Chemical Biophysics Symposium
May 2nd – 4th 2014
Welcome to the 13th Annual CBP Symposium!
Message from the Chair

It is my pleasure to welcome new guests, and welcome back our perennial members to CBP 2014. Having been a part of this community for many years, it is an honour to serve as Chair for the symposium, and I hope to meet you all throughout the weekend. Our team has prepared what promises to be an exciting program, and we are excited that you could all join us.

We have always taken much pride in our intimate atmosphere and the close-knit community we have built over the years, and it is our goal for you to truly make this symposium your own. We hope that you will take full advantage of this opportunity to meet our keynotes personally, make friends, connections, and be inspired by one another to form collaborations.

We also encourage you to join in during our panel discussion - a favourite amongst our committee members! We hope that this will be a catalyst for continued debate and discussion throughout the symposium, spilling over into coffee breaks, meals, and, of course, our annual banquet!

Being a collection of researchers from a wide variety of fields, it is always incredible to me to see the different paths that lead us to the same places. Our goal has always been to really engage you with an interdisciplinary spirit, and time and again this symposium is a fine display of how our fields of research are blending and relying on one another more and more.

We hope that you enjoy the symposium, and look forward to another great year! Please feel free to approach us with any questions, comments, ideas, and suggestions for the future. As the nature of interdisciplinary research moulds, so too do we hope to mould with it! We look forward to seeing you in 2015!

-- Richard Kil --
CBP 2014 Chair
Chemical Biophysics Symposium 2014
University of Toronto, Canada
May 2\textsuperscript{nd} - 4\textsuperscript{th}, 2014

Contact Information:
Richard Kil (Student Chair): richard.kil@mail.utoronto.ca
Prof. David McMillen (Faculty Advisor): david.mcmillen@utoronto.ca
CBP 2014 Organizing Committee

CHAIR
Richard Kil

SPONSORSHIP
Tracy Stone

PUBLICITY
Daniel Oblinsky

FINANCE
Nari Kim

ART
Ursula Florjanczyk

ACCOMODATIONS
Kris Kim

FACULTY ADVISOR
David McMillen

PROGRAMME
Ursula Florjanczyk
Tae Hun Kim
Hannah Morales
Samuel Yoshua

WEBSITE
Duncan Smith-Halverson
Zhenfu Zhang

LOGISTICS
Helen Fan
Hannah Howard
Angel Lai
Yang Li
Bryan Robertson
### Programme of Events

*Leslie L. Dan Pharmacy Building, 144 College Street, Toronto*

**Friday, May 2nd**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:00-2:50 PM</td>
<td>Registration</td>
<td>Main Lobby</td>
</tr>
<tr>
<td>2:50-3:00 PM</td>
<td>Opening Remarks – Prof. David McMillen &amp; Richard Kil</td>
<td>B250</td>
</tr>
<tr>
<td><strong>Session I</strong></td>
<td></td>
<td>B250</td>
</tr>
<tr>
<td>3:00-4:00 PM</td>
<td><strong>G. Julius Vancso</strong> <em>(University of Twente, Netherlands)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Smart Soft Matter”</td>
<td></td>
</tr>
<tr>
<td>4:00-4:20 PM</td>
<td><strong>Adam N. Raegen / John R. Dutcher</strong> <em>(University of Guelph)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Advances in Surface Plasmon Resonance Imaging Enable Quantitative Tracking of Nanoscale Changes in Thickness and Roughness”</td>
<td></td>
</tr>
<tr>
<td>4:20-4:40 PM</td>
<td><strong>Yi Liao</strong> <em>(University of Michigan)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Capturing the Dynamic Search for DNA Mismatches in Replicating Cells”</td>
<td></td>
</tr>
<tr>
<td>4:40-5:00 PM</td>
<td><strong>Meaghan Ward</strong> <em>(University of Guelph)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Structural Studies of <em>Anabaena</em> Sensory Rhodopsin in the <em>E. Coli</em> Inner Membrane”</td>
<td></td>
</tr>
<tr>
<td>5:00-5:15 PM</td>
<td>Coffee Break</td>
<td>Main Lobby</td>
</tr>
<tr>
<td><strong>Panel Discussion</strong></td>
<td></td>
<td>B250</td>
</tr>
<tr>
<td></td>
<td><em>Moderator:</em> David McMillen</td>
<td></td>
</tr>
<tr>
<td>6:15-7:15 PM</td>
<td>Dinner Buffet</td>
<td>Main Lobby</td>
</tr>
<tr>
<td><strong>Session II</strong></td>
<td></td>
<td>B250</td>
</tr>
<tr>
<td>7:15-8:15 PM</td>
<td><strong>Manu Prakash</strong> <em>(Stanford University)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Merging ‘Science’ and ‘Making’ to Explore the Micro-Cosmos (and Other Musings in Science)”</td>
<td></td>
</tr>
<tr>
<td>8:15-8:35 PM</td>
<td><strong>Joshua N. Milstein</strong> <em>(University of Toronto)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Mechanical Fluctuations and the Packaging of the Bacterial Chromosome”</td>
<td></td>
</tr>
<tr>
<td>8:35 PM</td>
<td>Informal Discussions and Poster Viewing <em>(refreshments served)</em></td>
<td></td>
</tr>
</tbody>
</table>
### Saturday, May 3rd

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-9:30 AM</td>
<td>Continental Breakfast</td>
<td>Main Lobby</td>
</tr>
<tr>
<td>9:30-10:30 AM</td>
<td><strong>Session III</strong></td>
<td>B250</td>
</tr>
<tr>
<td></td>
<td><strong>Jeffery J. Tabor</strong> (Rice University)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Characterizing Bacterial Gene Circuit Dynamics with Optically Programmed Gene Expression Signals”</td>
<td></td>
</tr>
<tr>
<td>10:30-10:50 AM</td>
<td><strong>Edouard Harris</strong> (University of Toronto)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Design and Bayesian Analysis of Small Activating RNAs Targeting a Synthetic Promoter in Human Cells”</td>
<td></td>
</tr>
<tr>
<td>10:50-11:10 AM</td>
<td><strong>Anil Kumar</strong> (University of Toronto)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Photo-control of Cap-dependent Translation”</td>
<td></td>
</tr>
<tr>
<td>11:10-12:30 PM</td>
<td>Poster Session I <em>(Odd numbered)</em> &amp; Coffee Break</td>
<td>Main Lobby</td>
</tr>
<tr>
<td>12:30-1:30 PM</td>
<td>Lunch</td>
<td>Main Lobby</td>
</tr>
<tr>
<td>1:30-2:30 PM</td>
<td><strong>Session IV</strong></td>
<td>B250</td>
</tr>
<tr>
<td></td>
<td><strong>Roman A. Melnyk</strong> (The Hospital for Sick Children, University of Toronto)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“The Pathogenic Toxins of <em>C. difficile</em>: Molecular Mechanisms &amp; Drug Discovery”</td>
<td></td>
</tr>
<tr>
<td>2:30-2:50 PM</td>
<td><strong>Amy Liu</strong> (University of Toronto)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Circular Dichroism Spectroscopy of Amyloid-β in the Presence of Aggregation Modulators: Towards Alzheimer’s Disease Therapy”</td>
<td></td>
</tr>
<tr>
<td>2:50-3:10 PM</td>
<td><strong>Erin Shelton</strong> (University of Guelph)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Quantifying Spatiotemporal Patterns in the Expansion of Twitching Bacterial Colonies”</td>
<td></td>
</tr>
<tr>
<td>3:10-4:30 PM</td>
<td>Poster Session II <em>(Even numbered)</em> &amp; Coffee Break</td>
<td>Main Lobby</td>
</tr>
<tr>
<td>4:30-5:30 PM</td>
<td><strong>Session V</strong></td>
<td>B250</td>
</tr>
<tr>
<td></td>
<td><strong>Jhih-Wei Chu</strong> (National Chiao Tung University, Taiwan)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“A Multiscale Approach for Biosystems Simulation”</td>
<td></td>
</tr>
<tr>
<td>5:30-5:50 PM</td>
<td><strong>Daniel G. Oblinsky</strong> (University of Toronto)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Supramolecular Biological Assemblies for Solar Light Harvesting”</td>
<td></td>
</tr>
<tr>
<td>5:50-6:10 PM</td>
<td><strong>Abdullah Mahboob</strong> (Brock University)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Reverse Engineering of the Donor side of Photosystem II: Second Generation of the Bacterioferritin ‘Reaction Centre’”</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>6:10-6:45 PM</td>
<td>Break &amp; travel to restaurant</td>
<td></td>
</tr>
<tr>
<td>6:45 PM --</td>
<td><strong>Symposium Banquet</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Forestview Chinese Restaurant, 466-468 Dundas St. West, 2nd floor</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Do you know your stuff?” Chemical Biophysics Game Show to follow!</td>
<td></td>
</tr>
</tbody>
</table>

**Sunday, May 4th**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-9:30 AM</td>
<td>Continental Breakfast</td>
</tr>
<tr>
<td></td>
<td>Main Lobby</td>
</tr>
<tr>
<td>Session VI</td>
<td>B250</td>
</tr>
<tr>
<td>9:30-10:30 AM</td>
<td><strong>Brian Kuhlman</strong> (<em>University of North Carolina at Chapel Hill</em>)</td>
</tr>
<tr>
<td></td>
<td>“Computational Design of Protein Interactions and Switches”</td>
</tr>
<tr>
<td>10:30-10:50 AM</td>
<td><strong>Loan Huynh</strong> (<em>University of Toronto</em>)</td>
</tr>
<tr>
<td></td>
<td>“Systematic Evaluation of the Native and Non-native Interactions of the Helical IM9 Protein Using All-Atom Molecular Dynamics Simulations”</td>
</tr>
<tr>
<td>10:50-11:10 AM</td>
<td><strong>Shaghayegh Vafaei</strong> (<em>University of Guelph</em>)</td>
</tr>
<tr>
<td></td>
<td>“Calculating Free Energy of the Aggregation of the Peptide (HHC-36) In Bulk”</td>
</tr>
<tr>
<td>11:10-11:30 AM</td>
<td>Coffee Break</td>
</tr>
<tr>
<td></td>
<td>Main Lobby</td>
</tr>
<tr>
<td>Session VII</td>
<td>B250</td>
</tr>
<tr>
<td>11:30-12:30 PM</td>
<td><strong>Yann R. Chemla</strong> (<em>University of Illinois at Urbana-Champaign</em>)</td>
</tr>
<tr>
<td>12:30-12:50 PM</td>
<td><strong>Laura Toppozini</strong> (<em>McMaster University</em>)</td>
</tr>
<tr>
<td></td>
<td>“The Structure of Cholesterol in Lipid Rafts”</td>
</tr>
<tr>
<td>12:50-1:00 PM</td>
<td>Closing Remarks</td>
</tr>
<tr>
<td></td>
<td>B250</td>
</tr>
</tbody>
</table>
Abstracts

Oral Presentations
The last decades have witnessed a tremendous growth in the field of science and technology of advanced macromolecular materials. During this period attention has shifted gradually from structural, and functional to smart (responsive) soft matter and to their integration in complex materials systems with inorganic and metallic (nanoscale) components, designed and prepared “bottom up” for function. How do sea cucumbers stiffen by the action of reversible polymeric bio-nanocomposites that form in their body when they get “poked” by external action? How do chameleons change their color and camouflage adapting to their environment? These animals possess stimulus responsive behavior, which helps them to survive. Artificial biomimetic strategies, learning from these and similar specii, are now increasingly being used in materials science to prepare advanced synthetic materials and open exciting avenues to prepare a new class of “smart” soft matter.

Achievements in polymer chemistry provided new, stimulus responsive macromolecules with hitherto unprecedented structural control, precision, and ease of synthesis. Materials surfaces with graft chains can now be functionalized with polymers using e.g. controlled free radical polymerizations in “grafting from” approaches, in addition to grafting chains to reactive substrates. Recently, even the first steps towards forming true 3D functional and responsive structures “bottom up” using polymer brushes as a starting platform have been made. In this presentation selected examples will be displayed to illustrate the versatility and application potential of smart surface grafts.

In the first example of this lecture temperature and pH responsive polymer grafts, so-called brushes, will be discussed. Their applications in controlling friction and adhesion of functionalized substrates will be elucidated. In case of two contacting brush systems, interdigitation of opposing brushes makes them prone to damage. We briefly show that immiscible brush systems can form slick interfaces, in which interdigitation is eliminated and dissipation strongly reduced.

We shall then turn our attention to cross-linked polymer gel-brushes, which can be used to first obtain metal nanoparticles in a controlled fashion, and then employ these in fluidic microreactor devices to control their catalytic activity. As an example for a nanotechnology compatible, i.e. nanoscale-addressable, stimulus responsive materials platform, redox active poly(ferrocenylsilane) (PFS) grafts will be presented. Chemically modified electrodes, decorated with covalently tethered PFS chains, will be introduced. An ascorbic acid electrochemical sensor based on surface-anchored PFS chains, exhibiting high sensitivity and stability, is discussed. The PFS layers described are easily derivatized, thus forming a platform for creating highly tailorable redox-active interfaces. At the end we present electrografting of robust, dense, redox active PFS films. These films can be formed by cathodic reduction of Au substrates, immersed in a solution of imidazolium–functionalized PFS chains in an ionic liquid. The electrografted polymer films are employed as an electrochemical sensor, exhibiting high sensitivity, stability and reproducibility.

