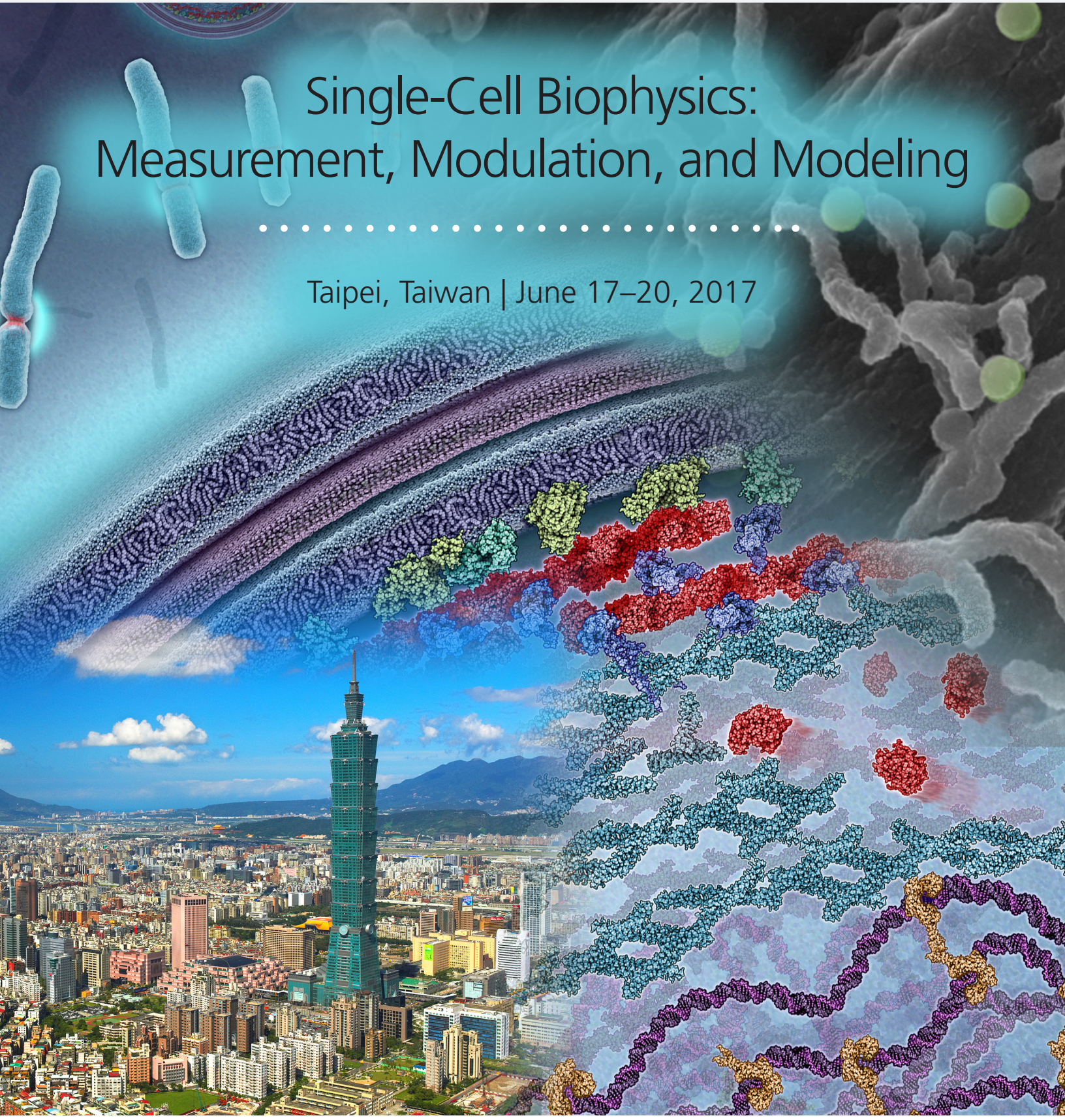
Single-Cell Biophysics: Measurement, Modulation, and Modeling

Taipei, Taiwan | June 17–20, 2017



APL Bioengineering

MAJOR | NSF | Georgia Institute of Technology | BITPLANE an Oxford Instruments company | 科技部 Ministry of Science and Technology | MOLECULAR DEVICES | 中央研究院 ACADEMIA SINICA | Biophysical Society

Organizing Committee

Jung-Chi Liao, Academia Sinica, Taiwan

Keng-Hui Lin, Academia Sinica, Taiwan

Christine Payne, Georgia Institute of Technology, USA

Jie Xiao, Johns Hopkins University, USA

Thank You to Our Sponsors

科技部
Ministry of Science and Technology

NSF

MAJOR

MOLECULAR DEVICES

MMI

BITPLANE
an Oxford Instruments company

Georgia Institute of Technology

APL Bioengineering

June 2017

Dear Colleagues,

We would like to welcome you to the Biophysical Society (BPS) Thematic Meeting, ***Single-Cell Biophysics: Measurement, Modulation, and Modeling***. BPS Thematic Meetings are an opportunity for scientists to gather and exchange ideas in different locations around the world. This meeting was made possible through funding from Taiwan's Ministry of Science and Technology (MOST) and the United States' National Science Foundation (NSF), as well as generous support from industry (APL Bioengineering, Bitplane, Major, Molecular Devices, and Molecular Machines & Industries) and Georgia Tech's College of Sciences.

Our meeting is aimed at bringing together physicists, biologists, chemists, and bioengineers to discuss the grand challenge of single cell biophysics. This is a truly global meeting with 170 participants from Australia, China, Denmark, Finland, Hong Kong, India, Japan, Netherlands, South Korea, Sweden, Taiwan, the United Kingdom, and the United States. During the meeting we hope to generate many informal discussions through coffee breaks, lunches, and multiple excursions. Two poster sessions will also provide an opportunity for one-on-one discussion. As an informal summer meeting, please dress casually and comfortably. Taipei can be warm in the summer and we expect temperatures >80°F/27°C.

Taiwan is a beautiful country with a rich culture. We hope to introduce you to some of this culture through an opening reception with traditional folk art, a tour of one of Taipei's famous night markets, and tours to two of the most famous landmarks in Taipei, the National Palace Museum and Taipei 101. A banquet on Monday night will highlight some of Taiwan's finest food.

Thank you for your participation. We look forward to four days of exciting science!

The Organizing Committee

Jung-Chi Liao, Institute of Atomic and Molecular Sciences, Academia Sinica, Taiwan
Keng-Hui Lin, Institute of Physics, Academia Sinica, Taiwan
Christine Payne, Georgia Institute of Technology, USA
Jie Xiao, Johns Hopkins University, USA

Table of Contents

GENERAL INFORMATION

Registration Hours/Information Location and Hours

Registration will be located at the Dr. Poe Lecture Hall Foyer of the Institute of Atomic and Molecular Science (IAMS) building, National Taiwan University (NTU). Registration hours are as follows:

Saturday, June 17	8:00 AM – 6:00 PM
Sunday, June 18	8:30 AM – 1:30 PM
Monday, June 19	8:30 AM – 6:00 PM
Tuesday, June 20	8:30 AM – 12:00 PM

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in the Dr. Poe Lecture Hall. 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 be held in the Courtyard of the Institute of Atomic and Molecular Sciences building, National Taiwan University.

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

3) There will be formal poster presentations on Saturday, June 17, and Monday, June 19, from 1:30 PM – 3:00PM. Odd-numbered posters should be set up on the morning of June 17 and removed by Noon on June 18. Even-numbered posters should be set up on the morning of June 19 and removed by 12:00 Noon on June 20.

4) During the assigned poster presentation sessions, 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.

Meals and Coffee Breaks

An Opening Reception will be held in the Courtyard on Saturday, June 17, from 6:00 PM – 7:30 PM.

Coffee breaks (June 17, 18, 19, and 20) and luncheons (June 17, 18 and 19) will be served in the Courtyard.

Social Events

On Saturday, June 17, there will be a night market tour where you can experience traditional Chinese street food from 7:30 PM – 9:30 PM. Transportation provided.

On Sunday, June 18, there will be a cultural tour where you will first visit the National Palace Museum followed by Taipei 101 from 2:00 PM – 7:00 PM.

On Monday, June 19, a banquet will be held at the Grand Hotel from 7:30 PM – 10:00 PM. Information regarding transportation will be provided at the registration desk.

All events are included in the registration fee, but advanced sign-up was required and tickets provided to those who completed sign-up. Tickets are required for admittance to transportation and functions. Advance sign-up deadline was May 18, 2017.

Smoking

Please be advised that smoking is not permitted on University Campus, including in the conference venue.

Name Badges

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

Internet

Wifi will be provided at the venue. Attendees will receive account number and password at registration.

University Map

Click here for a map of the campus.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from June 17 – June 20 during registration hours.

In case of emergency, you may contact the following:

Dr. Wei-Chun Huang
Email: chun5200@gmail.com
Cell: +886-911-103093

Dr. Jung-Chi Liao
Email: jcliao@iams.sinica.edu.tw
Cell: +866-979-074900

Dr. Ken-hui Lin
Email: kenghui@gmail.com
Cell: +866-933-365473

Dorothy Chaconas
Email: dchaconas@biophysics.org

Single-Cell Biophysics: Measurement, Modulation, and Modeling

Taipei, Taiwan
June 17-20, 2017

PROGRAM

Saturday, June 17, 2017

8:00 AM - 6:00 PM	**Registration/Information**	**Dr. Poe Lecture Hall Foyer**
8:55 AM - 9:00 AM	Jung-Chi Liao, Academia Sinica, Taiwan ***Opening Remarks***	**Dr. Poe Lecture Hall**
Session I	**Advanced Microscopy for Single Cell Studies** Jung-Chi Liao, Academia Sinica, Taiwan, Chair	
9:00 AM - 9:25 AM	Suliana Manley, École Polytechnique Fédérale de Lausanne, Switzerland ***The Tortoise and the Hare: Bacteria and Mitochondria Division Dynamics Revealed by Time-lapse Superresolution Microscopy***	
9:25 AM - 9:50 AM	Bi-Chang Chen, Academia Sinica, Taiwan ***Lattice Light Sheet Microscopy on Single-Cell Imaging***	
9:50 AM - 10:15 AM	Shean-Jen Chen, National Chiao Tung University, Taiwan ***Deep-Biotissue Imaging by Temporal Focusing Widefield Multiphoton Microscopy***	
10:15 AM - 10:35 AM	Masahiro Ueda, Osaka University, Japan* ***Automated Imaging System for Single-Molecule Analysis in Living Cells***	
10:35 AM - 11:05 AM	**Coffee Break**	**Courtyard**
Session II	**Single-Cell Mechanobiology I** Yujie Sun, Biodynamics Optical Imaging Center, Peking University, China, Chair	
11:05 AM - 11:30 AM	Taekjip Ha, Johns Hopkins University, USA ***Playing a Tug of War with Membrane Receptors Using the Double Helix***	
11:30 AM - 11:55 AM	Pakorn Kanchanawong, National University of Singapore ***Nanoscale Architecture of Cadherin-based Cell Adhesions***	
11:55 AM - 12:15 PM	Ashley Nord, Centre de Biochimie Structurale, France* ***Stator Stoichiometry and Mechanosensitivity of the Bacterial Flagellar Motor Probed by Load Manipulation***	
12:15 PM - 1:30 PM	**Lunch**	**Courtyard**
1:30 PM - 3:00 PM	**Poster Session I**	**Courtyard**
Session III	**Nanotechnology in Single Cell Biology** Takeharu Nagai, Osaka University, Japan, Chair	
3:00 PM - 3:25 PM	Bianxiao Cui, Stanford University, USA ***Membrane Curvature at the Nano-Bio Interface***	

3:25 PM - 3:50 PM	Haw Yang, Princeton University, USA ***The Multi-Resolution Imaging Approach to Nano-Bio Interactions at the Single-Cell Level***	
3:50 PM - 4:10 PM	Scott Thourson, Georgia Tech, USA * ***Localized Modulation of Single Cardiomyocytes Using PEDOT:PSS Conducting Polymer Microwires***	
4:10 PM - 4:40 PM	**Coffee Break**	**Courtyard**
Session IV	**Cellular Processes in Single Cells I: Cell Cycle** Jie Xiao, Johns Hopkins University, USA, Chair	
4:40 PM - 5:05 PM	Paul Wiggins, University of Washington, USA ***The Replisome Undergoes Multiple Rounds of Disassembly and Restart Every Cell Cycle***	
5:05 PM - 5:30 PM	Sheng-Hong Chen, Academia Sinica, Taiwan ***Protein Dynamics in Single Cells Unveil Regulatory and Therapeutic Principles***	
5:30 PM - 5:50 PM	Yuan Lin, University of Hong Kong* ***Shape Transformation of the Nuclear Envelope during Closed Mitosis***	
6:00 PM - 7:30 PM	**Opening Reception**	**Courtyard**
7:30 PM	**Night Market Tour**	**Shilin Night Market**

Sunday, June 18, 2017

8:30 AM - 1:30 PM	**Registration/Information**	**Dr. Poe Lecture Hall Foyer**
Session V	**Cellular Processes in Single Cells II: Transcription** Sua Myong, Johns Hopkins University, USA, Chair	
9:00 AM - 9:25 AM	Achillefs Kapanidis, Oxford University, United Kingdom ***Bacterial Transcription Meets Chromosome Organization: A Single-Molecule Perspective***	
9:25 AM - 9:50 AM	Nam Ki Lee, Pohang University of Science and Technology, South Korea ***Direct Observation of Transcription in a Living Bacterial Cell***	
9:50 AM - 10:15 AM	David Rueda, Imperial College London, United Kingdom ***Imaging Small Cellular RNAs with Fluorescent Mango RNA Aptamers***	
10:15 AM - 10:35 AM	Xiaoli Weng, Johns Hopkins University, USA* ***Spatial Organization of Transcription in E. coli via Superresolution Fluorescence Microscopy***	
10:35 AM - 11:05 AM	**Coffee Break**	**Courtyard**
Session VI	**Single-Cell Modeling** Wallace Marshall, University of California, San Francisco, USA, Chair	

11:05 AM - 11:30 AM	Phillip Nelson, University of Pennsylvania, USA ***Old and New News about Single-Photon Sensitivity in Human Vision***
11:30 AM - 11:50 AM	Po-Yi Ho, Harvard University, USA* ***Interrogating the Bacterial Cell Cycle by Cell Dimension Perturbations and Stochastic Modeling***
11:50 AM - 12:10 PM	Rosanna Smith, University of Southampton, United Kingdom* ***The Observer Effect in Cell Biology: Gene Expression Noise, Genetic Reporters, and the Problem of Measurement in Live Cells***
12:10 PM - 1:30 PM	**Lunch** **Courtyard**
1:30 PM	**Cultural Tour *(dinner on own)***

Monday, June 19, 2017

8:30 AM - 6:00 PM	**Registration/Information** **Dr. Poe Lecture Hall Foyer**
Session VII	**Cellular Processes in Single Cells III: Cell Shape and Size** Paul Wiggins, University of Washington, USA, Chair
9:00 AM - 9:25 AM	Wallace Marshall, University of California, San Francisco, USA ***Intrinsic and Extrinsic Noise in an Organelle Size Control System***
9:25 AM - 9:50 AM	KC Huang, Stanford University, USA ***Cell-size Determination by the Bacterial Actin Cytoskeleton***
9:50 AM - 10:15 AM	Chien-Jung Lo, National Central University, Taiwan ***How Fast Can Bacteria Grow Their Flagella?***
10:15 AM - 10:35 AM	Tony Yang, Academia Sinica, Taiwan* ***Intraflagellar Transport Proteins Undergo Nonaxonemal Staged Hindrance Between the Recruiting Distal Appendages and the Cilium***
10:35 AM - 11:05 AM	**Coffee Break** **Courtyard**
Session VIII	**Single-Cell Sequencing** Achillefs Kapanidis, Oxford University, United Kingdom, Chair
11:05 AM - 11:30 AM	Sunney Xie, Harvard University, USA; Peking University, Beijing Advance Innovation Center for Genomics, China ***Chromosomes as Single Molecules***
11:30 AM - 11:55 AM	Yanyi Huang, Biodynamics Optical Imaging Center, Peking University, China ***Microfluidic Single-Cell Sequencing***
11:55 AM - 12:15 PM	Jung-Ming Lin, University of California, Berkeley, USA* ***Single-Cell Proteotypic Analysis of Invasive Motility in Glioblastoma***
12:15 PM - 1:30 PM	**Lunch** **Courtyard**
1:30 PM - 3:00 PM	**Poster Session II** **Courtyard**

Session IX	**Cellular Processes in Single Cells IV: Chromosome Dynamics** Christine Payne, Georgia Institute of Technology, USA, Chair	
3:00 PM - 3:25 PM	Johan Elf, Uppsala University, Sweden ***Single-Molecule Studies of Cas9 Search Kinetics in Living Cells***	
3:25 PM - 3:50 PM	Melike Lakadamyali, Institute of Photonic Sciences, Spain ***Decoding Chromatin Organization with Superresolution Microscopy***	
3:50 PM - 4:15 PM	Yujie Sun, Biodynamics Optical Imaging Center, Peking University, China ***Single-Molecule Study of the Chromatin Structure and Dynamics***	
4:15 PM - 4:35 PM	Sangyoon Han, University of Texas Southwestern Medical Center, USA* ***Emerging Role of Differential Molecular Association in Force-transmitting Nascent Adhesions***	
4:35 PM - 5:05 PM	**Coffee Break**	**Courtyard**
Session X	**Single-Cell Mechanobiology II** Haw Yang, Princeton University, USA, Chair	
5:05 PM - 5:30 PM	Megan Valentine, University of California, Santa Barbara, USA ***Disruption of Cellular Force-sensing Triggers Systemic Tissue Collapse in the Botryllus Vasculature***	
5:30 PM - 5:55 PM	Chin-lin Guo, Institute of Physics, Academia Sinica, Taiwan ***Spontaneous Patterning of Cytoskeleton in Single Epithelial Cell Apicobasal Polarity Formation***	
5:55 PM - 6:15 PM	Poul Bendix, Niels Bohr Institute, Denmark* ***Rotation, Twisting, and Pulling: The Rich Dynamics of Filopodia***	
7:00 PM	Buses depart to Grand Hotel	
7:30 PM	**Banquet**	**Grand Hotel**

Tuesday, June 20, 2017

8:30 AM - 12:00 PM	**Registration/Information**	**Dr. Poe Lecture Hall Foyer**
Session XI	**Sensor and Probe Development** David Rueda, Imperial College London, United Kingdom, Chair	
9:00 AM - 9:25 AM	Amy Palmer, University of Colorado, Boulder, USA ***Quantitative Biology with Genetically Encoded Sensors – Opportunities and Challenges***	
9:25 AM - 9:50 AM	Takeharu Nagai, Osaka University, Japan ***Acid Resistant Monomeric GFP for Quantitative Single-Cell Analyses***	
9:50 AM - 10:15 AM	Sua Myong, Johns Hopkins University, USA ***Single mRNA Counting in Single Cell Reveals Function of RNA Stem Structure in Coupling Dicing and Gene Silencing***	

10:15 AM - 10:35 AM	Ralph Jimenez, University of Colorado, Boulder, USA* ***Systematic Evaluation of Cellular Zn^{2+} Sensors with Microfluidic Cytometry***
10:35 AM - 11:05 AM	**Coffee Break** **Courtyard**
Session XII	**Cellular Processes in Single Cells V: DNA Replication and Repair** Keng-Hui Lin, Institute of Physics, Academia Sinica, Taiwan, Chair
11:05 AM - 11:30 AM	Julie Biteen, University of Michigan, USA ***Single-Molecule Investigations of DNA Replication and Repair in Living Bacteria***
11:30 AM - 11:55 AM	Antoine van Oijen, University of Wollongong, Australia ***Single-Molecule Visualization of Bacterial DNA Repair in Live Cells***
11:55 AM - 12:15 PM	Nicholas Kurniawan, Eindhoven University of Technology, Netherlands* ***Local 3D Single-Cell–Matrix Interactions Underlie the Spatiotemporal Dynamics of Cell Populations***
12:15 PM - 12:20 PM	Keng-Hui Lin, Institute of Physics, Academia Sinica, Taiwan **Closing Remarks and *Biophysical Journal* Poster Awards**

**Contributed talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

The Tortoise and the Hare: Bacteria and Mitochondria Division Dynamics Revealed by Time-lapse Superresolution Microscopy

Suliana Manley, Ambroise Lambert, Aster Vanhecke, Anna Archetti, Seamus Holden, Tatjana Kleele, Lina Carlini, Dora Mahecic.
Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

Bacteria and mitochondria share a common ancient evolutionary history, and their division processes involve a similar sequence of shape changes, before they pass through a singular point on their way to becoming two. Nonetheless, as bacteria and mitochondria divide, their composite envelopes are shaped by dramatically different constraints and forces. Bacteria control and maintain their size from generation to generation, using a mechanism of constant elongation per cell cycle. Mitochondria dynamically become fragmented or form fused networks, depending on their metabolic state and that of the cell. However, fundamental questions remain as to how the rates and timing of these processes are controlled. We are using superresolution microscopy, both structured illumination- and single molecule localization-based, to elucidate the physical mechanisms behind these dynamic processes.

In the case of bacteria cell division, the control step for size homeostasis is unclear, and the relative roles of elongation and constriction and their coupling are poorly understood. Using genetic and pharmacological perturbations, we show that changing constriction rate alone can change the cell size. We also demonstrate that constriction duration compensates for elongation to allow for tighter homeostasis than either alone. We present a working model for how this may operate.

In the case of mitochondrial fission, while the cellular and molecular components implicated are known, little is known about the role of physical constraints. By comparing successful fission events with reversal events, we identify the roles of different physical parameters, such as bending energy and external pulling forces. We use existing models for membrane fission to begin to build a toy physical model for mitochondrial fission.

Lattice Light Sheet Microscopy on Single-Cell Imaging

Bi-Chang Chen.
Academia Sinica, Taipei, Taiwan.

Optical imaging techniques are constantly evolving with the desire to innovate an imaging tool that is capable of seeing sub-cellular processes in a biological system, especailly in three dimensions. We crafted ultra-thin light sheets derived from two-dimensional optical lattices that allowed us to image three-dimensional (3D) dynamics for hundreds of volumes, often at sub-second intervals, at the diffraction limit and beyond. We applied this tool to systems spanning four orders of magnitude in space and time, including the diffusion of single transcription factor molecules in a spheroid of stem cells, the 3D dynamic instability of microtubules during mitosis, the formation of the immunological synapse, neutrophil motility in a 3D matrix, and embryogenesis in Caenorhabditis elegans and Drosophila melanogaster.

Deep-Biotissue Imaging by Temporal Focusing Widefield Multiphoton Microscopy

Shean-Jen Chen[1], Chia-Yuan Chang[2,1], Yong-Da Sie[3].
[1]National Chiao Tung University, Tainan, Taiwan, [2]National Cheng Kung University, Tainan, Taiwan, [3]National Cheng Kung University, Tainan, Taiwan.

A developed temporal focusing-based multiphoton excitation microscope (TFMPEM) has a digital micromirror device (DMD) which is adopted not only as a blazed grating for light spatial dispersion but also for patterned illumination simultaneously. The TFMPEM has been extended to implement spatially modulated and digital holographic illumination to increase the beam coverage at the back-focal aperture of the objective lens. The axial excitation confinement (AEC) of TFMPEM can be condensed from 3.0 μm to 1.5 μm. By using the TFMPEM with HiLo technique, reconstructed deep-biotissue images according to the condensed AEC structured illumination are shown obviously superior in contrast and better scattering suppression.

Automated Imaging System for Single-Molecule Analysis in Living Cells

Masato Yasui[1], Michio Hiroshima[1], Jun Kozuka[1], Yasushi Sako[2], **Masahiro Ueda**[1,3].
[1]QBiC, RIKEN, Suita, Osaka, Japan, [3]Osaka University, Suita, Osaka, Japan.[2]Cellular Informatics Laboratory, RIKEN, Wako, Saitama, Japan,

Single-molecule imaging analysis has been applied to living cells and revealed molecular mechanisms of various intracellular events. However, technical expertise has been required for both microscope operation and data analysis, which has prevented the analysis from being a standard in medical and biological research. Here, we report a newly developed apparatus for single-molecule imaging analysis in living cells, by which single molecules on the plasma membrane can be observed without manual handling. Cell searching, focusing, and image acquisition were fully automated by utilizing a machine learning method to accomplish high accuracy, efficiency, and reproducibility. Furthermore, immersion-oil feeding, drug dispensing, and setting of the multi-well sample plate were also automated to observe many cells with different experimental conditions. The apparatus demonstrated that single-molecule imaging of EGF receptors in living CHO cells were completed for a 96-well plate within one day, in which about 600 cells were observed and analyzed automatically. Results revealed that EGF receptors adopt multiple states in their diffusion on membrane and undergo the state transition upon EGF stimulations, consistent with previous reports. The working efficiency was dramatically improved, showing that the automatically comprehensive single-molecule analysis in living cells is feasible.

Playing a Tug of War with Membrane Receptors Using the Double Helix

Taekjip Ha.
Johns Hopkins University, Baltimore, USA.

It is now widely appreciated that cancer cells and stem cells can change their cell fate (differentiation, metastasis, etc.) depending on their mechanical environment. Mechanical sensing is likely to be initiated by individual membrane receptor proteins that are in direct contact with the mechanical environment and are also linked to the cytoskeleton. In a sense, cells perform many single molecule mechanical measurements in parallel and process the information before making a critical cell fate decision. In order to understand how molecular level mechanical events trigger a cellular response, we need to examine the forces applied across individual cellular proteins during mechanical signaling. In order to study the mechanical requirements for integrin-mediated cell adhesion that regulates critical cellular functions in adherent cells we utilized our recently developed DNA tether called tension gauge tether (TGT) to study the mechanical requirements of integrin-mediated cell adhesion and activation of Notch receptors.

Nanoscale Architecture of Cadherin-based Cell Adhesions

Pakorn Kanchanawong.
National University of Singapore, Singapore

Cadherin-mediated cell adhesions are supramolecular complexes that play essential roles in ligating and mechanically integrating neighboring cells, supporting dynamic coupling between cell-cell adhesions and the contractile actin cytoskeletons. Despite well-documented functions in major aspects of tissue morphogenesis and multicellularity, the ultrastructural organization within cadherin-based adhesions remains unknown, thus obscuring insights into the underlying molecular mechanisms. We mapped the nanoscale organization of key cell-cell junction proteins within cadherin-based adhesions formed on planarized biomimetic cadherin substrate. The enhanced optical accessibility of the planar substrate together with interference-based nanoscopy methods enabled high precision (~10-nm) axial (z) position measurement using common fluorescent proteins. We observed a surprisingly well-organized molecular architecture that stratified along the z-axis, with the cadherin-catenin layer and the actin compartment separated by ~30 nm, interposed by a vinculin-containing interface zone. Our results indicated that vinculin can undergo a conformational activation to span between the cadherin-catenin layer and the actin compartment. The nanoscale positioning of vinculin is determined by alpha-catenin, while vinculin conformational state is controlled by contractility and Abl kinase phosphorylation on the residue Y822 of vinculin. Vinculin activation, in turn, modulates the positioning of VASP and zyxin, inducing VASP-mediated actin polymerization, that likely results in a positive feedback loop that regulates junction strengthening. In conclusion, our measurements reveal a modular nanoscale architecture of cadherin-based adhesions, suggesting a control principle whereby vinculin serves as a molecular clutch that integrates mechanical and biochemical signals to differentially engage the cadherin-catenin complexes to the actomyosin contraction machinery under different contexts such as developmental processes or diseases states.

Stator Stoichiometry and Mechanosensitivity of the Bacterial Flagellar Motor Probed by Load Manipulation

Ashley L. Nord, Emily Gachon, Alessandro Barducci, Francesco Pedaci.
Centre de Biochimie Structurale, Montpellier, France.

The bacterial flagellar motor (BFM) is the multi-component complex which powers the swimming and swarming of many motile bacteria. The BFM structure, many details of which are still unknown, displays a rich dynamic behavior in terms of exchange and conformational change of its internal components. The torque of this rotary motor is provided by stators, ion motive force powered ion channels which are known to assemble and disassemble dynamically in the BFM. Recently, it has been observed that this turn-over is mechano-sensitive, with the number of engaged stators dependent upon the external load acting on the motor. Despite their central role in the function of the BFM, a systematic study of the stator dynamics, as a function of the external parameters in unperturbed motors, is lacking. Here we provide a quantitative and non-invasive measurement of the temporal behavior of the stators active in the BFM of *E. coli*, by estimating stator stoichiometry from high-resolution single-motor torque traces, quantifying for the first time the dependence between stator number and external load at steady-state. Furthermore, a rapid and controlled change in the external load, applied via a magnetic field, allows us to directly probe BFM mechano-sensitivity, systematically triggering and detecting stator association and dissociation. We incorporate these results into an adsorption model of stator kinetics, providing the first step into understanding the mechanism of mechano-sensitivity of the BFM.

Membrane Curvature at the Nano-Bio Interface

Bianxiao Cui.
Stanford University, Stanford., USA.

The interaction between the cell membrane and the contacting substrate is crucial for many biological applications such as medical implants. We are interested in exploring nanotechnology and novel materials to improve the membrane-surface interactions. Recently, we and other groups show that vertical nanopillars protruding from a flat surface support cell survival and can be used as subcellular sensors to probe biological processes in live cells. Vertical nanopillars deform the plasma membrane inwards and induce membrane curvature when the cell engulfs them, leading to a reduction of the membrane-substrate gap distance. We found that the high membrane curvature induced by vertical nanopillars significantly affects the distribution of curvature-sensitive proteins and stimulates several cellular processes in live cells. Our studies show a strong interplay between biological cells and nano-featured surfaces, which is an essential consideration for future development of interfacing devices.

References

1. Zhao W, Hanson L, Lou HY, Akamatsu M, Chowdary P, Santoro F, Marks JR, Grassart A, Drubin DG, Cui Y, Cui B, Nanoscale manipulation of membrane curvature for probing endocytosis in live cells, Nature Nanotechnology, accepted (2017).
2. Hanson L, Zhao W, Lou HY, Lin ZL, Lee SW, Chowdary P, Cui Y, Cui B, Vertical nanopillars for in situ probing of nuclear mechanics in adherent cells, Nature Nanotechnology, 10, 554-562, (2015).
2. Lin ZL, Xie C, Osakada Y, Cui Y, Cui B, Iridium Oxide Nanotube Electrodes for Intracellular Measurement of Action Potentials, Nature Communications, 5, 3206 (2014).
3. Xie C, Lin ZL, Hanson L, Cui Y, Cui B, Intracellular recording of action potentials by nanopillar electroporation, Nature Nanotechnology, 7, 185-190 (2012).
4. Hanson L, Lin ZL, Xie C, Cui Y, Cui B, Characterization of the Cell-Nanopillar Interface by Transmission Electron Microscopy, Nano Letters, 12, 5815-5820 (2012).

The Multi-resolution Imaging Approach to Nano-Bio Interactions at the Single-Cell Level

Haw Yang.
Princeton University, Princeton, NJ, USA.

