Engineering Approaches to Biomolecular Motors: From in vitro to in vivo

Vancouver, Canada | June 14–17, 2016

Organizing Committee

Zev Bryant, Stanford University

Paul Curmi, University of New South Wales

Nancy Forde, Simon Fraser University

Heiner Linke, Lund University

Samara Reck-Peterson, University of California, San Diego

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June 2016

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting on *Engineering Approaches to Bimolecular Motors: From in vitro to in vivo*.

Over the past several decades, scientists and engineers in fields ranging from nanotechnology to cell biology have contributed to our understanding of the basic physical principles and biological functions of energy-consuming macromolecular machines. This meeting is bringing together researchers from diverse fields who are developing novel ways of measuring and controlling biomolecular motors inside and outside of cells, synthesizing artificial molecular motors inspired by biology, harnessing motors for applications in devices, and developing theories that cut across biological and synthetic systems.

We hope that the meeting will not only provide a venue for sharing recent and exciting progress, but also promote discussions and foster future collaborations in the area of biomolecular motors. The conference offers a full program with 36 talks and 36 posters, bringing together well recognized scientists from different fields and 16 countries, promising a truly international and multidisciplinary inspiring environment. We also encourage you to take part in social and cultural activities available in beautiful Vancouver, British Columbia.

Thank you all for joining our Thematic Meeting. We look forward to seeing you in Vancouver!

Best regards,

The Organizing Committee

Zev Bryant Paul Curmi Nancy Forde Heiner Linke Samara Reck-Peterson

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GENERAL INFORMATION

Registration Location and Hours

On Tuesday, registration will be held during the reception at Steamworks Brewpub located at 375 Water Street, Vancouver. On Wednesday, Thursday, and Friday registration will be located outside of Fletcher Challenge Theatre at the Harbour Centre concourse at SFU. Registration hours are as follows:

Tuesday, June 14	18:00 – 21:00	Steamworks Brewpub
Wednesday, June 15	8:00 – 17:30	Harbour Centre
Thursday, June 16	8:00 – 17:30	Harbour Centre
Friday, June 17	8:00 – 17:30	Harbour Centre

Instructions for Presentations

(1) Presentation Facilities:

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

(2) Poster Sessions:

1) All poster sessions will be held in Segal Centre.

2) A display board measuring 243.8 cm (8 feet) wide by 121.9 cm (4 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 Wednesday and Thursday, but all posters will be available for viewing during both poster sessions.

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

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

Meals and Coffee Breaks

A reception at Steamworks Brewpub (Tuesday evening), coffee breaks at the Harbour Center (Wednesday, Thursday, Friday), and a banquet (Friday evening) are included in the registration fee.

The address of Steamworks Brewpub is listed below:
375 Water St
Vancouver, BC

Information regarding dinner cruise location will be provided at the registration desk.

Smoking

Please be advised that smoking is not permitted inside the Harbour Centre.

Name Badges

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

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from June 14-June 17 during registration hours.

In case of emergency, you may contact the following BPS staff/meeting organizer:

Erica Bellavia, BPS Staff
ebellavia@biophysics.org

Nancy Forde, Thematic Meeting Organizer
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Engineering Approaches to Biomolecular Motors: From in vitro to in vivo

Vancouver, Canada
June 14-17, 2016

PROGRAM

Tuesday, June 14, 2016

18:00 – 21:00	**Registration/Information**	**Steamworks Brewpub**
18:00 – 21:00	**Reception**	**Steamworks Brewpub**

Wednesday, June 15, 2016

8:00 – 17:30	**Registration/Information**	**Outside Fletcher Challenge Theatre**
9:00 – 9:15	**Welcome** Organizers	**Fletcher Challenge Theatre**
Session I	**Synthetic Motors I: DNA-based Walkers** **Nancy Forde, Simon Fraser University, Canada, Chair**	
9:15 – 9:30	Session Introduction - Nancy Forde	
9:30 – 10:00	Andrew Turberfield, University of Oxford, United Kingdom ***Molecular Machinery from DNA***	
10:00 – 10:30	Jong Hyun Choi, Purdue University, USA* ***A Synthetic DNA Motor that Transports Nanoparticles along Carbon Nanotubes***	
10:30 – 10:50	**Coffee Break**	**Harbour Centre Concourse**
Session II	**DNA-based Walkers and General Theory** **Nancy Forde, Simon Fraser University, Canada, Chair**	
10:50 – 11:05	Jason Wagoner, Stony Brook University, USA* ***The Nonequilibrium Statistical Thermodynamics of Biomolecular Motors***	
11:05 – 11:35	Zhisong Wang, National University of Singapore, Singapore ***Biomimetic Nanowalkers: a Nano-engineering Path to the General Science behind Motor Proteins***	
11:35 – 11:50	Jieming Li, University of Michigan, USA* ***Rapid Unbiased Transport by a DNA Walker***	
11:50 – 12:05	Katharine Challis, Scion, New Zealand* ***Discretizing the Fokker-Planck Equation for Energy Conversion in a Molecular Motor to Predict Physical Observables***	
12:05 – 12:20	**Flash talks from posters**	

**Contributed talks selected from among submitted abstracts*

12:20 – 13:50	**Lunch Break** (on own)
Session III	**Synthetic Motors II: Proteins, Peptides, and Supramolecular Chemistry** **Paul Curmi, University of New South Wales, Australia, Chair**
13:50 – 14:05	Session Introduction – Paul Curmi
14:05 – 14:35	Amar Flood, University of Indiana, USA ***Artificial Molecular Switches and Motors by Synthetic Design***
14:35 – 15:05	Roberta Davies, Victor Chang Cardiac Research Institute, Australia ***Construction of a Synthetic Protein Motor Using a Covalent Self-Assembly System***
15:05 – 15:35	Elizabeth Bromley, University of Durham, United Kingdom ***Conformational Switching as a Driving Force for Designed Motors***
15:35 – 16:00	**Coffee Break** **Harbour Centre Concourse**
Session IV	**Biological Molecular Motors I: Mechanochemistry and Structural Dynamics** **Zev Bryant, Stanford University, USA, Chair**
16:00 – 16:15	Session Introduction – Zev Bryant
16:15 – 16:45	Ryota Iino, National Institutes of Natural Sciences, Japan* ***Direct Observation of Intermediate States during the Stepping Motion of Kinesin-1***
16:45 – 17:15	Borja Ibarra, IMDEA Nanoscience, Spain* ***Mechanical Tension vs. Force: Different Ways to Control the Activities of Molecular Motors Working on DNA.***
17:15 – 17:30	**Flash talks from posters**
17:30 – 19:30	**Dinner** (on own)
19:30 – 21:30	**Poster Session I** **Segal Centre**

Thursday, June 16, 2016

8:00 – 17:30	**Registration/Information** **Outside Fletcher Challenge Theatre**
Session V	**Biological Molecular Motors II: Modification and Redesign of Biological Motors** **Zev Bryant, Stanford University, USA, Chair**
9:00 – 9:15	Session Introduction – Zev Bryant
9:15 – 9:45	Hiroyuki Noji, University of Tokyo, Japan ***Robustness of Catalysis and Torque-Transmission of F1-ATPase Learned from Engineering Approach***

**Contributed talks selected from among submitted abstracts*

9:45 – 10:15	Lawrence Lee, University of New South Wales, Australia ***Artificial Synthesis of the Bacterial Flagellar Motor***
10:15 – 10:30	**Flash talks from posters**
10:30 – 10:50	**Coffee Break** **Harbour Centre Concourse**
Session VI	**Biological Molecular Motors II: Modification and Redesign of Biological Motors *(continued)*** **Zev Bryant, Stanford University, USA, Chair**
10:50 – 11:20	Robert Cross, University of Warwick, United Kingdom ***Tweaking the Kinesin-Microtubule Interface***
11:20 – 11:50	Kristen Verhey, University of Michigan, USA ***Engineering Inhibitable Kinesin Motors***
11:50 – 12:20	Ken'ya Furuta, NICT, Japan* ***Creating Novel Biomolecular Motors Based on Dynein and Actin-binding Proteins***
12:20 – 13:50	**Lunch Break** (on own)
Session VII	**Biological Molecular Motors III: Organization and Control of Motor Collection in vitro** **Samara Reck-Peterson, University of California, San Diego, USA, Chair**
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14:05 – 14:35	Andrej Vilfan, J. Stefan Institute, Slovenia ***Translational and Rotational Motion of Coupled Motor Proteins***
14:35 – 15:05	Sivaraj Sivaramakrishnan, University of Minnesota, USA ***Cooperativity in Myosin Ensembles Revealed by DNA Nanotechnology Platforms***
15:05 – 15:20	Nathan Derr, Smith College, USA* ***Cargo Rigidity Affects the Sensitivity of Dynein Ensembles to Individual Motor Pausing***
15:20 – 15:35	**Flash talks from posters**
15:35 – 16:00	**Coffee Break** **Harbour Centre Concourse**
Session VIII	**Nanodevices I: Using Devices to Study Motor Function** **Heiner Linke, Lund University, Sweden, Chair**:
16:00 – 16:15	Session Introduction – Heiner Linke
16:15 – 16:45	Philip Collins, University of California, Irvine, USA ***All-Electronic, Single-Molecule Monitoring of the Processive Activity of DNA Polymerase I***

**Contributed talks selected from among submitted abstracts*

16:45 – 17:15	Jens Gundlach, University of Washington, USA ***Subangstrom Single-Molecule Measurements of Motor Proteins Using a Nanopore***
17:15 – 17:30	Cassandra Niman, University of California, San Diego, USA* ***Tools for Control and Observation of Synthetic Molecular Motors Using Micro- and Nanofluidics***
17:30 – 19:30	**Dinner** (on own)
19:30 – 21:30	**Poster Session II** **Segal Centre**

Friday, June 17, 2016

8:00 – 17:30	**Registration/Information** **Outside Fletcher Challenge Theatre**
Session IX	**Nanodevices II: Harnessing Motors to Perform Functions in Devices** **Heiner Linke, Lund University, Sweden, Chair**
9:00 – 9:15	Session Introduction - Heiner Linke
9:15 – 9:45	Stefan Diez, B CUBE, Technische Universität Dresden, Germany ***Application of Biomolecular Transport Systems for Optical Imaging***
9:45 – 10:15	Henry Hess, Columbia University, USA ***Engineering with Kinesin Motors***
10:15 – 10:30	Janet Paluh, SUNY Polytechnic Institute CNSE, USA* ***Manipulating Kinesin-Tubulin-MTOC Interactions for Engineering Polarized Networks***
10:30 – 10:50	**Coffee Break** **Harbour Centre Concourse**
Session X	**From Nanodevices to General Theory to Living Cells** **Heiner Linke, Lund University, Sweden, Chair**
10:50 – 11:20	Dan Nicolau, Jr., Molecular Sense, Ltd., United Kingdom ***Doing Maths with Autonomous Biological Agents***
11:20 – 11:50	Alf Månsson, Linnaeus University, Sweden ***Chemomechanical Models for the Rational Design and Studies of Engineered Molecular Motors***
11:50 – 12:05	David Sivak, Simon Fraser University, Canada* ***Efficient Molecular-Scale Energy Transmission***
12:05 – 12:35	Lene Oddershede, Niels Bohr Institute, Denmark ***Measuring Force and Viscoelasticity inside Living Cells***
12:35 – 13:50	**Lunch break** (on own)

**Contributed talks selected from among submitted abstracts*

Session XI	**Motors in Context I: Reconstituted Systems** **Paul Curmi, University of New South Wales, Australia, Chair**	
13:50 – 14:05	Session Introduction - Paul Curmi	
14:05 – 14:35	Kiyoshi Mizuuchi, NIDDK/NIH, USA ***Transportation of Artificial Cargos by the Par and Min Systems***	
14:35 – 15:05	Margaret Gardel, University of Chicago, USA ***Mechanics and Design of Active Matter Constructed from Actomyosin***	
15:05 – 15:35	Shin'ichi Ishiwata, Waseda University, Japan ***Self-organization of Active Cytoskeletal Networks in a Cell-sized Confined Space***	
15:35 – 15:50	Barbara Haller. Max Planck Institute for Intelligent Systems, Germany* ***Reconstitution of Actomyosin Cortex and Adhesion-associated Proteins in Droplet-based Synthetic Cells***	
15:50 – 16:15	**Coffee Break**	**Harbour Centre Concourse**
Session XII	**Motors in Context II: Physical Measurements and Synthetic or Chemical Approaches in Living Cells** **Samara Reck-Peterson, University of California, San Diego, USA, Chair**	
16:15 – 16:30	Session Introduction - Samara Reck-Peterson	
16:30 – 16:45	Amy Oldenburg, University of North Carolina at Chapel Hill, USA* ***Optical Coherence Tomography for Depth-resolved Imaging of Intracellular Motility in Mammary Epithelial Cell Organoids and Excised Tissue***	
16:45 – 17:00	Kathleen Bickel, Northwestern University, USA* ***Src Phosphorylation Regulates the Human Kinesin-5, Eg5, and Disrupts the Binding of Eg5 Inhibitors***	
17:00 – 17:30	Bianxiao Cui, Stanford University, USA ***Light-mediated Motor Activities Control Cargo Distributions in Cells***	
17:30 – 17:45	**Closing Remarks and *Biophysical Journal* Poster Awards**	
17:45 – 18:15	**Break to reconvene at dinner cruise**	
18:15 – 21:45	**Dinner cruise and conclusion of conference**	**Harbour Cruise**

**Contributed talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

Molecular Machinery from DNA

Andrew J. Turberfield.
University of Oxford, Oxford, United Kingdom.

By exploiting programmable, sequence-dependent base-pairing interactions it is possible to design and build three-dimensional DNA scaffolds, to attach molecular components to them with sub-nanometre precision – and then to make them move. I shall describe our work on autonomous, biomimetic molecular motors powered by chemical fuels, hybrid DNA-kinesin devices, motors that compute, and the use of synthetic molecular machinery to control covalent chemical synthesis.

A Synthetic DNA Motor that Transports Nanoparticles along Carbon Nanotubes

Jing Pan, **Jong Hyun Choi**.
Purdue University, West Lafayette, IN, USA.

