FULL PROGRAM

Biophysical Society of Canada

6th Annual Meeting

May 25-28, 2021

Remote/Halifax

6th Annual
BSC Meeting
May 2021

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Welcome!

Welcome to the 6th Annual Meeting of the Biophysical Society of Canada! Because of the ongoing COVID-19 pandemic, this year's meeting will be held virtually. Despite this change in venue, this meeting offers attendees the opportunity to learn about exciting new developments in biophysics research and a great chance to network with peers from across North America.

The 2021 meeting is dedicated to biophysical techniques and discoveries that have revolutionized research leading to critical advancements in medicine, biotechnology, material sciences, and biosensing. The conference will commence with a Trainee Symposium on May 25th, and features the National Lecture (John Rubinstein), two plenary lectures (Catherine Royer and Albert Stolow), oral presentations given by both established and emerging researchers, as well as the recipients of BSC awards. All attendees are invited to participate in the Trainee Symposium on May 25th, which will consist of a career session and a venue for trainees to share their research accomplishments. With the pandemic, having engaging poster sessions has been exceptionally difficult, and we are excited to be hosting a poster session which will approximate the real thing and allow for easy interactions and networking to occur. At the end of the meeting there is a BYOB (bring your own beverage), so that we can all connect.

We are grateful to our many company sponsors for their generous support, as well as departments and faculties from Dalhousie and Saint Mary's University. Finally, we thank you for taking the time out of your busy schedule to join us. We are excited to meet you and look forward to a great meeting!

David Langelaan & Jan Rainey (Dalhousie University), Danielle Tokarz (Saint Mary's University), and Valerie Booth (Memorial University of Newfoundland)

President's message

Welcome to the 6th Annual Meeting of the Biophysical Society of Canada!
Thank you to the Organizing Committee lead by David Langelaan for their hard work organizing this meeting on line, and thank you all the participants joining us for the meeting.

I am looking forward to meet biophysics community and to celebrate another BSC meeting with a wonderful collections of speakers, Trainee's symposium, new awards and stimulating biophysics discussions.

I am serving my final year as President of the Biophysical Society, with Dr. Nancy Forde, our Vice-President, taking over as President of the BSC at the end of this meeting. We will be hosting AGM BSC meeting on Friday May 28, at 2:45-4 pm Atlantic time, all BSC members are welcome to join us.

Enjoy the meeting and stay in touch with Biophysics community in Canada!

Best wishes
Zoya Leonenko
President, Biophysical Society of Canada

BSC 2021 LOCAL ORGANIZING COMMITTEE

David Langelaan
Department of Biochemistry & Molecular Biology

Dalhousie University

Jan Rainey
Department of Biochemistry & Molecular Biology

Dalhousie University

Danielle Tokarz
Department of Chemistry

Saint Mary's University

Valerie Booth
Department of Biochemistry

Department of Physics and Physical Oceanography

Memorial University of Newfoundland

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BSC 2021 TRAINEE SYMPOSIUM LOCAL ORGANIZING COMMITTEE

Yanitza Trosel Arroyo
Memorial University of Newfoundland

Sheyla Montero Vega
Memorial University of Newfoundland

Kathleen Vergunst
Dalhousie University

CONFERENCE LOCATION

Dalhousie University

Dalhousie University is located on the Haifax peninsula, near historical landmarks such as Citadel Hill, the Halifax Public Gardens and the harbourfront Historic Properties. Dalhousie was founded in 1818, has over 19,000 students, and is the major academic research hub of the Maritimes with annual research funding of >$168 million dollars. The campus is diverse, with ~ 60% of students coming from out-of-province, and ~20% of students being international and coming from over 120 countries. One mission of Dalhousie is to be as environmentally sustainable as possible, and towards this goal, the Mona Cambell Building is one of only a handful of structures in North America that has been constructed using 'bubble deck' technology to reduce concrete usage.

Saint Mary's University

Saint Mary's University is nestled in the heart of Halifax and is marked by iconic buildings, green spaces and fresh ocean air. Saint Mary's is an urban, mid-sized university with over 7,000 full-time and part-time students from around the world making Saint Mary's a vibrant and culturally diverse community.

Memorial University of Newfoundland

Memorial University of Newfoundland hosts more than 18,000 students from over 100 countries. The main campus in St. John's, located a 2-hour flight north-east of Halifax, is famed for its proximity to exceptional natural beauty. Research activity at MUN is also vibrant, with research funding in 2019-2020 totalling more than $130 million dollars. In summer 2021, the Biochemistry, Biology and Chemistry Departments are moving to a brand-new building, the new Core Sciences Facility. Its architectural design is said to be evocative of the icebergs that often float past our shores.

POSTER AND FRIDAY BYOB INFORMATION

Posters are set up in Gather: https://gather.town/app/iTH0qPRENzYcLR8E/BSC2021 . Odd numbered posters will present on Wednesday and even numbered posters will present on Thursday. Your poster number is indicated with your abstract.

We will use the same space for informal networking between sessions and for the BYOB (Bring Your Own Beverage) social on Friday night.

If you're new to Gather, the best thing to do is to click on the link and take a look around! Use your arrows to navigate and take advantage of the hints on the screen (e.g. you'll press "x" to interact with posters). Just like an in-person conference, you will be able to see and hear people who are near to you in the room. Some spaces, like the areas around each poster, are set up so that only people standing in the marked area can hear each other (this cuts down on extra noise from people walking by). Have fun personalizing your avatar and interacting with other conference attendees.

ACCESSIBILITY AND INCLUSION

Accessibility

The talks will be carried out via Zoom and their accessibility tips are found here: https://zoom.us/accessibility. Gather (used for poster session and socials) has accessibility tips here: https://support.gather.town/help/accessibility . If there is something we can do to make the conference more accessible to You, please email vbooth@mun.ca and we'll do our best. If there are accommodations we should have thought about and baked into the conference planning earlier on, we'd appreciate it if you'd share your wisdom with us (vbooth@mun.ca) – we'd like to learn from you and will also pass the information on to future conference organizers.

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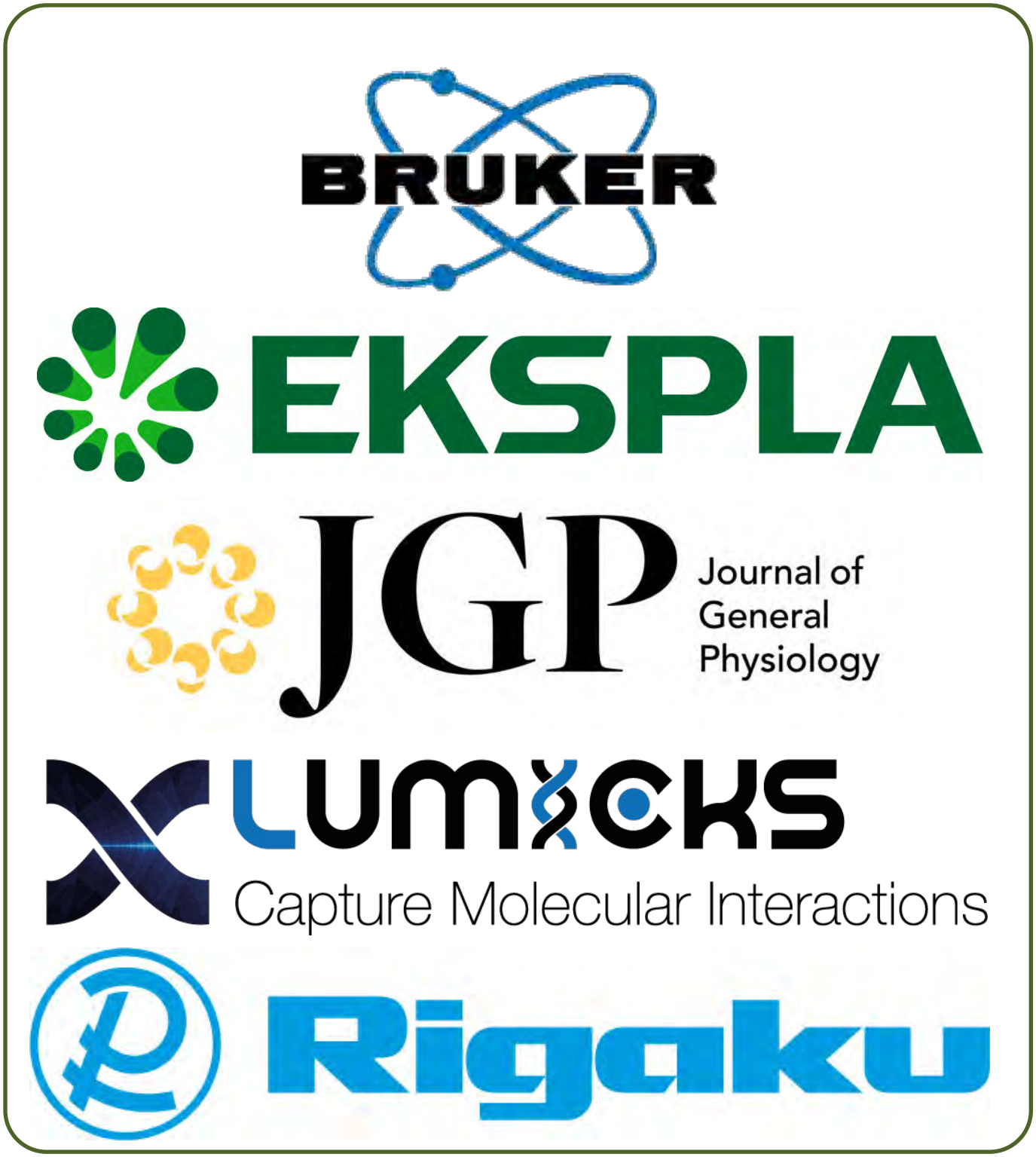

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Program Overview

All times are in **Atlantic Daylight Time (ADT)**. Presentations will take place via Zoom, while networking and poster sessions will occur in Gather.

Tuesday, May 25th – Trainee symposium – Zoom link	
12:30 – 12:35 pm	**Trainee symposium welcome & opening remarks**
12:35 – 2:10 pm	**Session 1: Career talks**
2:10 – 2:30 pm	**Break**
2:30 – 4:20 pm	**Session 2: Trainee talks**
Wednesday, May 26th – Zoom link	
12:30 – 12:45 pm	**Opening remarks & president's message**
12:45 – 1:35 pm	**Plenary talk:** Albert Stolow (University of Ottawa & National Research Council Canada)
1:35 – 2:25 pm	**Session 1: Molecular interactions & assembly** Trushar Patel (University of Lethbridge) Laurent Kreplak (Dalhousie University)
2:25 – 2:40 pm	**Contributed talk:** Rachael Mansbach (Concordia University)
2:40 – 4:00 pm	**Poster session and exhibits (Gather link)** ***Odd numbered poster presentation & judging***
4:00 – 5:15 pm	**Session 2: Lipids & membranes** Ruud Veldhuizen (Western University) Sharon Rozovsky (University of Delaware) Isabelle Marcotte (Université du Québec à Montréal)
5:15 – 5:30 pm	**Contributed talk:** Lucien Weiss (Polytechnique Montréal)
5:30 – 6:00 pm	**2020 Young Investigator Award:** Sabrina Leslie (University of British Columbia)
Thursday, May 27th – Zoom link	
12:30 – 1:45 pm	**Session 3: Biomolecule structure & dynamics** Sarah Rauscher (University of Toronto Mississauga) Katherine Borden (Institute for Research in Immunology and Cancer) Joanne Lemieux (University of Alberta)
1:45 – 2:30 pm	**BSC Trainee Award Session** Paper Award: Greg Gomes (University of Toronto Mississauga) Paper Award: Baljyot Parmar (McGill University) PhD Thesis Award: Ernest Awoonor-Williams (Memorial University of Newfoundland)
2:30 – 4:00 pm	**Poster session and exhibits (Gather link)** ***Even numbered poster presentation & judging***
4:00 – 4:50 pm	**Session 4: Single molecule biophysics** Vincent Tabard-Cossa (University of Ottawa) Isaac Li (University of British Columbia – Okanagan)

4:50 – 5:05 pm	**Contributed talk:** Aidan Brown (Ryerson University)
5:05 – 6:00 pm	**National Lecture:** John Rubinstein (The Hospital for Sick Children)
Friday, May 28th – Zoom link	
12:30 – 1:45 pm	**Session 5: Outside the box** Andreas Hilfinger (University of Toronto Mississauga) Tanya Dahms (University of Regina) Styliani Constas (Western University)
1:45 – 2:35 pm	**Plenary talk:** Catherine Royer (Rensselaer Polytechnic Institute)
2:45 – 4:00 pm	**BSC Annual General Meeting (Zoom link)**
4:00 – 5:15 pm	**Session 6: Spectroscopy & imaging** Chris Xu (Cornell University) Valerică Raicu (University of Wisconsin Milwaukee) Rikard Blunck (Université de Montréal)
5:15 – 5:45 pm	**2021 Young Investigator Award:** David Sivak (Simon Fraser University)
5:45 – 6:00 pm	**Awards and final remarks**
6:00 pm onwards	**BYOB social (Gather link)**

Scientific Program

All times are **Atlantic Daylight Time (ADT)**. All presentations will take place via Zoom, while networking and poster sessions will occur in Gather.

Tuesday, May 25th – Trainee Symposium – Zoom link	
12:30 – 12:35 pm	**Trainee symposium – Welcome & opening remarks**
12:35 – 2:10 pm	**Session 1: Career talks**
12:35	**Clarissa Sit** – Saint Mary's University (*Academia*)
1:05	**Camilo Martinez-Farina** – National Research Council (*Government*)
1:35	**Darren Anderson** – Vive Crop Protection (*Industry*)
2:10 – 2:30 pm	**Break**
2:30 – 4:15 pm	**Session 2: Trainee talks**
2:30	**Thomas Tsangaris** (University of Toronto) *"Protein Conformational Ensembles for a Disordered Protein Restricted by Separate Biophysical Experiments"*
2:45	**Lizzy Baker** (Dalhousie University) *"Functionalized Spider Silk Biomaterials Promote Neurite Outgrowth in PC12 Cells"*
3:00	**Sabastian Himbert** (McMaster University) *"Erythro-Vlps: Embedding Sars-Cov-2 Spike Proteins In Red Blood Cell Based Proteoliposomes Leads To Pronounced Antibody Response In Mouse Models"*
3:15	**James Otis** (University of Toronto) *"Correlating solvent interactions, structure and LLPS behaviour of resilin-derived polypeptides"*
3:30	**Jeffrey Simmons** (Dalhousie University) *"Probing Structural Modularity and Disorder in Recombinant Pyriform Silk"*
3:45	**Emily Prowse** (McGill University) *"Huntingtin S421 phosphorylation and overexpression differentially affect signalling and degradative intracellular cargoes"*
4:00	**Teo Ruijie** (University of Calgary) *"Modulation of Phospholipid Bilayer Properties by Simvastatin"*
4:15 – 4:20 pm	**Closing remarks**

All times are **Atlantic Daylight Time (ADT)**. All presentations will take place via Zoom, while networking and poster sessions will occur in Gather.

Wednesday, May 26th – Zoom link	
12:30 – 12:45 pm	**Opening remarks & president's message** David Langelaan (Dalhousie University & BSC2021 co-organizer) Zoya Leonenko (University of Waterloo & BSC President)
12:45 – 1:35 pm	**Plenary talk** Chair: Danielle Tokarz (Saint Mary's University) **Albert Stolow** (University of Ottawa & National Research Council Canada**)** "*Coherent Nonlinear Raman Microscopy"*
1:35 – 2:25 pm	**Session 1: Molecular interactions & assembly** Chair: David Langelaan (Dalhousie University)
1:35	**Trushar Patel** (University of Lethbridge) *"Towards developing treatment for hepatitis B virus infection"*
2:00	**Laurent Kreplak** (Dalhousie University) "*Mechanical signature of Collagen fibril's twist*"
2:25 – 2:40 pm	**Contributed talk: Rachael Mansbach** (Concordia University) "*The SARS-CoV-2 Spike Variant D614G Favors an Open Conformational State"*
2:40 – 4:00 pm	**Poster session and exhibits (Gather link)** ***Odd numbered poster presentation & judging***
4:00 – 5:15 pm	**Session 2: Lipids & membranes** Chair: Valerie Booth (Memorial University of Newfoundland)
4:00	**Ruud Veldhuizen** (Western University) "*Hijacking the biophysical properties of pulmonary surfactant to deliver therapeutic agents to the lung"*
4:25	**Sharon Rozovsky** (University of Delaware) "*The Unconventional Self-Cleavage of Selenoprotein K"*
4:50	**Isabelle Marcotte** (Université du Québec à Montréal) *"In-cell Solid-State NMR to Study the Action Mechanism of Antimicrobial Molecules"*
5:15 – 5:30 pm	**Contributed talk: Lucien Weiss** (Polytechnique Montréal) *"Three-dimensional localization microscopy in live flowing cells"*
5:30 – 6:00 pm	**2020 Young Investigator Award** Chair: Valerie Booth (Memorial University of Newfoundland) **Sabrina Leslie** (University of British Columbia) *"Single-molecule insights for drug discovery and development: the next level of resolution"*

All times are **Atlantic Daylight Time (ADT)**. All presentations will take place via Zoom, while networking and poster sessions will occur in Gather.

Thursday, May 27th – Zoom link	
12:30 – 1:45 pm	**Session 3: Biomolecule structure & dynamics** Chair: Jan Rainey (Dalhousie University)
12:30	**Sarah Rauscher** (University of Toronto Mississauga) *"All-Atom Molecular Simulations of Disordered and Flexible Proteins"*
12:55	**Katherine Borden** (Institute for Research in Immunology and Cancer) *"Cancer cells hijack RNA processing to modify the message: structural insights"*
1:20	**Joanne Lemieux** (University of Alberta) *"N-Terminal finger stabilizes the S1 pocket for the reversible drug GC376 in the SARS CoV-2 M^{pro} dimer"*
1:45 – 2:30 pm	**BSC Trainee Awards** Chair: Leonid Brown (University of Guelph)
1:45	**Paper Award: Greg Gomes** (University of Toronto Mississauga) *"Conformational ensembles of an intrinsically disordered protein consistent with NMR, SAXS, and single-molecule FRET"*
2:00	**Paper Award: Baljyot Parmar** (McGill Univeristy) *"Clusters of bacterial RNA polymerase are biomolecular condensates that assemble through liquid-liquid phase separation"*
2:15	**PhD Thesis Award: Ernest Awoonor-Williams** (Memorial University of Newfoundland) *"Modelling Covalent Modification of Druggable Cysteines in Enzyme Targets"*
2:30 – 4:00 pm	**Poster session and exhibits (Gather link)** ***Even numbered poster presentation & judging***
4:00 – 4:50 pm	**Session 4: Single molecule biophysics** Chair: David Langelaan (Dalhousie University)
4:00	**Vincent Tabard-Cossa** (University of Ottawa) *"Transport of DNA nanostructures through solid-state nanopores - Toward biosensing and molecular information storage applications"*
4:25	**Isaac Li** (University of British Columbia – Okanagan) *"Quantifying fast molecular adhesion by fluorescence footprinting"*
4:50 – 5:05 pm	**Contributed talk: Aidan Brown** (Ryerson University) *"Switch-like mRNA localization to mitochondria arises from nonequlibrium protein translation effects"*
5:05 – 6:00 pm	**National Lecture** Chair: David Langelaan (Dalhousie University) **John Rubinstein** (The Hospital for Sick Children & University of Toronto) *"Macromolecular machines at energized membranes"*

All times are **Atlantic Daylight Time (ADT**). All presentations will take place via Zoom, while networking and poster sessions will occur in Gather.

Friday, May 28th – Zoom link	
12:30 – 1:45 pm	**Session 5: Outside the box** Chair: Valerie Booth (Memorial University of Newfoundland)
12:30	**Andreas Hilfinger** (University of Toronto Mississauga) *"Can we analyze complex cellular processes two molecules at a time?"*
12:55	**Tanya Dahms** (University of Regina) *"Correlative atomic force-laser scanning confocal microscopy quantifies the impact of cell stressors in real-time"*
1:20	**Styliani Consta** (Western University) *"Stability of protein assemblies in droplets"*
1:45 – 2:35 pm	**Plenary talk** Chair: Jan Rainey (Dalhousie University) **Catherine Royer** (Rensselaer Polytechnic Institute) *"Pressure based mapping of biomolecular conformational landscapes"*
2:45 – 4:00 pm	**BSC Annual General Meeting (Zoom link)**
4:00 – 5:15 pm	**Session 6: Spectroscopy & imaging** Chair: Danielle Tokarz (Saint Mary's University)
4:00	**Chris Xu** (Cornell University) *"Multiphoton Microscopy for Imaging Deeper, Wider, and Faster"*
4:25	**Valerică Raicu** (University of Wisconsin-Milwaukee) *"Fluorescence fluctuations- and energy transfer-based methods for probing protein oligomerization in living cells"*
4:50	**Rikard Blunck** (Université de Montréal) *"Investigating fast inactivation in voltage-gated potassium channels using fluorescence spectroscopy"*
5:15 – 5:45 pm	**2021 Young Investigator Award** Chair: David Langelaan (Dalhousie University) **David Sivak** (Simon Fraser University) *"Flexible machine linkages maximize nonequilibrium energy transduction"*
5:45 – 6:00 pm	**Poster award presentations & final remarks** David Langelaan (Dalhousie University & BSC2021 co-organizer) Nancy Forde (Simon Fraser University & BSC President)
6:00 pm onwards	**BYOB social (Gather link)**

TALK ABSTRACTS

Plenary Talk – Dr. Albert Stolow

Albert Stolow is the Canada Research Chair in Molecular Photonics and Professor of Physics & Chemistry at the University of Ottawa. He founded the Molecular Photonics Group within the National Research Council Canada where he maintains an ongoing collaborative research program. He is Adjunct Professor of Chemistry and of Physics at Queen's University in Kingston. Stolow is also a Fellow of the Max-Planck-uOttawa Centre for Extreme and Quantum Photonics. His research interests include ultrafast molecular dynamics, quantum control, strong field physics of polyatomic molecules, and coherent non-linear optical microscopy.

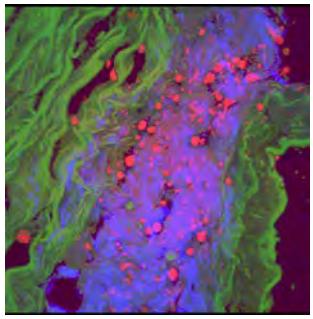
Albert Stolow studied at Queen's University and then obtained his Ph.D. degree in Chemical Physics from the University of Toronto in 1988, under Nobel Laureate John C. Polanyi. Stolow was an NSERC post-doctoral fellow at the University of California, Berkeley from 1989-1992 where he worked with Nobel Laureate Yuan T. Lee. In fall 1992, Stolow joined the National Research Council in Ottawa, rising to the position of Group Leader and Principal Research Officer. In 2014, he assumed the Canada Research Chair in Molecular Photonics at the University of Ottawa. Stolow is a Fellow of both the American Physical Society (2008) and the Optical Society of America (2009). He has won several national prizes including the Earle K. Plyler Prize of the American Physical Society, the Polanyi Award of the Canadian Society for Chemistry, the Queen Elizabeth II Diamond Jubilee Medal (Canada), the Laidler Award of the Canadian Society for Chemistry, and the Barringer Award of the Spectroscopy Society of Canada. Stolow sits on the editorial boards of numerous international journals and on the Advisory Boards of several international research institutions. Stolow was previously Chair of the American Physical Society's Division of Chemical Physics (DCP) and Member of the Executive Committee of the American Physical Society's Division of Laser Science (DLS).

Coherent Nonlinear Raman Microscopy

Albert Stolow[1]

[1]*University of Ottawa & National Research Council Canada*

Coherent Raman Microscopy (CRM), which includes both Coherent Anti-Stokes Raman Scattering (CARS) and Stimulated Raman Scattering (SRS) Microscopies, comprise a suite of label-free, chemical-specific imaging modalities wherein chemical contrast obtains from the natural Raman vibrations of the target molecule. Over the past decade, CRM has advanced from a laser laboratory experiment to a biomedical research and clinical tool. We discuss fundamentals of this nonlinear optical technique, its advantages and limitations and consider recent developments in coherent optical methods and Machine Learning which have improved sensitivity, speed, tuning range and specificity.