Nano materials hold great promise for fundamental biological research as well as biomedical applications. Mechanistic understandings for the nano materials actions, however, is often challenging due to the inhomogeneity in the nano materials themselves, and also to the complexity of biological media such as serum or live cells. Studying nano-bio interactions in real time at the single-particle level, in principle, should alleviate some of the difficulties and could bring about new insights that are unavailable otherwise. In this report, we will discuss some new tools that are being developed, e.g., 3D multi-resolution imaging, and their implications in future applications. Specifically, 3D multi-resolution imaging is achieved by combining real-time 3D single-particle tracking and 2-photon confocal microscopy to achieve 10 nm localization precision in all XYZ directions and 10 microsecond time resolution in the context of the sectioning confocal microscopy. This new approach has afforded the visualization of a single virus-like particle moving through the 3D space, finding a live call, landing on the cell, and eventually leaving the cell surface. Moreover, it enables tracing out cellular anatomy in 3D fidelity with a resolution commensurate with that of 2D electron microscopy, which can only operate on microtomed, fixed cells. We will also discuss some of the new developments following intra-cellular dynamics and trafficking.

Localized Modulation of Single Cardiomyocytes Using PEDOT:PSS Conducting Polymer Microwires

Scott Thourson[1,2], Christine K. Payne[1,3].
[1]Interdisciplinary Program in Bioengineering, Georgia Institute of Technology, Atlanta, GA, USA, [2]Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [3]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA.

Electrical modulation of single cells is needed to better understand and treat dysfunctional heart and brain cells. Current methods, such as patch clamping or microelectrode arrays, suffer from mechanical rigidity, complex fabrication methods, poor chronic stability, or spatial limitations. Conductive polymer wires have a small diameter (150 nm – 8 μm), high charge density (> 5 mC cm^{-2}) and relatively low Young's modulus (~ 1 GPa) that could enable long-term modulation of individual cells in a soft tissue environment. The present research used PEDOT:PSS microwires grown from gold electrodes to locally stimulate action potentials in single rat neonatal cardiomyocytes. These conductive polymer wires had an electrical conductivity of 33 ± 21 S cm^{-1}, determined with two point probe resistance measurements. Analysis of electrical current transients determined a charge storage density up to 6 mC cm^{-2} for the microwires. Successful cardiomyocyte stimulation was dependent on wire length, diameter, voltage, and wire separation. Since the local electric field stimulation was nonuniform, COMSOL was used in conjunction with experimental results to model the electric flux generated by PEDOT:PSS microwires near the cell membrane. The minimum electric flux needed for successful stimulation was found to be 1.71 ± 0.26 mV mm. These findings are important in developing small, tunable wires that can locally stimulate individual cells using nonuniform electric fields. We expect that these polymer wires will be useful for chronic, single cell level stimulation within brain, heart, or muscle tissue.

The Replisome Undergoes Multiple Rounds of Disassembly and Restart Every Cell Cycle

Paul Wiggins[1,2,3].
[1]University of Washington, Seattle, WA, USA, [2]University of Washington, Seattle, WA, USA, [3]University of Washington, Seattle, WA, USA.

The canonical model of replication describes a highly-processive and largely continuous process in which the genome is duplicated. This continuous model is based upon in vitro reconstitution and in vivo ensemble experiments. In this study, we characterize the replisome-complex stoichiometry and dynamics with single-molecule resolution in bacterial cells. These experiments reveal that the complex undergoes pervasive disassembly and reassembly every cell cycle (>5 times). Strikingly, a significant fraction of cells (>40%) have only a single helicase and polymerase complex. Many of the observed complexes have short lifetimes (<5 minutes). This instability is conflict-induced: transcription inhibition stabilizes these complexes and increases the replication rate. In contrast to the canonical model, DNA replication is a largely discontinuous process in vivo as a consequence of frequent replication-transcription conflicts.

Protein Dynamics in Single Cells Unveil Regulatory and Therapeutic Principles

Sheng-Hong Chen
Academia Sinica, Taipei, Taiwan

No Abstract

Shape Transformation of the Nuclear Envelope during Closed Mitosis

Qian Zhu, Chuanhai Fu, **Yuan Lin**.
The University of Hong Kong, Hong Kong, Hong Kong.

Lower eukaryotes, such as fission yeast , undergo closed mitosis during which the nuclear envelope (NE) stays intact but changes shape dramatically, usually from a sphere to an ellipsoid and then to a dumbbell for wild-type cells. In comparison, the NE in gene-deletion mutants of the yeast can undergo asymmetric division which often involves tethering or budding of the nuclear membrane.

Here we report a combined experimental and theoretical study to examine this intriguing phenomenon. Specifically, shape evolution of the cell nuclei in the wild-type and different mutants, with known gene defects, of fission yeast was closely monitored with live-cell imaging at high temporal resolution. Interestingly, it was found that structural deficiencies in one or both SPBs will cause the improper assembly and anchoring of mitotic spindle microtubules and ultimately lead to the formation of a single or multiple tethers. On the theoretical side, a physical model was also developed to predict the nuclear shape during mitosis based on energetic considerations. Our model suggests that, in addition to the bending rigidity and surface energy of the nuclear membrane, the spatial distribution of internally generated forces on the NE plays a key role in its shape transformation, with forces localized on both poles of the cell resulting in membrane tethering while a load distribution over a broad area typically leading to the formation of two equal-sized spherical daughter nuclei. These results provide physical explanations on how complex shapes of the nuclear envelope are developed during cell division as well as elucidate their correlations with structural alterations in the nuclear-cytoskeleton, as indicated in our experiments.

Bacterial Transcription Meets Chromosome Organization: A Single-Molecule Perspective

Achillefs Kapanidis, Mathew Stracy.
University of Oxford, Oxford, United Kingdom.

Despite the fundamental importance of transcription, a comprehensive analysis of RNA polymerase behavior and its role in the nucleoid organization in vivo is lacking, in part due to lack of sensitivity of conventional imaging and to its inability to resolve fine cellular structures. To address this challenge, my group is using superresolution fluorescence microscopy to study the localization and dynamics of the transcription machinery and the bacterial chromosome in live bacterial cells, both at the single-molecule and the population level.

Specifically, we use photo-activated single-molecule tracking to discriminate between diffusing RNA polymerases and RNA polymerases specifically bound to DNA, either on promoters or transcribed genes. We find that transcription can cause spatial reorganization of the nucleoid, with movement of gene loci out of the bulk of DNA as levels of transcription increase. We also studied the degree and mode of interaction of RNAP with the DNA during on the promoter search process, showing that RNAP interacts substantially with non-specific DNA. Current work focuses on identifying the identity and organisation of genes that are highly transcribed, as well as on developing assays to study the non-specific interactions of DNA-binding proteins with chromosomal DNA. Our work provides a global view of the organization of transcription and its interplay with chromosome organisation in living bacteria.

Direct Observation of Transcription in a Living Bacterial Cell

Nam Ki Lee.
Seoul National University, Seoul, South Korea.

Transcription, a process of mRNA generation by RNA polymerase (RNAP), is highly coupled with translation by ribosome in bacteria. The effect of the transcription-translation coupling on the transcriptional dynamics and the localization of genes in a living cell is poorly understood. Here, we directly observe the dynamics of transcription and the movement of the subcellular localization of genes actively transcribed by RNAP in living cells at the sub-diffraction limit resolution. The subcellular localizations of the non-membrane protein' genes, actively transcribed by RNAPs, move toward outside nucleoid or to plasma membrane by the effect of translation by ribosome. The movement of genes by transcription-translation coupling is general for both E. coli RNAP and T7 RNAP. Importantly, the subcellular movement of the gene is coupled with the enhancement of the transcription initiation rate, which is to a certain extent analogous to the chromatin decondensation in eukaryotes. Our observation demonstrates how two spatially separated processes of transcription and translation are coupled in bacteria and the movement of genes by the cooperation between transcription and translation plays a crucial role in the effective expression of genes in E.coli.

Imaging Small Cellular RNAs with Fluorescent Mango RNA Aptamers

Adam Cawte[1,2], Sunny Jeng[3], Alexis Autour[4], Michäel Ryckelynck[4], Peter Unrau[3], **David Rueda**[1,2].
[1]Imperial College London, London, United Kingdom, [2]MRC London Institute of Medical Sciences, London, United Kingdom, [3]Simon Fraser University, Burnaby, BC, Canada, [4]Université de Strasbourg, Strasbourg, France.

In recent years, there has been an explosion of fluorescent RNA aptamers that have been isolated using SELEX. Since their discovery, fluorogenic RNA aptamers, such as Spinach and Mango[1,2] have held great potential to enable the visualisation of RNA molecules in cells. However, their applicability has been limited primarily to bacterial cells.[3] Evolving new RNA aptamers with improved physicochemical properties (i.e., thermal stability, fluorescence brightness and ligand affinity) should better their use in cellular imaging.

Three new Mango-like aptamers have recently been evolved using microfluidic-assisted in vitro compartmentalization, mutagenesis and fluorescent selection,[4] which improved fluorescence brightness, ligand binding affinity and thermal stability. We show that these aptamers are readily useable to image small non-coding RNAs (such as 5S rRNA and U6 snRNA) in both live and fixed human cells with improved sensitivity and resolution. The imaging data show that the Mango tagged RNAs sub-cellular localisation pattern is conserved, as validated using immunofluoresence. Our data show that these new aptamers are vastly improved for cellular imaging over previous variants, and can in principle be incorporated into a wide range of coding and non-coding RNAs. We anticipate that these new aptamers will drastically improve RNA imaging in cells.

1. Paige, J. S., Wu, K. Y. & Jaffrey, S. R. RNA mimics of green fluorescent protein. Science. 333, 642–646 (2011).
2. Dolgosheina, E. V. et al. RNA Mango aptamer-fluorophore: A bright, high-affinity complex for RNA labeling and tracking. ACS Chem. Biol. 9, 2412–2420 (2014).
3. Zhang, J. et al. Tandem Spinach Array for mRNA Imaging in Living Bacterial Cells. Sci. Rep. 5, 17295 (2015).
4. Autour, A., Westhof, E., and Ryckelynck, M. (2016). iSpinach: a fluorogenic RNA aptamer optimized for in vitro applications. Nucleic Acids Research 44, 2491-2500.

Spatial Organization of Transcription in E. coli via Superresolution Fluorescence Microscopy

Xiaoli Weng[1], Christopher H. Bohrer[1], Arvin C. Lagda[2], Jie Xiao[1].
[1]Johns Hopkins School of Medicine, Baltimore, MD, USA, [2]Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Transcription, the process of converting genetic information stored in DNA to RNA, lies at the heart of gene expression. Transcription has been studied extensively in-vitro to probe its mechanistic detail, however, these conditions differ from the complex environment inside a living cell. Spatial distributions of molecular components have recently been shown to be an important facet of gene regulation in prokaryotic systems. We investigated the spatial distributions of various molecular components of transcription in *E. coli* and their physical correlation with each other, to gain insight into the regulation of gene expression at the global, cellular level. Using superresolution fluorescence microscopy, we found that RNA Polymerase (RNAP) forms distinct clusters under fast growth that are largely retained in cells under different global transcription perturbations. RNAP clusters are the most homogenously distributed in cells without active transcription via rifampicin treatment. Additionally, we used multi-color superresolution imaging to correlate the spatial localization of RNAP clusters with DNA sites, nascent rRNA and elongation factor NusA to further elucidate the underlying make-up of RNAP clusters. Our results show that RNAP clusters are highly co-localized with NusA, thus are likely composed of elongation complexes. Interestingly, while RNAP clusters have a certain level of colocalization with nascent rRNA, and rrn DNA sites in fast growing cells, RNAP clusters are retained under conditions where rRNA synthesis is reduced. This points to the independence of formation of RNAP clusters from active rRNA synthesis. These results suggest a high level of heterogeneity both in the spatial organization and functional role of RNAP clusters in *E. coli*.

Old and New News about Single-Photon Sensitivity in Human Vision

Philip Nelson.
University of Pennsylvania, Philadelphia, PA, USA.

One often hears that human vision is “sensitive to single photons,” when in fact the faintest flash of light that can reliably be reported by human subjects is closer to 100 photons. Nevertheless, there is a sense in which the familiar claim is true. Experiments conducted long after the seminal work of Hecht, Shlaer, and Pirenne now admit a more precise, and in some ways even more remarkable, conclusion to be drawn about our visual apparatus. A simple model that incorporates both old news (response of single rod cells) and newer news (loss at the first synapse) can account in detail for both old and new psychophysical data.
[Work supported in part by US NSF grant PHY--1601894.]

Interrogating the Bacterial Cell Cycle by Cell Dimension Perturbations and Stochastic Modeling

Hai Zheng[1,2], **Po-Yi Ho**[3], Meiling Jiang[1], Bin Tang[4], Weirong Liu[1,2], Dengjin Li[1], Xuefeng Yu[5], Nancy Kleckner[6], Ariel Amir[3], Chenli Liu[1,2].
[3]Harvard University, Cambridge, MA, USA, [1]Shenzhen Institutes of Advanced Technology, Shenzhen, China, [2]University of Chinese Academy of Sciences, Beijing, China, [4]Southern University of Science and Technology, Shenzhen, China, [5]Shenzhen Institutes of Advanced Technology, Shenzhen, China, [6]Harvard University, Cambridge, MA, USA.

Bacteria tightly regulate and coordinate the various events in their cell cycles to duplicate themselves accurately and to control their cell sizes. Growth of *Escherichia coli*, in particular, follows Schaechter's growth law. The law says that average cell volume scales exponentially with growth rate, with a scaling exponent equal to the time from initiation of a round of DNA replication to the cell division at which the corresponding sister chromosomes segregate. Here, we test the robustness of the growth law to systematic perturbations in cell dimensions achieved by varying the expression levels of mreB and ftsZ. We found that decreased mreB levels resulted in increased cell width, with little change in cell length, whereas decreased ftsZ levels resulted in increased cell length. In both cases, the time from replication termination to cell division increased with the perturbed dimension. Importantly, the growth law remained valid over a range of growth conditions and dimension perturbations. The growth law can be quantitatively interpreted as a consequence of a tight coupling of cell division to replication initiation. Its robustness to perturbations in cell dimensions strongly supports models in which the timing of replication initiation governs that of cell division, and cell volume is the key phenomenological variable governing the timing of replication initiation. These conclusions are discussed in the context of our recently proposed "adder-per-origin" model, in which cells add a constant volume per origin between initiations and divide a constant time after initiation.

The Observer Effect in Cell Biology: Gene Expression Noise, Genetic Reporters, and the Problem of Measurement in Live Cells

Rosanna C. Smith[1,3], Patrick S. Stumpf[1,3], Sonya J. Ridden[2], Aaron Sim[4], Sarah Filippi[5], Heather A. Harrington[6], Ben D. MacArthur[1,2,3].
[1]University of Southampton, Southampton, United Kingdom, [2]University of Southampton, Southampton, United Kingdom, [3]University of Southampton, Southampton, United Kingdom, [4]Imperial College, London, United Kingdom, [5]University of Oxford, Oxford, United Kingdom, [6]University of Oxford, Oxford, United Kingdom.

Fluoresence reporters allow investigation of temporal changes in protein expression in live cells and are consequently an essential measurement tool in modern molecular biology. However, their utility is dependent on their accuracy, and the effects of reporter constructs on endogenous gene expression kinetics are not well understood. Here, using a combination of mathematical modelling and experiment, we show that widely used reporter strategies can systematically disturb the dynamics they are designed to monitor, sometimes giving profoundly misleading results. We illustrate these results by considering the dynamics of the pluripotency regulator Nanog in embryonic stem cells, and show how reporters can induce heterogeneous Nanog expression patterns in reporter cell lines that are not representative of the wild-type. These findings help explain the range of published observations of Nanog variability and highlight the problem of measurement in cell biology in relation to genetic reporters.

Intrinsic and Extrinsic Noise in an Organelle Size Control System

Wallace Marshall
University of California, San Francisco, CA, USA

No Abstract

Cell-size Determination by the Bacterial Actin Cytoskeleton

Kerwyn C. Huang.
Stanford, Stanford, CA, USA.

Changes in bacterial cell size are associated with critical changes in fitness and growth rate at the single-cell level; at the population level, these changes affect biofilm formation and virulence. To explore the relationships among genotype, structural phenotype, and bacterial physiology, we constructed a library of *Escherichia coli* mutants with a range of cell sizes and used fluorescence-activated cell sorting to identify subtle changes in cell shape, allowing us to query the effect of cell shape on several physiological behaviors, growth rate, DNA content, and drug sensitivity. The quantitative relationships revealed by this strategy highlight the complex and dynamic links between bacterial morphology and physiology. In *E. coli*, the actin-like protein MreB localizes in a curvature-dependent manner and spatially coordinates cell-wall insertion to maintain cell shape across changing environments, although the molecular mechanism by which cell width is regulated remains unknown. I will show that the bitopic protein RodZ regulates the biophysical properties of MreB and alters the spatial organization of *E. coli* cell-wall growth. The relative expression levels of MreB and RodZ changed in a manner commensurate with variations in growth rate and cell width. We carried out molecular dynamics simulations and single-cell analyses to determine that RodZ alters the curvature-based localization of MreB, and therefore cell shape. Together, our results show that E. coli controls its shape and dimensions by differentially regulating RodZ and MreB to alter the patterning of cell-wall insertion, highlighting the rich regulatory landscape of cytoskeletal molecular biophysics.

How Fast Can Bacteria Grow Their Flagella?

Chien-Jung Lo,
National Central University, Taoyuan, Taoyuan, Taiwan.

Chien-Jung Lo
Department of Physics and Graduate Institute of Biophysics, National Central University, Jhongli, Taoyuan, Taiwan

Bacterial flagella are self-assembled external tubular filaments for swimming. During the assembly process, flagellins are transported unfolded through the central channel in the flagellum to the growing tip. We develop in vivo single-cell fluorescent imaging to monitor in real time the Vibrio alginolyticus polar flagella growth. The flagellar growth rate is found to be highly length-dependent. We characterized the key factors controlling the flagellar growth rate. We modeled flagellin transport inside the channel as a one-dimensional diffusive process. When the flagellum is short, its growth rate is determined by the loading speed at the base. Only when the flagellum grows longer does diffusion of flagellin become the rate-limiting step, dramatically reducing the growth rate. Our results and model shed new light on the dynamic building process of this complex extracellular structure.

Reference: M.T. Chen, Z.Y. Zhao, J.Yang, P. Kai, M.A.B. Baker, F. Bai and C.-J. Lo*, (2017) Length-dependent flagellar growth rate of Vibrio alginolyticus revealed by real time fluorescent imaging. eLife. e22140

Intraflagellar Transport Proteins Undergo Nonaxonemal Staged Hindrance between the Recruiting Distal Appendages and the Cilium

Tony Yang, Minh Nguyet T. Tran, Weng Man Chong, Chia-En Huang, Jung-Chi Liao.
Academia Sinica, Taipei, Taiwan.

The primary cilium is an essential organelle responsible for multiple sensory and signaling activities. Ciliogenesis is achieved by delivery of precursors such as tubulins along the axoneme through intraflagellar transport (IFT), which is mediated by molecular motors and various IFT particles. Distal appendages (DAPs) are known to serve as the recruiting site of IFT proteins. During ciliogenesis, IFT proteins must go through several different zones in cilia. One of missing links of the IFT dynamics is how IFT particles move between the DAPs and the ciliary axoneme. The major obstacle comes from the tiny volume surrounding the DAPs and TZ and the high density of IFT particles in this region, which is far smaller than the diffraction-limited spot. Here we performed live-cell sptPALM-based superresolution tracking of short trajectories to demonstrate IFT particle dynamics at the ciliary base with the optimization of particle density and trajectory duration suitable for IFT motion speed. Our results revealed the DAPs and TZ accommodate not only axonemal but also transverse IFT88 movement. IFT particles move slower at the base than in the ciliary compartment. Moreover, diffusion analysis revealed that IFT particle movement was confined at the distal TZ while superdiffusive at the proximal TZ. This heterogeneous diffusion characteristics was likely attributed to a complex organization at the DAPs, spatially partitioned into some obstructed regions and some unhindered areas. Together, our live-cell superresolution studies revealed that IFT proteins adopt location-dependent stochastic paths in different regions of the ciliary base, with newly reported dynamic characteristics of IFT particles to shed light on the mechanisms of IFT particle traffic and gating facilitating ciliogenesis.

Chromosomes as Single Molecules

Sunney Xie[1,2].
[1]Harvard University, Cambridge, MA, USA, [2]Peking University, Beijing Advanced Innovation Center for Genomics, Beijing, China.

Since the 1990s, developments in room-temperature single-molecule spectroscopy, imaging, and manipulation have allowed studies of single-molecule behaviors in vitro and in living cells. Unlike conventional ensemble studies, single-molecule enzymology is characterized by ubiquitous fluctuations of molecular properties. The understanding of such single-molecule stochasticity is pertinent to many life processes.

Applications of single-molecule technologies to biology and medicine have become a major force in life sciences. DNA exists as single molecules that carry genetic codes in each individual cell. For this reason, gene expression is stochastic, i.e probabilistic. Single-molecule gene expression experiments in live single cells have allowed quantitative description and mechanistic interpretations. The fact that there are 46 different individual DNA molecules (chromosomes) in a human cell and each chromosomal DNA has a different nucleotide sequence, dictates that genomic variation occurs stochastically and cannot be synchronized among individual cells. In fact, every germ cell of an individual is different because of recombination, and cancer cells in a primary tissue are highly heterogeneous because of drastic genome changes such as single-nucleotide variations and copy-number variations. Because these genomic changes in a single cell occur stochastically, different cells cannot be synchronized. Consequently, single-cell measurements are necessary, yet they have been hampered by technical difficulties. It is only recently that single-cell single-molecule measurements have been made possible, thus creating opportunities to investigate and diagnose cancer, and to avoid genetic disorders in newborns.

Microfluidic Single Cell Sequencing

Yanyi Huang.
Peking University, Beijing, China.

Biosystems are intrinsically heterogeneous. Conventional ensemble measurements heavily rely on 'average' values from multiple experimental replicates to study the quantitative difference between samples, or between conditions. However, the heterogeneity at the cellular level make such strategy impossible in many cases. Next generation sequencing technologies allow us to obtain large and informative data-set with relatively low cost and high throughput, and become the key driving force to facilitate our understanding of complex biosystems. However, prevalent methods are still problematic when handle highly heterogeneous samples because of the mix of real biological variations and technical variations or noises. I am going to present recent technology developments in my group, mostly the combination usage of microscopic imaging with microfluidics, to facilitate the approaches using next generation sequencing tools to analyze small number of cells or even single cells.

Single-Cell Proteotypic Analysis of Invasive Motility in Glioblastoma

Jung-Ming G. Lin[1,2], Chi-Chih Kang[2], Amy E. Herr[1,2], Sanjay Kumar[1,2].
[2]UC-Berkeley, Berkeley, CA, USA.[1]The UC-Berkeley-UCSF Graduate Program in Bioengineering, Berkeley, CA, USA,

Cellular invasion into the surrounding tissue is a critical step in many solid tumors. However, virtually nothing is known about how the protein profile of an individual tumor cell relates to its propensity to invade and metastasize. One type of cancer characterized by cellular invasion is glioblastoma (GBM). The extensive tissue infiltration of GBM cells makes complete surgical resection impossible and contributes to resistance to subsequent radiation and chemotherapy. To investigate the invasive capability of GBM cells, we present a microfluidic device that correlates differences in protein expression with cellular speed, persistence and aspect ratio. Our PDMS/polyacrylamide-based device supports chemotactic migration through channels of tissue-like stiffness, enabling separation of heterogeneous tumor cell populations based on migration speed. The isolated cells are then subjected to multi-target single-cell western blotting using the polyacrylamide device walls as the electrophoretic and immunoblotting matrix. Using this device, we analyzed patient-derived glioblastoma tumor-initiating cells (TICs), a subpopulation of GBM with stem-like properties. We show that TICs exhibit surprising heterogeneity with respect to marker expression, with an apparent enrichment of Nestin and depletion of STAT3 in the most motile cells. This Nestin enrichment is not observed when immunofluorescence is used to quantify Nestin expression. Furthermore, we identified correlations between specific biomarkers within single TICs, which would not have been possible with population-based assays. Specifically, we discovered a positive correlation between Nestin and β-tubulin, suggesting the possibility that β-tubulin can also be used as a TIC enrichment marker. Our system lays the groundwork for the development of single-cell protein profiles of tumor invasion and metastasis. These profiles would allow for the identification of proteins that can predict invasive capability, an insight with great fundamental and translational value.

Single-Molecule Studies of Cas9 Search Kinetics in Living Cells

Daniel Jones, Prune Leroy, Cecilia Unosson, David Fange, **Johan Elf**.
Uppsala University, Uppsala, Sweden.

Cas9-based DNA targeting is radically different from that of transcription factors. Cas9 can be programmed by a guide RNA to target any sequence. We have studied how much time it takes to located a singel target sequence in a living cell using single molecule fluorescence microscopy. We also measure how long time the bound dCas9 stays at a target sequences and what implications this has for repression of a targeted gene.

Decoding Chromatin Organization with Superresolution Microscopy

Melike Lakadamyali[1,2].
[1]ICFO-Institute of Photonic Sciences, Barcelona, Spain, [2]University of Pennsylvania, Philadelphia, PA, USA.

Nucleosomes help structure chromosomes by compacting DNA into fibers. Chromatin organizationin the kilo-to-megabase length scales plays an important role for regulating gene expression. However, these small length scales have been impossible to visualize using conventional microscopy methods, hence making it challenging to decipher the nanoscale organization of chromatin inside the nucleus. To gain insight into how nucleosomes are arranged in vivo, we have been using quantitative super-resolution nanoscopy to visualize and determine the organization of the chromatin fiber in single nuclei. We have been able to correlate the nanoscale organization of chromatin to cell state, enabling us to identify the level of pluripotency as well as the differentiation state of different cell types.

Single-Molecule Study of the Chromatin Structure and Dynamics

Yujie Sun
Biodynamics Optical Imaging Center, Peking University, China

No Abstract

Emerging Role of Differential Molecular Association in Force-transmitting Nascent Adhesions

Sangyoon J. Han[1], Alexia Bachir[4], Kevin Dean[1], Edgar Gutierrez[3], Alex Groisman[3], Alan R. Horwitz[2,4], Gaudenz Danuser[1].
[2]Allen Institute for Cell Science, Seattle, WA, USA, [1]University of Texas Southwestern Medical Center, Dallas, TX, USA, [3]University of California San Diego, San Diego, CA, USA, [4]University of Virginia, Charlottesville, VA, USA.

Adhesion dynamics play a critical role in cell migration, whose decision process is affected by lots of biochemical and biomechanical inputs. Adhesions and their coupling with mechanical force have been extensively characterized at the level of large, mature focal adhesions. However, it has been elusive whether the nascent adhesions (NAs), before they reach their full maturation, are able to transmit forces and how these forces, if exist, affect the recruitment of early adhesion molecules. Here, we investigated how the mechanical force and the molecular recruitment of talin, vinculin and paxillin synergistically affect the adhesion assembly using high-resolution traction force microscopy (TFM) and fluorescence imaging of each molecule with eGFP. We used the single-particle-tracking of all NAs, extracted kinematic and kinetic features from the tracks, and used human-in-the-loop machine learning to identify the sub-population of nascent adhesions that assemble and disassemble at the protruding cell edge. We report that about 60% of non-maturing NAs transmit forces during their average lifetime of 2 minutes. Via time-lag analysis between eGFP-intensity and force, we show distinct difference in the recruitment sequences of three molecules in between non-maturing NAs vs. maturing NAs. Talin was recruited ~7 sec before vinculin's recruitment to the adhesions complex in maturing NAs whereas the two molecules were recruited at the same time in non-maturing NAs. Moreover, vinculin's early assembly rate to the maturing NAs was higher than in non-maturing NAs whereas talin and paxillin have shown no difference in early assembly. Traction force growth rate in the maturing vinculin complex was also higher than that in non-maturing vinculin complex. Together, these findings suggest a pre-conditioning role of talin's early recruitment to promote faster vinculin recruitment and force transmission for successful adhesion maturation, possibly via effective stretching.

Disruption of Cellular Force-sensing Triggers Systemic Tissue Collapse in the Botryllus Vasculature

Megan Valentine, Delany Rodriguez, Aimal Khankhel, Anthony DeTomaso.
University of California, Santa Barbara, CA, USA.

We recently discovered we can directly apply physical forces and monitor the downstream responses in a living organism in real time through manipulation of the blood vessels of a marine organism called Botryllus schlosseri. The extracellular matrix (ECM) plays a key role in regulating vascular growth and homeostasis in Botryllus, a basal chordate which has a large, transparent extracorporeal vascular network that can encompass areas >100 cm2. We have shown that lysyl oxidase 1 (LOX1), which is responsible for cross-linking collagen, is expressed in all vascular cells and is critically important for vascular maintenance. Inhibition of LOX1 activity in vivo by the addition of a specific inhibitor, ß-aminopropionitrile (BAPN), causes a rapid, global regression of the entire vascular bed, with some vessels regressing >10 mm within 16 hrs. I will discuss the molecular and cellular origins of this systemic remodeling event, which hinges upon the ability of individual vascular cells to sense and respond to mechanical signals, while introducing this exciting new model system for cellular studies of mechanobiology.

Spontaneous Patterning of Cytoskeleton in Single Epithelial Cell Apicobasal Polarity Formation

Chin-Lin Guo.
Academia Sinica, Nankang, Taipei, Taiwan.

One important issue in developmental biology and regeneration medicine is how mammalian cells spontaneously arrange themselves into specific, 3-D forms of organs. Loss of such ordering is a hallmark of many diseases including cancer. To explain how such ordering emerges, for decades, emphasis has been placed on spatial pre-patterning and multi-cellular coordination of chemical signals. Not until rece1ntly, it is recognized that forces also play an important role in the spatiotemporal ordering of multi-cellular architecture. For example, we have shown that cells can use cell-matrix mechanical interactions to develop long-range multi-cellular coordination (up to 600 microns) in tissue formation and cancer invasion. Here, we report that single epithelial cells can spontaneously break symmetry and pattern cytoskeleton into a precursor form for multi-cellular coordination including the formation of apicobasal polarity. Such process occurs in the absence of spatial pre-patterning of chemical signals. Further, it provides a topological cue to guide the spatial patterning of intracellular signals, which is absent in cancer cells, mesenchymal cells, and stem cells. Through experimental and theoretical approach, we find that such spontaneous patterning arises from the mechanical instability of microtubule and its interactions with actin filaments, modulated by the stiffness of surrounding environment. Based on these results, we propose that mechanical instability of single cells is sufficient to create a topological precursor as a building block for chemical signaling and multi-cellular coordination in development, and failure in such a process might lead to diseases.

Rotation, Twisting, and Pulling: The Rich Dynamics of Filopodia

Natascha Leijnse, Lene B. Oddershede, **Poul M. Bendix**.
University of Copenhagen, Copenhagen, Denmark.

Filopodia allow the cell to sense and interact with their surroundings. Rigidity sensing and application of piconewton traction forces are key functions that filopodia employ to sense and regulate cellular interactions. However, despite their ubiquitous presence in most cells, their different modes of movement remains poorly characterized. Recently, we found surprising evidence for a new pulling mechanism originating from twisting of the actin within the filopodium [1,2]. This mechanism can only be present in filopodia which contain a rotating actin bundle. By imaging the actin, we clearly observed rotation and retrograde flow of small buckles which formed in filopodia from HEK293 cells. The movement of these buckles was found to be coupled to the pulling force exerted by the filopodium in a manner which indicated that torsional twist was accumulated in the actin bundle. Our results provide the first clear evidence of rotation of the actin within filopodia a key property which might facilitate both interaction and exploration of the cell's 3D environment.