Contact: g.j.vancso@utwente.nl
Advances in Surface Plasmon Resonance Imaging Enable Quantitative Tracking of Nanoscale Changes in Thickness and Roughness

Adam N. Raegen,1 Kyle Reiter,1 Alexander Dion,1 Anthony J. Clarke,2 Jacek Lipkowski,3 and John R. Dutcher1

1Department of Physics, 2Department of Molecular and Cellular Biology, and 3Department of Chemistry, University of Guelph, Guelph, ON, Canada N1G 2W1

To date, detailed studies of the thickness of coatings using surface plasmon resonance have been limited to samples that are very uniform in thickness, and this technique has not been applied quantitatively to samples that are inherently rough or undergo instabilities with time. We describe a significant improvement to surface plasmon resonance imaging (SPRi) that allows this sensitive technique to be used for quantitative tracking of the thickness and roughness of surface coatings that are rough on the scale of tens of nanometers. We tested this approach by studying samples with an idealized, one-dimensional roughness: patterned channels in a thin polymer film. We find that a novel analysis of the SPRi data collected with the plane of incidence parallel to the patterned channels allows the determination of the thickness profile of the channels in the polymer film, which is in agreement with that measured using atomic force microscopy. We have further validated our approach by performing SPRi measurements perpendicular to the patterned channels, for which the measured SPR curve agrees well with the single SPR curve calculated using the average thickness determined from the thickness profile as determined using AFM. We applied this analysis technique to track the average thickness and RMS roughness of cellulose microfibrils upon exposure to cellulolytic enzymes, providing quantitative determinations of the times of action of the enzymes that are of direct interest to the cellulosic ethanol industry.


Figure 1: (a) Representative SPR curves of reflectivity versus angle of incidence \( \theta_i \) for a cellulose microfibril-coated region at different times after exposure to a 21 \( \mu g/mL \) CBH2 enzyme solution.

(b) Dependence of average thickness on time of exposure to the CBH2 enzyme solution.
Capturing the Dynamic Search for DNA Mismatches in Replicating Cells

Yi Liao¹, Jeremy. W. Schroeder², Lyle A. Simmons² and Julie S. Biteen¹
¹Department of Chemistry, ²Department of Molecular, Cellular and Developmental Biology, University of Michigan

All cells possess the remarkable ability to replicate their genome with high fidelity, owing to the presence of various DNA repair systems which sense and correct replication errors when they arise. Deficiencies in any of these systems, including the mismatch repair system (MMR), can have severe consequences leading to increased antibiotic resistance in bacteria and certain types of cancers in humans. From prokaryotes to human cells, homologs of the highly conserved MMR protein MutS recognize mispaired nucleotides and recruit the proteins responsible for downstream repair. The initiation of MMR by MutS traveling on lesion-containing DNA strands has recently been visualized in vitro at single-molecule level, but the in vivo process, which must include overcoming the crowded cellular environment and the tightly packed chromosomal DNA, remains poorly understood.

To address this question, we have applied single-molecule fluorescence microscopy to reveal the in vivo distributions and dynamics of MutS in Bacillus subtilis, a Gram-positive bacterium that has served as the model organism for studying DNA replication and repair mechanisms due to its remarkable genetic competence and high level of homology with corresponding pathways in humans. Based on two-color photoactivated localization microscopy (PALM) and single-particle tracking, we have visualized the highly dynamic interplay between the MMR system and the replisome that stages MutS to sites of DNA replication, where MutS presumably has access to the newly synthesized, naked DNA, allowing for mismatch detection by sliding. To further our understanding of these processes, we then sequentially block three key steps in the MMR pathway, and study how each component affects the MutS/replisome interaction. Together, our results provide strong evidence that the recruitment of MutS to the replisome is a required step in MMR and precedes mismatch binding events in vivo.
Structural Studies of *Anabaena* Sensory Rhodopsin in the *E. Coli* Inner Membrane

Meaghan Ward†, Shenlin Wang†, Ivan Hung‡, Peter Gor’kov†, Hongjun Liang§, Leonid Brown†, Vladimir Ladizhansky†

†Department of Physics and Biophysical Interdepartmental Group, University Of Guelph
‡Beijing NMR Center, Peking University
§National High Magnetic Field Laboratory, University of Florida

*Anabaena* sensory rhodopsin (ASR) is a heptahelical retinal-binding membrane protein, whose oligomeric organization and high resolution structure have recently been solved using solid state nuclear magnetic resonance spectroscopy (SSNMR). While ASR was found to form a trimer lattice in proteoliposomes, a dimer structure has been solved using X-ray crystallography. Though the proteoliposome environment closely mimics the native environment of ASR, it is possible that the purification procedure, the high protein to lipid ratio, or the simplicity of the proteoliposome environment could alter the structure of the protein, and functionally relevant details could be obscured.

To study the structure and oligomeric organization of ASR in a more native environment, we created a sample of ASR in the *E. coli* inner membrane (IM-ASR). *E. coli* inner membrane vesicles were isolated from cells which overexpressed ASR. Removal of outer membrane proteins and selective isotopic labelling of ASR for solid state nuclear magnetic resonance (SSNMR) studies were monitored using Fourier transformed infrared spectroscopy (FTIR). Though circular dicroism (CD) reveals that the trimer structure remains intact in IM-ASR, the presence of a trimer lattice cannot be confirmed with small angle X-ray scattering (SAXS). To investigate site-specific structural changes, two and three dimensional SSNMR experiments were performed on selectively isotopically labelled samples. Despite the strong presence of additional proteins in IM-ASR, many isolated resonances corresponding to ASR can be identified in 2D spectra and are well resolved. Overlapping signals will be resolved through three dimensional experiments on IM-ASR, as well as two and three dimensional experiments performed on IM samples of uninduced cells. Structural changes in IM-ASR can then be detected through the analysis of chemical shift and peak intensity differences. Through these experiments it is predicted that sufficient information should be available to detect potential structural changes in IM-ASR.

References:
Merging ‘Science’ and ‘Making’ to Explore the Micro-Cosmos (and Other Musings in Science)

Manu Prakash
Department of Bioengineering, Stanford University, Stanford, California 94305, USA

The Makers movement sometimes struggles to integrate with the traditional science avenues. Similarly, biological and environmental questions are context dependent and need to be asked with field based science, where traditional approaches struggle to reach into the remote corners of the world. Citizen science sometimes struggles to engage people in broad range of scientific explorations since the goals are predefined. Using principles of "frugal science" - I will discuss a few ideas from our group where we are imagining how to enable "citizen" explorers to discover new biological, ecological and health related questions around the world. More specifically I will describe the history and making of Foldscope, a frugal approach to manufacture optical instruments using origami and how we are trying to make them widely available to encourage explorations of the micro-cosmos. Time permitting, I will dive into some scientific questions my lab is exploring in the field of marine organismic biophysics.

Contact: manup@stanford.edu

Mechanical Fluctuations and the Packaging of the Bacterial Chromosome

Joshua N. Milstein1,2
1Department of Physics, University of Toronto
2Department of Chemical and Physical Sciences, University of Toronto Mississauga

The bacterial chromosome is under varying levels of mechanical stress due to a high degree of crowding and dynamic protein-DNA interactions experienced within the nucleoid. DNA tension is difficult to measure in cells and its functional significance remains unclear although in vitro experiments have implicated a range of biomechanical phenomena. Using single-molecule tools, we have uncovered a novel protein-DNA interaction that responds to fluctuations in mechanical tension by condensing DNA. We combined tethered particle motion (TPM) and optical...
tweezers experiments to probe the effects of tension on DNA in the presence of the Hha/H-NS complex. The nucleoid structuring protein H-NS is a key regulator of DNA condensation and gene expression in enterobacteria and its activity in vivo is affected by the accessory factor Hha. We find that tension, induced by optical tweezers, causes the rapid compaction of DNA in the presence of the Hha/H-NS complex, but not in the presence of H-NS alone. Our results imply that H-NS requires Hha to condense bacterial DNA and that this condensation could be triggered by the level of mechanical tension experienced along different regions of the chromosome.

Session III, Sat 9:30 am

Characterizing Bacterial Gene Circuit Dynamics with Optically Programmed Gene Expression Signals

Jeffery J Tabor¹,²
¹Department of Bioengineering, Rice University, Houston, Texas, USA.
²Department of Biochemistry and Cell Biology, Rice University, Houston, Texas, USA.

The goal of the discipline of synthetic biology is to understand how to program systems-level biological processes, such as the growth of an artificial tissue, by writing unnatural DNA sequences. In order to gain better understanding of, and control over, the path from DNA to cell- and organism-level processes, synthetic biologists have adapted a modular design framework from electrical and systems engineering. In the ‘biological systems engineering’ framework, the manner in which individual genetic components, such as signaling proteins or transcription factors, transduce, transform, and transmit biological signals is first understood through rigorous characterization. The goal is then that components can be assembled into higher-order devices, and eventually systems, whose performance can be predicted from the properties of the components. Though synthetic biology has made notable progress, the actual utility of the biological systems engineering framework has been limited by an inability to directly characterize the dynamical performance features of biological components, and thus predict how they will behave when composed and deployed in different contexts. The result is that synthetic biology is not yet a mature engineering discipline. To address this limitation, we have recently developed an all-optical biological ‘function generator and oscilloscope’ framework that allows us to directly characterize the dynamical signal processing properties of genetic components in the native cellular environment. In particular, we have created sine waves and linear ramps of a transcriptional repressor in E. coli and shown that the promoter that it regulates transforms the repressor signal linearly with a seven-minute time delay. Our method is based upon our previously engineered light-switchable bacterial two component systems, mathematical models of their input/output dynamics, computational algorithms to design light inputs to program custom gene expression dynamics, and custom-built optical hardware for growing cells in well defined light conditions and making precise fluorescent protein measurements. Here, I will discuss how this and related methods can help overcome the major characterization bottlenecks that have limited synthetic biology, and along with future advances, enable a complete adaptation of the systems engineering framework to biology.

Contact: jeff.tabor@rice.edu
Design and Bayesian Analysis of Small Activating RNAs Targeting a Synthetic Promoter in Human Cells

Edouard Harris and David McMillen
Department of Physics, University of Toronto

As biological mechanisms and processes become better understood, they grow increasingly amenable to analysis using techniques originally developed for the quantitative sciences. We present the design, experimental investigation, and theoretical analysis of the first synthetic genetic construct to be targeted by RNA activation, a phenomenon whereby small double-stranded RNAs increase gene expression from sequence-targeted promoters in a poorly understood mechanism thought to be related to that of RNA interference. The selection of activating RNA sequences is informed by a custom-written computer program designed to choose target sites on the promoter of interest according to a set of previously determined optimality criteria. The consequent increase in gene expression by successful candidate RNAs is explored in substantial experimental detail. Bayesian inference is then used to populate a population-level Fokker-Planck model of RNA activation-dependent protein expression.

Photo-control of Cap-dependent Translation

Anil Kumar, Anna S. I. Jaikaran, and G. Andrew Woolley*  
Department of Chemistry, University of Toronto

Photo-control of translation initiation could be a powerful tool for probing the role of translational processes in cellular biology. Cap-dependent translation initiation requires the formation of the eukaryotic initiation factor-4F (eIF4F) complex that is composed of three subunits eIF4E, eIF4A and eIF4G. This is regulated by association/dissociation of hypophosphorylated/phosphorylated 4E-BP (eIF4E-binding protein) via competitive binding with eIF4G. Here, we report designs and initial characterization of photoswitchable 4E-BP chimeras. The flexible peptide linker GGSGGSGGGG of our previously designed circularly permuted PYP (cPYP) (Biochemistry, 2013) was replaced with active segments of 4E-BP. Three constructs were prepared, one with only the primary canonical site (cPYP-4E-BP-C0) and two longer constructs containing the secondary canonical site (cPYP-4E-BP-C1, cPYP-4E-BP-C1N1). All designed chimeras were expressed in E. coli and are highly soluble. UV-vis spectra of the dark adapted and irradiated state confirm the photoswitchable properties of all designed constructs. The recovery of the dark-state structure as monitored by UV-vis spectroscopy after removal of blue light irradiation indicates that cPYP-4E-BP chimeras recover slowly in minutes. Importantly, recovery rates of all of the constructs (cPYP-4E-BP-C0, cPYP-4E-BP-C1, cPYP-4E-BP-C1N1) were increased in the presence of 4E, consistent with a light dependent interaction of the chimeras with 4E. Initial activity
assays using an *in vitro* translation systems based on HeLa cell extracts shows inhibition of translation by cPYP-4E-BP-C1 and cPYP-4E-BP-C1N1. Further investigation using fluorescence polarization is in progress to directly characterize the binding ability of chimeras to 4E.

<table>
<thead>
<tr>
<th>Session IV, Sat 1:30 pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Pathogenic Toxins of <em>C. difficile</em>: Molecular Mechanisms &amp; Drug Discovery</td>
</tr>
<tr>
<td>Roman A. Melnyk¹,²</td>
</tr>
<tr>
<td>¹Program in Molecular Structure and Function, The Hospital for Sick Children, Toronto, ON</td>
</tr>
<tr>
<td>²Department of Biochemistry, University of Toronto, Toronto ON</td>
</tr>
</tbody>
</table>

Disease associated with *Clostridium difficile* infection is caused by the actions of the homologous toxins TcDA and TcDB on colonic epithelial cells. These large multi-domain proteins cross biological membranes with impunity, delivering their associated cytotoxic enzyme domain into the host cytosol to wreak havoc and induce cellular death. Our laboratory is interested in studying the molecular mechanisms of how these pathogenic toxins enter cells, and in applying this information towards the development of new therapeutics. Recently, we used a series of cellular, biochemical and biophysical methods to elucidate the architecture of the pore that is created by TcDB in the host membrane that allows membrane translocation. Using this and other information, we developed a high throughput image-based screen that allowed us to identify small molecule inhibitors of TcDB-mediated cellular toxicity. The discovery of both host- and toxin-targeted compounds that prevent toxicity will be described along with a discussion of their therapeutic potential to treat *C. difficile* disease.

