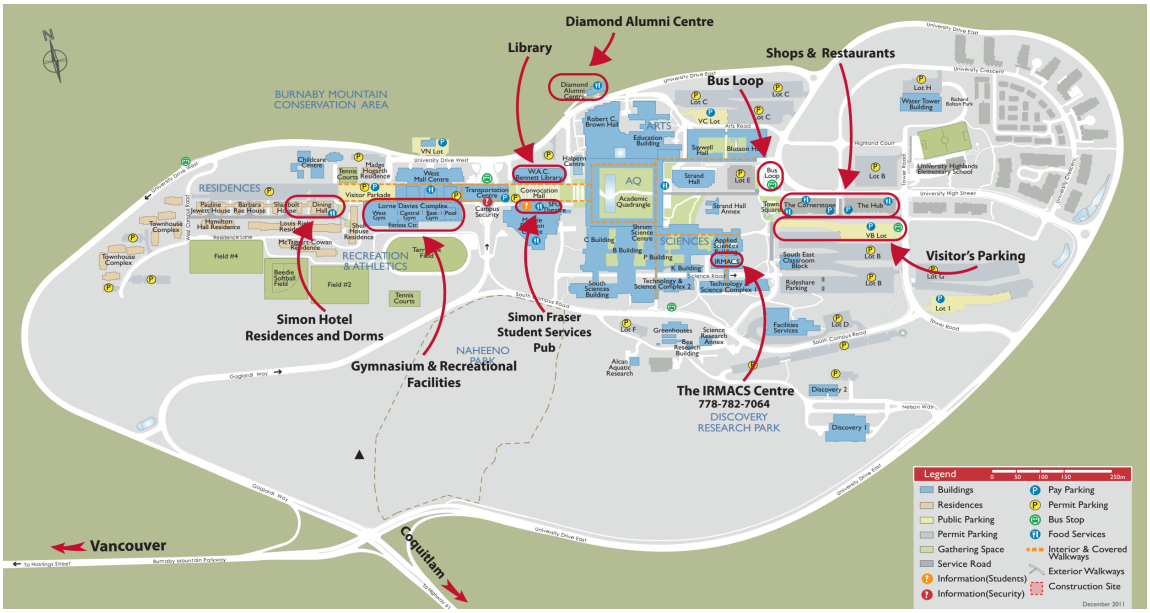
8:30	Registration and poster set up
8:50	Opening
9:00	**Talk session 1** 9:00 **Tuba Altindal** (Physics, SFU). Investigating the role of telopeptides in collagen self-assembly with microrheology 9:16 **Kelvin Lau** (MBB, UBC). Solving a jigsaw puzzle: Combining high-resolution crystal structures of ion channel domains and low-resolution cryo-EM 9:32 **Inigo Novales Flamarique** (Biology, SFU). Form and function of vertebrate photoreceptors 9:48 **Eric Mills** (Physics and Astronomy, UBC). Enthalpy-Entropy Compensation: Messy Data, Real Effect 10:04 **Leon H. Chew** (Life Science Institute, UBC). Structural characterization of the Atg1 kinase complex by single-particle electron microscopy
10:20	Surprise
10:45	Coffee break
11:00	**Keynote talk** **Régis Pomès** (Biochemistry, University of Toronto). Computer Simulations of Peptides and Proteins in Lipid Bilayers
12:15	Lunch break
13:00	**Talk session 2** 13:00 **Nathan Kuwada** (Physics and Bioengineering, University of Washington). Characterizing dynamic protein localization throughout the bacterial cell cycle at the proteome scale 13:16 **Shahzad Ghanbarian** (Physics and Astronomy, UBC). No title 13:32 **Heather Wiebe** (Chemistry, SFU). Volume Profiles as a Tool for Probing the Transition States of Protein Folding 13:48 **David Holloway** (Mathematics, BCIT). 3D reaction-diffusion modeling of conifer embryo development 14:04 **Julien Bergeron** (MBB, UBC). Architecture and assembly of the type 3 secretion system basal body
14:20	Coffee break

14:35	**Talk session 3** 14:35 **Joshua Scurll** and **Hildur Knutsdottir** (Mathematics and Statistics, UBC). An adaptive, patient-specific treatment approach for EGFR-driven, stage IV lung cancer 14:51 **Parvind Grewal** (Mechatronics, SFU). Time dependant Electrical Impedance Spectroscopy of Salivary glands 15:07 **Jeff Salvail** (Physics, SFU). Eigenmode super-resolution imaging in arbitrary optical systems 15:23 **Vaibhav Wasnik** (Physics, SFU). Criticality in neural ensembles: a mean field approach to expand network size from measured data
15:40	**Poster session**
17:10	Social at the Club Ilia

LOCATION

The IRMACS Centre is located on the Burnaby Campus of Simon Fraser University, at the south end of the Applied Science Building, Level 3 (Room 10905).



Contents

1 TALK SESSION 1 **5**
1.1 Investigating the role of telopeptides in collagen self-assembly with microrheology . . . 5
1.2 Solving a jigsaw puzzle: Combining high-resolution crystal structures of ion channel domains and low-resolution cryo-EM . . . 5
1.3 Form and function of vertebrate photoreceptors . . . 6
1.4 Enthalpy-Entropy Compensation: Messy Data, Real Effect . . . 7
1.5 Structural characterization of the Atg1 kinase complex by single-particle electron microscopy . . . 7

2 KEYNOTE TALK **8**
2.1 Computer Simulations of Peptides and Proteins in Lipid Bilayers . . . 8

3 TALK SESSION 2 **9**
3.1 Characterizing dynamic protein localization throughout the bacterial cell cycle at the proteome scale . . . 9
3.2 No title . . . 9
3.3 Volume Profiles as a Tool for Probing the Transition States of Protein Folding 10
3.4 3D reaction-diffusion modeling of conifer embryo development . . . 10
3.5 Architecture and assembly of the type 3 secretion system basal body . . . 11

4 TALK SESSION 3 **12**
4.1 An adaptive, patient-specific treatment approach for EGFR-driven, stage IV lung cancer . . . 12
4.2 Time dependant Electrical Impedance Spectroscopy of Salivary glands . . . 13
4.3 Eigenmode super-resolution imaging in arbitrary optical systems . . . 13
4.4 Criticality in neural ensembles: a mean field approach to expand network size from measured data . . . 14