1. Leijnse N, Oddershede LB, Bendix PM. An updated look at actin within filopodia. A Review, Cytoskeleton 2015, 72, 71-79.
2. Leijnse N, Oddershede LB, & Bendix PM. Helical buckling of actin inside filopodia generates traction. PNAS 2015, 112(1), 136-141.

Quantitative Biology with Genetically Encoded Sensors - Opportunities and Challenges

Amy Palmer.
University of Colorado Boulder, Boulder, CO, USA.

Fluorescent tools have launched biological research into a new realm of understanding of cellular processes and dynamics at the single-cell level. These tools are enabling characterization of stochasticity and heterogeneity exhibited by biological systems, which could not adequately be probed by techniques that rely on bulk analysis of populations of cells. Fluorescent sensors are increasingly providing insight into the "dark matter" of the cellular milieu: small molecules, secondary metabolites, metals, and ions. One of the great promises of such sensors is the ability to quantify cellular signals in precise locations with high temporal resolution. Yet this is coupled with the challenge of how to ensure that sensors are not perturbing the underlying biology and the need to systematically measure hundreds of individual cells over time. This talk will highlight our efforts to develop genetically encoded FRET-based sensors for quantitative mapping of zinc ions in cells. I will discuss approaches for defining whether sensors perturb cellular ions, and the specific challenges associated with quantifying ions in cellular organelles. Finally, I will discuss our efforts at systematic quantitative analysis of long-term imaging of ions during the cell cycle to highlight the need for sophisticated image analysis algorithms.

Acid Resistant Monomeric GFP for Quantitative Single-Cell Analyses

Takeharu Nagai.
Osaka University, Osaka, Japan.

Fluorescent proteins (FPs) cloned from diverse luminous organisms have made a great contribution as fusion tags and units of FRET-based indicators for visualizing biological phenomena. However, most FPs lose their fluorescence in an acidic environment due to their high pH sensitivity (pKa ≈ 6.0), and this has hampered their application to reliable imaging in compartments such as late-endosomes, secretory granules, lysosomes and vacuoles (pH ≈ 4.5-5.5). Up to date, some acid resistant FPs have been developed, including mTurquoise2 (cyan FP, pKa = 3.1) and TagRFP (red FP, pKa = 3.8), but there is little report of acid resistant green FPs [1]. Therefore, it has been restricted to perform multiple color imaging by FPs to track different proteins simultaneously, and to develop FRET-based indicators for observing ion dynamics. Here, we report an acid resistant monomeric GFP, Gamillus derived from flower hat jellyfish, Olindias formosa living in the Sea of Japan, and engineered by directed evolution. Compared with the most conventional GFP, EGFP (pKa = 6.0), Gamillus has much higher pH stability (pKa = 3.5). Gamillus also showed 1.9-folds higher brightness, 1.7-folds faster chromophore maturation speed and similar photo-stability compared with EGFP. Gamillus expressed in HeLa cells was properly localized in various cellular organelles including lysosomes. We applied Gamillus to develop a FRET-based ratiometric pH indicator by tandem fusion of acid resistant Gamillus with a pH-sensitive RFP, tdTomato. We also have determined a three dimensional structure of Gamillus at 1.7 Å resolution by X-ray crystallography, and been analyzing the structural characteristics. In near future, application of Gamillus to multi-color imaging and development of FRET-based indicators will accelerate revelation of mystery hiding in acidic organelles.

Single mRNA Counting in Single Cell Reveals Function of RNA Stem Structure in Coupling Dicing and Gene Silencing

Hye Ran Koh[1,2], Amirhossein Ghanbariniaki[3], **Sua Myong**[1,2,3].
[1]Department of Physics, University of Illinois, Urbana, IL, USA, [2]Institute for Genomic Biology, University of Illinois, Urbana, IL, USA, [3]Biophysics Department, Johns Hopkins University, Baltimore, MD, USA, [4]Center for Physics of Living Cells, University of Illinois, Urbana, IL, USA.

Micro (mi)RNAs possess secondary structures encompassing a loop and a stem with multiple mismatches, but how they contribute to dicing and subsequent gene silencing efficiency remains unclear. Using single molecule fluorescence in situ hybridization, we demonstrate that the number of nuclear mRNA remains unchanged while cytoplasmic mRNA undergoes silencing. Dicing rate and silencing efficiency both increase as a function of miRNA loop length in a correlated manner. In contrast, mismatches in a stem drastically diminishes silencing efficiency without reducing the dicing rate. We show that such decoupling effect is not due to the miRNA uptake, cellular dicing or RISC loading. We postulate that the mismatches in a stem perturbs the hand-over of cleaved miRNA from Dicer to Argonaute. Our result implies that the stem structure in cellular miRNAs is intended for suboptimal silencing efficiency.

Systematic Evaluation of Cellular Zn^{2+} Sensors with Microfluidic Cytometry

Brett Fiedler[1,2], Kyle P. Carter[2,3], Yan Qin[4], Margaret C. Carpenter[2,3], Amy E. Palmer[2,3], **Ralph Jimenez**[1,2].
[1]University of Colorado, Boulder, CO, USA, [2]University of Colorado, Boulder, CO, USA, [3]University of Colorado, Boulder, CO, USA, [4]University of Denver, Denver, CO, USA.

The accuracy of cellular ion concentration measurements with genetically-encoded biosensors depends heavily on heterogeneity of biosensor expression and on the reproducibility of the response as a function of environment. Studies with currently-available Zn^{2+} FRET-biosensors have reported widely varying Zn^{2+} concentrations in the endoplasmic reticulum (ER). It was previously observed that these sensors show a smaller dynamic range of FRET change in the ER relative to the cytosol. Furthermore, it has been observed that sensors with small dynamic ranges do not accurately report analyte concentrations. To investigate this discrepancy, we employed microfluidic flow cytometry to analyze the heterogeneity of the apo state of three Zn^{2+} biosensors (ZapCY1, eCALWY-4, eZinCh-2) localized to the cytosol and to the ER of HeLa cells. These sensors utilize three distinctly different moieties for Zn^{2+} binding. This instrument was also used to probe the responses of the sensors to perturbations of cellular Zn^{2+} concentrations on the timescales of several seconds. For each sensor, we find the absolute FRET value of the apo state shows an offset and larger heterogeneity in the ER relative to the cytosol. Secondly, the screening of the sensor dynamic responses revealed multiple subpopulations. Third, we found that sensor performance in the cytosol is not always correlated with high performance in other cellular compartments. Finally, we are using this microfluidic system to increase dynamic range of ER-localized FRET sensors by screening and sorting a cell-based library that targets the linker regions between the donor/acceptor FPs and Zn^{2+} binding domains.

Single-Molecule Investigations of DNA Replication and Repair in Living Bacteria

Julie Biteen.
University of Michigan, Ann Arbor, MI, USA.

By beating the diffraction limit that restricts traditional light microscopy, single-molecule fluorescence imaging is a precise, non-invasive way to sensitively probe position and dynamics. Our lab has been developing new methods to locate, track, and analyze single molecules to measure structure, dynamics, and cooperativity in live bacterial cells. I will discuss how we are measuring and understanding the dynamical interactions essential for DNA mismatch recognition and DNA replication in living *Bacillus subtilis* cells. In particular, we have integrated our nanoscopic biophysical tools with biochemical and genomic approaches to understand how the mismatch repair protein MutS efficiently identifies DNA mismatches during real time in living cells, to determine that the replicative polymerase PolC is continuously recruited to and released from a centrally located replisome, and to measure how the various components of the multi-protein replication machinery are organized in bacterial cells. Overall, our results provide a mechanistic model for the dynamical nature of these fundamental processes in live cells.

Single-Molecule Visualization of Bacterial DNA Repair in Live Cells

Antoine M. van Oijen,
University of Wollongong, Wollongong, NSW, Australia.

We are using single-molecule fluorescence methods to visualize the behaviour of individual, fluorescently labelled molecules inside living cells. Using these approaches, we aim to understand the relationship between DNA replication and repair. In particular, we study aspects related to the bacterial SOS damage response: how does the RecA protein coat single-stranded DNA after DNA damage stalls replication and how do the subsequently upregulated translesion synthesis DNA polymerases gain access to the lesions in the DNA? Further, we investigate the dynamics of transcription-coupled repair as mediated by the E. coli Mfd protein. These studies not only lead to new insights in molecular pathways, they also provide information on the critical role played by spatial and temporal regulatory mechanisms.

Local 3D Single-Cell–Matrix Interactions Underlie the Spatiotemporal Dynamics of Cell Populations

Nicholas Kurniawan.
Eindhoven University of Technology, Eindhoven, Netherlands.

It is increasingly accepted that the physical and mechanical properties of the extracellular environment play a guiding role in cell behavior, for instance during development, wound healing, and cancer metastasis. Cells sense physical and mechanical cues from the extracellular matrix (ECM) and transduce these cues into intracellular signals that can, in turn, affect cell response. However, surprisingly little is known about what the cells are actually sensing, especially in the context of the typically complex, nonlinear, and fibrillar ECM. We hypothesized that cells sense the local, rather than global, ECM properties, and base their decision-making on such local mechanosensing.

To test this hypothesis, we developed a spatially-resolved microrheological technique that provides quantitative information about the local viscoelasticity around single cells at sub-cellular resolution. We seeded cells in 3D collagen gels and found that cells created pockets of stiffened microenvironment, suggesting that cells 'prime' their surrounding ECM to support contractility. To determine whether cells rely on local mechanosensing for decision-making, we systematically modulated the ultrastructure of fibrin fibers, allowing us to independently vary the stiffness at network and fiber levels. We show that cell spreading and differentiation are determined by the stiffness of individual fibers, rather than by the bulk properties of the matrix. These two findings strongly suggest that the cell and the matrix can locally influence and adapt to each other in a reciprocal manner. We further speculated that such local and dynamic cell–matrix interactions may manifest in a heterogeneity of cell phenotype within a population. Indeed, we found a spatiotemporally-heterogeneous pattern of cell migration in 3D collagen gels that is mediated by cell-induced local ECM remodeling. These results reveal the role of cell–matrix interactions in single cell behavior and population dynamics.

POSTER ABSTRACTS

POSTER SESSION I

Saturday, June 17, 1:30 PM – 3:00 PM
Courtyard

Below are the formal presentations for Saturday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Posters should be set up the morning of June 17 and removed by noon June 18.

Aho, Vesa	**1-POS**	**Board 1**
Bai, Donglin	**3-POS**	**Board 2**
Bovyn, Matt	**5-POS**	**Board 3**
Chang, Chen	**7-POS**	**Board 4**
Chang, Kai-Chun	**9-POS**	**Board 5**
Chen, Ssu Ying	**11-POS**	**Board 6**
Chen, Yi-Lan	**13-POS**	**Board 7**
Chiu, Shao-Ting	**15-POS**	**Board 8**
Chu, Jen-Fei	**17-POS**	**Board 9**
Denzi, Agnese	**19-POS**	**Board 10**
Fang, Chao	**21-POS**	**Board 11**
Funatsu, Takashi	**23-POS**	**Board 12**
Garcia-Pelagio, Karla	**25-POS**	**Board 13**
Han, Sangyoon	**27-POS**	**Board 14**
Ho, Po-Yi	**29-POS**	**Board 15**
Huang, Wan-Chen	**31-POS**	**Board 16**
Huang, Yi-Fan	**33-POS**	**Board 17**
Iwane, Atsuko	**35-POS**	**Board 18**
Kato, Eiko	**37-POS**	**Board 19**
Kim, HyeMi	**39-POS**	**Board 20**
Kobayashi-Kirschvink, Koseki	**41-POS**	**Board 21**
Kuo, Yin-wei	**43-POS**	**Board 22**
Kwapiszewska, Karina	**45-POS**	**Board 23**
Lawson, Michael	**47-POS**	**Board 24**
Lee, Cho-yin	**49-POS**	**Board 25**
Lin, Jung-Ming	**51-POS**	**Board 26**
Lin, Sheng-Yao	**53-POS**	**Board 27**
Lindén, Martin	**55-POS**	**Board 28**
Liu, Yen-Liang	**57-POS**	**Board 29**
Lu, Chih-Hao	**59-POS**	**Board 30**
Macianskiene, Regina	**61-POS**	**Board 31**
Matsuoka, Satomi	**63-POS**	**Board 32**
Nirody, Jasmine	**65-POS**	**Board 33**
Paramanathan, Thayaparan	**67-POS**	**Board 34**
Paylaga, Giovanni	**69-POS**	**Board 35**

Qin, Xianan	**71-POS**	**Board 36**
Riani, Yemima	**73-POS**	**Board 37**
Runa, Sabiha	**75-POS**	**Board 38**
Scheideler, Olivia	**77-POS**	**Board 39**
Seeger, Stefan	**79-POS**	**Board 40**
Sepehri rad, Masoud	**81-POS**	**Board 41**
Shao, Xueying	**83-POS**	**Board 42**
Sun, Yi-Ren	**85-POS**	**Board 43**
Tian, Fang	**87-POS**	**Board 44**
Vallmitjana, Alex	**89-POS**	**Board 45**
Wadsworth, Gable	**91-POS**	**Board 46**
Wan-Shan, Que	**93-POS**	**Board 47**
Weng, Xiaoli	**95-POS**	**Board 48**
Wong, Felix	**97-POS**	**Board 49**
Yan, Zishen	**99-POS**	**Board 50**
Yeh, Jia-Wei	**101-POS**	**Board 51**
Zhu, Mingliu	**103-POS**	**Board 52**

1-POS Board 1

Modelling Intracellular Transport in Realistic Environments

Vesa Aho[1], Markko Myllys[1], Thomas Kühn[2,3], Jussi Timonen[1], Keijo Mattila[1,4], Carolyn A. Larabell[5,6], Maija Vihinen-Ranta[1].
[1]University of Jyväskylä, Jyväskylä, Finland, [4]Tampere University of Technology, Tampere, Finland, [2]University of Eastern Finland, Kuopio, Finland, [5]University of California, San Francisco, CA, USA, [6]Lawrence Berkeley National Laboratory, Berkeley, CA, USA.[3]Finnish Meteorological Institute, Kuopio, Finland,

The environment inside a cell is highly complex including, e.g., the chromatin network and membrane structures. In order to realistically model intracellular transport, the essential features of this environment must be captured and incorporated into a model. Here we describe our recent studies in computational modelling of intracellular transport utilizing realistic environments reconstructed from images obtained with microscopy methods. We first present our research of modelling diffusion through the nuclear envelope [1]. In this study the permeability of the nuclear envelope to a fluorescent molecule is determined by comparing simulated changes in fluorophore distribution to changes measured in a fluorescence microscopy experiment. Secondly, we present our modelling results for the diffusive motion of herpes simplex virus 1 capsids towards the nuclear envelope through the chromatin network that is characterized using soft x-ray tomography imaging [2,3]. In conclusion, we show that simulating the motion of particles in the reconstructions of their actual environments allows us to gain new insights into intracellular transport phenomena.

[1] Aho, V., K. Mattila, T. Kühn, P. Kekäläinen, O. Pulkkinen, R. Brondani Minussi, M. Vihinen-Ranta, and J. Timonen. 2016. Diffusion through thin membranes: Modeling across scales. Phys. Rev. E. 93: 043309
[2] Myllys, M., V. Ruokolainen, V. Aho, E. A. Smith, S. Hakanen, P. Peri, A. Salvetti, J. Timonen, V. Hukkanen, C. A. Larabell, and M. Vihinen-Ranta. 2016. Herpes simplex virus 1 induces egress channels through marginalized host chromatin. Sci. Rep. 6:28844
[3] Aho, V., M. Myllys, V. Ruokolainen, S. Hakanen, E. Mäntylä, J. Virtanen, V. Hukkanen, T. Kühn, J. Timonen, K. Mattila, C. A. Larabell, and M. Vihinen-Ranta. 2017. Chromatin organization regulates viral egress dynamics. Submitted for publication in Scientific Reports.

3-POS Board 2

Junctional Delay, Frequency, and Direction-Dependent Uncoupling of Human Heterotypic Cx45/Cx43 Gap Junction Channels.

Willy G. Ye[1], Benny Yue[1], Hiroshi Aoyama[2], John A. Cameron[1], Honghong Chen[1], **Donglin Bai**[1].
[1]University of Western Ontario, London, ON, Canada, [2]Osaka University, Osaka, Japan.

Gap junction (GJ) channels form low resistance passages between cardiomyocytes and play a role in the rapid propagation of action potentials in the heart. A GJ channel is formed by two properly docked hemichannels and each hemichannel is a hexamer of connexins. Connexin40 (Cx40) and Cx43 are the dominant connexins in atrial myocytes, while Cx45 is mostly expressed in the sinoatrial (SA) and atrioventricular (AV) nodes. Cardiac action potentials propagate from SA node to atrial myocytes and then to AV node, possibly via heterotypic Cx40/Cx45 and/or Cx43/Cx45 GJs. However, the functional status and channel properties of human heterotypic Cx40/Cx45 or Cx43/Cx45 GJs have not been studied. Here we investigated human Cx40/Cx45 and Cx43/Cx45 heterotypic GJs by recombinant expression. To our surprise, cell pairs expressing human Cx40 in one and Cx45 in the other failed to form functional GJs. Minor modifications in human Cx40 with designed variants (D55N, P193Q, or a double variant, D55N-P193Q) are sufficient to establish functional heterotypic GJs with Cx45. On the contrary, heterotypic human Cx43/Cx45 GJs are functional similar to that described on rodent Cx43/Cx45 GJs. Detailed characterizations of human heterotypic Cx43/Cx45 GJs revealed a rapid asymmetric Vj-gating and a much slower recovery, which can substantially reduce the GJ conductance in a junctional delay and frequency dependent manner. Dynamic uncoupling in Cx45-containing GJs might contribute not only in a slower action potential propagation in the AV node, but also in preventing high frequency atrial fibrillation from propagating into ventricles.

5-POS Board 3

Brownian Dynamics Simulation Reveals Freedom of Motors in the Cargo Membrane Can Influence Cargo Dynamics

Matt Bovyn[1,2], Steven Gross[3,1,2], Jun Allard[4,1,2].
[1]University of California - Irvine, Irvine, CA, USA, [4]University of California - Irvine, Irvine, CA, USA.[3]University of California - Irvine, Irvine, CA, USA, [2]University of California - Irvine, Irvine, CA, USA,

Active transport of subcellular cargos along microtubules is essential for organization and function of eukaryotic cells. Kinesin family molecular motors are responsible for the transport of some cargos, with multiple motors often active at once. These motors have been extensively studied, but it remains unclear how the motors function in groups, as well as how properties of the cargo itself can influence transport. We developed a Brownian dynamics simulator of cargo transport which includes cargo shape and motor location on the cargo. Simulations reveal that if motors are free to diffuse on the surface of a cargo, under certain circumstances motors self-organize into dynamic clusters. Furthermore, dynamic clustering can give rise to the cargo "memory" – when a travelling cargo becomes detached from the microtubule briefly, it will tend to return to its former state more quickly when it regains contact. This memory effect demonstrates that cargo geometry alone can influence cargo behavior and hints that dynamic motor organization on the cargo could be used by the cell to control intracellular transport.

7-POS Board 4

Mathematical Modeling of Mitochondrial Quality Control in Mitochondiral Life Cycle and its Role in Aging

Chen Chang[1], Yi-Ping Ho[2], An-Chi Wei[1].
[1]National Taiwan University, Taipei City, Taiwan, [2]The Chinese University of Hong Kong, Sha Tin, Hong Kong.

Mitochondria play an important role in cell viability, for providing most of the energy in cells. The dynamics and quality of these organelles are therefore critical for cell activities. Previous research has shown that mitochondria have two different morphologies, a network and an individual status, and that mitochondria, throughout their life cycles, constantly enter and reenter each of these statuses during fusion and fission events. This suggests that a combination of fusion, fission, and mitophagy events may serve as a mechanism of mitochondrial quality control. The increasing interest in the mitochondria life cycle has led to more attempts to create mathematical model of fusion/fission/mitophagy events. Recent modellings have demonstrated that a favorable fusion and fission balance may inhibit the clonal expansion of damaged mitochondrial DNA and may prove critical to the maintenance of mitochondrial quality.

This research implements mathematical modelling of the mitochondrial life cycle that accounts for several new findings regarding this cycle. In this stochastic model, the quality and size of individual mitochondria are described in terms of mitochondrial nucleic content. Simulations of fusion/fission/mitophagy are optimized to match the different cell statuses in different life periods. This model also considers the factor of spatial distribution of mitochondria within cells. We also assume that the event of mitophagy, followed by fission, is not random but is mediated to create an unbalanced distribution of mitochondrial contents. Implementations of a dynamic status then allow us the exploration of the role of fusion/fission/mitophagy in ageing, and its effects on the cell division, from a bio-energetic perspective. Our ultimate goal is gain greater insight into the differences in the dynamical equilibrium of fusion/fission and its relevance to cell survival.

9-POS Board 5

Structured Mrna Induces Ribosomal Rolling During Frameshifting

Kai-Chun Chang[1], Emmanuel Salawu[2,3], Yuan-Yu Chang[2], Po-Szu Hsieh[1], Jin-Der Wen[1], Lee-Wei Yang[2,3].
[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), where the ribosome slips backwards for one nucleotide (the "-1" frame), is promoted by a slippery sequence, usually with X-XXY-YYZ motif, and a road-blocking mRNA structure, such as hairpin or pseudoknot (PK). How the mRNA structure is unwound during translation and how these elements modify conformational dynamics of the ribosome to promote PRF remain largely unknown. However, the conformational dynamics of the ribosome occur at a scale unattainable by MD simulations ($\sim 1.6\times10^4$ atoms, 2.4 MDa, at millisecond regime). We circumvent this obstacle by modeling intrinsic dynamics with coarse-grained anisotropic network model (ANM), followed by predicting PK-induced perturbed dynamics by linear response theory (LRT). The external perturbation, which is the tension developed between PK and the ribosome, is obtained from a series of steps involving cryo-EM fittings and steered molecular dynamics simulations (SMD). Both ANM and SMD yield results well correlated with X-ray, cryo-EM, single-molecule Förster resonance energy transfer (smFRET) and mutational studies. Given that the intrinsic dynamics and perturbation forces are known, LRT predicts global conformational change of the ribosome upon encountering PK. Surprisingly, the 30S subunit seems to "roll" in a direction orthogonal to ratcheting, but parallel to the tension of PK. Rolling distorts A/P-tRNA, which is observed by cryo-EM previously. Subsequent MD simulations further indicate that A/P-tRNA bending disrupts codon-anticodon interaction. The resulting dissociation of A/P-tRNA and tRNA slippage are observed in the simulations. Our model, based on first principles, connects and rationalizes existing experimental data, providing a temporal and spatial description of PRF with unprecedented mechanistic details.

11-POS Board 6

The Correlation between Bacterial Transcription and Translation at Single Molecule Level in Living Cells

Ssu Ying Chen[1,2], Chia-Fu Chou[1], Yi-Ren Chang[2].
[1]Institute of Physics, Academia Sinica, Taipei, Taiwan, [2]Department of Physics, national Taiwan normal University, Taipei, Taiwan.

Although most or all transcription is co-translational in bacteria, ~ 10-15% of translation is co-transcriptional. Since the spatial distribution of ribosomes displays strongly segregated from the nucleoid, 30s/50s ribosomal subunits need a circulation between ribosome-rich region and the nucleoid. It's been suggested transertion enables expansion of the nucleoid and 30s/50s subunits moving into co-transcriptional translation region. To clarify if transertion plays a role in co-transcriptional translation, an in situ, time-resolved, single-molecule analysis would be beneficial in quantifying the syntheses of mRNA and polypeptide derived from a gene encoding a membrane protein in living cells. Hereby, such a real-time monitoring method was developed to investigate the spatiotemporal correlation between transcription and translation, and the role of transertion in co-transcriptional translation. We employed the labeling schemes of MS2 and SunTag to probe the syntheses of mRNA and polypeptides respectively, in which quantification is allowed by imaging the fluorescence emitted from the counter part of each tagging system, i.e., MCP-GFP and scFv-mCherry. Besides, exogenous, centromere-like DNA sequence parS was inserted to the flanking region of the gene encoding a specified membrane protein either in a single-copy plasmid or chromosome such that transcription events can be localized by imaging CFP-ParB fluorescence foci. In the present study, the events with co-localized fluorescence foci emitted from three tagging systems near the cell membrane that indicate the transertion were detected by TIRF microscopy and compared with other transcription and translation events. Our results demonstrate a feasible approach to probe the transertion events in living cells and further elucidate their spatiotemporal correlation in the process of co-transcriptional translation.

13-POS Board 7

How Mrna Wraps the 30S at the Beginning of Translation

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

Well begun is half done. In the cell, initiation is the rate limiting step in most protein synthesis events. At the early stage of initiation, the prokaryotic 30S subunit of the ribosome binds to the Shine-Dalgarno sequence of the mRNA, which then wraps around the 30S to form a pre-initiation complex in the presence of the initiator tRNA. However, the detailed mechanisms in this process are not clear. In order to see the dynamic signals between the 30S and mRNA, we use single-molecule FRET and optical tweezers to observe the translation initiation in each state. Here, we find that the mRNA reversibly wraps and unwraps around the 30S when the mRNA contains a structure downstream to the initiation site, and the structure is partially destabilized by the 30S during the wrapping-unwrapping process. Addition of the initiator tRNA opens some weak downstream structures nearby and stably locks this pre-initiation complex to the correct initiation site. In whole process, the initiation factors, IF1 and especially IF3 stabilize the binding of mRNA. In this study, we present a method to observe the interaction of mRNA and the 30S dynamically at the very beginning of translation which helps us to understand the mechanism of translation initiation in more detail.

15-POS Board 8

Information Transduction Capacity of Mitochondrial Retrograde Signaling

Shao-Ting Chiu[1], Jun-Yi Leu[2], An-Chi Wei[1].
[1]National Taiwan University, Taipei, Taiwan, [2]Academia Sinica, Taipei, Taiwan.

Mitochondrial retrograde signaling takes part in the communication between mitochondria and the nucleus, which is essential for mitochondrial quality control and maintaining energy production in eukaryotic cells. However, it is unclear how many different mitochondrial statuses can be distinguished via mitochondrial retrograde signaling under inevitable biochemical noise. To address this issue, we used the budding yeast *S.cerevisiae* as a model organism, and investigated the information transduction capacity of the retrograde pathway. Mitochondrial membrane potential ($\Delta\Psi m$) and translocation of Rtg3p/Rtp1p are considered to be the input and output of this noisy communication channel. We further used the parallel Gaussian channel with a common power constraint, based on the information theory, to model the retrograde signaling and to optimize the information-transmission rate based on the Kuhn-Tucker conditions and the water-filling method. The result implies the optimized $\Delta\Psi m$ probability distribution that maximizes the information-transmission rate under a power constraint contributed by the limited concentration of Rtg3p and Rtg1p. Therefore, the receiver located in the nucleus can distinguish maximum statuses of mitochondrial quality by the retrograde signaling pathway under the optimized $\Delta\Psi m$ probability distribution. In this study, we have provided an informatics view of mitochondrial retrograde signaling.

17-POS Board 9

A Single-Molecule Approach to Study the Dendritic Function of the Rna-Binding Protein Tdp-43

Jen-Fei Chu, Pritha Majumder, Biswanath Chatterjee, Che-Kun James Shen.
Academia Sinica, Taipei, Taiwan.

The RNA-binding protein TDP-43 regulates RNA metabolism at multiple levels, including transcription, RNA splicing, and microRNA metabolism, as well as a major component of the cytoplasmic inclusions characteristic of amyotrophic lateral sclerosis and some types of frontotemporal lobar degeneration. We previously found that TDP-43 represses the translation initiation of Rac1 mRNAs through binding of TDP-43 to mRNA(s) at specific UG/GU sequences and consequently the hippocampal spinogenesis. However, direct evidence that TDP-43 regulates Rac1 mRNAs in spatiotemporal manner has not been documented. We here conducted a multi-color localization based super-resolution microscopic and single particle tracking methods at the endogenous level to dissect the interaction of TDP-43 proteins and Rac1 mRNAs in live hippocampal neurons. It would allow us to extract messenger ribonucleoproteins (mRNPs) information that remains unclear about the TDP-43 protein-Rac1 mRNA complex in situ. This sheds new light on the pathology of mechanistic pathways induced by mis-metabolism of TDP-43 protein.

19-POS Board 10

Can Microsecond Pulse Affect Endoplasmic Reticulum? Theoretical Proof of Concept with a Realistic Microdosimetry Single-Cell Model

Agnese Denzi[1,2], Hanna Hanna[3], Frank M. Andre[3], Lluis M. Mir[3], Francesca Apollonio[2], Micaela Liberti[2].
[1]Istituto Italiano di Tecnologia, Rome, Italy, [2]University of Rome "La Sapienza, Rome, Italy, [3]CNRS, Univ. Paris-Sud, Université Paris-Saclay, Gustave Roussy,, Villejuif, France.

The use of electric pulses has become a promising application in cancer. Usually, pulses with duration in the range of μs and amplitude of kV/m are used to permeabilize the plasma membrane. Shorter pulses, with duration from few to hundreds of nanoseconds and amplitude of MV/m, are used to also permeabilize the cell internal structures, due to their higher frequency content [1].

Aim of this work is to theoretically demonstrate the efficacy of electroporation with a 100 μs pulse also on endoplasmic reticulum (ER). An accurate 2D microdosimetry model was obtained considering real images from an optical microscope: the nucleus in blue (Hoechst 33342) whereas the ER in green (Fluo-4). A MATLAB®2013 custom algorithm has been developed to recognize cell, ER and nucleus borders. Successively the images have been transformed in vector ones and imported in an electromagnetic simulator.

The parameters for the model were taken from [2]. The maximum of the transmembrane potential has been considered both for the plasma and the ER membranes. Considering a poration threshold value as in [3], the external electric fields necessary to obtain the cell (Eth_cell) and ER (Eth_ER) poration have been calculated (Table 1).

TABLE I
Parameter Values
Eth_cell (MV/m) ≈0.02
Eth_ER (MV/m) ≈0.07
Ratio=Eth_ER/Eth_cell ≈3.5

The efficacy of 100 μs pulse in ER poration has been numerically demonstrated and can be related to the higher frequency content present in the rise and fall times of the applied pulse. Furthermore, the electroporation of the external membrane could facilitate the electric field to penetrate inside the cell.

[1] M. Breton et al, Bioelectromagnetics, 2012.
[2] A. Denzi et al, Journal of membrane biology, 2013.
[3] K. C. Smith et al, Plasma Science, IEEE Transactions on, 2006.

21-POS Board 11

Modeling Cell Blebbing with Boundary Integral Method

Chao Fang, T.H Hui, Yuan Lin.
The University of Hong Kong, Hong Kong, Hong Kong.

