MISSISSAUGA
2019

FULL PROGRAM

Biophysical Society of Canada

5th Annual Meeting

May 28-31, 2019

University of Toronto Mississauga

Biophysical Society
Société de biophysique
du Canada

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WIFI INFORMATION

Username: BSC2019

Password: biophysics

Valid for the U of T Network.

Welcome to Mississauga!

Welcome to the 5th Annual Meeting of the Biophysical Society of Canada! This year's meeting will be held on the beautiful campus of the University of Mississauga (UTM), located just outside of downtown Toronto. The Biophysical Society of Canada annual meeting offers attendees the opportunity to learn about exciting new developments in biophysics research and to network with peers from across Canada and further afield.

The 2019 meeting is dedicated to biophysical techniques and discoveries that have revolutionized research leading to critical advancements in medicine, pharmaceutical sciences, biotechnology, material sciences and biosensing. The conference will commence with an opening reception on May 28th, and will feature four plenary lectures, oral presentations given by researchers from around the world, as well as poster sessions. The plenary lectures will be given by Samuel Hess from the University of Maine, Edward Lemke from Johannes Gutenberg University Mainz, Tanja Mittag from St. Jude Children's Research Hospital, and Johan Paulsson from Harvard University. In addition, all attendees are invited to participate in the trainee symposium that will be held throughout the afternoon on May 28th, just prior to the opening reception. The symposium will consist of a career session, as well as provide a venue for trainees to share their research accomplishments and network with their peers.

We are grateful to our many industrial sponsors for their generous support, as well as the departments and institutes from the University of Toronto and UTM that are supporting our meeting. Finally, we thank you for making the journey to the meeting, whether you are a local or a visitor to Toronto. We are proud to host you at our beautiful campus and we look forward to an exciting meeting!

Claudiu Gradinaru, Andreas Hilfinger, Joshua Milstein, and Sarah Rauscher
University of Toronto Mississauga

President's message

The annual meeting of the Biophysical Society of Canada provides a unique opportunity for students, researchers and industry partners to learn about the most recent advances in biophysics. The BSC meetings are recognized for both the quality and diversity of the invited speakers, as well as the outstanding social events. BSC2019 at the University of Toronto, Mississauga promises to surpass our expectations! The conference organizers Claudiu Gradinaru, Josh Milstein, Andreas Hilfinger, and Sarah Rauscher are to be congratulated for putting together an outstanding program with high profile invited speakers. The National Lecture will be delivered by Dr. Hue Sun Chan from the University of Toronto, the 2019 Fellow of the Biophysical Society of Canada. The BSC will also recognize the inaugural Young Investigator of the Biophysical Society of Canada, Dr. Roberto Chica from the University of Ottawa. Thank you to all organizers and volunteers for your hard work. BSC 2019 promises to be an exciting event. Enjoy!

John Baenziger
President
Biophysical Society of Canada

BSC 2019 LOCAL ORGANIZING COMMITTEE

Claudiu Gradinaru
Professor

Joshua Milstein
Associate Professor

Andreas Hilfinger
Assistant Professor

Sarah Rauscher
Assistant Professor

Michelle Bae
Department Coordinator

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

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Newsletter

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Awards & Student Travel

William Jennings
Trainee Representative

Benjamin Martial
Trainee Representative

Brenda Lee
Website Coordinator

BSC 2019 TRAINEE SYMPOSIUM LOCAL ORGANIZING COMMITTEE

Deniz Meneksedag Erol
Post-doctoral fellow

Emiel Visser
Post-doctoral fellow

Dennis Fernandes
Graduate student

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

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Benjamin Baylis
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Communications Director, University of Calgary

Morgan Robinson
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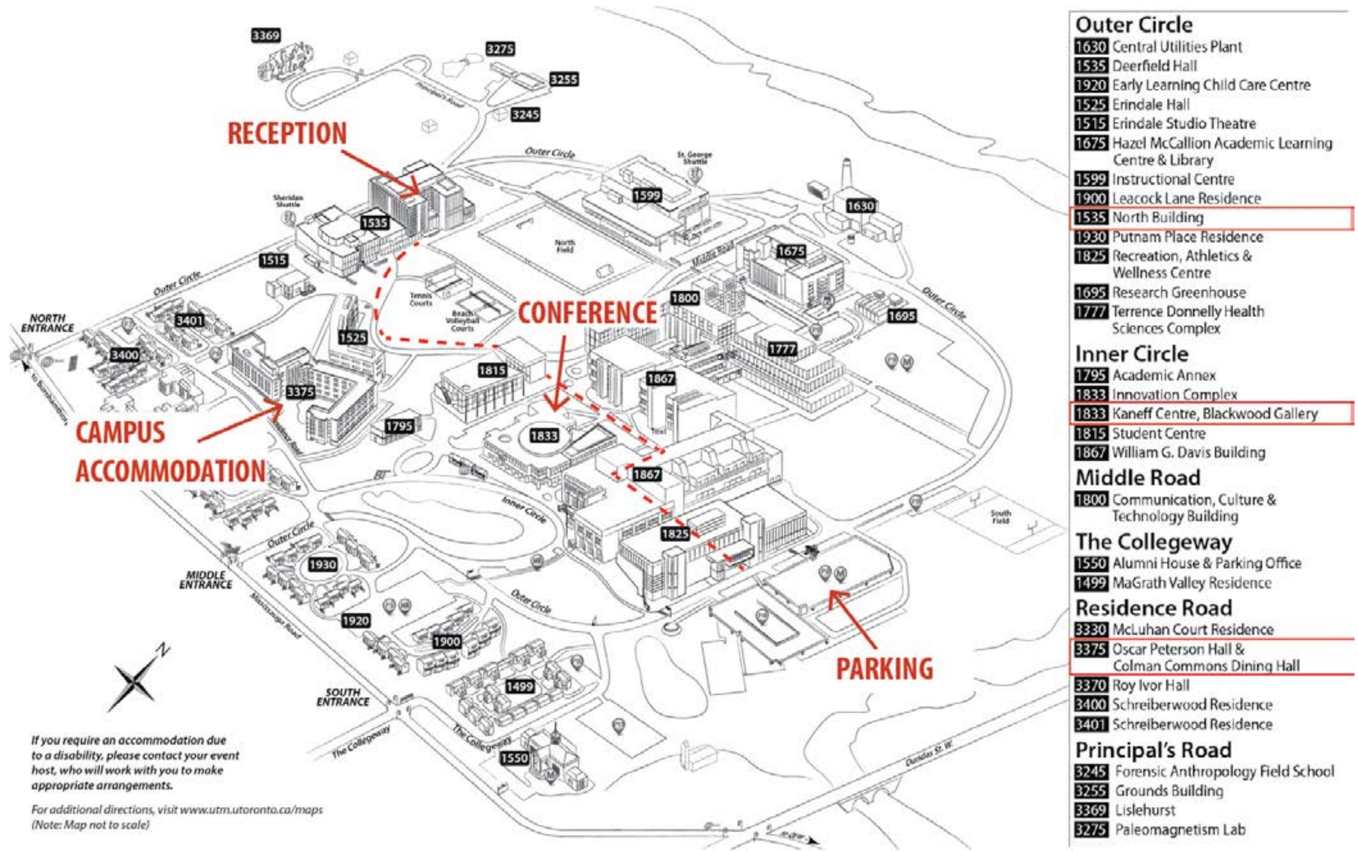
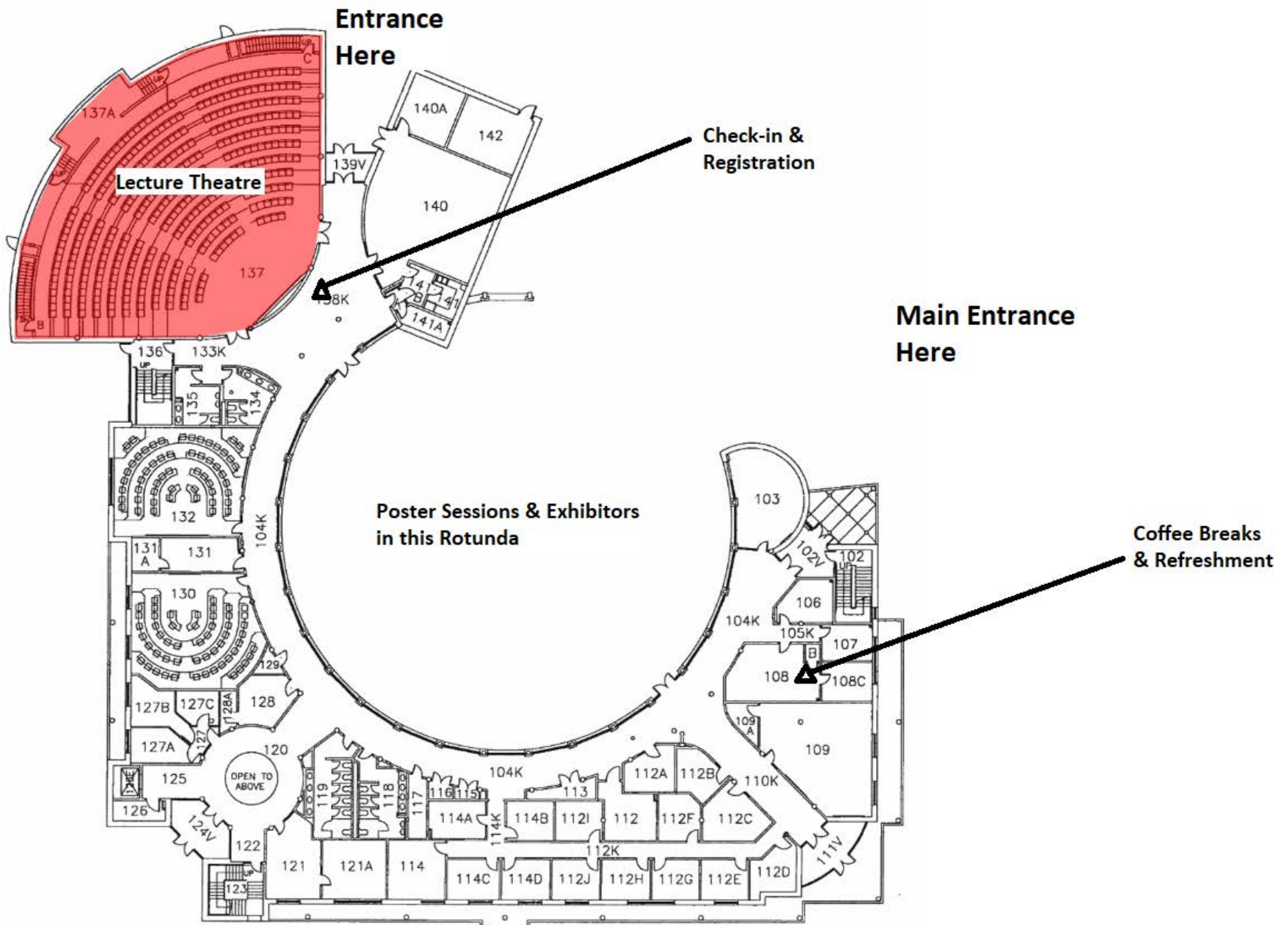
CONFERENCE LOCATION



CONFERENCE LOCATION



BANQUET LOCATION

The banquet will be in the **Port Credit Ballroom** of **The Waterside Inn hotel** (15 Stavebank Road South, Mississauga, L5G 2T2). How to get there:

By Car: Paid parking is available on Port Street in the Port Credit Harbour Marina.

By Bus:

1. Walk to the temporary MiWay bus stop as indicated on the UTM Campus Map.
2. Take bus number 110S and get off at Clarkson GO station (last stop of this bus route).*
3. Transfer to bus number 23E and get off at Lakeshore Road East at Stavebank Road South.*
4. Head south on Stavebank Road and The Waterside Inn will be on your lefthand side.

*The bus will announce each stop. If you aren't sure, ask the bus driver to alert you when you arrive at your destination.

MiWay bus fare: $3.75 if cash, $3.10 with PRESTO.
Paper transfer will be issued if the fare is paid with cash.
The transfer is valid for approximately 2 hours.

To check bus schedule and plan your trip, visit http://www4.mississauga.ca/planatrip/ or refer to the itinerary.

Thursday, May 30, 2019

41 min
(Walking 5 min)

05:21 PM — **University of Toronto at Mississauga**
Walk to stop #0991 on University Of Toronto At Mississauga Campus, Mississauga (2 min).

05:23 PM — **0991 - UNIVERSITY of TORONTO at MISSISSAUGA CAMPUS**
Stop Schedule
110 — Bus route 110 direction South (16 min).

05:39 PM — **0100 - CLARKSON GO STATION PLATFORM 9**
Walk to stop #0139 on Clarkson Go Station Platform 7, Mississauga (1 min).

05:44 PM — **0139 - CLARKSON GO STATION PLATFORM 7**
Stop Schedule
23 — Bus route 23 direction East (16 min).

06:00 PM — **0325 - LAKESHORE RD E at STAVEBANK RD S**
Walk to destination (2 min).

06:02 PM — **The Waterside Inn, Stavebank Road South, Mississauga, ON**

Please be at your stop 5 minutes early.
Thank you for using the trip planner. Have a good ride!

REGISTRATION INFORMATION

The registration desk is located in the atrium of the Kaneff Building (Innovation Complex) directly outside of the lecture hall (**KN137**) where all talks will take place.

<u>Hours of registration</u>

Tuesday May 28	**11:00 AM - 2:00 PM**
Wednesday May 29	**8:00 AM - 1:00 PM**
Thursday May 30	**8:00 AM - 11:00 AM**

<u>Hours of registration at Opening Reception (North Building Atrium)</u>

Tuesday May 28	**6:00 PM - 8:00 PM**

RECEPTION INFORMATION

Opening Reception: Tuesday May 28, 6:00 PM - 8:00 PM
North Building Atrium, University of Toronto Mississauga

Banquet: Thursday May 30, 6:00 PM - 10:00 PM
Waterside Inn, 15 Stavebank Rd S, Mississauga

Closing Reception: Friday May 31, 4:00 PM - 6:00 PM
North Building Atrium, University of Toronto Mississauga

POSTER INFORMATION

The poster boards will be available starting 12pm on Tuesday, May 28th. The poster board size is 4’ (width) x 6’ (height). Please post your poster on your assigned board by 9 am on Wednesday, May 29th. Please make sure to take down your poster by noon on Friday, May 31st. Poster presenters are expected to present their posters during the poster judging session. Poster judging will take place during Poster Sessions A and B on Wednesday, May 29th. Presenters with an **odd-numbered poster** should ensure they are present at their poster during Poster Session A. Presenters with an **even-numbered poster** should ensure they are present at their poster during Poster Session B. Everyone is welcome to visit other posters at their leisure.

ACCESSIBILITY AND INCLUSION

Accessibility
If you have any disability-related accommodation needs, please contact Sarah Rauscher (sarah.rauscher@utoronto.ca) to work with you to make appropriate arrangements. This may include, for example, information about accessible services on campus.

Parenting and Child Care Resources
If you are needing childcare services, please contact a member of the organizing committee. They will be able to provide you with information about options available.

The University of Toronto Family Care Office has prepared a list of places to breastfeed, chestfeed or pump. These places are comfortable, quiet, some are private, and some are open to pumping in addition to breastfeeding/chestfeeding. Please remember, however, it is your right to breastfeed or chestfeed anywhere on campus! Locations at UTM: http://familycare.utoronto.ca/childcare/breastfeeding-at-u-of-t/#utm

Washroom Inclusivity
An interactive campus map is available that lists different inclusivity features available in washrooms around campus including accessibility, change tables, all-gender, to increase inclusivity: Washrooms, Multi-Faith Spaces, Food Vendors: http://map.utoronto.ca/utm#

Multi-faith Spaces at UTM:
https://www.utm.utoronto.ca/utm-engage/student-groups/multi-faith/spaces

Campus Maps
Campus maps and directions with parking and buildings:
https://www.utm.utoronto.ca/about-us/contact-us/maps-directions

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UNIVERSITY OF
TORONTO

Faculty of Arts and Science - Pathways to Milestones program

PROGRAM OVERVIEW

All oral presentations and poster sessions will take place in the Kaneff Building.

Tuesday, May 28th

1:00 PM – 5:30 PM	Trainee Symposium
6:00 PM - 8:00 PM	Opening Reception (North Building Atrium)

Wednesday, May 29th

9:00 AM – 9:15 AM	Opening remarks
9:15 AM – 9:55 AM	Plenary Lecture 1: Samuel Hess
9:55 AM – 10:50 AM	Session 1: Biophotonics
10:50 AM – 11:15 PM	Coffee Break
11:15 PM – 12:30 PM	Session 2: Membranes
12:30 PM – 2:00 PM	Lunch
2:00 PM – 3:30 PM	Poster session
3:30 PM – 4:45 PM	Session 3: Cellular Biophysics
4:45 PM – 5:15 PM	Young Investigator of the BSC Award Lecture: Roberto Chica
5:15 PM – 7:00 PM	Poster Session and Mixer

Thursday, May 30th

9:00 AM – 10:15 AM	Session 4: Single Molecule Biophysics 1
10:15 AM – 10:45 AM	Coffee Break and Posters
10:45 AM – 12:00 PM	Session 5: Receptors and Signalling
12:00 PM – 1:40 PM	Lunch
12:30 PM – 1:30 PM	BSC Business Meeting
1:40 PM – 2:20 PM	Plenary Lecture 2: Tanja Mittag
2:20 PM – 3:20 PM	Session 6: Intrinsically Disordered Proteins
3:20 PM – 3:50 PM	Coffee Break and Posters
3:50 PM – 4:50 PM	National Lecture: Hue Sun Chan
4:50 PM – 6:00 PM	Travel to Banquet location
6:00 PM -10.00 PM	Conference Banquet at Waterside Inn
	7:30 PM Award Ceremony with Trainee awards

Friday, May 31st

9:00 AM – 9:40 AM	Plenary Lecture 3: Johan Paulsson
9:40 AM – 10:40 AM	Session 7: Cell and Systems
10:40 AM – 10:45 AM	Conference Photo
10:45 AM – 11:15 AM	Coffee Break
11:15 AM – 12:35 AM	Session 8: Biopolymers
12:35 PM – 2:00 PM	Lunch
2:00PM – 2:40 PM	Plenary Lecture 4: Edward Lemke
2:40 PM – 3:40 PM	Session 9: Single Molecule Biophysics II
3:40 PM – 3:50 PM	Closing Remarks
4:00 PM – 6:00 PM	Closing Reception (North Building Atrium)

TRAINEE SYMPOSIUM – TUESDAY MAY 28th

All oral presentations and poster sessions will take place in the Kaneff Building.

12:00 PM - 1:00PM	**Registration and Networking Lunch**
1:00 PM – 1:10 PM	**Opening Remarks**
1:10 PM - 3:10 PM	**Career session**
1:10 PM	Joshua Mogyoros, Research and Development Scientist, MesoMat
1:40 PM	Danielle Tokarz, Assistant Professor, Saint Mary's University
2:10 PM	Daaf Sandkuijl, Senior Optical Scientist, Fluidigm
2:40 PM	Ricky Ghoshal, Director, Strategic Development, Glysantis Inc. (Mirexus Biotechnologies)
3:10 PM - 3:30 PM	**Coffee Break**
3:30 PM - 5:30 PM	**Trainee Talks**
3:30 PM	Rashik Ahmed, Graduate Student, McMaster University *Atomic Resolution Map of the Soluble Amyloid Beta Assembly Toxic Surfaces*
3:45 PM	Aidan Tremblett, Graduate Student, Memorial University of Newfoundland *Systematic Coarse-Graining Method for Molecular Simulations using Relative Entropy*
4:00 PM	Jennifer Lou, Graduate Student, University of Toronto *Cryo-EM Study of Transient Protein Interactions in Fungal Type I FAS Catalysis*
4:15 PM	Adree Khondker, Undergraduate Student, McMaster University *A Molecular Mechanism for Polymyxin-induced Membrane Damage that predicts Bacterial Resistance*
4:30 PM	Anita Rágyanszki, Postdoctoral Fellow, York University *Artificial Neural Networks as a Tool to Describe Peptide Conformational Changes*
4:45 PM	Daryl Good, Graduate Student, University of Guelph *Characterizing the Hierarchy of Internal Dynamics in a Membrane Protein by Solid State NMR*
5:00 PM	Steven Chen, Graduate Student, University of Toronto *High-throughput Investigation of Rhodopsin Function using Yeast*
5:15 PM	Sara Molladavoodi, Postdoctoral Fellow, Wilfrid Laurier University *Collagen Contraction Induced by Annulus Fibrosus Cells of the Intervertebral Disc*
6:00 PM – 8:00 PM	**BSC 2019 Opening Reception** ***North Building Atrium***

SCIENTIFIC PROGRAM

Wednesday, May 29th	
9:00 AM	**Welcoming remarks** Kent Moore, Vice-Principal, Research, University of Toronto Mississauga Claudiu Gradinaru, Chair, Department of Chemical and Physical Sciences
9:15 PM – 9:55 AM	**Plenary Talk** Chair: Claudiu Gradinaru (University of Toronto Mississauga) Samuel Hess, University of Maine *Localization-Based Super-Resolution Microscopy: Technical Advances Coupled to Biological Applications*
9:55 AM - 10:50 AM	**Session 1: Biophotonics** Chair: Virgis Barzda (University of Toronto Mississauga)
9:55 AM	Paul Wiseman, McGill University *Mining the molecular noise via fluorescence fluctuation image analysis of microscopy and super-resolution microscopy*
10:15 AM	Danielle Tokarz, Saint Mary's University *Natural Compounds as Dyes for Nonlinear Optical Microscopy*
10:35 AM	Trainee talk: Pablo Dans, Institute for Research in Biomedicine Barcelona *From STORM Microscopy and Genome-Wide Analysis to Near Atomic Resolution of Human Genes*
10:50 AM - 11:15 AM	**Coffee Break and Posters**
11:15 AM - 12:30 PM	**Session 2: Membranes** Chair: Simon Sharpe (The Hospital for Sick Children)
11:15 AM	Voula Kanelis, University of Toronto *The influence of disease mutations and regulatory interactions in SUR proteins, the regulatory subunit in K-ATP channels*
11:35 AM	Suzana Straus, University of British Columbia *Novel alternatives to antibiotics: biophysical tools to study the function of host defense peptides*
11:55 AM	Jenifer Thewalt, Simon Fraser University *Order in the membrane*
12:15 PM	Trainee talk: Javier Porro-Suardiaz, Concordia University *GL13K, an antimicrobial peptide with anticancer activity: A story told through the interaction with model membranes*
12:30 PM - 2:00 PM	**Lunch Break**
1:30 PM – 2:00 PM	**Sponsor Presentation: SFR (Kaneff Building KN L1220)**

2:00 PM – 3:30 PM	**Poster Session A and Refreshments**
3:30 PM – 4:45 PM	**Session 3: Cellular Biophysics** Chair: Maikel Rheinstadter (McMaster University)
3:30 PM	Cecile Fradin, McMaster University *Visualizing transcription in the early fly embryo*
3:50 PM	Jon Rocheleau, University of Toronto *Islet-on-a-chip provides an optical window into cellular metabolism and insulin secretion*
4:10 PM	Anton Zilman, University of Toronto *Nuclear Pore Complex: simple physics of a complex biomachine*
4:30 PM	Trainee talk: Sebastian Himbert, McMaster University *The Bending Rigidity of Red Blood Cell Membranes Determined from Solid-Supported Multi-lamellar Membranes*
4:45 PM – 5:15 PM	**Award Lecture: Young Investigator of the BSC** Chair: John Baenziger (University of Ottawa)
	Roberto Chica, University of Ottawa *Rational Design of Protein Energy Landscapes*
5:15 PM – 7:00 PM	**Poster Session B and Mixer**

Thursday, May 30th

9:00 AM - 10:15 AM	**Session 4: Single Molecule Biophysics I** Chair: Claudiu Gradinaru (University of Toronto Mississauga)
9:00 AM	Sabrina Leslie, McGill University *Deconstructing biology with simple single-molecule imaging: Controlling conformation, confinement, and concentration*
9:20 AM	Michael Woodside, University of Alberta *Direct observation of formation of a knotted structure in single RNA molecules from Zika virus*
9:40 AM	Haw Yang, Princeton University *Unsupervised Statistical Learning in Multi-Resolution Dynamics*
10:00 AM	Trainee talk: Gregory-Neal Gomes, University of Toronto *Ensemble modelling of disordered proteins using single-molecule FRET*
10:15 AM - 10:45 AM	**Coffee Break and Posters**
10:45 AM - 12:00 PM	**Session 5: Receptors and Cell Signalling** Chair: Cecile Fradin (McMaster University)
10:45 AM	Kalina Hristova, Johns Hopkins University *Peering into cells with new imaging technologies*

