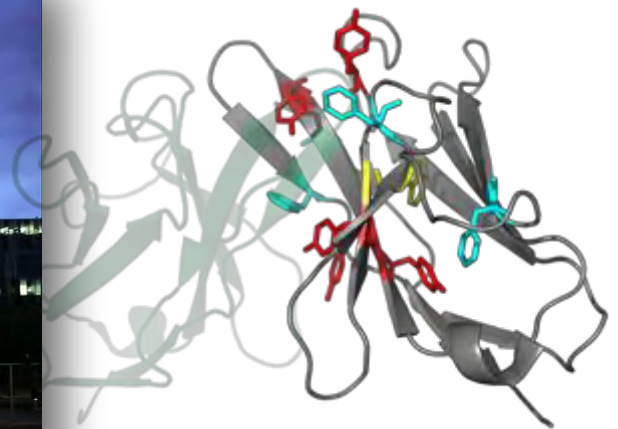




**MONTREAL
2017**

Biophysical Society of Canada
3rd Annual Meeting
May 24-26, 2017
Université du Québec à Montréal

FULL PROGRAM



Biophysical Society
Société de biophysique
du Canada

UQÀM

TABLE OF CONTENTS

Welcome.....	3
Message from the President.....	3
Organizing committee	4
Conference Location & Directions.....	5
Sponsors & Exhibitors.....	6
Program overview.....	8
Scientific program.....	9
National lecture & biography	14
Keynote lectures & biographies.....	15
Poster presentations.....	18
Abstracts – Oral presentations.....	26
Abstracts – Poster presentations.....	44

WIFI INFORMATION

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Bienvenue à Montréal!

It is a pleasure for us to welcome you to the 3rd Annual Meeting of the Biophysical Society of Canada. Since the first edition in Waterloo in 2015, the BSC meeting has become the main national conference in the field of Biophysics. This year, over 250 participants will gather on the beautiful science complex of the Université du Québec à Montréal (UQÀM) to discuss the latest developments in the field of biophysics; including protein structure and dynamics, biological nanoconfinement, computational biophysics and single-molecule spectroscopy. The 2017 BSC meeting includes 51 oral presentations and over 105 posters from academic groups all across Canada, renowned international scientists and industrial partners. This year, Professor Benoît Roux from the University of Chicago will address the National Lecture and will also be honored as the 2017 Fellow of the Biophysical Society of Canada. We will also have the privilege of three keynote lectures given by Professor Peter Davies from Queen's University, Professor Sarah L. Keller from the University of Washington and Dorothee Kern from Brandeis University.

We would like to thank our generous sponsors and partners who are supporting this exciting meeting and promoting the biophysical research in Canada. This year, many of our industrial sponsors will be present during the conference and we strongly encourage everyone to visit their booths during the mixer, coffee breaks, lunches and poster session. We are also very grateful to UQÀM, the Faculty of Sciences and the Chemistry Department for supporting this event. A special thanks to the members of the local organizing committee; Sabrina Leslie, Isabelle Marcotte, Guillaume Lamoureux and Anthony Mittermaier.

We wish you all an inspiring conference and a joyful time in *Montréal!* Bon symposium!

Steve Bourgault
Department of Chemistry
Université du Québec à Montréal

Peter Pawelek
Department of Chemistry and Biochemistry
Concordia University

President's message

Both the field of biophysics and the Biophysical Society of Canada have seen tremendous growth in recent years. Biophysical techniques have revolutionized research and have led to seminal advances in diverse research areas - materials research, biotechnology, biochemistry, bio-sensing, medicine, and pharmaceutical research. The annual meetings of the Biophysical Society of Canada provide a unique opportunity for students and researchers in academia and industry to learn about the most recent advances in biophysics. We have kept our meetings intimate, so that all participants are exposed to a wide range of novel experimental approaches used to solve biological problems. Our meetings are recognized for both the quality and diversity of the invited speakers, as well as the collegiality of all participants – and then there is the exceptional wine and food! BSC 2017 in *Montréal* promises to maintain, and perhaps even surpass all expectations for our annual meetings. I would like to acknowledge the conference organizers, including our co-chairs: Drs. Steve Bourgault and Peter Pawelek, and our organizing committee members: Drs. Guillaume Lamoureux, Sabrina Leslie, Isabelle Marcotte, and Anthony Mittermaier. Thank you for your hard work. Thank you for putting together such an exciting program! BSC 2017 promises to be an exciting conference. Enjoy!

John Baenziger
President
Biophysical Society of Canada

BSC 2017 LOCAL ORGANIZING COMMITTEE

Steve Bourgault (Co-chair)

Associate Professor

Department of Chemistry
Université du Québec à Montréal

Peter Pawelek (Co-chair)

Professor

Department of Chemistry and Biochemistry
Concordia University

Sabrina Leslie

Assistant Professor

Department of physics
McGill University

Isabelle Marcotte

Professor

Department of Chemistry
Université du Québec à Montréal

Guillaume Lamoureux

Associate Professor

Department of Chemistry and Biochemistry
Concordia University

Anthony Mittermaier

Associate Professor

Department of Chemistry
McGill University

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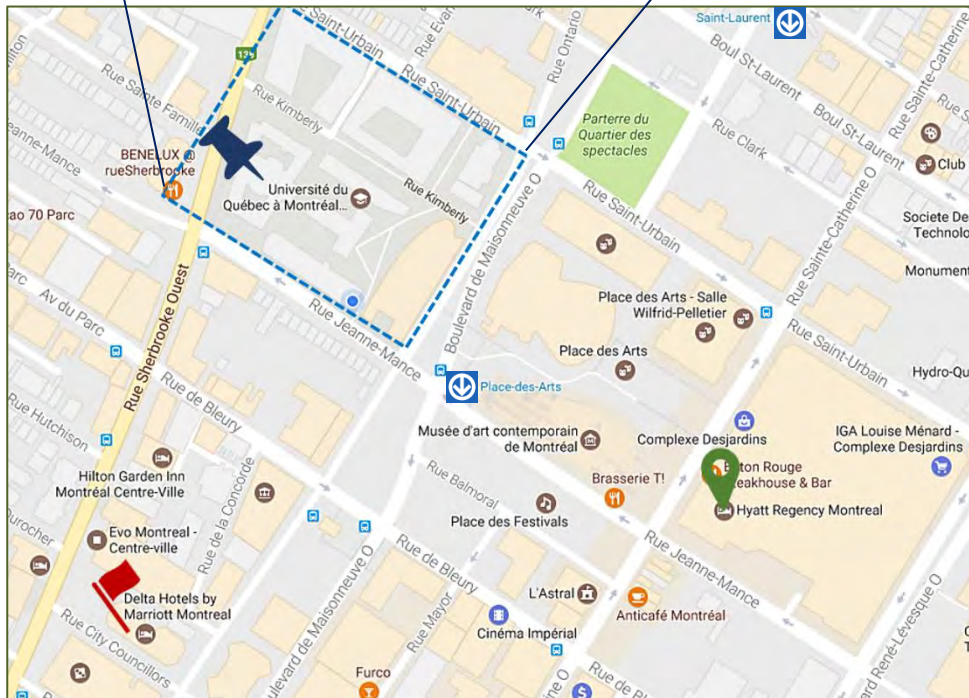
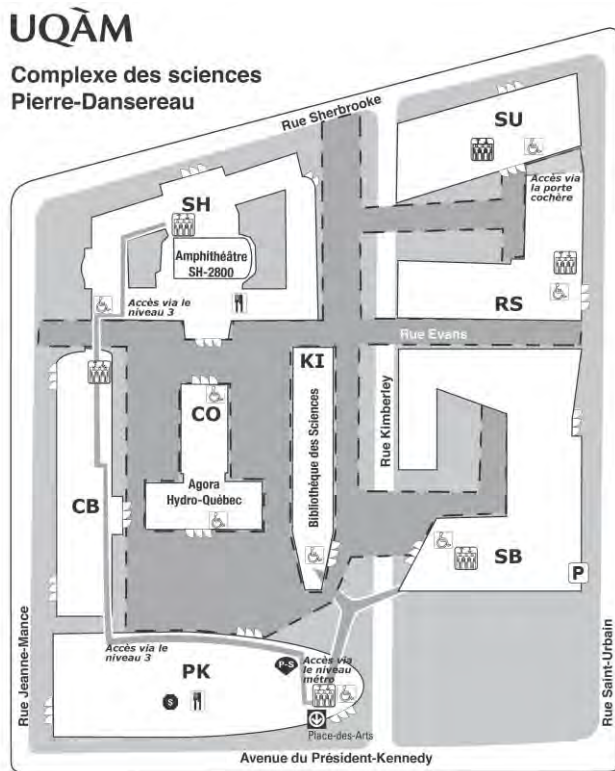
William Jennings





Trainee Representative

Zhenfu Zhang

Trainee Representative

CONFERENCE LOCATION & DIRECTIONS



-  Metro
-  SH Building
-  Delta Hotel
-  Reception (Hyatt)

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PROGRAM OVERVIEW

Wednesday, May 24th

1:00 PM - 1:20 PM	Opening Remarks
1:20 PM - 3:00 PM	Session 1
3:00 PM - 3:30 PM	Coffee & Beverage
3:30 PM - 4:50 PM	Session 2
4:50 PM - 5:30 PM	Keynote Lecture: Peter Davies, <i>Queen's University</i>
5:30 PM - 8:30 PM	Mixer & Poster Exhibition

Thursday, May 25th

8:00 AM - 10:00 AM	Session 3
10:00 AM - 10:30 AM	Coffee & Pastries
10:30 AM - 11:50 AM	Session 4
11:50 AM - 12:30 PM	Keynote Lecture: Sarah L.Keller, <i>University of Washington</i>
12:30 PM - 2:00 PM	Lunch (provided) & Poster Exhibition
2:00 PM - 4:05 PM	Session 5
4:05 PM - 4:35 PM	Coffee Break & Poster Exhibition
4:35 PM - 5:20 PM	Session 6: Student Talks
5:20 PM - 6:20 PM	National Lecture: Benoît Roux, <i>University of Chicago</i>
6:30 PM - 9:30 PM	Reception

Friday, May 26th

8:20 AM - 10:00 AM	Session 7
10:00 AM - 10:30 AM	Coffee & Pastries
10:30 AM - 11:50 AM	Session 8
11:50 AM - 12:30 PM	Keynote Lecture: Dorothee Kern, <i>Brandeis University</i>
12:30 PM - 2:30 PM	Lunch (on your own)
2:30 PM - 5:00 PM	Session 9
5:00 PM - 5:10 PM	Closing Remarks

SCIENTIFIC PROGRAM

Wednesday, May 24th

11:30 AM - 1:00 PM	Registration	SH-4800
1:00 PM - 1:20 PM	Welcoming Remarks Luc-Alain Giraldeau, Dean, Faculty of Sciences, UQÀM John Baenziger, President, Biophysical Society of Canada	SH-2800
1:20 PM - 3:00 PM	Session 1 Chair: Adam Hendricks, McGill University	SH-2800
1:20 PM	Andrea R. Tao, University of California, San Diego <i>Plasmonic nanoparticle probes for optical spectroscopy</i>	
1:40 PM	Andre Marziali, University of British Columbia <i>New methods to enable early detection of cancer</i>	
2:00 PM	Caroline Boudoux, École Polytechnique Montréal <i>Novel double-clad fiber couplers for microscopy and OCT</i>	
2:20 PM	Alexander Dunn, Stanford University <i>Vinculin forms a directional catch bond to F-actin</i>	
2:40 PM	Martin J. Zuckermann, Simon Fraser University <i>The bar-hinge motor: a synthetic protein design exploiting conformational switching to achieve directional motility</i>	
3:00 PM - 3:30 PM	Coffee & Beverage	SH-4800
3:30 PM - 5:30 PM	Session 2 Chair: Peter Pawelek, Concordia University	SH-2800
3:30 PM	Jenifer Thewalt, Simon Fraser University <i>Cholesterol: the versatile lipid</i>	
3:50 PM	Bruce Hill, Queen's University <i>Kinetics and thermodynamics of copper binding by the BsSCO protein - some lessons for understanding the mechanism of ligand transfer</i>	
4:10 PM	Mazdak Khajehpour, University of Manitoba <i>Ion-specific effects on hydrophobicity and protein stability</i>	
4:30 PM	Kalle Gehring, McGill University <i>Feed-forward amplification in the PINK1-parkin pathway acts as binary switch for mitochondrial quality control in Parkinson disease</i>	
4:50 PM	Keynote Lecture Peter Davies, Queen's University <i>Giant adhesin protein helps form a symbiotic biofilm on sea ice</i>	
5:30 PM - 8:30 PM	Mixer & Poster Exhibition	SH-4800

Thursday, May 25th

7:30 AM - 8:00 AM	Registration	SH-4800
8:00 AM - 10:00 AM	Session 3 Chair: Sabrina Leslie, McGill University	SH-2800
8:00 AM	Wesley P. Wong, Harvard Medical School <i>Approaches in mechanobiology: from DNA nanoswitches to single-molecule centrifugation</i>	
8:20 AM	Claudiu Gradinaru, University of Toronto <i>Single-molecule fluorescence approaches for GPCRs: oligomerization, conformational selection and dynamics</i>	
8:40 AM	Maxime Dahan, Institut Curie <i>Target search of DNA-binding proteins in mammalian cells: a single molecule study</i>	
9:00 AM	Jose M. Moran-Mirabal, McMaster University <i>The study of cellulase-cellulose interactions and cellulose structure through single-molecule fluorescence microscopy techniques</i>	
9:20 AM	Nancy Forde, Simon Fraser University <i>Single-molecule measurements of collagen's triple helical stability</i>	
9:40 AM	Stephen W. Michnick, University of Montréal <i>Endocytosis caused by liquid-liquid phase separation of proteins</i>	
10:00 AM - 10:30 AM	Coffee & Pastries	SH-4800
10:30 AM - 12:30 PM	Session 4 Chair: Isabelle Marcotte, UQÀM	SH-2800
10:30 AM	Paul G. Higgs, McMaster University <i>The emergence of RNA from the prebiotic mixture</i>	
10:50 AM	Dror Warschawski, Université Paris Diderot & UQÀM <i>A new method to assess lipid phases in lipid mixtures by ³¹P Magic Angle Spinning NMR</i>	
11:10 AM	Christine DeWolf, Concordia University <i>Biophysical impact of airborne pollution on model lung surfactant membranes</i>	
11:30 AM	Peter Tieleman, University of Calgary <i>Lipid-protein interactions are unique fingerprints for membrane proteins</i>	
11:50 PM	Keynote Lecture Sarah L. Keller, University of Washington <i>Latest experimental results on lipids and proteins demixing to form domains in model and cell-derived membranes</i>	
12:30 PM - 2:00 PM	Lunch (provided) & Poster Exhibition	SH-4800

Thursday, May 25th (continued)

2:00 PM - 4:05 PM	Session 5 Chair: Guillaume Lamoureux, Concordia University	SH-2800
2:00 PM	Joanne Lemieux, University of Alberta <i>Allosteric regulation of rhomboid intramembrane proteases</i>	
2:20 PM	Joelle Pelletier, University of Montréal <i>A combined computational/experimental approach to increase the efficiency of enzyme engineering</i>	
2:40 PM	Joerg Gsponer, University of British Columbia <i>Disorder in protein interactions</i>	
3:00 PM	Valter Zazubovich, Concordia University <i>Using high-resolution frequency-domain spectroscopy for studying protein dynamics and energy transfer in pigment protein complexes – photosynthesis and beyond</i>	
3:20 PM	George Okeyo, Nanion Technologies <i>Temperature modulation of thermo-TRP channels on a novel planar lipid bilayer recording system</i>	
3:35 PM	Marty Kurylowicz, Mirexus <i>Applications of phytoglycogen nanoparticles in cosmetics, nutrition and medicine</i>	
3:50 PM	Marc-André Gagnon, Agilent Technologies <i>FTIR imaging for biomedical research - Simultaneous chemical & spatial information</i>	
4:05 PM - 4:35 PM	Coffee Break & Poster Exhibition	SH-4800
4:35 PM - 5:20 PM	Session 6: Student talks Chair: John Baenziger, University of Ottawa	SH-2800
4:35 PM	Alexander C.Y. Foo, University of Ottawa <i>Solution NMR investigation of the lateral gate in GlpG rhomboid protease</i>	
4:50 PM	Tanja Kalstrup, University of Montréal <i>Probing the movement of the ball and chain during N-type inactivation in Kv channels</i>	
5:05 PM	Supratik Sen Mojumdar, University of Alberta <i>Partially native intermediates mediate misfolding of SOD1 in single-molecule folding trajectories</i>	
5:20 PM	Fellow of the Biophysical Society of Canada & National Lecture Benoît Roux, University of Chicago <i>The Molecular Mechanism of P-type ATPase Ion Pumps</i>	SH-2800
6:30 PM - 9:30 PM	Reception Hyatt Regency Montréal (1255 Rue Jeanne-Mance, H5B 1E5)	Terrace

Friday, May 26th

8:20 AM - 10:00 AM	Session 7 Chair: Steve Bourgault, UQÀM	SH-2800
8:20 AM	Corrie daCosta, University of Ottawa <i>Back to the future: adventures through acetylcholine receptor space and time</i>	
8:40 AM	Derek Bowie, McGill University <i>Title to be confirmed</i>	
9:00 AM	Matthew D. Shoulders, Massachusetts Institute of Technology <i>Folding and quality control mechanisms of collagen</i>	
9:20 AM	Yu-Shan Lin, Tufts University <i>Understanding and designing cyclic peptides</i>	
9:40 AM	Pierre Lavigne, University of Sherbrooke <i>Keep your C2H2 fingers off my DNA</i>	
10:00 AM - 10:30 AM	Coffee & Pastries	SH-4800
10:30 AM - 12:30 PM	Session 8 Chair: Anthony Mittermaier, McGill University	SH-2800
10:30 AM	Simon Sharpe, University of Toronto <i>Studying the phase separation and material properties of elastin using NMR spectroscopy</i>	
10:50 AM	Jan Rainey, Dalhousie University <i>Developing a mechanistic understanding of spider aciniform silk toughness</i>	
11:10 AM	Aron Broom, University of Waterloo <i>Improving proteins through point mutations is unreliable and may trade stability for solubility</i>	
11:30 AM	Roberto A. Chica, University of Ottawa <i>Rational design of proteins that exchange on functional timescales</i>	
11:50 PM	Keynote Lecture Dorothee Kern, Brandeis University <i>Recreating the evolution of enzyme catalysis and regulation over 3.5 billion years</i>	
12:30 PM - 2:30 PM	Lunch Break (on your own)	

Friday, May 26th (continued)

2:30 PM - 5:00 PM	Session 9 Chair: Vincent Tabard-Cossa, University of Ottawa	SH-2800
2:30 PM	Derek M. Stein, Brown University <i>Protein sequencing by nanopore mass spectrometry</i>	
2:50 PM	Meni Wanunu, Northeastern University <i>DNA capture and sequencing using nanopore-coupled zero-mode waveguides</i>	
3:10 PM	Marija Drndic, University of Pennsylvania <i>2D materials nanosculpting and bioelectronics nanopore applications</i>	
3:30 PM	Kyle Briggs, University of Ottawa <i>Kinetics of polymer translocation through nanopores under nanoscale pre-confinement</i>	
3:45 PM	Daniel Berard, McGill University <i>Squeezing New Information out of Macromolecules Using Adjustable Nanoconfinement</i>	
4:00 PM	Hendrick de Haan, Ontario Institute of Technology <i>Exploring the structure of phytospherix nanoparticles via atomistic and coarse-grained simulations</i>	
4:20 PM	Jerome Mathe, Université Paris-Saclay <i>Nanopores: from polymer physics to biological applications</i>	
4:40 PM	Craig Benham, University of California, Davis <i>Topology-mediated DNA dynamics</i>	
5:00 PM - 5:10 PM	Closing remarks	SH-2800

Fellow of the BSC & National Lecture – Professor Benoit Roux



Dr. Benoit Roux is Professor of Biochemistry and Molecular Biophysics at the University of Chicago. He has previously taught at University of *Montréal* and Weill Medical College of Cornell University. Benoit Roux was a recipient of the 1998 Rutherford Memorial Medal in Chemistry, awarded by the Royal Society of Canada.

His laboratory at the University of Chicago mostly uses theoretical techniques, such as classical molecular dynamics, to understand the functioning of biological systems at the molecular level. His research has investigated structure, dynamics, and the function of biological macromolecular systems such as ion channels, receptors, and protein kinases.

He is a pioneer in the study of membrane proteins using molecular dynamics with explicit phospholipid molecules and solvent. His laboratory has also developed novel computational methods to improve efficiency and applicability of theoretical investigations to molecular recognition phenomena. His work has bridged theory and experiment in biophysics by employing ever-increasing computational power to further the understanding of the molecular basis of life.

The Molecular Mechanism of P-type ATPase Ion Pumps Benoit Roux

Department of Biochemistry and Molecular Biophysics, University of Chicago, Illinois, USA

P-class ATPases ion pumps constitute a superfamily of cation transport enzymes, present both in prokaryote and eukaryote, whose members mediate membrane flux of all common biologically relevant cations. P-class pumps use ATP to transport ions against their electrochemical potential (they are also called E1-E2-type ATPase). The Na/K pump transports three Na^+ out of the cell by two K^+ into the cell at the expense of the hydrolysis of one molecule of ATP. The sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA) pumps two Ca^{2+} from the cytosol of muscle cells to the sarcoplasmic reticulum by exchanging two H^+ . From crystallography, we have a remarkable series of snapshots showing how these enzymes look at different states of their transport cycle. Using molecular dynamics simulation, the string method with swarms-of-trajectories, and free energy methods, we seek to understand the conformational dynamics involved as such pump transits through conformational states revealed by x-ray crystallography, the nature of the coupling between the binding of ATP, phosphorylation, and the movements of charged species across the core of the protein, the stepwise voltage-sensitive steps, and the origin of the ion binding specificity associated with different conformational states. A special attention is given to the protonation state of ionizable residues during the pumping cycle.

Keynote Lecture – Professor Peter L. Davies



Dr. Davies received his B.Sc. from the University of Wales and his Ph.D. from the University of British Columbia in biochemistry. He then went on to do postdoctoral research in Lund, Sweden and Calgary, Alberta, before joining Queen's as an MRC Scholar in 1977. Dr. Davies specializes in protein biochemistry and the study of protein structure-function relationships. He has been recognized for his work on fish and insect antifreeze proteins and their interaction with ice. This has included work on the structure, mechanism of action and evolution of these unusual proteins. His lab has recently begun to study other ice-binding and ice-nucleating proteins from plants and microorganisms. Another area of Dr. Davies' research is in the structure, function and mechanism of calpains, which are intracellular Ca^{2+} -dependent cysteine proteases that mediate Ca^{2+} signalling. His group was the first to discover the activation mechanism of calpain and its structure after activation by calcium and in the presence of its natural inhibitor, calpastatin. They are working to develop more potent and specific calpain inhibitors as drug leads for treating a number of diseases. A third area of interest is in bacterial adhesion proteins and their relationship to biofilm formation. Dr. Davies has published over 240 papers and reviews, with 10 them appearing in Nature or Science.

Giant adhesin protein helps form a symbiotic biofilm on sea ice

Peter L. Davies

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

Bacteria form biofilms on a wide variety of surfaces. We have recently described a marine bacterium (*Marinomonas primoryensis*) that can colonize the underside of sea ice. It attaches to the ice surface through a large 1.5-MDa adhesion protein (adhesin). In collaboration with other groups we have recently solved the structure of this giant adhesin. Of its >130 domains the majority are identical 11-kDa extender modules that project the ligand-binding region $\sim 0.6 \mu\text{m}$ away from the host cell surface. This C-terminal region includes ice-, sugar-, and peptide-binding domains. Binding to ice is aided by the motility of the bacterium, and is dependent on the functionality of its ice-binding domain. A polyclonal antibody raised against the ice-binding domain was shown to completely abolish bacterial ice adhesion. *M. primoryensis* also homes in on, and binds to, the Antarctic diatom *Chaetoceros neogracile* to form mixed cell clusters that the bacteria then attach to ice. These diatoms are bound to the bacteria through the peptide- and sugar-binding domains of the adhesin. We hypothesize the ice-binding function keeps its obligate aerobic host immediately under the surface ice in the phototrophic zone of the water column where photosynthetic organisms produce oxygen and carbon compounds for bacterial consumption. Furthermore, by recruiting diatoms to the ice-bound microcolony, *M. primoryensis* helps these photosynthesizers form a symbiotic community where light is most abundant. Our study also gives insight into how bacterial biofilms, including those of pathogens, can be disrupted by blocking key ligand-binding domains.

Keynote Lecture – Professor Sarah L. Keller



Dr. Keller is a biophysicist who investigates self-assembling soft condensed matter systems. Her group's primary research focus concerns how simple lipid mixtures within bilayer membranes give rise to complex phase behavior. Some of the awards that have recognized her research include the Biophysical Society's Avanti Award, Tom Thompson Award and Dayhoff Award; the ASBMB's Avanti Award; the Research Corporation's Cottrell Scholar Award; and the National Science Foundation's CAREER Award. In 2011 she was elected to the Washington State Academy of Sciences and was named a Fellow of the American Physical Society. She joined the Department of Chemistry at the University of Washington in 2000 after earning her Ph.D. from Princeton University and completing postdoctoral research as a Presidential Fellow at UC Santa Barbara and as an NIH NRSA Fellow at Stanford University. She is a recipient of UW's 2006 Distinguished Teaching Award and the UW Postdoctoral Association's 2012 Mentor Award. From 2010 to 2014 she served as the Associate Dean for Research Activities for the UW College of Arts and Sciences.

Latest experimental results on lipids and proteins demixing to form domains in model and cell-derived membranes

Sarah L. Keller

Department of Chemistry, University of Washington, Seattle, USA

A visually striking feature shared by simple and complex membranes alike is that they demix to form large, liquid domains enriched in particular proteins and lipids. These micron-scale domains appear below a distinct miscibility phase transition temperature and disappear above it. This keynote lecture will summarize key experimental results from the past year regarding demixing of lipids and proteins in model membranes and in cell-derived membranes.

Keynote Lecture – Professor Dorothee Kern



Dr. Kern is Professor of Biochemistry at Brandeis University and an Investigator of the Howard Hughes Medical Institute. She received her PhD at the Martin Luther University in Halle, Germany and then carried out her postdoctoral studies at UC Berkeley. She joined the faculty at Brandeis University in 1999. Her research group studies the dynamical nature of proteins with the goal to reveal the interplay between structure, dynamics and function. She has been a major contributor in the experimental characterization of protein dynamics during enzyme catalysis and signaling. Recently Dr. Kern is reconstructing the evolution of proteins over billions of years. Dr. Kern is the recipient of the Pfizer Award in Enzyme Chemistry from the American Chemical Society, the National Lecturer of the Biophysical Society, the Dayhoff Award from the Biophysical Society, the Young Investigator Award of the International Association for Protein Structure Analysis and Proteomics and the Strage Award for Aspiring Young Science Faculty. Before her professional scientific career, she was captain of the German National Basketball team for many years and won an MVP award.

Recreating the evolution of enzyme catalysis and regulation over 3.5 billion years

Dorothee Kern

Department of Biochemistry, HHMI/Brandeis University, Waltham, USA

Allosteric regulation, the process by which a protein's activity can be modulated by binding of an effector molecule distal to the active site, is vital for cellular signaling. However, its evolution is largely unexplored territory. I will describe our experimental exploration of the evolution over 1.5 billion years of two allosteric regulation mechanisms widely found in the modern protein kinase superfamily, phosphorylation of the activation loop and binding of a regulatory partner protein. Using Ancestral Sequence Reconstruction (ASR) we unravel the origins of allosteric activation including surprising mechanistic features. Moreover, ASR enabled identification of the underlying allosteric network that spans the kinase from the N-terminal to the C-terminal lobes. In the second part of the talk I describe how we exploit this new knowledge for the development of allosteric inhibitors and activators. This latter approach delivered novel kinase inhibitors and activators with extreme specificity and high affinity thereby opening the road to new cancer treatment. Third, I will address the evolution of enzyme catalysis in response to one of the most fundamental evolutionary drivers, temperature. Using ASR, we answer the question of how enzymes coped with an inherent drop in catalytic speed caused as the earth cooled down over 3.5 billion years. Tracing the evolution of enzyme activity and stability from the hot-start towards modern hyperthermophilic, mesophilic and psychrophilic organisms illustrates active pressure versus passive drift in evolution on a molecular level.