Although cell blebbing has been known to play important roles in processes like cell locomotion, spreading, and apoptosis, how the initiation and progression of such phenomenon are governed by physical factors such as membrane tension, bilayer-cortex cohesion and intracellular pressure remains poorly understood. In this study, we developed a computational model to describe this process by tracking the movement of cell membrane in a viscous medium with a boundary integral method. Simulations results showed good agreement with various experimental findings, obtained by us or reported in the literature. In particular, we verified the existence of a critical cortical tension for the formation of a bleb and showed how this threshold value is influenced by the strength of bilayer-cortex adhesion and the bending rigidity of lipid membrane. A linear relationship between the final volume of a bleb and its initial growth rate is also observed, in consistent with previous reports. Finally, an evolution map summarizing the blebbing dynamics was constructed where we showed that, depending on the level of intracellular pressure and the size of the weakened cortex, a bleb can either never be formed, or be formed but grows with a fixed width, or keeps growing in size as a result of the successive failure of adhesion between the bilayer membrane and the cortex.

23-POS Board 12

Culture-Independent Method for Identification of Microbial Enzyme-Encoding Genes by Single-Cell Sequencing Using a Water-in-oil Microdroplet Platform

Kazuki Nakamura[1], Ryo Iizuka[1], Shinro Nishi[2], Takao Yoshida[2], Yuji Hatada[2], Yoshihiro Takaki[2], Ayaka Iguchi[3], Dong H. Yoon[3], Tetsushi Sekiguchi[3], Shuichi Shoji[3], **Takashi Funatsu**[1].
[1]The University of Tokyo, Tokyo, Japan, [2]Japan Agency for Marine-Earth Science and Technology, Kanagawa, Japan, [3]Waseda University, Tokyo, Japan.

Environmental microbes are a major source of industrially valuable enzymes with potent and unique catalytic activities. Unfortunately, the majority of microbes remain unculturable or difficult to cultivate and thus are not accessible by culture-based methods. Recently, culture-independent metagenomic approaches have been successfully applied, opening access to untapped genetic resources. Here we present a methodological approach for the identification of genes that encode metabolically active enzymes in environmental microbes in a culture-independent manner. Our method is based on activity-based single-cell sequencing, which focuses on microbial cells exhibiting specific enzymatic activities. First, environmental microbes were encapsulated in water-in-oil microdroplets with a fluorogenic substrate for the target enzyme to screen for microdroplets that contain microbially active cells at the single cell level. Second, the microbial cells were recovered and subjected to whole genome amplification. Finally, the amplified genomes were sequenced to identify the genes encoding target enzymes. This method was successfully used to identify 14 novel beta-glucosidase genes from uncultured bacterial cells in marine samples. Our method contributes to the screening and identification of genes encoding industrially valuable enzymes.

25-POS Board 13

Sarcolemmal Biomechanics and Excitability in Cultured and Isolated Mechanically Muscle Fibers of Dystrophic Mice

Karla P. Garcia-Pelagio[1,2], Erick Hernandez-Ochoa[3], Stephen J. Pratt[4], Richard M. Lovering[4].
[1]Universidad Nacional Autonoma De Mexico, School Of Science, Physics Dept, Mexico City, Mexico, [2]University Of Maryland, School Of Medicine, Physiol Dept, Baltimore, MD, USA, [3]University Of Maryland, School Of Medicine, Biochem And Mol Biol Dept, Baltimore, MD, USA, [4]University Of Maryland, School Of Medicine, Orthopaedics Dept, Baltimore, MD, USA.

Duchenne muscular dystrophy (DMD), the most common and severe dystrophy, is caused by the absence of dystrophin. Muscle weakness and fragility (i.e. increased susceptibility to damage) are presumably due to structural weakness of the myofiber cytoskeleton, but recent studies suggest that malformed/split myofibers in dystrophic muscle may also play a role. We have studied the biomechanical properties of the sarcolemma in: 1) Single myofibers isolated mechanically from extensor digitorum longus (EDL) muscles, and 2) Enzymatically-dissociated myofibers (both normal and malformed) from the flexor digitorum brevis muscle (FDB) in wild-type (WT) and dystrophic (mdx, mouse model for DMD) mice. Suction pressures (P) applied through a pipette to the membrane generated a bleb, which increased in height with increasing P. Larger increases in P ruptured the costameres, the connections between the sarcolemma and myofibrils, and eventually caused the sarcolemma to burst. The results from dissociated FDB and dissected EDL myofibers was higher in separation P up to 14-fold higher in the FDB than EDL. P at which the sarcolemma separated from the underlying myofibrils was 27% lower in mdx myofibers and 50% less in branches of split fibers compared to the trunk. We also asked whether the abnormal biomechanical phenotype of the MDX myofibers is associated with further deficits on excitable properties. To this end we use high-speed confocal microscopy and the voltage-sensitive indicator di-8-butyl-amino-naphthyl-ethylene-pyridinium-propyl-sulfonate. We found that the AP amplitude is not altered in MDX ‘normal’ or MDX ‘split’ FDB muscle fibers when compared to WT. Data indicate a reduction in muscle stiffness, increased sarcolemmal deformability and instability in mdx muscle. Findings also suggest mechanical differences due to altered morphology and technique of getting the fibers.
Supported partially by PAPIIT-UNAM (IA210016) to KPGP and NIH to RML (R01AR059179).

27-POS Board 14

Emerging Role of Differential Molecular Association in Force-Transmitting Nascent Adhesions

Sangyoon J. Han[1], Alexia Bachir[4], Kevin Dean[1], Edgar Gutierrez[3], Alex Groisman[3], Alan R. Horwitz[2,4], Gaudenz Danuser[1].
[2]Allen Institute for Cell Science, Seattle, WA, USA, [1]University of Texas Southwestern Medical Center, Dallas, TX, USA, [3]University of California San Diego, San Diego, CA, USA, [4]University of Virginia, Charlottesville, VA, USA.

Adhesion dynamics play a critical role in cell migration, whose decision process is affected by lots of biochemical and biomechanical inputs. Adhesions and their coupling with mechanical force have been extensively characterized at the level of large, mature focal adhesions. However, it has been elusive whether the nascent adhesions (NAs), before they reach their full maturation, are able to transmit forces and how these forces, if exist, affect the recruitment of early adhesion molecules. Here, we investigated how the mechanical force and the molecular recruitment of talin, vinculin and paxillin synergistically affect the adhesion assembly using high-resolution traction force microscopy (TFM) and fluorescence imaging of each molecule with eGFP. We used the single-particle-tracking of all NAs, extracted kinematic and kinetic features from the tracks, and used human-in-the-loop machine learning to identify the sub-population of nascent adhesions that assemble and disassemble at the protruding cell edge. We report that about 60% of non-maturing NAs transmit forces during their average lifetime of 2 minutes. Via time-lag analysis between eGFP-intensity and force, we show distinct difference in the recruitment sequences of three molecules in between non-maturing NAs vs. maturing NAs. Talin was recruited ~7 sec before vinculin's recruitment to the adhesions complex in maturing NAs whereas the two molecules were recruited at the same time in non-maturing NAs. Moreover, vinculin's early assembly rate to the maturing NAs was higher than in non-maturing NAs whereas talin and paxillin have shown no difference in early assembly. Traction force growth rate in the maturing vinculin complex was also higher than that in non-maturing vinculin complex. Together, these findings suggest a pre-conditioning role of talin's early recruitment to promote faster vinculin recruitment and force transmission for successful adhesion maturation, possibly via effective stretching.

29-POS Board 15

Interrogating the Bacterial Cell Cycle by Cell Dimension Perturbations and Stochastic Modeling

Hai Zheng[1,2], **Po-Yi Ho**[3], Meiling Jiang[1], Bin Tang[4], Weirong Liu[1,2], Dengjin Li[1], Xuefeng Yu[5], Nancy Kleckner[6], Ariel Amir[3], Chenli Liu[1,2].
[3]Harvard University, Cambridge, MA, USA, [1]Shenzhen Institutes of Advanced Technology, Shenzhen, China, [2]University of Chinese Academy of Sciences, Beijing, China, [4]Southern University of Science and Technology, Shenzhen, China, [5]Shenzhen Institutes of Advanced Technology, Shenzhen, China, [6]Harvard University, Cambridge, MA, USA.

Bacteria tightly regulate and coordinate the various events in their cell cycles to duplicate themselves accurately and to control their cell sizes. Growth of *Escherichia coli*, in particular, follows Schaechter's growth law. The law says that average cell volume scales exponentially with growth rate, with a scaling exponent equal to the time from initiation of a round of DNA replication to the cell division at which the corresponding sister chromosomes segregate. Here, we test the robustness of the growth law to systematic perturbations in cell dimensions achieved by varying the expression levels of mreB and ftsZ. We found that decreased mreB levels resulted in increased cell width, with little change in cell length, whereas decreased ftsZ levels resulted in increased cell length. In both cases, the time from replication termination to cell division increased with the perturbed dimension. Importantly, the growth law remained valid over a range of growth conditions and dimension perturbations. The growth law can be quantitatively interpreted as a consequence of a tight coupling of cell division to replication initiation. Its robustness to perturbations in cell dimensions strongly supports models in which the timing of replication initiation governs that of cell division, and cell volume is the key phenomenological variable governing the timing of replication initiation. These conclusions are discussed in the context of our recently proposed "adder-per-origin" model, in which cells add a constant volume per origin between initiations and divide a constant time after initiation.

31-POS Board 16

Dynamic Analysis of DNA and Topoisomerase II Interaction Based on Fluorescence Fluctuation and Single Molecule Detection

Wan-Chen Huang[1], Chun-Ying Lee[2], Tao-Shih Hsieh[1,2].
[1]Academia Sinica, Taipei, Taiwan, [2]National Taiwan University, Taipei, Taiwan.

Topoisomerase 2 (Top2) is known as anticancer drug target due to its highly demanded on resolving DNA tangling problems during cell division. These enzymes regulate DNA topology by transiently breaking one DNA double strand (cleavage), allowing the second double strand to pass through the opened DNA gate (opening), and then closing the gate by rejoining the broken end. Previous studies defined the Top2 function mainly at DNA cleavage and re-ligation steps, however, whether the cofactors and Top2 drugs interfere the DNA dynamics at the opening and closing steps remain unclear. Here, we used fluorescence correlation spectroscopy (FCS) and Förster resonance energy transfer (FRET) in combination to examine the cofactor effects on the interaction of Top2 and DNA, and in addition used the single molecule FRET (smFRET) method to observe the opening and closing of DNA gate. Our results demonstrated that both types of anticancer drugs, teniposide (VM26) and dexrazoxane (ICRF187) can interfere the rate of DNA gate dynamics by shortening the dwell time at closing state. Moreover, binding of AMPPNP induced DNA gate dynamics, which is previously unaddressed. These findings unmask the Top2 dynamics after N-gate closure and reveal the potential mechanism of action of Top2 drugs.

33-POS Board 17

Label-Free, Ultrahigh-Speed, 3D Tracking Of Single Virus Particle on Cell Plasma Membranes by Coherent Brightfield (COBRI) Microscopy

Yi-Fan Huang[1], Cheng-Hao Lin[1], Wen Chang[2], Chia-Lung Hsieh[1].
[1]Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei, Taiwan, [2]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan.

Viral infection is a multistep process, starting with a virus particle landing on a cell surface followed by penetration of the plasma membrane. Due to the difficulty of measuring the rapid motion of small-sized virus particles on the membrane, little is known about how a virus particle reaches an endocytic site after landing at a random location. We used coherent brightfield (COBRI) microscopy to investigate early stage viral infection with ultrahigh spatiotemporal resolution[1]. By detecting intrinsic scattered light via imaging-based interferometry, COBRI microscopy allowed us to track the motion of a single vaccinia virus particle with nanometer spatial precision (< 3 nm) in 3D and microsecond temporal resolution (up to 210,000 frames per second). Through image post-processing, relatively stationary background scattering of cellular structures was effectively removed, generating a background-free image of the diffusive virus particle for precise localization. Using our method, we revealed single virus particles exploring cell plasma membranes with unprecedented clarity. We found that immediately after attaching to the membrane (within a second), the virus particle was locally confined within hundreds of nanometers. Surprisingly, within this confinement, the virus particle diffuses laterally with a very high diffusion coefficient (~1 μm2/s) in microsecond-timescale. We also observed numerous transient confinements of the virus particle in nano-sized zones on the membrane, presumably due to the rapid interaction between the virus and the cell membrane receptors. In order to elucidate the virus-membrane interaction with specificity, we further manipulated cell membrane receptors and the viral membrane proteins via molecular biological approaches. Critical interplays between viral membrane proteins and cell receptors that were thought to determine endocytic pathways (e.g., endocytosis and membrane fusion) were investigated at the molecular scales.

Keywords: coherent brightfield microscopy; single-virus tracking; early-stage infection; digital background removal, virus-membrane interaction.
References: Yi-Fan Huang et al., ACS Nano, Article ASAP.

35-POS Board 18

3D-Structural Modeling by Advanced Electron Microscopy at Whole Single Cell Level

Atsuko H. Iwane[1,2].
[2]Osaka University, Suita, Osaka, Japan.[1]Riken, QBiC, Suita, Osaka, Japan,

Recently several microscopy techniques have revealed the dynamics and structure of intracellular material such as organelles and supra molecular proteins for a better understanding of the fundamental properties of a cell. Several advanced electron microscopes provide information on extremely small biological specimens. Among them, cryo-TEM and tomography techniques are useful for visualization of fine structures but the observation area is limited. Conversely, FIB (Focus ion beam)-SEM and SBF (serial block face)-SEM theoretically are not affected by the thickness of the sample. Furthermore, UHVEM (Ultra-high voltage-TEM) tomography is expected to be able to visualize the detailed 3D-structure of thylakoid membrane in the plastid and of the micro-compartments (cristae) in mitochondria. In this meeting, while taking advantage of these characteristics using these advanced electron microscopy techniques I will introduce to 3D-structural modeling of the interaction of intracellular organelles and the internal fine structure of these organelles of *C. merolae* cells (2-5 microns), which is considered as the primitive eukaryotic organism during cell division at the whole cell level. In particular, I definitely would like to discuss about transitional changes in thylakoid membranes in plastid with the researcher of computer simulation modeling.

37-POS Board 19

NMDA Receptors and Large-Conductance Calcium-Activated Potassium Channels in Enkephalinergic Neurons of Mouse Spinal Superficial Dorsal Horn

Eiko Kato, Yuuichi Hori.
Dokkyo Medical University, Tochigi, Japan.

Enkephalin (Enk)-containing neurons are distributed at a high density in the superficial dorsal horn (SDH) of the spinal cord. In the present experiments, we analyzed whether Enk-containing neurons in the SDH express the *N*-methyl-*D*-aspartate receptor (NMDAR) 2B (NR2B) subunit and large-conductance calcium-activated potassium (BK) channels, and how the activity of Enk-containing neurons is regulated by NMDAR and BK channels.

The experiments were performed on 5- to 8-week-old male ICR mice. The lumbar spinal cord was dissected, and transverse slices were prepared. Tight-seal whole-cell recordings were obtained from neurons in the SDH. After the recordings, the neurons were collected, and single-cell real-time RT-PCR was performed to analyze the expression profile of genes in each SDH neuron.

Puff application of L-glutamate evoked an inward current at a holding potential of -70 mV. Upon depolarizing the holding potential to 0 mV, outward current of long duration appeared after initial inward current. The NR2B-selective NMDAR antagonist ifenprodil reduced the outward current. The outward current was also abolished by the selective BK channels antagonist iberiotoxin. Single-cell real-time RT-PCR analysis revealed that single SDH neurons expressing the preproenkephalin mRNA also expressed the BK channel α-subunit, NR1, and NR2B subunit mRNAs. Additionally, the neurons generating the outward current showed a significant tendency to express BK channels α-subunit mRNA, which is consistent with the pharmacological results of a BK channels antagonist.

Our experiments suggest that combining real-time RT-PCR with whole-cell recordings provides a useful tool to analyze the functions of genetically characterized central nervous neurons at the single-cell level and that Ca^{2+} influx through NMDAR may activate BK channels and hyperpolarize Enk-containing neurons in the SDH.

39-POS Board 20

3D Multi-Layered Blood Vessel/Inflamed Tissue Model for the Investigation of T Cell Tissue Infiltration

HyeMi Kim[3], Sang Min Park[1], Kwang Hoon Song[1], Seongsu Eom[1], HyoungJun Park[1], Dong Sung Kim[1], Junsang Doh[1,2].
[1]POSTECH, Pohang, South Korea, [2]POSTECH, Pohang, South Korea, [3]POSTECH, Pohang, South Korea.

Leukocyte infiltration, which plays critical roles in tissue inflammation for pathogen clearance and tumor eradication, is regulated by complex microenvironments in blood vessels, including inflamed endothelium, blood flow, and perivascular components. However, the role of perivascular components on the leukocyte infiltration has not been systematically investigated until recently. In this work, we developed a 3D multi-layered blood vessel/tissue model with a nanofiber membrane, enabling real-time visualization of dynamic T cell infiltration and subsequent interaction with perivascular macrophages. We directly fabricate a highly aligned, free-standing nanofiber membrane with an ultra-thin thickness of ~ 1 μm in a microfluidic system. Coating the nanofiber membrane with matrigel showed synergetic topographical and biochemical effects on the reconstitution of a well-aligned endothelial monolayer on the membrane, which is found in native blood vessels. Fine transparency of the ultra-thin membrane allowed real-time visualization of T cells that were flowing on endothelial layers, underwent transendothelial migration and further interacted with perivascular macrophages underneath the nanofiber membrane. Our 3D multi-layered blood vessel/tissue model will offer a powerful and versatile tool to investigate the mechanism of T cell tissue infiltration.

41-POS Board 21

The Correspondence between Raman Spectra and Total Omics

Koseki J. Kobayashi-Kirschvink[1], Hidenori Nakaoka[1], Arisa Oda[2], Kunihiro Ohta[2,3], Yuichi Wakamoto[1].
[1]The University of Tokyo, Meguro-ku, Tokyo, Japan, [2]The University of Tokyo, Meguro-ku, Tokyo, Japan, [3]The University of Tokyo, Bunkyo-ku, Tokyo, Japan.

Fluorescent reporters have been the de facto standard tool of *in vivo* research in the past two decades, allowing us to monitor specific molecules in living cells with high precision. However, the cell is a complex system with numerous molecules and interactions, and due to the specificity of fluorescent reporters, they often fail to elucidate the cellular behavior as a whole. On the other hand, omics techniques such as next generation sequencing or mass-spectrometry give us much more comprehensive molecular information about the cell, but are inherently destructive. No existing technique allows us to further analyze the dynamic molecular changes occurring at the single-cell level.

In contrast, Raman micro-spectroscopy has advanced in recent years, and is one of the very few imaging techniques that can potentially report on whole-cell molecular compositions at the single-cell level in both comprehensive and non-destructive manners. However, the complexity of Raman spectra has hampered its interpretation and thus its routine use. Previous attempts on interpreting spectra mostly relied on preparing Raman spectral databases of purified materials, which is usually time-consuming and laborious, especially when dealing with biological samples. Here, we propose a method that can possibly circumvent the preparation of such databases, and instead understand how Raman spectra and "total" omics correspond to each other. Our method is based on the linear relationship between the two data sets that holds in principle, and actively employs the intrinsic low-dimensionality of gene/protein expression profiles. Example data of Raman spectra and mRNA-seq data of *Schizosaccharomyces Pombe* under various stress conditions are obtained, and using supervised machine learning algorithms such as the partial least squares regression, it is shown that if certain conditions are met, mRNA-seq data can possibly be predicted from Raman spectra.

43-POS Board 22

Post-Translational Modifications Regulate Function and Self-Assembly of Axonemal Tubulin

Yin-wei Kuo[1], Ron Orbach[2], Jonathon Howard[2].
[2]Yale University, New Haven, CT, USA.[1]Yale University, New Haven, CT, USA,

Microtubules, cytoskeletal polymers assembled from building blocks comprising α- and β-tubulin heterodimers, are involved in many important intracellular functions including the maintenance of cell structure, the formation of the mitotic spindle, the transport of organelles, the sensation of force and the motility of cells. The diverse functions of microtubules require delicate control over the dynamics of assembly and disassembly, and the interactions with microtubule-associated proteins (MAPs). A long standing question is how do the evolutionally highly conserved tubulin building blocks differentially regulate microtubule dynamics and functions in different cellular processes.

Highly versatile post-translational modifications (PTMs) are present on tubulin, contributing to the so-called "tubulin code" and are suggested to be crucial for regulating the diverse functions of cellular microtubules. However, our current knowledge about microtubule dynamics is predominantly based on the highly heterogeneous mammalian brain tubulin system, whose complex isotypes and PTM combinations make it challenging to elucidate the effect and function of the individual modifications.

To reduce the complication of tubulin isotype, we selected biflagellated green algae *Chlamydomonas reinhardtii* as our model system. We recently developed a novel method to purify axonemal tubulin from various strains of *C. reinhardtii*, including a polyglutamylation-deficient mutant *tpg1*, to study the self-assembly dynamics of axonemal tubulin and how tubulin post-translational modifications (PTMs) contribute to the distinct properties of axonemal tubulin. We will do this using total-internal-reflection-fluorescence (TIRF) and other microscope techniques to determine the effects of PTMs on dynamics at the single-microtubule level.

45-POS Board 23

In Situ Quantitative Analysis of Protein Oligomerization in Living Cell

Karina Kwapiszewska[1], Tomasz Kalwarczyk[1], Bernadeta Michalska[2], Krzysztof Szczepanski[1], Jedrzej Szymanski[2], Jerzy Duszynski[2], Robert Holyst[1].
[1]Institute of Physical Chemistry of Polish Academy of Sciences, Warsaw, Poland, [2]Nencki Institute of Experimental Biology, Warsaw, Poland.

In this presentation we show that fluorescence correlation spectroscopy (FCS) can be used for quantification of protein oligomerization directly in cytoplasm of living cell. Nowadays, life-science researchers utilize plenty of methods aiming in quantification of protein-protein interactions. These methods range from simple biochemical experiments, through molecular biology methods, towards advanced proteomic analysis. In this way, myriads of valuable data for biology, medicine and pharmacology were provided. However, majority of experiments was performed on fixed cells or extracted proteins. Therefore, detailed information about *in vivo* dynamics of protein-protein interactions is still missing, but substantially needed.
We present an FCS-based method of protein oligomerization analysis. As a protein of interest, we chose dynamin-related protein 1 (Drp1) which is involved in mitochondrial fission process. Our method base on precise determination of length-scale dependent hydrodynamic drag of cytoplasm. It was proved, that cytoplasmic hydrodynamic drag (d_h, also interpreted as viscosity) depends on a probe's size. Therefore, first step of our research was determination of diffusion coefficients (D_{diff}) of probes of known sizes (GFP, Calcein-AM, dextrans) in cytoplasm of HeLa cells. These results were utilized for evaluation of a scaling equation, and, subsequently, for determination of D_{diff} expected for certain oligomers of Drp1. Next, D_{diff} of GFP-fused Drp1 was measured by FCS in HeLa. Different Drp1 mutants were investigated (monomer, dimer, wild type). Results indicate that there is an equilibrium between dimeric and tetrameric form of wild type Drp1 in cytoplasm. Length-scale dependence of d_h enabled separation of D_{diff} of these two forms (D_{diif} of dimer was 1.5 fold bigger than D_{diff} of tetramer, in contrast to constant viscosity conditions). Thus, quantity of dimer and tetramer forms could have been determined. Moreover, equilibrium constant of tetramer formation was calculated.

47-POS Board 24

Single-Cell Time-Series Measurements of a Library of Strains with Single Molecule Sensitivity

Michael J. Lawson, Daniel Camsund, Jimmy Larsson, Ozden Baltekin, David Fange, Johan Elf. Uppsala University, Uppsala, Sweden.

We have developed a method to perform sensitive, time-lapse imaging at the single-cell level for many different strains simultaneously. The method has three components. The first is library generation. We have created a library of plasmids, each with a constitutively expressed sgRNA and a uniquely associated barcode RNA (driven by an inducible and orthogonal expression system). We transform these plasmids into Escherichia coli containing chromosomally expressed dCas9 to allow for knockdown of the sgRNA-targeted gene.

The second component is single-cell phenotyping. We load the library of strains into a microfluidic device, which is mounted on a microscope. Here we can observe growth of isogenic microcolonies of every strain in the library over many generations, as well as count and localize single molecules and quantify any other phenotype discernable via microscopy.

The third and final step is to genetically identify each strain. The chip design allows for observing thousands of strains in one experiment, however the loading is random. We have developed a multiple round oligo-paint based approach to make an encoding between two fluorescently labeled primers (Cy3 and Cy5) and the unique barcode RNAs. In every round a probe with one of the fluorescent markers hybridizes to each barcode, thus providing a binary readout. After successive rounds, the cells in each trap have an associated binary word that uniquely identifies the strain. The encoding from fluorescent primers to barcode RNA sequence is achieved via template oligos, which are amplified by hybridization-round specific primers from an oligo pool.

As a proof of principle, we implemented a library of three strains with different levels of LacY-Ypet expression. Our method could differentiate between a strain with one molecule every second generation and a strain with one molecule every fifth generation.

49-POS Board 25

Mechano-Chemical Regulation of Actin Depolymerization Kinetics

Cho-yin Lee[1,2,3], Jizhong Lou[4], Kuo-Kuang Wen[5], Melissa McKane[5], Suzanne G. Eskin[1], Peter A. Rubenstein[5], Shu Chien[7], Ono Shoichiro[6], Cheng Zhu[1], Larry V. McIntire[1].
[1]Georgia Institute of Technology, Atlanta, GA, USA, [2]National Taiwan University Hospital, Taipei City, Taiwan, [3]Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan City, Taiwan, [4]Chinese Academy of Sciences, Beijing, China, [5]University of Iowa, Iowa City, IA, USA, [6]Emory University, Atlanta, GA, USA, [7]University of California at San Diego, La Jolla, CA, USA.

A fundamental yet unresolved issue in cell biology is how force regulates actin dynamics and how this biophysical regulation is modulated by biochemical signaling molecules. Here we show, by atomic force microscopy (AFM) force-clamp experiments, that tensile force regulates the kinetics of G-actin/G-actin and G-actin/F-actin interactions by decelerating dissociation at low forces (catch bonds) and accelerating dissociation at high forces (slip bonds). Steered molecular dynamics (SMD) simulations revealed force-induced formation K113: E195 salt bridge between actin subunits. This provided a structural mechanism for actin catch bonds, supported by the mutagenesis study showing that the K113S, K113E, E195S, E195K mutants of yeast actin suppressed the actin catch bonds; and that this suppression is rescued by a K113E/E195K double mutant (E/K) restoring the interaction in the opposite orientation. Moreover, formin controlled by RhoA-mediated auto-inhibitory module can serve as a "molecular switch", converting the catch-slip bonds to slip-only. These results demonstrate the mechano-chemical regulation of the actin depolymerization kinetics by catch-slip bonds. They also support biological significance of actin catch-slip bonds, as they corroborate reported observations that RhoA and formin switch force-induced actin cytoskeleton alignment and that either K113E or E195K induces yeast cell growth defects rescued by E/K. Our study may provide a mechanoregulatory mechanism to control cell functions by regulating the depolymerization kinetics of force-bearing actin filaments throughout the cytoskeleton.

51-POS Board 26

Single-Cell Proteotypic Analysis of Invasive Motility in Glioblastoma

Jung-Ming G. Lin[1,2], Chi-Chih Kang[2], Amy E. Herr[1,2], Sanjay Kumar[1,2].
[2]UC-Berkeley, Berkeley, CA, USA.[1]The UC-Berkeley-UCSF Graduate Program in Bioengineering, Berkeley, CA, USA,

Cellular invasion into the surrounding tissue is a critical step in many solid tumors. However, virtually nothing is known about how the protein profile of an individual tumor cell relates to its propensity to invade and metastasize. One type of cancer characterized by cellular invasion is glioblastoma (GBM). The extensive tissue infiltration of GBM cells makes complete surgical resection impossible and contributes to resistance to subsequent radiation and chemotherapy. To investigate the invasive capability of GBM cells, we present a microfluidic device that correlates differences in protein expression with cellular speed, persistence and aspect ratio. Our PDMS/polyacrylamide-based device supports chemotactic migration through channels of tissue-like stiffness, enabling separation of heterogeneous tumor cell populations based on migration speed. The isolated cells are then subjected to multi-target single-cell western blotting using the polyacrylamide device walls as the electrophoretic and immunoblotting matrix. Using this device, we analyzed patient-derived glioblastoma tumor-initiating cells (TICs), a subpopulation of GBM with stem-like properties. We show that TICs exhibit surprising heterogeneity with respect to marker expression, with an apparent enrichment of Nestin and depletion of STAT3 in the most motile cells. This Nestin enrichment is not observed when immunofluorescence is used to quantify Nestin expression. Furthermore, we identified correlations between specific biomarkers within single TICs, which would not have been possible with population-based assays. Specifically, we discovered a positive correlation between Nestin and β-tubulin, suggesting the possibility that β-tubulin can also be used as a TIC enrichment marker. Our system lays the groundwork for the development of single-cell protein profiles of tumor invasion and metastasis. These profiles would allow for the identification of proteins that can predict invasive capability, an insight with great fundamental and translational value.

53-POS Board 27

Comparison of Assembly Kinetics of Yeast Dmc1 and Rad51 Recombinases

Sheng-Yao Lin[1], Wen-Hsuan Chang[1], Chih-Yuan Kao[2], Peter Chi[2], Hung-Wen Li[1].
[1]National Taiwan University, Taipei, Taiwan, [2]National Taiwan University, Taipei, Taiwan.

Dmc1 and Rad51 recombinases are essential to the homologous recombination, the major pathway to repair double-strand break DNA damage. While only Rad51 is required in mitotic cells, both Rad51 and Dmc1 participate in meiotic cells. The mechanism underlying the differential requirement is unknown. Here, we compared the kinetics of the nucleoprotein filament assembly by Saccharomyces cerevisiae Rad51 and Dmc1 using single-molecule tethered particle motion experiments and in vitro biochemical assay. Ca^{2+} ions are found to stabilize the Dmc1 filaments by stimulating its nucleation step.Rad51 is found to form more stable nucleoprotein filaments than Dmc1 due to a faster nucleation rate. Both Rad51 and Dmc1 form stable nuclei on DNA in dimers. Dmc1 is found to nucleate at duplex/single-stranded (ss) DNA junctions with a higher rate, while Rad51 preferentially nucleates on long ssDNA. The differential substrate preference might be responsible for the different nucleation rates observed, and the requirement of both Rad51 and Dmc1 in meiotic homologous recombination.

55-POS Board 28

Analysis of Single Particle Tracking Data under Heterogeneous Conditions

Martin Lindén[1], Vladimir Curic[1,2], Elias Amselem[1], Johan Elf[1].
[1]Uppsala University, Uppsala, Sweden, [2]IMINT Image Intelligence, Uppsala., Sweden.

Recent advances in single particle tracking (SPT) microscopy make it possible to obtain tens of thousands of macromolecular trajectories from living cells in just a few minutes. Since molecules typically change their movement properties upon interactions, these trajectories contain information about locations and rates of intracellular reactions. Effectively extracting this information is a statistical challenge, and an active research area. A particular difficulty is heterogeneity in the data across and within nominally identical experiments. Unaccounted for, such variations can induce analysis artifacts and present a serious hurdle for quantitative analysis for large data sets. For example, the localization errors of single emitters may vary due to out-of-focus motion, drift, motion blur, fluorophore intensity fluctuations, heterogeneous background, or gradual photo-bleaching of the background or labeled molecule. To deal with such variations, we have extended localization methods to estimate both position and localization uncertainty from images of single emitters. We find that an uncertainty estimate based on the Bayesian posterior density and constrained by simple microscope properties performs well in a wide range of conditions relevant for live cell imaging. Furthermore, this additional information about localization uncertainty can be incorporated into trajectory analysis methods to improve diffusion constant estimates, detection of binding events, and the localization of single emitters. Here, we will discuss ongoing progress in this area, including attempts to estimate the number of diffusive states in multi-state SPT experiments.