Intracellular protein motors have evolved to perform specific tasks critical to the function of cells such as intracellular trafficking and cell division. Inspired by such biological machines, we demonstrate that motors based on RNA-cleaving DNAzymes can transport nanoparticle cargoes (CdS nanocrystals in this case) along single-walled carbon nanotubes. Our synthetic motors extract chemical energy from interactions with RNA molecules decorated on the nanotubes and use that energy to fuel autonomous, processive walking through a series of conformational changes along the one-dimensional track. However, their translocation kinetics is not well understood. In this work, the translocation kinetics of individual DNAzyme motors are probed in real-time using the visible fluorescence of the cargo nanoparticle and the near-IR emission of the carbon nanotube track. This visible/near-IR single-particle/single-tube spectroscopy allows us to examine the critical parameters in the motor design that govern the translocation kinetics, including DNA enzyme catalytic core type, upper and lower recognition arm lengths, and various divalent metal cations. Combined with spectroscopic single-motor measurements, a simple theoretical model, developed within the framework of stochastic single-molecule kinetics, describes the rates of individual intermediate reactions as well as the overall single turnover reaction. Our study provides general design guidelines to construct highly processive, autonomous DNA walker systems and to regulate their translocation kinetics, which would facilitate the development of functional DNA walkers.

The Nonequilibrium Statistical Thermodynamics of Biomolecular Motors

Jason A. Wagoner, Ken Dill,
Stony Brook University, Stony Brook, NY, USA.

Biomolecular motors operate through a complex sequence of transitions that transduce the chemical energy of nucleotide hydrolysis into work against some mechanical or chemical gradient. We use statistical physics to study motor operation. We integrate structural and dynamical information of molecular motors into this theory to understand the origins of fluctuations, dissipation, entropy production, etc. for these systems operating arbitrarily far from equilibrium. These analyses give insight into both biological mechanism and evolutionary design principles of molecular motors.

This presentation will discuss the difference between enthalpic driving forces (like the breaking of a high energy bond) and entropic driving forces (like a concentration gradient) for molecular motors. We show that motors can take large mechanical steps driven by enthalpic driving forces to operate not only faster but also more efficiently than a motor taking small steps. This gives an interesting perspective on the high-energy phosphate bond of ATP, the central driving force of nonequilibrium processes in the cell. We also discuss other characteristics of motor operation that are specific and fundamental to understanding small nonequilibrium systems: the role of fluctuations around mean behavior, the organization of conformational transitions, and the location and height of kinetic barriers. These characteristics have important consequences on performance metrics (power output, efficiency, etc.) of molecular motors.

Biomimetic Nanowalkers: a Nanoengineering Path to the General Science behind Motor Proteins

Zhisong Wang.
National University of Singapore, Singapore.

Motor proteins are a key player in enabling a great variety of biological functions from the molecular level to the cellular level, such as energy conversion, force generation in cell division, and long-range intracellular transport. It is a long-standing challenge to decipher the underlying science of these single-molecule motors and enabled functionalities at larger scale. Three strategies are being pursued thus far to tackle the challenge and harvest the science for biotechnology and nanotechnology. The first strategy is the heads-on biophysical study of the biological systems with ever new techniques of better spatial-temporal resolution. The second is a synthetic-biology strategy in which the biological systems are cast in a rationally engineered setup to mimic the original biological setup or extend for new bio/nanotechnological applications. In this talk, I shall discuss the third strategy that has a synthetic component too but shifted towards a thorough physical strategy. Here an entirely artificial nanomotor is invented following the lessons from biomotor study, and offers a parallel model system to study the same general science governing both biological and man-made nanomotors and related functionalities. As a specific example, I shall focus on our recent study of a new class of bioinspired bipedal DNA nanowalkers that capture some key aspects of cytoplasmic dyneins, especially a highly modularized construction and versatile regulation of directionality, speed and force generation. These rationally designed motors generate new insights to rationalize some biophysical findings of cytoplasmic dyneins, and also demonstrate a general modular design principle that potentially leads to many new track-walking nanomotors from simpler but widely reported switch-like nanodevices.

Rapid Unbiased Transport by a DNA Walker

Jieming Li[1], Alexander Johnson-Buck[4], Yuhe Renee Yang[2,3], Hao Yan[2,3], Nils Walter[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]Arizona State University, Tempe, AZ, USA, [3]Arizona State University, Tempe, AZ, USA, [4]Dana-Farber Cancer Institute, Boston, MA, USA.

Ever since the step-by-step movement of biomolecular motors such as myosin and kinesin super families was mechanistically characterized, attempts have been made to mimic their dynamic behavior in the form of synthetic molecular walkers. Several DNA-based molecular walkers have been synthesized, motivated by the long-term goal of controlling molecular transport processes with the programmability and structural robustness. Previous studies show that DNA walkers can walk directionally along a track upon sequential addition of a DNA strand as chemical "fuel". Despite this progress, the DNA walkers reported so far have been constrained by slow translocation rates, typically on the order of a few nm/min. By comparison, natural protein motors have translocation rates of ~1μm/s under saturating ATP conditions. It is desirable to reduce this gap if synthetic DNA walkers can serve as useful agents of molecular transport. Slow catalytic steps or slow release of cleavage products limits the translocation rate of many DNA walkers. In contrast, the displacement of one strand in a DNA duplex by another can be catalyzed by the nucleation of short single-stranded overhangs, or "toeholds", a process that can be very rapid when the reagents are present at high concentration. Here we report the design and single-molecule fluorescence resonance energy transfer characterization of a novel class of reversible DNA transporters that utilizes strand displacement mediated by toehold exchange. The fastest rate constant of stepping approaches 1 s^{-1}, which is ~100-fold higher than typical DNA-based transporters. We present evidence that the walking occurs by a rapid branch migration step followed by slower dissociation and rebinding of toehold sequences. While branch migration is rapid and may be treated as a rapid equilibrium process, the rate constant of stepping between adjacent track sites is dependent on the length of the associated toeholds.

Discretizing the Fokker-planck Equation for Energy Conversion in a Molecular Motor to Predict Physical Observables

Katharine J. Challis[1], Phuong Nguyen[1,2], Michael W. Jack[2].
[1]Scion, Rotorua, Bay of Plenty, New Zealand, [2]University of Otago, Dunedin, Otago, New Zealand.

Energy conversion in a molecular motor has been described in terms of Brownian motion on a free-energy surface. Free-energy surfaces for molecular motors such as F1-ATPase are emerging from single-molecule experiments and molecular dynamics simulations. Brownian motion on a free-energy surface is governed by a multidimensional Fokker-Planck equation that predicts physical observables. We have developed a suite of theoretical methods for systematically transforming the Fokker-Planck equation to simpler tractable discrete master equations. Our approach is to expand the Fokker-Planck equation in a localized basis of discrete states tailored to the free-energy potential surface. For periodic potentials with a single minimum and maximum per period we use a Wannier basis originally developed for quantum systems. For bichromatic potentials with multiple minima per period we generalize the Wannier basis to potentials with spatially fast- and slow-varying components. For more sophisticated potentials we expand in the lowest eigenstates of metastable approximations to the free-energy surface. The main benefits of our methods are that they take into account local details of the potential and make clear the validity regime of the discretization. We apply our methods to derive discrete master equations for a range of potential surfaces. This yields analytic expressions for the rate of thermal hopping between localized meta-stable states. We relate characteristics of the free-energy surface to physical observables including the drift and diffusion, the rate and efficiency of energy transfer, and single trajectories and hopping statistics.

Artificial Molecular Switches and Motors by Synthetic Design

Amar Flood.
University of Indiana, Bloomington, IN, USA.

Nature's biological motors and the engineered machines in our everyday world serve as inspirations for the creation of small-molecule systems that undergo controllable motion. That motion has historically relied upon the creation of molecules with simple moving parts, like, rings, rods, and rotors. The resulting synthetic systems have led to a plethora of molecular switches. These same switches now serve as the testing ground to consider more complex and synchronized motions needed for performing work. Yet, they must also reflect the operating principles seen in biology. To these ends, this talk will present the development of a class of voltage-driven molecular switches and outline a roadmap for its transformation into a molecular muscle. Our progress along that path will be described. Along the way, we also address interchangeable parts and the option to access Brownian ratchet motions.

Construction of a Synthetic Protein Motor Using a Covalent Self-Assembly System

Roberta B. Davies[1], Nancy R. Forde[2], Dek N. Woolfson[3], Heiner Linke[4], Paul M. Curmi[5].
[1]Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia, [5]University of NSW, Kensington, NSW, Australia.[2]Simon Fraser University, Burnaby, BC, Canada, [3]Bristol University, Bristol, United Kingdom, [4]Lund University, Lund, Sweden,

Nanotechnology is emerging as a powerful field in the attempt to harness nature's ability to work at the nanoscale through the use of protein machines. Working towards that end, considerable advances have been made in the construction of small molecule and DNA-based motors, however construction of synthetic protein based assemblies with motor properties is still in its infancy.
We present what is likely to be the first synthetic protein motor construction derived from non-motor protein components. These have been produced from DNA templates by expression in bacteria. Components have been designed to spontaneously self assemble into covalently linked branched protein structures capable of binding specific DNA sequences dictated by particular ligands.
Ultimately these assemblies will be tested for their ability to move along a DNA track bearing repeats of the specific DNA sequences required for binding by the protein modules. Movement will be controlled by supply of ligands using microfluidic devices.

Conformational Switching as a Driving Force for Designed Motors

Elizabeth Bromley[1], Lara Small[1], Asahi Cano-Marques[1], Richard Sessions[2], Martin Zuckermann[3].
[1]University of Durham, Durham, United Kingdom, [2]University of Bristol, Bristol, United Kingdom, [3]Simon Fraser University, Vancouver, BC, Canada.

Conformational switching is an important component of the operation of most biological motors, and can play a significant role in the processivity and speed with which such motors move. We have chosen to explore the function of nanoscale motors via the design of synthetic motors made from biomimetic components. One such motor design is the bar-motor, a bipedal motor designed to walk along an asymmetric DNA-based track. The motor comprises two ligand gated DNA binding domains linked by a coiled-coil segment. This motor would be expected to step randomly via the cyclic addition of ligands, however, processive motion can be encouraged via the introduction of a power stroke in which the conformation of the coiled coil is coupled to the ligand cycle.
In this work we use multiscale modelling to predict the function of a motor with the ascribed properties. We then explore the design of the coiled-coil region in relation to its ability to undergo conformational switching once coupled to a photosensitive azobenzene unit. We further present experimental data on the performance of various coiled-coil-azobenzene designs on exposure to light.

Direct Observation of Intermediate States during the Stepping Motion of Kinesin-1

Hiroshi Isojima[1], **Ryota Iino**[2,3], Yamato Niitani[1,4], Hiroyuki Noji[5], Michio Tomishige[1].
[2]National Institutes of Natural Sciences, Okazaki, Aichi, Japan, [1]The University of Tokyo, Bunkyo-ku, Tokyo, Japan, [3]The Graduate University for Advanced Studies (SOKENDAI), Hayama, Kanagawa, Japan, [4]The University of Tokyo, Bunkyo-ku, Tokyo, Japan, [5]The University of Tokyo, Bunkyo-ku, Tokyo, Japan.

The dimeric motor protein kinesin-1 walks along microtubules by alternatingly hydrolyzing ATP and moving two motor domains ("heads"). Nanometer-precision single-molecule studies demonstrated that kinesin takes regular 8-nm steps upon hydrolysis of each ATP; however, the intermediate states between steps have not been directly visualized. Here, we employed high-temporal resolution dark-field microscopy to directly visualize the binding and unbinding of kinesin heads to/from microtubules during processive movement. Our observations revealed that upon unbinding from microtubules, the labeled heads displaced rightward and underwent tethered diffusive movement. Structural and kinetic analyses of wild-type and mutant kinesins with altered neck linker lengths provided evidence that rebinding of the unbound head to the rear-binding site is prohibited by a tension increase in the neck linker, and that ATP hydrolysis by the leading head is suppressed when both heads are bound to microtubule, thereby explaining how the two heads coordinate to move in a hand-over-hand manner.

Mechanical Tension vs. Force: Different Ways to Control the Activities of Molecular Motors Working on DNA.

Borja Ibarra[1], Jose Morin[1], Francisco J. Cao[2], Margarita Salas[3].
[1]IMDEA Nanoscience, Madrid, Spain, [2]Universidad Complutense de Madrid, Madrid, Spain, [3]Centro Biologia Molecular-Severo Ochoa (CBMSO-CSIC), Madrid, Spain.

Single molecule force spectroscopy approaches have proven useful in studying the real time kinetics and mechano-chemical processes governing the operation of molecular motors. Mechanical force perturbs the interactions of the motor protein with its track and modulates the rates of the steps of the reaction located along the force application coordinate. Using optical tweezers and the multifunctional Phi29 DNA polymerase (a hybrid polymerase-helicase), we show how different pulling geometries on a single polymerase-DNA complex modulate the rate of DNA synthesis by acting on different steps of the reaction cycle:

1. Mechanical tension applied longitudinally along the DNA track modulates the equilibrium between the synthetic (pol) and degradative (exo) activities of the polymerase. Tension promotes the intramolecular transfer of the DNA primer strand from the pol to the exo active sites in a similar way to the incorporation of a mismatched nucleotide.
2. Tension applied to the ends of the complementary strands of a DNA hairpin favours the mechanical unwinding of the DNA duplex and modulates the coupling between the DNA synthetic and unwinding reactions of the polymerase.
3. Mechanical force, or load, applied directly on the polymerase acts specifically on the step of the reaction coupled to directional motion of the protein along the DNA and can be used as a variable to determine the coupling mechanism between chemical and mechanical energy during the DNA replication reaction.

Our results reveal that changing the pulling geometries on a single motor-track complex (polymerase-DNA) provides well-defined reaction coordinates to modify and quantify the kinetic rates, equilibrium constants and conformational changes of the steps of the reactions responsible for the motor operation.

Robustness of Allostery and Torque-transmission of F1-ATPase Learned from Engineering Approach

Hiroyuki Noji.
University of Tokyo, Japan.

