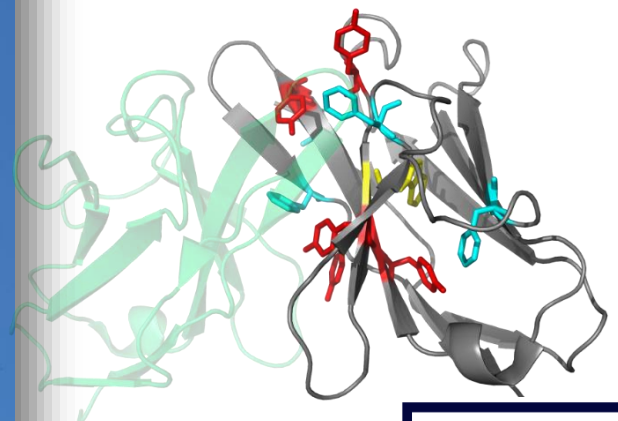
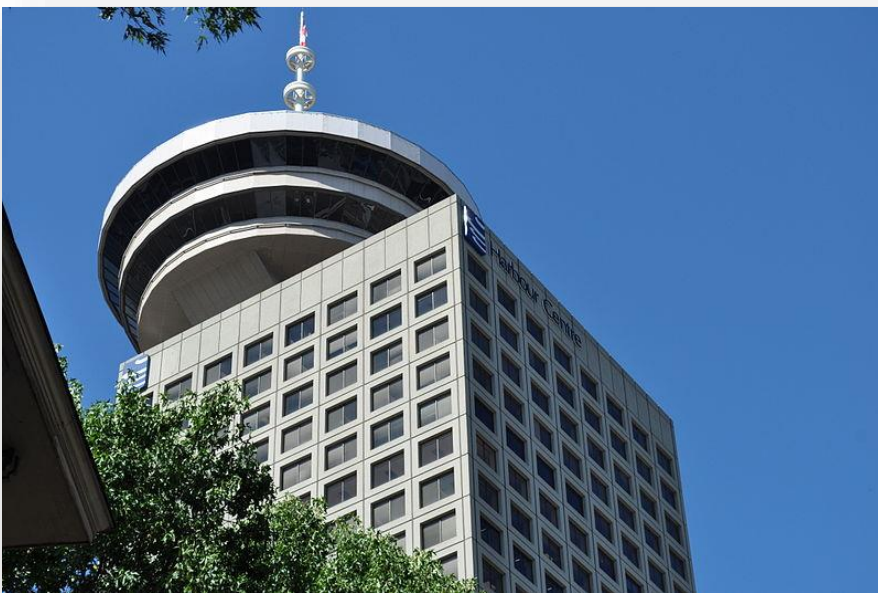




**VANCOUVER
2018**

Biophysical Society of Canada
4th Annual Meeting
May 22-25, 2018
Simon Fraser University, Vancouver

FULL PROGRAM



Biophysical Society
Société de biophysique
du Canada



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WIFI INFORMATION

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Valid for the SFUNET Network.

Welcome to Vancouver!

Bienvenue à Vancouver! We welcome you to the 4th Annual Meeting of the Biophysical Society of Canada. Following the first three meetings of the Society in Waterloo, Winnipeg and Montreal, the BSC meeting has become the main national conference in biophysics. This year's meeting reflects the diversity of biophysics research in British Columbia as well as nationally, with over 50 talks and 100 posters representing a broad range of areas at the molecular, cellular and organismal scales, including but by no means limited to membrane proteins, lipids, protein structure and function, a plethora of single molecule techniques, computational and conceptual modeling, imaging and cell mechanics. This year we have a new feature: the trainee symposium on Tuesday, with a session on academic-industrial interactions in biophysics as well as six trainee talks. Professor Natalie Strynadka of the University of British Columbia will be honoured as the 2018 fellow of the Society and will deliver the National Lecture. We are also very excited to host four plenary lectures by Mei Hong from MIT, Jennifer Lippincott-Schwarz from HHMI-Janelia, Barbara Baird from Cornell and Will Ryu from Toronto.

We are deeply grateful to our many sponsors for their support of this meeting and of biophysical research in Canada. We strongly encourage everyone to visit the booths and displays during the conference. We also wish to thank the many departments, institutes and faculties from Simon Fraser University and the University of British Columbia that are supporting our meeting. We are very thankful to our many volunteers for their time and enthusiasm, particularly high-school volunteer Callum Tinant for his many contributions.

Finally, we thank you for making the journey to the meeting, whether you are a local or a visitor to Vancouver. We are proud to host you in our beautiful city and we look forward to an eventful and exciting meeting!

Dan Coombs
University of British Columbia

Nancy Forde
Simon Fraser University

Suzana Straus
University of British Columbia

Jenifer Thewalt
Simon Fraser University

President's message

Biophysical techniques continue to revolutionize research, and continually lead to seminal advances in diverse areas, such as materials research, biotechnology, biochemistry, bio-sensing, medicine, and pharmaceuticals. The annual meeting of the Biophysical Society of Canada provides a unique opportunity for students, researchers and industry partners to learn about the most recent advances in biophysics. Our meetings are intimate, so that participants are exposed to the wide range of experimental approaches that are currently used to solve biological problems. Our meetings are recognized for both the quality and diversity of the invited speakers, as well as the collegiality of the participants - let's not forget the outstanding social events! BSC2018 in Vancouver from May 22 – 25 will build on our previous successes. Our conference organizers Nancy Forde, Jenifer Thewalt, Suzana Straus, and Dan Coombs have put together an outstanding program with high profile invited speakers. Along with our BSC trainees, they have incorporated a new feature into the conference, the Trainee Symposium on Tuesday afternoon, May 22. There will be increased time for poster presentations. Our conference banquet on Thursday, May 24, will be held while cruising the waterways of Vancouver. Thank you to all the organizers for their hard work. BSC 2018 promises to be an exciting conference. Enjoy!

John Baenziger
President
Biophysical Society of Canada

BSC 2018 LOCAL ORGANIZING COMMITTEE

Dan Coombs

Professor

Department of Mathematics
University of British Columbia

Nancy Forde

Professor

Department of Physics
Simon Fraser University

Suzana Straus

Professor

Department of Chemistry
University of British Columbia

Jenifer Thewalt

Professor

Department of Molecular Biology and Biochemistry
Department of Physics
Simon Fraser University

BSC 2018 TRAINEE SYMPOSIUM

Lead organizer

William Jennings

University of Ottawa

Program team

Benjamin Baylis

University of Guelph

Allen Leary

McGill University

Benjamin Martial

Université Laval

BIOPHYSICAL SOCIETY OF CANADA - EXECUTIVE TEAM

John E. Baenziger

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Awards & Student
Travel

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BSC Meetings

Claudiu Gradinaru

Councillor

Christopher M. Yip

Councillor

Benjamin Baylis

Trainee Representative

William Jennings

Trainee Representative

Allen Leary

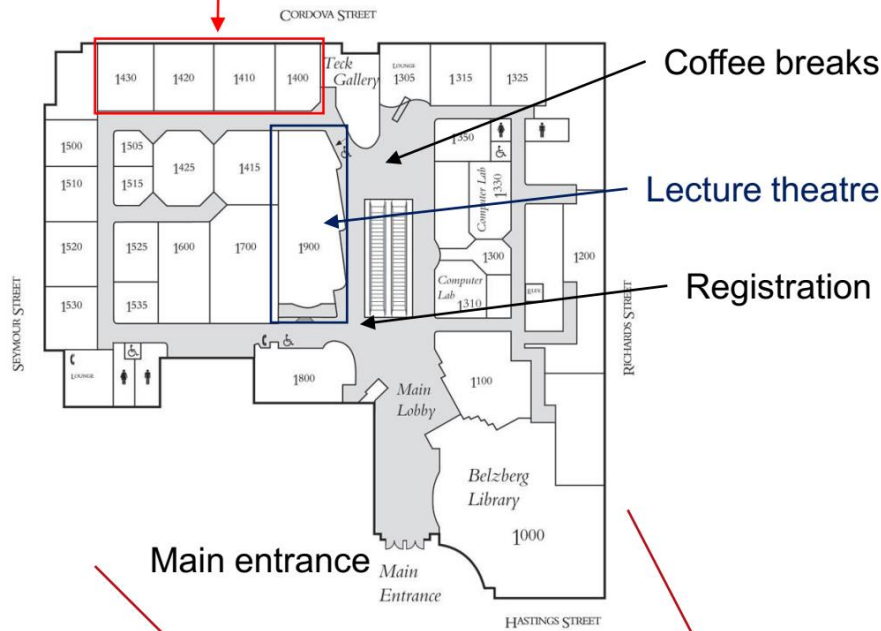
Trainee Representative

Benjamin Martial

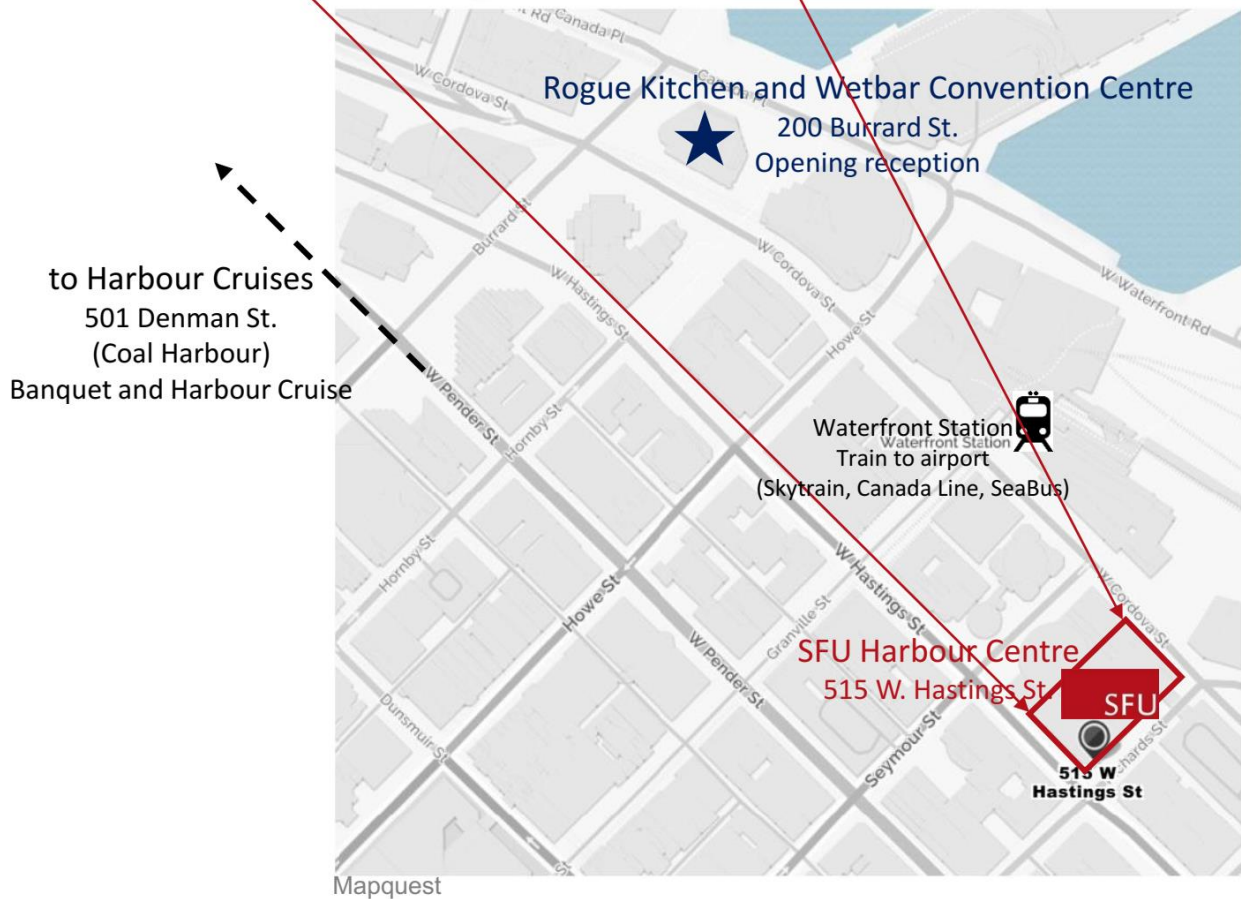
Trainee Representative

CONFERENCE LOCATION

Poster sessions + exhibitors



CONCOURSE



REGISTRATION INFORMATION

The registration desk is located in the lobby of SFU Harbour Centre, directly outside the Fletcher Challenge Theatre (room 1900), where all talks will take place.

Hours of registration at SFU Harbour Centre

Tuesday May 22 **12:00 PM - 5:00 PM**

Wednesday May 23 **8:00 AM - 5:00 PM**

Thursday May 24 **8:00 AM - 5:00 PM**

Registration will also be available at the conference Opening Reception, located at Rogue Kitchen and Wet Bar Convention Centre (200 Burrard Street).

Hours of registration at Opening Reception

Tuesday May 22 **6:00 PM - 8:30 PM**

RECEPTION INFORMATION

Opening Reception: Tuesday May 22, 6:00 PM - 9:00 PM

Rogue Kitchen and Wet Bar Convention Centre, 200 Burrard Street

Banquet and Boat Cruise: Thursday May 24, 6:15 PM (boarding) - 10:00 PM

MV Britannia, Harbour Cruises, 501 Denman Street

****Please note that the boarding location is a half-hour walk from SFU Harbour Centre****

POSTER INFORMATION

The maximum size of posters is 118 cm in the largest dimension.

Posters in Session A should be posted on Wednesday May 23 before the start of the morning session, and should be removed before heading to lunch on Thursday May 24.

Posters in Session B should be posted on Thursday May 24 during lunch, and should be removed by the end of the conference.

Presenters with an **odd-numbered poster** should ensure they are presenting for the first hour of the designated poster session. Presenters with an **even-numbered poster** should be present for the second hour of the designated poster session.

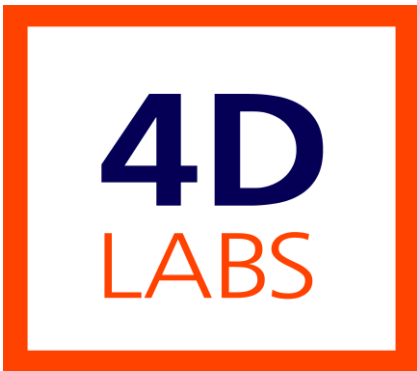
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JGFP Journal of
General
Physiology



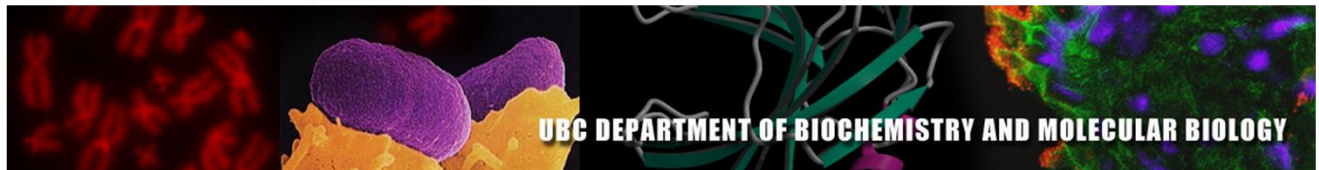
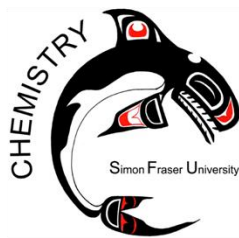
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Department of Mathematics



PROGRAM OVERVIEW

All oral presentation sessions are in the Fletcher Challenge Theater (room 1900) and all poster sessions are in rooms 1400-1430, SFU Harbour Centre.

Tuesday, May 22nd

1:00 PM - 3:20 PM	Trainee Symposium Session 1
3:20 PM - 3:50 PM	Coffee break
3:50 PM - 5:30 PM	Trainee Symposium Session 2
6:00 PM - 9:00 PM	Opening Reception, Rogue Kitchen and Wet Bar Convention Centre

Wednesday, May 23rd

8:50 AM - 9:00 AM	Opening remarks
9:00 AM - 10:40 AM	Session 1
10:40 AM - 11:10 AM	Coffee Break and Poster A viewing
11:10 AM - 12:10 PM	Session 2
12:10 PM - 1:40 PM	Lunch Break
1:40 PM - 3:00 PM	Session 3
3:00 PM - 3:30 PM	Coffee Break and Poster A viewing
3:30 PM - 4:30 PM	Session 4
4:30 PM - 5:20 PM	Plenary Lecture: Mei Hong, <i>Massachusetts Institute of Technology</i>
5:30 PM - 7:30 PM	Poster Session A and Refreshments

Thursday, May 24th

8:30 AM - 10:10 AM	Session 5
10:10 AM - 10:40 AM	Coffee Break and Poster B viewing
10:40 AM - 11:30 AM	Plenary Lecture: Jennifer Lippincott-Schwarz, <i>Janelia Research Campus, Howard Hughes Medical Institute</i>
11:30 AM - 12:10 PM	Session 6
12:10 PM - 1:40 PM	Lunch
1:40 PM - 3:40 PM	Poster Session B and Coffee
3:40 PM - 4:20 PM	Session 7
4:20 PM - 5:20 PM	National Lecture: Natalie Strynadka, <i>University of British Columbia</i>
5.45 PM	Walk (or bus or taxi) to Harbour Cruises Ltd, 501 Denman St.
6.15 PM - 10.00 PM	Banquet and Harbour Cruise

Friday, May 25th

8:45 AM - 10:05 AM	Session 8
10:05 AM	Conference photo
10:10 AM - 10:40 AM	Coffee Break and Poster B viewing
10:40 AM - 11:30 AM	Plenary Lecture: Barbara Baird, <i>Cornell University</i>
11:30 AM - 12:10 PM	Session 9
12:10 PM - 1:40 PM	Lunch
1:40 PM - 3:00 PM	Session 10
3:00 PM - 3:50 PM	Plenary Lecture: William Ryu, <i>University of Toronto</i>
3:50 PM - 4:00 PM	Poster Awards and Closing Remarks
4:15 PM - 5:30 PM	BSC Business Meeting

TRAINEE SYMPOSIUM – TUESDAY MAY 22nd

1:00 PM **Welcoming remarks**
William Jennings, University of Ottawa

1:00 PM - 3:20 PM **Session 1: Academic-Industry Interactions**

1:05 PM Dr. Martin Boulanger, Professor and Canada Research Chair, University of Victoria

Academic and Industry Perspectives in the Field of Biophysical Research

1:40 PM Dr. Norbert Maurer, Director, Research and Development, Evonik Transferra Nanosciences Inc.

Current Insights on My Transition from Academia to Industry

2:15 PM Dr. Allison Brennan, Business Development Specialist, MITACS and Megan Griffith, Research Partnerships Promotion Officer, NSERC
Utilizing Mitacs and NSERC to Support Innovative Academic and Industry Collaboration

2:50 PM Panel Discussion

3:20 PM - 3:50 PM **Coffee Break**

3:50 PM - 5:30 PM **Session 2 – Trainee Presentations**

3:50 PM Michelle Lam, University of Ottawa
Trapping DNA with Nanofiltered-Nanopore Devices

4:05 PM Sherry S.W. Leung, University of Illinois, Urbana-Champaign
Increased cationic lipid content in bilayer decreased lamellar repeat spacing

4:20 PM Morgan Robinson, University of Waterloo
Correlating biophysical studies of amyloid- β inhibitors for treating Alzheimer's disease with neuroprotection in vitro

4:35 PM Jessica Yu, University of Toronto
*Molecular asymmetries establish tissue boundaries during *Drosophila* axis elongation*

4:50 PM Parisa Zarkeshian, University of Calgary
Possible optical communication channels and biophoton sources in the brain

5:05 PM Harveer Singh, University of British Columbia
Pursuing structural characterization of membrane proteins in peptidisc

5:20 PM **Concluding Remarks**
William Jennings, University of Ottawa

SCIENTIFIC PROGRAM

Wednesday, May 23rd

8:50 AM **Welcoming remarks**
BSC 2018 Organizing Committee

9:00 AM - 10:40 AM **Session 1**

Chair: Dan Coombs (UBC)

- 9:00 AM David Sivak, Simon Fraser University
Allocating dissipation across a molecular machine cycle to maximize flux
- 9:20 AM Filip van Petegem, University of British Columbia
Structural investigation of components in muscle excitation-contraction coupling
- 9:40 AM Isaac Li, University of British Columbia Okanagan
Quantifying Molecular Forces with Serially Connected Force Sensors
- 10:00 AM Peter Stathopoulos, University of Western Ontario
A charge sensing region in STIM1 confers stabilization-mediated inhibition of SOCE in response to S-nitrosylation.
- 10:20 AM Joaquin Ortega, McGill University
Using cryo-EM to reveal the intricacies of ribosomal assembly in bacteria

10:40 AM - 11:10 AM **Coffee Break and Poster A viewing**

11:10 AM - 12:10 PM **Session 2**

Chair: Suzana Straus (UBC)

- 11:10 AM Rikard Blunck, Université de Montréal
Using fluorescent unnatural amino acids to study ion channel dynamics
- 11:30 AM Drew Marquardt, University of Windsor
Vitamin E: Miracle supplement or devil in disguise
- 11:50 AM Michael Murphy, University of British Columbia
Staphylococcus aureus IsdB-Associated Unfolding of the Heme Binding Pocket of Human Hemoglobin

12:10 PM - 1:40 PM **Lunch Break and Poster A viewing**

1:40 PM - 3:00 PM **Session 3**

Chair: Hongbin Li (UBC)

- 1:40 PM Tom Claydon, Simon Fraser University
Using biophysical tools to investigate cardio-protective gating mechanisms in potassium ion channels
- 2:00 PM Uttam Anand, University of Alberta
Differences in the folding dynamics of prion proteins from species with different disease susceptibility observed at the single-molecule level
- 2:20 PM Jean-Pierre Simorre, CNRS
Interaction between Cell-Wall and Biosynthetic Enzymes Using a Combination of Liquid- and Solid-State NMR Approaches
- 2:40 PM Lisa Craig, Simon Fraser University
The Ins and Outs of Type IV Pili

3:00 PM - 3:30 PM **Coffee Break and Poster A viewing**

3:30 PM - 4:30 PM **Session 4**

Chair: Lisa Craig (SFU)

- 3:30 PM Hongbin Li, University of British Columbia
Folding and Unfolding Mechanisms of Iron Sulfur Proteins Revealed by Single Molecule Force Spectroscopy
- 3:50 PM Olga Dudko, University of California San Diego
Space and Time in Genomic Interactions
- 4:10 PM Sarah Rauscher, University of Toronto Mississauga
The Liquid Structure of Elastin
-

4:30 PM - 5:20 PM **Plenary Talk**

Chair: Suzana Straus (UBC)

Mei Hong, Massachusetts Institute of Technology
Structure and Dynamics of the Influenza M2 Protein for Proton Transport and Virus Budding

5:30 PM - 7:30 PM **Poster Session A and Refreshments**

Thursday, May 24th

8:30 AM - 10:10 AM **Session 5**

Chair: David Sivak (SFU)

- 8:30 AM Raghuveer Parthasarathy, University of Oregon
Imaging Cholera's Intestinal Impact
- 8:50 AM Joshua Milstein, University of Toronto
Advances in Molecular Counting with Single-Molecule Localization Microscopy
- 9:10 AM Arpita Uphadhyaya, University of Maryland, College Park
Cytoskeletal dynamics and mechanosensing in immune cells
- 9:30 AM Sarah Veatch, University of Michigan
Functional ordered domains in intact B cell membranes
- 9:50 AM Steve Pressé, Arizona State University
Novel Statistical Tools for Single Molecule Imaging: A foray into Bayesian nonparametrics
-

10:10 AM - 10:40 AM **Coffee Break and Poster A viewing**

10:40 AM - 11:30 AM **Plenary Talk**

Chair: Dan Coombs (UBC)

Jennifer Lippincott-Schwartz, Janelia Research Campus, HHMI
Peering into cells with new imaging technologies

-
- 11:30 AM - 12:10 PM **Session 6**
Chair: John Bechhoefer (SFU)
- 11:30 AM Jun Allard, UC Irvine
Force-sensitivity and cooperativity arising from polymer properties of formins and other intrinsically disordered molecules
- 11:50 AM Delphine Gourdon, University of Ottawa
Altered mechanobiology of tumor-associated extracellular matrix proteins
-

12:10 PM - 1:40 PM **Lunch Break**

1:40 PM - 3:40 PM **Poster Session B and Refreshments**

- 3:40 PM - 4:20 PM **Session 7**
Chair: Eric Cytrynbaum (UBC)
- 3:40 PM Cecile Fradin, McMaster University
The role of diffusion in the establishment and interpretation of morphogen concentration gradients
- 4:00 PM David Langelaan, Dalhousie University
Characterization of the structure and function of the microphthalmia-associated transcription factor and its association with CBP/p300
-

- 4:20 PM - 5:20 PM **Fellow of the Biophysical Society of Canada & National Lecture**
Chair: John Baenziger
- Natalie Strynadka, University of British Columbia
Hybrid biophysical methods allow atomic resolution structure/function analysis of the multimembrane spanning Type III secretion nanomachine
-

5:45 PM **Walk (or bus or taxi) to Harbour Cruises Ltd, 501 Denman St.**

6:15 PM - 10:00 PM **Banquet and Harbour Cruise**

Friday, May 25th

- 8:30 AM - 10:10 AM **Session 8**
Chair: Isaac Li (UBC-O)
- 8:45 AM Steve Bourgault, Université du Québec à Montréal
Manipulating amyloid formation: from mechanisms of self-assembly to nanovaccine engineering
- 9:05 AM Michel Lafleur, Université de Montréal
Structure/permeability relationship in skin: working with more complex model systems leads to a less solid view
- 9:25 AM Glennis Rayermann, University of Washington
Reversible separation of living, unperturbed cell membranes into two liquid phases
- 9:45 AM Radek Sachl, J. Heyrovsky Institute of Physical Chemistry
Lipid driven nanodomains are fluid and inter-leaflet coupled
-
- 10:05 AM **Conference photo**
-

10:10 AM - 10:40 AM **Coffee Break and Poster B viewing**

10:40 AM - 11:30 AM **Plenary Talk**

Chair: Jenifer Thewalt (SFU)

Barbara Baird, Cornell University

How does the Plasma Membrane Participate in Receptor-Mediated Cell Signaling?

11:30 AM - 12:10 PM **Session 9**

Chair: Jenifer Thewalt (SFU)

11:30 AM Nicolas Touret, University of Alberta

Role of Lipid Nanodomains in CD36-Fyn Signal Transduction

11:50 AM Chi-Ming Chen, National Taiwan Normal University

Visualizing the GPCR Network: Classification and Evolution

12:10 PM - 1:40 PM **Lunch Break and Poster B viewing**

1:40 PM - 3:00 PM **Session 10**

Chair: Nancy Forde (SFU)

1:40 PM Eric Cytrynbaum, University of British Columbia

Cortical microtubules deflect in response to cell-surface curvature

2:00 PM Erika Plettner, Simon Fraser University

Molecular Recognition in Insect Odorant-Binding Proteins

2:20 PM Lisanne Rens, University of British Columbia

Hybrid cellular Potts model including focal adhesions as catch bond clusters explains cell response to substrate stiffness

2:40 PM John Bechhoefer, Simon Fraser University

Measurement of the functional form of Shannon entropy by partial erasure of a bit

3:00 PM - 3:50 AM **Plenary Talk**

Chair: Nancy Forde (SFU)

William Ryu, University of Toronto

The Physics of Behavior: measuring and modeling the sensorimotor response of C. elegans

3:50 PM - 4:00 PM **Awards Session (Poster Awards, Travel Awards and Service Awards) and Closing Remarks**

4:15 PM - 5:30 PM **Biophysical Society of Canada Business Meeting**

Fellow of the BSC & National Lecture – Professor Natalie Strynadka



Dr. Strynadka is a pioneer in the study of proteins and protein assemblies essential to bacterial pathogenicity and antibiotic resistance. Her ground-breaking dissection of complexes involved in infection, virulence and bacterial cell wall synthesis is having a major impact on the development of antibiotics and vaccines. Dr. Strynadka is unquestionably one of Canada's most accomplished biophysicists and is recognized worldwide for her unmatched contributions in defining the structural mechanisms underlying microbial diseases.

Many of the proteins studied by Dr. Strynadka, including penicillin-binding proteins and the Type III secretion apparatus, are membrane bound. To tackle this challenge, Dr. Strynadka has undertaken a multi-disciplinary approach, combining X-ray crystallography, NMR spectroscopy, single particle cryo-electron microscopy, mass spectrometry, and molecular modeling algorithms aiming to piece together structural insight into pathogen-specific membrane assemblies. Only a small number of groups worldwide are capable of such an integrated biophysical approach, and her success in unraveling the mysteries of such complex molecular structures is matched by few.

In addition to receiving numerous prestigious academic awards, Dr. Strynadka has been recognized as a CIHR Scientist, a Burroughs Wellcome Investigator, a MSFHR Senior Scholar, and a Killam Fellow of the Canadian Council of the Arts. She is one of only thirteen Howard Hughes Medical Institute Senior International Research Scholars worldwide. She is also a Fellow of the Royal Society of Canada, and was most recently elected as a Fellow of the Royal Society, London. Thus far, Dr. Strynadka has published over 160 papers, with an enviable number in top journals including Nature, Cell, and Science. To accomplish this research, Dr. Strynadka has mentored a large team of undergraduate and graduate students and postdoctoral fellows, and a major cohort of her trainees have secured leadership roles in both the academic and biotechnology sectors.

Hybrid biophysical methods allow atomic resolution structure/function analysis of the multimembrane spanning Type III secretion nanomachine

Natalie Strynadka

Department of Biochemistry, University of British Columbia

Bacteria have evolved several sophisticated assemblies to transport proteins across their biological membrane, including those required specifically for pathogenicity. Recent advances in our understanding of the molecular details governing the molecular action of these protein secretion systems has benefited from an integrated x-ray crystallography, NMR, mass spectrometry, electron microscopy, and molecular modeling toolbox. Highlights of recent advances in our piece wise structure/function analysis of the multi-membrane spanning Type III Secretion system "injectisome" will be presented. A molecular understanding of the Type III systems being garnered from these studies furthermore provides the foundation for the development of new classes of antibacterials and vaccines to combat infection in the clinic and community.

**Michèle Auger Awards for Exceptional Service to the BSC –
Professors Jenifer Thewalt and Nancy Forde**



Professor Jenifer Thewalt
Simon Fraser University



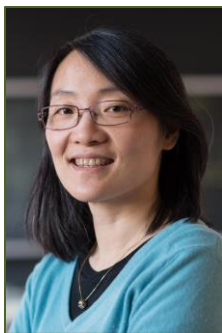
Professor Nancy Forde
Simon Fraser University

The Biophysical Society of Canada (BSC) is delighted to announce that Drs. Jenifer Thewalt and Nancy Forde are co-recipients of the 2018 Michèle Auger Award for Exceptional Service. Jenifer and Nancy are both executive members of the BSC. Jenifer currently serves as treasurer, while Nancy is in charge of the membership portfolio. During their tenure, the BSC has grown by more than an order of magnitude, from roughly 20 paying members in 2013 to roughly 350 in 2017. This outstanding growth is the result of long hours spent on outreach activities, as well as many more dedicated to coordinating membership subscriptions and payments, *etc.*

Both Jenifer and Nancy performed indispensable roles implementing the on-line membership system now available on the new BSC website. Nancy, in particular, worked tirelessly with the web developers to iron out bugs in the membership platform, as well as many more hours guiding BSC members through the web transformation. In addition, Jenifer served as BSC president in 2005 and organized the first BSC mixer at the American Biophysical Society meeting. This first mixer was a big success, and is now a well-established tradition that annually brings together Canadian biophysicists at the Biophysical Society meeting. Last but not least, Jenifer and Nancy are leading the organization of the 4th Annual BSC meeting in Vancouver. They have put together an outstanding program that is attracting a major cohort of national and international attendees.