57-POS Board 29

Effect of Epithelial-Mesenchymal Transition on the Dynamics of Egfrs of Prostate Cancer Cells Revealed by TSUNAMI 3D Tracking Microscope

Yen-Liang Liu[1], Aaron M. Horning[2], Evan P. Perillo[1], Cong Liu[1], Mirae Kim[1], Rohan Vasisht[1], Hannah Horng[3], Andrew K. Dunn[1], Chun-Liang Chen[2], Hsin-Chih Yeh[1].
[1]University of Texas at Austin, Austin, TX, USA, [2]University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [3]University of Maryland, College Park, MD, USA.

Dysregulated trafficking of receptor tyrosine kinases has been linked to oncogenesis. Here we study the dynamics and mechanisms of epidermal growth factor receptor (EGFR) trafficking of three epithelial prostate cancer cell with different degrees of metastatic potential: BPH1 (benign), LNCaP (non-invasive malignant), and PC-3 (highly invasive malignant) using a 3D single-particle tracking technique termed TSUNAMI (Tracking of Single particles Using Nonlinear And Multiplexed Illumination). TSUNAMI is capable of tracking fluorescent nanoparticle-tagged EGFR from 2 to 10 minutes and vertical tracking depth up to tens of microns. To analyze the long 3D trajectories generated by the TSUNAMI microscope, a trajectory analysis algorithm is developed to classify trajectories and extract the biophysical parameters, such as diffusivity, inward movement, and the linear dimension of compartments that restrict receptors. These parameters are used to quantify the metastatic potentials of prostate cancer cell lines. The diffusion coefficients of EGFRs on PC-3 cells (0.010±0.014 $\mu m^2/s$) are around one-quarter of those estimated from benign BPH1 cells (0.036±0.058 $\mu m^2/s$) due to the denser cortical actin networks on the apical side of PC-3 cells (revealed by 3D structured illumination microscope). The invasive cells also exhibit longer (2.83±0.23 μm vs. 1.45±0.16 μm) and faster (0.021±0.016 μm/s vs. 0.005±0.002 μm/s) inward movement than non-invasive prostate cancer cells, which implies that high endocytotic activity of metastatic cells. In addition, the biophysical properties of EGFR trajectories are correlated to the mRNA expression levels of epithelial-mesenchymal transition related genes. The results of single-cell gene analysis show that high expressions of EGFR and Arp2/3 are correlated to the decrease of EGFR diffusivity, and the increase of dynamins coheres with more active inward movement. This work demonstrates how EGFR trajectory-derived biophysical parameters are potentially linked to metastasis.

59-POS Board 30

Single-Molecule Biochemical Studies on the Stimulation Mechanism of SWI5-SFR1 Complex on RAD51 Presynaptic Filament Formation

Chih-Hao Lu[1], Hiroshi Iwasaki[2], Peter Chi[3], Hung-Wen Li[1].
[1]National Taiwan University, Taipei, Taiwan, [2]Tokyo Institute of Technology, Tokyo, Japan, [3]National Taiwan University, Taipei, Taiwan.

Eukaryotic RAD51 protein is essential for DNA homologous recombinational repair of DNA damage. RAD51 recombinases assemble onto ssDNA to form a presynaptic filament, the required functional component for homology pairing and strand exchange reactions. This filament assembly is the committed step of homologous recombination and is subjected to regulation. Nucleation step is kinetically slow, and several accessory proteins have been identified to regulate RAD51 nucleation. SWI5-SFR1 (S5S1) is a heterodimeric accessory protein, and previous biochemical work showed that S5S1 interacts with RAD51, and stimulates RAD51-mediated homologous recombination. Our single-molecule tethered particle motion (TPM) experiments demonstrate that mouse S5S1 interacts with mouse RAD51 to form complex, and the mRAD51-S5S1 complex efficiently stimulates the nucleation step. We also showed that mS5S1 stimulates mRAD51 nucleation by (i) reducing mRAD51 seed size, (ii) increasing mRAD51 ssDNA affinity and (iii) stabilizing mRAD51 nucleus on ssDNA. While nucleation stimulation by S5S1 is absent in fission yeast (Schyzosaccharomyce pombe, Sp) system in our single-molecule work, SpS5S1 is shown to prevent SpRad51 disassembly. Different regulation strategies among species allow S5S1 to stabilize Rad51 filament efficiently.

61-POS Board 31

Dual-Component Voltage Sensitivity of Indocyanine Green Fluorescence in the Heart

Regina Macianskiene, Mante Almanaityte, Rimantas Treinys, Antanas Navalinskas, Rimantas Benetis, Jonas Jurevicius.
Lithuanian University of Health Sciences, Kaunas, Lithuania.

Background: Voltage-sensitive fluorescent dyes (VSDs) have been used in heart electrophysiological studies for over 30 years. Nevertheless, to date, VSDs have not yet been approved for clinical use. It was reported that the widely used fluorescent dye indocyanine green (ICG), which has FDA approval, exhibits voltage sensitivity in various tissues.
Objective: The aim of this study was to explore the possibility of using ICG to monitor cardiac electrical activity.
Methods: A standard glass microelectrode and optical mapping, using a near-infrared ICG fluorescent dye, were used to simultaneously record electrical action potential (AP) and optical signal (OS) in a Langendorff-perfused rabbit heart that was fully stopped.
Results: We showed the first successful detection of voltage sensitivity of the ICG dye in a heart. The ICG OS is not caused by contraction or by Ca2+ transients, and reliably follows the AP changes induced by pharmacological compounds. The ICG OS has a dual-component (fast and slow) response to membrane potential changes that accurately tracks the time of electrical signal propagation but clearly differ in their kinetics and voltage-sensitive spectral properties. The voltage-sensitive fluorescence of ICG dye was not high relative to the fluorescence of standard VSDs. However, after averaging, the good signal-to-noise ratio (> 20 dB) of ICG rendered its signal suitable for observing cardiac electrical activity.
Conclusions: Our research confirms that ICG is a voltage-sensitive dye with a dual-component (fast and slow) response to membrane potential changes. We suggest that it can be used as a tool for examining excitation wave propagation in the heart.
This research was funded by a grant (No.SEN-15/2015) from the Research Council of Lithuania.

63-POS Board 32

Mutual Inhibition between PTEN and PIP3 Regulates Excitability for Eukaryotic Chemotaxis

Satomi Matsuoka[1,2], Masahiro Ueda[2,1].
[1]RIKEN Quantitative Biology Center, Osaka, Japan, [2]Osaka University, Osaka, Japan.

Excitable signal transduction in eukaryotic chemotaxis generates PI(3,4,5)P3-enriched domain asymmetrically on the cell membrane that serves as an all-or-none signal for cell migration. Here we report that PTEN, a PI(3,4,5)P3 phosphatase, regulates the excitability by constituting the mutual inhibition relationship with PI(3,4,5)P3. PTEN suppresses PI(3,4,5)P3 at the sub-threshold level by the phosphatase activity but is excluded from the membrane by PI(3,4,5)P3 at the supra-threshold level ensuring the PI(3,4,5)P3 excitation. We further demonstrates with single-molecule resolution that a subpopulation of PTEN adopting the stably-binding state with longer membrane-binding lifetimes acts as a regulatory point of the mutual inhibition as well as chemoattractant stimuli, thereby facilitating the PI(3,4,5)P3 excitation via the exclusion along the gradients. These observations illustrate multistate transitions of PTEN and the positive feedback regulations as mechanisms to regulate excitability in the cellular decision-making for chemotaxis and also suggest important implications in the tumor suppression.

65-POS Board 33

Direct Measurement of Coupling in the Bacterial Flagellar Motor

Jasmine A. Nirody[1,2], Jordan O. Juritz[2], Ren Chong Lim[3], Chien-Jung Lo[4], Richard M. Berry[2]. [1]University of California, Berkeley, Berkeley, CA, USA, [2]University of Oxford, Oxford, United Kingdom, [3]Universiti Brunei Darussalam, Bandar Seri Begawan, Brunei Darussalam, [4]National Central University, Taoyuan City, Taiwan.

The bacterial flagellar motor (BFM), which drives the rotation of the flagellar filament and drives locomotion in many species of bacteria, obtains its energy from the transmembrane gradient of ions (generally, either H^+ or Na^+). Most models have thus far assumed a tightly-coupled mechanism, in that each full revolution of the motor is driven by a fixed number of ions. However, recent experiments showing that the number of active torque-generating units (stators) in the BFM varies across applied loads has brought many previous assumptions, including the coupling between ion flux and motor rotation, into question. Here, we use a sodium ion fluorescence indicator, Sodium Green, that can be used to make rapid measurements of internal sodium concentration in single cells to quantify sodium flux in *Escherichia coli.* This system will provide the first direct measurement of the coupling ratio between ion flux and motor rotation in the flagellar motor.

67-POS Board 34

Threading Moiety Size Determines Locking Mechanism of DNA Threading Intercalators

Thayaparan Paramanathan[1,2], Andrew Clark[2], Fredrik Westerlund[3], Per Lincoln[3], Ioulia Rouzina[4], Mark C. Williams[2].
[1]Bridgewater State University, Bridgewater, MA, USA, [2]Northeastern University, Boston, MA, USA, [3]Chalmers University of Technology, Gothenburg, Sweden, [4]The Ohio State University, Columbus, OH, USA.

Threading intercalators are small molecules that bind to DNA by threading their ancillary motifs through DNA bases to intercalate a middle planar section between the DNA base pairs. These dumbbell-shaped molecules exhibit incredibly slow kinetics and high binding affinity compared to classical intercalators. These properties put them in the class of prospective anti-cancer drugs. We have been exploring a variety of ruthenium based threading intercalators using optical tweezers. In an optical tweezers set-up, a single DNA molecule is attached between two polystyrene beads and manipulated in the presence of various concentrations of intercalators to characterize their DNA binding properties. These intercalators are introduced to the system at different concentrations, while a single DNA molecule is held at a constant force. Measurements of DNA extension as a function of time provide the DNA equilibrium binding affinity and force-dependent binding kinetics for these molecules, revealing the structural rearrangements required for intercalation. In this study we explore the binding of a binuclear ruthenium complexes ΔΔ-[μ-bidppz(bipy)4Ru2]4+ (ΔΔ-B) in order to compare it with a previously studied sister molecule ΔΔ-P. These molecules have the same middle intercalating dppz component that interacts with the DNA and only their ancillary side chains, which must thread between the bases, are varied by size. ΔΔ-P previously showed an unusual locking mechanism, in which DNA must first increase in length for intercalation to occur, before decreasing in length as equilibrium is approached. The force dependence of the kinetics for ΔΔ-B suggests that the small reduction in the size of side chain in results in the elimination of the locking mechanism, fundamentally altering the overall structural rearrangements required for binding.

69-POS Board 35

Three-Dimensional Traction Force Microscopy

Giovanni Paylaga[1,2], Yu-chi Ai[1], Hsuan Yang[1], Hung-hui Lee[3], Jia-yang Juang[3], Keng-hui Lin[1]. [1]Academia Sinica, Taipei, Taiwan, [2]National Central University, Taoyuan, Taiwan, [3]National Taiwan University, Taipei, Taiwan.

Cells in vivo are grown in a three-dimensional context. In our lab, we found that when placing cells in a spherical pore, cells exhibit three-dimensional (3D) morphology and organization. In this poster, we present a method to measure 3D traction force of a cell placed in a pore. We embedded marker particles in the substrate and tracked their movement from stressed state to no-cell state. Based on the displacement of marker particles, we interpolate displacement field onto the spherical surface. Using finite element method, we computed the stress exerted by the cell. We also simulated traction stress exerted by a cell and checked the recovery stress at various particle density and accuracy. Based on our simulation results, we are confident that we can recover 3D traction stress exerted by the cell in a pore. Our result can be combined with our other investigation on pore size effect on cell volume. Together, we are building tools to study the mechanobiology in 3D.

71-POS Board 36

Track STIM1 and Orai1 Molecules in Live Cells

Xianan Qin[1], Sangkwon Lee[3], Kaitlin Chan[2], Min Zhang[2], Chenglong Yu[2], Jun Chu[4], Chan Young Park[3], Hyokeun Park[1,2].
[1]The Hong Kong University of Science and Technology, Kowloon, Hong Kong SAR, China, [2]The Hong Kong University of Science and Technology, Kowloon, Hong Kong SAR, China, [3]Ulsan National Institute of Science and Technology, Ulsan, South Korea, [4]Shenzhen Institutes of Advanced Technology, Chinese Academy of Science, Shenzhen, Guangdong, China.

Ca^{2+} inside cells plays important roles in biological processesy. The store operated calcium entry (SOCE) can be regulated by STIM1 (a calcium sensor in the membrane of ER) and Orai1 (a calcium channel in the plasma membrane). STIM1 binds to Orai1 , and activates the Orai1 channel after the calcium depletion in ER. Although there are several reports about the molecular mechanism of Orai1 and STIM1, the detailed dynamics of STIM1 and Orai1 assembly is not clearly understood yet. We tracked STIM1 and Orai1 particles using our real time single-particle tracking setup. Using single-particle tracking, we calculated the diffusion coefficients of individual STIM1 and Orai1 particles. The diffusion coefficients of STIM1 and Orai1 particles were widely distributed when the ER calcium was at resting state. The depletion of ER calcium store after the treatment of Thapsigargin (TG) decreased the diffusion coefficients drastically. We further obtained the diffusion coefficient map and the potential energy landscape of STIM1 and Orai1 inside cells using Bayesian inference analysis. We also found that the potential energy landscapes of STIM1 and Orai1drastically changed after ER calcium depletion. These studies imply the diffusion-trapping of STIM1 and Orai1 in their assembly.

73-POS Board 37

Green Variant of Monomeric Photosensitizing Fluorescent Protein for Photo-Inducible Protein Inactivation and Cell Ablation

Yemima D. Riani[1], Tomoki Matsuda[1,2], Takeharu Nagai[1,2].
[2]The Institute of Scientific and Industrial Research, Ibaraki, Osaka, Japan.[1]Osaka University, Ibaraki, Osaka, Japan,

Photosensitizing fluorescent protein, which generates reactive oxygen species (ROS) upon light irradiation, is known to be useful for protein inactivation and cell ablation. Those give us clues to elucidate protein function, intracellular signaling pathway and intercellular interaction. Red fluorescent protein based monomeric photosensitizer, SuperNova Red (SNR) [1], has been established and overcame the drawbacks of its original dimeric version, KillerRed [2]. Here, we established SuperNova Green (SNG), a green variant of SNR which will be useful to control ROS production spatially, temporally, specifically, and simultaneously when used in combination with SNR. *In vitro* and *in vivo* ROS measurement showed that SNG has higher phototoxic activity than the red variant. Specific cell ablation of co-culture HeLa cells expressing SNG and SNR was successfully performed. In conclusion, SNG is useful as a new color variant of photosensitizer and can be used in combination with red variant of SuperNova to perform protein inactivation or cell ablation.

References

[1] Bulina, M. E., Chudakov, D.M., Britanova, O.V., Yanushevich, Y.G., Staroverov, D.B., Chepurnykh, T.V., Merzlyak, E.M., Shkrob, M.A., Lukyanov, S., Lukyanov, K.A. A genetically encoded photosensitizer. *Nat. Biotechnol*. 24, 95-99 (2006).
[2] Takemoto, K., Matsuda, T., Sakai, N., Fu, D., Noda, M., Uchiyama, S., Kotera, I., Arai, Y., Horiuchi, M., Fukui, K., Ayabe, T., Inagaki, F., Suzuki, H., Nagai, T. SuperNova, a monomeric photosensitizing fluorescent protein for chromophore-assisted light inactivation. *Sci. Rep*. 3, 2629 (2013).

75-POS Board 38

Oxidation of Serum Proteins by Titanium Dioxide Nanoparticles Leads to Oxidative Stress

Sabiha Runa[1], Dhanya Jayaram[1], Melike Lakadamyali[2], Melissa L. Kemp[3,4].Christine K. Payne[1,4].
[3]Georgia Institute of Technology, Atlanta, GA, USA, [1]Georgia Institute of Technology, Atlanta, GA, USA, [4]Georgia Institute of Technology, Atlanta, GA, USA.[2]Institute de Ciencies Fotonics, Castelldefels, Barcelona, Spain,

The Payne Lab at Georgia Tech has shown that titanium dioxide (TiO_2) nanoparticles (NPs) lead to oxidative stress in human cells in the absence of light. Changes in gene expression of the peroxiredoxin family of antioxidant enzymes were observed after 24 hours of TiO_2 NP incubation using reverse transcription polymerase chain reaction and western blotting. To understand the mechanism behind these results, we investigated the protein corona, the serum proteins that adsorb onto the NP surface. TiO_2 NPs oxidized the protein corona as measured with a dinitrophenylhydrazine assay. This protein oxidation was found to mediate the observed changes in peroxiredoxin gene expression. While these experiments related TiO_2 NP incubation to oxidative stress, cell viability decreased only after NP incubation *in the absence* of serum proteins, suggesting that the protein corona serves as a protective layer. Without serum proteins, lipid peroxidation increased, indicating the NPs can directly oxidize the lipid membrane. This was also observed after 24 hours of NP incubation when serum proteins were present, prompting further characterization of the corona. We probed the spatial distribution of corona proteins along the NP surface using super-resolution fluorescent microscopy. Image analysis suggests a non-uniform arrangement with incomplete surface coverage, exposing much of the NP to the lipid membrane. These results confirm that the protein corona facilitates the multiple oxidation outcomes experienced by cells.

77-POS Board 39

A Scalable, DNA-Based Multicomponent Patterning Method to Model Multivariable Neural Stem Cell-Niche Interactions from a Single-Cell Perspective

Olivia Scheideler, Chun Yang, David Schaffer, Lydia Sohn.
University of California, Berkeley, Berkeley, CA, USA.

Biological processes are regulated by complex signaling networks that are challenging to dissect due to the multitude of extrinsic signals that coordinate to guide cell behavior. Understanding these extensive regulatory networks requires not only identifying contributing signaling components – including ligands that are soluble, presented from the extracellular matrix, or neighboring cell surfaces – but also investigating potential synergies or hierarchies between multiple components. In order to enable studies of the later, we have developed a broadly applicable high-throughput, high-resolution DNA-based patterning method that we employ to recapitulate multivariable cell-ligand signaling scenarios. To complement bulk approaches that offer lower resolution, population-level estimates of cell response, our platform offers the unique dual capability to recapitulate cell-cell interactions with single-cell resolution and enable precise spatial control of biologically-relevant "solid-phase" matrix cues. Using photolithographic techniques, we generate multicomponent DNA patterns with spatial and hierarchical complexity across different length scales. We demonstrate that these DNA patterns can instruct the organization of heterogeneous cell populations as well as immobilize multiple ligands with controlled spatial presentations. To demonstrate our method's unique ability to address complex biological questions, we reconstructed *in vitro* multifaceted signaling scenarios present within the adult neural stem cell (NSC) niche. Specifically, we generated large-scale arrays of three-component DNA patterns, where one DNA strand encodes the capture of single NSCs and the other two dictates the spatial presentation of two immobilized niche ligands, fibroblast growth factor and an ephrin-B2-mimetic peptide, that are known to promote opposing cell fates. We demonstrate the ability to vary independently the concentration and spatial organization of these two ligands, study their combined effects on single NSC differentiation after long-term culture, and map out the hierarchy of these two signals within the niche.

79-POS Board 40

Supercritical Angle Microscopy: Surface-Sensitive Nanoscale Fluorescence (SAF) and Raman Microscopy (SAR) for Imaging of Surfaces and Cells

Stefan Seeger, Diana Serrano.Valentin Dubois.
University of Zurich, Zurich, Switzerland.

Surface sensitive detection technologies are of great interest to study the interaction of biomolecules with surfaces, in particular. Some time ago, we have introduced Supercritical Angle Fluorescence Microscopy and applied it to study the realtime observation of biomolecules with lipid bilayers, in particular proteins involved in neurodegenerative diseases /1-4/. The simultaneous observation of light collected above and below the critical angle allows even the localization of the emitter down to a few nanometer (<10nm)/5/. Meanwhile, this technique has been combined with other superresolution techniques /6/. Now, we extend this technology to the Raman spectroscopic regime /7/. Raman spectroscopy is a non-invasive technique able to provide chemical and compositional information about a variety of materials and molecular entities. We show for the first time that the collection of Raman scattered signals exceeding the critical angle of total internal reflection leads to surface-confined nanometre axial resolution. This high axial selectivity gives access to the intrinsic fingerprint of surface-related molecular specimens and can provide evidence about their distinguished structural and functional features. The richness of the spectroscopic information obtained through the supercritical angle Raman (SAR) collection path is demonstrated by simultaneously comparing its output with that of a classical confocal collection path. Furthermore, the proposed SAR technique is a versatile microscopy approach which can be used alone or in combination with amplified Raman modalities such as SERS.

/1/ Ruckstuhl T, Rankl M, Seeger S: Highly sensitive biosensing using a Supercritical Angle Fluorescence (SAF) instrument,
Biosensors &Bioelectronics 18 (9) 1193-1199 (2003)
/2/ Ruckstuhl T., Verdes D., Winterflood C., Seeger S.: Simultaneous near-field and far-field fluorescence microscopy of single molecules,
Optics Express 19, 6836-6844 (2011)
/3/ Reynolds N., Soragni A., Rabe M., Verdes D., Liverani E., Handschin S., Riek R., Seeger S.: Mechanism of membrane interaction and
disruption by α-Synuclein, Journal of the American Chemical Society 133, 19366-19375 (2011)
/4/ Rabe M., Soragni A., Reynolds N., Verdes D., Liverani E., Riek R., Seeger S.: On-surface aggregation of α-Synuclein at nanomolar
concentrations, results in two distinct growth mechanisms, ACS Chemical Neuroscience 4, 408-417 (2013)
/5/ Winterflood C., Ruckstuhl T., Verdes D., Seeger S.: Nanometer Axial Resolution by Three-Dimensional Supercritical Angle Fluorescence
Microscopy (3D-SAFM), Physical Review Letters 105, 108103 (2010)
/6/ Bourg N, Mayet C, Dupuis G, Barroca T, Bon P, Lécart S, Fort E, Lévêque-Fort S: Direct optical nanoscopy with axially localized detection,
Nature Photonics 9, 587–593 (2015)
/7/ Serrano D, Seeger S: Supercritical angle Raman microscopy: a surface-sensitive nanoscale technique without field enhancement, in press 2017

81-POS Board 41

Imaging Membrane Potential of the Endoplasmic Reticulum with a Genetically-Encoded Voltage Indicator

Masoud Sepehri rad[1], Lawrence B. Cohen[1,2].Bradley J. Baker[1],
[1]Korea Institute of Science and Technology, seoul, South Korea, [2]Yale University School of Medicine,, New Haven, CT, USA.

In eukaryotic cells, the endoplasmic reticulum (ER) is the largest continuous membrane-enclosed network which surrounds a single lumen. Using a newly designed genetically encoded voltage indicator (GEVI), we applied the patch clamp technique to HEK293 cells and found that there is an electrical interaction between plasma membrane and ER membrane. We have optically monitored the voltage changes in both of these membranes simultaneously. The optical signal of the GEVI in the plasma membrane is consistent from trial to trial. However, the ER signal decreases in size with repeated trials while the plasma membrane resistance remains constant. This dynamic behavior of the internal signal suggests that voltage may stress the ER causing it to remodel and change its resistance. Our findings further suggest that the ER may transfer electrical signals from the plasma membrane to the nuclear envelope.

83-POS Board 42

Traumatic Retraction of Neurons Regulated by Cell-Extracellular Matrix Adhesion

Xueying Shao, Kwan Kei Wong, Yuan Lin.
The University of Hong Kong, Hong Kong, Hong Kong.

Damage to neural cells and retraction of axons during traumatic brain injury (TBI) are believed to trigger disintegration of the neural network and eventually lead to severe symptoms such as permanent memory loss and emotional disturbances. Unfortunately, a method allowing us to quantitatively characterize the traumatic retraction of neural cells as well identify key players involved is still lacking. In this study, we used a sharp atomic force microscope (AFM) probe to transect axons and trigger their retraction while, at the same time, utilizing a total internal reflection fluorescence microscope (TIRFM) to monitor alterations in the axon cytoskeleton and cell-ECM adhesion. Interestingly, it was observed that cortical neuron cells cultured on a poly-L-lysine (PLL) coated coverslip for 3 days retracted much faster than those cultured for 7 days, presumably because that stronger cell-substrate adhesion was formed after 7 days of culturing. Furthermore, a well-developed axon may not fully shrink back to the main cell body after the first axotomy. Instead, the retracting motion can be arrested/stopped. However, axon retraction will be re-triggered if a second transection is conducted. Finally, we showed that, in general, the retraction process can be divided into three stages: i) an initial stage with a high retracting speed of ~0.01 μm/s; ii) an adhesion-dominated stage with a shrinking velocity that is order of magnitude lower than the initial stage; and iii) the steady-state stage where no change in the axon length can be detected.

85-POS Board 43

Probing Cell-Wall Synthetic Dynamic Using Bacterial Membrane Protein-Complex

Yi-Ren Sun, Chien-Jung Lo.
National Central University, Chung-Li, Taiwan.

Self-Replication is one of most amazing process of living organism. Bacteria have to elongate their cell body as the preparation for division. Bacterial cell-wall is made by peptidoglycan (PG). As cell elongate, elongasome, a group of membrane associate enzymes, carry out the PG insertion on the cell-wall which is guided by the cytoskeletal protein MreB filament. We aim to understand the cell-wall synthetic dynamic as cells reproduce by observing the interaction among the PG insertion, elongasome/divisome and MreB/Ftsz filaments.
Bacterial flagellar motor is a protein-complex anchored on the cell membrane to rotate the extracellular flagellum for swimming. We use bacterial flagellar motor as markers to study the PG insertion. That is, when the new PG strand been inserted, the original cell-wall with the motors embedded in it will be pushed away from its original location. Therefore, by observe the movement of the motors as cells reproduce, we can observe the spatiotemporal coordination of the PG insertion. In our experiment, we observe the dynamics of fluorescent hook of flagella during the duplication process. We will discuss the implication of our observation.

87-POS Board 44

Observing the Dynamics of Actin in Live Breast Cancer Cells

Fang Tian[1], Jing Li[1], Sidong Chen[2], Hykeun Park[1,2].
[2]the Hong Kong University of Science and Technology, Hong Kong, Hong Kong.[1]the Hong Kong University of Science and Technology, Hong Kong, Hong Kong,

Actin filaments play an important role in maintaining cell morphology and dynamic processes including movement. In particular, the dynamics of actin is a key component in movement of cancer cells during metastasis. However, the dynamics of actin in live cancer cells is not clearly understood yet. We imaged actin filaments using Lifeact-GFP (a novel marker for actin filaments) in live MCF7 cells (breast cancer cells). We observed the dynamics of actin filaments during the movement of MCF7 cells. We also measured different dynamics of actin filaments under different forces in cancer cell, which were generated by a magnetic tweezers. Our studies demonstrate the dynamics of actin filaments in live cancer cells and provide a possible mechanism for metastasis of breast cancer cells to different tissues.

89-POS Board 45

Detection and Characterization of Spontaneous Calcium Release Events in Cardiac Myocytes

Alex Vallmitjana[1], Carmen Tarifa[2], Raul Benitez[1].Leif Hove-Madsen[2].
[2]Institut Català de Ciències Cardiovasculars, Barcelona, Spain.[1]Universitat Politècnica de Catalunya, Barcelona, Spain,

In cardiac function, calcium handling plays a critical role since it is responsible of the excitation-contraction coupling at the cellular level. Indeed, the spontaneous release of intracellular calcium in cardiac cells is a well-established mechanism underlying cardiac arrhythmias among other heart pathologies. An accurate, robust detection of such spatio-temporal patterns is key to further understand the cell physiology mechanisms underlying cardiac function and disease.

Most cell level studies in the past have focused in linescan (X-T) images which do not capture spatial characteristics such as the release area, spatial dynamics or the localization with respect to nearby subcellular structures.

We have developed an automatic image processing system that allows detecting, localizing and characterizing calcium release events from a sequence of live cell fluorescence microscopy images. The system is able to process both linescan and framescan (X-Y-T) image sequences and detect different event release types such as sparks and calcium waves.

The system uses a multilevel wavelet analysis in order to identify events with a duration within a given temporal range and a modified watershed segmentation algorithm in to determine the space-time shape and centroid of the event. The event candidates are further filtered out using morphological features such as amplitude, full width at half maximum or characteristic decay time.

The method has been applied to an experimental database previously validated by a human expert including a total of 621 events from 8 human atrial cardiomyocytes. As a measure of overall performance, the approach achieves an average area under the ROC curve (AUC) of 0.8 with a standard error of 0.017.

91-POS Board 46

Highly Specific mRNA Transcript Quantification in Budding Yeast via Strand Displacement Induced Hybridization Chain Reaction

Gable Wadsworth, Harold Kim.
Georgia Institute of Technology, Atlanta, GA, USA.

Quantitative determination of mRNA copy number distribution is essential for understanding phenotypic variability of single cells. Long mRNA transcripts (> 500 nt) can be detected using single-molecule Fluorescence In Situ Hybridization (FISH), where the target mRNA is hybridized with a large number of fluorescently labeled DNA probes. One downside to this method is the inability to interrogate short targets and short polymorphisms in sequence. We demonstrate a novel mRNA detection protocol in budding yeast based on strand displacement induced hybridization chain reaction (HCR). In this method we target a short 10 nucleotide sequence with an HCR amplifier which is comprised of a pair of DNA hairpins with an exposed input toehold and a sequestered output toehold. When the target mRNA is present, polymerization of the HCR amplifier is induced. Sensitive discrimination of the input mRNA sequence is demonstrated by placing mismatches in the stem of the hairpin. To our knowledge, this is the first demonstration of using an HCR amplifier to detect mRNA in yeast. Our method represents an efficient means to obtain quantification of either short mRNA transcripts or short polymorphisms in mRNA sequence.

93-POS Board 47

Real-Time Probing the Binding of Tumor Necrosis Factor-alpha (TNF-α) to TNF Receptor in Single Living Cell Using Biofuntionalized Quantum Dots

Que Wan-Shan[1], Liou Bing-Chun[2], Lu Long-Sheng[2,3], Hsu Che-Yu[4], Yang Tzu-Sen[2].
[2]Taipei Medical University, Taipei, Taiwan, [1]Taipei Medical University, Taipei, Taiwan, [3]Taipei Medical University Hospital, Taipei, Taiwan, [4]National Taiwan University Hospital, Taipei, Taiwan.