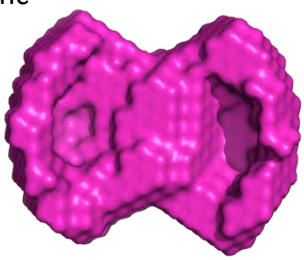
Session 1: Molecular interactions & assembly – Dr. Trushar Patel

TOWARDS DEVELOPING TREATMENT FOR HEPATITIS B VIRUS INFECTION

Maulik D Badmalia[1], Vanessa Meier-Stephenson[1,2] , Simmone D'Souza[2], Gerardo Balderas Figueroa[1], Carla S Coffin,[2,3] and Trushar R Patel[1,2]

[1] Alberta RNA Research and Training Institute, Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, T1K 3M4, Canada
[2] Department of Microbiology, Immunology and Infectious Diseases, Cumming School of Medicine, University of Calgary, Alberta, T2N 4N1, Canada
[3] Department of Medicine, Cumming School of Medicine, Calgary, AB, Canada

Over 250 million people worldwide have chronic infection with the hepatitis B virus (HBV), one of the leading causes of liver cancer. Current therapies block HBV at later steps in the virus life cycle but do not affect HBV cccDNA (covalently closed circular DNA), often leading to relapse once therapy is stopped. Previous studies have demonstrated that human protein Sp1 interacts with a specific site of the HBV cccDNA. This interaction is critical for the formation of the viral core protein. We have recently discovered that the Sp1-binding region of cccDNA forms a highly-ordered G-quadruplex structure using a variety of biophysical techniques. Next, we developed a panel of therapeutic candidates, modified them and have established protocols for their purification. Currently, we are investigating if the panel of therapeutic candidates can be utilized to inhibit HBV replication.



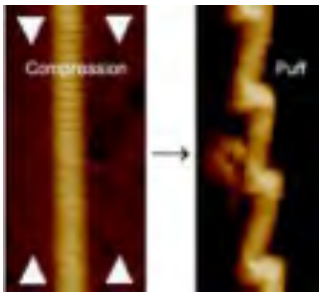
Session 1: Molecular interactions & assembly – Dr. Laurent Kreplak

Mechanical signature of Collagen fibril's twist

Chris J. Peacock[1], Eva Lee[1], Theo Beral[1] and Laurent Kreplak[1,2]

[1] *Department of Physics and Atmospheric Science, Dalhousie University.*

[2] *School of Biomedical Engineering, Dalhousie University.*



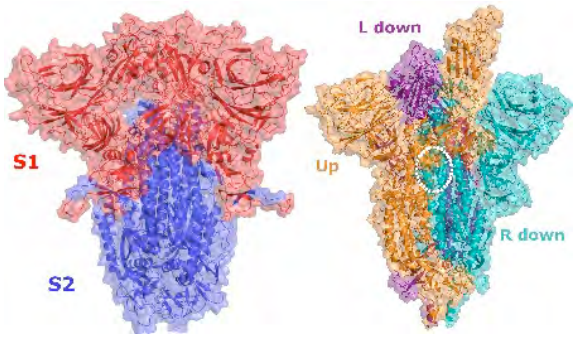
Collagen fibrils are good examples of nanoscale biological ropes. Like man-made laid ropes, fibrils are typically loaded in tension and due to their large aspect ratio, they are in principle prone to buckling and torsional instabilities. Tensile failure of single fibrils hints at the existence of a torsional instability due to axial compression. To explore this effect further we attach single collagen fibrils to a stretched elastic substrate that is then returned it to its original length. The observed behavior depends on the degree of hydration. By going from buckling in ambient condition to immersed in a buffer, fibrils go from the well-known sine wave response to a localized behavior reminiscent of the bird-caging of laid ropes. In addition, in ambient condition, the sine wave response coexists with the formation of loops along the length of the fibrils as observed for the torsional instability of a twisted filament when tension is decreased. This work provides direct evidence that single collagen fibrils are highly susceptible to axial compression because of their helical supermolecular structure. We think that this result provides a molecular mechanism for the development of mechanical damage in tendons due to repetitive physical activities.

Contributed Talk – Rachael Mansbach

The SARS-CoV-2 Spike Variant D614G Favors an Open Conformational State

Rachael A. Mansbach[1§], Srirupa Chakraborty[1], Kien Nguyen[1], David C. Montefiori[2], Bette Korber[1], Gnana Gnanakaran[1].

[1]*Theoretical Biology and Biophysics, Los Alamos National Laboratory*
[2]*Surgery, Duke Human Vaccine Institute, Durham, NC, USA.*
[§]*Current affiliation: Physics, Concordia University, Montreal, Quebec, Canada*



The COVID-19 pandemic is an international emergency caused by the SARS-CoV-2 virus, which binds with its Spike protein to the human ACE2 receptor. In 2020, researchers observed the emergence of a single amino acid variant at residue 614 of the Spike, in which the aspartic acid of the original "D-form" was replaced by a glycine in the emergent "G-form." The G-form rapidly became dominant, displaying heightened infectivity and transmissibility. To understand the molecular mechanisms governing such a drastic change, we performed extensive all-atom simulations of the Spike. For both forms, we simulated the "all down" conformational state, which is not infection-capable, and the "one up" state, which is. We show that an emergent symmetry in the inter-protomer contacts likely causes a heightened population of Spikes in the one up state in the G-form. While there is no significant difference between the exposure of the ACE2 binding site between the D- and G-forms, there is a difference when comparing the all down and one up states: a heightened population in the one up state corresponds to higher infectivity. Overall, this work presents molecular-level understanding of the differences between the D- and G-forms that is of crucial importance for vaccine design.

Session 2: Lipids & membranes – Dr. Ruud Veldhuizen

Hijacking the biophysical properties of pulmonary surfactant to deliver therapeutic agents to the lung

Ruud Veldhuizen[1,2,3], Brandon Baer[1], Lynda McCaig[3]

[1]*Department of Physiology and Pharmacology, Western University*
[2] *Department of Medicine, Western University*
[3]*Lawson Health Research Institute, London Ontario.*

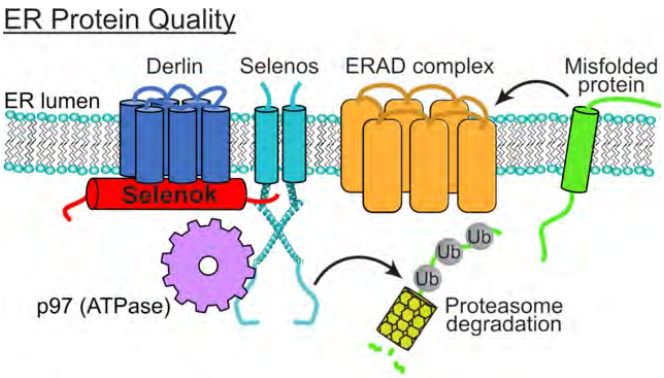
Pulmonary surfactant system is a lipid – protein mixture that lines the inner surface of the lung where it reduces the surface tension to low values thereby enabling breathing with minimal effort. Surfactant has biophysical properties that allow it to adsorb and spread rapidly at an air-liquid interface, which, in vivo, translates to distributing throughout the lung and opening-up collapsed airways and alveoli. This ability of surfactant has inspired the hypothesis that this material can be utilized as to deliver pulmonary therapeutics to remote areas of the lung. To test this hypothesis, we utilized a wet-bridge between two Teflon-wells to assess the functionality of the surfactant to spread to across an air-liquid interface as well as the function of the therapeutic at a remote location. Two therapeutics were examined: glucocorticoids and host defense peptides. Our results showed excellent surfactant spreading and function of glucocorticoids. Although surfactant allowed for spreading of host defense peptides, the function of these peptides was inhibited by the surfactant. This inhibition could be overcome by alterations of the surfactant lipid composition. It is concluded that due to its biophysical properties, pulmonary surfactant can be utilized as a delivery vehicle for respiratory drugs.

Session 2: Lipids & membranes – Dr. Sharon Rozovsky

The Unconventional Self-Cleavage of Selenoprotein K

Rujin Cheng[1], Sharon Rozovsky[1]

[1]*Department of Chemistry and Biochemistry, University of Delaware*



Through known association with other proteins, human selenoprotein K (selenok) is currently implicated in the palmitoylation of proteins, degradation of misfolded proteins, innate immune response, and the life cycle of SARS-CoV-2 virus. However, neither the catalytic function of selenok's selenocysteine (Sec), which, curiously, resides in an intrinsically disordered protein segment nor selenok's specific role in these pathways are known to date. This report casts these questions in a new light as it describes that selenok is able -both *in vitro* and *in vivo*- to cleave some of its own peptide bonds. The cleavages not only release selenok segments that contain its reactive Sec, but as the specific cleavage sites were identified, they proved to cluster tightly near sites through which selenok interacts with protein partners. Furthermore, it is shown that selenok's cleavage activity is neither restricted to itself nor promiscuous but selectively extends to at least one of its protein partners. Together, selenok's cleavage ability and its features have all hallmarks of a regulatory mechanism that could play a central role in selenok's associations with other proteins and its cellular functions overall.

Session 2: Lipids & membranes – Dr. Isabelle Marcotte

In-cell Solid-State NMR to Study the Action Mechanism of Antimicrobial Molecules

Zeineb Bouhel[1,2], Marwa Laadhari[1], Alexandre A. Arnold[1], Réjean Tremblay[2], Dror E. Warschawski[3] and Isabelle Marcotte[1]

[1]Department of Chemistry, Université du Québec à Montréal
[2]Institut des Sciences de la Mer, Université du Québec à Rimouski
[3]Laboratoire des Biomolécules, CNRS UMR 7203, Sorbonne Université

Solid-state nuclear magnetic resonance (SS-NMR) can provide valuable insights on the structure and dynamics of cell constituents, without alteration of the cell integrity. To circumvent the complexity of intact cells, specific labelling can be performed to obtain molecular-level information on particular parts of the cell. In our work, deuteration of the lipid chains in bacterial membranes was combined to ^{2}H SS-NMR to determine the action mechanism of antimicrobial agents. More specifically, we have investigated the effect of the antimicrobial peptides (AMPs) caerin 1.1 and aurein 1.2, on *E. coli* and *B. subtilis*, and the action of the microalgal pigment marennine on the marine bacteria *V. splendidus*. The deuteration protocol established for these bacteria using deuterated palmitic acid will be presented. Our approach evidences the effect of AMPs on the lipid chain order and, in the case of *B. subtilis*, a likely interaction with negatively-charged teichoic acids. *A contrario* to polymyxin B – a well-characterized Gram(-)-interacting antibiotic used in aquaculture - marennine was shown to act through a stiffening mechanism, possibly implying an interaction with lipopolysaccharides. Overall, the nature of the cell wall is shown to play a key role in AMP-membrane interactions, thus highlighting the importance of studying intact bacteria.

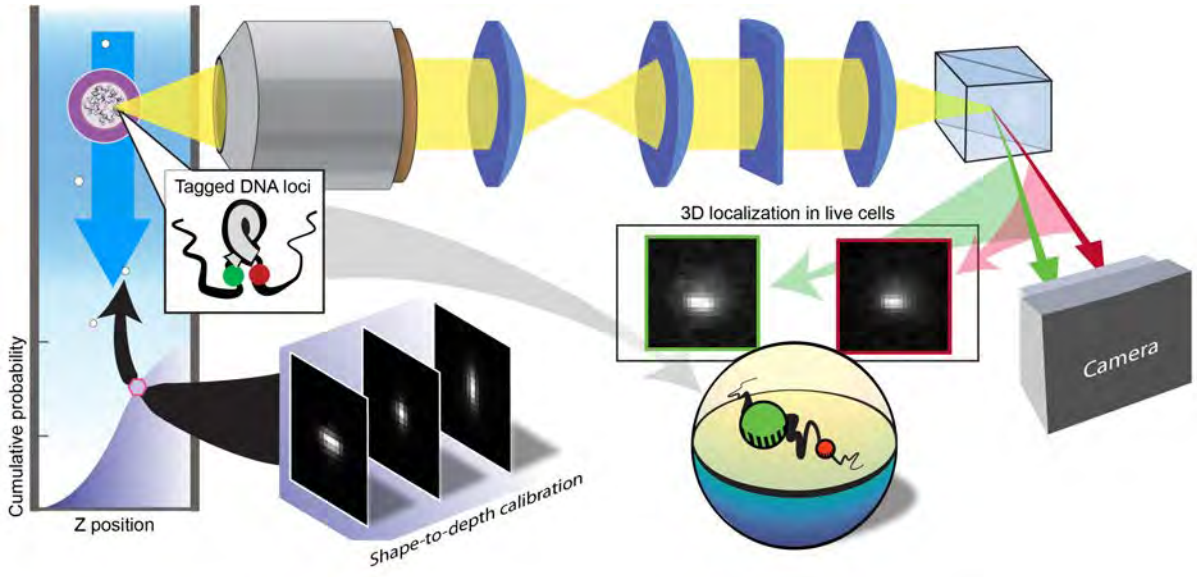
Contributed Talk – Lucien Weiss

Three-dimensional localization microscopy in live flowing cells

Lucien E. Weiss[1,2], Yael Shalev Ezra[2], Sarah Goldberg[2], Yael Shalev Ezra[2], Boris Ferdman[2,3], Omer Adir[3,4], Avi Schroeder[4], Onit Alalouf[2], Yoav Shechtman[2]

[1]*Department of Engineering Physics, Polytechnique Montreal*
[2]*Department of Biomedical Engineering, Technion – Israel Institute of Technology*
[3]*Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology*
[3]*Department of Chemical Engineering, Technion – Israel Institute of Technology*

Localizing subcellular objects in 3D at high throughput is both a major challenge in microscopy and essential for robustly characterizing nanoscale dynamic changes in populations of cells. Here we demonstrate an extremely high throughput approach for 3D colocalization of fluorescent emitters by combining two technologies, point-spread function engineering and imaging flow cytometry. We first encode the depth of fluorescent emitters into the image shape formed on the camera of a flow-imaging system and leverage the statistical properties of laminar flow to calibrate each measurement. This is accomplished by adding a calibration standard, i.e. nanoscale fluorescent beads, to the imaging buffer, then analyzing the distribution of image shapes present in collected images. This calibration is then applied to sample images thus attaining 3D localizations. We validate our approach by co-localizing fluorescent beads in six color channels with tens of nanometer precision. Next, to demonstrate the method in live-cell imaging, we characterize chromatin compaction states in live yeast cells at unprecedented throughput: 1000s of cells per minute. Finally, we show that our technique is generally applicable to other engineered PSFs which can be used to further improve the depth range.



Young Investigator Award 2020 - Sabrina Leslie

Professor Sabrina Leslie studied physics and mathematics at the University of British Columbia before moving to UC Berkeley where she obtained her PhD in optical and atomic physics, followed by a post-doctoral fellowship in biophysics at Harvard University. There she invented a tether-free, high-throughput single-molecule imaging technology called Convex Lens-induced Confinement (CLiC), which established her as a pioneer in single-molecule investigations. In 2012, she became an Assistant Professor at McGill University and founded her research group in the Physics Department. There she developed CLiC into a platform technology and used it for new single-molecule studies of nucleic acids, proteins, polymers, nanomaterials, biologics and cells. After promotion to Associate Professor with tenure in 2017, she decided to expand and commercialize her technology and key applications by co-founding a start-up company ScopeSys, with a technical and business team based in Canada. In 2019 she was honored with the Young Investigator Award from the Biophysical Society of Canada, and in 2020 she was elected to the Royal Society of Canada (RSC) College of New Scholars. In 2021 she has joined the Michael Smith Labs and Department of Physics at UBC as an Associate Professor.

Single-molecule insights for drug discovery and development: the next level of resolution

Sabrina Leslie, Ph.D.

UBC Physics Dept and Michael Smith Biotechnology Labs, Vancouver, Canada

Molecular interactions lie at the core of biochemistry and biology, and their understanding is crucial to the advancement of biotechnology, therapeutics, and diagnostics. Most existing tools make “ensemble” measurements and report a single result, typically averaged over millions of molecules or more. These measurements can miss rare events, averaging out the natural variations or sub-populations within biological samples, and consequently obscure insights into multi-step and multi-state reactions. The ability to make robust and quantitative measurements on single molecules, cellular complexes and cells is a critical unmet need. In this talk, I will introduce a general method called “CLiC” imaging to image molecular interactions one molecule at time with precision and control, and under cell-like conditions. CLiC works by mechanically confining molecules to the field of view in an optical microscope, isolating them in nanofabricated features, and eliminates the complexity and potential biases inherent to tethering molecules. By directly imaging the trajectories of many single molecules simultaneously and in a dynamic manner, CLiC allows us to investigate and discover the design rules and mechanisms which govern how therapeutic molecules (or molecular probes) interact with target sites on nucleic acids; and how molecular cargo is released inside cells from lipid nanoparticles. In this talk, I will discuss applications of our single-molecule platform to help develop and understand emerging classes of genetic medicines as well as gene editing and drug delivery systems; and highlight current and potential future applications to connect our observations from the level of single molecule to single cells.

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References:

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Leslie, et. al. Single-molecule imaging of the biophysics of molecular interactions with precision and control, in cell-like conditions, and without tethers. *Current Opinion in Biomedical Engineering* 2019, 12:75–82.
S. Scott, et. al. Single-molecule visualization of the effects of ionic strength and crowding on structure-mediated interactions in supercoiled DNA molecules. *Nucleic Acids Research* 2019, 0305-1048.

M. Shayegan, et. al. Probing inhomogeneous diffusion in the microenvironments of phase-separated polymers under confinement. *JACS* 2019, 141 (19), 7751-7757.

S. Scott, et. al. Visualizing Structure-mediated Interactions in Supercoiled DNA Molecules. *Nucleic Acids Research* 2018, 46(9), 4622-431.

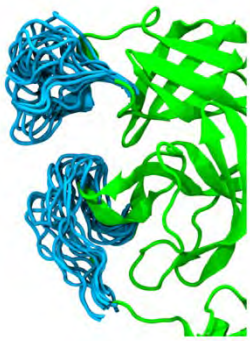
Session 3: Biomolecule structure & dynamics – Dr. Sarah Rauscher

All-Atom Molecular Simulations of Disordered and Flexible Proteins

Ethan Lee[1, 3], Sarah Rauscher[1, 2, 3]

[1] Department of Chemical and Physical Sciences, University of Toronto Mississauga
2 Department of Physics, University of Toronto
3 Department of Chemistry, University of Toronto

Molecular dynamics (MD) simulations are a useful tool to investigate the structure and dynamics of proteins because they can provide an all-atom view of their complex conformational landscapes. Using MD simulations, we can identify highly populated conformational states and determine the effects of perturbations (e.g. mutation, ligand binding, solution conditions) on their populations. I will present recent and ongoing simulation studies of disordered and flexible proteins, including the disordered loops of the main protease of SARS-CoV-2.



Session 3: Biomolecule structure & dynamics – Dr. Katherine Borden

Cancer cells hijack RNA processing to modify the message: structural insights

Laurent Volpon, Lucy Coutinho de Oliveira*, Michael J. Osborne, Katherine L.B. Borden

Institute for Research in Immunology and Cancer, University of Montreal, QC
**Current address: NMX Research and Solutions, Laval, QC*

Cancer cells hijack a variety of RNA processing mechanisms to hijack the cell. These can induce proliferation and survival programming. We use the eukaryotic translation initiation factor eIF4E as a model factor that modifies multiple steps in RNA metabolism. eIF4E amplifies transcriptional signals by increasing the nuclear RNA export and translation of specific transcripts that engage oncogenic programmes. However, recent studies reveal that eiF4E also changes the chemical nature of the transcript. Specifically, eIF4E alters the capping of transcripts with 7-methyl guanosine (m^7G), splicing and polyadenylation of selected transcripts. Recently, m^7G capping was shown to be a “tunable” property of RNAs which can be dysregulated in cancer. Here, we studied the biochemical and structural principles related to eIF4E-dependent m^7G RNA capping. We found that eIF4E directly binds the key enzyme involved in cap maturation and positioned to modify its enzymatic activity. We also found, for the first time, evidence that a protein could substitute for the m^7G cap using a potyviral protein-RNA conjugate. This suggests that there are multiple means for RNAs to engage eIF4E. In all, our studies provide a biochemical basis for novel aspects of RNA metabolism. These are directly related to oncogenic properties associated with eIF4E.

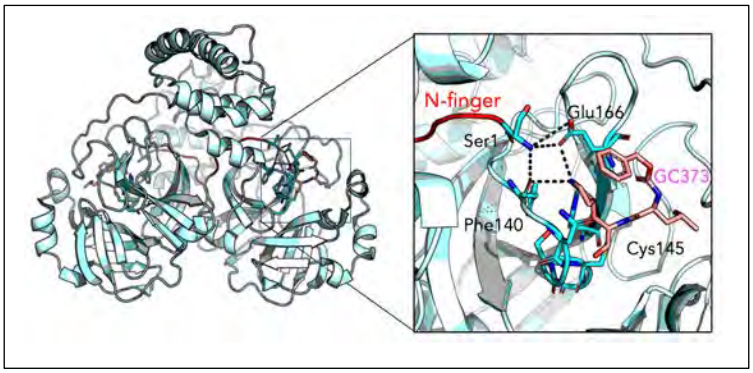
Session 3: Biomolecule structure & dynamics – Dr. Joanne Lemieux

N-Terminal finger stabilizes the S1 pocket for the reversible drug GC376 in the SARS-CoV-2 M^{pro} dimer

Elena Arutyunova[1,4], Muhammad Bashir Khan[1], Conrad Fischer[2†], Jimmy Lu[1,4], Tess Lamer[2], Wayne Vuong[2], Marco J. van Belkum[2], Ryan T. McKay[2], D. Lorne Tyrrell[3,4], John C. Vederas[2], Howard S. Young[1], M. Joanne Lemieux[1,4]

[1] Department of Biochemistry, Faculty of Medicine and Dentistry, Membrane Protein Disease Research Group, University of Alberta, Edmonton T6G 2R3, Alberta, Canada
[2]Department of Chemistry, Faculty of Science, University of Alberta, Edmonton T6G 2G2, Alberta, Canada
[3]Department of Medical Microbiology and Immunology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton T6G 2E1, Alberta, Canada
[4]Li Ka Shing Institute of Virology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton T6G 2E1, Alberta, Canada
†Present address: Department of Physical Sciences, Barry University, 11300 NE 2nd Ave., Miami Shores FL 33161, USA

The main protease (M^{pro}, also known as 3CL protease) of SARS-CoV-2 is a high priority drug target in the development of antivirals to combat COVID-19 infections. A feline coronavirus antiviral drug, GC376, has been shown to be effective in inhibiting the SARS-CoV-2 main protease and live virus growth. As similar drugs move into clinical trials, further characterization of GC376 with the main protease of coronaviruses is required to gain insight into the drug's properties, such as reversibility and broad specificity. Here we demonstrate that GC376 has nanomolar K_i values with the M^{pro} from both SARS-CoV-2 and SARS-CoV strains. Restoring enzymatic activity after inhibition by GC376 demonstrates reversible binding with both proteases. Stability and thermodynamic parameters of both proteases revealled higher stability for SARS-CoV-2 M^{pro}. The comparison of a new X-ray crystal structure of M^{pro} from SARS-CoV complexed with GC376 reveals similar molecular mechanism of inhibition compared to SARS-CoV-2 M^{pro}, and gives insight into the broad specificity properties of this drug. In both structures, we observe domain swapping of the N-termini in the dimer of the M^{pro}, which facilitates coordination of the drug's P1 position. These results validate that GC376 is a drug with an off-rate suitable for clinical trials.



BSC Awards – Trainee Paper Award (1/2) - Greg Gomes

Dr. Gregory-Neal Gomes received a B.Eng. in Engineering Physics from McMaster University and his Ph.D. in Physics from the University of Toronto under the supervision of Dr. Claudiu Gradinaru. After his Ph.D., he began postdoctoral research at Yale University in the Department of Pathology in the lab of Dr. Zachary Levine. Here, he is studying protein aggregation diseases by integrating single-molecule fluorescence measurements and computational models of lab-derived and patient-derived pathological aggregates. His research interests are single-molecule fluorescence techniques, integrative structural biology, and neurodegenerative diseases.