POSTERS PRESENTATIONS

- P01** **Biochemical role of threonine-77 in atlantic salmon tropomyosin Tpm1.1**
A. Madhushika M. Silva and David H. Heeley
- P02** **Brighter red fluorescent proteins display reduced structural dynamics**
Adam M. Damry, Natalie K. Goto and Roberto A. Chica
- P03** **Understanding skin impermeability: structure characterization and permeability measurements**
Adrian Paz Ramos, Gert Gooris, Joke Bouwstra and Michel Lafleur
- P04** **Free energy of a polymer in slit-like confinement from the Odijk regime to the bulk**
Albert Kamanzi, Jason Leith, David Sean, Daniel Berard, Andrew C. Guthrie, Christopher M.J. McFaul, Gary W. Slater, Hendrick W. de Haan and Sabrina R. Leslie
- P05** **Studying protein energy landscapes in dimeric cytochrome b6f with optical spectroscopy**
Alexander Levenberg, Golia Shafiei, Rafael Picorel and Valter Zazubovich
- P06** **Transcriptional foci assembly by phase separation in prokaryotes**
Anne-Marie Ladouceur and Stephanie C. Weber
- P07** **Determination of lipid phase behaviour in drug delivery systems via small angle X-ray scattering**
Bashe Y.M. Bashe, Sherry S.W. Leung, Joanne E. Mercer, Miranda L. Schmidt, Pieter R. Cullis, D. Peter Tieleman and Jenifer L. Thewalt
- P08** **Towards a model of the cell envelope of Mycobacterium tuberculosis: Martini coarse-grained force field parameters for mycolic acids**
Beibei Wang and D. Peter Tieleman
- P09** **Atomic Force Microscopy Imaging and Particle Size Determination of Soft Phytoglycogen Nanoparticles**
Ben Baylis and John R. Dutcher
- P10** **Study of the functional interaction between RNF-167 Ubiquitin ligase and a library of conjugating ubiquitin E2**
Kim Ghilarducci, Billel Djerir, Camille Desroches, Steve Bourgault and Marc P. Lussier
- P11** **The effects of a cationic lipid on a phospholipid bilayer: a deuterium NMR and small-angle X-ray scattering study**
Iulia Bodnariuc, Miranda L. Schmidt, Mohsen Ramezanpour, Joanne E. Mercer, Sherry S.W. Leung, Pieter R. Cullis, D. Peter Tieleman and Jenifer L. Thewalt
- P12** **Probing Peptide Ligand-GPCR Interactions By ¹⁹F NMR Spectroscopy**
Calem Kenward, Kyungsoo Shin, Muzaddid Sarker and Jan K. Rainey
- P13** **Structure Determination of a Class IB Hydrophobin by NMR Spectroscopy**
Calem Kenward and David N. Langelan

- P14** **DNA Translocations through Nanopores coated with an Antifouling Layer**
Caroline Tippins and Vincent Tabard-Cossa
- P15** **A Putative Nucleolar Signal Sequence Directs the Localization of TRPM7 Kinase**
Ceredwyn Hill, Adenike Ogunrinde, Christiane Whetstone and Evalina Williamson.
- P16** **Functional Characterization of Kir6.1 (A88G)/SUR2A) Channels Expressed in HEK293H cells and the Association with J Wave Syndromes.**
Cinthia Rangel-Sandoval, Hector Barajas-Martínez, Enrique Sanchez-Pastor, Tania Ferrer-Villada and Carlos G Onetti
- P17** **Biophysical Approaches in the Development of a Coupled Glycosyltransferase Assay Employing E. coli UDP-glucose Dehydrogenase**
Cory Campbell and Peter D. Pawelek
- P18** **The Dynamical Zinc Fingers of Miz-1**
Cynthia Tremblay, Mikaël Bédard, Martin Montagne, Danny Letourneau and Pierre Lavigne
- P19** **Counting Molecules with Localization Microscopy using Fluorophore Photophysics Statistics**
Daniel Nino, Nafiseh Rafiei, Daniel Djayakarsana, Anton Zilman and Joshua N. Milstein
- P20** **Effects of Phylogenetic Distance on Protein Dynamics, Antibacterial Activity and Cytotoxicity in Members of the Ribonuclease 3 Subfamily**
David Bernard, Myriam Létourneau, Donald Gagné, Marie-Christine Groleau, Éric Déziel and Nicolas Doucet
- P21** **Structural and functional characterization of oncogenic protein-protein interactions involving the microphthalmia-associated transcription factor**
Makenzie Branch and David N. Langelan
- P22** **Allosteric regulation of rhomboid intramembrane proteases**
Rashmi Panigrahi, Elena Arutyunova and M. Joanne Lemieux
- P23** **Quantifying Spatiotemporal Patterns in the Expansion of Twitching Bacterial Colonies**
Erin Shelton, Maximiliano Giuliani, Lori Burrows, and John R. Dutcher
- P24** **P-gp lipid uptake pathways determined by coarse-grained molecular dynamics simulation**
E. Barreto-Ojeda, V. Corradi, R-X. Gu and D. P. Tieleman
- P25** **Inter-Sarcomere Dynamics within Skeletal Muscle Myofibrils**
Felipe de Souza Leite and Dilson Rassier
- P26** **Untangling the hairball: fitness based asymptotic reduction of biological networks**
Félix Proulx-Giraldeau, Thomas Rademaker and Paul François

- P27** **Dimensions and dynamics of disordered proteins from single molecule fluorescence measurements**
Gregory-Neal W. Gomes, Julie Forman-Kay and Claudiu C. Gradinaru
- P28** **More than a pore: The role of the dimeric intermediate in the pore-forming mechanism of fragaceotoxin C, an actinoporin from the sea anemone *Actinia fragacea***
Haydeé Mesa-Galoso, Karelia Delgado-Magnero, Uris Ros, Pedro A. Valiente and D. Peter Tieleman
- P29** **QM/MM Simulations Reveal Molecular Mechanism of ATP Hydrolysis in an ABC Transporter**
Hendrik Göttsche, Marten Prieß, Gerrit Groenhof and Lars V. Schäfer
- P30** **Artificial Crown Ether Ion Channel as Promising Therapeutic Agents**
Jean-Daniel Savoie, François Otis, Jochen Bürck, Anne S. Ulrich, Christophe Moreau, Michel Vivaudou and Normand Voyer
- P31** **Methodological development to study lipid membranes of intact microalgae by solid-state NMR**
Jean-Philippe Bourgoignie, Alexandre Poulhazan, Francesca Zito, Alexandre A. Arnold, Dror E. Warschawski and Isabelle Marcotte
- P32** **Investigating the Phase Behaviour of a Model Lipid Nanoparticle System with Dlin-KC2-DMA/Distearoylphosphatidylserine and the Addition of Cholesterol**
Joanne Mercer, Sherry Leung, Bashe Bashe, Ismail M. Hafez, Pieter R. Cullis, D. Peter Tieleman and Jenifer Thewalt
- P33** **Long Chain Sphingolipids (C24) Suppress Membrane Domains by Concentrating Cholesterol in the Inner Leaflet**
Kevin C. Courtney and Xiaohui Zha
- P34** **Long DNA as a Genomic Platform**
Daniel Berard, Francis Stabile, Gil Henkin, Marjan Shayegan, Jason Leith, Kim Metera and Sabrina Leslie
- P35** **A Mathematical Model for Vertebrate Somitogenesis**
Laurent Jutras-Dubé and Paul François
- P36** **Bundling of acetylated microtubules drives enhanced kinesin-1 motility**
Linda Balabanian, Christopher L. Berger and Adam G. Hendricks
- P37** **Design of amyloid-based nanostructures as a novel vaccination platform**
Margaryta Babych, Ximena Zottig, Geneviève Bertheau Mailhot, Jessica Dion, Denis Archambault and Steve Bourgault
- P38** **A Single-particle detection and tracking algorithm for fluorescence microscopy as demonstrated on *Thermobifida fusca* cellulases**
Markus Rose, Jake Bolewski, Cecile Fradin and Jose Moran-Mirabal
- P39** **An instrument to Quantify DNA using Solid-State Nanopores**
Martin Charron, Matthew Waugh, Kyle Briggs, Samuel Berryman, Simon King, Dylan Gunn and Vincent Tabard-Cossa

- P40** **Thioflavin T fluorescence to detect amyloid assembly: effect of the frequency of measurement in microplate assays**
Mathew Sebastiao, Noé Quittot and Steve Bourgault
- P41** **Towards a nanoscale description of the interactions between the amyloid peptide A β ₍₁₋₄₂₎ and mutants with membranes**
Mehdi Azouz, Christophe Cullin, Michel Lafleur and Sophie Lecomte
- P42** **²H NMR studies of bacterial membrane perturbation by antimicrobial polypeptides**
Nury P. Santisteban, Michael R. Morrow, and Valerie Booth
- P43** **Super-Resolution Imaging of Native Cellulose Nanostructure**
Mouhanad Babi, Ayodele Fatona and Jose Moran-Mirabal
- P44** **Release of mesoporous silica particles by free standing pH sensitive membranes**
Nan Jia, Erica Rosella and Jesse Greener
- P45** **Effects of Cellular Tension Stress on Time-Lag Response of YAP Movement During Cell Migration**
Newsha Koushki, Rosa Kaviani and Allen J. Ehrlicher
- P46** **New insights into the mechanism of islet amyloid polypeptide self-assembly by mean of a split tetra-Cys motif approach**
Noé Quittot, Médina Hicheur, Mathew Sebastiao and Steve Bourgault
- P47** **Structural-functional relationship of single skeletal and cardiac thin filaments visualized by high-speed Atomic Force Microscopy**
Oleg Matusovsky, Malin Persson, Yu-Shu Cheng and Dilson E. Rassier
- P48** **Biophysical effects of the interaction between hydrophilic nanoparticles and pulmonary lung surfactants: influence of nanoparticle concentration and charge**
Olga Borozenko, Abdullah Khan, Antonella Badia and Christine De Wolf
- P49** **Design and Validation of Multi-Charged Dendrimers to Modulate Amyloid Assembly**
Phuong T. Nguyen, Rishi Sharma, Rabindra Rej, Carole Anne De Carufel, René Roy and Steve Bourgault
- P50** **Gel/Liquid-ordered Phase Coexistence in Bilayers Containing Palmitoyl Sphingomyelin, Palmitoyl Ceramide and Cholesterol**
Reza Siavashi, Felix Goni, and Jenifer Thewalt
- P51** **Kinetics Investigations of Peptide Crystallization Using Coarse-Grained α Molecular Dynamics Simulations with Polymorphically Biased Force Fields**
Robert J Girardin and Apichart Linhananta
- P52** **RNA transcription modulates phase transition-driven nucleolar assembly**
Stephanie C. Weber, Joel Berry, Nilesh Vaidya, Mikko Haataja and Clifford P. Brangywnne

- P53** **LSPR study of interactions of Amyloid β (1-42) with model lipid membranes**
Stephen Turnbull, Nanqin Mei, Elizabeth Drolle, Morgan Robinson, Brenda Yasia Lee and Zoya Leonenko
- P54** **Metadynamics: Speeding up conformational sampling of intrinsically disordered peptides**
Trang Nhu Do, Elio A. Cino, Wing-Yiu Choy and Mikko Karttunen
- P55** **Covalent immobilization of protein onto self-assembled nanostructures via an enzymatic-mediated ligation**
Ximena Zottig, Marie-Jeanne Archambault, Isabelle Rouiller and Steve Bourgault
- P56** **Integration of Optical Instrumentation and Microfluidics for CBD-Fabricated Solid State Nanopore Sensors**
Zachary Roelen, Jose Bustamante, Autumn Carlsen, Kyle Briggs and Vincent Tabard-Cossa
- P57** **Cross Correlation Spectroscopy and CLiC**
Zhiyue Zhang, John Ross, Paul Wiseman, Mark Sutton and Sabrina Leslie
- P58** **Reconstructing developmental gene regulatory networks using ϕ -evo, an *in-silico* evolution software**
Adrien Henry, Mathieu Hemery, Jeremy Rothschild and Paul François
- P59** **Mechanistic formation of a bipolar spindle: A fast and irreversible process that sequentially integrates Cin8's varied motility and microtubule crosslinking properties.**
Allen Leary, Elena Nazarrova, Shannon Sims, Kristy Shulist, Paul Francois and Jackie Vogel
- P60** **Structural study of α -synuclein 71-82, a peptide derived from a protein involved in Parkinson's disease: interactions with model membranes**
Benjamin Martial, Thierry Lefèvre, Émilie Bruneau, Laurie Bédard, Thierry Buffeteau and Michèle Auger
- P61** **A Novel Model of Daptomycin Inhibition by Lung Surfactant: Biophysical Studies using Model Lipid Membranes**
Brenda Y. Lee, Maureen M. W. Li, Jeff H. Y. Lam and Zoya Leonenko
- P62** **Identifying the key parameters in biological models using parameter compression**
Chieh Ting Hsu, Gary Brouhard and Paul Francois
- P63** **Interaction of Tetryl, a Nitroaromatic Explosive, with Bacterial Reaction Center in *R. sphaeroides***
Daniel Modafferi, Valter Zazubovich and Laszlo Kalman
- P64** **The Role of G-protein-Coupled Receptor Activation by Conformational Selection as revealed by Single-molecule Fluorescence**
Dennis D. Fernandes, Libin Ye, Yuchong Li, R. Zhenfu, Zhang, Gregory-Neal W. Gomes, Scott Prosser and Claudiu C. Gradinaru
- P65** **On the selectivity vs. conductivity paradox in narrow biological ion channels**
D. G. Luchinsky, W.A.T. Gibby, I. Kh. Kaufman, D.A. Timucin and P.V.E. McClintock
- P66** **Identifying Structure in Short DNA Scaffolds using Solid-State Nanopores**
Eric Beamish, Vincent Tabard-Cossa and Michel Godin

- P67** **SRP rapidly scans translating ribosomes until it binds the signal anchor sequence emerging from the peptide exit tunnel**
Evan Mercier, Wolf Holtkamp, Marina V. Rodnina, and Wolfgang Wintermeyer
- P68** **SERS – Optophysiology: Toward *in-vitro* neurotransmitters monitoring of dopaminergic neurones**
Lussier, F., Brulé, T., Ducrot, C., Bourque, M.-J., Trudeau, L.-E. and Masson, J.F.
- P69** **Structural investigations of supercontracted spider dragline silk**
Justine Dionne, Thierry Lefèvre, Philippe Bilodeau, Mathieu Lamarre and Michèle Auger
- P70** **Molecular Views of a Eukaryotic Plasma Membrane Model**
Karelia H. Delgado-Magnero, Valentina Corradi, Gurpreet Singh and Peter Tieleman
- P71** **Distinct differences in membrane interaction for two peptide ligands for the same GPCR**
Kyungsoo Shin, Muzaddid Sarker, Shuya K Huang, Calem Kenward, and Jan K Rainey
- P72** **Stoichiometry of Kv2.1/Kv6.4 heterotetramers revealed by fluorescent single channel subunit counting**
Lena Moeller, Glenn Regnier, Alain J. Labro, Dirk J. Snyders and Rikard Blunck
- P73** **Characterization of voltage sensitive dyes with free-standing lipid bilayers**
Maria Tsemperouli and Kaori Sugihara
- P74** **Scaling of Phase-Separated Polymer Viscoelastic Properties Under Confinement**
Marjan Shayegan, Francis Stabile, Cynthia Shaheen, Stephen Michnick and Sabrina Leslie
- P75** **Separation of Polymer Mixtures by Length Using a Series of Nanopores Connected by Nanochannels**
Martin Magill, Ed Waller and Hendrick W. de Haan
- P76** **Evolving Logical-Gates Through Phenotypic Evolution**
Mathieu Hemery, Adrien Henry and Paul François
- P77** **Atomistic Simulations of the Structure of Phytoglycogen Nanoparticles**
Michael Greenberg and Hendrick W. de Haan
- P78** **Spectroscopic Imaging in Microchannels for Studies of Biofilms**
Mohammad Pousti, François Paquet-Mercier and Jesse Greener
- P79** **AS Simple AS Possible but not Simpler: On the Reliability of Protein Coarse-Grained Models**
Mona Habibi, Jörg Rottler and Steven S. Plotkin
- P80** **High-speed AFM of complex lipid membranes captures video of protein-lipid interactions**
Morgan Robinson, Loren Picco, Mervyn Miles and Zoya Leonenko
- P81** **Direct observation of transition-state dynamics during folding reactions**
Krishna Neupane, Noel Q. Hoffer and M.T. Woodside

- P82** **Single-Molecule Detection Using Various Integrated Nanopore-Microfluidic Arrays**
Radin Tahvildari, Eric Beamish, Vincent Tabard-Cossa and Michel Godin
- P83** **Mechanistic Insight into a Possible Regulatory Region of an Intramembrane Enzyme Diacylglycerol acyltransferase**
Rashmi Panigrahi, Kristian Mark P. Caldo, Jeella Acedo, Tsutomu Matsui, John C. Vederas, Randall J. Weselake and M. Joanne Lemieux
- P84** **Filamin A has a role in mechanotransduction of cells in shear**
Rosa Kaviani, Chris Sitaras, Haruka Yoshie and Allen Ehrlicher
- P85** **Discovery of key molecular recognition sites between JAZ transcriptional repressors and MYC transcription factors involved in plant defense Jasmonate hormone signaling, a computational study**
Samara Oña and Miguel Méndez
- P86** **DNA Invasion: How the Rate of Oligo Binding to Plasmids Provides DNA Structural Information**
Shane Scott, Cindy Shaheen, Xhi Ming Xu, Fedor Kouzine, Laura Saunders, Barbara Gravel, Daniel Berard, Alexander Hofkirchner, Jill Laurin, Catherine Leroux, David Levens, Craig J. Benham and Sabrina R. Leslie
- P87** **A Nanofluidic Knot Factory based on Compression of Single DNA in Nanochannels**
Susan Amin, Ahmed Khorshid, Lili Zeng, Philip Zimny and Walter Reisner
- P88** **Curvature-induced lipid sorting in plasma membrane tethers**
Svetlana Baoukina, Helgi I. Ingolfsson, Siewert J. Marrink and D. Peter Tieleman
- P89** **Comparing the folding dynamics of prion proteins from species with different disease susceptibility at the single-molecule level**
Uttam Anand, Craig Garen and Michael T. Woodside
- P90** **Characterization of Huntingtin's N-terminal via all-atom simulation: a solution and membrane study**
Vincent Binette and Normand Mousseau
- P91** **Kinetic model of conduction through an open narrow channel**
W.A.T Gibby, D. Luchinsky, A. Ward, I. Kh. Kaufman and P.V.E McClintock
- P92** **Ion Conduction through the Cardiac Ryanodine Receptor (RyR2): All-atom Molecular Dynamics Simulation Studies**
Williams E. Miranda, Van A. Ngo and Sergei Y. Noskov
- P93** **Theoretical Elucidation for Sequence-Specific Behaviors of Charged Intrinsically Disordered Proteins in Liquid-Liquid Phase Separation**
Yi-Hsuan Lin, Julie D. Forman-Kay and Hue Sun Chan

- P94** **Dynamic Compression of a Nanoslit Confined Polymer Solution**
Yue Qi, Lili Zeng, Ahmed Khorshid and Walter Reisner
- P95** **Strategies for the *in vivo* characterisation of the marine bacteria *Vibrio splendidus* by ²H NMR**
Zeineb Bouhleb, Dror E. Warschawski, Alexandre A. Arnold, Réjean Tremblay and Isabelle Marcotte
- P96** **Bacterial Ribosome Selective Antibacterials**
Dev P. Arya
- P97** **Insights about the effect that the J-domain has on the substrate binding domain (SBD) of 70 kDa heat shock protein Hsp70 from a chimeric human SBD-J polypeptide**
Ana O. Tiroli-Cepeda, Thiago V. Seraphim, Júlio C. Borges and Carlos H.I. Ramos
- P98** **S-Nitrosylation suppresses stromal interaction molecule-1 activation and ameliorates cardiomyocyte hypertrophy**
Jinhui Zhu, Le Gui, Xiangru Lu, Wei-Yang Lu, Qingping Feng and Peter B. Stathopoulos
- P99** **Délivrance de médicaments par effet de tampon via des transporteurs moléculaires**
Arnaud Desrosier
- P100** **DNA-protein conjugates for electrochemical biosensing applications**
Xiaomeng Wang and Alexis Vallée-Bélisle
- P101** **Monitoring Enzyme Activity at the Nanoscale with DNA Probes**
Scott G. Harroun, Arnaud Desrosiers and Alexis Vallée-Bélisle
- P102** **Tau directs intracellular trafficking by regulating the forces exerted by kinesin and dynein teams**
Abdullah R. Chaudhary and Adam G. Hendricks
- P103** **Why protein oligomer complexes allow more precise regulation mechanisms over dimers and monomers?**
Dominic Lauzon and Alexis Vallée-Bélisle
- P104** **A Suit of Methods to Measure Enzyme Kinetics Using Isothermal Titration Calorimetry**
Justin Di Trani, Nicolas Moitessier and Anthony Mittermaier
- P105** **Rapid characterization of folding and binding interactions with thermolabile ligands by DSC**
R. W. Harkness, V. S. Slavkovic, P. E. Johnson and A. K. Mittermaier
- P106** **Kinetic Study of APH(3')-IIIa Inhibition Mechanism by Isothermal titration calorimetry (ITC)**
Yun Wang, Justin Di Trani and Anthony Mittermaier

ORAL PRESENTATION ABSTRACTS

Plasmonic Nanoparticle Probes for Optical Spectroscopy

Andrea R. Tao

Department of NanoEngineering, University of California, San Diego, California, USA

Many optical spectroscopy and imaging techniques are limited by the ability to fabricate nanoscale probes that are robust, reproducible, and support high quality optical resonances. I will describe how colloidal plasmonic nanoparticles overcome these obstacles and serve as enabling materials for spectroscopy and imaging. First, I will present our recent work on the synthesis and self-assembly of colloidal nanoparticles for the fabrication of resonant optical nanojunctions. Previously, we demonstrated that shaped colloidal nanoparticles can be organized into nanojunctions that possess intense “hot spots” due to electromagnetic field localization. Here, I will describe how colloidal nanoparticles can be assembled and used as scanning near-field optical probes for Raman nanospectroscopy. Nanoparticles are assembled onto atomic force microscope cantilevers that can then be raster scanned across a surface during optical measurements. Nanoparticle size and shape can be used to modulate the optical response of the scanning probe. Second, I will present spectroscopic data for plasmonic nanodisks that are capable of upconverting near-infrared (NIR) light. These two-photon absorbing (TPA) inorganic nanoparticles support plasmon resonances in the NIR to mid-infrared wavelengths have the potential to exhibit extraordinary two-photon action cross-sections. We measure the two-photon action cross-sections for CuS nanodisks whose LSPR wavelengths are tuned on and off the NIR excitation wavelength, spanning a large range of operating wavelengths covering the tissue transparency window.

New methods to enable early detection of cancer

Andre Marziali

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Cancer remains one of the most challenging diseases to treat, primarily due to its ability to spread and its clonal diversity when it reaches advanced stages. Early detection and treatment continues to be one of the most effective approaches to preventing cancer mortality. Despite various adopted screening methodologies, many patients still present with cancer at advanced stages due to lack of symptoms. Advances in less invasive, less expensive, and more accurate early detection methods are required to continue to decrease cancer mortality. At UBC Engineering Physics and Boreal Genomics, we have developed an electrophoretic method for enriching cancer DNA from blood that may enable a blood test capable of detecting cancers early even when they are asymptomatic. In this presentation, I will outline the enrichment technology, the underlying non-linear electrophoretic mechanism, the development of the cancer detection assay, and the clinical trial underway to determine its utility.

Novel double-clad fiber couplers for microscopy and OCT

Caroline Boudoux

Department of Engineering Physics, École Polytechnique, Montréal, Canada

Double-clad fiber couplers combine the properties of single-mode fiber light delivery with high efficiency collection provided by multimode fibers. They are used in multiple sensing and imaging applications including surface plasmon resonance, optical coherence tomography, confocal and nonlinear microscopy. Recent work from our laboratory allowed increasing their performance to quasi-lossless transmission through the single-mode core combined with >85% transfer efficiency of multimode light. This presentation will focus on novel double-clad fiber coupler designs as well as on the brief history of their commercialization.

Vinculin forms a directional catch bond to F-actin

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Vinculin is an actin-binding protein thought to reinforce cell-cell and cell-matrix adhesions. However, how mechanical load affects the vinculin/F-actin bond has not been examined. Using a single-molecule optical trap assay, we find that vinculin forms a force-dependent catch bond with F-actin via its tail domain, but with lifetimes that depend strongly on the direction of the applied force. Force toward the pointed end of the actin filament results in a bond that is maximally stable at 8 pN, with a mean lifetime (12 s) that is 10-fold longer than the mean lifetime when force is applied toward the barbed end. A computational model of lamellipodial actin dynamics suggests that the directionality of the vinculin/F-actin bond can establish long-range (~10 μm) order in the actin cytoskeleton. The directional and force-stabilized binding of vinculin to F-actin may represent a novel mechanism by which adhesion complexes maintain front-rear asymmetry in migrating cells.

The Bar-Hinge Motor: A synthetic protein design exploiting conformational switching to achieve directional motility

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NanoLund and Solid State Physics, Lund University, Lund, Sweden##

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Basic approaches to synthetic biology have taken advantage of the various benefits of available building blocks. Our approach has been to use the building blocks of nature, amino acids, and the peptide motif frequently observed in natural systems, the coiled coil, to design and produce synthetic molecular motors. Here we investigate a novel bipedal stepping motor concept, the Bar-Hinge Motor (BHM), which combines ligand cycling and binding to a one dimensional DNA track with a peptide-based system able to undergo externally controlled conformational switching. The latter mechanism emulates the dynamics of biological bipedal nano-motors such as kinesin and myosin V, which employ conformational changes induced by ATP hydrolysis to achieve directed motion. The BHM design is unique in that it executes a random walk in the absence of conformational switching, allowing us to investigate the power of conformational switching to drive directed motion in isolation from other motor functions. We describe a proposed experimental realization and a minimal computational model for the BHM. We then present results of numerical simulations for the model using overdamped Lagrangian dynamics, which show that the addition of a conformational switch enables the BHM to execute directional motion. The interplay between thermal fluctuations and conformational rigidity is examined, with the aim of determining the stringency of design parameters necessary for directional motion of the BHM.

Cholesterol: The Versatile Lipid

Jenifer Thewalt

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Most of the cholesterol in our bodies serves a structural role, strengthening and leak-proofing plasma membranes. Using biophysical techniques - with a heavy emphasis on solid state deuterium NMR spectroscopy - we have explored the physical attributes of a wide range of cholesterol-containing lipid environments. Three lines of inquiry will be introduced. 1) Cholesterol enables the formation of the *liquid ordered (l_o)* phase, thought to be critical to proper membrane function. How does ceramide affect the *l_o* phase of sphingomyelin/cholesterol? 2) Can other sterols substitute for cholesterol to form the *l_o* phase with unsaturated lipids such as 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)? 3) Aside from its job forming the *l_o* phase in plasma membranes, cholesterol plays a big part in lipid-mediated drug delivery. Biopharmaceuticals like siRNA can be shepherded to their sites of action by cholesterol-containing *lipid nanoparticles*. To successfully deliver siRNA to the cytosol, the endosome-encased lipid nanoparticle must be destabilized. This destabilization is thought to involve the formation of non-bilayer lipid structures. What is cholesterol's contribution to this process?

Kinetics and thermodynamics of copper binding by the BsSCO protein - some lessons for understanding the mechanism of ligand transfer

Bruce Hill

Department of Biomedical and Molecular Sciences, Queen's University

Efficient assembly of the Cu_A centre of cytochrome *c* oxidase requires the intervention of the accessory protein SCO. The **S**ynthesis of **C**ytochrome **O**xidase, or **SCO**, protein was discovered in yeast, and homologs have been recognized in higher eukaryotes and in many prokaryotic species. The primary sequence of SCO places it in the thioredoxin family of proteins, and as such it has a pair of cysteine residues that can engage in redox reactions. However, SCO is also able to bind copper using the redox active thiols as inner sphere ligands, and it is through such complexes that SCO is proposed to transfer copper to the Cu_A site of cytochrome *c* oxidase. We have used SCO from *Bacillus subtilis* (*i.e.*, BsSCO) to characterize interactions with copper by spectroscopic, kinetic and thermodynamic approaches. The dissociation constant of BsSCO for copper (II) is nearly pM, with the high-affinity conferred by a two-step mechanism in which the rate of copper dissociation is slow. Can such a stable species participate effectively in copper transfer? Our results show that the reduction of BsSCO-Cu(II) to BsSCO-Cu(I) is chemically facile, reduces the affinity of BsSCO for copper by six orders of magnitude, and accelerates the dissociation rate by a factor of one hundred. Thus we propose a catch and release mechanism for copper transfer in which Cu(II) is captured by BsSCO with high affinity, and its release is triggered by reduction to Cu(I). Such a redox-triggered, copper-affinity switch is distinct from other copper-based electron transfer proteins.

Ion-specific effects on hydrophobicity and protein stability

Olga Sorokina, Hayden Glor, Courtney Clark and Mazdak Khajehpour

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Since Hofmeister's discovery of his eponymous series, it has become well-established fact that ions affect biomolecules in a specific manner that is not consistent with interpretations based on classical continuum electrostatic theory. However, the underlying origins of the ion specific effects observed in biophysical phenomena have not yet been established unambiguously. In this work we have investigated effects of a group of simple monovalent cations and anions on the micellization of a simple amphiphile molecule, 1,2-hexanediol. The diol headgroup of this molecule is uncharged, therefore counterion-headgroup interactions play a minimal role in the micelle formation process. Therefore, any observed specific ion effects on the micellization process are to great extent caused by ion-induced changes to the hydrophobic contribution to the free energy of micelle formation. The significant result we have obtained is the discovery that cations and anions affect hydrophobic interactions through two different mechanisms. "Hard" monovalent cations interact minimally with hydrophobic molecules, however, adding them to the aqueous solvent causes a perturbation in bulk solvent properties. This perturbation manifests itself in "the free energy of cavitation" as defined by scaled particle theory. In other words, cation addition makes it more difficult to create a solvent cavity to accommodate the hydrophobic solute; thus, promoting hydrophobic aggregation. On the other hand, "soft" monovalent anions can weakly associate with hydrophobic moieties, thereby inhibiting hydrophobic aggregations that lead to the micelle forming process. We have then investigated the effects of the same group of cations and anions on the unfolding free energy of the protein RNase A as measured by differential scanning calorimetry (DSC). Our results suggest that ion specific effects on RNase A stability strongly mirror their effects on 1,2-hexanediol micellization. Indicating that ions mediate their specific effects on protein stability largely through the mechanism by which they affect hydrophobic interactions.

Feed-Forward Amplification in the PINK1-Parkin Pathway Acts as Binary Switch for Mitochondrial Quality Control in Parkinson Disease

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Genetic forms of Parkinson disease have revealed that removing damaged mitochondrial components through autophagy is essential for the long-term survival of neurons. Mutations in PINK1, a mitochondrial kinase, or Parkin, an E3 ubiquitin ligase, lead to an early onset form of Parkinson disease. Studies of Parkin have revealed that the ligase is natively in an autoinhibited state but becomes active upon phosphorylation by PINK1. I will summarize our past and current structural studies of Parkin and show how feed-forward activation of Parkin leads to an all-or-nothing switch that controls autophagy of mitochondria.

New Approaches in Mechanobiology: From DNA nanoswitches to single-molecule centrifugation

Wesley P. Wong

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Mechanical force plays a critical role in regulating many aspects of biological function and structure, particularly at the nanoscale. My research group develops and applies methods in single-molecule manipulation to understand the force-dependent dynamics of biological interactions. I will present some methods that we are developing, including massively parallel single-molecule force measurements using centrifugal force, and nanoscale devices built using DNA origami, and demonstrate how these approaches can bring new insights into biological processes ranging from blood clotting to hearing.

Single-Molecule Fluorescence Approaches for GPCRs: Oligomerization, Conformational Selection and Dynamics

Claudiu Gradinaru

Department of Chemical & Physical Sciences, University of Toronto Mississauga

G Protein-coupled receptors (GPCRs) constitute the largest family of transmembrane signaling proteins and the largest pool of drug targets, yet their mechanism of action remains obscure. We characterized the oligomeric status of eGFP-tagged M₂ muscarinic receptor (M₂R) and G_{i1} by single-particle photobleaching of immobilized complexes. The method was calibrated with multiplexed controls comprising 1–4 copies of fused eGFP. The photobleaching patterns of eGFP-M₂R were indicative of a tetramer and unaffected by muscarinic ligands; those of eGFP-G_{i1} were indicative of a hexamer and unaffected by GTP-γS. A complex of M₂R and G_{i1} was tetrameric in both, and activation by a full agonist plus GTP-γS reduced the oligomeric size of G_{i1} without affecting that of the receptor. The oligomeric nature of G_{i1} in live CHO cells was demonstrated by means of Förster resonance energy transfer (FRET) and dual-color fluorescence correlation spectroscopy dcFCS) in studies with eGFP- and mCherry-labeled G_{i1}. These results suggest that the complex between M₂R and holo-G_{i1} is an octamer comprising four copies of each, and that activation is accompanied by a decrease in the oligomeric size of G_{i1}. The structural feasibility of such a complex was demonstrated in molecular dynamics simulations.

Target search of DNA-binding proteins in mammalian cells: a single molecule study

Maxime Dahan

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For many cellular functions, DNA-binding proteins (DBPs) need to find specific target sites in the genome. Facilitated diffusion (FD), namely the combination of one-dimensional motion along non-specific DNA and three-dimensional exploration, is the dominant model for the target search (TS) of DBPs. Yet, this model has hardly been tested *in vivo*, particularly in the complex environment of a mammalian nucleus, and it is still controversial whether it accelerates association to specific DNA binding sites. To address that question, we have implemented a TS assay using human cells with a unique target locus for an inducible exogenous searcher, the tetracycline repressor (TetR). Using single-molecule tracking and *in situ* biochemical measurements of association kinetics, we directly characterize the mobility of TetR, its transient interaction with non-cognate DNA and the kinetics of binding to the specific locus. Overall, we find that the searcher follows a FD strategy but that the search kinetics is not limited by the diffusive motion but by the low association efficiency to non-specific DNA sites. Importantly, we observe a lack of delimitation between specific and non-specific binding kinetics, with a broad, power-law distribution of off-target binding times. Similar results are obtained for other DNA-binding proteins (TALE, Cas9, LacI,...) and we will present a simple model to account for this general observation. Finally, we will discuss novel microscopy approaches for single molecule studies in cells.

