concentration.

37-POS Board 37

Optomechanical Actuators for Controlling Mechanotransduction in Living Cells

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The most desirable approaches for characterization and manipulation within biological systems are optical-based. This is because of the non-invasive and high-resolution nature of optical techniques which has led to the widespread adoption of optical microscopy in biology. Therefore, the development of methods to harness light for delivering precise physical inputs to biological systems could potentially transform the study of mechanotransduction. To acheive this goal, we developed an approach for optically controlling receptor tension at the surface of living cells. This is achieved using optomechanical actuator nanoparticles that are controlled with non-invasive near-infrared light. Illumination leads to particle collapse within 1.2 msec, delivering ~13 pN piconewton forces to specific cell surface receptors with high spatial and temporal resolution. We specificaly decorate the surface of nanoactuators using the RGD peptide and immobilize the particles to conventional glass coverslips. Upon culturing of fibroblasts onto these surfaces, near-infrared illumination was used to exert pictonewton forces through the integrin receptors, thus mechanically controlling focal adhesion formation, cell protrusion, and cell migration in living cells. We demonstrate that 10-100 Hz frequency stimulation leads to paxillin and vinculin recruitment, as well actin polymerization in a Rho kinase independent manner. This material shows the first example of optically controlling cell migration without the use of genetic engineering.

38-POS Board 38

Lighting Up the Force: Molecular Tension Sensors that Fluoresce in Response to Piconewton Forces

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Mechanical stimuli profoundly alter cell fate, yet the mechanisms underlying mechanotransduction remain obscure due to a lack of methods for molecular force imaging. In this presentation, I will describe the development of mechanophores (molecules that fluoresce upon experiencing force) for imaging molecular forces exerted by individual integrin cell surface receptors. Specifically, we developed a new class of molecular tension probes that function as a switch to generate a 20-30-fold increase in fluorescence upon experiencing a threshold piconewton force. The probes employ immobilized DNA-hairpins with tunable force response thresholds, ligands, and fluorescence reporters. Quantitative imaging reveals that integrin tension is highly dynamic and increases with an increasing integrin density during adhesion formation. Mixtures of fluorophore-encoded probes show integrin mechanical preference for cyclized-RGD over linear-RGD peptides. Multiplexed probes with variable guanine-cytosine content within their hairpins reveal integrin preference for the more stable probes at the leading tip of growing adhesions near the cell edge. DNA-based tension probes are among the most sensitive optical force reporters to date, overcoming the force and spatial-resolution limitations of traction force microscopy. The application of these sensors to image forces associated with a range of mechano-regulatory processes that occur at the lipid membrane of the cell, such as endocytosis, Notch receptor activation, and integrin adhesion receptors will be described as well.

39-POS Board 39

Withdrawn

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40-POS Board 40

A FRET-based Calcium Biosensor based on Calmodulin (CaM) and Its Newly Identified Binding Peptide from c-FLIPL

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Genetically encoded calcium biosensors are valuable tools in cell biology and neuroscience. A FRET-based calcium biosensor Cameleon has received a great deal of optimization since it was released in 1997. Despite all the extensive improvements, the sensing and functional domains of Cameleon were solely based on engineering of the fusion protein CaM and its binding peptide from myosin light-chain kinase (M13). Whether there exist other feasible CaM-binding peptides that can replace M13 in sensor construction has not been explored. Here, we report a FRET-based calcium biosensor comprising CaM and its newly identified binding peptide from c-FLIPL. We tested the performance of this new CaM-binding peptide from c-FLIPL in construction of functional FRET-based biosensors. We also performed preliminary optimization and application in imaging of Ca2+ dynamics in living cells.