5 POSTER SESSION **15**
5.1 Solving a jigsaw puzzle: Combining high-resolution crystal structures of ion channel domains and low-resolution cryo-EM . . . 15
5.2 Structural characterization of the Atg1 kinase complex by single-particle electron microscopy . . . 15
5.3 Architecture and assembly of the type 3 secretion system basal body . . . 16
5.4 Mechanically Tightening A Protein Slipknot into A Trefoil Knot . . . 16
5.5 Introducing a Synthetic Kinesin-Inspired Protein Motor . . . 17
5.6 Phase Coexistence in Ternary Lipid Mixtures Containing POPC and Phytosterols, Ergosterol or 7-Dehydrocholesterol . . . 17

5.7 The HCN C-terminus speeds channel activation rates independently of autoinhibition 18
5.8 Modular organization and conformational variability of the yeast SAGA complex 19
5.9 Modeling dorsal closure during Drosophila embryogenesis 19
5.10 A Biomechanical Model for Cell Polarization and Intercalation 20
5.11 Proline scan of the hERG cardiac potassium channel S6 helix reveals the location of the activation gate 21
5.12 The Origin and Spread of Cooperative Replicators in the Presence of Parasitic Template Sequences 21
5.13 Characterizing the viscoelasticity of collagen systems: from molecular solutions to fibrillar networks 22
5.14 External protons destabilize the activated voltage sensor in hERG channels 23
5.15 A mechanical model for fluidization of cells under dynamic strain 24
5.16 S4-S5 Linker Flexibility Stabilizes hERG Channel Closed States 24
5.17 Design and development of a compact Centrifuge Force Microscope 25
5.18 Investigation of the role of SeqA in force generation and chromosome segregation in E. coli 26

TALK SESSION 1

Investigating the role of telopeptides in collagen self-assembly with microrheology

Tuba Altindal, Marjan Shayegan and Nancy R. Forde
Department of Physics, Simon Fraser University

Collagen-based structures are what confer to connective tissues in vertebrates their integrity and strength. Self-assembly of collagen into fibrils and higher-order structures can be mimicked in vitro when the appropriate conditions are met. It has long been known that fibril formation kinetics can be slowed down considerably by proteolytic removal of telopeptides short non-helical fragments flanking the triple-helical domain of a collagen. Based on competitive binding assays, it has also been suggested that there could be transient interactions between telopeptides and the collagen triple-helix, conferring telopeptides their catalytic role in fibrillogenesis. However, the mechanism of interaction between telopeptides and collagen molecules is still unknown.

Here, we describe the results of the experiments where we probed the local viscoelasticity of collagen solutions with intact and removed telopeptides using optical-tweezers-based microrhelogy. We find that the removal of telopeptides significantly reduces elasticity of collagen solutions at timescales from 10 msec to 1 sec. Telopeptides have previously been postulated to bind transiently to specific sites on the collagen triple helix in solution, and thus may facilitate the otherwise less probable encounter of two collagen molecules in proper register. Our microrheology experiments provide direct evidence of increased strength and duration of interprotein contact arising from the presence of telopeptides, critical in catalyzing self-assembly of fibrillar collagen systems.

Solving a jigsaw puzzle: Combining high-resolution crystal structures of ion channel domains and low-resolution cryo-EM

Kelvin Lau and Filip Van Petegem
Department of Molecular Biology and Biochemistry, University of British Columbia

The ryanodine receptor is the largest ion channel known. It is responsible for the release of calcium ions from the intracellular stores of the sarcoplasmic/endoplasmic reticulum. The release of calcium signals for a wide assortment of cellular processes, most importantly, muscle contraction in skeletal and cardiac tissue. Only two regions of this receptor have been described by high-resolution crystal structures. In addition these two domains have been docked into low-resolution cryo-EM structures. Here, I will present a novel domain x-ray crystal structure of the ryanodine receptor from both skeletal and cardiac isoforms.

Stability of the wild-type versus those of mutants will be discussed. The docked location of the domain within the whole channel may suggest its functional properties.

Form and function of vertebrate photoreceptors

Novales Flamarique, Inigo
Department of Biological Sciences, Simon Fraser University

Vertebrate photoreceptors are commonly distinguished based on the shape of their outer segments: those of cones taper, whereas the ones from rods do not. The functional advantages of cone taper, a common occurrence in vertebrate retinas, have remained elusive. Here, this topic was investigated using theoretical analyses aimed at revealing structure-function relationships in photoreceptors. Geometrical optics combined with spectrophotometric and morphological data were used to support the analyses and to test predictions. Three functions were considered for correlations between taper and functionality. The first function proposed that outer segment taper serves to compensate for self-screening of the visual pigment contained within. The second function linked outer segment taper to compensation for a signal-to-noise ratio decline along the longitudinal dimension. Both functions were supported by the data: real cones taper more than required for these compensatory roles. The third function related outer segment taper to the optical properties of the inner compartment whereby the primary determinant was the inner segments ability to concentrate light via its ellipsoid. In support of this idea, the rod/cone ratios of primarily diurnal animals were predicted based on a principle of equal light flux gathering between photoreceptors. In addition, ellipsoid concentration factor, a measure of ellipsoid ability to concentrate light onto the outer segment, correlated positively with outer segment taper expressed as a ratio of characteristic lengths, where critical taper was the yardstick. Depending on a light-funneling property and the presence of focusing organelles such as oil droplets, cone outer segments can be reduced in size to various degrees. The main conclusion from this study was that outer segment taper is but one component of a miniaturization process that reduces metabolic costs while improving signal detection. Compromise solutions in the various retinas and retinal regions occur between ellipsoid size and acuity, on the one hand, and faster response time and reduced light sensitivity, on the other.

Enthalpy-Entropy Compensation: Messy Data, Real Effect

Eric Mills
Physics and Astronomy Department, University of British Columbia

In a wide variety of soft systems the measured enthalpy and the entropy changes upon some perturbation move in step with each other, so that the change in free energy is much less than either. This may indicate some deep principle in the thermodynamics of soft or biological systems, but efforts to understand this have been confounded by the nature of the uncertainty in the enthalpy and entropy, which is so large and correlated that it renders many measurements statistically insignificant. We look at this problem using data on two-state proteins, to shed light on enthalpy-entropy compensation and the perils of reading too much into your data.

Structural characterization of the Atg1 kinase complex by single-particle electron microscopy

Leon H. Chew, Dheva Setiaputra, Daniel J. Klionsky Calvin K. Yip
Life Science Institute, UBC

In eukaryotes, autophagy is an evolutionarily conserved and essential self-degradative process used to maintain cellular homeostasis. Central to autophagy is the formation of double-membrane vesicles termed autophagosomes. The process of autophagosome formation is coordinated by over 35 autophagy- related (Atg) proteins. The Atg1 kinase complex constitutes one group of proteins required for the initial induction step of autophagosome formation. The Atg1 kinase complex is composed of the kinase Atg1, a regulatory phosphoprotein Atg13, and a protein scaffold Atg17 that forms a ternary complex with Atg31 and Atg29.