F1-ATPase is a rotary motor protein in which the inner subunit rotates against the surrounding stator ring upon ATP hydrolysis. The stator ring is composed of 3 alpha and 3 beta subunits, and the catalytic reaction centers are located on the 3 alpha-beta interfaces, mainly on the beta subunits. The unique feature of F1-ATPase that discriminates F1-ATPase from other molecular motors is the high energy conversion efficiency and the reversibility of the chemomechanical coupling; when the rotation is forcibly reversed, F1-ATPase catalyzes ATP synthesis reaction against large free energy of ATP hydrolysis. The experimental verification that the rotary angle of the rotary shaft controls the chemical equilibrium of ATP hydrolysis/synthesis was thought to suggest that the 3 reaction centers communicate via the atomically fine-tuned molecular interaction of the beta subunits with the rotary shaft subunit. However, recent experiments showed the rotation mechanism is far more robust than we thought before; even after removing the rotary shaft, the remaining stator ring undergoes cooperative power stroke motion among 3 beta subunits (Uchihashi et al. Science 2013). This finding suggests that the allostery is programmed in the stator ring, pointing the possibility that an artificial rod-shaped molecule would be rotated in the stator ring of F1-ATPase. We tested this hypothesis by incorporating a xenogeneic protein in the stator ring. The artificial molecule showed unidirectional rotation although the generated torque is evidently lower than the wild-type F1-ATPase (Iwamoto et al. unpublished data).

Artificial Synthesis of the Bacterial Flagellar Motor

Lawrence Lee.
University of New South Wales, Sydney, Australia.

Large protein complexes assemble spontaneously, yet their subunits do not prematurely form unwanted aggregates. This paradox is epitomized in the bacterial flagellar motor, a sophisticated rotary motor and sensory switch consisting of hundreds of subunits. Here we demonstrate that FliG from Escherichia coli, one of the first motor proteins to assemble, forms ordered ring structures via domain-swap polymerization, which in other proteins has been associated with uncontrolled and deleterious protein aggregation. Solution and crystal structural data, in combination with in vivo biochemical crosslinking experiments and evolutionary covariance analysis, reveal that FliG exists predominantly as a monomer in solution but only as domain-swapped polymers in assembled flagellar motors. We propose a general structural and thermodynamic model for self-assembly, where a structural template controls assembly and shapes polymer formation into rings. We will then discuss our approach to artificially construct the flagellar motor on synthetic DNA scaffolds.

Tweaking the Kinesin-Microtubule Interface

Daniel R. Peet[1], Nigel Burroughs[2], **Robert A. Cross**[1].
[1]Warwick Medical School, Coventry, United Kingdom, [2]Warwick University, Coventry, United Kingdom.

The kinesin binding site lies entirely within a single tubulin heterodimer, and for some kinesins, unpolymerised tubulin heterodimers can fully activate the kinesin ATPase. Other kinesins require that tubulin assemble into microtubules before it can activate their ATPase - for example, the classical combination of brain tubulin and brain kinesin is like this. These behaviours show that kinesin sensitively reads the conformation of its binding site on tubulin. We have asked the obvious question, can motile kinesins feedback on the conformation of tubulin and on microtubule dynamics? We find that strong-state kinesin motor domains (apo or AMPPNP states of kinesin-1, or a rigor mutant, T93N) can dramatically alter microtubule dynamics. The action of kinesin-13 (MCAK) to destabilise the GTP-caps of dynamic microtubules is familiar; we find that strong-state binding of the kinesin-1 motor domain to microtubules has close to the opposite effect, stabilising the GDP-lattice against disassembly. GDP-microtubules are ordinarily extremely unstable and depolymerise endwise at ~200 heterodimers per second, via the unzipping and disassembly of curved GDP-protofilaments. The binding of strong-state kinesins reduces the off-rate of GDP-tubulins from the shrinking tip to <2 per second. The stabilising action of the kinesin and the disassembly rate of the microtubules can be controlled by titrating in GDP so that some of the kinesins are switched into weak binding. Further, if we anchor GDP-microtubules by their ends in a flow cell, and introduce strong-state kinesin motor domains via hydrodynamic flow, the microtubules bend in the flow, and kinesin binding locks their curvature. We speculate that kinesin does this by binding preferentially to the convex side of curved microtubules, stabilising thereby the increased lattice spacing and preventing recoil. With our cryoEM collaborators, Carolyn Moores and Ottilie von Loeffelholz, we are currently exploring whether kinesin binding can alter the subunit spacing of various microtubule lattices.

Engineering Inhibitable Kinesin Motors

Kristen Verhey.
University of Michigan, Ann Arbor, MI, USA.

The human genome encodes 45 kinesin motor proteins that drive cell division, cell motility, intracellular trafficking, and ciliary function. Determining the cellular function of each kinesin would benefit from specific small molecule inhibitors. However, screens have yielded only a few specific inhibitors. Here we present a novel chemical-genetic approach to engineer kinesin motors that can carry out the function of the wildtype motor yet can also be efficiently inhibited by small, cell-permeable molecules. Using kinesin-1 as a prototype, we developed two independent strategies to generate inhibitable motors, and characterized the resulting inhibition in single molecule assays and in cells. We further applied these two strategies to create analogously inhibitable kinesin-3 motors. These inhibitable motors will be of great utility to study the functions of specific kinesins in a dynamic manner in cells and animals. Furthermore, these strategies can be used to generate inhibitable versions of any motor protein of interest.

Creating Novel Biomolecular Motors Based on Dynein and Actin-binding Proteins

Akane Furuta[1], Kazuhiro Oiwa[1,2], Hiroaki Kojima[1], **Ken'ya Furuta**[1].
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Biomolecular motors have the potential to be used as molecular-scale actuators, switches, and robots in nanoscale devices. Protein engineering is a key technology for such applications; however, it is currently not possible to design a novel biomolecular motor from scratch. Here, we present an alternative strategy where the existing functional modules (as protein building blocks) are combined through protein engineering techniques such as domain swapping and circular permutation to create a new series of biomolecular motors. According to the new bottom-up strategy, we successfully created novel actin-based motors; we show that the hybrid motors — combinations of a motor core derived from the microtubule-based dynein motor and non-motor actin-binding proteins — robustly drive the sliding movement of an actin filament. Furthermore, the direction of actin movement is reversible by simply changing the geometric arrangement of these building blocks. Our results emphasize that our strategy is useful for rapidly obtaining biomolecular motors with desired properties. At the same time, the new strategy combined with structural studies can be a powerful tool to investigate the design principles of biomolecular machines.

Translational and Rotational Motion of Coupled Motor Proteins

Andrej Vilfan.
J. Stefan Institute, Ljubljana, Slovenia.

The dynamics of groups of coupled motor proteins is interesting both due to its relevance for cargo transport in vivo and as a possibility to study motor features that are not accessible in single molecule experiments. But the relationship between collective properties of motor ensembles and those of individual motors is often not straightforward.

We will first look at pairs of myosin-V motors, coupled through an elastic linkage. The randomness of their motion leads to a buildup of tension, which in turn slows the pair down. It also leads to an increased detachment rate of the motors. The run length of a pair is longer than that of a single motor, but the enhancement is surprisingly small (~50%). This has important implications for the nature of detachment events.

In the second part we investigate the working stroke of kinesin-14 (ncd) motors based on ensemble measurements in a gliding motility assay with simultaneous recording of translational and rotational motion of microtubules as a function of ATP and ADP concentrations. The measured velocity is zero below a threshold [ATP] and increases abruptly above that concentration. The rotational pitch also depends on the ATP concentration and is shortest at low [ATP]. Combined with a simple mechanical model, both findings indicate that the main power stroke of kinesin-14 takes place after ATP binding and that each step also comprises a small off-axis component. By fitting the measured data with the model solution we show that the working stroke starts with a small movement of the motor's stalk in lateral direction when ADP is released. When ATP binds it is followed by a second, main stroke with a primarily longitudinal direction and a small lateral component in a direction that is opposite to the initial lateral step.

Cooperativity in Myosin Ensembles Revealed by DNA Nanotechnology Platforms

Sivaraj Sivaramakrishnan
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No Abstract

Cargo Rigidity Affects the Sensitivity of Dynein Ensembles to Individual Motor Pausing

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Cytoplasmic dynein is a minus-end directed microtubule-based motor protein that drives intracellular cargo transport in eukaryotic cells. While many intracellular cargos are propelled by small groups of dynein motors, many of the biophysical mechanisms that govern ensemble motility remain unknown. We have designed a programmable DNA origami synthetic cargo "chassis" that allows us to control the number of dynein motors in the ensemble and vary the rigidity of the cargo chassis itself. On this chassis, motors within an ensemble are conjugated together through variable length cargo "linkers" comprised of parallel segments of either single- or double-stranded DNA. These regions determine the number of independent steps each motor can take before exerting forces on the other motors within the ensemble. This design enables investigation of how motor steps and pauses are "communicated" through the cargo structure and how they affect the emergent behavior of the ensemble. Using TIRF microscopy, we have observed dynein ensembles transporting these cargo chassis along microtubules in vitro. We find that ensembles of dynein on flexible cargos move faster as more motors are added, whereas ensembles on rigid cargos move slower as more motors are added. Using the slowly-hydrolyzable ATP analog ATP-gamma-S, we have observed that ensembles connected through flexible cargos are less sensitive to individual motor pausing. Our results suggest that the role of cargo rigidity in the communication of motor pausing plays an important role in determining the collective motility of dynein motor ensembles. The ability for cargos propelled by dynein ensembles to maintain their motility despite the pausing of individual motors may allow the cargo to maintain productive transport regardless of pauses induced by single motors encountering obstacles on the microtubule.

All-Electronic, Single-Molecule Monitoring of the Processive Activity of DNA Polymerase I

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Nanoscale electronic devices like field-effect transistors have long promised to provide sensitive, label-free detection of biomolecules and their activity. In particular, single-walled carbon nanotube transistors have the requisite sensitivity to monitor single molecule events, and they have sufficient bandwidth to directly monitor single molecule dynamics in real time.

Recent measurements have successfully demonstrated this premise by monitoring the dynamic, single- molecule processivity of three different enzymes: lysozyme [1,2], protein Kinase A [3], and the Klenow fragment of DNA polymerase I [4,5]. With all three enzymes, single molecules were electronically monitored for 10 or more minutes, allowing us to directly observe rare transitions to chemically inactive and hyperactive conformations. The high bandwidth of the nanotube transistors further allow every individual chemical event to be clearly resolved, providing excellent statistics from tens of thousands of turnovers by a single enzyme. Besides establishing values for processivity and turnover rates, the measurements revealed variability, dynamic disorder, and the existence of intermediate states.

This presentation will focus on this new single-molecule technique as it has been applied to the catalytic cycle of DNA polymerase I incorporating nucleotides into single-stranded DNA templates [4,5]. The nanotube transistor technique observes the binding and processing of individual template molecules with base-by-base precision. After processing as few as 10 template molecules, template length has been correctly determined with <1 base pair resolution, even in the presence of short tandem repeat motifs and in solutions containing mixtures of templates. Unique electrical signals generated during the accommodation and incorporation of certain nucleotide analogs reveal the transistor's sensitivity to slight conformational changes and suggest new strategies for all-electronic DNA sequencing.

[1] Y. Choi et. al., Science **335** 319 (2012). [2] Y. Choi et. al., JACS **134** 2032 (2012). [3] P. Sims et. al., JACS **135** 7861 (2013). [4] T. Olsen et. al., JACS **135** 7855 (2013). [5] K. Pugliese et. al, JACS **137** 9587 (2015).

Subangstrom Single-Molecule Measurements of Motor Proteins Using a Nanopore

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We have developed a high-resolution nanopore sensor to study enzyme activity with unprecedented positional and temporal sensitivity. In this new method, single stranded DNA (or RNA) that is bound to an enzyme is drawn into the nanopore by an applied electrostatic potential. The single stranded DNA passes through the pore's constriction until the enzyme comes into contact with the pore. Further progression of the DNA through the pore is then controlled by the enzyme. The pore we use is an engineered version of the protein pore MspA in which nucleotides of the DNA strongly affect the ion current that flows through the pore's constriction. Analysis of this ion current indicates the precise position of the DNA and thereby provides a real-time record of the enzyme's activity. The motion of DNA can be measured on millisecond time scales with a position resolution as small as ~40 picometers, while simultaneously providing the DNA's sequence within the enzyme. We demonstrate the extraordinary potential of this new single molecule technique on a Hel308 helicase, where we observe two distinct sub-states for each nucleotide processed. One of these about half-nucleotide long steps is ATP-dependent and the other is ATP-independent. The spatial and temporal resolution of this low-cost single molecule technique allows exploration of hitherto unobservable enzyme dynamics in real-time.

Tools for Control and Observation of Synthetic Molecular Motors using Micro- and Nanofluidics

Cassandra S. Niman[1], Martin J. Zuckermann[2], Martina Balaz[3,4], Andrew Hudson[5], Kara Van Aelst[6], Jason P. Beech[3,4], Jonas O. Tegenfeldt[3,4], Paul M G. Curmi[7,8], Dek N. Woolfson[6,9], Mark Dillingham[6], Nancy R. Forde[2], Heiner Linke[3,4].
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This project aims to develop synthetic motors based on proteins and protein-DNA interactions. Specifically, we have developed two approaches to synthetic molecular motors that each isolate certain mechanisms for stepping observed in natural motors [1,2], namely rectification of diffusion and a power stroke. For both designs, the processivity of the motor is critically dependent on the coordination of feet-to-track binding. To this end, we induce coordinated binding between repressor proteins and DNA via changes of the ligands in solution surrounding the motors. We have fabricated and tested micro- and nanofluidic devices allowing for precise control over solute changes in the channels and simultaneous observation of motor movement via standard microscopy techniques [2,3]. These devices are designed either to minimize external forces due to fluid flow, or to enable testing of the motors' performance against a load force. With a natural helicase motor, we have used a similar experimental scheme to demonstrate control of the motor motion via addition and withdrawal of the fuel supply, ATP, in the surrounding solution. Using total internal reflection fluorescence microscopy, we have monitored single helicase motors as they move and halt along DNA tracks.

The micro- and nanofluidics, in combination with single molecule detection, set the stage for future measurements of synthetic molecular motors. These fluidic devices have a number of other potential applications, as they enable the chemical environment surrounding single molecules to be changed whilst causing minimal disturbance to these.

[1] Bromley et al. 2009, HFSP Journal, 3:204.
[2] Niman et al. 2014, Nanoscale, 6(24):15008.
[3] Niman et al. 2013, LabChip, 13:2389.

Application of Biomolecular Transport Systems for Optical Imaging

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B CUBE - Center for Molecular Bioengineering, Technische Universität Dresden, Dresden, Germany.