11:05 AM	R. Scott Prosser, University of Toronto *Molecular and Mechanistic Underpinnings of Signal Transduction in Membranes: NMR Inspired Studies of the Conformational Landscape in Enzymes & GPCRs*
11:25 AM	Trainee talk: Wendy Wang, University of Toronto *A spatial-temporal map of the Protocadherin expression code in the CNS*
11:40 AM	TBA
12:00 PM – 1:40 PM	**Lunch Break**
1:00 PM – 1:30 PM	**Sponsor Presentation: Lumicks (Kaneff Building KN L1220)**
12:30 PM – 1:30 PM	**BSC Business Meeting (Davis Building Room 3130)**
1:40 PM - 2:20 PM	**Plenary Talk** Chair: Sarah Rauscher (University of Toronto Mississauga) Tanja Mittag, St. Jude Children's Research Hospital *Phase separation and mesoscale assembly for functional compartmentalization*
2:20 PM - 3:20 PM	**Session 6: Intrinsically Disordered Proteins** Chair: Sarah Rauscher (University of Toronto Mississauga)
2:20 PM	Andrea Soranno, Washington University in St. Louis *Single-molecule fluorescence spectroscopy reveals heterogeneous conformations in Apolipoprotein E*
2:40 PM	Joan-Emma Shea, University of California San Diego *Aggregation and coacervation of the tau peptide*
3:00 PM	Julie Forman-Kay, The Hospital for Sick Children *Phase Separation Biophysics, Prediction and Role in Biological Regulation*
3:20 PM - 3:50 PM	**Coffee Break and Posters**
3:50 PM - 4:50 PM	**Fellow of the Biophysical Society of Canada & National Lecture** Chair: John Baenziger Hue Sun Chan, University of Toronto *A Physicist's Journey in Molecular Biology*
4:55 PM – 6:00 PM	**Travel to Waterside Inn (15 Stavebank Rd S, Mississauga)**
6:00 PM - 10:00 PM	**Conference Banquet at Waterside Inn**

Friday, May 31st

9:00 AM - 9:40 AM	**Plenary Talk** Chair: Andreas Hilfinger (University of Toronto Mississauga)
	Johan Paulsson, Harvard University *General principles for timing and oscillations in stochastic reaction systems*
9:40 AM - 10:40 AM	**Session 7: Cell and Systems** Chair: Sid Goyal (University of Toronto)
9:40 AM	Andrew Rutenberg, Dalhousie University *Collagen fibrils: liquid-crystals, crystals, and rubber bands*
10:00 AM	Lisa Manning, Syracuse University *Biophysical modeling of the collective behavior of cells in tissues*
10:20 AM	Erdal Toprak, University of Texas Southwestern *Exploiting trade-offs in the evolution of antibiotic resistance*
10:40 AM	**Conference photo**
10:45 AM - 11:15 AM	**Coffee Break and Posters**
11:15 AM - 12:35 PM	**Session 8: Biopolymers** Chair: Joan-Emma Shea (University of California San Diego)
11:15 AM	Régis Pomès, The Hospital for Sick Children *Molecular Mechanisms of Gating, Permeation, and Leakage in Ion Channels*
11:35 AM	Gary Slater, University of Ottawa *Two recent "proofs" that the theory of diffusion is not dead*
11:55 AM	Justin MacCallum, University of Calgary *Binding of cannabinoids and fatty acids to fatty acid binding proteins*
12:15 AM	Christopher Rowley, Memorial University of Newfoundland *Modeling Covalent Modifiers of Kinase Proteins*
12:15 PM – 2:00 PM	**Lunch Break**
2:00 PM - 2:40 PM	**Plenary Talk** Chair: Sarah Rauscher (University of Toronto Mississauga)
	Edward Lemke, Johannes Gutenberg University Mainz *Tools to Decode Molecular Plasticity in the Dark Proteome*
2:40 PM - 3:40 PM	**Session 9: Single Molecule Biophysics II** Chair: Josh Milstein (University of Toronto Mississauga)
2:40 PM	Matthew Lew, Washington University in St. Louis *New Methods for Visualizing Single Amyloid Protein Dynamics at the Nanoscale*
3:00 PM	Ilya Finkelstein, University of Texas Austin *Playing with Knives: Regulation of Human DNA Resection*

3:20 PM	Jingyi Fei, University of Chicago *Dynamic interactions between the RNA chaperone Hfq, small regulatory RNAs and mRNAs in live bacterial cells*
3:40 PM - 3:50 PM	**Closing Remarks** BSC Organizaing Committee
4:00 PM - 6:00 PM	**Closing Reception** ***North Building Atrium***

Fellow of the BSC & National Lecture – Dr. Hue Sun Chan

Dr. Chan has made major contributions to the field of protein folding and continues to design and apply computational tools to a wide variety of biophysical problems.

Dr. Hue Sun Chan obtained his B.Sc. degree in Physics (First Class Honors) from the University of Hong Kong in 1981, after which he moved to the University of California at Berkeley where he earned a M.A. in Physics in 1983 and a Ph.D. in Theoretical Physics in 1987. He then joined the University of California at San Francisco initially as postdoctoral fellow with Professor Ken A. Dill and then as adjunct faculty. In 1998 he joined the faculty of the Departments of Biochemistry, and of Medical Genetics & Microbiology at the University of Toronto, where he has been a full professor since 2003.

Dr. Chan is an exceptional biophysicist, who, in the last three decades, has made major seminal contributions to the field of protein folding, and who continues to be extraordinarily productive in designing and applying computational tools to a wide variety of biophysical problems. He has published more than 120 papers that have been cited nearly 15,000 times, resulting in an h-index of 56.

While working with Ken Dill at UCSF, Dr. Chan focused on a long-standing problem in biophysics, i.e. the protein folding problem. He developed transformative computational approaches to model protein folding mechanisms. His 1997 paper in Nature Structural Biology has become standard material in biophysics and biochemistry textbooks, including an iconic schematic figure illustrating the concept of a funneled energy landscape in protein folding. More recently, Dr. Chan has implemented computational methods and developed polymer theory to study new systems and processes, such as protein folding cooperativity, role of nonnative interactions in folding, protein evolution, DNA topology and topological simplification by topoisomerases, intrinsically disordered proteins and protein condensates. He has been at the top of a crowded field for many years and has impacted the careers of many scientists all over the world.

A Physicist's Journey in Molecular Biology

National Lecture

Young Investigator of the BSC – Dr. Roberto Chica

Dr. Chica has been pushing the boundaries of protein engineering through his pioneering work in computational protein design.

Dr. Roberto Chica received his B.Sc. in biochemistry from the Université de Montréal in 2001 and his Ph.D. from the same institution in 2007 under the mentorship of Drs. Joelle N. Pelletier and Jeffrey W. Keillor. After working at Caltech as postdoctoral fellow with Dr. Stephen L. Mayo, he joined the Department of Chemistry of the University of Ottawa in 2010, where is now an associate professor. In 2018, he was a visiting scholar at the Department of Bioengineering and Therapeutic Science, UCSF.

Dr. Chica is developing an innovative research program in protein engineering and computational design and is emerging as a leader in this field. Since establishing his independent research program in 2010, Dr. Chica has been pushing the boundaries of protein engineering through his pioneering work in the incorporation of multistate approaches into computational protein design (CPD). These methods allow proteins to be modeled, not as a single structure as is traditionally done, but as structural ensembles that more realistically represent the range of conformations accessed within their energy landscapes. Dr. Chica also set a new standard in the discipline by being the first to rationally design protein dynamics. His contributions have been commended by F1000 for “pushing the frontiers of CPD with respect to designing functionally diverse proteins”. These methods have entered into the mainstream of the protein engineering community, having been incorporated into the commercial-grade CPD platforms Triad and Rosetta. Dr. Chica has also emerged as a leading figure in the protein engineering community. For example, he created the biennial Protein Engineering Canada (PEC) Conference (now in its 3rd edition), and is currently building a multi-institutional training program for the protein engineers of the future (funded by NSERC CREATE).

Rational Design of Protein Energy Landscapes

Award Lecture

Plenary Lecture – Dr. Samuel Hess

Localization-Based Super-Resolution Microscopy: Technical Advances Coupled to Biological Applications

Samuel Hess

Department of Physics and Astronomy, University of Maine

Localization microscopy circumvents the diffraction limit by identifying and measuring the positions of numerous subsets of individual fluorescent molecules, ultimately producing an image whose resolution depends on the uncertainty and density of localization. Now that the diffraction barrier has been broken, what defines the best possible resolution that can be obtained? Photophysical studies coupled with a theoretical framework reveal that there is an optimal resolution for a given fluorescent marker imaged under a given set of conditions. Dark state transitions, fluorescence background, acquisition frame rate and illumination intensity all play a role in defining the spatial resolution for a given probe. Spectral resolution can be improved by incorporating a dispersive element in the detection path of a localization microscope, which can be useful for separation of multiple probes imaged simultaneously, and also for detection of changes in emission spectra of fluorophores resulting from changes in their (cellular) environment. Neural networks and new illumination geometries can improve the rate of identification of individual molecules, thus improving localization density and overall resolution. These methodological advances enable new biological applications, which in turn motivate new questions and technical innovations. In one such example, live- and fixed-cell imaging of the influenza viral protein hemagglutinin (HA), and host cell components, have revealed a relationship between HA, host cell actin, actin binding proteins, and the lipid phosphatidylinositol (4,5)bisphosphate (PIP2). Results will be discussed in the context of several existing models of plasma membrane organization.

Plenary Lecture – Dr. Edward Lemke

Tools to Decode Molecular Plasticity in the Dark Proteome

Edward Lemke

Departments of Biology and Chemistry, Biocentre Johannes Gutenberg-University Mainz
Institute of Molecular Biology Mainz
European Molecular Biology Laboratory

The mechanisms by which intrinsically disordered proteins (IDPs) engage in rapid and highly selective binding is a subject of considerable interest and represents a central paradigm to nuclear pore complex (NPC) function, where nuclear transport receptors (NTRs) move through the NPC by binding disordered phenylalanine-glycine-rich nucleoporins (FG-Nups). In the first part of my talk, I will present a combined single molecule, ensemble spectroscopy, solvation approach that paired with atomic simulations and microfluidics reveals unique features that reveal an unexpectedly high plasticity of IDPs uniquely possible due to their disordered and multivalent nature, which we argue is key to selective transport in the living cell. Since site-specific labeling of proteins with small but highly photostable fluorescent dyes inside cells remains the major bottleneck for directly performing such high resolution studies in the interior of the cell, I will demonstrate an approach how to overcome this limitation in the second part of my talk. We have now developed a semi-synthetic strategy based on novel artificial amino acids that are easily and site-specifically introduced into any protein by the natural machinery of the living cell via a newly developed synthetic organelle which equips the living cell with two genetic codes. This allows rapid, specific "click" labeling and even multi-color studies of living cells and subsequent super resolution microscopy.

Plenary Lecture – Dr. Tanja Mittag

Phase separation and mesoscale assembly for functional compartmentalization

Tanja Mittag

St. Jude Children's Research Hospital

Liquid-liquid phase separation of proteins leads to demixing from solution and results in a dense, protein-rich phase, which co-exists with a light phase that is depleted of protein. Recent findings support a model in which phase separation is the biophysical driving force for the formation of membrane-less organelles in cells, such as stress granules, nucleoli and nuclear speckles. Current open questions are: (i) How is phase separation propensity encoded in the protein sequence, (ii) are dense liquid droplets used as reaction compartments in the cell, and (iii) is physiological phase separation disrupted in disease states? To address these, we study two systems, the tumor suppressor Speckle-type POZ protein (SPOP) and the RNA-binding protein hnRNPA1.

SPOP, a substrate adaptor of a ubiquitin ligase, localizes to different liquid membrane-less organelles in the nucleus, where it encounters its substrates, but it is never found diffuse in the cell. However, its recruitment mechanism to these organelles is not understood. Here, we show that SPOP undergoes LLPS with substrate proteins, and that this mechanism underlies its recruitment to membrane-less organelles. Multivalency of SPOP and substrate for each other drive their ability to phase separate. We present evidence that the SPOP/substrate assemblies are active ubiquitination compartments in vitro and in cells. SPOP cancer mutations reduce the propensity for phase separation. We propose that SPOP has evolved a propensity for phase separation in order to target substrates localized in membrane-less compartments.

Intrinsically disordered protein regions with low-sequence complexity, so-called low-complexity domains (LCDs), are autonomously phase-separating domains in RNA-binding proteins. Recent mutagenesis experiments have revealed the importance of aromatic residues for their ability to undergo LLPS. Here, we investigate the interactions that mediate phase separation of the intrinsically disordered LCD of hnRNPA1. Phase separation of hnRNPA1 promotes the fibrillization of mutants of hnRNPA1 that cause ALS and other neurodegenerative diseases. We find that aromatic side-chains cluster and lead to compaction of the LCD, and that this compaction is coupled to LLPS. The patterning of aromatic residues in the sequence is an evolutionarily conserved feature that is key to prevent aggregation. Understanding the interactions that mediate phase separation has the potential to provide mechanistic insight into membrane-less compartmentalization in cell

Plenary Lecture – Dr. Johan Paulsson

General principles for timing and oscillations in stochastic reaction systems

Johan Paulsson

Department of Systems Biology, Harvard University

Precise timing can be advantageous in many biological processes but is difficult to achieve because chemical reactions often produce exponentially distributed waiting times for individual events. I will discuss hard bounds on timing and the phase-drift of oscillators in reaction networks with finite numbers of molecules, based on analytical theory for classes of stochastic processes. Based on this theory, I then show how we designed and built a series of ultra-precise synthetic gene oscillators, some of which can oscillate for 500 cell generations without entrainment before drifting out of phase with even half a period. Finally, I will show how some natural systems use the same simple but counterintuitive principles to achieve timing in cell fate decisions, and how these are easily missed in deterministic modeling frameworks.

POSTER PRESENTATIONS

- odd numbers: *Poster session A*, Wed 2:00 PM – 3:30 PM
- even numbers: *Poster Session B,* Wed 5:15 PM – 7:00 PM

P1 **2D NMR investigation of water dynamics at the interface of carbon nanotubes**

P2 **Implication of cholesterol in regulating the pore-formation mechanism of Vibrio cholerae cytolysin, a beta-barrel PFT**

P3 **Solid-state NMR spectroscopy detection following thermal unfolding of a seven-helical membrane protein**

P4 **Characterizing the hierarchy of internal dynamics in a membrane protein by solid state NMR**

P5 **Functional studies of the human cholesterol transporter ABCG5/ABCG8 in lipid-bilayer nanodiscs**

P6 **Membrane binding of S100A10 protein and AHNAK peptide involved in cell membrane repair**

P7 **Melatonin Changes Nanoscale Lipid Domains to Protect Neuronal Model Membranes Against Amyloid Toxicity**

P8 **Impact of muscular dystrophy causing mutations on the folding and stability of dysferlin C2A domain**

P9 **The role of the beta subunit in P4-ATPase function**

P10 **Whole cell 2H Solid-State NMR of Antimicrobial Peptides Interacting with cell envelopes: Role of lipopolysaccharide**

P11 **Atomic Resolution Map of the Soluble Amyloid Beta Assembly Toxic Surfaces**

P12 **A Molecular Mechanism for Polymyxin-induced Membrane Damage that predicts Bacterial Resistance**

P13 **Evidence Supporting Self-Assembly of UCP2 in the Membrane: Monomeric, Dimeric or Tetrameric?**

P14 **The effects of different fatty acids on phospholipid bilayers during necroptosis**

P15 **CryoEM structural investigation of two calcium ion channels: IP3R and Orai**

P16 **Mechanisms by which pore lining mutations alter nicotinic acetylcholine receptor function leading to disease**

P17 **Non-Additivity of Ligand Binding Affinities and Partial Agonism for Protein Kinase G (PKG)**

P18 **Probing the Binding Mechanism of a Competitive Inhibitor of EPAC1, I942, using NMR Spectroscopy**

P19 **Mapping the allosteric pathway leading from a pore-lining mutation in the nicotinic acetylcholine receptor to CMS**

P20 **Understanding the Structural Basis of cAMP Signal Termination in PKA**

P21 **Activation of G protein by the adenosine A2A receptor**

P22 **Increased ubiquinone content confers respiratory protection to Escherichia coli under osmotic stress**

P23 **Toward a structural model for osmosensing by transporter ProP of Escherichia coli**

P24 **Investigating the Mechanism of the cAMP-Dependent Signalling Pathway Termination in Type 1 Acrodysostosis**

P25 **How Reactive are Druggable Cysteines in Protein Kinases? — A Computational Study**

P26 **Investigating the structure and self-assembly potential of diverse class IB hydrophobins**

P27 **Determination of the Structures of SUR NBD1/Drug Complexes with NMR**

P28 **Substrate-based Allosteric Regulation of a Homodimeric Enzyme**

P29 **Fragment-based drug discovery for the oncogenic protein KRas**

P30 **Structural and functional investigations of KH domains in cancer antigen helicases DDX43 and DDX53**

P31 **High-throughput Investigation of Rhodopsin Function using Yeast**

P32 **Is the Hydrophobic Gasket a Secondary Selectivity Filter in The Human Voltage Gated**

P33 **All-atom molecular dynamics simulations of the Hco-ACC2 ion channel homodimer binding site**

P34 **Molecular Mechanism of Selective Cholesterol Uptake in Class B Scavenger Receptor LIMP-2**

P35 **Role of Dynamics in the Autoinhibition and Activation of the P. Falciparum Protein Kinase G by Cyclic GMP**

P36 **Sequence-specific polymer theory for intrinsically disordered proteins in liquid-liquid phase separation**

P37 **Dynamic Interactions Between a Disordered Protein and its Target at the Single-Molecule Level**

P38 **Local Chain Dynamics of Intrinsically Disordered Sic1 From Fluorescence Anisotropy Decay Measurements**

P39 **Liquid-liquid phase separation in Disordered Proteins: Effect of Interaction Potentials and Charge Pattern Parameters**

P40 **Structural Studies Elucidating Substrate Transport and the Role of the Disordered Regulatory Region of Ycf1p**

P41 **Investigating the role of G-quadruplex RNA binding on the specificity of phase separation**

P42 **Artificial Neural Networks as a Tool to Describe Peptide Conformational Changes**

P43 **MD Simulations of IDP Phosphorylation Parameters and Comparison to Experiment**

P44 **Hamiltonian Replica Exchange for Enhanced Sampling of the Conformational Landscape of Intrinsically Disordered Proteins**

P45 **Fragment-based Identification of Functional Hot Spots in Enzymes**

P46 **Combining simulation and experiment to achieve a detailed analysis of structure of elastin-like peptides**

P47 **Role of π-π Interactions in Liquid-Liquid Phase Separation**

P48 **Using Simulations to Understand the Self Assembly and Mechanical Properties of Elastin-like Peptides**

P49 **Observing Single Molecule DNA Transcription and Protein-DNA interactions in-vitro Using Tethered Particle Motion**

P50 **Vibrational Circular Dichroism Reveals Supramolecular Chirality Inversion of α-Synuclein Peptide Assemblies**

P51 **Systematic Coarse-Graining Method for Molecular Simulations using Relative Entropy**

P52 **Cryo-EM study of transient protein interactions in fungal type I FAS catalysis**

P53 **TEM and SAXS Reveal Structural Similarities Between Lipid-Free and Lipid-Bound Serum Amyloid A**

P54 **Can the RNA World still function without cytidine?**

P55 **Induced memory effects in single-molecule force spectroscopy measurements of biomolecular folding**

P56 **Effective screening platform of α-synuclein monomers in solution using computational and experimental methods**

P57 **Multi-scale ligand-induced dynamics in the A2A Adenosine Receptor**

P58 **Versatile Tools Towards Real-Time Single-Molecule Biology**

P59 **Defining Conformational States of Proteins Using Dimensionality Reduction and Clustering Algorithms**

P60 **Uncovering the Molecular Basis for the Clinical N642H Mutation in STAT5B Using Atomistic Molecular Simulations**

P61 **Single-Molecule Counting and Stoichiometric Analysis using Super-Resolution Localization Microscopy**

P62 **Optical biosensors engineered for imaging of monovalent copper ion in mammalian cell culture**

P63 **Dynamic Imaging of Biological Specimens Using Wide-Field Second Harmonic Generation Microscopy**

P64 **Organization and Composition of Collagen in Biological Tissue Revealed With Polarimetric Nonlinear Microscopy**

P65 **Examination of Drosophila Musculature using Second Harmonic Generation Microscopy**

P66 **Exploring the Mechanical and Structural Properties of Recombinant Spider Pyriform Silk**

P67 **Collagen contraction induced by annulus fibrosus cells of the intervertebral disc**

P68 **Dendritic Morphology and Mechanical Modulus of Soft Phytoglycogen Nanoparticles Revealed By AFM Force Spectroscopy**

P69 **Effect of spinning rate on the molecular structure and dynamics in native and supercontracted spider silk**

P70 **Constructing All-atom Structure of Phytoglycogen-like Nanoparticles Using Molecular Dynamics Simulations**

P71 **All-Atom Molecular Dynamics Simulations to Study the Structure and Dynamics of Phytoglycogen-like Nanoparticles**

P72 **Uncovering the structural basis for the mechanical properties of elastin**

P73 **All-atom Molecular Dynamics Simulations of Single Amylose-like Chains: Exploring the Secondary Structure**

P74 **Studying the interactions of elastin-like polypeptides using all-atom molecular dynamics simulations**

P75 **The physical bases of forming a smooth boundary between an expanding actin network and a contracting actomyosin network**

P76 **Push and Pull: regulation of the actin cytoskeleton by myosin-dependent activation of Dia1**

P77 **Biased protrusion dynamics steer cells towards stiffer extracellular matrix**

P78 **Calcium Sparks in Focal Adhesions Drive Cell Migration Along ECM Stiffness Gradients**

P79 **Self-Assembly of Rod-Like Colloids**

P80 **Using Coarse-Grained Simulations to Investigate the Structure of a Dendritic Nanoparticle**

P81 **Development of a novel assay for quantifying the forces involved in rolling adhesion**

P82 **The Effect of Lithium on Neuronal Cell Electrophysiology and Phosphorylation of Biomolecules Implicated in Alzheimer's Disease Progression**

P83 **Active multi-point microrheology of biopolymer networks**

P84 **Single-molecule visualization of the effects of ionic strength and crowding on structure-mediated interactions in supercoiled DNA molecules**

P85 **Biomechanics of Tuvan Throat Singing**

P86 **Crowding in the nuclear pore complex does not slow down transport**

P87 **Population-level signatures of the co-evolutionary arms race between bacteria and viruses**

P88 **Modelling Signal Cross-talk in Type I Interferon**

P89 **The Origin of Life: Bridging the Gap Between Nucleotides and Protocells**

P90 **Conformational Properties of an Equimolar Mixture of Complementary DNA Strands from c-MYC Promoter Region**

P91 **Potential role of oxygen molecules and cytochrome c oxidase proteins in optical communication in the brain**

P92 **Cell type stability in the context of intercellular signalling**

P93 **Characteristic dynamics of co-regulated genes with different intrinsic properties**

P94 **Explicit parameters in deterministic equations are insufficient to calculate extinction times**

P95 **Exploring the dynamics of extranuclear inheritance**

P96 **On MicroRNA and Protein Noise Models**

P97 **Solving First Passage Problems in Nanofluidic Devices with Deep Neural Networks**

TALK ABSTRACTS

1

Mining the molecular noise via fluorescence fluctuation image analysis of microscopy and super-resolution microscopy images

P. Wiseman

Departments of Chemistry and Physics, McGill University

The transport properties of biomolecules in cells can reveal a great deal about the functional interactions regulating cells at the molecular level. Various biophysical methods have been developed to measure these properties in cells, although most have relied on fluorescence microscopy imaging as the window for measurement of labeled macromolecules in living cells. Image correlation methods are an extension of fluorescence fluctuation spectroscopy that can measure protein-protein interactions and macromolecular transport properties from input fluorescence microscopy images of living cells. These approaches are based on space and time correlation analysis of fluctuations in fluorescence intensity within images recorded as a time series using a fluorescence microscope. We previously introduced spatio-temporal image correlation spectroscopy (STICS) which measures vectors of protein flux in cells based on the calculation of a spatial correlation function as a function of time from an image time series. Here we will describe the application of time window STICS and its two color extension, spatio-temporal image cross-correlation spectroscopy (STICCS), for measuring cellular waves of adhesion related macromolecules talin and vinculin as well as cytoskeletal actin between assembling and disassembling podosomes in dendritic immune cells. Podosomes are cylindrical membrane complexes with an integrin adhesive ring and an actin rich core that are associated with cellular migration and invasion in specific cell types. EM and super-resolution microscopy of cells shows radial actin filaments that connect neighboring podosomes so we applied pair vector correlation analysis to further characterize the transport waves within connected podosome clusters. These analyses combined with pharmacological perturbation experiments show that podosome turnover is coordinated within local clusters in cells with a correlation length scale extending to next nearest neighbor podosomes. As well I will introduce future extensions of ICS including new extensions to super-resolution fluorescence microscopy.