41-POS Board 41

Imaging Subcellular Voltage Dynamics in vivo with Improved Genetically Encoded Indicators

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Nervous systems encode information as spatiotemporal patterns of membrane voltage transients, so accurate measurement of electrical activity has been of long-standing interest. Recent engineering efforts have improved our ability to monitor membrane voltage dynamics using genetically encoded voltage indicators. In comparison with electrophysiological approaches, such indicators can monitor many genetically defined neurons simultaneously; they can also more easily measure voltage changes from subcellular compartments such as axons and dendrites. Compared with genetically encoded calcium indicators, voltage sensors enable a more direct, accurate, and rapid readout of membrane potential changes. However, several challenges remain for in vivo voltage imaging with genetically encoded indicators. In particular, current voltage sensors are characterized by insufficient sensitivity, kinetics, and/or brightness to be true optical replacements for electrodes *in vivo*.
As a first step towards addressing these challenges, we developed new voltage indicators, ASAP2f and ASAP2s, that further improve upon the sensitivity of the fast voltage sensor Accelerated Sensor of Action Potentials 1 (ASAP1). We also describe here how these novel sensors are able to report stimulus-evoked voltage responses in axonal termini of the fly visual interneuron L2. In this system, ASAP sensors enabled the monitoring of neural activity with greater temporal resolution than three recently reported calcium and voltage sensors. Overall, our study reports novel voltage indicators with improved performance, illustrates the importance of sensor kinetics for accurately reporting neural activity, and suggests L2 as an *in vivo* platform for benchmarking neural activity sensors. We anticipate that ASAP2f and ASAP2s will facilitate current and future efforts to understand how neural circuits represent and transform information.

42-POS Board 42

Curvature-Generating Proteins and Subcellular Pattern Formation

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Ordered assembly of cellular processes in the form of oscillations and waves is an emerging theme in living cells. Occurrences of such patterns indicate the presence of local and global coupling mechanisms. However, the nature of the coupling remains to be determined. In our recent work, we discovered a striking appearance of FBP17, one of the Bin1/amphiphysin/Rvs167 (BAR) domain proteins, in actin waves of stimulated mast cells. In addition to being a reporter, FBP17 is essential for wave formation. BAR domain proteins are widely known for their curvature sensing and inducing capabilities, motivating us to directly visualize membrane shape and test its function. We will discuss our findings on the role of physical parameters such as membrane curvature and plasma membrane tension in the propagation of waves. Collectively, our work suggests a mechanochemical basis for pattern formation, which regulates the dynamic reorganization of cell cortex in response to external stimulation, the first and essential step of cellular activation.

43-POS Board 43

Imaging Intraorganellar Ca^{2+} at Subcellular Resolution Using CEPIA

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The endoplasmic reticulum (ER) and mitochondria accumulate Ca^{2+} within their lumens to regulate numerous cell functions. However, determining the dynamics of intraorganellar Ca^{2+} has proven to be difficult. Here, we developed a family of genetically-encoded Ca^{2+} indicators, named calcium-measuring organelle-entrapped protein indicators (CEPIA), which can be utilized for intra-organellar Ca^{2+} imaging. CEPIA, which emit green, red or blue/green fluorescence, were engineered to bind Ca^{2+} at intra-organellar Ca^{2+} concentrations. They can be targeted to different organelles and may be used alongside other fluorescent molecular markers, expanding the range of cell functions that can be simultaneously analyzed. The spatiotemporal resolution of CEPIA made it possible to resolve Ca^{2+} import into individual mitochondria while simultaneously measuring ER and cytosolic Ca^{2+}. We used these imaging capabilities to reveal differential Ca^{2+} handling in individual mitochondria. CEPIA will provide a useful new approach to further the understanding of organellar functions.

44-POS Board 44

Study of Min Protein-Induced Membrane Species Waves in vitro

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In the bacterium Escherichia coli, the proper placement of the division site selection is regulated in part by the pole-to-pole oscillations of Min proteins. In vitro, the oscillation dynamics emerges from the self-organization of MinD, MinE and ATP. However, it is still unclear how the Min proteins affect the E. coli lipid membrane and interact with the other E. coli membrane proteins. We hypothesized that the spatial oscillations of Min protein systems could play a crucial role in changing lipid membrane dynamics and therefore indirectly influence the dynamics of other membrane associated species. We developed supported E. coli lipid bilayer platforms in order to systematically explore the underlying mechanism between lipid membranes and Min proteins. We observed fluorescently labeled lipids in the E. coli membranes moving in a spiral wave pattern after introducing unlabeled MinD, MinE and ATP to the membranes. Fluorescence recovery after photobleaching (FRAP) and kymograph analyses showed that dynamics of the labeled lipid membrane waves had different characteristics from those of the Min protein waves. The simultaneous observation of the labeled Min proteins and the labeled lipids in the membrane further suggested that the labeled lipid membrane pattern dynamics was directly influenced by the binding concentration gradients of the Min proteins. We will further incorporate some E. coli signaling proteins into the membrane to examine whether their movement or clustering can be influenced by the Min proteins-induced membrane pattern. The result could provide us insights into the long-standing question about the possible function of the E. coli Min protein oscillation phenomenon.