Conformational Ensembles of an Intrinsically Disordered Protein Consistent with NMR, SAXS, and Single-Molecule FRET

Gregory-Neal W. Gomes[1,2], Mickaël Krzeminski[3], Ashley Namini[2], Erik W. Martin[4], Tanja Mittag[4], Teresa Head-Gordon[5], Julie D. Forman-Kay[3], Claudiu C. Gradinaru[1,2]

[1]Department of Physics, University of Toronto
[2]Department of Chemical and Physical Sciences, University of Toronto Mississauga
[3]Molecule Medicine Program, Hospital for Sick Children
[4]Department of Structural Biology, St. Jude Children's Research Hospital
[5]Department of Chemistry, University of California Berkley

Protein structures modelled at atomic resolution can provide valuable mechanistic and functional information. For intrinsically disordered proteins (IDPs), which exhibit high conformational entropy and extreme conformational dynamics, an ensemble of atomic resolution structures is necessary for their description. However, the large number of degrees of freedom necessary to specify these ensembles requires the integration of multiple solution biophysical experiments. To date, the determination of these ensembles has focused primarily on Small-Angle X-ray Scattering (SAXS) and Nuclear Magnetic Resonance (NMR) data. In this work, we showed how single-molecule Förster Resonance Energy Transfer (smFRET) data provides important complementary information to SAXS and NMR data. Using the disordered N-terminal region of the Sic1 protein as a test case, we calculated ensembles jointly consistent with SAXS, NMR and smFRET data. Applying this approach to Sic1 and multisite phosphorylated Sic1 (pSic1) revealed subtle changes in the conformational ensemble upon phosphorylation that would be difficult to quantify without an integrative approach.

BSC Awards – Trainee Paper Award (2/2) Balijyot Parmar

I did my undergrad at UofT in physics where I became interested in biophysics during my senior year. I wanted to experience an experimental side of biophysics so I completed my Masters degree in biology at McGill, and am currently a physics PhD student at McGill. Apart from looking at *E. coli* all day, I enjoy playing soccer and painting.

Clusters of bacterial RNA polymerase are biomolecular condensates that assemble through liquid-liquid phase separation

Anne-Marie Ladouceur[a], Baljyot Singh Parmar[1b],Stefan Biedzinski[a], James Wall[a], S. Graydon Tope[a], David Cohn[a], Albright Kim[a], Nicolas Soubry[a], Rodrigo Reyes-Lamothe[a], and Stephanie C. Weber[a,b]

[a]*Department of Biology, McGill University*
[b]*Department of Physics, McGill University*

Once described as mere “bags of enzymes,” bacterial cells are in fact highly organized, with many macromolecules exhibiting non- uniform localization patterns. Yet the physical and biochemical mechanisms that govern this spatial heterogeneity remain largely unknown. Here, we identify liquid–liquid phase separation (LLPS) as a mechanism for organizing clusters of RNA polymerase (RNAP) in Escherichia coli. Using fluorescence imaging, we show that RNAP quickly transitions from a dispersed to clustered localization pattern as cells enter log phase in nutrient-rich media. RNAP clusters are sensitive to hexanediol, a chemical that dissolves liquid-like compartments in eukaryotic cells. In addition, we find that the transcription antitermination factor NusA forms droplets in vitro and in vivo, suggesting that it may nucleate RNAP clusters. Finally, we use single-molecule tracking to characterize the dynamics of cluster components. Our results indicate that RNAP and NusA molecules move inside clusters, with mobilities faster than a DNA locus but slower than bulk diffusion through the nucleoid. We conclude that RNAP clusters are biomolecular condensates that assemble through LLPS. This work provides direct evidence for LLPS in bacteria and demonstrates that this process can serve as a mechanism for intra- cellular organization in prokaryotes and eukaryotes alike.

BSC Awards – Doctoral Thesis Award – Dr. Ernest Awoonor-Williams

Ernest completed his B.Sc (Hons) at Mount Allison University. During his time at MtA, he was fortunate to dabble in multiple research projects in the labs of Khashayar Ghandi, Steve Westcott, and Glen Briand. In 2014, Ernest moved to St. John's, NL to pursue his doctorate at Memorial University. There he worked under the tutelage of Christopher Rowley exploring the use of computational methods in modelling the covalent modification of druggable cysteines in enzyme targets. During his PhD, Ernest was supported by an NSERC Vanier Canada Graduate Scholarship and an advanced research computing fellowship by ACEnet.

Modelling Covalent Modification of Druggable Cysteines in Enzyme Targets

Ernest Awoonor-Williams[1] and Christopher N. Rowley[2]

[1]Department of Chemistry, Memorial University of Newfoundland, St. John's, Canada
[2]Department of Chemistry, Carleton University, Canada

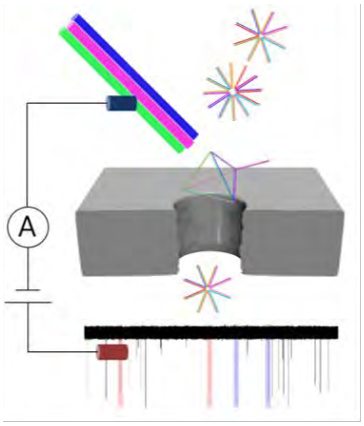
Drugs that bind to their targets covalently have over recent years garnered significant interest in drug discovery owing to their benefits of improved potency and sustained target engagement. Computational modelling plays a crucial role in drug discovery but has not been extensively employed in modelling covalent modifier drugs. In this brief talk, I will highlight our efforts in leveraging advanced computational methods to predict the reactivity and binding mechanism of druggable cysteines in enzyme targets.

Session 4: Single molecule biophysics – Dr. Vincent Tabard-Cossa

Transport of DNA Nanostructures through Solid-State Nanopores - Toward Biosensing and Molecular Information Storage Applications

Vincent Tabard-Cossa

Department of Physics, University of Ottawa, Ottawa, ON, Canada



Many emerging applications in diverse areas such as medical diagnostics and molecular information storage make use of DNA nanotechnology (nanostructures self-assembled via specific base pairing of DNA) to deliver small molecules, detect disease biomarkers, or encode digital information. Here, I present an experimental study of the capture and translocation kinetics of DNA origami nanostructures through solid-state nanopores that has helped us develop a deeper understanding of their behavior in confined environments. Solid-state nanopores are versatile single-molecule sensors to electrically characterize biological molecules at the single-molecule level. Nanopores operate on the simple premise that when a voltage is applied across a pore immersed in a salt solution, the passage of a biomolecule results in a transient blockage in the ionic current that provide information about the identity (size, shape, charge, etc.) of the molecule. Using solid-state nanopores fabricated by controlled dielectric breakdown, ranging from 3 to 20 nm in diameter, the transport of different DNA nanostructures, including 2D star-shaped molecules (multiple-way junctions) and 3D ribbon-like origami structures (helix bundles), are investigated. These nanostructures produce unique translocation signatures modulated by their shape and mechanical properties. These results are used to inform several biosensing and digital data storage applications.

Session 4: Single molecule biophysics – Dr. Isaac Li

Quantifying fast molecular adhesion by fluorescence footprinting

Isaac T.S. Li[1]

[1]*Department of Chemistry, University of British Columbia - Okanagan*

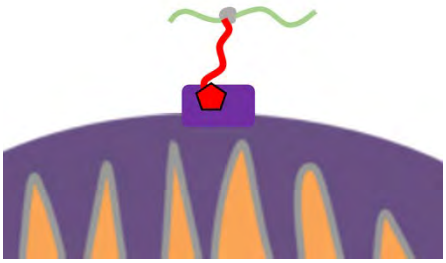
Rolling adhesion is a unique process in which the adhesion events are short-lived and operate under highly non-equilibrium conditions. These characteristics pose a challenge in molecular force quantification, where in situ measurement of such forces cannot be achieved with molecular force sensors that probe near equilibrium. Here, we demonstrated a quantitative adhesion footprint assay combining DNA-based non-equilibrium force probes and modelling to measure the molecular force involved in fast rolling adhesion. We were able to directly profile the ensemble molecular force distribution in our system during rolling adhesion with a dynamic range between 0 – 18 pN. Our results showed that the shear stress driving bead rolling motility directly controls the molecular tension on the probe-conjugated adhesion complex. Furthermore, the shear stress can steer the dissociation bias of components within the molecular force probe complex, favouring either DNA probe dissociation or receptor-ligand dissociation.

Contributed Talk – Aidan Brown

Switch-like mRNA Localization to Mitochondria Arises from Nonequlibrium Protein Translation Effects

Aidan I. Brown[1], Ximena G. Garcia-Arceo[2], Tatsuhisa Tsuboi[2], Brian Zid[2], Elena F. Koslover[3]

[1]*Department of Physics, Ryerson University*
[2]*Department of Chemistry and Biochemistry, University of California, San Diego*
[3]*Department of Physics, University of California, San Diego*



Many mitochondrial genes are encoded in the nucleus, translated in the cytosol, and the proteins imported into mitochondria. In yeast, the fraction of cell volume occupied by mitochondria changes with growth conditions. Some nuclear-encoded mRNA switch from low to high mitochondrial localization as the mitochondrial volume fraction increases, while the localization of other genes remains consistently low or high. mRNA can be effectively tethered to mitochondria via the mitochondrial import of nascent, incompletely translated polypeptides, enhancing mRNA localization to mitochondria. To understand the distinct localization behaviours for mRNA of nuclear-encoded mitochondrial genes, we use a quantitative model of mRNA diffusion around the cell, protein translation along mRNA, and nascent polypeptide tethering. Using this model, we explain how the nonequilibrium nature of protein translation combines with asymmetric diffusive search times to allow for switch-like, consistently low, or consistently high mitochondrial localization.

National Lecturer – Dr. John Rubinstein

A native of Toronto, John Rubinstein received his BSc from the University of Guelph, graduating in 1998. For his PhD he moved to England to work with Dr. Richard Henderson and Sir John Walker at the MRC laboratories in Cambridge. He completed his PhD research and a postdoctoral fellowship before returning to Canada for a second short postdoctoral fellowship at U of T. He started his own research group at The Hospital for Sick Children in 2006, where he holds a Canada Research Chair, and studies the structural biology of bioenergetics and develops new methods in cryoEM to facilitate this work. He has been recognized by the Burton Medal of the Microscopy Society of America, the New Investigator award of the Canadian Society of Molecular Biosciences, the Lars Ernster Lectureship in Bioenergetics, and most recently a Doctorate of Philosophy *honoris causa* from Stockholm University.

Macromolecular machines at energized membranes

John L. Rubinstein[1,2]

[1.] *Molecular Medicine Program, The Hospital for Sick Children*

[2.] *Departments of Biochemistry and Medical Biophysics, The University of Toronto*

In biology, energy is often stored as an electrochemical transmembrane proton gradient. These gradients are established by the electron transport chain during cellular respiration, or proton pumps such as V-ATPases. The proton motive force from these gradients is used to generate ATP or drive secondary transport. We have used cryoEM to study the structure and dynamics of membrane protein complexes involved in these bioenergetic processes. The studies reveal the mechanism of proton translocation in V-ATPases, ATP synthases, and electron transport chain supercomplexes. The discovery of new subunits in V-ATPases links them to multiple processes in mammalian cells. Structures of the electron transport chain supercomplexes and ATP synthase from mycobacteria opens new routes for developing antibiotics to treat mycobacterial infections such as the *Mycobacterium tuberculosis* infection that cause the disease TB.

Session 5: Outside the Box – Dr. Andreas Hilfinger

Can We Analyze Complex Cellular Processes Two Molecules at a Time?

Andreas Hilfinger[1,2,3], Brayden Kell[1], Nava Leibovich[1], Euan Joly-Smith[1], Jerry Wang[4], Timon Wittenstein[1,5]

[1]Department of Physics, University of Toronto
[2]Department of Mathematics, University of Toronto
[3]Department of Cell & Systems Biology, University of Toronto
[4]Division of Biology and Biological Engineering, California Institute of Technology, USA
[5]Department of Physics, Johannes Gutenberg University, Mainz, Germany

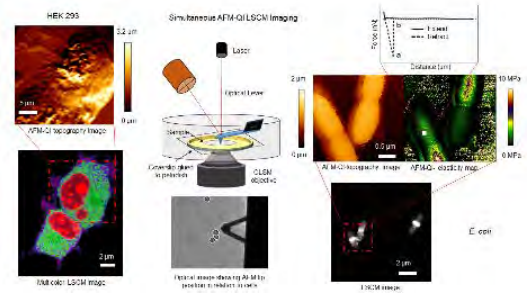
Cellular function is achieved through complex processes that involve many components with many uncharacterized interactions. Our work addresses how to possibly make sense of such systems if only a handful of components are observed at a time. Instead of ignoring or guessing unknown details, we utilize a theoretical framework to rigorously characterize incompletely specified biochemical interaction networks. Analyzing broad classes of systems that share some features but are left to vary arbitrarily in all unknown features allows us to derive fundamental trade-offs that shape biological processes such as bacterial cell fate switches or robust adaptation. Additionally, I will show how invariants for classes of incompletely specified systems can be exploited to infer gene expression regulation dynamics from static fluctuation snapshots of just two genetic reporters. This promises to turn seemingly intractable complex systems into a sequence of solvable inference problems.

Session 5: Outside the Box – Dr. Tanya Dahms

Correlative atomic force-laser scanning confocal microscopy quantifies the impact of cell stressors in real-time

Tanya E. S. Dahms[1], Supriya V. Bhat[1]

[1]*Department of Chemistry and Biochemistry, University of Regina, Regina, SK*



The exponential increase in anthropogenic chemicals in our environment urgently necessitates methods to assess their impact prior to their release for predicting potential environmental and health impacts. Atomic force microscopy (AFM) and laser scanning confocal microscopy (LSCM) each provide abundant information on cell behaviour. In addition to determining cell morphology and surface ultrastructure, AFM in quantitative imaging (QI) mode probes cellular mechanics and surface biochemistry with minimal applied force, and LSCM offers a window into the cell for imaging fluorescently tagged macromolecules. Data from correlative AFM-LSCM is thus complimentary, providing a comprehensive picture of cellular physiology.

I will present a correlative AFM-QI-LSCM assay for the simultaneous real-time imaging of living cells *in situ* that generates real-time multiplexed data, including cell morphology and mechanics, surface adhesion and ultrastructure, along with the localization of intracellular macromolecules. The broad applicability of the assay is demonstrated using disparate cell types, showing altered surface properties, internal molecular arrangement and oxidative stress in model bacterial, fungal and human cells exposed to 2,4-dichlorophenoxyacetic acid. This correlative microscopy assay is broadly applicable to a variety of cell types and can be used to assess the impact of any multitude of contaminants, alone or in combination.

Session 5: Outside the Box – Dr. Styliani Costa

Stability of protein assemblies in droplets

Styliani Consta[1]

[1]*Department of Chemistry, Western University*

Macroion–droplet interactions play a critical role in many settings such as ionization techniques used in native mass spectrometry and ion mobility experiments. These methods are used to detect the conformations and assemblies of biological macromolecules originating from the bulk solution. The droplets under investigation are composed of a polar solvent, primarily water, a charged macroion, and, possibly, buffer ions. The macroion-droplet interactions discovered in ``in-silico'' experiments in the Consta group will be presented. We have determined that, depending on the charge on the macroion and certain macroscopic properties of the solvent, such as its dielectric constant and surface tension, a droplet may obtain striking conformations such as droplets with extruded tails, ``pearl-necklace'' conformations, and multipoint ``star'' shapes. The shapes of the droplet containing the macroion influence the charging mechanism of the macroion and the stability of protein complexes in a reciprocal manner. The studies provide insight in the stabilization of assemblies of proteins and nucleic acids studied in biomolecular mass spectrometry methods.

Plenary Talk – Dr. Catherine Royer

Dr. Royer obtained her Bachelors (Licence) degree in 1979 at the University of Pierre and Marie Curie - Paris 6 in Biochemistry and Chemistry. She obtained her Ph.D. in 1985 in the Department of Biochemistry in the School of Chemical Sciences at the University of Illinois at Urbana-Champaign under the direction of Professor Gregorio Weber. She then carried out postdoctoral studies at the University of Paris 7, the CNRS at Gif-sur-Yvette and at LURE under the direction of Bernard Alpert, Guy Hervé and Jean-Claude Brochon. Upon returning to the US, she took a position as User Coordinator and Research Physicist at the Laboratory for Fluorescence Dynamics in the Department of Physics at the University of Illinois - Urbana Champaign under the direction of Enrico Gratton. In 1990 she moved to an Assistant Professorship in the School of Pharmacy at the University of Wisconsin-Madison, where she was promoted to Associate Professor with tenure in 1995. In 1997 she took the position of INSERM Director of Research in the Center for Structural Biochemistry in Montpellier France where, in 2002, she became Associate Director of the Center and in 2007, Director. In 2013 She moved to Rensselaer Polytechnic Institute as a Professor of Biological Sciences and chaired Constellation Professor in Bioinformatics and Biocomputation.

Dr. Royer is an internationally recognized expert in biological fluorescence and biomolecular interactions. She developed fluorescence anisotropy-based assays for quantification of protein-nucleic acid and protein-peptide interactions and the modulation of these interactions by ligands and drugs. She also developed numerically-based biomolecular interaction analysis software for complex and allosteric effects in protein oligomers. She developed and implemented novel methodologies for quantifying proteins and their interactions in live cells, and the effects of environmental changes and ligands thereon. Her elucidation of the molecular mechanisms of pressure effects on biomolecular structure and stability has allowed for structural and energetic characterization of low-lying excited states of proteins implicated in allosteric regulation of their function.

Pressure based mapping of biomolecular conformational landscapes

Catherine A. Royer[1].

[1]*Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy NY USA 12180*

The mechanisms underlying biomolecular function often implicate structural “excited” states that are less ordered than that obtained from crystallographic studies. Thus, understanding and modulating function requires characterization of functionally relevant excited states. Hydrostatic pressure destabilizes biomolecular structure due to differences in system volume between ordered and disordered states, leading to significant population of excited states. Coupling pressure perturbation with NMR, fluorescence, small angle x-ray scattering and simulations allows for their characterization.

Session 6: Spectroscopy and Imaging – Dr. Chris Xu

Multiphoton Microscopy for Imaging Deeper, Wider, and Faster

Chris Xu[1]

[1]*School of Applied and Engineering Physics, Cornell University*

Multiphoton microscopy has changed how we visualize neurons by providing high-resolution, non-invasive imaging capability deep within intact brain tissue. Multiphoton imaging will likely play a major role in understanding how the brain works at the level of neural circuits. In this talk, in vivo structural and functional imaging of mouse brain using long wavelength excitation and three-photon microscopy will be presented. By quantitative comparison to two-photon microscopy, the application space where 3-photon microscopy outperforms conventional 2-photon microscopy will be defined. In addition, a number of interesting directions, including new laser sources, new spectral windows, optimum illumination schemes, etc., will be presented, and their impact on further improving the imaging depth, volume, or speed in biological tissues will be quantified.

Session 6: Spectroscopy and Imaging – Dr. Valerică Raicu

Fluorescence fluctuations- and energy transfer-based methods for probing protein oligomerization in living cells

Valerică Raicu[1,2]

[1]*Physics Department, University of Wisconsin-Milwaukee*
[2]*Department of Biological Sciences, University of Wisconsin-Milwaukee*

Fluorescence-based methods for probing association of proteins or other biological macromolecules within living cells fall roughly within two groups. One family of methods, based on *Förster Resonance Energy Transfer (FRET)*, probes molecular association by measuring relative distances between molecules within a complex (or oligomer) via transfer of energy from an optically excited to un-excited fluorescent tags attached to the molecules of interest. A second class of methods, generically known as *Fluorescence Fluctuation Spectroscopy (FFS)*, probes fluctuations in fluorescence intensities from pixel to pixel in an image (i.e., spatial fluctuations) or from time to time (i.e., temporal fluctuations) to determine whether the molecules that produced the fluctuations diffuse around as monomers, dimers, or higher order oligomers. In this talk, I will overview our contributions to the development of such methods and their application to the study of oligomerization of membrane receptors in the presence and absence of their natural or artificial ligands. Our studies aim to provide **(i)** biophysicists and life scientists with tools for understanding cellular signaling, and **(ii)** pharmacologists with in-cell assays for probing the effects of natural or artificial ligands (or drugs) on protein-protein interactions.

Session 6: Spectroscopy and Imaging – Dr. Rikard Blunck

Investigating fast inactivation in voltage-gated potassium channels using fluorescence spectroscopy

Rikard Blunck

Department of Physics, Université de Montréal, Montréal, Québec

Voltage-gated potassium channels Kv are widely expressed in many tissues in the organism. In neurons, they are responsible for the repolarization of action potentials as well as determining the excitability of membranes. Several family members of the Kv channel family undergo fast inactivation caused by the block of the conduction pathway by a peptide attached to the N-terminus of either the main (alpha) or auxiliary subunits. Using voltage-clamp fluorometry, we studied two features of fast inactivation in the Shaker Kv channel. We determined the location and activation of the inactivation peptide during the resting and early activation steps. For this purpose, we employed fluorescent unnatural amino acids and Lanthanide-based resonance energy transfer (LRET). We also investigated how the proximity of Kv channels alters fast inactivation.

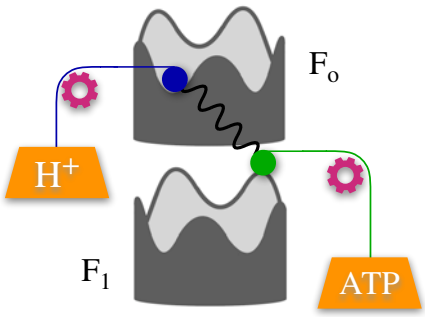
Young Investigator Award 2021 – David Sivak

Dr. David Sivak is Associate Professor of Physics at Simon Fraser University in Vancouver, Canada, an Associate member of the Chemistry and Molecular Biology & Biochemistry departments, and a Tier-II Canada Research Chair. His academic peregrination has included undergraduate degrees in Applied Math from Harvard, and Philosophy, Politics, and Economics from Lincoln College, Oxford; a PhD in biophysics from UC Berkeley; a Physicist Postdoctoral Fellow at Lawrence Berkeley National Lab; and a Systems Biology Fellow at UC San Francisco. His research is in nonequilibrium statistical biophysics, with particular focus on free-energy transduction in biology.