2

Natural Compounds as Dyes for Nonlinear Optical Microscopy

K. Purvis, K. Brittain, A. Joseph, R. Cisek and D. Tokarz

Department of Chemistry, Saint Mary's University

Information regarding the function of living tissue is instrumental to the advancement of science. Nonlinear optical microscopy, including third harmonic generation (THG) microscopy, can provide such information however, only certain biological structures generate THG signals. Structural specificity can be achieved by designing dyes for THG microscopy that demonstrate large third-order optical nonlinearities.

The third-order nonlinear optical properties of a number of natural compounds including natural phenols such as curcumin, photosynthetic pigments such as phycobilins and flavonoids as well as light-harvesting pigment-protein complexes such as phycobiliproteins were investigated. The third-order nonlinear optical properties of these compounds and pigment-protein complexes were obtained at 1030 nm wavelength using the THG ratio technique, combined with index of refraction measurements. The third-order nonlinear optical properties of these compounds and pigment-protein complexes were found to be similar to other dyes previously identified as labels for THG microscopy. Since these compounds and pigment-protein complexes have high third-order optical nonlinearities and they are nontoxic, they can potentially be useful in clinical applications of visualizing the structural changes in cellular components.

3

From STORM Microscopy and Genome-Wide Analysis to Near Atomic Resolution of Human Genes

P.D. Dans, M.V. Neguembor, D. Buitrago, J. Walther, R. Lema, P. Romero, I. Brun-Heath, P. Cosma, and M Orozco

Institute for Research in Biomedicine, Computational Biology

The linear sequence of DNA provided invaluable information about the nature of genes and regulatory elements and their distribution along chromosomes. However, to fully understand gene function and gene regulation we need to place the linear genome in the right context: the cell nucleus. Inside the nucleus, genes are organized forming complex three-dimensional structures that change over time. The GENSTORM team is focused on the mechanisms that influence gene regulation, from local changes in nucleosome positioning to global three-dimensional rearrangements of chromatin subject to differentiation/reprogramming in human cells. We produced Hi-C data to obtain chromatin and chromosome contacts, along with Mnase-seq data to determine nucleosome positioning (IMR90 and hiPSC cells). These genome-wide analyses were combined with optical microscopy and super-resolution imaging allowing us to get the first super-resolution images of individual genes together with their regulatory regions (GAPDH, STELLA, and NANOG). In addition, a strategy has been set up for the simultaneous visualization of genes and chromatin-associated proteins which localize protein binding in specific genes with nanometric precision. All these experimental techniques were integrated into two state-of-the-art coarse-grained models of chromatin to unravel the three-dimensional conformation and dynamics of genes and chromosomes in different conditions right into the nucleus.

4

The influence of disease mutations and regulatory interactions in SUR proteins, the regulatory subunit in K-ATP channels

V. Kanelis

Department of Chemical and Physical Sciences, University of Toronto Mississauga
Departments of Chemistry and Cell & Systems Biology, University of Toronto

ATP sensitive potassium (K-ATP) channels play crucial roles in the pancreas, brain, and cardiovascular system. K-ATP channels consist of four pore-forming Kir6.2 proteins and four regulatory sulphonylurea receptor (SUR) proteins. KATP channels are of vast medical importance, as mutations in K-ATP channels causes cardiovascular disease, neonatal diabetes, hyperinsulinism, or epilepsy. Recent high-resolution structures of the pancreatic KATP channel provide insights into the mechanism of pore closing, but not pore opening, which involves MgATP binding and hydrolysis at the nucleotide binding domains (NBDs). Further, structural information is lacking for some of the regulatory regions in the SUR protein, some of which are also sites of disease-causing mutations and post-translational modifications that regulated channel opening. Thus, additional structural studies are necessary to determine how the action of the NBDs regulates channel gating and the molecular basis by which NBD mutations cause diseases.
Using nuclear magnetic resonance, CD, and fluorescence spectroscopies, we have characterized structural changes in the SUR NBDs and regulatory regions imparted by disease-causing mutations and/or phosphorylation. Phosphorylation alters the structure of a regulatory linker and its interactions with the NBDs. NBDs bearing disease-causing mutations have altered binding to the membrane-spanning domains, even with the disease-causing mutation is not at the NBD/membrane domain interface, suggesting that mutations likely disrupt allosteric pathways linking the action of the NBDs to the membrane-spanning domains and ultimately K-ATP channel opening. Thus, our data shed light on the underlying molecular basis by which K-ATP channels are regulated by phosphorylation and how several SUR mutations cause disease.

5

Novel alternatives to antibiotics: biophysical tools to study the function of host defense peptides

S. Straus

Department of Chemistry, University of British Columbia

Antibiotic resistance is projected as one of the greatest threats to human health in the future and hence there is a great need to find alternatives. Antimicrobial peptides (AMPs) have shown great promise, because bacteria develop no or low resistance to AMPs. However, only few antimicrobial peptides are used as therapeutics, due to problems such as toxicity, short circulation half-life, and rapid kidney clearance.

This contribution describes a strategy to circumvent such challenges by conjugating the peptides to polymers to alter residence time and biodistribution in the body, without significant loss in activity. Hyperbranched polyglycerol (HPG) has gained attention due to its excellent biocompatibility, multifunctionality and plasma half-life, which can be tuned by changing its molecular weight. An alternative strategy to the challenges posed by AMPs is to formulate them with pegylated phospholipid micelles. In order to understand how these systems function, new biophysical tools are required. Results of conjugation and formulation of the natural aurein 2.2 and more active derivatives to HPG will be presented. New labelling approaches and whole cell NMR experiments will also be discussed.

6

Order in the membrane

J. Thewalt

Departments of Molecular Biology & Biochemistry and Physics, Simon Fraser University

What do we mean by membrane order? In liquid crystalline membranes, deuterium NMR gives a direct measurement of this property of lipid chains. Fluorescence can also be used to measure the degree of lipid organization. One particular fluorescence method is the measurement of "general polarization" using a probe such as Laurdan. The two techniques are measuring different physical quantities. Are these determinations of "order" related?

7

GL13K, an antimicrobial peptide with anticancer activity: A story told through the interaction with model membranes

J. Porro-Suardiaz

Department of Chemistry and Biochemistry, Concordia University

GL13K is a thirteen amino acid peptide with broad-spectrum bactericidal and antibiofilm activity at low minimum inhibitory concentrations while not showing hemolytic activity, which makes it an attractive candidate for antibiotic applications. Recently, it has emerged that many antimicrobial peptides additionally show anticancer activity; herein, we present the first assessment of anticancer activity for both D- and L-GL13K enantiomers in lung and breast cancer cell lines. D-GL13K showed greater selective toxicity towards cancer cells attributed to their ability to evade the action of proteases. To probe the roles of membrane composition and peptide chirality, we have studied the interaction of D- and L-GL13K with model membranes representing bacterial and human cancer cells using a combination of bulk and surface-specific biophysical characterization techniques including circular dichroism, PM-IRRAS and X-ray scattering techniques. Both D- and L-GL13K displayed a crystalline beta-sheet conformation in presence of bacterial models, whereas there is no crystallinity when they interact with the cancer cell model membranes. In the latter case, the lower surface charge led to greater proportion of unstructured peptide and to lower peptide insertion into monolayers. Despite this, the model membranes exhibited differential interactions with the two enantiomers, i.e. in the absence of proteases, the molecular origins of which will be discussed.

8

Visualizing transcription in the early fly embryo

C. Fradin

Department of Physics and Astronomy, McMaster University

In embryos, the expression of many important genes is under the control of morphogens, which are proteins forming spatio-temporal concentration gradients in developing tissues. Each morphogen acts as a letter in a postal code, providing information to the cells about their location, and allowing them to differentiate accordingly. We use the fly embryo as a model system to study the transcription of a particular gene, hunchback, which we image live using the MS2 system and laser-scanning confocal microscopy. Hunchback is expressed in response to the presence of the Bicoid morphogen, a transcription factor which forms a concentration gradient along the anterior-posterior axis of the fly. Observing hunchback transcription along this axis therefore provides a natural titration experiment, which shows how the parameters of the transcription vary with Bicoid concentration. Our data suggests that factors other than Bicoid must play a role in order to explain the very sharp switch-like response of hunchback expression to change in morphogen concentration.

9

Islet-on-a-chip provides an optical window into cellular metabolism and insulin secretion

J. Rocheleau

Institute of Biomaterials and Biomedical Engineering, University of Toronto

Living pancreatic islets are difficult to study due to a limited amount of tissue and the complexity of the cell types. To measure cell metabolism within living islets, we combine quantitative fluorescence microscopy (e.g. two-photon NAD(P)H imaging and genetically encoded sensors) with microfluidics engineering. We first designed microfluidic devices to simply hold islets stationary against a glass coverslip during media changes (e.g. glucose bolus) while imaging the metabolic and Ca2+ responses. However, our devices quickly expanded to take advantage of other aspects of microfluidic flow: (i) enhanced media transfer to the center of the tissue, (ii) controlled non-turbulent flow, and (iii) the ability to place novel sensors to measure individual islet responses. We are now leveraging these features to design a multiparametric islet-on-a-chip device to dynamically assay the metabolism (oxygen consumption and extracellular acidification rates) and function (insulin secretion) of individual islets. In essence, I will describe our efforts to measure the metabolism and function of individual cells and islets using a growing “toolkit” of quantitative fluorescence microscopy and islet-on-a-chip devices.

10

Nuclear Pore Complex: simple physics of a complex biomachine

A. Zilman

Department of Physics and Institute for Biomaterials and Biomedical Engineering, University of Toronto

Nuclear Pore Complex (NPC) is a biomolecular "nanomachine" that controls nucleocytoplasmic transport in eukaryotic cells, and is operation is central for a multitude of health and disease processes in the cell. The key component of the functional architecture of the NPC is the assembly of the polymer-like intrinsically disordered proteins that line its passageway and play a central role in the NPC transport mechanism. Due to the unstructured nature of the proteins in the NPC passageway, it does not possess a molecular "gate" that transitions from an open to a closed state during translocation of individual cargoes. Rather, its passageway is crowded with multiple transport proteins carrying different cargoes in both directions. It remains unclear how the NPC maintains selective and efficient bi-directional transport under such crowded conditions. Remarkably, although the molecular conservation of the NPC components is low, its physical transport mechanism appears to be universal across eukaryotes - from yeast to humans.

Due to the paucity of experimental methods capable to directly probe the internal morphology and the dynamics of NPCs, much of our knowledge about its properties derives from in vitro experiments interpreted through theoretical and computational modeling. I will present the current understanding of the Nuclear Pore Complex structure and function arising from the analysis of in vitro and in vivo experimental data in light of minimal complexity models relying on the statistical physics of molecular assemblies on the nanoscale.

11

The Bending Rigidity of Red Blood Cell Membranes Determined from Solid-Supported Multi-lamellar Membranes

S. Himbert and M. Rheinstädter

Department of Physics and Astronomy, McMaster University

The preparation of Red Blood Cell (RBC) Ghosts is a well-known protocol in biological and medical research and describes the extraction of the membrane from RBCs. Another well-known protocol is the preparation of highly ordered stacks of artificial lipid bilayers on silicon wafers. There are various attempts to adapt this protocol to a native cell membrane. For the first time, we were able to combine both techniques and prepare highly ordered stacks of RBC membranes on silicon wafers [1]. This assay can now be used as an inexpensive and safe platform for testing the effect of drugs and bacteria on RBC membranes in-vitro using biophysical techniques, such as X-ray and Neutron diffraction. We present direct experimental evidence that these RBC membranes consist of nanometer-sized domains of integral coiled-coil peptides, as well as liquid ordered (lo) and liquid disordered (ld) lipids. This patchy nature of the red blood cell membrane has a significant effect on the bending rigidity of the membranes. We present a novel method to determine the bending modulus and membrane interaction modulus of a biological membrane on the molecular scale from 2-dimensional X-ray diffraction measurements. These measurements are complemented by Molecular Dynamics simulations and Neutron Spin-Echo measurement which allow a direct determination of the membrane undulations.
[1] Himbert S. et al., Scientific Reports 7, Article number: 39661 (2017)

12

Rational Design of Protein Energy Landscapes

R. A. Chica

Department of Chemistry and Biomolecular Sciences, University of Ottawa

Proteins have found widespread application in research, industry, and medicine because they can mediate complex molecular processes with extreme precision and efficiency. Even so, continued engineering of proteins with tailored functions is essential to enable novel biotechnological applications. Computational protein design (CPD) has enjoyed considerable success in creating protein sequences that stably adopt a single targeted structure. However, attempts to use these methods to generate proteins that can carry out specific functions have mostly failed to match the efficiencies that are found in nature. This is partly due to the fact that most CPD methods evaluate sequence energies in the context of a single structure even though protein function is dictated by the energetic contributions of many conformational states. To increase the accuracy of CPD predictions and thereby design more efficient proteins, we are developing multistate CPD methods that can evaluate sequences in the context of any number of protein conformational states, allowing energy landscapes to be engineered for desired functions. I will show how these methods can be used to design a specific mode of conformational exchange into a stable globular fold, and modulate the conformational equilibrium of an enzyme for enhanced activity with a non-native substrate.

13

Deconstructing biology with simple single-molecule imaging: Controlling conformation, confinement, and concentration

S. Leslie

Department of Physics & Quantitative Life Sciences Program, McGill University

Molecular biology is messy and complex. The future of life sciences research, drug development, and many other fields depends on the ability of physicists and engineers to unravel the complex, biophysical phenomena that underlie cellular function with a finer level of resolution. Using currently available technologies, it is still challenging to conduct quantitative measurements that can potentially reveal the true complexity of life at the molecular scale. In this talk, I will introduce Convex Lens-induced Confinement (CLiC) microscopy, a simple, general method to image molecular interactions one molecule at a time, while emulating "cell like" conditions, with precision and control. By mechanically confining molecules to the field of view, CLiC eliminates the complexity and potential biases inherent to "tethering" molecules, often used in standard methods. By visualizing the individual trajectories of many molecules at once, and for long time periods, CLiC allows us to investigate important biophysical questions about their behaviour, such as how higher-order structures in DNA molecular and a crowded environment can regulate the dynamic unwinding of specific target sites, and the kinetic rates of binding to these sites. Beyond new insights into the statistical mechanics of DNA, I will discuss key applications of our work to advancing medicine and biologics drug development. I will share new and emerging areas of exploration with CLiC, including visualizing protein aggregation, nanoparticle dynamics, CRISPR-Cas9 targeting dynamics, and therapeutics applications.

14

Direct observation of formation of a knotted structure in single RNA molecules from Zika virus

M. Woodside

Department of Physics, University of Alberta

RNA takes on diverse structures related to its many biological roles. Recently, the first knotted structure was identified in exoribonuclease-resistant RNA (xrRNA) from Zika virus. This knot is proposed to be a mechanical roadblock halting digestion of viral RNA by host exonucleases, leading to production of essential sub-genomic RNA. Using force spectroscopy, we characterized the mechanical stability and folding mechanism of Zika xrRNA. The occurrence of the knotted structure—the most stable structure seen in RNA—correlated well with exonuclease resistance in wild-type and mutant xrRNAs. Knot formation involved threading the 5′ end into a cleft coordinated by Mg^{2+}-dependent tertiary contacts before closure of the encircling loop by a pseudoknot. This work reveals how folding and function are related in a new class of RNAs.

15

Unsupervised Statistical Learning in Multi-Resolution Dynamics

H. Yang

Department of Chemistry, Princeton University

Nanoscale dynamics—be it changes in protein or DNA conformation, or movements of biological macromolecules in cellular/biomolecular milieu—spans several spatiotemporal scales. As such, it is extremely challenging to quantify the underlying dynamics to uncover governing physical principles. Advances in experimental techniques allow the study of these processes at the most fundamental single-molecule and single-particle level. The measurements, however, are complicated by readout noise on top of the ever-presenting thermal fluctuations. Worse, experimental data often contains insufficient information to afford educing the physical principles. In this presentation, we discuss unsupervised statistical leaning frameworks we developed that overcome these challenges. For systems exhibiting discrete states, clustering algorithms and Bayesian information criterion are used as a model selection scheme for determining the number of states. For observations involving continuous stochastic dynamics such as protein conformational changes, the likelihood functional and the trajectory entropy are used to determine the potential of mean force and intra-molecular diffusion coefficient as two fundamental dynamics parameters for protein motions. We further introduce a Bayesian data augmentation framework to infer the conformational modes of a protein from limited number of experimental observables such as single-molecule FRET. On a larger scale, we describe a high-precision structural reconstruction method for cellular components that utilizes the components' shape and mechanical properties. When used with 3D multi-resolution imaging, it promises the elucidation of the fast dynamics of particles or molecules in the context of the large, slow cellular dynamics. These data-driven methods capitalize on the advances in modern computation such that the new and otherwise hidden information emerges as a result of statistical learning. It is hoped that these frameworks will help make new discoveries from the multiscale data.

16

Ensemble modelling of disordered proteins using single-molecule FRET

G-N. W. Gomes[1, 2], M. Krzeminski[3, 4], E. Martin[5], T. Mittag[5], J. Forman-Kay[3, 4], and C. C. Gradinaru[1, 2]

1 Department of Physics, University of Toronto
2 Department of Chemical and Physical Sciences, University of Toronto Mississauga
3 Molecular Structure and Function Program, Hospital for Sick Children
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In the experimental characterization of intrinsically disordered proteins (IDPs), a major objective is to define and characterize the nature and significance of any identified (transient) structure. The concept of transient structure in IDPs can only be meaningfully defined in relation to the random coil (RC) reference state. Paradoxically, the RC polymer physics properties are assumed a priori in order to interpret and compare various biophysical measurements of IDP dimensions, e.g., end-to-end distance from single molecule Förster Resonance Energy Transfer (smFRET) and radius of gyration from Small Angle X-Ray Scattering (SAXS). The sequence-encoded differences from the RC state are precisely what is interesting about IDPs, yet may also be key to reconciling the apparent inconsistency between IDP dimensions as determined by SAXS and smFRET.
A solution to this paradox is to use computational approaches to calculate structural ensembles consistent with diverse experimental input. We calculate, to our knowledge for the first time, ensembles consistent with smFRET, SAXS, Paramagnetic Relaxation Enhancement, Cα and Cβ chemical shifts and hydrodynamic data for an IDP in native conditions (N-terminal Sic1 1-90). By contrasting the polymer physics behaviour of the experimentally-restrained ensembles to that of the reference RC state, we obtain new clues on how sequence-to-ensemble relationships shape the conformational landscape of Sic1 and enable its biological functions.

17

Direct measurements of VEGF:VEGFR2 binding affinities reveal the coupling between ligand binding and receptor dimerization

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Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) controls angiogenesis, and is critically important for normal human development and cancer progression. A recent finding that VEGFR2 can dimerize in the absence of ligand raises the question whether VEGF binds to either VEGFR2 monomers or dimers, or to both. While VEGF-VEGFR2 effective binding constants have been measured, these prior measurements have not discriminated between the association state of the receptor. Since ligand binding is coupled to receptor dimerization, this coupling lends complexity to a seemingly straight-forward problem. Here we unravel this complexity by applying a rigorous thermodynamics approach and performing binding measurements over a broad range of receptor and ligand concentrations. By applying a global fitting procedure to a large data set, we reveal a 45-fold difference between VEGF binding affinities for monomeric and dimeric forms of VEGFR2.

18

Molecular and Mechanistic Underpinnings of Signal Transduction in Membranes - NMR Inspired Studies of the Conformational Landscape in Enzymes & GPCRs

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In the last decade, X-ray crystallography and cryo-electron microscopy have brought about a renaissance in structural biology. Recent atomic-resolution structures of both soluble and membrane associated proteins provide the possibility of mechanistic explanations of function and have renewed efforts in drug discovery. However, the “structure-function” perspective ignores the reality of protein dynamics and the fact that most proteins adopt a fluid ensemble of functional conformers (states). We are interested in the role of this ensemble along the reaction coordinate pathway. This talk will review studies associated with a homodimeric enzyme, fluroacetate dehalogenase, and the role of the ensemble in accomplishing catalysis. We will then present recent NMR data aimed at elucidating the key functional states associated with the adenosine A2A G-Protein-Coupled Receptor. The free-energy landscape is tremendously informative with regard to mechanistic explanations of activation and signal transduction and provides new opportunities for drug discovery.

19

A spatial-temporal map of the Protocadherin expression code in the CNS

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The assembly of neuronal circuits is dependent on complex and selective cell-cell interactions, mediated by cell-surface receptors. In addition to regulation of neuronal connectivity at the level of cell-types, individual neurons within the same cell-type may also be shaped by distinct receptors. The clustered Protocadherins (Pcdhs) are a large family of cell-surface receptors with the potential to endow each neuron with a unique cell surface signature. Profiling studies of single neurons suggest that the 58 Pcdh genes are subject to stochastic and combinatorial expression, producing millions of distinct Pcdh barcodes for establishing single neuron identities. It remains unknown if combinatorial Pcdh expression is a general feature in the nervous system, and whether Pcdh signatures change during development. To tackle these questions, I devised a novel strategy using multiplexed, single-molecule fluorescence in situ hybridization (multiplex FISH) techniques to map the spatial-temporal expression profiles of all 58 Pcdh isoforms across neuron types in the mouse nervous system. To increase the number of targets that can be imaged simultaneously, I am employing sequential barcoding techniques to combinatorially label each isoform with a colored (fluorophore) barcode, which will be decoded through sequential rounds of single molecule RNA imaging. This work aims to gain insight into the logic behind molecular diversity and complexity, and their roles during neuronal wiring.

20

Single-molecule fluorescence spectroscopy reveals heterogeneous conformations in Apolipoprotein E

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The e4-allele isoform of apolipoprotein E (ApoE4) plays a key-role in Alzheimer’s disease and cardiovascular pathologies. A large body of evidence support that conformations of the monomeric protein are instrumental in its contribution to function and disease; yet, much remains unknown about the conformational ensemble of the full-length ApoE4 and its role in protein function, largely because of its elevated propensity for oligomerization and its inherent flexibility.

Here, we combine state-of-the-art single-molecule fluorescence spectroscopy and molecular dynamic simulation to access the structural ensemble of the monomeric full-length ApoE4 and develop a quantitative model of its conformations. Our data reveal that ApoE4 does not adopt a unique stable structure but, instead, rapidly explores a conformational heterogeneous ensemble where multiple distinct conformers coexist in equilibrium. Importantly, single-point mutations that are known to alter protein function and neurotoxicity act as a molecular rheostat of ApoE conformational ensemble, reshaping long-range interactions between the folded N-terminal domain and the flexible C-terminal half.

21

Aggregation and Coacervation of the Tau Peptide

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Tau is an intrinsically disordered peptide that plays an important role in the cell by binding to microtubules. Under pathological conditions, Tau can form fibrillar aggregates, a process that has been linked with Alzheimer's disease. In addition to forming fibrils, the Tau protein can also phase separate into a protein rich and a protein depleted phase, a process known as liquid-liquid phase separation (LLPS), or coacervation. I will present field theoretic simulations that map out the phase diagram for Tau coacervation. The phase diagram is then used to predict the conditions under which Tau can be driven towards coacervation under live cell coculturing conditions.