45-POS Board 45

Self-Referenced Quantitative FRET with Dual-Switchable Donor-Acceptor Pair

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Fluorescence resonance energy transfer (FRET), especially with fluorescent proteins of donor and acceptor, has been widely used to measure biomolecular interactions. To overcome limitations of existing approaches for quantitative FRET, we here put forward a novel platform of dual-switching FRET (dsFRET), with a photoswitchable donor as well as a photoswitchable acceptor. This way, neither donor-only nor acceptor-only samples would be required as control reference for calculation of FRET efficiency. Traditional 33-FRET and dsFRET were compared side by side for both intra- and inter-molecular interactions. Our data demonstrate that dsFRET exhibits higher accuracy and stability than 3-cube FRET, mainly benefited from in-situ references. Also, with confocal microscopy, dsFRET is able to achieve self-referenced quantitative FRET at subcellular levels, with which key molecular interactions in the cell could be quantified with spatial and temporal information, e.g., the dynamic interactions between subunits in heteromultimeric ion channels. Further development of dsFRET has been pursued to extend its applications, such as in-vivo FRET at organism levels.

46-POS Board 46

Spectrally Differential Imaging of Nuclear and Cytoplasmic Actin Filaments in Live Cells by Multicolor LifeAct-BiFC

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Actin participates in many fundamental cellular processes, which take place not only in the cytoplasm, such as cell motility, cell division, and vesicle movement, but also in the nucleus, including chromatin remodeling, RNA processing, and transcription regulation. However, live cell imaging of nuclear actin with high spatial resolution remains to be a challenging task, partially due to the low concentration of nuclear actin compared to its cytoplasmic counterpart. Here we developed a method that combines LifeAct, a 17-amino-acid F-actin probe and multicolor Bimolecular Fluorescence Complementation (BiFC) to spectrally separate nuclear F-actin from cytoplasmic F-actin, achieving differential imaging of F-actin in distinct subcellular compartments with high signal-to-noise ratio. With this method, we revealed that nuclear F-actin is also present in the cell nucleus at physiological condition, arguing against the current notion that canonical nuclear actin filaments are absent from the nucleus under physiological conditions. This method enables direct visualization of nuclear F-actin with high resolution in live cells and provides implications for the biological functions of nuclear F-actin.

47-POS Board 47

Sensing and Structure Investigations of Nucleic Acid Systems Using Fluorescent Base Analogue FRET-probes

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Utilization of the tricyclic cytosine family, tC/tCO/tCnitro, is increasing in nucleic acid applications and the fluorescent members of this family have unique properties among fluorescent base analogues.[1] In contrast to other fluorescent base analogues tCO, for example, has i) a high quantum yield in duplex being virtually insensitive to neighboring base combination, ii) an emission in DNA being characterized by a single-exponential decay in duplexes, and iii) an average brightness in duplex being among the highest reported and up to 50 times higher than 2-aminopurine.[2] Furthermore, the tricyclic bases form stable base pairs with guanine and give minimal perturbations to DNA native structure.[1-3] Importantly, we have utilized tCO as a donor and developed tCnitro as an acceptor and, thus, established the first base analogue förster resonance energy transfer (FRET)-pair.[3] As a consequence of the exact and rigid positioning, this FRET-pair enables high control of orientation factor. DNA strands containing the FRET-pair will, thus, make it possible to accurately distinguish distance- from orientation-changes. To enable optimized use of our probes we have developed the freeware FRETmatrix[4], globally fitting FRET-data to obtain the best overall structure/dynamics of the nucleic acid under investigation. Recently we successfully utilized our FRET-pair in studies on DNA structural changes[5] and have ongoing investigations using it in combination with FRETmatrix. We envision our method, possibly in combination with single-molecule FRET on longer distances, to be a powerful complement for techniques like NMR and X-ray crystallography. References: [1] Wilhelmsson Q. Rev. Biophys. 2010, 43, 159. [2] Sandin et al. Nucleic Acids Res. 2008, 36, 157. [3] Börjesson et al. J. Am. Chem. Soc. 2009, 131, 4288. [4] Preus et al. Nucleic Acids Res. 2013, 41, e18. [5] Shi et al. P.N.A.S. 2012, 109, 16510.