The BSC thanks both Jenifer and Nancy for their hours of dedication and commitment to the BSC!

Plenary Lecture – Professor Mei Hong



Mei Hong is a Professor of Chemistry at the Massachusetts Institute of Technology. She received her PhD from the University of California at Berkeley and carried out postdoctoral studies at MIT. Before MIT, she held positions at the University of Massachusetts in Amherst and at Iowa State University, where she was the John D. Corbett Professor of Chemistry. Her research group develops and uses high-resolution solid-state NMR spectroscopy to determine the structures and dynamics of biological macromolecules. Particular biomolecular systems under investigation include virus membrane proteins, amyloid fibrils, and plant cell walls with relevance to influenza infection, Alzheimer's disease and plant development. Dr. Hong has been the recipient of many honours and fellowships during her career and she is a Fellow of the AAAS.

Structure and Dynamics of the Influenza M2 Protein for Proton Transport and Virus Budding

Mei Hong

Department of Chemistry, Massachusetts Institute of Technology

Membrane proteins carry out a myriad of biological functions such as ion conduction, substrate transport, and signaling. Solid-state NMR allows us to obtain functionally relevant structural information of these proteins in lipid bilayers. In this talk, I will present our investigations of the structure and dynamics of a multifunctional influenza membrane protein, matrix protein 2 (M2), which forms a proton channel for virus uncoating and mediates membrane scission during virus budding. ^{13}C , ^{15}N , and ^1H chemical shifts provided detailed information about pH-dependent conformations of the open and closed states of the proton channel. Motionally averaged NMR spectra revealed microsecond-timescale dynamics of the proton-selective histidine residue and the gating tryptophan residue, while 2D exchange NMR spectra revealed millisecond-timescale motion of the entire tetrameric complex. Hydrogen bonding between water and histidine are observed in ^{15}N NMR spectra, which give insight into the proton transfer mechanism of the hydrated channel. In the second function, M2 interacts with membrane cholesterol to cause scission of the emerging virus from the host cell in the last step of virus budding. By measuring protein-cholesterol distances and cholesterol orientations, we have determined the cholesterol-binding site structure of M2. The result gave unexpected insight into how the M2 protein is attracted to the neck of the budding virus to cause membrane scission. These studies of protein-ligand binding are enabled by new solid-state NMR methods to measure distances up to ~ 2 nm using ^{19}F NMR.

Plenary Lecture – Dr. Jennifer Lippincott-Schwarz



Jennifer Lippincott-Schwarz is a Senior Group Leader at the Howard Hughes Medical Institute Janelia Research Campus near Washington, DC. She received her PhD from Johns Hopkins University and was a postdoctoral researcher at the NIH. She stayed on at the NIH, becoming a primary investigator and chief of the Section on Organelle Biology, before moving to Janelia in 2016. While at the NIH she played a leading role in the development and application of super-resolution fluorescence microscopy techniques, including the creation of photoactivatable GFP. Her primary research focus at Janelia is on live cell imaging in the context of the brain. Her group studies neurobiology on a cellular level, looking into processes such as organelle trafficking and metabolism, to better understand how nerve cells communicate and behave in normal and diseased brain function. Among many awards, fellowships and distinctions, Dr. Lippincott-Schwarz is a member of the National Academy of Sciences, a Fellow of the Biophysical Society and a Fellow of the AAAS.

Peering into cells with new imaging technologies

Jennifer Lippincott-Schwarz

Janelia Research Campus, Howard Hughes Medical Institute

Powerful new ways to image the internal structures and complex dynamics of cells are revolutionizing cell biology and bio-medical research. In this talk, I will focus on how emerging fluorescent technologies are increasing spatio-temporal resolution dramatically, permitting simultaneous multispectral imaging of multiple cellular components. Using these tools, it is now possible to begin constructing an “organelle interactome” describing the interrelationships of different cellular organelles as they carry out critical functions. The same tools are also revealing new properties of the cell’s largest organelle, the endoplasmic reticulum, and how disruptions of its normal function due to genetic mutations may contribute to important diseases.

Plenary Lecture – Professor Barbara Baird



Barbara Baird is the Horace White Professor of Chemistry and Chemical Biology at Cornell University. Her PhD degree is also from Cornell University, and she completed postdoctoral work at the National Cancer Institute of the NIH. The Baird laboratory employs a range of biophysical and biochemical methods to investigate the structure and molecular mechanisms of cell surface receptors that operate in immunological and other responses. She is particularly well-known for work on FcεRI signaling pathways in the immune system, including extensive research into the nanoscale organization of lipids, receptors and signaling proteins. Dr. Baird is a Fellow of the AAAS and of the Biophysical Society.

How does the Plasma Membrane Participate in Receptor-Mediated Cell Signaling?

Barbara Baird

Department of Chemistry and Chemical Biology, Cornell University

Cells are poised to respond to their physical environment and to chemical stimuli in terms of collective molecular interactions that are regulated in time and space by the plasma membrane and its connections with the cytoskeleton and intracellular structures. Small molecules may engage specific receptors to initiate a transmembrane signal, and the surrounding system efficiently rearranges to amplify this nanoscale interaction to microscale assemblies, yielding a cellular response that often reaches to longer length scales within the organism. A striking example of signal integration over multiple length scales is the allergic immune response. IgE receptors (FcεRI) on mast cells are the gatekeepers of this response, and this system has proven to be a valuable model for investigating receptor-mediated cellular activation. My talk will describe our efforts with quantitative fluorescence microscopy and modeling to investigate the poised, “resting state” of the plasma membrane and how signaling mediated by specific receptors is regulated and targeted within this milieu.

Plenary Lecture – Professor William Ryu



William Ryu is an Associate Professor in the Department of Physics at the University of Toronto, where he is also a member of the Donnelly Centre for Cellular and Biomolecular Research. He is also appointed to the Department of Cellular and Systems Biology. His PhD degree was received from Harvard University. He was subsequently a Fellow at the Lewis-Sigler Institute for Integrative Genomics at Princeton University. Dr. Ryu's laboratory studies how biochemical and neuronal networks encode and process sensory information to produce adaptive locomotive behaviour, with particular focus on *E. coli* and *C. elegans* as model organisms.

The Physics of Behavior: measuring and modeling the sensorimotor response of *C. elegans*

William Ryu

Department of Physics, University of Toronto

The roundworm, *C. elegans* is a relatively simple organism with only 300 neurons but can generate complex adaptive behavioral responses to a wide range of sensations including taste, touch, and temperature. *C. elegans* locomotion consists of a number of stereotyped behavioral states such as forward and reverse, pausing, turning, etc. In general, the worm moves randomly by making stochastic transitions between these states, and in response to sensory measurements it performs adaptive locomotory changes (behaves) by biasing the probability of these transitions. However under certain conditions the worm will respond deterministically with a specific behavioral sequence or motif, such as in the escape response to a “painful” or noxious stimulus. One of the grand goals in science is to understand how neural, genetic, and biochemical circuits produce these behaviors. While a great deal of work has been done in the development of tools to perturb and measure the circuits underlying sensory behavior, advances in the study of behavior itself has lagged behind. Here I will describe some attempts to close this gap with focus on *C. elegans* locomotion and its response to thermal stimuli. We've developed simple desktop experiments to programmatically stimulate *C. elegans* and quantitatively capture its behavioral response. Using these data we have shown that *C. elegans* moves through a “shape space” that is low dimensional in which four dimensions capture approximately 95% of the variance in body shape. Here I will give two examples of modeling that take advantage of this low dimensionality and stereotypy. In the first we show that stochastic dynamics within this shape space predicts transitions between attractors corresponding to abrupt reversals in crawling direction. With no free parameters, our inferred stochastic dynamical system generates reversal timescales and stereotyped trajectories in close agreement with experimental observations. In the second we use Sir Isaac, an algorithm that allows inference of the dynamical equations underlying a noisy time series, even if the dynamics are nonlinear—to analyze the thermal “pain” response of *C. elegans*. Both examples show that it is possible to learn “equations of behavior” of the worm, and that these equations give an interpretable, complementary perspective to traditional biological studies.

POSTER PRESENTATIONS

Poster Session A: Posters should be posted and available for viewing from Wednesday May 23 8:45 am - Thursday May 24 12:15 pm.

- A1** **Modulation of Kv1.2 activation-gating by the Sigma-1 Receptor: novel insights to the pathophysiology of ALS16**
Madelyn Abraham
- A2** **Altered mobility and nanoscale spatial organization of IgM-BCRs is associated with increased tonic BCR signaling in marginal zone B cells**
Libin Abraham
- A3** **Structural and kinetic analysis of penicillin-binding protein 4 (PBP4)-mediated antibiotic resistance in Staphylococcus aureus**
J. Andrew Alexander
- A4** **Exploring the Molecular Properties of Collagen Type IV with Atomic Force Microscopy**
Alaa Al-Shaer
- A5** **Amino Acid Formation Energy and Frequency in Different Species and Adinkra Model**
Sahar Arbabimoghadam
- A6** **Solving High Resolution Structures using Cryo-Electron Microscopy at HR-MEM**
Claire Atkinson
- A7** **Unusual Reaction Catalysed by Cytochrome P450_{cam} (CYP101A1) and Its Significant Role in the Chemotaxis by Pseudomonas Putida (ATCC 17453)**
Priyadarshini Balaraman
- A8** **Miniaturized flow cell with pneumatically-actuated vertical nanoconfinement**
Daniel Berard
- A9** **Arp2/3 complex-dependent spatial organization of the B cell receptor (BCR) impacts immune synapse formation, BCR signalling output and B cell activation**
Madison Bolger-Munro
- A10** **Non-local modeling of cellular adhesions: Applications and Theory**
Andreas Buttenschoen
- A11** **Characterizing the Catalytic Domain of the Deubiquitinase scOTU1 from S. cerevisiae**
Herby Cadet
- A12** **Beyond the beta-lactam: Structural insights into L,D-transpeptidase mediated beta-lactam resistance in Escherichia coli.**
Nathanael Caveney
- A13** **Tuning Length-Scales of Small Domains in Cell-Derived Membranes and Synthetic Model Membranes**
Caitlin Cornell

- A14** Pursuing structural characterization of membrane proteins in peptidisc
Harveer Dhupar
- A15** Translocation and interaction of PAR proteins explain oscillation and ratcheting mechanisms during *Drosophila* dorsal closure
Clinton Durney
- A16** Phosphorylation-enhanced Protein Phase Separation of the Regulatory Module of a *Mycobacterium tuberculosis* ABC transporter
Florian Heinkel
- A17** Identifying unique observations in stochastic optical reconstruction microscopy (STORM) with a spatiotemporal model
Alejandra Herrera
- A18** Near-atomic cryo-EM analysis of the *Salmonella* T3SS needle complex reveals the molecular basis of substrate induced gating in the giant outer membrane secretin portals
Jinhong Hu
- A19** Microfluidic Fabrication of Asymmetric Lipid Vesicles
Tina Huang
- A20** Investigating implications of Stochastic Pattern formation for chemistry of the Min System
Alastair Jamieson-Lane
- A21** Plasmid behaviour under Par system control
Lavisha Jindal
- A22** Optimization of Single-Molecule Assay for Proteolytic Susceptibility: Force-Induced Collagen Destabilization
Michael Kirkness
- A23** A conserved inter-domain linker of CCT mediates allosteric communication between regulatory and catalytic domains
Daniel Knowles
- A24** Probable Structures of DNA-Protein Complexes
Andrew Koenig
- A25** Virtual histology with multimodal nonlinear imaging for quantitative analysis of vocal fold structure and injury
Ksenia Kolosova
- A26** Synthesis and characterization of the Lawnmower: an artificial protein-based molecular motor
Chapin Korosec
- A27** On the Formation and Morphology of Lipid Nanoparticles Containing Ionizable Cationic Lipids and siRNA
Jayesh Kulkarni

- A28** **Novel antimicrobial peptides derived from aurein 2.2 and their conjugates**
Prashant Kumar
- A29** **Trapping DNA with Nanofiltered-Nanopore Devices**
Michelle Lam
- A30** **Expression, purification, and characterization of Class IB hydrophobins**
David Langelaan
- A31** **Using a System's Equilibrium Behavior to Reduce Its Energy Dissipation in Non-Equilibrium Processes**
Steven Large
- A32** **Increased cationic lipid content in bilayer decreased lamellar repeat spacing**
Sherry S. W. Leung
- A33** **Extensions of the wave-pinning model for cellular polarization and localized patterns**
Yue Liu
- A34** **ALS mutations in the TDP-43 low-complexity domain can either enhance or repress its liquid-liquid phase separation**
Luke McAlary
- A35** **Effect of Melatonin on Binding of Amyloid- β to Model Lipid Membranes**
Nanqin Mei
- A36** **A Rho-GTPase based model explains spontaneous collective migration of neural crest cell clusters**
Brian Merchant
- A37** **Ionizable Amino Lipid's Confinement in the POPC Bilayer Interior: A Potential Obstacle for siRNA Release from Endosomes**
Mohsen Ramezanpour
- A38** **Using Isothermal Titration Calorimetry (ITC) to Reassess Calcium and Magnesium Binding to Cardiac Troponin C**
Kaveh Rayani
- A39** **Correlating biophysical studies of amyloid- β inhibitors for treating Alzheimer's disease with neuroprotection in vitro**
Morgan Robinson
- A40** **StormGraph: A graph-based algorithm for quantitative clustering analysis of single molecule localization microscopy data**
Joshua Scurll
- A41** **Characterization of Amyloid Beta Oligomer Populations Using Size Exclusion Chromatography and Oligomer-Specific Antibody**
Chih Chieh Shyu
- A42** **Probing the Conformational Dynamics of the Disordered 4E-BP2 Protein in Different Phosphorylation States Using Single-Molecule Fluorescence**
Spencer Smyth

- A43** **Quinolinic acid amyloid-like fibrillar assemblies seed α -synuclein aggregation**
Omid Tavassoly
- A44** **SbnI is a free serine kinase that generates O-phospho-L-serine for staphyloferrin B biosynthesis in *Staphylococcus aureus***
Meghan Verstraete
- A45** **The Peptidisc: a Simple Approach for Stabilizing Membrane Proteins in Detergent-free Solution**
Irvinder Wason
- A46** **Molecular asymmetries establish tissue boundaries during *Drosophila* axis elongation**
Jessica Yu
- A47** **Differential mechanisms of bacterial adhesion in response to substrate stiffness**
Alexa Zayadi
- A48** **Structural characterization of feeding-tube channel components essential to spore formation in bacteria**
Natalie Zeytuni

Poster Session B: Posters should be posted and available for viewing from Thursday May 24 12:15 pm - Friday May 25 4:00 pm.

- B1** **Sequence modification through charge addition improves the cyclic peptide permeability in LUVs of *S. aureus* phospholipid composition**
Simone C. Barbosa
- B2** **Mathematical modeling of oral Epstein-Barr virus shedding**
Catherine Byrne
- B3** **Two-state Diffusion Analysis with Measurement Errors**
Rebeca Cardim Falcao
- B4** **Examining the Nanosecond-to-Millisecond Dynamics of Sic1 by Fluorescence Techniques**
John Darvy Castroverde
- B5** **Phase Diagrams of Ternary Model Membrane Systems with Phosphatidylethanolamine (PE) Lipids**
Catherine Chang
- B6** **Amino acids bind to and influence the structure of fatty acid vesicles: A mechanism for the co-evolution of membranes and polymers**
Zachary Cohen
- B7** **Structure and function of an Ntn-hydrolase from *Staphylococcus aureus***
Brigid Conroy
- B8** **Versatile Tools Towards Real-time Single-molecule Biology**
Rosalie Driessen
- B9** **Binding Pose Prediction Using Coarse Grained Molecular Dynamics**
Pranav Garg

- B10** **Wanted: Small molecule inhibitors of ETV6 PNT domain polymerization**
Chloe Gerak
- B11** **Optical Mapping of Human Induced Pluripotent Stem Cell-derived Cardiac Tissues as a Platform for in vitro Drug Testing**
Marvin Gunawan
- B12** **Talin autoinhibition regulates cell-ECM adhesion dynamics and wound healing in vivo**
Amanda Haage
- B13** **Towards detergent-free purification of the P4-ATPase phospholipid transporters**
William Jennings
- B14** **Confinement-assisted DNA Translocation Through a Nanopore**
Albert Kamanzi
- B15** **hERG K⁺ channel activator compounds enhance repolarizing cardio-protective currents.**
Jacob Kemp
- B16** **Structural determinants of substrate specificity in Staphylococcus aureus siderophore synthesis.**
Maxim Kolesnikov
- B17** **A feedback trap based on optical tweezers**
Avinash Kumar
- B18** **The thermodynamics of molecular machines**
Emma Lathouwers
- B19** **Probing dynamic intermolecular interactions between an intrinsically disordered protein and its binding partner at single-molecule level**
Taehyung Lee
- B20** **Investigating the Role of Ergosterol in Phase Separation of Yeast Vacuole Membranes**
Chantelle Leveille
- B21** **Biophysical Characterizations of the hypertrophic cardiomyopathy related-I79N TnT mutation**
Yueh Alison Li
- B22** **Structural and Biochemical Characterization of Wall Teichoic Acid Biosynthetic and Degradative Enzymes**
Franco Li
- B23** **Measuring LDLR-PCSK9 Interactions on Cell Membrane Using Image Correlation Spectroscopy (ICS)**
Yanning Liu
- B24** **Cryo-EM analysis of the type III secretion system ATPase-inner stalk complex and insights into its rotary catalysis**
Dorothy Majewski
- B25** **Interactions of amyloid peptide AS₇₁₋₈₂ with model membranes: structural and morphological study via FTIR and ssNMR**
Benjamin Martial

- B26** **Toward the characterization of the interactions between the TPR domain of O-linked N-acetylglucosaminyl transferase (OGT) and its substrates by NMR spectroscopy**
Stacy Maynard
- B27** **Investigating Structure-mediated Dynamics and Interactions in Supercoiled DNA**
Brendon McGuinness
- B28** **Understanding the pore-forming mechanism of peptides derived from the N-terminus of sticholysin**
Haydee Mesa Galloso
- B29** **Single cell tracking is crucial to study cell rolling adhesion**
Yousif Murad
- B30** **Estimating Molecular Counts using Fluorophore Blinking Statistics**
Daniel Nino
- B31** **Tumor cell invasion in mammary tissue and tumor growth in the cervical epithelia using a 3-D Individual Cell Based model**
Eirikur Palsson
- B32** **Structure and Mechanism of Action of Two Active Derivatives of Aurein 2.2**
Nigare Raheem
- B33** **Kir6.1-A88G and Kir6.2-S365C/SUR2A Channels, Biophysical Characteristics and Possible Association With Brugada Syndrome.**
Cinthia Rangel Sandoval
- B34** **Fluid Response: Species-specific Variations in Bacterial Membrane Stiffness in Response to Environmental Stresses**
Kunal Samantaray
- B35** **Atrial differentiation of hiPSC derived cardiomyocytes by activation of retinoid pathways.**
Sarabjit Sangha
- B36** **Investigating a biological specificity conundrum: the role of dynamics in transcription factor DNA-binding**
Karlton Scheu
- B37** **Investigating Interactions Between Lipids Involved in Drug Delivery**
Miranda Schmidt
- B38** **Comparing the stability and cellular inclusion formation of ALS-associated and in silico designed SOD1 mutants**
Mine Sher
- B39** **Protons accelerate hERG K⁺ channel deactivation gating by destabilizing the relaxed state of the voltage sensor**
Yu Patrick Shi
- B40** **Gel/Liquid-ordered phase coexistence in sphingomyelin and PC bilayers in the presence of palmitoyl ceramide and cholesterol**
Reza Siavashi

- B41** **p53 protein contains an intrinsic regulatory element that modulates its protein-protein interactions**
Qinyan Song
- B42** **Single-molecule platform for real-time manipulation and visualization of protein-DNA interactions**
Francis Stabile
- B43** **Testing for localized unfolding as a trigger of aciniform silk fibrillization**
Anamika Sulekha
- B44** **Confinement microscopy of highly-branched functional nanoparticles**
Radin Tahvildari
- B45** **Pheromone-Binding Protein-Ligand Equilibrium and Kinetics Constants Correlation with Electroantennogram Response**
Mailyn Terrado
- B46** **Tracking the Movements of Tumor Cells That Express Different Cell Adhesion Molecules During Collective Migration**
Alannah Wilson
- B47** **Crystal structure of an intramembranal phosphatase central to bacterial cell wall peptidoglycan biosynthesis and lipid recycling**
Sean Workman
- B48** **Possible optical communication channels and biophoton sources in the brain**
Parisa Zarkeshian
- B49** **Site-selective Optical Spectroscopy in Systems of Coupled Chromophores: Spectral Hole Burning Modeling Meets Excitonic Calculations**
Valter Zazubovits
- B50** **Transformation of a protein nano-walker into a nano-motor by feedback**
Martin Zuckermann

ORAL PRESENTATION ABSTRACTS

Allocating dissipation across a molecular machine cycle to maximize flux

A.I. Brown and D.A. Sivak

Department of Physics, University of California-San Diego; Department of Physics, Simon Fraser University

Biomolecular machines consume free energy to break symmetry and make directed progress. Nonequilibrium ATP concentrations are the typical free energy source, with one cycle of a molecular machine consuming a certain number of ATP, providing a fixed free energy budget. Since evolution is expected to favor rapid-turnover machines that operate efficiently, we investigate how this free energy budget can be allocated to maximize flux. Unconstrained optimization eliminates intermediate metastable states, indicating that flux is enhanced in molecular machines with fewer states. When maintaining a set number of states, we show that---in contrast to previous findings---the flux-maximizing allocation of dissipation is not even. This result is consistent with the coexistence of both 'irreversible' and reversible transitions in molecular machine models that successfully describe experimental data, which suggests that in evolved machines different transitions differ significantly in their dissipation.

Structural investigation of components in muscle excitation-contraction coupling

Siobhan Wong King Yuen(1), Jocelyn Lu(1), Ching-Chieh Tung(1), Marta Campiglio (2), Bernhard E. Flucher (2), Filip Van Petegem (1)

(1) University of British Columbia, Dept. of Biochemistry and Molecular Biology

(2) University of Innsbruck, Austria

The contraction of skeletal muscle requires the release of calcium ions, stored in the sarcoplasmic reticulum, into the cytosol. It is initiated by an electrical signal, the depolarization of the plasma membrane. This event, termed "excitation-contraction coupling", requires the concerted action of two different ion channels: the voltage-gated calcium channel (CaV) located in the plasma membrane, and the Ryanodine Receptor (RyR) in the Sarcoplasmic Reticulum membrane. They are thought to couple mechanically, but the exact contact points between these two channels have remained elusive for decades.

Here we present structural and functional studies of STAC3, a small protein essential for excitation-contraction coupling. It is the target for a severe mutation causative of Native American myopathy, characterized by muscle weakness, skeletal deformations, and malignant hyperthermia. We identified the binding site for STAC3 on CaV and show a crystal structure of the complex. Two SH3 domains in STAC3 interact with a cytosolic loop of the CaV. The interaction is disrupted by the Native American Myopathy mutation and is crucial for excitation contraction coupling. Two other STAC isoforms, STAC1 and STAC2 are also able to bind CaV channels. Importantly, non-skeletal muscle CaVs like CaV1.2 also form targets for STAC proteins, which slow calcium-dependent and voltage-dependent inactivation. This latter process does not involve the SH3 domains, indicating that STAC proteins have multiple interaction sites on CaV channels.

Quantifying Molecular Forces with Serially Connected Force Sensors

Isaac T.S. Li

Department of Chemistry, University of British Columbia Okanagan, Kelowna, BC, Canada

Adhesion molecules on cell surfaces help cells attach to their environment and transduce mechanically-coupled biochemical signal. To understand the mechanical forces involved at the molecular scale, molecular force sensors have been used to probe forces at cell-substrate interfaces. Several classes of force sensors have been developed in the past 5 years including the Tension Gauge Tether (TGT). TGTs rely on the force-dependent dissociation of complementary dsDNA strands to probe forces. The mechanical stability can be semi-quantitatively tuned by dsDNA sequence and pulling geometry. TGTs offer high signal-to-noise ratio and is ideal for studying fast / single molecular adhesion processes. However, a significant drawback using TGT lies in the difficulties in quantitative interpretation of experimental results. Here, we developed a theoretical framework using serially connected TGTs to offer better defined molecular force interpretation in experiments.

A charge sensing region in STIM1 confers stabilization-mediated inhibition of SOCE in response to S-nitrosylation.

Jinhui Zhu, Xiangru Lu, Qingping Feng and Peter B. Stathopoulos

Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, 1151 Richmond St. N, London, Ontario, Canada. N6A 5C1.

Store-operated calcium (Ca²⁺) entry (SOCE) drives cytosolic Ca²⁺ influx from the extracellular space in response to the depletion of endoplasmic reticulum (ER) Ca²⁺ stores. Stromal interaction molecule 1 (STIM1) is the Ca²⁺ sensor which activates SOCE following ER luminal Ca²⁺ depletion. The EF-hand together with the sterile a motif domains (EFSAM) of STIM1 are essential for detecting changes in ER Ca²⁺, where low Ca²⁺ levels trigger the destabilization and oligomerization of STIM1, culminating in the assembly of plasma membrane Orai1 subunits into open Ca²⁺ channels. Nitric oxide (NO) regulates myriad protein functions by S-nitrosylating cysteine thiols, but the effects of NO on the structural mechanisms underlying SOCE are unclear. Here, we demonstrate that S-nitrosylation of STIM1 Cys49 and Cys56 enhances the thermodynamic stability of the luminal domain resulting in suppressed hydrophobic exposure and diminished Ca²⁺-depletion-dependent oligomerization. We pinpoint a structural mechanism for the stabilization driven by complementary charge interactions between an electropositive patch on EFSAM and the S-nitrosylated non-conserved region of STIM1. Finally, we demonstrate that the enhanced luminal domain stability conferred by S-nitrosylation suppresses puncta formation and SOCE in live cells. Collectively, our data reveal that S-nitrosylation inhibits SOCE via electrostatic EFSAM interactions which modulate the thermodynamic stability of the luminal domain.

Using cryo-EM to reveal the intricacies of ribosomal assembly in bacteria

Aida Razi¹, Joseph H. Davis², Brett Thurlow¹, Jean Philippe Cotte³, Eric Brown³, James R. Williamson² and Joaquin Ortega¹

1Department of Anatomy and Cell Biology, McGill University, Montreal, Canada. 2Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA, USA. 3Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada

The ribosome is the enzyme responsible for protein synthesis and the most complex macromolecule existing in bacterial cells. In *Escherichia coli*, the ribosome is made from more than 50 different components organized into the small (30S) and large (50S) ribosomal subunits. In spite of its complexity, each bacterial cell assembles more than 20,000 ribosomes in less than 30 minutes. Cells are able to maintain this assembly rate because a number of auxiliary factors make the process extremely efficient. Our recent work focuses on the Era GTPase assembly factor. To gain new key insights regarding the function of Era, we created an *E. coli* strain in which the era gene is under the control of an arabinose inducible promoter. Under Era depletion conditions this strain accumulates a 30S subunit assembly intermediate. We hypothesized that this assembly intermediate constitutes the substrate for the Era protein and that the biochemical and structural analysis of this assembly intermediate would be informative on the function of Era. We have obtained the 3D structure of these immature subunits at 3.8Å resolution using cryo-electron microscopy. The map shows a 30S subunit at the late stages of maturation with helices 44 and 45 in the decoding center still in an immature state. Similarly, helices 23 and 24 in the platform region are still not adopting the mature conformation. Density for late entry ribosomal proteins uS2, uS3 and bS21 is also missing and that is consistent with our analysis of these particles using quantitative mass spectrometry. These results indicate that Era is an important factor acting at the late stages of assembly of the 30S subunit playing a role in the maturation of the platform region and decoding center in the 30S ribosomal subunit. The theme that emerges from this work and our previous studies in YjeQ and RimM, other factors assisting the assembly of the 30S subunit, is that these assembly factors work as "local chaperones" dedicated to fold regions of the rRNA that have a native folding which is not thermodynamically favored or that tend to fall into local energy minima during folding. We believe the analysis of atomic resolution structures of immature subunits by themselves and in complex with assembly factors has the potential to ultimately describe the sequence of events leading to a mature 30S subunit and elucidate the role of these assembly factors.

Using fluorescent unnatural amino acids to study ion channel dynamics

Rikard Blunck

Université de Montréal

With the availability of an increasing number of high resolution structures of membrane proteins, it is essential to link these structures to functional data in order to understand membrane protein function. Voltage-clamp fluorometry (VCF), the simultaneous measurement of measurement of structural rearrangements via fluorescence and function via electrophysiology has proven very powerful for any electrogenic membrane proteins such as ion channels, transporters or pumps. An efficient and highly-specific targeting of the fluorescent label is key to the success of VCF experiments. Here, we will show how incorporation of the fluorescence unnatural amino acid Anap into channel proteins overcomes previous limitations in the labeling. Using this technique, we studied the gating mechanisms of the voltage-gated potassium channel Shaker.

Vitamin E: Miracle supplement or devil in disguise

Mitchell DiPasquale, Michael H.L. Nguyen and Drew Marquardt

University of Windsor Chemistry and Biochemistry Department

A growing number of scientists are seeking to determine what purpose VitE serves in the human body and if, in fact there is a health benefit to supplementing it. Currently, many nutrition companies and some health professionals advocate for the supplementation of VitE despite its biological role is shrouded in mystery. VitE deficiency can lead to several health disorders including infertility and neuromuscular dysfunction, but detailed picture of how these effects arise are mostly unknown. In the face of its well-known antioxidant behaviour, recent assertions have implied that VitE may not perform the same function in living systems. The lack of a clear antioxidant health benefit from supplementing vitamin, and its naturally low concentrations in the human body, cast doubt on its action as a direct defense against oxidants. The case for VitE supplementation should be based on evidence.

Staphylococcus aureus IsdB-Associated Unfolding of the Heme Binding Pocket of Human Hemoglobin

C.F.M. Bowden, A.C.K. Chan, E.J.W. Li, A.L. Arrieta, M.E.P. Murphy

Department of Microbiology and Immunology, Life Sciences Institute, The University of British Columbia

IsdB is a receptor on the surface of *Staphylococcus aureus* that extracts heme from hemoglobin (Hb) to enable growth on Hb as a sole iron source. We present a crystal structure of a complex between human Hb and IsdB. The α subunits of Hb are refolded with the heme displaced to the interface with IsdB. Atypical His residues of Hb coordinate to the heme iron, which is poised for transfer into the heme-binding pocket of IsdB. An IsdB variant was produced and is defective in heme transfer yet formed a stable complex with Hb in solution with spectroscopic features of the bis-His species observed in the crystal structure. Haptoglobin binds to a distinct site on Hb to inhibit heme transfer to IsdB and growth of *S. aureus*. A model for IsdB heme transfer from Hb is proposed that involves unfolding of Hb and heme-iron ligand exchange.