Contact: roman.melnyk@sickkids.ca
Circular Dichroism Spectroscopy of Amyloid-β in the Presence of Aggregation Modulators: Towards Alzheimer’s Disease Therapy

Amy Liu1, Kagan Kerman1,2*
1Department of Chemistry, University of Toronto
2Department of Physical and Environmental Sciences, University of Toronto Scarborough

Alzheimer’s Disease (AD) is a complex neurodegenerative disorder that can influence neurological functions and cause impairments to the brain through the accumulation of neuronal senile plaques resulting from the dysregulation and overproduction of the protein amyloid-beta (Aβ).1 Due to the multifactorial nature of AD, multi-target directed ligands (MTDLs) have emerged as a novel drug design strategy to circumvent complications of adverse drug reaction and conflicting bioavailabilities associated with the simultaneous administration of single-target drug entities.1 Novel compounds AM-1 and AM-2 based off previously synthesized sym-triazine-based MTDLs by our group were designed to target Aβ-aggregation and acetylcholinesterase activity.2 Circular dichroism (CD) spectroscopy is a fast and powerful technique that allows us to observe the conformational transitions and mutual interactions of Aβ with our compounds (Fig. 1). In addition, fluorescence spectroscopy of Aβ incubated with Thioflavin T (ThT) and Congo Red have been implemented to support our data. Characterization of the interaction between small molecules and Aβ remains a central step in identifying the mechanism of aggregation and may provide information on the structural aspects of Aβ for designing future drugs.

Quantifying Spatiotemporal Patterns in the Expansion of Twitching Bacterial Colonies

Erin Shelton,1 Maximiliano Giuliani,1 Lori Burrows2 and John R. Dutcher1
1Department of Physics, University of Guelph, Guelph, ON N1G 2W1
2Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON L8S 4K1

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 PAO1 [1]. These bacteria use T4P to move across viscous interfaces, referred to twitching motility. Twitching can occur for isolated cells or in a collective manner [2]. We have developed a custom-built, temperature and humidity controlled environmental chamber and customized data analysis techniques to quantify the expansion of the bacterial colony at the agar-glass interface. Using this well-defined experimental geometry and data analysis, we have observed an interesting transition in the spatiotemporal patterns formed by the bacteria at the expanding front of the colony as a function of the stiffness of the agar.

Figure 1: Optical microscopy image of the edge of an expanding colony of P. aeruginosa PAO1 bacteria. In this experiment, the colony expanded from right to left. The width of the image corresponds to 200 um.


A Multiscale Approach for Biosystems Simulation

Jhih-Wei Chu1,2,3
1Department of Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA 94720, USA,
2Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan,
3Institute of Bioinformatics and Systems Biology, National Chiao Tung University, Hsinchu, Taiwan.

The hallmark of biosystems is achievement of delicate functionalities through orchestration of biomolecular structures and dynamics. Elucidating the underlying principles of molecular design enabled by evolution is a core objective of biological science. To confront the issue of complexity, we develop and apply a multiscale computational framework to take a systems view of protein conformational dynamics and enzyme machines. In this talk, I will introduce the idea and applications of this framework with a couple of specific examples.

Contact: jwchu@nctu.edu.tw
Supramolecular Biological Assemblies for Solar Light Harvesting

Daniel G. Oblinsky, Pu Qian, Neil Hunter, and Gregory D. Scholes.
Department of Chemistry, University of Toronto

Evolutionary pressure has aided in the development of various light-harvesting antenna with a broad diversity of micro and macrostructures in order to increase the efficiency of light absorption. Recently, in purple bacterium Chromatium purpuratum distinctive biological vesicles of a homogenous nature were observed and consisted solely of LH2 light-harvesting complexes. Such a large assembly of strongly interacting pigments poses significant interest for theoreticians and experimentalists, providing a unique perspective into harvesting solar energy. In this work we modeled the natural vesicles by a synthetic system composed of 32 light-harvesting complexes containing 768 chromophores. Excitation energy transfer was then simulated using modified Redfield theory. Quantum chemical calculations show, that energy can be funnelled across potential energy surfaces in a biologically relevant timeframe. Simulations accounting for static disorder demonstrate, that excitation energy preferentially chooses a path that is unique for each supramolecular complex. These strategies of light-harvesting-only assemblies can be successfully applied in synthetic biologically inspired devices and help in search for the most efficient and robust solar fuel production technology.

Reverse Engineering of the Donor side of Photosystem II: Second Generation of the Bacterioferritin “Reaction Centre”

Abdullah Mahboob1, Art van der Est2, Nick LeBrun3, Sergey Vasssiliev1, Doug Bruce1
1Centre for Biotechnology, Brock University, St. Catharines, Canada: am03ph@brocku.ca
2Department of Chemistry, Brock University, St. Catharines, Canada
3School of Chemistry, University of East Anglia Norwich, United Kingdom

Photosystem II (PSII) of oxygenic photosynthesis has the unique ability to photochemically oxidize water and evolve oxygen. In 2009, an engineered Bacterioferritin photochemical ‘reaction center’ (BFR-RC) using a zinc chlorin pigment (ZnCe6) in place of the native heme was suggested to oxidize a bound manganese ion through a tyrosine residue, thus mimicking two of the key reactions on the donor side of PSII. Based on our calculations and experimental work, we propose that ZnCe6 oxidizes the manganese cluster and tyrosine separately due to its low oxidation potential (640mV). In order to develop a BFR reaction centre capable of oxidizing tyrosine and the manganese cluster in succession, we proposed using phosphorus porphyrin pigment instead of ZnCe6. We aimed at oxidizing a tyrosine residue in absence of the manganese cluster using this protein-phosphorous pigment complex. The phosphorus pigment was cross-linked to
BFR-RC through two cysteine residues axially, we call this arrangement the second generation of the Bacterioferritin ‘reaction centre’ BFR-RC2. Fluorescence decay kinetics studies suggest that charge separation is occurring in BFR-RC2. Transient and CW-EPR evidence suggests that in absence of the manganese cluster, the pigment oxidizes a tyrosine residue forming a radical pair in which the triplet state of the pigment dominates. DFT calculations suggest that the redox potential for the pigment in the protein is ~1.2V, similar to the potential of P680 in PSII.

Session VI, Sun 9:30 am

Computational Design of Protein Interactions and Switches

Brian Kuhlman1,2
1Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
2Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

The molecular modeling program Rosetta has been used to design protein-protein interactions and protein switches that respond to light.

Interface design. Multi-state design simulations, that incorporate explicit positive and negative design, were used to generate antibody heavy and light chains with orthogonal Fab interfaces. Parental monoclonal antibodies incorporating these interfaces, when simultaneously coexpressed, assemble into bispecific IgG with the desired heavy chain-light chain pairing. Because the can bind two separate antigens simultaneously, these bispecific antibodies can be used to block or inhibit multiple pathways simultaneously, and they can be used to recruit different cell types to each other.

Photoactivatable protein switches. The engineered photoactivatable proteins incorporate the naturally occurring LOV domain from plants, and show increased binding affinity for specified binding partners in the light. These binding events have been used to control gene transcription and protein localization with blue light.

Contact: bkuhlman@email.unc.edu

Session VI, Sun 10:30 am

Systematic Evaluation of the Native and Non-native Interactions of the Helical IM9 Protein Using All-Atom Molecular Dynamics Simulations

Loan Huynh1, Chris Neale2,3, and Régis Pomès2,3 and Hue Sun Chan3
1Department of Biochemistry, University of Toronto
2Molecular Structure and Function, The Hospital for Sick Children, Toronto

Protein folding research aims to decipher how the amino acid sequence of a protein encodes its structure (which may, in some cases, be intrinsically disordered). The research reported here aims to gain insight into the thermodynamic and
dynamic processes involved in folding, how misfolding occurs, and how potential misfolding is avoided by natural proteins. To address these fundamental questions, we study the helical colicin immunity protein 9 (Im9) as a model system. Im9 has been characterized extensively by biochemical, biophysical, and molecular simulation techniques. Here we take a systematic computational approach to examine the effect of helical tilt and rotation on the folding mechanism of Im9. Using equilibrium and non-equilibrium molecular dynamics simulations on the microsecond timescale at atomistic resolution, we find that the folding transition state of Im9 is likely characterized by the initial association of helices 1 and 4, which together control the docking of helix 2. Furthermore, we find that initial association of helices 1 and 2 may lead to protein misfolding. Our ongoing investigation is focused on elucidating how the Im9 sequence drives helical association through the particular intermediate conformations that lead most smoothly to the folded structure. Notably, the quantitative atomistic descriptions of transient nonnative interactions derived from our studies point us beyond the simple notion that pairs of nonpolar residues can always form strong contacts.

Calculating Free Energy of the Aggregation of the Peptide (HHC-36) In Bulk

Shaghayegh Vafaei¹, Mostafa NateghoEslam¹, Matthew Nichols³, Masoud Jelokhani-Niaraki², Bruno Tomberli¹, Chris Gray¹

¹University of Guelph, Guelph, ON, Canada, ²Wilfrid Laurier University, Waterloo, ON, Canada, ³Dalhousie University, Halifax, NS, Canada.

The increasing demand for antibiotics has contributed to the investigation of possible novel antibiotics by many researchers. For this purpose, experimental and theoretical studies have been carried out to draw scientists' attention to antimicrobial peptides and their interaction with the surface of bacterial membranes. Their ability to disrupt the functioning of bacterial membranes has been probed from different perspectives. The best possible choices of antimicrobial peptides are those which do not harm plant or animals' membranes but which disrupt bacterial membranes. It has been found that some cationic antimicrobial peptides (CAPs) satisfy these requirements. CAPs interacting with the outer membrane of gram-negative bacteria and the membrane of gram-positive bacteria have been studied recently.

We conduct a Molecular Dynamics simulation study of peptide-peptide interactions in the physiological solutions and investigate the mechanism of CAPs aggregation, since aggregation of the peptides usually precedes formation of a pore in the membrane. Different algorithms will be applied to calculate the potential mean force of the aggregation process of peptides to select the most efficient one. Also, we have run CD spectroscopy and Calorimetry experiments to predict the structure of the peptide and measure the peptide-peptide binding enthalpy, and we compare the results with our simulation data. The particular CAP studied is HHC-36, a peptide selected by high throughput screening (A. Cherkasov et al, ACS Chem. Biol., 2009, 4 (1), pp 65–74), which has 9 amino acid residues and charge +5.

Yann R. Chemla\textsuperscript{1,2,3}
\textsuperscript{1}Center for Biophysics and Computational Biology, \textsuperscript{2}Center for the Physics of Living Cells, \textsuperscript{3}Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Single-molecule techniques have emerged as powerful tools in molecular biology, biochemistry and biophysics. Recent advances in optical traps or “tweezers”, which utilize focused light to trap and manipulate microscopic objects, have made it possible to monitor protein motion along DNA with a resolution of only a few ångstroms. Despite these advances, the information that can be obtained is often limited. In this talk, I will focus on our work developing the “next generation” of single-molecule instruments to overcome these limitations: hybrid instruments that allow simultaneous high-resolution detection of multiple, orthogonal observables.

I will discuss new measurements of \textit{E. coli} UvrD helicase that reveal directly how protein stoichiometry and conformation regulate DNA unwinding activity. UvrD is a model for understanding Superfamily 1 helicases, important for maintaining genomic integrity. Using an instrument that combines optical tweezers with single-molecule fluorescence microscopy, we record UvrD unwinding activity with base pair-scale resolution (via optical tweezers) simultaneously with helicase stoichiometry and conformation (via fluorescence). Our measurements settle a longstanding debate over which UvrD conformational states are required for unwinding. I will discuss the biological implications of our findings.

Contact: ychemla@uiuc.edu

The Structure of Cholesterol in Lipid Rafts

Laura Toppozini\textsuperscript{1}, Sebastian Meinhardt\textsuperscript{2}, Clare Armstrong\textsuperscript{1}, Zahra Yamani\textsuperscript{3}, Norbert Kučerka\textsuperscript{3}, Friederike Schmid\textsuperscript{2}, and Maikel C. Rheinstädter\textsuperscript{1}
\textsuperscript{1}Department of Physics & Astronomy, McMaster University \textsuperscript{2}KOMET 331, Institute of Physics, Johannes Gutenberg-Universität Mainz \textsuperscript{3}Canadian Neutron Beam Centre, Chalk River

Rafts, or functional domains, are transient nano- or mesoscopic structures in the plasma membrane and are thought to be essential for many cellular processes such as signal transduction, adhesion, trafficking and lipid/protein sorting. Experimental observations of these membrane heterogeneities have proven challenging, as they are thought to be both small and short-lived. With a combination of coarse grained molecular dynamics simulations and neutron diffraction using
deuterium labeled cholesterol molecules we observe raft-like structures and, for the first time, determine the ordering of the cholesterol molecules in binary cholesterol lipid membranes.

First evidence of highly ordered lipid domains in the liquid-ordered (l_0) phase of cholesterol-rich DPPC membranes has recently been reported from molecular dynamics simulations [1] and neutron diffraction [2]. From coarse-grained computer simulations, heterogeneous membrane structures were observed and characterized as small, ordered domains. Neutron diffraction was used to study the lateral structure of the cholesterol molecules. We find pairs of strongly bound cholesterol molecules in the liquid-disordered phase, in accordance with the umbrella model. Bragg peaks corresponding to ordering of the cholesterol molecules in the raft-like structures were observed and indexed by two different structures: a monoclinic structure of ordered cholesterol pairs of alternating direction in equilibrium with cholesterol plaques, i.e., triclinic cholesterol bilayers [3]. These small-scale domains can be speculated to be the nuclei of ‘rafts’ in biological membranes.