Our lab is interested in the development and the application of novel optical techniques to investigate molecular transport in cell biology and nanotechnology. Building on our experience in single molecule biophysics and in the in vitro reconstruction of subcellular mechano-systems we study cooperative effects in motor transport and cell motility. Moreover we aim to apply biomolecular motor systems in synthetic, engineered environments for the generation, manipulation and detection of nanostructures. Towards this end, the talk will focus on how kinesin-propelled microtubules can be used as probes to measure nanoscopic surface topographies and optical near fields. In contrast to many alternative methods, microtubules possess the advantages that they can be applied in a highly parallel manner and that they do not alter the fields to be measured.

Engineering with Kinesin Motors

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Motor proteins, such as kinesin, can serve as biological components in engineered nanosystems. A proof-of-principle application is a "smart dust" biosensor for the remote detection of biological and chemical agents, which is enabled by the integration of recognition, transport and detection into a submillimeter-sized microfabricated device. The development of this system has revealed a number of challenges in engineering at the nanoscale, particularly in the guiding, activation, and loading of kinesin-powered molecular shuttles. Overcoming these challenges requires the integration of a diverse set of technologies, illustrates the complexity of biophysical mechanisms, and enables the formulation of general principles for nanoscale engineering. Molecular motors also introduce an interesting new element into self-assembly processes by accelerating transport, reducing unwanted connections, and enabling the formation of non-equilibrium structures. The formation of nanowires and nanospools from microtubules transported by kinesin motors strikingly illustrates these aspects of motor-driven self-assembly. Our most recent work aimed to create a molecular system that is capable of dynamically assembling and disassembling its building blocks while retaining its functionality, and demonstrates the possibility of self- healing and adaptation. In our system, filaments (microtubules) recruit biomolecular motors (kinesins) to a surface engineered to allow for the reversible binding of the kinesin motors. These recruited motors perform the function of propelling the microtubules along the surface. When the microtubules leave the kinesin motors behind, the kinesin track can either disassemble and release the motors back into solution with the possibility of being reassembled into another track, or recruit other microtubules onto itself, reinforcing the track and thus creating a molecular 'ant trail'. We show that this allows for more efficient use of the molecular building blocks, and demonstrate that this system is defect tolerant, self-healing, and adaptive.

Manipulating Kinesin-Tubulin-MTOC Interactions for Engineering Polarized Networks

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In the semiconductor industry, photolithography through a reticle generates mesoscale and larger fixed patterns in contrast to the dynamic self-assembling and adaptable systems in Nature. To advance novel nanomanufacturing strategies we are interested in generating dynamic mesoscale self-assembling patterns by the ability to control polarized networks in vitro utilizing Kinesin-Tubulin and Microtubule organizing center (MTOC) linkages. Here we define the MTOC as the g-tubulin ring complex (g-TuRC) that nucleates microtubules and in vivo attaches to the mammalian centrosome or fission yeast spindle pole body. To fulfill our goal we biochemically purified MTOC g-TuRC from fission yeast and mammalian cells, demonstrated cross-compatibility in vivo of g-TuRC and Kinesin-14 and Kinesin-5 proteins from human to yeast, established in vitro nucleation assays from g-TuRC, and identified and minimally isolated Kinesin control elements for regulation of g-TuRC allowing us to separate MTOC control from Kinesin-tubulin roles. We are experimenting with nanoprinting of the g-TuRC through antibody tethers and designing photoactivatable microtubule connectors based on known sequences from Kinesin-14's and non-motor microtubule associated proteins, MAPs. Printing capture molecules-antibodies, peptides, proteins or g-TuRC- is accomplished using PDMS stamps with 3um pillar features at 3um spacing. The substrate on which they are printed is silanized to facilitate surface bonding. The printing of g-TuRC into desired patterns is a critical step towards setting polarity points from which to anchor self-assembling networks. We are validating printing by immunofluorescence probing and have patterned antibodies, peptides, and WCE and FPLC purified g-TuRC, and antibody orienting protein A-Fc tethers. The next step is to optimize nucleation control from tethered g-TuRC. The introduction of g-TuRC to in vitro kinesin-tubulin assays represents an important next generation step that extends previous elegant studies by numerous groups in an effort to more fully exploit self-assembling systems.

Doing Maths with Autonomous Biological Agents

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Electronic computers are very good at performing a high number of operations at very high speeds - one-at-a-time. As a result, they struggle with combinatorial tasks that can be only be practically solved if many operations are performed in parallel. This has led to ideas about harnessing the parallelism inherent in biological signal processing for producing a new kind of computing system, such as DNA and molecular computing. In recent work, we presented a proof-of-concept for a parallel molecular-motor driven computer that solves a classic "combinatorial" NP-complete problem - Subset Sum. The device consists of a specifically designed, nanostructured network explored by a large number of molecular-motor-driven, protein filaments. This system is highly energy efficient, avoiding the heating issues limiting electronic computers. In this talk, we will discuss the potential and the challenges involved in developing this kind of technology and how it might be able to tackle problem classes that vex existing computational devices

Chemomechanical Models for the Rational Design and Studies of Engineered Molecular Motors

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Molecular motors and cytoskeletal filaments may be genetically and/or chemically engineered for external modulation and controlled switching of their properties, e.g. the direction of filament transport by the motors. In addition to forming the basis for entirely new nanotechnological applications, such engineering opens for functional insights beyond those obtainable in conventional biophysical studies. In order to aid insights and design, a new chemomechanical modelling approach is here reported that connects kinetic and elastic properties of the motors with observable variables such as force, velocity and direction of motion of motor propelled filaments (1). The model performance is illustrated by Monte-Carlo simulations of force-, and motion-generation by the actin-myosin motor system of muscle. Using independent parameter values from published data, it is shown that a given model accounts well for experimental observations of force-, and motion-generation from single molecules to the large motor ensembles of muscle. It is further demonstrated how the model may be modified to simulate changes in actin-myosin function achievable by genetic or chemical engineering. This includes changes in kinetics or motor elasticity with effects on sliding velocity and/or processivity as well as changed power-stroke direction with altered transportation direction. Finally, a strategy is described for combining modelling of motor properties with simulation of filament elasticity and prediction of motor propelled filament paths on flat, as well as nanostructured surfaces. To conclude, it is shown that one given model accounts for the function of muscle actomyosin from single molecules to the large ensembles in muscle cells. Furthermore, the usefulness of this model in aiding design and studies of engineered myosins is demonstrated.

(1) Mansson, A. (2010). Biophys. J. 98:1237-1246.

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Efficient Molecular-scale Energy Transmission

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Given the centrality of energy transmission in the function of molecular motors, it seems plausible that evolution has sculpted these rapid-turnover machines to efficiently transmit energy in their natural contexts, where stochastic fluctuations are large and nonequilibrium driving forces are strong. But what are the physical limits on such nonequilibrium efficiency? And what machine designs would actually achieve these limits? Toward a systematic picture of efficient stochastic nonequilibrium energy transmission, I address two related fundamental questions in nonequilibrium statistical mechanics: How do we predict the response of molecular-scale soft-matter systems to rapid nonequilibrium driving? And how do we identify the driving that most efficiently (yet rapidly) carries such a noisy system from one state to another? These abstract theoretical considerations have immediate consequences for the design of single-molecule biophysical experiments and molecular simulations, and nontrivial yet intuitive implications for the design principles of molecular-scale energy transmission out of equilibrium, which I illustrate through application to simple model systems.

Measuring Force and Viscoelasticity inside Living Cells

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Niels Bohr Institute, Copenhagen, Denmark.

Much progress has been done in understanding the action of single molecules in vitro, where single parameters affecting the system can be tested one at a time. However, results obtained in vitro face the criticism that the conditions are too far from being physiologically relevant. Therefore, it is important to take the next step, namely to investigate the systems under in vivo conditions, inside the living organism. Optical tweezers are excellent tools for such investigations, because they allow for quantitative exploration of biological systems, both at the level of the single molecule, the cell, and the whole organism [1]. An optical trap can be used to measure forces and distances, however, such measurements are not trivial inside the living cell as the cytoplasm is viscoelastic and the normal calibration procedures, which rely on a purely viscous environment, cannot be used. Instead, in order to reliably measure forces inside a living cell, a combination of active and passive calibration must be employed. In the talk, an active-passive calibration procedure inside living cells will be demonstrated [2] and there will be examples of how to perform forces measurements and how to determine the viscoelastic landscapes inside living cells. Among these results, focus will be on embryonic stem cells, where optical tweezers measurements show that the mechanical properties of the cells' cytoplasm correlate with the cells' stage of differentiation. Also, there will be measurements of the force generated by filopodia while cells interact with their surroundings, this traction force being mediated by polymerization and helical buckling of actin inside the protruding filopodia of living cells [3].

[1] Oddershede, Nature Chemical Biology 8, 879 (2012)
[2] Mas et al., Physical Biology 10, 046006 (2013)
[3] Leijnse et al., PNAS 112, 136 (2015)

Transportation of Artificial Cargos by the Par and Min Systems

James A. Taylor[1], Anthony G. Vecchiarelli[1], Keir C. Neuman[2], **Kiyoshi Mizuuchi**[1].
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Spatial organization and active transportation of cellular contents are essential for cellular division. This is true even in bacteria, which were once thought too simple to require much internal organization. The ubiquitous deviant Walker ATPase family of proteins has been shown to be involved in systems important for spatial coordination in bacteria, the two most highly studied examples of which are the DNA partitioning Par system and the divisome positioning Min system. Both these systems contain the eponymous deviant Walker A ATPase (ParA or MinD), a protein which stimulates the ATPase's hydrolysis activity (ParB or MinE) and a cargo to be transported or localized (the "centromere" DNA site cluster bound by ParB or the divisome inhibitor MinC bound to MinD). Despite the similarities of their components these systems appear to behave quite differently in vivo: the Par system segregates large clusters of ParB bound DNA cargo into sister cells during division by displacing ParA non-specifically bound to the nucleoid whereas the Min proteins bind the inner membrane and are seen to oscillate from one cell pole to the other. These differences may arise due to the nature of the binding surface (DNA vs lipid) or the nature of the cargo (a large cargo bound by a large number of stimulator protein molecules in Par vs small protein molecules individually bound to membrane-bound MinD dimers). Here we use TIRF microscopy demonstrating that the Min system is capable of transporting a magnetic bead (a large cargo) along a supported lipid bilayer in a manner strikingly similar to our previous reconstitution of the Par system. This demonstrates that despite their clear differences in their system dynamics under conditions for their natural functions, Par and Min systems operate based on common underlying mechanistic principles.

Mechanics and Design of Active Matter Constructed from Actomyosin

Margaret Gardel
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No Abstract

Self-organization of Active Cytoskeletal Networks in a Cell-sized Confined Space

Shin'ichi Ishiwata, Makito Miyazaki, Masataka Chiba, Kazuya Suzuki.
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Biomolecular machines generally fulfil their functions within a cell, which is a microscopic space confined by a deformable cell membrane. Among the biomolecular machines, bio-motile systems composed of molecular motors and cytoskeleton (actin filaments and microtubules) are exceptional in the sense that they produce force to change the cell shape. Conversely, the dynamics of bio-motile systems will be influenced by the size and shape of cell, and the physical properties of the cell membrane. To understand the relationship between the structure/function of the bio-motile systems and the physical properties of cell membrane, i.e., to understand the role of cell membrane as a boundary condition for the dynamics of bio-motile systems, we have examined how the spatio-temporal dynamics of the bio-motile systems are realized within a cell-sized space confined by a phospholipid membrane. In the present talk, two to three topics will be introduced. The first topic will be the demonstration that polymerization of actin filaments inside a cell-sized water-in-oil droplet surrounded by a monolayer phospholipid membrane induces spontaneous formation of a ring-shaped contractile actin bundle in the presence of appropriate amount of myosin molecules and bundling factors. This strongly suggests that the spherical boundary condition promotes the formation of contractile ring. The second topic will be that when cytoplasmic extracts prepared from Xenopus eggs are encapsulated in a droplet, microtubule network induced accumulation of cellular components and rotational cytoplasmic flow, depending on the level of dynein activity. Those results were obtained in a cell-sized confinement surrounded by a monolayer of phospholipids self-organized in oil. When those phenomena are reproduced in a giant unilamellar liposome prepared by the inverted emulsion method, the present experimental system is expected to be useful for designing a model system of artificial cells.

Reconstitution of Actomyosin Cortex and Adhesion-associated Proteins in Droplet-Based Synthetic Cells

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Cellular interactions with the extracellular matrix or other cells are involved in nearly every cellular response in vivo. These responses, in turn, affect many facets of cell's life including directional migration, cell proliferation, differentiation, survival and gene expression. Therefore, adhesion/interaction-mediated processes are playing a crucial role in physiological conditions as well as in the regulation of a wide variety of disease states. To understand the complex interplay of different extracellular and intracellular factors bottom-up synthetic approaches have become increasingly important in modern cell biology. Towards this end, lot of efforts have been focused on creation of protocell systems that are on the one hand mechanically and chemically stable and easy to manipulate, and on the other hand possessing biologically relevant features in a reduced molecular complexity.
In the lecture we will describe our bottom-up synthetic approaches to dissect complex cellular adhesion-mediated processes by means of an automated, high-throughput droplet-based microfluidic technology. Towards this end, water-in-oil nanostructured emulsion droplets[1,2] were developed as cell-like compartments and the droplet-based microfluidic systems[3] were implemented for precise delivery of cytoskeletal and adhesion-associated proteins. Similarly to living cells, but with reduced molecular complexity, droplet-based cells showed the capability to self-assemble different cytoskeletal networks and adhesion associated proteins. Furthermore, as a consequence of the organization of an actomyosin network, droplet-based protocells showed migration and self-propulsion. This developed protocell system has a strong potential to contribute to the understanding of mechanisms underlying the ability of cells to perform "intelligent" missions, such as acquiring, processing and responding to environmental information.

Optical Coherence Tomography for Depth-resolved Imaging of Intracellular Motility in Mammary Epithelial Cell Organoids and Excised Tissue

Amy Oldenburg[1,2,3], Xiao Yu[1], Liza Makowski[3], Melissa Troester[3].
[1]University of North Carolina at Chapel Hill, Department of Physics & Astronomy, Chapel Hill, NC, USA, [2]University of North Carolina at Chapel Hill, Department of Biomedical Engineering, Chapel Hill, NC, USA, [3] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Optical Coherence Tomography (OCT) is a method for real-time, depth resolved imaging with micrometer-scale resolution. OCT employs low-coherence interferometry which enables imaging several mean-free scattering path lengths into tissues and 3D cell cultures (typically >1 mm). Here we use OCT to study the intracellular motility of mammary epithelial cell (MEC) organoids grown in 3D tissue culture, as well as freshly excised mouse mammary tissue. This motility is apparent as a speckle fluctuation in OCT corresponding to nanoscale motions of light scattering cellular features. The power spectrum of the observed speckle fluctuations exhibits 1/f power law scaling over the range from 0.01 – 1 Hz, and is notably inconsistent with the Lorentzian profile expected for Brownian motion.