Our work has determined the structure of a minimal assembly of the Saccharomyces cerevisiae Atg1 kinase complex by single-particle electron microscopy. The Atg17-Atg31-Atg29 sub-complex forms an S-shaped dimer. Atg17 was found to form the central scaffold while Atg31 and Atg29 formed two globular densities tethered to the arcs formed by Atg17. Further analysis of purified Atg17 dimers showed that Atg17 mediated dimerization of the complex while Atg31 and Atg29 had a structural role in maintaining the distinct curvature of the complex. Minimal domains from Atg1 and Atg13 were found to localize to the terminal regions of Atg17. Together this work supports an important role for Atg17 in mediating complex integrity and complex formation.These structures provide information as to how the Atg1 kinase complex functions in autophagosome formation.

KEYNOTE TALK

Computer Simulations of Peptides and Proteins in Lipid Bilayers

Régis Pomès
Molecular Structure and Function, Hospital for Sick Children, and Department of Biochemistry, University of Toronto

Integral membrane proteins fulfill vital functions involving signalling, recognition, and the exchange of ions and nutrients. Thus, the rapid passage of cations in and out of excitable cells through selective pathways in specialized membrane proteins called ion channels underlies the generation and regulation of electrical signals in all living organisms. Accordingly, the malfunction of membrane proteins is linked to numerous diseases such as cystic fibrosis and membrane proteins are top targets of drug design efforts. Biological membranes are also the arena in which many battles of bacterial infection and immune response are played out, which may hold clues for the design of new antibiotics. Finally, the interaction of amyloid-forming peptide oligomers with membranes is thought to play a role in the neurotoxicity of severe degenerative pathologies such as Alzheimers and Parkinsons diseases.

Despite the importance of these processes to human health and disease, elucidating the molecular mechanisms underlying the interaction of peptides and proteins with lipid membranes has remained challenging. The combination of high-performance computing and efficient sampling algorithms makes it possible to access length- and time-scales relevant to the structure and function of peptides and proteins in lipid bilayers at the atomic level of detail. I will present recent and ongoing molecular simulation studies aimed at elucidating (1) the mechanism of ion transport and selectivity in voltage-gated sodium channels, (2) the interaction of antimicrobial peptides with lipid bilayers, and (3) the self-organization of amyloidogenic peptides at the membrane-water interface.

TALK SESSION 2

Characterizing dynamic protein localization throughout the bacterial cell cycle at the proteome scale

Nathan J. Kuwada and Paul A. Wiggins
Departments of Physics and Bioengineering. University of Washington, Seattle, WA, USA

Bacteria exhibit a surprising complexity of subcellular organization despite the absence of membrane-bound organelles and cytoskeletal motor proteins. To characterize localization dynamics throughout the cell cycle at a proteome scale, we combine time-lapse fluorescence microscopy and automated image analysis to capture the cell-cycle localization dynamics of nearly every protein in E. coli with non-diffuse localization. For each protein we capture hundreds of complete cell cycles, which facilitates both the quantitative analysis of cell cycle dynamics and cell-to-cell variation in protein localization. Global analysis reveals many subtle yet significant variations in spatiotemporal localization behavior. In addition, although cell division in E. coli was long believed to be essentially symmetric, we have discovered many examples of non-trivial protein partitioning at cell division. This observation in E. coli suggests that processes like asymmetric cell division, which plays a central role in development, have primitive precursors in bacterial cells with even the simplest life cycles.

No title

Shahzad Ghanbarian
Department of Physics and Astronomy, University of British Columbia

We present the results of molecular dynamics simulations of electrostatic interaction between two DNA double strands. In particular we study a simple model for B-DNA with helical charge pattern in the presence of divalent mobile ions. The effective force on each molecule depends on the central distance and on the relative orientation of two DNAs. We explore the role of solvation effects and the resulting deviations from Coulombs law on the nanoscale on the interaction between two DNA strands. A coarse- grained ion-phosphate potential can be constructed from all-atom simulations. This function can be parameterized in order to reproduce the structure of counter ions in detailed atomistic solvent model and in the presence of DNA. Successes and limitations of this approach for capturing ion-ion correlation effects will be discussed.

Volume Profiles as a Tool for Probing the Transition States of Protein Folding

Heather Wiebe and Noham Weinberg
Department of Chemistry, Simon Fraser University

Within the framework of transition state theory (TST), the kinetics of large-scale conformational changes in proteins and other biomolecules is described in terms of transition states (TS). The volumetric properties of TSs are expressed as the logarithmic pressure derivatives of the rate constants, known as activation volumes V(TS) = -RT(lnk P). Experimental activation volumes are available for a number of protein systems. According to TST, activation volumes can be identified as the difference in volume between the TS and reactant species V(TS) = V(TS) - V(R). The concept of volume profile V(y), describing how the volume of a molecular system varies along its reaction coordinate y, is widely used in discussing the mechanisms of high pressure reactions. Volume profiles can be calculated theoretically using our recently developed method based on molecular dynamics (MD) simulations. If the position y(TS) of the TS along the reaction coordinate is unknown, it can be found by locating V(TS) on the MD-generated volume profile: V(y) = V(TS). We illustrate this approach by its successful application to the unfolding of a model chain system.

3D reaction-diffusion modeling of conifer embryo development

David Holloway
Mathematics, British Columbia Institute of Technology

Experimental work at UBC, UVic and BCIT has shown that cotyledons (embryonic seed leaves) in conifers form with a constant spacing, such that larger embryos have more cotyledons. We have also used hormone disruption to alter the patterning. I will describe recent work on modelling the spacing phenomena as two coupled reaction-diffusion mechanisms, with the first mechanism establishing an annular pattern within which the cotyledons can be formed by the second (hormone-dependent) mechanism.

Architecture and assembly of the type 3 secretion system basal body

Julien R. C. Bergeron (1), Liam J. Worrall (1), Nikolaos G. Sgourakis (2), David Baker (2), Natalie C. J. Strynadka (1)
1 Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada.
2 Department of Biochemistry, University of Washington, Seattle, USA.

The type 3 secretion system injectisome is a large (3.5 MDa) syringe-shaped macromolecular assembly found in pathogenic Gram-negative bacteria, that allows for the direct delivery of virulence effectors into host cells. It is composed of a basal body, a lock-nut structure spanning both bacterial membranes, and a needle that protrudes away from the bacterial surface. A hollow channel spans throughout the apparatus, permitting the translocation of effector proteins from the bacterial cytosol to the host plasma membrane. The basal body is composed largely of three membrane-embedded proteins, that form oligomerized concentric rings.