Using biophysical tools to investigate cardio-protective gating mechanisms in potassium ion channels

Patrick Shi, Samrat Thouta, Christina Hull, May Cheng, Danielle Jeong, Jacob Kemp, Raj Johal, Zhaokai Pang, Tom Claydon

Molecular Cardiac Physiology Group, Biomedical Physiology and Kinesiology, Simon Fraser University

Cardiac potassium channels contribute to repolarization of the cardiomyocyte action potential. Their functional diversity produces heterogeneous repolarization in different regions of the heart, which contributes to the orchestration of rhythmic excitation of the myocardium. This diversity arises from variations in biophysical gating mechanisms, which produces markedly different channel behaviours. Cardiac Kv11.1 potassium channels are of particular interest, because they have unique and unusual gating, their dysfunction predisposes sudden cardiac death, and their high affinity for a diverse range of pharmacological compounds presents a significant challenge to pharmaceutical drug development. We have used conventional and fluorescence-based electrophysiological approaches to study potassium channel gating mechanisms. Measuring ion current flow through the channel pore and dynamics of the voltage sensing domain in Kv11.1 channels, we have characterized important gating mechanisms and explored their molecular determinants. We highlight the role of these biophysical events in the protection against cardiac arrhythmias and suggest potential novel therapeutic strategies for ameliorating the effects of inherited or acquired channel dysfunction.

Differences in the folding dynamics of prion proteins from species with different disease susceptibility observed at the single-molecule level

Uttam Anand, Craig Garen and Michael T. Woodside

Department of Physics, University of Alberta

PrP is highly conserved across the animal kingdom, yet disease susceptibility varies widely, with some species being highly susceptible whereas others are resistant to varying degrees. These species differences appear to be driven by sequence differences in just a few amino acids, but how these changes alter the misfolding dynamics remains unclear. We explored the effects of species-related sequence differences on PrP folding by using force spectroscopy to observe the folding dynamics of single molecules held in optical tweezers. We compared the behaviour of PrP from hamsters (HaPrP) and bank voles (BvPrP) to that of PrP from rabbits (RaPrP) and dogs (CaPrP): hamsters are disease-susceptible, rabbits are quite resistant, dogs appear to be immune, and bank voles are amongst the most susceptible of all species. Unfolding and refolding trajectories were measured while ramping the force applied by the tweezers up and down. The resulting force-extension curves (FECs) revealed the existence of any on-pathway intermediates or misfolded (off-pathway) states, reflecting also the energetics and kinetics of the different states. In contrast to HaPrP, which was found previously to exhibit two-state folding, the folding of RaPrP and CaPrP involved multiple on-pathway intermediates; in both cases, native folding was rapid and misfolded states were not detected in FECs. For BvPrP, FECs included zero or at most one on-pathway intermediate, but the folding kinetics were much slower. Notably, metastable misfolded states with lifetimes on the order of seconds were observed for BvPrP. Hysteresis between unfolding and refolding FECs was seen for both HaPrP and BvPrP but not RaPrP and CaPrP, suggesting that disease resistance is associated with smaller energy barriers. Finally, by relating the contour-length changes observed in the FECs to structural features of the proteins, possible intermediates in the native folding pathways for RaPrP, CaPrP, and BvPrP were deduced. These results show that the subtle sequence differences between PrP from different species produce significant differences in the folding dynamics.

Interaction between Cell-Wall and Biosynthetic Enzymes Using a Combination of Liquid- and Solid-State NMR Approaches

C. Bougault; I. Ayala; P. Schanda, W. Vollmer, J.-P. Simorre

Univ. Grenoble Alpes, CEA, CNRS, Institute for Structural Biology (IBS), 71 avenue des martyrs, 38044 Grenoble, France

The Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle University, Richardson Road, Newcastle upon Tyne, NE2 4AX, United Kingdom

The cell wall is essential for the survival of bacteria. It gives the bacterial cell its shape and protects it against osmotic pressure, while allowing cell growth and division. The machinery involved in the synthesis of this envelope is crucial and is one of the main antibiotic target. Different proteins as transpeptidases, transpeptidase activators or hydrolases are recruited to maintain the morphogenesis of this polymer during the bacterial cell cycle. Based on few examples involved in the machinery of synthesis of the peptidoglycan, we will present a combination of liquid and solid-state NMR that can be a powerful tool to screen for cell-wall interacting proteins in vitro and on cell.

In particular, we have explored the possibilities to study the PG with ultra-fast (100 kHz) magic-angle spinning NMR. We show that highly resolved spectra can be obtained, and we have developed strategies to obtain site-specific resonance assignments and distance information. We have also in parallel investigated the potential of Dynamic Nuclear Polarization (DNP) to investigate cell surface directly in intact cells.

The Ins and Outs of Type IV Pili

Lisa Craig

Department of Molecular Biology and Biochemistry, Simon Fraser University

Many bacteria display on their surfaces long thin filaments called pili or fimbriae that are involved in adhesion to host cells and other substrates. The Type IV pili are unique in that they are retractile, a feature that facilitates a diverse array of pilus functions in addition to adhesion. These include microcolony formation, twitching motility, DNA and phage uptake and in some cases secretion. The pili assemble, adhere to receptors or substrates, and retract to pull the bacteria along or pull substrates such as DNA and phage into the bacterium. Type IV pili are comprised of thousands of copies of the major pilin subunit, which polymerize to form flexible yet remarkably strong filaments capable of withstanding forces of >100 pN. The major pilin protein has a conserved hydrophobic N-terminal alpha-helix that anchors its globular C-terminal domain in the bacterial inner membrane prior to pilus assembly, and forms a helical array in the assembled pilus, displaying the C-terminal domains on the filament surface. Type IV pili are assembled at the inner membrane by a trans-envelope machinery that includes a cytoplasmic assembly ATPase, an inner membrane platform complex, a periplasmic alignment complex and a gated outer membrane secretin channel. Pilus assembly is primed by several minor pilins, which are structurally related to the major pilin. The Type IV pili of *Vibrio cholerae* and enterotoxigenic *E. coli* (ETEC) are among the simplest of the Type IV pilus systems, with only a single minor pilin and no retraction ATPase. Here I will describe our recent cryoEM structures of Type IV pili, which reveal structural features that explain the strength and flexibility of these pili. We show that the *V. cholerae* Type IV pili are retractile despite lacking a retraction ATPase, and that retraction is essential for their functions in microcolony formation, secretion and phage uptake. We further show that the single minor pilin initiates both assembly and retraction of *V. cholerae* Type IV pili, and provide a generalizable model to describe filament dynamics in Type IV pili and the closely related Type II secretion systems.

Folding and Unfolding Mechanisms of Iron Sulfur Proteins Revealed by Single Molecule Force Spectroscopy

Hongbin Li

Department of Chemistry, University of British Columbia

Metal ions play important roles in biology. In metalloproteins, metal centers serve as active sites, as well as important structural elements to facilitate protein folding and assembly. However, it is challenging to investigate the unfolding-folding of metalloproteins due to the loss or decomposition of the metal center. Here, we combine single molecule force spectroscopy and protein engineering to investigate the unfolding-refolding mechanism of small iron sulfur proteins rubredoxin and ferredoxin. Our results revealed that the unfolding of both proteins are characterized by the initial partial unfolding of the protein followed by the rupture of the iron sulfur center and complete unraveling of the protein. However, differences in the iron chelation motif in rubredoxin and ferredoxin gave rise to distinct rupture patterns of the iron sulfur center. After complete unfolding, rubredoxin and ferredoxin were observed to refold to its holo-native form with the fully reconstituted iron sulfur center. Our results open new avenues towards investigating the folding mechanism of metalloproteins at an unprecedented resolution.

Space and Time in Genomic Interactions

Nimish Khanna (1), Yaojun Zhang (2), Olga K. Dudko (3), Cornelis Murre (1)

(1) Division of Biological Sciences, University of California San Diego, USA

(2) Princeton Center for Theoretical Science, Princeton University, USA

(3) Department of Physics, University of California San Diego, USA

Many processes in biology, from antibody production to tissue differentiation, share a common fundamental step — establishing physical contact between distant genomic segments. How fast this step is accomplished sets the “speed limit” for the larger-scale processes this step enables. A key outstanding question is then: How do genomic segments that are strung out over millions of base pairs along the DNA find each other in the crowded cell on a remarkably short timescale? This question, fundamental to biology, can be recognized as the physics problem of the first-passage time. We show how concepts from statistical mechanics and polymer physics help reveal the physical principles by which cells solve this first-passage problem with astonishing efficiency. We illustrate these ideas in the context of V(D)J recombination – the genetic mechanism that allows the human immune system to respond to millions of different antigens.

The Liquid Structure of Elastin

S. Rauscher(1) and R. Pomès(2)

(1) Department of Chemical and Physical Sciences, University of Toronto Mississauga

(2) Molecular Medicine, Hospital for Sick Children

The protein elastin imparts extensibility, elastic recoil, and resilience to tissues including arterial walls, skin, lung alveoli, and the uterus. Elastin and elastin-like peptides are hydrophobic, disordered, and undergo liquid-liquid phase separation upon self-assembly. Despite extensive study, the structure of elastin remains controversial. We use molecular dynamics simulations on a massive scale to elucidate the structural ensemble of aggregated elastin-like peptides. Consistent with the entropic nature of elastic recoil, the aggregated state is stabilized by the hydrophobic effect. However, self-assembly does not entail formation of a hydrophobic core. The polypeptide backbone forms transient, sparse hydrogen-bonded turns and remains significantly hydrated even as self-assembly triples the extent of nonpolar side-chain contacts. Individual chains in the assembly approach a maximally-disordered, melt-like state which may be called the liquid state of proteins. These findings resolve long-standing controversies regarding elastin structure and function and afford insight into the phase separation of disordered proteins.

Structure and Dynamics of the Influenza M2 Protein for Proton Transport and Virus Budding

M. Hong

Department of Chemistry, Massachusetts Institute of Technology

Membrane proteins carry out a myriad of biological functions such as ion conduction, substrate transport, and signaling. Solid-state NMR allows us to obtain functionally relevant structural information of these proteins in lipid bilayers. In this talk, I will present our investigations of the structure and dynamics of a multifunctional influenza membrane protein, matrix protein 2 (M2), which forms a proton channel for virus uncoating and mediates membrane scission during virus budding. ^{13}C , ^{15}N , and ^1H chemical shifts provided detailed information about pH-dependent conformations of the open and closed states of the proton channel. Motionally averaged NMR spectra revealed microsecond-timescale dynamics of the proton-selective histidine residue and the gating tryptophan residue, while 2D exchange NMR spectra revealed millisecond-timescale motion of the entire tetrameric complex. Hydrogen bonding between water and histidine are observed in ^{15}N NMR spectra, which give insight into the proton transfer mechanism of the hydrated channel. In the second function, M2 interacts with membrane cholesterol to cause scission of the emerging virus from the host cell in the last step of virus budding. By measuring protein-cholesterol distances and cholesterol orientations, we have determined the cholesterol-binding site structure of M2. The result gave unexpected insight into how the M2 protein is attracted to the neck of the budding virus to cause membrane scission. These studies of protein-ligand binding are enabled by new solid-state NMR methods to measure distances up to ~ 2 nm using ^{19}F NMR.

Imaging Cholera's Intestinal Impact

Raghuveer Parthasarathy

Department of Physics, The University of Oregon

Our digestive tracts are home to trillions of microbes that immigrate, emigrate, reproduce, and compete with one another. Little is known about the physical structure and temporal dynamics of gut microbial communities, which must necessarily influence the function not only of normal, commensal communities but also community invasion by pathogens. To address this, my lab applies light sheet fluorescence microscopy to a model system that combines a realistic *in vivo* environment with a high degree of experimental control: larval zebrafish with defined subsets of commensal bacterial species. Light sheet microscopy enables three-dimensional imaging with high resolution over the entire intestine, providing visualizations that would be difficult or impossible to achieve with other techniques. I will describe this approach and focus in this talk on experiments in which a native bacterial species is challenged by the invasion of a second species, specifically *Vibrio cholerae*, the pathogen that causes cholera. We find that responses of bacteria to the mechanical contractions of the gut and to specific bacterial machineries, namely *Vibrio*'s Type VI secretion system, can dictate competition between microbes, suggesting a major role for physical mechanisms in guiding the composition of the gut microbiota and its susceptibility to invasion.

Advances in Molecular Counting with Single-Molecule Localization Microscopy

D. Nino, D. Djayakarsana, N. Rafiei and J. N. Milstein

Department of Physics, University of Toronto

Institute of Biomaterials and Biomedical Engineering, University of Toronto

Department of Chemical and Physical Sciences, University of Toronto Mississauga

Cell biology is becoming increasingly quantitative with advances in light microscopy strongly driving this trend. Beyond imaging structure, significant effort has gone into developing microscopy based approaches to determining the abundance of proteins and nucleic acids in cells. Molecular counting experiments can yield additional insight into cellular structure and define the stoichiometry of interacting protein complexes. Moreover, since microscopy provides information at the single-cell level, it may be used to study stochastic variation within a population due to varying levels of mRNA and protein copy number, which is inaccessible to bulk techniques. Single-molecule localization microscopy (SMLM) has the potential to serve as an accurate, single-cell technique for counting the abundance of intracellular molecules. However, the stochastic blinking of single fluorophores can introduce large uncertainties into the final count. Here we provide a foundation for applying SMLM to the problem of molecular counting based on the distribution of blinking events from a single fluorophore.

Cytoskeletal dynamics and mechanosensing in immune cells

King Lam Hui (1), David Garcia (1), Arpita Upadhyaya (1,2)

1. *Department of Physics, University of Maryland, College Park, MD 20742*

2. *Institute for Physical Science and Technology, University of Maryland, College Park, MD 20742*

Lymphocyte activation is an essential step in the adaptive immune response, and involves the binding of specialized receptors with antigen on the surface of antigen presenting cells. This leads to changes in cell morphology and assembly of receptors and signaling proteins into microclusters, which are essential for immune cell activation. During activation, immune cells interact with structures possessing a diverse range of physical properties and respond to physical cues such as stiffness, topography and ligand mobility. We have used traction force microscopy to measure the forces exerted by T cells during activation on elastic substrates. I will discuss the distinct roles of the actin and microtubule cytoskeleton in the exertion of mechanical stresses that support signaling activation, microcluster assembly and receptor movement in T cells. Forces exerted are largely due to actin dynamics and T cells are mechanosensitive to substrate stiffness. Further, these forces are regulated by microtubule dynamics through Rho activity and myosin filament assembly. Our studies highlight the importance of cytoskeletal forces in immune cell receptor activation.

Functional ordered domains in intact B cell membranes

Sarah A. Shebly, Matthew B. Stone, Sarah L. Veatch

Department of Biophysics, University of Michigan

Both synthetic and isolated biological membranes can separate into coexisting liquid-ordered and liquid disordered phases at low temperatures, and it is often speculated that this miscibility phase transition plays some role in organizing plasma membrane proteins on the cell surface at elevated physiological temperature. In this talk, I will present evidence that ordered membrane domains sort proteins on the B cell surface to establish local regions that favor receptor activation, based largely on super-resolution fluorescence localization imaging of chemically fixed and live B cells. I will also present theoretical models based on experimental observations in vesicles that describe how protein clustering can stabilize extended domains that resemble low temperature phases even when membranes are examined above their miscibility transition temperature.

Novel Statistical Tools for Single Molecule Imaging: A foray into Bayesian nonparametrics

Steve Pressé

ASU Department of Physics and School of Molecular Sciences

One route to modeling biophysical dynamics involves the bottom-up, molecular simulation, approach. In this approach, approximate classical potentials are used to simulate short time local motions in order draw insight on dynamics at longer time and larger length scales. Here we take a different route. Instead we present a top-bottom approach to building models of single molecule conformational dynamics and diffusion. The approach we present exploits a novel branch of Statistics called Bayesian nonparametrics (BNPs) first proposed in 1973 and now widely used in data science as the important conceptual advances of BNPs have become computational feasible in the last decade. BNPs are new to the physical sciences. They use flexible (nonparametric) model structures to efficiently learn models from complex data sets. Here we will show how BNPs can be adapted to address important questions in biophysics directly from the data which is often limited by factors such as finite photon budgets as well as other fluorophore artifacts in addition to data collection artifacts (e.g. aliasing, drift). More specifically, we will show that BNPs hold promise by allowing complex spectroscopic time traces (e.g. smFRET, photon arrivals) or images (e.g. single particle tracking) to be analyzed and turned into principled models of single molecule motion from diffusion to conformational dynamics and beyond.

Peering into cells with new imaging technologies

Jennifer Lippincott-Schwartz

Janelia Research Campus, HHMI, Ashburn, VA

Powerful new ways to image the internal structures and complex dynamics of cells are revolutionizing cell biology and bio-medical research. In this talk, I will focus on how emerging fluorescent technologies are increasing spatio-temporal resolution dramatically, permitting simultaneous multispectral imaging of multiple cellular components. Using these tools, it is now possible to begin constructing an “organelle interactome” describing the interrelationships of different cellular organelles as they carry out critical functions. The same tools are also revealing new properties of the cell’s largest organelle, the endoplasmic reticulum, and how disruptions of its normal function due to genetic mutations may contribute to important diseases.

Force-sensitivity and cooperativity arising from polymer properties of formins and other intrinsically disordered molecules

Jun Allard, Lara Clemens, Derek Bryant

UC Irvine

Proteins with intrinsically disordered regions, that lack a dominant structure, appear often in cell signaling and cell mechanics. In signaling, examples include the T cell receptor zeta chain. In mechanics, examples include the formin family that creates and elongates actin structures. Here, we develop models of intrinsically disordered regions with a simplified theta-solvent freely-jointed chain model coupled to idealized spherical binding enzymes. Many actin structures are nucleated and assembled by the formin family, including filopodia, focal adhesions, the cytokinetic ring and cell cortex. These structures respond to forces in distinct ways. Formins typically have profilin-actin binding sites embedded in disordered FH1 domains, hypothesized to diffusively explore space to rapidly capture actin monomers for delivery to the barbed end. Recent experiments demonstrate that formin-mediated polymerization accelerates when under tension. The acceleration has been attributed to modifying the state of the FH2 domain of formin. Intriguingly, the same acceleration is reported when tension is applied to the FH1 domains, ostensibly pulling monomers away from the barbed end. In this work we ask whether this behavior emerges from basic entropic polymer properties by simulating a model of formin-mediated actin polymerization. The binding of actin monomers to their specific sites on FH1 is entropically disfavored by the high disorder. We find that this penalty is attenuated when force is applied to the FH1 domain by revealing the binding site, increasing monomer capture efficiency. Overall polymerization rates can decrease or increase with increasing force, depending on the length of FH1 domain and location of binding site. Our results suggest that the widely varying FH1 lengths and binding site locations found in known formins could be used to differentially respond to force, depending on the actin structure being assembled. This nonlinear force response is part of a growing body of work demonstrating the emergence of nonlinear behavior, including cooperativity, anticooperativity, and sequentialization of enzymatic events, all arising from polymer properties alone.

Altered mechanobiology of tumor-associated extracellular matrix proteins

Maryam Asadishekari, Elie Ngandu Mpoyi, Delphine Gourdon

Mechanobiology and Tribology Lab, Department of Physics, University of Ottawa

The extracellular matrix (ECM), a complex network of proteins including collagen (COL) and fibronectin (FN) couples a cell with its environment and directly regulates the cell’s fate via physical and biochemical signals. Although the ECM was often considered a static structure providing cohesion and mechanical integrity to tissues, it has recently been shown that (i) the nano-structure, (ii) the nano/micro mechanics, and (iii) the signaling capacity of the ECM are affected by cell-generated forces. Our work has focused on investigating and controlling the material properties of ECM networks and the synergistic roles of FN and COL in 3D environments. In a first example, I will show how the integrated method used in our lab allows us to diagnose early dysregulation of the ECM materials properties in tumors. In a second example, I will present 3D matrix-mimicking polymeric platforms we developed to control both COL and FN properties over macroscopic volumes. These platforms enable a better understanding of the critical link between protein structure and function, with the ultimate goal of controlling cellular functions (responsible for tumor growth) through cell-matrix interactions. As such, they represent a new tool for biophysical research with many potential applications in basic research, medical diagnostics, and tissue engineering.

The role of diffusion in the establishment and interpretation of morphogen concentration gradients

C. Fradin¹, C. Perez-Romero¹, H. Tran², M. Coppey³, A. Walczak², and N. Dostatni³

1Department of Physics and Astronomy, McMaster University, Canada; 2Ecole Normale Supérieure, PSL Research University, France; 3Institut Curie, PSL Research University, France.

Morphogens are proteins that form concentration gradients in embryos and developing tissues and act as postal codes, providing cells with positional information. Bicoid was the first discovered morphogen, and remains one of the most studied. It regulates segmentation in flies, forming an exponential gradient in early embryos, and activating the transcription of multiple target genes in a concentration-dependent manner. Bicoid, and its main target, hunchback, provide an unparalleled system for quantitative analysis of the processes at work during morphogen gradient formation and readout. We use fluorescence correlation spectroscopy to characterize the mobility of Bicoid in *D. melanogaster* embryos, and fluorescence imaging of transcription in live embryos to assess the precision and the timing of hunchback transcriptional response. Our experiments show that the diffusion of Bicoid plays a central role played in both the establishment of the gradient and the activation of target genes. However, they also show that it is not sufficient to explain the observed precision of the hunchback transcriptional response. This leads us to consider the different way in which the precision of the transcriptional response could be improved beyond that predicted by a simple diffusion-activation model.

Characterization of the structure and function of the microphthalmia-associated transcription factor and its association with CBP/p300

M.A. Branch, K. Vergunst and D. Langelaan

Department of Biochemistry & Molecular Biology, Dalhousie University

Transcription factors control gene expression and coordinate fundamental processes such as cell growth and differentiation. The microphthalmia-associated transcription factor (MITF) is a melanocyte-specific protein essential for melanocyte development and differentiation. MITF has also been identified as a lineage-specific oncoprotein in melanoma, and knockdown of MITF function results in the activation of cellular senescence. MITF contains N-terminal and C-terminal activation domains as well as a central basic helix-loop-helix DNA binding motif. Both the N-terminal and C-terminal activation domains directly interact with the homologous histone acetyltransferases CBP/p300 and are required for MITF-dependent transcriptional activation. We have used a combination of pull-down experiments, NMR spectroscopy, biophysical studies, and functional transactivation assays to show that the N-terminal activation domain of MITF is intrinsically disordered in solution and directly interacts with the TAZ2 domain of CBP/p300 with high affinity. NMR-based titrations indicate that MITF also interacts with the KIX and TAZ1 domains of CBP/p300, raising the possibility of co-operative interactions between these domains. These results provide insight as to how MITF may control gene expression in melanoma and support a model in which MITF interacts with multiple domains of CBP/p300 to activate transcription of MITF-target genes.

Hybrid biophysical methods allow atomic resolution structure/function analysis of the multimembrane spanning Type III secretion nanomachine

Natalie Strynadka

Department of Biochemistry, University of British Columbia

Bacteria have evolved several sophisticated assemblies to transport proteins across their biological membrane, including those required specifically for pathogenicity. Recent advances in our understanding of the molecular details governing the molecular action of these protein secretion systems has benefited from an integrated x-ray crystallography, NMR, mass spectroscopy, electron microscopy, and molecular modeling toolbox. Highlights of recent advances in our piece wise structure/function analysis of the multi-membrane spanning Type III Secretion system "injectisome" will be presented. A molecular understanding of the Type III systems being garnered from these studies furthermore provides the foundation for the development of new classes of antibacterials and vaccines to combat infection in the clinic and community.

Manipulating amyloid formation: from mechanisms of self-assembly to nanovaccine engineering

S. Bourgault

Department of Chemistry, Université du Québec à Montréal, Montreal, Canada

Endogenous proteins are known for their ability to self-associate in living organisms into supramolecular structures that perform crucial physiological functions. Over the last decade, amyloid fibrils that accomplish biological activities have been identified in almost all species, from bacteria to mammals. Amyloids are organized proteinaceous assemblies characterized by a cross-beta-sheet quaternary conformation. The mechanical, physical and biological properties of amyloids suggest that they hold great potential as biomaterials for medical applications. However, the usage of amyloids is still today limited by a number of issues, which include; (i) partial understanding of the mechanisms of self-assembly, (ii) difficulty of controlling amyloid formation, (iii) problem of predicting the final supramolecular architecture from the primary sequence and (iv) potential toxicity of conformational intermediates. In this context, our research group aims at developing (bio)chemical approaches to control amyloid assembly and design functionalized proteinaceous nanostructures. By using the islet amyloid polypeptide, whose deposition in the pancreatic islets is associated with type II diabetes, we are investigating the early steps of amyloidogenesis and the relation between structure and cellular toxicity. Besides, we are developing chemical strategies to modulate the (supra)molecular architecture of amyloid assemblies in order to design novel nanovaccines against the Chikungunya virus.

Structure/permeability relationship in skin: working with more complex model systems leads to a less solid view

M. Lafleur, and A. Paz Ramos

Department of Chemistry, Université de Montréal.

Human life requires a barrier between the internal (regulated) environment and the outside world. The majority of this barrier is located in the skin. The outermost layer of mammalian skin (the epidermis) produces the Stratum corneum (SC), an impermeable composite material. The unusual lipid membranes in SC determine both the rate of water loss through the skin, and absorption of exogenous molecules into the body. A significant fraction of SC lipids exist in crystalline phases and this unusual organization is believed to be a key feature associated with the very limited permeability of this biological material. Our studies on model systems, using solid-state ^2H NMR, and vibrational spectroscopy, participate in establishing the crystallinity of lipids in SC model mixtures and bring some insights in the mixing properties of SC lipids. However, recently, we have discovered, using a more complex lipid model mixture, that SC lipid structure likely includes hydrocarbon nanodrops. The droplets are proposed to result from steric constraints imposed on the acyl chain of Cer EOS (a ceramide bearing an oleate linked to a very long saturated acyl chain) by the solid/crystalline structure. This finding could profoundly modify our understanding of the skin barrier as it is proposed that the hydrocarbon nanodrops could play a central role in SC impermeability and in the mechanism associated with some penetration enhancers.

Reversible separation of living, unperturbed cell membranes into two liquid phases

G.E. Rayermann, S.P. Rayermann, C.E. Cornell, A.J. Merz and S.L. Keller

Department of Chemistry and Department of Biochemistry, University of Washington

Scientists have been arguing for decades whether living, unperturbed cells employ spontaneous lateral demixing of membranes to organize proteins and lipids on micron scales. Since the 1960s, researchers have observed domains within yeast vacuolar membranes that are consistent with, but not conclusive of, liquid-liquid phase separation. Therefore, we tested whether in vivo yeast vacuole membranes labeled with a Vph1-GFP fusion protein exhibited both hallmarks of reversible phase separation into coexisting liquid phases: (1) domain coalescence on short time scales and (2) the existence of a miscibility temperature, T_{mix} . We observed domains with rounded, smooth shapes that coalesce in seconds and subsequently change shape within tens of seconds, characteristic of fluid phases. In both in vivo and cell-free vacuoles, we found a distinct T_{mix} above which domains disappear and below which they reappear over multiple temperature cycles. Our results show that large-scale membrane organization in living cells under physiologically relevant conditions can be controlled by tuning a single thermodynamic parameter. Cells may access this same phase transition through internal or external molecular cues. Recent work by Alexandre Toulmay, William Prinz, Ted Powers, and Jodi Nunnari (*Journal of Cell Biology*, 2017) shows that membrane domains control a growth signaling pathway that is conserved across the broad evolutionary diversity of eukaryotes.

Lipid driven nanodomains are fluid and inter-leaflet coupled

A. Koukalová¹, M. Amaro¹, I. Vinklársek¹, L. Velás¹, G. Gröbner², P.T.F. Williamson³, M. Hof¹ and R. Šachl¹

1 J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

2 Department of Chemistry, University of Umeå, Sweden

3 Centre for Biological Sciences/Institute for Life Sciences, University of Southampton, United Kingdom

It is a fundamental question in cell biology and biophysics whether nanodomains enriched by sphingomyelin (SM) and cholesterol (Chol) exist in living cells and in model membranes. Biophysical studies on model membranes revealed SM and Chol driven micrometer-sized liquid-ordered domains. Although the existence of such microdomains has not been proven for the plasma membrane, such lipid mixtures have been often used as a model system for 'rafts'. On the other hand, recent super resolution and single molecule results indicate that the plasma membrane might organize into nanocompartments. In this work, a novel combination of Förster resonance energy transfer and Monte-Carlo simulations (MC-FRET) identifies directly 10 nm large nanodomains in liquid-disordered model membranes composed of lipid mixtures containing SM and Chol. Combining MC-FRET with solid-state wide-line and high resolution magic angle spinning NMR as well as with fluorescence correlation spectroscopy we demonstrate that these nanodomains containing hundreds of lipid molecules are fluid, disordered with subtle differences in respect to the surrounding disordered phase, and importantly inter-leaflet coupled. In terms of their size, fluidity, order and lifetime these nanodomains may represent a relevant model system for cellular membranes and are closely related to nanocompartments suggested to exist in cellular membranes.

How does the Plasma Membrane Participate in Receptor-Mediated Cell Signaling?

Barbara Baird

Department of Chemistry and Chemical Biology, Cornell University

Cells are poised to respond to their physical environment and to chemical stimuli in terms of collective molecular interactions that are regulated in time and space by the plasma membrane and its connections with the cytoskeleton and intracellular structures. Small molecules may engage specific receptors to initiate a transmembrane signal, and the surrounding system efficiently rearranges to amplify this nanoscale interaction to microscale assemblies, yielding a cellular response that often reaches to longer length scales within the organism. A striking example of signal integration over multiple length scales is the allergic immune response. IgE receptors (FcεRI) on mast cells are the gatekeepers of this response, and this system has proven to be a valuable model for investigating receptor-mediated cellular activation. My talk will describe our efforts with quantitative fluorescence microscopy and modeling to investigate the poised, “resting state” of the plasma membrane and how signaling mediated by specific receptors is regulated and targeted within this milieu.