The study of cellulase-cellulose interactions and cellulose structure through single-molecule fluorescence microscopy techniques

Jose Moran-Mirabal,

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The ability to efficiently break down lignocellulosic biomass into nanocrystalline particles (cellulose nanocrystals, CNCs) or soluble carbohydrates that can be used to produce the next generation of bioproducts and biofuels has become the focus of much experimental and theoretical research. Yet, limited understanding exists on how the native cellulosic structure impacts the production of CNCs or how the enzymes that catalyze the biochemical conversion of biomass, such as cellulases, interact with crystalline cellulose. This has spurred the application of a number of high-resolution imaging techniques to obtain a glimpse of the changes in the cellulosic structure and biomolecular interactions that take place at the nanoscale. Along these lines, we have used single molecule super-resolution microscopy to study microcrystalline cellulose and found that the size of the CNCs that are produced through acid hydrolysis is dictated by the native structure of the crystalline microfibrils. Furthermore, we have used single molecule tracking to study cellulase-cellulose interactions and elucidate the ability of cellulases to diffuse along cellulose surfaces. In this presentation, I will give an overview of our experimental findings and will discuss our ongoing work on the study of cellulose structure and cellulase-cellulose interactions at the nanoscale.

Single-molecule measurements of collagen's triple helical stability

Nancy Forde

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Collagen is the fundamental structural protein in vertebrates and is widely used as biomaterial, for example as a substrate for tissue engineering. Assembled from individual triple-helical proteins to make strong fibres, collagen is a beautiful example of a hierarchical self-assembling system. In spite of its prevalence and mechanical importance in biology, surprisingly little is known about how or even whether the mechanics of the triple helix vary according to type, source or sequence. The flexibility of the triple helix is unresolved, as is its response to stress. In this presentation, I will describe the tale of collagen's mechanics that my research group is unravelling using a variety of single-molecule analysis techniques (optical tweezers, atomic force microscopy and centrifuge force microscopy). These complementary single-molecule studies are providing new insight into the molecular basis for mechanical response.

Endocytosis caused by liquid-liquid phase separation of proteins

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Clathrin-mediated endocytosis (CME) underlies intra- and extracellular material trafficking in eukaryotes, and is essential to protein metabolism, intercellular signaling, membrane remodeling and other cell regulatory processes. Although CME is usually driven by F-actin polymerization, membrane invagination can also occur through unknown actin independent mechanisms. Here, we present evidence that CME is driven by the accumulation of proteins at sites of endocytosis initiation that undergo liquid-liquid phase separation to form viscoelastic droplets. The surfaces of these droplets, through contact adhesion with the membrane and surrounding cytosol, generate the work required to drive membrane invagination. The proposed mechanism expands the repertoire of functions of membraneless organelles that form via liquid-liquid phase separation to include their ability to do work due to soft interfaces that shape and organize cellular matter.

The Emergence of RNA from the Prebiotic Mixture

Paul G Higgs

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According to the RNA World theory for the Origin of Life, the first replicating molecules were nucleic acids that had the ability to act as both a gene and a catalyst. We are studying the way that a self-replicating biological system can emerge from a non-living chemical system that is able to synthesize a mixture of random sequences. Biological RNA shows several kinds of ordered properties - it uses only 'right-handed' nucleotides rather than a mixture of both chiralities, it uses regular 5'-3' bonds between ribose sugars rather than a mixture of different bond types, and it uses a specific set of four nucleotides rather than a mixture of many other similar molecules. We will show that if template-directed replication is important, we can explain the emergence of all these ordered properties by the same mechanism in terms of symmetry breaking phase transitions.

A new method to assess lipid phases in lipid mixtures by ^{31}P Magic Angle Spinning NMR

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There are over 40,000 different lipids in the LIPID MAPS database, differing by their chains and headgroups. Such a stable diversity during evolution must reflect a specific role for each lipid, and the scarcity of knowledge on this topic is puzzling. A striking example is the lipid composition of the inner membranes of gram negative bacteria such as *Escherichia coli* which is 77% phosphatidylethanolamine (PE) and 20% phosphatidylglycerol (PG), while that of gram positive bacteria such as *Bacillus subtilis* is 18% PE and 78% PG. The main difference between PE and PG is their propensity to form different phases. While PG is a "lamellar phase" lipid, PE is known to go from lamellar to inverse hexagonal phases depending on temperature. Lipid phases are commonly studied by static solid-state ^{31}P NMR, each lipid possessing a single phosphorus, whose chemical shift anisotropy (CSA) reflects the phase it is located in. In the case of lipid mixtures, various lipid spectra overlap and it may become difficult to disentangle the various phases associated with each lipid. Here, we suggest a magic-angle spinning (MAS) approach which relies on CSA recoupling. The obtained 2D spectra reflect the composition of lipid mixtures, and the phase of each lipid is easily read. We have successfully applied this technique to mixtures of PE, PG and cardiolipin. The extension of this technique to whole cells will be discussed.

Biophysical impact of airborne pollution on model lung surfactant membranes

Christine DeWolf

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Lipid monolayers have long been used as models of biological membranes as interfacial interactions govern many important biological processes. The lung surfactant membrane serves to lower the air-alveolar surface tension to reduce the work of respiration and prevent alveolar collapse. Lung surfactant films achieve near-zero surface tensions (high collapse pressures) and recover their surface activity with each breathing cycle (reversibility on compression and expansion), achieved via a lipid-protein film existing in dynamic monolayer-multilayer equilibrium. Our work focuses on determining the extent to which inhalation of airborne pollutants (from small molecule oxidants to nanoparticulate) may interfere with the functional properties of lung surfactant using Langmuir monolayers as model membranes. The impact of ozone and silica nanoparticles on the collapse, morphology, structure and viscoelastic properties of lipid and lipid-protein films will be presented and the intercorrelation of these properties discussed. These findings will be correlated to the potential for pollutant exposure to induce immediate impairment of lung surfactant function.

Lipid-protein interactions are unique fingerprints for membrane proteins

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Cell membranes function as physical barriers for the cell and control the exchange of ions, peptides, and small molecules between the interior and the exterior of the cell. The main constituents of cell membranes are lipid molecules, whose hydrocarbon tails provide the barrier-like properties, and membrane proteins, which carry out specific functions. Complex lipid-protein interactions take place in the membrane, where proteins and lipids affect each other, strictly regulating a wide range of cellular tasks. Here, we use coarse grained (CG) molecular dynamics (MD) simulations to characterize the lipid environment of ten membrane proteins, which include examples of receptors, transporters, channels, and enzymes. To provide a realistic lipid environment, the proteins are embedded in a model plasma membrane, where more than 60 lipid species are represented, asymmetrically distributed between upper and lower leaflet (JACS, 2014, 136, 14554-59). The simulations show in detail how each protein modulates its local lipid environment in a unique way through local lipid composition, thickness, curvature, lipid dynamics and other properties.

Allosteric regulation of rhomboid intramembrane proteases

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The rhomboid superfamily of intramembrane serine proteases is highly conserved throughout evolution. The most basic form of the rhomboid protease is found in prokaryotes and consists of six – seven transmembrane domains. Crystallographic studies indicate these helices form a bundle with a buried active site consisting of a catalytic serine-histidine dyad (PNAS 2007). Our recent data revealed that rhomboid proteases from *E. coli*, *Haemophilus influenza* and *Providencia stuartii* form dimers in membrane (EMBOJ 2014). Furthermore, catalytic activity was dependent on dimerization, and transmembrane substrates were cleaved with positive cooperativity. Competitive inhibition studies demonstrated substrate recognition proceeds through the recognition of an exosite. Since crystallographic analysis revealed a monomeric enzyme, the nature of this functional dimer remained elusive. Here we show SAXS data to reveal the nature of this dimer within the rhomboid superfamily, and use mutagenesis to probe the functional nature of the dimer. This study provides insight into the dynamic nature of intramembrane proteases that is needed to achieve substrate cleavage.

A combined computational / experimental approach to increase the efficiency of enzyme engineering

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Despite key advances in our capacity to engineer enzymes, we remain very much in the dark with respect to predicting the effects of mutations on function. A central aspect of our incapacity to predict sequence-function relationships is the fact that proteins are dynamic, yet we rarely treat them as such. Using the β -lactamase system, we examine the effects of sequence alterations on protein dynamics. Comparison of the CPMG NMR backbone dynamics and molecular dynamics simulations of several β -lactamases reveals them to be unusually rigid, with some motions centered about the active site region. This is consistent with evolutionary conservation of dynamics and suggests a functional role. Sequence changes in the active-site area altered the dynamics, yet catalytic function was maintained. Our results indicate that β -lactamases are highly adaptable. Furthermore, we show that enzyme engineering need not preserve native-like dynamics in order to maintain function. We then apply computational methods to a cytochrome P450 system, to predict the trajectory of ligand binding and entry into the active-site cavity. We apply the Implicit Ligand Sampling and Adaptive Biasing Force methods to successfully predict, in one single simulation, all residues known to be important for fatty acid substrate binding, thus confirming predictive accuracy. In addition, a new binding residue was identified and experimentally confirmed, and a mechanism for evolutionary protection against CO poisoning is proposed. These new computational biology approaches show great promise to guide efforts to identify functional hotspots for mutation.

Disorder in protein interactions

Jörg Gsponer

Michael Smith Laboratories, UBC, Vancouver, Canada

Intrinsically disordered regions (IDRs) play an essential role in the regulation of function and interaction of many signaling proteins. I will discuss insights that we recently gained on their regulatory mechanism and how this knowledge can be exploited in the prediction of IDR function.

Using High-Resolution Frequency-Domain Spectroscopy for Studying Protein Dynamics and Energy Transfer In Pigment Protein Complexes – Photosynthesis and beyond

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Pigment-protein complexes involved in photosynthesis offer a unique opportunity to explore native protein environments using optical spectroscopy methods, as chromophores are built into them by Nature, without any extraneous manipulations that could potentially alter the structure or dynamics of the protein. Single Molecule (or single complex) Spectroscopy has recently been a technique of choice for studying spectral dynamics in photosynthetic complexes. However, Non-Photochemical Spectral Hole Burning (NPHB) is capable of providing additional or competing information. In particular, I will show that most of the spectral line shifts observed in single complex experiments are in fact light-induced (and not occurring anyway whether one observes them or not) and constitute NPHB on a single-molecule level. Inspired by our early results on LH2 complex, we undertook a detailed NPHB study of spectral dynamics in several other systems, such as CP43 antenna complex and Cytochrome b_6f . We also developed a unified approach to modeling NPHB and spectral hole. This approach relies on the argument that in the presence of “spectral memory” (spectral holes recovering mostly due to “burnt” pigment-protein systems returning to the pre-burn configuration) the barrier distributions encoded into the non-saturated spectral holes and manifesting during the hole recovery differ from the full true barrier distributions. These partial barrier distributions are vastly different for different shapes of the true full distributions, and one can easily distinguish their manifestations. Quantitatively, all complexes we have explored so far exhibit barriers in the same range, distinct from that of simple organic glasses explored by similar methods. Qualitatively, however, barrier distribution shapes show great variability. I will also discuss the possible nature of the entities responsible for NPHB in pigment-protein complexes, possible cooperative effects, how understanding protein dynamics helps understand issues more directly related to photosynthesis such as excitation energy transfer, etc.

Solution NMR Investigation of the Lateral Gate in GlpG Rhomboid Protease

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The rhomboid family of intramembrane serine proteases has a unique ability to catalyze proteolysis below the surface of the cell membrane, making them key players in numerous biological processes such as parasitic host cell invasion and growth factor signaling. High-resolution X-ray crystal structures of the *E. coli* GlpG rhomboid reveal six transmembrane helices which form an active site that is sequestered from the membrane environment. Previous studies demonstrate a correlation between the dynamics of transmembrane helix 5 (TM5) and activity, suggesting a conformation change in this region is required to facilitate substrate access to the active site. However, the structural nature of substrate gating and the physiological relevance of open structures captured by x-ray crystallography remain a matter of debate. Here, solution-state NMR has been used for the first time to characterize a TM5-open conformation. Furthermore, interactions involving a peptide-based inhibitor provide evidence that this open conformation represents a physiologically-relevant state involved in substrate gating. We also provide kinetic evidence that catalytic activity of the open state can be compromised, potentially by the incursion of lipids or detergent molecules into the active site, and that this can be attenuated by the presence of a transmembrane segment in the substrate. This preference for transmembrane substrates imposed by the sensitivity of the open state to the lipid phase provides a mechanism through which rhomboid selectivity could be enhanced in-vivo.

Probing the Movement of the Ball and Chain during N-type Inactivation in Kv Channels

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N-type inactivation is a mechanism in certain Kv channels where the N-terminal peptide occludes the pore upon depolarization resulting in block of ionic currents (ball-and-chain). Numerous mutational studies have characterized N-type inactivation functionally, while X-ray crystal structures have yet to include the ball-and-chain structure. It still remains unknown how far the N-terminus travels during N-type inactivation and where it is located in the resting state. Does it reside in a fixed position near the T1 window or is it randomly floating in the cytosol? By incorporating a fluorescent unnatural amino acid (Anap) into the N-terminus and into receptor sites (T1 window and S4-S5 linker) we directly tracked the movement of the ball peptide using voltage clamp fluorometry in *Xenopus* oocytes.

Partially native intermediates mediate misfolding of SOD1 in single-molecule folding trajectories

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Prion-like misfolding of superoxide dismutase 1 (SOD1) is associated with the disease ALS, but the mechanism of misfolding remains unclear, partly because misfolding is difficult to observe directly. We used optical tweezers to study the most misfolding-prone form of the protein, reduced un-metalled monomers. Measuring unfolding and refolding of isolated monomers, we found that the folding is more complex than suspected, resolving numerous previously undetected intermediate states corresponding to the sequential formation of each β -strand in the native structure. We identified a stable core of the protein that always unfolded last and refolded first, and directly observed several distinct misfolded states that branched off from the native folding pathways at specific points after the formation of the stable core. Partially-folded intermediates thus play a crucial role mediating between native and non-native folding. Comparing the dimer to the monomer suggests that the dimerization enhances the cooperativity and prevent misfolding. These results help explain the propensity of SOD1 for prion-like misfolding and point to targets for therapeutic intervention.

Back to the future: adventures through acetylcholine receptor space and time

Corrie J.B. daCosta

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Acetylcholine receptors (AChRs) are members of a superfamily of proteins called pentameric ligand-gated ion channels, which are found in almost all forms of life, and thus have a rich evolutionary history. Muscle-type AChRs are heteropentameric complexes assembled from four related subunits (α , β , δ , and ϵ). Here we reconstruct the amino acid sequence of a β -subunit ancestor shared by humans and cartilaginous fishes (i.e. Torpedo). Then, by resurrecting this ancestral β -subunit and co-expressing it with human α -, δ -, and ϵ -subunits, we show that despite 132 substitutions, the ancestral subunit is capable of forming human/ancestral hybrid AChRs. Whole cell currents demonstrate that the agonist acetylcholine has reduced potency for hybrid receptors, while single channel recordings reveal that hybrid receptors display reduced conductance and open probability. Our results outline a promising strategy for studies of AChR evolution aimed at identifying the amino acid origins of AChR structure and function.

Folding and Quality Control Mechanisms of Collagen

Matthew D. Shoulders

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Collagen is the molecular scaffold for multicellular life and by far the most abundant protein in the human body. Collagen presents a uniquely complex and still poorly elucidated proteostasis challenge to cells. Imbalances in collagen proteostasis are related to diverse, currently incurable diseases ranging from osteogenesis imperfecta (brittle bone disease) to fibrosis. Our approach focuses on illuminating molecular details of intracellular collagen folding and quality control, followed by using what we learn to identify potential new therapeutic approaches for the collagenopathies. We will discuss: (1) A molecular code that critically controls collagen assembly, based on the identity of a single atom in the globular C-propeptide domain; and (2) Mass spectrometry-based proteomics studies leading to the discovery that the endoplasmic reticulum proteostasis network can be remodeled to resolve collagen-I production defects associated with disease.

Understanding and designing cyclic peptides

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Cyclic peptides (CPs) are highly sought after for several unique applications. For example, CPs can target protein surfaces with high affinity and selectivity, thereby inhibiting specific protein-protein interactions that cannot be easily targeted with other molecules. New inhibitors will enable mechanistic studies to dissect the functions of individual protein-protein interactions in the complicated cellular interactome. However, robust application of this fundamentally interesting class of molecules for these and other purposes is limited by our poor capacity to predict CP structures and the resulting inability to rationally design functional CPs. In this talk, we describe an efficient enhanced sampling method to simulate CPs, using which we aim to fill the knowledge gap of CP sequence-structure relationships, and enable rational design of CPs with desired structures.

Keep your C2H2 fingers off my DNA

Pierre Lavigne

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The C2H2 Zinc Finger (ZF) motif is the most conserved protein fold in the annotated human genome. Since the discovery of their ($\beta\beta\alpha$) 3D structure, ZFs have been assigned DNA binding and recognition roles in transcriptional regulation. Genes that contain ZFs have evolved by duplication from an ancestor gene coding for only a few (two or three) ZFs. In fact, more than 700 hundred genes in the annotated human genome possess ZFs and the average number of ZFs in these genes is 8.5 with some encoding for more than 30. Why did these genes evolve to possess so many ZFs? The first reason that comes to mind is to promote highly specific DNA binding. However, this is unlikely, considering that a specific sequence of 12 base pairs, that would be recognized by four ZFs, will only occur once every Giga base pairs in a genome. Therefore, poly-ZF proteins may have also evolved to limit their DNA binding abilities. I will present the biophysical characterization of our prototypical poly-ZF protein; c-Myc Interacting Zinc finger protein 1 (Miz-1) which contains 13 ZFs and report that; **1-** only a subset of ZFs can bind DNA specifically, **2-** four of the ZFs undergo unsuspected conformational exchange, **3-** a compact inter-ZF structure exists and **4-** that one inter-ZF linker introduces electrostatic repulsions with the phosphodiester DNA backbone. Collectively, our results support the notion that poly-ZF proteins have evolved to limit their DNA binding capabilities. I will discuss emerging and alternative biological roles for poly-ZF proteins.

Studying the phase separation and material properties of elastin using NMR spectroscopy

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The liquid-liquid phase separation of proteins, resulting in the formation of dynamic and responsive droplets, is becoming rapidly recognized as an important process underlying many critical aspects of biology. In the case of elastin, phase separation occurs in response to salt and heat, and is an essential step in formation of the elastic fibre. The atomistic details of the coacervation process, and of the structural and dynamic properties of the resulting phase-separated state, are of significant interest, but remain poorly understood. We have recently designed a set of elastin-like polypeptides (ELPs) that accurately mimic the assembly and mechanical properties of tropoelastin, and that are ideally suited for detailed structural analysis using NMR spectroscopy. Using a combination of solution and solid state NMR spectroscopy, we have been able to directly observe the phase transition of these ELPs and obtain site-specific details of structure and dynamics throughout the coacervation process. Our data confirm the presence of highly disordered hydrophobic domains in all states from monomer to crosslinked material, while also revealing important local structural elements within both hydrophobic and crosslinking domains that are likely tied to the elastic properties of the ELPs. Likewise, we are able to discern changes in global and local dynamics that occur during the coacervation process, which shed new light on the nature of coacervation and of the phase-separated state of elastin.

Developing a Mechanistic Understanding of Spider Aciniform Silk Toughness

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Of the fibrous spider silks, aciniform silk is the toughest due to a combined high strength and extensibility. The major component of spider silk proteins ("spidroins") is a repetitive domain unique to a given silk-type. In most spidroins, this consists of numerous copies of short motifs typically ≤ 10 residues in length. Conversely, the aciniform spidroin repetitive domain contains far longer, internally heterogeneous repeat units - 200 residues in the *Argiope trifasciata* aciniform spidroin we study (the "W unit"). Most silk fibres lack α -helical content, with strength typically associated with oriented β -sheet domains and extensibility with β -turn-rich disordered domains. Aciniform silk, conversely, contains approximately equal proportions of α -helix and β -sheet alongside disordered structuring. Facilitated by recombinant production of proteins ranging in length from 1-4 concatenated W units in *Escherichia coli*, we have been characterizing the structural transition between the soluble and fibrous states. Using solution-state NMR spectroscopy, we demonstrated that the W unit behaves as a compact series of beads (α -helical bundles)-on-a-string (*intrinsically disordered linkers*). Compellingly, locally increased backbone dynamics directly correlates with a helical region more readily denatured than the remainder of the helical bundle. Hypothetically, localized denaturation during fibrillogenesis would enable the transition to mixed α -helical/ β -sheet structuring, with distinct segregation of units rich in each class of secondary structural element. To test and refine this hypothesis, we are targeting an atomic-level understanding of all stages of aciniform silk fibre formation and the features giving rise to its extraordinary toughness.

Improving proteins through point mutations is unreliable and may trade stability for solubility

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Increasing protein stability is a highly desirable yet elusive goal, leading to higher production yields, longer shelf-life, improved activity, and reduced immunogenicity. Yet, random mutagenesis yields increased stability in only ~2% of cases, and often of marginal magnitude. Computational tools predicting stability change upon mutation have been developed to address this problem. Accuracies of ~80% are often reported, yet experimental success rates when engineering increased stability are ~25%. Analyzing 21 prediction tools reveals that destabilizing mutations - making up the overwhelming majority of existing datasets - are predicted with >80% accuracy, whereas correct identification of stabilizing mutations is rare. In effect, these tools filter out a large proportion of the worst mutations, leaving behind a pool of moderately destabilizing, neutral, and stabilizing mutations to randomly select from. Furthermore, many of the stabilizing mutations that are correctly identified result in increased hydrophobicity at the protein surface. As such, even when thermodynamic stability is increased, solubility is often lost. Using robust performance metrics, methods showing promise for future protein engineering are highlighted, and their particular advantages discussed.

Rational Design of Proteins that Exchange on Functional Timescales

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Proteins are the molecular machines of life, carrying out complex physical and chemical processes that often require concerted motions of local protein structural elements. Previous efforts to design new proteins for applications in research, industry, and medicine have focused on the creation of sequences that stably adopt a single target structure, ignoring the potential impact of protein dynamics in function. Although computational protein design has enjoyed considerable success in creating new proteins using this approach, most have failed to match the efficiencies that are found in nature because standard methods do not allow for the design of exchange between necessary conformational states on a functionally-relevant timescale. Here, we develop a broadly-applicable computational method to engineer protein dynamics that we term *meta*-multistate design. We used this methodology to design spontaneous exchange between two novel conformations introduced into the global fold of Streptococcal protein G domain β 1. The designed proteins, named DANCERs, for *Dynamic And Native Conformational ExchangeRs*, are stably folded and exchange between predicted conformational states on the millisecond timescale, as evidenced by nuclear magnetic resonance structures and ZZ-exchange experiments. The successful introduction of defined dynamics on functional timescales paves the way to new applications requiring a protein to spontaneously access multiple conformational states.

Protein Sequencing by Nanopore Mass Spectrometry

Derek Stein

Department of Physics, Brown University

Our group is developing a single-molecule protein sequencing technology that aims to combine the benefits of nanopores with the speed, sensitivity, and robustness of chemical analysis by mass spectrometry. The basic idea is to cleave the individual amino acids from a protein molecule as they transit a small hole in sequence, and to identify each one by determining its charge-to-mass ratio in a mass spectrometer. This talk will describe the concept, the development of a prototype nanopore mass spectrometer, and the results of studies of the transfer of single ions from liquid into vacuum from the nanoscale orifice of a charged needle.

DNA Capture and Sequencing using Nanopore-coupled Zero-Mode Waveguides

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We are developing a new platform for high-throughput single molecule, real-time (SMRT) sequencing of DNA and RNA molecules. The system integrates the familiar zero-mode waveguides (ZMWs), optical wells in which single-dye fluorescence signals are acquired during DNA replication, with pore-containing membranes, which allow electrophoretic molecular focusing of charged analytes into the ZMWs for subsequent interrogation. We observe an interesting packaging process of DNA into the waveguides, length dependence, and anomalously long residence times, which we attribute to entanglement of the DNA inside the ZMWs.

2D Materials Nanosculpting and Bioelectronics Nanopore Applications

Marija Drndic

University of Pennsylvania, United States

Electron beams constitute powerful tools to shape materials with atomic resolution. I will describe experiments that push the limits of device size to atomic scale in 2D materials and expand their function and precision, while addressing fundamental questions about structure and properties at atomic scales. Experiments include fabrication of nanoribbons and field-effect-transistors from novel two-dimensional materials down to sub-nm widths and the ultrafast, all-electronic detection and analysis of biomolecules with nanopores. As molecules are driven through nanopores in solution, they block the ion current flow resulting in current reductions from which molecule's physical and chemical properties are inferred. DNA, proteins, microRNA and other biomolecules can be analyzed. The temporal, spatial resolution and sensitivity in these experiments have been improved over the last few years thanks to advanced materials, device designs and new electronics.

Kinetics of polymer translocation through nanopores under nanoscale pre-confinement

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We present a comprehensive experimental and simulation study of a novel nanosensor comprising a two-membrane system, wherein a highly porous nanofilter membrane is separated from a single solid-state nanopore by a nanoscale gap. We demonstrate that the presence of the nanofilter has dramatic effects on the kinetics of dsDNA passage through the sensing pore, reducing the spread in passage times and in some cases linearizing dsDNA and preventing folding. We also show that the presence of the nanofilter completely removes the dependence of passage time distributions on pore size and allows for pore size instability without compromising signal quality. This study experimentally verifies that the molecular passage time distribution depends critically on the conformation of dsDNA prior to translocation through a solid-state nanopore, reveals fundamental polymer physics details about the capture process which have been inaccessible until now, and provides practical tools with which researchers can control the kinetics of biomolecular passage through solid-state nanopores.

Squeezing New Information out of Macromolecules Using Adjustable Nanoconfinement

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Our goal is to understand the physical principles that determine the structure and dynamics of macromolecules such as DNA; principles that are central to researching essential information storage and replication functions which underlie life, as well as to enabling new biotechnologies. This goal has motivated the development of single-molecule methods that allow us to visualize spatiotemporal dynamics under applied nanoconfinement. While existing methods often face challenges in gently formatting, chemically modifying, and visualizing delicate DNA polymers and protein-DNA complexes in nanofluidic environments, we present a new platform for gently loading and reacting delicate biopolymers and complexes in a wide range of nanostructures. Our single-molecule manipulation and visualization platform uses the principle of “Convex Lens-induced Confinement” (CLiC). In CLiC, we can continuously adjust the height of a nanofluidic imaging chamber to gently and dynamically unravel long DNA polymers into embedded nanostructures, such as nanogroove or dimple arrays, from above (Berard et al, PNAS 2014 and APL 2016). Recently, we have integrated controlled, in-situ chemistry procedures within the CLiC nanofluidic device, allowing us to first format DNA polymers in embedded nanostructures, and subsequently introduce reagent molecules with exquisite temporal control. In this talk, we explore how the configurations of DNA molecules, introduced through either enzymatic processes, or through applied nanoconfinement, can influence their dynamics and interactions with other molecules. Beyond studies of DNA molecules, the flexibility of our assay opens the door to performing new measurements of weak and slow molecular interactions in a wide range of biophysical, biochemical, and biotechnological contexts.

Exploring the Structure of PhytoSpherix Nanoparticles via Atomistic and Coarse-Grained Simulations

Hendrick W. de Haan

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PhytoSpherix nanoparticles are a promising green nanotechnology. Derived from sweet corn, not only are they biodegradable but are in fact biocompatible. They also exhibit some remarkable properties such as high monodispersity, high water absorption and retention, and interesting viscous properties when used as an additive. However, fundamental questions about the structure of the nanoparticles remain. At a basic level, estimates for the size of the particles vary greatly between different experimental approaches. Likewise, the density profile of the particles and characterization of the surface are open questions. At an even more detailed level, the nature of the interactions between the polysaccharide chains and the resulting porous nature of the particles are of interest to understand the both the interactions between the particles and water and for the development of new applications such as drug delivery. In this talk I will present simulations of PhytoSpherix nanoparticles across a range of scales extending from detailed, atomistic simulations of sections of particles up to coarse-grained models that simulate entire particles. Results from all levels of detail are combined to present a holistic view of the nanoparticles and to give new insight into the structure of the particles and why different experimental methods might give different answers for the particle size. Results concerning details of water inside of the particle and the implications of the structure for drug delivery applications will also be presented.

Nanopores : from polymer physics to biological applications.

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The nanopore technique is now widely used to study transport of polymers and to develop new probes for molecules, for molecules conformation, for biological objects such as bimolecular complexes. The simple experimental concept based on the resistive pulse technique has been extensively broadened toward multiple applications. In this presentation I will present how the nanopore can be used as a ideal tool to explore some basic concepts of polymer physics which were theoretically predicted 30 years ago by de Gennes. The nanopore is also a good choice for the conformation probing of biological object. I will then focus on biological applications like sensing conformational change of a biological structure by interpreting complex nanopore signals. The concept of the technique will be presented with experiments using DNA. Finally, the manipulation of single protein using nanopore, to study sequential unfolding, will be exposed.

POSTER PRESENTATION ABSTRACTS

P01 - Biochemical role of Threonine-77 in Atlantic salmon tropomyosin Tpm1.1

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Tropomyosin is a dimeric, coiled coil protein in the thin filament of striated muscle. Atlantic salmon contains a single (Tpm1.1) isoform that is >92% identical (20 substitutions) to the more conformationally-stable mammalian homologue. The basis of the relative instability of salmon Tpm1.1 was investigated by mutagenically reversing the only charge substitution; Thr-77 in salmon, Lys in rabbit. The introduction of a positive charge (confirmed by mass spectrometry) increases electrophoretic mobility (of the mutant and fragments that encompass residue-77) in the presence of SDS, a detail that serves as a convenient reference. Thermal stability was analyzed by calorimetry (0.1 M KCl, 1 mM DTT; pH 7.0). Two unfolding transitions are observed, the mid-points (Tms) of which are approximately 5 °C higher in the case of the mutant, 35 (minor) and 44 °C (major), than the control. Under the same conditions, the Tms of rabbit Tpm1.1 are: 42 (minor) and 53 °C (major). Unfolding of the Nt CNBr fragment (CN1A, residues 11-127) is monophasic, Tms 45 (mutant) vs. 40 °C (control). The sites of chymotrypsin digestion (0.1M NaCl, pH 8.5; Temp 10-30 °C) were characterized by Edman-sequencing of Western blotted peptides and mass spectrometry. Compared to the control, mutant tropomyosin displays marked resistance to proteolysis of the 11th peptide bond, the initial cleavage site, as well as the 169th, the secondary site. The main chymotryptic site in the mutant CN1A, between residues 88-89, is also less susceptible to digestion. Molecular modeling (PyMOL) indicates that Lys-77 ion pairs to Glu-82 in the adjacent chain. The loss of this ionic-interaction (in salmon Tpm1.1) is concluded to be a key factor in modulating the conformational stability of more-than-half (>150 amino acids) of the molecule. Since we also show that salmon Tpm1.1 has high affinity for F-actin at temperatures below 10 °C, the resulting destabilization is proposed to provide sufficient flexibility to achieve an optimum interaction in a cold marine environment.