In order to further characterize the architecture and assembly of the basal body, we have solved the structures of four individual domains from the three proteins composing the prototypical Salmonella typhimurium SPI-1 basal body, namely InvG, PrgH and PrgK, using a combination of X-ray crystallography and NMR. We next have developed a new approach incorporating symmetric flexible backbone docking and EM data, to produce refined models for the oligomeric assembly of these individual domains within the functional T3SS injectisome. Finally, we have used a combination of NMR, EM and molecular modelling to investigate the discrete steps leading to the formation of a secretion-competent basal body.

TALK SESSION 3

An adaptive, patient-specific treatment approach for EGFR-driven, stage IV lung cancer

Philip Gerlee, Ben Creelan, Lori Hazelhurst, Jill Gallaher, **Joshua Scurll**, **Hildur Knutsdottir**, Olya Grove, Dan Nichol, Marc Sturrock
Department of Mathematics and Statistics, University of British Columbia

Lung cancer is the leading cause of cancer-related mortality in Canada and the United States. It can typically be classified at the molecular level by oncogene mutations that drive the cancer, with one such mutation occurring in the EGFR oncogene. Standard of care for patients suffering from stage IV (metastatic) non-small cell lung cancer (NSCLC) that is being driven by an EGFR oncogene mutation is to give the patient an EGFR tyrosine kinase inhibitor (TKI) such as Erlotnib, but unfortunately most patients develop acquired resistance to the drug within a year or so, and survival rates are poor, with median survival time less than two years. Hildur Knutsdottir and I will present a $50,000-grant-winning model aimed at tackling this problem, which we developed as part of a team at the Integrated Mathematical Oncology (IMO) Workshop on Personalized Medicine at the Moffitt Cancer Center, Tampa, Florida in November 2013. We first simplified the EGFR pathway down to five key genes and subsequently developed a model that describes the evolutionary dynamics of the number of cell clones harbouring various combinations of gene mutations or amplifications. Using a threshold tumour burden as an indicator of patient death, we proceeded to use a genetic algorithm to predict a locally optimal sequence of drug combination therapies to maximize patients survival times. When simulated on a cohort of 100 virtual patients, our models selected treatment schedule predicted a prolongation of survival by an average of 45 days compared with standard of care Erlotnib. Moreover, our model allows for new patient data to be fed back into the model every time new data (e.g. imaging) is available from a patient, thus allowing the model to be continually refined and increasingly personalized for individual patients.

Time dependant Electrical Impedance Spectroscopy of Salivary glands

Parvind K Grewal (1,2), Steven Thomas (2), Anand Karvat (2), Farid Golnaraghi (1), Jeff Liu (2), Krishnan Kalpagam (2) and Kirpal S Kohli (2,3)
1 Mechatronic Systems Engineering, Simon Fraser University, Surrey, Canada
2 BC cancer Agency, Fraser Valley Cancer Centre, Surrey, Canada
3 E-mail any correspondence to: kkohli@bccancer.bc.ca

Xerostomia is a known side effect of radiation therapy patients undergoing head and neck radiotherapy. Electrical Impedance Spectroscopy (EIS) has a potential to better understand the mechanism of salivary production and related cellular changes due to radiation tissue damage. In present study the electrical impedance is measured using an impedance spectroscope HF2IS from Zurich instruments (Zurich Instruments). The electrodes are circular Ag/AgCl 2mm electrodes from Vermed. These are non-polarizable and generate less than 10V noise, hence preferred for skin surface measurements. This paper discusses the use of superficially placed cutaneous electrodes for EIS measurement of salivary glands output and the challenges related to signal drift that needs to be better accounted for.

Eigenmode super-resolution imaging in arbitrary optical systems

Jeff Z. Salvail
Department of Physics, Simon Fraser University

Imaging is one of the most basic and fundamental tools in science, having nearly countless applications across chemistry, biology, physics and engineering. While the limit to imaging resolution was originally considered the Abbe diffraction limit, it is now well known that sub-diffraction imaging is possible. In this talk I will discuss a new approach to super-resolution imaging, based on optical eigenmode reconstruction. The technique is robust and versatile, in principle combinable with other super-resolution techniques. I will present experimental and numerical data showing that eigenmode super-resolution imaging offers measurable improvement in diffraction-limited imaging systems, and can be used for arbitrary imaging systems (i.e., with arbitrary diffraction and aberrations) and for arbitrary objects.

Criticality in neural ensembles: a mean field approach to expand network size from measured data

Vaibhav Wasnik (1), Barack Caracheo (2), Jeremy Seamans (2) and Eldon Emberly (1)
1 Department of Physics, Simon Fraser University,
2 Brain Research Center, University of British Columbia.

At the point of a second order phase transition also termed as a critical point, systems display long range order and their macroscopic behaviours are independent of the microscopic details making up the system. This makes the idea of criticality interesting for studying biological systems which even though are different microscopically still have similar macroscopic behaviours. Recent high-throughput methods in neuroscience are making it possible to explore whether criticality exists in neural networks. Despite being high-throughput, many data sets are still only a minute sample of the neural system and methods towards expanding these data sets have to be considered in order to study the existence of criticality. Using measurements of firing neurons from the pre-frontal cortex (PFC) of rats, we map the data to a system of Ising spins and calculate the specific heat as a function of the measured network size, looking for the existence of critical points. In order to go to the thermodynamic limit, we propose a mean field approach for expanding such data.

POSTER SESSION

Solving a jigsaw puzzle: Combining high-resolution crystal structures of ion channel domains and low-resolution cryo-EM

Kelvin Lau and Filip Van Petegem
Department of Molecular Biology and Biochemistry, University of British Columbia

The ryanodine receptor is the largest ion channel known. It is responsible for the release of calcium ions from the intracellular stores of the sarcoplasmic/endoplasmic reticulum. The release of calcium signals for a wide assortment of cellular processes, most importantly, muscle contraction in skeletal and cardiac tissue. Only two regions of this receptor have been described by high-resolution crystal structures. In addition these two domains have been docked into low-resolution cryo-EM structures. Here, I will present a novel domain x-ray crystal structure of the ryanodine receptor from both skeletal and cardiac isoforms. Stability of the wild-type versus those of mutants will be discussed. The docked location of the domain within the whole channel may suggest its functional properties.