Tumor necrosis factor (TNF-α) is an inflammatory cytokine produced mainly by macrophages. Binding of TNF-α to TNF receptor (TNFR) activates nuclear factor kappa B (NF-kB) transcription factors, leading to oscillations in nuclear NF-kB and target gene expression by negative feedback loops. However, it is difficult to probe real-time binding of TNF-α to TNFR because TNF-α needs its specific membrane-derived receptors to form a trimeric complex. To this end, we have developed a platform in which the temperature-controlled microfluidic flow cell is incorporated into single-molecule fluorescence microscopy systems. In addition, we utilize the biofunctionalized quntum dots conjugated with TNF-α to monitor the cellular distribution of TNF-α, which can provide a more direct access to probing the spatio-temporal distribution of TNF-α- TNFR complex and the corresponding transport pathway. Furthermore, we applied biotin anti-human TNF-α antibody to bind with TNF-α followed by chemical bonding for streptavidin- quantum dots or labeled biotin-TNF-α with streptavidin conjugated quantum dots to identify the space structure between TNF-α and TNFR. The observation demonstrated that antibody-functionalized quntum dots are more adequate for live monitoring the binding of TNF-α to TNFR in living NIH-3T3 cells, which implied that the choice of marker material may affect the success rate that TNF-α binding to its receptor in space. Furthermore, we also established an optimized method for labeling TNF-α, it can help us to explore its signal transduction pathway and the spatiotemporal interaction between binding of TNF-α to TNFR and the corresponding NF-kB dynamics.

95-POS Board 48

Spatial Organization of Transcription in E. coli via Superresolution Fluorescence Microscopy

Xiaoli Weng[1], Christopher H. Bohrer[1], Arvin C. Lagda[2], Jie Xiao[1].
[1]Johns Hopkins School of Medicine, Baltimore, MD, USA, [2]Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Transcription, the process of converting genetic information stored in DNA to RNA, lies at the heart of gene expression. Transcription has been studied extensively in-vitro to probe its mechanistic detail, however, these conditions differ from the complex environment inside a living cell. Spatial distributions of molecular components have recently been shown to be an important facet of gene regulation in prokaryotic systems. We investigated the spatial distributions of various molecular components of transcription in *E. coli* and their physical correlation with each other, to gain insight into the regulation of gene expression at the global, cellular level. Using superresolution fluorescence microscopy, we found that RNA Polymerase (RNAP) forms distinct clusters under fast growth that are largely retained in cells under different global transcription perturbations. RNAP clusters are the most homogenously distributed in cells without active transcription via rifampicin treatment. Additionally, we used multi-color superresolution imaging to correlate the spatial localization of RNAP clusters with DNA sites, nascent rRNA and elongation factor NusA to further elucidate the underlying make-up of RNAP clusters. Our results show that RNAP clusters are highly co-localized with NusA, thus are likely composed of elongation complexes. Interestingly, while RNAP clusters have a certain level of colocalization with nascent rRNA, and rrn DNA sites in fast growing cells, RNAP clusters are retained under conditions where rRNA synthesis is reduced. This points to the independence of formation of RNAP clusters from active rRNA synthesis. These results suggest a high level of heterogeneity both in the spatial organization and functional role of RNAP clusters in *E. coli*.

97-POS Board 49

Shape Recovery through Mechanical Strain-Sensing in *Escherichia Coli*

Felix Wong[1], Lars D. Renner[2,3], Gizem Özbaykal[4], Jayson Paulose[5], Douglas B. Weibel[3,6], Sven Van Teeffelen[4], Ariel Amir[1].
[1]Harvard University, Cambridge, MA, USA, [2]Leibniz Institute of Polymer Research and the Max Bergmann Center of Biomaterials, Dresden, Germany, [3]University of Wisconsin-Madison, Madison, WI, USA, [4]Institut Pasteur, Paris, France, [5]Leiden University, Leiden, Netherlands, [6]Department of Biomedical Engineering, Madison, WI, USA.

The shapes of most bacteria are imparted by the structures of their peptidoglycan cell walls, which are determined by many dynamic processes that can be described on various length-scales ranging from short-range glycan insertions to cellular-scale elasticity. Understanding the mechanisms that maintain stable, rod-like morphologies in certain bacteria has proved to be challenging due to an incomplete understanding of the feedback between growth and the elastic and geometric properties of the cell wall. Here we probe the effects of mechanical strain on cell shape by modeling the mechanical strains caused by bending and differential growth of the cell wall. We show that the spatial coupling of growth to regions of high mechanical strain can explain the plastic response of cells to bending and quantitatively predict the rate at which bent cells straighten. By growing filamentous *E. coli* cells in donut-shaped microchambers, we find that the cells recovered their straight, native rod-shaped morphologies when released from captivity at a rate consistent with the theoretical prediction. We then measure the localization of MreB, an actin homolog crucial to cell wall synthesis, inside confinement and during the straightening process and find that MreB localization only weakly depends on cell geometry but not on strain: MreB localization by itself cannot explain the plastic response to bending or the observed straightening rate. Our results implicate mechanical strain-sensing, implemented by components of the elongasome yet to be fully characterized, as an important component of robust shape regulation in *E. coli*.

99-POS Board 50

A Platform for Fast Drug-Resistance Identification of Cancer Cells

Zishen Yan, Yuan Lin, Tsz Hin Hui.
The University of Hong Kong, Hong Kong island, Hong Kong.

A major challenge in today's cancer treatment is that patients undergoing therapy can develop resistance to previously effective drugs. As such, finding new and faster ways to achieve drug resistance identification of cancer cells has always been an area of great interest. Interestingly, our recent study showed that the drug-resistance of tumor cells can be evaluated by their membrane resealing response. Specifically, micron-sized membrane pores, introduced by mechanical puncturing, in tumor cells can reseal ~2-3 times faster than those in the corresponding normal cells. Furthermore, the membrane resealing time in cancer cell lines exhibiting resistance to several leading chemotherapeutic drugs was also found to be substantially shorter than that in their drug-sensitive counterparts. Following these interesting findings, an electroporation-based device was designed and fabricated that allows us to introduce membrane pores on multiple cells simultaneously and then observe their resealing via fluorescent dyes. The efficiency and accuracy of our prototype system will be demonstrated on several lung and nasopharyngeal carcinoma cell lines.

101-POS Board 51

Stretching Single Native Chromain Fiber in Nanofluidic Channel for Detection of Epigenetics Marks

Jia-Wei Yeh[2,1], Kylan Szeto[2].
[1]Academia Sinica, Taipei, Taiwan, [2]Cornell University, Ithaca, NY, USA.

Stretching single chromosomal DNA fibers in nanofluidic devices has become a valuable tool for studying the genome and epigenetics. Although nanofluidic technology has been extensively used in single molecular DNA analysis applications, compared to bare DNA, much less work has been done to elongate and analyze native eukaryotic chromatin fibers. Here, we provide a method for stretching and imaging individual chromatin fibers. A micro- and nanofluidic device was used to electrophoretically stretch and image single native chromatin fibers attached to microspheres held at the entrance of a nanoslit. Chromatin fragments extracted from human cancer cells (HeLa cells) were stretched and held in nanoslits, and the histone modification H3k79me2 was optically detected by fluorescence microscopy.

103-POS Board 52

Measuring Single Suspension Cell Mechanical Properties and Cytoskeleton Contribution by AFM

Mingliu Zhu, Fen Liu, Dong Sun, Jian LU,
City University of Hong Kong, Kowloon, Hong Kong

Living cell is confronting to different forces from the outer environments. During the life processes, cell mechanics is involved and closely related to different functions. Studying the cell mechanical properties will help us well understand different physical phenomenon. Cytoskeleton underneath the cell membrane serves as the main supporting part to the whole cell. It contributes most to cell mechanical properties. Studying the cytoskeleton mechanical properties contribution is the key to understand cell mechanics.

This work concentrates on the acute myeloid leukemia (AML) suspension cell mechanical properties and contribution of cytoskeleton to the whole cell mechanical properties.
We established a measuring methodology to quantify the mechanical properties of the single suspension by Atomic Force Microscope (AFM). AFM experiments are conducting to extract cell and cytoskeleton elastic mechanical properties. The cell anchoring method, detailed testing method parameters like ramp sizes, tip velocities, trig thresholds, and the indenting times will all be determined in this methodology. According to the deformation depth, a proper fitting model will be used to extract cell's elastic properties.

This methodology can be applied in the following two parts: first, using AFM to quantify different types of AML cell lines mechanical properties is conducted. We can use mechanical properties to distinguish different species of AML cell lines. These results will be useful parameters when distinguishing different stages of cell lines.

Second by the aid of cytoskeleton distribution agents, we can quantify the contributions of cytoskeletons to the whole cell mechanical properties. The experiment results shows that as the increasing concentrations of cytoskeleton distribution agents in the culture medium, the measuring mechanical properties of certain AML cell line will decrease. These results will be useful parameters when conducting the cell modeling work.

POSTER SESSION II

Monday, June 19, 1:30 PM – 3:00 PM
Courtyard

Below are the formal presentations for Monday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Posters should be set up the morning of June 19 and removed by noon June 20.

Bae, Joseph	**2-POS**	**Board 1**
Bendix, Poul	**4-POS**	**Board 2**
Cao, Francisco	**6-POS**	**Board 3**
Chang, Jian	**8-POS**	**Board 4**
Chen, Geng-Yuan	**10-POS**	**Board 5**
Chen, Ya-Na	**12-POS**	**Board 6**
Ching-Fen, Jiang	**14-POS**	**Board 7**
Chou, Wen-hung	**16-POS**	**Board 8**
Del Favero, Giorgia	**18-POS**	**Board 9**
Denzi, Agnese	**20-POS**	**Board 10**
Feng, James	**22-POS**	**Board 11**
Galajda, Peter	**24-POS**	**Board 12**
Gynnå, Arvid	**26-POS**	**Board 13**
Ho, Han	**28-POS**	**Board 14**
Huang, Pei-Tzu	**30-POS**	**Board 15**
Huang, William Y. C.	**32-POS**	**Board 16**
Huber, Roland	**34-POS**	**Board 17**
Jones, Daniel	**36-POS**	**Board 18**
Kidoaki, Satoru	**38-POS**	**Board 19**
Kim, Kyung Sook	**40-POS**	**Board 20**
Konopka, Michael	**42-POS**	**Board 21**
Kurniawan, Nicholas	**44-POS**	**Board 22**
Lavaisse, Lucia	**46-POS**	**Board 23**
Lazarte, Ivan Alex	**48-POS**	**Board 24**
Li, Qingsen	**50-POS**	**Board 25**
Lin, Shan-Shan	**52-POS**	**Board 26**
Lin, Shin-Shiou	**54-POS**	**Board 27**
Liu, Allen	**56-POS**	**Board 28**
Liu, Zheng	**58-POS**	**Board 29**
Lu, Shao-Ju	**60-POS**	**Board 30**
Marklund, Emil	**62-POS**	**Board 31**
Meiners, Jens-christian	**64-POS**	**Board 32**
Nord, Ashley	**66-POS**	**Board 33**
Park, HyoungJun	**68-POS**	**Board 34**

Pei, Li	**70-POS**	**Board 35**
Rajagopal, Vijay	**72-POS**	**Board 36**
Rosendahl, Philipp	**74-POS**	**Board 37**
Sakuma, Morito	**76-POS**	**Board 38**
Schlierf, Michael	**78-POS**	**Board 39**
Seita, Akihisa	**80-POS**	**Board 40**
Shannon, Michael	**82-POS**	**Board 41**
Smith, Rosanna	**84-POS**	**Board 42**
Szczepanski, Krzysztof	**86-POS**	**Board 43**
Tsai, Wei-Chen	**88-POS**	**Board 44**
Von Ahnen, Inga	**90-POS**	**Board 45**
Wang, Chung Kang	**92-POS**	**Board 46**
Wei, Xi	**94-POS**	**Board 47**
Whyte, Graeme	**96-POS**	**Board 48**
Yamauchi, Shunpei	**98-POS**	**Board 49**
Yang, Tony	**100-POS**	**Board 50**
Yu, Che-Hang	**102-POS**	**Board 51**
Raja, Sufi	**104-POS**	**Board 52**

2-POS Board 1

Analyzing the Role of CXCR4 in Metastatic Prostate Cancer through Single-Cell Analysis

Joseph Bae, Anna Sandstrom Gerdtsson, Peter Kuhn.
University of Southern California, Los Angeles, CA, USA.

Metastasis to the bone marrow and other sites comprises the most lethal phase of prostate cancer progression. Circulating tumor cells (CTCs) are present in the blood at a low concentration, and play a role in the mechanism of metastasis. Homing and retention of cancer cells from the primary tumor to the bone marrow niche is thought to be mediated by the CXCR4 chemokine receptor and its ligand, CXCL12. The High-Definition Single-Cell Analysis (HD-SCA) assay utilizes fluorescent staining and high-throughput microscopy to identify and characterize CTCs among the patient's leukocyte population in blood and bone marrow samples based on expression of cytokeratin (epithelial cell marker), DAPI (nuclear stain) and CD45 (white blood cell marker). Additionally, this novel platform is useful in identifying various subpopulations of CTCs that are not characterized by other similar assays. The HD-SCA assay can also be expanded upon to accommodate analysis of additional biomarkers of interest by introducing new assays to the platform. CTCs that have been identified using the HD-SCA assay can then be individually isolated and further analyzed through genomic or proteomic profiling. In this study, we have used prostate cancer PC3 cells to develop a HD-SCA compatible fourth color assay for identifying the expression of CXCR4. The assay will be used to elucidate the role of CXCR4 in the homing of tumor cells to metastatic sites such as the bone marrow, and to characterize the relative levels of expression among tumor cells in primary, circulatory, and metastatic compartments.

4-POS Board 2

Rotation, Twisting and Pulling: The Rich Dynamics of Filopodia

Natascha Leijnse, Lene B. Oddershede, **Poul M. Bendix**.
University of Copenhagen, Copenhagen, Denmark.

Filopodia allow the cell to sense and interact with their surroundings. Rigidity sensing and application of piconewton traction forces are key functions that filopodia employ to sense and regulate cellular interactions. However, despite their ubiquitous presence in most cells, their different modes of movement remains poorly characterized. Recently, we found surprising evidence for a new pulling mechanism originating from twisting of the actin within the filopodium [1,2]. This mechanism can only be present in filopodia which contain a rotating actin bundle. By imaging the actin, we clearly observed rotation and retrograde flow of small buckles which formed in filopodia from HEK293 cells. The movement of these buckles was found to be coupled to the pulling force exerted by the filopodium in a manner which indicated that torsional twist was accumulated in the actin bundle. Our results provide the first clear evidence of rotation of the actin within filopodia a key property which might facilitate both interaction and exploration of the cell's 3D environment.

1. Leijnse N, Oddershede LB, Bendix PM. An updated look at actin within filopodia. A Review, Cytoskeleton 2015, 72, 71-79.
2. Leijnse N, Oddershede LB, & Bendix PM. Helical buckling of actin inside filopodia generates traction. PNAS 2015, 112(1), 136-141.

6-POS Board 3

Mechanics, Thermodynamics, and Kinetics of Ligand Binding to Biopolymers

Francisco Cao.
n/a, Madrid, Spain.

Ligands binding to polymers regulate polymer functions by changing their physical and chemical properties. This ligand regulation plays a key role in many biological processes. We propose here a model to explain the mechanical, thermodynamic, and kinetic properties of the process of binding of small ligands to long biopolymers. These properties can now be measured at the single molecule level using force spectroscopy techniques. Our model performs an effective decomposition of the ligand-polymer system on its covered and uncovered regions, showing that the elastic properties of the ligand-polymer depend explicitly on the ligand coverage of the polymer (i.e., the fraction of the polymer covered by the ligand). The equilibrium coverage that minimizes the free energy of the ligand-polymer system is computed as a function of the applied force. We show how ligands tune the mechanical properties of a polymer, in particular its length and stiffness, in a force dependent manner. In addition, it is shown how ligand binding can be regulated applying mechanical tension on the polymer. Our model will be useful to understand ligand-binding regulation of biological processes, such as the metabolism of nucleic acid. In particular, this model allows estimating the coverage fraction and the ligand mode characteristics from the force extension curves of a ligand-polymer system. We illustrate the power of the method based in this model with the analysis of experimental results of Human mitochondria SSB (HmtSSB) binding to single stranded DNA (ssDNA), which has allowed to characterize the binding modes and coverage of HmtSSB-ssDNA complexes in several configurations, including ssDNA generated during DNA replication.

8-POS Board 4

Formation of Cytoophidia Studied by Superresolution Microscopy

Jian Chang[1], Ömür Y. Tastan[2], Ji-Long Liu[2,3], Yan-Wen Tan[1].
[1]Fudan University, Shanghai, China, [2]University of Oxford, Oxford, United Kingdom, [3]ShanghaiTech University, Shanghai, China.

CTP synthase (CtpS) is an essential metabolic enzyme. It catalyses CTP production from UTP, ATP, glutamine and acts as an attractive target for various cancer and parasitic diseases in drug development. Recently, CtpS has been observed to polymerize into "Cytoophidia" in different organisms. Cytoophidia is an interesting evolutionarily-conserved filamentous structure and plays an essential role in regulation of enzymatic activity. However, current knowledge of cytoophidia are coming from conventional methods and a lot are still unknown, especially the mechanism of cytoophidia polymerization. We use fluorescence superresolution microscopy to observe the filaments. Our results indicate that CtpS from Drosophila can assemble into cytoophidia in vitro without participation of other proteins and these filaments exhibit interesting twisting pattern. Whether cytoophidia can co-ploymerize with other components from Drosophila is also studied.

10-POS Board 5

Contribution of Kinesin-5 Mediated Microtubule Stability to the Regulation of Mitotic Spindle Size

Geng-Yuan Chen, William O. Hancock.
Pennsylvania State University, University Park, PA, USA.

In addition to their capacity to slide apart antiparallel microtubules during spindle formation, the mitotic kinesin-5 motor Eg5 has been shown to pause at microtubule plus-ends and enhance microtubule polymerization (Chen and Hancock, Nature Comm. 2015). This Eg5-induced microtubule stability and mitotic spindle integrity were eliminated by conventional loop-5 inhibitors, but enhanced by the rigor inhibitor BRD9876 (Chen et al., ACS CB, 2017). The goal of the present work is to understand the Eg5 microtubule polymerase mechanism by studying how the motor alters the lateral and longitudinal tubulin-tubulin interactions that stabilize the microtubule lattice. Transient kinetics and single-molecule tracking experiments demonstrate that dimeric Eg5 motors reside predominantly in a two-head-bound strong-binding state while stepping along the microtubule (Chen et al., JBC 2016). When the motor pauses at a growing microtubule plus-end, the motor is hypothesized to act as a "staple" to stabilize the longitudinal bonds of incoming tubulin dimers. The on-rate for Eg5 binding to free tubulin is slow, suggesting that end-bound Eg5 motors do not bind free tubulin in solution; rather they stabilize incoming tubulin dimers that have bound to the plus-end.
Because tubulin in the microtubule lattice resides in a "straight" conformation, while free tubulin resides in a "kinked" conformation, an attractive model is that Eg5 stabilizes the straight conformation of tubulin. Consistent with this, monomeric Eg5 bound to the microtubule lattice stabilizes microtubules against depolymerization. Furthermore, the affinity of Eg5 for free tubulin is reduced in the presence of "wedge inhibitor" drugs that stabilize the kinked conformation of tubulin. Thus, we propose a microtubule polymerase mechanism in which Eg5 motor domains straighten tubulin to stabilize lateral tubulin-tubulin interfaces in the lattice, while the tethered head binds the incoming tubulin to stabilize longitudinal tubulin-tubulin bonds.

12-POS Board 6

Structural Stability of Rhodopsin Analyzed by Single-Molecule Force Microscopy

Ya-Na Chen[1], Maria Kamenetska[1], Jacob Black[1], Kelly J. Culhane[2], Ziad Ganim[1], Elsa C. Y. Yan[1].
[1]Yale University, New Haven, CT, USA, [2]Yale University, New Haven, CT, USA.

Rhodopsin is a representative member of family A G-protein coupled receptors (GPCRs). Its structural components include seven transmembrane helices and a chromophore molecule, 11-cis retinal. As a biological dim-light detector, rhodopsin possesses exceptional photon-detecting characteristics: high quantum yield and low dark noise. To elucidate how the structure of rhodopsin give rise to its astounding biophysical properties, single-molecule force microscopy (SMFM) experiments were carried out. Among different SMFM techniques, optical tweezers (OT) is an ideal method due to its high force resolution and the versatility it offers in force extension geometries. Being a membrane protein, to study rhodopsin *in vitro*, sample preparation procedures such as protein engineering, expression and purification, have been carefully optimized. A rhodopsin mutant has been designed to facilitate DNA linkage via cysteine-maleimide covalent bonds at the N- and the C- termini. Rhodopsin-DNA handle complexes have been successfully conjugated and characterized. Silica microspheres have been bio-functionalized for the attachment of DNA handles through biotin / streptavidin or digoxigenin / anti-digoxigenin interactions. Through optically trapping silica microspheres, force can thus be applied to the entire assembled construct. Force-extension data presented display evidence for reversible unfolding of rhodopsin. Analysis of more single-molecule force-extension traces will provide a high molecular resolution model uncovering structure-function relations of rhodopsin. Future studies involve altering protein environmental components to quantify effects on rhodopsin structural stability. This study will not only impact and expand current understanding of GPCRs but will also set the foundation for applying optical tweezers-based SMFM experiments on a wide variety of transmembrane receptor, channel, and gate proteins.

14-POS Board 7

Inspect the Dynamic Variations in Cytoskeleton Arrangement during Cell Aggregating in Time Lapse Microscopy

Jiang Ching-Fen.
I-Shou University, Kaohsiung, Taiwan.

The cytoskeleton is an intricate network of protein filaments that support the cell architecture and dominate cell motility by performing contractility. The structure of cytoskeleton is continuously reorganized as a cell changes shape, divided, and responds to its environment. Therefore, investigation into its dynamic structural variation gives a clue to understand the biological functional change of the cell, such as differentiation, growth, metastasis, and apoptosis. However, current fluorescence microscopic techniques limit to observation on static structure of the cytoskeleton. This study developed a series of image processing techniques to extract the cytoskeleton in the cell images obtained from time-lapse microscopy, and allowed the investigation into the dynamic structure of cytoskeleton during cell migration. Two types of cells, mesenchymal stem cells (MSCs) and lung cancer cells (LCCs), cultured in chitosan-HA membranes were investigated in this study. Microscopic videos were provided by the Biomaterials Laboratory in the Institute of Polymer Science and Engineering at National Taiwan University. The videos were recorded by a ASTEC® CCM-1.4XZY/CO2 system with a CCD camera mounted on a time-lapse microscope with a magnification ratio of 100:1. The acquisition rate was fixed at one acquisition every 15 min. We first verified the consistency between textural patterns extracted by our method and the cytoskeleton distribution viewed by fluorescence microscopy. The dynamic variations in cytoskeleton patterns of the MSCs and LCCs during their aggregation were then compared. The results show that the aggregated LCCs without merging contained concentric textural pattern, while the aggregated MSCs with merging revealed directional texture pattern which corresponded to their moving directions. Hence, the developed cell image processing techniques may provide a direct and convenient way to inspect the dynamic arrangement of cytoskeleton during cell movement without immuno-fluorescence staining

16-POS Board 8

Robust Three-Dimensional Traction Force Recovery of Micro-Patterned Epithelial Cell Colonies

Wen-hung Chou[1,2], Hsuan Yang[2], Giovanni J. Paylaga[3,2], Jia-yang Juang[4], Keng-hui Lin[2].
[1]National Taiwan University, Taipei, Taiwan, [2]Academia Sinica, Taipei, Taiwan, [3]National Central University, Taoyuan, Taiwan, [4]National Taiwan University, Taipei, Taiwan.

Epithelial cells move collectively and exert force to their surrounding and to each other. In previous studies on collective cell migration, most traction force measurements are done on very large epithelial cell sheets. In this work, we study confined cells in small patterned regions and performed three-dimensional traction force microscopy (TFM). TFM is carried out by imaging the positions of fluorescent nanoparticles with and without cells adhering to the substrate, and finite element method (FEM) is used to recover stress from displacement field. To validate our FEM calculation, we performed numerical simulation to estimate the accuracy of our traction recovery results based on experimental parameters such as nanoparticle density and tracking accuracy. Criteria for robust force recovery is then determined in terms of the parameters. Experimentally, we observe that traction forces of Madin-Darby canine kidney (MDCK) cell colonies are concentrated at the periphery, but occasional non-vanishing forces at cell-cell junctions exist. Time-lapse observations can shed light on the transient dynamics of cell-cell interaction. Finally, the magnitude sum of normal traction force under a cell colony is independent of cell density but dependent on colony area, which agrees with previous shear force findings.

18-POS Board 9

The Intestinal Challenge: Combinatory Effect of Shear Stress and Alternaria Alternata Mycotoxin Altertoxin II (ATXII)

Giorgia Del Favero, Doris Marko.
University of Vienna, Faculty of Chemistry, Vienna, Austria.

Intestinal cells are continuously subject to biomechanical stimulation, both as a result of the movement of the fluids into the lumen, and due to the peristaltic contraction of the organ. In this respect, the performance of cytotoxicity studies in a mechanically stimulated environment is of crucial importance. Mycotoxins are prevalent food contaminants, in fact, being produced as secondary metabolites of molds, they can easily contaminate food commodities. Among the emerging challenges of food toxicology there is ATXII, a genotoxic compound produced by fungi of Alternaria spp. In human colon carcinoma cells (HT-29), ATXII is severely cytotoxic and triggers the activation of the Nrf2/ARE pathway [1]. Being Nrf2 translocation particularly sensitive to shear stress [2-4], the cross-talk between ATXII and the Nrf2 pathway was investigated in two different models of intestinal cells (HT-29 and HCEC-1CT human colonic epithelial cells) in static conditions and in presence of shear stress. The effect on cellular morphology and on Nrf2 localization was monitored by confocal microscopy. ATXII proved to have a direct effect on Nrf2 translocation, as well as an impact on the actin cytoskeleton, which appeared to be modulated by shear stress. Moreover, differential response was observed between tumor and non-transformed cells. Taken together, these findings suggest that the effect of ATXII can interplay with shear stress and open new perspectives into the understanding of the mechanisms of action of this emerging mycotoxin.

[1]. Jarolim, K., et al., Arch Toxicol, 2017. 91(1): p. 203-216. [2]. Chen, X.L., et al., J Biol Chem, 2003. 278(2): p. 703-11.
[3]. Healy, Z.R., et al., Proc Natl Acad Sci U S A, 2005. 102(39): p. 14010-5. [4]. Hosoya, T., et al., J Biol Chem, 2005. 280(29): p. 27244-50.

20-POS Board 10

Study of Microthermal Effects Due to Nanosecond Pulsed Electric Field on a Single Realistically Shaped Neuron

Agnese Denzi[1,2], Francesca Apollonio[2], Riccardo Di Stefano[2], Marco Leonetti[1], Giancarlo Ruocco[1], Micaela Liberti[2].
[1]Istituto Italiano di Tecnologia, Rome, Italy, [2]University of Rome "La Sapienza, Rome, Italy.

In the last decades, the use of short (order of nanosecond) and intense (order of MV/m) electric pulses, has become a promising tool in different applications and in particular in cancer treatment [1]. Though a macroscopic thermal effect can be experimentally excluded, if the effect of the local electric field, in particular on irregular cell [2], can produce a microthermal effect is still under discussion.

Aim of this work is to numerically study the local temperature increment during a 10 nanosecond pulse with amplitude able to determine the poration of the cell membrane. In order to consider an irregularly shaped cell, a realistic model of single neuron has been considered.
The parameters for the thermal model have been taken from [3] and the electrical ones from [4], considering two different external medium with high (DMEM) and low (Sucrose) conductivity. The microdosimetry model has been extracted starting from a fluorescence image using a clusterization method. The border of the neuron has been imported in a multiphysics software (COMSOL Multiphysics®) in which we have been able to couple the electric and the thermal problem.

The results are reported in terms of external field necessary for poration ($E_{poration}$) and the increment of temperature (ΔT):

DMEM $E_{poration} \approx 0.91$ MV/m and $\Delta T \approx 0.03$ K
Sucrose $E_{poration} \approx 9.3$ MV/m and $\Delta T \approx 0.04$ K

The electric field, as expected, is higher in a non conductive medium but the ΔT is very low in both the conditions.

[1] M. Breton et al, Bioelectromagnetics, 2012.
[2] A. Denzi et al., Journal of membrane biology, 2016.
[3] R.P. Croce et al., Plasma Science, IEEE Transactions on, 2010.
[4] A. Denzi et al, Journal of membrane biology, 2013.

22-POS Board 11

A Rho Gtpase Based Model Explains the Spontaneous Directional Migration of Neural Crest Cells

Brian Merchant, Leah Keshet, **James Feng**.
University of British Columbia, Vancouver, BC, Canada.

During early vertebrate embryogenesis, neural crest cells (NCCs) migrate in clusters from the neural tube to various target locations over long distances. Their collective migration is tightly regulated by environmental signals and intercellular interactions. Strikingly, NCC clusters are capable of spontaneously developing a persistent migration down migratory corridors in the absence of chemoattractants. To understand this phenomenon, we built a vertex-dynamics model that predicts the key modes of cell interactions—contact inhibition of locomotion (CIL) and co-attraction (COA)—through the modulation of Rho GTPase biochemistry. Using such a signaling-based model, as opposed to implementing hard-coded simulation rules, we find that CIL and COA conspire to suppress Rac1 activity, leading to the persistence of polarization (POP) of clustering cells and thus the spontaneous directional migration in the absence of chemical gradients.

24-POS Board 12

Trapping Single Bacterial Cells and Their Progeny to Study the Emergence of Phenotypic Heterogeneity

Ágnes Ábrahám, Krisztina Nagy, Lóránd Kelemen, **Peter Galajda**.
Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary.

Despite their genetic identity cells in a clonal population exhibit a variation in their phenotypic characteristics. This heterogeneity contributes to the fitness of the population especially in a changing environment. Such variability have been observed for various different cell types, from bacteria to mammalian cells. However, the origin and the processes behind the emergence of phenotypic heterogeneity remain largely unknown.

We have developed a microfluidic device consisting of an array of single cell traps to study the emergence of phenotypic heterogeneity in bacteria. Following division of a trapped cell one daughter cell remains in the trap while the other drops out and falls into the next empty trap. After several divisions all the traps are filled with the progeny of a single cell. All trapped cells may be imaged and characterized using high resolution microscopy, Phenotypic characteristics such as cell size, cell shape, division rate or gene expression levels may be precisely measured in an experimental timeframe reaching 100 generations. By analyzing this data along with the information on the relatedness of each cell we are exploring the basic principles and processes behind the emergence of phenotypic heterogeneity in a population originating from a single cell.

26-POS Board 13

Tracking Orthogonal Fluorescent Ribosomes inside Living Cells

Arvid H. Gynnå, Ivan L. Volkov, Johan Elf.Magnus Johansson.
Uppsala University, Uppsala, Sweden.

The kinetic parameters of protein synthesis have been successfully studied by in vitro methods for decades. There are however many differences between the cellular environment and reconstituted systems that might affect these parameters. Among those are molecular crowding, confinement, uncertain intracellular ionic strength and pH, and possibly even the presence of yet unknown factors. Current methods for in vivo investigation of protein synthesis are limited. They are normally indirect, and/or give average numbers for several different molecular species. A method to directly measure the translation rate of individual ribosomes performing well-defined functions inside living cells is hence needed. To define the function of the ribosomes under observation inside a cell, a subset of the ribosomes should preferentially be orthogonal so they will only translate the mRNA of interest. In an organism with several rRNA loci, this can be achieved by modifying the anti-Shine-Dalgarno sequence of one of them and expressing a correspondingly orthogonal mRNA. For use in single-particle tracking experiments, the orthogonal ribosome subset must also be fluorescently labeled. We have developed such a system for *E. coli*, where the orthogonal small ribosomal subunits are labeled with an organic dye and thus can be monitored in singulo while operating inside the cell interacting with all natural components. This opens up the possibility to measure in vivo translation rates on defined mRNAs, as well as mapping the physical locations of the synthesis of different proteins.