References
Abstracts

Poster Presentations

Sessions: I – May 3rd, 11:10 am – 12:30 pm (Odd Numbered Posters)
II – May 3rd, 3:10 pm – 4:30 pm (Even Numbered Posters)
List of Posters

P1  Characterizing Transcription Factories between Stem Cells and Differentiated Cells with Super-Resolution Localization Microscopy  
    Nafiseh Rafiei, Amir Mazouchi and Joshua N. Milstein

P2  Evolution of the Min Protein Oscillation in *E. coli* Bacteria During Cell Growth and Division  
    Benjamin Baylis, Maximiliano Giuliani and John R. Dutcher

P3  A Single-Molecule Approach to Studying Gene Transcription  
    Tai-Wei Su, Yih-Fan Chen, and Joshua N. Milstein

P4  Super-resolution imaging of the DNA replisome in live *Bacillus subtilis*  
    Yilai Li, Jeremy Schroeder, Yi Liao, Lyle A. Simmons and Julie S. Biteen

P5  Focus Drift Compensation for Extended Duration Single Molecule Detection  
    Ho Fung Sam Siu, Gregory-Neal W. Gomes, Claudiu C. Gradinaru

P6  Two-dimensional Electronic Spectroscopy of Photosystem II Light Harvesting Complex and Reaction Center  
    Samansa Maneshi, Amy L. Stevens, Lu Chen, Valentyn I. Prokhorenko, Oliver P. Ernst, and R. J. Dwayne Miller

P7  Supercontinuum generation in the deep-UV wavelength range using polycrystalline calcium fluoride  
    Amy L. Stevens, Valentyn I. Prokhorenko, and R. J. Dwayne Miller

P8  Understanding the Pathogenicity of *Vibrio Cholerae* via Two-Color Live-Cell Super-Resolution Microscopy  
    Chanrith Siv, Beth L. Haas, Andrew I. Perault, Victor J. DiRita, and Julie S. Biteen

P9  Biomechanical Aspects of Gene Silencing in Bacteria  
    Samuel Yoshua, Haowei Wang, Nanak Singh, William W. Navarre and Joshua N. Milstein

P10  The Conformations of the DrkN SH3 Domain Studied by Single Molecule Fluorescence Spectroscopy  
    Zhenfu Zhang, Amir Mazouchi, Andrew Chong, Julie Forman-Kay, Claudiu C. Gradinaru

P11  Multivariate Analysis of Hyperspectral Coherent Anti-Stokes Raman Scattering (CARS) Images  
    Joel Tabarangao, Aaron Slepkov

P12  Reproducibility of Self-Organising Maps for the Comparison of Protein Ensembles  
    Michelle A. Eisner

P13  Electrostatics-Dependent Shape, Size and Dynamics of the Intrinsically-Disordered Protein SIC1  
    Gregory-Neal W. Gomes, Patrick Farber, Julie Forman-Kay, and Claudiu C. Gradinaru
P14 Blue light induced domain swapping
   Jakeb M. Reis, Darcy. C. Burns and G. Andrew Woolley

P15 Mechanical activation of the Hha/H-NS protein complex to condense bacterial DNA
   Haowei Wang, Samuel Yoshua, Sabrina S. Ali, William W. Navarre and Joshua N. Milstein

P16 Fluorescent imaging of PYP photo-switching in vivo and in vitro
   Katherine Brechun, Vitali Borisenko, Lori Yin, Asim Rashid, Huixin Lu, Andrew Woolley

P17 Interplay of native topology and specific nonnative interactions in the different folding mechanisms of bacterial immunity proteins Im7 and Im9
   Tao Chen and Hue Sun Chan

P18 Osmolytes TMAO and Urea Acting at a Hydrophobic Interface: Structural Insights from Single Molecule Studies
   Duncan Halverson, Isaac T.S. Li, Gilbert C. Walker

P19 Effects of TMAO (Trimethylamine-N-oxide) and GdmCl (Guanidinium chloride) on aqueous hydrophobic contact-pair interactions
   Ryan D. Macdonald, Mazdak Khajehpour

P20 Estimating the Oligomeric Status of G-Protein Coupled Receptors (GPCRS) and G-Proteins in Vitro by Photobleaching Analysis
   Dennis Fernandes, Rabindra Shivnaraine, Yuchong Li, Huiqiao Ji, James W. Wells, and Claudiu C. Gradinaru

P21 Functional Relevance of Oligomers in a Fusion Protein of the M2 Muscarinic Receptor and Ga11
   John Y. Dong, Fei Huang, Amy W.-S. Ma, James W. Wells

P22 Dynamic Regulation of Hexokinase by Myristoylation in Absence of Specified Sequence Motifs
   Sujeet Kumar, Sreejit Parameswaran, Rajendra K. Sharma

P23 The effect of BH3-only protein Bad on cBid binding to mitochondrial-like liposomes
   Obaidullah Khan, Cecile Fradin

P24 Investigating Lipid Domain-Specific Cytoskeletal Organization in Living Cell
   Carolin Madwar, Gopakumar Gopalakrishnan and R. Bruce Lennox

P25 Nature of the M2 Muscarinic Receptor Signaling Complex Revealed by Dual-Color FCS and FRET
   Yuchong Li, Rabindra V. Shivnaraine, Huiqiao Ji, Dennis Fernandes, Fei Huang, James W. Wells and Claudiu C. Gradinaru

P26 Conformational dynamics of a seven transmembrane helical protein Anabaena Sensory Rhodopsin probed by solid-state NMR
   Daryl Good, Shenlin Wang, Meaghan Ward, Jochem Struppe, Leonid Brown, Josef Lewandowski and Vladimir Ladizhansky

P27 Dynamic Equilibria between Monomeric and Oligomeric Misfolded States of the Mammalian Prion Protein Measured by 19F NMR
   Sacha Thierry Larda, Karen Simonetti, M. Sameer Al-Abdul-Wahid, Simon Sharpe, and R. Scott Prosser
P28  Structural Dynamics in the Activation of Protein Kinase G I (PKG I)
     Bryan VanSchouwen, Rajeevan Selvaratnam, Choel Kim and Giuseppe Melacini

P29  Conformational dynamics in the regulation of β2-adrenergic receptor signaling
     Tae Hun Kim, Aashish Manglik, Christian Altenbach, Zhongyu Yang, Daniel Hilger, Foon Sun Thian, Tong Sun Kobila, Wayne L Hubbell, R Scott Prosser, and Brian K Kobila

P30  Human Aquaporin1 Protein
     Sanaz Emami, Shenlin Wang, Ying Fan, Rachel Munro, Vladimir Ladizhansky & Leonid S. Brown

P31  Residue patterning determines the lipid compatibility of transmembrane peptides
     Tracy Stone and Charles M. Deber

P32  1+1=3? Concerted Action of Membrane Permeabilizers
     Hiren Patel, Quang Huynh, Dominik Bärlehner, Heiko Heerklotz

P33  Fluorescence Spectroscopy and 1H NMR as Tools to Investigate the Physical Properties of Spherical Supported Bilayer Lipid Membranes
     Amélie Tessier, Carolin Madwar, R. Bruce Lennox

P34  Age-Related Effects on the Interaction between Amyloid-Beta Peptides and Anionic Lipid Membranes
     Hannah Dies, Laura Toppozini, Maikel Rheinstädter

P35  Interaction Of Digitonin and Cholate with Complex Membranes
     Helen Y. Fan, Dar'ya S. Redka, and Heiko Heerklotz

P36  A Structural Basis for Cholesterol Inhibition of Outer Mitochondria Membrane Permeabilization
     Kelly Cathcart, Aisha Shamas-Din, Norbert Kucerka, Maikel Rheinstadter, Cecile Fradin

P37  Preliminary Investigation of Monodisperse Polysaccharide Nanoparticles Using Inelastic and Small Angle Neutron Scattering
     John Atkinson, Jonathan Nickels, Erzsi Papp-Szabo, John Katsaras, and John R. Dutcher

P38  Polymer Stencil Lift-Off: A Simple and Rapid Method for Patterning Arrays of Single or Stacked Lipid Bilayers Containing Phase-Segregated Domains
     Yujie Zhu, Jose Moran-Mirabal

P39  Specific Ion Effects on the Micellization of a Non-Ionic Surfactant
     Hayden Glor, Mazak Khajepour

P40  Two photon fluorescence microscopy for the analysis of trabecular bone architecture
     Hemanth Akkiraju, Christopher Price, Liyun Wang, Jeff Caplan, Anja Nohe

P41  Treatment of osteoporotic patient samples using peptide CK2.3 induces bone mineralization
     Miho Maeda, Christopher M. Bowens, Jeremy C. Bonor, Anja G. Nohe
P42 Intracellular Routing in Breast Cancer Cells of Streptavidin-Conjugated Trastuzumab Fab Fragments Linked to Biotinylated Doxorubicin-Functionalized Metal Chelating Polymers
Peng Liu, Hyungjun Cho, Yijie Lu, Sachdev Sidu, Raymond M. Reilly, Mitchell A. Winnik

P43 Reporter-Assisted Assembly of Gold Nanorod Complexes
Alexander F. Stewart, Gilbert C. Walker

P44 Polymer Substrates for Waveguide Evanescence Field Microscopy
Ronny Sharon, Silvia Mittler

P45 Evidence for electron-transfer among Polyvinylamine bound TEMPO moieties during the oxidation of cellulose membranes
Qiang (Sean) Fu, Robert Pelton

P46 Decomposition of Surface Enhanced Raman Spectroscopy (SERS) Data using Target Factor Analysis and Band Selection
Frauke Breitgoff, Christina MacLaughlin, Brandon Gagnon, Gilbert Walker

P47 Distribution of amino acid heterodoublets in native proteins: Dependence on chain length, residue polarity, and location
Gustavo Arteca and Chunhang Gong

P48 Aspirin Increases the Solubility of Cholesterol in DMPC Membranes
Richard J. Alsop, Matthew A. Barrett, Songbo Zheng, Hannah Dies, Maikel C. Rheinstäder

P49 Energetics of the interaction of an antimicrobial peptide with phospholipid membranes
Mostafa Nategholeslam, Bruno Tomberli and Chris G. Gray

P50 Interactions of Complexes of Macromolecules in Charged Aqueous Nanodroplets
Falana Sheriff, Styliani Constas

P51 Solvation of a Polyethylene Glycol Chain in Aqueous Nanodroplets
Myong In Ob, Styliani Constas

P52 Escape from adaptive conflicts in protein evolution: bi-stability, mutational robustness, and gene duplication
Tobias Sikosek, Erich Bornberg-Bauer, Hue Sun Chan

P53 Towards Multiplexed Ultrabright J-Aggregate – Nanoparticle SERS
Brandon P. Gagnon, Christopher M. Walters, Gilbert C. Walker

P54 Solvation of Poly(ethylene glycol) in Aqueous Nanodroplets
Sepideh Soltani, Styliani Constas

P55 Polycation-π interactions are a likely driving force for molecular recognition by an intrinsically disordered oncoprotein family
Jianhui Song, Sheung Chun Ng, Peter Tompa, Kevin A. W. Lee, Hue Sun Chan
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P56</td>
<td>Minimum Nanoindentation Methods and Their Application to Biomaterials</td>
<td>Chuan Xu, Erika F Merschrod S</td>
</tr>
<tr>
<td>P57</td>
<td>Hierarchical, Self-Similar Structure in Native Squid Pen</td>
<td>Fei-Chi Yang, Robert Peters, Hannah Dies, and Maikel C. Rheinstädter</td>
</tr>
<tr>
<td>P58</td>
<td>Nanomechanical Response of Bacterial Cells to Cationic Antimicrobial Peptides</td>
<td>Shun Lu, Grant Walters, Richard Parg, John R. Dutcher</td>
</tr>
<tr>
<td>P59</td>
<td>Aqueous based process of fabricating nanostructured block copolymer films as effective marine antifouling coatings</td>
<td>Kris S. Kim, Drew MacNeil, Nikhil Gunari and Gilbert C. Walker</td>
</tr>
<tr>
<td>P60</td>
<td>Preparation and Surface Modification of NaLnF4 NPs as Potential Elemental Tags for Mass Cytometry</td>
<td>Lemuel Tong, Guangyao Zhao, Pengpeng Cao, Elsa Lu, Mark Nitz, Mitchell A. Winnik</td>
</tr>
<tr>
<td>P61</td>
<td>Design and Synthesis of Potential Theranostic Metal-Chelating Polymers Chelating Cu$^{64}$ Radioisotope with Reactive End-Group for Bioconjugation for PET Imaging and Treatment of Pancreatic Cancer</td>
<td>Hyungjun Cho, Amanda J. Boyle, Peng Liu, Yijie Lu, Raymond M. Reilly, Mitchell A. Winnik</td>
</tr>
<tr>
<td>P62</td>
<td>Stochastic and Spatial Effects in the Origin and Stability of RNA Replicators</td>
<td>Christopher Huynh, Paul Higgs</td>
</tr>
<tr>
<td>P63</td>
<td>Informatic Prediction of Microbial Secondary Metabolomes</td>
<td>Michael Skinnider, Nathan A. Magarvey</td>
</tr>
<tr>
<td>P64</td>
<td>A phylogenetic model to predict the patterns of presence and absence of genes in bacterial genomes and estimate the frequency of horizontal gene transfer</td>
<td>Alireza Zamani, Paul Higgs</td>
</tr>
<tr>
<td>P65</td>
<td>Reduced dimensional stochastic simulation of biochemical systems</td>
<td>Midhun K S, Marc R Roussel</td>
</tr>
<tr>
<td>P66</td>
<td>Simulations of a sphere-dimer nanomotor in an oscillating chemical environment</td>
<td>Bryan Robertson, Raymond Kapral</td>
</tr>
<tr>
<td>P67</td>
<td>Derivative-free methods for stochastic models of biochemical kinetics</td>
<td>Monjur Morshed, Silvana Ilie</td>
</tr>
<tr>
<td>P68</td>
<td>Membrane Leakage and Antimicrobial Action of Polymers and Surfactants</td>
<td>Sara G. Hovakeemian, Runhui Liu, Samuel H. Gellman, Heiko Heerklotz</td>
</tr>
<tr>
<td>P69</td>
<td>Cellular Mechanisms of Force Generation in Antibody-Mediated Phagocytosis</td>
<td>Maria Goiko, John R. de Bruyn, Bryan Heit</td>
</tr>
</tbody>
</table>
Characterizing Transcription Factories between Stem Cells and Differentiated Cells with Super-Resolution Localization Microscopy

Nafiseh Rafiei1,2, Amir Mazouchi2 and Joshua N. Milstein1,2

1Institute for Biomaterials and Biomedical Engineering, University of Toronto, Toronto ON 2Department of Chemical and Physical Sciences, University of Toronto Mississauga, Mississauga ON

A stem cell has the potential to transform into almost any cell type found in the body. One of the great challenges of modern biomedical engineering is to understand how differential RNA transcription determines the fate of stem cells, so that this fate can be controllably altered and/or engineered. There is strong evidence suggesting that genes migrate to preassembled subnuclear units called transcription factories that are essentially clusters of active RNA Polymerase II and other transcriptional components. The estimated size of a transcription factory varies between 40-200 nm, which is beyond the reaches of conventional light microscopy. In this study, we will quantitatively map, count and measure the size of the active transcription factories in mouse embryonic stem cells and correlate these results with observations in differentiated cell lines. In conjunction with immunofluorescence labeling, we use direct stochastic optical reconstruction microscopy (dSTORM), which can circumvent the diffraction limit and resolve fine structures as small as ~20 nm.