Role of Lipid Nanodomains in CD36-Fyn Signal Transduction

S.M. Khaing, J.M. Githaka, S. Ungarian and N. Touret

Department of Biochemistry, University of Alberta, Edmonton, AB, Canada

CD36, a multi-ligand plasma membrane receptor, has been implicated in immunity, metabolism and angiogenesis. We have recently demonstrated that CD36 nanoclustering at the plasma membrane is key to the initiation of CD36 signaling. In endothelial cells (ECs), the binding of thrombospondin-1 (TSP-1, an endogenous extracellular matrix anti-angiogenic factor) to CD36 nanoclusters activates an associated Src family kinase, Fyn, leading to ECs apoptosis, hence, inhibiting angiogenesis. Our project centralized in elucidating the mechanisms of CD36-Fyn enrichment on the lipid nanodomains during TSP-1 induced signaling in ECs. We hypothesized that lipid nanodomains play a role in bringing together CD36-Fyn to form a signaling platform. Using microscopy methods to visualize Fyn, various fluorescent lipid biosensors and F-actin (Phalloidin-AF647), we determined that Fyn is enriched on F-actin area at sites of phosphatidylinositol 4,5-bisphosphate enrichment (PIP₂). During TSP-1 stimulation on Human Microvascular Endothelial Cells (HMEC), the CD36-Fyn-F-actin enrichment shift to domains containing PI(3,4,5)P₃, suggesting a role for the phosphoinositide 3-kinase in signaling. To test the role of PI3K in Fyn activation and in CD36 nanocluster enhancements, we employed pharmacological inhibition of PI3K (LY294002) to arrest the production of PIP₃ on the plasma membrane and depletion of membrane PI(4,5)P₂, a precursor for PI(3,4,5)P₃ using ionomycin. Using Immunoblotting and super-resolution fluorescence microscopy (TIRF-PALM), we determined that PI3K is important for Fyn activation and in CD36 nanocluster enhancements in CD36-Fyn signaling upon stimulation with TSP-1. Additionally, we employed a unique optogenetic tool (LARIAT) to facilitate in understanding the role of lipid nanodomains in CD36-Fyn signaling. Upon clustering of CD36 molecules using LARIAT, Fyn activation enhanced within these clusters and this activation is reduced by treatment with LY294002 which further supported our hypothesis that engagement with PI3K (lipid nanodomains PI(4,5)P₂ and PI(3,4,5)P₃) play a significant role in Fyn activation and CD36 nanoclustering. With this, we proposed a model in which CD36 nanoclusters are located within PI(4,5)P₂ domains and upon TSP-1 stimulation, PI3K is engaged, producing PI(3,4,5)P₃ within the CD36 nanoclusters and enhances the nanoclusters and downstream Fyn activity.

Visualizing the GPCR Network: Classification and Evolution

Geng-Ming Hu, Te-Lun Mai, and Chi-Ming Chen

Department of Physics, National Taiwan Normal University

In this study, we delineate an unsupervised clustering algorithm, minimum span clustering (MSC), and apply it to detect G-protein coupled receptor (GPCR) sequences and to study the GPCR network using a base dataset of 2770 GPCR and 652 non-GPCR sequences. High detection accuracy can be achieved with a proper dataset. The clustering results of GPCRs derived from MSC show a strong correlation between their sequences and functions. By comparing our level 1 MSC results with the GPCRdb classification, the consistency is 87.9% for the fourth level of GPCRdb, 89.2% for the third level, 98.4% for the second level, and 100% for the top level (the lowest resolution level of GPCRdb). The MSC results of GPCRs can be well explained by estimating the selective pressure of GPCRs, as exemplified by investigating the largest two subfamilies, peptide receptors (PRs) and olfactory receptors (ORs), in class A GPCRs. PRs are decomposed into three groups due to a positive selective pressure, whilst ORs remain as a single group due to a negative selective pressure. Finally, we construct and compare phylogenetic trees using distance-based and character-based methods, a combination of which could convey more comprehensive information about the evolution of GPCRs.

Cortical microtubules deflect in response to cell-surface curvature

E.N. Cytrynbaum, R. Froese, and S. Bachmann

Department of Mathematics, University of British Columbia

In growing plant cells, parallel ordering of microtubules (MTs) influences the direction of cell expansion. Models of MT growth in the plane and on polyhedral surfaces have shown that growing-MT encounters lead to the formation of ordered arrays. The polyhedral surfaces models assume that when a MT crosses an edge, it emerges on the adjacent face at the same angle with the edge as the incident angle (i.e. following geodesics). This assumption ignores the MT mechanics - an elastic rod constrained to a rigid surface ought to deflect away from a geodesic when such a deflection decreases its energy. Here, we show this principle for a growing elastic rod on a cylindrical surface with one end clamped. We write down an energy functional that accounts for the bending energy of the rod and derive the associated Euler-Lagrange equation getting a two-variable boundary value problem. Minima and their stability can be found analytically in some cases. The system has a locus of saddle-nodes with a pitchfork in the symmetric case. In general, growing rods deflect away from high curvature directions and toward the flat axial direction, as expected. A rod growing circumferentially continues to grow circumferentially until a critical length (the pitchfork) after which it buckles up or down the cylindrical wall. Our results indicate that mechanics complicate the ordering process by biasing the arrays to orientations that are not the ones seen in real cells.

Molecular Recognition in Insect Odorant-Binding Proteins

E. Plettner and M. Terrado

Department of Chemistry, Simon Fraser University

Insects have a tremendously selective and sensitive sense of smell that is involved in key stages of their life cycles. E. g. female moths attract mates using species-specific pheromones. The gypsy moth, *Lymantria dispar*, has a chiral hydrocarbon epoxide, 2-methyl-(7R, 8S)-epoxyoctadecane ((+)-disparlure), as its pheromone. Recognition of this compound by male moths is highly specific: even a few percent of the opposite enantiomer ((-)-disparlure) will cancel male upwind flight behavior. The sensor for these pheromone compounds is on the antennae, in the form of hollow sensory hairs. These hairs are innervated with the dendritic projections from 2-3 olfactory neurons, and the dendrites in the hair are surrounded by sensory lymph. The lymph contains an odorant-binding protein (OBP) that is the first gene product to selectively interact with the pheromone. The actual sensor on the dendritic membrane consists of a pair of transmembrane proteins, the odorant receptor with its co-receptor. OBPs could serve two roles that are not mutually exclusive: 1) solubilization of the hydrophobic pheromones (or general odorants) in the lymph and/or 2) scavenging of excess odorant molecules to prevent sensory saturation. We have studied OBP ligand association and dissociation kinetics with the pheromone and with closely related analogs, to understand the role OBPs play in molecular recognition in the exquisitely selective and sensitive pheromone detection system of the gypsy moth.

Hybrid cellular Potts model including focal adhesions as catch bond clusters explains cell response to substrate stiffness

Lisanne Rens and Roeland Merks

Scientific Computing, CWI, Amsterdam, The Netherlands

Mathematical Institute, Leiden University, Leiden, The Netherlands

Cell behavior depends on extracellular matrix (ECM) stiffness. For instance, on soft substrates, cells are generally small and rounded, while on stiffer substrates cells assume spindle-like shapes, and on glass-like substrates cells generally spread out. Cells also generally move upwards a stiffness gradient (durotaxis). Stiffness sensing is mediated through transmembrane integrin molecules, which behave as 'catch bonds' whose strength increases under tension. Focal adhesions, large assemblies of integrins, grow larger on stiffer substrates. We extended a hybrid cell-based continuum model (van Oers, Rens et al. PCB 2014, Rens and Merks BJ 2017) to describe such molecular mechanics. The model includes 1) A finite-element model for the ECM; 2) a cellular Potts model, to describe cell shape changes; 3) a set of ordinary-differential equations describing the growth and decay of individual focal adhesions (Novikova and Storm, BJ 2013). In this model, cells move and pull on the ECM. This leads to a slow build-up of tension on the FA, which changes the FA's size. Substrate stresses generated by the cells then further strengthen cell-matrix adhesion. The focal adhesions finally inhibit the cell's pseudopod retractions from the ECM. These minimal model assumptions reproduce the observed cell shape behavior on substrates of varying stiffness, as well as durotaxis. Our model result increases our understanding of the molecular mechanism behind cell response to ECM stiffness.

Measurement of the functional form of Shannon entropy by partial erasure of a bit

M. Gavrilov, R. Chérite, and J. Bechhoefer

Department of Physics, Simon Fraser University

We use a feedback trap to erase a fraction of a bit of information from a memory whose states are encoded in the two states of a double-well potential. The system consists of a colloidal particle in water in a "virtual" potential. We show experimentally that the minimum amount of work required is proportional to the Shannon entropy function for a two-state system, for arbitrary state probabilities. This is the first experimental confirmation that the Shannon function is the appropriate definition for nonequilibrium system entropy as it relates to thermodynamics. In particular, the Shannon entropy is a typical starting point for the analysis of biophysical processes in the cell, such as sensing systems. Interestingly, both sensing systems and some of the protocols we explored can be inherently more dissipative than thermodynamic bounds suggest. That is, the work required can exceed the change in free energy, no matter how slowly the transformation is carried out.

The Physics of Behavior: measuring and modeling the sensorimotor response of *C. elegans*

W. Ryu

Department of Physics, University of Toronto

The roundworm, *C. elegans* is a relatively simple organism with only 300 neurons but can generate complex adaptive behavioral responses to a wide range of sensations including taste, touch, and temperature. *C. elegans* locomotion consists of a number of stereotyped behavioral states such as forward and reverse, pausing, turning, etc. In general, the worm moves randomly by making stochastic transitions between these states, and in response to sensory measurements it performs adaptive locomotory changes (behaves) by biasing the probability of these transitions. However under certain conditions the worm will respond deterministically with a specific behavioral sequence or motif, such as in the escape response to a “painful” or noxious stimulus. One of the grand goals in science is to understand how neural, genetic, and biochemical circuits produce these behaviors. While a great deal of work has been done in the development of tools to perturb and measure the circuits underlying sensory behavior, advances in the study of behavior itself has lagged behind. Here I will describe some attempts to close this gap with focus on *C. elegans* locomotion and its response to thermal stimuli. We’ve developed simple desktop experiments to programmatically stimulate *C. elegans* and quantitatively capture its behavioral response. Using these data we have shown that *C. elegans* moves through a “shape space” that is low dimensional in which four dimensions capture approximately 95% of the variance in body shape. Here I will give two examples of modeling that take advantage of this low dimensionality and stereotypy. In the first we show that stochastic dynamics within this shape space predicts transitions between attractors corresponding to abrupt reversals in crawling direction. With no free parameters, our inferred stochastic dynamical system generates reversal timescales and stereotyped trajectories in close agreement with experimental observations. In the second we use Sir Isaac, an algorithm that allows inference of the dynamical equations underlying a noisy time series, even if the dynamics are nonlinear—to analyze the thermal “pain” response of *C. elegans*. Both examples show that it is possible to learn “equations of behavior” of the worm, and that these equations give an interpretable, complementary perspective to traditional biological studies.

POSTER PRESENTATION ABSTRACTS

Poster A1

Modulation of Kv1.2 activation-gating by the Sigma-1 Receptor: novel insights to the pathophysiology of ALS16

M.J. Abraham, K. Ferguson, A.Y.C. Wong and R. Bergeron.

Department of Cellular and Molecular Medicine, Neuroscience Program, University of Ottawa; Department of Cellular and Molecular Medicine, Neuroscience Program, University of Ottawa; Ottawa Hospital Research Institute, Ottawa ON; Department of Cellular and Molecular Medicine, Neuroscience Program, University of Ottawa and Ottawa Hospital Research Institute, Ottawa ON.

Expression of Kv1.2 within Kv1 potassium channel complexes is critical in maintaining appropriate neuronal excitability and determining the threshold for action potential firing. This is attributed to the interaction of Kv1.2 with an unidentified molecule that confers bimodal channel activation gating, allowing neurons to adapt to repetitive trains of stimulation and protecting against hyperexcitability. It is known that the Sigma-1 receptor (Sig-1R) regulates other members of the Kv1 channel family at baseline and upon ligand-activation, but the biophysical nature of the interaction between Sig-1R and Kv1.2 has not been elucidated. We hypothesized that Sig-1R may regulate Kv1.2 biophysical properties and may further act as the unidentified modulator of Kv1.2 activation. In transiently transfected HEK293 cells, we show that ligand-activation of Sig-1R modulates Kv1.2 current amplitude and inactivation, and that Sig-1R interacts with Kv1.2 in baseline conditions to influence bimodal activation gating. These effects are abolished in the presence of the auxiliary-subunit Kv β 2 and when the Sig-1R mutation underlying ALS16 (Sig-1R-E102Q) is expressed. These data respectively suggest that Kv β 2 physically occludes the interaction of Sig-1R with Kv1.2, and that E102 may be a residue critical for Sig-1R modulation of Kv1.2. This work provides a new role for Sig-1R in the regulation of neuronal excitability and introduces a novel mechanism of pathophysiology in Sig-1R dysfunction.

Poster A2

Altered mobility and nanoscale spatial organization of IgM-BCRs is associated with increased tonic BCR signaling in marginal zone B cells

L. Abraham 1,2,3, J. Scurl 3, R. Tafteh 4, HY. Lu 1,2, R.Chappell 3, RC. Falcão 3, KC. Chou 4, D.Coombs 3, M. Gold 1,2,3

1 Department of Microbiology & Immunology, University of British Columbia, Vancouver, Canada

2 Life Sciences Institute I3 and Cell Research Groups, University of British Columbia, Vancouver, Canada

3 Department of Mathematics, University of British Columbia, Vancouver, Canada

4 Department of Chemistry, University of British Columbia, Vancouver, Canada

B cells integrate signals from multiple activating and inhibitory receptors in a highly regulated spatio-temporal manner to regulate strength of B cell receptor (BCR) signaling and B cell activation. Marginal Zone (MZ) B cells are unique subset of B cells that reside in the marginal sinus of the spleen and exist in a partially activated 'primed' state, with higher antigen-independent 'tonic' BCR signaling. The molecular basis for this priming is not fully understood. We used single particle tracking and super-resolution microscopy (SRM) to quantify receptor mobility and organization on the plasma membrane of resting circulating follicular (FO) B cells and MZ B cells. We found that IgM-BCRs and co-receptor CD19 on the cell surface of MZ B cells exhibit greater lateral mobility, when compared to FO B cells. In contrast, the mobility of IgD-BCRs was similar on MZ and FO B cells. IgM-BCRs and CD19 were more dispersed and exhibited less clustering in MZ B cells when compared to FO B cells. In contrast, IgD-BCRs were organized into larger nanoclusters in MZ B cells. Using three-color STED SRM, we showed that pCD79 nanoclusters (a read-out for signaling BCRs) predominantly overlapped IgM-BCRs, than with IgD-BCRs on FO B cells. This IgM-pCD79 overlap was even greater in MZ B cells. Our results suggest that the increased tonic BCR signaling in MZ B cells is associated primarily with changes in the mobility and spatial organization of IgM-BCRs and CD19, as opposed to IgD-BCRs.

Poster A3**Structural and kinetic analysis of penicillin-binding protein 4 (PBP4)-mediated antibiotic resistance in *Staphylococcus aureus***

J.A.N. Alexander^{1,2}, S.M. Hamilton³, S.S. Chatterjee³, L.D. Eltis^{1,4}, H.F. Chambers³, and N.C.J. Strynadka^{1,2}

1 Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada; 2 Centre for Blood Research, University of British Columbia, Vancouver, British Columbia, Canada; 3 Division of Infectious Disease, Department of Medicine, San Francisco General Hospital, San Francisco, CA; 4 Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes serious community-acquired and nosocomial infections around the world. MRSA infections are resistant to a wide variety of β -lactam antibiotics, making them intractable to treatment. While β -lactam resistance in MRSA has traditionally been ascribed to the action of the *mecA* gene product, Penicillin Binding Protein 2a, it has recently been observed that resistance can also be mediated via Penicillin Binding Protein 4 (PBP4) in some strains with *pbp4* mutations. The highly β -lactam resistant CRB strain of *S. aureus* has two missense mutations in *pbp4* as well as a mutation in the *pbp4* promoter. These *pbp4* missense and promoter mutations have been found to play an instrumental role in the PBP2a independent β -lactam resistance observed in the CRB strain. In order to better understand PBP4 and its role in antibiotic resistance we structurally and kinetically characterised it with clinically relevant β -lactam antibiotics. We present the first apo and acyl-enzyme intermediate wild-type PBP4 and CRB PBP4 X-ray crystallographic structures in complex with three late-generation β -lactam antibiotics: ceftobiprole, ceftaroline, and nafcillin. With ceftobiprole the missense mutations impair the Michaelis constant (K_M) 170-fold, decreasing the proportion of inhibited PBP4 present. However, ceftaroline resistance appears to be mediated by other factors, possibly including mutation of the *pbp4* promoter.

Poster A4**Exploring the Molecular Properties of Collagen Type IV with Atomic Force Microscopy**

A. Al-Shaer, A. Lyons and N. R. Forde

Department of Physics and Department of Molecular Biology and Biochemistry, Simon Fraser University

Collagen type IV is a network-forming collagen that provides support and anchorage to cells. Its basic structural unit is a 410 nm long and 1.5 nm in diameter triple helix, with natural discontinuities in the triple-helical defining Gly-X-Y sequence. The C-terminal globular domain (NC1) in a collagen IV molecule plays an important role in forming networks, and has recently been reported to be structurally triggered by chloride ions to form hexamers outside the cell. How this hexamer assembles in vitro remains unknown. Here, I aim to use atomic force microscopy (AFM) to investigate the molecular basis of collagen type IV network assembly by studying the effects of different solvent conditions on the stability of the NC1 domain. Studying the dissociation of this hexameric domain can shed light onto how it assembles in solution and under what ionic conditions. The flexibility of the collagen type IV molecule is also investigated by performing statistical analysis of AFM-imaged chains and estimating persistence length, a mechanical property that quantifies the flexibility of a polymer. Here, I investigate the effects of triple helix interruptions on the flexibility of the molecule, by comparing collagen type IV to other fibrillar collagens that are entirely triple helical. In addition, I determine a position-dependant flexibility profile of the molecule showcasing the effects of over-lapping interruptions on persistence length.

Poster A5**Amino Acid Formation Energy and Frequency in Different Species and Adinkra Model**

S. Arbabimoghadam, J. Tuszynski

Department of Physics, University of Alberta

Proteins, made of amino acids, play key roles in almost all biological processes. There are 20 amino acids, each having its unique physico-chemical properties. The genetic code includes 64 possible permutations/combinations of three-letter nucleotide sequences that can be made from the four nitrogenous nucleotide bases: Adenine(A), Guanine(G), Cytosine(C) and Thymine(T); 61 of these combinations represent amino acids and the remaining three are stop signals. Theoretical physicists were inspired by Adinkra symbols, which are geometric objects encode mathematical relationships of graph theory and supersymmetric algebras. In this project, based on Adinkra graphs, we were able to generate a 6-dimensional hypercube with all 64 codons as nodes. The lengths of the edges were obtained based on codons/amino acids Tanimoto coefficients. Moreover, we categorized amino acids based on their characteristics and their positions in our model. We also studied the amino acids formation Gibbs free energy of triplets and the occurrence frequency for different species in the phylogenetic tree from the simplest species(E.coli) to the most complex(Human). Results are pretty consistent and show that leucine is the highest probable amino acid while tryptophan is the least probable. Interestingly, both amino acids have a high formation energy. Further analysis indicates an amino acids frequency does not correlate with the number of synthesis pathways. In future, we will add their energy to our graph.

Poster A6**Solving High Resolution Structures using Cryo-Electron Microscopy at HR-MEM**

C.E. Atkinson and N.J. Strynadka

Department of Biochemistry, University of British Columbia

Cryo-electron microscopy (Cryo-EM) has undergone a “resolution revolution” over the last 5 years as a result of the introduction of direct electron detector cameras and advances in processing software. This has allowed researchers to obtain cryo-EM structures of biological molecules to near atomic resolution. The University of British Columbia has created the High-Resolution Macromolecular Cryo-Electron Microscopy (HRMEM) facility to capitalize on these techniques. The facility is equipped with a Titan Krios microscope and a Falcon III camera. Here, we introduce the facility and present structures that have been solved at HR-MEM, including the Type III secretion system from Salmonella typhimurium with the needle present.

Poster A7**Unusual Reaction Catalysed by Cytochrome P450_{cam} (CYP101A1) and Its Significant Role in the Chemotaxis by Pseudomonas Putida (ATCC 17453)**

P. Balaraman, E. Plettner

Department of Chemistry, Simon Fraser University

The camphor-degrading microorganism, *Pseudomonas putida* strain (ATCC 17453), is an aerobic, gram-negative soil bacterium that uses camphor as carbon and energy source. The genes responsible for degradation of camphor are encoded on the extra-chromosomal CAM plasmid. A monooxygenase, cytochrome P450_{cam}, mediates hydroxylation of camphor to 5-exo-hydroxycamphor as the first and committed step in the camphor degradation pathway, requiring oxygen from air. When O₂ levels are low, P450_{cam} catalyzes the formation of borneol by an unusual reduction, and borneol downregulates the expression of P450_{cam}. To understand the non-catalytic function of P450_{cam} and the consequences of down-regulation by borneol under low O₂ conditions, we have studied two aspects of borneol formation: 1) the properties of P450_{cam} required for formation of borneol and 2) chemotaxis of *P. putida* (strain ATCC 17453) in the presence of camphor and borneol. We found a P450_{cam} variant that does not produce borneol, and this helps us understand how the wild-type enzyme reduces camphor. We also found that camphor is a chemoattractant, which turns toxic and chemorepellent when P450_{cam} is inhibited or downregulated by borneol. This is the first report of the chemotactic behaviour of *P. putida* ATCC 17453 and the essential role of P450_{cam} in this process.

Poster A8**Miniaturized flow cell with pneumatically-actuated vertical nanoconfinement**

D. J. Berard and S. R. Leslie

Department of Physics, McGill University

Developing simple, easy-to-use single-molecule imaging technologies is critical to advancing the fields of diagnostics, pharmaceuticals, and materials processing. This work builds on a technique which we have introduced, called Convex Lens-induced Confinement (CLiC) microscopy, which is an easy-to-use single-molecule imaging method. CLiC uses a deformable glass flow cell with dynamically adjustable vertical nanoconfinement to gently trap and image single molecules within a variety of micro- and nano-structures. We demonstrate trapping of single DNA molecules in closed micropits and linearization of DNA in nanogrooves, opening new doors in pharmaceutical and genomics applications. We employ concentric circular nanogrooves to eliminate DNA drift due to confinement gradients inherent to the CLiC chamber geometry. In this work, we present a miniaturized, pneumatically-actuated CLiC flow cell with reduced confinement gradient and improved mechanical stability, while maintaining low autofluorescence and proper refractive index-matching with oil-immersion objectives. The device size has been greatly reduced to enable fabrication of hundreds of flow cells on a pair of bonded glass wafers, and to reduce the internal volume from microliters to nanoliters - an advancement which is critical to working with precious biomolecules, typically available in small quantities. We demonstrate DNA linearization in sub-50 nm nanogrooves in the miniaturized flow cell, confirming the advantages of CLiC confinement and imaging in the miniature format.

Poster A9

Arp2/3 complex-dependent spatial organization of the B cell receptor (BCR) impacts immune synapse formation, BCR signalling output and B cell activation

M. Bolger-Munro ¹, K. Choi ¹, R. Chappell ², L. Abraham ^{1, 2}, J. Scurll ², M. Dang-Lawson ¹, D. Sheen ¹, X. Wu ³, D. Coombs ², J. A. Hammer ³, M. R. Gold ¹

¹Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia

²Department of Mathematics, University of British Columbia, Vancouver, British Columbia

³Cell Biology and Physiology Centre, National Heart, Lung and Blood Institute, National Institutes for Health, Bethesda, Maryland

The immune synapse (IS) is a contact-dependent communication platform that amplifies antigen receptor signaling and initiates immune responses. A B cell IS is formed when antigens displayed on the surface of an antigen-presenting cell (APC) are recognized by the B cell receptor (BCR). This triggers dynamic, multi-scale spatial reorganization of the BCR and associated signal transduction machinery. BCRs are gathered into microclusters that are initially dispersed throughout the B cell:APC contact site but then coalesced into a central supramolecular activation cluster (cSMAC). The molecular mechanisms controlling BCR organization during IS formation are not fully understood. Importantly, how spatial patterning of the BCR promotes the translation of extracellular information (recognition of membrane-associated antigens) into functional outcomes (B cell activation) is not clear. Therefore, we tested the hypothesis that the dynamic spatial reorganization of the BCR optimizes BCR signaling and enhances B cell responses to membrane-associated antigens. By imaging the B cell:APC contact site in real time, we showed that inhibition of the actin-related protein 2/3 (Arp2/3) complex, which nucleates branched actin networks, is important for both immune synapse formation and for amplifying proximal BCR signaling reactions that are required for B cell activation. Although microcluster formation and antigen gathering were unaffected by inhibition of the Arp2/3 complex, both Arp3 siRNA and the Arp2/3 complex inhibitor CK-666 severely impaired the coalescence of BCR microclusters into a cSMAC and reduced BCR signaling output, as assessed by the phosphorylation of CD79 and Syk. We found that Arp2/3 complex activity was important for BCR signaling in response to spatially-restricted arrays of membrane-bound antigens but not for uniformly-distributed soluble antigens. Consistent with these findings, Arp2/3 complex-dependent cSMAC formation is important for APC-associated antigens to stimulate transcriptional responses, induce the expression of CD69 and CD86, and stimulate proliferation. Therefore, our data indicate that the dynamic spatial patterning of BCRs is controlled by Arp2/3 complex-dependent branched actin networks and that this is important for B cell responses to spatially-restricted antigens that are presented to B cells by APCs. Overall, our work shows that the spatial patterning of receptors can encode information that impacts cellular decision-making.

Poster A10**Non-local modeling of cellular adhesions: Applications and Theory**

A. Buttenschoen, T. Hillen, K.J. Painter, and A. Gerisch

Department of Mathematics, University of British Columbia; Department of Mathematical and Statistical Sciences, University of Alberta; Department of Mathematics, Heriot-Watt University; Fachbereich Mathematik, Technische Universitaet Darmstadt

Cellular adhesions are one of the fundamental biophysical interactions between cells and their surroundings. For instance, cellular adhesions are the main drivers of cell-sorting, and play a prominent role in the onset of metastasis (through the epithelial-mesenchymal transition). The inclusion of cellular adhesions in continuum models (i.e. partial differential equations) of biological tissues has remained a challenge. In 2006 Armstrong et. al. proposed a continuum model in the form of a non-local partial differential equation (integro-PDE). Most significantly this model successfully reproduced Steinberg's cell-sorting experiments, and since has shown popular in models of cancer invasion and morphogenesis.

The advantage of the non-local cell-cell adhesion model is that it can be rigorously analyzed using the computational and analytical tools that the mathematical community has built up over many decades. In this talk, I give an overview of the modeling successes of the non-local model of cell-cell adhesion using examples from cell-sorting, morphogenesis, and cancer cell invasion. I will outline the model's derivation from both an equation of motion of a single cell, and a stochastic position-jump process. Key to both derivations is the formulation of a continuum adhesion force. Next, I will give a flavour of the development of the mathematical theory of non-local models of cell-cell adhesion, in this part of the talk I will focus on bifurcations that lead to pattern formation. I will conclude this talk with a brief discussion on the insights that are gained by such a rigorous mathematical treatment.

(joint work with T. Hillen (University of Alberta), K.J. Painter (Heriot-Watt University), A. Gerisch (TU-Darmstadt)).

Poster A11**Characterizing the Catalytic Domain of the Deubiquitinase scOTU1 from *S. cerevisiae***

H. Cadet, J. O'Neil and M Khajehpour

Department of Chemistry, University of Manitoba

Otu1 is a member of a major subfamily of deubiquinating enzymes that have been shown to play a key role in a number of cell signalling processes including those that protect cells from apoptosis. The expression of recombinant construct of Otu1 containing only the catalytic domain (scOTU1) and lacking two of its other major domains called the Ubiquitin Like Domain "UBX" and the Zinc Binding Domain "Zn" was successfully over-expressed in *E. coli* and subsequently purified. The procedure will be presented including a general discussion of the required steps with supporting figures including SDS-PAGE, Gel Filtration and Absorbance Spectra. Previous work has shown that these deletions do not affect its enzymatic activity towards artificial substrates. The biophysical results that will be presented include the monitoring of the pH of the scOTU1 by running assays that will enable us to calculate the specificity constant and subsequently investigate the stability of the free Enzyme as it undergoes changes between various ionization states. The next steps going forward will include performing amino acid mutations in the active site of the scOTU1 and later the measurement of the enzymatic activity will be taken and overall stability of these new constructs will be tested using Differential Scanning Calorimetry, Temperature Jump Experiments, and Fluorescence Spectroscopy.

Poster A12**Beyond the beta-lactam: Structural insights into L,D-transpeptidase mediated beta-lactam resistance in Escherichia coli.**

N. A. Caveney, G. Caballero, L. J. Worrall, A. P. Niciforovic, N. C. J. Strynadka
Department of Biochemistry and Molecular Biology, University of British Columbia

Synthesis of peptidoglycan has been a crucial drug target since the introduction of β -lactam antibiotics such as penicillin. In *Escherichia coli*, this crosslinking reaction is mainly carried out by proteins with D,D-transpeptidase activity. Recently it has been shown that an alternate crosslinking mechanism can lead to bypass of PBP mediated D,D-transpeptidation in the presence of β -lactam antibiotics. The L,D-transpeptidase YcbB, with remarkably few other factors - alarmone, PBP5 and PBP1b, is able to undertake recovery of peptidoglycan crosslinking. Despite the resistance of L,D-transpeptidases to most β -lactam antibiotics, they are inhibited by carbapenem antibiotics such as meropenem. We report the structure of YcbB acylated with meropenem. YcbB is seen have a novel domain architecture consisting of a conserved L,D-transpeptidase domain, with the notable addition of a subdomain on the substrate release loop, a PG binding domain, and a large scaffolding and potential interaction domain. This architecture is unique in comparison to characterised Gram positive and *Mycobacterium* L,D-transpeptidases. In addition, we report the structures of PBP5-meropenem acyl enzyme and PBP1b-meropenem acyl enzyme and describe the molecular interactions which facilitate the inhibition of YcbB, PBP5 and PBP1b. Our results provide the first structure of a Gram negative PG L,D-transpeptidase and insight into D,D-transpeptidase bypass in *E. coli*.

Poster A13**Tuning Length-Scales of Small Domains in Cell-Derived Membranes and Synthetic Model Membranes**

C.E. Cornell, A.D. Skinkle, S. He, I. Levental, K.R. Levental, and S.L. Keller

Department of Chemistry, University of Washington, Seattle WA 98195 USA; Department of Integrative Biology and Pharmacology, The McGovern Medical School at The University of Texas Medical Center, Houston TX 77030 USA

Micron-scale, coexisting liquid-ordered (Lo) and liquid-disordered (Ld) phases are straightforward to observe in giant unilamellar vesicles (GUV) composed of ternary lipid mixtures. Experimentally, uniform membranes undergo demixing when temperature is decreased: domains subsequently nucleate, diffuse, collide, and coalesce until only one domain of each phase remains. The sizes of these two domains are limited only by the size of the system. In contrast, in membranes with excess area, small domains are expected when coarsening is hindered or when a microemulsion or modulated phase arises. Here, we test predictions of how the size, morphology, and fluorescence levels of small domains vary with the membrane's temperature, tension, and composition. Using GUVs and cell-derived giant plasma membrane vesicles (GPMVs), we find that: (1) the characteristic size of domains decreases when temperature is increased or membrane tension is decreased, (2) stripes are favored over dots for lipid compositions with low energy per unit interface, (3) fluorescence levels are consistent with domain registration across both monolayer leaflets of the bilayer, and (4) small domains form in GUVs composed of lipids both with and without ester-linked lipids. Our results are consistent with several features of current theories for microemulsions and modulated phases, with caveats.

Poster A14**Pursuing structural characterization of membrane proteins in peptidisc**

Harveer Dhupar, Michael Carlson, Franck Duong

University of British Columbia Department of Biochemistry and Molecular biology

Classical methods for reconstitution of membrane proteins in detergent-free buffer require optimization such as precise amount of scaffold and lipids, and multiple purification steps. The new membrane mimetic system - termed peptidisc - uses a short amphipathic peptide for fast and effective trapping of membrane proteins in a single step, such as density gradient centrifugation. Given its compositional homogeneity and packing density, the peptidisc may be advantageous for structural studies.