28-POS Board 14

Single-Molecule Imaging in Live Cells Reveals Kinetics of Transcription-Coupled Repair

Han N. Ho, Harshad Ghodke, Antoine M. van Oijen.
University of Wollongong, Wollongong, NSW, Australia.

Transcription-coupled repair (TCR) is a highly conserved process responsible for the targeted removal of lesions that stall RNA polymerases on the transcribed strand. In *Escherichia coli*, TCR is mediated by the repair factor Mfd. On binding to stalled RNA polymerases, Mfd displaces the polymerase and subsequently recruits the nucleotide excision repair machinery. The molecular mechanisms of Mfd recruitment to transcription complexes remain to be elucidated in live cells. Using single-molecule imaging, we imaged Mfd in live *E. coli* cells and found that a significant fraction of Mfd associates with transcription elongation complexes even in the absence of genotoxic stress. Further, we identify two populations of bound Mfd: a long-lived fraction representing Mfd in TCR and a sub-second fraction, presumably representing Mfd in the search mode. Using inhibitors of RNAP that promote stalling, we found that Mfd displays a new kinetic mode, reflecting the kinetics of drug-stalled RNA polymerases. These results provide insights into the mechanism of transcription-coupled repair in *E. coli*.

30-POS Board 15

Mechanism of Microtubule Growth Promotion by the Kinesin Kip2

Pei-Tzu Huang, Anneke Hibbel, Jonathan Howard.
Yale University, New Haven, CT, USA.

Microtubules are dynamic structures formed by α- and β-tubulin heterodimers. They play essential roles in the cell cycle, cell motility, and cellular transport, and are regulated by a diverse array of microtubule associated proteins (MAPs). One of these important MAPs is the budding yeast kinesin Kip2. Kip2 can translocate processively along the microtubules to the plus-end, and is involved in mitotic spindle positioning and stabilization of microtubules during cell cycle telophase. Previously, it was shown that Kip2 regulates microtubules indirectly by transporting microtubule positive regulators Bik1 and dynein to the microtubule plus end; recently, it was shown that Kip2 regulates microtubules directly by acting as a polymerase. However, the molecular mechanism by which Kip2 polymerizes microtubules is unclear. One of the hypotheses is that Kip2 functions as a processive polymerase that adds multiple tubulin heterodimers to the microtubule plus end. In this case, it is expected that there are multiple tubulin binding sites on each Kip2 molecule. Therefore, I aim to map the putative binding interfaces of Kip2 with tubulin heterodimers by using gel filtration binding assays, and perform microtubule dynamic assays of full-length and truncated Kip2 to identify the tubulin binding sites involved in the polymerization mechanism.

32-POS Board 16

Activation Time Distribution of Membrane Recruitment Process Revealed by First-Passage Analysis of Full-Length SOS

William Y. C. Huang[1], Steven Alvarez[1], Young Kwang Lee[1], Yasushi Kondo[2], Jean K. Chung[1], Hiu Yue Monatrice Lam[1], John Kuriyan[1,2], Jay T. Groves[1].
[1]University of California, Berkeley, Berkeley, CA, USA, [2]University of California, Berkeley, Berkeley, CA, USA.

Many cytosolic signaling proteins are autoinhibited in the cytosol and only activate upon membrane recruitment. Release of autoinhibition generally involves structural rearrangements within the protein and therefore leads to a time interval between initial recruitment and activation. The physical mechanism of activation establishes both the mean time to activation as well as distribution of activation times. The form of the activation time distribution, not its mean, plays a critical role in enabling regulatory processes such as kinetic proof reading. Here, we develop a single-molecule assay to temporally map the activation process of the Ras guanine nucleotide exchange factor SOS on membrane surfaces. Simultaneous imaging of individual SOS molecules and localized Ras activation on supported membrane microarrays reveals both the activation time and rejection time distributions resulting from Grb2-mediated membrane recruitment of SOS. The gamma-like shape of the activation time distribution indicates the existence of rate-limiting kinetic intermediates in the release of autoinhibition, and establishes a basis for kinetic proofreading in the activation of Ras. Using an analytical kinetic model, we demonstrate how competition between the SOS activation pathway and dissociation from the membrane together shape the Ras activation response to receptor-mediated SOS recruitment. Finally, we proposed a framework to reconstruct signaling timescale of proximal signaling from single-molecule activation timing.

34-POS Board 17

Integrative Models of Complete Viruses – Combining Structure, Genomics and Modeling

Roland G. Huber[1], Jan K. Marzinek[1,2], Daniel Holdbrook[1], Ana de Suoto Martins[3], Ivo Martins[3], Yue Wan[4], Peter J. Bond[1,2].
[1]A*STAR Bioinformatics Institute (BII), Singapore, Singapore, [2]NUS Department of Biological Sciences (DBS), Singapore, Singapore, [4]A*STAR Genome Institute Singapore (GIS), Singapore, Singapore.[3]Instituto de Medicina Molecular, Lisboa, Portugal,

Dengue and Zika viruses belong to the Flavivirus family of mosquito-borne infections prevalent in tropical and subtropical regions. They are enveloped, single-stranded positive-sense RNA viruses with a genome of approximately 11kb and form virions of ~45 nm in diameter. In recent years, near-atomistic cryo-EM structures have become available. While these structures reveal the precise organization of the protein envelope, they are not able to resolve either the lipid membrane or the viral core in detail.

To probe deeper into the viral particle, we created detailed models of the viral envelope including the lipid vesicle circumscribed by the envelope proteins. These models reveal a tensioned membrane structure that follows the icosahedral pattern given by the symmetry of the surrounding proteins. We show how anionic lipids within the lipid vesicle anchor the membrane to the protein coat. We propose that this tension within the deformed lipid vesicle may store energy that is used to overcome the activation barrier for fusion with the late endosomal membrane and subsequent release of the viral genome.

Within the envelope, the viral genome interacts with a highly charged capsid protein. Cryo-EM structures do not show a simple geometric capsid as commonly found in non-enveloped viruses. In order to determine interaction sites of this capsid protein with the viral genome we performed a nuclease digestion assay: protein-bound segments are better protected from digestion and can be sequenced. Subsequent alignment against the full genome reveals the location of these protected fragments.

Integrating structural, biochemical, genomic, and computational methods thus allowed us to create a near-atomistic, dynamic model of the mature virion. We are currently modeling the fusion of these particles with endosomal membrane models, which may allow us to identify novel targets for therapeutics against these and other Flaviviruses.

36-POS Board 18

Kinetics of dCas9 Target Search in *Escherichia Coli*

Daniel Jones, Cecilia Unoson, Prune Leroy, Vladimir Curic, Johan Elf.
Uppsala University, Uppsala, Sweden.

How fast can a cell locate a specific chromosomal DNA sequence specified by a single stranded oligonucleotide? To address this question we study the CRISPR-associated protein Cas9 which can be programmed by guide RNAs to bind essentially any DNA sequence. This targeting flexibility requires Cas9 to unwind the DNA double helix to test for correct base pairing to the guide RNA. Here we study the search mechanisms of the catalytically inactive dCas9 in living *Escherichia coli* cells using complementary single molecule fluorescence microscopy and bulk restriction protection assays. We find that it takes a single dCas9 ~10 h to find and bind a specific target, in stark contrast to transcription factors such as LacI, which takes 5 minutes to locate its target. Thus, the price dCas9 pays for flexibility in targeting is time. By introducing transcription factor "roadblocks" adjoining dCas9's binding site, we demonstrate that dCas9 undergoes short-range (5-10 bp) sliding along DNA while searching for potential binding sites. The physical limitations (*i.e.*, slow search) for Cas9 are likely applicable to other systems that are programmed by single stranded oligonucleotides to locate sequences in dsDNA, such as the homologous repair machinery.

38-POS Board 19

Negative Mechanotaxis of iPS Cells Observed on Microelastically-Patterned Hydrogels

Satoru Kidoaki, Kenta Mizumoto.
Kyushu University, Fukuoka, Japan.

Feeder-free dissociating culture of iPS cells has been required for stable and easier supply of them for further clinical applications. One of the problems with the dissociating culture is that iPS cells undergo apoptosis not only due to the loss of cell-cell adhesion but also depending on the quality and strength of cell-substrate adhesion. To clarify the optimized physicochemical conditions for the dissociating culture of iPS cells, we have scrutinized the effect of laminin-density and stiffness of the hydrogel gel surfaces on proliferation activity of iPS cells. Laminin 511-E8 fragments were chemically-fixed onto photocrosslinked-gelatin gels through water-soluble carbodiimide-NHS condensation reaction. Laminin-density and elasticity were changed by regulating feed concentration of laminin in the reaction and photoirradiation conditions, respectively. As for the effect of laminin density, proliferation of iPS cells was attained over the feed concentration of laminin of 0.3 mg/ml. Concerning the effect of stiffness of gel surface, significant difference in proliferation activity was not observed for the elasticity conditions between several kPa and 100 kPa. On the other hand, packing density of iPS cells in the proliferating colonies became significantly higher on the gels with lower elasticity. To check actual responses of iPS cells for elasticity of culture hydrogels, time-lapse observation of iPS cells was performed on the microelastically-patterned gel with 400 μm-wide square hard domains (270 kPa) in softer surroundings (10 kPa). Interestingly, iPS cells preferred to and transferred into soft region to form cluster to survive and proliferate on the hydrogel surfaces. i.e.,iPS cells exhibit biased movement toward softer region. These observations suggest that in the case of iPS cells, negative mechanotaxis toward softer region occurs to inhibit apoptosis induced by the lack of cell-cell adhesions.

40-POS Board 20

Irritating Effects of Sodium Lauryl Sulfate on Human Primary Keratinocytes at Sub-Toxic Level Exposure

Kyung Sook Kim[1,1,1], Hyeongwon Choi[3,3,3], Min Kyung Shin[2,2,2].
[1]Kyung Hee University, Seoul, Seoul, South Korea, [2]Kyung Hee Unoversity, Seoul, South Korea, [3]Kyung Hee University, Seoul, South Korea.

Chemical agents that can potentially cause skin irritation are tested in vivo in an animal and in vitro by cell viability or cytokine expression. These methods do not always provide translatable results and are not sensitive enough to sub-toxicity detection. In this work, we introduce the mechanical properties of keratinocytes as novel endpoints for safety assessment of chemical agents in sub-toxicity level. Human primary keratinocytes were treated with various concentrations of sodium lauryl sulfate (SLS). The response of keratinocytes to SLS was investigated by both biological and biomechanical methods. Cell proliferation, membrane integrity, inflammatory response, and cell morphology were observed using biological methods. Changes in stiffness and surface roughness as biomechanical parameters were investigated by atomic force microscopy. Morphophysiological changes were clearly seen at a relatively high dose of SLS ($\geq$ 25 μM), while the mechanical properties of keratinocytes responded linearly to SLS at lower doses ($\leq$ 10 μM).

42-POS Board 21

Single-Cell Respiration Rate Measurements on Bacteria

Krishna Ojha, John Ertle, **Michael C. Konopka**.
The University of Akron, Akron, OH, USA.

Respiration can be an indicator of physiological state and therefore is an excellent target for analysis of cell-to-cell variation within a sample. Single-cell respiration rate measurements are made by first sealing cells in 2-500 pL wells in a glass chip designed to isolate single cells and then monitoring the oxygen consumption over time. A phosphorescent Pt-porphyrin dye acts as the oxygen sensor since the lifetime of the dye's excited state depends on the oxygen concentration in the sample. Microspheres embedded with the Pt-porphyrin are loaded into the wells and all measurements are made on a fluorescence microscope. Samples can also be reoxygenated to replicate measurements or perform measurements on the same cells with altered conditions.

The respiration rates of individual bacterial cells from the same isogenic culture can have as much as a four-fold difference. Separate measurements also show large variations in membrane diffusion coefficients between different cells. We are currently investigating the role membrane fluidity may play in the variation in respiration rates found between individual cells. In particular, determining if it is the direct mobility of a component of the electron transport chain (ETC), such as the electron carrier ubiquinone, that is limiting the respiration rates. Alternatively, heterogeneity in respiration rates could be caused by differences in the segregation of oxidative phosphorylation complexes caused by variations in membrane composition.

44-POS Board 22

Local 3D Single-Cell–Matrix Interactions Underlie the Spatiotemporal Dynamics of Cell Populations

Nicholas Kurniawan.
Eindhoven University of Technology, Eindhoven, Netherlands.

It is increasingly accepted that the physical and mechanical properties of the extracellular environment play a guiding role in cell behavior, for instance during development, wound healing, and cancer metastasis. Cells sense physical and mechanical cues from the extracellular matrix (ECM) and transduce these cues into intracellular signals that can, in turn, affect cell response. However, surprisingly little is known about what the cells are actually sensing, especially in the context of the typically complex, nonlinear, and fibrillar ECM. We hypothesized that cells sense the local, rather than global, ECM properties, and base their decision-making on such local mechanosensing.

To test this hypothesis, we developed a spatially-resolved microrheological technique that provides quantitative information about the local viscoelasticity around single cells at sub-cellular resolution. We seeded cells in 3D collagen gels and found that cells created pockets of stiffened microenvironment, suggesting that cells ‘prime’ their surrounding ECM to support contractility. To determine whether cells rely on local mechanosensing for decision-making, we systematically modulated the ultrastructure of fibrin fibers, allowing us to independently vary the stiffness at network and fiber levels. We show that cell spreading and differentiation are determined by the stiffness of individual fibers, rather than by the bulk properties of the matrix. These two findings strongly suggest that the cell and the matrix can locally influence and adapt to each other in a reciprocal manner. We further speculated that such local and dynamic cell–matrix interactions may manifest in a heterogeneity of cell phenotype within a population. Indeed, we found a spatiotemporally-heterogeneous pattern of cell migration in 3D collagen gels that is mediated by cell-induced local ECM remodeling. These results reveal the role of cell–matrix interactions in single cell behavior and population dynamics.

46-POS Board 23

Surface Properties of Saccharomyces Cerevisiae during Fermentative and Respiratory Cycles

Lucia Lavaisse[1,2], Axel Hollmann[1,3], Monica Nazareno[2], Anibal Disalvo[1].
[1]Laboratory of Biointerphases and Biomimetic Systems, CITSE UNSE CONICET, Santiago del Estero, Santiago del Estero, Argentina, [2]Laboratory of Antioxidants and Oxidative Process CITSE UNSE CONICET, Santiago del Estero, Santiago del Estero, Argentina, [3]National University of Quilmes, Bernal, Buenos Aires, Argentina.

The aim of this work is evaluate the zeta potential (ZP) evolution during the growth of *Saccharomyces cerevisiae* under different stresses to understand the physiological states in connection to changes in cell surface properties.
Yeast growth was evaluated following the electrophoretic mobility of cells in an electric field, together with CFU, OD_{600nm}, pH and size variations.
S.cerevisiae spp cultures show different ZP mean values along the growth phases. A single cell method permits to identify different subpopulations with defined ZP values coexisting at each growth stage, indicating that cells in different states of growth are present at each of them. Accordingly, cells can be classified into subpopulations considering their surface charge that anticipates growth phase changes.
In the fermentative cycle, the release of acid to media produces a decrease in the negative surfaces charges. In contrast, in the respiratory cycle, the zeta potential decreases abruptly at constant pH as a consequence of changes in the chemical composition of the cell wall or a cell volume change. ZP measurements were applied on cells exposed at different stresses and showed that cells in exponential phase were more sensitive to stresses. These data can be correlated with size information obtained by DLS.
It is concluded that the ZP is a useful technique for monitoring cell culture, and to sense changes in the physiological state of the cell.

48-POS Board 24

Quantifying Tight Junction Morphology of MDCK Epithelial Cells and Its Implications in Cell-Cell Interactions

Ivan Alex P. Lazarte[1,2], Chen-Ho Wang[1], Ching chung Hsueh[3], Li-fan Wu[1], Yu-Chieh Kuo[1], Keng-hui Lin[1].Wan-jung Lin[1].
[1]Academia Sinica, Taipei, Taiwan, [2]National Central University, Taoyuan, Taiwan, [3]National Taiwan University, Taipei, Taiwan.

Epithelium comprises the majority of metazoan structures and perform important physiological functions such as protection barrier, secretion, and selective absorption. They are highly polarized and the plasma membranes are separated into apical, lateral, and basal sides. Tight junctions form a continuous belt at the sub-apical location at the borders of two cells as a fence function to maintain the polarity of membrane proteins and seal the paracellular space between cells. The tight junctions are linked to actin cytoskeleton through an adaptor proteins. We found that the tight junctions form tortuous structure as Madin Darby Canine Kidney (MDCK) cells grow into higher confluency on a 2D transwell or as a cyst in 3D matrigel. When we perturbed actin-myosin contractility of MDCK cells by small molecules Y27632 and blebbistatin, the tight junctions become less tortuous, and cell shape changes in terms of height and the apical area. We developed 3D image analysis to quantify the tortuosity of tight junctions and proposed that the morphological change of tight junctions can be indication of apical constriction force and cell-cell tension. By constructing a simple theoretical model by surface evolver to explain the morphology of tight junction affected by the interplay between apical, and lateral tension. Using the endogenous cellular structure for quantifying intercellular force is non-perturbative and the gained knowledge can be used to test current theoretical models which explains the epithelial cell shapes based on basal, lateral, and apical tensions.

50-POS Board 25

Cell Stretching Dish

Qingsen Li, Stefano Marchesi, Gururaj Rao, Giorgio Scita, Marco Foiani.
IFOM, Milan, Milano, Italy.

Cells in the body constantly experience mechanical stimuli in various organ movement, development and functions. These mechanical stimuli are often essential to proper cell function, such as migration, proliferation, differentiation[1-4]. Cells sense such mechanical stimuli through a process called mechanotransduction [1, 5, 6]. Abnormal mechanotransduction leads to several patho-genesis, such as cancer, asthma, heart disease[1, 7, 8]. In Mechanobiology studies, the advance of in vitro technologies for the application of physiologically mimicking mechanical force to cultured cells and tissue are vital due to the complex in vivo biological system. The growing interest in Mechanobiology and technology gap have motivated the development of cell stretching device in various research labs and companies [10]. However, most cell stretching systems are bulky and limited in functions. In particular, there is no cell culture chamber/dish capable of multifunctional cell stretching. A novel cell stretching device in the size of a cell culture petri dish has been invented, which is called cell stretching dish. It is capable of uniaxial as well as biaxial stretching. The cell stretching dish is characterized by high level of stretching, which can reach up to 100% (and more). It is portable and easy to be used for both cell culture and microscope imaging experiment. The device has been tested to be compatible with the high resolution objectives, for live cell as well as fixed cell immunofluorescence imaging, and can be scaled up and down to fit any inverted microscope. The device can be 3D printed or made from plastic, thus further reducing the cost of production, bringing down the complicated cell stretching system to the consumable level. The unique design, high level stretch, cheap cost will open variety of possibilities not only to researchers in mechanobiology but also broad field of biologist.

52-POS Board 26

The Function of Dynamin 2 in Podosome-Regulated Postsynaptic Neuromuscular Junction Maturation

Shan-Shan Lin, Ya-Wen Liu.
National Taiwan University Institute of Molecular Medicine, Taipei City, Taiwan.

Podosomes are dynamic, actin-rich structures and best-characterized for cell-matrix interactions, migration, and matrix degradation in monocytic lineage and cancer cells. Recently it has been reported that podosomes are also present in aneurally formed postsynaptic neuromuscular junction (NMJ), and a GTPase, dynamin-2, is enriched at the postsynaptic podosome. Dynamin-2 is a mechanochemical enzyme, best-known for mediating endocytosis at presynaptic neuron membrane. Therefore, the presence of dynamin-2 in postsynaptic NMJ is novel and remains to be investigated. To understand the function of dynamin-2 at postsynaptic NMJ, we utilized cultured C2C12 myotubes and found that dynamin-2 is enriched at the peripheral of the podosomes and tightly surrounds the actin core. Podosomes with more dynamin-2 surrounding are taller than those without or with partial dynamin-2 ring. Interestingly, treatment with GTPase inhibitor, dynasore, can further increase the height of podosomes in myotubes. Through in vitro actin bundling assay, we demonstrated that dynamin-2 has promising actin bundling activity, and dynamin-2 mutations with altered assembly ability have abnormal actin bundling efficiency. Finally, we found that expression of the hyper-assembly dynamin-2, but not the wild type or membrane fission defective mutations, result in disturbance to the cytoskeleton of postsynaptic NMJ in Drosophila. Taken together, our finding suggests that dynamin-2 plays a structural role in podosome maturation through actin bundling activity, and therefore affect the cytoskeleton organization in podosome-regulated postsynaptic NMJ maturation.

54-POS Board 27

A Robust and Sensitive Method to Quantify Receptor Responses in the Nano Range

Shin-Shiou Lin, Huai-hu Chuang.
Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan.

In the brain, many hormones and neurotransmitters activate cellular signal transduction pathways via G-protein coupled receptors. G alpha proteins are speedily activated subsequent to the formation of a ternary complex from the direct interaction of receptors with their cognate ligands. Among a host of PLC-coupled pathways, despite all signaling through the canonical Gαq as well as downstream effectors thereof, each receptor is still capable of eliciting events of a distinct, or even more so, unique spatiotemporal profile. To elucidate the mechanism with which the complexity arises, we performed single cell analysis to monitor GPCR activation in real time. The GPCR of interest was made a channel fusion to facilitate receptor counting, with the ligand-induced response tracked simultaneously. By this method, we quantitatively compared the receptors for acetylcholine (muscarinic type) with those for angiotensin. Our results underpin the robustness of GPCR signaling, which might explain the prevalence of such transduction network in Nature.

56-POS Board 28

A Microfluidic Pipette Array and Compression Device for Mechanical Perturbation of Single Cells

Kenneth Ho[1], Lap Man Lee[1], **Allen Liu**[1,2,3].
[1]University of Michigan, Ann Arbor, MI, USA, [2]University of Michigan, Ann Arbor, MI, USA, [3]University of Michigan, Ann Arbor, MI, USA.

The proper responses of the cells to mechanical stimuli are important in numerous physiological processes. With the development of microsystem engineering tools, controlled and repeatable application of active mechanical input to single cells is becoming more available. Several microfluidic platforms have been developed for mechanotransduction research over the last decade that mainly focus on applying a single mechanical perturbation and often to a population of cells. Here we develop a multilayer microfluidic device using soft lithography with the goal of applying controlled aspiration and compression to single cells. The device, called microfluidic pipette array and compression (μFPAnC), consists of a flow channel with trapping cups that have narrow microchannels to the side to serve as aspiration micropipettes. Two independent pneumatically controlled valves above the flow channel serve to facilitate single cell loading and compression when they are actuated. The design enabled us to perform mechanical measurements of single cells at a higher throughput compared to manual micropipette aspiration. We characterized the stiffness of normal breast epithelial cells and breast cancer epithelial cells and found the cancer cells are two times softer than their normal counterpart. Compression in the normal direction is also a unique feature of this novel setup and we were able to perform static and cyclic compression at various amplitudes and frequencies. The development of μFPAnC will provide ample opportunities for single cell mechanotransduction research.

58-POS Board 29

Manipulation of Receptor Tension in Living Cells by Nanoscale Optomechanical Actuators

Zheng Liu[1,2], Yang Liu[2], Khalid Salaita[2].
[1]Wuhan University, Wuhan, Hubei, China, [2]Emory University, Atlanta, GA, USA.

Mechanical force has a critical role in many cellular functions, including cell division, gene expression, differentiation and motility. Despite its fundamental importance to cell biology, significant gaps remain in our understanding of the coupling between chemical and mechanical signals. As a first step to understanding mechanotransduction circuits operating within cells, a number of techniques have been developed to investigate the response of individual cells to spatially confined physical perturbations. The current state-of-the-art tools for force manipulation of living cells, such as the atomic force microscope and optical and magnetic tweezers have been hampered by their low experimental throughput. Another general approach involves using magnetic actuation of nanoparticles and micropillars are lack of spatial and tempo resolution in mechanical stimulation and molecular specificity.
To address these challenges, we developed an approach involving optomechanical actuator (OMA) nanoparticles that are controlled with near-infrared light. Illumination leads to OMA particle collapse, delivering piconewton forces to specic cell surface receptors with high spatial and temporal resolution. OMA nanoparticles were comprised of a plasmonic gold nanorod core coated with a thermo-responsive polymer shell (poly(N-isopropylmethacrylamide, pNIPMAm) . With this design, the gold rod functions as a photothermal transducer, converting NIR excitation to localized heat that drives a drastic and transient collapse of the polymer shell. OMA nanoparticles rapidly shrink upon photo-illumination, and exclusively apply forces to cell receptors that are bound to their cognate ligands on the nanoparticle surface. The amplitude, duration, repetition and loading rate of the mechanical input are controlled by the NIR illumination profile. We demonstrate optomechanical actuation by controlling integrin-based focal adhesion formation, cell protrusion and migration, and t cell receptor activation.

60-POS Board 30

Quantitative Single Analysis of C-Jun/AP-1 for Clinical Studies

Shao-Ju Lu, Keith Willison, David Klug.
Imperial college, London, United Kingdom.

Both c-Jun and its dimerization partners in transcription factor AP-1 formation play important roles in cell proliferation, apoptosis and cell transformation. AP-1/c-Jun is documented to be one of the key activators in breast cancer. AP-1-mediated regulation determined cell fates critically depends on the relative abundance of AP-1 subunits. However, bulk measurements of AP-1/c-Jun levels do not record the intrinsic and extrinsic variation in cellular responses across a population of cells, thereby masking the individual details.

We have developed a label-free, microfluidic antibody chip platform called the MAC chip[1] , to quantify precisely the copy numbers of many proteins from a single cell. Utilizing microfluidic strategies along with optical trapping and lysis techniques, we have established c-Jun assay which is capable of quantification of c-Jun, phospho-c-Jun and its homodimer/ heterodimer complex individually at the single cell level. Furthermore, the protein assays can now be accomplished in a multiplexed single assay format.

We are working on investigations into protein noise in tumor cells isolated from biopsies of cancer patients through the identification of biomolecular signatures.[2] Using patient-derived colorectal cancer xenografts as proof-of-concept, we have demonstrated a potential route for moving single cell proteomics into medical diagnostics. Our aim is to integrate the protein measurements with single cell transcriptomics. In the future, we hope to gain deep insights into c-Jun transcriptional and translational regulation at the single cell level.

1. E. Burgin, A. Salehi-Reyhani, M. Barclay, A. Brown, J. Kaplinsky, M. Novakova, M. A. A. Neil, O. Ces, K. R. Willison, and D. R. Klug, Analyst, 2014, 139, 3235–44.
2. K. R. Willison and D. R. Klug, Curr. Opin. Biotechnol., 2013, 24, 745–51.

62-POS Board 31

Measuring the Orientation of Proteins Sliding on DNA

Emil Marklund, Elias Amselem, Kalle Kipper, Magnus Johansson, Sebastian Deindl, Johan Elf. Uppsala University, Uppsala, Sweden.

Many transcription factors and restriction enzymes slide on DNA when searching for their specific target sites. Indirect evidence suggests that some of them also rotate around the DNA in a helical path while sliding. Little is however known about the orientation of the proteins while sliding and how it changes in time. To bridge this gap, we are measuring the orientation of the *lac* repressor labeled with a fluorophore that is rigidly attached to the DNA binding domain . In our experiments we measure polarization of the light emitted from single transcription factor molecules when they are bound specifically or sliding on flow stretched DNA. The polarization data acquired is shifted towards the direction of DNA with relatively small changes in the signal over several seconds when using camera tracking with an integration time of ~100 ms. This shows that the transcription factor has a preferred and maintained orientation on this timescale. For transcription factors sliding on DNA, very little polarization preference is observed in the direction that would be averaged out by rotation of the fluorophore around the DNA. We have also studied the sliding process at the faster timescale expected for one revolution of the protein around the DNA. For this we have developed a tracking confocal microscope with real time position feedback and a photon time tagging resolution of 5 ns. By doing fluorescence correlation spectroscopy we get a decay time in the range expected for transcription factor molecules rotating around the DNA while sliding. Taken together these observations strongly support the model of rotational sliding for the *lac* repressor.

64-POS Board 32

Dynamics of DNA in Living Cells Probed by Fluorescence Correlation Microrheology

Cameron Hodges[1], Rudra Kafle[2], **Jens-christian Meiners**[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]Worcester Polytechnic Institute, Worcester, MA, USA.

DNA inside a living cell is highly dynamic. In order to fulfil its biological functions, it has to move to come in contact with distant sites or protein complexes. Yet surprisingly little is known about how DNA actually moves around inside a cell, and what is driving and constraining that motion. We are using fluorescence correlation spectroscopy (FCS) to study the fluctuations of bacterial chromosomal DNA in live and ATP-depleted *E. Coli* with high temporal resolution. We find that the bacterial chromosome in fully metabolically active cells is softer and more fluid than in the ATP-depleted ones. While this is at odds with the notion of DNA as an entropic spring in the presence of active fluctuations, we can explain our observations with a model in which proteins that weakly crosslink the bacterial DNA are constantly driven off the DNA by processive motor enzymes like RNA polymerase, which keeps the chromosome more liquid than the gel-like state that it assumes in the absence of these processes.

For these experiments, we have developed a quantitative live-cell FCS microrheology technique that allows us to measure the viscous and elastic modulus of the DNA over a broad frequency range from hundred microseconds to seconds. We will discuss pitfalls of quantitative live cell FCS, and methods to effectively overcome sources of artifacts, especially photobleaching. We will also present a first application of our technique to HeLa cells.

66-POS Board 33

Stator Stoichiometry and Mechanosensitivity of the Bacterial Flagellar Motor Probed by Load Manipulation

Ashley L. Nord, Emily Gachon, Alessandro Barducci, Francesco Pedaci.
Centre de Biochimie Structurale, Montpellier, France.

The bacterial flagellar motor (BFM) is the multi-component complex which powers the swimming and swarming of many motile bacteria. The BFM structure, many details of which are still unknown, displays a rich dynamic behavior in terms of exchange and conformational change of its internal components. The torque of this rotary motor is provided by stators, ion motive force powered ion channels which are known to assemble and disassemble dynamically in the BFM. Recently, it has been observed that this turn-over is mechano-sensitive, with the number of engaged stators dependent upon the external load acting on the motor. Despite their central role in the function of the BFM, a systematic study of the stator dynamics, as a function of the external parameters in unperturbed motors, is lacking. Here we provide a quantitative and non-invasive measurement of the temporal behavior of the stators active in the BFM of *E. coli*, by estimating stator stoichiometry from high-resolution single-motor torque traces, quantifying for the first time the dependence between stator number and external load at steady-state. Furthermore, a rapid and controlled change in the external load, applied via a magnetic field, allows us to directly probe BFM mechano-sensitivity, systematically triggering and detecting stator association and dissociation. We incorporate these results into an adsorption model of stator kinetics, providing the first step into understanding the mechanism of mechano-sensitivity of the BFM.