22

Phase Separation Biophysics, Prediction and Role in Biological Regulation

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There is growing awareness of the role of phase separation of protein and nucleic acid in responsive cellular organization and formation of biomaterials. Dynamic, multivalent interactions drive phase separation, often involving intrinsically disordered protein regions (IDRs). We have shown the significance of planar pi:pi contacts in the propensity for IDR phase separation, based on the predictive ability of long-range pi contact frequencies mined from folded protein structures. Building on this, we are developing more comprehensive predictors of IDR phase separation including contributions from other feature and energetic terms. Post-translational modifications have significant effects on phase behaviour of reconstituted minimal mimics of cellular membraneless organelles with IDRs and RNA. Phase behaviour, including multi-phase compartmentalization, in turn affects enzymatic rates in biochemical assays of mRNA translation and deadenylation, essential RNA processing in all cells but having particularly significant roles in neuronal function. These biophysical and biochemical results provide clear mechanisms for how phase separation can regulate biological organization and function.

23

Collagen fibrils: liquid-crystals, crystals, and rubber bands

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Collagen fibrils are versatile self-assembled structures that provide mechanical integrity to mammalian tissues. Collagen fibrils are structurally diverse: their radii vary widely depending on anatomical location; the tilt of individual molecules with respect to the fibril axis varies with radial distance in a “double-twist” configuration; and fibrils exhibit a robust periodic modulation of density along the axial direction -- the “D-band”. Since collagen molecules are chiral, we can use a liquid-crystal free-energy for cholesterics to describe how the double-twist stabilizes the fibril phase and determines the fibril radius. By adding phase-field crystal terms we can capture the periodic D-band, and show how the double-twist affects elastic properties of collagen fibrils -- and vice-versa.

24

Biophysical modeling of the collective behavior of cells in tissues

L. Manning

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How do individual cells generate large-scale patterns on the scale of organs and tissues, and how does this process break down in disease? While patterning of biochemical signaling molecules such as morphogen gradients play an important role, physical forces can also help to execute and control the pattern proscribed by genetics. In multicellular tissues, cell-scale physical forces can interact with one another to generate surprising and robust behaviors that span many cell diameters. I will discuss our recent work using mathematical models to predict how cells migrate and change shape in response to physical forces in in vitro systems, including cultured human bronchial cells and breast cancer cell lines, as well as in vivo systems, such as the zebrafish left-right organizer and cancer tumors from human patients. Understanding these emergent behaviors helps us to generate unexpected new hypotheses about the molecular mechanisms that drive pattern formation in these systems.

25

Exploiting trade-offs in the evolution of antibiotic resistance

E. Toprak

Green Center for Systems Biology, University of Texas Southwestern Medical Center

Antimicrobial resistance is a burgeoning global health threat. Pathogenic bacteria quickly evolve antibiotic resistance either by spontaneous mutations in their genomes or horizontal transfer of mobile resistance genes. In my talk, I will summarize what we learned from evolving antibiotic-resistant bacteria in our lab and how we used these findings for developing novel drug molecules.

26

Molecular Mechanisms of Gating, Permeation, and Leakage in Ion Channels

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By providing atomic-level information on nanosecond to microsecond time scales and beyond, molecular dynamics simulations are a useful tool to investigate the structure and function of ion channels embedded in lipid bilayers. I will present recent and ongoing simulation studies of gating, permeation, and leakage in various ion channels, including voltage-gated sodium and proton channels, and calcium release activated calcium channel Orai1.

27

Two recent "proofs" that the theory of diffusion is not dead

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Diffusion is obviously of great importance in biophysics. The theory explaining the process is rather old, and includes the famous 1905 Einstein paper, Brown's discovery of moving pollen particles (1827), Fick's law (1855), and the like. Does it mean that the field is dead or dying? NO! In this presentation, I will discuss two problems from which we have recently learned interesting lessons. 1) Lattice Monte Carlo (LMC) methods are simple and widely used. But are they right? Oddly, the standard LMC algorithm fails to reproduce the solution of the diffusion equation for short times, a fact that affects LMC's usefulness in some cases. I will show how to fix this problem and a few results we recently obtained that could connect the steady-state and anomalous diffusion regimes in random systems. 2) Drug release systems are often modeled using LMC simulations and characterized using the Weibull function. However, modelling inhomogeneous systems where both the porosity and the diffusivity are space-dependent is ambiguous in LMC simulations. Furthermore, the meaning of the Weibull exponent is unclear. I will clarify how LMC models should be designed and how one can actually predict the Weibull exponent from fundamental principles.

28

Binding of cannabinoids and fatty acids to fatty acid binding proteins

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Fatty acid binding proteins are critical intracellular transport proteins capable of binding hydrophobic molecules, including fatty acids, endocannabinoids, and phytocannabinoids from marijuana. FABPs are implicated in a variety of cellular processes and have been linked to a number of diseases. Given the recent legalization of cannabis, it is important to understand the interactions between FABPs and phytocannabinoids and, ultimately, to understand how these interactions alter function. I will present both computational and experimental work on characterizing the binding of fatty acids and cannabinoids to fatty acid binding proteins. The experimental results include microscale thermophoresis and fluorescence displacement experiments. We observe that in addition to binding to individual fatty acid molecules, these proteins also bind to fatty acid micelles, which complicates analysis. Our computational work predicts that several phytocannabinoids bind with similar poses and that binding is coupled to the dynamics of the protein, which may influence intracellular trafficking.

29

Modeling Covalent Modifiers of Kinase Proteins

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Covalent modifier drugs act by forming a chemical bond with their targets. This class of drugs includes kinase inhibitors such as ibrutinib, which has improved selectivity and affinity through the addition of its electrophilic acrylamide substituent to an active site cysteine thiol of its target. Modeling the activity of these drugs requires new computational methods to describe the mechanism of their reactions with their targets. We have developed an integrated workflow to model the deprotonation of the protein into the reactive thiolate state, the binding of the drug to the binding site using free energy perturbation, and QM/MM methods to calculate the kinetics and reaction energies of the covalent bond formation. We have applied these methods to model the binding of an acrylamide-containing covalent inhibitor of Bruton's tyrosine kinase (BTK). Non-covalent interactions still account for the bulk of the protein–ligand binding energy, but the formation of the covalent bond results in a higher degree of affinity for kinases that possess a cysteine residue in the front pocket of the ATP binding site.

30

New Methods for Visualizing Single Amyloid Protein Dynamics at the Nanoscale

M. Lew

Electrical and Systems Engineering, Washington University in St. Louis

Oligomeric amyloid structures are crucial intermediates within the aggregation pathway of Alzheimer's and other amyloid diseases. However, these oligomers are too small to be resolved by standard light microscopy. We are developing new optical and chemical imaging tools to visualize the morphology and structure of amyloid aggregates without the need for covalent labeling or immunostaining. First, we have designed a Tri-spot point spread function (PSF) to measure the orientation and rotational motion of single fluorescent molecules. Using the Tri-spot PSF, we have observed orientational order and disorder of YOYO-1 transiently bound to DNA that is not resolvable by standard super-resolution microscopy. Second, we have developed super-resolution Transient Amyloid Binding (TAB) microscopy, which uses dynamic binding of single thioflavin T molecules to generate photon bursts for localization microscopy. Its robustness to photobleaching enables TAB microscopy to record amyloid dynamics over minutes to days. We imaged oligomeric and fibrillar structures formed during different stages of amyloid-β aggregation, as well as the structural remodeling of amyloid-β fibrils by the compound epi-gallocatechin gallate.

31

Playing with Knives: Regulation of Human DNA Resection

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Genetic recombination in all kingdoms of life initiates when helicases and nucleases resect the free DNA ends to expose single-stranded (ss) DNA overhangs. Resection regulation in bacteria is programmed by a DNA sequence, but a general mechanism limiting resection in eukaryotes has remained elusive. I will describe our recent results characterizing the human DNA resectosome—a multi-enzyme machine consisting of BLM helicase and EXO1 or DNA2 nucleases. We show that the human resectosome catalyzes kilobase-length DNA resection on nucleosome-coated DNA. The resulting ssDNA is rapidly bound by RPA, which further stimulates DNA resection. RPA is phosphorylated during resection as part of the DNA damage response (DDR). Remarkably, pRPA inhibits DNA resection in cellular assays and in vitro via inhibition of BLM helicase. pRPA suppresses BLM initiation at DNA ends and promotes the intrinsic helicase strand-switching activity. These findings establish that pRPA provides a feedback loop between DNA resection and the DDR. More broadly, RPA phosphorylation may be a critical regulatory signal for multiple replication and repair enzymes.

32

Dynamic interactions between the RNA chaperone Hfq, small regulatory RNAs and mRNAs in live bacterial cells

J. Fei

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RNA binding proteins play myriad roles in controlling and regulating RNAs and RNA-mediated functions, often through simultaneous binding to other cellular factors. In bacteria, the RNA chaperone Hfq modulates post-transcriptional gene regulation. Absence of Hfq leads to the loss of fitness, and compromises the virulence of bacterial pathogens. Using live-cell super-resolution imaging, we demonstrate that under normal growth conditions, Hfq exhibits widespread mRNA binding activity, and recruits RNase E to promote turnover of these mRNAs. Stress induced small regulatory RNAs (sRNAs), once expressed, can either co-occupy Hfq with the mRNA or displace the mRNA from Hfq, suggesting mechanisms through which sRNAs rapidly access Hfq to induce sRNA-mediated gene regulation. Finally, we observe that locally condensed ribosomes, together with mRNAs, can trap Hfq in a slow diffusivity state, leading to the formation of large Hfq clusters. Our data collectively demonstrate that Hfq dynamically changes its interactions with different RNAs in response to changes in cellular conditions.

POSTER ABSTRACTS

P1

2D NMR investigation of water dynamics at the interface of carbon nanotubes

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Material Science, National Center for Scientific Research, Athens
National Center for Scientific Research, Athens

Several theoretical studies using molecular dynamics MD simulations showed an enchantment of water dynamics inside hydrophobic channels and reported a peak of the liquid self-diffusion coefficient at certain carbon nanotube sizes. However, experimental confirmation of this size-dependent liquid-dynamics was so far lacking. Here, we report two-dimensional NMR spectroscopy diffusion relaxation 2D D-T2, relaxation-relaxation 2D T1-T2 measurements as well as MD simulations of water in carbon nanotubes (CNTs) of different diameters (1.1 nm to 6nm) in the temperature range of 305-265 K. These NMR methods provide unique ways to distinguish water in the interior of CNTs from bulk water and water adsorbed on the external surface of the CNTs. Experiments show that water inside CNTs is further resolved into two components with different diffusion coefficient (D) values; water in proximity with the CNT walls, and water along the CNT axis. We were able to confirm experimentally, for the first time, a favorable diameter range (3-4.5nm) in which maximum water dynamics occur as predicted by MD simulation works.

P2

Implication of cholesterol in regulating the pore-formation mechanism of Vibrio cholerae cytolysin, a beta-barrel PFT

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Vibrio cholerae cytolysin (VCC) is a beta-barrel pore-forming toxin with potent cell-killing cytolytic/cytotoxic activity. To exert its cytolytic effect, VCC binds to the target cell membranes and forms transmembrane oligomeric beta-barrel pores. Earlier studies have indicated that cholesterol might play a critical role in regulating the mode of action of VCC. However, the exact mechanistic basis of the process still remains unknown. In the present study, we have explored the role of cholesterol in the different steps of VCC mode of action by employing the liposomes containing varying amount of cholesterol, we have shown that cholesterol governs the efficient interaction of the toxin with the membrane lipid bilayer that in turn facilitates the subsequent oligomeric pore-formation process. We have also studied the physiological relevance of membrane cholesterol in the context of the biomembranes of the eukaryotic cells. Our results have shown that the depletion of membrane cholesterol from erythrocytes compromises the pore-forming activity of VCC. In the biomembrane of erythrocytes, where accessory interactions are available, binding of VCC is not affected to any significant extent. However, upon depletion of membrane cholesterol in the erythrocytes membranes, membrane-bound fraction of VCC remains trapped in the form of abortive oligomeric assembly, and does not form the functional pores. Our study has also revealed that VCC shows marked propensity to associate with lipid rafts.

P3

Solid-state NMR spectroscopy detection following thermal unfolding of a seven-helical membrane protein

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Department of Physics and Biophysics Interdepartmental Group, University of Guelph

Our research aims to investigate the driving forces behind membrane protein folding and to provide insight into the molecular nature of the unfolding/folding pathway. We combine hydrogen-deuterium exchange and solid-state NMR detection to site-specifically follow the sequence of the thermal unfolding events in a lipid-embedded transmembrane protein of seven-helical (7TM) architecture, Anabaena Sensory Rhodopsin. The thermal unfolding of ASR was induced by incubating the protein sample packed in a NMR rotor in a D2O buffer in a range of 20-80°C, which results in a gradual temperature-dependent increase of the solvent-accessible surface, with amide protons at exposed sites exchanging for deuterons. Following each incubation, a set of multidimensional correlation NMR spectra were collected to site-specifically detect the extent of H/D exchange by monitoring the intensities of cross-peaks which strongly depend on the protonation states of the backbone amides. Relative signal-to-noise ratio were extracted from each spectrum to determine the exchanged sites, and these exchanged sites were further mapped onto a structure model. A series of H/D exchange pattern at different temperatures were constructed to analyse the dynamics of unfolding, such as the nature of stabilization factors and the inter-residue interactions, and to further deduce possible folding/unfolding models.

P4

Characterizing the hierarchy of internal dynamics in a membrane protein by solid state NMR

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Protein dynamics play critical role in protein function. Membrane proteins reside in a highly anisotropic environment of a lipid bilayer, and experience a myriad of interactions with solvent, lipids and other proteins. Understanding how these interactions affect the energy landscape of internal motions is a major challenge. Here, we use solid-state Nuclear Magnetic Resonance (SSNMR) to probe temperature-dependent nuclear spin relaxation rates to probe internal dynamics of a seven transmembrane (7TM) helical light-driven proton pump Green Proteorhodopsin (GPR). We used two samples of GPR reconstituted in DMPC/DMPA and DOPE/DOPG liposomes with distinctly different melting points. Following methodology proposed by Lewandowski et al. (1), we measured a total of seven relaxation rates in the temperature range from 104 K - 289 K, and observed both commonalities and significant variations between the two samples. Using model free analysis, we directly determine activation energies of motional modes shared between the two samples, and representing common sidechain methyl rotations and reorientations as well as backbone motions. Remarkably, we were also able to determine motional modes with distinctly different activation energies that likely result from the thermodynamic properties of lipid bilayers, thereby highlighting the influence of the surrounding environment on protein motions.

(1) Lewandowski, J. R.; Halse, M. E.; Blackledge, M.; Emsley, L. Science 2015, 348, 578-581.

P5

Functional studies of the human cholesterol transporter ABCG5/ABCG8 in lipid-bilayer nanodiscs

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Cholesterol is a key component of biological membranes. Dysregulated cholesterol homeostasis can risk cardiovascular disease development. In the liver, a plasma membrane protein ABCG5/ABCG8 (G5/G8) mediates excess cholesterol excretion into biliary fluids and is a key regulator of cholesterol homeostasis. Rare genetic mutations causing loss of G5/G8 function can result in sitosterolemia, a disease characterized by elevated plasma plant sterols, hypercholesterolemia and early coronary artery disease. Upon analysis of a previous structural study that revealed the first three-dimensional amino acid architecture of G5/G8, we located several disease-causing mutations to a hypothetical cholesterol binding region; The current study aims to investigate the effect of these pathological mutations on the physiological cholesterol-transport of G5/G8 using in vitro lipid bilayer mimetic nanodiscs. We have developed a nanodisc reconstitution protocol for G5/G8. The G5/G8 nanodiscs purified by size exclusion chromatography were evaluated by native gel electrophoresis, and function determined by an in vitro ATP hydrolysis assay. We will perform a systematic examination of cholesterol/lipid environment on the ATPase function of G5/G8 mutants. Ultimately, our research will further mechanistic understanding of G5/G8 cholesterol transport, providing a platform for pharmacological discovery to modulate the protein function for cardiovascular and metabolic diseases.

P6

Membrane binding of S100A10 protein and AHNAK peptide involved in cell membrane repair

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Cholesterol is a key component of biological membranes. Dysregulated cholesterol homeostasis can risk cardiovascular disease development. In the liver, a plasma membrane protein ABCG5/ABCG8 (G5/G8) mediates excess cholesterol excretion into biliary fluids and is a key regulator of cholesterol homeostasis. Rare genetic mutations causing loss of G5/G8 function can result in sitosterolemia, a disease characterized by elevated plasma plant sterols, hypercholesterolemia and early coronary artery disease. Upon analysis of a previous structural study that revealed the first three-dimensional amino acid architecture of G5/G8, we located several disease-causing mutations to a hypothetical cholesterol binding region; The current study aims to investigate the effect of these pathological mutations on the physiological cholesterol-transport of G5/G8 using in vitro lipid bilayer mimetic nanodiscs. We have developed a nanodisc reconstitution protocol for G5/G8. The G5/G8 nanodiscs purified by size exclusion chromatography were evaluated by native gel electrophoresis, and function determined by an in vitro ATP hydrolysis assay. We will perform a systematic examination of cholesterol/lipid environment on the ATPase function of G5/G8 mutants. Ultimately, our research will further mechanistic understanding of G5/G8 cholesterol transport, providing a platform for pharmacological discovery to modulate the protein function for cardiovascular and metabolic diseases.

P7

Melatonin Changes Nanoscale Lipid Domains to Protect Neuronal Model Membranes Against Amyloid Toxicity

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Alzheimer's disease (AD) is a neurodegenerative disease resulting from the cleavage of the transmembrane amyloid-beta (Abeta) precursor protein into toxic Abeta peptides. These peptides can oligomerize and contribute to neuronal damage manifesting as memory loss, dementia, and neuronal degradation. Melatonin is a naturally occurring compound produced by the pineal gland of the brain. Due to its lipophilic and anti-oxidant properties melatonin is capable of interacting and inserting into membranes. The goal of this study is to observe the structural changes induced by melatonin insertions into neuronal model membranes and to assess whether these changes offer protection against toxic Abeta peptides. Through atomic force microscopy (AFM) and Kelvin probe force microscopy (KPFM) studies, we have determined that melatonin inserts into the membranes and changes both topographical and electrostatic nanoscale domains within neuronal model membranes. Preliminary data using Localized Surface Plasmon Resonance (LSPR) demonstrates the protective role of melatonin against amyloid binding. This study contributes to understanding the protective role of melatonin in Alzheimer's disease.

P8

Impact of muscular dystrophy causing mutations on the folding and stability of dysferlin C2A domain

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Failure to repair injured sarcolemmal membranes leads to muscular dystrophy, a degenerative disorder that results in increasing weakness and gradual wasting of skeletal muscles. Dysferlin is a membrane repair protein involved in trafficking of proteins and vesicles around injured membranes in skeletal muscle cells. The C2A domain is the major portion of dysferlin responsible for responding to calcium ion influx upon membrane damage and the activation of dysferlin. Here we show the role of calcium in stabilizing the secondary structure of the C2A domain and mediating its phospholipid binding. Two mutations, V67D and W52R, in the C2A domain prevent membrane repair, resulting in two forms of muscular dystrophy (MD): Limb-girdle muscular dystrophy type 2B (LGMD2B), and Miyoshi myopathy (MM). Substituted dysferlin V67 and W52 proteins were expressed, and NMR spectroscopy and circular dichroism thermal unfolding experiments were conducted, showing that the V67D and W52R substitutions decrease the stability of the C2A domain. Calcium titration experiments by isothermal titration calorimetry (ITC) and lipid binding assays will be performed to characterize the calcium and lipid binding properties of the mutants compared to the wild-type. We predict that the unfolding of the C2A domain of dysferlin encompassing the V67D and W52R substitutions is responsible for impaired dysferlin function in the membrane repair process, and consequently the wasting of skeletal muscles in MD patients.

P9

The role of the beta subunit in P4-ATPase function

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Biological membranes are organized as bilayers with an asymmetric distribution of lipid molecules between the two leaflets of lipid. Lipid bilayer asymmetry is critical for regulating membrane curvature, a prerequisite for membrane trafficking events such as endocytosis and vesicle-mediated transport in the secretory pathway while controlled disruption of membrane asymmetry initiates critical biological processes such as blood coagulation, apoptosis, cytokinesis and cell fusion. Lipid asymmetry is generated and maintained by the type IV P-type ATPase (P4-ATPase) family of phospholipid flippases that harness energy from ATP to unidirectionally transport lipids to the designated leaflet. P4-ATPases appear to operate in conjunction with an accessory beta-subunit, however, the requirement of this component for function has been debated. We developed a detergent-free purification for beta-subunit-less Drs2 P4-ATPase from Saccharomyces cerevisiae in order to systematically compare the enzymatic properties of purified Drs2 in the presence and absence of its beta-subunit. We aim to characterize the lipid determinants of Drs2 ATPase activity and substrate specificity in the presence and absence of its beta-subunit. Our future directions also include structural determination of P4 ATPase using X-ray crystallography and single-particle cry-electron microscopy. This work will facilitate a better understanding of the biophysical and biochemical basis of lipid transporter function.

P10

Whole cell 2H Solid-State NMR of Antimicrobial Peptides Interacting with cell envelopes: Role of lipopolysaccharide

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Antimicrobial Peptides (AMPs) have been studied for a long time, but still, there exist gaps in our understanding of their molecular mechanism of action. AMPs are well known to permeabilize model lipid membranes. However, it is likely that interactions between AMPs and non-lipid components of the cell envelope also important to their mechanisms of action. Specifically, the focus of this study is to find out if the lipopolysaccharide (LPS) outer membrane layer of Gram-negative bacteria promotes or inhibits AMP-induced membrane disruption. We disrupt the lipopolysaccharide layer of Gram-negative bacteria (JM109) via chelation of the stabilizing divalent cations. Then, we use deuterium NMR of deuterated bacteria to observe the level of membrane disruption by AMPs with and without EDTA treatment. In addition to 2H NMR, we do flow cytometry to analyze the physiological response of bacteria after treatment.

P11

Atomic Resolution Map of the Soluble Amyloid Beta Assembly Toxic Surfaces

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Soluble amyloid beta assemblies (Aβn) are neurotoxic and play a central role in the early phases of the pathogenesis cascade leading to Alzheimer's disease. However, the current knowledge about the molecular determinants of Aβn toxicity is at best scant. Here, we comparatively analyze Aβn prepared in the absence or presence of a catechin library that modulates cellular toxicity. By combining solution NMR with dynamic light scattering, fluorescence spectroscopy, electron microscopy, wide-angle X-ray diffraction and cell viability assays, we identify a cluster of unique molecular signatures that distinguish toxic vs. nontoxic Aβ assemblies. These include the exposure of a hydrophobic surface spanning residues 17-28 and the concurrent shielding of a highly charged N-terminus. We show that the combination of these two dichotomous structural transitions promotes the colocalization and insertion of beta-sheet rich Aβn into the membrane, compromising membrane integrity. These previously elusive toxic surfaces mapped here provide an unprecedented foundation to establish structure-toxicity relationships of Aβ assemblies.

P12

A Molecular Mechanism for Polymyxin-induced Membrane Damage that predicts Bacterial Resistance

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With the advent of polymyxin B (PmB) resistance in bacteria, the mechanisms for mcr-1 resistance are of crucial importance in the design of novel therapeutics. The mcr-1 phenotype is known to decrease membrane charge and increase membrane packing by modification of the bacterial outer membrane. We used X-ray diffraction, Molecular Dynamics simulations, electrochemistry, and leakage assays to determine the location of PmB in different membranes and assess membrane damage. By varying membrane charge and lipid tail packing independently, we show that increasing membrane surface charge promotes penetration of PmB and membrane damage, whereas increasing lipid packing decreases penetration and damage. The penetration of the PmB molecules is well described by a phenomenological model that relates an attractive electrostatic and a repulsive force opposing insertion due to increased membrane packing. The model applies well to several gram-negative bacterial strains and may be used to predict bacterial resistance strength. Together, our results suggest a basic mechanism of bacterial resistance to polymyxins through minimizing membrane charge and increasing membrane toughness [1,2].
[1] Khondker et al., Communications Biology. 2019 Feb 18;2(1):67.
[2] Khondker et al., Biophysical journal. 2017 Nov 7;113(9):2016-28.

P13

Evidence Supporting Self-Assembly of UCP2 in the Membrane: Monomeric, Dimeric or Tetrameric?