We studied changes in the apparent motility of an MEC line (MCF10DCIS.com) in 3D culture longitudinally (1hr – 6 days) after exposure to a variety of substances: doxorubicin and taxol (chemotherapeutics), estrogen (a hormone), and blebbistatin (a myosin II inhibitor). Significant changes in the motility spectra compared to control were found ($p<0.01$). Furthermore, freshly excised mouse mammary tissue displayed significantly different motility spectra from formalin-fixed ($p<0.001$).

OCT provides a new capability for visualizing the amplitude and fluctuation spectrum of intracellular motility over the entire cross-section of an organoid or tissue. Future experiments aim for a better understanding of subcellular components, including molecular motors, giving rise to the observed non-Brownian speckle fluctuations. This represents a novel tool for understanding changes in nonequilibrium cell motion in organized tissues.

Src Phosphorylation Regulates the Human Kinesin-5, Eg5, and Disrupts the Binding of Eg5 Inhibitors

Kathleen G. Bickel[1], Joshua S. Waitzman[1], Barbara Mann[2], Melissa C. Gonzalez[1], Patricia Wadsworth[2], Sarah E. Rice[1].
[1]Northwestern University, Chicago, IL, USA, [2]University of Massachusetts-Amherst, Amherst, MA, USA.

The human kinesin-5 motor, Eg5, is required to establish and maintain the mitotic spindle in organisms ranging from yeast to humans. Several phosphoregulatory mechanisms tightly control the localization and activity of Eg5 during mitosis. Phosphorylation of the Eg5 tail domain by Cdk1 is well known to regulate its localization to the mitotic spindle. Phosphoregulation of yeast and *Drosophila* Eg5 motor domains has also been reported, but the majority of the reported sites are not conserved in human or mammalian Eg5. Here, we examined human Eg5 motor domain phosphorylation. Using an anti-phospho-tyrosine antibody we show that Eg5 tyrosines are phosphorylated in human and mammalian-derived cells. Furthermore, using a chemical genetic approach we show that this phosphorylation is dependent on Src family kinase activity. Results from *in vitro* kinase assays and transfection experiments in mammalian-derived cells suggest that c-Src kinase binds to a SRC Homology 3 domain targeting motif (-PXXP-) within the Eg5 microtubule binding site and phosphorylates residues Y125, Y211, and Y231. These results implicate human Eg5 as a potential direct mitotic target of Src family tyrosine kinases. Additionally, we have evaluated the functional effects of this phosphoregulation. Phosphomimetic mutations within these three sites alter the *in vitro* motility characteristics of Eg5 motor domains relative to wild-type and non-phosphorylatable mutant proteins. Furthermore, mammalian cells expressing phosphomimetic Eg5 motors exhibit increased spindle polarity defects. Lastly, we report that phosphomimetic mutations reduce Eg5 affinity for the inhibitor S-trityl-L-cysteine (STLC). In cells with high Src activity, including many types of cancers, the same mechanism could provide rapid resistance to therapy using Eg5 inhibitors.

Light-mediated Motor Activities Control Cargo Distributions in Cells

Bianxiao Cui
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No Abstract

POSTER ABSTRACTS

POSTER SESSION I

Wednesday, June 15, 19:30 – 21:30
Segal Centre

All posters are available for viewing during both poster sessions; however, below are the formal presentation times when presenters are required to remain in front of their poster boards to meet with attendees.

Altman, David	**1-POS**	**Board 1**
Brozik, James	**3-POS**	**Board 3**
Driller-Colangelo, Amalia	**5-POS**	**Board 5**
Grant, Barry	**7-POS**	**Board 7**
Jeon, Tae-Joon	**9-POS**	**Board 9**
Kakugo, Akira	**11-POS**	**Board 11**
Large, Steven	**13-POS**	**Board 13**
Mickolajczyk, Keith	**15-POS**	**Board 15**
Nettesheim, Guilherme	**17-POS**	**Board 17**
Nitta, Takahiro	**19-POS**	**Board 19**
Rahman, Mohammad	**21-POS**	**Board 21**
Ryu, Sangjin	**23-POS**	**Board 23**
Singh, Shree	**25-POS**	**Board 25**
Toleikis, Algirdas	**27-POS**	**Board 27**
Verardo, Damiano	**29-POS**	**Board 29**
Wei, Kathy	**31-POS**	**Board 31**
Zajdel, Tom	**33-POS**	**Board 33**
Tuzel, Erkan	**35-POS**	**Board 35**
Qiu, Weihong	**37-POS**	**Board 37**

Posters should be set up on the morning of June 15 and removed by 22:00 June 16.

1-POS Board 1

Force Dependence of Phagosome Trafficking in RPE Cells

David Altman,
Willamette University, Salem, OR, USA.

Retinal pigment epithelial (RPE) cells play an integral role in the renewal of photoreceptor disk membranes. As rod and cone cells shed their outer segments, they are phagocytosed and degraded by the RPE, and a failure in this process can result in retinal degeneration. We have studied the role of myosin VI in nonspecific phagocytosis in a human RPE primary cell line (ARPE-19), testing the hypothesis that this motor generates the forces required to traffic phagosomes in these cells. Experiments were conducted in the presence of an external force through the use of an optical trap. To quantify applied forces, the extracellular environment was index-matched to the interior of the cell, allowing for in vitro measurements of the trap stiffness. Our results support a role for myosin VI in phagosome trafficking and demonstrate that applied forces modulate rates of phagosome trafficking.

3-POS Board 3

Single Molecule Dynamics and Hidden Markov Models for P2X1 Receptors

Adam O. Barden[1], Brian N. Webb[1], Andrew J. Thompson[2], **James A. Brozik**[1].
[1]Washington State University, Pullman, WA, USA, [2]Cambridge University, Cambridge, United Kingdom.

Purinergic receptors are ubiquitous throughout the human body and participate in the regulation of vast numbers of physiological processes. In particular, ATP binds to P2X1 receptors causing a two stage allosteric modulation for each bound ATP. This modulation opens an integral pore causing the entry of calcium into cells and initiates numerous downstream processes. We will present results from stochastic single molecule fluorescence studies that show multiple discrete ATP binding states associated with individual P2X1 receptors. These observations have been incorporated into a Hidden Markov Model(s) that: (1) takes into account discrete ATP binding states, (2) accounts for the photophysical properties of the probe molecules, (3) extracts the most likely elementary rate constants, and (4) predicts the most likely state occupancies. These results have been used to create a minimal potential energy surface that describes the operation P2X1 as a molecular machine. Some recent super-resolution data that maps out the spatial arrangement of ATP on individual receptors will also be presented.

5-POS Board 5

Cargo Rigidity Affects the Sensitivity of Dynein Ensembles to Individual Motor Pausing

Amalia Driller-Colangelo, Jessica Morgan, Karen Chau, Nathan D. Derr.
Smith College, Northampton, MA, USA.

Cytoplasmic dynein is a minus-end directed microtubule-based motor protein that drives intracellular cargo transport in eukaryotic cells. While many intracellular cargos are propelled by small groups of dynein motors, many of the biophysical mechanisms that govern ensemble motility remain unknown. We have designed a programmable DNA origami synthetic cargo "chassis" that allows us to control the number of dynein motors in the ensemble and vary the rigidity of the cargo chassis itself. On this chassis, motors within an ensemble are conjugated together through variable length cargo "linkers" comprised of parallel segments of either single- or double-stranded DNA. These regions determine the number of independent steps each motor can take before exerting forces on the other motors within the ensemble. This design enables investigation of how motor steps and pauses are "communicated" through the cargo structure and how they affect the emergent behavior of the ensemble. Using TIRF microscopy, we have observed dynein ensembles transporting these cargo chassis along microtubules in vitro. We find that ensembles of dynein on flexible cargos move faster as more motors are added, whereas ensembles on rigid cargos move slower as more motors are added. Using the slowly-hydrolyzable ATP analog ATP-gamma-S, we have observed that ensembles connected through flexible cargos are less sensitive to individual motor pausing. Our results suggest that the role of cargo rigidity in the communication of motor pausing plays an important role in determining the collective motility of dynein motor ensembles. The ability for cargos propelled by dynein ensembles to maintain their motility despite the pausing of individual motors may allow the cargo to maintain productive transport regardless of pauses induced by single motors encountering obstacles on the microtubule.

7-POS Board 7

Engineering Structural Dynamic Mechanisms in Molecular Motors and Switches

Xin-Qiu Yao, Guido Scarabelli, **Barry J. Grant**,
University of Michigan, Ann Arbor, MI, USA.

Molecular motors and switches lie at the heart of key biological processes, from the division and growth of cells to the muscular movement of organisms. Our approach to studying these fascinating nanomachines couples bioinformatics, molecular simulations and experimental protein engineering. Here we describe our most recent results that exemplify the power of this approach when applied across evolutionary related motors and switches. This includes: dissection of allosteric mechanisms in G protein switches, prediction and experimental engineering of the first constitutively active heterotrimeric G protein, computational and experimental dissection of microtubule-kinesin motor interactions, and the rational modulation of the key molecular motor property of processive motility - the ability of an individual motor to take multiple steps along its microtubule filament.

Software and other material related to this work can be found at: http://thegrantlab.org/

Selected References:
1. Yao, X. et al. J Biol Chem. 291, 4742-4753 (2016).
2. Scarabelli, G et al. Biophys J. 109, 1537–1540 (2015).
3. Skjærven, L. et al. BMC Bioinformatics. 15, 399, (2014).
4. Scarabelli G, Grant BJ. Biophys J.107, 2204-13, (2014)
5. Scarabelli G, Grant BJ. PLoS Comp Biol. 9, e1003329, (2013)
6. Yao XQ, Grant BJ. Biophys J. 105, L08-10, (2013).

9-POS Board 9

Bio-functionalized Vesicles for the Measurement of Biological Activities and their Automated Generation

Huisoo Jang, Sunhee Yoon, Sun Min Kim.**Tae-Joon Jeon**,
Inha University, Incheon, South Korea.

We have functionalized polymeric vesicles with antibodies to measure their activities to the target molecules. Polymeric vesicles are made of polydiacetylene that has chromatic transition from blue to red upon external stimuli, such as pH, heat, and perturbation of its structure. These vesicles are further encapsulated within hydrogel matrix to achieve more enhanced biological activities. This process is fully automated using a microfluidic device. The target molecule is phosphinothricin acetyltransferase (PAT) that is one of most abundant protein in genetically modified organisms (GMOs). To recognize PAT, anti-PAT antibody was conjugated onto the surface of the PDA vesicles using EDC/NHS coupled reaction. PDA vesicles were encapsulated into the poly(ethylene) glycol diacrylate (PEG-DA) hydrogel at high concentration to achieve amplified the signal of color. Moreover, we generated a bead type hydrogel using a microfluidic device in an automated manner. With our system, we were successfully able to detect PAT protein in a very low concentration with amplified singals.

11-POS **Board 11**

Compression Stress Induced Buckling of Microtubule and It's Effect on Kinesin-based Cargo Transportation in vitro

Akira Kakugo, Arif Md. Rashedul Kabir, Tanjina Afrin, Daisuke Inoue, Kazuki Sada.
Hokkaido University, Sapporo, Hokkaido, Japan.

Microtubule (MT) and associated motor proteins play a key role in intracellular transport which is crucial to survival of living organisms. Although MT is the most rigid component of cytoskeleton, in cell it is often subjected to compression stress and undergo mecanical deformation manifested by buckling. MT buckling may interrupt the intracellular transport which has been suspected to be linked to neurodegenerative diseases. However, the buckling mechanism of MT and the effect of buckled MT track on the motor protein-based cargo transportation is yet to be understood. In this work, we have demonstrated compression stress induced mechanical deformation of MTs in vitro on a two-dimentional elastic medium and investigated the role of compression strain, strain rate, and interaction of MT with the elastic medium on the MT deformation. Compression strain resulted in MT buckling, extent of which is dependent on applied strain, although compression rate has no substantial effect on the buckling of MTs. Most importantly, the interaction between the MT and the elastic medium is found to play the key role in determining the buckling mode of MTs. By monitoring the kinesin driven transportation along the buckled MTs, we investigated the role of MT buckling on the cargo transportation. Velocity of kinesin-based cargo transportation was found to be decelerated along the buckled MTs which suggests that MT buckling plays an important role in the interruption of kinesin driven cargo transport. This work might help understand the buckling mechanism of MTs and the connection of MT buckling to kinesin driven cargo transportation in cells.

13-POS Board 13

Efficient Operation of Stochastically Driven Biomolecular Systems

Steven J. Large, David A. Sivak.
Simon Fraser University, Burnaby, BC, Canada.

Biomolecular motors convert different forms of energy into work, performing specific functions in their natural fluctuating environments through effective use of cellular resources. However, a theoretical understanding of biomolecular machines' operational principles has proven difficult due to an essential characteristic of living organisms: they must operate far from thermodynamic equilibrium. Motivated in part by the hypothesis that evolution has provided selective pressure toward minimizing energy loss during molecular machine's nonequilibrium operation, researchers have developed theoretical models predicting minimum-dissipation methods to transition a stochastic system between two states. These theories typically assume deterministic driving (convenient for single-molecule experimental tests), but *in vivo* molecular motors are driven by stochastic events such as the ATP hydrolysis cycle. We extend the theory of optimal nonequilibrium control to accommodate stochastic driving forces, leading to notably different minimum-dissipation control regimens. In particular, stochastic control imposes additional costs associated with slow operations. The novel characteristics of minimally dissipative stochastic driving processes point to previously underappreciated design principles for efficient molecular machinery.

15-POS Board 15

Kinetics of Nucleotide-dependent Structural Transitions in the Kinesin-1 Hydrolysis Cycle

Keith J. Mickolajczyk[1], Nathan C. Deffenbaugh[1], Jaime Ortega Arroyo[2], Joanna Andrecka[2], Philipp Kukura[2], William O. Hancock[1].
[1]Penn State University, University Park, PA, USA, [2]Oxford University, Oxford, United Kingdom.