Evolution of the Min Protein Oscillation in E. coli Bacteria During Cell Growth and Division

Benjamin Baylis, Maximiliano Giuliani and John R. Dutcher

Department of Chemistry, University of Waterloo

Cell division is a key step in the life of a bacterium. This process is carefully controlled and regulated so that the cellular machinery is equally partitioned into two daughter cells of equal size. In E. coli, this is accomplished, in part, by the Min protein system, in which Min proteins oscillate along the long axis of the rod-shaped cells. We have used high magnification, time-resolved fluorescence microscopy to characterize in detail the oscillation in E. coli cells in which the MinD proteins are tagged with green fluorescent protein (GFP-MinD). We have used the “mother machine” microfluidic device [1] to confine the bacteria into microchannels that allows us to track the evolution of the oscillation in cells as they grow and divide in LB growth media.
Figure 1: Optical microscopy image of *E. coli* bacterial cells confined in the channels of the microfluidic device. The width of each channel is 1 μm. Corresponding fluorescence microscopy images allow the tracking of the GFP-MinD oscillation for a large number of cells.


**A Single-Molecule Approach to Studying Gene Transcription**

Tai-Wei Su¹,², Yih-Fan Chen², and Joshua N. Milstein¹

¹Department of Chemical and Physical Sciences, University of Toronto Mississauga, Mississauga, ON, Canada

²Department of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan

Transcription is a fundamental process in gene expression that produces RNA for protein synthesis and regulatory control. We have combined tethered particle motion (TPM) microscopy with TIRF microscopy to study transcription at a single-molecule level. Our assay allows us to watch RNA transcription occurring on dozens of single DNA molecules in parallel. Currently, we have been exploring the procession rate of T7 polymerase as a function of dNTP concentration, but our setup is amenable to the study of a host of different regulatory factors.

**Super-resolution imaging of the DNA replisome in live Bacillus subtilis**

Yilai Li, Jeremy Schroeder, Yi Liao, Lyle A. Simmons and Julie S. Biteen

Department of Chemistry, University of Michigan

DNA replication happens in all living organisms, and assures that the genome is accurately copied and maintained. The replisome is the molecular machine in cells that replicates DNA, and it is composed of several different proteins, including DNA polymerases, which directly synthesize DNA by adding nucleotides. Although there have been a lot of studies on the replisome *in vitro*, little is known about the dynamics and architecture of replisome components *in vivo*. Here we used *Bacillus subtilis*, a Gram-positive bacterium which is commonly found in soil, as a model organism in which to study the architecture and dynamics of several replisome components *in vivo*. Photo-activated localization microscopy (PALM) and single-molecule tracking was used to give a resolution of 10-20 nm, far below the diffraction limit of conventional microscopy, enabling us to localize and track every single protein molecules. Thus, we can watch the behavior of different
replisome components during the DNA synthesis process in real time, and study them quantitatively. A number of replisome components were investigated under different conditions in this study, including the DNA polymerases PolC and DnaE, and the β-clamp loader DnaX.

Focus Drift Compensation for Extended Duration Single Molecule Detection

Ho Fung Sam Siu [1], Gregory-Neal W. Gomes [1,2], Claudiu C. Gradinaru  
[1] Department of Chemical and Physical Sciences, University of Toronto Mississauga  
[2] Department of Physics, University of Toronto

An autofocus system was designed and implemented onto a home-built multicolor confocal microscope. Autofocusing allows for accurate collection of data by compensating for the effects of focus drift that typically occurs for long measurement times. In the detection of fluorescent, surface-immobilized single molecules, focus drift moves the molecules out of the illumination volume, causing blurred images and decreasing the signal-to-noise ratio of the single-molecule trajectories. It is therefore important to design a system which keeps the illumination-detection volume within 100 nm of the surface for extended periods of time, i.e. hours and days.

First, the focus drift was characterized using two methods: an image processing and a backscattering intensity method. It was verified that the two methods produce similar results and the backscattering intensity method was chosen for its fast processing speed (seconds vs. minutes). The software required for the autofocusing routine was written using LabView and appended onto the existing data acquisition program. Additional optical components were added to the microscope setup, including a diode laser with a lateral focus offset from the excitation beam, a pinhole and a photomultiplier tube for detection of the autofocusing signal.

The autofocusing algorithm was tested using 200 nm fluorescent beads, using one of the existing excitation lasers and an avalanche photodiode (APD) as a proof of principle, as the final design is being further refined. The axial drift measured in our setup was on the order of 10 nm/min. The effect of focus drift on a single molecule experiment was measured using spin-coated Alexa488 dye and His-tagged GFP molecules. The autofocus system was then used on the same samples to quantify the effectiveness of drift correction.
Two-dimensional Electronic Spectroscopy of Photosystem II Light Harvesting Complex and Reaction Center

Samanssa Maneshi¹, Amy L. Stevens¹, Lu Chen², Valentyn I. Prokhorenko¹, Oliver P. Ernst², and R. J. Dwayne Miller¹,³

¹ Max Planck Institute for Structure and Dynamics of Matter Luruper Chaussee 149, Hamburg, Germany ² Departments of Biochemistry and Molecular Genetics, University of Toronto, 1 King’s College Circle, Medical Sciences Building, Toronto, Canada ³ Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Canada

Our goal in this project is to unambiguously determine the time scales of energy transfer and charge separation processes in the photosystem II (PSII) subunits at ambient temperature and to distinguish between the role of excitonic, vibronic, and vibrational states in these processes.

Past experiments on PSII subunits have been performed at cryogenic temperatures (77K) and long-lasting oscillations have been observed in light-harvesting complex (LHCII) [1], attributed to long-lived coherences of excitonic states of coupled pigments. The presence of long-lived electronic coherence was then linked to efficient energy transfer from LHCII to the reaction center. In a measurement on the PSII reaction center [2], the observed oscillatory features were attributed to electronic and vibrational superposition states. While many theoretical models predict that purely excitonic coherences are responsible for the observed oscillations, other recent models find vibrational superpositions to be the dominant contributing factor [3].

In our group, previous measurements on LHCII and the Reaction Center at room temperature [4] also verified the presence of beating frequencies. We are continuing this work to properly attribute the observed frequencies to their relevant electronic, vibrational and/or vibronic energy levels.

For our experiments, LHCII and the PSII reaction centre (D1D2-cyt.b559) are extracted from fresh, locally-grown spinach. A strong detergent followed by a combination of salts [5] is used to precipitate out LHCII from its PSII complex solution. Column chromatography then separates the RC from its closely-associated antenna complexes [6]. The LHCII has been successfully isolated from spinach and its purity tested by circular dichroism (CD). The CD data clearly show the presence of trimeric LHCII with no aggregation. Pump-probe spectroscopy on the LHCII sample clearly demonstrates the process of fast energy transfer between Chlb and Chla pools. However, fully resolving the energy transfer pathways requires performing 2D-spectroscopy for a wide range of “waiting time” delays.

Supercontinuum generation in the deep-UV wavelength range using polycrystalline calcium fluoride

Amy L. Stevens, Valentyn I. Prokhorenko, and R. J. Dwayne Miller

1 Max Planck Institute for Structure and Dynamics of Matter, Luruper Chaussee 149, Hamburg, Germany.
2 Dept. of Chemistry, University of Toronto, 80 St George Street, ON, Canada.

The focus of spectroscopic experiments, such as transient absorption and two-dimensional electronic spectroscopy, is shifting to biomolecules such as DNA and proteins [1]. Mutagenesis and other pathological processes of these molecules can be investigated through their energy and charge dynamics. Such investigations require probe wavelengths in the deep-UV range. Calcium fluoride (CaF2) is known to generate a supercontinuum that approaches the UV. The crystal broadens incident laser light through nonlinear effects such as self-phase modulation, which arise from the Kerr nonlinearity [2]. However, CaF2 has a low damage threshold and must be moved continuously during measurements. The extra motion-induced fluctuations contribute to the experiment noise. Other complications include intensity and polarization dependence of the generated supercontinuum on the rotation angle of the crystal [3, 4].

We have examined these problems by comparing the supercontinua generated in rotated and circularly translated single- and polycrystalline CaF2. In the experiment, a 2mm-thick CaF2 plate is pumped with the frequency-doubled output of an ultrafast amplifier, as displayed in Figure 1. The spectral shape and stability of the emitted supercontinuum is controlled mainly by the iris (P), the variable optical density filter (VOD), and the 100 or 150 cm lens (L). The prisms (Pr1/2), combined with the cut-off screen (S), disperse the supercontinuum and filter out the 400 nm generating light. The resulting spectrum, measured with a fiber spectrometer, is shown at the top of Figure 1. We achieved a broad UV spectrum down to $\sim 240$ nm in both types of CaF2 with a stability of $\sim 1\%$ – the first time this has been demonstrated in polycrystalline CaF2. The potential for extensive utilization of stable, broadband, CaF2-generating supercontinuum probes in pump-probe spectroscopies is vast.


Fig. 1. Bottom left: Schematic diagram of the supercontinuum generation setup. Top right: The graph shows the observed spectrum from a 2mm-thick polycrystalline calcium fluoride disc rotated at 30° per second.
Understanding the Pathogenicity of *Vibrio Cholerae* via Two-Color Live-Cell Super-Resolution Microscopy

Chanrith Siv\(^1\), Beth L. Haas\(^2\), Andrew I. Perault\(^3\), Victor J. DiRita\(^3\), and Julie S. Biteen\(^2\)

\(^1\)Program in Biophysics, \(^2\)Department of Chemistry, University of Michigan, Ann Arbor, MI 48109 \(^3\)Department ofMicrobiology & Immunology, University of Michigan Medical School, Ann Arbor, MI 48109

Cholera afflicts more than 5 million people annually. Here, we investigate the virulence pathway of this epidemic disease with live-cell single-molecule fluorescence imaging in *Vibrio cholerae* cells, the bacterium responsible for producing the cholera toxin. In the Gram negative pathogen *V. cholerae*, virulence gene expression is under control of an unusual set of membrane proteins. Here, a membrane complex including two activators, ToxR and TcpP, binds the toxT promoter, recruits RNA polymerase, and activates toxT gene expression leading to activation of ToxT-controlled virulence genes. To circumvent the diffraction limit of light, which bounds the resolution of optical microscopy to ~250 nm, we use single-molecule tracking and super-resolution techniques like Photoactivated Localization Microscopy (PALM) to achieve resolutions more than an order of magnitude better than the diffraction limit. We have created fusions of the membrane-bound transcription activators TcpP and ToxR with orthogonal photo-(re)activatable fluorescent proteins, and in this study, we examine the dynamics and co-localization patterns of single PAmCherry-TcpP and mCitrine-ToxR molecules in the virulence pathway. This work aims to identify characteristics of TcpP and ToxR motion to understand their regulatory behavior in the transcriptional activation of the gene toxT and subsequent activation of downstream virulence genes, and to establish a model for the formation of the ToxR/TcpP/toxT protein-DNA complex important in early pathogenesis. In addition to elucidating the regulatory pathway of *V. cholerae*, the impact of this work will be to further provide a general model for outer-membrane-bound transcription control in bacteria and nuclear-membrane-bound transcription in eukaryotic cells.

Biomechanical Aspects of Gene Silencing in Bacteria

Samuel Yoshua\(^1\,2\), Haowei Wang\(^2\), Nanak Singh\(^2\), William W. Navarre\(^3\) and Joshua N. Milstein\(^1\,2\)

\(^1\)Department of Physics, University of Toronto, Toronto, ON, Canada
\(^2\)Department of Chemical and Physical Sciences, University of Toronto Mississauga, Mississauga, ON, Canada
\(^3\)Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

Horizontal gene transfer (HGT) is an evolutionary process within bacteria that permits the exchange of genetic material, allowing rapid adaptation to environmental changes. Expression of foreign genes can be detrimental to the host as well
as a waste of energy that could otherwise be used for cellular growth. Bacteria have evolved a variety of ways to selectively silence undesired genes. For example, the histone-like nucleoid structuring protein (H-NS), found in some enterobacteria, silences foreign genetic material by binding to DNA with a high ratio of AT to GC content as compared to native DNA. H-NS is able to form extended nucleoprotein filaments along the DNA that interfere with transcription, although the exact biophysical mechanism is still a matter of debate. Using single-molecule techniques, we have discovered that tension can alter the mechanical properties of these filaments, which may have important physiological implications.