Flexible machine linkages maximize nonequilibrium energy transduction

Emma Lathouwers, Joseph N. E. Lucero, David A. Sivak

Department of Physics, Simon Fraser University



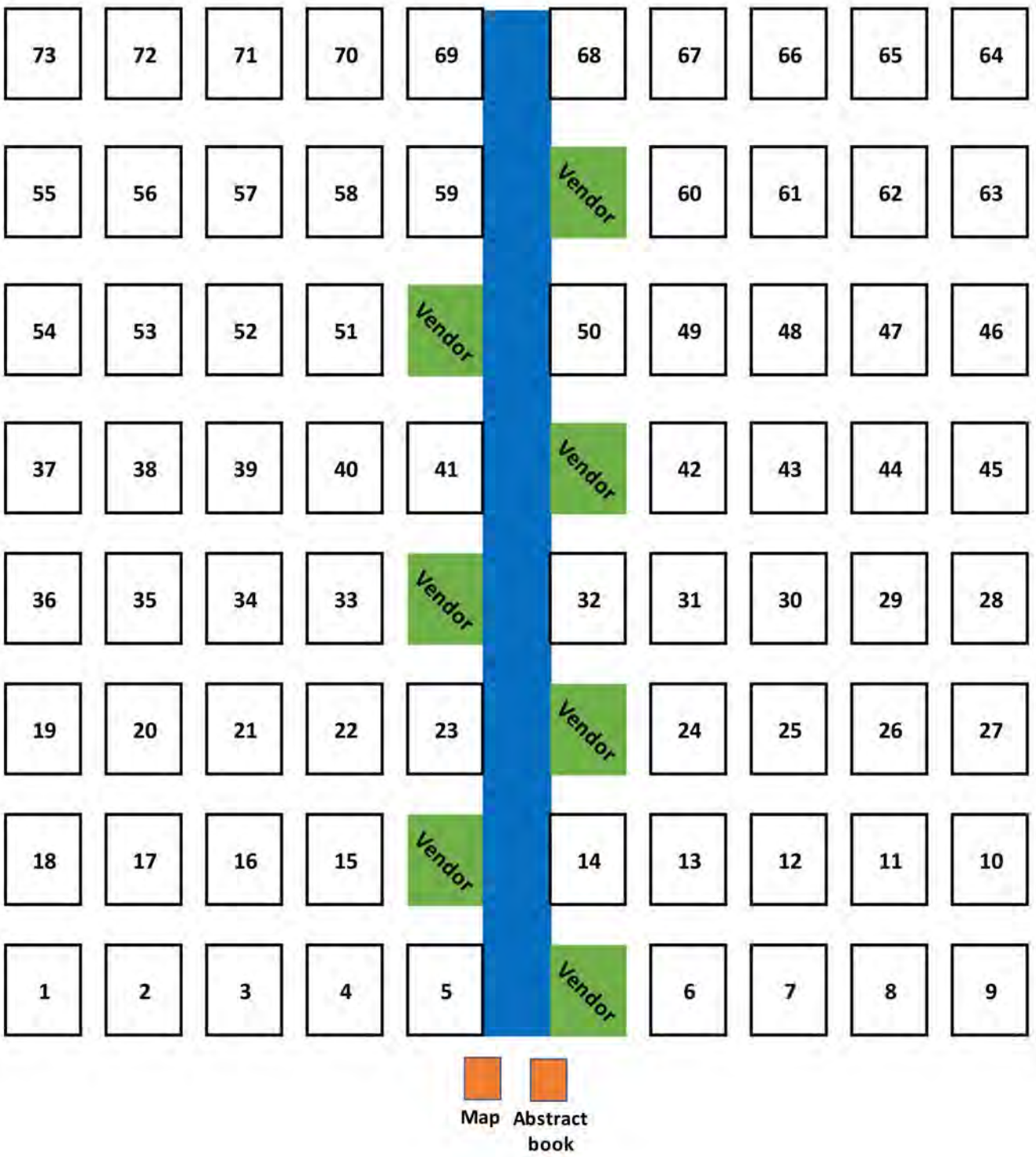
Living systems at the molecular scale are composed of many constituents with strong and heterogeneous interactions, operating far from equilibrium, and subject to strong fluctuations. These conditions pose significant challenges to efficient, precise, and rapid free energy transduction, yet nature has evolved numerous biomolecular machines that do just this. In this talk, I present a simple model of the ingenious rotary machine that makes ATP (the predominant portable energy currency of the cell), where one can investigate the interplay between nonequilibrium driving forces, thermal fluctuations, and the strength of interactions between subsystems. This model reveals nontrivial yet intuitive design principles for effective molecular-scale free energy transduction. Most notably, while tight coupling between machine components is intuitively appealing, output power is in fact maximized at intermediate-strength coupling, which permits lubrication by stochastic fluctuations with only minimal slippage.

ABSTRACTS FOR POSTERS AND TRAINEE SYMPOSIUM TALKS

Note: Presentations are scheduled for odd-numbered posters on Wednesday, May 26 (2:40-4:00 pm ADT) and even-numbered posters on Thursday, May 27 (2:30-4:00 pm ADT).

But, all posters will be available any time throughout the meeting so please feel free to browse the posters and arrange to meet at a poster at any time.

Poster Room Map



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67 - Anna Ananchenko	18 - Hannah Krivic	27 - Emily Prowse
53 - Lizzy Baker	26 - Sarika Kumari	9 - Jayatee Ray
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Poster #59:
Bundling of Rod-Like Colloids via Depletion Forces

Jonathan Adams, Hendrick W. de Haan

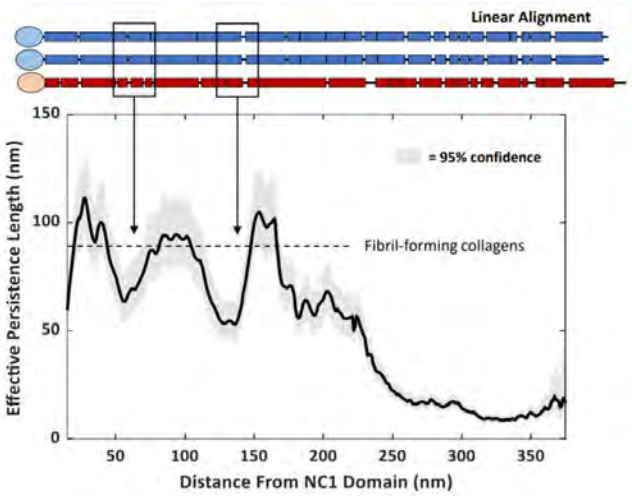
Ontario Tech University

Self assembly of rod-like colloids in to bundles can be driven by entropic processes. We use Coarse Grained Molecular Dynamics simulations to investigate using depletion forces to induce bundling in a suspension of rod-like colloids. The colloids are approximated as bead-spring polymers, with persistence lengths much larger than the length of the polymer. Depletion forces are an effective attractive force between colloids due to the presence of particles significantly smaller than the colloids, called depletants. Depletion forces are modelled as an implicit entropic force between colloids. Bundling begins to occur when the occupied volume fraction occupied by depletants is above a critical value. This volume fraction is dependent on the length and stiffness of the rod-like colloids. We investigate using this property to induce bundling only in rods above a certain length or certain persistence length.

Poster #44:
Sequence-dependent analysis of collagen mechanics

Alaa Al-Shaer[1], Aaron Lyons[2], Yoshihiro Ishikawa[3], Billy G. Hudson[4], Sergey P. Boudko[4] and Nancy R. Forde[1,2]

[1]Department of Molecular Biology and Biochemistry, Simon Fraser University
[2]Department of Physics, Simon Fraser University
[3]Department of Ophthalmology, University of California San Francisco
[4]Center for Matrix Biology, Vanderbilt University Medical Center

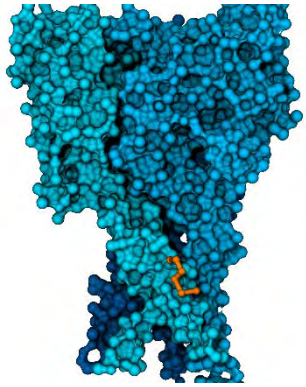


Extracellular matrix mechanics influence diverse cellular functions, yet surprisingly little is known about the mechanical properties of their constituent collagens. While collagen type IV is an integral component of basement membranes, it has received far less attention than the more abundant fibrillar collagens. In this work, we used atomic force microscopy to image different collagen types and analyze their sequence-dependent mechanics. By analyzing flexibility along their contour, we learned that discontinuities in the triple-helix-defining sequence (Gly-X-Y) in collagen IV lead to a generally more flexible polymer with notable flexible "hinges" that correlate with non-helical regions. A simple model in which flexibility is dictated only by the presence of interruptions fit the extracted profile reasonably well, providing insight into the alignment of chains and supporting the role of interruptions in instilling flexibility. However, limitations of this model were illuminated by our determination of variable flexibility along continuously triple-helical collagen III, which we found to possess a high-flexibility region around its matrix-metalloprotease (MMP) binding site. Our result demonstrates a unique mechanical signature of the MMP site along collagen and offers the opportunity to examine the interplay between sequence, mechanics, and stability in these proteins.

Poster #67:
Arachidonic Acid Interactions with Human Acid-Sensing Ion Channel 3

Anna Ananchenko[1], Dana Kneisley[2], David MacLean[2], Maria Musgaard[1]

[1]*Department of Chemistry and Biomolecular Science, University of Ottawa*
[2]*University of Rochester Medical Centre (United States)*



Acid-sensing ion channels (ASICs) are trimeric, proton-gated cation channels and are important to pain sensation in relation to tissue acidification (Wemmie et al. Nat Rev Neurosci 2013). Although known to be proton-gated, recent evidence suggests that the inflammatory membrane lipids, arachidonic acid (AA) (Smith et al. J Neurosci 2007) and lysophosphatidylcholine (LPC) (Marra et al. EMBO J 2016), activate the ASIC3 subtype through a direct effect without a change in pH. However, this mechanism of activation and possible binding sites are unknown. Using computational homology modelling and molecular dynamics (MD) simulations, we have simulated models of human ASIC1 and ASIC3 channels in a membrane environment containing AA, LPC and POPC. Exploiting the MARTINI coarse grained model and force field (Marrink et al., J. Phys. Chem. B 2007), we have compared lipid interactions with the two subtypes of ASICs. We have identified putative binding sites for AA through analysing lipid residence times and binding poses. Our data propose that specific residues, which differ between ASIC1 and ASIC3, are crucial for the AA interaction patterns observed in hASIC3. The identified binding sites are further explored through atomistic resolution MD simulations for more mechanistic insight.

Poster #53 & Trainee Symposium Talk: Functionalized Spider Silk Biomaterials Promote Neurite Outgrowth in PC12 Cells

Lizzy A. Baker[1], Lingling Xu[1], John P. Frampton[1,2] and Jan K. Rainey[1-3]

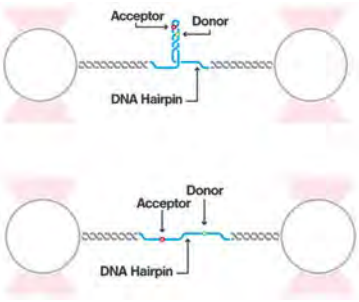
[1]*Department of Biochemistry & Molecular Biology, Dalhousie University*
[2]*School of Biomedical Engineering, Dalhousie University*
[3]*Department of Chemistry, Dalhousie University*

Spinal cord injury is often accompanied by a lifetime of physical, psychological and economic burden due to the limited natural capacity for nerve regeneration in adults. Biomaterials have been developed to treat peripheral nerve injury, but have shown little success in treating injury to the central nervous system. Spider silks have shown promise as biomaterials due to their robust mechanical properties and low immunogenicity. Various types of silk proteins are produced by spiders in nature, with the toughest being aciniform silk used in prey wrapping. Previous work has elucidated mechanisms for the production and spinning of recombinant aciniform silk, including fusion constructs. This enables the rational modification of recombinant aciniform silk to enhance neuronal growth. The present work explores the functionalization of recombinant aciniform silk with domains capable of tethering the neurotrophic factor, nerve growth factor-β (NGF-β). We test the hypothesis that the presence of NGF-β-binding domains within the silk construct will support neurite outgrowth. To do this, a novel aciniform construct was expressed in *E. coli*, purified using chromatography, cast into protein films and characterized. PC12 cells were then cultured on the recombinant silk films to assess neurite outgrowth and molecular markers of differentiation in response to NGF-β signalling.

Poster #14:
Correlative Single-Molecule Force-FRET Measurements of DNA Hairpin Conformational Dynamics

Zsombor Balassy[1], Nastaran H. Yazdi[2], Ann Mukhortava[3], Raymond Pauszek III[3], Sheema Rahmanseresht[1], Rosalie P.C. Driessen[3]

[1]*LUMICKS, Waltham, MA, USA*
[2]*LUMICKS, Emoryville, CA, USA*
[3]*LUMICKS, Amsterdam, Netherlands*



The biological function of many macromolecules is tightly coupled to their conformation and their conformational dynamics. In particular DNA/RNA hairpins play essential regulatory roles *in vivo,* since they can regulate the interaction with nucleic acid processing proteins in transcription, recombination, or replication. Single-molecule force spectroscopy represents an ideal tool for understanding the principles of their structure formation because of its unique capability to isolate individual biomolecules and observe conformational transitions in real-time, while providing the control over the population distribution. Here we report a study on FRET-labelled DNA hairpin folding transitions using high-resolution optical tweezers combined with fluorescence microscopy. We present equilibrium conformational state measurements at unprecedent nanometer trap distance stability over hours without altering the energy landscape. We could capture the rare conformational states that might be overlooked over a timescale that is limited by instrumental drift with integrated trap-distance and temperature stabilization feedback loops. Kinetic, energetic and structural properties of these states could be determined quantitatively from the correlative force and fluorescence data, providing new insights into folding pathways and energy landscape. This study shows how correlating force spectroscopy measurements with fluorescence microscopy could complement the investigation of the prevalence and energy landscape of certain conformational states.

Poster #42:
Effects of macromolecular crowding on protein folding thermodynamics

Saman Bazmi[1], Stefan Wallin[1]

[1]*Department of Physics and Physical Oceanography, Memorial University of Newfoundland*

The high concentration of macromolecules inside living cells leads to the emergence of an effect which is called macromolecular crowding. It influences various processes in cells including protein folding. Here we investigate the effects of repulsive crowders on protein folding with a sequenced-based coarse-grained model. The crowders are modeled as spheres with two independent parameters representing size and softness. Using simulated tempering Monte Carlo, we determine the thermodynamic behaviors of two sequences with 35 amino acids folding into a helical hairpin and a 5-stranded beta barrel, respectively, over a range of crowder concentrations and crowder sizes. The behaviors of the two proteins are analyzed in terms of the thermal stability of the folded states, the radius of gyration, and folding cooperativity. For the alpha-helical protein, we find that the native state stability increases monotonically with decreasing crowder size, as expected from the entropic effect of repulsive crowders. By contrast, the stability of the beta-barrel protein first increases with decreasing crowder size, and thereafter increases. This non-monotonic dependence of crowder size is analyzed in terms of the cooperativity of the folding transition.

Poster #38:
Investigating the structure and function of coactivator recruitment by the melanogenic transcription factor MITF

Alexandra Brown[1], Kathleen Vergunst[1], Denis Dupre[2], David Langelaan[1]

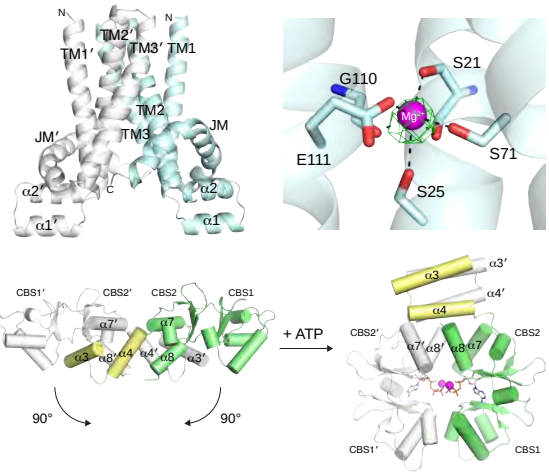
[1]Department of Biochemistry and Molecular Biology, Dalhousie University
[2]Department of Pharmacology, Dalhousie University

The microphthalmia-associated transcription factor (MITF) is a master regulator of development within the melanocyte lineage. However, aberrant MITF activity can lead to multiple malignancies including skin cancer, where it plays a key role in modulating the progression of melanoma. To perform its function, MITF recruits transcriptional co-activators including histone acetyltransferase CREB- binding protein (CBP) through an N-terminal transactivation domain (TAD), however details of their interactions are still not known. We investigated the structure and functional interaction between MITF-TAD and the TAZ2 domain of CBP. In luciferase-based reporter assays, MITF transcriptional activity was enhanced in the presence of CBP and abolished upon deletion of residues within the MITF-TAD. Deletion of these residues also ablated its ability to bind TAZ2 using isothermal titration calorimetry and protein pulldown assays. NMR-based chemical shift mapping experiments determined that MITF-TAD interacts with the same surface of TAZ2 as the adenoviral protein E1A, which has been shown to inhibit MITF function. We determined that MITF-TAD directly competes for the E1A binding surface of CBP using NMR-based titrations and pulldown assays. Understanding the interactions between MITF and its co-activators is fundamental to our understanding of gene regulation by MITF and may outline new targeted strategies to treat melanoma.

Poster #8:
Crystal Structure of an Archaeal CorB Magnesium Transporter

Yu Seby Chen[1], Guennadi Kozlov[1], Brandon E. Moeller[2], Ahmed Rohaim[3], Rayan Fakih[1], Benoît Roux[3], John E. Burke[2], Kalle Gehring[1]

[1]Department of Biochemistry & Centre de Recherche en Biologie Structurale, McGill University, Montréal, Quebec, Canada
[2]Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada
[3]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois, USA



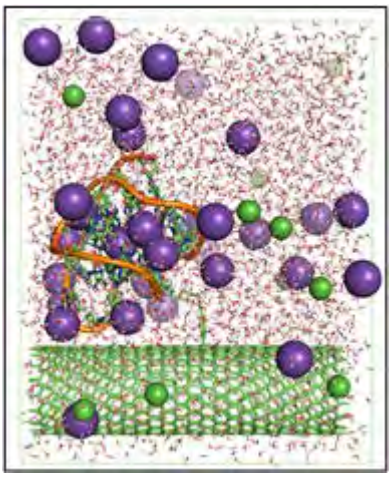
CNNM/CorB proteins are a broadly conserved family of integral membrane proteins with close to 90,000 protein sequences known. They are associated with Mg^{2+} transport but it is not known if they mediate transport themselves or regulate other transporters. Here, we determined the near complete structure of an archaeal CorB protein in two conformations (apo and Mg^{2+}-ATP bound). The structure reveals a novel transmembrane fold (DUF21), representing the largest family of domains of unknown function. The transmembrane domain exists in an inward-facing conformation with a Mg^{2+} ion coordinated by a conserved π-helix. The CBS-pair domain in absence of Mg^{2+}-ATP exists in a novel dimeric configuration with previously unobserved domain-domain contacts. Supported with HDX-MS, AUC, and MD simulations, major structural rearrangements in the cytosolic domains suggest involvement in Mg^{2+}-ATP sensing. Lastly, with an *in vitro*, liposome-based assay, we unambiguously demonstrated direct Mg^{2+} transport function by CorB proteins. Together, these structural and functional insights provide a framework for understanding function of CNNMs in Mg^{2+} transport and associated diseases.

Poster #37:
Simulations of DNA-carbon nanotube interactions for the design of field-effect transistor biosensors

Sébastien Côté[1,2]

[1]Département de physique, Université de Montréal
[2]Département de physique, Cégep de Saint-Jérôme

Bioanalytical sensors based on field-effect transistors (bioFETs) are emerging as promising tools to measure the kinetics of biopolymers such as proteins and DNA strands. This class of biosensors is based on an ultra-miniaturized electronic circuit whose conductance is very sensitive to the variations of the electrostatic potential in its environment caused by conformational changes in the biopolymer. Here, we investigate the working of a specific bioFET made of a single carbon nanotube to which is covalently grafted a single DNA strand of the G-quadruplex motif. More specifically, we use advanced sampling techniques based on molecular dynamics simulations to unveil the interactions and kinetics between the biopolymer and the carbon nanotube. We observed that, while the structural stability of the G-quadruplex motif is not significantly altered by the carbon nanotube, some interactions could modify its folding kinetics. Our observations from computational simulations complement the experimental measurements obtained by our collaborators who characterized the same setup. Together, they support the development of this promising biosensor for monitoring the kinetics of biopolymers.



Poster #39:
Comparative roles of charge, π , and hydrophobic interactions in Liquid-liquid phase separation: A case study with DEAD-box helicase Ddx4

Suman Das[1], Yi-Hsuan Lin[1,2], Robert M. Vernon[2], Julie D. Forman-Kay[1,2] and Hue Sun Chan[1]*

[1]*Department of Biochemistry, University of Toronto, Toronto*
[2]*Molecular Medicine, Hospital for Sick Children, Toronto*

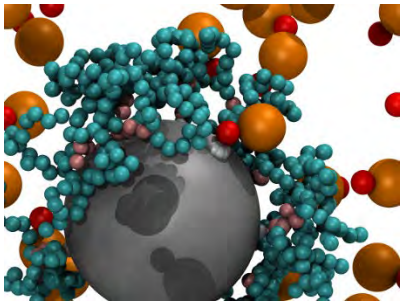
Biomolecular condensates underpinned by Liquid-liquid phase separation (LLPS) has garnered intense interest due to their versatile role in organismal functions. Compared to a plethora of experimental studies, theoretical and simulation studies are still limited. Endeavoring toward a transferable, predictive coarse-grained explicit-chain model of LLPS, we conducted simulations of the N-terminal intrinsically disordered region (IDR) of DEAD-box helicase Ddx4 to assess roles of electrostatic, hydrophobic, cation–π, and aromatic interactions in amino acid sequence-dependent LLPS. We evaluated three different residue–residue interaction schemes with a common electrostatic potential. Neither a common hydrophobicity scheme nor one augmented with arginine/lysine-aromatic cation–π interactions consistently accounted for available experimental LLPS data. In contrast, interactions based on PDB statistics among folded globular protein structures reproduce the overall experimental trend. Consistency between simulation and experiment was also found for RtoK mutants of P-granule protein LAF-1, underscoring that, to a degree, important LLPS-driving π-related interactions are embodied in classical statistical potentials. Protein–protein electrostatic interactions are also adjusted by computing relative permittivity from all-atom simulations, which in general depends on aqueous protein concentration. Our systematic evaluations have deepen the understanding of the molecular-level interactions that drive the formation of biomolecular condensates.

Poster #69:
Using a Coarse-Grained Model to Investigate the Molecular Sieving Effect of Comb Polymers Grafted on a Protein Surface

Nicole Drossis[1], Marc A. Gauthier[2], Hendrick W. de Haan[1]

1Faculty of Science, Ontario Tech University

[2]Institut National de la Recherche Scientifique



Advances in polymer chemistry have allowed us to make a wide variety of synthetic polymers that can be grafted to protein surfaces to create complex bioconjugates that are able to be tuned to address specific challenges. One such challenge is that therapeutic antibodies, when administered in the human body, are degraded rapidly by the body's immune response. A solution to this issue is to block out the antibodies responsible for this degradation without blocking the activity of the protein. It has been hypothesized that when ramified structures like comb polymers or dendritic polymers are grafted to the surface of a protein, the gaps in them act as a semi permeable membrane that allows small particles to pass through, allowing the protein to maintain its activity while blocking larger particles like antibodies that cause degradation. This work uses a coarse-grained model of α-chymotrypsin with the comb polymer POEGMA grafted to its surface to investigate the ability of grafted comb polymers with different dimensions to block particles of different sizes from reaching the active site via steric behaviours.

Poster #40:
How did first life emerge on terrestrial planets?

Alix Dujardin [1, 2, 3], Renée-Claude Bider [1, 2], Breanna Crompvoets [1, 2], Sebastian Himbert [1, 2], Hannah V. Rheinstädter [1, 2], Ralph Pudritz [1,2], Maikel Rheinstädter [1,2]

[1]*Origins Institute, McMaster University, Hamilton, Ontario, Canada*

[2]*Department of Physics and Astronomy, McMaster University, Hamilton, Ontario, Canada*

[3]*Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario, Canada*

How did the first genetic code and the first forms of cellular appear in the early life, about 3.5 billion years ago, of terrestrial and Earth-like exoplanets? This question has become especially timely with the discovery of an ever-increasing number of rocky exoplanets where liquid water is present.

We investigated how specific conditions on terrestrial planets, such as water, temperatures, radiation, atmospheres, and the presence of certain minerals and organic molecules, can potentially drive polymerization of RNA-like polymers. Experiments were conducted using the Planet Simulator, a custom-built simulation chamber.

Our current results show that the formation of the first genetic assemblies would have occurred in shallow wells that undergo hot-cold and wet-dry cycles. Nucleic acids may have evolved in contact with salt, such as ammonium chloride, and in particular phospholipids and simple membranes, for the formation of protocells when the concentration of those elements would have been optimal. We found that long RNA polymers form spontaneously in the presence of membranes in these warm little ponds and that these polymers are spontaneously incorporated into liposomes. We observe RNA-chains of hundreds of nucleotides whose length depends on the exact temperature and humidity cycles due to daily and seasonal change.

Poster #68:
Physical mechanisms of tissue compartmentalization in the Drosophila embryo

Gonca Erdemci-Tandogan[1,2], Jessica C. Yu[1,2], Negar Balaghi[1,2], Veronica Castle[1,2], Rodrigo Fernandez-Gonzalez[1,2]

[1]*Institute of Biomedical Engineering, University of Toronto*
[2]*Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, University of Toronto*

Compartment boundaries prevent cell mixing and are essential for embryonic development. Cables formed by actin and the molecular motor myosin II are often found at compartment boundaries. How boundaries are established and maintained remains unclear. In the *Drosophila* embryo, the mesectoderm separates ectoderm and mesoderm, forming the ventral midline. Eventually, mesectoderm cells are internalized becoming part of the central nervous system. We found that ectoderm and mesectoderm remained separated as the mesectoderm was internalized, suggesting the presence of a boundary between the tissues. Using live microscopy, we found an enrichment of myosin at the mesectoderm-ectoderm boundary (MEB), forming a supracellular cable. Myosin levels at the MEB decreased as the mesectoderm was internalized. To study the role of myosin cables at the MEB, we simulated mesectoderm internalization using a vertex model. Our model predicted that tension at the MEB maintains the linearity of the interface, prevents cell mixing, and controls the timing of mesectoderm internalization. Consistent with this, pharmacological inhibition of myosin disrupted the MEB, leading to mesectoderm-ectoderm cell mixing and premature mesectoderm internalization. Our model also predicted that cell divisions in the ectoderm play an unexpected role in maintaining the linearity of the MEB, a hypothesis that we are testing.