To dissect the kinetics of structural transitions underlying the stepping cycle of kinesin-1 at physiological ATP, we used interferometric scattering microscopy to track the position of gold nanoparticles attached to individual motor domains in processively stepping dimers. The high spatiotemporal resolution of this method enabled real-time recording of structural changes in the protein as it walked at ~100 steps per second. Labeled heads resided stably at positions 16.4 nm apart, corresponding to a microtubule-bound state, and at a previously unseen intermediate position, corresponding to a tethered state. The chemical transitions underlying the structural transitions to and from this one-head-bound intermediate were identified by varying nucleotide conditions and carrying out parallel stopped-flow kinetics assays. At saturating ATP, kinesin-1 spends half of each stepping cycle with one head bound, meaning that there is one rate-limited step in each the one- and two-heads bound states. Analysis of stepping kinetics in varying nucleotides shows that ATP binding is required to properly enter the one-head-bound state, and hydrolysis is necessary to exit it at a physiological rate. These transitions differ from the standard model in which ATP binding drives full docking of the flexible neck linker domain of the motor, and show that the mechanism underlying stepping is a two-step process. Thus, this work defines a consensus sequence of mechanochemical transitions that can be used to understand functional diversity across the kinesin superfamily.

17-POS **Board 17**

Teams of Kinesin-1 Motors are Slowed Down by Macromolecular Crowding

Guilherme Nettesheim[2], Gabriel Jaffe[2], Stephen J. King[3], George T. Shubeita[1,2].
[1]New York University Abu Dhabi, Abu Dhabi, United Arab Emirates, [2]The University of Texas at Austin, Austin, TX, USA, [3]University of Central Florida, Orlando, FL, USA.

The cytosol is crowded by a high concentration of macromolecules. Crowding can alter protein conformation, binding rates, and reaction kinetics, yet it is not known how crowding affects cargo transport by molecular motors. Here, we report on experiments in which we mimicked cellular crowding *in vitro* to study its consequences on cargo transport by kinesin-1 motors. Surprisingly, we find that crowding alters transport by teams of motors more dramatically than it does single motors. A mechanistic model supported by experiments allows us to reconcile this unexpected finding with the properties of the single motor.

19-POS Board 19

Simulating Motor Protein-based Microtransporters

Takahiro Nitta, Koki Kawauchi, Yuki Ishigure.
Gifu University, Gifu, Gifu, Japan.

Motor proteins, such as myosin and kinesin, are attractive motive powers for nanoscale engineering. Integration of the motor proteins may offer new driving methods of microdevices, such as MEMS and biosensors. In pursuing the integration, gliding assay serves as a basis, where cytoskeletal filaments glide over surfaces covered with their associated motor proteins. In order to design microdevices integrated with motor proteins, by developing and using a computer simulation, we investigated the detail mechanisms of the gliding movements.

Cytoskeletal filaments were modeled as inextensible elastic rods. Time evolutions of conformations of the filaments were computed with a Brownian dynamics simulation. Kinesin and myosin motors were modeled as linear springs. Once bound, motor heads were assumed to move toward designated ends of the cytoskeletal filaments. This led that the filaments were propelled toward the opposite direction. In addition, normal forces were applied when the filaments collided against the microfabricated guiding walls and the track surfaces. The filaments were also subjected to external forces. The external forces induced by electric field and fluid flow are used for directing gliding movements of the filaments.

As well as reproducing previous experimental results, the simulation revealed detail mechanisms of the gliding movements of the filaments. For example, at chemical edges, zipping of motor proteins located at the edges was found to occur. And, under strong external forces, filaments were found to be detached from the leading and trailing ends of cytoskeletal filaments. Such findings were difficult to obtain in experiments due to their limited spatial and temporal resolutions.

In summary, our simulation study revealed the detail mechanisms of the gliding movements. Insights obtained here would be useful in designing microdevices integrated with motor proteins.

21-POS Board 21

Modifying Actomyosin Function by Small-molecular Inhibitor Blebbistatin

Mohammad A. Rahman, Alf Månsson.
Linnaeus University, Kalmar, Småland, Sweden.

Addition of small molecular substances could substitute genetic engineering for temporarily changing myosin motor function. One interesting compound is blebbistatin that inhibits myosin II motor activity. In muscle cells, the effect of actin-myosin sliding velocity is modulated by myosin regulatory light chain (RLC) phosphorylation1. Otherwise, evidence exists that blebbistatin stabilizes a start of the power-stroke, strongly attached, actomyosin state2. Here, in vitro motility assay studies are performed to investigate (a) whether the drug effect varies with myosin RLC phosphorylation in absence of the myofilament lattice of muscle and (b) if the effect on velocity is consistent with stabilization of the mentioned actomyosin state. Heavy meromyosin (HMM) from Rabbit skeletal muscle, with either phosphorylated or un-phosphorylated RLCs, was adsorbed to silanized surfaces for in vitro motility assays. We found that blebbistatin (1μM) inhibits sliding velocity to similar degree (~50 %) independent of phosphorylation status. The inhibition induced by blebbistatin (2μM) was doubled for an increase in ionic strength (60 to 130 mM) whereas the effect on velocity was similar at different MgATP concentrations (0.1 – 1 mM). The findings suggest that the blebbistatin effect on velocity does not depend on myosin RLC phosphorylation, consistent with the idea that such dependence in vivo is fully attributed to myosin head – backbone interactions (absent in vitro). Furthermore, modelling suggests that blebbistatin increases the population of a start-of-power stroke actomyosin state with possible physiological roles e.g. in eccentric contractions (stretch of active muscle). 1.Stewart, M., Franks-Skiba, K. & Cooke, R. (2009) J. Muscle Res. Cell Motil. 30, 17–27.2.Takács, B. et al. (2010). Proc. Natl. Acad. Sci. 107, 6799–6804 Supported by the FET-program of EU-FP7 (grant agreement 613044; ABACUS)

23-POS Board 23

Contractility Measurements of Calcium-powered Biomotors of the *Vorticella* Spasmoneme

Sangjin Ryu[1], Eun-gul Chung[2].
[1]University of Nebraska-Lincoln, Lincoln, NE, USA, [2]University of Nebraska-Lincoln, Lincoln, NE, USA.

Vorticella is a sessile protist, and its slender stalk anchors the cell body (zooid) on a solid surface. Powered by calcium ions, the contractile organelle spasmoneme coils the stalk in ~5 ms. Thus, the zooid is translated toward the surface at the maximum speed of ~50 mm/s. Because of its ultrafast contraction and unique energy source, the spasmoneme serves as a model organelle for calcium-powered cell motility and biomimetic actuators.

The spasmoneme consists of 3-4 diameter fibrils, which appear to be the motor unit composed of the calcium-binding protein, spasmin. These spasmonemal fibrils are thought to coil upon calcium binding due to protein conformation change, leading to the organism-level contraction. Thus, characterizing the spasmonemal motors requires tension measurements of the spasmoneme, and we measured the spasmonemal tension employing engineering approaches.

First, we developed computational and theoretical fluid dynamics models of contracting *Vorticella*, where the zooid was modeled as a sphere moving toward a solid plane following experimentally measured speed profiles of the zooid. Because of the negligible inertia of the zooid, the spasmonemal tension was equated to the zooid drag. The evaluated peak tension ranges 15-50 nN, with a linear dependence on the spasmoneme length.

Second, we utilized microfluidic channels to measure the isometric tension. Tethered to the channel surface and laid in the flow, *Vorticella* showed stalled stalk contractions due to drag on the zooid. Consequently, as the drag increased, the stalk contracted over shorter distances. Therefore, we could estimate the isometric tension to be 100-350 nN, which also shows a linear dependence on the spasmoneme length.

Last, based on the measured tension values and their linear dependence on the spasmoneme length, we developed a concenptual model of the spasmoneme and evaluated the contractilty of its motor element.

25-POS Board 25

Development of a Tissue-engineered Skin Model for Wound Healing

Saurabh Dixit, **Shree Singh**, Vida Dennis.
Alabama State University, Montgomery, Al, USA.

Biomedical tissue engineering, particularly 3D printing, is rapidly emerging as an alternative model system in the biomedical field to study the molecular interactions between cells and biomaterials, drugs and pathogens; genetic and proteomics analyses, transplantation and understanding transplantation immunology. The synthetic tissues are attractive since they have potential to generate individualized biomolecules to treat various disease conditions. Our research has been at the forefront in investigating molecules that potentially could control inflammatory disorders. We use the similar technology to study the skin tissue regeneration and wound healing which are regulated by the cytokines and other growth factors. We routinely use lipopolysaccharide (LPS) as a model molecule to study inflammation in epithelial cells. We have identified interleukin 10 (IL-10) and silver-polyvinilpirrolidone (Ag/PVP) nanoparticles that effectively control adverse immune functions following exposure to LPS. We are studying human keratinocytes to generate 3D tissue-engineered skin in presence of LPS as a model system to test and understand the molecular mechanisms that mediate development of the skin system. We have shown that the levels of LPS-induced cytokines [tumor-necrosis factor (TNF), interleukin (IL)-6 or IL-12p40] are significantly reduced in cells. In addition, IL-10 encapsulated within the biodegradable nanoparticles, PLGA (poly (lactic-co-glycolic acid) coated with chitosan or PEGylated poly lactic acid (PLA) markedly modulate the LPS-induced inflammation in cells. Our system provides the basis to develop the 3D skin model system that will enable researchers to generate skin tissues for wound healing and various other synthetic biomolecules ready for use in in vivo systems.

27-POS **Board 27**

Forestep-Backstep Ratio Determines Stall Force for both Fast and Slow Kinesin-1

Algirdas Toleikis, Nicholas J. Carter, Robert A. Cross.
University of Warwick, Coventry, United Kingdom.

Kinesin-1 is a motor protein responsible for molecular transport inside cells. It consumes ATP as an energy source required to do work and transports cargos while walking on microtubule tracks. Kinesin-1 is a processive motor and can walk ~100 steps before falling off. It walks in ~8 nm steps in a hand-over-hand fashion and stalls at ~7 pN backwards (hindering) force. At this stall force, steps are infrequent so backstepping and forward stepping are equally probable. In this work, we are using optical trapping to apply directional force on single kinesins in vitro to study the mechanism of stepping. Particularly, we are focusing on the directionality of stepping, counting forward vs backward steps at a particular force, and interrogating the mechanism underlying the switch of direction. We are comparing kinesin-1 isoforms from Drosophilla and Neurospora to better understand how nature has engineered changes in amino acid sequence that influence the stepping behaviour. We present preliminary data which we hope in the future might help us understand the mechanistic determinants of stepping directionality.

29-POS Board 29

Synthesis and Characterization of the Lawnmower: An Artificial Protein-based, Burnt-bridges Molecular Motor

Damiano Verardo[1], Chapin Korosec[2], Martin J. Zuckermann[2], Heiner Linke[1], Nancy R. Forde[2].
[1]Lund University, Lund, Sweden, [2]Simon Fraser University, Vancouver, BC, Canada.

In this work we report progress towards constructing an artificial protein motor dubbed Lawnmower, based on the burnt-bridges concept and inspired by matrix metalloproteinases (MMPs), involved in the degradation of collagen fibrils.

The Lawnmower consists of a quantum dot hub (16nm diameter), conjugated to multiple trypsin protease blades via flexible PEG linker molecules [1]. It is expected to undergo biased motion on a peptide track through enzymatically cleaving peptides into product and seeking substrate-rich areas, and will remain processive as long as the individual binding events occur before complete detachment. To guide experiments, Langevin simulations are carried out to provide insight into Lawnmower design criteria.

To demonstrate the motor's properties, 2D ensemble experiments on peptide covered, lightguiding III-V nanowires will be performed. Nanowires are grown via metalorganic vapour deposition, coated with silicon oxide, and alkyne-silanized in order to allow subsequent click chemistry functionalization by modified peptides. It was recently observed that light emitted by molecules close to the surface of these nanowires is waveguided to the tip due to coupling of optical modes. Nanowires coated with peptides that emit fluorescent photons upon cleavage can then be used as sensors to assess the speed and processivity of the Lawnmower in two dimensions.

Following optimization of Lawnmower construction, 1D experiments are planned on peptide-functionalized DNA, where biased motion of the Lawnmower should be clearly evident. This DNA-supported peptide lawn has controllable average spacing between peptides, allowing evaluation of processivity as a function of substrate density. Successful implementation of the Lawnmower in the above assays would result in the first protein-based artificial molecular motor.

[1] Kovacic, S., *et al.*, Design and Construction of the Lawnmower, An Artificial Burnt-Bridges Motor. IEEE Transactions on NanoBioscience, 14(3), 305–312.

31-POS Board 31

Computase: Computing with a Self-assembling 3D Lattice of De Novo Designed Biomolecular Machines

Kathy Y. Wei, David Baker.
University of Washington, Seattle, WA, USA.

Although silicon-based computers continue to improve, alternative models of computation have the potential to supply more energy-efficient and parallelized solutions to domains difficult for sequential instruction-based semi-conductor computers. While traditional computers require energy to hold the state of each transistor, proteins will only require energy to change state. We propose a protein-based computer that self assembles into a 3D cubic lattice of nodes. Each node is a computationally designed 3-input/3-output protein logic gate "wired" to its 6 lattice neighbors. The connectedness allows computing processes to be parallel - throughout the volume of the assembled lattice - and be scalable. For example, the proposed protein computer could process an image by taking one pixel per node along an entire face of the lattice and thus process an entire image simultaneously, as opposed to sequentially on traditional computers. Physically, each node is composed of two components: a scaffold to support self-assembly, and a molecular machine that integrates and transfers information between lattice neighbors. We have demonstrated the ability to build multicomponent self-assembling nanostructures and 2D protein arrays, and to design de novo proteins with atomic accuracy. As a first step, we will design bistable protein switches that can 1) toggle between two states, and 2) toggle in such a way as to influence the states of neighboring nodes. The protein design goals of 3D self-assembly, bistable switches, and directed protein-protein information transfer are important next steps addressable with computational protein design. Protein-based 3D lattice computers bear great potential for the future of energy efficient parallel computing.

33-POS Board 33

Impedance-based Electrochemical Readout of Bacterial Flagellar Rotation

Tom J. Zajdel[1], Alexander N. Walczak[1], Debleena Sengupta[1], Victor Tieu[1], Caroline M. Ajo-Franklin[2], Michel M. Maharbiz[1].
[1]University of California, Berkeley, Berkeley, CA, USA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

The objective of this work is to construct a low-power biosensor suitable for use in microrobotics applications. The target detection limit is 1 nM and the target response time is on the order of 30 seconds or less.