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Uncoupling proteins (UCPs) are members of the mitochondrial carrier superfamily, and uncouple the electron transport process from ATP synthesis. Among the five human UCP homologs, UCP2 is known for its ubiquitous expression and potential role in ROS regulation. In this study, UCP2 is recombinantly expressed in E. coli bacterial strains and purified either as monomers or as a mixture of monomeric, dimeric and tetrameric molecular forms in mild detergents; and then reconstituted in phosphatidylcholine vesicles. Structure and proton transport function of UCP2 were analyzed by a combination of analytical and spectroscopic methods, such as circular dichroism and fluorescence spectroscopies, and gel electrophoresis. Protein structure, intermolecular interactions and self-association in membranes were further analyzed by molecular dynamics simulations. Regardless of its molecular form prior to reconstitution, UCP2 self-assembles and functions as an asymmetric tetramer in liposomes. Tetrameric UCP2 is a dimer of dimers with stabilizing salt-bridges between the monomeric units of each dimer. Neighboring lipids act as bridging agents to further stabilize the tetrameric structure of UCP2. The results of this experimental and computational study support the stability and functionality of oligomeric UCPs within the model biological membranes, which can have important implications in understanding a broader scope of the physiological function and regulation of these proteins.

P14

The effects of different fatty acids on phospholipid bilayers during necroptosis

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It was previously shown that saturated very long chain fatty acids (VLCFAs) accumulate during necroptosis, a form of regulated cell death, and that inactivating fatty acid biosynthesis reduced cell death during necroptosis. In present study, the effects of varying the levels of VLCFAs by inactivating ELOVL7 on the necroptotic membrane permeabilization and cell death is investigated. It is shown that increasing the levels of VLCFAs permeabilizes membranes. Similarly, introducing C24 FA disrupts membrane integrity in liposomes whereas C16 FA does not. Molecular dynamics simulations suggest clear differences between fatty acids and bilayers based on length. Specifically, the interdigitation of C24 FA between individual leaflets results in disorder in the region and, consequently, membrane disruption. Moreover, C16 and C24 FAs have different effects on the phospholipid acyl chains, with the latter significantly disturbing the bilayer. Furthermore, the interactions of cholesterol, which is known to have ordering effects, with C16 and C24 are also different. This is of a particular interest since balance between different types of interactions can balance molecules in membrane. Overall, the present results suggest very long chain fatty acids disturb membrane packing due to the mismatch in size with respect to abundant membrane phospholipids and that protein fatty acylation by these unusual very long chain fatty acids can mediate membrane recruitment.

P15

CryoEM structural investigation of two calcium ion channels: IP3R and Orai

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The element calcium flows across cellular membranes transducing information in diverse and flexible cellular signalling networks involving various calcium ion channels. The chemical messenger inositol 1,4,5-trisphosphate (IP3) binds to the receptor IP3R in the endoplasmic reticulum (ER) and calcium flows through through ion channel down a concentration gradient spanning three orders of magnitude. Subsequent additional calcium enters the cytosol from outside the cell through another membrane protein, Orai, mediated by the ER protein STIM. Recently investigators have been studying the role of calcium signalling proteins in cancer and immune cell activation, and we are interested to better understand their role from a structure-function perspective. Over the past several years single particle electron cryomicroscopy (cryoEM) has become a tractable technique for high resolution structural investigation of membrane proteins like IP3R and Orai. Comparing and contrasting IP3R and Orai with cryoEM is interesting because they represent relative extremes in size: a tetramer of 4 x 2758 amino acids, and a hexamer of 6 x 301, respectively. Here we summarize our ongoing efforts to solve their high resolution structures. Our long term goal is to visualize heterogeneous structural conformations of open and closed ion channels, their sufficiently populated transition states and thereby enable better design of specific channel inhibitors to ease the burden of human disease.

P16

Mechanisms by which pore lining mutations alter nicotinic acetylcholine receptor function leading to disease

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Mutations at the 9’ pore position in the nicotinic acetylcholine receptor (nAChR) potentiate channel function by unknown mechanisms and in some cases lead to disease. To understand underlying mechanisms, we first generated L9’S in each of the human α (2 per subunit), β, δ, and ε subunits, and tested using electrophysiology whether potentiation was independent and thus additive. L9’S in β and δ led 130- and 30-fold gains-in-channel function, respectively, but neither the α nor ε mutations expressed. The L9’T and L9’G mutations in each of the subunits gave roughly 10-fold and 50-fold potentiations, respectively. The L9’T mutation in four of the five subunits (α2δε) potentiates function by 1400- fold, a value close to that expected (~104-fold) if the mutations act independently, while the L9’G mutations in four sites (α2βε) yield only a 3000-fold potentiation, which is lower than expected (~6x106-fold). We detected large coupling energies (up to 15 kJmol-1) between Leu9’ residues on adjacent subunits, suggesting that these interactions stabilize the resting state and that their elimination contributes to potentiation. In contrast a loss of energetic couplings between the Leu9’ residues with the Thr mutation does not explain potentiation, suggesting that the polar Thr residues lead to novel interactions in the open state. Our preliminary data suggest that Thr and Gly residues at the 9’ position lead to potentiation of channel function through distinct mechanisms.

P17

Non-Additivity of Ligand Binding Affinities and Partial Agonism for Protein Kinase G (PKG)

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Protein kinase G (PKG) is a major protein involved in eukaryotic cyclic GMP (cGMP) dependent intracellular signaling, playing a regulatory role in such processes as cell differentiation, platelet activation, memory formation and vasodilation. Notably, the signaling pathways controlled by PKG are often distinct from those regulated by cyclic AMP (cAMP), and so the selective activation of PKG by cGMP rather than cAMP is critical. However, the mechanism of cGMP-vs.-cAMP selectivity in PKG is only limitedly understood. Previously, we showed that cAMP is a partial agonist for PKG, and we elucidated the mechanism of cAMP partial agonism through comparative NMR analysis of the apo, cGMP- and cAMP-bound forms of PKG CNB-B. In the current work, we assessed the contributions of two key cGMP-specific interactions to PKG CNB-B binding and activation, and addressed an apparent non-additivity observed among the contributions of the cGMP-specific interactions.

P18

Probing the Binding Mechanism of a Competitive Inhibitor of EPAC1, I942, using NMR Spectroscopy

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A novel partial agonist of the exchange protein activated by cAMP isoform 1 (EPAC1), I942, was recently discovered by Parnell et al. Although Parnell et al established that I942 reduces the GEF activity of cAMP-bound EPAC1 to approximately 10%, the binding mechanism between EPAC1 and I942 remains unknown. Here, we utilize NMR spectroscopy to probe the inhibitory I942 - EPAC1 interactions at atomic resolution. The EPAC1 - I942 interface was mapped through intermolecular NOEs by NOESY-HSQC with extensive 15N and 13C filtering in order to suppress signals from protein intramolecular NOE. Other NMR experiments, such as saturation transfer difference and chemical shift mapping, were also performed to identify the binding site of I942. It was discovered that I942 interacts with the phosphate binding cassette (BBR) and base binding region (PBC) of EPAC1- CNB, similar to cAMP, which is the endogenous allosteric effector for EPAC1. The PBC controls the conformation of the hinge region, and subsequently, allosterically shifts the hinge region between its active/inactive state. In addition, the R2 relaxation rates of 15N I942 bound EPAC1 were compared to those of the apo and cAMP-bound EPAC1 samples and an increase in the inhibitory ionic latch region compared to the inactive state was revealed, which explains why I942 partially activates EPAC1.

P19

Mapping the allosteric pathway leading from a pore-lining mutation in the nicotinic acetylcholine receptor to CMS

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The nicotinic acetylcholine receptor is a heteropentameric ligand-gated ion channel responsible for facilitating fast synaptic transmission. Mutations to the ion channel gate, comprising of L9' residues, have been shown to lead to a disease state. My project aims to address how pore-lining mutations change channel function by characterizing the change in residue interactions between L9' and surrounding residues in the wild-type and mutant receptors. Using electrophysiology, the effects of a leucine-to-serine mutation in the βL9' residue (βL9'S) on channel function were assessed and served as a model mutation. The largest degree of potentiation was observed when replacing βL9' with small, and polar residues, suggesting a destabilization of the closed state. Residues αS248 and αS252 were the closest residues to βL9' and therefore suspected of forming a stable interaction with βL9' in the closed state. The serine and leucine residues were mutated to Ala individually, and then simultaneously to remove potential interactions. Comparing the single to the simultaneous mutations, the single mutations were shown to be additive, revealing no interaction between βL9' and αS248/αS252, suggesting that βL9'S likely forms new interactions with one or more unknown residues in the open state. Other nearby residues are currently being assessed for potential interactions with βL9' in the open state.

P20

Understanding the Structural Basis of cAMP Signal Termination in PKA

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Protein kinase A (PKA) binds the cyclic 3'- 5' adenosine monophosphate (cAMP) for transducing downstream effects of hormones and neurotransmitters in mammalian cells. PKA includes two components, a catalytic (C) subunit and a regulatory (R) subunit spanning two homologous cAMP binding domains, which are known to bind cAMP with high affinity (KD ~ nM). The molecular mechanism underlying the activation of PKA by cAMP is well-understood. However, much less is known about how termination of the cAMP signal occurs in PKA. Though it is known that phosphodiesterases (PDEs) catalyze the hydrolysis of the 3'-5' phosphodiester bond in cAMP to generate 5'-AMP, signal termination through PDEs is expected to be kinetically limited by the very slow off-rate for the dissociation of cAMP from the PKA R subunit in the absence of PDE-PKA R interactions. Recently, the Anand group reported that the termination of cAMP signaling is initiated through formation of a PDE-PKA R subunit complex. To further investigate the PDE-PKA R subunit communication, we analyzed by NMR the RIa subunit of PKA in its agonist and antagonist-bound state in the presence and absence of PDE. The observed NMR changes suggest a direct and specific interaction between the PKA RIa subunit and PDE. The atomic resolution provided by NMR allowed us to analyze the residues perturbed by the PDE:PKA complex formation.

P21

Activation of G protein by the adenosine A2A receptor

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Thirty-four percent of current pharmaceuticals target G protein-coupled receptors (GPCRs), a class of membrane proteins that regulate diverse biological processes including sensory perception and immune response. Structurally, GPCRs adopt a dynamic ensemble of conformations spanning inactive, intermediate, and active states capable of engaging G proteins. The adenosine A2A receptor (A2AR) is a prototypical GPCR and a drug target for the treatment of inflammation, cancer, and Parkinson's disease. While X-ray crystal structures reveal only an inactive and an active conformation, nuclear magnetic resonance (NMR) spectroscopy show that detergent-reconstituted A2AR exhibits at least four functional states including two inactive and two active-like states. Similarly, a G protein exhibits at least three functional states characterized by nucleotide binding (GDP-bound, GTP-bound, and nucleotide- free). Here, we employ fluorine NMR to characterize the A2AR conformational ensemble as a function of drug binding and G protein coupling in synthetic lipid bilayers. We show that receptor activation occurs through biasing of its conformational landscape (by both agonist and G protein) to active-like conformations. Simultaneously, a change in the conformational landscape of G protein towards nucleotide-free form is induced and reinforced through receptor-G protein coupling. This knowledge furthers our understanding of GPCR signaling in the context of receptor-G protein allostery.

P22

Increased ubiquinone content confers respiratory protection to Escherichia coli under osmotic stress

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Ubiquinone 8 (Q8) is dimethoxymethylbenzoquinone linked to a side chain of eight isoprenoid units. Published work showed that Q8 accumulated in E. coli cultivated at high osmolality and thereby improved bacterial osmotic stress tolerance. Q8 was proposed to promote osmotolerance by enhancing membrane stability. Given that Q8 is a respiratory electron carrier, and that respiration is temporarily inhibited by osmotic stress, we hypothesized that increased levels of Q8 may contribute to E. coli osmotolerance by restoring aerobic respiration. To test this hypothesis, the effects of Q8 on bacterial growth, on respiration, and on the activities of the H+-symporters ProP and LacY were assessed in cells that were Q8 deficient due to a ubiG deletion, or had elevated Q8 levels due to growth at high osmolality. Q8 deficiency impaired bacterial growth, osmotolerance, respiration, and the activities of ProP and LacY. Diphenylhexatriene fluorescence anisotropy measurements showed that Q8 decreased the fluidity of liposomes prepared from E. coli lipid. However, Q8 had no effect on the osmotic activation of ProP in proteoliposomes, a system in which the protonmotive force can be imposed without respiration. These results indicate that elevated Q8 confers osmoprotection by stimulating aerobic respiration rather than by affecting membrane physical properties. This work provides the first evidence that the regulation of Q8 synthesis can afford respiratory protection under osmotic stress.

P23

Toward a structural model for osmosensing by transporter ProP of Escherichia coli

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Increasing osmotic pressure threatens cells with dehydration. Osmosensing transporters detect increasing osmotic pressure and respond by mediating organic solute uptake; they thereby prevent cellular dehydration. Data suggest that osmosensing transporter ProP of Escherichia coli activates as cellular dehydration elevates cytoplasmic cation concentration, modulating electrostatic interactions. The cytoplasmic C-terminal domains (CTDs) of adjacent ProP molecules form antiparallel, intermolecular alpha-helical coiled-coils in vivo and in vitro. Molecular dynamics simulations suggested that association of the monomeric ProP CTD with an E. coli membrane surface and homodimeric coiled-coil formation are mutually exclusive. We proposed that ProP activates as cellular dehydration increases cytoplasmic cation concentration, releasing the CTD from the membrane surface. To create a more realistic system, homology modeling was used to attach the CTD to Transmembrane Helix XII (TMXII) of ProP. No template was available for the loop connecting the CTD to TMXII, so unbiased and biased molecular dynamics simulations were used to examine the effect of this loop on peptide-peptide and peptide-lipid interactions. The dynamics of the ProP peptide/E. coli lipid systems, with and without TMXII, were compared at different salt concentrations. Our data identify relevant salt bridges and complement biochemical analyses of ProP sequence variants to further develop our structural model for osmosensing.

P24

Investigating the Mechanism of the cAMP-Dependent Signalling Pathway Termination in Type 1 Acrodysostosis

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Acrodysostosis is a skeletal disorder characterized by brachydactyly and facial dysostosis. Patients also have impairment in signalling pathways due to their resistance to multiple G protein-coupled receptor signalling hormones. Several point mutations in the PRKAR1A gene coding for the cAMP-dependent type 1-a regulatory subunit of protein kinase A (PKA RIa) were identified in Type 1 Acrodysostosis. These loss-of-function mutations impair the activation of the cAMP signalling pathway. However, the mechanism of this perturbation is still unknown. This project will investigate the R333L loss of function mutation in RIa in Type 1 Acrodysostosis, which is hypothesized to act as a reinforcer of PKA inhibition via attenuation of signal termination involving phosphodiesterases (PDEs). Nuclear Magnetic Resonance is used to explore the phosphodiesterase activity of both the free and RIa bound- state cAMP of the mutated PKA over time. 1H and 1H15N NMR are used to monitor the chemical reaction from the perspective of the cAMP ligand and the PKA protein, further characterizing the conformational changes and respective states at the structural level upon cAMP hydrolysis. The presence of R333L PKA unexpectedly accelerates hydrolysis of both free and cAMP-bound RIa. These results indicate that the R333L mutation in PKA elicits a long-range signal that alters PDE's activity and further suggests that the interaction between these two proteins is crucial in the signal termination mechanism.

P25

How Reactive are Druggable Cysteines in Protein Kinases? — A Computational Study

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The protein kinase family of signalling enzymes is a popular target for enzyme inhibition by therapeutic drugs, particularly for cancer treatment purposes. Traditionally, most drugs bind to their targets through non-covalent interactions like hydrogen bonding and dispersion forces. Recently, there has been renewed interest among drug developers and medicinal chemists to design drugs that bind covalently to their targets, since these drugs tend to be more therapeutically potent than conventional non-covalent binding drugs. Cysteine-targeting covalent inhibitors are a promising branch of kinase drug development that has the potential to increase potency and residence time. Cysteines reactivity towards a drug molecule depends on its acidity, a property quantified by a pKa value. Experimentally identifying a targetable cysteine residue and determining its pKa is a difficult task because of the need to express and purify the protein, and the presence of many ionizable residues. We have developed a computational method to predict the reactivity of cysteine residues in proteins based on their pKa's. We have used this method together with other rigorous computational approaches to predict the reactivity of selected druggable cysteines across the protein kinase family of popular drug targets.

P26

Investigating the structure and self-assembly potential of diverse class IB hydrophobins

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Hydrophobins are low molecular weight self-assembling proteins secreted by fungi that accumulate at hydrophobic-hydrophilic interfaces. Hydrophobins may undergo structural rearrangement and oligomerize to form rodlets, which are an insoluble functional amyloid that coats fungal spores to act as a water repellent, facilitate spore dispersal into the air, and prevent immune recognition. Due to their unusual biochemical properties hydrophobins have a range of potential commercial applications. To better understand which sequence characteristics determine hydrophobin properties, we have characterized the structure and properties of class IB hydrophobins from Serpula lacrymans (SL1), Wallemia ichthyophaga (WI1), and Phanerochaete carnosa (PC1). Uniformly $^{13}C/^{15}N$-labelled protein was expressed in E. coli and then purified to homogeneity using Ni^{2+} affinity and HPLC. The high- resolution structure of each hydrophobin was then determined using NMR spectroscopy. These hydrophobins all have a β-sheet that folds upon itself to form a β-barrel-like structure, however an intervening loop is disordered in WI1 while it is an α-helix in SL1 and PC1. Amyloid formation assays indicate that SL1, WI1, and PC1 have differing propensities to form rodlets and atomic force microscopy has provided a preliminary characterization of hydrophobin assemblies. Overall, this work establishes a fundamental correlation between the sequence, structure, and self-assembly properties of hydrophobins.

P27

Determination of the Structures of SUR NBD1/Drug Complexes with NMR

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ATP sensitive potassium (KATP) channels are found in a number of biological tissues. These channels are composed of four inward rectifying K+ (Kir6) channel proteins and four sulfonylurea receptors (SURs) surrounding the pore. Mutations in SUR proteins cause various diseases, such as diabetes, hyperinsulinism, epilepsy, and heart disease. The SUR proteins are members of the ATP binding cassette (ABC) family of proteins, and as such possess a core structure of two membrane-spanning domains and two cytoplasmic nucleotide binding domains (NBDs). ATP binding and hydrolysis at the SUR NBDs regulates gating of the Kir6 channel pore. The SUR NBDs are the sites of binding for KATP channel opener (KCO) drugs that are used to treat a variety of diseases associated with KATP channel function. The binding of the KCO pinacidil to SUR NBD1 was investigated using NMR spectroscopy of NBD1 in absence and presence of pinacidil. NMR drug titrations enable identification of specific NBD1 residues involved in drug binding, and the requirement of key NBD1 residues was verified by mutagenesis. The NMR titration data using wild type and mutant NBD1 proteins was used to calculate models of the NBD1/drug complex. These data will be used for optimization of pinacidil and other lead compounds into novel drugs.

P28

Substrate-based Allosteric Regulation of a Homodimeric Enzyme

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Many homodimeric enzymes exclusively require an empty protomer to accomplish catalysis. Here, the homodimer, fluoroacetate dehalogenase, utilizes a regulatory cap domain to prevent second substrate binding to the empty protomer. At high substrate concentrations, a second substrate binds instead to a site along the binding channel of the occupied protomer, inhibiting catalysis. While a mutation (K152I) abrogates second site binding and removes inhibitory effects, it also precipitously lowers the maximum catalytic rate, implying a role for the allosteric pocket at low substrate concentrations. We show that the allosteric pocket first desolvates the substrate, whereupon it is deposited in the active site. This triggers the empty pocket to serve as an inter-protomer allosteric conduit, enabling sampling of activation states needed for catalysis. These results illustrate the role of dynamics along allosteric networks in facilitating function.

P29

Fragment-based drug discovery for the oncogenic protein KRas

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Ras proteins are important signalling proteins that control crucial cellular processes like proliferation, cell survival and differentiation. Gain-of-function Ras mutations are found in ~30% of human cancers, most frequently in the KRas isoform at codon 12. An effective Ras inhibitor would, therefore, be beneficial to many cancer patients. However, despite numerous attempts, there is no effective KRas inhibitor available in the clinic yet. Ras has proven to be an extremely challenging drug target due to its lack of classical binding pockets. Here, we performed a virtual screen of 10,000 'fragments', i.e., small molecules about half the size of typical oral drugs, for their potential to bind the KRas mutant G12V. Promising hits from this in silico screen were then validated in vitro using nuclear magnetic resonance (NMR) spectroscopy and surface plasmon resonance (SPR) to map their binding sites and determine binding affinities.

P30

Structural and functional investigations of KH domains in cancer antigen helicases DDX43 and DDX53

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DDX43 and DDX53 are two cancer/testis antigens that are overexpressed in various tumors. Both belong to DEAD-box RNA helicase, and contain a KH domain in their N-terminal regions. Although the KH domain has been reported in many proteins, the function of the KH domain in helicases is not clear. In this project, we have purified DDX43-KH and DDX53-KH domain proteins using Ni-NTA column chromatography and size exclusion chromatography. Using electrophoretic mobility shift assay (EMSA), we found that DDX43 KH domain binds ssDNA and ssRNA substrates efficiently but not blunt-end dsDNA and dsRNA substrates. We performed 1H-15N HSQC NMR spectroscopy to determine the binding affinity of DDX43-KH protein with dT5, dA5, dC5, dG5 and rU5 oligonucleotides, and found that there are significant chemical shifts for pyrimidines (dT5, dC5, and rU5), but not for purines (dA5 and dG5). Titrations with dT10 and dT5 oligonucleotide revealed several residues, especially in the GXXG loop, are involved in protein-nucleic acids interactions. We have generated mutants A81I and G87D that are located in the GXXG loop to determine their role in nucleic acids binding. To further determine the specific nucleotides bound by the DDX43-KH and DDX53-KH domains, we have used two approaches: SELEX (Systematic evolution of ligands by exponential enrichment) and ChIP-seq (Chromatin Immunoprecipitation Sequencing) experiments. Currently, all libraries have been constructed and are ready for deep sequencing.

P31

High-throughput Investigation of Rhodopsin Function using Yeast

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G protein-coupled receptors (GPCRs) make up the largest family of druggable receptors in humans. To study the relationship between GPCR structure and function, we focus on rhodopsin, a light-activated cell surface receptor that is critical for initiating the first step in the visual transduction cascade. To study rhodopsin activation, we engineered a high-throughput platform for measuring GPCR function via a fluorescent readout by coupling human rhodopsin to the yeast mating pathway. Using flow cytometry, we validated our platform against 33 well-characterized rhodopsin mutants known to promote misfolding, are asymptomatic, affect receptor internalization, disrupt post-translational modifications or increase activation. Our results show that light-activated signal transduction in yeast correlates with published assays of rhodopsin function.
Collectively, we demonstrate that our yeast-based rhodopsin platform conforms to a variety of well-characterized mutations. Thus, providing a scalable platform for studying rhodopsin function in high-throughput using deep mutational scanning approaches.

P32

Is the Hydrophobic Gasket a Secondary Selectivity Filter in The Human Voltage Gated Proton Channel hHV1?

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A large family of membrane proteins, the voltage gated ion channels, regulate a vast array of physiological functions in essentially all life forms. The mechanism by which these molecules sense membrane potential and respond by creating ionic conduction is incompletely understood. These channels contain a ring of hydrophobic amino acids near the center of the voltage sensing domain in the membrane, the “hydrophobic gasket,” HG, which is anchored by a highly conserved Phe in nearly all voltage-dependent ion channels and phosphatases. Various functions of this structure have been suggested in addition to its role of separating internal and external aqueous solutions. Here we identify the HG in voltage- gated H+ channels and test the hypothesis that it functions as a secondary selectivity filter. Selectivity is ensured primarily by the interaction of D112 and one or more Arg in S4. However, molecular dynamics simulations indicate that cation exclusion may not be complete at the Asp-Arg selectivity filter. Potential of mean-force calculations show that the top of the free energy barrier opposing Na+ permeation coincides with the HG region. We replaced amino acids of the HG with less hydrophobic residues and measured reversal potentials at various pH and in the presence of Na+. All mutants tested were selective for H+ and did not conduct Na+. We conclude that proton selectivity is accomplished by the primary selectivity filter, comprising the interaction of Asp and Arg.