Poster #13:
Ligand modulation of the conformational dynamics of the A2A Adenosine Receptor revealed by Single-molecule Fluorescence

Dennis D. Fernandes[1,2], Chris Neale[3], Gregory-Neal W. Gomes[1,2], Yuchong Li [1,2], Aimen Malik[2], Aditya Pandey[2,4], Alexander Orazietti[2,4], Xudong Wang[5], Libin Ye[2,5,6] , R. Scott Prosser [2,4] , and Claudiu C. Gradinaru[1, 2]

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G protein-coupled receptors (GPCRs) are the largest class of transmembrane proteins, making them an important target for therapeutics. Activation of these receptors is modulated by orthosteric ligands, which stabilize one or several states within a complex conformational ensemble. The intra- and inter-state dynamics, however, is not well documented. Here, we used single-molecule fluorescence to measure ligand-modulated conformational dynamics of the adenosine A2A receptor (A2AR) on nanosecond to millisecond timescales. Experiments were performed on detergent-purified A2R in either the ligand-free (apo) state, or when bound to an inverse, partial or full agonist ligand. Single-molecule Förster resonance energy transfer (smFRET) was performed on detergent-solubilized A2AR to resolve active and inactive states via the separation between transmembrane (TM) helices 4 and 6. The ligand-dependent changes of the smFRET distributions are consistent with conformational selection and with inter-state exchange lifetimes ≥ 3 ms. Local conformational dynamics around residue $229^{6.31}$ on TM6 was measured using fluorescence correlation spectroscopy (FCS), which captures dynamic quenching due to photoinduced electron transfer (PET) between a covalently-attached dye and proximal aromatic residues. Global analysis of PET-FCS data revealed *fast* (150–350ns), *intermediate* (50–60µs) and *slow* (200–300µs) conformational dynamics in A2AR, with lifetimes and amplitudes modulated by ligands and a G-protein mimetic (mini-Gs). Most notably, the agonist binding and the coupling to mini-Gs accelerates and increases the relative contribution of the sub-microsecond phase. Molecular dynamics simulations identified three tyrosine residues (Y112, $Y288^{7.53}$, and $Y290^{7.55}$) as being responsible for the dynamic quenching observed by PET-FCS and revealed associated helical motions around residue $229^{6.31}$ on TM6. This study provides a quantitative description of conformational dynamics in A2AR and supports the idea that ligands bias not only GPCR conformations but also the dynamics within and between distinct conformational states of the receptor.

Poster #41:
The Mosquito Protein AEG12 as a Scaffold for Novel Antiviral Therapeutics

Alexander C.Y. Foo[1], Shih-Heng Chen[2], Negin Martin[2], and Geoffrey A. Mueller[1].

[1]Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences
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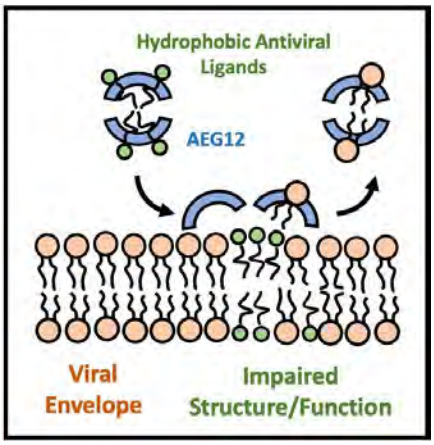

The mosquito protein AEG12 is upregulated in response to viral infection, though its specific contribution and mechanism of action remained unknown. The structure of AEG12 is defined by a large central cavity which can bind a range of hydrophobic ligands. Here we identified a mixture of saturated and unsaturated fatty acids as the likely native ligands of AEG12. AEG12-mediated delivery of these ligands via an elegant lipid-exchange mechanism disrupts the lipid bilayer of both mammalian cells and enveloped viruses, resulting in broad-spectrum viral suppression with micromolar IC_{50}'s against flaviviruses, lentiviruses, and human coronaviruses albeit at the cost of significant cytotoxic activity. The large cavity of AEG12 can also bind a range of non-natural ligands such as lysophosphocholine (LPC) and ginkgolic acid (GA); both of which have been shown to inhibit viral membrane fusion, reducing infectivity independent of membrane destabilization and its associated cytotoxic effects. The resulting complexes displayed robust antiviral activity. In contrast to free-LPC or Free-GA, these AEG12 complexes retained their activity even in the presence of serum albumins and other lipid-sequestering moieties. These studies clarify the biophysical mechanisms underlying AEG12's biological functions and highlight the potential of AEG12 as a versatile delivery vehicle for hydrophobic therapeutic compounds.

Poster #58:
Development and characterization of recombinant hybrid spider silks

Anupama Ghimire[1], Jeffrey R. Simmons[1], Jan K. Rainey[1-3]

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[2]*Department of Chemistry, Dalhousie University, Halifax, NS, Canada*
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Spider silks are natural protein-based biomaterial secreted by spiders for purposes such as web construction, locomotion, wrapping of prey and protection of eggs. They are renowned for their mechanical properties and hold great promise for applications ranging from high-performance textiles to vehicles to the biomedical sector. This study focuses on development and use of materials made up of mixtures of aciniform and pyriform silks. Each silk on its own has distinctive mechanical behaviour and physicochemical properties, with materials produced using combinations of these silks currently unstudied. Recombinant aciniform and pyriform silk proteins are being expressed in *Escherichia coli*, with downstream purification achieved by Ni-NTA affinity chromatography. Building on methods previously introduced in our lab, wet-spinning approaches are being developed and used to compare fibres spun from the individual silks to those from mixtures of aciniform and pyriform proteins. FTIR spectroscopy is being applied to infer fibre-state secondary structuring, with structural differences being observed from one type of fibre to another. These structural differences are directly related back to mechanical behaviour measured through tensile testing. Through the investigation and characterization of structural and mechanical properties of hybrid aciniform-pyriform silk materials, their suitability and tunability for disparate applications will be determined.

Poster #61:
Building a polymer model of bacterial chromosomes: chain heterogeneity, supercoiling, and crowding

Chanil Jeon[1], Youngkyun Jung[2], and Bae-Yeun Ha[1]
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Chromosomes in living cells are strongly confined but show a high level of spatial organization. If constructed carefully, a polymer-chromosome model will be useful for understanding the way chromosomes are spatially organized and for interpreting chromosome experiments. Here we discuss chromosome-like polymers in a crowded and confined space: (heterogeneous) ring polymers and bottle-brush polymers. We focus on clarifying the role of bimolecular crowding and confinement in organizing bacterial chromosomes, using molecular dynamics simulations. Our simulations results are consistent with the observation that crowding promotes clustering of transcription-active sites into transcription foci. Also, we find that crowding is essential for distributing the two “arms" of a ring polymer in the way observed with *E. coli* chromosomes. Finally, cylindrical confinement can induce helical organization of bottle-brush polymers. We discuss how our results can be used to interpret chromosome experiments. For instance, they suggest that experimental resolution has unexpected consequences on writhe measurements (e.g., narrowing of the writhe distribution and kinetic separation of opposite helical states).

Poster #7:
Spectral Broadening of Ultrashort Laser Pulses in Optical Fiber for Applications in Nonlinear Optical Microscopy

MacAulay Harvey[1,2*], Richard Cisek[1,2] and Danielle Tokarz[2]

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Saint Mary's University, Halifax, Canada

Wavelength-tunable ultrafast laser sources can be used in combination with polarization-resolved harmonic generation microscopy techniques to probe the ultrastructure of biological materials such as collagen without alteration of the tissue. Polarization second harmonic generation in particular, has been shown to have applications in automated quantitative diagnosis of cancer in different human pathological tissue samples including breast, pancreas and thyroid. Further specificity leading to earlier diagnosis will likely be achieved by additional measurements at different wavelengths. While single wavelength ultrafast lasers are increasingly affordable, their tunable counterparts are still not, and they additionally require annual maintenance which further prohibits the research for most biophysics research groups. To this end, the spectral broadening of ultrashort pulses with a center wavelength of 1030 nm using commercially available polarization-maintaining large mode area photonic crystal fibers is investigated. When an ultrashort optical pulse propagates in optical fiber its spectrum is broadened by nonlinear optical effects such as self-phase modulation and stimulated Raman scattering. Spectral broadening of up to 230 nm was achieved, and its application to nonlinear microscopy was investigated.

Poster #12:
Non-equilibrium Structural Dynamics of Supercoiled DNA Plasmids

Cynthia Shaheen[1], Cameron Hastie[1], Shane Scott[2], Lisa Weber[3], Brian Munsky[3], Fedor Kouzine[4], David Levens[4], Craig Benham[5], and Sabrina Leslie[1*]

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[2]*Institute of Materials Science, Kiel University*
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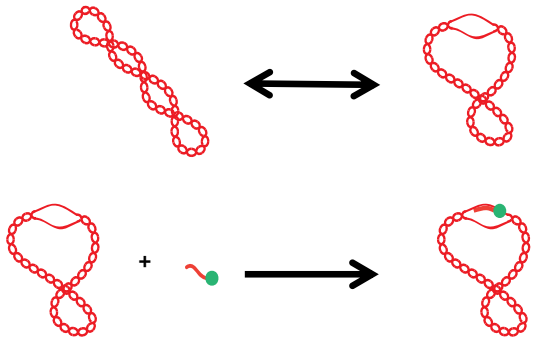
[4]*Center for Cancer Research, National Cancer Institute*
[5]*Genome Center, University of California Davis*

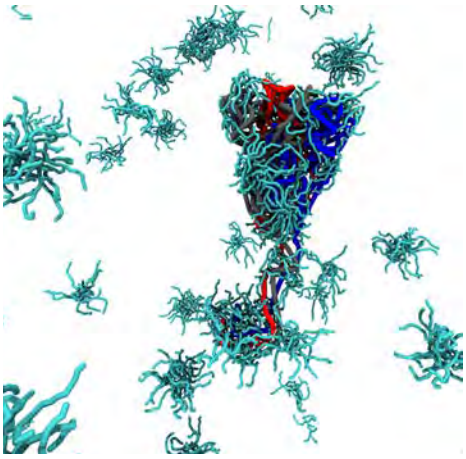
Many cellular processes occur out of equilibrium. This includes the site-specific unwinding of supercoiled DNA, which may play an important role in gene regulation. Here, we use convex lens-induced confinement (CLiC) microscopy to study plasmid unwinding using a fluorescent oligo probe complimentary to the unwinding site. We studied two model plasmid systems, pFLIP-FUSE and pUC19, and investigated the dynamics of DNA unwinding after a temperature perturbation. We used Monte Carlo Markov Chain (MCMC) methods to estimate the transition rates of the oligo-plasmid system. We found that structural transitions can be slow, and plasmids approaching the experimental temperature from a higher temperature had a slower transition rate than plasmids approaching from a lower temperature. Our findings highlight the importance of non-equilibrium effects when characterizing the complex structural dynamics of DNA and the mechanisms of gene regulation.



Poster #71 & Trainee Symposium Talk: Erythro-Vlps: Embedding Sars-Cov-2 Spike Proteins In Red Blood Cell Based Proteoliposomes Leads To Pronounced Antibody Response In Mouse Models

Sebastian Himbert, Maikel Rheinstädter

Department of Physics and Astronomy, McMaster University, 1280 Main Street West, Hamilton, Canada.



Novel therapeutic strategies are urgently needed to control the SARS-CoV-2 pandemic. Here, I present the fabrication and characterization of Erythro-VLPs: Erythrocyte-Based Virus Like Particles, i.e., red blood cell (RBC) based proteoliposomes carrying the SARS-CoV-2 spike protein.

Erythrocytes can present antigens to the immune system when senescent cells are being phagocytized in the spleen. This capacity together with their high biocompatibility make RBCs effective vehicles for the presentation of viral immunopathogens, such as the SARS-CoV-2 S-protein, to the immune system. Epi-fluorescent and confocal microscopy, dynamic light scattering (DLS), and Molecular Dynamics (MD) simulations were used to characterize the liposomes and the insertion of the S-proteins. The Erythro-VLPs exhibit dose-dependent binding to ACE-2 (angiotensin converting enzyme 2) in biolayer interferometry assays.

We present experimental evidence of a pronounced immunological response in mice after 14 days, and the production of antibodies was confirmed in ELISA. These antibodies were found to be specific for the S-protein RBD sub-domain. This immunological response was observed in the absence of any adjuvant which is usually required for protein-based vaccines.

[1] Himbert et al., “Erythro-VLPs: Embedding SARS-CoV-2 spike proteins in red blood cell based proteoliposomes leads to pronounced antibody response in mouse models”, submitted

Poster #36:
Elucidating the conformational landscape of a G protein-coupled receptor by fluorine NMR

Shuya Kate Huang[1], Aditya Pandey[1,2], Duy Phuoc Tran[3], Nicolas L Villanueva[4], Akio Kitao[3], Roger K Sunahara[4], Adnan Sljoka[1,5], R. Scott Prosser[1,2]

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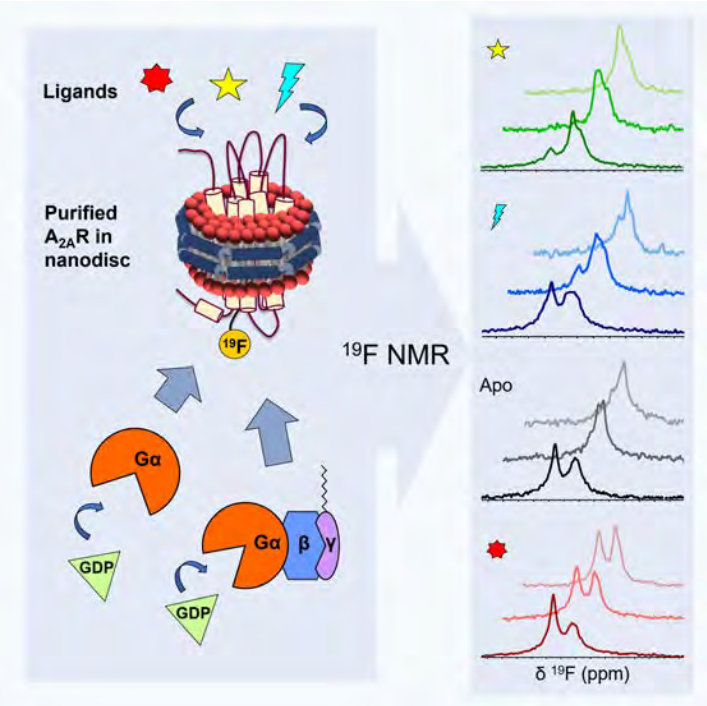
G protein-coupled receptors (GPCRs) represent a ubiquitous membrane protein family and are important drug targets. Their diverse signaling pathways are driven by complex pharmacology arising from a conformational ensemble rarely captured by structural methods. Here, fluorine nuclear magnetic resonance spectroscopy (^{19}F NMR) is used to delineate key functional states of the adenosine-A_{2A} receptor ($A_{2A}R$) complexed with heterotrimeric G protein ($G\alpha_s\beta_1\gamma_2$) in a phospholipid membrane milieu. Analysis of $A_{2A}R$ spectra as a function of ligand, G protein, and nucleotide identifies an ensemble represented by inactive states, a G protein-bound activation intermediate, and distinct nucleotide-free states associated with either partial- or full-agonist driven activation. The $G\beta\gamma$ subunit is found to be critical in facilitating ligand-dependent allosteric transmission, as shown by ^{19}F NMR, biochemical, and computational studies.



Poster #24:
Simulation and Modelling to Develop a Nanopore-Based Device to Detect Ricin

Timothy B. Hurlburt[1], Hendrick W. de Haan[1].

[1]*Department of physics, Ontario Tech University*

The goal of the research is to enhance our ability to detect ricin via nanopores (nano-scale pores). Ricin is a remarkably toxic protein - limited exposure can be lethal. Due to its toxicity, it has been used in bioterrorism attacks and is classified as a category B biothreat agent. Computer simulations are used to explore a variety of pore geometries and assay conditions to optimize diagnostic accuracy by maximizing the magnitude of current blockage. Several coarse-grained protein, ion, and aptamer-encoded nanopore systems are developed to model and simulate their associated dynamics and, in particular, to measure the magnitude of the blockade. Ultimately the research attempts to inform the design of devices capable of ricin detection for medical, food safety, and biosecurity applications.

Poster #73:
Inferring gene regulation dynamics from static snapshots of gene expression variability

Euan Joly-Smith[1], Zitong Jerry Wang[2], Andreas Hilfinger[1,3,4]

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[2]*Division of Biology and Biological Engineering, California Institute of Technology*
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[4]*Department of Cell & Systems Biology, University of Toronto*

A key aim of systems biology is to understand how intracellular reaction networks generate dynamic cellular behaviours. This requires an understanding beyond statistical associations of the components making up these networks, but high-throughput methods in cell biology like flow-cytometry or single-cell sequencing typically produce static population snapshots which are difficult to translate into dynamic interactions. Here we show how static population snapshots of co-variability can be exploited to rigorously infer properties of gene expression dynamics. For instance, we derive correlation conditions that detect the presence of closed-loop feedback regulation in gene regulatory networks from snapshots of transcript-levels. Furthermore, we show how genes with cell-cycle dependent transcription rates can be identified from the variability of co-regulated fluorescent proteins with unequal maturation times. Our approach exploits the fact that unequal fluorescent reporters effectively probe their upstream dynamics on separate time-scales such that their correlations implicitly encode information about the temporal dynamics of their upstream regulation.

Poster #54:
Multi-parameteric single-particle microscopy platform for high-throughput, tether-free measurements of drug-delivery nanoparticle size, loading, and dynamics in cell-like conditions

Albert Kamanzi[1], Radin Tahvildari[1], Yifei Gu[1], Zach Friedenberger[1], Raffles Xu[1], Romain Berti[1,2], Marty Kurylowicz[1,2], Dominik Witzigmann[3], Jayesh A. Kulkarni[3], Jerry Leung[3], Andreas Dahlin[4], Fredrik Hook[5], Mark Sutton[1], Pieter R. Cullis[3], and Sabrina Leslie[1,2]

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[3]Department of Biochemistry and Molecular Biology, University of British Columbia,
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Lipid nanoparticles (LNP) are a promising solution for delivery of nanomedicines and vaccines. Optimizing their design depends on being able to resolve, understand, and predict their biophysical and therapeutic properties. While existing tools have made great progress, gaps in understanding remain because of the inability to make measurements of multiple correlated properties. This work develops and applies a new method for characterizing nanoparticles with single-particle resolution, high throughput, no tethers and controlled cell-like conditions. This method uses CLiC microscopy to isolate and quantify trajectories and intensities of nanoparticles in microwells for long times. First, we benchmark detailed measurements of polystyrene nanoparticles against prior data, to validate our approach. Second, we apply our method to investigate size and loading properties of LNPs containing siRNA, as a function of lipid formulation, solution pH, and loading. We gain new insights into LNP structure and distribution of siRNA in the LNP by taking a detailed look at the correlation between intensity and size measurements. The impact of this work is to help create a through-line of understanding between microscopic and macroscopic properties of drug-delivery vehicles as a function of design parameters, and by enabling these insights into nanomedicines, significantly accelerate drug development and discovery.

Poster #15:
Using Gate-based Quantum Computing for Protein Design

Mohammad-Hassan Khatami[1], Nathan Wiebe[2,3,4], Philip M. Kim[1,2,5]

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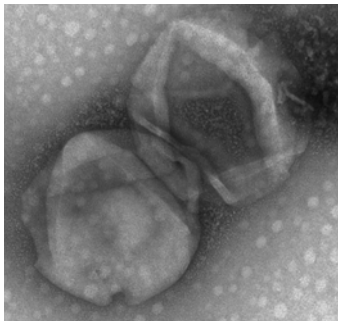
[4]*Department of Physics University of Washington, Seattle WA USA*
[5]*Department of Molecular Genetics, University of Toronto*

In general, protein design is categorized under the Non-deterministic Polynomial-time (NP)-hard class of problems in computer science. Suppose a protein has “S” possible designable sites and “A” possible individual amino acids. In this case, exploring all states and combinations of amino acids for protein design ($N = A^S$) is hard, if not impossible. The current conventional computational methods do not explore all possibilities but sample a probability distribution. As an alternative, quantum computation methods are proposed to solve the NP-hard class problems. In the present work, we implement Grover’s algorithm, a pure quantum computation approach, to address the protein design problem. Grover’s algorithm provides quadratic speed up in searching for answer states in an unsorted list. Due to the noisy state of current quantum computers, we use quantum computer simulators for this study. Our results show that using $O(\sqrt{N})$ iterations, the algorithm chooses the correct results among all N possibilities with more than 97% probability. While for conventional methods, the number of iterations is O(N). Our results also show that even a single iteration of our implementation of Grover’s algorithm identifies the correct states from incorrect ones, with low but still distinguishable probability.

Poster #18:
Novel Drug Delivery System for Antibiotic Therapy Using Modified Erythrocyte Liposomes

Hannah Krivic[1], Ruthie Sun[1], Sebastian Himbert[1], Maikel Rheinstadter[1]

[1]*Department of Physics and Astronomy, McMaster University Hamilton ON, Canada*



As a result of the growing world-wide antibiotic resistance crisis, many currently existing antibiotics have become ineffective due to bacteria developing resistive mechanisms. There are a limited variety of potent antibiotics that are successful at suppressing microbial growth, such as polymyxin B, however are deemed as a last resort due to being highly toxic to healthy cells. Previous literature has focused on the use of nanoparticles as an antibiotic delivery system to minimize the negative side effects. Despite their potential, an antibiotic delivery system has yet to be established, due to the nanoparticles lacking specificity and lack of biocompatibility and rejection. Here, we present a novel antimicrobial drug delivery method that uses modified red blood cells (RBCs) that are encapsulated around polymyxin B. These RBC-based antibiotics have been made specific to certain bacteria through the addition of the corresponding antibodies to their cell membranes. We investigate whether this drug delivery system is effective at inhibiting bacterial growth and selective, which is important to minimize the negative side effects seen with conventional polymyxin B treatment. This RBC-based platform is potentially advantageous to synthetic nanoparticle-based approaches because of their biocompatibility and bioavailability, resulting in a longer retention time in the human body.

Poster #26:

Whole cell ^{2}H Solid-state NMR of Antimicrobial Peptides Interacting with Cell Envelopes: Role of Lipopolysaccharide

Sarika Kumari[1], Michael Morrow[2], Valerie Booth[1, 2]

Department of Biochemistry[1] and Department of Physics and Physical Oceanography[2], Memorial University of Newfoundland

Antimicrobial Peptides (AMPs) have been studied for more than two decades because they promise to help overcome the problem of resistance conventional antibiotics. However, AMPs have not been as successful as hoped, perhaps because we lack a detailed understanding of their mechanisms of action. To understand these mechanisms, numerous biophysical techniques, including solid-state ^{2}H NMR, have been used to study membrane disruption both in model lipid system and in intact bacteria. In the real biological context of AMPs, studies suggests that, in addition to interaction with lipids, it is essential to consider non-lipid components. Gram-negative bacteria have a lipopolysaccharide layer (LPS), a outer membrane of cell envelope component that protects bacteria from AMPs. This study investigated how LPS affects AMP-induced membrane disruption. MSI-78 is an AMP that has been shown to disrupt lipid membranes of target bacteria. We disrupt the LPS layer of *Escherichia coli* cells (*E. coli*) via chelation of the stabilizing divalent cations. However, the ^{2}H NMR spectra of *E. coli* demonstrated that the 2.5 mM and 9.0 mM EDTA concentration used does not affect the lipid acyl chain order in a major way. Interestingly, we found that the ^{2}H NMR spectra of *E. coli* with a 9.0mM concentration of EDTA in the presence of MSI-78 show a slight increase of the lipid acyl chain disorder compared to MSI-78 alone. Thus, our results suggest that disruption of the LPS layer very slightly sensitizes bacteria to membrane-disruption by MSI-78.