P02 - Brighter red fluorescent proteins display reduced structural dynamics

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Red fluorescent proteins (RFPs) are extensively used genetically-encoded fluorophores. For all imaging applications, brighter variants are desired. Brightness is directly proportional to quantum yield (QY), and QY improvements can theoretically be achieved by decreasing dynamics of the chromophore. Although it has been demonstrated that optimization of local packing interactions intended to reduce the chromophore's conformational freedom can provide brighter RFPs, backbone dynamics at sites both proximal and distal to the chromophore can also influence QY but the magnitude and extent of this effect has never been systematically evaluated. Here, we study this relationship using nuclear magnetic resonance (NMR) spectroscopy and molecular dynamics (MD) simulations of the mCherry RFP (QY = 0.22) and its dim variant mRojoA (QY = 0.02). A residue-by-residue comparison using ¹H-¹⁵N HSQC spectra showed line-width broadening in roughly 20% of backbone amide peaks in the dim mRojoA variant's spectrum when compared to their mCherry equivalent. Since peak line-widths are influenced by microsecond-second timescale motions, this suggests that mRojoA exhibits conformational flexibility in regions that are not dynamic on this timescale in mCherry. CPMG relaxation dispersion experiments revealed evidence of millisecond-timescale motion in 22 residues in mRojoA, versus only 7 in mCherry. However, relaxation dispersions magnitudes were small, suggesting that dynamics in these RFPs are dominated by shorter-timescale motions. To probe faster timescales, we ran 20-nanosecond MD simulations on mCherry and mRojoA, as well as an mRojoA mutant possessing a QY of 0.05. Root-mean-square fluctuations in residues surrounding the chromophore correlate very strongly ($R^2 = 0.81$) to their QY. To validate this result, we are now investigating nanosecond-picosecond timescale dynamics of these RFPs by NMR. In the long term, we will use this information to rationally design bright RFP variants.

P03 - Understanding skin impermeability: structure characterization and permeability measurements

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The *Stratum corneum* (SC), the top layer of skin, is an impermeable composite material consisting of hydrophobic protein blocs glued together with stacks of unusual membranes that determine both the rate of water loss through the skin, and absorption of exogenous molecules into the body. The crystalline organization of SC is believed to be a key feature associated with the very limited permeability of skin. Recently we characterized the organization of SC lipid models that include, as real SC, a free fatty acid (FFA) series (saturated with C16-C24 chains), as well as an unusual SC lipid: a ceramide bearing an oleate linked to a very long acyl chain (CER-EOS). The latter is reported to be essential for the native SC lipid structure. Our findings propose a novel structural role of CER-EOS as our ²H-NMR data indicate that this lipid leads to a very fluid fraction in the SC model; therefore this lipid is a strong modulator of SC solid/fluid balance. We examined the possibility to bring this structural information to the functional level. We are currently developing a Raman microspectroscopic approach to measure the permeability of model SC lipid systems. The method presents several advantages including a full control of the lipid composition of SC models, and hydration conditions similar to real skin. The permeability and structural measurements bring insights into the correlation between organization and impermeability of SC, leading to a significant advance for understanding (and controlling) the skin barrier.

P04 - Free Energy of a Polymer in Slit-like Confinement from the Odijk Regime to the Bulk

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We directly measure the free energy of confinement for semiflexible polymers from the nanoscale to bulk regimes in slit-like confinement. We use convex lens-induced confinement (CLIC) microscopy of DNA to directly count molecules at equilibrium in a single chamber of smoothly increasing height. Our data (Leith, Kamanzi et al, *Macromolecules* 2016), acquired across a continuum of confinement regimes, provide a bridge with which to connect scaling theories established for qualitatively different regimes. We present new experimental data and simulations that connect the Odijk theory describing sub-persistence-length confinement, the interpolation model by Chen and Sullivan extending Odijk to moderate confinement, and the Casassa theory describing the transition from moderate confinement to bulk. Further, this work establishes a robust, quantitative platform for understanding and manipulating biopolymers at the nanoscale, with key applications toward optical genomic analysis tools and emerging nanopore electrical single-molecule detection.

P05 - Studying Protein Energy Landscapes in Dimeric Cytochrome b_6f with Optical Spectroscopy

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Hole burning spectroscopy was employed to explore low-temperature protein dynamics, protein energy landscapes and energy transfer processes in Cytochrome b_6f . Cyt b_6f mediates the transfer of electrons between Photosystem II and Photosystem I, while also transferring protons across the thylakoid membrane. Most of the cofactors in Cyt b_6f are involved in these charge transfer processes. Just two chlorophyll molecules are present in Cyt b_6f dimer. Weak inter-chlorophyll coupling and lack of excitonic effects makes Cyt b_6f a very useful system for spectroscopic studies of protein energy landscapes. Unlike in CP43 core antenna complex of PSII, the distributions of barriers between protein sub-states involved in light-induced conformational changes (non-photochemical spectral hole burning) appear to be glass-like $\sim 1/\sqrt{V}$ (V is the barrier height), and not Gaussian. Quantitatively, the barriers in Cyt b_6f fall in the same range as in CP43 and other antenna complexes. Although the glass-like barrier distribution could indicate denaturing of the protein, with chlorophylls being exposed to the surrounding (glassy) buffer-glycerol matrix, in fact the protein remains intact. There is evidence of energy transfer between the chlorophylls with the rate in agreement with (intact) structural data. Deuteration of the environment surrounding the protein also has very little effect on protein dynamics. Results also indicate that two tiers of the protein energy landscape are likely simultaneously probed in spectral hole burning experiments, possibly just creating an illusion of $\sim 1/\sqrt{V}$ barrier distribution. These two tiers possess nearly degenerate distributions of the tunneling parameter, originating, however, from different combinations of barrier heights and the mass of the entity experiencing tunneling. Cooperative small conformational changes are also likely.

P06 - Transcriptional foci assembly by phase separation in prokaryotes

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Recently, it was found that eukaryotic membrane-less organelles assemble by phase separation (i.e. formation of liquid drops within a liquid mixture). Bacteria do not have membrane bound vesicles so we can suppose that phase separation drives cytoplasmic granule assembly. Intrinsically-Disordered Proteins (IDPs) tend to drive molecules to phase separate and it is predicted that *E. coli*'s genome has a relatively low proportion of IDPs (4%) compared to mammals. However, transcriptional proteins are surprisingly enriched in IDPs. Interestingly, RNA polymerase (RNAP) localization changes following growth in different media (from diffuse in poor growth media to formation of transcriptional foci (TF) in rich growth media). I have developed a quantitative assay to measure the formation of TF in living cells through imaging of a *rpoC::mCherry E. coli* strain (RNAP). Harralick texture analysis shows that cells grown in rich media have more fluorescence contrast than cells grown in minimal media. Currently, I am performing a targeted screen on a list of *E. coli* transcriptional proteins that we predict to be disordered (DP). I generated DP deletion mutants in the *rpoC::mCherry* strain in order to analyze TF assembly. The Harralick texture analysis assay will be used to establish a phase diagram of TF assembly in relation to environmental conditions and in the DP mutants. Thus, I am using high-resolution microscopy of a simplified bacteria system and a reverse genetic screen to determine how growth conditions affect TF assembly.

P07 - Determination of Lipid Phase Behaviour in Drug Delivery Systems via Small Angle X-Ray Scattering

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The use of lipid nanoparticles (LNPs) as a delivery mechanism for small interfering RNA (siRNA) offers exciting new avenues in gene therapy. While exogenous RNA is typically degraded in the bloodstream due to interaction with proteins, RNase and immune system cells, the LNP offers protection while en route to its target location. During endocytosis, the LNP enters an endosome that will experience a decrease in pH. The increased acidity will further protonate the cationic lipid of the LNP, allowing for interaction with anionic lipids found in the endosomal membrane. These interactions are thought to result in the formation of non-bilayer phases, which disrupt the endosomal membrane and release siRNA into the cytoplasm for participation in the RNA interference mechanism. To investigate the formation of such non-bilayer phases, I use small angle X-ray scattering (SAXS) on systems containing DLin-K5-C2-DMA (XTC2, a cationic lipid found in the LNP) and Lysobisphosphatidic acid (LBPA, an endosomal, anionic lipid). Comparing our mixture to known non-bilayer phases allows us to determine whether LBPA:XTC2 systems form a single phase or a mixture of phases. This could include the inverted hexagonal (H_{II}) phase or a bicontinuous cubic phase such as the Pn3m or Im3m phase. By analyzing the SAXS data, we determine lattice parameters needed for computer simulations which will aid in the design of future nanoparticles. These results lay a foundation that will help improve the release efficacy of therapeutic siRNA resulting in gene silencing.

P08 - Towards a model of the cell envelope of *Mycobacterium tuberculosis*: Martini coarse-grained force field parameters for mycolic acids

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Mycolic acids (MAs) are unique long chain fatty acids that are the major component of the outer membrane of mycobacteria, including *Mycobacterium tuberculosis*. The properties and conformations of MAs, however, are poorly understood. Here, we parameterized coarse-grained models for two major MAs (α -MA and keto-MA) following the philosophy of the Martini force field. The parameters were optimized to reproduce atomistic results of monolayers directly and experimental results with different areas per lipid. Structural properties of monolayers such as distributions of bonds and angles, thickness, order parameters, and ratios of different conformations, and dynamical properties such as the lateral diffusion constant can be modeled by our CG force field, and match the atomistic quantities. These simulations revealed that decreasing the area per lipid unfolds α -MAs, but affects Keto-MAs only slightly. We further modeled bilayer membranes with different compositions and different initial conformations. The population of conformations and bilayer thickness were analyzed, and compared with experimental results. Furthermore, the best characterized porin in the outer membrane, MspA, was inserted into the bilayer model, which leads to curvature of the bilayer to fit the thickness of the hydrophobic part of MspA. These models will be a good start for large scale CG simulations of the *Mtb* cell envelope.

P09 - Atomic Force Microscopy Imaging and Particle Size Determination of Soft Phytoglycogen Nanoparticles

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Phytoglycogen nanoparticles, extracted and purified from sweet corn, are a promising sustainable nanomaterial with many applications in the personal care, nutraceutical and biomedical industries. These applications rely on the size of the nanoparticle as well as exceptional properties that emerge from its highly branched structure and unique interaction with water. We have used atomic force microscopy (AFM) imaging to resolve a large discrepancy in the nanoparticle radius measured using dynamic light scattering (DLS) and small angle neutron scattering (SANS), and have calculated the effect of hydration on the nanoparticle size. The AFM measurements are challenging because of the “stickiness” and deformability of the soft nanoparticles. By significantly reducing the interaction between the AFM tip and the “sticky” nanoparticles, we were able to obtain high quality images in both air and water. By measuring the distribution of the volume of isolated particles in air, we calculated the corresponding average effective spherical radius of the particles. By comparing the volumes of nanoparticle aggregates measured in air and water, we determined the effect of hydration on individual nanoparticles. The values of the radius of phytoglycogen nanoparticles measured using AFM in both air and water are in excellent agreement with the value determined using SANS. The much larger particle radius determined using DLS indicates that the diffusion of the nanoparticles is significantly slower than expected. Our AFM measurements of the soft phytoglycogen nanoparticles illustrate the distinct advantages of AFM over other imaging techniques for visualizing nanoscopic soft objects in a liquid environment.

P10 - Study of the functional interaction between RNF-167 Ubiquitin ligase and a library of conjugating ubiquitin E2

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Brain functions depend on the ability of neuronal cells to efficiently and accurately communicate for various processes such as memory and learning. Knowing that the abundance of the AMPA-type glutamate receptor (AMPA) at synapses determines neurotransmission efficacy, there is consequently a critical need for research aimed at elucidating mechanisms regulating AMPARs at hippocampal synapses. Accordingly, recent evidences show that AMPAR are modified by ubiquitin (UB), a functionally relevant posttranslational modification that acts to regulate receptor trafficking, endocytosis and lysosomal degradation. In this manner, it was recently reported that RNF167, an UB ligase enzyme who brings specificity to the ubiquitination process by recognizing the substrate protein, controls AMPAR membrane expression. However, our understanding the molecular characteristics and intrinsic properties of RNF167 are exceptionally limited. The main objective of this study is to identify and characterize UB-conjugating enzymes (UBE2) functionally interacting with RNF167. To reach this goal, we implemented biochemical and biophysical assays. First, we optimized the coding sequence of the C-terminal portion of RNF167 (aa 196-350) that includes the RING domain (aa 230-271) but excludes the transmembrane domain. Then, we optimized the expression of the recombinant protein HA-RNF167-6xHis in BL21-GOLD (DE3) pLysS bacteria and then purified the protein by affinity chromatography. Then, we developed an *in-vitro* ubiquitination assay to determine which E2 functionally pair with purified RNF167. Our result shows that our purified RNF167 is functionally active with a subset of the twenty-nine UBE2s functionally pairing with RNF167. Moreover, the RNF167/UBE2s interactions are validated by Surface Plasmon Resonance (SPR) to gain key insights regarding the kinetics of interaction. We covalently immobilized an anti-HA antibody on the surface of a CM5 chip to capture our recombinant protein HA-RNF167-6xHis, which can then be used to determine the UBE2s kinetic parameters of interaction at different concentrations. The results demonstrate so far that the kinetic parameters correlate well with a transient physiological interaction. In conclusion, our study demonstrates that RNF167 can selectively bind to some UBE2s and that characteristic functional interactions are performed according to kinetic parameters that can be associated with physiological processes.

P11 - The Effects of a Cationic Lipid on a Phospholipid Bilayer: A Deuterium NMR and Small-Angle X-ray Scattering Study

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Lipid nanoparticles (LNPs) provide a fundamental mechanism for drug delivery in gene therapy. Small interfering RNA (siRNA) can be encapsulated within LNPs for delivery into target cells. LNPs contain several components: distearoylphosphatidylcholine (DSPC), a cationic lipid, cholesterol, and a polyethylene glycol-lipid. Each of these constituents plays a role in the successful delivery of the siRNA. The cationic lipid, DLin-KC2-DMA, facilitates the release of the siRNA into the cytoplasm via attractive electrostatic interactions between itself and the anionic lipids found in the cell membrane. These interactions are thought to induce a phase change from bilayer to inverted hexagonal which releases the drug. The inefficiency of this process is one of the barriers to the potency of siRNA drugs. Optimization of the LNP composition to improve drug delivery efficiency is important. Computer models of LNP systems can provide insight into this optimization, however the complex nature of DLin-KC2-DMA has made it challenging to model this lipid. In this work, we characterize the interactions between DLin-KC2-DMA and a phospholipid bilayer using both deuterium nuclear magnetic resonance (²H NMR) and small angle X-ray scattering (SAXS). We used ²H NMR to study the effects of the cationic lipid concentration and pH on the phospholipid bilayer as a function of temperature. SAXS provides additional information about the long-range correlations within the sample (bilayer repeat spacing). These data will be used to validate computer simulations of DLin-KC2-DMA, leading to a stronger understanding of the behaviour of the cationic lipid in more complex systems including LNPs.

P12 - Probing Peptide Ligand-GPCR Interactions By ¹⁹F NMR Spectroscopy

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The apelin receptor (AR) is a class A G-protein-coupled receptor (GPCR) activated by two peptide hormones, apelin and apela. In addition to its high gyromagnetic ratio and broad chemical shift range, ¹⁹F is not naturally occurring within proteins. This allows for the incorporation of ¹⁹F-labels to characterize conformational changes in response to membrane mimetics, ligands, and other environmental factors. Using a recently introduced method showing efficient and cost-effective 5-fluorotryptophan protein labeling through addition of 5-fluoroindole to M9 minimal medium (Crowley et al. (2012) *Chem Commun* 48: 10681), we have incorporated tryptophan ¹⁹F-labelled at the 4-, 5-, 6-, and 7-positions into AR. Solution-state NMR spectroscopy was used to probe conformation and dynamics of two portions of AR: the N-terminus and first transmembrane (TM) segment (residues 1-55, AR55) and the first three TM segments of AR (residues 1-137, AR TM1-3). Our results demonstrate that ¹⁹F NMR chemical shift, peak pattern and dynamics are highly variable, depending upon both the NMR conditions and the ¹⁹F position on the indole ring. Perturbations in the ¹H-¹⁵N HSQC spectra of AR segments were also apparent in a ¹⁹F configuration dependent manner, primarily localized to cross-peaks corresponding to residues proximal to the Trp. Addition of AR ligand peptides resulted in perturbations to the ¹H-¹⁵N HSQC spectra, with corresponding ligand concentration-dependent changes in ¹⁹F peak intensity. From this study, strategies are suggested for effective fluorotryptophan incorporation and application for ¹⁹F NMR studies of membrane protein topology, structure, dynamics, and ligand binding.

P13 - Structure Determination of a Class IB Hydrophobin by NMR Spectroscopy

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Hydrophobins are low molecular weight (5-20 kDa) self-assembling proteins secreted by fungi that accumulate at hydrophobic-hydrophilic interfaces and are extremely surface-active. 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 dispersal into the air, and prevent immune recognition. Due to their biochemical properties hydrophobins are a target for commercial applications, where they could be incorporated as biodegradable foam stabilizers or emulsifiers. To better understand what sequence characteristics determine hydrophobin properties, we are characterizing the structure and properties of a class IB hydrophobin from *Serpula lacrymans* (SlaHyd1). SlaHyd1 has only one charged amino acid, meaning it may have unusual properties compared to other hydrophobins. We expressed uniformly $^{13}\text{C}/^{15}\text{N}$ -labeled SlaHyd1 in *E. coli* and then purified it to homogeneity using Ni^{2+} affinity and ion exchange chromatography. We then determined the high-resolution structure of SlaHyd1 using NMR spectroscopy. SlaHyd1 contains a four strand anti-parallel β -sheet that is connected by three loop sequences (L1-L3). The β -sheet folds upon itself to form a β -barrel-like structure, L1 contains an α -helix, L2 is a dynamic loop, and L3 is a three residue β -hairpin. Overall, the structure of SlaHyd1 is consistent with SC16, which is another class IB hydrophobin. This data indicates that class IB hydrophobins have a consistent three-dimensional structure despite having a variety of sequence properties and will form the basis of future mutagenesis experiments and examination of rodlet properties to further characterize these proteins.

P14 - DNA Translocations through Nanopores coated with an Antifouling Layer

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Nanopores are nanometer-sized holes fabricated in a thin insulating membrane used as an electrical single molecule sensor. While biological molecule translocation (e.g. DNA, proteins) through nanopores can be performed, clogging of the nanopore can occur from interactions of the molecules with the membrane. This is limiting the lifetime of the sensor and consequently the number of single-molecule translocations that can be acquired with a given nanopore. This poses a real challenge for many applications. To solve this problem, we coat a polyethylene glycol silane (PEG-silane) self-assembled monolayer (SAM) on top of a silicon nitride (SiN) membrane. We demonstrate pore fabrication by CBD through the PEG-silane coated SiN membrane. Controlled breakdown (CBD) is a novel technique to fabricate such nanopores developed by our group, allowing a sub-nanometer control on the size of pores. After pore fabrication, noise analysis is conducted to characterize the pore and it is shown that coated pores have reduced $1/f$ noise and stable current baselines. We validate that this coating can effectively reduce nonspecific adsorption of biomolecules by conducting translocation experiments that show prolonged lifetime of the nanopore. This technique will allow more complex molecules that would otherwise clog the pore, to be studied by solid-state nanopore sensors.

P15 - A Putative Nucleolar Signal Sequence Directs the Localization of TRPM7 Kinase

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The channel-kinase TRPM7 supports the survival, proliferation, and differentiation of many cell types. Both plasma membrane channel activity and kinase function have been implicated in these roles. Identified proteinaceous substrates are localized throughout the cytoplasm and nucleus, lending credence to the observation that the soluble kinase domain can be cleaved from the channel (Desai et al. 2012). Further, a kinase construct (1510-1863), heterologously expressed in HEK293 cells, appears in the nucleus (Krapivinsky et al. 2014). We recently showed that hepatoma cells express a soluble C-terminal immunoreactive domain of TRPM7 in the nucleus and nucleolus, whereas non-dividing hepatocytes express nuclear envelope but not nucleoplasmic chanzyme. To further our understanding of the cellular role(s) of the kinase domain and establish how the kinase is targeted to specific nuclear compartments, we used genetic and immunofluorescence approaches to identify the subcellular location(s) of TRPM7 kinase constructs containing putative subcellular localization sequences. HEK293T cells heterologously expressing the isolated kinase domain (1510-1863) confirmed the nucleoplasmic localization. Contrary to the endogenous labelling in hepatoma, this protein did not appear in the nucleolus. However, a larger construct containing a putative nucleolar signalling sequence (K1147-K1151) labelled the nucleoplasm and the nucleolus. Western blotting of fractionated nuclei confirmed these results. Thus the TRPM7 kinase appears to take different forms, allowing it to localize to specific nuclear locations. We surmise that TRPM7 and its cleavage products distribute between the plasma membrane and nucleus in proliferating hepatoma cells. Nucleolar kinase activity may be involved in nucleolar functions supporting cellular proliferation.

P16 - Functional Characterization of Kir6.1 (A88G)/SUR2A Channels Expressed in HEK293H cells and the Association with J Wave Syndromes

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ATP-sensitive potassium channels (K_{ATP}) belong to the family of inward rectifying potassium channels (Kir) and can be found in pancreatic β cells, heart, brain, smooth and skeletal muscle. These channels are inhibited by intracellular ATP and sulfonylurea drugs and are activated by potassium channel openers (KCOs). Nowadays, mutations in genes that encode for Kir6.x and SUR subunits have been associated with the J wave phenomenon including Brugada syndrome (BrS) and early repolarization syndrome (ERS). Thus, in the present study, we aimed to evaluate the functional characterization of the A88G variation in KCNJ8, gene that encodes the Kir6.1 subunit, expressed in HEK293H cells, by using whole cell and inside-out patch clamp techniques. The same missense mutation A88G (c.263 C>G) in KCNJ8, was identified in 3 BrS patients but was absent in 390 alleles from ethnically matched healthy controls. Whole cell patch-clamp studies show an increased gain of function of glibenclamide-sensitive ATP-sensitive potassium channel current when KCNJ8-A88G was coexpressed with SUR2A wild type. Current-voltage relationship for KCNJ8-WT and KCNJ8-A88G channels was obtained from membrane patches in inside-out configuration. The results for single channel conductance were 53.3 ± 3.6 pS ($n=6$) for wild type and 84.4 ± 4.0 pS ($n=6$) for KCNJ8-A88G ($p<0.001$); and, for the open probability-voltage relationship, the results for slope were: 0.0046 ± 0.0004 mV^{-1} WT and 0.0043 ± 0.0004 mV^{-1} (no significant difference). Our results support the hypothesis that KCNJ8 may be a susceptible gene for BrS and suggest that A88G induced gain of function in ATP sensitive potassium channel current with an increased conductance but with no difference in open probability.

P17 - Biophysical Approaches in the Development of a Coupled Glycosyltransferase Assay Employing *E. coli* UDP-glucose Dehydrogenase

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In certain *E. coli* strains, the C-glycosyltransferase IroB glycosylates enterobactin prior to its secretion. Glycosylated enterobactin (aka salmochelin) facilitates bacterial iron uptake in a host while evading the mammalian immune system. To characterize its enzymatic properties, recombinant hexahistidine-tagged IroB was expressed and purified to near-homogeneity. We developed a coupled enzyme assay utilizing UDP-glucose dehydrogenase (Ugd) to quantitate UDP-glucose consumption, and thus IroB activity, more rapidly and efficiently than a previously reported HPLC-based assay. Purified IroB and recombinant *E. coli* H6-Ugd were then employed in a coupled assay where the glycosylation of enterobactin, catalyzed by IroB using the substrate UDP-glucose, was quenched at appropriate time points. Enzymatic assays using H6-Ugd demonstrated that its activity was stimulated by ATP, and inhibited by $MgCl_2$, as previously reported. Upon NAD^+ addition, H6-Ugd was used to convert all remaining UDP-glucose to UDP-glucuronate and NADH. The consumption of UDP-glucose by IroB was then quantitated from NADH production, which was measured spectrophotometrically. Initial rates were obtained and Michaelis-Menten steady-state kinetic parameters were determined for wild type IroB, which agreed with previously reported values. The kinetic parameters of the mutant variant W264L, hypothesized to be impaired in UDP-glucose binding, were then determined. An equilibrium-binding assay employing spin columns demonstrated that the K_D for UDP-glucose binding was higher than the observed K_m value from the coupled assay, likely due to a lack of Mg^{2+} in the binding assay. As expected, saturation of the W264L variant with UDP-glucose was not observed, confirming the role of W264 in UDP-glucose binding.

P18 - The Dynamical Zinc Fingers of Miz-1

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The C2H2 Zinc Finger (ZF) motif is the most conserved protein fold with a least 2% of the annotated human genome encoding for ZFs. Since the discovery of their $\beta\beta\alpha$ fold, ZFs have been assigned DNA binding and recognition roles in transcriptional regulation, with at least 45% of our transcription factor containing one or more ZFs. More precisely, over 700 hundred genes in the human genome possess ZFs, with an average of 8.5 ZFs per protein, where some of them encode for more than 30 ZFs. Despite their abundance, the function of only a few of those poly-ZF proteins is beginning to be understood. In order to contribute our understanding of the functions of those poly-ZF proteins, we study the structure, dynamics and DNA binding of Miz-1, a transcription factor containing 13 ZF. Through the structural study of ZF 1 to 10 (plus 13) of Miz-1 and their dynamical properties through NMR spectroscopy, we noticed that some of its ZFs show divergences from the conserved residues in the $\beta\beta\alpha$ core and inter-ZF linkers. Those divergences lead to unsuspected conformational exchange within 3 ZFs, to a compact inter-ZF structure and to predicted repulsion with the phosphodiester DNA backbone. We will present the structural and dynamical properties of the remaining ZFs, *i.e.* 10 to 12, in absence of DNA. Our results show that ZF 10 and 11 undergo structural fluctuations in the ns- μ s-ms timescale. Collectively, our results allow us to propose that Miz-1 could possess functions different than the canonical ZF-DNA binding.

P19 - Counting Molecules with Localization Microscopy using Fluorophore Photophysics Statistics

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Super-resolution localization microscopy (SRLM) has been a powerful tool for expanding our understanding of cell biology. By taking advantage of the stochastic blinking that fluorophores naturally exhibit and controlling the dynamics of this process, SRLM pushes the level of resolution an order of magnitude beyond the diffraction limit allowing light microscopy to visualize cellular components. There is also tremendous interest in using the technique to count single molecules. The main challenge of molecular counting in SRLM is largely a result of the multiple and random blinking of fluorophores, which leads to over-counting the number of molecules. We propose a method for counting biomolecules based on the blinking statistics and labeling efficiency of the dye. We show how our theory may be used by analyzing simulated data using two popular open-source programs to generate a localization table: rapidSTORM and ThunderSTORM. Comparing the results obtained from these two programs shows that ThunderSTORM shows more potential for filtering out false localizations at lower photon thresholds. Accurately determining the number of proteins or nucleic acids in a cell has wide-ranging applications, from systems biology, to proteomics/genomics, to fundamental cell biology. As a potential application, we show how such an approach could be applied to counting plasmid DNA without the need to individually resolve the plasmids.

P20 - Effects of Phylogenetic Distance on Protein Dynamics, Antibacterial Activity and Cytotoxicity in Members of the Ribonuclease 3 Subfamily

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Enzymes are key players of many important biological processes and understanding their mechanism of action is mandatory for proper pharmaceutical or industrial applications of these macromolecules. In fact, 3D structure, function and dynamics appear to be closely related, recent experimental evidence suggesting that conformational exchange may be involved in promoting catalysis in many enzyme systems, although the mechanisms underlying this atomic flexibility remain unclear. It is still unknown whether sequence and/or structure are evolutionarily conserved to promote flexibility events linked to biological function among protein homologs. In order to tackle these interrogations, we have used NMR to characterize the millisecond timescale conformational exchange in various members of the ribonuclease A superfamily. While these enzymes display very similar structure, their evolutionary distance and diversified biological activities complicate flexibility-function analyses. To solve this issue, we have investigated mammalian homologs of human ribonuclease 3 (Eosinophil Cationic Protein, ECP), comparing the human enzyme with its close ECP homologs from monkeys *Macaca fascicularis*, *Pongo pygmaeus*, *Pongo abelii* and *Aotus trivirgatus*. Our findings show that conformational exchange in the monkey enzymes strongly resembles that of their human counterpart, with subtle changes in exchange rates and/or localization, thus providing insights into the effects of sequence and phylogenetic diversity on protein dynamics. In parallel, antibacterial assays against *E. coli* and *S. aureus* have been performed on these proteins, and we have found that the more the protein sequence diverges from the common ancestor, the more potent its antibacterial activity is. Finally, cytotoxicity of these proteins was evaluated on HeLa cells, and a stark difference was found between human ECP and the monkey enzymes, which were still more potent than RNase A. These experiments are the ground to establish the interdependence that could exist between the functions of these proteins and their atomic flexibility.

P21- Structural and functional characterization of oncogenic protein-protein interactions involving the microphthalmia-associated transcription factor

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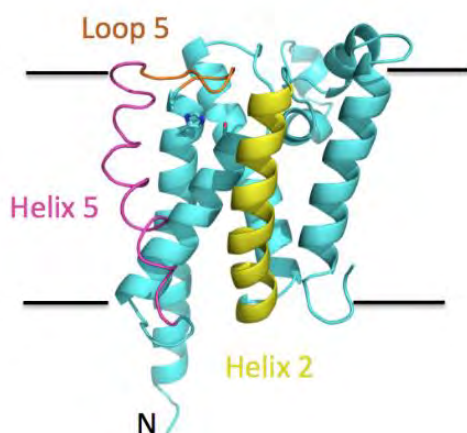
Transcription factors control gene expression and coordinate fundamental processes such as cell growth and differentiation. The microphthalmia-associated transcription factor (MITF) is a melanocyte-specific protein essential for melanocyte development and differentiation. MITF has also been identified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

P22 - Allosteric regulation of rhomboid intramembrane proteases

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The rhomboid superfamily of intramembrane serine proteases is highly conserved throughout evolution. The most basic form of the rhomboid protease is found in prokaryotes and consists of six – seven transmembrane domains. Crystallographic studies indicate these helices form a bundle with a buried active site consisting of a catalytic



serine-histidine dyad (*PNAS* 2007). Our recent data revealed that rhomboid proteases from *E. coli*, *Haemophilus influenzae* and *Providencia stuartii* form dimers in membrane (*EMBOJ* 2014). Furthermore, catalytic activity is dependent on dimerization, and transmembrane substrates are cleaved with positive cooperativity. Competitive inhibition studies demonstrated that substrate recognition proceeds through the recognition of an exosite. Since crystallographic analysis revealed a monomeric form of enzyme, the nature of dimerization remains elusive. We used mutagenesis to probe monomer-monomer interactions of *Haemophilus influenzae* rhomboid enzyme and SAXS to reveal the mode of dimer assembly. This study provides insight into organization of a functional dimer and its importance for substrate cleavage.

P23 - Quantifying Spatiotemporal Patterns in the Expansion of Twitching Bacterial Colonies

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Type IV pili (T4P) are very thin (5-8 nm in diameter) protein filaments that can be extended and retracted by certain classes of Gram-negative bacteria including *P. aeruginosa*. These bacteria use T4P to move across viscous interfaces, referred to twitching motility. Twitching can occur for isolated cells or in a collective manner. The advancing front consists of finger-like protrusions consisting of many bacteria, with the cells within the expanding colony arranged in a lattice-like pattern. The fingers consist of aligned bacteria 5 to 30 cells across, which move radially outward across the surface; although the average motion is radially outward, cells within rafts may reverse direction. Using a custom-built, temperature and humidity controlled environmental chamber, we have studied the motion of fingers at high spatial and temporal resolution. We have developed a bacterial segmentation and tracking technique to identify the trajectories of individual bacteria within the densely packed fingers. We have characterized the distance, displacement, orientation and direction reversals of the leading bacteria, as well as those within the fingers and the lattice network.