Structural characterization of the Atg1 kinase complex by single-particle electron microscopy

Leon H. Chew, Dheva Setiaputra, Daniel J. Klionsky Calvin K. Yip
Life Science Institute, UBC

In eukaryotes, autophagy is an evolutionarily conserved and essential self-degradative process used to maintain cellular homeostasis. Central to autophagy is the formation of double-membrane vesicles termed autophagosomes. The process of autophagosome formation is coordinated by over 35 autophagy- related (Atg) proteins. The Atg1 kinase complex constitutes one group of proteins required for the initial induction step of autophagosome formation. The Atg1 kinase complex is composed of the kinase Atg1, a regulatory phosphoprotein Atg13, and a protein scaffold Atg17 that forms a ternary complex with Atg31 and Atg29.

Our work has determined the structure of a minimal assembly of the Saccharomyces cerevisiae Atg1 kinase complex by single-particle electron microscopy. The Atg17-Atg31-Atg29 sub-complex forms an S-shaped dimer. Atg17 was found to form the central scaffold while Atg31 and Atg29 formed two globular densities tethered to the arcs formed by Atg17. Further analysis of purified Atg17 dimers showed that Atg17 mediated dimerization of the complex while Atg31 and Atg29 had a structural role in maintaining the distinct curvature of the complex. Minimal domains from Atg1 and Atg13 were found to localize to the terminal regions of Atg17. Together this work supports an important role for Atg17 in

mediating complex integrity and complex formation.These structures provide information as to how the Atg1 kinase complex functions in autophagosome formation.

Architecture and assembly of the type 3 secretion system basal body

Julien R. C. Bergeron (1), Liam J. Worrall (1), Nikolaos G. Sgourakis (2), David Baker (2), Natalie C. J. Strynadka (1)
1 Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada.
2 Department of Biochemistry, University of Washington, Seattle, USA.

The type 3 secretion system injectisome is a large (3.5 MDa) syringe-shaped macromolecular assembly found in pathogenic Gram-negative bacteria, that allows for the direct delivery of virulence effectors into host cells. It is composed of a basal body, a lock-nut structure spanning both bacterial membranes, and a needle that protrudes away from the bacterial surface. A hollow channel spans throughout the apparatus, permitting the translocation of effector proteins from the bacterial cytosol to the host plasma membrane. The basal body is composed largely of three membrane-embedded proteins, that form oligomerized concentric rings.

In order to further characterize the architecture and assembly of the basal body, we have solved the structures of four individual domains from the three proteins composing the prototypical Salmonella typhimurium SPI-1 basal body, namely InvG, PrgH and PrgK, using a combination of X-ray crystallography and NMR. We next have developed a new approach incorporating symmetric flexible backbone docking and EM data, to produce refined models for the oligomeric assembly of these individual domains within the functional T3SS injectisome. Finally, we have used a combination of NMR, EM and molecular modelling to investigate the discrete steps leading to the formation of a secretion-competent basal body.

Mechanically Tightening A Protein Slipknot into A Trefoil Knot

Chengzhi He, Adam Xiao, Guillaume Lamour, Joerg Gsponer* and Hongbin Li*

Knotted polypeptide chain is one of the most surprising topological features found in some proteins. The topological difficulty in the folding of knotted proteins has become a challenging problem. It was reported that a structure of slipknot could serve as an important intermediate state during the folding of knotted proteins. Here we use single molecule force spectroscopy (SMFS) as well as steered molecular dynamics (SMD) simulations to

investigate the mechanism of transforming a slipknot protein AFV3-109 into a tightened trefoil knot by pulling. Our results show that by pulling the N-terminus and the threaded loop of AFV3-109, the protein can be unfolded via multiple pathways and the slipknot can be transformed into a tightened trefoil knot. The SMD simulation results, which are consistent with our experimental findings, provide detailed molecular mechanism of mechanical unfolding and knot tightening of AFV3-109.

Introducing a Synthetic Kinesin-Inspired Protein Motor

Martin J. Zuckermann, Elizabeth H.C. Bromley, Christopher N. Angstmann, Gerhard A. Blab, Heiner Linke, **Nancy R. Forde**, Paul M.G. Curmi

Based on a recent approach to understanding biological protein-based molecular motors (1), we present computer simulations of a novel protein-based motor dubbed the Synthetic Kinesin Inspired Protein (SKIP). SKIP is a purely diffusive linear motor consisting of four ligand gated DNA-binding (repressor) proteins of two types, A and B, linked by three rigid coiled-coils (rods) to form a complex A1-A2-B2-B1, the length of the central rod being shorter than the outer two. Directional stepping along a periodic DNA track is maintained by a temporally periodic external chemical supply. Due to the shorter central rod, SKIP makes use of a mechanism analogous to that used by kinesin which involves docking of a neck linker onto a motor domain. This mechanism allows kinesin to reduce its diffusional search time for a binding site. We use coarse-grained Langevin Dynamics simulations in the overdamped limit to study the detailed motion of SKIP when subject to a load force and SKIP acting as a shuttle.

1. Bromley et al. HFSP Journal 3 204-213 (2009).

Phase Coexistence in Ternary Lipid Mixtures Containing POPC and Phytosterols, Ergosterol or 7-Dehydrocholesterol

Mehran Shaghaghi, Ming-Yen Kuo, Ya-Wei Hsueh, Martin Zuckermann, Jenifer Thewalt

2H-NMR spectroscopy was used to investigate the occurrence of phase coexistence in multilamellar vesicles of DPPC and POPC (33mol%: 33mol%) with either stigmasterol, brassicasterol, ergosterol or 7-DHC (each 33mol%). In all cases, the sn-1 chains of DPPC and POPC were deuterated in turn, and 2H-NMR spectra were measured for both lipid components as a function of temperature between 5 oC and 48 oC. The chain order of DPPC was found to be greater than that of POPC in all the above mixtures. An equimolar

DPPC/POPC membrane exhibits a clear solid-ordered (so)-liquid disordered (ld) transition between 5 oC and about 33 oC as observed for each lipid by a fairly steep reduction in the average spectral width. Adding sterol generally results in a more gradual change in average spectral width over this temperature range. Depending on the particular sterol, NMR spectra taken in this temperature range either appeared poorly resolved (blurry) or displayed coexisting liquid ordered (lo) and ld spectra. Liquid-liquid phase coexistence is observed in DPPC/POPC/ergosterol mixtures. The spectra for DPPC/POPC/ brassicasterol only exhibited liquid-liquid coexistence when a small concentration (0.02 mol%) of the fluorescent lipid dye TR-DHPE was added to the system. Only one liquid phase was observed for either DPPC/POPC/7DHC or DPPC/POPC/stigmasterol. In summary, our interest was to study how liquid phase coexistence in DPPC/POPC/sterol is modified by small changes in the sterol structure. These studies show that ergosterol is the most effective sterol in fostering phase coexistence. Note that in comparison to cholesterol, which does not cause micron scale phase coexistence in DPPC/POPC, ergosterol has an extra double in the fused ring, as well as a double bond at C22. Possible connections between the detailed sterol structures and these observations will be discussed.