Funding agency: Natural Sciences and Engineering Research Council of Canada

Poster #43:
Inferring Transition Rates from Stationary Distributions within Complex Networks

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[4] *Department of Mathematics, University of Toronto*

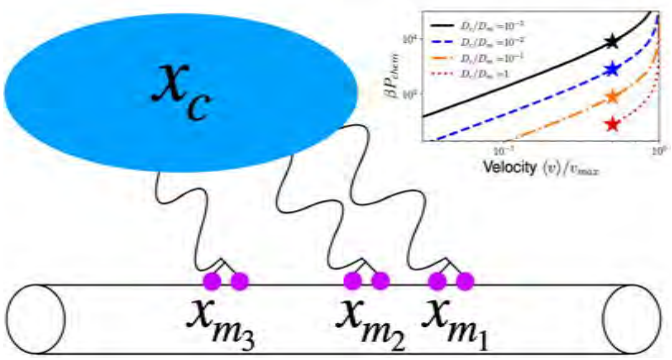
[5] *Department of Cell & Systems Biology, University of Toronto*

I will introduce an approach for estimating the transition rates between discrete states of a stationary Markov process. In general, when given the birth and death rates of a process, one can obtain its stationary distribution. In the "inverse problem", we aim to reconstructing biochemical rates from observed stationary data. The method is general, and can be applied to other processes as well. For a given reaction network, our method allows us to extract the reactions rates between system components only from a "snapshot" of the concentration of the relevant species. This approach has three key features; First, we use for the birth-rate inference only the stationary PDF, without any dynamical information. Second, the production rate of a given molecule might depend on the number of other molecules in the system. Third, the structure/topology of the entire reaction network may remain arbitrary, thus the only specified part of the network is for the relevant species. We examine the validity of the approach for different properties of the systems.

Poster #20:
Performance Trade-offs in Collective Cellular Transport Systems

Matthew P. Leighton[1], David A. Sivak[1]

[1]*Department of Physics, Simon Fraser University*



Intracellular transport of organelles, vesicles and other molecular cargo is often accomplished with the use of motor proteins like kinesin and myosin. These motors consume chemical energy in order to achieve directed motion. Within cellular transport systems, molecular cargo are often hooked up to teams of transport motors. In fact, observations of different systems both *in vivo* and *in vitro* have found widely varying numbers of motors coupled to a single cargo, from only one to well over 200. To explore the rich behaviour of these systems we present a stochastic model for motor-driven transport of molecular cargo with a variable number N of motors. Using this model we investigate how different quantities vary with N including the velocity, efficiency and chemical power consumption, and identify key performance trade-offs.

Poster #47:
Investigating the effects of cytosine methylation on dynamics and stability of the nucleosome

Shuxiang Li[1], Yunhui Peng[2], David Landsman[2], Anna R. Panchenko[1]

[1]*Department of Pathology and Molecular Medicine, Queen's University, ON, Canada*
[2]*National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA*

Cytosine methylation at the 5-carbon position is an essential DNA epigenetic mark in many organisms. Although many structural and functional studies of cytosine methylation have been reported, our understanding of how it influences the nucleosome assembly, structure, and dynamics remains obscure. Here we investigated the effects of cytosine methylation on nucleosome dynamics and stability. By applying long molecular dynamics simulations, we generated extensive atomic level conformational nucleosome ensembles. Our results revealed that 5mC induces pronounced changes in methylated DNA geometry, leading to a more curved, under-twisted DNA. This conformational change allows the nucleosomal DNA to accommodate a considerably larger number of contacts with the histone octamer than unmethylated conventional nucleosomes. These effects prevent a spontaneous DNA unwrapping of 3-4 helical turns for the tailless system, otherwise observed in the unmethylated system on several microsecond time scales. We can conclude that 5-cytosine methylation might not only make available specific binding sites or occlude binding of regulatory proteins, but in addition, have a direct role in inhibiting the DNA unwrapping and regulating DNA accessibility. This additional mechanism could be employed by factors that can bind to nucleosomes.

Poster #72:
Brownian-Smoluchowski Simulation of the Accelerated Evolution of Antibiotic Resistant Bacteria in Microhabitats with Drug and Nutrient Gradients

Michael Frood and Apichart Linhananta

Department of Physics
Lakehead University

Spatial inhomogeneity can have a profound influence on how organisms evolve and colonize new environment. Experiments have found that bacteria can developed resistance to anti-biotic drugs within a few hours. This work is inspired by the statistical physics model of Greulich, Waclaw, and Allen (PRL 109, 088101, 2012), which studies bacteria of six different genotypes in a one-dimensional microhabitat with a drug gradient. They found that the drug gradient usually accelerates growth and colonization of microhabitats. The model is adapted to include both drug and nutrient gradients, by using an inhomogeneous growth rate that depends on the Monod function times the pharmacodynamic function. The model is simulated using a Brownian/Smolucowski algorithm where the bacteria are freely diffusing point particles that can divide or mutate or die. The result shows that when non-resistant bacteria are introduced at the center of the microhabitat, there is a dormant period where the bacteria do not grow. This is followed by a period where they mutate to genotypes more resistant to the drug, as they move along the drug gradient to colonize regions with higher drug concentration. We will describe efforts to use available biological data with the model to understand how bacteria can become drug resistant in a few hours.

Poster #57:
Impedimetric Membrane-Based Biosensor: A Machine Learning Approach

Tetyana Loskutova[1,2], Sebastian Himbert[1,2], Maikel Rheinstadter[1,2].

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This study explores the potential of using Machine Learning (ML) to improve the accuracy and specificity of biosensors based on lipid biomembranes. Previous studies have shown the potential of using biomembranes for detection of pathogen contamination in liquid samples using Electrochemical Impedance Spectroscopy (EIS) of biomembranes, which allows the detection of a variety of processes in biomembranes linked to active contamination, such as chemical binding of proteins, nucleic acids, cells, antibodies, and antigens among others.

For industrial applications speed, ease of use, and cost remain challenging. ML has the potential to improve the sensitivity and specificity of the EIS method due to the enhanced ability to filter out the noise and consequently detect minor changes in composition. Additionally, the processing on the server allows continuous improvement and growth of the range of applications without the need to modify the electronics.

Preliminary simulation and fitting of experimental data show that non-linear change in the membrane structure due to contamination processes can be associated with alternative circuits using ML. These circuits characterize different stages of biomembrane modification due to the presence of a contaminant. Further work will focus on the discrimination of the contaminants based on the structure of the alternative circuits.

Poster #31:
Thermodynamics of the transition-path ensemble

Miranda D. Louwerse[1], David A. Sivak[2]

[1]*Department of Chemistry, Simon Fraser University*
[1]*Department of Physics, Simon Fraser University*

The reaction coordinate describing a transition between reactant and product states is a fundamental concept in the theory of chemical reactions. Within transition-path theory, a quantitative definition of the reaction is found in the committor, the probability that the system initiated from a given microstate first reaches the product before the reactant. In this work, we consider the information-theoretic origin of the committor, showing how it naturally arises from selecting out the transition-path ensemble from the equilibrium ensemble, and further derive the effect of selecting out the transition-path ensemble on system entropy production. Our results provide parallel thermodynamic and information-theoretic measures of the relevance of any system coordinate to the reaction, as well as a notion of dynamical distance between the reactant and product states.

Poster #33:
Domain-Specific Interactions with Heparin Promote Tropoelastin Coacervation

Robert Lu[1], Sean Reichheld[1,2], Min Jin[1], Simon Sharpe[1,2]

[1]*Department of Biochemistry, University of Toronto, Canada*
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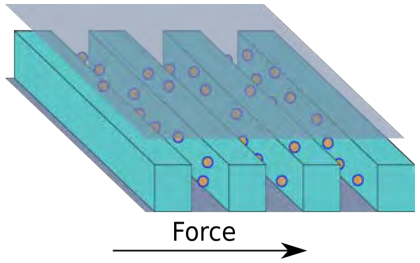
Elastin is the extracellular matrix (ECM) protein responsible for the elastic recoil property of certain tissues, found mostly in the skin, arteries, and lung. Assembly of soluble monomeric tropoelastin into a mature elastic fibre is initiated by liquid-liquid phase separation, or coacervation. This process results in a high local concentration of tropoelastin and is driven by interactions between disordered hydrophobic low sequence complexity domains. Interactions between tropoelastin and other ECM components such as proteoglycans and glycosaminoglycans have been proposed to play a role in both tropoelastin coacervation and the subsequent crosslinking of elastin. While these interactions are thought to regulate the process of tropoelastin assembly and maturation into elastin fibres, the molecular details regarding their specificity and their spatiotemporal relationship with elastin assembly are unclear. To address this, we have used NMR and other biophysical methods to demonstrate that a specific interaction between the cell-surface glycosaminoglycan heparin and the C-terminal domain of tropoelastin strongly promotes coacervation, supporting a role for ECM glycosaminoglycans in the assembly of elastin.

Poster #34:
Solving Electric Fields in Nanofluidic Devices with Deep Neural Networks

Martin Magill, Andrew M. Nagel, Hendrick W. de Haan

Faculty of Science, Ontario Tech University

The driven motion of particles in confinement is of widespread relevance in biophysics. For instance, many micro- and nanofluidic devices operate by driving nanoparticles or biopolymers through fabricated geometries. The interplay of diffusive and driven motion with the shape of the devices can induce useful and interesting behaviours, such as separation of analytes by size.



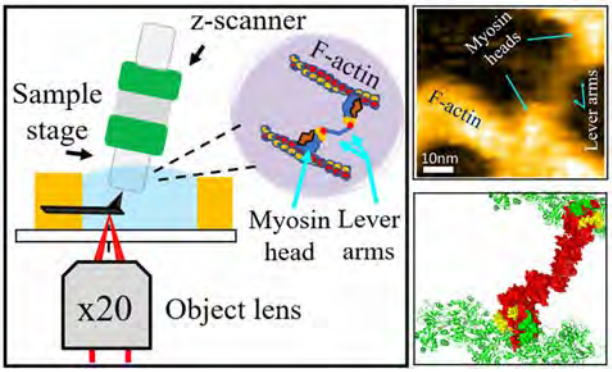
Simulations are often useful in understanding such devices. Commonly, these consist of molecular dynamics simulations, which require numerical solutions of the driving force fields. However, traditional methods for solving such fields (e.g. finite element method) have limitations when used for this application. For one, because they approximate the solution on a spatial discretization of the domain, they exhibit reduced accuracy at sub-grid length scales. Additionally, it is very expensive to use such methods to solve for dynamic coupling between the force fields and the motion of the analytes.

In this poster, we discuss a novel technique for solving force fields: the neural network method. Instead of approximating the forces on a mesh, the solution is approximated globally by a deep neural network. The method is applied to particle simulations in a typical nanofluidic device, and its accuracy is quantified in several metrics.

Poster #50:
Conformational dynamics of the actin-myosin complex studied by high-speed atomic force microscopy.

Oleg S. Matusovsky, Dilson E. Rassier

Department of Kinesiology and Physical Education, McGill University, Montreal, QC, Canada



A common feature of molecular motors is the ability to generate force using energy of ATP hydrolysis while interacting with the polarized tracks, such as actin filaments or microtubules. The force generation is caused by structural alterations within the motors. Despite extensive research the details of the force-generating mechanism is poorly understood, due to the difficulty to directly visualize the fast myosin power stroke at high spatiotemporal resolution. Insight into the mechanism will help us to better understand the fundamental basis of cell contractility powered by molecular motors in health and disease. In our study we visualize the actin-myosin complex in the presence of different nucleotides, providing new details in the force-generating mechanism of myosin II at high spatiotemporal resolution. We showed a two-step motion of the double-headed myosin fragment lever arm, coupled to an 8.4nm working stroke observed in the presence of ATP. Myosin heads attached to an actin filament worked independently, exhibiting different lever arm configurations. A lever arm rotation was associated with several non-stereospecific long-lived and short-lived (~1ms) conformations. We concluded that the presence of free Pi increased the short-lived stereospecific binding events, followed by release of Pi after the power stroke.

Poster #16:
Q277 stabilizes the cASIC1 desensitized state and retards recovery from desensitization

Megan Miaro[1], Matthew Rook[2], Tyler Couch[2], Dana Kneisley[3], David M. MacLean[3], Maria Musgaard[1]

[1]Department of Chemistry and Biomolecular Sciences, University of Ottawa
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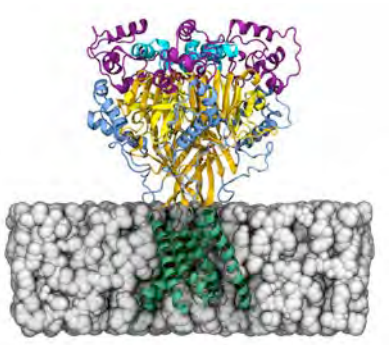
[3]Department of Pharmacology and Physiology, University of Rochester



The desensitization of ASIC1, a proton-gated ion channel, is linked to the β11-12 linker composed of L414 and N415 in the palm domain that undergo an isomerization during desensitization and recovery. A Q276G mutation in the human ASIC1 has been reported to abolish desensitization. It was proposed that this mutation destabilizes the desensitized state and that this state in the wild type is stabilized through a sterics-based mechanism involving the larger Q277 side chain and the β11-12 linker (Wu et al., 2019). We find through electrophysiology that this mutation instead quickens desensitization and recovery. Using molecular dynamics simulations, we observe that Q277 in chicken ASIC1 mediates a hydrogen bond network with the backbone carbonyl of L414 and the side chain of E80, which we hypothesize to stabilize the desensitized state. Q277N and Q277L do not satisfy hydrogen-bond requirements and experimental data confirm an accelerated recovery from desensitization, like the Q277G mutant. This suggests that the stabilization of the desensitized state is more dependent on the observed electrostatic interactions than the previously hypothesized steric mechanism.

Wu, Y., Chen, Z. & Canessa, C. M. A valve-like mechanism controls desensitization of functional mammalian isoforms of acid-sensing ion channels. eLife 8, e45851, doi:10.7554/eLife.45851 (2019).

Poster #22: Podosome Fluctuation Dynamics revealed by Radial Spatiotemporal Image Correlation Spectroscopy

Thomas J. G. Mosher[1], Paul W. Wiseman[1,2], Alessandra Cambi[3], Koen Van den Dries[4]

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[2]*Department of Chemistry, McGill University*
[3]*Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center*
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Podosomes are dynamic cytoskeleton associated structures localized to the cell membrane that play a mechanosensory role in cell protrusion and migration dynamics in macrophages, dendritic cells, osteoclasts and endothelial cells. Podosomes organize into macroscale clusters that modulate migratory behaviour. They are composed of a bipartite structure; the first consists of a dense actin core and actin-regulating proteins that exert forces on the membrane via actin polymerization. The second is a ring of membrane integrins and associated proteins that control cell adhesion. Podosomes have been shown to dynamically regulate their organization in clusters and their individual protein composition of both the core and ring. This multiscale regulation facilitates protrusion and membrane detachment, and drives cell migration. However, the mechanism driving these processes remains poorly understood. Radial spatiotemporal image correlation spectroscopy (radial STICS) takes advantage of intensity fluctuations in fluorescently labelled proteins to probe dynamics on scales ranging from single podosomes to entire clusters. Analysis of Airy scan confocal images of actin and vinculin in human dendritic cells has revealed underlying periodic oscillations of actin recruitment and dispersal and coordinated fluctuations in protein composition. Our measurements report substrate stiffness dependent protein dynamics indicative of substrate sensing behavior integrated on a mesoscopic scale.

Poster #17:

Understanding voltage sensing in the hERG cardiac potassium ion channel

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Ion channels are important for creating and shaping the cardiac action potential, which underlies every heartbeat. The hERG ion channel facilitates repolarization, and is of significant interest because dysfunction due to drug block or inherited mutations results in arrhythmia and sudden death. The structure of hERG channels was recently resolved by cryo-EM, but this does not reveal the dynamic mechanisms that regulate how the channel opens and closes. We have studied how the voltage sensing domain of the channel controls opening and closing, and therefore repolarization in the heart. We aimed to identify molecular interactions important for stabilizing the activated and resting state of the voltage sensing domain. To do this, we introduced charge reversal mutations at a number of sites and performed electrophysiological characterization of their effects on hERG channel opening and closing. We also performed double mutant cycle analysis to determine functional interaction sites by comparing the effects of mutant pairs on the free energy of channel opening. Our findings identify several functional interactions between these residues that stabilize either open or closed states of the hERG channel. These findings will help build fundamental insight into how hERG channels operate and how their function might be manipulated.

Poster #46:
Small Angle X-Ray Scattering Study of Lipid-Free and Lipid-Bound Serum Amyloid A

Asal Nady[1,2*], Sean Reichheld[1], Simon Sharpe[1,2]

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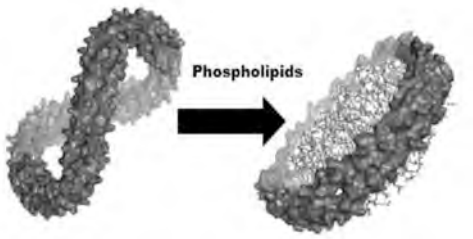


Serum amyloid A (SAA) is a highly conserved apolipoprotein that binds high-density lipoprotein (HDL) particles in plasma by displacing apolipoprotein A-I (Apo A-I) from HDL during the inflammatory response. During an acute inflammatory response, SAA plays beneficial roles that help alleviate the cause of inflammation. However, in patients with chronic inflammatory diseases, constantly elevated levels of SAA contribute to the worsening of inflammation. While structures for lipid-free forms of SAA have been reported, their relationship with the HDL-bound form of the protein, and with the mechanism of SAA binding to lipids, has not been established. Here, we have used multiple biophysical techniques, including SAXS, TEM, SEC-MALS, and native PAGE, to characterize the lipid-free and lipid-bound states of SAA. The SAXS and TEM data show the presence of soluble octamers of SAA with structural similarity to the ring-like structures reported for lipid-free Apo A-I, and that are distinct from the previously reported SAA structures. These SAA octamers are capable of scaffolding lipid nanodiscs that exhibit similar morphology to those formed by Apo AI, and that have similar dimensions to lipid-free SAA, suggesting that relatively few conformational rearrangements may be required to allow SAA interactions with HDL.

Poster #4:
Characterizing the structure and function of rhodoquinone biosynthesis enzymes

Trilok Neupane[1], Jamie E. Spawn[2], Sophia Whitworth[2], Jennifer N. Shepherd[2] & David N. Langelaan[1]

[1]*Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, NS, Canada*
[2]*Department of Chemistry and Biochemistry, Gonzaga University, Spokane, WA, USA*

Rhodoquinone (RQ) is essential to the bioenergetics of organisms that survive in low oxygen environments. RQ allows the electron transport chain to function with fumarate as a final electron acceptor instead of oxygen. Rhodoquinone biosynthesis protein A (RquA) is a putative methyltransferase-like enzyme that is essential for RQ biosynthesis in many bacteria and protists, making RquA a drug target specific to pathogens that require RQ. RquA uses ubiquinone as a substrate; however, the enzymatic mechanism of RquA is unknown. In this study, we expressed RquA from Rhodospirillum rubrum (RquA-Rr) in Escherichia coli as MBP fusion protein. The fused protein is purified in the presence of Brij-35 (0.1% w/v) using Ni-NTA affinity chromatography and is cleaved by TEV protease. The soluble RquA is isolated by size exclusion chromatography and detergent exchanged by ion-exchange chromatography. We are currently optimizing the detergent conditions before protein crystallization. In addition, the structure of RquA-Rr was predicted using trRosetta to have a Rossmann-fold similar to that of S-adenosyl-L-methionine (SAM) dependent methyltransferases. Molecular dynamics simulation and the I-TASSER server predicted that SAM binds with RquA-Rr via two aspartic acid residues. Mutation of those residues to alanine disrupted RQ production, confirming that SAM is required for RquA activity.

Poster #66:
On the dynamic range of molecular counting with quantitative single-molecule localization nanoscopy

Daniel F. Nino[1,2], Joshua N. Milstein[1,2]

[1]*Department of Physics, University of Toronto,*

[2]*Department of Chemical and Physical Sciences, University of Toronto Mississauga,*

The advent of single-molecule localization microscopy (SMLM) allowed for qualitative studies of the nanoscale in biological systems. There has also been significant effort devoted to developing techniques which use the principle of SMLM to extract quantitative properties of such systems. These include quantities such as molecule copy number and protein stoichiometry. Many of the techniques developed for this purpose infer these quantities from the properties of the detected emissions in SMLM, such as number of blinks, number of localizations, or the ratio of ON to OFF time. However, the accurate measurement of these properties pre-supposes a fluorophore density that is low enough that emission events from distinct fluorophores within a diffraction-limited area do not overlap. Despite its importance to the experimental design of counting experiments, the necessary conditions for this have not been quantitatively explored. We have developed a method which can estimate the relative number of missed localizations/ blinks given fluorophore densities. This method not only accounts for the effects of discretization of emissions into camera frames but also offers insight into how fluorophore photophysics affects how accurately the number of localizations/blinks can be measured and by extension how well molecule copy numbers can be inferred from these quantities.

Poster #30 & Trainee Symposium Talk:
Correlating solvent interactions, structure and LLPS behaviour of resilin-derived polypeptides

James B. Otis, Simon S. Sharpe

Department of Biochemistry, University of Toronto

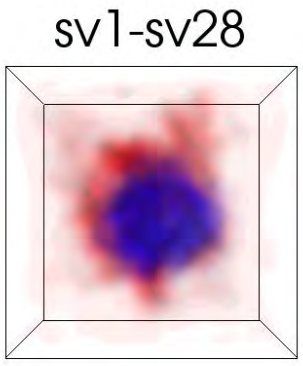
The insect protein pro-resilin is an intrinsically disordered protein (IDP) that self-assembles to form a rubber-like material (resilin) with exceptional mechanical properties. Self-assembly via a liquid-liquid phase transition (LLPS) is controllable through alteration of solution conditions, making it promising for *in-situ* delivered biomaterials for tissue engineering. Here we studied the effects of pH and Hofmeister salts (Na_2SO_4, NaCl, NaI) on the LLPS of two resilin like poly-peptides, RLP_17GQ and RLP_38, constructed from consensus motifs of pro-resilin's domains 1 and 3 respectively. Using turbidity assays, NMR spectroscopy, and SAXS, we correlated the pH and salt dependent LLPS behaviour of RLPs with structural and dynamic properties of the monomeric peptides. The phase transition temperatures followed expected trends for the salts used, but differed in magnitude when changing pH and salt. Interestingly, Rg and Rh did not change significantly at high salt but did evidence a partially collapsed conformation, in agreement with previous studies of domain 1. NMR data were then used to relate LLPS and conformational changes to amino acid specific differences in hydration and salt anion binding. The results show how global conformation, local solvation, and syntax of RLPs are correlated with unique dependencies of their LLPS behaviour on solution conditions.

Poster #19:
Sub-compartmentalization of polyampholyte species in organelle-like condensates is promoted by charge pattern mismatch and strong excluded volume interaction

Tanmoy Pal[1], Jonas Wessén[1], Suman Das[1] and Hue Sun Chan[1]

[1]*Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada.*

In addition to displaying liquid droplet like behaviour as a whole, membraneless organelles such as nucleolus, stress granules contain sub-compartments that are enriched with specific proteins. Aiming to better understand the physics of selective compartmentalization in membraneless organelles, we studied coarse-grained models of multi-species Intrinsically Disordered Proteins (IDPs) solutions where the residues interact via short-range excluded volume and long-range Coulomb interactions. Our polyampholyte Field Theoretic Simulations (FTS) and explicit chain molecular dynamics (MD) simulations of two-species IDP systems show consistently that a substantial polymer excluded volume and a significant mismatch of the IDP sequence charge patterns can act in concert, but not in isolation, to demix the two IDP species upon condensation. This finding reveals an energetic-geometric interplay in a stochastic, "fuzzy" molecular recognition mechanism that may facilitate sub-compartmentalization of membraneless organelles. Here we present results published in our recent publication Pal *et al.*, Physical Review E **103**, 042406 (2021).