In this study, using two multi-subunit membrane-bound protein complexes: the 538 kDa photosynthetic reaction center from *Rhodobacter Sphaeroides* and the 138kDa ABC transporter MsbA. I show that the peptidisc preparations are homogeneous and amenable to structural characterization. I will present my preliminary results on x-ray crystallography and negative stain electron microscopy. Altogether, my data indicates that the peptidisc may be a valuable tool for the structural characterization of membrane proteins.

Poster A15**Translocation and interaction of PAR proteins explain oscillation and ratcheting mechanisms during *Drosophila* dorsal closure**

C.H. Durney, T.J.C. Harris, J.J. Feng

Department of Mathematics, University of British Columbia, Department of Cell & Systems Biology, University of Toronto; Department of Mathematics and Department of Chemical and Biological Engineering, University of British Columbia

We have developed a mechanochemical model that is able to recapitulate the *Drosophila* dorsal closure (DC) phenomenon. During DC, an opening on the dorsal side of the embryo is sealed in a great feat of coordination at the cellular and tissue scales of the embryo. The amnioserosa tissue, in the dorsal opening, exhibits three distinct phases of dynamic behavior: an early phase characterized by cellular oscillations, a late phase distinguished by dampening oscillations and loss of area, and a final late phase which is marked by rapid tissue contraction. Based on recent experimental observations, we couple the kinetics and transport of 3 key signaling proteins with cell mechanics to establish a delayed-negative feedback network that reproduces the three phases and provides a natural transition between them. In particular, the model explains the origin of the cell oscillation in the early phase, and that of the subsequent "ratcheting" action that allows the cells and tissue to shrink progressively over cycles of oscillation.

Poster A16**Phosphorylation-enhanced Protein Phase Separation of the Regulatory Module of a Mycobacterium tuberculosis ABC transporter**

Florian Heinkel, Libin Abraham, Gagandeep Narula, Joseph Chao, Yossef Av-Gay, Lawrence P McIntosh, Joerg Gsponer

University of British Columbia

Protein phase separation has been recently shown to be a fundamental mechanism underlying the clustering of some proteins at eukaryotic cell membranes. The virulence-linked ABC transporter Rv1747 from *Mycobacterium tuberculosis* (Mtb) possesses a cytoplasmic regulatory module containing two phosphothreonine-binding FHA (Forkhead associated) domains connected by an intrinsically disordered (ID) linker. Upon multi-site phosphorylation of the ID linker by several Mtb serine/threonine protein kinases including PknF, the isolated regulatory module readily phase separates into dynamic liquid droplets with diagnostic properties similar to those exhibited by eukaryotic proteins. The process is reversed by the sole Mtb serine/threonine phosphatase PstP. In the absence of phosphorylation, the Rv1747 regulatory module can still undergo phase separation, albeit at higher protein concentrations and with more dynamic properties of the resulting droplets. This points to a synergy between specific FHA-pThr binding and additional weak association of the ID linker and/or the FHA domains leading to the pre-requisite multivalent interactions for phase separation. Droplet formation of the regulatory module was replicated in a pseudo-two-dimensional system on a model supported lipid bilayers. Potential clusters of Rv1747 were also detected in Mtb using ultra-high resolution Direct Stochastic Optical Reconstruction Microscopy (dSTORM). This is the first reported example of phase separation by both a bacterial protein and an ABC transporter, and suggests possible mechanisms for the regulation of Rv1747.

Poster A17**Identifying unique observations in stochastic optical reconstruction microscopy (STORM) with a spatiotemporal model**

A. Herrera-Reyes, D. Coombs

Department of Mathematics and Institute of Applied Mathematics, University of British Columbia

STORM is a super-resolution technique that uses photoswitchable fluorophores to achieve resolutions at or below 20nm. A downside of STORM is the possibility of recording several blinks from one fluorophore, affecting the estimation of the number of molecules detected in the image. I constructed a mathematical model to identify unique fluorophores in STORM images by independently using the localization and the time series of the observations. The temporal sequence is described with a Markov chain approach and their spatial distribution with a Gaussian mixture model. To estimate the parameter values, I implemented a maximum likelihood procedure which requires a mixed optimization. Initially, I solved the mixed optimization problem with an extensive search algorithm on integers and a continuous optimizer for the rest of the parameters. I am currently investigating MCMC and Bayesian methods to speed up the optimization. I have tested my protocol in simulated data and I will use it to improve STORM images of B-cell surface receptors. B-cell receptors distribution on the membrane has been related to B-cell activation. This model will enhance a microscopy technique that is already widely used in biological applications and will allow to deeper analyze immune cells signaling.

Poster A18**Near-atomic cryo-EM analysis of the Salmonella T3SS needle complex reveals the molecular basis of substrate induced gating in the giant outer membrane secretin portals**

J. Hu, L.J. Worrall, C. Hong, C.E. Atkinson, M. Vuckovic, N. Caveney, Z. Yu, N.C.J. Strynadka

*Department of Biochemistry and Molecular Biology and the Center for Blood Research, University of British Columbia, Vancouver, British Columbia, Canada.**HRMEM facility, University of British Columbia, Vancouver, British Columbia, Canada**CryoEM Shared Resources, Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA, United States*

The bacterial type III secretion system, or injectisome, is a syringe shaped nanomachine essential for the virulence of many disease causing Gram-negative bacteria. At the core of the injectisome structure is the needle complex, a continuous channel formed by the highly oligomerized inner and outer membrane hollow rings and a polymerized helical needle filament which spans through and projects into the infected host cell. We present here the near-atomic resolution structure of a needle complex from the prototypical Salmonella Typhimurium SPI-1 type III secretion system, with local masking protocols allowing for model building and refinement of the major membrane spanning components of the needle complex in addition to an isolated needle filament. This work provides significant new insight into injectisome structure and assembly and importantly captures the molecular basis for substrate induced gating in the giant outer membrane secretin portal family.

Poster A19**Microfluidic Fabrication of Asymmetric Lipid Vesicles**

Tina Huang, David A. Weitz

Harvard University School of Engineering and Applied Sciences Department of Applied Physics

Lipid vesicles are aqueous volumes surrounded by a bilayer of lipid molecules, which are amphiphilic molecules with their head groups facing water and tail groups facing oil. These vesicles are simple models that mimic cell membranes and can be used for drug delivery. One interesting type of lipid vesicle is the asymmetric vesicle, in which its bilayer is composed of two dissimilar lipid monolayers. Importantly, all eukaryotic cell membranes exhibit this type of asymmetry and asymmetry is also proposed to enhance mechanical properties of the membrane. Here, we use microfluidics to fabricate mono disperse and highly controllable asymmetric lipid vesicles, which unlike the conventional methods that often end up with highly poly disperse samples. To achieve this, asymmetric vesicles are produced using water/oil1/oil2/water emulsions in a glass capillary device, with different lipids immersed in two different volatile oil phases. Using the asymmetric vesicles, we are trying to measure how mechanical properties are affected by this asymmetry and also how to improve the degree of asymmetry in our vesicles even more. In future, we envision asymmetric lipid vesicles could open a new door in the field lipid based drug delivery systems.

Poster A20**Investigating implications of Stochastic Pattern formation for chemistry of the Min System**

A. Jamieson-Lane

Department of Mathematics, University of British Columbia

The Min system is one of several systems regulating the division of E-coli cells, when working correctly it enables cells to identify their middles and divide into two equal daughter cells. The exact chemical reactions involved in this process are however unclear- at present there are multiple competing hypotheses in the literature. Recent experiments by Vecchiarelli et al. (2016) have demonstrated a wide array of dynamical patterns, including waves, spirals, and the previously un-observed "Burst" pattern formation. By examining what mathematical terms are necessary for burst patterns to occur in silico, we explore the types of chemical reaction these patterns imply must be occurring in the actual Min System.

Poster A21**Plasmid behaviour under Par system control**

Lavisha Jindal and Eldon Emberly

Simon Fraser University

The underlying mechanism of the ParA-ParB protein interaction that causes plasmid translocation and equidistant spacing between multiple plasmid foci has been widely discussed but a comprehensive study that describes how system parameters lead to either oscillatory behaviour or stable plasmid arrangements requires further explanation. In this study we provide a deterministic model that includes finite substrate size effects on protein concentrations and reaction rates to reproduce the wide range of observed plasmid behaviour. System parameters like cell length and plasmid numbers affect the organization and dynamics of plasmid foci. Since these parameters change during the cell-cycle, simulations that include cell-cycle durations and plasmid replication events are required to provide a complete explanation of observed plasmid behaviour in-vivo. We find that increasing plasmid numbers pushes a system with oscillatory plasmid behaviour toward non-oscillating trajectories while increasing nucleoid length has the opposite effect on the system. Furthermore, we outline the in-vivo conditions required to observe plasmid oscillations and find that realistically it is impossible to observe three plasmid oscillation in a E. Coli cell of regular size.

Poster A22**Optimization of Single-Molecule Assay for Proteolytic Susceptibility: Force-Induced Collagen Destabilization**

Michael W.H. Kirkness and Nancy R. Forde

Department of Molecular Biology and Biochemistry, and Department of Physics, Simon Fraser University, Burnaby, BC, Canada

Collagen has a triple helical structure, which allows for hierarchal assembly and tension resistance. Determining collagen's structure under load is a complex problem; it has been a hotly debated topic with studies finding contradictory results. We use a centrifuge force microscope to perform single-molecule measurements and find that collagen's triple helix is destabilized by an external load. We also report on our recent efforts towards a new surface chemistry based on an end-labelled block copolymer, F127 Pluronic - NHS. The surface chemistry is protein-free, reduces non-specific interactions with proteins, beads, quantum dots and peptides, is force-stable and is reproducible. The surface chemistry has a wide variety of uses in the fields of single-molecule force spectroscopy, lab-on-chip applications, and fluorescence microscopy.

Poster A23**A conserved inter-domain linker of CCT mediates allosteric communication between regulatory and catalytic domains**

Daniel Knowles, Kady Tishyadhigama, Rosemary Cornell

Department of Molecular Biology and Biochemistry, Simon Fraser University

Folding landscapes of linker segments between ligand-binding and catalytic/functional domains have evolved to facilitate transmission of inter-domain signals. We are investigating the structure/function of a conserved linker between the catalytic and membrane-binding (M) domains of the enzyme CCT, which regulates phosphatidylcholine synthesis. The structure of inactive, soluble CCT is solved, but the structure of active, membrane-bound CCT is still elusive. Activity of membrane-bound CCT is very sensitive to mutations in the linker connecting domain M to the αE helices extending from the base of the active site. MD simulations of inactive CCT revealed that dynamics of the αE helices is constrained by interactions with an auto-inhibitory (AI) helix in domain M, and that when the AI helices are displaced by membrane binding the αE helices may bend to contact the active site, bringing the catalytic domain close to the membrane surface. Tryptophan fluorescence quenching revealed that the linker lies superficially on the membrane surface. FRET analysis of distances between engineered Trps and the surface of vesicles containing Dansyl-PE support a bent αE helix, but not the straight, rigid helix conformation observed in soluble CCT. The linker may communicate membrane binding signals to enhance CCT activity by directly stabilizing a bent αE . This work is elucidating the mechanism of CCT activation by membrane binding, and more broadly, how allosteric inter-domain linkers function.

Poster A24**Probable Structures of DNA-Protein Complexes**

A.J. Koenig and Nancy R. Forde

Department of Physics, Simon Fraser University

DNA-protein complexes are dynamic, mobile systems, with much of our insight into their structures coming from the rigid views of X-ray crystallography. In this project, we develop a method for calculating probable DNA structures within a dynamic complex of DNA with the restriction enzyme EcoRV. We are interested in understanding the force-dependent suppression of DNA cleavage by EcoRV, as measured by single molecule force spectroscopy (SMFS). For this analysis, we model DNA base pairs as planks with six covariant degrees of freedom (pitch, roll, tilt, rise, shift, slide), which oscillate harmonically about their mean positions. This allows us to model the cognate DNA sequence as a multivariate Gaussian distribution with 6 variables for each distinct base pair step. From this model, we compute a force dependent probability distribution of different DNA conformations and compute this distribution's force derivative. By computer simulation, we show that the measured SMFS cleavage rates of DNA are linearly proportional to the probability that a single DNA molecule obtains a configuration which allows EcoRV to begin cleavage. Then we fit the force derivative of SMFS cleavage rates with the force derivative of the conformation probability distribution to find the most probable base pair step parameters. Our method identifies the conformation of DNA prior to hydrolysis by EcoRV and provides a new method for computing the transfer of free energy between EcoRV and DNA during cleavage. This procedure bridges the gap between DNA-protein complex dynamics and rigid X-ray crystallography, and we expect it will be widely applicable to other DNA-protein interactions.

Poster A25**Virtual histology with multimodal nonlinear imaging for quantitative analysis of vocal fold structure and injury**

K. Kolosova, A. Kazarine, H. Wang, A. Rammal, R. Tahara, K.M. Kost, N.Y.K. Li-Jessen, L. Mongeau, P.W. Wiseman

Department of Physics, McGill University; Department of Chemistry, McGill University; Department of Mechanical Engineering and School of Communication Sciences and Disorders, McGill University; Department of Otolaryngology, Head and Neck Surgery, McGill University; Redpath Museum, McGill University; Department of Otolaryngology, Head and Neck Surgery, McGill University; School of Communication Sciences and Disorders and Department of Otolaryngology, Head and Neck Surgery and Department of Biomedical Engineering, McGill University; Department of Mechanical Engineering, McGill University; Department of Physics and Department of Chemistry, McGill University

Vocal fold injury and disease can lead to tissue scarring and impairment resulting in vocal dysfunction. Scarred tissue exhibits markedly different distribution and organization of extracellular matrix components including collagen and elastin compared to healthy tissue. In histopathological analysis, these components need to be visualized to understand the scarring process and evaluate the outcomes of potential treatments such as injectable vocal fold biomaterials. We used nonlinear laser-scanning microscopy (NLSM) supported by solvent-based tissue clearing, and nano-computed tomography (nano-CT) imaging, to visualize the structure of intact vocal folds and surrounding laryngeal tissue in a rabbit model. The label-free NLSM technique uses the high second-order nonlinear susceptibility of organized collagen fibers, and two-photon autofluorescence of other tissue components including elastin fibers and blood vessels, to visualize tissue structure with high resolution in three dimensions by optical sectioning multiphoton imaging. The complementary nano-CT technique uses intrinsic density differences between tissues, enhanced with staining, to image the excised larynx. This virtual histology approach utilizes intact tissue, circumventing artifacts introduced by traditional histological slicing. Qualitative and quantitative information was acquired from these images to inform treatment progression for vocal fold scarring.

Poster A26**Synthesis and characterization of the Lawnmower: an artificial protein-based molecular motor**

C. Korosec*, D. Verardo**, M. Zuckermann*, N.R. Forde* and H. Linke**

**Department of Physics, Simon Fraser University*

***Department of Solid State Physics & NanoLund, Lund University, Sweden*

A Burnt-bridges ratchet (BBR) is a mechanism for directional molecular transport whereby the molecular machine rectifies motion through cleavage of its substrate track, thereby introducing an asymmetry that prevents backwards stepping. This mechanism is found throughout cellular systems, from MMPs that degrade collagen in the extracellular matrix to the ParA/ParB system that segregates low-copy plasmids in bacterial systems. The physical principles that dictate BBR kinetics remain unknown. We take a de novo approach towards understanding BBRs by constructing an artificial protein-based modular BBR called the Lawnmower. The Lawnmower consists of a controllable number of trypsin protease blades connected to adjustable polymer legs that connect to a single hub of adjustable size.

To understand the mechanochemical coupling and kinetics of the nano-hub lawnmower we engineered lightguiding nanowires into a bio-sensing assay. Light emitted by molecules close to the surface of these nanowires is guided to the tip due to coupling of optical modes. Nanowires coated with peptides that become fluorescent upon cleavage can then be used as sensors to assess the speed and processivity of the nano-hub Lawnmower. Successful implementation of the Lawnmower in the nanowire assay would result in the first protein-based artificial molecular motor.

Poster A27**On the Formation and Morphology of Lipid Nanoparticles Containing Ionizable Cationic Lipids and siRNA**

Jayesh A. Kulkarni¹, Maria M. Darjuan¹, Joanne E. Mercer², Sam Chen^{1,3}, Roy van der Meel^{1,4}, Jenifer L. Thewalt², Yuen Yi C. Tam^{1,3}, and Pieter R. Cullis^{1*}

1Department of Biochemistry and Molecular Biology, University of British Columbia, 2350 Health Sciences Mall, Vancouver, British Columbia, Canada, V6T 1Z3

*2Department of Physics, Simon Fraser University, 8888 University Drive
Burnaby, British Columbia, Canada, V5A 1S6*

3 Integrated Nanotherapeutics, 2350 Health Sciences Mall, Vancouver, British Columbia, Canada, V6T 1Z3

4 Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands

Lipid nanoparticles (LNPs) containing short interfering RNA (LNP-siRNA) and optimized ionizable cationic lipids are now clinically-validated systems for silencing disease-causing genes in hepatocytes following intravenous administration. However, the mechanism of formation and certain structural features of LNP-siRNA remain obscure. These systems are formed from lipid mixtures (cationic lipid, distearoylphosphatidylcholine, cholesterol, and PEG-lipid) dissolved in ethanol that is rapidly mixed with siRNA in aqueous buffer at a pH (pH 4) where the ionizable lipid is positively charged. The resulting dispersion is then dialyzed against a normal saline buffer to remove residual ethanol and raise the pH to 7.4 (above the pKa of the cationic lipid) to produce the finished LNP-siRNA systems. Here we provide cryogenic transmission electron microscopy (cryo-TEM) and X-ray evidence that the complexes formed between siRNA and ionizable lipid at pH 4 correspond to tightly packed bilayer structures with siRNA sandwiched between closely apposed monolayers. Further, it is shown that ionizable lipid not complexed to siRNA promotes formation of very small vesicular structures at pH 4 that coalesce to form larger LNP structures with amorphous electron dense cores at pH 7.4. A mechanism of formation of LNP-siRNA systems is proposed whereby siRNA is first sandwiched between closely apposed lipid monolayers at pH 4 and subsequently trapped in these structures as the pH is raised to 7.4, whereas ionizable lipid not interacting with siRNA moves from bilayer structure to adopt an amorphous oil phase located in the center of the LNP as the pH is raised. This model is discussed in terms of previous hypotheses and potential relevance to the design of LNP-siRNA systems.

Poster A28**Novel antimicrobial peptides derived from aurein 2.2 and their conjugates**

P. Kumar*, J. Kizhakkedathu, S. Straus

Centre for Blood Research, Department of Pathology and Laboratory Medicine, University of British Columbia, and Department of Chemistry, University of British Columbia

Antibiotic resistance is projected as one of the greatest threats to human health in the future with an estimated 10 million deaths per year by 2050, hence there is a great need to find alternatives. Antimicrobial peptides (AMPs) have shown great promise, because bacteria develop no or low resistance to AMPs. However, only few antimicrobial peptides are used clinically, due to problems such as toxicity, protease degradation and short half-life. This contribution describes a strategy to circumvent such challenges by conjugating the peptides to polymers to alter the toxicity, degradation profile and biodistribution in the body, without significant loss in activity. Hyperbranched polyglycerol (HPG) has gained attention due to its excellent biocompatibility, multifunctionality and plasma half life. HPGs have been used as scaffolds for the development of long circulating drug conjugates, anticoagulant neutralizing agents, for cell surface modification, making them an excellent candidate to conjugate AMPs. Results of conjugation to the natural aurein 2.2 and more active derivatives to HPG will be presented.

Poster A29**Trapping DNA with Nanofiltered-Nanopore Devices**

M.H. Lam, K. Briggs, G. Madejski, M. Magill, K. Kastritis, H.W. de Haan, J.L. McGrath, and V. Tabard-Cossa

Department of Physics, University of Ottawa; Department of Biomedical Engineering, University of Rochester; Faculty of Science, University of Ontario Institute of Technology; Department of Physics & Astronomy, University of British Columbia

We present an advanced nanopore device, which consists of an ultra-thin nanoporous membrane positioned within nanometer distances from a sensing nanopore. This dual membrane device creates a nanodevice with two pores in series, separated by a cavity which can confine DNA at the nanoscale. We have previously demonstrated how capture through the nanoporous membrane can minimize the distribution of passage times by controlling molecular conformation of DNA of varying lengths [1]. Here, we will show how the device can be operated in reverse configuration. The initial capture from the single sensing pore side can trap polymers in the cavity, acting as an entropic cage before subsequent recapture and reanalysis. We will present results describing how the probability of recapture varies after loading a single DNA molecule in the cavity as a function of polymer length. The effect of the trapping time delay at zero voltage (i.e. no electrophoretic force) before recapturing, during which the molecule could stay in the cavity or diffuse out through the nanoporous membrane, will be discussed. We will demonstrate that the number of DNA molecules that can be trapped in the cavity depends on the period of time for which voltage is applied and its strength for a given cavity size and nanoporous membrane porosity. Finally, the distribution of passage times when capturing DNA from free solution versus an entropic cage will be compared. The recapture efficiency and translocations kinetics can be manipulated by changing the porosity and distance of the nanoporous membrane from the single sensing pore. This study expands on the newly revealed capabilities offered by nanofiltered nanopore device to explore kinetics of biomolecular transport through nanopores.

Briggs et al. "DNA Translocations Through Nanopores Under Nanoscale Pre-Confinement" *Nano Letters* 18, 2, 660-668 (2018)

Poster A30**Expression, purification, and characterization of Class IB hydrophobins**

C. Kenward and D. Langelaan

Department of Biochemistry & Molecular Biology, Dalhousie University

Hydrophobins are low molecular weight (5-20 kDa) self-assembling proteins that are secreted by fungi. They accumulate at hydrophobic-hydrophilic interfaces (e.g., air-water), and undergo structural rearrangement to oligomerize as rodlets, which are an insoluble functional amyloid that coats fungal spores to act as a water repellent, facilitate spore dispersal into the air, and prevent immune recognition. Due to their unusual biochemical properties, hydrophobins are a target for commercial applications as emulsifiers, surface modification agents, and drug delivery systems. To understand sequence characteristics that determine hydrophobin properties, we are characterizing the structure and properties of diverse class IB hydrophobins from various fungal sources. One example from *Serpula lacrymans* (SlaHyd1), has only one charged amino acid. We expressed uniformly ¹³C/¹⁵N-labeled SlaHyd1 in *E. coli* and purified it to homogeneity using Ni²⁺ affinity and ion exchange chromatography. The high-resolution structure of SlaHyd1 was determined using NMR spectroscopy. SlaHyd1 contains a four strand anti-parallel β -sheet that is connected by three loop sequences (L1-L3), which is consistent with SC16, a previously characterized class IB hydrophobin. This data suggests that class IB hydrophobins form a subgroup of hydrophobins with consistent three-dimensional structure. We are currently pursuing functional studies of SlaHyd1 and comparing it to other hydrophobins, including W11 from *Wallemia ichthyophaga*, which contains >25% charged residues. These experiments will form the basis of future mutagenesis experiments to inform on the self-assembly mechanism of hydrophobins, allowing for the rational modification of their properties.

Poster A31**Using a System's Equilibrium Behavior to Reduce Its Energy Dissipation in Non-Equilibrium Processes**

S. Tafoya, S.J. Large, S. Liu, C. Bustamante and D.A. Sivak

Department of Physics, Chemistry, Molecular and Cell Biology, University of California, Berkeley; Physical Biosciences Division, Lawrence Berkeley National Laboratory; Department of Physics, Simon Fraser University; Laboratory of Nanoscale Biophysics and Biochemistry, The Rockefeller University

Cells must operate far from equilibrium, utilizing and dissipating energy continuously to maintain their organization and to avoid stasis and death. However, they must also avoid unnecessary waste of energy. Recent studies have revealed that molecular machines are extremely efficient thermodynamically when compared to their macroscopic counterparts. There are also tantalizing hints of molecular machines conserving energy while operating out of equilibrium. However, the principles governing the efficient out-of-equilibrium operation of molecular machines remain a mystery. A theoretical framework has been recently formulated in which a generalized friction coefficient quantifies the energetic efficiency in non-equilibrium processes. Moreover, it posits that to minimize energy dissipation, external control should drive the system along the reaction coordinate with a speed that is informed by the equilibrium fluctuations of the system. Here, we test and validate the predictions of this theory by probing the non-equilibrium energetic efficiency of a single DNA hairpin subjected to unfolding and refolding protocols using a dual-trap optical tweezers.

Poster A32**Increased cationic lipid content in bilayer decreased lamellar repeat spacing**

Sherry S. W. Leung, Cecilia Leal

Department of Materials Science and Engineering, University of Illinois Urbana-Champaign

Charged lipids play important biological roles, such as signalling programmed cell death and inducing membrane fusion, and are key components in nano-capsules designed for gene therapy. The repeat spacing between lipid bilayers in a lamellar phase is established by attractive van der Waals forces balanced by undulation repulsion. When bilayers are charged there is an additional repulsive electrostatic force. The conventional observation is that as the charge density of the bilayer increases the lamellar spacing increases as well. Here, we present evidence to the contrary for a complex lipid mixture that has great potential as materials with enhanced gene delivery capabilities. The ternary lipid mixture 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), monoolein (GMO), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000 (DOPE-PEG) forms multilamellar structures when cationic lipid DOTAP content is high. Using small angle X-ray scattering (SAXS), we show that lamellar repeat spacing decreases with increasing charge lipid content. This data suggests that a closer look at the balance between inter-bilayer repulsive (electrostatic and undulations) and attractive van der Waals forces is needed. Understanding the magnitude and origin of inter-membrane forces is essential to understanding the role of charged lipids in cellular functions and will impact the design principles used in engineering gene delivery vehicles.

Poster A33**Extensions of the wave-pinning model for cellular polarization and localized patterns**

Y. Liu, L. Edelstein-Keshet

Department of Mathematics, University of British Columbia

Cellular polarization is essential for a variety of cell behaviors, including chemotaxis and directed motion. Understanding the mechanism behind polarization is therefore of great interest. The wave-pinning model was proposed by Mori et al (2008) as a caricature of cellular polarization in eukaryotic cells. The model is simple enough to understand mathematically. For the types of cells of interest, polarization of regulatory proteins (GTPase) plays an important role in triggering morphological response in the cell. In this project, we explore the possible behaviors exhibited by two extensions to the wave-pinning model that include interactions of GTPase with the actin cytoskeleton. We study the extended model numerically, and explore its bifurcation properties. We also explain its biological significance in real cells.

Poster A34**ALS mutations in the TDP-43 low-complexity domain can either enhance or repress its liquid-liquid phase separation**

L. McAlary[1,2], J. Hoggarth[1], X. Peng[1], M. Sher[2], Z. Gidden[2], N. Cashman[2], S. Plotkin[1]

*[1] Department of Physics and Astronomy, University of British Columbia, Vancouver, Canada**[2] Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, Canada*

Background: TAR DNA binding protein 43 (TDP-43) contains over 40 mutations associated with amyotrophic lateral sclerosis (ALS) that are localized primarily to its c-terminal low-complexity domain (LCD). The TDP-43 LCD is responsible for the proteins ability to undergo liquid-liquid phase separation, and the concentration of mutations within suggests that aberrant LLPS dynamics may play a role in ALS pathogenesis.

Methods: Purified TDP-43 LCD (residues 267-414) had their LLPS propensities examined using increasing NaCl concentrations with both turbidimetry (OD600) and visual inspection. We chose 8 variants for further in-cell analysis using the light activated optoDroplets method developed by Shin et al (2017).

Results: The LLPS propensities of the purified TDP-LCDs were found to be highly variable dependent upon mutation. Mutants were split into three LLPS categories dependent on their difference to WT at the highest salt concentration: (i) promoting (ii) similar (iii) deficient. From these data we chose 8 variants to examine using the optoDroplet method. Our optoDroplet data shows a general trend of decreased LLPS propensity of TDP-LCD mutants in cells compared to WT with lower saturation concentration observed for most mutants.

Conclusions: Our data suggest that mutations in TDP-43 have variable effects on its LLPS propensity, calling into question the hypothesis of prolonged LLPS leading to formation of the pathological aggregates observed in disease.

Poster A35**Effect of Melatonin on Binding of Amyloid- β to Model Lipid Membranes**N. Mei ¹, S. Turnbull ¹, C. Filice ², B.Y. Lee ^{1,3} and Z. Leonenko^{1,2,3}*Department of Physics and Astronomy ¹, Department of Biology ², Waterloo Institute of Nanotechnology ³,
University of Waterloo, Waterloo, Ontario, Canada*

Alzheimer's Disease (AD) is a neurodegenerative disease that causes dementia, memory loss and cognitive decay. There is currently no known cure or preventive strategies for AD. Soluble amyloid- β (A β) oligomers are toxic to neuronal cells and act through a non-specific interaction with the neuron's cellular membrane. Our previous study indicated that, this toxic effect depends on changes in membrane's lipid compositions ¹. Melatonin is a pineal hormone produced in the brain and has been shown to be protective against A β in cellular and animal studies ³, but the molecular mechanism of this protection remains unclear. We previously reported melatonin partitions into the DPPC membrane and changes its properties ², this membrane action results in protection from amyloid binding ⁴. In this work, we used Localized Surface Plasma Resonance (LSPR), to study effect of melatonin on binding kinetics of A β to model neuronal membranes (composed of DPPC, POPC, Cholesterol, SM and GM1 ¹) that mimic healthy and AD-degraded neurons and Atomic Force Microscopy (AFM) and Kelvin Probe Force Microscopy (KPFM) to study effect of melatonin on the structure and properties of these lipid membranes. Our LSPR results demonstrate that high affinity of A β to neuronal model membranes significantly decreases in the presence of melatonin, while our AFM and KPFM results show that the presence of melatonin results in changes in structure and size of lipid domains model lipid monolayers.

Poster A36**A Rho-GTPase based model explains spontaneous collective migration of neural crest cell clusters**

Brian Merchant, Leah Edelstein-Keshet and James J. Feng

Department of Mathematics, University of British Columbia; Department of Mathematics, University of British Columbia; Department of Mathematics and Department of Chemical and Biological Engineering, University of British Columbia

Neural crest cells (NCCs) are an embryonic cell group that engage in long distance migration during embryogenesis. Surprisingly, experimental observations suggest that NCC clusters are able to migrate directionally without the presence of typical gradients such as chemoattractants. If NCC clusters are conceptually modelled as diffusive particles with short range repulsion (to model contact inhibition of locomotion) and long-range attraction (due to autocrine production of C3a/C3aR chemoattractant), then it is difficult to understand why such a cluster of particles should choose a particular direction to migrate in persistently. We propose a model to explain this directional migration in the absence of traditional symmetry breaking mechanisms such as chemoattractants. Fundamentally, the model captures the dynamics between Rac1 and RhoA known to regulate cell polarization and intercellular interactions within the neural crest. Coupling the reaction-diffusion equations for active and inactive Rac1 and RhoA on the cell membrane with a mechanical model for the overdamped motion of membrane vertices, we show that co-attraction and contact inhibition cooperate to produce persistence of polarity in a cluster of neural crest cells by suppressing the random onset of Rac1 hotspots that may mature into new protrusion fronts. Our model confirms a prior hypothesis that co-attraction and contact inhibition are key to spontaneous collective migration, and provides an explanation of their cooperative working mechanism in terms of Rho GTPase signaling. The model shows that geometric confinement and size of the cluster are key for robust directional migration.