The HCN C-terminus speeds channel activation rates independently of autoinhibition

Kaylee E. A. Magee and Edgar C. Young

The C-terminal Cyclic Nucleotide Binding (CNB) fold of HCN channels preferentially stabilizes the closed state, and this "autoinhibition" is relieved by cAMP binding or by CNB fold truncation [Wainger et al. (2001) Nature 411: 805]. Whereas the autoinhibition model was discovered in excised-patch conditions, we investigated the ability of the C-terminal region to regulate gating thermodynamics and kinetics with two-electrode voltage clamp on Xenopus oocytes. We examined wildtype channels (HCN2) with endogenous cAMP bound, C-terminal-truncated channels (HCN2 ΔCNB), and channels with autoinhibition imposed due to a mutation preventing cAMP binding (HCN2 R591E). Hyperpolarized V1/2 values were observed for HCN2 R591E, verifying the presence of autoinhibition in intact oocytes. Surprisingly, we found conditions where the autoinhibition model is insufficient to explain the hyperpolarization-dependent activation kinetics. First, channels with autoinhibition relieved by cAMP binding exhibited faster activation (HCN2, 81 $\pm$ 17 ms at -150 mV) than channels with autoinhibition wholly removed by CNB fold truncation (HCN2 ΔCNB, 215 $\pm$ 110 ms at same voltage). Second, autoinhibited channels opened at a relatively fast rate (HCN2 R591E, 77 $\pm$ 16 ms) similar to the autoinhibition-free HCN2. We thus propose that in intact oocytes channels possessing a CNB fold are in a quickening conformation"" which speeds activation kinetics. Full length channels can be in this conformation regardless of

whether or not they are autoinhibited. Activation rates at the less hyperpolarized voltage of -100 mV were faster for full length liganded HCN2 compared to unliganded HCN2, as predicted by the autoinhibition model. Therefore in intact oocytes, the autoinhibition and the C-terminal quickening conformation exert two opposing influences on HCN activation speeds with a balance that changes with voltage.

Modular organization and conformational variability of the yeast SAGA complex

Dheva Setiaputra, James D. Ross, Calvin K. Yip

Spt-Ada-Gcn5-Acetyltransferase (SAGA) is a conserved histone acetyltransferase complex that regulates transcription through acetylation and deubiquitination of nucleosomal histones. The 19 subunits constituting SAGA are thought to organize into four modules. To further elucidate the relationship between these modules, we developed an improved purification method that enhances the stability of SAGA and used single-particle electron microscopy to examine the overall architecture and organization of this complex. Our two- and three-dimensional analyses revealed that SAGA is flexible and adopts three major conformations involving large-scale rearrangements of its subunits. Subunit deletion and labeling showed that the acetyltransferase module is located in the most mobile region of the complex, and that the loss of the deubiquitination module reduces SAGAs ability to adopt one specific conformation. These results lead to a working model in which the different modules of SAGA coordinate to mediate structural remodeling, which in turn accommodates binding of various substrates and transcriptional activators.

Modeling dorsal closure during Drosophila embryogenesis

A. Mafi (1), W. Lou (2), T. J. C. Harris (3) and J. J. Feng (1,4)
1 Department of Chemical and Biological Engineering, UBC
2 Department of Physics, UBC
3 Department of Cell and Systems Biology, University of Toronto
4 Department of Mathematics, UBC

We report a mathematical model for the dynamic interaction among signaling proteins that control dorsal closure of Drosophila. Recent experiments have implicated the Par-family proteins as prominent players. In particular, aPKC suppresses the apicomedial actomyosin network, while Bazooka tends to inhibit aPKC. Their spatial recruitment from the cortex to the apicomedial surface introduces a delayed negative feedback loop. Our

model represents the interaction among the signaling proteins through delayed differential equations, and further couples these kinetic equations with mechanical equations governing the dynamic contraction of the cells due to the actomyosin network. Thus, we are able to predict sustained oscillations of the cells in the early stage of dorsal closure, and a natural transition to a later stage of sustained areal contraction. These predictions are in good agreement with observations.

A Biomechanical Model for Cell Polarization and Intercalation

Haihan Lan (1), Qiming Wang (2), J.J. Feng (3,4)
1 Department of Electrical and Computer Engineering, University of British Columbia, Vancouver, BC V6T 1Z4, Canada
2 Department of Mathematics and Statistics, York University, Toronto, ON M3J 1P3, Canada
3 Department of Mathematics, University of British Columbia, Vancouver, BC V6T 1Z2, Canada
4 Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, BC V6T 1Z3, Canada

In this poster we present a biomechanical model for planar cell polarization and dynamic tissue remodelling during intercalation in Drosophila embryo. Our model is based on the myosin positive feedback mechanism observed by Fernandez-Gonzalez et al. Dev. Cell 17, 736743 (2009). We model the kinetics of three chemical species: Rho-kinase, non-muscle myosin-II and Bazooka/Par-3 in a cluster of initially hexagonal cells. The mechanical deformation of the cells is governed by contraction and elongation of cell edges subject to passive elasticity, active myosin contraction and adhesion via adherens junctions. After setting the initial perturbations and allowing the system to evolve, the model predicts the correct polarization of signalling proteins between the anterior-posterior and dorsal-ventral axes, as well as cell intercalation through a neighbor-swapping T1 process. Our current work focuses on improving the model based on experimental evidence and extracting insight from model predictions.