The Conformations of the DrkN SH3 Domain Studied by Single Molecule Fluorescence Spectroscopy

Zhenfu Zhang¹, Amir Mazouchi¹, Andrew Chong², Julie Forman-Kay², Claudiu C. Gradinaru¹
¹Department of Chemical and Physical Sciences, University of Toronto Mississauga
²Molecular Structure and Function Program, The Hospital for Sick Children Toronto

SH3 domains are highly involved in signal transduction and cellular localization. The N-terminal SH3 domain of Drosophila adaptor protein Drk is found to be marginally stable, exchanging between folded and unfolded states under non-denaturating conditions. The high unfolded state population makes DrkN SH3 a useful model system to study the physical polymeric properties of disordered protein states and to advance the understanding of the mechanism of protein folding.

Single-molecule techniques have the unique capability to resolve populations of protein conformations and also the rates of exchange dynamics among them. Here, single-molecule Förster resonance energy transfer (smFRET) is performed in order to study the conformational distribution and dynamics of the DrkN SH3 domain, using fluorophores attached to two cysteines mutated at the N- and the C-termini of the 61 amino acid chain. Freely-diffusing proteins in diluted solutions give rise to fluorescence bursts which can be quantitatively characterized. Multiparameter fluorescence analysis reveals two populations with different end-to-end distances, attributed to the folded and unfolded states coexisting under normal conditions. Conformational populations and internal chain dynamics are measured in both physiological and non-physiological conditions in order to understand the role of solvent-protein interactions for the structural stability. Fluorescence correlation spectroscopy (FCS) and FRET-FCS are applied to investigate local chain dynamics and the inter-conversion kinetics between the ordered and disordered conformations of DrkN SH3 in different solvent conditions.
Multivariate Analysis of Hyperspectral Coherent Anti-Stokes Raman Scattering (CARS) Images

Joel Tabarangao, Aaron Slepkov
Materials Science Graduate Program, Trent University

Coherent Anti-Stokes Raman Scattering (CARS) Microscopy is an emerging nonlinear imaging modality that probes the inherent vibrational resonances in molecules. This makes it a practical alternative to conventional confocal fluorescence microscopy, which uses staining dyes as contrast, for studying biological samples and biomaterials. In particular, its simultaneous integration with other nonlinear imaging modes such as second harmonic generation (SHG) and two-photon excitation fluorescence (TPEF), makes this label-free imaging tool very powerful. Broadband CARS microscopy also allows for vibrational spectroscopy at each pixel to produce a hyperspectral image stack. There is growing interest in analyzing these complex chemically-specific spectra and utilizing them as a contrast mechanism for label-free optical imaging. We explore the use of multivariate statistical analyses tools such as Principal Component Analysis (PCA) and Vertex Component Analysis (VCA) for the spectral differentiation of hyperspectral CARS Images with examples from atherosclerotic tissue samples.

Reproducibility of Self-Organising Maps for the Comparison of Protein Ensembles

Michelle A. Eisner
Department of Chemistry, Brock University

We are using self-organising maps (SOM) to compare two different conformational distributions of proteins obtained by computer simulations. The SOM applications are being developed by comparing conformational populations of the pentapeptide Met-Enkephalin obtained by molecular dynamics simulations utilizing different sampling techniques. It would be desirable that the clusters produced by the SOM be reproducible. The large number of clusters produced by a SOM is the main reason for lack of reproducibility. Furthermore, the two dimensional organization of the trained SOM clusters also inhibits inter-map comparisons. We are exploring both the Cramers V and a similarity index as metrics for SOM reproducibility as a function of parameters such as map size and boundary conditions (bordered vs toroidal). We have found by implementing toroidal boundaries the maps (i.e. clusters) produced are more reproducible.
Electrostatics-Dependent Shape, Size and Dynamics of the Intrinsically-Disordered Protein Sic1

Gregory-Neal W. Gomes [1], Patrick Farber [2], Julie Forman-Kay [2], and Claudiu C. Gradinaru [1]

[1] Department of Physics, University of Toronto, and Department of Chemical and Physical Sciences, University of Toronto Mississauga
[2] Molecular Structure and Function Program, Hospital for Sick Children, Toronto, Ontario M5S 1A8 and Department of Biochemistry, University of Toronto, Toronto Ontario M5G 1X8, Canada

Sic1 is a highly positively charged intrinsically disordered protein (IDP) in the budding yeast Saccharomyces Cerevisiae which prevents the cell cycle from entering the S-phase from the G1-phase. Sic1’s binding affinity for Cdc4 is highly phosphorylation-dependent, although the corresponding physical basis is not fully understood. NMR data supports the presence of a dynamic complex of Sic1:Cdc4 and a poly-electrostatic model has been proposed by Forman-Kay and coworkers.

We studied the Sic1 N-terminal targeting region (1-90) to better understand the role of intrachain electrostatics, and to compare with the structural ensembles calculated from NMR and SAXS data. Sic1 exists in a dynamic ensemble of conformations and ensemble-averaged experiments have limitations in identifying and characterizing static and/or dynamic inhomogeneity in the motional dynamics. Therefore, we probed the molecule’s shape, size, flexibility and dynamics, using a combination of burst spectroscopy and Fluorescence Correlation Spectroscopy (FCS) on freely diffusing Sic1 in different phosphorylation states. The degree of intrachain repulsion screening was modified by adjusting salt concentrations and described within a polymer physics framework, the polyelectrolyte model. To differentiate between global and local properties, Sic1 was labelled with a fluorescent dye at 6 different sites along the chain.

This multiparameter single-molecule data will be incorporated as a constraint in future conformational ensemble calculations, complementary to existing SAXS and NMR data. Additionally, our measurements can also be used to derive more accurately the radius of gyration of IDP’s and other charged polymers from the distribution of end-to-end distances measured by smFRET, replacing the default assumption of a Gaussian chain model for the end-to-end distance probability distribution.
Blue light induced domain swapping

Jakeb M. Reis, Darcy. C. Burns and G. Andrew Woolley
Department of Chemistry, University of Toronto.

The design of new optogenetic tools would be facilitated by the development of protein scaffolds that undergo large, well defined structural changes upon exposure to light. We describe here a variant of the blue light photoreceptor photoactive yellow protein (PYP), in which a surface loop is replaced by a heterodimeric coiled-coil forming sequence (E-helix). The protein forms domain swapped dimers with a dimerization affinity of $K_d \sim 10 \mu$M in the dark. These interconvert with monomers on the timeframe of weeks. Blue light irradiation decreases the dimerization affinity ($K_d \sim 300 \mu$M) and dramatically enhances the rate of domain swapping, leading to the production of monomers on a time frame of <1 min. Whereas the dimer form of the protein specifically binds a partner K-helix sequence in a coiled-coil motif, the monomeric form is unable to do so. Blue light induced domain swapping thus provides a mechanism for control of protein activity with very low thermal background activation.

Mechanical activation of the Hha/H-NS protein complex to condense bacterial DNA

Haowei Wang¹, Samuel Yoshua¹,², Sabrina S. Ali³, William W. Navarre³ and Joshua N. Milstein¹,²
¹Department of Chemical and Physical Sciences, University of Toronto Mississauga, ²Department of Physics, University of Toronto, ³Department of Molecular Genetics, University of Toronto

The bacterial chromosome must be under varying levels of mechanical stress due to a high degree of crowding and repeated protein-DNA interactions experienced within the nucleoid. DNA tension is difficult to measure in cells and it is not known if its effects have any functional significance. However, in vitro experiments have implicated a range of biomechanical phenomena for DNA. The histone-like nucleoid structuring protein, H-NS, is a key regulator of DNA condensation and gene expression in enterobacteria and is affected by a variety of cofactors with which it may form a complex, such as the protein Hha. By combining tethered particle motion (TPM) and optical tweezers experiments we probed the effects of tension on DNA in the presence of the Hha/H-NS complex. We find that a brief fluctuation in DNA tension, induced by optical tweezers, causes the rapid and irreversible compaction of DNA when in the presence of H-NS and Hha. Our results imply that the Hha/H-NS complex may selectively condense bacterial DNA based upon the level of mechanical tension that is experienced along different regions of the chromosome.
Fluorescent imaging of PYP photo-switching in vivo and in vitro

Katherine Brechun¹, Vitali Borisenko¹, Lori Yin¹, Asim Rashid², Huixin Lu¹, Andrew Woolley¹

¹Department of Chemistry, University of Toronto.
²Molecular, Cellular and Systems Cognition, Hospital for Sick Children.

Photoactive yellow protein (PYP) is a bacterial protein that changes its structure in response to irradiation with blue light. From a biotechnological standpoint, PYP is useful because it can be used to develop synthetic proteins with activity that can be controlled by light; these light-controlled proteins would enable in vivo studies with a high degree of spatial and temporal control. In this project, a system was developed to monitor the photo-cycle of PYP using fluorescence, allowing the activity of PYP to be confirmed in eukaryotic cell culture. This is an important step to confirm the feasibility of in vivo studies with PYP-controlled proteins.

To monitor the photo-cycle, PYP was fused with blue fluorescent protein (BFP). When PYP is in its dark-adapted state, it absorbs blue light; therefore, in the constructed fusion protein the blue fluorescence from BFP is absorbed by PYP resulting in an observed quench of fluorescence. When PYP absorbs blue light it changes conformation, assuming its light-adapted state. In this light-adapted state, the absorbance characteristics of PYP change and the protein no longer absorbs blue light. Therefore after the initial quench in blue fluorescence, PYP light-adapts and ceases to quench the blue fluorescence, resulting in a corresponding increase in blue fluorescence from BFP. The time-dependent changes in fluorescence produced by this BFP-PYP fusion protein were characterized in vitro, as well as in E. coli and in eukaryotic cell culture. The changes in blue fluorescence were able to confirm the PYP photocycle in vivo.

Interplay of native topology and specific nonnative interactions in the different folding mechanisms of bacterial immunity proteins Im7 and Im9

Tao Chen and Hue Sun Chan

Departments of Biochemistry, Molecular Genetics and Physics, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Despite sharing the same overall fold and ~ 60% sequence identity, extensive experiments have demonstrated that the bacterial immunity proteins Im7 and Im9 fold with significantly different kinetic mechanisms. Whereas Im7 populates a transient folding intermediate at pH 7.0 and 10°C, Im9 folds in an essentially two-state manner under the same conditions. Experimental data suggested that nonnative interactions are at play in Im7 folding. To gain insight into the biophysics of this intriguing contrast between Im7 and Im9, we developed several classes of coarse-grained native-centric
models that account for elementary desolvation barriers and are augmented by sequence-dependent hydrophobic interaction terms to simulate folding of this pair of proteins. We found that when the nonnative hydrophobic interactions are modeled by a common statistical potential of heterogeneous contact energies, folding kinetics of our Im7 and Im9 models exhibit trends very similar to those observed experimentally, namely that the folding arm of the model chevron plot for Im7 shows a serious rollover but that for Im9 is much closer to being linear by comparison. Many of the amino acid residues participating in stable nonnative contacts in this class of models also coincide with those experimentally deduced to engage in nonnative interactions. However, this clear distinction between the simulated folding of Im7 and Im9 vanishes when a uniform strength was used for the nonnative hydrophobic interactions in the models, indicating that the differences in hydrophobicity among the different nonpolar residues in Im7 and Im9 is a crucial cause of their divergent folding properties.

Osmolytes TMAO and Urea Acting at a Hydrophobic Interface: Structural Insights from Single Molecule Studies

Duncan Halverson, Isaac T.S. Li, Gilbert C. Walker
Department of Chemistry, University of Toronto.

The effects of trimethylamine oxide (TMAO) and urea on protein folding are well known, but the mechanisms underlying those effects are less so. Simulations offer conflicting predictions as how these solutes will alter the strength of the hydrophobic interaction, and experimental studies have been minimal due to the difficulties inherent in studying hydrophobic compounds in water. Using single molecule force microscopy (SMFS), we find evidence for TMAO induced hydrophobic collapse, contrasted against urea induced hydrophobic solvation. Using a ball-and-chain model, these results are contrasted with macroscopic interfacial tensions. The resulting picture involves a conformationally dependent surface excess, which has implications for protein folding and hydrophobic hydration.
Effects of TMAO (Trimethylamine-N-oxide) and GdmCl (Guanidinium chloride) on aqueous hydrophobic contact-pair interactions

Ryan D. Macdonald, Mazdak Khajehpour
Department of Chemistry, University of Manitoba

Trimethylamine-N-oxide (TMAO) and guanidinium chloride (GdmCl) are both highly studied molecules in the field of protein folding/unfolding. Thermodynamic studies have shown that TMAO is a strong stabilizer of the protein folded state, while GdmCl is known to be one of the most effective protein denaturants. Although TMAO and GdmCl are well studied the mechanism by which they stabilize and denature proteins, respectively, is not well understood. In fact there are few studies looking at their effect on hydrophobic interactions. In this work we determine the effect of TMAO and GdmCl on hydrophobic interactions, by looking at the model system of phenyl and alkyl hydrophobic contact pairs. Contact pair formation is monitored through the use of fluorescence spectroscopy, i.e., measuring the intrinsic phenol fluorescence being quenched by carboxylate ions. Hydrophobic interactions are able to be isolated from other interactions through a developed methodology. The results show that TMAO addition to the aqueous solvent destabilizes hydrophobic contact-pairs form between phenol and carboxylate ions. The TMAO acts as a “denaturant” for hydrophobic interactions. For GdmCl the data shows that for small alkyl groups, acetate and propionate, hydrophobic contact-pairs are promoted due to cavity formation energetics dominating. For the larger alkyl groups van der Waals interactions dominate, letting GdmCl disrupt contact pair formation by solvating the alkyl groups.