48-POS Board 48

Direct Visualization of Nanoscopic Diffusion Path of Single Lipid Molecules in Raft-Containing Bilayer Membranes with Microsecond Temporal Resolution

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Lipid rafts are lipid domains that float dynamically on cell plasma membranes. They are believed to play an important role in cell signaling processes due to their capability of recruiting receptor molecules into domains through interaction between lipids and proteins. Experimental evidence of how such interaction takes place at single-molecule level is limited because it is challenging to obtain sufficient spatiotemporal resolution for observing rapid and nanoscopic motion of individual molecules in the heterogeneous membranes.

Here, we employ an advanced high-speed optical microscope along with the single-particle tracking method to directly observe the partition and diffusion of single molecules in raft-containing reconstituted bilayer membranes. The bilayer membranes consisted of micron-sized liquid-ordered (L_o) domains coexisting with liquid-disordered (L_d) phases can be visualized in fluorescence. The distinct L_o domains provide a platform for studying lipid rafts as they share features with raft domains in cell membranes. Using interferometric detection of scattering signal (iSCAT) technique, we image and track individual small gold particles (20 nm in diameter) attached to the head groups of the probe lipid molecules. The iSCAT detection provides optimal spatial localization precision down to a few nanometers at very high speed (50 kHz), which allows us to continuously follow the motion of single lipid molecules with microsecond temporal resolution. The precise diffusion trajectories of the probe lipids were overlaid on the corresponding fluorescence image indicating the location of the L_o domains. The high spatiotemporal resolution reveals lipid diffusion in and out of membrane domains with unprecedented clarity. By further analyzing the trajectories, subdiffusion of lipids in L_o raft domains is detected in sub-milliseconds, suggesting the membrane heterogeneities in the nanoscopic scale contribute to the regulation mechanisms of lipid rafts.

49-POS Board 49

RecA E38K Mutant Displaces SSB without Apparent ssDNA Length Dependence

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RecA recombinases catalyze the homology pairing and strand exchange reactions in homologous recombinational repair. RecA must compete with single-stranded DNA binding proteins (SSB) for single-stranded DNA (ssDNA) substrates to form RecA nucleoprotein filaments as the first step of the repair process. Previously, it has been suggested that RecA competes with SSB by binding and extending onto the free ssDNA region. However, the detailed displacement process is not clear. In this study, we monitored individual RecA filament formation by single-molecule tethered particle motion (TPM) technique in real-time. Binding of RecA on the SSB wrapped-ssDNA extends the DNA substrate, visible by the increase in bead Brownian motion in the TPM imaging. We found a point mutation on RecA (E38K) accelerates the SSB removal rate. By varying the length of ssDNA gap from 60 to 100 nucleotides (60, 65, 70, 72, 75, 78, 80, 90, 100), in which only one SSB binding is allowed using the 65mer wrapping mode, we found the nucleation time of RecA E38K to form extended nucleoprotein filament is roughly constant over the ssDNA gap size used. This and control experiments suggest that the length of ssDNA is not critical for the nucleation process, an observation different from the long-believed, expected passive nature of RecA nucleation.

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Gymnastics in Translation—Deciphering Ribosomal Frameshifting Dynamics