Proline scan of the hERG cardiac potassium channel S6 helix reveals the location of the activation gate

Samrat Thouta, Stanislav Sokolov, Yen M. Cheng and Tom W. Claydon
Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, British Columbia, Canada

The human-ether-a-go-go-related gene (hERG) channels have a crucial role in regulating the electrical activity of the heart. Unlike other voltage-gated potassium (Kv) channels, hERG channels display unusual gating characteristics: slow activation (opening) and deactivation (closing) with rapid inactivation and recovery from inactivation. These unusual gating properties provide a resurgent repolarizing current (Ikr) that is essential for repolarization and termination of the cardiac action potential. In the archetypal Kv channel, Shaker, the activation gate is formed by the convergence of the inner S6 transmembrane helices near a conserved proline-valine-proline (PVP) motif, which introduces a kink in the helices that allows for electromechanical coupling with motions of the voltage sensing unit via a short connecting S4-S5 linker. hERG channels lack the PVP motif and therefore the location of the activation gate and how it is coupled to voltage sensor movement is less clear. Here, we performed a proline scan of the inner S6 helix, from I655 to Y667, to determine the position of the gate. The scan revealed that proximal (I655P-Q664P), but not distal (R665P-Y667P) substitutions trapped the activation gate open, leading to constitutive channel current. This suggests that Q664 marks the position of the activation gate in hERG channels. Using voltage clamp fluorimetry and gating current analysis to report on motion of the voltage sensor, we show that proline substitutions trap the activation gate open by disrupting the coupling between the voltage sensor unit and the pore of the channel. We characterize the voltage sensor movement in one such trapped-open channel and show that a component of voltage sensor movement appears to be intrinsically slow in hERG channels

---

The Origin and Spread of Cooperative Replicators in the Presence of Parasitic Template Sequences

Julie A. Shay (1,2), Christopher Huynh (1), Paul G. Higgs (1)
1 Department of Physics and Astronomy, McMaster University, Hamilton, Ontario L8S 4M1, Canada
2 Present address: Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

The origin of life requires the emergence of a system of autocatalytic polymers such as self-replicating RNA. We envisage a combination of a single-molecule replication process (template directed replication) and a two-molecule catalytic replication process (where one

molecule is a catalyst and the other is a template). Non-catalytic molecules that can be templates act as parasites of the catalysts. Previous work by other groups showed that spatial clustering of catalysts allows them to survive in the presence of parasites, but did not consider the question of the origin of life. Our previous work emphasized that the origin of life is a stochastic transition involving a small number of molecules. In this paper, we present a model which can simulate both the origin and maintenance of a system of replicating molecules in the presence of parasites. Two additional problems faced by the replicators are that the fraction of functional sequences in sequence space, f, may be very small, and that replication is error prone. Parasites are continually produced by inaccurate replication of catalysts. In the well-mixed case, there are stable solutions corresponding to living and dead situations (autocatalysis or prebiotic chemistry). However, when f is very small, only the dead state exists. In contrast, in spatial simulations on a lattice with local diffusion, it is possible for a small patch of replicators to be initiated from a single initial catalyst. Once the patch is large enough to be stable, it spreads to cover the whole surface. Thus the model demonstrates that a stochastic transition from the dead state to the living state can occur even when parasites are present, even when replication is inaccurate, and even when f is very small. Spatial structure and limited diffusion are essential because they allow the concentration fluctuations that create a localized living patch as well as preventing the system being over-run by parasites.

Characterizing the viscoelasticity of collagen systems: from molecular solutions to fibrillar networks

Marjan Shayegan (1) and Nancy R. Forde (1,2)
1 Simon Fraser University, Department of Chemistry, 8888 University Dr., Burnaby, BC, V5A 1S6, e-mail: msa98@sfu.ca
2 Simon Fraser University, Department of Physics, 8888 University Dr., Burnaby, BC, V5A 1S6, e-mail: nforde@sfu.ca

Collagen, the most abundant protein in the extracellular matrix and many connective tissues, provides integrity and mechanical strength through its hierarchical organization. Defects in collagen can lead to serious connective tissue diseases. Given that mechanical properties and the structure of materials are related, the main goal of our research is to understand the correlation between collagens structure and its physical and mechanical properties.

In order to determine the viscoelastic properties of collagen systems at different hierarchical scales, we performed optical-tweezers-based microrheology. Here, optical tweezers was used to trap and monitor thermal fluctuations of probe particles located within our sample of interest. We found that viscoelastic response is altered by collagen concentration,

which particularly influences the high-frequency response. We then evaluated viscoelastic properties of collagen while it self-assembles into fibrils, properties which vary by an order of magnitude at different locations within fibrillar collagen gels. By making measurements as collagen self-assembled into fibrils, we evaluated the development of mechanical heterogeneity during this process.

External protons destabilize the activated voltage sensor in hERG channels

Yu Patrick Shi, Yen May Cheng, Aaron C. Van Slyke, Tom W. Claydon
Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, British Columbia, Canada

Human ether--go-go related gene (hERG) encodes for a voltage-dependent potassium channel that is important for the repolarization phase of the cardiac action potential. hERG channels possess unique gating mechanisms that ideally serve this purpose: upon depolarization, channels activate slowly and inactivate quickly, so that little current passes early in the action potential; however as the membrane repolarizes, channels quickly recover from inactivation and deactivate slowly, so that robust repolarizing potassium current passes causing termination of the action potential. The importance of hERG channels is underscored by the observation that loss-of-function congenital mutations predispose individuals to cardiac arrhythmia and sudden death. Acidosis is a consequence of myocardial ischemia and is associated with suppression of hERG channel function that may be pro-arrhythmogenic. We have therefore investigated the site and mechanism of action of protons on hERG channels. External acidic pH shifted the voltage-dependence of hERG channel activation towards more depolarized potentials and accelerated deactivation; both of which points to a relative stabilization of the closed state over the open state. Interestingly, the divalent cation, Cd2+, mimicked the effects of protons. Cd2+ is known to coordinate with a metal ion binding pocket formed by residues D456, D460, and D509 within the voltage sensing unit of the channel. We hypothesized, therefore, that a common site may be responsible for the effects of external protons on hERG gating. We show that the pH-sensitivity of hERG channel activation, but not deactivation, was greatly reduced in the presence of 0.1 mM Cd2+, suggesting that protons and Cd2+ compete for a common binding site to mediate their effects on activation, but that deactivation is mediated by a separate mechanism. Mutational analysis confirmed that D509 and the cooperative action of D456 and D460 play a critical role in the pH-dependence of hERG channel activation. Importantly, neutralization of all three acidic residues abolished the shift of activation induced by protons, suggesting that the metal ion binding pocket alone accounts for the proton-induced shift of activation in hERG channels. Furthermore, using a fluorescence

probe to track voltage sensor movement, we show that the effect of protons on the voltage-dependence of activation is due to a destabilization of the activated configuration of the voltage sensor. These data therefore elucidate the site and mechanism of action of acidic pH on hERG channel activation.