P24 - P-gp lipid uptake pathways determined by coarse-grained molecular dynamics simulation

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P-glycoprotein (P-gp) belongs to the ATP-binding cassette transporters (ABC) superfamily of membrane proteins. The transport mechanism in ABC transporters is triggered by ATP binding and hydrolysis at cytosolic nucleotide binding domains (NBDs). NBDs dimerization and dissociation are coupled with conformational changes in the transmembrane domains (TMDs), which form the translocation pathway for the substrates. Due to its ability to recognize and pump drugs to the extracellular medium, P-gp is a key player in cancer multidrug resistance. Among its substrates are also lipids and lipid-like molecules, as, for example, miltefosine, a phospholipid-based anticancer drug. For the development of strategies targeting P-gp inhibition, a better understanding of the steps involved in substrate recognition and uptake is needed. In this work, we use coarse-grained (CG) molecular dynamics (MD) simulations to explore possible pathways of lipid uptake in the inward-facing conformation of P-gp. Five different lipid environments were considered: a pure POPC bilayer, a pure POPE bilayer, a symmetrical POPC:POPE bilayer, and two additional bilayers with different ratios of POPE and POPC lipids. The simulations, each of the duration of 20 μ s, were carried out using the MARTINI force field (Chem. Soc. Rev., 2013,42, 6801-22). The results highlight the role of specific transmembrane helices and residues in the initial steps of the uptake and for the interactions established with a given lipid once inside the cavity. We suggest possible uptake pathways in P-gp in the different lipid environments used in this study.

P25 - Inter-Sarcomere Dynamics within Skeletal Muscle Myofibrils

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Introduction: We used microfluidic perfusions to control the microenvironment of sarcomeres within single myofibrils in order to explore inter-sarcomere dynamics. **Methods:** Micro-needles were used to hold single myofibrils from rabbit skeletal muscles. Micro-perfusions were used to control individual sarcomeres or groups of sarcomeres. **Results: (A)** We tested length dependence of inter-sarcomere dynamics. Three initial average sarcomere lengths (SLi) were tested: (I) between 2.4-2.65 μm , (II) between 2.65-2.9 μm , and (III) above 2.9 μm . Activation of the target (half-)sarcomere propelled displacement of the adjacent sarcomeres and the micro-needles towards the activation point. Force produced by the myofibril, and the displacements of sarcomeres situated adjacent to local activation were larger at increasing SLi ((I) 1.01 \pm 0.03 μm , (II) 1.17 \pm 0.03 μm , (III) 1.28 \pm 0.04 μm). **(B)** The displacement of adjacent sarcomeres was further increased when the myofibril was tested in rigor conditions in comparison with relaxing conditions (0.92 \pm 0.04 μm), suggesting that the interaction among sarcomeres is regulated by myofibril stiffness. **(C)** We measured the force of several sarcomeres comprising a myofibril. Sarcomeres produced similar active forces (21.07 \pm 0.56nN/ μm^2) within a myofibril at a fixed SLi. **(D)** We measure force of fully activated myofibrils comprised of 4 to 48 sarcomeres. We found that long myofibrils produce higher forces, but present higher levels of non-uniformity among sarcomeres, affecting shortening magnitude. We found that sarcomeres following on the descending limb of the force-sarcomere length relationship negatively affect shortening and force production. **Conclusion:** Inter-sarcomere dynamics requires the cooperative work of the contractile apparatus with structural and elastic protein complex that interconnects sarcomeres in order to transmit force.

P26 - Untangling the hairball: fitness based asymptotic reduction of biological networks

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Complex mathematical models of interaction networks are routinely used for prediction in systems biology. However, it is difficult to reconcile network complexities with a formal understanding of their behavior. Here, we propose a simple procedure (called FIBAR) to reduce biological models to functional subunits, using statistical mechanics of complex systems combined with a fitness-based approach inspired by *in silico* evolution. We illustrate FIBAR on biochemical adaptation and on absolute discrimination in early immune recognition. A model for immune recognition with close to a hundred individual transition rates is reduced to a simple two-parameter model. FIBAR identifies three different mechanisms for discrimination, belonging to two branches in the space of possible models, and automatically discovers similar functional modules in other discrimination models. Our procedure can be applied to biological networks based on rate equations using a fitness function that quantifies phenotypic performance.

P27 - Dimensions and dynamics of disordered proteins from single molecule fluorescence measurements

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A large number of proteins, termed intrinsically disordered proteins (IDPs), fail to fold into well-defined three-dimensional structures. Many of these IDPs are associated with diseases, such as cancer or neurodegenerative disorders. The polymer properties of IDPs are often crucial aspects of their functions. Correspondingly, to develop a better understanding of how disorder is used in function, we used a quantitative polymer-physics-based approach to analyze data from single-molecule FRET (smFRET) spectroscopy experiments. A challenge when dealing with ensemble models of IDPs is to determine if they provide accurate, realistic and biologically relevant representations of the IDPs. For example, the Protein Ensemble Database (pE-DB, <http://pedb.vib.be>) contains ensemble models restrained by SAXS and/or NMR measurements. These models can be cross-validated by using independent data from smFRET. We study Sic1, a cyclin-dependent kinase inhibitor which must be phosphorylated on at least six sites (termed Cdc4 phosphodegrons, CPDs) to allow its recognition by the WD40 binding domain of Cdc4. The highly-cooperative switch-like dependence on the number of phosphorylated sites on Sic1 cannot be accounted for by traditional thermodynamic models of cooperativity. Further experimental attention is necessary to determine the basis of its highly cooperative binding.

P28 - More than a pore: The role of the dimeric intermediate in the pore-forming mechanism of fragaceatoxin C, an actinoporin from the sea anemone *Actinia fragacea*

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Actinoporins are produced by sea anemones, and are excellent models of eukaryotic α -pore forming toxins. Recent research on actinoporins has mainly focused on the oligomerization step on the membrane and the detachment of the N-terminal region. Crystallographic data suggested the key role of a small hydrophobic protein-protein interaction surface for actinoporin oligomerization and pore formation in membranes. Here, we demonstrate that disrupting the key hydrophobic interaction between V60 and F163 (fragaceatoxin C (FraC) numbering scheme) in the oligomerization interface of FraC, equinatoxin II (EqII) and sticholysin II (StII) impairs the pore formation activity of these proteins. We combined molecular dynamics simulations with biochemical and biophysical experiments to design, obtain and characterize the double mutants FraCV60D/F163D, EqIIIV60D/F163D and StIII58D/I161D, which contain mutations in the oligomerization interface. We predicted that these mutations in the oligomerization interface would disrupt dimerization and therefore pore formation. Consequently, the double mutants completely lacked the activity of the wild type proteins, although they maintained the main structural properties of actinoporins and the capacity to bind to membranes. We also performed atomistic molecular dynamics simulations to determine the membrane disruption mechanism of the dimeric intermediate in model membranes. Our results suggest that the dimeric and monomeric intermediates are able to destabilize model membranes and induce curvature. Moreover, our findings support the importance of dimer formation, which seems to be a functional intermediate in the assembly pathway of different pore-forming proteins. These findings support the hybrid pore proposal mechanism as a universal model of actinoporin pore formation.

P29 - QM/MM Simulations Reveal Molecular Mechanism of ATP Hydrolysis in an ABC Transporter

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Hydrolysis of nucleotide triphosphate (NTP) plays a key role in the function of many proteins. However, the chemistry of the catalytic reaction in terms of an atomic-level understanding often remains elusive. We studied the molecular mechanism of adenosine triphosphate (ATP) hydrolysis in the ATP-binding cassette (ABC) transporter BtuCD-F. Free energy profiles obtained from hybrid quantum mechanical/molecular mechanical (QM/MM) molecular dynamics (MD) simulations reveal that the hydrolysis reaction proceeds in three steps. The first step is the nucleophilic attack of an activated lytic water molecule at the ATP-phosphate to yield ADP + HPO₄²⁻ as intermediate product. A glutamate residue that is located very close to the phosphate transiently accepts a proton and thus acts as catalytic base. In the second and third step, this proton is transferred back to HPO₄²⁻, yielding ADP + H₂PO₄⁻ as final products. The rate estimated from the computed free energy barriers is in very good agreement with experiments. The overall free energy change associated with the reaction is positive, suggesting that ATP hydrolysis itself does not provide the power stroke for substrate transport in BtuCD-F. The proposed mechanism is likely relevant for all ABC transporters and might have implications also for other NTPases, thus representing a key step towards understanding chemo-mechanical energy conversion in NTP-driven molecular machines.

P30 - Artificial Crown Ether Ion Channel as Promising Therapeutic Agents

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Ion channels are transmembrane proteins that regulate the flow of ions through cell membranes and are required for the proper functioning of the cell. However, some ion channels such as non-gated nanopores may act as toxins by enabling the uncontrolled passage of ions, destroying the usual electrochemical gradients of a cell and leading to its death. Targeting non-gated nanopores towards cancer cells would be very promising for the development of new nanochemotherapeutic agents to treat resistant cancer cells. In this intent, a new family of synthetic ion channels was developed in our laboratory using a transmembrane helical peptide as framework bearing six crown ethers to create a transmembrane channel for ions. Even though biophysical studies have shed light on several aspects of these channels, the mechanism of action by which they incorporate into membranes remains unclear. Therefore, in an attempt to assess what drives the incorporation of crown ether-modified peptides into bilayer membranes, we have used oriented circular dichroism (OCD) spectroscopy and the two-electrode voltage clamp method (TEVC). Studies in OCD showed a transmembrane orientation at very low peptide/lipid ratios in lipid bilayers and aggregation at higher ratios, while TEVC showed ionic current in genuine *Xenopus laevis* oocytes cells. Furthermore, oocytes tend to die after a short period of incubation with the peptide, probably from depletion of energy, thus revealing its potential as a cytotoxic agent.

P31 - Methodological development to study lipid membranes of intact microalgae by solid-state NMR

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Microalgae are unicellular organisms at the basis of the aquatic food chain. They are protected by a cell wall which composition varies depending on the species, and which constitutes one of the first barriers crossed or targeted by contaminants. Our laboratory has developed a range of experiments designed to characterize microalgal cells in vivo by ^{13}C solid-state NMR with magic-angle spinning (MAS), which was applied to the study of the green unicellular microalgae *Chlamydomonas reinhardtii* (Arnold et al. 2015, *Biochim. Biophys. Acta* 1848:369-377). Considering the importance of the lipid membrane in various biological processes as well as its possible role in the action of water contaminants, it is of great importance to develop tools to specifically look at the microalgal cell membranes. In this work, new strategies were tested to selectively ^2H -label membrane lipids for ^2H solid-state NMR study of membrane perturbations. Their efficiency was probed and quantified. The membrane rigidity of *C. reinhardtii* was assessed in vivo under various cell states and temperatures. This work shows that ^2H solid-state NMR with MAS is a valuable diagnostic tool to monitor the health state of microalgae.

P32 - Investigating the Phase Behaviour of a Model Lipid Nanoparticle System with DLin-KC2-DMA/Distearoylphosphatidylserine and the Addition of Cholesterol

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Lipid nanoparticles (LNPs) are a favoured delivery device for short pieces of RNA (siRNA) used in gene silencing. Whilst LNPs can easily undergo endocytosis into targeted cells, the actual release of siRNA into the cytoplasm has an efficiency of only 1%. Understanding the molecular interactions between both LNP and endocytic components is essential for improving LNP efficiency. The release trigger for LNPs is an ionisable cationic lipid, which becomes protonated in an acidifying endosome. The hypothesis is that the protonated cationic lipid will electrostatically interact with anionic lipids in the endosomal membrane, inducing the formation of the inverted hexagonal (H_{II}) phase and releasing siRNA into the cytoplasm. To study this, a model system comprised of the cationic lipid XTC2 and an anionic lipid DSPS- d_{70} was investigated using ^2H NMR spectroscopy. Experiments were conducted at an acidic pH (pH 4.7) to establish full protonation of XTC2 and observe the resulting interaction with DSPS- d_{70} . Using a temperature range from 5-40°C, we observed a phase transition from gel to H_{II} with a transition temperature (T_{H}) of $\sim 17^\circ\text{C}$. We then introduced 10 mol% cholesterol (found in LNPs) which reduced the T_{H} and increased order in the H_{II} phase. Small angle X-ray scattering (SAXS) experiments will allow identification of phase coexistence within each system, and enable measurement of lattice spacings. Experimental results are being fed into computational simulations at the University of Calgary to develop a comprehensive model of LNP interactions for improved efficiency in the future.

P33 - Long Chain Sphingolipids (C24) Suppress Membrane Domains by Concentrating Cholesterol in the Inner Leaflet

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In mammalian cells, sphingolipids are almost exclusively in the outer leaflet of the plasma membrane. Yet little is known how this bilayer asymmetry affects lateral microdomains. Here, we provide evidence that, in the plasma membrane of live cells and asymmetric model membranes, C24 sphingomyelin, the major mammalian sphingolipid, suppresses microdomains. Molecular dynamics simulations suggest a strong preference for cholesterol in the inner leaflet when C24 sphingomyelin is in the outer leaflet. We indeed detect 75-80% of the cholesterol in the cytoplasmic leaflet of live cell plasma membrane and also in the inner leaflet of large unilamellar vesicles asymmetric with C24 sphingomyelin. C16 sphingomyelin does not have an effect on cholesterol partitioning, promoting microdomains in live cell plasma membranes and asymmetric model membranes. Asymmetrically placed C24 sphingolipids, therefore, limit microdomains in model membranes and in the plasma membrane of live cells by generating cholesterol asymmetry.

P34 - Long DNA as a Genomic Platform

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Significant advancements in genome sequencing and mapping techniques have accelerated research in human health, forensics, and the agricultural and fisheries industries. Most current techniques fragment native DNA into small pieces, but this generally results in computational complexity and makes it difficult to analyze repetitive sequences, structural variations, and haplotypes. It is thus a critical challenge for emerging sequencing technologies to control and manipulate intact, delicate DNA molecules for genomic analysis. In addition, biophysical and biomedical studies are hindered by the inability to monitor, in real time and with single-molecule resolution, the reactions/interactions of freely diffusing biopolymers with small molecules. We address these challenges using Convex Lens-induced Confinement (CLiC) and nanoscale surface-Embedded Topographies (nanoSET). These methods let us gently coax long, intact biomolecules like DNA into open-face nanogrooves and image them with single-fluorophore sensitivity. The nanogrooves serve as a massively parallel platform for templating biomolecules in linear, concentric circular, and ring formats. Here we show examples of real-time observation of kinetics and dynamics of confined DNA. Control over the confinement geometry allows us to monitor delicate biomolecules for very long times and at reaction concentrations inaccessible to conventional microscopy techniques. Reagents can be introduced to the confined biopolymers and their reactions (eg, salt-, surfactant- or enzyme-induced) can be monitored in real time. Together, CLiC and nanoSET technologies will enable critical advancements in the study of long and intact biopolymers like DNA – both for fundamental biophysical studies and to advance genomics and biotechnological research.

P35 - A Mathematical Model for Vertebrate Somitogenesis

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Somitogenesis refers to the highly dynamic process during which blocks of mesoderm, called somites, form in a periodic fashion along the antero-posterior axis of the embryo. Somites play an important role in the patterning of the adult embryo: different somites will differentiate into distinct specialized structures, such as vertebrae. Using computational tools based on non-linear physics that allow the modeling of cellular regulatory systems, we build a mathematical phase model relying on a minimal set of parameters that can effectively recover the latest experimental observations relevant to somite formation. In our model, cyclic expressions of genes associated with the Notch signaling pathway, which were shown to be linked to somitogenesis, define a clock mechanism that allows individual cells to acquire positional information. We model explicitly the slowing down of these internal genetic oscillations, and their coordination within neighboring cells generates a sweeping wave of cyclic gene expression across the embryo, as observed experimentally. Furthermore, our model predicts that somite size scales exponentially when growth stops, which is also observed experimentally. Simulations of our model are also fully consistent with the characteristic modulation in the somite formation pattern that result from FGF-bead introduction experiments. This is not the case for simulations of a simple Clock and Wavefront model, which is the most widely accepted model for vertebrate somitogenesis.

P36 - Bundling of acetylated microtubules drives enhanced kinesin-1 motilityLinda Balabanian¹, Christopher L. Berger² and Adam G. Hendricks¹¹ *Dept. of Bioengineering, McGill University, Montréal, QC, Canada*² *Dept. of Molecular Physiology and Biophysics, University of Vermont, Burlington, VT*

The motor proteins kinesin and dynein transport organelles, mRNA, proteins, and signalling molecules along the microtubule cytoskeleton. In addition to serving as tracks for transport, the microtubule cytoskeleton directs intracellular trafficking by regulating the activity of motor proteins through the organization of the filament network, microtubule-associated proteins, and tubulin post-translational modifications. However, it is not well understood how these factors influence motor motility, and *in vitro* assays and live cell observations often produce disparate results. To systematically examine the factors that contribute to cytoskeleton-based regulation of motor protein motility, we extracted intact microtubule networks from cells and tracked the motility of single fluorescently-labeled motor proteins on these cytoskeletons. We find that tubulin acetylation alone does not directly affect kinesin-1 motility. However, acetylated microtubules are predominantly bundled, and bundling enhances kinesin binding and processivity. The neuronal MAP tau binds preferentially on the highly curved regions of microtubules where it strongly inhibits kinesin motility. Taken together, these results suggest that the organization of the microtubule network is a key contributor to the regulation of motor-based transport.

P37 - Design of amyloid-based nanostructures as a novel vaccination platform

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The development of new vaccination strategies is an important challenge for modern research. In most cases, the protection induced by vaccines is only partial. The efficiency of immunotherapies is often complicated by problems related to the limited immunogenicity of certain antigens. The antigen size and geometry are key elements that contribute to the efficiency of a vaccine by favoring their internalization by dendritic cells via the endosomal pathway. This work aims at developing a novel vaccine platform based on natural self-assembling peptides. The nanofibrils take the form of a cross- β -sheet conformation as observed in amyloid³ and the N-terminal amine group can be used as a fusion site for the functional epitope. This approach brings the following advantages: the assembly process of the amyloids is spontaneous, the nanostructures exhibit a strong mechanical rigidity and the capacity to form highly ordered structures³. The modulation of amino-acid sequences allows to adjust the physico-chemical properties of the amyloid substance. Equally, a self-assembling peptide domain can be easily added to an epitope by using solid phase peptide synthesis and the synthetic peptides can be prepared with a precise chemical composition for optimal epitope exposure. Moreover, the use of self-assembling structures can present the epitopes in a multivalent fashion, leading to an increased recognition by immune cells. Finally, the use of chemically synthesized peptides allows us to avoid the difficult purification and characterization processes. These nanostructures can serve as a platform to anchor heterologous antigens and the incorporation of these materials should improve the delivery efficiency to increase immunogenicity.

P38 - A Single-particle detection and tracking algorithm for fluorescence microscopy as demonstrated on *Thermobifida fusca* cellulases

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The dynamics of cellulase play a significant role in unraveling enzymatic hydrolysis used in the production of biofuels from cellulosic biomatter. The interaction of enzymes with their substrates cannot be observed directly and so one must rely on the knowledge of the enzyme structure combined with measurements of their dynamics. Single-molecule tracking of fluorescently labelled particles allows for a more detailed analysis of the movement, revealing multiple enzymatic states while ensemble averaging can be avoided. For this purpose, we developed an algorithm that allows for detection and tracking of diffraction-limited spots and is capable of localization uncertainties of about 10nm, depending on signal-to-noise ratio, as well as a stable frame-to-frame linking method which uses fiducial markers to eliminate stage drift. The program is benchmarked by means of simulated videos and applied to a known system of diffusing lipids in a supported lipid bilayer. Finally, we present preliminary tracking results of *Thermobifida fusca* cellulases on bacterial micro-crystalline cellulose. The tracks display various movements, including slow diffusion, immobility, and fast hopping motions.

P39 - An instrument to Quantify DNA using Solid-State Nanopores

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The ability to fabricate solid-state nanopores by controlled-breakdown (CBD) has the potential to democratize the field of nanopore sensing. Leveraging this method, we have developed a small benchtop instrument that enables users to fabricate solid-state nanopores of any desired size in a fully automated fashion, and immediately perform single-molecule sensing experiments. We will present the features of this new instrument. Also discussed is our effort to use nanopores as sensors capable of accurately quantifying different DNA concentrations in solution. The capture and detection of DNA across a nanopore depend on a high number of parameters. Undesired variations in translocation rates from pore to pore are therefore observable and render the counting process less reliable. We present a method for improving the quantification of DNA in solution extracted from capture rate data. We hope that through the development of a cost-effective, rapid, easy-to-use solid-state nanopore fabrication and sensing tool, a greater number of researchers will be able to develop applications that take advantage of the sensitivity and versatility offered by solid-state nanopores.

P40 - Thioflavin T fluorescence to detect amyloid assembly: effect of the frequency of measurement in microplate assays

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Misfolding of proteins in the form of amyloid fibrils is associated with numerous pathological states such as the Alzheimer's disease, spongiform encephalopathies and type II diabetes. The development of pharmaceutical inhibitors of amyloid formation is a very active area of research and requires routine and high-throughput assays to evaluate amyloid fibrillization. Still today, the most common experimental approach to monitor amyloid fibril formation *in vitro* relies on thioflavin T (ThT), a benzothiazole amyloid-sensitive fluorescent dye. However, it is known that assembly is sensitive to a wide array of parameters, making comparison of experimental results between studies and research groups problematic. We observed that even when all parameters, *i.e.* temperature, buffer, surface, quiescent condition, are carefully maintained constant, discrepancies among kinetics of amyloid assembly monitored in microplate are observed. These differences between assays, performed under quiescent conditions, were associated with the frequency at which the fluorescence is measured. Monitoring the kinetics of self-assembly of the amyloidogenic peptide islet amyloid polypeptide (IAPP) at shorter intervals dramatically accelerates the rate of fibrillization. This observation was confirmed by transmission electron microscopy and circular dichroism spectroscopy. By means of the 8-anilino-1-naphthalenesulfonic acid (ANS) dye, we observed that this effect is independent of ThT. This effect was attributed to the displacements of the microplate between measurements. Overall, this study emphasizes that amyloid assembly is very sensitive to environmental factors and reinforces the importance of a better standardization in kinetic assays.

P41 - Towards a nanoscale description of the interactions between the amyloid peptide A β ₍₁₋₄₂₎ and mutants with membranesMehdi Azouz^{1,2}, Christophe Cullin¹, Michel Lafleur² and Sophie Lecomte¹¹*Chimie et Biologie des Membranes et Nanoobjets, CNRS UMR 5248, Institut Polytechnique de Bordeaux, Université de Bordeaux, 33607 Pessac, France.*²*Department of Chemistry, Center for Self-Assembled Chemical Structures, Université de Montréal, Montreal, Quebec, Canada.*

Alzheimer's disease is considered as the major form of dementia, with an estimated number of 130 million of people living with the pathology by 2050, worldwide. In the project, we aim at determining the interactions between Alzheimer's neurotoxic peptide A β ₁₋₄₂ with some lipid components of neuron membranes. We focus our study on the effects of cholesterol and GM1 on the interaction with the peptide. Polarized Attenuated Total Reflection Infrared Spectroscopy (pATR-FTIR) allowed us to observe the impact of the membrane composition on the interaction while high speed AFM in aqueous condition is used to define the evolution of the lipid bilayer morphology upon the addition of the peptide. Key information about the kinetics of the bilayer fragmentation, peptide auto-assembly, and domain formation were collected. Composition dependant lipid bilayer extraction was observed and quantified as the ν_{CH_2} band intensity, observed by IR spectroscopy, decreased after the peptide was introduced. Small amounts (~10 mol%) of cholesterol, or GM1 in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) membranes led to significant disruptions unlike pure POPC lipid bilayers where no effect was observed. These results highlight the pivotal role of membrane composition in the effect of A β ₁₋₄₂.

P42 - ²H NMR studies of bacterial membrane perturbation by antimicrobial polypeptidesNury P. Santisteban¹, Michael R. Morrow¹ and Valerie Booth²¹*Department of Physics and Physical Oceanography, Memorial University of Newfoundland, St. John's, NL, Canada*²*Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada*

Small amphipathic polypeptides that can reduce the viability of bacterial cells are known as antimicrobial polypeptides (AMPs). Permeabilization of lipid bilayers by AMPs has been previously investigated in model lipid systems that mimic aspects of bacterial membrane composition. Model studies, however, do not address the possibility that polypeptide antimicrobial activity might also involve the disruption of non-membrane targets or might be enhanced by interaction with non-membrane cell components. In order to learn how AMPs interact with more complex structures within real bacterial cell envelopes, we have deuterium-labeled membranes of the gram positive bacteria *Bacillus subtilis* and have used ²H NMR to study how lipid acyl chain orientational order in the membranes of these bacteria is affected by the presence of specific AMPs. In the absence of AMP, the ²H NMR spectrum of the labeled bacterial membrane is a superposition of doublets characteristic of fast axially symmetric chain reorientation. Prominent shoulders, at +/-12 kHz, reflect an order parameter profile having a plateau near the headgroup end of the chain. In the presence of CAME (a hybrid of cecropin and melattin), intensity shifts from the plateau spectral region to smaller quadrupole splittings suggesting a peptide-induced disordering of the bacterial membrane. Spectra have also been obtained from deuterated *B. subtilis* in which the peptidoglycan layer has been disrupted using lysozyme. The capacity to observe such spectra will facilitate studies of how the bacterial peptidoglycan layer affects AMP perturbation of the *B. subtilis* membrane.

P43 - Super-Resolution Imaging of Native Cellulose Nanostructure

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Cellulose – a major and critical component of plant cell walls – constitutes the largest component of Earth's biomass and is an attractive raw material to exploit in the production of biodegradable and renewable products, such as biocomposites, biofuels and other biomaterials. Manufacturing these products is limited by the recalcitrance of cellulose, which stems from its highly crystalline structure. We thus require insight into the nanoscale structure of cellulose and the mechanism of its depolymerization to improve the efficiency of its industrial processing. In this work, direct stochastic optical reconstruction microscopy (dSTORM) was used to study the structure of fluorescently-labelled bacterial microcrystalline cellulose (BMCC) at the nanoscale. Super-resolution imaging unveiled regular repeating patterns of high and low fluorophore density regions on BMCC microfibrils that are hypothesized to represent disordered and crystalline regions of cellulose. Grafting cellulose using different dyes or labelling reactions produced similar patterns, evidencing that the fluorescent patterns are labeling chemistry-independent and are instead encoded within the native cellulose structure. The length of the dark regions was measured and their distribution was found to correlate with the length of cellulose nanocrystals produced by short hydrolysis treatments. A fluorescently-labeled cellulase – CBHI-Cy5 – bound to labelled BMCC was imaged in the same way and the results show that the enzyme does not exhibit any preferential binding to either the crystalline or disordered cellulose regions. Understanding the nanostructure of cellulose will allow us to improve cellulose manufacturing processes and give insight into how plant cells assemble, restructure and degrade their cell wall.

P44 - Release of mesoporous silica particles by free standing pH sensitive membranes

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Since Whitesides et al. used laminar flow patterning in microfluidics for in situ microfabrication, many methods have been exploited for polymer membranes fabrication in microfluidic devices. In some cases, membranes from the polymerization of non-toxic monomers has been demonstrated using a microfluidic flow-templating approach. Another, more generalizable approach for creating biocompatible, freestanding membranes has been demonstrated by the precipitation of preassembled chitosan polymer molecules from solution. This was accomplished at the interface between two co-flowing laminar flow streams where a local pH gradient could be established. In this work, we demonstrate the ability to impart new functionality to biomimetic materials. Here, we form chitosan biomembranes with embedded mesoporous silica nanoparticles which can be released by exposure to acidic pH values. This approach opens the way for delivery of molecular agents, catalysis, biosensors and imaging applications applied to either the interior of the membrane or the surrounding liquid. As a proof of concept, triggered release of nanoparticles in slightly acidic conditions demonstrates the potential of microfluidics to form “enhanced” biomimetic materials with integrated drug delivery functionality.

P45 - Effects of Cellular Tension Stress on Time-Lag Response of YAP Movement During Cell Migration

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The physical and mechanical signals received by cells significantly impact many aspects of cellular behavior through mechanotransduction. One key mechanotransduction protein is the Yes Associated Protein (YAP), which is related to cell proliferation, survival and metastasis. Recently, it was found that YAP localization is dependent on mechanical tension in the actin cytoskeleton, however, the dynamic movement of YAP in response to forces remains poorly understood. Here, we track and quantify the spatial distribution of YAP in living cells during cell migration and spreading on PDMS substrates with different stiffness. To quantitatively track YAP distribution in time, we transfect NIH-3T3 cells with YAP-eGFP and NLS2-mcherry, plate the cells on compliant silicone substrates with different moduli, and perform time-lapse imaging using confocal microscopy. We quantify the YAP distribution as a function of cellular traction stress using Traction Force Microscopy (TFM). Our results demonstrate that substrate stiffness alone does not control YAP localization, however, cellular contractile stress appears to determine the YAP distribution. As cell morphology and contractile forces change in time, YAP nuclear localization is not constant, but increases with increasing cell contractile forces. These results implicate YAP as a mechanosensor regulated by the amount of stress and strain in the cytoskeleton rather than substrate stiffness. We hypothesized that the degree of YAP translocation and the time required is correlated to the amount of cell contractile stress.

P46 - New insights into the mechanism of islet amyloid polypeptide self-assembly by mean of a split tetra-Cys motif approach

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A large number of peptides and proteins are recognized for their ability to self-assemble into amyloid fibrils, whose tissue deposition is associated with degenerative diseases such as type II diabetes (T2D) and Alzheimer's disease (AD). The mechanism of self-assembly of amyloidogenic polypeptides has been studied by various techniques and one of the most widely used is an assay based on a fluorescent dye, thioflavin T, which binds to amyloid fibrils. However, this technique is insensitive to the formation of oligomers. In this context, we propose to develop a new detection method. This method relies on a fluorescent dye, Fluorescein Arsenical Hairpin (FIAsH), whose emission of fluorescence is amplified following its binding to a tetra-cysteic motif. Thus, a monomeric unit possessing two cysteines may lead to the reformation of the tetra-cysteic epitope during its oligomerization. In our study, we are interested in the self-assembly of islet amyloid polypeptide (IAPP), whose amyloid deposits are associated with T2D. Using native cysteines in the N-terminal region of IAPP, we were able to follow the self-assembly of IAPP. By this method, we have determined that IAPP does not form stable oligomers during the lag phase, which reinforces the hypothesis of a nucleated polymerization mechanism. Moreover, this assay allows us to evaluate the impact of amyloid co-factors, such as glycosaminoglycans, on the fibrillization process.

P47 - Structural-functional relationship of single skeletal and cardiac thin filaments visualized by high-speed Atomic Force Microscopy.

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High-speed atomic force microscopy (HS-AFM) is a powerful tool to study dynamic biological processes. Currently, the spatial and time resolutions of HS-AFM have levels of nanometers and milliseconds, respectively, and allow structural and functional characterization of biological samples at the single-molecule level. In this study, we purified native skeletal and cardiac thin filaments consisting of actin, alpha-actinin, tropomyosin, troponin complex, nebulin / nebulin, and measured their Ca^{2+} -sensitivity in ATPase and motility assays. We then visualized and tracked single thin filament using ultra-fast detector and contact-mode cantilever (0.2 N/m spring constant) with a high-resonant frequency (the first resonant frequency = 0.6 MHz), in real time mode, without fixation of the samples. We obtained high-resolution images and movies of individual molecules at a 100-2000 ms time frame acquisition, which revealed the structural and positional stability of thin filament complex. Skeletal and cardiac thin filaments visualized by HS-AFM in the absence of Ca^{2+} showed similar structural features, however their Ca^{2+} -sensitivity and myosin ATPase activation were different. This might reflect to different conformational alterations of the troponin complex in skeletal and cardiac thin filaments in the presence of Ca^{2+} .