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The genetic content of a messenger RNA (mRNA) can be recoded and hence expanded when the translating ribosomes are programmed to switch reading frames. For instance, the *Escherichia coli dnaX* mRNA programs ribosomes to decode not one but two protein products: the 0-frame τ and the -1-frameshifted γ subunits for DNA polymerase III. It was thought that the latter product is translated via a probabilistic -1-nucleotide slip midway during translation across a slippery sequence, AAAAAAG. mRNA structural barriers flanking the slippery sequence—i.e. a Shine-Dalgarno:anti-Shine-Dalgarno mini-helix and a stable hairpin—can further "actuate" the ribosome to frameshift with an efficiency as high as 80% (= γ/(γ+τ)). However, the mechanism, including the timing and location of such a -1-slip, remain unresolved. Here, we determine when within one translation cycle a slippage occurs by following a single ribosome translating a frameshift-programming mRNA held on optical tweezers. In complement, by mass spectroscopy, we survey the entire pool of synthesized polypeptides to identify on which codon the ribosome slipped.
Mass spectrometry of translated products shows that ribosomes enter the -1 frame from not one specific codon but various codons along the slippery sequence and slip by not just -1 but also -4 or +2 nucleotides. Coincidentally, single-ribosome translation trajectories detect distinctive codon-scale fluctuations in ribosome-mRNA displacement across the slippery sequence, representing multiple ribosomal translocation attempts during frameshifting. Flanking mRNA structural barriers mechanically stimulate the ribosome to undergo back-and-forth translocation excursions, thereby permitting the ribosome to explore alternative reading frames. Both experiments reveal aborted translation around mutant slippery sequences, indicating that subsequent fidelity checks on newly adopted codon position base pairings lead to either resumed translation or early termination. What has then emerged from our results is a versatile ribosomal frameshifting scheme during mRNA translocation, mediating broad branching of frameshift pathways.

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Three-Dimensional Cellular Traction Force Measurement and Simulation on a Flat and Compliant Substrate

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Measuring cellular traction force is essential to understand how cells process mechanical cues from microenvironment and respond to it. Most traction force measurements focus on the shear stress on a two-dimensional flat and compliant substrate. Recently more and more studies investigate three-dimensional (3D) traction stress including the normal stress whose magnitude is not negligible and play a role for cell migration speed. We present a 3D traction force method based on finite element method to analyze the displacements of marker beads embedded in the substrate under cell traction. We found the downward normal stress is sometimes located near the nucleus and sometimes near the proximal of focal adhesions which both are reported in the literature. We also perform simulation which we impose upward normal traction stress at the distal site of focal adhesion and downward normal traction stress at the proximal site of the focal adhesions and distribute the focal adhesions evenly on a circular cell edge. We found when the interparticle distance between marker beads are more than focal adhesion size or the uncertainty to displacement tracking increases, the recovered normal traction stress will deviate from the proximal site of focal adhesions toward the nucleus. Our results suggests a high resolution 3D traction force microscopy is necessary for consistent traction force measurement.

52-POS Board 52

Chemical Biology Sensors for Membrane Curvature and Lipid Composition

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Elevated microvesicle (MV) shedding is an indication of cancer metastasis. Selectively sensing the highly curved membrane of MVs may provide a novel strategy for their detection, which renders the potential of developing diagnostic, prognostic, and therapeutic agents. We employed a multidisciplinary approach, utilizing our expertise in computational modeling, membrane biophysics, and cellular assay development to rationally design novel tools that sense and regulate membrane curvature and lipid components. Curved membranes are a common and important attribute in exosomes and microvesicles. Our interest focuses on identifying and designing peptides that can sense membrane curvature based on established elements observed in natural curvature-sensing proteins. We have successfully identified a 25-mer peptide, MARCKS-ED, as well as its D-isomer that can both recognize PS with preferences for highly curved vesicles in a specific manner. These rationally designed agents not only help us to understand the critical protein-lipid interactions but also provide prototypes that may eventually lead to novel cancer biomarkers. These studies provide coveted specific probes for exosomes and microvesicles that may be of diagnostic and prognostic potentials.

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RFP Tags for Labeling Secretory Pathway Proteins

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Many RFPs are prone to form artificial puncta when labeling proteins in secretory pathway, which may severely impede their further uses in living cell imaging and multi-color imaging. Here we used an environment sensitive membrane protein Orai1 to report the fusion properties of several commonly used RFPs and revealed that intracellular artificial puncta are actually colocalized with lysosome, indicating pKa value of RFPs is also a key factor for artificial puncta appearance besides monomeric properties. In summary, our current study established a fast and easy method to evaluate RFPs fusion properties and provides a useful guide for choosing appropriate RFP for labeling secretory membrane proteins. Among RFPs tested, mOrange2 is highly recommended based on excellent monomeric property, appropriate pKa and high brightness.