A mechanical model for fluidization of cells under dynamic strain

Tenghu Wu, James J. Feng

Recently, Fredberg and coworkers have investigated the response of human airway smooth muscle cells under a transient stretch-unstretch (SC) and compress-uncompress (CS) maneuvers. Their results indicate that the transient SC maneuver causes a sudden fluidization of the cell followed by a slow resolidification. Interestingly, the CS maneuver does not induce fluidization at all. We have built a model to probe the asymmetric behavior of the stress fibers (SFs) under the CS and SC maneuvers. The model couples the crossbridge cycle of myosin motors with a viscoelastic Kelvin-Voigt element representing the SF. Simulation results suggest that the sensitivity of the myosin detachment rate to the SF tension may have caused the asymmetric behavior of the SF under the CS and SC maneuvers. For the SC maneuver, the stretch increases the tension of the SF and suppresses the myosin detachment rate. The subsequent compression then causes a large proportion of the myosin population to disengage from actin filaments. This leads to the disassembly of the stress fibers and the observed fluidization.

S4-S5 Linker Flexibility Stabilizes hERG Channel Closed States

C.M. Hull, A.C. Van Slyke, J. Qi and T.W. Claydon

The hERG potassium channel, found in cardiac tissues, is an important contributor to repolarization of the cardiac action potential. Loss-of-function of hERG channels is associated with prolonged action potential durations that predispose to Long-QT syndrome, a life-threatening cardiac arrhythmia. Although hERG channels resemble other voltage-gated potassium (Kv) channels, the activation (opening) and deactivation (closing) kinetics are unusually slow and this is critical to their unique role in repolarization. Despite this, the mechanistic basis for slow gating kinetics in hERG is unknown. In previous work, our group has shown that flexibility afforded by a key residue (Gly546) in the S4-S5 ?-helical linker, which connects the voltage sensing domain to the pore domain in hERG channels, dictates the relative stability of the open state of the channel. Mutation of Gly546 to a helix-stabilizing leucine (G546L) resulted in a relative destabilization of

the closed state of the channel (i.e. the voltage-dependence of hERG channel activation was dramatically shifted by ∼-50mV). Here, using a combination of site-directed mutagenesis, two-electrode voltage clamp electrophysiology and voltage clamp fluorimetry, we have investigated whether flexibility at position 546 in particular is key, or if flexibility of the S4-S5 linker per se is a critical determinant of the closed-open equilibrium in hERG channels. To do this, we have tested the effects of re-introducing flexibility at different sites within the S4-S5 linker, by substituting a glycine residue at different positions in the background of the G546L mutation. This enabled evaluation of the role of flexibility at each position within the linker. We found that glycine re-introduced at positions in the N-terminal end of the S4-S5 linker restored the WT-like voltage-dependence of activation and stability of the closed state in G546L channels, whilst many other glycine mutants tested were unable to restore WT-like gating. These data suggest that flexibility of the S4-S5 linker, particularly in the N-terminal region, contributes to stabilization of hERG channels in the closed state and that the native glycine, G546, affords this flexibility in WT channels. Our glycine scan of the S4-S5 linker highlighted an additional interesting finding. All mutations within the linker greatly attenuated the coupling of voltage sensor relaxation with the pore of the channel. Relaxation of the voltage sensor is a poorly understood, but physiologically relevant gating step that involves immobilization of the voltage sensor in its activated position. Our data show for the first time that translation of the relaxation process to the pore of hERG channels, which results in stabilization of the open state, involves the S4-S5 linker. Interestingly, a similar uncoupling of voltage sensor relaxation with the pore was shown to be caused by mutations in the N-terminus suggesting that the S4-S5 linker may form functional interactions with the N-terminus to regulate closing of the hERG channel pore.

Design and development of a compact Centrifuge Force Microscope

Michael W.H. Kirkness (1,2) and Nancy R. Forde (2)
1 Department of Molecular Biology and Biochemistry
2 Department of Physics, Simon Fraser University, Burnaby BC V5A 1S6, Canada

Whether developing new or advancing existing technology, there are several considerations: higher productivity, easier use, and/or faster and more economical instruments and techniques. The idea of introducing a light microscope into a centrifuge to monitor cells, molecules and other objects under force is not new [1]. Further development of the centrifuge force microscope has occurred through time, leading to the development of a high-throughput single-molecule stretching instrument, which involves tethering single molecules of interest between microspheres and a sample chamber [2]. Our goal was to reduce cost and create a very simple instrument with similar capabilities, which would

be accessible for labs even without prior single-molecule experience. By using a wireless camera, custom sample chambers and an infinity-corrected microscope objective lens, we were able to develop a centrifuge force microscope that is small and light enough to fit inside a benchtop swinging bucket centrifuge, for a total cost of under $1000. The field of view enables parallel visualization of hundreds of tethered particles. Instrument stability was a major concern throughout the design process; the current version is stable from 200-1500 RPM (8-450g), corresponding to an ability to exert forces from fN-?N on silica or polystryene microspheres through the use of different rotation speeds . Microspheres are monitored in real time through video; post-processing particle tracking of the Brownian motion of the beads allows the extraction of the forces they are experiencing [3]. Currently, tethering of DNA between the sample chamber and microspheres is being explored, for further testing of the instrument capabilities.

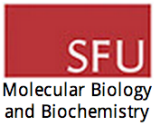
References:
1. E.N. Harvey and A.L. Loomis, A microscope-centrifuge. Science 72, 42-44 (1930).
2. K. Halvorsen and W.P. Wong, Massively parallel single-molecule manipulation using centrifugal force. Biophysical Journal 98, L53-55 (2010)
3. T. Strick et al., The elasticity of a single supercoiled DNA molecule. Science 271, 1835-1837 (1996)

Investigation of the role of SeqA in force generation and chromosome segregation in E. coli

Julie A. Cass, Nathan J. Kuwada, Paul A. Wiggins

While chromosome segregation is essential for the survival of a species, the mechanism for force generation in this process is completely unknown for the model organism Escherichia coli. While most bacterial species possess a particular set of genes known to be involved in structuring and partitioning the chromosome, homologues of these genes are completely absent in E. coli. These bacteria posses their own unique set of genes, suggesting one, or a combination of the resulting unique gene products is responsible for force generation during chromosome segregation. Among these is the non-essential DNA-biding protein SeqA, known to be responsible for the regulation of the initiation of DNA replication. Although consistent with SeqAs known function in replication regulation, many of its characteristics, such as preferentially binding new DNA near oriC, are suggestive of an additional role in segregation. We therefore aim to measure cell-cycle dependent chromosome forces in a SeqA-deletion strain and obtain a super-resolution structure of SeqA clusters within E. coli to investigate the potential role of SeqA in force generation during chromosome segregation.

Sponsored by:



Organized by:

Momcilo Gavrilov, Pau Farré, Jason Wang, Tristan Hansen and Sherry Leung