Poster A37**Ionizable Amino Lipid's Confinement in the POPC Bilayer Interior: A Potential Obstacle for siRNA Release from Endosomes**

M. Ramezanpour, M.L. Schmidt, I. Bodnariuc, J.A. Kulkarni, P.R. Cullis, J.L. Thewalt and D.P. Tieleman

Centre for Molecular Simulation, Department of Biological Sciences, University of Calgary;

Department of Physics, Simon Fraser University;

Department of Biochemistry and Molecular Biology, University of British Columbia

Lipid nanoparticles (LNPs) based on ionizable cationic lipids are currently the leading systems for siRNA delivery in liver disease, with a major limitation of low release efficacy in the cytoplasm. Ionizable cationic lipids (e.g. DLin-KC2-DMA also known as KC2) are known to be of a critical importance in LNP structure and stability, siRNA loading, and the siRNA release stage.

In this study, we investigated the physical properties of POPC:KC2 bilayers using molecular dynamics simulation, deuterium NMR, and SAXS, and Cryo-TEM experiments.

Simulations strongly suggest that the neutral KC2 lipid is confined to the POPC bilayer hydrophobic core, a finding which was further supported by the experiments. The morphology of these confined lipids depends on both temperature and concentration. Moreover, adding a small amount of KC2 lipids to POPC bilayers induces chain order in POPC lipids, in a pH, temperature, and concentration-dependent way. These findings might explain why the KC2 lipids have more limited interactions with the negatively charged lipids of endosome than expected. Lack of lipid-lipid interactions might cause a delay in the formation of the non-lamellar phase required for drug release, resulting in a low siRNA release efficacy. Based on these findings, a new model for LNPs containing KC2 lipids was proposed. The results are of great importance in the rational design of new ionizable amino lipids and LNPs with enhanced drug release profiles for siRNA delivery.

Poster A38**Using Isothermal Titration Calorimetry (ITC) to Reassess Calcium and Magnesium Binding to Cardiac Troponin C**

K. Rayani, E. Muñoz, A.M. Spuches, F. Van Petegem, G.F. Tibbitts

Department of Biomedical Physiology and Kinesiology, Simon Fraser University; Affinimeter, Software for Science Developments; Department of Chemistry, East Carolina University; Department of Biochemistry, University of British Columbia; Department of Biomedical Physiology and Kinesiology and Cardiovascular Science, Simon Fraser University and British Columbia Children's Hospital

Cardiac Troponin C (cTnC) is the calcium (Ca²⁺) sensing component of the myofilament. Ca²⁺ or magnesium (Mg²⁺) binding to sites III and IV tethers cTnC to the rest of the troponin complex (cTn) and the thin filament (TF). With a dissociation constant (K_d) of 10⁻⁵ M, the N-terminal site II is unbound at diastolic [Ca²⁺] and subsequently bound at systolic [Ca²⁺]. This interaction acts as a switch, initiating conformational changes that culminate in myocardial force production.

ITC has been used to thermodynamically quantify the Ca²⁺-cTnC interaction (K_d = 15.2 ± 0.4 μM). Contrary to expectation, the data suggests that physiologically relevant concentrations of Mg²⁺ bind site II (K_d = 649.4 ± 25.5 μM). Both cations appear to interact with the same site as pre-incubation with Ca²⁺ significantly decreased Mg²⁺ binding and a similar effect was seen for pre-incubation with Mg²⁺ (p < 0.0001). Moreover, D67A and D73A mutations in site II lowered Ca²⁺ affinity 11-fold (K_d = 170.9 ± 41.5 μM) and Mg²⁺ affinity 1.7-fold (K_d = 1114.8 ± 227.0 μM). Studies in the absence and presence of the C-terminal domain of cTnC provided corroborative results. It must be noted that while these parameters can be compared between conditions, they may not translate in absolute terms when the cTnC is incorporated into a more complex system such as the cTn or TF.

Normally, ~95% of cellular Mg²⁺ is buffered, largely to ATP to yield a free cytosolic [Mg²⁺] of ~1 mM. Although [Mg²⁺] likely remains constant under normal physiological conditions, we posit that certain stressors such as ischemia may significantly deplete ATP, increasing [Mg²⁺] and allowing for binding to site II. Pending further studies, our findings suggest that Mg²⁺ may impact Ca²⁺ binding to site II of cTnC thus affecting regulation of heart contractility.

Poster A39**Correlating biophysical studies of amyloid- β inhibitors for treating Alzheimer's disease with neuroprotection in vitro**

Morgan Robinson 1,2, Jennifer Lou 3, Arvi Rauk 4, Michael Beazely 1, Zoya Leonenko2,3,5

1 School of Pharmacy and 2 Department of Biology and 3 Department of Physics and Astronomy, University of Waterloo; 4 Department of Chemistry, University of Calgary; 5 Waterloo Institute of Nanotechnology, University of Waterloo.

Alzheimer's disease (AD) is a devastating neurodegenerative disorder with no cures and limited treatment options. AD is characterized by dementia and the accumulation of amyloid- β ($A\beta$) plaques, the definitive molecule hallmark of AD. $A\beta$ has a strong self-affinity causing it to aggregate into neurotoxic oligomers that bind to nanoscale features of brain cell (neuron) membranes inducing membrane damage that interferes with neuron function and ultimately causes cell death. One potential strategy to prevent neurotoxicity associated with $A\beta$ is to inhibit the formation of toxic oligomers. Previously, SG pseudo-peptide aggregation inhibitors were designed and optimized for affinity to the $A\beta$ using molecular dynamics simulations in Rauk's group. In this work, we experimentally tested lead candidates using single-molecule force spectroscopy and cell viability assays. We demonstrated that all SG inhibitors prevent dimerization of $A\beta$ on a single-molecule level and shift the distribution of $A\beta$ binding forces distinctly. With MTT cell viability assays we demonstrated that several SG inhibitors can ameliorate $A\beta$ toxicity to mHT22 cells, in vitro. In comparing the results of these two studies with previously performed molecular dynamics simulations, we identify important structure-function relationships of effective peptide inhibitors. We show that SG inhibitors may be a promising preventative treatment for early stages AD and should be pursued further in pre-clinical trials.

Poster A40**StormGraph: A graph-based algorithm for quantitative clustering analysis of single molecule localization microscopy data**

J.M. Scurll 1, L. Abraham 2, D.W. Zheng 1, R. Tafteh 3, K. Chou 3, M. Gold 2 and D. Coombs 1

1 Department of Mathematics, UBC; 2 Department of Microbiology & Immunology, UBC; 3 Department of Chemistry, UBC.

The ability of cellular proteins to cluster in response to stimuli is important for the function of many cells, but uncontrolled clustering can lead to diseases such as cancer. Quantitative analysis of protein clustering in cells can therefore help to advance understanding of cellular function and provide insight into related diseases. This is possible at the nanoscale thanks to super-resolution microscopy techniques such as dSTORM, but efficient and accurate methods are needed to analyze the resulting data.

To this end, we have developed StormGraph, a graph-based clustering algorithm for the analysis of clustering in 2- or 3-dimensional super-resolution microscopy data. Using simulated data, we found that StormGraph recovers ground-truth clusters more accurately than DBSCAN and ClusterViSu, two current leading algorithms. Additionally, we have demonstrated StormGraph's use on actual dSTORM data by imaging B-cell receptors (BCRs), cell membrane proteins, on the surface of normal and cancerous B-cells, using dSTORM, and subsequently quantifying the areas of BCR clusters found by StormGraph. Our results support data reported previously by other authors using diffraction-limited microscopy. Moreover, some super-resolution microscopes provide uncertainties in the calculated positions of localized fluorophore "blinks"; unlike most existing algorithms, StormGraph can integrate this information into the clustering analysis, while remaining relatively fast.

Poster A41**Characterization of Amyloid Beta Oligomer Populations Using Size Exclusion Chromatography and Oligomer-Specific Antibody**

C.C. Shyu, E. Gibbs, N. Cashman

*Department of Medicine, University of British Columbia**Center for Brain Health, University of British Columbia*

One of the greatest challenges in the study of Alzheimer's disease (AD) is the elucidation of causative agent in disease. Evidence implicates A β oligomers (A β O) as the causative agent leading to progressive neurodegeneration in AD, particularly low molecular weight (LMW) populations, which have been shown to impair synaptic transmission and cause cell death. A β Os can also travel long distances in the brain, leading to seeding of new disease foci and inducing cytotoxic insults. Great efforts are being made in understanding the structural and functional biology of the various A β O isoforms, with the goal of developing targeted therapeutics. However, rapid aggregation of monomeric A β in solution hampers efforts to identify specific disease-causing A β O. It is thus imperative to develop a method to reliably identify and isolate individual subpopulations of A β Os. In my project, size exclusion chromatography (SEC) will be used to separate the heterogeneous mixture of A β monomers and oligomers and collect the different populations according to apparent sizes. Subsequently, surface plasmon resonance (SPR), a state-of-the-art label-free technology that can monitor protein-protein interactions in real-time, will be employed to determine the specificity of an A β O-specific monoclonal antibody (mAb), 5E3, a proprietary antibody developed in-house targeting the unique cSNK loop that is solvent-exposed only in A β Os.

Poster A42**Probing the Conformational Dynamics of the Disordered 4E-BP2 Protein in Different Phosphorylation States Using Single-Molecule Fluorescence**

Spencer Smyth 1,2 Zhenfu Zhang 1,2, Alaji Bah 3,4, Julie D. Forman-Kay 3,4 and Claudiu C. Gradinaru 1,2

1 Department of Physics, 2 Chemical and Physical Sciences, 3 Biochemistry, University of Toronto, 4 Molecular Structure and Function Program, The Hospital for Sick Children, Toronto, CANADA

Cap-dependent initiation of translation is regulated by the interaction of 120-residue eukaryotic initiation factor 4E (eIF4E) with disordered eIF4E binding proteins (4E-BPs) in a phosphorylation-dependent manner.

Fluorescence correlation spectroscopy (FCS) and fluorescence anisotropy decay (FAD) were used to study the conformations, dynamics and binding of 4E-BP2. Anisotropy data informed the local chain flexibility at various points within the 4E-BP2. The segmental flexibility is hindered in the folded 18-62 region upon phosphorylation, whereas the rest of the chain becomes more flexible. The local segments become more flexible upon denaturation, which suggests that the native protein, whereas not having a stable 3D fold, contains significant secondary structure. Segmental rotational correlation times and wobbling cone angles extracted for different labelling sites along the chain provide a rigidity map of 4E-BP2 and are essential for elucidating the binding mode to eIF4E.

Longer range intra-chain reorganization dynamics were assessed by FCS via photoblinking/quenching of the fluorophore by aromatic residues. Heterogeneous quenching kinetics were observed: multi-site phosphorylation of the protein slows the proximal chain motions and modulates the kinetics of the distal regions. Our results paint a complex behavior of 4E-BP2 upon phosphorylation and binding and suggest that electrostatics play a crucial role in modulating its dimensions and compactness and thus its activity.

Poster A43**Quinolinic acid amyloid-like fibrillar assemblies seed α -synuclein aggregation**

O. Tavassoly (1†), D. Sade (2†), S. Bera (2), D. Vocadlo (1,3) and E. Gazit (2,4,5*)

*1-Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, V5A 1S6 Canada**2-Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv 6997801, Israel**3-Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, V5A 1S6, Canada**4-Sagol Interdisciplinary School of Neurosciences, Tel Aviv University, Tel Aviv, 6997801 Israel**5-Blavatnik Center for Drug Discovery, Tel Aviv University, Tel Aviv, 6997801, Israel*

† First co-authors

*To whom correspondence should be addressed: ehudg@post.tau.ac.il

Quinolinic acid (QA), a downstream neurometabolite in the kynurenine pathway, the biosynthetic pathway of tryptophan, is associated with neurodegenerative diseases pathology. Mutations in genes encoding kynurenine pathway enzymes, that control the level of QA production, are linked with elevated risk of developing Parkinson's disease (PD). Recent findings have revealed the accumulation and deposition of QA in post-mortem samples, as well as in cellular models of Alzheimer's disease (AD) and related disorders. Furthermore, intrastriatal inoculation of mice with QA results in increased levels of phosphorylated α -synuclein and neurodegenerative pathological and behavioral characteristics. However, the cellular and molecular mechanisms underlying the involvement of QA accumulation in protein aggregation and neurodegeneration remain elusive. We recently established that self-assembled ordered structures are formed by various metabolites and hypothesized that these "metabolite amyloids" may seed amyloidogenic proteins. Here we demonstrate the formation of QA amyloid-like fibrillar assemblies and seeding of α -synuclein aggregation by these nanostructures both in vitro and in cell-culture. Notably, α -synuclein aggregation kinetics was accelerated by an order of magnitude. Additional amyloid-like properties of QA assemblies were demonstrated using ThT assay, powder X-ray diffraction and cell apoptosis analysis. Moreover, fluorescently-labelled QA assemblies were internalized by neuronal cells and co-localized with α -synuclein aggregates. Additionally, we observed cell-to-cell propagation of fluorescently-labelled QA assemblies in a co-culture of treated and untreated cells. Our findings suggest that excess QA levels, for example due to mutations in the kynurenine pathway, may lead to metabolite assemblies formation that seed α -synuclein aggregation, resulting in neuronal toxicity and induction of PD.

Poster A44**SbnI is a free serine kinase that generates O-phospho-L-serine for staphyloferrin B biosynthesis in *Staphylococcus aureus***

M.M. Verstraete 1, C. Perez-Borrajero 2, K.L. Brown 1, D.E. Heinrichs 3, and M.E.P. Murphy 1

1 Department of Microbiology and Immunology, 2 Genome Sciences and Technology Program Life Sciences Institute, University of British Columbia, Vancouver, BC, V6T 1Z3, Canada; 3 Department of Microbiology and Immunology, University of Western Ontario, London, ON, N6A 5C1, Canada

Staphyloferrin B (SB) is an iron-chelating siderophore produced by *Staphylococcus aureus*. Proteins for SB biosynthesis are encoded by SbnABCDEFGHI, in which SbnI acts as a heme-dependent transcriptional regulator of the Sbn locus. Here, a crystal structure of SbnI revealed striking structural homology to an ADP-dependent free serine kinase, SerK, from the archaea *Thermococcus kodakarensis*. We found that the active site architecture is conserved, and biochemical assays, and 31P NMR and HPLC analyses indicated that SbnI is also a free serine kinase but uses ATP rather than ADP to generate O-phospho-L-serine (OPS), which serves as the substrate for SbnA and is a precursor for SB biosynthesis. SbnI consists of two domains, and elevated B-factors in domain II were consistent with the open-close reaction mechanism previously reported for SerK. Mutagenesis of Glu20 and Asp58 in SbnI revealed that they are required for kinase activity. The only known OPS source in bacteria is from the serine biosynthesis pathway through the phosphoserine aminotransferase activity of SerC and we demonstrate that an *S. aureus* SerC mutant is a serine auxotroph, consistent with a function in serine biosynthesis. However, the SerC mutant strain could produce SB when provided L-serine, suggesting that SbnI produces OPS for SB biosynthesis in vivo. These findings indicate that in addition to transcriptionally regulating the Sbn locus, SbnI also has an enzymatic role in the SB biosynthetic pathway.

Poster A45**The Peptidisc: a Simple Approach for Stabilizing Membrane Proteins in Detergent-free Solution**

M.L. Carlson, J.W. Young, Z. Zhao, L. Fabre, D. Jun, J. Li, J. Li, H.S. Dhupar, I. Wason, A.T. Mills, J.T. Beatty, J.S. Klassen, I. Rouiller, F. Duong

Department of Biochemistry and Molecular Biology, Faculty of Medicine, Life Sciences Institute, University of British Columbia; Department of Anatomy and Cell Biology, McGill University, Strathcona Anatomy and Dentistry Building; Department of Microbiology and Immunology, University of British Columbia; Glycomics Centre and Department of Chemistry, University of Alberta

Functional and structural studies of purified membrane proteins can be limited by the use of detergents. To circumvent the problem, researchers replace detergent micelles for amphipathic scaffolds designed to recreate the membrane environment. The reconstitution can be laborious however, with limitations such as cost, effectiveness, unmatched scaffold dimension and need for specific amount of lipids. The peptidisc system we present is straightforward, a “one step fits all” method to capture of membrane proteins into functional, heat-stable, water-soluble particles. Addition of lipids or engineering of the scaffold is not necessary. The peptide is simple to produce and its flexibility allows to capture proteins of various fold and architecture. The reconstitution can be embedded within the membrane protein purification protocol. We demonstrate its effectiveness of the method using 5 different membrane protein assemblies using “on-column”, “in-gel”, and “on-bead” reconstitution protocols.

Poster A46**Molecular asymmetries establish tissue boundaries during *Drosophila* axis elongation**

J.C. Yu 1,2 and R. Fernandez-Gonzalez 1,2,3,4

1 Institute of Biomaterials and Biomedical Engineering, University of Toronto; 2 Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research; 3 Cell and Systems Biology, University of Toronto; 4 Developmental and Stem Cell Biology Program, Hospital for Sick Kids

Animals establish the head-to-tail body axis in a process known as axis elongation, driven by directional cell rearrangements and oriented cell divisions. We showed that during *Drosophila* axis elongation, mesectoderm cells, a group of glial precursors, divide parallel to the head-to-tail axis to reduce strain generated by cell rearrangements, facilitating axis elongation. Using spinning disk confocal microscopy and image analysis, we found that the polarity factor Par-3 localized to new junctions between daughter mesectoderm cells, thus becoming planar polarized. The molecular motor non-muscle myosin II and its upstream activator Rho-kinase were also asymmetrically distributed after mesectoderm divisions, forming supracellular cables parallel to the head-to-tail axis on either side of the tissue, thus establishing a boundary between the ectoderm and mesectoderm. The polarization of both myosin and Par-3 was lost when embryos were treated with Y-27632, a Rho-kinase inhibitor. Loss of the myosin cables resulted in invasion of the mesectoderm by ectoderm cells, and the premature internalization of the mesectoderm. Our results suggest that redistribution of Rho-kinase and Par-3 after oriented cell division organize mesectoderm cell polarity to establish tissue boundaries. We are investigating the interdependency of myosin and Par-3 polarity in the mesectoderm, and the impact on junctional stability, tissue mechanics, and architecture when either polarity is impaired.

Poster A47**Differential mechanisms of bacterial adhesion in response to substrate stiffness**

A. Zayadi, R. Hengge, R.M.A. Sullan

*Department of Chemistry, University of Toronto; Department of Microbiology, Humboldt University of Berlin;
Department of Chemistry, University of Toronto*

Biofilms confer bacterial resistance to antimicrobial agents and host defenses and, once mature, are difficult to eradicate making prevention of biofilm formation the optimal mitigation strategy. Biofilms begin with bacterial adhesion to a substrate, mediated by sticky cell surface appendages including flagella and type 1 fimbriae. Recent evidence indicates bacteria can use surface appendages to sense and respond to the properties of an underlying substrate, including its stiffness. Understanding the molecular mechanisms of stiffness-dependent adhesion is an important part of informed materials design and selection for surfaces vulnerable to bacterial colonization. In this work, the extent of adhesion by isogenic *Escherichia coli* mutants, lacking either flagella or type 1 fimbriae, is shown to differentially depend on the stiffness of the underlying substrate.

Poster A48**Structural characterization of feeding-tube channel components essential to spore formation in bacteria**N. Zeytuni ¹, C. Hong ², K.A. Flanagan ³, L.J. Worrall ¹, M. Vuckovic ¹, A.H. Camp ³, Z. Yu ²,
N.C.J. Strynadka ^{1*}*¹ Department of Biochemistry and Molecular Biology and the Center for Blood Research, University of British Columbia, Vancouver, British Columbia, Canada.**² CryoEM Shared Resources, Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, Virginia, United States.**³ Department of Biological Sciences, Mount Holyoke College, South Hadley, Massachusetts, USA.*

Poster B1**Sequence modification through charge addition improves the cyclic peptide permeability in LUVs of *S. aureus* phospholipid composition**

SC Barbosa, TM Nobre, EM Cilli, ON Oliveira Jr

Instituto de Física de São Carlos, IFSC-USP, 13560-970, São Carlos-SP, Brazil; Department of Natural Sciences, Mid Sweden University, SE-851 70 Sundsvall, Sweden.

Antimicrobial resistance has reached alarming levels in many countries, and to overcome it is the next great global challenge. Antimicrobial peptides (AMPs) are considered a novel class of antibiotics since their mode of action can be efficient against super-resistant bacteria. Recently, we investigated the molecular-level mechanism of action of Labaditin (Lo), a 10-amino acid residue cyclic peptide (VWTVWGTIAG) from *Jatropha multifida* with known bactericidal activity against *Staphylococcus aureus*, a Gram-positive bacterium¹. A comparison was made with its linear counterpart (L1), which did not show activity. It was then shown that the conformational restriction of the cyclic structure is essential for the Labaditin antimicrobial activity. As Labaditin differs from the class of AMPs since it is highly hydrophobic and has no charge, two new cyclic peptides were designed through a single lysine addition in each Labaditin: AC1 (VWTVWGTKAG) and AC2 (KWTWGTIAG). In this work, we analyzed the affinity of these cyclic charged peptides to large unilamellar vesicles (LUVs) composed of *S. aureus* membrane phospholipid. Fluorescent dyes encapsulated in LUVs were used as a probe to identify the peptide ability to induce cell leakage. Both peptides induced vesicle leakage in the LUVs. From circular dichroism experiments, no modification of the secondary structure of these modified peptides was noted upon interacting with the LUVs. This appears to contribute to preserving the peptide activity, although differences were identified among them (Lo, AC1 and AC2). The charge position affected the peptide action, with AC2 where the charge is at the terminal position being much stronger and forming pores. Therefore, a cationic substitution position does affect peptide activity, which depends on the position of the charge in the peptide sequence, even in a cyclic peptide.

1. Barbosa, S. C.; Nobre, T. M.; Volpati, D.; Ciancaglini, P.; Cilli, E. M.; Lorenzon, E. N.; Oliveira, O. N., Jr., The importance of cyclic structure for Labaditin on its antimicrobial activity against *Staphylococcus aureus*. *Colloids Surf B Biointerfaces* 2016, 148, 453-459.

Poster B2**Mathematical modeling of oral Epstein-Barr virus shedding**Byrne C^{1,2,3}, Coombs D^{3,4}, Schiffer JT^{6,7}, Corey L^{6,7}, Casper C⁵, Johnston C^{6,7}, Gantt S^{1,2}

1 Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC; 2 BC Children's Hospital Research Institute, University of British Columbia, Vancouver, BC; 3 Institute of Applied Mathematics, University of British Columbia, Vancouver, BC; 4 Department of Mathematics, University of British Columbia, Vancouver, BC; 5 Infectious Disease Research Institute, Seattle, WA; 6 Department of Medicine, University of Washington, Seattle, WA; and 7 Fred Hutchinson Cancer Research Center, Seattle, WA

EBV infects >90% of humans and is a major cause of malignancy worldwide. EBV establishes latent infection in B cells, with intermittent reactivation in tonsils and oral epithelium. Transmission results from viral shedding in saliva, which occurs more frequently and at higher EBV viral loads in HIV-1-co-infected people. We hypothesize that increased shedding with HIV-1 co-infection may be due to greater reactivation of EBV-infected B cells and/or impaired immune control of EBV replication. To address this hypothesis, we constructed a stochastic, mechanistic mathematical model to describe chronic oral EBV shedding. We fit this model to daily quantitative oral EBV shedding data from a cohort of 42 HIV-1-infected and 42 HIV-1 uninfected individuals who self-collected oral swabs for 28 days. Our model predicts that frequent shedding and higher EBV viral loads in saliva is due to increased reactivation of EBV-infected B cells, rather than impaired EBV-specific immunity. Increased B cell reactivation created larger plaques that were often not cleared by the immune response, resulting in stable, high viral loads. These results explain the dynamics of oral EBV infection and explain increased shedding by HIV-1-co-infected individuals as due to higher levels of B cell activation. This reveals B cell reactivation as a potential therapeutic target to minimize the likelihood of patients developing EBV associated malignancies.

Poster B3**Two-state Diffusion Analysis with Measurement Errors**

R. C. Falcao, D. Coombs

Department of Mathematics and Institute of Applied Mathematics, University of British Columbia

Single particle tracking is a powerful tool to study the mobility of molecules in the cell membrane. The most common approaches in analyzing these kind of data are mean square displacement and analyses with one or more hidden Markov states. However, in most experiments, positional measurements contain systematic and random errors, and to achieve proper fits, we must take these errors into account. In this work, we develop a hidden Markov model with two diffusive states. Our goal is to estimate the diffusion coefficients and transition probabilities between the different states, incorporating uncertainty due to measurement error in a rational way. We test our methods using simulated data.

Poster B4**Examining the Nanosecond-to-Millisecond Dynamics of Sic1 by Fluorescence Techniques**

J.D.M. Castroverde, T.C. Lee, G.W. Gomes, J.D. Forman-Kay and C.C. Gradinaru

Department of Physics and Department of Chemical and Physical Sciences, University of Toronto; Department of Chemical and Physical Sciences, University of Toronto; Department of Physics and Department of Chemical and Physical Sciences, University of Toronto; Molecular Structure and Function Program, Hospital for Sick Children and Department of Biochemistry, University of Toronto; Department of Physics and Department of Chemical and Physical Sciences, University of Toronto

As part of the cell cycle of the budding yeast *Saccharomyces cerevisiae*, the cyclin-dependent kinase inhibitor Sic1 is degraded by Cdc4 of the SCF complex upon the phosphorylation of Sic1 on at least six sites (known as Cdc4 phosphodegrons, or CPDs). More specifically, the binding affinity of Sic1 to the WD40 binding domain of Cdc4 increases as the number of phosphorylated sites increases, especially after the sixth site. Experimental work is necessary to study the structural dynamics of Sic1 as it undergoes phosphorylation and binding.

We used fluorescence techniques to study the dynamics of the disordered N-terminal targeting tail of Sic1 (residues 1 to 90), phosphorylated Sic1, and the Sic1-WD40 dynamic complex. Using a combination of fluorescence correlation spectroscopy and time-resolved fluorescence anisotropy, we measure the chain dynamics of Sic1 in the nanosecond-to-millisecond timescales. Additionally, we perform these experiments on six different single cysteine Sic1 mutants to probe the segmental dynamics along the regions near the CPDs. Data from these initial fluorescence experiments would form a basis for the interpretation of subsequent single-molecule data to elucidate the physical basis for the ultrasensitive phosphorylation-dependent binding of Sic1 to Cdc4.

Poster B5**Phase Diagrams of Ternary Model Membrane Systems with Phosphatidylethanolamine (PE) Lipids**

C. Chang, C.E. Cornell and S.L. Keller

Department of Chemistry, University of Washington

Many membranes spontaneously demix into coexisting liquid phases. These include membranes that are as simple as three-component lipid vesicles and as complex as protein-rich vacuole membranes within living yeast cells. The resulting membrane phases are enriched in particular lipids and proteins. Membrane phase separation is one of several mechanisms proposed to explain the observation of heterogeneous distributions of lipids and proteins in cell membranes ("rafts"), which are thought to be important in cell signaling, apoptosis, and protein regulation. The overwhelming majority of experiments that probe phase separation in membranes uses only one type of lipid (namely phosphatidylcholine). However, it is known that biological membranes also contain significant fractions of other kinds of lipids (e.g. phosphatidylethanolamine, or PE-lipids). An experimental challenge is that membranes composed of high fractions of PE-lipids are unstable - the lipids assemble in tubes instead of lying in sheets. In my project, I am [1] determining the maximum amount of saturated and unsaturated PE-lipids that can be incorporated into stable membranes, [2] measuring the temperature at which the membranes demix into coexisting phases, and [3] imaging the vesicles by fluorescence microscopy. Results from Aim 1 will inform future researchers which lipid ratios they can use to produce stable membranes. Results from Aim 2 will provide data against which the scientific community can calibrate molecular dynamics simulations. Results from Aim 3 will provide a direct test of a recent prediction that PE-lipids should be anti-registered across fluctuating membranes.

Poster B6**Amino acids bind to and influence the structure of fatty acid vesicles: A mechanism for the co-evolution of membranes and polymers**

Z.R. Cohen, A. Ramsay, C. E. Cornell, S. L. Keller, R.A. Black

Dept. of Chemistry, University of Washington

The essential structures for cellular life are the two polymers, nucleic acid (e.g. RNA) and protein, and a surrounding membrane composed of amphiphilic molecules. We propose that these three structures evolved from a self-assembled aggregate of their building blocks: nucleobases, sugars, amino acids, and simple amphiphiles. We present four lines of evidence that amino acids interact with decanoic acid membranes. (1) Both the concentration-dependence and the duration of the effect on light scattering indicate that serine, in particular, interacts potently with decanoic vesicles. (2) Based on fluorescence microscopy, serine's effect on light scattering is due to the formation of vesicles with extensive internal structure. (3) Serine raises the pH at which decanoic acid transitions from micelles to vesicles, i.e. it facilitates vesicle formation. (4) Several amino acids bind to decanoic acid vesicles, based on an assay in which the vesicles - and compounds bound to them - are retained in the upper chamber of a centrifugal filtration device. Overall, our four new lines of evidence support the hypothesis that like the building blocks of RNA, amino acids spontaneously bind to and influence the structure of fatty acid membranes. This scheme provides an explanation for the prebiotic selection, concentration and co-localization of the essential components of cellular life.

Poster B7**Structure and function of an Ntn-hydrolase from *Staphylococcus aureus***

B.S. Conroy, A.L. Arrieta, A.C.K. Chan and M.E.P. Murphy

Department of Microbiology and Immunology, University of British Columbia

Staphylococcus aureus is a commensal on human skin and in the upper respiratory tract of mammals and is the cause of severe illnesses, including soft tissue infections, pneumonia, and endocarditis. We identified an Ntn-hydrolase encoded in the *S. aureus* genome that is annotated as a member of the CG-hydrolase family. This family is composed of the bile acid hydrolases and the penicillin V acylases. These classes of enzymes both cleave C-N bonds in linear amides, but act on chemically distinct substrates. Bile acid hydrolases are important in the modification of bile acids in the gut, while the physiological role of penicillin V acylases is not clear. The protein was produced in *E. coli*, purified to homogeneity, and crystallized. Diffraction data were collected at the Canadian Light Source and the structure was solved by molecular replacement using the penicillin V acylase from *Bacillus subtilis* (PDB entry 2oqc) as the search model. The Ntn-hydrolase is a tetramer with an N-terminal cysteine residue that acts as the probable nucleophile in the active site. Preliminary assays indicated that the hydrolase removes the acyl chain from penicillin V; however, the enzyme is predicted to be localized in the cytoplasm. The biological function of the *S. aureus* Ntn-hydrolase is under continued investigation.

Poster B8**Versatile Tools Towards Real-time Single-molecule Biology**

R.P.C. Driessen, J. Cabanas-Danés, A. Ramaiya, P. Rauch and A. Candelli

LUMICKS, Amsterdam, The Netherlands

Biological processes performed by proteins interacting with and processing DNA and RNA are key to cell metabolism and life. Detailed insights into these processes provide essential information for understanding the molecular basis of life and the pathological conditions that develop when such processes go awry.

The next scientific breakthrough consists in the actual, direct, real-time observations and measurements of the individual mechanisms involved, in order to validate and complete the current biological models.

Single-molecule technologies offer an exciting opportunity to meet these challenges and to study protein function and activity in real-time and at the single-molecule level.