54-POS Board 54

Developing Genetically Encoded Actuators with an Engineered Photocleavable Protein, PhoCle

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Here, we report a photocleavable protein (PhoCle) engineered in Campbell Lab from green-to-red photoconvertible fluorescent protein, mMaple. PhoCle is a circularly permutated variant of mMaple. Like all known green-to-red photoconvertible fluorescent proteins, PhoCle can be converted by violet light (~400 nm). This photoconversion involves a covalent bond break of peptide backbone at the chromophore. Upon photoconversion, PhoCle spontaneously dissociates into two fragments. Improved variants of PhoCle are engineered by using directed evolution. Genetically encoded actuators are being developed with PhoCle. One strategy is to combine PhoCle with the well-established approach of conditional inactivation of protein by genetic fusion to a steroid receptor (SR). Briefly, the protein-of-interest is fused to SR via a PhoCle linker such that it is in an inactive (caged) state until it is cleaved away from SR by illumination. Using this approach we have demonstrated photo-uncaging of Cre recombinase and the Gal4-VP16 transcription factor. Another strategy is activating protein by PhoCle-mediated release of a tethered inhibitory domain. PhoCle-caged protease has be developed by using this approach. Overall, PhoCle shows its potential to be a powerful and versatile new technology to develop genetically encoded actuators.

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Slowing Down DNA Translocation Through Solid-State Nanopores by Pressure

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Charged single molecules of DNA can be detected and characterized with a voltage-biased solid-state nanopore immersed in an electrolyte solution. This has stimulated intense research towards understanding and utilizing this nano-sensor device for the analysis of a wide variety of charged polymer molecules, and for the ultimate goal: DNA sequencing. As one of its fundamental challenges, DNA translocation speed through solid-state nanopores (~30 base/us) is too fast for instruments to "read" each base signal compared to their protein counterparts.

By taking advantage of the ability of solid-state membranes to sustain large pressure drops without breaking, we show here that a pressure-induced fluid flow, in and near the nanopore, provides an additional force to control the motion of the molecule through the pore. This pressure-derived force, combined with the voltage bias, enables solid-state nanopores to detect and characterize very short molecules, and near-neutral molecules, and to discriminate two different lengths of DNA molecules (600 bp and 1200 bp). For uniformly charged polymers like DNA, the pressure-derived force can be countered by the voltage-derived force to slow the molecule motion without reducing the ionic current signal by at least an order of magnitude. Modest pressures applied to a voltage-biased nanopore greatly extend their utility as single molecule detectors by enabling neutral molecule capture and detection, as well as control of molecule translocation speeds through the pore. We demonstrate nearly an order-of-magnitude improvement in length discrimination. This broader range of detectable molecule sizes, charge states, and spatial conformations considerably expands the applicability of nanopore detection technologies.

Publications:
1. Pressure-Controlled Motion of Single Polymers through Solid-State Nanopores, Bo Lu, David P. Hoogerheide, Qing Zhao,* et al. Nano Letters, 13, 3048 (2013)

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Single-Biomolecule Fluorescence Enhancement by Gold-Nanoantenna

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Single-molecule experiment can resolve molecular details of dynamic processes and has been applied extensively in biological studies and medical examinations. However, fluorescence from single biological macromolecules suffers from feeble signals that are not easily detected. We aim to utilize plasmonic nano-antennas, which can create highly enhanced local fields when pumped resonantly, leading to increased fluorescence intensities. Furthermore, to make the fluorescence enhancing process controllable and specific, we offered a novel method to conjugate fluorescent proteins of interests to gold nano-structures directly. Our method contains three parts: antenna-fabrication, surface passivation and gold-protein interaction. To replace conventional surface passivation procedure and facilitate nano-structure fabrication on insulating glass substrate, we demonstrated that CVD-graphene can be used to screen non-specific fluorescence signal while reducing charging effect during e-beam writing processes. For gold-protein conjugation, fluorescent proteins with Cysteine-tag can bind with gold-particle specifically via coordination bond between gold and thiol. In this step, pH is an important factor for binding process between gold-layer and protein molecules. The experimental fluorescence enhancement factors of fluorescent proteins coupled with gold nano-antenna are consistent with our simulation results. According to fano-effect, asymmetric-structures with controllable emission rate will provide stronger enhancement than bowtie, offering a potential improvement to our nano-antenna.