Estimating the Oligomeric Status of G-Protein Coupled Receptors (GPCRS) and G-Proteins in Vitro by Photobleaching Analysis

Dennis Fernandes¹, Rabindra Shivnaraine², Yuchong Li¹, Huiqiao Ji, James W. Wells², and Claudiu C. Gradinaru¹
¹Department of Chemical and Physical Sciences, University of Toronto Mississauga,
²Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto

The oligomeric size of family 1A GPCRs is uncertain despite considerable attention in recent years. Techniques such as FRET, PCH, and TIRF intensity analysis have been employed and have produced varying estimates of size. The G-protein to which these receptors couple have received comparatively less attention. Of particular interest are the respective sizes of the receptor and the G protein in the coupled form that initiates signalling. Here, we have evaluated the oligomeric size using an antibody-based immobilization assay and GFP-tagged proteins to count the number of photobleaching steps
of single molecules (complexes). Immobilized single-molecules were studied using TIRF microscopy using optimal laser excitation power and frame exposure time in order to accurately capture step-wise variations in individual intensity-time trajectories. M₂ muscarinic receptors were either fused at the N- or C-terminus with both 6x His and GFP, and the Gαᵢ₁ subunit were tagged with GFP and 6xHis at position 91. As control samples, His-tagged GFP and His-tagged fusions of GFP as dimers, trimers and tetramers were measured. Receptors and G-proteins were expressed in Sf9 cells, washed membranes containing receptors were solubilised in digitonin and those containing G-proteins were solubilised in cholate. The solubilised samples were purified via the 6x His tag on a Ni²⁺ column. The oligomeric fusions of GFP gives rise to a distribution of photobleaching steps that never exceeds its size (i.e. trimeric-GFPs gave only 1, 2 and 3 steps but never 4). GFP-tagged receptors and G-proteins show a predominance of 4 steps, which is indicative of a significant presence of tetramers. We are using statistical methods to estimate the distribution of the number of photobleaching steps that result from the G protein or receptor when bound in the signalling complex. Next, we will extend this type of analysis to purified samples containing the Gαᵢ₁ subunit bound to GFP-tagged M₂ receptors, or the Gαᵢ₁-GFP subunit bound to M₂ receptors.

**Functional Relevance of Oligomers in a Fusion Protein of the M₂ Muscarinic Receptor and Gαᵢ₁**

**John Y. Dong, Fei Huang, Amy W.-S. Ma, James W. Wells**

*Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto*

G protein-coupled receptors can exist as oligomers, but monomers can activate G proteins when reconstituted in small discoidal membranes; moreover, the receptor–G protein complex (RG) emerges as transient in some reports and stable in others. To examine the functional relevance of oligomers and transience, we fused the M₂ muscarinic receptor to Gαᵢ₁ and compared monomers and oligomers of the fusion protein in terms of the interaction between agonists and GMP-PNP. The fusion protein was expressed in Sf9 cells, extracted primarily as an oligomer in digitonin–cholate plus DTT and purified as a monomer. The oligomeric status was determined by electrophoretic mobility after cross-linking with BS⁵. Both forms of the fusion protein revealed two classes of sites differing in affinity for the agonist oxotremorine-M (Kᵢₜ, Kᵢₗ). With oligomers, graded concentrations of GMP-PNP progressively increased the apparent fraction of low-affinity sites (Fᵢₗ) from 23% to 100% without affecting Kᵢₜ. Such an upward shift in the binding profile has been reported previously for cardiac muscarinic receptors and reconstituted tetramers of the purified M₂ receptor, and it can be explained in terms of co-operativity between interacting sites. With monomers, GMP-PNP progressively increased Kᵢₜ toward an upper limit equal to Kᵢₗ without affecting the apparent distribution of sites between the two states (Fᵢₜ = 71%). The nucleotide-insensitive low-affinity sites appear not to communicate with the fused Gαᵢ₁-subunit, perhaps owing to inadequate protection by DTT. The rightward shift effected by GMP-PNP on the binding of oxotremorine-M at the high-affinity sites
is consistent with an intramolecular interaction within a monomer. Whereas oligomers of the fusion protein resemble muscarinic receptors in myocardial membranes, the behaviour of monomers is atypical. These observations suggest that the \( \text{M}_2 \) receptor signals as an oligomer and that the native RG complex remains intact during signalling per se. (Supported by HSFO and CIHR)

Dynamic Regulation of Hexokinase by Myristoylation in Absence of Specified Sequence Motifs

Sujeet Kumar, Sreejit Parameswaran, Rajendra K. Sharma
Department of Pathology and Laboratory Medicine, Cancer Cluster, College of Medicine, University of Saskatchewan

The diverse functional regulation of proteins is often mediated by specific interactions. Specified motifs/residues are involved in such functional interactions and novel modulation of these interactions requires complex sequence change. Here we observe that diversity of protein function can arise by the virtue of specialized lipidic modification. Binding of hexokinase to mitochondria is an integral part of its functional regulation, which is mediated by a 15-20 amino acid long hydrophobic domain, located at N-terminus of the enzyme. We observe that, in a novel isoform lacking this hydrophobic domain, the membranous interaction is modulated by addition of “greasy finger”, a 14-carbon long myristoyl moiety. The synthetic peptides corresponding to the N-terminal region of the novel hexokinase isoform were found to be substrates of purified N-myristoyltransferase in enzymatic assays. The authenticity of the myristoylated peptide was verified by MALDI-MS analysis. The full-length gene was cloned under CMV promoter in tandem with red fluorescent protein (RFP) and the constructs were transfected into HEK293 cells. In microscopic imaging, we observe a localization pattern reminiscent of membranous compartmentalization. A single glycine to alanine mutation of the myristoylation site is sufficient to switch the membranous localization to diffused cytoplasmic pattern. The effects were echoed in constructs with the N-terminus portion of protein fused to the RFP. The novel myristoylation seems to win out the loss of hydrophobic domain to retain the cellular localization and thus provides a unique functional module. It is imperative to understand the mechanisms of such re-functionalization in regulation of protein functions. Further insights into the role of myristoylation on the process of ATP generation, if any, and on regulation of glycolytic pathway should improve our understanding of previously un-described functions.
The effect of BH3-only protein Bad on cBid binding to mitochondrial-like liposomes

Obaidullah Khan, Cecile Fradin

Department of Biochemistry and Biomedical Sciences, McMaster University

The Bcl-2 antagonist of cell death (Bad) is an unstructured BH3-only protein of the Bcl-2 protein family that plays vital roles in glucose metabolism and apoptosis. It inhibits another Bcl-2 protein, Bcl-XL, to sensitize the cell to apoptosis in the mechanism of mitochondrial outer membrane permeabilization (MOMP). As the first step towards apoptosis execution, MOMP serves as a critical “point-of-no-return”, after which cell death is imminent. If not regulated tightly, cell death can be resisted, a phenomenon that is a hallmark of cancer cells. In prior studies, Bad has been observed to interact with Bcl-XL and Bcl-2 while no obvious interaction was observed with Bax. Little research has described its interaction with Bid. Unpublished data in our lab have indicated an interaction between murine Bid and human his-tagged Bad in liposome-binding hydrophobicity experiments. To understand this interaction, we measured the targeting of fluorescently-labeled cleaved Bid (cBid) to mitochondrial-like (mito-like) liposomes in the presence of Bad using a chromatography column to separate ‘free’ and ‘bound’ cBid. We also measured the interaction between fluorescently-labeled cBid 126C and 1mol% NBD-PE mito-like liposomes in the presence of Bad using Förster Resonance Energy Transfer (FRET) in order to create a simple binding curve that measures the dissociation constant (K_d) between cBid and mito-like liposomes. Targeting experiments indicated a large increase in unbound cBid in the presence of 200nM Bad, while our model of a simple binding curve indicated very little change in the K_d between cBid and mito-like liposomes in the presence of 85nM Bad. Further experiments shall examine this phenomenon using a tagless Bad construct. Also, a single-cysteine Bad shall be purified using an optimized purification protocol so as to fluorescently label Bad and observe its direct interaction with cBid using FRET.
Investigating Lipid Domain-Specific Cytoskeletal Organization in Living Cell

Carolin Madwar, Gopakumar Gopalakrishnan and R. Bruce Lennox
Department of Chemistry, McGill University

Lipid membrane domains (also known as lipid rafts) are dynamic assemblies of membrane components (phospholipids, cholesterol, proteins, etc) that are fundamental for many cellular functions including signalling, trafficking and membrane fusion. In this study, we report an experimental platform where co-existing lipid microdomains are formed on a solid, spherical substrate. This spherically supported bilayer membrane (SS-BLM) is used to establish a spatial correlation between lipid microdomains of a well characterized model system and the organization of cellular cytoskeletal networks in response to membrane heterogeneity at physiological conditions. Confocal fluorescence microscopy and cryo-EM are used to characterize the SS-BLM formation and the co-existence of lipid microdomains. The effect of tethering/spacers on their bilayer fluidity is investigated using fluorescence techniques. SS-BLMs with co-existing lipid microdomains are co-cultured with live cells under physiological conditions and then examined using confocal microscopy combined with immunofluorescence in order to examine the respective ordering of cellular cytoskeletal networks around the lipid microdomains. Both actin filaments and microtubules, two major cytoskeletal components, are found to preferentially extend and assemble around the fluid lipid domains. Because of its simplicity, robustness and experimental versatility, the SS-BLM platform is an attractive complement to lipid vesicles and planar S-BLMs in membrane biophysical studies.


Nature of the M2 Muscarinic Receptor Signaling Complex Revealed by Dual-Color FCS and FRET

Yuchong Li1, Rabindra V. Shivnaraine2, Huiqiao Ji2, Dennis Fernandes1, Fei Huang2, James W. Wells2 and Claudiu C. Gradinaru1
1Department of Chemical and Physical Sciences, University of Toronto Mississauga,
2Department of Pharmaceutical Sciences, University of Toronto

The mechanism of signaling via G protein-coupled receptors (GPCRs) remains unresolved, and two questions in particular have been the focus of much attention and debate: namely, the oligomeric status of the receptor, and the nature of its interaction with the G protein. Here, we examine those questions with the M2 muscarinic receptor using a combination
of dual-color fluorescence correlation spectroscopy (dcFCS) and Förster resonance energy transfer (FRET). The unambiguous identification of a complex afforded by cross-correlation in dcFCS allows recruitment to be distinguished from conformational effects as the basis of changes in FRET. Both FRET and dcFCS were measured using a spectrally well-separated pair of fluorescent proteins, eGFP and mCherry (mCh), which were fused at the N- or the C-terminus of the receptor and at position 91 of the αi3-subunit of Gi1. Insertion of the fluorophore in Gi1 did not disrupt function, as measured by ligand-induced changes in the fluorescence of Trp 211 in switch region II. Oligomers of the receptor (eGFP-M2 and mCh-M2) and of the G protein (eGFP-αi1β1γ2 and mCh-αi1β1γ2) were identified at the membrane of live CHO cells. In the absence of an agonist, there is neither FRET nor cross-correlation between receptor and G protein (M2-mCh and eGFP-αi1β1γ2). Activation of the receptor by carbachol leads to a rapid increase in FRET and the appearance of cross-correlation, which suggests that G proteins bind only to activated receptors. Cross-correlation also revealed oligomers of the G protein (eGFP-αi1β1γ2 and mCh-αi1β1γ2) in solution and persisted upon the addition of AlF4−. Taken together, our data suggest that an agonist promotes the transient interaction of an oligomer of receptors with an oligomer of heterotrimeric G proteins, and that the oligomeric status of the G protein is conserved upon activation by AlF4−. (Supported by CIHR, HSFO, NSERC and OGS)

Conformational dynamics of a seven transmembrane helical protein Anabaena Sensory Rhodopsin probed by solid-state NMR

Daryl Good1,2, Shenlin Wang1, Meaghan Ward1,2, Jochem Struppe3, Leonid Brown1,2, Josef Lewandowski4 and Vladimir Ladizhansky1,2

1Department of Physics and 2Biophysics Interdepartmental Group, University of Guelph 3Bruker Biospin Ltd., Billerica, Massachusetts 4Department of Chemistry, University of Warwick, Coventry, United Kingdom

Recent advances in the field solid state Nuclear Magnetic Resonance (NMR) have made it possible to directly measure the site specific dynamics of large membrane proteins in a native like lipid environment. Here we report the amplitude and time scale of backbone motions in the seven trans membrane alpha helical (7TM) protein Anabaena Sensory Rhodopsin (ASR) using dipolar order parameters and 15N rotating frame spin-lattice (R1ρ) relaxation rates. We find from dipolar order parameters that the backbone of the protein undergoes small amplitude motions on the submicrosecond time scale. Additionally large variations in the R1p relaxation rates between the interhelical loop regions and the TM regions suggest that the motions occurring in the loop regions occur on a much slower timescale.
than in the TM regions. Using a model where we assume that the observed motions are as a result of the collective motions of molecular fragments we find that motions in the TM helices A, B, C, D, E and F occur on the order of 10ns while motions in the inter helical B-C loop and F-G loop as well as the TM helix G occur on the 100-200ns time scale.

Dynamic Equilibria between Monomeric and Oligomeric Misfolded States of the Mammalian Prion Protein Measured by 19F NMR

†Sacha Thierry Larda, Karen Simonetti‡, M. Sameer Al-Abdul-Wahid†, Simon Sharpe,*†§ and R. Scott Prosser*,†§
†Department of Chemistry, University of Toronto ‡Molecular Structure and Function Program, The Hospital for Sick Children, Toronto §Department of Biochemistry, University of Toronto

The assembly of misfolded proteins is a critical step in the pathogenesis of amyloid and prion diseases, although the molecular mechanisms underlying this phenomenon are not completely understood. Here, we use 19F NMR spectroscopy to examine the thermodynamic driving forces surrounding formation of β-sheet-rich oligomers early in the misfolding and aggregation pathway of the mammalian prion protein. We show that initial assembly of a small octameric intermediate is entropically driven, while further assembly to putative prefibrillar aggregates is driven by a favorable change in enthalpy. Kinetic data suggest that formation of the β-octamer represents a rate-limiting step in the assembly of prion aggregates. A disease-related mutation (F198S) known to destabilize the native state of PrP was also found to stabilize the β-octamer, suggesting that it can influence susceptibility to prion disease through two distinct mechanisms. This study provides new insight into the misfolding pathway leading to critical oligomers of the prion protein and suggests a physical basis for increased assembly of the F198S mutant.
Structural Dynamics in the Activation of Protein Kinase G I (PKG I)

Bryan VanSchouwen,¹ Rajeevan Selvaratnam,¹ Choel Kim²,³ and Giuseppe Melacini¹,⁴
¹Department of Chemistry and Chemical Biology, and ⁴Department of Biochemistry and Biomedical Sciences, McMaster University. ⁵Verna and Marrs McLean Department of Biochemistry and Molecular Biology, and ⁶Department of Pharmacology, Baylor College of Medicine, Houston, TX, USA.