Here, we present our efforts for further enabling discoveries in the field of biology and biophysics using both the combination of optical tweezers with single-molecule fluorescence microscopy (C Trap).

We show the latest applications of these technologies that can enhance our understanding not only in the field of DNA/RNA-protein interactions but also in the fields of molecular motors, protein folding/unfolding, cell membranes and genome structure and organization.

These experiments show that the technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new venues in many research areas.

Poster B9**Binding Pose Prediction Using Coarse Grained Molecular Dynamics**

Pranav Garg, Mahdi Ghelichi, Sabrina Semmler, Christine Vande Velde, Steven Plotkin

Department of Physics and Astronomy, University of British Columbia; Department of Neurosciences, Université de Montréal

We present a method to predict the binding pose between two protein chains and identify residues mediating the interaction. The system consists of two spatially separated protein chains enclosed in a sphere modeled using the AWSEM-MD coarse-graining scheme. Several randomly oriented copies of this system are allowed to run. Frames from these trajectories are collected and preferred orientations are identified using HDBSCAN clustering on the coordinates. Concurrently, the biased simulation method, metadynamics, is used to calculate the potential of the mean force between the chains as a function of two polar angles on the surface of each chain. This highlights the residues on each chain important for the interaction. Further checks are done by studying the effect of mutations on these results.

The method is being used as part of a project examining interactions between the proteins SOD1 and TRAF6 in the context of the disease ALS. We provide computational justification for results from biochemical assays conducted by our collaborators and suggest mutational tests to verify our predictions.

Poster B10**Wanted: Small molecule inhibitors of ETV6 PNT domain polymerization**

C. A. N. Gerak, M. Okon, R. B. Sessions, M. Roberge and L. P. McIntosh

Department of Biochemistry and Molecular Biology, University of British Columbia; Department of Biochemistry, University of Bristol

ETV6 is a modular transcriptional repressor for which head-to-tail self-association of its PNT domain facilitates cooperative binding to tandem DNA sites by its ETS domain. Chromosomal translocations that fuse the PNT domain to the catalytic domains of several protein tyrosine kinases create chimeric oncoproteins. These fusion proteins undergo ligand-independent autophosphorylation, and aberrantly stimulate downstream signaling pathways leading to a variety of cancers. Accordingly, our research goal is to identify and characterize small molecule inhibitors of ETV6 PNT domain self-association to prevent the constitutive activation of these oncoproteins. Protein-protein interactions are challenging to disrupt therapeutically, and thus we are following a multi-pronged approach for lead compound discovery. In particular, we are conducting alanine scanning mutagenesis and amide hydrogen exchange (HX) experiments to obtain structural, dynamic and thermodynamic insights of the self-association interface to guide rational inhibitor design. To complement these experimental approaches, we have also used in silico screening to identify potential inhibitors.

Poster B11**Optical Mapping of Human Induced Pluripotent Stem Cell-derived Cardiac Tissues as a Platform for in vitro Drug Testing**

Marvin Gunawan, Sarabjit Sangha, Sanam Shafaattalab, Eric Lin, Zachary Laksman and Glen F. Tibbits

Department of Biomedical Physiology and Kinesiology, Simon Fraser University; Faculty of Medicine, University of British Columbia; Children Hospital Research Institute

Preclinical drug screening of cardiac compounds is hampered by limited experimental models that do not fully recapitulate human cardiac physiology. As such, human induced pluripotent stem cell (hiPSC)- derived cardiac tissue has emerged as a promising approach in characterizing pharmacological effects in vitro. Furthermore, the ability to distinguish drug effects between atrial and ventricular sub-types add a tremendous value to future treatment of atrial fibrillation and ventricular arrhythmias. Here, we present a custom-built medium-throughput optical mapping to interrogate the voltage and calcium dynamics of hiPSC-derived cardiomyocytes (hiSPC-CMs) cultured in monolayer format as a physiologically relevant tissue structure. First, atrial-like and ventricular-like cardiomyocyte monolayers are generated from hiPSCs using two distinct differentiation protocols by modulation of the retinoic pathway. We then investigated the cardiac subtype specific pharmacology using atrial-selective compounds AVE0118 and UCL1684 to dissect the ultra rapid (I_{Kur}) and calcium activated (I_{SK}) potassium currents, respectively. Additionally, the hiPSC-CMs are electrically paced to investigate rate dependent properties of drug response. Overall, optical mapping coupled with hiPSC differentiation techniques offers a powerful tool for a more selective and suitable method to develop cardiac active compounds in vitro.

Poster B12**Talin autoinhibition regulates cell-ECM adhesion dynamics and wound healing in vivo**

Amanda Haage 1, Katharine Goodwin 1, Austin Whitewood 2, Darius Camp 1, Aaron Bogutz 3, Christopher T. Turner 4, David J. Granville 4, Louis Lefebvre 3, Sergey Plotnikov 5, Benjamin T. Goult 2, Guy Tanentzapf 1*

Department of Cellular and Physiological Sciences, 2350 Health Sciences Mall, University of British Columbia, Vancouver, Canada

School of Biosciences, Canterbury, University of Kent, Kent, UK

Department of Medical Genetics, 2350 Health Sciences Mall, University of British Columbia, Vancouver, Canada

Department of Pathology and Laboratory Medicine, 2211 Wesbrook Mall, Vancouver V6T 2B5, BC, Canada.

Department of Cell and Systems Biology, 25 Harbord Street, University of Toronto, Toronto, Canada

Cells in multicellular organisms are arranged in complex three-dimensional patterns. This requires both transient and stable adhesions with the ExtraCellular Matrix (ECM). Integrin adhesion receptors bind ECM ligands outside the cell and then, by binding the protein talin inside the cell, assemble an adhesion complex connecting to the cytoskeleton. The activity of talin is controlled by several mechanisms, but these have not been well studied in vivo. By generating mice containing the activating point mutation E1770A in talin (Tln1), which disrupts autoinhibition, we show that talin autoinhibition controls cell-ECM adhesion, cell migration, and wound healing in vivo. In particular, blocking autoinhibition gives rise to more mature, stable focal adhesions that exhibit increased integrin activation. Consequently, cells show stronger binding to the ECM and decreased traction force. Overall, these results demonstrate that modulating talin function via autoinhibition is an important mechanism for regulating multiple aspects of integrin-mediated cell-ECM adhesion in vivo.

Poster B13**Towards detergent-free purification of the P4-ATPase phospholipid transporters**

W.J. Jennings, B. M. Xavier and J.Y. Lee

Department of Biochemistry, Microbiology and Immunology, University of Ottawa

Biological membranes are tremendously diverse structures comprised of hundreds of different lipid species. Membranes are organized as bilayers consisting of two leaflets with a distinct asymmetric distribution of lipid molecules. The cytosolic leaflet is heavily enriched with phosphatidylserine and phosphatidylethanolamine, whereas the extracellular leaflet is almost devoid of these lipids. Maintaining lipid bilayer asymmetry is critical for regulating membrane curvature, a prerequisite for membrane trafficking events such as endocytosis and vesicle-mediated transport in the secretory pathway. Alternatively, controlled disruption of membrane asymmetry initiates critical processes such as blood coagulation, apoptosis, cytokinesis and cell fusion. Lipid asymmetry is generated by members of the type IV P-type ATPase (P4-ATPase) family of phospholipid flippases that utilize ATP as an energy source to unidirectionally transport lipids to the designated leaflet. Unfortunately, little is understood at the molecular level regarding lipid substrate recognition and ATPase driven phospholipid transport by P4-ATPases. To study the mechanism of P4-ATPase mediated phospholipid transport, we overexpressed recombinant *Saccharomyces cerevisiae* P4-ATPase Drs2p/CDC50 by exploiting a *Pichia Pastoris* system. Using the novel technique of styrene maleic acid copolymer lipid particles (SMALP), we are establishing a protocol for the detergent-free and large-scale purification of yeast P4-ATPase complexes from microsomal preparations. Our future directions include 1) functional characterization of the purified P4-ATPase in its membrane mimetic environment and 2) structural determination of P4-ATPase using X-ray crystallography and single-particle cryo-electron microscopy. Ultimately, this work will facilitate an understanding of the biophysical and biochemical basis of lipid transporter function and establish the molecular basis underpinning the consequences of transporter inactivation on cell function and survival.

Poster B14**Confinement-assisted DNA Translocation Through a Nanopore**

A. Kamanzi, D. Berard, R. Tahvildari, Z. Zhang, V. Tabard-Cossa, S. R. Leslie

Department of Physics, McGill University; Department of Physics, University of Ottawa

We explore new ways to dynamically squeeze and thread single DNA macromolecules through a nanopore, with new degrees of control. Threading a biomolecule from free solution through a nanoscale hole is challenging due to the changes in entropy that the molecule experiences while transitioning between several orders of magnitude of confinement. Our approach is to use the Convex Lens-induced Confinement (CLiC) technique (Leslie et al, *Anal Chem.* 2010; Berard et al, *APL* 2016) to controllably load, squeeze, and guide DNA into custom, nano-confined regions. This technique allows us to directly visualize dynamic changes in the structure of DNA macromolecules under applied confinement as they transition from “bundled” to “rod-like” states, and ultimately while they translocate through pores. By combining two novel technologies - CLiC visualization and manipulation tools with Controlled Breakdown nanopore fabrication (Briggs, K. et al, *Small* 2014) - we can regulate the DNA conformations as they enter the nanopores; for instance, by suppressing folded conformations. Our approach will allow for simplified interpretation of current blockage signals, and ultimately reduce the anticipated spread in nanopore transit times compared to other methods. Here, we present steps towards the first prototype device of this kind, and related microscopy data to support its performance.

Poster B15**hERG K⁺ channel activator compounds enhance repolarizing cardio-protective currents.**

J.M. Kemp, R. Johal, Z.K. Pang, Y.M. Cheng, and T. Claydon

Department of Biomedical Physiology and Kinesiology, Simon Fraser University

hERG K⁺ channels play a key role in cardiac action potential repolarization. hERG channel loss-of-function due to inherited mutations or high affinity drug block result in delayed repolarization and Long QT Syndrome (LQTS2), which predisposes cardiac arrhythmia. As such, there is significant interest in compounds that activate hERG channels. One compound, RPR260243, reduces hERG deactivation gating kinetics, which would be expected to enhance repolarizing hERG current. Surprisingly, RPR260243 has minimal influence on cardiac repolarization unless repolarization reserve is depleted. Here, we have studied the effects of activator compounds on hERG channel availability early in the action potential refractory period, which has been suggested to protect against premature membrane excitation. We demonstrate that hERG protective current in response to premature stimulations in early refractory is significantly reduced by the LQTS2 mutation, R56Q, which accelerates channel deactivation. Application of RPR260243 slowed deactivation kinetics and increased the protective current in R56Q mutant hERG channels, effectively rescuing the R56Q loss-of-function. These data show that LQTS2-causing mutations that accelerate deactivation kinetics impair hERG protective current in early refractory and that activator compounds, which slow deactivation, can restore the protective current. These findings suggest alternative therapeutic potential for hERG activator compounds.

Poster B16**Structural determinants of substrate specificity in *Staphylococcus aureus* siderophore synthesis.**

M. Kolesnikov, J.C. Grigg, A.L. Arrieta, M. Ikehata, M.E.P. Murphy

Department of Microbiology and Immunology, University of British Columbia

Staphylococcus aureus is a gram-positive opportunistic pathogen, found as a commensal on human skin and in the upper respiratory tract. *S. aureus* is the causative agent of illnesses of varying severity including pneumonia, endocarditis and toxic shock syndrome. Successful infection requires the acquisition of iron from the host. This is mediated by several iron-uptake systems including two siderophores: Staphyloferrin A (SA) and Staphyloferrin B (SB). These small, iron-chelating molecules are secreted into the environment and taken up after binding iron. Using a combined structural and biochemical approach we determined substrate specificities of several enzymes in SA and SB synthesis located on the Sfa and Sbn loci respectively. High resolution crystal structures of wild-type and active site mutants of the synthetases SfaD and SbnF as well as the decarboxylase SbnH were obtained. Reactivity of these enzymes with several alternative substrates was measured using phosphate release assays and potential products were detected and purified by HPLC. Identity of the enzyme products was confirmed by mass spectrometry and NMR analysis. The ability of the new products to support *S. aureus* growth was examined by bioassays. Modification of enzymes in both pathways to accept a wider range of substrates could be used for production of new siderophores that could have antibacterial or industrial applications.

Poster B17**A feedback trap based on optical tweezers**

A. Kumar and J. Bechhoefer

Department of Physics, Simon Fraser University

After the pioneering work by Ashkin and collaborators on optical tweezers, many experimental techniques have been proposed to trap and control mesoscopic particles. In 2005, Cohen and Moerner developed the Anti-Brownian Electrokinetic (ABEL) trap, or feedback trap, a new tool for trapping and manipulating small objects in solution by applying feedback forces. Here, we introduce an instrument that combines both techniques, optical tweezers and a feedback trap. We apply feedback forces on optically trapped particles by moving the trap position rapidly in response to observed fluctuations with the help of an acousto-optic deflector (AOD). The control loop is based on an FPGA card and has been run at cycle rates approaching 250 kHz. Preliminary experiments show that we can confine a silica bead in a “virtual potential” that is 35-40 times stiffer than the underlying optical trap, whose laser power is kept constant. Stiffer traps at low laser power may help to perform biological experiments without damaging the specimen and reduce photobleaching effects wherever fluorescence is involved. The combined instrument can also be used in stochastic thermodynamics experiments based on virtual potentials that can have complicated shapes. Traditional thermodynamic limits apply to very slow, quasistatic processes, but applications generally favour fast processes. We will use this instrument to explore optimal protocols for tasks such as information erasure in small, fluctuating systems.

Poster B18**The thermodynamics of molecular machines**

E. Lathouwers and D.A. Sivak

Department of Physics, Simon Fraser University

Molecular machines are important for many cellular processes, such as producing high-energy chemicals to fuel other processes and transporting cargo across the cell. These machines are embedded in a strongly fluctuating, non-equilibrium environment that imposes significant physical limitations on their performance, in turn suggesting design principles for effective behavior in such a setting. We examine simple models that capture the essential physics of multi-component machines transducing between nonequilibrium stores of free energy. Such models indicate fundamental tradeoffs between speed and efficiency, and considerations of Pareto optimality point to effective ways of transmitting energy out of equilibrium.

Poster B19**Probing dynamic intermolecular interactions between an intrinsically disordered protein and its binding partner at single-molecule level**

T.C. Lee, G.-N. Gomes, J. Castroverde, C. C. Gradinaru

Department of Chemical and Physical Sciences, University of Toronto Mississauga; Department of Physics, University of Toronto

Sic1 is a cell cycle regulator in *S. cerevisiae* that inhibits the cell cycle progression from G1 to S phase. It works by binding to cyclin dependent kinases (CDKs) to inhibit their activity and is removed by degradation via ubiquitination. To be ubiquitinated, it needs to bind the WD40 domain of Cdc4, a component of SCF complex, and this binding affinity dramatically increases when Sic1 is phosphorylated on 6 or more sites. Previous studies have shown that the N-terminal intrinsically disordered domain of Sic1 is responsible and sufficient enough for this modulation of binding affinity, whereas it contains 7 of 9 phosphorylation sites of Sic1. Here, we investigated the changes in conformational ensemble of Sic1 upon binding to the WD40 domain, and the dynamic interactions of this unique “fuzzy complex” using multi-parameter single-molecule fluorescence techniques, including intra- and inter-molecular smFRET and FCS. To enable such studies, a novel chemical fluorophore labeling method has been developed and applied. Our results are interpreted within the framework of previous structural studies of Sic1 and provide new insights towards a physical mechanism for the phosphorylation-modulated binding between this intrinsically disordered protein and its target.

Poster B20**Investigating the Role of Ergosterol in Phase Separation of Yeast Vacuole Membranes**

C.L. Leveille, C.E. Cornell, A.J. Merz, S.L. Keller

Department of Chemistry, University of Washington; Department of Biochemistry, University of Washington

Membranes of vacuoles, the lysosomal organelle in yeast, phase separate into two coexisting liquid phases. This phenomenon provides a physical mechanism for lateral organization of the membrane in vivo. The yeast growth cycle follows a characteristic sequence of events: a logarithmic phase of rapid growth, followed by a stationary phase in which nutrients become scarce and vacuole membranes exhibit coexisting liquid domains. Recent studies show that the resulting domains play a key role in a central eukaryotic signaling pathway. However, how the cell adapts and tunes its vacuole membrane in order to invoke phase separation in the stationary phase is still largely unknown. Sterols play a significant role in phase separation within model membranes, and it has been shown that Ltc, a sterol transport protein, is pivotal for the formation of domains in glucose-starved yeast. Here, we directly investigate the role of ergosterol, the main sterol in yeast, in domain formation in vacuoles. We isolate vacuoles from yeast in the logarithmic phase of growth, in which the membranes do not natively exhibit domains. Using fluorescence microscopy, we observe changes in vacuole phase separation as a function of ergosterol content, which we manipulate by using a known sterol exchanger, methyl- β -cyclodextrin. We find the surprising result that domains appear in vacuole membranes with reduced ergosterol. We conduct control experiments in model lipid membranes in order to confirm that the methyl- β -cyclodextrin reduces ergosterol content under our conditions.

Poster B21**Biophysical Characterizations of the hypertrophic cardiomyopathy related-I79N TnT mutation**

Li AY (1), Shafaattalab S (1), Lin E (1,2), Liang B (1,2), Gunawan M (1), Rayani K (1), Barszczewski T (1), Davis J (3), Tibbits GF (1,2)

(1) *Molecular and Cellular Cardiology Group, Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada*

(2) *Cardiovascular Sciences, Child and Family Research Institute, Vancouver, BC, Canada;*

(3) *Department of Physiology & Cell Biology, The Ohio State University, Columbus, OH, USA*

Cardiac troponin (cTn) is a heterotrimeric complex that regulates cardiac contractility. Each cTn is composed of a Ca²⁺ binding subunit (cTnC), an inhibitory subunit (cTnI), and a tropomyosin binding subunit (cTnT). This study focuses on the I79N TnT mutation, which is associated with a high incidence of sudden cardiac death in young population. The biophysical properties of the mutation were investigated in reconstituted thin filaments (RTF), skinned cardiomyocytes, and human-induced pluripotent stem cell derived-cardiomyocytes (hiPSC-CM). At the RTF level, the mutation significantly slows the Ca²⁺ dissociation rate (80 s⁻¹) compared to the WT (102 s⁻¹) ($p < 0.05$). Higher Ca²⁺ sensitivity was observed for the mutant cardiomyocytes as demonstrated through a leftward shift of the pCa curve with ΔpCa of 0.65 ($p < 0.05$). This mutation was also incorporated into hiPSC-CM by genome editing, and the cells were characterized by optical mapping. Simultaneous voltage and Ca²⁺ transient were recorded using potentiometric (RH-237) and Ca²⁺ indicator (Rhod-2) dyes. The mutant hiPSC-CMs exhibited action potential remodelling and triangulation, which is considered a predictor of arrhythmogenicity.

Poster B22**Structural and Biochemical Characterization of Wall Teichoic Acid Biosynthetic and Degradative Enzymes**

F.K.K. Li, R.T. Gale, C. Myers, E.D. Brown and N.C.J. Strynadka

Department of Biochemistry and Molecular Biology, The University of British Columbia; Department of Biochemistry and Biomedical Sciences, McMaster University

Wall teichoic acids (WTAs) are phosphate-rich polymers covalently attached to peptidoglycan (PG) of gram-positive bacteria. WTA modification of PG has been shown to have important advantages for bacteria including biofilm formation, host cell adherence, and coordination of cell division and peptidoglycan synthesis. Because these processes are crucial to pathogenesis and normal bacterial physiology, WTA biosynthetic enzymes are recognized as drug targets for combating bacterial infections. Although WTA is not an essential cell wall component, the inactivation of certain downstream enzymes can induce bacterial cell death through disruption of peptidoglycan synthesis. This occurs through the sequestration of undecaprenyl phosphate, the lipid carrier in which both WTA and PG are synthesized on in the cytoplasm. To advance our understanding in the biosynthesis and recycling of WTAs, and to facilitate drug development, we are working towards the structural and biochemical characterization of WTA biosynthetic and degradative enzymes.

Poster B23**Measuring LDLR-PCSK9 Interactions on Cell Membrane Using Image Correlation Spectroscopy (ICS)**

Y. Liu, J. Mayne, D. Figeys, P.W. Wiseman

Department of Chemistry, McGill University; Department of Biochemistry, Microbiology and Immunology, University of Ottawa; Department of Biochemistry, Microbiology and Immunology, University of Ottawa; Department of Chemistry and Physics, McGill University

Image correlation spectroscopy (ICS) is a family of fluorescence fluctuation methods that use correlation function analysis to extract molecular information from fluorescence microscopy images. ICS analysis can be performed in the spatial or temporal domain on image time series to measure molecular distribution and transport properties. We report the application of ICS methods to quantitatively study the interaction between Low Density Lipoprotein Receptor (LDLR) and Proprotein Convertase Subtilisin Kexin-like 9 (PCSK9) within the cell membrane. LDLR predominantly resides on liver cell membranes and is responsible for clearing the so-called “bad” cholesterol from blood circulation. PCSK9, another protein secreted by liver cells, was found to bind to LDLR and modulate this process. In our study, LDLR was labelled with GFP and imaged in living Huh7 cells using Total Internal Reflection Microscopy (TIRFM) at 37C. Spatial ICS analysis showed a gradual decrease of LDLR membrane density under PCSK9 treatment, confirming that PCSK9 promotes LDLR internalization. The dynamics of membrane LDLR was also characterized using temporal ICS (TICS), in which temporal autocorrelation function of time series images was calculated, fitted with a 2D diffusion decay model to extract diffusion coefficients. These dynamic studies will provide a new window into the complex biological process of how PCSK9 regulates membrane LDLR distribution within cells.

Poster B24**Cryo-EM analysis of the type III secretion system ATPase-inner stalk complex and insights into its rotary catalysis**

D. D. Majewski 1, C. Hong 2, Z. Yu 2, N.C.J. Strynadka 1

*Department of Biochemistry and Molecular Biology and the Center for Blood Research, University of British Columbia**CryoEM Shared Resources, Janelia Research Campus, Howard Hughes Medical Institute*

Many pathogenic Gram-negative bacteria rely on an intricate macromolecular nanomachine - the type III secretion system (T3SS) - to secrete virulent “effector” proteins into host cells, subverting host signalling pathways to the benefit of the pathogen. The T3SS is a multi-megadalton complex made up of over 20 different proteins, forming a continuous effector-translocating pore through the Gram-negative envelope and host cell membrane. As limited pore diameter prevents secretion of folded proteins, dedicated chaperones must transport partially-unfolded effectors to the cytosolic face of the T3SS, where a vital ATPase separates them from their cargo. We present the high-resolution cryo-EM structures of a hexameric T3SS ATPase, with and without density for the bound inner stalk protein, at 3.9 Å and 3.4 Å respectively. The ATPase displays remarkable similarity to the hetero-hexameric F-type ATPases, despite being homo-hexameric; it is asymmetric, with a prominent cleft between two subunits demarcating the “nucleotide-exchange” catalytic state, and a gradient of other catalytic conformations in the remaining subunits. Analysis of the inner stalk binding pocket in the ATPase pore suggests an electrostatic mechanism for the propagation of ATP-derived torque to the rotor. The structure of a T3SS ATPase sheds light on the evolution of highly conserved ATPases, and on the rotary catalytic mechanism employed by the T3SS.

Poster B25**Interactions of amyloid peptide AS₇₁₋₈₂ with model membranes: structural and morphological study via FTIR and ssNMR**

B. Martial, T. Lefèvre, G. Raiche-Marcoux and M. Auger

Department of Chemistry, PROTEO, CERMA, CQMF, Université Laval, Québec

α -Synuclein (AS) is an amyloid protein involved in Parkinson's disease. In pathological cases, aggregates of this protein form in the dopaminergic neuronal network, leading to its progressive degeneration accompanied with a dramatic decrease in dopamine levels. Under physiological conditions, AS is disordered in solution or weakly bound to neuronal membranes, via the formation of α -helices. The triggers and steps underlying the formation of insoluble β -sheet rich fibrils are still unclear. In our work, we focus on a central 12 amino acids segment of AS in the amyloidogenic part of the protein that is believed to be responsible for the fibrillization of the whole protein: AS₇₁₋₈₂.

Interactions between AS and neuronal membranes are thought to be the starting point of the fibrillization process. In order to investigate and probe the mechanisms responsible for this fibrillization, model membranes composed of different ratios of zwitterionic (PC) and anionic (PG) phospholipids were used in our work. Infrared spectroscopy allowed the identification of irreversible changes in the β -sheet structure of AS₇₁₋₈₂ upon the gel-fluid phase transition of the lipids, underlining the critical role of peptide/membrane interactions. Furthermore, we studied via the ³¹P 2D solid-state NMR pulse sequence PROCSA the impact of AS₇₁₋₈₂ on phospholipid headgroups, in order to identify the preferentially interacting phospholipid in membranes composed of a PC-PG mixture.

Poster B26**Toward the characterization of the interactions between the TPR domain of O-linked N-acetylglucosaminyl transferase (OGT) and its substrates by NMR spectroscopy**

Stacy Maynard, Laura Packer, Lawrence McIntosh

*University of British Columbia**Department of Biochemistry and Molecular Biology*

O-linked N-acetylglucosaminylation (O-GlcNAc) is a single sugar post-translational modification reversibly regulated by two enzymes, OGT and OGA, which add and remove the modification from Ser/Thr residues, respectively. A myriad of proteins involved in transcription, translation, metabolism, stress response, and the cytoskeleton are O-GlcNAc-modified, and aberrant O-GlcNAcylation is associated with many diseases spanning cancer to diabetes and neurodegeneration. Although structural studies of the OGT catalytic domain do explain a preference for acceptor sequences such as PV(S/T), the molecular basis by which GlcNAc is targeted to many protein sites lacking these motifs remains unclear. Preceding its C-terminal catalytic domain, OGT contains 13.5 tetratricopeptide repeats (TPRs, a 34 residue antiparallel helical motif) arranged in series to form an extended solenoid. These TPRs are hypothesized to serve as multivalent docking interfaces for regions of substrate proteins distinct from their acceptor Ser/Thr; this docking then directs acceptor modification by the catalytic domain. To test this hypothesis, we have expressed TPR fragment amenable for NMR spectroscopy and are characterizing their interactions with known OGT substrates.

Poster B27**Investigating Structure-mediated Dynamics and Interactions in Supercoiled DNA**

B. McGuinness, S. Scott, C. Shaheen, F. Kouzine, Z. Xu, D. Levens, C. J. Benham, and S. Leslie

Department of Physics, McGill University, Montreal, Canada; Center for Cancer Research, National Institute of Health, Bethesda, USA; Genome Center, University of California, Davis, USA

While extensive investigation has revealed much about the higher-order structure of DNA, the dynamics of structural transitions have been challenging to observe experimentally. To understand these dynamics with in vitro techniques, it is important to explore conditions that approach those of the cellular environment. For example, ionic strengths in the cell lie in the 100-150 mM range, and organelles and other structures crowd 20-40% of the cytoplasm. Previously, we presented a single-molecule confinement microscopy method that allows molecules to freely explore their structural states, and used it to observe supercoil-induced unwinding of a specific target site in pUC19 DNA plasmids. By introducing oligonucleotides (oligos) designed to bind to the site when open, we were able to study unwinding and binding as a function of temperature, supercoiling, and oligo sequence. In this work, we study how these dynamics are influenced by environmental conditions such as ionic strength and the concentration of crowding agents. Our experiments demonstrate that increasing ionic strength can impede oligo binding to the unwinding site. Further, they demonstrate that the oligo-plasmid binding can increase with crowding agent concentration. The results from this work demonstrate the importance of considering the role of ionic strength and crowding agents in describing DNA interactions and dynamics in the nuclei of living cells.

Poster B28**Understanding the pore-forming mechanism of peptides derived from the N-terminus of sticholysin**

H. Mesa-Galloso, U. Ros, P.A. Valiente and D.P. Tieleman

Department of Biological Sciences and Centre for Molecular Simulation, University of Calgary; Centre for Protein Studies, University of Havana; Interfaculty Institute of Biochemistry, University of Tübingen.

Actinoporins are pore-forming toxins found in sea anemones that are able to bind and oligomerize and form pore in model and natural in membranes. These toxins can be used in the design of vaccines capable of forming pores and cross the plasma and endosomal membranes to enhance the immune response in anti-cancer treatments. Sticholysin I and II (StI and StII) are actinoporins produced by the Caribbean sea anemone *Stichodactyla helianthus*. These actinoporins exhibit differences in hemolytic activity that could be related to those found in their N-termini. StII1-30 and StI1-31 are peptides that are spanning the first thirty (St II) or thirty-one (St I) N-terminal amino acid residues of StII and StI respectively. Experimental evidence suggests that StII1-30 is more active than StI1-31 in cells. However, the molecular determinants that explain this differences in functional activity are still unknown. Here, we used molecular dynamics (MD) simulations to characterize the mechanism of pore formation by StII1-30 and the effect of membrane curvature on its permeabilizing activity. Based on our MD simulations, we proposed that StII1-30 aggregation properties and its ability to induce lipid reorganization are key determinants in its lytic activity. This was tested by differential scanning calorimetry and transbilayer movement experiments to confirm our simulation predictions. Our results show an integrated picture of cell membrane pore formation by these StII and StI derived peptides.

Poster B29**Single cell tracking is crucial to study cell rolling adhesion**

Y. Murad and I. T.S. Li

Department of Chemistry, University of British Columbia Okanagan

Cell rolling adhesion is a dynamic process involved in many physiological functions such as tissue remodelling and inflammation as well as cancer metastasis. For leukocytes, this process has been shown to depend on receptors called selectins and their interactions with specific ligands. Flow chambers as well as in vivo models have been used to study the P-selectin and PSGL-1 interaction by various groups in the literature with a variety of reported findings. Past studies have mainly focused on how cell rolling at the population level is affected by shear stress and other factors. Our data has shown that many key features of cell rolling behavior are disguised or significantly distorted when looking at the overall population behavior. This leads us to direct our effort towards in-depth single cell biophysical studies thus eliminating the influence of cell-to-cell variability and different phenotypic traits. Here, we used precision controlled syringe pumps to vary shear stress patterns affecting single cells in a rationally designed barrage of shear ramp and oscillation experiments with the purpose of unmasking previously unseen cell rolling behavior. We have also adapted a PEG surface functionalization protocol to allow for control of P-selectin density on the surface and prevent the non-specific adhesion of proteins and cells to the glass surface.