P33

All-atom molecular dynamics simulations of the Hco-ACC2 ion channel homodimer binding site

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The parasitic nematode Haemonchus contortus's increasing resistance to existing anti-parasitic drugs presents a growing threat to animal welfare and the economic viability of key cultural ruminants such as sheep in endemic areas. Haemonchus contortus's acetylcholine-gated ion channel (Hco-ACC2) is an attractive target for novel anti-parasitic drugs based on experimental results. However, the binding mechanism of this channel is not fully explored. In this study, we perform all-atom molecular dynamics simulations to investigate the Hco-ACC2 homodimer binding site with its known activator, acetylcholine, as well as two partial activators, nicotine and pyrantel. Our results indicate that hydrophobic interaction between the ligands and specific Trp and Tyr residues in the binding site play an important role in their binding. Additionally, hypothesized polar interactions with Glu or Trp may explain the increased activity of acetylcholine seen in experiments. These results provide hypothesized interactions that enhance our understanding of how different ligands interact with the Hco-ACC2 homodimer binding site to guide novel anti-parasitic drugs.

P34

Molecular Mechanism of Selective Cholesterol Uptake in Class B Scavenger Receptor LIMP-2

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Class B scavenger receptor proteins have a variety of functions in immunity and homeostasis, including key roles in preventing heart disease, currently the second leading cause of death for Canadians. One class B receptor, LIMP-2, is a proposed transporter of cholesterol from bound lipoproteins to the outer leaflet of the cell membrane and vice versa, and may therefore have a role in regulating cholesterol homeostasis. This transport by LIMP-2 is hypothesized to occur through the predominantly hydrophobic cavity that spans its luminal domain.
To test this hypothesis, we perform extensive atomistic molecular dynamics simulations of cholesterol and LIMP-2 in solution and in a simplified membrane mimetic. We first analyze dozens of independent, brute-force simulations to determine the spatial distribution and potential binding modes of cholesterol, suggesting possible pathways. We then compute the free energy profile of cholesterol movement through the cavity by using steered molecular dynamics and umbrella sampling. Our results provide insight into the structural and physical basis of cholesterol translocation through LIMP-2 and open the way to deciphering the molecular mechanisms for selective lipid transport in class B scavenger receptors, with implications in reducing heart disease risk.

P35

Role of Dynamics in the Autoinhibition and Activation of the P. Falciparum Protein Kinase G by Cyclic GMP

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The Plasmodium falciparum cGMP-dependent protein kinase (PfPKG) is essential in multiple steps of the Pplasmodium's whole life cycle who can causeand is a drug target for malaria. Among all four cGMP-binding domains (CBDs), CBD-D plays the most crucial role in PfPKG regulation, having similar activity as the entire structure. Although the apo versus holo conformational changes of CBD-D have been reported, no information is presented on the intermediates of the activation pathway. Here, we employed molecular dynamic simulations to model four key states along the thermodynamic cycle for the cGMP-dependent activation of the most necessary functional structure of the PfPKG , which is CBD-D domain, and the. The simulations were validated by NMR experimental data. The simulations and show that cGMP binding stabilizes the C- terminal lid region, forming hydrogen bond interactions with residues around it. In the absence of cGMP, the structure becomes more flexible, and the N- terminal region approach to the β-core, which displays a different behavior from the holo structure.

P36

Sequence-specific polymer theory for intrinsically disordered proteins in liquid-liquid phase separation

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Recently, liquid-liquid phase separation (LLPS) of biomolecules has attracted great scientific interest. In the cell, LLPS underpins the formation of various intracellular membraneless organelles, which are critical in many biological processes such as cellular integrity, homeostasis, gene regulation, and cell growth. Intrinsically disordered proteins (IDPs), proteins that do not fold into a stable tertiary structure in isolation because of their lack in hydrophobic but enrichment in polar, charged, and aromatic residues, play an important role in many LLPS phenomena. LLPS behavior of an IDP is often determined by not only its overall amino acid composition but also its sequence pattern. To understand the sequence specificity in biological LLPS, we develop a random-phase-approximation (RPA) theory. Our theory predicts that the LLPS of IDP Ddx4 is strongly influenced by its unique charge sequence and pi-electron interactions, which is consistent with experiments. We apply the theory to 30 model sequences that have the same residue composition but different charge patterns, and demonstrate a strong correlation between IDP LLPS and single-chain compactness. We also investigate solutions of two IDP species and find a "fuzzy" molecular recognition mechanism that determines whether two IDPs will coalesce or exclude each other based on the similarity of their charge patterns. Our theory provides a general framework for studying sequence-dependent phase separation of biomolecules.

P37

Dynamic Interactions Between a Disordered Protein and its Target at the Single-Molecule Level

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Cap-dependent initiation of translation is regulated by the interaction of the eukaryotic translation initiation factor 4E (eIF4E) with the 120-residue disordered eIF4E binding protein 2 (4E-BP2) in a phosphorylation-dependent manner. Previous NMR studies have shown that 4E-BP2 interacts with eIF4E at two distinct sites, via an α-helical structure at the canonical binding site and a disordered secondary binding site ~20 residues away. Phosphorylation of the 4E-BP2 at 5 distinct sites decreases its binding affinity for the eIF4E by ca. 4000 times, partially due to the formation of a 4-stranded β-sheet that sequesters the canonical binding site.
Single-molecule fluorescence resonance energy transfer (smFRET) studies of the 4E-BP2 between residues 32 and 91 show an increase in FRET efficiency upon binding to the eIF4E. This confirms that the 4E-BP2 adopts extended conformations in the bound state that wrap around eIF4E. Intermolecular smFRET with evanescent-field excitation was used to study the interaction between donor-labelled surface-immobilized eIF4E and acceptor-labelled free-diffusing 4E-BP2. FRET efficiency-time trajectories from thousands of individual molecules were used to derive the distribution of on- and off-binding times at the sub-ensemble level. Finally, the kinetic binding data is analyzed using several candidate models to gain an understanding of the physical mechanism of interaction in the 4E complex.

P38

Local Chain Dynamics of Intrinsically Disordered Sic1 From Fluorescence Anisotropy Decay Measurements

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As part of the cell cycle of the budding yeast Saccharomyces cerevisiae, the cyclin-dependent kinase inhibitor Sic1 is an intrinsically disordered protein (IDP) that is degraded by the Cell division control protein 4 (Cdc4) substrate of the SCF ubiquitin ligase complex upon multi-site phosphorylation. Previous studies have shown that the overall compaction of Sic1 is not altered significantly upon phosphorylation and/or binding. In fact, the Sic1 protein remains disordered upon binding and it is thought to form a “fuzzy” complex with Cdc4.
The internal chain dynamics of the disordered region of Sic1 were examined by fluorescence anisotropy decay (FAD) measurements using site-specific labelling with Alexa Fluor-488 at six different positions along the chain. Anisotropy decays for each labelled construct were fit with two rotational correlation times (~0.3 ns and ~2 ns), much faster than the tumbling rate of the whole protein. Conversely, these data provide a map of the dye/linker rotational freedom and the intrinsic backbone torsional mobility of separate regions in the Sic1 sequence. Differential quenching as measured by the fluorescence lifetime points to local/distant contacts between some of the labelling sites and the only aromatic residue in Sic1 (Y14). Going forward, chain dynamics data obtained from fluorescence anisotropy decays can be incorporated as an additional (local) constraint in ENSEMBLE to complement existing constraints from NMR (local) and SAXS (global).

P39

Liquid-liquid phase separation in Disordered Proteins: Effect of Interaction Potentials and Charge Pattern Parameters

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Recent experimental studies have revealed that membraneless organelles are multi-component viscous liquid droplets, assembled through weak, dynamic, multivalent interactions among biomolecules such as proteins and RNAs. Understanding their formation and physio-chemical properties is challenging due to the intrinsic complexity of cellular environment and heteropolymeric nature of the biomolecules. Extensive Langevin dynamic simulations have been performed with coarse-grain model to understand how phase separation propensity depends on the charge pattern of the amino acid sequences. Our studies indicate that liquid-liquid phase separation propensity for a previously-studied set of sequences correlates well with two well-known charge pattern parameters, the “blockiness measure” κ and the “sequence charge decoration” SCD. Moreover, quantitative analysis suggests that overall phase separation propensity is enhanced by background residue-residue attraction. However, for a novel set of sequences we designed to exhibit an anti- correlation between κ and $-$SCD, the simulated critical temperatures are quite insensitive to κ or SCD. In general, our results reveal both the utility and limitations of analytical theory as well as the charge pattern parameters, and point to several fruitful future directions in the development of theory and simulation for the phase behaviors of disordered proteins.

P40

Structural Studies Elucidating Substrate Transport and the Role of the Disordered Regulatory Region of Ycf1p

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The yeast cadmium factor 1 protein (Ycf1p) is an ATP binding cassette (ABC) protein that transports toxic metals from the yeast cytoplasm into the vacuole. ABC proteins contain a core ABC structure of two membrane spanning domains (MSD1 and MSD2) and two nucleotide binding domains (NBD1 and NBD2). Binding and hydrolysis of MgATP at the NBDs results in transport of glutathione-metal conjugates. Ycf1p also contains an additional membrane spanning domain (MSD0) that is connected to the ABC core by the cytoplasmic L0 linker.
As with other ABC transporters, Ycf1p is regulated by phosphorylation. Phosphorylation of the L0 inhibits transport, while phosphorylation of the regulatory region (RR) – which connects NBD1 and MSD2 - enhances transport. However, the molecular basis underlying the phosphorylation-dependent regulation of Ycf1p transport remains unclear. Recently, structure of the homologous protein MRP1, in substrate-loaded and unloaded states, was solved by electron cryo-microscopy (cryo-EM). Although, these studies provide information on the conformation of the substrate bound in the MSDs, the role of the disordered regulatory regions in transport remains unknown. I will present cryo-EM and NMR studies of Ycf1p that probe phosphorylation-dependent changes to Ycf1p structured and disordered regions. Together, these studies elucidate how phosphorylation of disordered segments leads to regulation of substrate transport in Ycf1p and other multi-drug resistant proteins.

P41

Investigating the role of G-quadruplex RNA binding on the specificity of phase separation

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Cells use phase separation to form membraneless organelles, also referred to as biomolecular condensates, as a way to organize many biological processes. Phase separation is driven by multivalent interactions often involving intrinsically disordered regions (IDRs) within RNA-binding proteins and RNA. Despite the growing appreciation of IDR and RNA interactions underlying phase separation, how specific RNA structures are targeted to distinct condensates is not fully understood. Here, I characterize the binding and phase separation properties of a model G-quadruplex forming RNA with the IDR of Fragile X Mental Retardation Protein (FMRPIDR), a characterized G-quadruplex binding protein region. Using a combination of biophysical tools including circular dichroism, isothermal calorimetry, and fluorescence resonance energy transfer experiments, I examine the biophysical properties of G-quadruplexes within FMRPIDR condensates. Together, results from this work suggest a possible role of stabilization of G-quadruplexes within FMRPIDR condensates. More broadly, since G-quadruplexes are involved in condensate trafficking and transcriptional and translational regulation, my work has implications for targeting of these structures for therapeutic purposes, such as in neurodevelopmental disorders.

P42

Artificial Neural Networks as a Tool to Describe Peptide Conformational Changes

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The amino acid sequence of a protein is believed to contain all information necessary to predict its three-dimensional structure. This prediction requires an understanding of the relationship between the conformation of peptides and their energy. However, accurate energy computations of most protein structures are impractical because of the enormous computational cost. To initiate protein folding studies based on quantum chemistry, proteins can instead be gradually built from smaller models like single amino acids and di- and tri-peptides. We prepared a dataset, including the structures and the corresponding energies of many conformations of all the amino acids and for different small peptides. We used quantum chemical methods to develop new multivariable mathematical functions and to train machine learning algorithms and predict the new conformations. Here we report the performance of ANN in fitting and interpolating over our data set. New geometry descriptor variables were developed and analysed for input into the ANN, and a more effective method was implemented for optimization. Our application of ANN will be used in future studies to compute, at a very modest computational cost, atomic forces in peptides and proteins. This method will allow us to simulate conformational changes and predict the conformational network for peptides or small proteins from amino acid sequences.

P43

MD Simulations of IDP Phosphorylation Parameters and Comparison to Experiment

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Phosphorylation of proteins can have a marked influence on protein structure, dynamics, and function. In addition to being functionally important, phosphorylation is common in naturally occurring proteins and thus a framework with which phosphorylated proteins can be studied is valuable for studies of protein dynamics. Currently, it is not known how well molecular dynamics force field parameters for phosphorylated intrinsically disordered proteins agree with experimental measurements. Serine is the most commonly phosphorylated residue (at 90% prevalence) with the other 10% occurring on tyrosine and threonine. Thus, the addition of correctly parametrized phosphoserine to a current force field would provide a framework for most computational studies of phosphorylated proteins. To this end, we add phosphoserine to a current force field, and test the predictions of these parameters against experimental measurements. Preliminary results show that it is difficult to obtain adequate sampling for our test system – a 24 residue intrinsically disordered protein – and enhanced sampling techniques are required. Once adequate sampling is acquired, phosphoserine parameters may need to be tuned to improve agreement between experimental measurements and simulated data. This will allow for accurate numerical studies of phosphorylated intrinsically disordered proteins.

P44

Hamiltonian Replica Exchange for Enhanced Sampling of the Conformational Landscape of Intrinsically Disordered Proteins

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Intrinsically disordered proteins (IDPs) are a class of proteins that lack a unique native three-dimensional structure. Molecular dynamics (MD) is an invaluable method to study the dynamics of folded proteins. However, there have been challenges in its application to simulating IDPs. One of these challenges is the need to sample a vast number of structural conformations. To help overcome this challenge, one of the methods that were introduced is the temperature replica exchange (TREX) algorithm. This method uses a random walk in temperature space to help overcome energy barriers. However, this method can require vast computing resources and the efficiency of the TREX algorithm is not well characterized. We use a variant of Hamiltonian replica exchange (HREX), which instead of temperature uses the scaling of the Lennard-Jones protein-water interactions. In conducting the algorithm in this manner, the computational requirements are reduced compared to TREX. Here, we used MD simulations on an IDP system to compare the sampling efficiency between these sampling algorithms. We tested this algorithm on the RS1 region of the serine/arginine-rich splicing factor 1; an IDP with many consecutive repeating RS residues.

P45

Fragment-based Identification of Functional Hot Spots in Enzymes

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A rational method to create substrate- specific binding sites and tunnels in enzymes would be an invaluable tool for the design of new biocatalysts. Using only experimental screening methods, researchers typically must screen several thousand to millions of mutants to engineer a new substrate tunnel in an enzyme. It is possible to use computational methods to relieve the experimental burden of engineering tunnels. Mutants will be predicted according to their ability to accept a new substrate and the most likely candidates will be screened first; computational methods could reduce the experimental requirement to screening between one hundred and one thousand mutants. By using cytochrome p450 BM3 as our model, BM3 is an industrially attractive enzyme with a promiscuous active site, implicit ligand sampling was used to screen for hot spots by measuring the binding affinity of oxygen. Biased molecular dynamics simulations were used to find energy barriers impeding imidazole to refine the search for hot spots.

P46

Combining simulation and experiment to achieve a detailed analysis of structure of elastin-like peptides

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Elastin confers elasticity to various tissues. Tropoelastin, the monomer of elastin, has alternating hydrophobic and cross-linking domains. The hydrophobic domains are disordered and confer self-assembly and elasticity of elastin. The cross-linking domains contain lysine for cross-linking tropoelastin, imparting stability to elastin. Elastin-like peptides (ELPs) are intrinsically disordered proteins (IDPs) and adopt an ensemble of conformations including Î±-helices, beta-turns, beta-strands, and random coils.1,2 To tackle the structural heterogeneity of ELPs, we combine molecular dynamics (MD) simulations, NMR, and small-angle X-ray scattering (SAXS) to achieve a detailed analysis of structure of an ELP with alternating hydrophobic and cross-linking domains. Atomistic simulations in explicit water yield a detailed description of the structural ensemble of monomers. Comparing experimental observables from simulations to experiments gives excellent cross-validation of simulation and NMR data, consistently showing that the cross-linking domains form helices, while the hydrophobic domains form sparse and transient hydrogen bonded turns. Our results underline the complementarity of MD, NMR, and SAXS methods in the study of IDPs and ELPs. The ability of MD simulations to generate a high-resolution ensemble allows more precise determination of structural propensities from NMR observables, while discrepancies between computed and measured SAXS data will inform future force field refinement.

P47

Role of π-π Interactions in Liquid-Liquid Phase Separation

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Protein liquid-liquid phase separation (LLPS) is an important feature of cellular function that involves self-association of proteins into biomolecular condensates. Many proteins that undergo LLPS contain low complexity intrinsically disordered regions (IDRs) that are enriched in π-orbital-containing groups. Data mining of the protein data bank (PDB) showed that planar π-π interactions occur more frequently in highly solvated regions not involved in regular secondary structure, pointing to the likely importance of π-π interactions in IDRs. Further, many phase-separating proteins can be identified based on predicted long-range π-π contacts, suggesting that π-π interactions play a significant role in phase separation (Vernon et al., Elife 2018). Despite these findings, the energetic properties of π-π interactions of IDRs that enable phase separation are poorly understood. To better understand the structural and physico-chemical basis of LLPS of IDRs, we investigate π-π interactions between amino acid residues.
We extract statistical information from the PDB regarding the relative positions and orientations of pairs of amino acids. Next, we conduct quantum chemical calculations to characterize the energies of π-π interactions between residues. Linking calculations to our statistical data will provide insights into the contribution of π-π stacking to intra- and inter-molecular interactions of proteins, enabling a quantitative description of IDRs and their involvement in LLPS.

P48

Using Simulations to Understand the Self Assembly and Mechanical Properties of Elastin-like Peptides

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Elastin belongs to an important but poorly-understood class of structural proteins that impart extensibility and elastic recoil to major arteries, skin, and other elastic human tissues. Its monomeric precursor, tropoelastin, is intrinsically disordered. Although it is highly hydrophobic, it is soluble at room temperature and undergoes liquid-liquid phase separation upon temperature increase. Some elastin-derived peptides are able to coacervate while others form amyloid-like fibrils. However, this fibril formation is impeded by depositing the amyloid- like peptides in methanol. In this study, we use molecular dynamics simulations to uncover the structural and chemical basis for the self-assembly of elastin-derived peptides into liquid-like or amyloid-like aggregates. To this end, we examine the structural ensemble of a set of model elastin-derived peptides in explicit water and methanol. Results obtained with three force fields suggest that compared to water, methanol reduces the burial of nonpolar groups in all peptides, promotes β-sheet formation in amyloid-like peptides, and lowers the elastic modulus of elastin-like peptides. The latter result is confirmed by tensile mechanical measurements of cross-linked elastin materials. Together, these findings provide an explanation as to why methanol prevents fibril formation in amyloid-like peptides and indicate that the hydrophobic effect is a significant driving force for the elastic recoil of elastin.

P49

Observing Single Molecule DNA Transcription and Protein-DNA interactions in-vitro Using Tethered Particle Motion

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The regulation of genes acquired through horizontal gene-transfer plays an important role in the spread of antibiotic resistance in bacteria. However, transcriptional regulation of horizontally acquired genes by DNA associated proteins like H-NS remain little understood. We investigate the molecular mechanism of transcription and gene silencing by DNA associated proteins in-vitro at a single molecular level using Tethered Particle Motion (TPM). Utilizing 3rd generation click-chemistry we have established a TPM platform that is remarkably stable, easily prepared, adjustable, self-contained, inherently anti-fouling and highly parallelized. Over 100 particles can be observed simultaneously using a microscope over a time span of 12 hours, each representing an individual single molecule system allowing for highly efficient experimentation. In our transcription experiments each reporter particle is anchored to a substrate using a 500 bp – 5 kbp DNA tether. So called gene DNA strands are attached to the particle, containing a promotor sequence, the gene to be transcribed and an optional termination site. T7 RNA Polymerase is attached to the substrate and repeatedly transcribes the DNA gene sequence attached to the particles. We determine the transcriptional dynamics from the motion of the reporter particle. We report our progress on in-vitro DNA transcription and the effect of DNA associated proteins on transcription, and discuss the investigative avenue.

P50

Vibrational Circular Dichroism Reveals Supramolecular Chirality Inversion of α-Synuclein Peptide Assemblies

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Parkinson's disease is an incurable neurodegenerative disorder caused by the aggregation of α-synuclein (AS). This amyloid protein contains a 12-residue-long segment, AS71−82, that triggers AS pathological aggregation. This peptide is then essential to better understand the polymorphism and the dynamics of formation of AS fibrillar structures. In this work, vibrational circular dichroism showed that AS71−82 is random coil in solution and forms parallel β-sheet fibrillar aggregates in the presence of anionic vesicles. Vibrational circular dichroism, with transmission electronic microscopy, revealed that the fibrillar structures exhibit a nanoscale tape-like morphology with a preferential supramolecular helicity. Whereas the structure handedness of some other amyloid peptides has been shown to be driven by pH, that of AS71−82 is controlled by peptide concentration and peptide-to-lipid (P:L) molar ratio. At low concentrations and low P:L molar ratios, AS71−82 assemblies have a left-twisted handedness, whereas at high concentrations and high P:L ratios, a right-twisted handedness is adopted. Left-twisted assemblies interconvert into right-twisted ones with time, suggesting a maturation of the amyloid structures. As fibril species with two chiralities have also been reported previously in Parkinson's disease Lewy bodies and fibrils, the present results seem relevant to better understand AS amyloid assembly and fibrillization in vivo.

P51

Systematic Coarse-Graining Method for Molecular Simulations using Relative Entropy

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Computer simulations have become a powerful tool for studying the structure, dynamics, or other characteristics of a wide variety of physical systems. The goal of coarse-grained (CG) models is to simplify the representation of the physical system while still maintaining enough information to capture the desired properties of the system. A main challenge in the development of CG models is determining the interaction potential, which often depends on a large number of unknown model parameters. Different methods for determining these model parameters have been proposed, (potential of mean force, multi-scale coarse-graining), but they rely on determining quantities that are computationally difficult to calculate, such as the free energy. Here we develop a systematic method to determine the optimal parameters for coarse-grained models of molecular systems. The method is based on generalized ensemble simulations in which the model parameters are dynamic, meaning they are allowed to change under a Monte Carlo update criteria. These simulations allow for the calculation of the relative entropy, which is used as a metric to compare a CG ensemble with a target ensemble. The relative entropy was then minimized to obtain the parameter set for the optimal CG ensemble. The novel systematic method was applied to a CG model for protein folding to determine the optimal model parameters that allowed a protein to fold to its native structure.

P52

Cryo-EM study of transient protein interactions in fungal type I FAS catalysis

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Fatty acid synthase (FAS) catalyzes de novo synthesis of fatty acids through a series of iterative chemical reactions, which is relevant for antifungal, biofuel and disease applications. S. cerevisiae has a Type I FAS complex: two genes encode proteins that assemble into a macromolecular machine with 48 catalytic domains and 6 tethered acyl carrier protein (ACP) domains which bind and shuttle fatty acid intermediates to active sites. X-ray crystallography and electron cryo-microscopy (cryo-EM) structures show that the 2.6 MDa heterododecameric FAS complex in S. cerevisiae forms a barrel bisected into two chambers by a central disc, each containing 3 complete sets of reaction centers. However, the transient ACP interactions that are necessary for S. cerevisiae FAS catalysis are poorly understood. We aim to use cryo-EM, a powerful technique capable of resolving high-resolution structures of proteins in solution, to study the shuttling mechanism of fungal FAS. We have purified endogenous FAS from S. cerevisiae and the pathogenic yeast C. albicans and show that the purified enzymes are pure, catalytically active and sensitive to an inhibitor. Cryo-EM analysis of the apoenzyme and inhibited enzyme reveal striking differences in the localization of the mobile ACP, suggesting an effect of substrates on the ACP interaction landscape. These findings may have implications in efforts to utilize S. cerevisiae for biofuel production, as well as design of novel inhibitors of fungal FAS.

P53

TEM and SAXS Reveal Structural Similarities Between Lipid-Free and Lipid-Bound Serum Amyloid A

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Serum amyloid A (SAA) is a highly conserved apolipoprotein that binds high-density lipoprotein (HDL) in plasma by displacing apolipoprotein A-I (Apo A-I) from HDL particles during the inflammatory response. In acute or short-term inflammation, SAA is proposed to play several 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 and can result in systemic amyloidosis caused by SAA misfolding and aggregation. While structures for lipid-free, or apo-, forms of SAA have been reported, their relationships with the HDL-bound form of the protein, and with the mechanism of SAA binding to lipids, have not been established. We have used multiple biophysical techniques, including SAXS, TEM, SEC-MALS, and native gel electrophoresis, to characterize the lipid-free and lipid-bound forms of human 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. These SAA octamers are capable of scaffolding lipid nanodiscs with similar morphology to those formed by Apo A-I, and with similar dimensions as the lipid-free SAA, suggesting that relatively few conformational rearrangements may be required to allow SAA interactions with lipid-containing particles such as HDL.