When detecting bioanalytes, speed, sensitivity, and sensor size are subject to fundamental physical constraints set by diffusion noise (Berg & Purcell 1977). The chemical sensors and signaling pathways used by *Escherichia coli* during chemotaxis - the organism's motility control in response to its surroundings - are known to approach the fundamental limits on response time and sensitivity for its volume (approximately 1 fL) (Bialek & Setayeshgar 2005, Kaizu et al. 2014). Despite this capability, no modern engineered biosensing system approaches these limits within a volume approaching that of the bacterium (Su et al. 2011, Arlett et al. 2011).

We demonstrate a biosensor that can monitor chemotactic motor switching in a single *Escherichia coli* cell tethered by a single flagellum. A nanoelectrode array detects the electrical impedance perturbation of an *E. coli* cell as it passes between electrode pairs. With this array, we are able to obtain an electronic report of the cell's motor bias in response to the presence of chemoeffectors within 30 seconds. Our results suggest that this platform enables biosensing that approaches the performance of a chemotactic bacterium while minimizing power consumption and instrumentation overhead.

35-POS **Board 35**

Cooperative Transport by Populations of Fast and Slow Kinesins Reveals Important Family-dependent Motor Characteristics

Goker Arpag[1], Shankar Shastry[2], David Arginteanu[2], Stephen R. Norris[4], Kristen Verhey[3], William O. Hancock[2], **Erkan Tuzel**[1].
[1]Worcester Polytechnic Institute, Worcester, MA, USA, [2]Pennsylvania State University, University Park, PA, USA, [3]University of Michigan, Ann Arbor, MI, USA, [4]Vanderbilt University, Nashville, TN, USA.

Intracellular cargo transport frequently involves multiple motor types, either having opposite directionality or having the same directionality but different speeds. Although significant progress has been made in characterizing kinesin motors at the single-molecule level, predicting their ensemble behavior is challenging and requires tight coupling between experiments and modeling to uncover the underlying motor behavior. To understand how diverse kinesins attached to the same cargo coordinate their movement, we carried out microtubule gliding assays using pairwise mixtures of motors from the kinesin-1, 2, 3, 5 and 7 families engineered to have identical run lengths and surface attachments. Uniform motor densities were used and microtubule gliding speeds were measured for varying proportions of fast and slow motors. A coarse-grained computational model of gliding assays was developed and found to recapitulate the experiments. The simulations show that the force-dependence of detachment is the key parameter that determines gliding speed in multi-motor assays and provide estimates for force-dependent dissociation rates suggesting that kinesin-1 and the mitotic motors kinesin-5 and −7 maintain microtubule association against loads, while kinesin-2 and −3 readily detach. Using these predictions, we investigated how these motors carry scaffold proteins in teams to carry out distinct mechanical tasks in cells. Our work uncovers unexpected motor behavior in multi-motor ensembles and clarifies functional differences between kinesins.

37-POS Board 37

Engineering a Processive Minus End-directed Kinesin-14 for Long-range Transport on Microtubules

Yuh-Ru Julie Lee[1], Kuo-Fu Tseng[2], Pan Wang[2,3], Joel Bowen[4], Lijun Guo[3], **Weihong Qiu**[2,6], and Bo Liu[1]

[1]Department of Plant Biology, University of California, Davis, CA, USA, [2]Department of Physics, Oregon State University, Corvallis, OR, USA, [3]School of Physics and Electronics, Henan University, Kaifeng, Henan, China, [4]Department of Mathematics, Oregon State University, Corvallis, OR, USA, [5]Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA.

Retrograde transport along microtubules in animal and fungal cells is carried out mostly by cytoplasmic dynein, a minus end-directed motor with high intrinsic processivity. In contrast, land plants lack cytoplasmic dynein and contain instead a large number of minus end-directed kinesins. However, none of these minus end-directed kinesins in plants has been found to be intrinsically processive. We report here that the minus end-directed kinesin OsKCH2 from the rice plant (Oryza sativa) is intrinsically processive on the microtubule, which is enabled by a nonmotor domain (CC2) that enhances its affinity for the microtubule. Our findings suggest that plants have evolved unconventional minus end-directed kinesins as efficient substitutes for cytoplasmic dynein. Interestingly, substituting CC2 in OsKCH1 – a nonprocessive kinesin-14 from the rice plant – with that from OsKCH2 results in a kinesin-14 chimera (OsKCH1P) that moves processively on the microtubule. We suggest that OsKCH1P is an important tool for dissecting the in vivo functional significance of OskCH1 processivity.

POSTER SESSION II

Thursday, June 16, 19:30 – 21:30
Segal Centre

All posters are available for viewing during both poster sessions; however, below are the formal presentation times when presenters are required to remain in front of their poster boards to meet with attendees.

Brown, Aidan	**2-POS**	**Board 2**
Deshpande, Ria	**4-POS**	**Board 4**
Goldstein, Alina	**6-POS**	**Board 6**
Harvey, Stephen	**8-POS**	**Board 8**
Jindal, Lavisha	**10-POS**	**Board 10**
Kasper, Alexandra	**12-POS**	**Board 12**
Lindberg, Frida	**14-POS**	**Board 14**
Naufer, M. Nabuan	**16-POS**	**Board 16**
Nirody, Jasmine	**18-POS**	**Board 18**
Park, Yoonkook	**20-POS**	**Board 20**
Rauch, Philipp	**22-POS**	**Board 22**
Sakamoto, Takeshi	**24-POS**	**Board 24**
Small, Lara	**26-POS**	**Board 26**
Tsekouras, Konstantinos	**28-POS**	**Board 28**
Urnavicius, Linas	**30-POS**	**Board 30**
Xu, Jing	**32-POS**	**Board 32**
Zuckermann, Martin	**34-POS**	**Board 34**
Lin Hsin-Yu	**36-POS**	**Board 36**

Posters should be set up on the morning of June 15 and removed by 22:00 June 16.

2-POS Board 2

Maximizing Irreversibility and Minimizing Energy Dissipation for Simple Models of Mechanochemical Machines

Aidan I. Brown, David A. Sivak.
Simon Fraser University, Burnaby, BC, Canada.

Driven reactions and processes at microscopic scales must overcome fluctuations to proceed forwards more than they go in reverse. It is well known that some free energy dissipation is required to achieve irreversible forward progress, but the detailed relationship between irreversibility and free energy dissipation is not well understood. We present results for the irreversibility-dissipation relationship of a model system which captures the basic physics of energy storage. Such a system can represent many examples – ATP synthase completing unfavourable rotations to synthesize ATP, linear walking motors changing conformation prior to a power stroke, and viral packing motors translocating against the pressure of a polymer trying to escape. Our analysis reveals that reactions which do not carry a system far beyond the energy storage step can achieve equal irreversibility while reducing dissipation. Our results also suggest scenarios where a system must pay a higher dissipation cost for a relatively low irreversibility. We discuss how our investigation points towards general principles of microscopic machine operation and process design.

4-POS Board 4

Isolation of Subdomain Mechanism in Cytoplasmic Dynein with Engineered Chimeric Proteins

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Cytoplasmic dynein is a microtubule-associated minus-end directed molecular motor with several functions, including the segregation of chromosomes during mitosis and the transport and distribution of intracellular cargo. A member of the AAA^+ ATPase family of proteins, the dynein heavy chain has a complex structure consisting of a ring with 6 AAA^+ domains, four of which can bind ATP. A coiled-coil stalk projects from this ring, containing the microtubule binding domain (MTBD) at its end. Two of these heavy chains come together to form the dynein homodimer, which is necessary for motor function and processive motility. The MTBD binds to microtubules, forming the interface between the motor and its track. In vitro experiments aimed to discern the mechanism of dynein revealed that despite significant structural conservation, yeast and mammalian dynein have different motile properties. The mammalian dynein requires accessory proteins like dynactin and BICD2, to convey processivity, whereas yeast dynein is processive on its own. We hypothesized that the motile differences between dyneins from different species could provide opportunities to reengineer dynein for targeted mechanistic investigations. Specifically, a re-engineered chimeric dynein in which a single subdomain of the yeast dynein is replaced with the equivalent subdomain from mammalian dynein could isolate and elucidate the subdomain's contributions to the overall motor mechanism. Our initial work focuses on the MTBD as it may directly determine many of the motile behaviors of the motor. To this end, we genetically engineered yeast to express dynein with the endogenous MTBD replaced by the mammalian MTBD. Using TIRF microscopy and single molecule biophysical assays, we are currently characterizing the chimeric motor's motile behavior in vitro.

6-POS Board 6

Study of Phospho-regulation of a Mitotic Motor Protein by Systematic Mutagenesis

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The *S. cerevisiae* Cin8 belongs to the kinsesin-5 family of mitotic motor proteins. During mitosis, Cin8 orchestrates the mitotic spindle assembly and its elongation. Recent work from our laboratory indicated that phosphorylation of Cin8 at cyclin-dependent kinase 1 (Cdk1) sites located in its catalytic domain governs its localization to the mitotic spindle during mitosis. Here we tested the flexibility of phosphoregulation of Cin8, and examined whether phosphorylation at newly created Cdk1 sites can mimic the known phospho-regulation or create new regulation. For this purpose, we first generated a phospho-deficient mutant of Cin8 and then introduced new Cdk1 sites by single amino acid replacement. We examined the mutants by viability test, live imaging and quantitation of phosphorylation by Cdk1 *in vitro*. We found that out of 32 novel sites, only one site at position 276, which is located in high proximity to a native Cdk1 phosphorylation site (S277), recapitulated the original phospho-regulation of Cin8. Although several sites were created nearby, and some of them were found to undergo phosphorylation *in vitro*, only this site exhibits localization and viability phenotypes similar to those of the wt Cin8. This result indicates that phospho-regulation of Cin8 by Cdk1 is rigid and highly dependent on the structural context. However, several additional mutants bearing novel Cdk1 sites which are not adjacent to native sites, exhibited new phenotypes, suggesting that phsopho-regulation by Cdk1 at additional sites of Cin8 can affect its activity. The mechanism and physiological significance of phospho-regulation at these new sites needs to be further investigated.

8-POS Board 8

The Scrunchworm Hypothesis: DNA Conformational Changes are Responsible for Force Generation in Viral Packaging Motors

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The motors that drive double-stranded DNA (dsDNA) genomes into viral capsids are among the strongest of all biological motors for which forces have been measured, but it is not known how they generate force. Previous models all assume that viral proteins constitute the motor and treat the DNA as a passive substrate, pushed forward by lever-like protein motions. We previously proposed that the DNA is not a passive substrate, but that it plays an active role in force generation. This "scrunchworm hypothesis" holds that the motor proteins repeatedly dehydrate and rehydrate the DNA, which then undergoes cyclic transitions between the A-DNA and B-DNA conformations. A-DNA is 23% shorter than B-DNA. The cyclic shortening and lengthening motions are captured by a coupled protein-DNA grip-and-release cycle to rectify the motion and translocate the DNA into the capsid. In this study we examined the interactions of dsDNA with the dodecameric connector protein of bacteriophage φ29, using molecular dynamics simulations on four different DNA sequences, starting from two different conformations (A-DNA and B-DNA). In all four simulations starting with the protein equilibrated with A-DNA in the channel, we observed transitions to a common, metastable, highly scrunched conformation, designated A*. This conformation is very similar to one recently reported by Kumar and Grubmüller in MD simulations on B-DNA docked into the φ29 connector. These scrunched conformations occur spontaneously, without requiring lever-like protein motions often believed to be necessary for DNA translocation.

10-POS Board 10

Microscopic Model for Force Generation in the ParA-ParB System

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Reconstitution of the ParA-ParB system in vitro has produced conclusive evidence that ParB bound cargo can undergo directed motion when interacting with DNA bound ParA. Previously we developed a continuum deterministic model for the in vitro system consisting of a ParB decorated bead moving on a ParA decorated DNA substrate. The model involved only two parameters that resulted from a mean field treatment of the net force acting on the bead due to the ParA-ParB interaction. It predicted that for certain parameter values, constant speed motion of the bead was possible with the possibility of an optimal speed. In order to determine the validity of the continuum approximation made in the model, we have now performed stochastic simulations of a microscopic model consisting of a ParB decorated polymer interacting with a ParA substrate. We have explored the dependence of force generation in this model as a function of the polymer size, the fraction of ParB, the density of ParA and the interaction energy. Recently, it has been speculated that the elasticity of the DNA substrate may also facilitate force generation. We also consider the effects of ParA substrate elasticity on the motion of the ParB bound polymer. Our findings should help to bridge the gap on how forces are generated in the ParA-ParB system from the length scales relevant in vitro all the way down to in vivo.

12-POS **Board 12**

Geometrical Theories of Optimization of Molecular Motors

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Molecular motors, whether biological or synthetic, operate in highly fluctuating environments and are perpetually operating in a nonequilibrium state. As a result, equilibrium statistical mechanics is not a sufficient framework for discussing the operation and efficiency of molecular motors. In the pursuit of a general framework for these stochastic, nonequilibrium systems, significant theoretical research has focused on systems driven by time-dependent control parameters. Considering an operating motor as a system driven through state space by external controls is a promising framework for predicting average system response, identifying minimum-dissipation control parameter schedules, and potentially elucidating the design of minimally-dissipative systems for given external controls. The diverse collection of recent work spans from an analog of the quantum mechanical geometrical Berry phase that predicts the average number of rotations of F1-ATPase driven by a rotating magnetic field, to a geometric view of thermodynamic state space that predicts the dissipation associated with particular control schedules. I will highlight the current ideas in this area and provide an argument for unifying existing geometrical theories of molecular motors.

14-POS Board 14

Parallel Biocomputational Devices Based on Molecular Motors in Nanostructures

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Solving mathematical problems of a combinatorial nature requires the exploration of a large solution space. As the number of possible solutions grows, this task becomes intractable for traditional, serial computation and therefore, calls for parallel computation techniques. Here we demonstrate an approach to solve a combinatorial problem by parallel computation based on molecular-motor driven biomolecules to explore physical networks of nanoscaled channels in a highly energy-efficient manner (Nicolau et al. 2016).