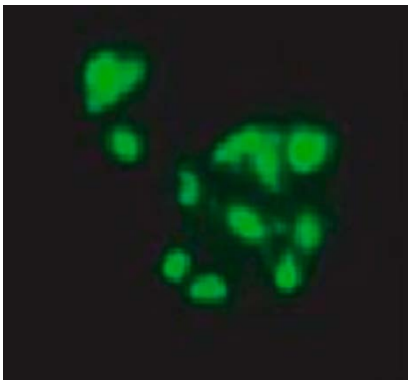


Poster #5:
Modeling protein fluctuations in mitochondrial networks

Rutvik J. Patel and Aidan I. Brown

Department of Physics, Ryerson University

Mitochondria form tubular networks that are dynamic, including fusion events that form larger mitochondria, fission events resulting in multiple smaller mitochondria, and import of newly-synthesized proteins. The concentration of proteins can vary greatly between the individual mitochondria in a cell. We use quantitative modeling to explore how the stochasticity in protein delivery to mitochondria can lead to variation in mitochondrial protein concentrations in yeast cells. mRNA for many mitochondrial proteins can be tethered by a nascent protein to a mitochondrion as the nascent protein is simultaneously synthesized by a ribosome and imported into the mitochondrion. Stochasticity thus arises from these binding events and the production of individual proteins when combined with the small number of mRNA present in a yeast cell. We compare mitochondrial protein concentration variation to experimental measurements across mitochondrial sizes and cell conditions.



Poster #2:
Anti-Prion Chaperone Pentosan Polysulfate Interacts Avidly with Unfolded States of the Prion Protein

Shubhadeep Patra, Rafayel Petrosyan, Uttam Anand, Craig R. Garen, Michael T. Woodside

Department of Physics, University of Alberta, Edmonton, AB, T6G 2E1, Canada

Prion diseases involve propagated misfolding of the protein PrP. Small-molecule pharmacological chaperones can impede conversion of protein, but how they do so is often unclear. Previous studies of the interactions of two different pharmacological chaperones with hamster PrP (HaPrP) showed that they bound not only to native PrP, but also to partially or fully unfolded PrP, indicating that unfolded states play a crucial role. However, it is challenging to observe the interaction of ligands with unstable and transient unfolded conformers. Here we use single-molecule force spectroscopy to destabilize bank vole PrP (BVPrP) and observe interactions between unfolded BVPrP and the chaperone pentosan polysulfate (PPS). Unlike HaPrP, BVPrP unfolds and refolds natively via intermediates. We found that PPS stabilized certain partially unfolded intermediates of BVPrP, as well as the fully unfolded state, interacting heterogeneously as for HaPrP. By examining the extent of the interactions at different concentration, we estimated that PPS bound 10-fold more tightly to unfolded states than to the native state of PrP. These results reinforce the likely importance of unfolded states in the mechanism for propagated misfolding: when natively folded PrP molecules unfold partially or fully during conversion, PPS binds avidly to the unfolded portions, inhibiting conversion.

Poster #64:
Elucidating the roles of histone tails in nucleosome recognition

Yunhui Peng[1], Shuxiang Li[2], Alexey Onufriev[3], David Landsman[1] and Anna R. Panchenko[2]*

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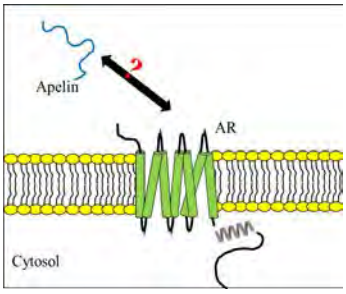
[2] Department of Pathology and Molecular Medicine, School of Medicine, Queen's University, ON, Canada
[3]Physics Department, Virginia Tech, VA, USA

Histone tails play critical roles in epigenetic regulation. However, little is known about the mechanisms of how histone tails modulate the nucleosomal and linker DNA solvent accessibility and recognition of nucleosomes by other macromolecules. Here, we generate extensive atomic level conformational ensembles of histone tails in the context of the full nucleosome, totaling 50 microseconds of molecular dynamics simulations. We explore the histone tail binding modes with the nucleosomal and linker DNA and observe rapid conformational transitions between bound and unbound states allowing us to estimate kinetic and thermodynamic properties of the histone tail-DNA interactions. Our results show that different histone types exhibit distinct binding modes, and each histone type occludes specific DNA regions from the solvent. Furthermore, we utilize experimental data on nucleosome structural complexes to explore how tail dynamics may mediate the interactions of nucleosomes with partners. We find that most of the studied nucleosome-binding proteins and histone tails target mutually exclusive regions on nucleosomal or linker DNA. This finding is explained within the generalized competitive binding and tail displacement models of partners recruitment to nucleosomes. Finally, we demonstrate how histone tails' post-translational modifications and mutations may influence this process through comprehensive simulations of modified histone tails.

Poster #63:
Applying ^{19}F-labelled apelin to characterize binding to bicelles en route to on-cell NMR studies

Trần Thanh Tâm Phạm[1], Alexandre Murza[2], Éric Marsault[2] and Jan K. Rainey[1,3,4]

[1] *Department of Biochemistry and Molecular Biology, Dalhousie University*
[2] *Département de Pharmacologie-Physiologie, Institut de Pharmacologie de Sherbrooke, Uversité de Sherbrooke*
[3] *Department of Chemistry, Dalhousie University*
[4] *School of Biomedical Engineering, Dalhousie University*

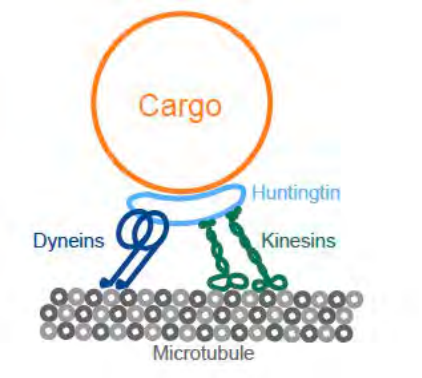


The interaction between a G-protein coupled receptor, the apelin receptor (AR), and its peptidic apelin ligands elicits critical physiological responses but is poorly understood. Apelin may be processed from a 55-residue peptide into shorter versions (isoforms), all of which share the C-terminal segment but with a wide range of bioactivity and tissue localization. Little is known about the regulation leading to preferential isoform production nor differential activation of AR. These are important questions to answer both fundamentally and for therapeutic application in chronic conditions. To understand these interactions, apelin peptides with ^{19}F-labels at defined positions are being applied for nuclear magnetic resonance (NMR) spectroscopy in: (i) two forms of phospholipid bicelle (zwitterionic bicelles either without or with incorporation a fraction of lipids having negatively charged headgroups); and, (ii) AR-transfected HEK 293 cells. Using ^{19}F-labelled apelin-17 analogues in bicelle conditions, we find that each analogue was able to bind both classes of bicelle. All analogues showed a decrease in diffusion coefficient with both bicelle classes although this decrease was more prominent among zwitterionic bicelles without negatively charged headgroups. This correlates to our hypothesis that the cell membrane enhances and, perhaps, provides isoform specificity of peptide binding to the AR.

Poster #27 & Trainee Symposium Talk: Huntingtin S421 phosphorylation and overexpression differentially affect signalling and degradative intracellular cargoes

Emily N. P. Prowse[1], Abdullah R. Chaudhary[1], David Sharon[1], Adam G. Hendricks[1]

[1]*Department of Bioengineering, McGill University*



Huntingtin phosphorylation and expression levels differentially regulate the motility of early endosomes and lysosomes. Huntingtin acts as a scaffold that interacts with both motors and cargoes, enabling it to modulate intracellular transport. Polyglutamine expansions in huntingtin cause Huntington's Disease, a debilitating neurodegenerative disease. Previous studies in neurons showed that huntingtin phosphorylation at S421 increased signalling vesicle trafficking toward the cell periphery. To mimic phosphorylation and maintain endogenous stoichiometry of interactions, we used CRISPR to generate a HEK293T cell line with serine 421 to aspartate (S421D) mutation. Early endosomes demonstrate no significant differences in motility between the wild-type (WT) and S421D cells. Upon overexpression of either WT or S421D huntingtin, the processivity of early endosomes is increased, while their run lengths decrease from the control condition. Interestingly, overexpression of WT or S421D huntingtin constructs had no significant effect on either processivity or run length of lysosomes. Lysosomal transport demonstrated a significant increase in run length upon S421D mutation at the endogenous level, while processivity remained unaffected. This indicates a longer microtubule association time in cells endogenously expressing S421D huntingtin, without changing apparent motor protein activity. Huntingtin overexpression appears to affect early endosomes exclusively, increasing the number of rapid, short runs.

Poster #9:
Comparing Interactions Between Four Distinct Apelin Isoforms and the Class A GPCR Apelin Receptor Through Molecular Dynamics Simulations

Jayatee Ray[1], Trần Thanh Tâm Phạm[1], Jan K. Rainey[1-3]

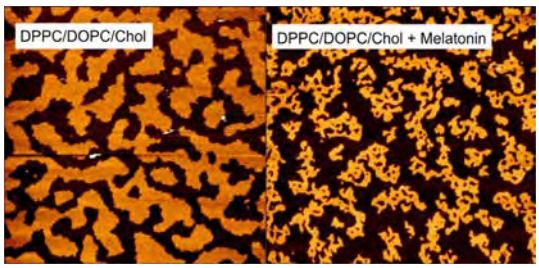
[1]Department of Biochemistry & Molecular Biology, [2]Department of Chemistry, and [3]School of Biomedical Engineering, Dalhousie University, Halifax NS

The apelinergic system is involved in many protective physiological roles; however is also active in numerous pathophysiological states. This system comprises a class A G-protein coupled receptor (GPCR), the apelin receptor, and two families of peptide ligands, apelin and apela. The intricacies of the system are not yet fully known; therefore, to develop treatments targeting the apelinergic system, greater information regarding how various ligands are able to differentially activate a single GPCR is essential. We present molecular dynamics (MD) simulation-based studies of complexes of the apelin receptor with apelin-13 (i.e., the 13-residue isoform of apelin), -17, -36 and -55, evaluating the changes to these complexes over 1.1 μs atomistic simulations with the receptor embedded in a lipid bilayer solvated by water. Findings show that across all four ligand-receptor complexes, the transmembrane region of the apelin receptor remains stable throughout the simulation. In contrast, the N and C-termini of the receptor are quite dynamic. Each of the four apelin isoforms studied display a decreasing gradient from relatively flexible N-termini to restrained C-termini. As the isoforms increase in length, greater structural change is observed. Future studies with longer simulation times alongside independent replicates must be implemented to determine if these behaviors persist.

Poster #48:
The Interaction of Melatonin with Model Lipid Membranes by AFM

Morgan Robinson[1], Erik Herz[1], Minh Phung[1], Carina T. Filice[2], Brenda Lee[1], Zoya Leonenko[1,2]

[1]Department of Physics and Astronomy, University of Waterloo
[2]Department of Biology, University of Waterloo



The biophysical properties and structure of the cell membrane are crucial for normal cellular function. The composition of the lipid bilayer and its interactions with small molecules affect these properties thus playing a role in health and disease. One such molecule is melatonin, a pineal gland hormone involved in circadian rhythm regulation, as well as a potent antioxidant and neuroprotectant against toxic amyloid-β in Alzheimer's disease. Melatonin is also a membrane active molecule and is known to partition into the lipid membrane. In this work, we use atomic force microscopy (AFM) to show the interaction of melatonin with model lipid systems. We show that melatonin can incorporate into DPPC/DOPC/Chol containing lipid bilayers and affect domain structure. Melatonin also affects membrane nanomechanical properties, such as breakthrough force, as demonstrated by atomic force spectroscopy. We also show that melatonin affects model neuronal lipid monolayer electrostatic properties. This work demonstrates melatonin's ability to affect membrane properties and structure which may play a key role in its physiology including its neuroprotective action.

Poster #62:
Behavioural Phases of Immigration-Birth-Death Processes in Competitive Ecosystems

Jeremy B Rothschild[1], Nava Leibovich[1], Sidhartha Goyal[1], Anton Zilman[1]

[1]*Department of Physics, University of Toronto*

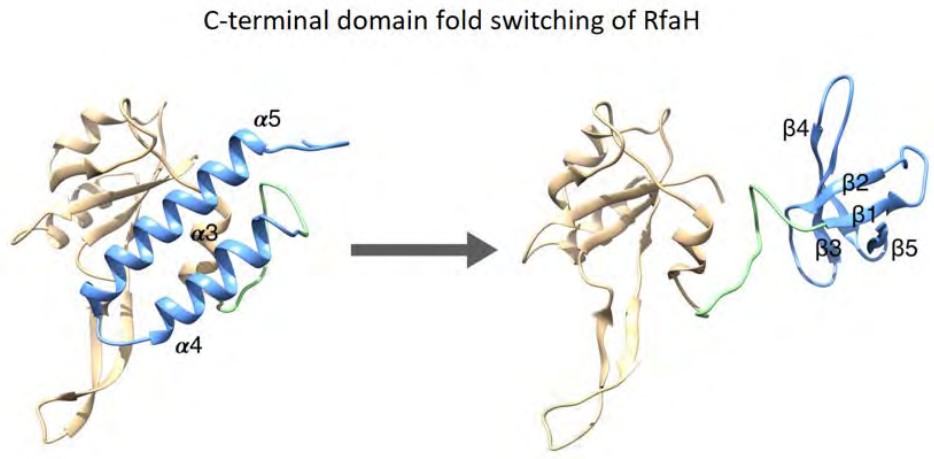
Neutral theories of biodiversity assume that all individuals are functionally identical regardless of the species, whereas symmetrical non-neutral (i.e “niche”) theories forego the assumption of equivalent individuals and distinguish solely between self and non-self interactions. Although stochastic dynamics mediate species survival in both theories, a competitive niche overlap in non-neutral theories affects the species abundance distribution; coexistence and dominance regimes have been observed in both theories in previous works. Using a minimal model of interacting species, we have comprehensively investigated how the species abundance distribution changes between different regimes defined by immigration rate and the niche overlap transitions. We also identify a previously unknown regime where the abundance distribution is multimodal. We investigate the presence and absence of dominant species, illustrating how the number of dominant individuals and species change in different regimes. Furthermore, we show that transitions between different coexistence regimes are controlled by the balance of mean first passage times for transmission and reflection between different abundance levels. Our results provide a framework for interpreting the discrepancies of abundances in ecological data and inferring the underlying dynamics that shape communities of interacting species.

Poster #29:
Structural fluctuations and mechanical stabilities of the bacterial transcription factor RfaH

Bahman Seifi[1], Stefan Wallin[1]

Department of Physics and Physical Oceanography, Memorial University of Newfoundland, St John's, Newfoundland and Labrador, Canada

RfaH is a two-domain bacterial transcription factor that functions both as a regulator of transcription and an enhancer of translation. To carry out these dual functions the ~50-amino acid C-terminal domain (CTD) undergoes a large-scale structural transition from a helical hairpin fold to a 5-stranded beta-barrel, triggered by the binding of RfaH to RNA polymerase (RNAP). The ~100 amino acid N terminal domain (NTD), On the other hand, remains structurally unchanged by the switch in fold. In this study, we use all-atom Monte Carlo simulations to investigate the size of structural fluctuations and local mechanical stabilities of both full-length RfaH and the CTD as an isolated fragment. Our simulations show that the α-fold of the CTD is only stable in the presence of interactions with the NTD. Hence, in agreement with experiment, we find that inter-domain interactions play a crucial role in stabilizing the native state of free RfaH. To probe the mechanical stabilities, we measure the work required to impose small local structural deformations at different positions along the chain, which provides a measure of the local structural rigidity. The resulting mechanical stability profile reveals that free RfaH is divided into a rigid NTD part, a soft CTD part, and a flexible unstructured linker which joins these two domains to each other. Similarly, structural fluctuations are more pronounced in the NTD than in the CTD. We consider how these structural and mechanical features may trigger fold switching in RfaH upon binding to RNAP.



Poster #32:
Structure and Dynamics of Non-mammalian Tubulin

Madeline Shred, Gary Brouhard

Department of Biology, McGill University

All eukaryotic organisms rely on microtubules for their role in important cellular processes like cell division, intracellular transport, and cell shape. Until recently, tubulin could only be purified from mamallian brains. I propose to use recent advances in tubulin purification to study the structure of *Drosophila melanogaster* microtubules via cryo-EM and characterize the dynamics of these microtubules by Interference-Reflection Microscopy. *Drosophila* tubulin is suspected to polymerize faster than mammalian tubulin suggesting subtle structural differences between the polymers. Comparing *Drosophila* tubulin dynamics and microtubule structure with a control mammalian data set will allow for a better understanding of the role and function of microtubules across evolutionary distance.

Poster #6 & Trainee Symposium Talk: Probing Structural Modularity and Disorder in Recombinant Pyriform Silk

Jeffrey R. Simmons[1], Geneviève Seabrooke[2], Jan K. Rainey[1,3,4]

[1]Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, NS [2]Princess Margaret Cancer Research Center, PMH, High-Field NMR Core Facility, Toronto, ON [3]Department of Chemistry, Dalhousie University, Halifax, NS

[4]School of Biomedical Engineering, Dalhousie University, Halifax, NS

Spider silks are biomaterials used for many diverse adaptations by spiders, with mechanical properties comparable to Kevlar and high strength steel. Orb-weaver spiders create up to seven distinct types of silk, including pyriform silk, which is used with a glue-like substance to attach silks together and anchor the spider web. Although filling a critical role in web formation, prior to our work neither the structural nor mechanical properties of pyriform silk had been widely investigated. Based on the central pyriform silk repetitive domain from *Argiope argentata*, we successfully engineered recombinant pyriform silk-based proteins. Silk fibres formed from these proteins showed a relatively high combination of strength and extensibility, in contrast to extremes of one vs. the other as seen in most silks. To understand the structure-function relationships for this distinctive class of silk, we are performing atomic-level solution structural studies in parallel with fibre structural studies. Our initial results suggest the solution state of this protein contains an ordered, 5-6 helix bundle with long disordered linkers at each terminus that is retained with addition of an extra unit from the pyriform central repetitive domain. This allows us to provide the first basic model for pyriform silk.

Poster #49:
Investigation of the Determinants of Agonism in a Ligand-Gated Ion Channel using Statistical Coupling Analysis

Mykhaylo Slobodyanyuk[1], Jesús Banda-Vázquez[1], Mackenzie J. Thompson[2], John E. Baenziger[2], Roberto A. Chica[1], Corrie J. B. daCosta[1]

[1]*Department of Chemistry and Biomolecular Sciences, University of Ottawa*
[2]*Department of Biochemistry, Microbiology and Immunology, University of Ottawa*

Acetylcholine is the native agonist of acetylcholine receptors, a subfamily of eukaryotic pentameric ligand-gated ion channels. The structurally related prokaryotic *Erwinia chrysanthemi* ligand-gated ion channel (ELIC) is competitively inhibited by acetylcholine. To understand the opposite effect upon acetylcholine binding between ELIC and acetylcholine receptors, we used statistical coupling analysis to predict mutations necessary for installing acetylcholine agonism into ELIC. To perform statistical coupling analysis, we acquired thousands of homologous structures and sequences that resembled the *Lymnaea stagnalis* acetylcholine binding protein. This protein is a structural surrogate for the agonist binding domain of acetylcholine receptors, for which a high-resolution structure in complex with acetylcholine is available. Our analysis identified a group of statistically coupled residues that comprises several amino acids previously implicated in acetylcholine agonism of acetylcholine receptors. Mapping these residues onto ELIC revealed several residue discrepancies which formed the basis for ELIC mutagenesis. Electrophysiological characterization of ELIC mutants indicates that acetylcholine is still unable to activate the receptor. However, by installing two residues present in acetylcholine receptors we were able to convert acetylcholine from a competitive antagonist into a potentiator. This work demonstrates the ability of statistical coupling analysis to identify functionally important residues in ligand-gated ion channels.

Poster #52:
Dynamic and Disordered: Local and Global Chain Motions in the 4E-BP2 protein are Tuned by Phosphorylation and Binding

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

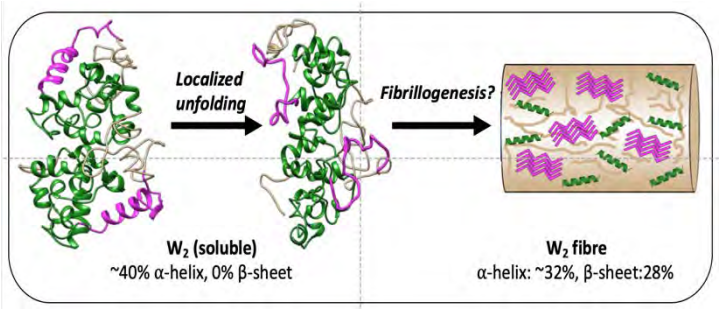
Cap-dependent translation initiation is regulated by the interaction of eukaryotic initiation factor 4E (eIF4E) with disordered eIF4E binding proteins (4E-BPs) in a phosphorylation-dependent manner. Single-molecule fluorescence resonance energy transfer (smFRET), fluorescence correlation spectroscopy (FCS), and fluorescence anisotropy decay (FAD) were used to monitor the structural changes and sequence-specific local chain motions of 4E-BP2 upon phosphorylation and binding to eIF4E.

FAD was used to study the local segmental dynamics and chain flexibility of 4E-BP2. The segmental flexibility is hindered in the folded 18-62 region upon phosphorylation, whereas the rest of the chain becomes more flexible. In the eIF4E:4E-BP2 complex local motion of segments N-terminal to the canonical binding site are hindered while those C-terminal do not change. Longer range intra-chain reorganization dynamics were assessed by FCS via photoblinking/quenching of the fluorophore by aromatic residues. Heterogeneous quenching kinetics were observed: 4E-BP2 in the eIF4E:4E-BP2 complex shows an increased quenching rate compared to the apo state. Global conformation changes were assessed by two distinct FRET constructs, with probes flanking the folded and N-terminal disordered regions, respectively. An increase in the dimension of the folded region was observed upon complexation with eIF4E suggesting 4E-BP2 adopts extended conformations in the bound state.

Poster #25:
A DISULPHIDE LOCK REVEALS THE TRIGGER FOR FIBRILLOGENESIS IN ACINIFORM SPIDER SILK

Anamika Sulekha[1], Lingling Xu[1], Paul Xiang-Qin Liu[1] & Jan K. Rainey[1,2,3]

[1]*Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, NS, Canada.*
[2]*Department of Chemistry, Dalhousie University, Halifax, NS, Canada.*
[3]*School of Biomedical Engineering, Dalhousie University, Halifax, NS, Canada.*



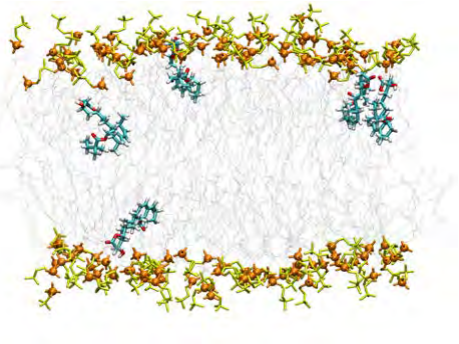
A variety of silks are produced by spiders for disparate ecological needs, each differing in its amino acid content, morphology, and mechanical properties, making these highly desirable biomaterials. Aciniform silk from *Argiope trifasciata* (AcSp1), used in prey wrapping and egg-casing, contains a core repetitive domain of ≥14 identical 'W units'. This protein adopts an α-helix-rich *bead-on-a string* architecture in the soluble state. AcSp1 fibres, unlike other most silks, retain significant α-helical content alongside transformation to a substantial proportion of β-sheet. Relative instability and elevated dynamics of the C-terminal α-helix (H5) in the soluble state led us to hypothesize that its localized unfolding would decrease compactness, favouring protein entanglement and intermolecular interactions to promote fibrillogenesis. Two Ser-to-Cys substitutions were introduced to act as a disulphide staple. Comparison of fibre formation, fibre properties and atomic-level structural and dynamics analysis of the oxidized vs. the reduced W unit are presented to test this hypothesis. While the mutant cannot form fibres in the oxidized state, the reduced form is found to be fully functional. Loss of H5 helicity along with variations in backbone dynamics correlate with these functional changes to reveal a key trigger of AcSp1 fibrillogenesis, in turn facilitating protein engineering.