P54

Can the RNA World still function without cytidine?

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Most scenarios for the origin of life assume that RNA played a key role in both catalysis and information storage. The A, U, G, and C nucleobases in modern RNA all participate in secondary structure formation and replication. However, the rapid deamination of C to U and the absence of C in meteorite samples suggest that prebiotic RNA may have been deficient in cytosine. Here we assess the ability of RNA sequences formed from a three-letter AUG alphabet to perform both structural and genetic roles in comparison to sequences formed from the AUGC alphabet. Despite forming less thermodynamically stable helices, the AUG alphabet can find a broad range of structures and thus appears sufficient for catalysis in the RNA world. However, in the AUG case, longer sequences are required to form structures with an equivalent complexity. Replication in the AUG alphabet requires GU pairing. Sequence fidelity in the AUG alphabet is low whenever G's are present in the sequence. We find that AUG sequences evolve to AU sequences if GU pairing is rare, and to RU sequences if GU pairing is common (R denotes A or G). It is not possible to conserve a G at a specific site in either case. These problems do not rule out the possibility of an RNA World based on AUG, but they show that it would be significantly more difficult than with a four-base alphabet.

P55

Induced memory effects in single-molecule force spectroscopy measurements of biomolecular folding

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In single-molecule force spectroscopy (SMFS) experiments, force is applied by a probe to a molecule to cause it to unfold. The observable is not the motion molecule of interest itself, but rather that of the force probe tethered to the molecule. Although biomolecular folding is generally assumed to be memoryless, existing theory predicts that coupling the molecule to a force probe should induce memory in the overall system. By explicitly accounting for the effect of the apparatus in optical tweezer measurements, we developed a method to quantify the induced memory by analyzing its effect on the position autocorrelation function, which can be readily obtained in SMFS experiments. We show that the timescale of induced memory is dependent on the apparatus, as expected, but also on the intrinsic molecular parameters. By applying this method to Brownian dynamics simulations and experimental extension measurements of DNA hairpins, we determine the timescale of the induced memory, validating the prediction that the experimental apparatus induces memory in the overall system. Thus, memory effects are a general feature of SMFS measurements, arising from the mechanical connection between the molecule and the force probe. Since the presence of memory convolves intrinsic molecular parameters with the properties of the measurement apparatus, induced memory in SMFS experiments should be considered when interpreting experimental data.

P56

Effective screening platform of α-synuclein monomers in solution using computational and experimental methods

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Aggregates of misfolded α-synuclein, a disordered protein, are a distinctive feature of Parkinson's disease. Misfolded α-synuclein is neurotoxic, but small oligomeric forms may be the most important for disease, enhancing toxicity and acting as seeds to accelerate and propagate aggregation. Attempts to find pharmacological chaperones to inhibit oligomer formation have been mostly unsuccessful, in part because the methods used to screen drug candidates are less effective than needed for probing the early stages of aggregation. Here we present a platform using a combination of computational and experimental methods to screen for effects on the earliest stages of aggregation, near the single-molecule level. Simulations of α-synuclein dimer structures (as minimal oligomers) were used to build a pharmacophore model for high-throughput screening of the ZINC library to find oligomerization inhibitors. The 26 highest-scoring compounds that were commercially available were then tested in vitro, using fluorescence cross-correlation spectroscopy to assay the effects of ligands on oligomer nucleation and ThT fluorescence to assay their effects on fibril formation and growth. We identified several compounds that delayed nucleation, inhibited fibrils, or acted on both early and late stages of aggregation. These results demonstrate the viability of integrating computation with single-molecule and ensemble assays as a platform to discover aggregation inhibitors.

P57

Multi-scale ligand-induced dynamics in the A2A Adenosine Receptor

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G-protein-coupled receptors (GPCRs) are the largest class of transmembrane proteins, making them an attractive target for therapeutics. At the heart of drug discovery is understanding the structural implications that ligands pose on the activation states of receptors. Two models are typically used to explain the basis of ligand-activation: induced-fit and conformational selection. Recent evidence incline towards the second model, suggesting the coexistence of multiple receptor states, which, depending on the nature of the ligand bound (full, partial or inverse agonist), will be preferentially populated. We labelled the A2A adenosine receptor (A2A) with a small dye (BODIPY-FL) to probe transmembrane reorientation dynamics between helixes TM5 and TM6. Fluorescence Correlation Spectroscopy measurements were performed on A2A under various ligand and cation conditions. Global data analysis shows that the basal state of A2A can be described as a superposition of three dynamic conformations. The population and switching kinetics of these states is modulated by binding of full, partial, and inverse agonist or cations (Na+or Mg2+) to A2A. Single-molecule Förster resonance energy transfer (smFRET) monitored distance changes between these states when bound to different ligands and provided a means to monitor reorientation of TM6. This single-molecule study provides new information on the multi-scale kinetics of transmembrane motions in GPCRs, which is relevant for activation processes.

P58

Versatile Tools Towards Real-Time Single-Molecule Biology

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LUMICKS USA Inc.

Biological processes performed by proteins interacting with and processing DNA and RNA, or protein complexes walking along microtubules for intracellular transport, are key to cell metabolism and life. Detailed insights into these processes provide essential information for understanding the molecular basis of life and the pathological conditions that develop when such processes go awry. Single-molecule technologies offer an exciting opportunity to simultaneously study protein function, activity and structures in real-time. Here, we present our efforts for further enabling discoveries in the field of biology and biophysics. The C-Trap technology integrates optical tweezers, confocal/STED microscopy, and an advanced microfluidics system enabling live-correlative visualization and manipulation of molecular interactions with sub-piconewton force resolution and a kilo- to megahertz temporal resolution. The setup can also be integrated with label-free IRM (Interference Reflection Microscopy) and TIRF microscopy to visualize surface assays such as microtubule and motor-protein interactions. We show how these technologies allow for the measurement of conformational changes of proteins folding and unfolding; DNA-protein interactions and genome modifications; effects of mechanical stress on DNA/RNA structure; motility of cytoskeletal molecular motors; protein droplet and aggregation dynamics; cell receptor force activation; as well as various other intracellular dynamic processes.

P59

Defining Conformational States of Proteins Using Dimensionality Reduction and Clustering Algorithms

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Molecular dynamics (MD) simulations of proteins produce large data sets - long trajectories of atomic coordinates - and provide a representation of the sampling of a given molecule's structural ensemble. A deep quantitative analysis using advanced machine learning techniques is a means to interpret MD trajectories. To visualize the conformational space of the molecule and properly identify conformational states, we suggest combining clustering methods and dimensionality reduction algorithms. We investigate different choices of features to represent individual structures, clustering algorithms, similarity metrics, and methods to assign the number of clusters.

P60

Uncovering the Molecular Basis for the Clinical N642H Mutation in STAT5B Using Atomistic Molecular Simulations

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The cytosolic protein STAT5 (signal transducer and activator of transcription 5) is important in cell proliferation and differentiation. Hyperactivation of STAT5 occurs in leukemia and lymphoma; a mutation within STAT5 has been linked to increased drug resistance in patients with leukemia. Despite the importance of STAT5 as a therapeutic target, drug design efforts have been limited due to a lack of structural and dynamic information. Here, we use atomistic molecular dynamics simulations to elucidate the dynamics of the wild type and oncogenic N642H mutant of human STAT5B dimers, and to provide a molecular basis for the increased oncogenicity of STAT5B N642H. In all of our tested systems, the wild type STAT5B dimer exhibited marked instability and dissociated rapidly. In contrast, STAT5B N642H dimer remained intact during 1 *μ*s-long simulations. The STAT5B N642H dimer was observed to be highly flexible, populating different dimer interfaces. Additional inter-chain contacts formed in the STAT5B N642H compared to the wild type may account for the enhanced dimer stability of STAT5B N642H. Notably, these contacts are not close to the N642H mutation site, suggesting a possible allosteric communication pathway between the interface domains and the SH2 domain bearing the N642H mutation. Ongoing structural and dynamic analysis will reveal the intra and interchain contacts that provide enhanced stability to the oncogenic STAT5B N642H dimer.

P61

Single-Molecule Counting and Stoichiometric Analysis using Super-Resolution Localization Microscopy

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Single-molecule localization microscopy (SMLM) revolutionized the field of biophysics by surpassing the diffraction-limit of fluorescence microscopy by an order of magnitude. Since its invention there has been increasing interest and effort to develop quantitative tools which capitalize on the large amount of information available in a typical SMLM experiment. One major field of interest is the molecular counting problem, which aims to accurately and precisely determine the copy number of proteins and nucleic acids in cells as well as the stoichiometry of membrane proteins. This has wide-ranging applications in systems biology, proteomics/genomics, and fundamental cell biology. We have developed a technique based on the blinking statistics of organic dyes and fluorescent proteins typically used in SMLM which can infer the copy number or the stoichiometry of molecules based on the total number of fluorophore blinks detected. We show how our technique may be applied using an in vitro DNA origami structure to model a molecule of interest. As a potential application, we've begun to apply this technique to the challenging problem of accurately determining plasmid copy number in individual bacteria.

P62

Optical biosensors engineered for imaging of monovalent copper ion in mammalian cell culture

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This poster set out to present a toolbox for imaging cellular monovalent copper ion — a family of optical biosensors engineered with a red fluorescent protein backbone. In this family of red fluorescent Cu1+ biosensor, a circularly permuted red fluorescent protein is grafted into Cu1+ binding protein AmtI to form a chimera red fluorescent protein. Three sites were chosen in order to screen for the optimal construct. It was envisioned that the restoration of Amt1 structure upon the binding of Cu1+ would influence cpRFP's barrel and in turn change its fluorescence. After the mass production of those three novel RFP, they were characterized for their conformational and optical response to the presence of different concentration of Cu1+. With the increasing of the molar ratio of Cu1+ over sensor, the secondary structure of those novel RFPs gradually disappeared as indicated by circular dichroism, resulting in the corresponding decreasing of its emission intensity. And this change of fluorescence reached its maximum when Cu1+ saturated, all of which point to the fluorescent readout being an indicator for the Cu1+ presence. The affinity of those sensors to Cu1+ is roughly hundreds of zeptomolar concerning dissociation constant as determined from test carried out in vitro. Those sensors possessed minimal response to other metal ions. Demonstrating the sensors' quality specificity as well as sensitivity, paving the way for their cellular application, which was also shown here.

P63

Dynamic Imaging of Biological Specimens Using Wide-Field Second Harmonic Generation Microscopy

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In this work, we report a wide-field second harmonic generation (SHG) microscope, suitable for fast imaging of live samples and large area imaging of histopathology sections. High peak power lasers are required to obtain SHG contrast in a wide-field microscope. For this purpose, we use a high power, high repetition rate, SESAM mode-locked Yb:KGW oscillator, with 12 W average power and 400 fs pulse duration.
Here we exhibit imaging of histology sections and live recording of moving Drosophila melanogaster larvae. It is demonstrated that high spatial resolution wide-field SHG microscopy clearly distinguishes anisotropic A-bands and isotropic I-bands in the striated muscle structure, allowing accurate determination of the sarcomere length and SHG intensity from the individual sarcomeres. The wide-field imaging of biological tissue was achieved with optimal 4 W of laser power at the sample over 450x450 μm area.

P64

Organization and Composition of Collagen in Biological Tissue Revealed With Polarimetric Nonlinear Microscopy

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Polarimetric second-harmonic generation (P-SHG) microscopy enables label-free imaging of non-centrosymmetric structures in collagenous and muscular biological tissues. P-SHG provides detailed information regarding the structural properties of the underlying sample, in the form of second order nonlinear susceptibility element ratios.
Imaging of the sample is conducted with a home-built Yb:KGW laser at 1028nm, with 400 fs pulses. Measurement of beam polarization before and after the interaction of laser light with the sample enables the extraction of two important second order nonlinear susceptibility element ratios, C-ratio (Xxyz/Xzxx) and R-ratio (Xzzz/Xzxx), which are highly sensitive to the organization of the imaged material. In addition, in-plane fiber orientations are used to fully characterize the ultrastructural organization of the tissue components. It is shown that R-ratio ranges from 0.5 to 3.0 in muscle fibers, as compared to 1.5 to 3.0 in collagen, illustrating a clear difference between the tissue types. C-ratio which appears due to the chiral nature of collagen, is used to study out-of-plane fiber orientation and collagen polarity. P-SHG microscopy allows for precise investigations of subtle structural variations in the biological tissues, with applications reaching beyond SHG image intensity-dependent tissue characterization. It has been shown that P-SHG can be used in histopathology, offering great potential for advanced biomedical diagnostics.

P65

Examination of Drosophila Musculature using Second Harmonic Generation Microscopy

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Nonlinear optical microscopy has been shown to be a superior imaging modality where imaging can be done without prior staining, providing structural and functional information in a biological system. SHG Microscopy as a parametric process, also has the added benefit of having a significantly reduced photo-bleaching effect. SHG is observed in non-centrosymmetric cylindrical molecules like myosin and can be used to study tissue development and physiology in a non-invasive manner. The focus of this project is to use SHG microscopy and polarimetric techniques to obtain quantitative information on the biological structure by measuring its SHG response at different incoming and outgoing polarizations. Drosophila muscles were used since its structure and physiology is conserved in vertebrates. We found that the SHG response was affected by sarcomere size. Second order susceptibility values were found to change whether the muscle was in a rigor or relaxed state. Changes in the SHG response were compared in various types muscles to observe changes in SHG response due to myofibril organization. We also use polarimetric SHG microscopy to study changes in myosin accumulation by inhibiting the expression of myosin chaperones. This provides insight on how second harmonic properties changes with myosin filament structure. This opens new perspective on the dynamic properties of contraction.

P66

Exploring the Mechanical and Structural Properties of Recombinant Spider Pyriform Silk

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Spider silks are biomaterials mechanically comparable to high strength steel and Kevlar, and used for many diverse adaptations by spiders. Orb weaving spiders produce up to seven distinct types of silk, including pyriform silk. In nature, pyriform silk is spun as part of a glue-coated attachment disc, connecting web silks to disparate materials. Even with its critical role in web formation, neither the structural nor mechanical properties of pyriform silk have been widely investigated. By engineering recombinant proteins based on the Argiope argentata pyriform silk repetitive domain, we have shown that recombinant pyriform silk fragments are both strong and extensible, in contrast to mechanical extremes seen in most silks. To develop the structure-function relationship for this silk, we have initiated solution-state structural studies for this recombinant silk protein. Circular dichroism spectroscopy implies a predominantly α-helical structure in solution, with differing number of repeat units showing minimal line shape changes, indicating minimal changes in total structural content upon addition of repetitive units. NMR analysis has also implied the presence of distinct regions of order and disorder in the repetitive unit, based upon degrees of resonance dispersion correlated to the sign of 1H-15N nuclear Overhauser effect enhancements, consistent with a sequence that has segregated Gln-rich (likely structured) and Pro- rich (likely disordered or polyproline-II) regions.

P67

Collagen contraction induced by annulus fibrosus cells of the intervertebral disc

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Intervertebral disc (IVD) degeneration is one of the main causes of low back pain; a chronic condition affecting up to 80% of the global population. IVDs have a heterogeneous structure comprised of two main regions: a central nucleus pulposus (primarily type II collagen) and surrounding annulus fibrosus (AF; primarily type I collagen). AF cells have a fibroblast morphology and can attach to collagen fibres. There is limited knowledge available on the mechanobiology of these cells and how this may affect IVD mechanical integrity. In this study, AF cells (live and fixed) from the IVD of Sprague Dawley rats were cultured within a type I collagen matrix (3 mg/ml); images of the matrices were taken at 24h intervals post culture and processed by ImageJ. Uniaxial tensile testing was also performed to quantify the mechanical properties. Live AF cells were able to induce a robust contraction on collagen as early as 24 hours and contracted up to 40% of the initial size; fixed cells did not induce collagen contraction. This contraction is likely due to attachment of AF cells to the collagen fibres. Upon attachment, cells form focal adhesions and apply forces that can collectively induce a noticeable deformation on the matrix. Tensile mechanical testing revealed that constructs contracted by live cells are mechanically stronger than fixed-cell constructs. This work highlights the mechanical interaction between AF cells and their surrounding extracellular matrix.

P68

Dendritic Morphology and Mechanical Modulus of Soft Phytoglycogen Nanoparticles Revealed By AFM Force Spectroscopy

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Phytoglycogen is a naturally occurring glucose polymer that is produced in the form of soft, highly-branched, compact nanoparticles by sweet corn. Because of their deformability, unique hydration and safety profile, they have promising applications in personal care, nutrition and biomedicine. We have used atomic force microscopy (AFM) to measure the morphology and mechanical properties of phytoglycogen nanoparticles. By successfully immobilizing the phytoglycogen nanoparticles on the terraces of annealed gold substrates by using a thiolated boronic acid-based self-assembled monolayer, we have been able to measure the properties of the fully hydrated phytoglycogen nanoparticles in water. To overcome the challenges of measuring these soft and deformable particles, we used the Quantitative Imaging mode of our JPK AFM, which is an optimized mapping of many force-distance curves. This allowed us to obtain high resolution measurements of the particle morphology and stiffness, while minimizing the lateral forces exerted on the delicate particles. By measuring the sample height at different applied forces, we observed the inner dendrimeric structure of the particle. By analyzing the indentation portion of the force-distance curves, we determined the distribution of mechanical modulus values within the particles. I will also quantify changes in the particle morphology, volume and mechanical modulus when the particles are removed from water and measured in air.

P69

Effect of spinning rate on the molecular structure and dynamics in native and supercontracted spider silk

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Spider dragline silk is a strong, extensible and mechanically resistant proteinaceous fiber. It is particularly attractive for various fields of applications. The structure of silk is dictated by the primary structure of the spidroins and by the spinning process, notably by the speed at which the fiber is spun. The final structure obtained can differ in terms of protein conformation, molecular orientation and chain packing. When exposed to water or high humidity, dragline silk contracts longitudinally (supercontraction). The shrinking amplitude is altered by spinning speed as the latter influences the molecular orientation of the polypeptide chains. Despite several works in this domain, some fundamental questions remain. To answer these questions we have used Raman spectromicroscopy and NMR spectroscopy to investigate the secondary structure, molecular orientation and the dynamics of proteins of native and supercontracted spider silk fibers as influenced by spinning speed and relative humidity.
To this aim, we analyzed globally and at the amino acid level the molecular structure of dragline silk of two spider species, Nephila clavipes and Araneus diadematus. The fibers were obtained by forced spinning at speeds varying between 0.3 and 20 cm/s and were subsequently subjected to a relative humidity above 90%. For NMR experiments, two amino acids labeled solution (1-13C-Gly and 1-13C-Ala) were administered to two groups of spiders.

P70

Constructing All-atom Structure of Phytoglycogen-like Nanoparticles Using Molecular Dynamics Simulations

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PhytoSpherix (Phytoglycogen) is a biodegradable carbohydrate-based nanoparticle, produced by Mirexus Biotechnologies from sweet corn. This particle is made of simple sugar (α-D-glucose) units, connected in a dendritic (highly branched) fashion, which acts as an energy storage unit in plants. PhytoSpherix consists of roughly 22,000 glucose molecules, with an overall experimental radius of ~17 nm. Despite the extensive experimental studies, the detailed structure of this particle is unknown. In this work, we employ a combination of growth algorithms (using customized C++ codes) and large-scale molecular dynamics simulations (using the GROMACS package) to construct the structures of phytoglycogen-like particles in atomistic detail. Here, we start with an initial seed structure and grow it in different cycles. In each cycle, glucose molecules are added to the free ends of the particle, and MD simulations in the presence of explicit water molecules are performed. We built particles containing up to ~10,000 glucose molecules (~45% of the actual size), where the simulation box contains more than 3 million atoms. Details of the structure in the form of the radial density of the particle are provided. We use our structures to study the interactions and dynamics of the phytoglycogen particle.

P71

All-Atom Molecular Dynamics Simulations to Study the Structure and Dynamics of Phytoglycogen-like Nanoparticles

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PhytoSpherix is a phytoglycogen nanoparticle composed of glucose units joined in a dendritic manner. These particles are of interest as they are a biodegradable and biocompatible nanomaterial. In this work we present results from all-atom molecular dynamics simulations (GROMACS) of a PhytoSpherix like particle. The particle is dynamically grown until it contains ~1100 glucose units. Analysis of structural features is conducted as the particle equilibrates to a relaxed state. The complex internal structure is characterized by hydrophobic interactions between chains and water-containing areas in contact with hydrophilic regions of the glucose chains. Furthermore, the relaxed molecule exhibits a dense core and a sparse corona. Results concerning the dynamics inside of the particle are also examined. This characterization can assist the development of new applications for PhytoSpherix particles.

P72

Uncovering the structural basis for the mechanical properties of elastin

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Elastin is an extracellular matrix protein that imparts elasticity of vertebrate tissues and is formed from tropoelastin monomers. Tropoelastin is composed of alternating cross-linking domains, which contribute to stiffness and stability, and hydrophobic domains that are responsible for self-assembly and elastic recoil. Mechanical properties of elastin include elastic recoil (revert to original shape once deformed), resilience (energy lost in extension-recoil cycles) and stress relaxation (work needed to keep an elastic material extended). Recent studies that showed elastin-like peptides recapitulate the mechanical properties of elastin spurred interest in designing elastic biomaterials. To design such materials, it is essential to understand the structural basis that modulates protein elasticity. However, this remains poorly understood. The proposed research will carry out molecular dynamic simulation of elastin-like peptides to investigate how the structure determines the mechanical properties. We will study variants with systemic sequence changes in the hydrophobic and cross- linking domains that alter hydrophobicity, polarity, charge and secondary structure. Results will be used to build a quantitative model of how structural changes of elastin affect its mechanical properties. This essential knowledge can then be used towards rational design of biomaterials for use in tissue replacement and repair, with defined combinations of stiffness, resilience and stress relaxation.

P73

All-atom Molecular Dynamics Simulations of Single Amylose-like Chains: Exploring the Secondary Structure

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Amylose is a linear polymeric chain of simple glucose molecules (α-D-glucose), bound through α-1, 4 glycosidic linkages. It usually adopts helical structures in the presence of iodine, DMSO, alcohols or fatty acids. However, amylose can assume a variety of conformations in water – the structure and dynamics of which are of primary interest in this research. Despite well-defined secondary structures of amylose chains in experimental studies, the structures obtained through molecular dynamics simulations lack such well-defined conformations (i.e., helical structures). Presented are results from all-atom MD simulations of single amylose chains (V- amylose) in water. From these simulations, criteria for helix-like structures are developed, and the prevalence of these structures is explored. Dynamics such as bond flipping and other structural changes (e.g., the tertiary structures) and their effects on helices are also discussed.

P74

Studying the interactions of elastin-like polypeptides using all-atom molecular dynamics simulations

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Elastin-like polypeptides (ELP) are a class of biomaterials that are engineered to be used for different applications, especially as the replacement of natural tissues. Tuning their biochemical and biomechanical properties could change their behaviour, e.g., elasticity, proteolytic remodeling, cell binding, and signaling, which introduces a variety of potential applications for these materials. In this project, we use a mixture of cell-binding, structural binding, and QK peptides, in addition to THPC ligand to act as our ELP system. We use the GROMACS package to run all-atom molecular dynamics simulations of our system. In this project, we study the interactions between different peptides in the ELP, along with the effect of THPC ligand on the peptide mixture. These results could help us better understand and explain the behaviour of our system in experimental studies.

P75

The physical bases of forming a smooth boundary between an expanding actin network and a contracting actomyosin network

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Smooth boundaries commonly form for cell and tissue morphogenesis. One example is the cytokinetic ring which drives cell division, and another is the leading edge purse string for wound healing. In addition, smooth boundaries form between growing Arp2/3 actin networks and encircling actomyosin networks to bend the plasma membrane and form nuclear compartments during syncytial Drosophila embryogenesis. To understand the interaction between these two materials, we utilize mathematical modeling. The contractile actomyosin material is depicted by spring-node dynamics such that hundreds of myosin nodes connect randomly and pull on each other. Our model mimics reported in vitro behavior of reconstituted actomyosin networks, including shape changes following patterned activation. The Arp2/3 network is modelled based on the properties of these branched networks. These networks also mimic reported in vitro behavior, such as increased density with physical restriction. By reconstituting an Arp2/3 network encircled by a myosin network, we found that Arp2/3 network growth disrupted the smoothness of the myosin border, and that seemingly unnaturally high myosin network contractility was needed to counteract this effect. We are now testing a more natural configuration of an actomyosin network embedded with an array of Arp2/3 networks, and effects of local remodeling of the myosin network.