P48 - Biophysical effects of the interaction between hydrophilic nanoparticles and pulmonary lung surfactants: influence of nanoparticle concentration and charge

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Human exposure to nanomaterials is inevitable given the increasing presence in the environment of nanoparticulates generated by industrial activities. Colloidal silica is the most abundant air pollutant in industrial regions. Due to their exceptional chemical reactivity, silica nanoparticles (NPs) can potentially inhibit lung surfactant and cause difficulties in breathing. The present study investigates the biophysical effects of NP charge and concentration on the phase behavior of model lipid mixtures and clinical pulmonary surfactant. A surfactant monolayer was formed at the air-water interface over a NPs-containing subphase. The Langmuir film balance was used to examine the lipid surface activity, while Brewster angle microscopy and Atomic Force Microscopy allowed the study of the overall morphological changes to the film, while the structural changes were detected with x-ray diffraction. We obtained clear evidence that silica NPs influence the lipid monolayer at concentrations as low as 0.001%. The revealed biophysical mechanism of NP interaction with pulmonary surfactant brings new insight in understanding how inhaled NPs impact pulmonary function.

P49 - Design and Validation of Multi-Charged Dendrimers to Modulate Amyloid Assembly

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The deposition of the islet amyloid polypeptide (IAPP) as insoluble amyloid fibrils in the pancreatic islets is associated with type II diabetes. Recent studies have revealed that pre-fibrillar proteospecies and/or the amyloidogenic process mediate b-cell degeneration whereas amyloid fibrils are poorly cytotoxic. Thus, therapeutic strategies that aim at preventing b-cell death associated with amyloid deposition should either sequester prefibrillar species and/or modulate the initial steps of fibrillization. In this view, low generation flexible dendritic scaffolds harboring 4 to 16 hydroxyl, amine, carboxylate or sulfate functional groups were designed and evaluated for their effects on IAPP self-assembly and cytotoxicity. Whereas neutral polyhydroxylated and polycationic dendrimers did not affect the kinetics of amyloid assembly, carboxylated dendrimers accelerated IAPP fibrillization proportionally to surface group density. Interestingly, as revealed by thioflavin T fluorescence, circular dichroism spectroscopy and atomic force microscopy, the G0 sulfated dendrimer inhibited amyloid formation by maintaining the peptide in a random coil conformation. In contrast, G1 sulfated dendrimers potentiated IAPP self-assembly into long amyloid fibrils by a scaffold-based mechanism. Anionic dendrimers attenuated IAPP-induced toxicity on pancreatic b-cells. Our results indicate that sulfated dendrimers can alter the fibrillization pathway of IAPP and inhibit its proteotoxicity, either by accelerating amyloid formation or by trapping the peptide in a non-aggregating and non-toxic state. This study offers novel mechanistic insights for the design of a nanomolecular scaffold to manipulate the self-assembly of natively disordered amyloidogenic peptides.

P50 - Gel/Liquid-ordered Phase Coexistence in Bilayers Containing Palmitoyl Sphingomyelin, Palmitoyl Ceramide and Cholesterol

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Elucidation of the molecular mechanisms of apoptosis have significant therapeutic benefits since it reveals how cell death can be modulated. One of the unknown aspects of apoptosis is its effects on the phase behavior of the plasma membrane (PM). Due to apoptotic signaling, enzymatic activity of sphingomyelinase (SMase) converts SM to ceramides (Cer) in the PM. It has been suggested that SMs are localized in domains within the PM. Accumulations of Cer in the vicinity of SM induce gel phase in the SM: Cer vesicles. On the other hand, cholesterol (Chol) is one of the major components of the PM and it forms liquid-ordered (l_o) phase in SM: Chol bilayers. Therefore, SM: Chol: Cer interactions seem to be crucial in determining the cell's fate. In this study, we are interested in determining the physical effects of Cer accumulation in SM/Chol vesicles to give an insight on the phase behaviour of the PM during apoptosis. Using $^2\text{H-NMR}$, we have characterized the phase behavior of palmitoyl-SM (PSM) and palmitoyl-Cer (PCer) in their ternary mixtures with Chol. According to our results, there is l_o -gel phase coexistence in the PSM: Chol: PCer bilayers with the molar ratios of (7:3:1), (7:3:2) and (7:3:3) at room and physiological temperatures. These results are consistent with the combined fluorescent microscopy data using NAP and DiI18 as the fluorescence dyes for the (7:3:3) mixture (Busto J V, et al. Lamellar gel (L_β) phases of ternary lipid composition containing ceramide and cholesterol. *Biophys J.* 2014).

P51 - Kinetics Investigations of Peptide Crystallization Using Coarse-Grained $C\alpha$ Molecular Dynamics Simulations with Polymorphically Biased Force Fields

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Proteins and peptides can aggregate into insoluble fibril plaques which are found in a host of ailments, including Alzheimer's Disease (AD) with its hallmark amyloid-beta ($A\beta$) peptide. The Tycko group has demonstrated parallel / antiparallel $A\beta$ polymorphism AD Iowa mutant whereas the wild type is predominately parallel, potentially implicating polymorphism as a contributing factor to the virulence of the disease. Debate exists about whether the plaques or soluble oligomers are the pathogenic agents and current experimental techniques can only show us the end result of amyloidosis, providing little insight into the oligomerization process. This requires theoretical modelling to fill the gap; but simulating full length $A\beta$ is computationally expensive. Fortunately, recent work from the Eisenberg group has observed polymorphism within a variety of microcrystals generated from relatively short $A\beta$ fragments, and demonstrated that these microcrystals can be used as representative models of full length fibrils; indicating that insights achieved from modelling short peptides may be applicable to larger fibrils. In our study, we conduct coarse-grained $C\alpha$ molecular dynamics simulations of systems of 20 peptides with 8 beads per peptide to investigate the kinetics of peptide crystallization with Go-like Lennard Jones and specialized hydrogen bond potentials to bias the systems towards either parallel or antiparallel association to resemble the Eisenberg structures. Our results indicate that small oligomers (dimers, trimers) have higher probabilities of occurring during antiparallel rather than parallel association and are present for longer periods of time, particularly at higher concentrations, and larger oligomers (6-18) have low probabilities of occurring.

P52 - RNA transcription modulates phase transition-driven nucleolar assembly

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Cells are partitioned into functional compartments called organelles. Some organelles, such as germ granules and nucleoli, lack enclosing membranes and instead consist of local concentrations of RNA and protein that dynamically exchange with the surrounding cytoplasm or nucleoplasm. These structures behave like liquid droplets whose assembly appears to reflect a type of intracellular phase separation. While phase transition models can accurately predict organelle size and assembly, they assume thermodynamic equilibrium. However, cells actively consume chemical energy and it is unclear how such nonequilibrium activity might impact classical liquid-liquid phase separation. Here, we combine *in vivo* and *in vitro* experiments with theory and simulation to characterize the assembly and disassembly dynamics of nucleoli in early *C. elegans* embryos. In addition to classical nucleoli, which assemble at the transcriptionally active nucleolar organizing regions, we observe dozens of "extranucleolar droplets" (ENDs) that condense in the nucleoplasm in a transcription-independent manner. We show that growth of nucleoli and ENDs is consistent with a first-order phase transition in which late-stage coarsening dynamics are mediated by Brownian coalescence and/or Ostwald ripening. Our results demonstrate that classical phase separation mechanisms long associated with non-living condensed matter can mediate organelle assembly in living cells, while nonequilibrium activity such as transcription may serve to regulate these processes in response to developmental or environmental conditions.

P53 - LSPR study of interactions of Amyloid β (1-42) with model lipid membranes

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Alzheimer's disease (AD) is a neurodegenerative condition with no treatment or cure causing cognitive impairment, memory loss and death. In the current leading hypothesis amyloid- β ($A\beta_{(1-42)}$), in its soluble oligomer form, is expected to be the causal neurotoxic species. The toxic nature of $A\beta$ is directly related to their interactions with cellular membranes. $A\beta$ binding has been shown, with lipid monolayer and membrane models, to be membrane composition dependent. In this work we use a Localized Surface Plasmon Resonance (LSPR) technique with OpenSPR instrument (Nicoya Lifesciences) to characterize real time binding kinetics of $A\beta$ -lipid-membrane interactions. We studied $A\beta_{(1-42)}$ binding to complex neuronal lipid membrane models that mimic healthy and AD degraded neurons and report the differences in binding curves depending on membrane models. Results from these LSPR studies will be compared and correlated to AFM images of the same systems, as well AFM imaging was used to verify the supported lipid membrane on the LSPR sensor surface. Protective effects of melatonin against $A\beta$ binding to membrane models are also discussed.

P54 - Metadynamics: Speeding up conformational sampling of intrinsically disordered peptides

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Intrinsically disordered proteins (IDPs) are proteins that lack the textbook secondary structure. They are characterized by shallow free energy landscape with multiple local minima which allows them to bind to multiple targets. Their typical functions include signaling and cellular regulation. The absence of well-defined folded structures makes them difficult to characterize since a conformational ensemble is needed. This ensemble is also highly dependent on conditions such as pH. Computer simulations can provide information of conformational ensembles but proper sampling requires enhanced sampling techniques beyond conventional simulations. Metadynamics is a family of techniques that can provide such information. One of the main issues in metadynamics simulations is the choice collective variables (CVs) that are required for efficient sampling. We have studied the choices and number of CVs together with several variants of metadynamics, namely well-tempered metadynamics (WT-META), and bias-exchange metadynamics (BE-META). We then study β -hairpin conformation of the Neh2 peptide and the binding of nrf2 and PTMA to Kelch. The latter is a key player in cellular response to oxidative stress and binding hub for multiple intrinsically disordered proteins. The extensive sampling enabled by metadynamics shows that the coupled folding-binding mechanism may be the dominant mode in binding to Kelch.

P55 - Covalent immobilization of protein onto self-assembled nanostructures via an enzymatic-mediated ligation

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Specific polypeptide sequences that auto-assemble into amyloid fibrils that are characterized by a cross- β -sheet quaternary structure appears attractive for nanomaterials development. These fibrils showed mechanical strength comparable to steel and silk while demonstrating structural plasticity. These properties contribute to the potential of amyloid nanofibrils as promising materials for biomedical and biotechnological applications. In this study, we investigate an enzymatic approach using a sortase to functionalize amyloid nanofibrils. *Staphylococcus aureus* sortase (SrtA) is a bacterial transpeptidase that covalently anchors surface proteins to the bacterial cell wall. This is accomplished by cleaving between threonine and glycine at an LPXTG recognition motif to generate an acyl-enzyme intermediate that reacts with a terminal amino group of pentaglycine on the cell wall. The amyloid scaffold was prepared by using peptide sequences derived from known amyloidogenic polypeptides, such as the islet amyloid polypeptide. Peptides were synthesized by solid phase synthesis and the amyloid formation was monitored/screened using thioflavin T (ThT) fluorescence. In order to follow the fibrillization conformational transitions, circular dichroism (CD) was employed whereas transmission electron microscopy (TEM) was used for morphological investigation. The ligation approach was validated using synthetic peptides, high performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC/MS). To verify our enzymatic-mediated ligation of pre-assembled amyloid structure, green fluorescence protein (GFP) was employed as a model and fused to the surface of fibrils. With this approach, amyloid scaffolds can similarly be decorated using multiple proteins. Overall, the ultimate goal of this project is to develop biocompatible scaffolds for applications in drug delivery and tissue repair/engineering.

P56 - Integration of Optical Instrumentation and Microfluidics for CBD-Fabricated Solid State Nanopore Sensors

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Solid state nanopore sensors, (nanometer-sized holes in thin dielectric membranes), have emerged as a versatile tool for detecting single biomolecules (e.g. proteins, DNA) and investigating their structural properties. Commonly, they operate by electrically sensing changes in background ionic current as voltage-driven molecules pass through the pore, using high-bandwidth current amplifiers. The inclusion of an additional fluorescence-based optical detection method in parallel, for instance through the use of intercalating YOYO-1 dye on DNA, offers the exciting possibility of accessing independent and complementary biomolecular information to electrical detection alone. However, in incorporating the equipment necessary to illuminate and record such fluorescent tags, significant noise can be introduced to the original ionic current signal. Here we describe the instrumentation of a synchronous optical-electrical nanopore sensor setup as well as noise reduction strategies required to bring the signal-to-noise ratio of ionic current measurements down to levels compatible with DNA detection. Our instrument utilizes nanopores fabricated in thin (10-30 nm) SiN membranes by controlled breakdown (CBD), a technique developed by our group as a low-cost, high-yield alternative to traditional focused ion-beam/TEM drilling methods. In short, CBD uses the controlled application of high electric fields ($>0.5\text{V/nm}$) to break down the dielectric membrane in a single, cylindrical pore of tunable diameter. With the strategies implemented here, in particular using PDMS to isolate the membrane surface and de-couple its conductivity and surface charge from incident laser illumination, we report a $>10\text{x}$ reduction in RMS current noise under optical sensing conditions. Other complications to consider, including the reduction and distortion of translocation signals due to access resistance of the optofluidic cell microchannels connecting the nanopore to the biasing electrodes, are also discussed.

P57 - Cross Correlation Spectroscopy and CLiC

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Understanding the relationship between the dynamics of biomolecules and their biological functions is of importance in biophysics, biotechnology, and cell biology. Fluorescence Correlation Spectroscopy (FCS) has proven to be a viable tool to study translational and rotational diffusion coefficients, chemical kinetic rate constants, flow rates, and molecular aggregation of fluorescently tagged biomolecules. The common setups of FCS such as confocal microscopy and total internal reflection fluorescence (TIRF) microscopy, however, suffer from poor signal-to-noise ratio, limited field view, and limited observation times. To address these issues, we propose to leverage Leslie's novel Convex Lens-induced Confinement (CLiC) technology to improve the performance of FCS. The CLiC technology can dynamically confine biomolecules within a nanoscale region without destroying the molecules' structural integrity, thus allowing them to diffuse more slowly while also dramatically suppressing background noise and enabling a wide observation field. An improved signal-to-noise ratio is expected, and furthermore, this approach allows us to probe slower protein-DNA interactions which were previously not available to study.

P58 - Reconstructing developmental gene regulatory networks using ϕ -evo, an *in silico* evolution software

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Patterning of body parts is a complex process that involves multiple genes connected via mutual interactions (transcription factor regulations, protein-protein interaction). Inferring in a systematic way the exact connectivity between the genes that govern a given function in an organism is a difficult task. For instance, the gap gene network, responsible for patterning the fruit fly embryo, is one of the most studied dynamic network and yet there is still a lot of uncertainty on the wiring between the genes that control this patterning. ϕ -evo is a tool that help to construct hypothesis regarding the structure of a gene network that could produce an objective function. By function we mean qualitative behavior, a given phenotype and not necessarily fitting data. ϕ -evo implements a genetic algorithm that mimics evolution by adding or removing genes or gene interactions in a population of networks or by tuning parameters. The selection of the networks is made on their ability to reproduce a desired function. We present several successes of ϕ -evo in reconstructing a network capable of producing known biological functions. Although fitting data is not the first purpose of ϕ -evo, it can also be used for it. To test the software on this task, we use expression time series for the different gap genes in mutant fly embryos as target for the algorithm in order to retrieve the gap gene network.

P59 - Mechanistic formation of a bipolar spindle: A fast and irreversible process that sequentially integrates Cin8's varied motility and microtubule crosslinking properties

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The bipolar architecture of the mitotic spindle is a common and essential design feature required for chromosome alignment and accurate equipartitioning of genetic material. Any collapse or instability of the bipolar architecture of the spindle leads to profound mitotic defects. The kinesin-5 motor protein is a critical actor in the formation of a bipolar spindle but a mechanistic model for this process is still lacking. Employing the simplicity of the budding yeast mitotic spindle we capture the in-vivo dynamics of this process and find it to be one of the fastest steps in spindle assembly. Intriguingly using a Cin8 motility mutant (Cin8R196K) we find this process to be driven by microtubule crosslinking. Equally we report asymmetric loading of Cin8 to the pre-existing spindle pole body prior to bipolar spindle formation. In order to investigate the bipolar spindle's initial state we used electron tomography to characterize its microtubule architecture. We find that high angle overlaps dominate the microtubule interactions emanating from early mitotic monopolar spindles. These monopolar spindles exhibit one highly self-aligned microtubule array emanating from one spindle pole and a splayed one emanating from the other. We propose a mechanistic model for the formation of a bipolar spindle which sequentially employs the minus ended motility of Cin8 to asymmetrically load and align the microtubule array of the pre-existing spindle pole which can then be captured and crosslinked by its splayed counterpart. This mechanism is independent of the canonical plus ended processive motion of Kinesin-5 and illustrates its profound role on the earliest stages of spindle architecture and assembly.

P60 - Structural study of α -synuclein 71-82, a peptide derived from a protein involved in Parkinson's disease: interactions with model membranes

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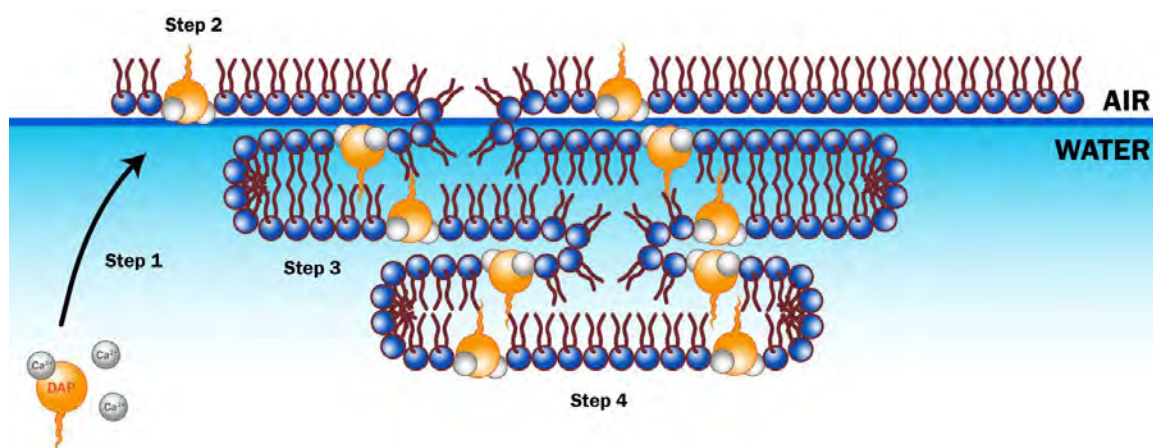
α -synuclein is an amyloid protein involved in Parkinson's disease. Aggregates of this protein are found in the brain of the patients, more specifically in nerve tissues. This protein can be found in several forms, from monomers to fibrils. The secondary structure of the latter has been described as a parallel β -sheet structure. Along this 140 amino acids protein, a specific portion plays an important role in the aggregation process: the non-amyloid- β component (NAC, sequence 61-95). Within the very core of this NAC, the sequence 71-82 appears to be crucial in the fibril formation process. It has been shown that α -syn71-82 shares several general properties and structural similarities with its parent protein. However, the behavior of this peptide in the proximity of cellular membranes remains unclear. In the present study, we have investigated the peptide structure when interacting with phospholipid model membranes. While no change in the structure (random coil) was observed upon interactions with neutral membranes, the peptide displays a dramatic structural change and adopts a parallel β -sheet structure in the presence of negatively charged membranes. To further investigate this structure, vibrational circular dichroism (VCD) spectroscopy was used to determine its supramolecular chirality. VCD revealed the presence of twisted fibrils, where the fibril twist chirality (left/right) depends on both the peptide concentration and the peptide-to-lipid molar ratio. The structural similarities shared by the peptide with the parent protein suggest that the former may overall influence the behavior and properties of the latter.

P61 - A Novel Model of Daptomycin Inhibition by Lung Surfactant: Biophysical Studies using Model Lipid Membranes

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Daptomycin is an antimicrobial peptide that is clinically used to treat severe infections caused by highly-resistant Gram-positive bacteria. However, in cases of pneumonia, daptomycin is curiously inhibited by lung surfactant and is rendered ineffective against Gram-positive *Streptococcus pneumoniae*, the primary cause of this illness. The reason for this is currently not understood. We developed lipid model systems to mimic *S. pneumoniae* membranes, tissue cell membranes, and both synthetic and natural (BLES) lung surfactant. Using the Langmuir-Blodgett trough, constant-area insertion assays were performed. It was discovered that daptomycin inserts into lung surfactant to a greater extent than it does with bacterial membrane at physiologically-relevant calcium concentrations. Meanwhile, compression isotherms provided data on monolayer compressibility. We found that daptomycin and calcium can improve surfactant activity and performance, suggesting the formation of multilayers at lower pressures. Atomic force microscopy and Kelvin probe force microscopy imaging provided visual evidence of multilayer formation induced by the presence of daptomycin and calcium, confirming this mechanism of inhibition. Based on these results, we propose a novel model of daptomycin sequestration by lung surfactant (see figure), where (1) daptomycin inserts into surfactant, (2) lowers its compressibility, (3) induces multilayer formation, and (4) reinforces its sequestration, rendering it unable to exert its bactericidal activity against *S. pneumoniae*.



P62 - Identifying the key parameters in biological models using parameter compressionChieh Ting Hsu¹, Gary Brouhard^{1,2} and Paul Francois^{1,2}*Department of Biology, McGill University, Montreal, Canada**Department of Physics, McGill University, Montreal, Canada*

Building physical models of systems is usually difficult due to the number of parameters required. In the end, the models themselves usually depend on and are sensitive only to a very small number of parameters. Various works have explored the method of parameter compression to identify those necessary parameters. In the literature, systems are often solved analytically, but this method is usually impossible when analyzing biological systems because of the complexity of the observables taken from experiments. Most observables are strongly determined by one parameter, meaning that complete parameter inference from realistic biological data is fundamentally challenging. Here we will present a numerical approach to the parameter compression method, working with example biological models such as particle diffusion, protein production-degradation and a simple dynamic microtubule model. The analysis done here shows how the importance of a given parameter is strongly dependent on the measured experimental observable.

P63 - Interaction of Tetryl, a Nitroaromatic Explosive, with Bacterial Reaction Center in *R. sphaeroides*

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Upon illumination, photosynthetic bacterial reaction centres can transfer an electron from an electron donating pigment to a final electron acceptor, creating an electric current or a potential difference across a membrane. Because of this property, these proteins are under study to find models for solar energy production or to create bio-hybrid solar batteries and capacitors. Additionally, some explosives can impact the kinetics of the separation and recombination of the charge in the protein. An electrochemical biosensor using this protein could be designed with detection of explosives based on the kinetics of photocurrents. The kinetics of the charge separation and recombination in the protein can be monitored by UV-Vis-NIR spectroscopy. The reduced bacteriochlorophyll dimer, the initial electron donor, absorbs at 865 nm. The disappearance of this absorbance band indicates that chlorophyll molecule has been oxidized. In the presence of tetryl, a nitroaromatic explosive, the speed of both the charge separation and the recovery decreases. Additionally, the reaction centre appears to catalyze the hydrolysis of tetryl into picric acid, which has absorption peaks at 345 nm and 415 nm. This research focuses on identifying the particular sites within the protein that are interacting with tetryl. It was found that tetryl is optimally hydrolyzed at pH 9.4, suggesting the involvement of deprotonated amino acids. Additionally, in the dark, tetryl is hydrolyzed at a higher rate by the R26 mutant of the protein. This mutant is missing the carotenoid pigment, suggesting that tetryl binds to the protein near the carotenoid binding site.

P64 - The Role of G-protein-Coupled Receptor Activation by Conformational Selection as revealed by Single-molecule Fluorescence

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G-protein-coupled receptors (GPCRs) are the largest class of transmembrane receptors, 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 mechanistic basis of ligand-activation: induced-fit and conformational selection. Recent evidence from our groups and others (Ye, L. *et al.*, *Nature* **533**, 265–268, May 2016) incline towards the second model, suggesting the coexistence of multiple receptor states, which, depending on the nature of the ligand bound, *i.e.*, full, partial or inverse agonist, will be more or less populated. We acquired single-molecule fluorescence (SMF) correlation, lifetime and anisotropy data of Alexa488-labelled A_{2A} adenosine receptors (A_{2A}R) to probe local conformational fluctuations at TM1, TM5, TM6, and H8 domains of A_{2A}R. We designed functional single-cysteine mutants for dye attachment near native amino-acid quenchers (*i.e.*, histidine, tryptophan, and tyrosine). The global analysis of the intensity and anisotropy decay curves show that the basal state of A_{2A}R can be described as a superposition of three states, whose relative abundance shift upon binding of the full agonist, NECA, the partial agonist, LUF5834, or the inverse agonist, ZM241385. To study the implications of this model on the binding of G proteins, a peptide mimic of the α -helical domain of the G α_s subunit, α 374-394, was labelled with Alexa488 to study the binding kinetics to unlabelled and Alexa647-labelled A_{2A}R, via SMF correlative methods and time-resolved Förster Resonance Energy Transfer (tr-FRET), respectively. Collectively, the data provided information on the binding dynamics and on the variation in peptide-efficacy toward A_{2A}R in the presence of full, partial, or inverse agonists.

P65 - How can channels selectively conduct ions at diffusion limited rate: first principles analytical solution

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The ability of biological channels to conduct ions selectively at the rate $\sim 10^8$ ions per second is critical for living systems. Although the mechanism of this phenomenon was revealed experimentally [1] its theoretical explanation remains elusive [2]. Here we propose first principle analytical solution to the problem. We obtain analytical presentation of the effective grand canonical ensemble [3] of the KcsA selectivity filter taking into account its known structure, calculate probabilities of the filter to bind ions from mixed intra- and extra-cellular solution, and evaluate filter's conductivity in the linear response regime. It allows us to introduce the conditions for barrier-less

conduction through the KcsA selectivity filter and to show that the Eisenman selectivity relations follows from these conditions. We provide estimate of the filter's conductivity under physiological conditions and demonstrate an existence of narrow bands of filter's simultaneous strong selectivity and high conductivity. The results are applicable to a wide range of biological channels and artificial nanopores.

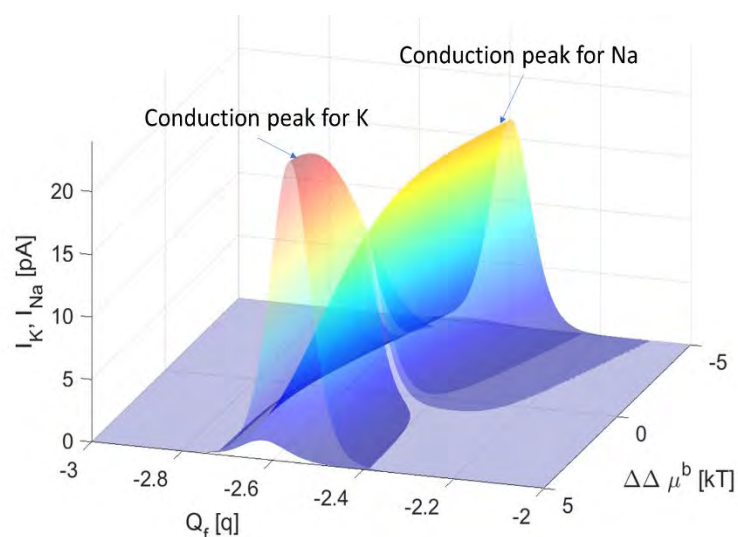


Figure. Potassium and sodium peaks of conduction through KcsA filter as a function of the total fixed charge on the channel wall Q_f and the difference in excess chemical potentials $\Delta\Delta\mu$ at the site and in the water for K^+ and Na^+ ions at $\Delta V = 50$ mV.

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[3] B. Roux, T. Allen, S. Berneche, and W. Im, *Quart. Rev. Biophys.* 37, 15-103 (2004)

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P66 - Identifying Structure in Short DNA Scaffolds using Solid-State Nanopores

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The detection of molecular features in DNA origami complexes has many potential applications in nanobiotechnology, disease biomarker detection and DNA sequencing. The inherent sensitivity of solid-state nanopore sensors enables the identification of substructure in such complexes, thus providing an avenue for proposing novel molecular sensing assays. We present an approach of molecular assembly in which solid-state nanopores are capable of differentiating DNA scaffolds containing zero, one and two dsDNA protrusions in origami complexes which are over an order of magnitude smaller than those used in typical nanopore experiments. This highly scalable technique requires minimal sample preparation and is customizable for a wide range of targets and applications. As a proof-of-concept, an aptamer-based DNA displacement reaction is performed in which a dsDNA protrusion is formed along a DNA scaffold in the presence of ATP. While ATP is too small to be directly sensed using conventional nanopore methods, our approach allows us to detect ATP by identifying molecular substructure along the DNA scaffold.

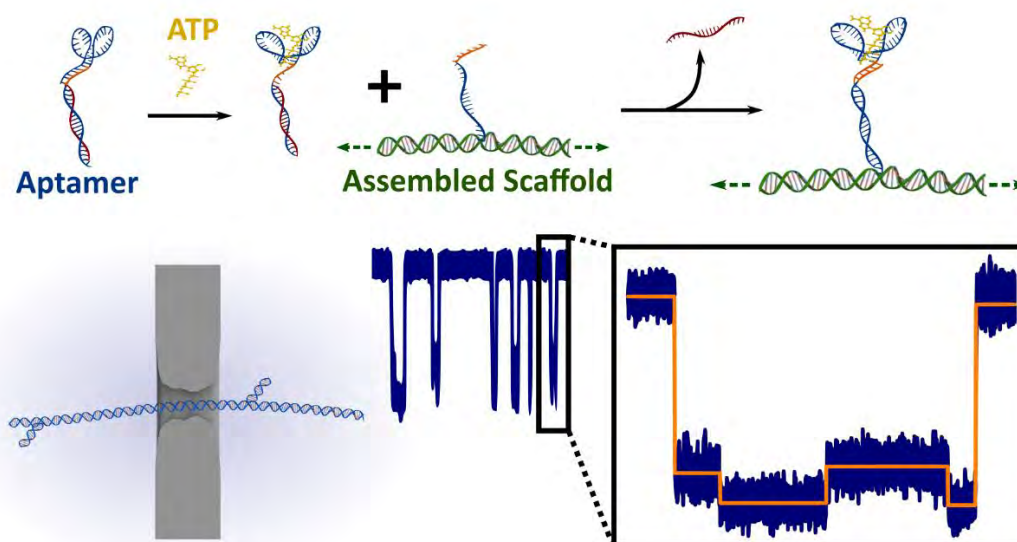


Figure: Scaffolds assembled using the principles of DNA origami contain customisable functional regions for the addition of molecular tags. In this case, a ssDNA overhang hybridises to an aptamer sequence in the presence of a small target molecule (ATP) via a DNA displacement reaction. The resulting biomolecular structures are resolved in ionic current measurements using solid-state nanopores.

P67 - SRP rapidly scans translating ribosomes until it binds the signal anchor sequence emerging from the peptide exit tunnel

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The signal recognition particle (SRP) targets ribosomes synthesizing membrane proteins to the membrane-embedded translocon in a cotranslational manner. Recognition of membrane proteins occurs by virtue of a hydrophobic signal-anchor sequence (SA) contained in the nascent chain. Previous studies with purified RNCs have revealed that SRP rapidly scans ribosomes and is partially stabilized in a standby mode by the presence of short nascent chains within the ribosomal peptide exit tunnel. Upon emergence of the nascent chain, kinetic stabilization of SRP is maintained only for RNCs bearing an SA, while SRP rapidly dissociates from non-substrates. Recent ribosome profiling and single molecule fluorescence studies, both of which use actively translating ribosomes, challenge this mechanism as they only detect SRP interaction with the ribosome after emergence of an SA. This discrepancy has been attributed to a difference between stalled RNCs and ribosomes actively engaged in translation. Here we use fluorescence-based stopped-flow to monitor SRP-ribosome interactions as an SRP substrate is synthesized in a purified *in vitro* translation system from *E. coli*. The detailed kinetic analysis of this process by global fitting of time courses reveals that, at cellular concentrations, SRP rapidly binds to and dissociates from ribosomes until the signal sequence has partially emerged from the peptide exit tunnel. At this length of the nascent peptide (45-50 amino acids), SRP undergoes a conformational change which is likely associated with signal sequence binding and stabilizes SRP on the ribosome.