68-POS Board 34

Migration of Densely Packed T Cells in Microchannel

HyoungJun Park[1], HyeMi Kim[3], Junsang Doh[1,2].
[1]POSTECH, Pohang, Kyung-buk, South Korea, [2]POSTECH, Pohang, Kyung-buk, South Korea, [3]POSTECH, Pohang, Kyung-buk, South Korea.

T cell antigen recognition is a key initial step for the antigen-specific immune responses. T cell antigen recognition occurs in secondary lymphoid organs such as spleens and lymph nodes. In the lymph node, there are T cell regions where densely packed T cells continuously migrate to survey antigens presented by dendritic cells. The movement of T cells in the T cell region is important for efficient antigen recognition, but the migration of T cells in such densely packed microenvironments has not been studied so far. To study the migration of densely packed T cells, we fabricated a microchannel system filled with high density T cells. Migration of T cells within the microchannels was quantitatively analyzed by particle imaging velocimetry (PIV) methods, and further processed to extract various motility parameters, including mean velocity, vorticity, and order parameter. Roles of cytoskeletons and membrane tension were assessed by treating with pharmacological inhibitors and regulating tonicity, respectively. In addition, computer simulation was performed to further understand mechanisms of T cell motility in densely packed microenvironments.

70-POS Board 35

Blue-light Dependent Conformational Change of Cryptochromes

Li Pei[1], Chongjun Ma[1], Huaqiang Cheng[1], Xuanxuan Li[2], Haiguang Liu[2], Yanwen Tan[1].
[1]Fudan University, Shanghai, China, [2]Beijing Computational Science Research Center, Beijing, China.

Cryptochromes are a kind of blue-light photoreceptors, which entrain the circadian rhythms in diverse organisms. Cryptochromes have also been reported to sense magnetic fields for some species such as fruit fly, butterfly and pigeons. Algae, plant and animal cryptochromes possess conserved photolyase homology region (PHR) domain and vastly different size carboxyl-terminal (C-terminal) extensions. The C-terminal conformations have been hypothesized to participate in the blue-light response signaling mechanism. Here, we use single molecule Förster Resonance Energy Transfer (smFRET) and Small-Angle X-ray Scattering (SAXS) to investigate the C-terminal conformational changes of cryptochromes. The FRET efficiency distributions indicate that cryptochromes prefer to stay in close conformation in dark. After exposure to blue-light, C-terminal will be released from PHR domain. SAXS measurements are consistent with this result, which reveal the radius of gyration changes of cryptochromes after sensing the blue light. Furthermore, the blue-light dependent homodimerization has been confirmed by smFRET assays, size exclusion chromatography and Native-PAGE assay.

Key Words： Cryptochromes, smFRET, SAXS, Conformational change, Dimerization

72-POS Board 36

The Cardiac Cell under the Mathematical Microscope

Vijay Rajagopal[1], Gregory Bass[4], Shouryadipta Ghosh[1], Eric Hanssen[5], Edmund Crampin[2,3,4]. [1]University of Melbourne, Melbourne, VIC, Australia, [2]University of Melbourne, Parkville, Australia, [5]University of Melbourne, Melbourne, VIC, Australia.[3]University of Melbourne, Melbourne, Australia, [4]University of Melbourne, Melbourne, Australia,

The cells that make up our hearts have a highly specialised organisation. This organisation can undergo drastic changes in patients with heart disease, but a fundamental understanding of the significance of these changes and how they develop is lacking. We are developing methods to integrate state-of-the-art structural microscopy data and biophysical modeling techniques in order to gain new insights into the role of spatial organization in cardiac cell systems biology.

Here we present a new method to computationally integrate electron microscopy and immunofluorescence data of heart cell ultrastructure to build a detailed model of the heart cell. We applied this method to computationally combine confocal-scale (~ 200 nm) data of RyR clusters with 3D electron microscopy data (~ 30 nm) of myofibrils and mitochondria that were collected from rat left ventricular myocytes. Using this hybrid-scale spatial model, we simulated reaction-diffusion of Ca^{2+} during the rising phase of the transient (first 30 ms after initiation).

We demonstrate in this study that: (i) heterogeneities in the Ca^{2+} transient are not only due to heterogeneous distribution and clustering of mitochondria; (ii) but also due to heterogeneous distribution of RyR clusters; Further, we show that: (iii) these structure-induced heterogeneities in Ca^{2+} can appear in line scan data. Using our unique method for generating RyR cluster distributions, we demonstrate the robustness in the Ca^{2+} transient to differences in RyR cluster distributions measured between rat and human cardiomyocytes.

We also discuss our on-going development of a complete 3D model of a heart cell and our investigations into the impact of cardiac ultrastructural remodeling on function in diabetic cardiomyopathy.

74-POS Board 37

Deformability Cytometry and 1D Fluorescence Imaging in Real-Time

Philipp Rosendahl[1], Katarzyna Plak[1], Angela Jacobi[1], Nicole Töpfner[1,2], Jochen Guck[1].
[1]Biotechnology Center, Technische Universität Dresden, Dresden, Germany, [2]University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany.

During the last decades, tools for rapid characterization of large cell quantities have become indispensable not only for basic research but also clinical diagnostics. The gold standard for cell characterization is flow cytometry. Its success is closely tied to the availability of fluorescent labels. But what if there is no molecular marker known for the cells of interest? Or if the label changes cell function? Or cells shall be used for transplantation? As an attractive alternative, deformability cytometry exploits cell mechanics as a sensitive, inherent, label-free functional marker, but lacks the specificity provided by a fluorescent signal. Here we present real-time fluorescence deformability cytometry (RT-FDC), the ideal, combined system. It facilitates fluorescence detection as in conventional flow cytometry, extended by 1D analysis of spatial information encoded in the fluorescence pulse shape, and adds bright field imaging for mechanical phenotyping of single cells — all in real-time at rates of 100 cells/s. We show utility of RT-FDC for the most common fluorescent labels: Fluorescent surface markers (CD34) are used to separate human hematopoietic stem and progenitor cells (HSPCs) from an unpurified apheresis sample as harvested for bone marrow transplantations. Membrane permeant dyes identify reticulocytes by their ribonucleic acid (RNA) content in a blood sample. And endogenously expressed fluorescent proteins (FUCCI) reveal cell cycle phases in an unsynchronized sample of retinal pigment epithelial cells (RPE1). In addition, we can now also directly correlate mechanical characteristics with fluorescence intensity and localization for each single cell to improve correct classification. In future, this combined approach could establish mechanical phenotyping as equivalent to fluorescent labeling, or even identify subpopulations invisible to molecular labels. RT-FDC is destined to find wide-spread utilization and will help to further improve the applicability of flow cytometry.

76-POS Board 38

Damage of Cancer Cells Evaluated by Intensity Fluctuation of Images under Phase Contrast Microscope

Morito Sakuma, Yuichi Kondo, Hideo Higuchi.
The University of Tokyo, Tokyo, Japan.

Selective removal of cancer cell without side effects is necessarily for cancer therapy. Phototoxic dyes such as IR700 can specifically be delivered to cancer cells, and the cells are damaged by reactive oxygen species (ROS). But surviving cancer cells damaged by oxidative stress could obtain resistant to the therapy. Here to understand the effects of phototoxic dyes and detect resistant cancer cells, we developed a method to measure quantitatively cell damages induced by ROS of IR700. We evaluated cell damage by calculating the intensity fluctuation of each pixel in cell images under phase contrast microscopy. We succeeded in quantifying the change in motility of cell organelles by this method (Sakuma et al. Sci Technol Adv Mater. 2016). IR700 was labeled with the antibody which binds specifically to target cells. IR700-antibody complex was endocytosed into cultured cells and then cell was photodamaged by illumination of red laser (650 nm). The degree of cell damage was controlled by adjusting the irradiation time of red laser. The motility of cell organelles detected by the intensity fluctuation method decreased gradually with a progression of cell damage. To elucidate the mechanisms of decrease in the organelles motility, we analyzed the effects of photoactivation of IR700 on activities of organelles and motor proteins. Lysosomes containing IR700 in cells were damaged quickly by the photoactivation of IR700 and the contents in lysosomes were diffused out into cytoplasm. The contents diffused from lysosomes caused the dysfunction of transporter kinesin and mitochondria. Therefore, these damages of organelles and motors may be a primary factor for causing decrease of organelles motility. These results suggested that the cell organelles motility is a primary indicator of cell viability.

78-POS Board 39

Superresolution Imaging Reveals Protein-Templated Patterns for Biosilica Formation

Philip Gröger[1], Nicole Poulsen[1], Jennifer Klemm[1], Nils Kröger[1,2], **Michael Schlierf**[1].
[1]TU Dresden, Dresden, Germany, [2]TU Dresden, Dresden, Germany.

The intricate, genetically controlled biosilica nano- and micropatterns produced by diatoms are a testimony for biology's ability to control mineral formation (biomineralization) at the nanoscale and regarded as paradigm for nanotechnology. Several recently discovered protein families involved in diatom biosilica formation remain tightly associated with the final biosilica structure. Determining the locations of biosilica-associated proteins with high precision is therefore expected to provide clues to their roles in biosilica morphogenesis. To achieve this, we introduce single-molecule localization microscopy to diatoms based on photo-activated light microscopy (PALM) to overcome the diffraction limit. We identified six photo-convertible fluorescent proteins (FPs) that can be utilized for PALM in the cytoplasm of Thalassiosira pseudonana. However, only three FPs that share a common molecular conversion-mechanism were also functional when embedded in diatom biosilica and localized with a mean precision of 25 nm to resolve structural features. Further co-localization studies on proteins of the Cingulin family when extracted from the biosilica using a combined two-color PALM+STORM approach revealed characteristic protein filaments with distinct protein specific patterns. The enhanced microscopy techniques introduced here for diatoms will aid in elucidating the molecular mechanism of silica biomineralization as well as other aspects of diatom cell biology.

80-POS Board 40

Heterogeneous Response of Lymphocytic Cells to Mitomycin-C Revealed by Microfluidic Single-Cell Time-Lapse Microscopy

Akihisa Seita[1], Yuichi Wakamoto[1,2].
[1]University of Tokyo, Bunkyo-ku, Tokyo, Japan, [2]University of Tokyo, Bunkyo-ku, Tokyo, Japan.

Over the past years, single-cell analysis has revealed that cellular phenotypes are heterogeneous even for genetically identical cells. It has been also shown that at least some types of phenotypic heterogeneity contribute to adaptation of cellular population without apparent genetic mutations from bacterial cells to cancer cells. However, understanding which single-cell phenotypic states are related to growth and death of individual cells is generally difficult due to time-course fluctuation of cellular phenotypes and transitions of environmental conditions caused by the proliferation of cells. Microfluidic single-cell time-lapse microscopy is a promising technique to achieve the reliable measurements on time-course fluctuations of cellular phenotype and tight control of environmental conditions, yet such methods have been still limited to the measurements for bacterial cells.
Here, we have created a microfluidics device for mammalian cells based on the "Mother machine" scheme, that have allowed the long-term tracking of single bacterial cells in controlled environments. With this device, we observed the response of single lymphocytic cells (L1210) to Mitomycin-C for five days prior to drug treatment and six days under the continuous exposure to the drug. The result showed that the cell lineages that divided more frequently before the drug exposure had lower chances of survival after the exposure, which indicates that pre-exposure growth states are correlated with the survival fates of individual cell lineages against Mitomycin-C. In addition, pre-exposure interdivision times were positively correlated between the neighboring generations (~0.6), suggesting some epigenetic factors affecting the growth characteristics of cell lineages.

82-POS Board 41

Linking Actin Engagement with Integrin Nanoclustering Using Superresolution in PTPN22 Deficient Migrating T Cells

Michael Shannon[1], Georgina Cornish[2], Andrew Cope[2], Dylan Owen[1].
[1]King's College London, London, United Kingdom, [2]King's College London, London, United Kingdom.

T cells navigate the body using constant actin flow, which transiently engages with a spatio-temporally controlled 'molecular clutch' to translate forward cell movement (Ishibashi et al., 2015). The clutch regulates cell speed through its link to actin and the substrate, using integrin affinity, avidity and colocalization with effectors. We posit that reorganisation of this clutch on the nanoscale results in an observed speed increase in PTPN22 mutant cells, which predispose human patients for autoimmune disease.

Nano-adhesions are too small to see with conventional fluorescence microscopy, so we use super resolution localisation microscopy to characterise clustering, and live-TIRF microscopy to image actin flow and engagement. To analyse the point data, we developed new tools to reduce the need for human decision making, allowing us to extract precise metrics on the size, composition and spacing of nanoclusters in cells (Rubin-Delanchy et al., 2015). For actin flow, we adapt Spatio Temporal Image Correlation Spectroscopy (STICS: Hebert, Costantino, & Wiseman, 2005) for fast cells by changing the reference frame.

We observe that to migrate, hundreds of ~40 nm membrane LFA-1 clusters gather in the leading edge/front of substrate proximal membrane, which condense and group closer together in the focal zone/middle. This is coupled to a high degree of actin engagement (70 %), retrograde slippage (30 %) and front only anterograde flow. On removal of PTPN22, or treatment of wildtype cells with SDF-1 chemokine, migration speed is increased; this is coupled to reduced actin engagement and a maintained integrin nanoclustering schema. Slow moving cells (Manganese added to increase LFA-1 affinity, or Cytochalasin D to reduce actin polymerisation) show the reverse. Finding out what links an active T cell integrin nanocluster to the cytoskeleton is the next step in this project.

84-POS Board 42

The Observer Effect in Cell Biology: Gene Expression Noise, Genetic Reporters and the Problem of Measurement in Live Cells

Rosanna C. Smith[1,3], Patrick S. Stumpf[1,3], Sonya J. Ridden[2], Aaron Sim[4], Sarah Filippi[5], Heather A. Harrington[6], Ben D. MacArthur[1,2,3].
[1]University of Southampton, Southampton, United Kingdom, [2]University of Southampton, Southampton, United Kingdom, [3]University of Southampton, Southampton, United Kingdom, [4]Imperial College, London, United Kingdom, [5]University of Oxford, Oxford, United Kingdom, [6]University of Oxford, Oxford, United Kingdom.

Fluoresence reporters allow investigation of temporal changes in protein expression in live cells and are consequently an essential measurement tool in modern molecular biology. However, their utility is dependent on their accuracy, and the effects of reporter constructs on endogenous gene expression kinetics are not well understood. Here, using a combination of mathematical modelling and experiment, we show that widely used reporter strategies can systematically disturb the dynamics they are designed to monitor, sometimes giving profoundly misleading results. We illustrate these results by considering the dynamics of the pluripotency regulator Nanog in embryonic stem cells, and show how reporters can induce heterogeneous Nanog expression patterns in reporter cell lines that are not representative of the wild-type. These findings help explain the range of published observations of Nanog variability and highlight the problem of measurement in cell biology in relation to genetic reporters.

86-POS Board 43

Cell Cycle-Dependent Fluctuations of Protein Mobility in Cytoplasm of Hela Cell

Krzysztof Szczepanski, Karina Kwapiszewska, Robert Holyst.
Institute of Physical Chemistry PAS, Warsaw, Poland.

Great deal of life-sustaining processes are diffusion-limited and viscosity of cytoplasm may influence the diffusion rates. Up to date no one attempted to measure how viscosity of cytoplasm changes throughout the cell cycle. Current research focuses rather on short time-stamps in cell life. Our research aims to measure fluctuations of rate of diffusion of example protein during cell cycle and to give possible explanations of any changes observed.
We studied diffusion of enhanced green fluorescent protein (eGFP) in cell cytoplasm of HeLa eukaryotic cell line using fluorescence correlation spectroscopy (FCS). Monomeric eGFP was chosen as well-known and biologically-neutral probe which has size comparable to cell cytoplasm content. Observations made on this basis could be easily transferred to other cytoplasmic proteins. To ensure uniform progress through cell cycle, cells were first synchronized at the G1/S phase transition using aphidicolin and then allowed to grow.
Our results clearly show fluctuations in diffusion coefficients of eGFP travelling through the cytoplasm of cells (as compared to diffusion in buffer). These fluctuations seem to be connected with events happening inside cells as they prepare for division and undergo the mitosis. Throughout S and G2 phases diffusion decreases relatively slowly until the division, when the decrease is sharp. Then, during G1 phase, it slowly but steadily increases. Our results could hint at possible not yet studied mechanism of modulation of cellular activities through changes in rates of diffusion of proteins in cytoplasm. Alternatively these changes could also originate from cellular volume fluctuations. This could be the first step towards understanding whether viscosity could regulate internal processes of the cell.

88-POS Board 44

The Modulation of Min and Nucleoid Occlusion Systems in Escherichia Coli

Wei-Chen Tsai[1], Jie-Pan Shen[1], Yi-Ren Chang[2], Chia-Fu Chou[1].
[1]Academia Sinica, Taipei, Taiwan, [2]National Taiwan Normal University, Taipei, Taiwan.

Spatial control of prokaryotic cell division is solely achieved by directing Z-ring assembly at specified position. In E. coli, Min oscillation, together with nucleoid occlusion (NO), destabilize Z-ring in the regions away from midcell to ensure faithful septation. Min oscillation, driven by MinD-ATPase hydrolysis via MinE activation on the membrane, establishes a time-averaged MinCD gradient to yield a pronounced minimum of MinC's inhibition on Z-ring at midcell. Because early-division proteins ZipA and ZapA/B, along with FtsZ, assemble into complexes that counter-oscillate with Min system, stable oscillations to suppress Z-ring assembly is expected to allow MinC residing in each polar zone longer than the half-time of FtsZ turnover. Even though Min oscillation has been displayed by synthetic systems, it's unclear the interplays of Min proteins and compartment geometry are sufficient to bolster oscillation stability in vivo. Here the Min-nucleoid interaction is reported as the physicochemical element missing in previous researches to commit stable Min oscillation in vivo. We found, compared with unperturbed cells, Min oscillation in anucleate and nucleoid-perturbed cells was deviated up to a quarter-cycle, but the frequency was higher in anucleate and lower in nucleoid-perturbed cells. Enhanced stability and lowered frequency were observed in cells expressing excess NO factor SlmA. Further, cell filamentation by excess SlmA and associated DNA-binding sites was antagonized by overproduced Min proteins, and as such asymmetric septation was enabled to reduce cell size. Our results reveal an unanticipated role of the nucleoid in the modulation of frequency and stability of Min system; moreover, SlmA is indicated to facilitate such modulations, potentially via directly interacting with Min system. we propose a fresh perspective that frequency modulation of Min system is mediated via the act of nucleoid-associated factor; and envision a model of Min-nucleoid interaction.

90-POS Board 45

Chemically Functionalized Vertical Nanowire Arrays as Molecular Probes for Single-Cell Analysis

Inga Von Ahnen[1,2], Karl Adolfsson[1,2], Damiano Verardo[1,2], Laura Abariute[1,2], Mercy Lard[1,2], Christelle N. Prinz[1,2].
[1]Div. Solid State Physics, Lund University, Lund, Sweden, [2]NanoLund, Lund, Sweden.

Chemically functionalized vertical nanowire (NW) arrays have the potential to provide versatile tools for the investigation of living cells in real time on a single cell level. For this aim it is essential to further investigate and understand the behavior of the cell membrane and its proteins around NWs, such as whether the membrane is pierced or not by the NWs.
We are using fluorescence microscopy and stimulated emission and depletion (STED) microscopy to investigate the interactions between the cell membrane and vertical gallium phosphide (GaP) NW arrays. STED microscopy offers sufficient resolution to resolve fluorescently labeled membrane proteins and their distribution around the NWs. To probe the NW intracellular access, we functionalized the nanowires with fluorescein diacetate (FDA). FDA is a non-fluorescent molecule, that becomes fluorescent once inside the cytosol due to acetate group cleavage by intracellular esterase. The NWs were successfully chemically functionalized using a silane linker with a terminal alkyne group, to which different azide-terminated fluorophores or molecular probes can be clicked via the highly specific alkyne azide cycloaddition. [1]

The activation of FDA immobilized on NWs was investigated using isolated esterase in solution and the results suggest that a possible steric hindrance of the NWs influences the catalysis of the hydrolysis.

Overall vertical NW arrays functionalized with FDA have the potential to provide a simple and straight-forward assay to confirm access of NWs to the cytosol on a single cell level. The chemical functionalization method can be adapted to several different fluorophores and molecular probes to further investigate various properties of living cells on a single cell level.

References

[1] Y. Maidenberg et al. Langmuir, 29 (38), (2013) 11959.
[2] Financed by NanoLund, the Swedish Research Council (VR), and the ERC consolidator grant NanoPokers (682206).

92-POS Board 46

Investigation of Metabolic Regulation of Cell Growth and Division in Escherichia Coli

Chung Kang Wang[1], Chung Hsien Hung[1], Yu Ling Shih[1,2,3].
[1]Institute of Biological Chemistry, Academia Sinica, Taipei City, Taiwan, [2]Institute of Biochemical Sciences, National Taiwan University, Taipei City, Taiwan, [3]Department of Microbiology, College of Medicine, National Taiwan University, Taipei City, Taiwan.

6-Phosphofructokinase I (PFK I, encoded by *pfkA*) is a key enzyme of the glycolysis pathway that catalyzes the irreversible conversion of fructose 6-phosphate to fructose 1,6-bisphosphate thereby regulating the energy production in *Escherichia coli*. Interestingly, our recent study suggested that membrane association of PFK I was regulated by the Min system, a protein machine that mediates placement of the division septum at the midcell, through direct protein-protein interaction. We therefore set out to investigate the role of PFK I in cell growth and division by analyzing the wild-type, *ΔpfkA* and *Δpgi* cells cultured in the presence of different carbon sources. The examined phenotypes include cell morphology and dimension, as well as positioning of the FtsZ ring that plays a critical role in septal wall growth prior to cell division. Our results showed strong correlations between PFK I and the cell size control. Meanwhile, we found that oscillation of the Min system could be tuned by nutrient availability that controls the energy status in cells. We are currently investigating the underlying mechanisms that mediate the metabolic regulation of cell size, morphology, Min oscillation and the interplay between them.

94-POS Board 47

Rheological Behavior of Filamentous Networks Governed by the Binding Kinetics of the Cross-Linking Molecules

Xi Wei[1], Vivek B. Shenoy[2], Yuan Lin[1].
[1]The University of Hong Kong, Hong Kong, Hong Kong, [2]University of Pennsylvania, Philadelphia, PA, USA.

Cells employ the actin cytoskeleton, a highly dynamic and complex network of actin filaments inter-connected by various cross-linking proteins, to withstand mechanical load. Although accumulating evidence has shown that the binding kinetics of cross-linkers can significantly influence the rheology of such biopolymer networks, the underlying mechanisms remain poorly understood.

Here we present a computational study to address this important issue. Specifically, the rheological behavior of randomly cross-linked actin networks was examined via a combined finite element – Langevin dynamics (FEM-LD) approach where, besides bending and stretching, thermal fluctuations of individual filament have also been taken into account. Each crosslinking molecule was modeled as a combination of linear and rotational springs, resisting both separation and relative rotation between two filaments, whose association/dissociation was assumed to take place in a stochastic yet strain energy – dependent manner. Interestingly, the frequency spectrum of a random network was found to exhibit three distinct regimes. The high-frequency response is dominated by the behavior of independent filaments while the crosslinks can be treated as "static". At intermediate and low frequencies, unbinding of individual cross-linkers starts to contribute significantly to energy dissipation. In particular, we showed that, under such circumstance, the elasticity of the network mainly depends on the unbroken cross-links while the viscous dissipation is largely determined by the continuous unbinding and rebinding events occurring during the deformation process. Furthermore, we demonstrated that the interplay between the rate of loading and how fast a cross-linker can rupture/reform dictate whether such networks will undergo strain hardening or softening, in good agreement with experimental observations.

96-POS Board 48

Simultaneous Measurement of Cellular and Nuclear Optical Deformability for Single Cell Mechanics

Kai Skodzek, **Graeme Whyte**.
Heriot Watt University, Edinburgh, United Kingdom.

Measurement of cell mechanics can give insight into the current state of individual cells by measuring their inherent physical properties, without requiring the addition of any markers. Changes in the mechanical properties have been linked to a wide variety in changes in the type, function and health of individual cells, with both the cytoskeletal and nuclear stiffness being variable, however the ability to measure both of those at the same time, in the same cell have been limited. To counter this, we have developed a novel form of optical stretcher which is capable of measuring both properties simultaneously, and allows comparison of the relative contributions to overall cell stiffness. By adding the additional dimension to cell mechanics data, more detailed understanding can be sought of the changes cells undergo when they modulate their physical properties, and may lead to more accurate detection of changes in cell function or disease.

98-POS Board 49

Differences in Growth Statistics in Different Schemes of Long-Term Single-Cell Measurements

Shunpei Yamauchi, Hidenori Nakaoka, Yuichi Wakamoto.
n/a, Meguro City, Tokyo, Japan.

When cells with the same genotype are in the same environment, phenotypes such as cell size, growth rate, and expression of genes are heterogeneous from cell to cell. Recently, techniques of microfluidics have advanced greatly, enabling us to easily detect the heterogeneity and stochasticity of single cell dynamics.

In this study, we compared the growth statistics in two different microfluidics devices for long-term single-cell measurement. One is called dynamics cytometer (DC), in which each channel can harbor about 50 *E.coli* cells by constantly flushing out the cells at the both ends of channels. In DC, which cell lineages remain in growth channels becomes non-deterministic. The other device is Modified Mother Machine (MMM), which is similar to Mother Machine but has wider channels. Cells at the bottom of channels push out other cells and are more likely to stay in channels for a long time.

The growth statistics are expected to depend not only on micro-environments around cells including pressure from narrow channels, efficiency of exchanging medium and so on, but also on the selection effect within a small population determined by cellular growth heterogeneity and the geometries of channels. We calculated the age-dependent division rates and estimated the inherent growth rates and the relative selection effects with respect to the expected dynamics of infinite population. The results show that the growth rate in DC was greater than that in MMM, and the selection effect in DC was also greater than that in MMM. The difference of the growth rates suggests that DC allows cells to grow faster than MMM for the same medium and temperature. The result on the selection effect implies that the geometries of growth channels matters to growth statistics in different types of microfluidic devices.

100-POS Board 50

Intraflagellar Transport Proteins Undergo Nonaxonemal Staged Hindrance between the Recruiting Distal Appendages and the Cilium

Tony Yang, Minh Nguyet T. Tran, Weng Man Chong, Chia-En Huang, Jung-Chi Liao.
Academia Sinica, Taipei, Taiwan.

The primary cilium is an essential organelle responsible for multiple sensory and signaling activities. Ciliogenesis is achieved by delivery of precursors such as tubulins along the axoneme through intraflagellar transport (IFT), which is mediated by molecular motors and various IFT particles. Distal appendages (DAPs) are known to serve as the recruiting site of IFT proteins. During ciliogenesis, IFT proteins must go through several different zones in cilia. One of missing links of the IFT dynamics is how IFT particles move between the DAPs and the ciliary axoneme. The major obstacle comes from the tiny volume surrounding the DAPs and TZ and the high density of IFT particles in this region, which is far smaller than the diffraction-limited spot. Here we performed live-cell sptPALM-based superresolution tracking of short trajectories to demonstrate IFT particle dynamics at the ciliary base with the optimization of particle density and trajectory duration suitable for IFT motion speed. Our results revealed the DAPs and TZ accommodate not only axonemal but also transverse IFT88 movement. IFT particles move slower at the base than in the ciliary compartment. Moreover, diffusion analysis revealed that IFT particle movement was confined at the distal TZ while superdiffusive at the proximal TZ. This heterogeneous diffusion characteristics was likely attributed to a complex organization at the DAPs, spatially partitioned into some obstructed regions and some unhindered areas. Together, our live-cell superresolution studies revealed that IFT proteins adopt location-dependent stochastic paths in different regions of the ciliary base, with newly reported dynamic characteristics of IFT particles to shed light on the mechanisms of IFT particle traffic and gating facilitating ciliogenesis.

102-POS Board 51

Microtubules Push Chromosomes Apart in Anaphase

Che-Hang Yu[1], Stefanie Redemann[2], Hai-Yin Wu[1], Tae Yeon Yoo[1], Thomas Mueller-Reichert[2], Daniel Needleman[1].
[1]Harvard University, Cambridge, MA, USA, [2]Technische Universität, Dresden, Germany.

The processes which drive chromosome motion in anaphase are poorly understood and different mechanisms have been proposed to operate in meiosis and mitosis and in different organisms, including depolymerization of kinetochore microtubules, pulling apart spindle poles by cortical pulling forces, and pushing apart spindle poles by the elongation of microtubules which extend from the pole to the center of the spindle.

We have investigated anaphase in the first mitotic division of C. elegans and found that, contrary to previous speculations, spindle pole separation and chromosome separation are two mechanistically distinct processes. We argue that while spindle pole separation is driven by cortical pulling forces, chromosome separation results from a new population of microtubules which appear between chromosomes at the onset of anaphase and push chromosomes apart. We characterized the behaviors of this newly discovered population of microtubules using a combination of laser ablation, nonlinear microscopy, fluorescence recovery after bleaching, larger scale electron tomography reconstructions, and mathematical modeling. Our results suggest that these microtubules continually nucleate in a small region between chromosomes throughout anaphase, and push chromosomes apart by a combination of polymerization and sliding. Additional preliminary results indicate that the same processes could be the primary driver of chromosome motion in anaphase in C. elegans meiosis and in mitotic human tissue culture cells. Thus, a common underling mechanism may drive chromosome segregation in anaphase in diverse systems: newly generated microtubules between chromosomes push them apart.

104-POS Board 52

Visualizing Mitochondrial Dynamics and Cellular States with Environmentally Sensitive Fluorescence Probes

Sufi O. Raja[1], Gandhi Sivaraman[1], Ananya Mukherjee[1], Shikha Sharma[1], Sunny Kataria[2], Akash Gulyani[1].
[1]Institute for Stem Cell Biology and Regenerative Medicine, Bengaluru, India, [2]National Center for Biological Sciences, Bengaluru, India.

Mitochondria are known as power house of the cell as they efficiently produce ATP through Oxidative Phosphorylation (OxPhos). Apart from this, mitochondria also play significant role in regulating cellular metabolism, calcium and ROS signaling as well as in programmed cell death. Despite decades on research, precise and real-time information on mitochondrial dynamics and functionality, is still limiting. For example, recently it has been shown that cellular migration during metastasis relies, in part, on mitochondrial motility and precise positioning within the cell. Similarly, morphology and activity of mitochondria are linked with maintenance of stemness as well as triggering of differentiation event. Therefore, unraveling the spatio-temporal localization as well as functional heterogeneity of mitochondria during various cellular states appears to be crucial for understanding the coherent behavior of cell. To better visualize the functional dynamics of mitochondrion, we have developed red-emitting, multi-functional, novel mitochondrial probes that are sensitive to local environment, specifically parameters like viscosity, pH, ROS, etc. The developed dyes have low toxicity and very high photo-stability, allowing their use in long term imaging. In this presentation, we will show these dyes have yielded new insights into mitochondrial dynamics in embryonic stem cells as well as onset of differentiation. In a different example, we have also used our new dyes to probe mitochondrial heterogeneity within primary ‘activated’ cells during cell migration. These results would be placed in the context of our larger efforts to build new ways of probing ‘cellular dynamics’ with a focus on physico-chemical changes in the cell.