Poster #45 & Trainee Symposium Talk: Modulation of Phospholipid Bilayer Properties by Simvastatin

Ruijie D. Teo[1,2], Peter Tieleman[1,2]

[1]*Department of Biological Sciences, University of Calgary, Calgary AB T2N 1N4, Canada*
[2]*Center for Molecular Simulation, University of Calgary, Calgary AB T2N 1N4, Canada*

Simvastatin (Zocor©) is one of the most prescribed drugs for reducing high cholesterol. However, some of the side effects associated with the intake of simvastatin and other lipophilic statins at higher doses include statin-associated myopathy (SAM) and in more severe cases, kidney failure. It is hypothesized that these side effects are dependent on the localization of statins in lipid bilayers and their impact on bilayer properties. In this work, we carry out all-atomistic molecular dynamics simulations on both the lactone and dihydroxyheptanoate forms of simvastatin (termed 'SN' and 'SA' respectively) with a POPC lipid bilayer as the model membrane. Additional simulations were carried out on multiple simvastatin molecules in order to mimic experimental conditions. Both SN and SA spontaneously diffused into the lipid bilayer, and a longer simulation time of up to 2 μs was needed for complete incorporation of multiple SAs into the bilayer. We also find that SN localizes deeper within the hydrophobic interior of the bilayer, and that SA has a greater tendency to form hydrogen bonding interactions with neighboring water molecules. Both SN and SA increase the lateral surface area and membrane order, causing greater perturbations when more drug molecules are added.

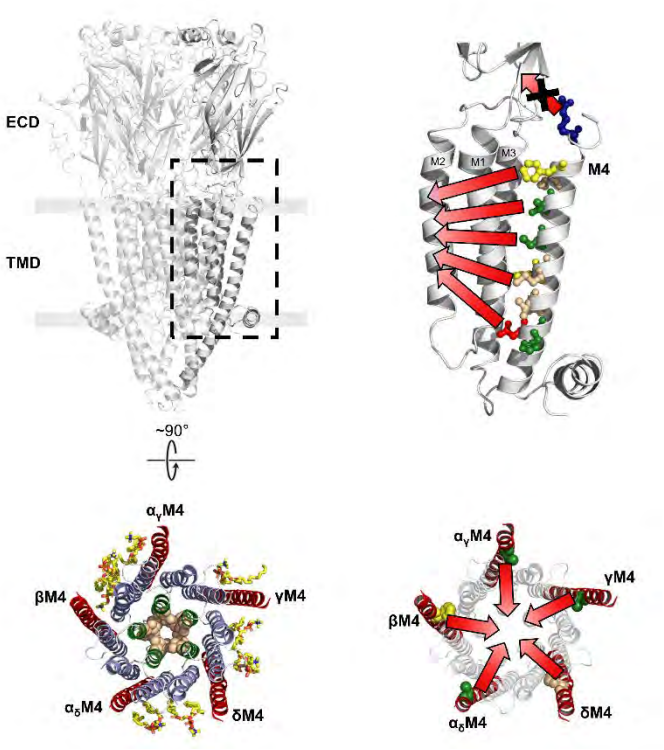


Poster #60:
Functional role of the M4 lipid-sensor in the muscle nicotinic acetylcholine receptor

Mackenzie J. Thompson[1], Jaimee A. Domville[1], John E. Baenziger[1]

[1]*Department of Biochemistry, Microbiology, and Immunology, University of Ottawa*

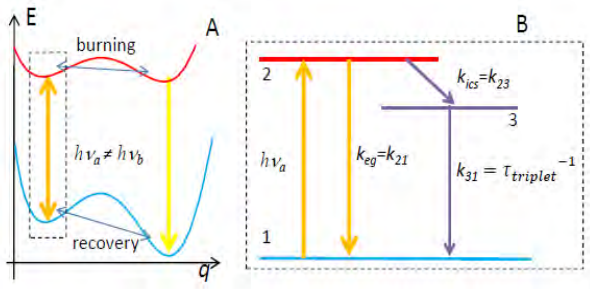
The muscle nicotinic acetylcholine receptor (nAChR) displays an exquisite functional sensitivity to its surrounding lipid environment, but the structural basis of this sensitivity has remained poorly defined. The peripheral M4 α-helix in the transmembrane domain (TMD) of each nAChR subunit has been proposed to act as a lipid-sensor, relaying changes in lipid composition to altered function. As a first step in deciphering a structural basis for how lipids modulate function, we probed the functional role of each M4 α-helix through a combination of site-directed mutagenesis and two-electrode voltage-clamp electrophysiology. We found that residues along M4 that orient toward the M1 and M3 α-helices of the TMD were most sensitive to mutation. On the other hand, deleting extracellular domain (ECD) interacting residues in the M4 C-termini had little to no effect on nAChR function. Finally, mutations to each individual M4 α-helix were shown to influence function independently and thus additively. These results suggest lipid induced modulation of nAChR function occurs primarily through altered interactions between M4 and the TMD, rather than through interactions between M4 and the ECD. The sum of subtle changes at each M4-M1/M3 interface likely add up to define the exquisite functional sensitivity of the nAChR to lipids.



Poster #55:
Effects of Chlorophyll Triplet States on the Kinetics of Spectral Hole Growth

Alexandra Trempe[1], Alexander Levenberg[1], Angel David Gonzalez Ortega[1], Maria A. Lujan[2], Rafael Picorel[2], Valter Zazubovich[1]

[1]*Department of Physics, Concordia University, Montreal*
[2]*Estacion Experimental de Aula Dei (CSIC), Zaragoza, Spain*



Chlorophyll (Chl) triplet states can affect the results of optical spectroscopy experiments in pigment-protein complexes involved in photosynthesis. Chl *a* triplet lifetimes increase to several milliseconds at cryogenic temperatures.

One type of experiments performed near absolute zero is persistent non-photochemical hole burning (NPHB). The process is normally explained using double-well potentials / two-level systems. In NPHB experiments on cytochrome b_6f and chemically modified LH2, we observed 1) mismatch between hole growth kinetics (HGK) hole depths and post-burn hole depths, and 2) the slowdown of HGK with increased light intensity, with stronger effect in deuterated-solvent samples.

Including the triplet states requires modifying several terms in the NPHB master equation. Allowing for transient holes explains the discrepancy between persistent hole depths observed with different NPHB modalities. Triplet states would indeed lead to slower hole burning, playing a bigger role at higher light intensities. We also simulated the solvent deuteration effect as solvent deuteration is known to increase Chl *a* triplet lifetimes. However, even the five-fold increase of triplet lifetimes could not fully account for our observations. Therefore, local heating of the pigment-protein complexes due to poor heat conduction though the protein-solvent interface may be present as well.

Poster #10:
How do intracellular crowders affect the behaviour of polymers: Diffusion NMR of polyethylene glycol in the presence of bacteria cell lysate crowders

Yanitza Trosel[1], Valerie Booth[1,2], and Anand Yethiraj[1]

[1]*Department of Physics and Physical Oceanography*
[2]*Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada*

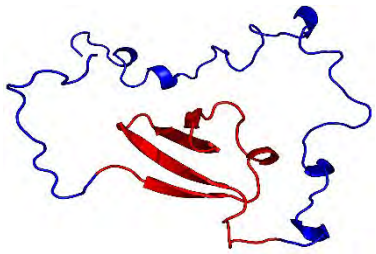
The volume occupied by biomolecules inside living cells is estimated to be around 40%, making the environment within cells crowded and heterogenous. Macromolecular crowding affects protein- protein interactions, protein folding, ligand binding and aggregation. These effects can be caused by aspects of crowding including viscosity, excluded volume and non-specific interactions. Crowding studies have typically involved artificial crowders like ficoll and dextran, and less is known about the effects of biological macromolecules as crowders. It has been suggested that the nature of the crowders, like size, shape and charge affect the properties of biomolecules, such as diffusion within a cell. The purpose of this study is to compare the translational diffusion of polyethylene glycol (PEG), using pulsed gradient NMR, in the presence of cell lysate as a crowder. While PEG is not a biomolecule it is a useful analog of protein, especially intrinsically disordered proteins. To differentiate the effects of different types of crowding, the cell lysate is manipulated to modify its size and charge profile, and the effects on PEG diffusion compared. Moreover, we examine the flow properties between biological and artificial crowders. These results will provide a better understanding of the non-specific interactions in complex biological crowders.

Poster #3 & Trainee Symposium Talk: Protein Conformational Ensembles for a Disordered Protein Restricted by Separate Biophysical Experiments

Thomas Tsangaris, Claudiu C. Gradinaru

Department of Chemical and Physical Sciences, University of Toronto Mississauga

Conformational ensembles consistent with NMR, SAXS and smFRET were calculated for the translation regulator protein 4E-BP2. This is a disordered protein having an active, non-phosphorylated (NP) state and an inactive, multi-phosphorylated (5P) state. ENSEMBLE method (Krzeminski et al, Bioinformatics, 2013) was used to sample conformations within a large initial pool that are consistent with experimental data. NMR and SAXS data were used as restraints while smFRET data were used as validation criteria.



The NP 4E-BP2 ensemble restrained only by SAXS showed bimodality of global dimensions such as radius of gyration and end-to-end distance. The back-calculated FRET efficiency and the hydrodynamic radius were lower than the respective experimental values. These inconsistencies are likely due to lack of short-range (5-20 Å) distance restraints, which are currently being addressed by using newly acquired PRE data. Such data is available for 5P 4E-BP2, however this is partly folded, spanning residues 18-62. To address this challenge, we used the FastFloppyTail (FFT) program (Ferrie et al, JPCB, 2020) to generate the initial pool. Hence, folds from the Protein Databank and disordered tails generated through FFT were concatenated to model the folded region. Analysis of these ensembles reveal new insights into the biological function of 4E-BP2.

Poster #51:
Interaction Between Antimicrobial Peptides and Non-lipid Components in Bacterial Outer Membrane

Sheyla Montero Vega[1], Valerie Booth[1], Christopher Rowley[2]

[1] Department of Biochemistry, Memorial University of Newfoundland
[2] Department of Chemistry, Carleton University

Antimicrobial peptides (AMPs) offer advantages over conventional antibiotics; for example, bacteria develop less resistance to AMPs. The interaction of the AMPs with the lipopolysaccharide layer of the gram-negative bacteria cell envelope is not well understood. This first interaction is essential for the peptide uptake and specificity to the target organism. We simulated the AMPs interactions with the bacterial envelope. We build a coarse-grained system formed by a gram-negative bacterial outer membrane, including the LPS layer and the AMP Magainin 2. We perform 20us of molecular dynamics simulation on the free system. During the simulation, the AMP stays at 5nm in the Z axis, corresponding to the LPS layer of the cell envelope, this result suggests interactions between the positive charge of the Magainin 2 and the LPS layer. Finally, we calculated the Free Energy Profile for the insertion of the Magainin 2 in the membrane. For the Free Energy Profile construction, the AMP was restrained to 200 different positions across the membrane performing molecular dynamic simulation to each window. The minimum of energy was found around the 4nm, suggesting an interaction between the AMP and the negative charge groups of the LPS layer of the bacterial cell envelope.

Poster #28:
Exploring the Structural Stability and Assembly Mechanism of Hydrophobin Proteins

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[1]*Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, NS, Canada*

Hydrophobins are small, globular proteins with amphiphilic character that are produced and secreted by filamentous fungi. At hydrophobic-hydrophilic interfaces they self-assemble into durable amyloid-containing structures, called rodlets, which create protective, water repellent coatings for fungal spores. Current models of hydrophobin self-assembly predict that hydrophobin monomers undergo a conformational change at a hydrophobic-hydrophilic interface and integrate into a growing rodlet, however the mechanistic details of rodlet assembly are unknown. To investigate the assembly mechanism of hydrophobins, we carried out stability studies with SC16, a hydrophobin isolated from *Schizophyllum commune.* SC16 was recombinantly expressed using *E. coli* and purified by immobilized Ni^{2+} affinity chromatography. NMR-based relaxation experiments were used to identify the termini and one loop region as dynamic. The NMR data and X-ray crystal structure suggest that SC16 is a monomer in solution, which is consistent with current assembly models. Mutant forms of SC16 are being employed to determine the sequences and conformational changes required for rodlet assembly, with thioflavin T assays used to quantify amyloid formation and atomic force microscopy used to visualize rodlet formation. Determining which hydrophobin sequences are responsible for self-assembly will allow the rational modification of hydrophobins to add new functionalities or influence their self-assembly.

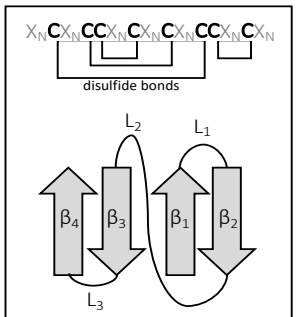
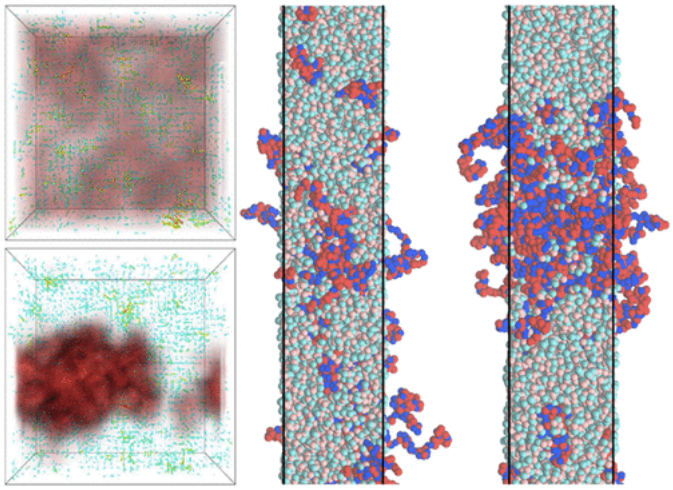


Fig. 1. A generalized hydrophobin sequence and structure.

Poster #70:
A Simple Model for Polyampholyte Phase Behaviour in Presence of Dipolar Solvent

Jonas Wessén[1], Tanmoy Pal[1], Suman Das[1], Yi-Hsuan Lin[1], Hue Sun Chan[1]

[1]*Department of Biochemistry, University of Toronto*



Biomolecular condensates, underpinned by liquid–liquid phase separation (LLPS), are important for physiological function, with electrostatics, among other interaction types, being a prominent force in their assembly. Because the relative permittivity of protein is significantly lower than that of water, the interior of an intrinsically disordered protein (IDP) condensate is expected to be a relatively low-dielectric regime, which should facilitate stronger electrostatic interactions among the scaffold IDPs. To address whether a low-dielectric condensed phase entails more favorable LLPS than that posited by assuming a uniform dielectric background, we consider a simplified multiple-chain model of polyampholytes immersed in explicit solvents that are either polarizable or possess a permanent dipole. Combined efforts of explicit chain simulations, analytical calculations in the random phase approximation and polymer field-theoretic simulations indicate that these systems exhibit only minor to moderate differences from those using implicit-solvent models with a uniform relative permittivity equals to that of pure solvent. This can be understood through a partial compensation of effects between favorable solvent-mediated interactions among the polyampholytes in the condensed phase and favorable polyampholyte–solvent interactions in the dilute phase, netting only a minor enhancement of overall LLPS propensity from the very dielectric heterogeneity that arises from the LLPS itself.

Poster #65:
Learning Cryo-EM in the Lockdown:
An Online Mathematics and Theory Study Group

Geoffrey Woollard[1,2]

[1]*Medical Biophysics, University of Toronto, Toronto, Ontario, Canada*
[2]*Princess Margaret Cancer Centre, University Health Network,Toronto, Ontario, Canada*

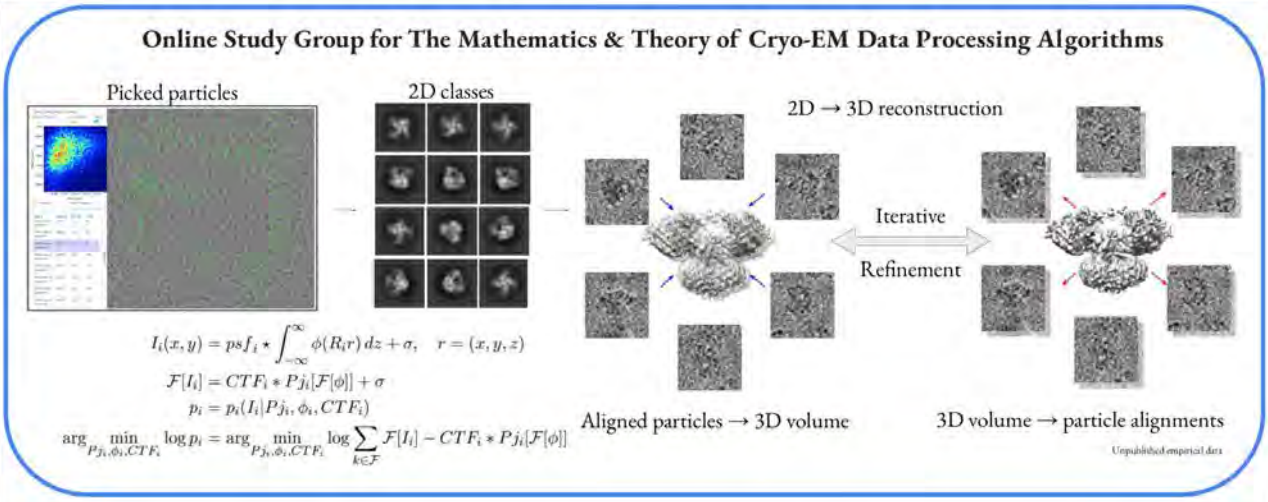
During the Great Plague of London Isaac Newton had to work from home, too. Cambridge University sent their students home, and Newton had an *annus mirabilis.* While we may not work on calculus, optics and gravity, there is plenty to learn about cryo-EM that requires no access to the bench, and is fun to do with others online!

In the spirit of solidarity during the pandemic, I launched a free online cryo-EM study group in November 2020 for graduate students, long term staff, and principal investigators. Many structural biologists want to learn more math and theory to become a cryo-EM power user and follow recent literature.

The learning style is a flipped classroom, with pre-lecture readings provided beforehand, and online meetings spent answering questions, working out problems, and building intuition between data, equations, code, as well as experimental and data processing choices.

We have had 6 months of weekly meetings on topics like Fourier transforms, phase contrast imaging, advanced forward models of image formation, Nyquist, expectation-maximization, rotations, 2D to 3D reconstruction via back propagation, deep generative models, and normal mode analysis.

A syllabus, annotated bibliography, and online coding notebooks can be found at
https://github.com/geoffwoollard/learn_cryoem_math



Poster #56:
Protective Effect of Trehalose Sugar Against Amyloid β Toxicity in Model Lipid Membranes

Yue Xu[1], Carina T. Filice[2], Zoya Leonenko[1,2,3]

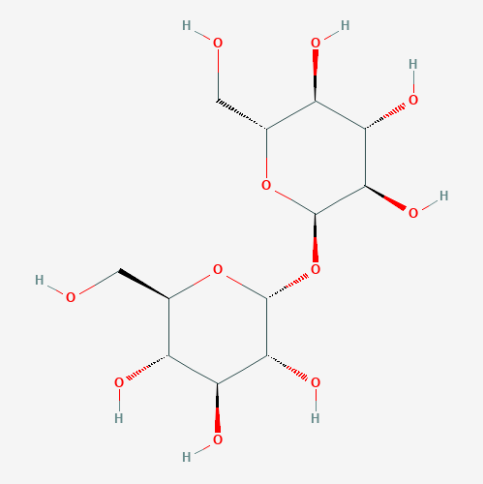
[1]*Department of Physics and Astronomy, University of Waterloo*

[2]*Department of Biology, University of Waterloo*

[3]*Waterloo Institute for Nanotechnology, University of Waterloo*

The amyloid-β(1-42) peptide (Aβ) is a major player in Alzheimer's disease—a debilitating neurodegenerative disorder that causes memory loss and neuronal damage in elderly patients. Aβ forms toxic oligomers that bind to neuronal membranes and result in damage to these membranes through the insertion of the peptide and its subsequent formation into an ion channel or pore (Drolle et al, J. Drug Metabolism Research, 2014).

Trehalose, a disaccharide, has been shown to protect plant membranes in extreme conditions and modify protein misfolding processes, including those seen in Aβ. We hypothesise that trehalose can protect the membrane from amyloid toxicity. In this work we studied the protective effect of trehalose against Aβ-induced damage in model lipid membranes (DPPC/POPC/Cholesterol in mass ratio of 4:4:2). In this study, the Black Lipid Membrane (BLM) technique was used to measure the changes in permeability (ion currents) through suspended model lipid bilayers upon addition of Aβ and trehalose. Our results demonstrate that Aβ increases ion current across lipid bilayers as a result of channel incorporation and membrane damage while the presence of trehalose reduces the current caused by Aβ insertion. This may indicate potential protective effects of trehalose against Aβ toxicity in model membranes.



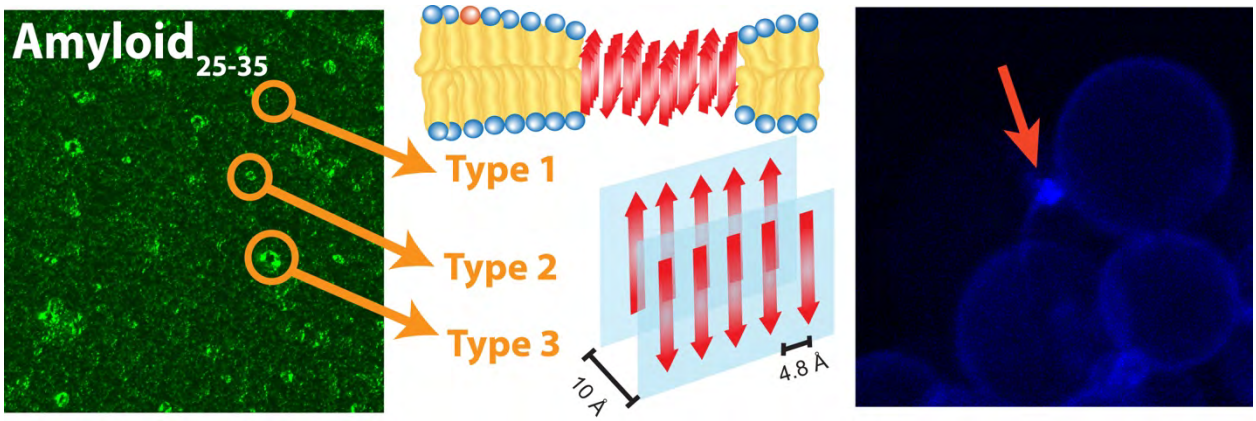
Trehalose

Poster #11:
Curcumin and Homotaurine Suppress Amyloid-β25−35 Aggregation in Synthetic Brain Membranes

Xingyuan Zou[1], Sebastian Himbert[1], Alix Dujardin[1], Maikel C. Rheinstädter[1]

[1.] *Department of Physics and Astronomy and Origins Institute, McMaster University*

More than 30 million individuals worldwide are living with Alzheimer's Disease. To further the current understanding on this neurodegenerative disease, we developed a technique to create amyloid peptide clusters in synthetic, brain-like membranes, which mimic the senile plaques found in the brains of Alzheimer's patients. I compared the molecular functioning of homotaurine, a peptic antiaggregant that binds to amyloid peptides directly, and curcumin, a non-peptic molecule that can inhibit aggregation by changing membrane properties. By using microscopy, x-ray diffraction, and UV-vis spectroscopy, we found that both curcumin and homotaurine significantly reduce the number of small, nanoscopic amyloid aggregates and the corresponding β- and cross-β sheet signals. This research shows that membrane active drugs can be as efficient as peptide targeting drugs in inhibiting amyloid aggregation in-vitro [1]. The findings can open new pathways for the developments of drugs to slow down first occurrence and progression of the disease.



[1] Xingyuan Zou, Sebastian Himbert, Alix Dujardin, Janos Juhasz, Samantha Ros, Harald D. H. Stöver, and Maikel C. Rheinstädter, "Curcumin and homotaurine suppress amyloid-b25-35 aggregation in synthetic brain membranes", *ACS Chemical Neuroscience* **2021** *12* (8), 1395-1405, DOI: 10.1021/acschemneuro.1c00057