P68 - SERS – Optophysiology: Toward *in-vitro* neurotransmitters monitoring of dopaminergic neurones

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Signaling mechanisms between living cells are governed by the secretion of small molecules triggering specific stimuli. During these events, the local concentration of these chemical messengers can be elevated, albeit still at low concentration, for a short period of time requiring highly sensitive sensor. The evaluation of both, identity and relative concentration of those messengers hold a promising use in medical diagnostic. Although, the current analytical tools are not typically suited for monitoring fast and heterogeneous variation in concentration during secretion events. In one example of success, micro and nanometric sized electrodes have been extensively used for *in vivo* electrochemical sensing. Yet, electrochemistry is still limited to electroactive targets, and offers a lower sensitivity for a broader range of relevant biochemical targets. Alternatively, plasmonic nanomaterials lead to enhanced spectroscopy such as surface enhanced Raman spectroscopy (SERS) which enable identification of the messenger while allowing detection at single molecule level. To address these current limitations, we developed a plasmonic nanosensor based on nanometric sized glass *patch clamp* pipettes, which were recently applied as nanoprobe to locally and selectively monitor cellular secretion nearby single, or multiple living cells. Using dynamic SERS (D-SERS), we locally monitored the secretion of dopaminergic neurons upon multiple depolarization stimulus in physiological conditions. Furthermore, we analyzed the complex and heterogeneous optical response by SERS-Optophysiology, a novel tool allowing a selective multiplex sensing of Raman active molecule. With these tools, we successfully monitored the secretions of several neurotransmitters (e.g. Dopamine and ATP) *in vitro*. Selectivity and sensitivity of both, chemometric and the SERS nanosensor will be discussed while biological results and potential applications in neurodegenerative diseases research will be presented.

P69 - Structural investigations of supercontracted spider dragline silk

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Dragline spider silk is composed of fibrous proteins organized into crystalline nanodomains embedded in an amorphous matrix. This assembly confers to silk mechanical properties that surpass those of industrial fibers. In the presence of liquid water or high humidity, the amorphous phase is plasticized, which results in the shrinking of the fiber up to 50% of its initial length. This phenomenon, known as supercontraction, can be reversibly induced and thus paves the way for new applications such as artificial muscles. However, before developing this type of biomaterial, it is imperative to have a better understanding of the supercontraction process at the molecular level. Actually, the impact of supercontraction on the conformation of the proteins constituting the dragline is not well defined. Furthermore, although it has already been established that supercontraction induces a disorientation of the molecular units, this effect has not been quantified yet. The main objective of the project is thus to investigate and quantify structural variations upon shrinking for two orb-web spiders. The experiments were carried out by Raman spectromicroscopy, an efficient tool to probe the molecular structure of a unique silk thread. The data revealed a decrease in the orientation level within the fibers and a slight modification of the β -sheet and unordered structure content upon supercontraction. This analysis also constitutes one of the first studies to quantitatively compare the molecular structure of the dragline silk of two spider species. Besides, it would appear that a greater disorientation is associated to a higher percentage of shrinking.

P70 - Molecular Views of a Eukaryotic Plasma Membrane Model

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Plasma membranes are composed of hundreds of lipid species and different types of membrane proteins. Lipid-lipid and lipid-protein interactions play a crucial role in regulating cell membranes functions and are involved in many diseases when altered. Despite advances in experimental methods, the interplay between lipids and proteins is not completely understood. In this context, computer simulations have become a powerful tool to investigate the dynamic behaviour of plasma membrane components and their interactions at a near atomic resolution. The goal of our research is to work towards large-scale modeling of plasma membranes using computer simulations. In this study, we placed ten different types of eukaryotic membrane proteins in a membrane model containing various lipid species found in the plasma membrane of eukaryotic cells. In our simulation setup, membrane proteins are present in different ratios, for a total of ca. 150 protein molecules, embedded in more than 60,600 lipids. The protein-membrane system was prepared using the Martini force field, with in house software. Using molecular dynamic simulations, we investigate the local lipid environment around proteins, and provide a molecular view of a complex membrane model.

P71 - Distinct differences in membrane interaction for two peptide ligands for the same GPCR

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The membrane catalysis hypothesis suggests that ligands associate with the membrane prior to interacting with their cell surface receptors. This initial membrane interaction step increases the local concentration of ligand, reduces diffusion from a 3D to a 2D process, and/or induces conformational change for receptor recognition, all of which enhance the rate of ligand-receptor interaction. Since membrane composition can vary, ligands likely encounter a variety of environments, with potential for lipid-dependent binding and/or conformational change. To study this, we employed apelin and apela, two peptide hormones that are processed to multiple bioactive isoforms and share a class A GPCR (the apelin receptor). Apelin exists as 55, 36, 17, and 13-residue isoforms, apela as 32, 21, and 11-residue isoforms. Each isoform retains the C-terminal region of the respective preproprotein for receptor binding and activation. According to far-UV CD and solution-state NMR spectroscopy, all apelin isoforms exhibited β -turn characteristics in the presence of anionic, but not zwitterionic, micelles. This behaviour is suggestive of preferential interactions with anionic lipids. Apela-32, conversely, demonstrated a similar level of conformational change with both zwitterionic and anionic micelles; but, upon truncation to apela-11, zwitterionic micelle-mediated changes were lost. Thus, apelin and apela exhibit distinct micelle interaction behaviour as a function of both isoform and detergent headgroup. Therefore, in accordance with the membrane catalysis hypothesis, hormonal signalling in the apelinergic system may be directly regulated by the relative availability of each ligand, its processing state, and target cell membrane composition under physiological settings.

P72 - Stoichiometry of Kv2.1/Kv6.4 heterotetramers revealed by fluorescent single channel subunit countingLena Moeller¹, Glenn Regnier², Alain J. Labro², Dirk J. Snyders², Rikard Blunck³¹*Department of Biochemistry, Université de Montréal, Montréal, Canada*²*Biomedical Sciences, University of Antwerp, Antwerpen, Belgium*³*Department of Physics, Université de Montréal, Montréal, Canada*

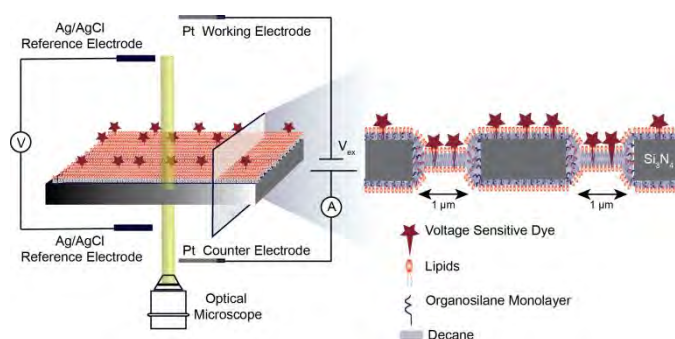
It was previously shown that electrically silent voltage-gated channel subunits (KvS) do not form functional homotetramers but can assemble as heteromeric Kv2/KvS channels with rendered biophysical properties compared to Kv2 homotetramers. So far only few of these heteromeric channels have been studied regarding their stoichiometry. FRET measurements have shown that for example Kv2.1/Kv9.3 channels have a fixed stoichiometry of 3:1 (Kerschensteiner et al., 2005, PNAS). In order to assess if other heteromeric Kv2.1/KvS channels also adopt this arrangement of subunits, we previously investigated the Kv2.1/Kv6.4 heteromer by examining the functional channel properties of different concatemers. We found that the stoichiometry in these heterotetramers can be either 3:1 or 2:2. Here, we co-express Kv2.1 and a fluorescently tagged Kv6.4-GFP in *Xenopus* oocytes and verify the stoichiometry by single-molecule fluorescence imaging. Co-expressed Kv6.4-GFP is subjected to a fluorescence intensity recording. The number of fluorescently labelled subunits within a single heteromer can then be determined by counting the photobleaching steps observed as each fluorescent label loses permanently its fluorescence by photochemical destruction (Ulbrich and Isacoff, 2007, Nature Methods). We confirm that there are maximally two Kv6.4 subunits per heteromeric complex. Furthermore, our results suggest that this arrangement of subunits is independent of the availability of Kv6.4 subunits in the cell.

P73 - Characterization of voltage sensitive dyes with free-standing lipid bilayers

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Voltage sensitive dyes (VSDs) are powerful tools for membrane potential monitoring. The characterization of VSDs is commonly performed either directly with living cells or with vesicles where the membrane potential is established by incorporating ion selective channels. However, both characterization methods lack the possibility to precisely control the voltage sequences, thus are unable to study the kinetics of the dyes. In this work, we demonstrate a new approach for the VSD characterization using free-standing lipid bilayers. First, pores ($\phi = 1 \mu\text{m}$) were fabricated in silicon nitride thin (200 nm) membranes by focused ion beam (FIB). Second, the membrane with pores was mounted in a home-made electrochemical cell where both sides of the membrane is electrically accessible. Third, free-standing lipid bilayers were formed over the pores by the well-known painting method. After the incorporation of VSDs (e.g. di-4-ANNEPS), the fluorescence signal from the bilayer is monitored by fluorescence microscopy while applying different voltage sequences. This platform offers the possibility to apply any voltage sequences, to modify bilayer composition freely, and to acquire two-dimensional mapping of the VSD activities, allowing more detailed studies of VSDs.

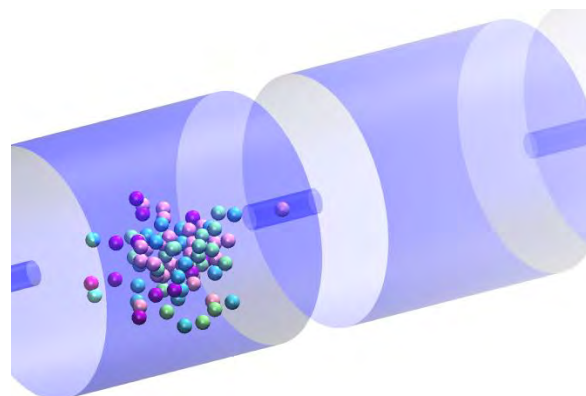


P74 - Scaling of Phase-Separated Polymer Viscoelastic Properties Under ConfinementMarjan Shayegan¹, Francis Stabile¹, Cynthia Shaheen¹, Stephen Michnick² and Sabrina Leslie¹¹Department of Physics, McGill University, Montréal, Canada²Département de Biochimie, Université de Montréal, Canada

How do protein-protein interaction networks regulate the formation of higher-order protein structures such as non-membranous organelles, and what is the influence of nanoscale confinement in regulating these structural dynamics? We aim to understand the underlying molecular interactions and physical properties of important cellular structures called non-membranous organelles (NMOs). NMOs result from intracellular phase separation of proteins and RNA. Such phase transitions within cells are involved in adapting cells to environmental changes. To date, however, studies of physical properties of reconstituted NMOs *in vivo* have been complicated by the fact that reconstituted droplets can be as much as 1000 times larger than natural NMOs formed *in vitro*. Sensitive visualization and conformational control of biopolymer interactions at mesoscale (tens to hundreds of nanometers, between molecular and cellular lengths) dimensions is important because it is at these scales that biopolymers undergo liquid-liquid phase separation. We are attempting to overcome this challenge using Convex Lens-induced Confinement (CLiC) technology to gently load polymers and biopolymers into an array of nanofabricated pits. We have tested this approach using solutions of two water-soluble polymers, polyethylene glycol and dextran, as a model system. Our tools enable us to confine polymeric solutions in 10-100 nanometer scales and visualize the phase separation directly. Additionally, we are performing particle tracking microrheology to study the mesoscale properties of dextran liquid droplets in order to explore the relationship between droplet sizes and their viscoelastic properties. We are now performing the same experiments on models of RNA-induced protein NMOs.

P75 - Separation of Polymer Mixtures by Length Using a Series of Nanopores Connected by NanochannelsMartin Magill¹, Ed Waller², and Hendrick W. de Haan¹¹ Faculty of Science, University of Ontario Institute of Technology, Oshawa, Canada² Faculty of Energy Systems and Nuclear Science, University of Ontario Institute of Technology, Oshawa, Canada

The ability to separate polymer mixtures by their lengths is an important experimental technique in modern biological analysis. Gel electrophoresis, the modern gold standard for this purpose, requires manual labour and cannot easily be miniaturized. Alternative separation methods that could be incorporated into lab-on-a-chip designs are thus being investigated. However, it has proven challenging to reproduce the performance of gel electrophoresis in such devices. Synthetic nanopores have potential for separation applications, since the translocation time of polymers passing through a nanopore grows with their length. Unfortunately, the variation of translocation times is generally large, negatively impacting separation performance. If several nanopores are placed in series, additional variation arises from diffusion between consecutive nanopores, offsetting the improvements that might otherwise be expected from multiple passes. In this work, coarse-grained simulation methods are used to study the effect of connecting consecutive nanopores by cylindrical nanochannels. These nanochannels are intended to limit the diffusion of polymers as they transit between nanopores. Langevin dynamics are used to simulate polymers near the nanopores, and Brownian dynamics are used to simulate dynamics farther from the nanopores. The simulation drift-diffusion balance is tuned to experimentally relevant conditions. The effects of nanochannel dimensions on separation effectiveness are explored.



P76 - Evolving Logical-Gates Through Phenotypic Evolution

Mathieu Hemery, Adrien Henry and Paul François

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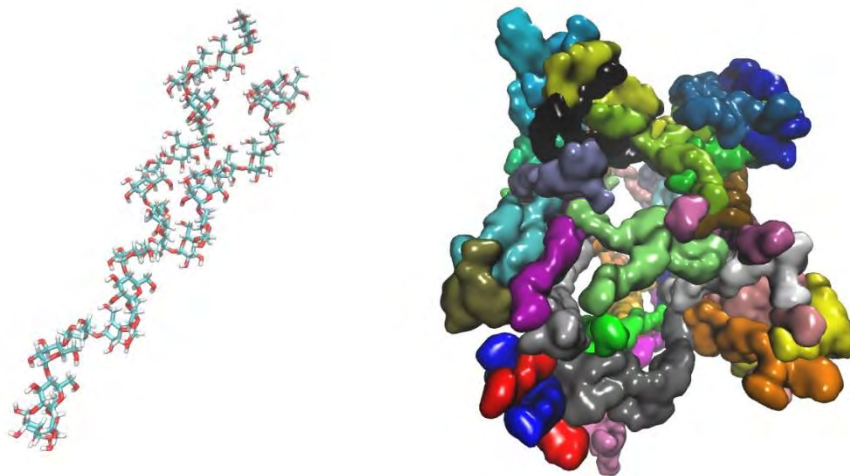
Making decisions based on cues in the environment is the very basis of cell behaviour. A text-book example is the lactose-operon: it allows bacteria to produce costly enzymes needed to consume lactose solely when a more efficient source, such as glucose, is not present. From a logical standpoint this can be viewed as a “lactose and no glucose” logical binary gate. Using the ϕ -evo software, that evolves models of gene regulatory networks (GRN) based on a genetic algorithm to reproduce Darwinian evolution. We explore how different logical gates may be implemented in biology. We will start with lac-operon and present results for the AND, NAND and XOR gates, the last of them being known to be difficult to implement because of its inherent All gates can be recovered using protein-protein interactions and transcriptional regulation only. Surprisingly, for each of these gates, only a few solutions are actually found by the evolution. This may be an important point toward the tenants of predictive evolution and for building an actual grammar of GRN.

P77 - Atomistic Simulations of the Structure of Phytoglycogen Nanoparticles

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Phytoglycogen is a water-soluble nanostructure made out of glucose monomers. It is a dendritic structure with branches consisting of glucose compounds bonded through covalent bonds. This compound is used to store energy in plants such as maize. Based on its interaction with other compounds it is believed to that phytoglycogen can be used as a biodegradable capsule for delivering medicine. Simulations are used to determine structural features of phytoglycogen that would otherwise be difficult to determine experimentally. Simulations were made using the AMBER MD simulation package, consisting of up to 50 repeatable branching subunits solvated in water. These simulations demonstrate that phytoglycogen branches are held together mainly by hydrophobic interactions, but maintain enough space for hydrophilic pockets to form and interact with water.



P78 - Spectroscopic Imaging in Microchannels for Studies of Biofilms

Mohammad Pousti, François Paquet-Mercier and Jesse Greener

Department of chemistry, Université Laval, Quebec, Canada

Biofilms are surface-attached communities of bacteria, encased in an extracellular matrix of secreted proteins, carbohydrates and DNA. The vast majority of all bacteria on Earth exist in a biofilm format. They are also becoming an important biomaterial for applications ranging from bioreactors to energy production. Microfluidics is accelerating biofilm research as a general platform for manipulation and study of biofilms. However, for further development, new in situ analytical methodologies are needed. Specifically, for chemical analysis biofilms and chemical by-products at different positions are required. Here we introduce a fast, low-cost and reliable method for acquiring 1D maps with microfluidic devices by attenuated total reflection infrared (ATR-IR) spectroscopy. The system includes a home-built automatic ATR-IR stage for moving the microfluidic/ATR assembly to different positions relative to the probe beam. Furthermore, a customized micro-imaging system is coupled to the platform to add complementary data. We demonstrate this approach in two acquisition modes: parallel measurements of multiple microchannels containing biofilms at different ionic strength environments and 1D maps of biofilms down the length of a single channel. Future directions will be discussed.

P79 - AS Simple AS Possible but not Simpler: On the Reliability of Protein Coarse-Grained Models

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Mechanical unfolding of a single domain of loop-truncated superoxide dismutase protein has been simulated via force spectroscopy techniques with both all-atom (AA) models and several coarse-grained models having different levels of resolution: A Gō model containing all heavy atoms in the protein (HA-Gō), the associative memory, water mediated, structure and energy model (AWSEM) which has 3 interaction sites per amino acid, and a Gō model containing only one interaction site per amino acid at the C α position (C α -Gō). To systematically compare results across models, the scales of time, energy, and force were suitably renormalized in each model. TM alignment, native contact, and clustering analysis show that all models consistently predict a similar single pathway unfolding mechanism for early force-induced unfolding events, but these models diverge in their predictions for late stage unfolding events when the protein is more significantly disordered. When the protein is about half-unfolded, the unfolding pathways of the AA, HA-Gō, C α -Gō models bifurcate repeatedly to multiple branches. The AWSEM model has a single dominant unfolding pathway over the whole range of unfolding, in contrast to all other models. However, the AWSEM pathway has the most structural similarity to the AA model at high nativeness, but the least structural similarity to the AA model at low nativeness. In comparison to the AA model, the sequence of native contact breakage is best predicted by the HA-Gō model.

P80 - High-speed AFM of complex lipid membranes captures video of protein-lipid interactionsMorgan Robinson¹, Loren Picco³, Mervyn Miles³ and Zoya Leonenko^{1,2}*Department of Biology¹, Physics and Astronomy², Waterloo Institute of Nanotechnology, University of Waterloo, Waterloo Ontario Canada; HH Wills Physics Laboratory³, University of Bristol, Bristol U.K.*

The lipid membrane plays a critical structural role facilitating interactions between the cell and the environment. The cell membrane not only anchors key receptors, but changes in lipid composition and non-specific interactions with the membrane serve important roles in basic biology and disease mechanisms. Unfortunately, topographical analysis of lipid membranes using atomic force microscopy (AFM) is limited by long scan times, making large high-resolution images difficult and observations of membrane processes unfeasible. A high-speed AFM (HS-AFM) platform that operates in contact mode at frame rates as high as 60fps has been developed in recent years that can be used to capture dynamic processes and mm^2 areas efficiently. Despite extremely high contact forces for extended periods of time, we show that complex multi-component lipid bilayers can be imaged using contact-mode HS-AFM in liquid with no apparent damage to the sample and that processes involving protein-lipid interactions can be visualized in real-time. Amyloid- β interactions with model lipid bilayers are shown in which lateral rearrangement of lipid domains – very likely corresponding to cholesterol and sphingomyelin enriched regions – are observed. This work represents an important step towards future HS-AFM studies visualizing biological processes at the cell membrane interface.

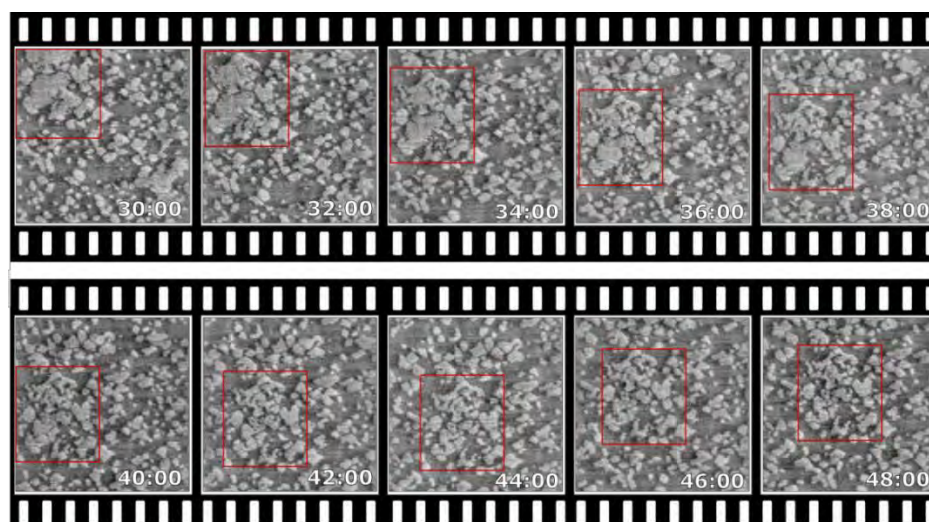


Figure 1: Amyloid- β added to complex neuronal model membrane causes lateral rearrangement of lipid domains.

P81 - Direct observation of transition-state dynamics during folding reactionsKrishna Neupane^{1*}, Noel Q. Hoffer^{1*}, M.T. Woodside^{1,2}¹*Department of Physics, University of Alberta, Edmonton AB, T6G 2E1, Canada*²*National Institute for Nanotechnology, National Research Council, Edmonton AB, T6G 2M9, Canada*

The formation of complex structures in biomolecules typically involves thermally activated crossing of an energy barrier. The unstable transition states in the barrier region dominate the folding dynamics and are thus of critical importance for understanding folding mechanisms. Because of their brief lifetime, however, it has not heretofore been possible to observe them directly, and their properties have thus only been deduced indirectly. Recent work has begun to probe the properties of the transition paths—the paths taken across the barrier region—such as the crossing time. Here we report the first observation of the dynamics within the transition states, using high-resolution optical tweezers to probe the folding of single molecules held under tension. From folding trajectories of DNA hairpins, we measured the variations in the velocity as the molecule moved over the barrier separating folded and unfolded states. The velocity distribution agreed well with simple one-dimensional (1D) diffusive theories, and the velocity profile across the barrier was found to be a sensitive probe of the barrier shape. Most intriguingly, we detected brief but ubiquitous pauses along the transition paths that allowed transient, high-energy transition states to be observed directly. Remarkably, the dynamics within the transition states reflected by these pauses agreed well with the predictions of a microscopic theory of folding in terms of a search through non-native conformations. Moreover, the diffusion coefficient governing the folding timescale calculated from the pausing statistics matched the value found from macroscopic kinetics, showing that the folding dynamics can be described quantitatively in a consistent way across all observable timescales. These results suggest we can detect the most elemental steps of the folding as individual bases pair up, opening an exciting new approach for visualising the key microscopic events governing folding reactions.

P82 - Single-Molecule Detection Using Various Integrated Nanopore-Microfluidic Arrays

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Nanopore sensors are a relatively new technology capable of detection and analysis with single-molecule sensitivity. This work highlights the integration of solid-state nanopores within various microfluidic networks with the aim of enhancing the analytical capabilities required to analyze biomolecular samples. The first generation of devices (Figure 1) contained five independently addressable microfluidic channels supporting the fabrication of nanopore arrays using controlled breakdown (CBD). In the second generation (Figure 2) pneumatic microvalves were utilized to manipulate electrical and fluidic access through connected microfluidic channels. This inclusion enabled the introduction and sequestration of multiple samples to be analyzed by different nanopores within a single device.

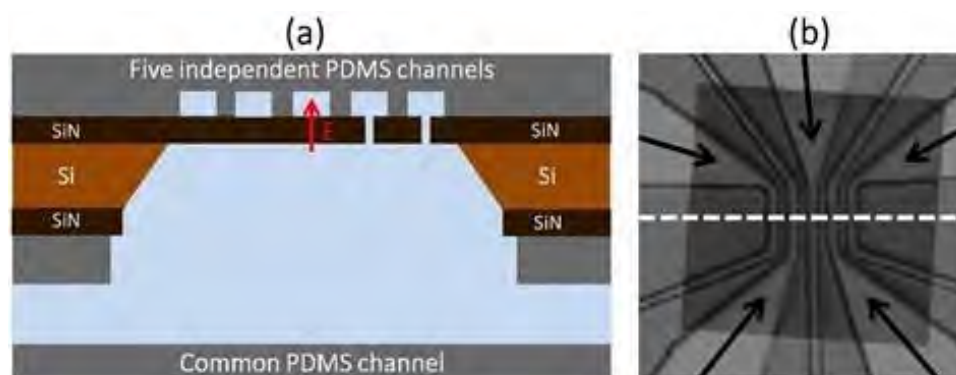


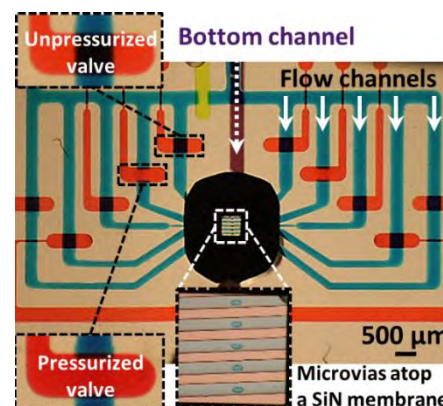
Figure 1. Cross-sectional view and reflected optical image of the device containing five independent channels cross over a $500 \times 500 \mu\text{m}^2$ SiN membrane

Figure 2. On-chip microvalves regulate electrical and fluidic access to the integrated nanopore-microfluidic arrays

It was also demonstrated that inclusion of the microfluidic via (microvia) limited the exposed area of the embedded silicon nitride membrane to the solution. This helped in localizing nanopore formation by confining the electric field to specific regions of the thin insulating membrane while significantly reducing high frequency noise in the ionic current signal through the reduction of chip capacitance.

As a proof-of-concept, various biomolecules (proteins, single- and double-stranded DNA) were successfully detected.

This approach is scalable and supports the creation of larger nanopore arrays. Such an integration will offer exciting new applications for solid-state nanopores with control over sample flow, including sample preparation and mixing, and complex manipulations for analysis.



P83 - Mechanistic Insight into a Possible Regulatory Region of an Intramembrane Enzyme Diacylglycerol acyltransferase

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Diacylglycerol acyltransferase (DGAT) catalyzes the rate limiting step of acyl-CoA-dependent formation of triacylglycerol (TAG) thus the activity of the enzyme may have a substantial effect on the flow of carbon into the seed oil of oleaginous plants. DGAT1 is a unique and essential membrane-bound enzyme that shares no homology with other family members involved in TAG production. Recombinant DGAT1 from oilseed rape (*Brassica napus*) (BnaDGAT1) was used for the study. The active enzyme reconstituted in phospholipids exhibited a hyperbolic response in activity to increasing bulk concentration of *sn*-1, 2-dioleoylglycerol revealing positive cooperativity. Various deletion constructs of the protein showed that the hydrophilic N-terminal region could act as an allosteric exosite crucial for enzyme activity. Bioinformatics analysis assisted by circular dichroism studies revealed an intrinsic disordered nature of this region. Interestingly, the disordered nature was reduced significantly upon interaction with oleoyl-CoA. Isothermal titration calorimetry was used to assess the affinity of oleoyl-CoA towards this N-terminal region. The K_d was found to be ~17 micromolar. Further, truncation of this N-terminal region demonstrated that the necessary attributes for ligand binding is localized in its C-terminal region. The three-dimensional structure of the truncated N-terminus with and without oleoyl-CoA was obtained using NMR spectroscopy confirming the mode of binding. Finally, solution x-ray scattering studies were performed to understand the structure and dynamics of full length N-terminal region upon ligand binding. Our findings pave way towards gaining mechanistic insight into the unstructured regulatory domain of BnaDGAT1, which may lead to the development of novel strategies for increasing TAG content in plant biomass.

P84 - Filamin A has a role in mechanotransduction of cells in shear

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Force-sensing and mechanical adaptation can be seen in nearly every aspect of our physiology, including the adaptation of vascular cells to shear flow, differentiation of stem cells according to the stiffness of their environment, or changes in cell traction forces and stiffness with respect to their invasiveness. Despite many landmark findings in this area, the feedback relationship between mechanics and biochemistry is still unclear, as is exactly how cells measure mechanical signals. Recent studies on reconstructed networks suggest that Filamin A and changes in its interaction with its two binding partners (FilGAP and integrin) has a potential role in mechanosensory responses of cells in shear. In this study, to test whether filamin A has a role in cell mechanotransduction responses in shear, we used two cell lines, Filamin A deficient human melanoma cells (M2) and M2 cells re-transfected with Filamin A (A7). The two cells lines were exposed to different flow shear stress and the changes in their contractile forces during time were measured. Our results show that M2 cells contractile forces are irresponsive to shear flow magnitude while A7 cells increase contractile forces to shear in a time and magnitude dependent manner. The results of this study helps describe the role of Filamin A in mechanosensory response of cells, providing new insight on how cells perceive and sense the mechanical signals.

P85 - Discovery of key molecular recognition sites between JAZ transcriptional repressors and MYC transcription factors involved in plant defense Jasmonate hormone signaling, a computational study.

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Jasmonic acid (JA) is a volatile organic compound which plays an important role in plant immunity. JA activates plant defense associated with necrotrophic and herbivorous pathogens. When the threshold level of JA is exceeded, it acts like a competitive inhibitor of JAZ protein and MYC transcription factor binding site. This activates the gene expression of plant defense molecular pathways. The main goal of this study is to identify the hotspots of the protein/protein interaction MYC-JAZ; these proteins are involved in the inactivation or potentiation of plant defense ultimately triggering or decreasing the protective response in the plant. We selected JAZ1/MYC3 and JAZ9/MYC3 complexes to analyze critical recognition sites via *in silico* alanine scanning mutagenesis. The criteria for a preliminary selection of highly important residues was affinity changes, homology, and conservation. We predicted 13 critical residues for MYC3, 8 for JAZ1 and 7 for JAZ9. Our results are in great extend similar with experimental mutagenesis yeast 2 hybrids results previously published. We address our study to use data mining machine learning algorithms to make a more robust prediction model for protein/protein interaction hotspots for this system. As a natural continuation of this work, we expect to find candidate compounds via molecular docking for the inactivation or potentiation of this mechanism.