We solve a combinatorial problem known as the subset sum problem, by encoding it into physical networks of channels patterned by lithography. These networks encode binary addition computers. The channel floors are covered with molecular motors that propel protein filaments fed into the network at one end, exploring the network. The filaments' exit-points correspond to different solutions. Each filament explores one solution, thus, a large number of proteins can be used to compute problems in a massively parallel, energy-efficient manner.

We present a proof-of-principle demonstration of the parallel-computation technique, and the status of our ongoing work to optimize and up-scale this system. We test different designs to optimize the individual architectural elements, reducing error rates and increase computing efficiency. We also aim to incorporate switchable junctions into the networks, providing programmable “gates” that can be switched on and off, controlling passage of protein filaments, enabling a high variability of networks. Furthermore, we develop different processing methods for fabricating devices.

Our approach is scalable using existing nanofabrication technology. Because one NP complete problem can be converted into another, this technique can be used, in principle, to solve many NP complete problems, with applications in, drug design, scheduling activities, checking of electronic circuit designs, etc.

Nicolau, D.V.J. et al., 2016. Massively-parallel computation with molecular motor-propelled agents in nanofabricated networks. PNAS 113(10), pp. 2591–2596.

16-POS Board 16

Single-molecule Characterization of *E. coli* Pol III Core Polymerase Activity

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Antibiotic resistance is a growing health problem. Hence new antibiotics and new antibiotic targets are critically needed. Bacterial DNA polymerases are potentially attractive targets for the development of new antibiotics because, while the process of DNA replication is conserved, the structures and interactions of the proteins involved vary considerably between prokaryotes and eukaryotes. DNA polymerase III (Pol III) is the replicative DNA polymerase in *E. coli* and is composed of 10 subunits that tightly coordinate leading and lagging strand synthesis. The core of the polymerase (Pol III core) contains the catalytic polymerase subunit, α, the $3' \rightarrow 5'$ proofreading exonuclease, ε, and a subunit of unknown function, θ. Here we employ optical tweezers to characterize Pol III core activity on a single DNA molecule. We quantify the transitions between single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) during Pol III core activity via constant force measurements, in which the change in extension can be used to determine the rate of polymerization or exonucleolysis at forces below the melting transition force. These experiments are performed at forces greater than the force at which dsDNA and ssDNA stretching curves cross; therefore, while polymerization is inhibited, exonucleolysis is favored by force. We show that the application of tension facilitates the inter-molecular transfer of the primer between the polymerase and exonuclease subunits of the Pol III core assembly. Here we report the dependence of these catalytic rates on template tension and find the zero-force polymerization rate of Pol III core to be 38 ± 9 nts/s. We show that the dwell times of processive events between pauses for polymerization and exonucleolysis are 0.7 ± 0.1 s and 0.3 ± 0.1 s, respectively, suggesting distinct DNA binding dynamics during the polymerase and exonuclease activities.

18-POS **Board 18**

Dynamics of the Bacterial Flagellar Motor: Theoretical Model and Validation

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The bacterial flagellar motor (BFM) drives swimming in a wide variety of bacterial species. Understanding the dynamics of the BFM has a broad range of applications, including drug design and engineering biosensors because of this motor's fundamental role in a variety of fundamental biological processes, such as chemotaxis and community formation. We put forward a model for motor rotation that implicates a steric interaction between the rotor and the torque-generating complexes (stators). Ours was the first mechanically-specific model of torque generation in the BFM, pinpointing critical residues and structures for motor function. An important feature of our model is that motor rotation is loosely coupled to ion flow across the membrane. As a consequence, we predict that the maximum speed of the motor increases as additional stators are recruited to the motor. While this is contrary to the current widely-held belief that there is a universal upper limit to the speed of the BFM, we show that this is consistent with current experimental evidence. We also put forward several further experiments and measurements designed to test the validity of this model and its implications.

20-POS **Board 20**

Influence of Net Charge Value on the Second Virial Coefficients of BSA Solutions

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Using a Wescor colloidal membrane osmometer the osmotic pressures of bovine serum albumin (BSA) aqueous solutions were measured under two different salts of ammonium sulfate and potassium sulfate. The experimental osmotic pressure made it possible to estimate the osmotic virial coefficient for BSA. The type of cation with same anion, pH, and ionic strength played a significant role in forces between protein molecules. The magnitude of the coefficient seems to be affected by the net charge value of the BSA and depth of the square well.

22-POS Board 22

Investigation of Molecular Force Generation Mechanisms Using Optical Tweezers

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Optical tweezers (OT) are being applied in a variety of research fields, ranging from material characterization to the tracking of proteins as they actively or passively move inside living and reconstituted biological systems. They allow the parallel detection of nanoscale particle positions and forces at µs time resolution which renders OT an ideal tool for investigating motor protein dynamics and cytoskeletal force generation mechanisms.
A novel experimental set-up featuring flexible force-clamp mechanisms has been developed, capable of the adaptive force-clamping of bi-directional motor proteins like the Kinesin5 variant Cin8 found in S. cerevisiae. Single Cin8 motors were shown to move towards the (-)-end of microtubules (MTs) in in-vitro experiments. However, if multiple molecules cross-link anti-parallel MTs, they switch direction. It's MT cross-linking capability and force-dependent reversal of motion direction indicates that Cin8 can not only generate but also adapt its motile properties to different forces.

Other mechanisms of cytoskeletal organization are based on the kinetics of diffusible cross-linker molecules that do not per se have the ability to perform directed movement, but as an ensemble generate substantial forces. OT-based force detection combined with quantitative TIRF microscopy revealed that Ase1 molecules cross-linking MTs counteract the anti-parallel sliding of filaments. The overlap of the cross-linked microtubules reduces during sliding, and thus, confines the available space for Ase1 diffusion. The resulting entropic forces aiming to increase the overlap length were found to be in the range of several piconewtons, comparable to the force generation by conventional motor proteins.

In collaboration with leading research groups, the OT platform has been continuously optimized for motor protein and cytoskeleton dynamics applications. This was achieved by integrating high-end optical methods and advanced software features for the automated execution of complex experimental schemes, including high-speed (50kHz) closed-loop feedback implementations.

24-POS Board 24

Nonprocessive Motor Ensemble for Large Vesicle Trafficking

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Molecular motors are protein machines that perform numerous cellular functions including cell motility, contraction, organelle transport, and cell division. Cargo transport is an essential cellular process indispensible for life across phylogeny. Class V myosin (MyoV) is the actin-based molecular motors implicated in organelle and vesicle transport and has been found in many species ranging from yeast to mammals. In vertebrates, there are three class V myosin genes encoding, named MyoVa, MyoVb, and MyoVc. MyoVc is kinetically weak affinity for actin filament, called non-processive, than MyoVa / Vb, though MyoVc binds on vesicles and believed involving vesicle trafficking.
MyoVc is a low duty ratio, non-processive motor unable to move continuously along actin filaments though it is believed to participate in secretory vesicle trafficking in vertebrate cells. Previously two dimers of Myo5c molecules on a DNA scaffold increased the probability of rebinding to F-actin and enabled processive steps along actin filaments, which could be used for collective cargo transport in cells. We measured the processivity and velocity (100 nm/s) of purified Zymogen granules in vitro. Mass spectrometry analysis showed a few myosins (MyoVc and MyoI) binds on ZGs. Kinesin and/or Dynine may bind the surface of ZGs. To understand the ZG's vesicle transportation and the function of molecular motors in cells, we observed the ZGs movement in acinar cells.

26-POS Board 26

The Use of Coiled-coil Peptides for Synthetic Molecular Motor Constructs

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A vast array of biological processes, in the human body and beyond, are reliant on the successful function of molecular motors. These protein-based molecular machines are involved in many fundamental cellular activities, from muscle contraction to cell division. The complexity of interactions between the amino acids which make up these proteins make them both the preferred building materials of nature, and difficult systems to fully explore. In addition to improving our knowledge of how these natural proteins perform their individual tasks, understanding and replicating these impressive abilities is highly attractive for bionanotechnology applications.

One means of understanding such systems is through bottom-up design of synthetic molecular motors. Our aim is to combine coiled-coil peptide designs with other molecular components to devise and produce synthetic motors. These include the Tumbleweed, a synthetic molecular motor designed to progress via rectified diffusion along a DNA track. One design for the formation of the Tumbleweed motor is reliant on the self-assembly of orthogonal coiled coils, a peptide motif commonly found in nature. I will discuss the requirements of such a system, and the biophysical characterisation of a set of designed peptides with the ability to form a suitable structure, demonstrated using circular dichroism, dynamic light scattering, analytical ultracentrifugation and disulphide exchange reactions.

Reference
Bromley et al., The Tumbleweed: towards a synthetic protein motor, HFSP Journal. 2009 Jun; 3(3): 204–212

28-POS Board 28

Increased Diffusion of Enzymes Catalyzing Exothermic Reactions

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We recently demonstrated (Riedel et al. Nature 2015) that enzymes catalyzing exothermic reactions exhibit increased diffusion in the presence of their substrate, confirming experiments done by the group of A. Sen. We concluded that the energy released by the turnover event briefly accelerates the enzyme's center of mass resulting in a short ballistic motion of the enzyme; this transient increase registers as an observed diffusion coefficient rise. Although our theory explains observations, many questions remain open. What displacement is necessary to explain the observations? Why is the energy not dissipated among all thermal modes? What leads us to reject global or local heating of the solution as an explanation for the diffusion coefficient rise? We present our theory on enhanced diffusion of enzymes that catalyze exothermic reactions that addresses these key questions.

30-POS Board 30

Velocity Control of Dynein-based Transport by Bicaudal D Family Adaptor Proteins

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Most if not all cytoplasmic dynein-1 transport requires the activity of its cofactor dynactin. Additional proteins, cargo adaptors, are required to stabilize the interaction between dynein and dynactin. How different cargo adaptors activate dynein is still poorly understood. It was previously shown in vivo that vesicular transport activated by two Bicaudal D family adaptor proteins, BICD2 and BICDR-1, differ in velocity, which affects the distribution of transport carriers. Vesicular transport activated by the BICDR-1 has twice the velocity compared to the BICD2. We find the same difference in velocity in vitro using a single-molecule motility assay. In order to understand the control of the velocity of dynein-based transport we combine engineered complexes based on a 6Å cryo-EM structure of the dynein-dynactin-BICDR-1 and high spatial-precision measurements of fluorescently labelled complexes to analyse processivity and stepping behaviour.

32-POS **Board 32**

Understanding the Role of ATP in Active Self-organization of Microtubule Spools

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Self-organization of microtubules into ring-shaped structures ("spools") constitutes a model system for studying active material. To form microtubule spools, biotinylated microtubules are shuttled atop a motor-coated surface as in standard gliding assays; binding between microtubules is mediated by streptavidin. While the mechanisms underlying microtubule spool formation remain unclear, it is known that considerable energy (10^5 k_BT) is required to deform microtubules to form spools. Here, we investigated the role of kinetic energy input (ATP) in microtubule spool formation. We systematically varied the ATP concentration in our experiments and examined the steady state size and number density of microtubule spools formed under otherwise identical conditions. Surprisingly, we found that although ATP is necessary to initiate spool formation and to sustain spool rotation, the steady state of microtubule self-organization is not sensitive to ATP concentration. We detected no significant changes in the morphology (spool circumference) or the probability of microtubule spools formed (number density) over a 20-fold range in ATP concentration (0.05 – 1 mM). Our study indicates that microtubule spool formation is not directly coupled to any particular level of kinetic energy input. Lowering the ATP concentration, which slows the kinetic rate of microtubule self-organization, may therefore be a useful experimental approach for understanding the mechanisms underlying microtubule spool formation.

34-POS **Board 34**

Transform Ation of a Protein Nano-walker into a Nano-motor by Feedback

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Biological protein nano-motors are vital to the survival and function of cells. The objective of our research is to design synthetic protein motors which mimic such biological nano-motors. To this purpose we have conceived a concept for a protein nano-walker dubbed Synthetic Kinesin Inspired Protein (SKIP), so called because it is inspired by kinesin's use of restricted diffusional search for forward head binding. The SKIP model is composed of coiled coils and ligand-gated repressor proteins; its two types of repressors each bind to specific sequences patterned symmetrically along a linear double-stranded DNA track. SKIP is designed to undergo biased directional motion along this track in the presence of a temporally symmetric ligand pulse sequence. Thus, in this design directionality is achieved by persistence rather than asymmetry. Furthermore its symmetry allows it to reverse through the influence of a sufficient rearward force or the use of specific track termini.

Here, we explore the role of feedback in enhancing SKIP's performance. First, we alter the direction of the applied force, in order to maximize the work being done by SKIP as it walks (Zuckermann et al., New J. Phys. 2015). In an alternative approach, we alter the duration of ligand pulses, switching to a new potential binding state as soon as forward motion is detected. In this scheme we can maximize output power. We evaluate the information cost and benefits of feedback in these two scenarios. The properties of this new construct demonstrate that SKIP can indeed be engineered by feedback to become a directional and processive nano-motor.

36-POS **Board 36**

Fungal Space Searching Can Outperform Standard Algorithms

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The ecological success of basidiomycetous fungi, accounting for ~30% of known fungal species, can be attributed to the efficient expansion of branched filaments (hyphae) when seeking out nutritional resources in the surrounding environment. Despite the fact that these fungi naturally colonize 3D micro-structured media, their growth behaviour has been primarily studied on flat surfaces. Fortunately, microfluidics provides a versatile methodology for the probing the fungal various space search strategies.
Solving mazes is a difficult algorithmic exercise, which is why mazes are used to estimate the optimality of the behavioural response, or intelligence, of many higher organisms including ants, bees, mice, rats, octopi, and humans, as well as artificial intelligence-enabled robots. When presented to "intelligence-testing" geometries, e.g., mazes, fungi use a natural program for searching the available space. While different species present different variants of this fungal program, its framework is common and it consists of the interplay of two 'sub-routines': collision-induced branching, and directional memory. These studies also demonstrated that the natural program comprising the two 'sub-routines' is markedly superior to variants where one of these is, or both are suppressed.
A comparison of the performance of the natural algorithm against those of several standard space searching ones revealed that fungi consistently outperforms Depth-First-Search (DFS) algorithm. Although the performance of the natural algorithms is inferior to that of' informed algorithms', e.g., A*, this under-performance does not importantly increase with the increase of the size of the maze. These findings encourage a systematic effort to harvest the natural space searching algorithms used by microorganisms, which, if efficient, can be reverse-engineered for graph and tree search strategies