P76

Push and Pull: regulation of the actin cytoskeleton by myosin-dependent activation of Dia1

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Cells are truly the ultimate “smart material” regulating their physical properties to renew, repair and defend through precise regulation of the cytoskeleton. Myosin exerted forces on actin filaments are known to regulate cytoskeleton dynamics, but the mechanism remains unclear. We hypothesize that myosin contractility regulates actin cytoskeleton by enhancing the activity of actin polymerization factors (APFs). By combining live-cell imaging and pharmacological manipulations, we assessed how suppression of APFs affects the rate of actin polymerization at focal adhesions (FAs). We found that formin inhibition decreased polymerization by ~ 60%. By depleting individual formins, we identified Dia1 as the major APF at FAs. Inhibition of myosin decreased polymerization rate by ~30%, suggesting contractility activates APFs. However, this effect was lost in Dia1-depleted cells suggesting myosin-dependent activation of Dia1. By using a novel super-localization technique, we visualized instantaneous rate of polymerization and showed that Dia1 activity is not constant but undergoes myosin-dependent cyclical events. To investigate the biological role of myosin-dependent Dia1 activation, we perturbed Dia1 and myosin in a cell spread assay and showed that both treatments decreased mechanosensitive response, implicating myosin-dependent Dia1 activation in cellular mechanosensing. These results provide important insights into mechanical regulation of formin-mediated cytoskeleton dynamics.

P77

Biased protrusion dynamics steer cells towards stiffer extracellular matrix

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Many biological processes, such as developmental morphogenesis and immune responses, require a coordinated, directional movement of cells along gradients of extracellular matrix (ECM) stiffness, i.e. cell durotaxis. Yet, durotaxis can also contribute to several pathological conditions including atherosclerosis and cancer metastasis. Despite the biological and clinical importance of durotaxis, the mechanisms by which cells sense and respond to ECM stiffness are unclear. This is, in part, due to the lack of a technique to synthesize ECMs that recapitulate mechanical gradients in native tissues. Here, we developed a quantitative durotaxis assay using photo-polymerized hydrogels with a controlled gradient of stiffness that is confirmed by atomic force microscopy. We used this assay to show that cells migrating on an ECM with a gradient of stiffness exhibit a biased distribution of protrusions towards stiff region of the substrate. We found that suppression of calcium signaling through a stretch-activated Ca^{2+} channel TRPV4 abrogates biased protrusions and also suppresses durotaxis. To dissect the role of TRPV4 in durotaxis, we assessed the effect of TRPV4 depletion on the dynamics of protrusion/retraction. We found that TRPV4 is dispensable for the leading edge protrusion, but is essential for local contraction of the leading edge. Together, these data suggest ECMs with a gradient of stiffness steer migrating cells by regulates cell edge dynamics via Ca^{2+} signaling.

P78

Calcium Sparks in Focal Adhesions Drive Cell Migration Along ECM Stiffness Gradients

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The ability of cells to detect the stiffness of the extracellular matrix (ECM) is critical for directed migration in both physiological and disease processes. Despite its importance, how cells detect local ECM stiffness and transduce this information across the whole cell is unclear. To address this question, we characterized calcium signalling events at integrin-based focal adhesions (FAs) of mouse embryonic fibroblasts. FAs physically couple the cell cytoskeleton with the ECM. Here, we show that FAs are centres of transient, local calcium entry ("calcium sparks"). These sparks require extracellular calcium and are suppressed by stiff ECM. By screening the TRP family of mechanosensitive ion channels, we identified a single channel, TRPV4, responsible for calcium sparks. Calcium sparks are dependent on intracellular actomyosin activity; mutations that affect the ability of TRPV4 to interact with actin suppress FA calcium sparks, indicating that intracellular forces directly open TRPV4. By analyzing FA dynamics, we revealed that TRPV4 has a functional role in efficient FA disassembly. Finally, consistent with more calcium sparks on soft ECM, cell migration from soft toward stiff ECM requires TRPV4. Together, we show that TRPV4 establishes a gradient of focal adhesion disassembly across the cell, promoting focal adhesion disassembly on soft ECM and inducing preferential cell attachment to and migration toward stiff ECM.

P79

Self-Assembly of Rod-Like Colloids

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The purpose of this research is to examine the effects of the depletion force on rod-like colloids. The depletion force is an attractive force between colloids in a solution with depletants, the clustering of colloids together produces an increase in the entropy of the system, which yields an attractive entropic force between colloids. With rod-like colloids, this force causes the formation of bundles of colloids. In order to investigate this process, we use Molecular Dynamics computer simulations to model the colloids and their behaviour. Results show that depletion forces can be used to bundle rod-like colloids together in a solution. By more closely examining the effects of depletion forces on rod-like colloids, it's possible to selectively bundle colloids based on certain characteristics such as length or stiffness. In addition, many biological systems experience depletion forces naturally, and we can gain insight into the nature of these systems.

P80

Using Coarse-Grained Simulations to Investigate the Structure of a Dendritic Nanoparticle

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Phytoglycogen is a naturally occurring dendritic nanoparticle found in sweet corn. It is composed of repeatedly branching units of glucose. Despite glucose being a common form of energy storage in plants, many questions still remain about the structure of this naturally occurring particle. In this work pytoglycogen was modelled with a coarse-grained approach, simplifying each glucose unit to a single particle. A hydrophobic attraction between chains was observed in atomistic simulations and this was modelled in the coarse-grained simulations with a simple attraction between glucose particles. The strength of this attraction was used as a free parameter to investigate how changing it changes the structure of the particle. The simulated particles with radii similar to those seen in experiment were observed to have a hairy colloid shape, with a dense core and hairs extending out from the core.

P81

Development of a novel assay for quantifying the forces involved in rolling adhesion

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Rolling adhesion is crucial for the recruitment of immune cells to the site of infection. It is a highly physical phenomenon mediated by the interaction between p-selectin and p-selectin glycoprotein ligand 1 (PSGL1). The p-selectin/PSGL1 interaction is force dependent with bond lifetime first increasing upon force application, and then decreasing as force continues to rise. Studying these forces has proven to be difficult and they cannot be accurately represented by single molecule studies. Here, we use a new approach to study these forces by rolling PSGL1 coated polystyrene beads on p-selectin functionalized tension gauge tethers (TGTs). This creates a fluorescent track with intensity proportional to force. TGTs are DNA duplexes that dissociate when enough force is applied, leaving behind single stranded DNA that can be imaged with a fluorescently labeled complementary strand. The PSGL1 beads were rolled on the TGT surface at rolling velocities ranging from 2 to 20 μm/s. This resulted in fluorescent track intensities ranging from 4 to 24 respectively. The change in fluorescence intensity is proportional to the number of ruptured TGTs. At higher forces, more TGTs rupture and a higher fluorescence intensity is observed. Currently, a relationship between rolling velocity and track intensity has been established. Further work will be done to develop a model that uses fluorescence intensity as a metric to calculate the force on the p-selectin/PSGL1 bonds.

P82

The Effect of Lithium on Neuronal Cell Electrophysiology and Phosphorylation of Biomolecules Implicated in Alzheimer's Disease Progression

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Approved use of lithium in treatment of mental illnesses like bipolar disorder, major depressive disorder, and schizophrenia dates to the mid 20th century. Research indicates that the symptoms of Alzheimer's Disease can also be lessened by lithium treatment, though the complete mechanisms are unknown. The ability of lithium to reduce amyloid plaques and oligomer accumulation has been demonstrated in the brains of model mice with Alzheimer's disease-like neural pathology. Our research aims to characterize the change in the phosphorylation of biomolecules involved in Alzheimer's disease progression as well as characterize the change in membrane electrophysiology as a result of lithium treatment. To accomplish this task; first, cell toxicity assays will determine the dose-dependant toxicity of lithium on the in-vitro neural cell; second, Western blotting will be performed to detect the effect of lithium on the phosphate-mediated activity of intracellular molecules GSK-3-β, TAU, CaMKII, as well as NMDA membrane receptors; lastly, cell patch clamping will be used to characterize the effects of lithium on cell membrane electrophysiology. This research will shed light on how lithium affects the activity of these so called "memory molecules" as well as cell membrane potential in in-vitro cell lines. This will greatly increase our understanding of lithium's effects on neuronal physiology, mental illnesses, and degenerative brain diseases.

P83

Active multi-point microrheology of biopolymer networks

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Rheology is the field that can describe viscoelastic behavior of a material in response to applied force or deformation. Active microrheology is a technique in which particles can be manipulated by an external force, in contrast to the passive one, in which thermal fluctuations of particles are recorded. One experimental approach to active microrheology uses optical tweezers, which trap a μm-sized particle located within the material and excite it with an oscillating force. In this study, we use optical tweezers to oscillate a particle inside reconstituted cytoskeletal networks and by measuring the response of multiple neighboring particles to the excitation of a reference particle, we are able to measure the frequency-dependent viscoelastic response of the material at various length scales.

P84

Single-molecule visualization of the effects of ionic strength and crowding on structure-mediated interactions in supercoiled DNA molecules

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DNA unwinding is an important cellular process involved in DNA replication, transcription and repair. In cells, molecular crowding caused by the presence of organelles, proteins, and other molecules affects numerous internal cellular structures. Here, we visualize plasmid DNA unwinding and binding dynamics to an oligonucleotide probe as functions of ionic strength, crowding agent concentration, and crowding agent species using single-molecule CLiC microscopy. We demonstrate increased probe- plasmid interaction over time with increasing concentration of 8 kDa polyethylene glycol (PEG), a crowding agent. We show decreased probe-plasmid interactions as ionic strength is increased without crowding. However, when crowding is introduced via 10% 8 kDa PEG, interactions between plasmids and oligos are enhanced. This is beyond what is expected for normal in vitro conditions, and may be a critically important, but as of yet unknown, factor in DNA's proper biological function in vivo. Our results show that crowding has a strong effect on the initial concentration of unwound plasmids. In the dilute conditions used in these experiments, crowding does not impact probe- plasmid interactions once the site is unwound.

P85

Biomechanics of Tuvan Throat Singing

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"Throat singers" from central Asia are well known for their unique song vocalizations. In particular, singers from Tuva (a federal subject of Russia) have become popularized in part due to the exploits of the late physicist Richard Feynman. A salient example is "Khoomei", a style that creates an otherworldly yet organic sound. Deeply steeped in folk tradition, Tuvan "overtone singing" is achieved without the aid of external apparatus (e.g., a lamellophone). However, the underlying vocal tract biomechanics required for throat singing are not well understood. To elucidate such mechanisms, this study combines several approaches: detailed spectral analysis of a variety of song from several Tuvans, dynamic and volumetric structural MRI of a Tuvan singer, and computational airway modulation modeling. Preliminary results indicate that singers produce source patterns that give rise to a dense array of harmonics (i.e., "overtones"), which are kept relatively stable across time. Singers simultaneously modify their vocal tract to create narrowly "focused" filter states [i.e., highly accentuated formant(s)] that can be modulated independent of the source and other focused states (if present). Model results suggest a focused state arises from a singer's ability to merge two formants to (greatly) enhance the amplitude of one or two harmonics.

P86

Crowding in the nuclear pore complex does not slow down transport

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The nuclear pore complex (NPC) facilitates the selective transport of materials between the nucleus and cytoplasm in eukaryotic cells. Typically, many cargo are simultaneously present within the NPC during transport, and it is not fully understood how the NPC can function efficiently despite the crowding in the channel. In this study, we simulate transport through an NPC-like channel with coarse-grained cargoes and NPC-associated intrinsically disordered proteins (FG nucleoporins). From the results of this simulation, we show that cargo densities and trajectories along the direction of transport can be captured by a model of 1-dimensional diffusion through a potential, which is an effective potential arising through the interactions between cargoes and NPC components, and is modified by the non-equilibrium density profiles of cargoes inside the channel. With this framework, we are able to account qualitatively for previously unexplained experimental data which show that an increased number of cargo results in both increased efficiency and speed of transport. Our model therefore provides an explanation for why crowding does not necessarily affect the functioning of the NPC in a negative way, which can explain how the NPC can achieve both high specificity and high throughput.

P87

Population-level signatures of the co-evolutionary arms race between bacteria and viruses

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Complex communities of microorganisms are important ecological forces and phages (viruses which infect bacteria) are integral components of microbial populations. Among the many bacterial defense mechanisms against phages, CRISPR-Cas is unique in its ability to learn from past infections by storing pieces of phage DNA (called spacers) in its own genome to neutralize future infections. Here we present a model of interacting bacteria and phages to explore both the effects of stochasticity and co-evolution on the population as a whole.
Even with limited phage diversity, spacer abundance is highly stochastic and variable. In spite of this, the population-level rank-abundance distribution of spacers is time invariant, a surprising prediction that we test with dynamic spacer-tracking data from literature.
When phage diversity increases through mutations, we find that the overall population reaches a stable state of constant “motion” through sequence space: phages continually mutate to new sequences, and bacteria follow with a constant lag that depends on the effectiveness of the CRISPR system. Counterintuitively, the lag is larger when the CRISPR system is more effective.
This study lays out a path toward a phenomenological framework for understanding microbial dynamics and may provide insights into complex and diverse natural populations where microscopic modeling is plagued by overparameterization and overfitting.

P88

Modelling Signal Cross-talk in Type I Interferon

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Signaling networks have historically been studied as direct input-output relationships. This view is now undermined by frequent observation of signal cross-talk, a phenomenon wherein several signals are transmitted through interacting signaling pathways. Cross-talk makes signal discrimination a highly underdetermined problem, raising the question of how cells discriminate between different ligands in the presence of cross-talk. We analyse a stochastic model of signaling to formulate theoretical bounds on the accuracy and specificity of signaling in the presence of cross-talk. We show that receptor pleiotropy overcomes fundamental limitations in sensing multiple ligands in the presence of cross-talk. To study a real-world example, we systematically investigate factors responsible for specificity of Type I IFN signaling, an innate immune signaling system which prominently features cross-talk. We use a nonlinear dynamical model validated by quantitative flow cytometry data to show that specificity arises from the interplay of receptor binding affinity, the numbers of receptor subunits and their in-membrane mobility, amplified by transcriptional negative feedback. Crucially, we find that IFN-specific negative feedback such as receptor internalization is necessary to explain observed differences in signal response between IFN signals. Our results provide a theoretical framework and working model for further investigation into signal specificity in the presence of cross-talk.

P89

The Origin of Life: Bridging the Gap Between Nucleotides and Protocells

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The hydrothermal field hypothesis for the origin of life suggests that cyclical wetting and drying of the edge of volcanic ponds, due to tides, seasons, or day night cycles, coupled with extreme temperature promoted the synthesis of the first RNA and the formation of first protocells. Compounds found in those ponds such as clay, inorganic salts and amphiphiles are important to align nucleotides into pre-polymers. While RNA has been polymerized from nucleotides in experiments using rudimentary hydration-dehydration cycles (HD cycles), these experiments were limited by possible number of cycles and precise environmental control. To mimic the contents of these volcanic ponds, lipids, clays and inorganic salts were mixed with adenosine 3'-phosphate (AMP) and uridine 3'-phosphate (UMP). Solutions were dried on silicon wafers and fast HD cycles run in the Planet Simulator in McMaster's newly constructed Origins of Life Lab. Gel electrophoresis was used measure the length and volume of synthesized RNA. Conjointly, the samples were analyzed through microscopic imaging and with X-ray diffraction, which allowed us analyze changes in the structure of the free nucleotides in different environments and compare them to uncycled samples. I will present first results of a pathway for the formation of first RNA and protocells under prebiotic conditions.

P90

Conformational Properties of an Equimolar Mixture of Complementary DNA Strands from c-MYC Promoter Region

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Sequences that are capable of folding into the G-quadruplex and i-motif conformations often co-exist as complementary strands in biologically important loci of the genome, including the promoter regions of oncogenes. The regulatory role of tetraplex DNA structures in gene expression depends on subtle equilibria between the duplex and the tetraplex conformations. Here, we employ circular dichroism spectroscopy to characterize the populations of duplex, G-quadruplex, i-motif, and coil conformations within an equimolar mixture of the complementary 22-meric DNA strands from the promoter region of the c-MYC oncogene. We conducted our studies as a function of pH, KCl concentration, and temperature. Our results reveal that, at low KCl concentrations (0-1 mM), the system predominantly exists as a duplex within the pH range of 5 to 7. On the other hand, high KCl concentrations (10-50 mM) promoting the G-quadruplex state result in the conformational equilibrium shift towards the tetraplex states in a pH-dependent manner. These findings suggest that, at high KCl concentrations, the genomic DNA with sequences that potentially adopt the G-quadruplex and/or i-motif conformations is able to spontaneously dissociate and form (partially or exclusively) these tetraplex conformations. Our findings have clear biological implications given the high intracellular concentrations of potassium and provide a further evidence for the biological significance of the G-quadruplex and i-motif structures.

P91

Potential role of oxygen molecules and cytochrome c oxidase proteins in optical communication in the brain

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Great discoveries have been made in the field of neuroscience; however, the origin of consciousness and many mental abilities of the brain are still unanswered. Quantum physics may help provide some answers. In particular, there is some experimental evidence of spins and photons playing a role in biology. We explore the possibility of the existence of an optical communication network connecting spins in addition to the well-known electro-chemical network in the brain. Photon emission by neurons has been observed experimentally. Also, it has been shown that photons can be guided by myelinated axons. We investigate the possible role of oxygen molecule and cytochrome c oxidase (cco) as biophoton sources and receivers, respectively. The oxygen molecule not only has non-zero spin, but also emits light which matches spectrally with the observed biophotons. Cco in mitochondria is a light absorbing metalloprotein. This protein has a peak in its absorption spectrum around the frequency of dimol emission of oxygen. The role of this protein in generating singlet oxygen (an excited state of oxygen) after photon absorption and the fact that this protein has electron and nuclear spin make the possibility of a spin-photon interface stronger, resembling quantum repeaters (key components of quantum communication networks). We propose experimental tests for many important aspects of these ideas, including spin-light coupling, absorption cross sections and spin properties of oxygen and cco.

P92

Cell type stability in the context of intercellular signalling

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The development of single-cell RNA sequencing (scRNA-seq) has led to widespread interest in dynamical modelling of cell state. Clustered scRNA-seq data reveals stable transcriptomes associated with known cell types. A fundamental question is how to specify a dynamical system, in this case a gene regulatory network, for which the identified cell types correspond to attractors in gene expression space. Hopfield networks offer an elegant solution and have been used to describe reprogramming in individual cells. Given a set of cell type transcriptomes, they prescribe a matrix of gene-gene interactions which form the basis of an Ising model. While this provides an exciting framework to model cell fate, its applications have been restricted to the level of single cells. A natural and theoretically interesting question is the following: given a dynamical model of intracellular gene regulation for which known cell types are fixed points, what happens when copies of the model interact via intercellular signalling? To address this, we propose to model interacting cells using a lattice of interacting Hopfield networks. We consider cell-cell interactions mediated by both ligand-receptor signalling and exosomes. We investigate under what conditions the single- cell attractors remain stable, and whether cell-cell interactions can facilitate the emergence of new stable single-cell states. Our results offer new perspectives on cell type stability in natural and engineered contexts.

P93

Characteristic dynamics of co-regulated genes with different intrinsic properties

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Intracellular reaction networks are often sparsely characterized and are made of many parts, making the reductionist approach ineffective when trying to make strong statements like identifying sources of cellular noise. A different way to identify sources of noise uses correlations between two identical and independent genes subject to the same environment. This technique relies on the two gene reporters being identical and distinguishable. Motivated by the experimental reality that any two distinguishable systems can't be exactly the same, we analyze the dynamics of co-regulated pairs of stochastic components with different intrinsic lifetimes, subject to arbitrary upstream influences. In doing so, we find that variance and covariance measurements of such dual reporters can potentially be used to obtain information on upstream components. In particular, we show that the constrained space of possible correlations depends critically on whether or not there is feedback control in the system, suggesting a way to experimentally detect the existence of feedback without making upstream measurements. We also show that a sub region of the no-feedback constrained correlation space can only be accessed if the reporters are subject to oscillatory driving, suggesting a way to obtain dynamical upstream information and differentiate between types of upstream variability via static downstream measurements.

P94

Explicit parameters in deterministic equations are insufficient to calculate extinction times

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We have found that "hidden" parameters present in the stochastic birth and death rates have a notable contribution to the MTE. Increasing the strength of competitive interaction in the death rate and similarly decreasing this competitive interaction in the birth rate decreases non-linearly the MTE by several orders of magnitude. Of the various common approximation techniques investigated, only the Fokker-Planck and WKB approximations compare well to the exact MTE in a wide regime of the parameter space. Additionally, we've developed a framework to describe the relaxation time, the decorrelation time between measurements of a population's size, which also depends on the different birth and death rates. Incorrect conclusions may be drawn about underlying dynamics of a system if the choice of stochastic rates is uninformed. Notably, the wrong timescales are derived. Knowing the correct system timescales, such as extinction and relaxation times, is invaluable to experimentalists sampling from bacterial colonies to establish population distributions. As such, it is important to carefully define rates to properly study the behaviour of the system.

P95

Exploring the dynamics of extranuclear inheritance

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Nuclear inheritance has been studied extensively by scientists in a variety of fields. The traits of cells that inherit nuclear genes follow simple rules due to the segregation and mixing of chromosomes. However, when sources of genetic information exist both inside and outside of the nucleus, the rules of genetic inheritance become more complex. Extranuclear DNA often exists in multiple copies and is regulated on multiple levels; At the cell level, extranuclear DNA replication and cell division are asynchronous, so extranuclear DNA undergoes its own mutation and selection dynamics before cell division. At a population level, cells transmit extranuclear DNA to progeny under less strict segregation rules, and are selected for or against depending on the quality of their total DNA pool. Evidently, the extranuclear DNA in a population of cells is a rich dynamical system, with selection acting at multiple levels (intracellular vs. population) and on different timescales (extranuclear DNA replication vs. cell division). In this work we propose a multi-level selection model to describe the distribution of mitochondrial DNA (mtDNA) in yeast populations, which is an example of extranuclear DNA that is particularly amenable to experiments. In efforts to validate predictions of this model, we will also demonstrate how a simple computer-vision pipeline can be used to extract a coarse measure of mtDNA content by analyzing yeast colony morphology.

P96

On MicroRNA and Protein Noise Models

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Gene expression noise plays an important role in many cellular processes. Recent studies suggest that the role of microRNA-mRNA interactions is to reduce gene expression noise. Here we show that the previously applied quasi-steady state approximation gives incorrect results in the biologically relevant regime. We find that microRNA binding is not effective at noise reduction when compared to other mechanisms that reduce mRNA lifetimes. In contrast to the previously reported conclusions, this analysis suggests that the role of microRNA binding might be to increase – rather than decrease – gene expression variability.

P97

Solving First Passage Problems in Nanofluidic Devices with Deep Neural Networks

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First passage processes are ubiquitous in biology. These problems can be formulated as partial differential equations (PDEs). Nanobiophysical systems often exhibit complicated geometries; solving PDEs in such domains is traditionally accomplished with mesh-based numerical solvers, like the finite element method. Unfortunately, for complicated molecules these PDEs are very high-dimensional, and mesh-based solution methods are prohibitively expensive for such problems. Instead, these systems are often modelled with stochastic differential equations (SDEs), which can be efficiently studied using particle simulations. Nonetheless, to establish how observables (like radius of gyration, molecular mobility, etc.) depend on problem parameters (like molecular size, temperature, etc.), the simulations must be conducted repeatedly for different parameter choices. A relatively new method of solving PDEs is to train deep neural networks (DNNs) directly to satisfy the conditions of a PDE, without using any external data. This method has been shown to work even on certain high-dimensional PDEs. Moreover, it can learn solutions as continuous functions of the problem parameters. In this talk, I will discuss our application of this technique to the PDEs describing particle motion through the slitwell nanofluidic device. Unlike finite element and particle simulation solutions, the DNNs can directly yield the mean first passage time as a function of the applied force.