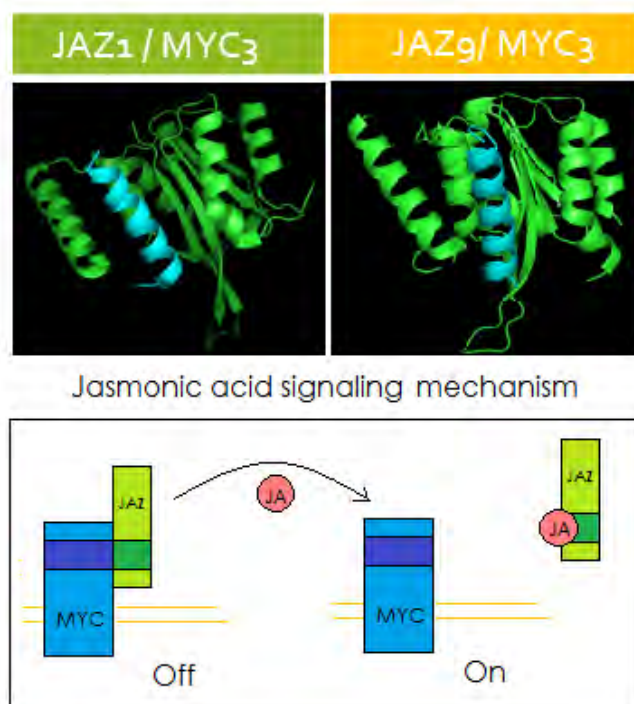


Figure 1: JAZ1-MYC3 and JAZ9-MYC3 crystallography structures. Jasmonic acid hormone signaling mechanism

P86 - DNA Invasion: How the Rate of Oligo Binding to Plasmids Provides DNA Structural Information

Shane Scott¹, Cindy Shaheen¹, Xhi Ming Xu¹, Fedor Kouzine², Laura Saunders¹, Barbara Gravel¹, Daniel Berard¹, Alexander Hofkirchner¹, Jill Laurin¹, Catherine Leroux¹, David Levens², Craig J. Benham³ and Sabrina R. Leslie¹

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³*Genome Center, University of California, Davis, USA*

While the higher order, static structure of DNA has been widely investigated, the dynamics of structural transitions and the DNA-DNA interactions permitted by them have been more challenging to investigate. We have previously demonstrated the interactions between specific unwinding sites on supercoiled DNA, and site-specific probes designed to bind to these sites using Convex Lens-induced Confinement (CLIC) microscopy to confine the molecules to nanoscale pits embedded in a coverslip. Using this methodology, we have recently been able to measure the interaction rates of these molecules as functions of supercoiling, temperature, oligo sequence, and salt concentrations. This is advantageous over other techniques, such as optical/magnetic tweezers or total internal reflection (TIRF) microscopy, in that the DNA molecules are free to explore all possible configurations without mechanical or chemical constraints. At low temperatures, we observe lower binding rates, consistent with predictions of Z-DNA formation in our sample plasmids playing an important role in structural regulation. Supercoil-induced unwinding is suppressed by alternate structures, such as Z-DNA, which are predicted to occur for the entire physiological range of superhelicities at low temperatures. Surprisingly, the target sequence for oligo binding does not affect oligo-plasmid binding rates under our experimental conditions. Binding rate dependence on oligo size is shown to be important only below a certain size of oligo. This work provides the first experimental exploration of the dynamics of torsionally-induced, unconstrained DNA unwinding, paving way for a new host of potential biophysical measurements.

P87 - A Nanofluidic Knot Factory based on Compression of Single DNA in Nanochannels

Susan Amin, Ahmed Khorshid, Lili Zeng, Philip Zimny and Walter Reisner

Department of Physics, McGill University, Montréal, Canada

Knots can form during DNA packaging in chromosome and obstruct mapping of DNA molecules in vitro in nanochannels. We demonstrate that DNA molecules can be knotted following hydrodynamic compression against nanofabricated barriers in nanochannels. In particular, we measure the probability of forming single or multiple knots on a chain as a function of compression and waiting time in the compressed state. We find knotting probability increases as the chain is compressed. In addition, we observe that knot formation probability increases with waiting time, enabling direct measurement of knot formation kinetics. Using a free energy derived from scaling arguments, we show that the enhanced knotting probability at high compression arises by avoiding the free energy cost of high self-exclusion interactions due to contour stored in the knot.

P88 - Curvature-induced lipid sorting in plasma membrane tethersSvetlana Baoukina¹, Helgi I. Ingolfsson², Siewert J. Marrink² and D. Peter Tieleman¹¹ *Department of Biological Sciences and Centre for Molecular Simulation, University of Calgary, Canada.*² *Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for Advanced Materials, University of Groningen, The Netherlands.*

Membrane tethers are nanotubes formed by lipid bilayers. They are efficient structures for cellular transport and communication, and for storage of excess membrane area. Previous tether pulling experiments provided insights on membrane mechanical properties, and the curvature effects on phase behaviour and distribution of coexisting phases. However, detailed information on tether properties and variations in composition is challenging to obtain experimentally due to the small diameters and dynamic nature of tethers. Here we provide a molecular view on curvature-induced lipid sorting in plasma membrane tethers. We pulled tethers from an idealized plasma membrane model using molecular dynamics simulations with the coarse-grained Martini model. The membrane consists of 63 lipid types with an asymmetric distribution of components between the leaflets [JACS, 2014, 136, 14554]. The tethers are formed by applying an external constant force to a lipid patch in the direction normal to the bilayer plane [Biophys J, 1012, 102, 1866]. Pulling is performed both from the inner and outer leaflets, corresponding to the direction in and out of the cell, respectively. As a result of pulling, we observe re-distribution of different lipid types along the regions of different curvature without macroscopic phase separation. Depending on the direction of pulling, the distribution of lipids and the tether properties differ.

P89 - Comparing the folding dynamics of prion proteins from species with different disease susceptibility at the single-molecule levelUttam Anand,¹ Craig Garen¹ and Michael T. Woodside^{1,2}¹*Department of Physics, University of Alberta, Edmonton, Alberta, Canada*²*National Institute for Nanotechnology, National Research Council, Edmonton, Alberta, Canada*

The prion protein PrP misfolds to cause the family of prion diseases, including 'mad cow' disease, scrapie in sheep, chronic wasting disease in cervids, and Creutzfeldt-Jakob disease in humans. PrP is highly conserved across the animal kingdom, yet disease susceptibility varies widely, from highly-susceptible species like deer and bank voles to highly resistant species like rabbits and horses. These species differences appear to be driven by changes in just a few amino acids, but how these changes alter the misfolding remains unclear. We explored the effects of species-related sequence differences using force spectroscopy to observe the folding dynamics of single PrP molecules held by optical tweezers. We compared the behavior of hamster PrP (HaPrP) to that of rabbit PrP (RbPrP) and bank vole PrP (bvPrP): hamsters are disease-susceptible, rabbits are quite resistant, and bank voles are amongst the most susceptible of all species. Unfolding and refolding trajectories were measured while ramping the applied force up and down. The resulting force-extension curves (FECs) revealed the existence of any on-pathway intermediates or misfolded (off-pathway) states, reflecting also their energetics and kinetics. In contrast to HaPrP, which was found previously to exhibit two-state folding, the folding of RbPrP involved multiple on-pathway intermediates; in both cases, native folding was rapid and misfolded states were not detected in FECs. For bvPrP, FECs included zero or at most one on-pathway intermediate, but the folding kinetics were much slower. Notably, metastable misfolded states with lifetimes on the order of seconds were observed for bvPrP. By relating the contour-length changes observed in the FECs to structural features of the proteins, we propose possible intermediates in the native folding pathways for RbPrP and bvPrP. These results show that the subtle sequence differences between PrP from different species produce important differences in the folding dynamics.

P90 - Characterization of Huntingtin's N-terminal via all-atom simulation: a solution and membrane study.

Vincent Binette and Normand Mousseau

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The huntingtin protein has attracted considerable attention because its aggregation into amyloid fibrils is linked with the neurodegenerative Huntington disease. Although huntingtin is a very large protein of more than 3000 amino acids, research has mainly focused on its N-terminal region (Htt^{NT}), composed of an amphipathic sequence of 17 amino acids (Htt17), a polyglutamine repeat domain (Q_N) and a proline rich domain (C₃₈), because it is sufficient to reproduce Huntington's phenotype *in vitro* and *in vivo*. Most notably, the Htt17 fragment plays crucial role in the acceleration of the fibrillation process and the interaction with organelle's membrane. Thus, it is essential to gain detailed insights into Htt^{NT} atomic structure. However, Htt^{NT}'s intrinsic flexibility in solution makes it difficult to experimentally characterize its structure and one must turn to numerical approaches to develop a microscopic understanding about this fragment. In this project, we use state-of-the-art numerical techniques such as Metadynamics and Hamiltonian replica exchange to characterize the dynamics and thermodynamics of HttNT in solution, refining the recently proposed experimental model of Htt17 in micelles with simulations in phospholipid bilayer. In solution, we find that Htt17 samples a large ensemble of helix, coil and two-helix bundle structure in agreement with NMR chemical shifts. The addition of QN allows the sequestration of the hydrophobic residues of Htt17. Finally, the addition of the poly-proline domain stabilizes more extended and helical conformations. In membrane, we find that Htt17 could be more structured inside the membrane than the proposed experimental model. The insertion of Htt17 induces deformation of the membrane compatible its potential dimerization.

P91 - Kinetic model of conduction through an open narrow channel

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Paradoxically, narrow K⁺ channels allow fast conduction (near to the diffusion rate), coexisting with high selectivity amongst mono-valent species. We introduce a multi-species kinetic theory of charged (Q_f) narrow channels, based on an equilibrium statistical theory. [1,2] Non-equilibrium transition rates are formed implementing the Grand Canonical Monte Carlo scheme [3], as functions of: voltage, concentration, Q_f and the species (i) dependent excess chemical potential. The theoretical current displays a good agreement with experimental data [4], Coulomb blockade phenomena vs Q_f , and large selectivity (only given vs Q_f) between K⁺ and Na⁺.

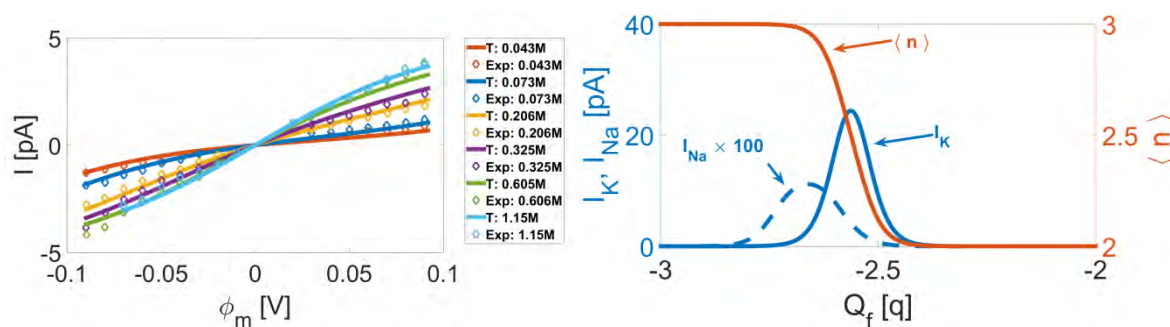


Figure 1 : Left: Theoretical current-voltage curves are compared against experimental data (diamonds) [4], showing a good agreement. Right : Coulomb blockade is observed due to resonant transitions occurring at degeneracies in the energy spectrum, displaying large selectivity in K⁺ vs Na⁺ currents.

P92 - Ion Conduction through the Cardiac Ryanodine Receptor (RyR2): All-atom Molecular Dynamics Simulation Studies

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Centre for Molecular Simulations and Department of Biological Sciences, University of Calgary, Alberta, Canada

The imbalance of calcium levels inside cardiomyocytes (heart muscle cells) contribute to the onset of arrhythmias. This pathological condition may lead to sudden cardiac deaths, resulting in 15% of all global fatalities. The ryanodine receptor (RyR2) is a “gatekeeper” of the sarcoplasmic reticulum (SR), storing a significant concentration of calcium ions to be used for muscle contractions. Therefore, understanding the molecular mechanisms that regulate calcium levels in myocytes through RyR2 is essential for the development of new, safe and inexpensive therapies. Several mutations in RyR2 are responsible of inherited lethal arrhythmias. Although electrophysiology and mutagenesis experiments have shed some light on the role of residues in the pore domain contributing to the functions of RyR2, a full atomistic description of the ion conduction is still lacking. In this work, we used computational electrophysiology approaches based on all-atom molecular dynamics (MD) simulations to study ion permeation processes in RyR2. We used an open state of RyR2 solved by Cryo-EM at 4.2-Å resolution. A constant electric field was applied to simulate ion conduction through RyR2 from the luminal side to the cytosol and vice versa under symmetric ionic conditions. A preliminary result shows that the conductance of the wild-type channel calculated from the simulated I-V curve is in fair agreement with experimental data. We also performed electrophysiology simulations of mutated RyR2 isoforms to provide atomistic insights into the role of critical sidechains in regulating the ion conduction and selectivity, which are linked to cardiac arrhythmias.

P93 - Theoretical Elucidation for Sequence-Specific Behaviors of Charged Intrinsically Disordered Proteins in Liquid-Liquid Phase Separation

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Intrinsically disordered proteins (IDPs) are depleted in hydrophobic and enriched in polar, charged, and aromatic residues. Thus they do not have a unique folded structure and often remain disordered in the context of function, including regulatory interactions. Recently, some IDPs have been found to undergo liquid-liquid phase separation (LLPS) in aqueous solution, forming membraneless organelles or proteinaceous bodies with nucleic acids and/or other proteins in the cell. These bodies can rapidly respond to environmental stimuli and play critical roles in many biological functions. Among various properties of the IDPs forming LLPS, charge sequence pattern has been identified as one of the most important characteristics. To understand this sequence-specific phenomenon, we present a theoretical polymer physics approach, applying the random-phase-approximation (RPA) theory of polyampholytes to a collection of RNA helicase Ddx4 proteins with a charged and aromatic-rich IDP region. Our theory predicts that the LLPS of Ddx4 is determined by its charge sequence pattern as well as pi-electron interactions mediated by aromatic rings, consistent with recent experiments on wild-type and a charge-scrambled mutant of Ddx4. In addition, we present a comprehensive investigation of 30 model sequences that are different permutations of an equal number of positively and negatively charged residues, in which a strong correlation between phase separation and single-chain compactness has been demonstrated. Our analytical theory can be applied to arbitrary charged biopolymers as a general framework for the research on biological phase separation.

P94 - Dynamic Compression of a Nanoslit Confined Polymer Solution

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Single nanoconfined polymers have been extensively studied over the past decade, but many systems of biophysical and technological interest consist of *many* interpenetrating chains in a confined volume, i.e. a confined solution. Here we use nanofluidics to create a confined effective “solution” of DNA coils and study how the effective solution responds to applied compressive forcing. A nanoslit structure is terminated at one end via a slit-barrier that permits fluid flow but traps single coils. Lambda DNA molecules are then pumped into the nanoslit building up a solution of coils. The solution is compressed by flow, forming a ramp-like concentration profile. We find, for sufficiently high concentration, that the profile can be described by a mean-field polymer model based on Doi's two fluid approach. We also study the hindered diffusion of nano-sized colloids in the packing; the particle diffusion can be accessed as a function of solution concentration by accessing diffusion at different positions along the ramp.

P95 - Strategies for the *in vivo* characterisation of the marine bacteria *Vibrio splendidus* by ²H NMRZeineb Bouhlel^{1,2}, Dror E. Warschawski³, Alexandre A. Arnold¹, Réjean Tremblay² and Isabelle Marcotte¹¹ *Department of Chemistry, Pharmaqam/NanoQAM, Université du Québec à Montréal, P.O. Box 8888, Downtown Station, Montréal, Canada H3C 3P8*² *Institut des Sciences de la Mer, Université du Québec à Rimouski, P.O. Box 3300, Rimouski, Québec, Canada G5L 3A1*³ *Institut de Biologie Physico-Chimique, CNRS - Université Paris Diderot, 13 rue Pierre et Marie Curie, F-75005 Paris, France*

²H solid state NMR is a useful tool to probe the organisation and dynamics of phospholipid membranes. The application of this technique to intact bacteria is further advantageous as it allows the study of lipid acyl chains in their natural environment. However, due to the structure of bacteria and complexity of their metabolic pathways, selective isotopic labelling remains a challenge. In this work, we develop protocols to selectively deuterate membrane phospholipids of the aquatic pathogenic bacteria *Vibrio splendidus*- thus enabling their *in vivo* study by ²H solid-state NMR. Labelling was optimised by growing bacteria at 25° C in culture medium enriched with deuterated palmitic acid (PA-*d*₃₁) and an appropriate detergent such as Tween-20. The latter ensures an efficient incorporation of PA-*d*₃₁ in the membrane without affecting bacterial growth. Samples were analysed using ²H NMR with Magic Angle Spinning (MAS) to improve sample viability by shortening acquisition times. Using this strategy, cell viabilities were measured to be as high as 95% after MAS experiments. In order to prevent any alteration of membrane fluidity due to an excess of saturated PA-*d*₃₁, we supplemented the growth media with unsaturated fatty acids. The resulting bacterial lipid profile was verified by GC-MS analysis of the extracted acyl chains and shown to be closer to the one of non-labelled bacteria. Subsequently, lipid phases and dynamics of *V. splendidus*' membranes with both labelling diets were characterized through the analysis of their deuterium spectral moments. A methodology is thus established for the *in vivo* study of *V. splendidus* in diverse growth conditions. The approach can be extended to the study of the interaction of these bacteria with antimicrobial agents.

P96 - Bacterial Ribosome Selective Antibacterials

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Aminoglycosides inhibit bacterial growth by binding to the A-site decoding region of the bacterial 16s ribosomal RNA (rRNA) within the 30S ribosomal subunit. Previous work has shown that there is approximately a five-fold difference in the affinity of aminoglycosides (neomycin) for the human A-site model and the E. coli model (Figure 1). Herein, we describe an approach to identify novel compounds that are selective for bacterial rRNA and include motifs such as aminosugars, amino acids and nucleobases. To accomplish such a task, we have first developed a pH sensitive fluorescent assay that rapidly identifies compounds that discriminate between the two model rRNA structures (Figure 2). This approach, coupled with a rapid solid phase synthesis method, has identified active antimicrobials (against wild type and mutant strains) that show large differences in binding affinity for the E. coli A-site and the human A-site than that of neomycin (~30 fold). The methodology for synthesizing, screening for both ribosomal binding/selectivity and bacterial growth inhibition, and rapid analysis of the data provides a systematic method for identification of bacterial ribosome specific antibacterial that can evade bacterial resistance pathways.

P97 - Insights about the effect that the J-domain has on the substrate binding domain (SBD) of 70 kDa heat shock protein Hsp70 from a chimeric human SBD-J polypeptide

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DnaJ/Hsp40 chaperones deliver unfolded proteins and via their J-domain stimulate the ATPase activity of DnaK/Hsp70, a crucial event to aid folding. The interaction between Hsp40 and Hsp70 is weak and thus difficult to study because mixing the binding partners might lead to quick dissociation due to their low affinity, challenging detailed analysis. As a consequence, many important aspects of the mechanism of interaction is still lacking, as for instance the effect that the binding of the J-domain has on Hsp70. We asked whether it would be possible to increase the current knowledge about this interaction by engineering a chimeric polypeptide where the substrate binding domain (SBD) of Hsp70 was covalently attached to the J-domain of Hsp40 by a flexible linker. The rationale is that an increase in the proximity between the interacting partners in the chimera will promote the natural interaction and facilitate the characterization of protein-protein interactions, a requirement to gain further understanding of biological processes. The resultant chimera, named here J-SBD, was produced folded and exhibited chaperone activity as showed by aggregation protection and substrate binding assays. Contrary to free SBD, which oligomerizes, the chimera behaved mainly as a monomer in all conditions tested. Collectively, our results suggest that the Hsp40 binding on Hsp70 thru the J-domain shifts the Hsp70 equilibrium towards the monomer to expose hydrophobic sites prone to accommodate substrates.

P98 - S-Nitrosylation suppresses stromal interaction molecule-1 activation and ameliorates cardiomyocyte hypertrophy.

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Calcium (Ca²⁺) is a universal signaling entity in eukaryotic cells mediating diverse processes such as the immune response, hypertrophy, apoptosis, platelet aggregation and memory, to name a few. These processes require a sustained elevation of cytosolic Ca²⁺ levels which is mediated by store operated Ca²⁺ entry (SOCE). SOCE is the process whereby endoplasmic reticulum (ER) luminal Ca²⁺ depletion signals the opening of Ca²⁺ channels on the plasma membrane (PM), facilitating the movement of Ca²⁺ down the concentration gradient from the extracellular space into the cytosol. The principal molecules that mediate SOCE include ER-resident stromal interaction molecule-1 (STIM1) and PM Orai1 protein subunits which assemble into a channel pore. Upon ER luminal Ca²⁺ depletion, STIM1 undergoes a destabilization-coupled oligomerization which leads to translocation of this Ca²⁺ sensor to ER-PM junctions where it couples to Orai1 subunits and opens these PM Ca²⁺ channels. Despite progress in elucidating the structural mechanisms of STIM1 activation, the mechanistic consequences of post-translational modifications are poorly understood. Here, we apply solution NMR spectroscopy, optical spectroscopies and cellular assays to assess how S-nitrosylation affects the STIM1 Ca²⁺ sensing mechanism with respect to pathophysiological cardiomyocyte hypertrophy. We show that nitric oxide specifically modifies two luminal Cys residues within STIM1, leading to increased stability of the domain coupled with a resistance to oligomerization and suppressed SOCE. Ultimately, these STIM1 biophysical changes endow cardiomyocytes with protection against agonist-induced hypertrophic response and pinpoint a new therapeutic target in heart disease.

P99 - Délivrance de médicaments par effet de tampon via des transporteurs moléculaires

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Le développement de mécanismes ou stratégies permettant de délivrer des médicaments directement à des sites affectés à l'intérieur du corps humain et de manière contrôlée permettrait d'augmenter grandement la performance des médicaments tout en diminuant leurs effets secondaires. À cet égard, depuis des millions d'années, la nature a développé une multitude de nano-transporteurs à base de protéines qui permettent de maintenir une concentration finement régulée de biomolécules dans la circulation sanguine. La thyroid-binding globulin (TBG), par exemple, est une protéine qui transporte l'hormone thyroïdienne thyroxine, mais dont seulement une très faible proportion (0,06%) sera libre et donc active. Cela permet à cette protéine d'agir comme un réservoir tampon et d'absorber ou de libérer l'hormone en fonction des fluctuations de concentrations pour maintenir la concentration de cette hormone stable. Le développement de transporteurs moléculaires semblables à la TBG, mais qui transporterait des médicaments permettrait de développer des systèmes versatile qui contrôleraient tel un tampon la concentration de médicament dans la circulation sanguine et de fait permettrait de maintenir une concentration idéale durant une longue période de temps. Pour ce faire, nous tirerons avantage des récentes avancées en nanotechnologie à base d'ADN, un polymère beaucoup programmable que les protéines. Dans un premier temps, nous construirons une nanomachine d'ADN se basant sur un aptamère de doxorubicine dans le but d'illustrer le mécanisme de délivrance de médicament contrôlée. À partir de ces connaissances, nous développeront les principes de bases inhérents à la programmation de ce réservoir tampon pour optimiser la délivrance du médicament doxorubicine.

P100 - DNA-protein conjugates for electrochemical biosensing applicationsXiaomeng Wang,¹ Alexis Vallée-Bélisle^{1,2}*¹Laboratoire de Biosenseurs & Nanomachines, Département de Chimie, et ²Département de Biochimie et Médecine Moléculaire, Université de Montréal, Montréal, Québec, Canada*

Protein-oligonucleotide conjugates (POCs) possess unique properties with broad applications ranging from biomedical diagnostic assays to fundamental research on molecular recognition. The future of this class of molecules is bright, but the tools for making them are far from generic. Different approaches of conjugation, including non-covalent and covalent attachment, typically require modification of the protein. Here, we explore a cheap and universal covalent labelling approach to synthesize a wide range of POCs from non-modified proteins. By using a heterobifunctional cross-linker, we have successfully attached a thiol-modified, redox-labeled single-stranded DNA (ssDNA) to lysine residues of a bacterial fimbriae protein. Using careful choice of reaction conditions (e.g. stoichiometry, time, buffer), we show that we can synthesize a range of conjugated proteins containing different numbers of DNAs. Future efforts will investigate site-specific labelling for different research topics in our lab. Possible approaches to achieve this goal include kinetic control, or by bringing two reactive groups linked to oligonucleotides into proximity by specific DNA hybridization. Then we will use these conjugates to detect bacteria in a single step for the first time using a DNA-based electrochemical sensor.

P101 - Monitoring Enzyme Activity at the Nanoscale with DNA ProbesScott G. Harroun,¹ Arnaud Desrosiers^{1,2} and Alexis Vallée-Bélisle^{1,2}*¹Laboratoire de Biosenseurs & Nanomachines, Département de Chimie, Université de Montréal, Montréal, Québec, Canada**²Département de Biochimie et Médecine Moléculaire, Université de Montréal, Montréal, Québec, Canada*

Several studies have recently reported enhanced diffusion of enzymes during exothermic catalysis, but explaining this phenomenon remains controversial. How does heat produced at the active site affect the enzyme and surrounding medium? Is the enzyme's structure destabilized? These questions are a matter of debate. Here we employ programmable DNA switches to measure structural destabilization, and possibly local temperature rise, in the vicinity of an enzyme. The unfolding temperature of DNA stem-loops can be readily tuned by varying their nucleobase composition. By attaching a fluorophore/quencher pair at the extremities of these stem-loops, we obtain a library of fluorescent switches that can act as nanothermometers. We selected a DNA switch with optimal signal sensitivity around 37 °C, and anchored it onto an enzyme via the strong biotin-streptavidin interaction. We then measure the effects of heat released during enzyme catalysis by monitoring fluorescence variation. Alkaline phosphatase was chosen because its conversion of para-nitrophenylphosphate to para-nitrophenol is highly exothermic, and this enzyme undergoes enhanced diffusion during this reaction. We find that the DNA switches attached to the enzyme are destabilized during enzyme catalysis, while control DNA switches not attached to the enzyme (i.e. free in solution) do not undergo destabilization. Along with distance-dependent destabilization, these results suggest that enzyme activity may destabilize structures located in their near vicinity.

P102 - Tau directs intracellular trafficking by regulating the forces exerted by kinesin and dynein teams

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Organelles, proteins, and mRNA are transported bidirectionally along microtubules by plus-end directed kinesin and minus-end directed dynein motors. Microtubules are decorated by microtubule-associated proteins (MAPs) that organize the cytoskeleton, regulate microtubule dynamics and modulate the interaction between motor proteins and microtubules to direct intracellular transport. Tau is a neuronal MAP that stabilizes axonal microtubules and crosslinks them into bundles. Dysregulation of tau leads to a range of neurodegenerative diseases known as tauopathies including Alzheimer's disease (AD). Tau reduces the processivity of kinesin and dynein by acting as an obstacle on the microtubule. Single-molecule assays indicate that kinesin-1 is more strongly inhibited than kinesin-2 or dynein, suggesting tau might act to spatially modulate the activity of specific motors. To investigate the role of tau in regulating bidirectional transport, we isolated phagosomes driven by kinesin-1, kinesin-2, and dynein and reconstituted their motility along microtubules. We find that tau biases bidirectional motility towards the microtubule minus end in a dose-dependent manner. Optical trapping measurements show that tau increases the magnitude and frequency of forces exerted by dynein through inhibiting opposing kinesin motors. Mathematical modeling indicates that tau controls the directional bias of intracellular cargoes through differentially tuning the processivity of kinesin-1, kinesin-2, and dynein. Taken together, these results demonstrate that tau modulates motility in a motor-specific manner to direct intracellular transport, and suggests that dysregulation of tau might contribute to neurodegeneration by disrupting the balance of plus- and minus-end directed transport.

P103 - Why protein oligomer complexes allow more precise regulation mechanisms over dimers and monomers?

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Proteins have mutated over millions of years and up to 75% of human enzymes listed have evolved into multimeric complexes. We already know that protein complexes can improve biological input by, for example, increasing the activity of enzyme or by helping regulation by combining specificity, allostery, activation and inhibition. On the other hand, less is known about the thermodynamic advantage or cost related to the use of protein complexes and how their assembly may regulate their function. In this study, we employ a synthetic biochemistry approach to compare the performance of monomeric, dimeric and trimeric complexes. We do so by designing a simple DNA structure that can be formed using one, two or three DNA strands. DNA represents a material of choice because it enables to control every thermodynamic parameters of the structure through simple mutations (e.g. modify the trimer affinity). This contrasts with protein systems where the impacts of mutations are often unpredictable. Using mathematical simulation and experimental studies, we show that trimeric complex can exhibit a much larger window of regulation mechanism compared to dimeric complexes or monomers. Our DNA trimers illustrate binding behaviors going from positive to negative cooperativity with K_{obs} that cover 4 fold of magnitude. We also identify the ratio of dimeric affinity over trimeric affinity as the key parameter for programming the assembly of the active complexes. Results provided by this study shine a new light on possible regulation mechanism of trimeric system and may help understanding why some proteins have evolved into oligomers.

P104 - A Suit of Methods to Measure Enzyme Kinetics Using Isothermal Titration Calorimetry

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Isothermal titration calorimetry (ITC) is a powerful tool for acquiring both thermodynamic and kinetic data for biological systems. ITC offers several advantages over other experimental kinetics methods as it can be performed entirely in solution under physiological conditions, does not require spectroscopically-active (eg. fluorescent) molecules, it is compatible with spectroscopically opaque solutions, and can be applied to relatively dilute samples. Despite its long history and technical advantages, kinetic applications of ITC remain fairly rare. In order to expand the use of ITC kinetics we have developed several techniques in order to measure physical properties of enzymes and enzyme inhibitors. These techniques allow us to measure both Michaelis-Menten and non-Michaelis-Menten properties of rapidly evolving enzyme reactions, extract the mode and strength of enzyme inhibitors in a single experiment and, lastly, directly measure the association and dissociation rates of enzyme inhibitors. These experiments are simple, allow for rapid and complete characterization of enzymes and enzyme inhibitors, and will broaden the overall applicability of ITC.

P105 - Rapid characterization of folding and binding interactions with thermolabile ligands by DSC

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Differential scanning calorimetry (DSC) is a powerful technique for measuring tight biomolecular interactions. However, many pharmaceutically relevant ligands are chemically unstable at the high temperatures used in DSC analyses. Thus, measuring binding interactions is challenging because the concentrations of ligands and thermally-converted products are constantly changing within the calorimeter cell. Using experimental data for two DNA aptamers that bind to the thermolabile ligand cocaine, we present a new global fitting analysis that yields the complete set of folding and binding parameters for the initial and final forms of the ligand from a pair of DSC experiments, while accounting for the thermal conversion. Furthermore, we show that the rate constant for thermolabile ligand conversion may be obtained with only one additional DSC dataset.

P106 - Kinetic Study of APH(3')-IIIa Inhibition Mechanism by Isothermal titration calorimetry (ITC)

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The enzyme aminoglycoside phosphotransferase (APH) inactivates aminoglycoside antibiotics by phosphorylation, thereby conferring bacterial resistance. APH inhibitors could potentially re-sensitize resistant bacteria, and are therefore of clinical interest. Enzyme kinetic studies of APH suggest that the substrate ATP is required to bind first, followed by aminoglycoside, and that the product phospho-aminoglycoside dissociates rapidly while ADP dissociates slowly. The previous study suggested that the accumulation of ADP might compete with ATP for the enzyme active site. However, the inhibition process cannot be studied by the commonly used coupled assay method; in which the product ADP is converted to ATP by the pyruvate kinase. We therefore developed a new method using Isothermal titration calorimetry (ITC) to study the kinetic and mechanism of APH product inhibition by ADP. We demonstrate for the first time that ADP is a potent competitive inhibitor of APH and determined K_{iADP} via a multiple injection ITC assay, giving the unexpected result that APH(3')-IIIa binds more tightly to ADP than ATP.