Poster B30**Estimating Molecular Counts using Fluorophore Blinking Statistics**

D. Nino, D. Djayakarsana, N. Rafiei, A. Zilman and J.N. Milstein

*Department of Physics, University of Toronto and Department of Chemical and Physical Sciences, University of Toronto Mississauga;**Department of Chemical and Physical Sciences, University of Toronto Mississauga; Institute of Biomaterials and Biomedical Engineering, University of Toronto and Department of Chemical and Physical Sciences, University of Toronto Mississauga; Department of Physics, University of Toronto; Department of Chemical and Physical Sciences, University of Toronto Mississauga and Department of Physics, University of Toronto and Institute of Biomaterials and Biomedical Engineering, University of Toronto*

Single-molecule localization microscopy (SMLM) has been a powerful tool for expanding our understanding of cell biology. By taking advantage of the stochastic blinking that fluorophores naturally exhibit and controlling the dynamics of this process, SMLM pushes the level of resolution an order-of-magnitude beyond the diffraction limit allowing light microscopy to visualize cellular components with improved resolution. There is also tremendous interest in using the technique to count single molecules. The main challenge of molecular counting in SMLM is largely a result of the multiple and random blinking of fluorophores, which leads to over-counting the number of molecules. We propose a method for counting biomolecules based on the blinking statistics of fluorophores, and taking into account the labeling efficiency to the target molecule. We show how our theory may be used by analyzing simulated data, and in vitro data based on DNA origami structures. Accurately determining the number of proteins or nucleic acids in a cell has wide-ranging applications, from systems biology, to proteomics/genomics, to fundamental cell biology. As a potential application, we show how such an approach could be applied to counting plasmid DNA without the need to individually resolve the plasmids.

Poster B31**Tumor cell invasion in mammary tissue and tumor growth in the cervical epithelia using a 3-D Individual Cell Based model**

F. Mohseni*, A. Payamara*, E. Palsson

Department of Physics and Department of Biology, Simon Fraser University;

A 3-D Individual Cell Based Model (ICBM) is used to study tumor cell invasion in mammary tissues and tumor growth in the cervical epithelia. The ICBM consists of deformable volume conserving ellipsoidal cells. It simulates and visualizes the effect of cell adhesion, stiffness, active force generation and chemotaxis on movement and signaling of cells in multicellular systems. The cell's response depends on its internal parameter state and external environment (neighbor cells, extracellular matrix, and chemical signals)

i) To better understand the different stages of cervical cancer we simulate the equilibrium state of the multilayered stratified cervical epithelium by using 3 cell types: stem cells (SC), transit amplifying (TA) cells and differentiated cells (DC). SC divide into TA cells and TA cells divide a few times before they differentiate into DC cells. We explore how growth rate, cell-cell and cell-boundary adhesion, pressure on the cell, and cell volume, affect the equilibrium state.

ii) EGF/CSF-1 paracrine signaling between macrophages and tumor cells facilitates the invasion of tumor cells. Tumor cells/macrophages secrete CSF-1/EGF and chemotact towards EGF/CSF-1, respectively. Here we show that the invasion and migration of tumor cells and macrophages depend on the degree of non-uniformity both of receptor density and EGF/CSF-1 secretion.

Our goals are to i) visualize cervical cancer and ii) find the degree of non-uniformity that minimizes tumor cell invasion.

Poster B32**Structure and Mechanism of Action of Two Active Derivatives of Aurein 2.2**

N. Raheem and S. K. Straus

Department of Chemistry, University of British Columbia.

With bacteria becoming increasingly resistant to antibiotics, it has become increasingly important to look for therapeutic alternatives such as antimicrobial peptides (AMPs). AMPs are short peptides with 10 - 50 amino acid residues, an overall positive charge and a significant portion of hydrophobic residues. Aurein peptides are AMPs secreted from the Australian southern bell frog *Litoria aurea*. Specifically, aurein 2.2 has a net charge of +2 and an amidated C-terminus. A number of biophysical experiments have demonstrated that aurein 2.2 functions via toroidal pore formation [1-4]. Although aurein 2.2 shows good activity towards Gram positive bacteria (e.g. *S. aureus*), we sought to find more active peptides by screening a library of peptides generated through SPOT synthesis [5, 6].

From this library, two active derivatives of aurein 2.2 were identified. Data demonstrating the antimicrobial as well as the antibiofilm activity will be presented. Experiments to elucidate the mechanism of action of these peptides (as compared to aurein 2.2), such as solution and oriented CD spectroscopy, NMR spectroscopy, DiSC35 leakage assay and pyranine leakage assay, will also be discussed. Overall, the data suggests that the two new peptides do not function by forming well-defined pores, as aurein 2.2 does [4]. The implications of these results in terms of future AMP design will be discussed.

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Poster B33**Kir6.1-A88G and Kir6.2-S365C/SUR2A Channels, Biophysical Characteristics and Possible Association With Brugada Syndrome.**

C. Rangel, H. Martinez, E. Sanchez, T. Ferrer and C. Onetti

*1 Centro Universitario De Investigaciones Biomédicas, Universidad de Colima, México**2 Global Genetics Corporation, Genetics for life, Ventura, CA.*

ATP- sensitive potassium channels (KATP) belong to the family of inward rectifying potassium channels (Kir).

Nowadays, mutations in genes that encode for Kir6.x and SUR subunits have been associated with Brugada syndrome (BrS). Thus, in the present study, we aimed to evaluate the functional characterization of the A88G variation in KCNJ8 (Kir6.1 subunit) and the S385C variation in KCNJ11 (Kir6.2 subunit), using whole cell and inside-out patch clamp techniques.

Whole cell patch-clamp studies show a 2-fold gain of function of glibenclamide-sensitive IKATP when KCNJ8-A88G or KCNJ11-S385C was co-expressed with SUR2A. Current-voltage relationship for KCNJ8-A88G and KCNJ11-S385C channels was obtained from membrane patches using the inside-out configuration. The result conductance was 48.02 pS WT vs 80.10 pS A88G ($p < 0.001$; $n=6$); 74.4 pS WT vs 65.1 pS S365C ($p > 0.001$; $n=6$). The open probability (P_o) was also affected showing an increase in the open state for the variants ($\tau = 12$ ms WT vs 26 ms A88G; $\tau = 9.9$ ms WT vs 14.9 ms S365C). Finally, Inside-out patch clamp evaluation shows that a significantly greater inhibitory concentration for ATP in the mutant channels is needed, leading to an incomplete closing of the KATP channels under normal conditions.

Our results support the hypothesis that KCNJ8 and KCNJ11 may be susceptible genes for BrS and suggest that these variations induce gain of function in IKATP, with a mayor conductance and P_o and a reduced sensitivity to intracellular ATP.

Poster B34**Fluid Response: Species-specific Variations in Bacterial Membrane Stiffness in Response to Environmental Stresses**

Kunal Samantaray, Bikash Ranjan Sahu, Mrutyunjay Suar, Suresh K. Bhat, and Priti Sundar Mohanty*

*School of Biotechnology and School of Applied Sciences, KIIT University, Bhubaneswar 751024, India**Polymer Science & Engineering Division, CSIR-National Chemical Laboratory, Pune 411008, India*

Several studies 1,2, including those from our own group 3,4, have quantified genetic and proteomic changes in bacteria exposed to environmental stressors. However, as the first line of defence, membrane dynamics is a crucial determinant of bacterial survival and propagation. We have studied membrane fluidity in several bacteria, both gram negative (*Salmonella Typhimurium*, *Escherichia coli*, *Vibrio parahemolyticus*, *Shigella flexneri*, etc.) and gram positive (*Pseudomonas aerogenosa*, *Staphylococcus aureus*, *Micrococcus luteus*, *Lactobacillus* spp., *Bacillus subtilis*, etc.) using confocal microscopy-based fluorescent recovery after photobleaching (FRAP), fluorescent loss in photobleaching (FLIP) and flow cytometry-based lipid dye excitation/emission analysis (DI-4-ANEPPDHQ). We have inferred membrane dynamics under multiple stresses (pH, salt and antimicrobials) and bacterial states (nutritional deprivation, planktonic and biofilm). Proteomics data suggests that membrane components are dynamically modified by gene level and transcriptional changes to cope with specific environmental stress. However, we have studied instantaneous changes that bacterial membranes undergo in response to stress. We also identified adaptation conditions that enable better survival during future stresses. Our results relate bacterial survival to their membrane dynamics and investigate this first line of bacterial defense against various environmental stresses.

Poster B35**Atrial differentiation of hiPSC derived cardiomyocytes by activation of retinoid pathways.**

Sarabjit Sangha*¹, M. Gunawan*¹, S. Shafaattalab¹, Thomas Claydon¹, Zachary Laksman^{1,2}, Glen Tibbits^{1,3}

** authors contributed equally*

1 Department of Biomedical Physiology and Kinesiology, Simon Fraser University

2 Department of Medicine, University of British Columbia

3

BC Children's Hospital Research Institute

The advent of pluripotent stem cell (PSC) derived cardiomyocytes has revolutionized the field of cardiac research. For the first time, we are able to study human disease in human models while avoiding the challenges of obtaining biopsy tissue. Additionally, we are able to study a patient's disease in a personalized manner by the use of the patient-derived induced pluripotent stem cells (iPSCs). Current differentiation protocols result in a mixed cardiac population that consists of mostly ventricular cells. This makes the study of chamber-specific diseases, like atrial fibrillation (AF), difficult. As such, the development of atrial-specific differentiation protocols is vital. Retinoic acid has long been identified as a modulator of cardiac subtypes in animal models. Recent work has shown that the addition of retinoic acid during the cardiac mesoderm phase in embryonic stem cells increased atrial lineage significantly. These differentiations however, were completed using Activin A/BMP (Act/BMP) protocols which are difficult and costly to run. Here we take lessons learned from previous protocols and attempt to apply them to a more accessible CHIR (GiWi) protocol. We hypothesized that the addition of retinoic acid during the days in which cells are in the cardiac committed mesoderm phase, days 3-5, will direct them towards an atrial-like fate. Cells were then characterized by quantitative real-time PCR (qRT-PCR), flow cytometry and optical mapping.

Poster B36**Investigating a biological specificity conundrum: the role of dynamics in transcription factor DNA-binding**

K. Scheu, S. De and L. McIntosh

Department Biochemistry and Molecular Biology, University of British Columbia; School of Bioscience; Indian Institute of Technology, Kharagpur, India; Department of Biochemistry and Molecular Biology and Department of Chemistry, University of British Columbia

A biological specificity conundrum challenges our understanding of the DNA-binding mechanisms underlying many transcription factor families. Family members often share a highly conserved DNA-binding domain, yet individual members are often able to recognize specific DNA sequences. This has been corroborated with an increasing number of genome-wide DNA-protein interaction studies showing that static X-ray crystallographic structures of isolated DNA-binding domains with consensus DNA oligonucleotides do not fully explain the different *in vivo* specificities exhibited by members of the same transcription factor families. This leads to the hypothesis that the intrinsic dynamic properties of individual transcription factor family members modulate their specificities for binding a continuum of DNA sequences. To test this hypothesis, I am implementing an unbiased Bind-N-Seq approach with Next-Gen sequencing to examine how mutations that alter the structural and dynamic properties of the ETS transcription factor, ETV6, affect its relative affinities for DNAs ranging from those with a consensus ETS binding site to those with completely "non-specific" sequences.

Poster B37**Investigating Interactions Between Lipids Involved in Drug Delivery**

M.L. Schmidt ¹, B.Y.M. Bashe ², Y. Atsmon-Raz ³, N. Subramanian ³, P.R. Cullis ⁴, D.P. Tieleman ³ and J.L. Thewalt ^{1,2}

Department of Physics, Simon Fraser University;

Department of Molecular Biology and Biochemistry, Simon Fraser University;

Centre for Molecular Simulation, Department of Biological Sciences, University of Calgary;

Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada

Lipid nanoparticles (LNPs) designed to encapsulate small interfering RNA (siRNA) are a promising method for drug delivery in the treatment of diseases through gene silencing mechanisms. These LNPs are composed of several components, each with a role to play in the successful delivery of the siRNA to the target site. LNPs are made up of a phospholipid, an ionizable cationic lipid (in this case DLin-KC2-DMA (KC2), pKa ~6.7), cholesterol, and a polyethylene glycol-lipid. It is proposed that the electrostatic interactions between the ionizable cationic lipid and the anionic lipids (predominantly lysobisphosphatidic acid (LBPA)) found in the endosomal membrane are able to disrupt the membrane by inducing a phase change from bilayer to non-bilayer (inverted hexagonal or bicontinuous cubic) phases facilitating the release of the siRNA cargo into the target cell's cytoplasm. One of the obstacles to the potency of siRNA drugs is the inefficiency of the release mechanism. Computer simulations can provide valuable insights for optimizing the LNP composition to overcome this issue. In this work, we investigated the effect of LBPA on a model bilayer forming lipid, POPC, using ²H NMR to validate the simulation parameters of LBPA for future molecular dynamics simulations. In addition, we investigated the interactions between LBPA and the ionizable cationic lipid of the LNP, KC2, using both ³¹P NMR and small angle X-ray scattering (SAXS). NMR and SAXS provide complementary information about the lipid phase(s) present on different length scales. Non-bilayer phases were observed using both techniques when KC2 was added to LBPA. These systems provide a foundation for further experimental and computational studies of the LNP drug delivery mechanism that will help improve the siRNA release efficacy of these therapeutics.

Poster B38**Comparing the stability and cellular inclusion formation of ALS-associated and in silico designed SOD1 mutants**

L. McAlary^[1,2], M. Sher^[2], M. Habibi^[1], M. Duval ^[3], N. Cashman^[2], T. Allison^[3] S. Plotkin^[1]

[1] Department of Physics and Astronomy, University of British Columbia, Vancouver, Canada

[2] Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, Canada

[3] Department of Biological Sciences, University of Alberta, Edmonton, Canada

Background: Mutations in superoxide dismutase 1 (SOD1) are associated with amyotrophic lateral sclerosis (ALS). SOD1-associated ALS is characterized by the deposition of the protein into insoluble inclusions in motor neurons. Understanding the biophysical properties of SOD1 mutants may shed light on ALS pathogenesis. Therefore, we compare the stability and cellular inclusion formation of ten disease associated SOD1 mutants along with two in silico designed SOD1 mutants.

Methods: The stabilities of purified apo-SOD1 mutants were investigated by measuring their melting temperature (T_m) using differential scanning fluorimetry. We then compared inclusion formation by over-expressing GFP-tagged SOD1 mutants in U2OS cells, imaging the cells, and analyzing the images using machine learning to count the number of cells that contained inclusions.

Results: Most SOD1 mutants were more susceptible to thermal denaturation than WT and a low melting temperature was observed, indicating the mutants were significantly less stable than WT. Only H46R and the in silico designed K128N (predicted to be a stabilizing mutation) had stabilities similar to WT. Interestingly, the mutants with lower stabilities were more prone to form inclusions in cells.

Conclusions: Our data suggests there is a correlation between SOD1 mutant stability and inclusion formation, and that mutant stability can be predicted in silico.

Poster B39**Protons accelerate hERG K⁺ channel deactivation gating by destabilizing the relaxed state of the voltage sensor**

Yu Patrick Shi, Samrat Thouta, and Tom W. Claydon

Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, V6A 1S6, Canada

hERG K⁺ channel slow deactivation (closing) contributes to cardiac repolarization and rhythm. Repolarization is reduced by external protons, which accelerate hERG channel deactivation kinetics by an unknown mechanism. We hypothesized that protons accelerate deactivation by destabilizing the relaxed state of the hERG voltage sensor domain, because relaxation stabilizes the activated voltage sensor and limits its return during closing. We used cut-open voltage clamp and voltage clamp fluorimetry techniques to measure hERG channel voltage sensor movement. Both measures reported an energetic separation of channel activation and deactivation (mode-shift), which reflects transition of activated voltage sensors into the stable relaxed state. Increasing the proton concentration reduced the mode-shift by specifically reducing the energy of voltage sensor return without affecting that of voltage sensor activation. Neutralization of an external acidic residue, Asp509, also abolished the voltage sensor mode-shift, mimicking the effects of protons. These results show that the relaxed state of the voltage-sensor is destabilized by protons, and that Asp509 is critical for stabilizing the relaxed state. We conclude that protonation of Asp509 disrupts salt-bridge interactions with voltage sensor counter charges destabilizing the relaxed state and accelerating deactivation. These data reveal key intra voltage sensor domain interaction sites that control the stability of the relaxed state.

Poster B40**Gel/Liquid-ordered phase coexistence in sphingomyelin and PC bilayers in the presence of palmitoyl ceramide and cholesterol**

R. Siavashi, T. Phaterpekar, S Leung, F.M. Goni, and J. Thewalt

*Department of Physics and Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, Canada**Instituto Biofisika (CSIC, UPV/EHU) and Departamento de Bioquímica, Universidad del País Vasco, Bilbao, Spain*

Elucidation of the molecular mechanisms of apoptosis might have significant therapeutic benefits by revealing how cell death can be modulated. The putative early effects of apoptosis on the phase behavior of the plasma membrane (PM) have not been explored in detail. Due to apoptotic signaling, the enzymatic activity of sphingomyelinase (SMase) converts SM to ceramides (Cer) in the PM. It has been suggested that SMs are localized in domains within the PM. Accumulation of Cer in the vicinity of SM induces gel-phase domains in the SM: Cer vesicles. Moreover, cholesterol (Chol) is one of the major components of the PM. Chol is known to give rise to liquid-ordered (L_o) phase domains in SM: Chol bilayers. Thus SM: Chol: Cer interactions could play a role in determining cell fate. In this study, we are interested in determining the physical effects of Cer accumulation in SM/ Chol vesicles to give an insight into the phase behavior of the PM at the start of apoptosis. Using ²H-NMR, we have characterized the phase behavior of selectively deuterated palmitoyl-SM (PSM) and palmitoyl-Cer (PCer) in their ternary mixtures with Chol. According to our results, there is L_o-gel phase coexistence in the PSM: Chol: PCer bilayers at molar ratios of (7:3:1), (7:3:2) and (7:3:3) both at room and physiological temperatures. In addition, replacing PSM with DPPC in vesicles with the same molar ratios of Chol and PCer reveals that PCer molecules show a higher tendency to accumulate in gel phase domains in PSM- than in DPPC-containing vesicles. These results are consistent with the combined fluorescence microscopy data using NAP and DiI C18 as the fluorescent dyes for the (7:3:3) vesicles (Busto J V, et al. Lamellar gel (L_β) phases of ternary lipid composition containing ceramide and cholesterol. Biophys J. 2014).

Poster B41**p53 protein contains an intrinsic regulatory element that modulates its protein-protein interactions**

Q.Y. Song, J.K. Rainey and P. Liu

Department of Biochemistry and Molecular Biology, Dalhousie University

Tumor suppressor p53 is the central regulator of diverse cellular pathways and is essential for maintaining genome integrity while preventing development of cancer. The DNA binding domain of p53 interacts with p53-specific promoters and is required for transcription of downstream genes. Moreover, numerous proteins and protein domains have been demonstrated to directly interact with the DNA binding domain to promote or inhibit p53 activity. Despite that many of the interactions have been thoroughly studied, it is not known whether or not the other domains of p53 are involved in the interaction. In this study, we utilized nuclear magnetic resonance spectroscopy to explore the role of the N-terminal region of p53, including a transactivation domain and a proline-rich domain, in the interaction with plakoglobin C-terminal domain or MDM2 acidic domain. We demonstrated that the transactivation domain inhibits the interaction whereas the proline-rich domain has no effect on the interaction. More interestingly, we found that the N-terminal region of p53 directly interact with its DNA binding domain. Together, our results suggest p53 possess an intrinsic regulatory element that modulates protein-protein interactions.

Poster B42**Single-molecule platform for real-time manipulation and visualization of protein-DNA interactions**

F. Stabile, G. Henkin, K. Metera, D. Berard, S. Leslie

McGill University

Whether to detect the origin of a disease, or understand the effectiveness of a drug treatment, single-molecule imaging and manipulation techniques are at the forefront of emerging biotechnology research and development. We present a single-molecule imaging platform for visualizing and controlling the interactions and dynamics of molecules - in real time, with high statistics, and without necessitating surface-immobilization of molecules. This work is motivated by enabling new single-molecule studies of "gene-editing biotechnologies", such as CRISPR/Cas9 - where new mechanistic insights, at the level of single molecules, are needed to bring these technologies to practice. Here, we combine the CLiC imaging and manipulation method with a deep embedded microchannel, in order to 1.) gently load and linearize DNA into open-faced nanogrooves, and 2.) subsequently exchange reagents without disturbing the entrapped DNA molecules. This allows us to visualize the response of the DNA to the inserted reagent molecules - which we demonstrate for several kinds of interactions (ions, enzymes, proteins). Further to this, we present a method to selectively deposit the DNA complexes from the embedded grooves onto one surface of the flow cell - which we can subsequently remove for high-resolution analysis. This allows us to first observe solution-phase interactions and dynamics using fluorescence microscopy, and subsequently perform high-resolution microscopy (such as AFM) of surface-immobilized complexes - using the same constituent molecules. We present preliminary results with an eye to contributing new mechanistic insights to the interactions and dynamics of CRISPR/Cas9 gene editing systems.

Poster B43**Testing for localized unfolding as a trigger of aciniform silk fibrillization**

A. Sulekha, L. Xu, P. X. Liu and J. K. Rainey

Department of Biochemistry and Molecular Biology, Dalhousie University

Aciniform silk is employed by araneid spiders to wrap prey and make egg-cases. This silk protein has an unique domain architecture and mechanical behaviour. The *Argiope trifasciata* aciniform silk repetitive domain is made up of ≥ 14 identical units, each 200 residues long ("W units"). Based on solution-state NMR spectroscopy-studies and hydrodynamics measurements, our group proposed a compact "beads-on-a-string" structure, rich in α -helical character, for the soluble W unit. Retention of significant α -helical character in the fibrous form, unlike other silks, along with lower stability of one α -helix relative to the others led to a hypothesis: localized unfolding of this less stable helix will lengthen the "string-like" linker, decreasing protein compactness. This, in turn, would favour protein entanglement and intermolecular interactions, triggering β -sheet formation. To test this hypothesis, we engineered W units with one Ser on the unstable helix and another on the core mutated to Cys to form a disulfide "staple", linking the unstable helix to the globular core. Compellingly, when oxidized, the mutant cannot form fibres, while the reduced state is fully functional. Comparing the atomic-level structure and dynamics of the stapled to the reduced and native forms of the W unit, we are testing the hypothesis that denaturation and decompaction of this helix allows aciniform silk fibrillogenesis. This, in turn, will facilitate engineering of W units with modified mechanical behaviour.

Poster B44**Confinement microscopy of highly-branched functional nanoparticles**

R. Tahvildari, M. Shayegan, H. Liao, Y. Patel, M. Kurylowicz and S.R. Leslie

*Physics Department, McGill University, Montreal, Quebec, Canada H3A 2T8**Mirexus Biotechnologies Inc., 361 Southgate Drive, Guelph, Ontario, Canada N1G 3M5*

Improved understanding of the biophysical properties and interactions of individual nanoparticles is crucial to developing new functional nanomaterials, such as pharmaceuticals and cosmetics. We report the use of Convex Lens-induced Confinement (CLiC) microscopy[1] with embedded micron-sized wells, to investigate the biophysical properties as well as interaction kinetics and stoichiometries of Phytoglycogen nanoparticles[2]. This unique imaging technique confines the particles within reaction volumes as small as femto-liters and allows prolonged monitoring of reaction trajectories over seconds to minutes long timescales.

We pioneer CLiC imaging of nanoparticle-nanoparticle and probe-nanoparticle interactions, including investigations of binding/unbinding kinetic rates, as well as encapsulation and adsorption dynamics. We demonstrate direct, real-time visualization and monitoring of the response of nanoparticles to introducing reagents in solution.

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Poster B45**Pheromone-Binding Protein-Ligand Equilibrium and Kinetics Constants Correlation with Electroantennogram Response**

M. Terrado and E. Plettner

Department of Chemistry, Simon Fraser University

Olfaction is very important in the reproductive cycle of moths. Females release pheromones to signal that they are ready for mating. Male moths smell these molecules through sensory hairs (sensilla) on their antennae. Perireceptor events occur within the sensillar lymph fluid that bathes the dendritic membrane. Abundant in the sensillar lymph are water-soluble proteins called pheromone-binding proteins (PBPs). These are small (~15 kDa) proteins believed to transport the hydrophobic pheromones across the lymph fluid to the neural receptors or to scavenge the pheromone, thereby preventing neuronal adaptation. There are two known PBPs in gypsy moth (PBP1 and PBP2). Both PBPs recognize the pheromones, (+)- and (-)-disparlure.

Equilibrium dissociation constants (K_d) indicate the extent of protein-ligand binding. K_d values of each PBPs with the natural pheromones and various neurally active but behaviourally inactive analogues were determined. In addition, apparent kinetics constants, k'_{on} and k'_{off} , were determined through fluorescence assay. Responses of moth antennae to the odorants were determined by electroantennogram (EAG) recording. We hypothesize that the PBPs should affect the rates of EAG lag, depolarization and repolarization, if they are involved in pheromone transport and/or scavenging. Therefore, correlation of K_d , k'_{on} and k'_{off} values with EAG parameters will be important in deeper understanding of PBP function.

Poster B46**Tracking the Movements of Tumor Cells That Express Different Cell Adhesion Molecules During Collective Migration**

A. Wilson, T. Poon, D. Bhaskar, P. Dean, C. Ozdemir, H. Knutsdottir, L. Edelstein-Keshet and C. Roskelley

Department of Cellular and Physiological Sciences and Department of Mathematics, University of British Columbia

A loss of cell-cell adhesion contributes to the ability of tumor cells that have undergone an epithelial to mesenchymal transition to become migratory and invasive when they move as single cells. However, it is not clear how alterations in cell-cell adhesion contribute to the ability of tumor cells to move through a collective cell mass towards a migratory front. To address the latter issue we generated fluorescently tagged breast tumor cells that express E-cadherin only, N-cadherin only, or both E-cadherin and N-cadherin together. We also developed cell tracking strategies that make it possible to follow the movement of the fluorescent cells within confluent tumor cell monolayers. The goal of the project is to determine if changes in cell-cell adhesion molecule expression affect the ability of tumor cells to move through the monolayer toward a migratory front after the confluent monolayer is scratched. Additionally, we will determine if changes in cell-cell adhesion molecule expression alter the ability of cells to become leaders once they reach that front.

Poster B47**Crystal structure of an intramembranal phosphatase central to bacterial cell wall peptidoglycan biosynthesis and lipid recycling**

S.D. Workman, L.J. Worrall, N.C.J. Strynadka

Department of Biochemistry and Molecular Biology and the Center for Blood Research, University of British Columbia

Undecaprenyl pyrophosphate phosphatase (UppP) is an integral membrane protein that recycles the lipid carrier essential to the ongoing biosynthesis of the bacterial cell wall. Individual building blocks of peptidoglycan are assembled in the cytoplasm on undecaprenyl phosphate (C55-P) before being flipped to the periplasmic face where they are polymerized and transferred to the existing cell wall sacculus, resulting in the side product undecaprenyl pyrophosphate (C55-PP). Interruption of UppP's regeneration of C55-P from C55-PP leads to the buildup of cell wall intermediates and cell lysis. We present the crystal structure of UppP from *Escherichia coli* at 2.0 Å resolution, which reveals the mechanistic basis for intramembranal phosphatase action and substrate specificity using an inverted topology repeat. In addition, the observation of key structural motifs common to a variety of cross membrane transporters hints at a potential flippase function in the specific relocalization of the C55-P product back to the cytosolic space.

Poster B48**Possible optical communication channels and biophoton sources in the brain**

Parisa Zarkeshian, Christoph Simon

Institute for Quantum Science and Technology, Department of Physics and Astronomy, University of Calgary, AB, Canada

Despite great progress in neuroscience, there are still fundamental unanswered questions about the brain, including the origin of subjective experience and consciousness. Right answers might rely on unexplored physical mechanisms. Given that biophotons have been discovered in the brain, it is interesting to explore if neurons use photonic communication in addition to the well-studied electro-chemical signals. Such photonic network in the brain would require sources, detectors and physical waveguides to connect all these spatially separated agents in a selective way. Myelinated axons have been proposed as potential biophoton waveguides in the brain. Myelin sheath has a higher refractive index than both the inside of the axon and the interstitial fluid outside which let the myelin sheath to guide the light inside itself even with its imperfections. Among the electronically excited species which are proposed to be responsible for the biophoton emission, we investigate the mechanism of radiation of the excited oxygen molecules. The recently discovered spectrum of the biophoton emission from the brain is consistent with the measured frequency range of emission of a pair of oxygen molecules. Besides, the resemblance of electronic structure of the molecular oxygen to that of an NV center in diamond, which has been studied extensively for quantum information applications, has inspired us to study oxygen selection rules and radiative transitions, possibly with the use of plasmonics.

Poster B49

Site-selective Optical Spectroscopy in Systems of Coupled Chromophores: Spectral Hole Burning Modeling Meets Excitonic Calculations

V. Zazubovich and R. Jankowiak

Department of Physics, Concordia University, 7141 Sherbrooke Str. West, Montreal H4B 1R6, Quebec, Canada;
Department of Chemistry, Kansas State University, Manhattan KS, USA.

Spectral hole burning has been used to determine important details about energy transfer, charge transfer and protein dynamics in photosynthetic pigment-protein complexes for decades. Some common interpretations of SHB results may, however, be erroneous. Improvements in computer performance finally allowed us to combine excitonic effects and high spectral resolution using a personal computer, enabling modeling various light-induced alterations of the optical spectra in a consistent manner. We present the results of simulations of Non-Photochemical Spectral Hole Burning (NPHB), Fluorescence Line Narrowing (FLN) and D-FLN spectra of chromophore dimers and trimers embedded in a protein at cryogenic temperatures. The model includes calculation of Excitation Energy Transfer (EET) rates for individual chromophore dimer or trimers generated by Monte-Carlo excitonic calculations and converting them to a set of non-identical sub-site distribution functions (sub-SDF) for different EET rates; NPHB in pigments excited via excitation energy transfer, and, for the first time, the effects of the NPHB of the lowest-energy pigment in the multi-pigment / protein complex on the higher exciton states. The NPHB of the lowest-energy pigment in the coupled dimer or trimer (following the EET from the higher states, which are excited directly) causes the shifts of the higher-energy states. These shifts contribute to the apparent rate of the resonant hole burning at higher excitation frequencies as well as affect the spectral hole widths and energy transfer times derived from these widths by traditional means. Also affected are the shapes of the hole burning action spectra, commonly utilized for determination of the site-distribution functions of the absorption bands of particular chromophores.

Poster B50

Transformation of a protein nano-walker into a nano-motor by feedbackM.J. Zuckermann 1, R. Schmitt 4, N.R. Forde 1, E.H.C. Bromley 3, H. Linke 4, C.N. Angstmann 2,
P.M.G. Curmi 5

1 Department of Physics, Simon Fraser University and IRMACS, 2 School of Mathematics, UNSW 3 Department of Physics, Durham University, 4 Solid state Physics and NanoLund, Lund University, 5 School of Physics, UNSW

The objective of our research is to design, create and measure the performance of synthetic protein constructs which mimic biological nano-motors. In this poster, we examine a synthetic nano-walker concept based on kinesin called Synthetic Kinesin Inspired Protein (SKIP). In contrast to kinesin, SKIP motor's movement is controlled by pulses of ligands that activate and deactivate binding of repressor proteins onto a track made of DNA. Furthermore, the symmetry of the SKIP construct allows it to reverse, through the influence of a sufficient rearward external (load) force or the use of specific track ends. Examples of such repressor proteins include TrpR and Pur R. (1)

In our initial simulations, SKIP proved to be a persistent nanowalker, being able to proceed along the track. Here, we explore the role of feedback in enhancing SKIP's performance. First (FEEDBACK 1), we alter the direction of the applied force, in order to maximize the work being done by SKIP as it walks (2). In an alternative approach (FEEDBACK 2), 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 against a low enough load force under Feedback 1 and against load forces of any magnitude under Feedback 2.

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