Protein kinase G (PKG) is a major protein involved in eukaryotic cyclic GMP (cGMP) dependent intracellular signaling, playing a regulatory role in such processes as cell differentiation, platelet activation, memory formation and vasodilation.¹⁻⁵ However, the mechanism by which cGMP controls PKG I activity is not fully understood. Therefore, the current research work sought to probe key cGMP-controlled allosteric features of PKG I by examining a fragment of PKG Iβ that was previously shown to play a key role both in allostery and in selectivity for cGMP over cyclic AMP (cAMP).¹⁻⁷ Using a combination of NMR-based analyses, previously unknown features of PKG I allostery were revealed, including features that distinguish it from the cyclic-nucleotide-regulated proteins EPAC and PKA,⁸⁻¹² and features that may explain the differing response of PKG I to ligand analogues tested previously on EPAC and PKA.⁸,⁹

References:
Conformational dynamics in the regulation of β2-adrenergic receptor signaling

Tae Hun Kim1*, Aashish Manglik2*, Christian Altenbach3, Zhongyu Yang3, Daniel Hilger2, Foon Sun Thian2, Tong Sun Kobilka2, Wayne L Hubbell3, R Scott Prosser3, and Brian K Kobilka2

1Department of Chemistry, University of Toronto, Mississauga, Ontario L5L 1C6, Canada, 2Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, California 94305, USA, 3Jules Stein Eye Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095, USA

* Both authors contributed equally to this work.

G protein-coupled receptors (GPCRs) transduce signals from the extracellular environment to intracellular proteins1. GPCR function relies on allosteric regulation of the intracellular G protein-coupling domain by the extracellular facing ligand-binding domain3. Despite recently acquired structures of active and inactive conformations of a prototypical GPCR, the β2 adrenergic receptor (β2AR)1,2, it remains unclear how ligands regulate conformational changes in receptors. Here, we utilize 19F-fluorine NMR to directly examine the conformations and dynamics of the G protein-coupling domain of the β2AR4. These studies show that β2AR conformational plasticity is highly regulated by ligand efficacy and affinity. While NMR reveals a wealth of information regarding the influence of ligand on population and conformational dynamics, double electron-electron resonance (DEER) spectroscopy provides detail on the distribution of states5. Taken together, the ligand both influences on-pathway states associated with β2AR activation and lifetimes of specific spectroscopically detected intermediates. This “fluid” exchange between distinct intermediates stands in sharp contrast to the tight regulation of protein conformation observed for rhodopsin, and may be responsible for the complex signaling behavior observed for many GPCRs5.

**Human Aquaporin1 Protein**

Sanaz Emami, Shenlin Wang, Ying Fan, Rachel Munro, Vladimir Ladizhansky & Leonid S. Brown  
*Departments of Physics, and Biophysics Interdepartmental Group, University of Guelph*

AQP1 is one of the important proteins in human tissue that selectively transfer water through cell membrane. It is functional in its tetramer structure and each monomer consist of six transmembrane alpha helices with the number H1 to H6 and two half membrane spanning helices HA and HB. Each monomer transfer water separately and the helices surround a central water filled channel. There is highly conservative sequence which consists of Asn-Pro-Ala (NPA) sequence in the center of each segment. In order to investigate structure of this membrane protein we used a solid-state NMR (ssNMR) experiment which is well suited in this case. The most critical step for studying a protein structure with ssNMR is to achieve a functional protein in its proper folding with a very good stability. Methyotrophic yeast Pichia pastoris is one of the reliable systems to produce the isotopically labeled protein for this purpose. In this attempt we show that AQP1 can be doubly ($^{13}$C/$^{15}$N) isotopically labeled and be functionally reconstituted into the phospholipids. We began our studying by running several set of NMR experiments. The obtained NMR spectra had an excellent quality, dispersion and resolution. The linewidth for both canrbon and nitrogen was 0.5 ppm. All the spectra showed a very high resolved cross peaks related to the backbone atoms and side chain of different amino acid types in which it helped us to build our spin system and to identify each spin system according to its chemical shift value. As a result we related these spin systems to the amino acid residues that they belong to. Thus we could identify most of the aliphatic resonances related to each amino acid and confirm the high validity of our linking system.

**Residue patterning determines the lipid compatibility of transmembrane peptides**

Tracy Stone and Charles M. Deber  
*Division of Molecular Structure & Function, Research Institute, Hospital for Sick Children, Toronto MSG 1X8*  
*Department of Biochemistry, University of Toronto, Toronto M5S 1A8, Ontario, Canada*

Incorporation of transmembrane (TM) sequences into the cytoplasmic membrane may be described in terms of partitioning between aqueous and lipid bilayer compartments. Partitioning relies on compatibility of the TM sequence with bilayer properties; if a sequence is sufficiently hydrophobic and helical, it becomes membrane embedded. It is unclear, however, whether hydrophobicity is recognized as an averaged value along the length of a TM segment, or if regions of highly hydrophobic character direct partitioning. Here we have undertaken biophysical analyses of 11
compositionally identical peptides comprised of polar (Ser) and hydrophobic (Leu) residues – such as the Lys-tagged peptides SLSLSLSWLSSLSSL, SLLSSLSSWLSLLSSL – that have localized regions of hydrophobic character in the form of Leu blocks and faces along various surfaces of the proposed TM segment. Secondary structure assessment in SDS micelles using circular dichroism showed that sequences containing contiguous Leu residues are more helical than sequences containing discontinuous stretches of hydrophobic residues. Such ‘Leu-block’ sequences travel slowest on SDS-PAGE, and exhibit fluorescence spectra in SDS micelles consistent with increased burial of a centrally located Trp residue, implying preferential detergent coating of sequences containing hydrophobic blocks. Variations in retention time in a C18 column show that hydrophobicity is measured as localized areas of hydrophobic character rather than an averaged value, as sequences containing a Leu-block have the longest retention times. Our results indicate that residue patterning, rather than averaged hydrophobicity, modulates peptide partitioning into a variety of membrane-mimetic apolar phases and adoption of helical secondary structure.

1+1=3? Concerted Action of Membrane Permeabilizers

Hiren Patel1,2, Quang Huynh1, Dominik Bärlehner1, Heiko Heerklotz1,3
1 Leslie Dan Faculty of Pharmacy, University of Toronto. 144 College St, Toronto ON, M5S3M2, Canada
2 Department of Biophysics and Chemistry, University of Michigan, Ann Arbor MI
3 Institut für Medizinische Physik und Biophysik, Universität Leipzig, Germany

Certain antibiotic peptides are thought to permeabilize membranes of pathogens by effects that are also observed for simple detergents, such as membrane thinning and disordering, asymmetric bilayer expansion, toroidal pore formation, and micellization. Here we test the hypothesis that such peptides act additively with detergents when applied in parallel. Additivity is defined analogously to a fractional inhibitory concentration index (FICI) of unity, and the extent and mechanism of leakage are measured by the fluorescence lifetime-based vesicle leakage assay using calcein-loaded vesicles. Concerted action was tested for the detergent C12EO8 with the detergents octyl glucoside and CHAPS, the antimicrobial peptide magainin 2, and the fungicidal lipopeptides surfactin, fengycin and iturin from Bacillus subtilis QST713, respectively. The results are discussed in terms of an optimum heterogeneity of the system that governs the leakage mechanism, extent of leakage, and additivity of action with another agent. The results are important for understanding the nature of detergent-like actions of peptides as well as for optimizing formulations of such antimicrobials for medical applications or crop protection.
Fluorescence Spectroscopy and $^1$H NMR as Tools to Investigate the Physical Properties of Spherical Supported Bilayer Lipid Membranes

Amélie Tessier, Carolin Madwar, R. Bruce Lennox
Department of Chemistry, McGill University

Spherical supported lipid bilayer membranes using biotin-avidin tethering on silica beads are interesting due to their resemblance to biological systems. They are therefore ideal candidates for modelling cell membrane interactions and have been shown to trigger presynaptic vesicle assembly when introduced in neuronal cultures. [1] Research on the subject is mainly focused on the structure of the lipid bilayer itself and its interaction with cells. However, little is known about the interface between the lipid bilayer and the silica support. Conventional techniques for their observation such as confocal fluorescence microscopy and transmission electron microscopy (TEM) do not offer much information about the interface. On the other hand, fluorescence spectroscopy has the potential to provide insight into its properties. 5(6)-Carboxyfluorescein – a self-quenching fluorophore – is used to determine the nature of the water layer that is believed to be between the lipid bilayer and the silica bead. The use $^1$H Nuclear Magnetic Resonance ($^1$H NMR) shift reagents, which provide differentiation between the inner and outer bilayer leaflets, is also being explored.


Age-Related Effects on the Interaction between Amyloid-Beta Peptides and Anionic Lipid Membranes

Hannah Dies, Laura Toppozini, Maikel Rheinstädter
Department of Physics and Astronomy, McMaster University

The symptoms of Alzheimer’s disease do not manifest in most patients until after the age of 60, suggesting a strong link between age-related changes in the composition of brain tissue and progression of the disease. In particular, age-related changes in cholesterol and melatonin levels are currently highly discussed in the literature. We studied the interaction between two amyloid-$\beta$ peptides, amyloid-$\beta_{1-42}$ and amyloid-$\beta_{25-35}$, with anionic lipid model membranes containing cholesterol and melatonin. Our high resolution X-ray setup enabled us to determine the molecular structure of the membranes with sub-nanometer resolution in-situ, under physiological conditions. We determined the location and orientation of the molecules in the bilayers, and their lateral organization in the plane of the membranes. Both peptides were found to embed in the membrane core, which is believed to be crucial for the formation of oligomers. Moderate levels of cholesterol (30mol%) led to the formation of cholesterol plaques in the anionic membranes. The A$\beta_{25-35}$ peptides
were found to strongly interact with the membrane, displacing cholesterol molecules from the lipid regions into the plaques and increasing the total fraction of plaques in the membrane. The melatonin molecules were found to reside in the head group region of the membranes and increase the fluidity of the anionic membranes, in effect inhibiting the insertion of Aβ25-35 into the hydrocarbon core of the bilayers. We, therefore, present direct experimental evidence for an interaction between Aβ peptides, melatonin and cholesterol on the level of lipid membranes and suggest an important role for age-related effects in membrane-peptide interactions [1].


Interaction Of Digitonin and Cholate with Complex Membranes

Helen Y. Fan, Dar’ya S. Redka, and Heiko Heerklotz
Leslie Dan Faculty of Pharmacy, University of Toronto. 144 College St, Toronto ON, M5S3M2, Canada

The non-ionic detergent digitonin is widely used in the solubilization and reconstitution of membrane proteins, although knowledge of its aggregation behaviour is limited. In contrast, the bile salt sodium cholate which is also commonly used in the solubilization of membrane proteins, has been studied extensively. In order to understand the role of detergent-lipid interactions in the reconstitution of the muscarinic M2 receptor, we have studied the interactions between a particular digitonin-cholate mixture and a mixed membrane composed of phosphatidylcholine, phosphatidylserine and cholesterol. Isothermal titration calorimetry was used, along with time-resolved fluorescence leakage assays and light scattering, to study the self-assembly of the mixed surfactant system and its interactions with lipid membranes.

A Structural Basis for Cholesterol Inhibition of Outer Mitochondria Membrane Permeabilization

Kelly Cathcart1, Aisha Shamas-Din2, Norbert Kucerka3, Maikel Rheinstadter1,3, Cecile Fradin1,2
1 Department of Physics and Astronomy, and 2 Department of Biochemistry and Biomedical Sciences, McMaster University
3 Canadian Neutron Beam Center, Chalk River, Ontario, Canada

The permeabilization of the mitochondrial outer membrane is an important and irreversible step in apoptosis. The point of no return occurs when Bcl-2 family proteins interact with the membrane to form pores and release toxic molecules into the cytosol of the cell. Addition of cholesterol to mitochondrial-like membranes has been shown to inhibit the pore
formation process. The fact that the enantiomer of cholesterol also has this inhibitory effect provides support for an inhibition mechanism based on structural changes in the membrane as opposed to one based on direct interaction between the proteins and cholesterol. Structural investigations of phospholipid membranes consistently show that cholesterol increases both the thickness and rigidity of lipid bilayer. However, available studies were done for membranes containing, at most, three lipid components. We thus asked the question of whether cholesterol affects more complex membranes, such as the outer mitochondrial membrane, in the same way. We used both x-ray and neutron diffraction to probe the structure of a complex five component mitochondria-like membrane as a function of cholesterol content. X-ray diffraction was used to investigate the lamellar and in plane structure of the membrane, providing membrane thickness and average area per lipid as a function of cholesterol content. Neutron reflectivity was used to probe the position of cholesterol within the membrane. We find that, as for less complex membranes, cholesterol increases the head-to-head thickness of the membrane, the area per lipid, and the order parameter associated with lipid tail orientation.

Preliminary Investigation of Monodisperse Polysaccharide Nanoparticles Using Inelastic and Small Angle Neutron Scattering

John Atkinson,1 Jonathan Nickels,2 Erzsi Papp-Szabo,1 John Katsaras,2 and John R. Dutcher1

1Department of Physics, University of Guelph, Guelph, ON N1G 2W1
2Joint Institute for Neutron Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831

Phytoglycogen is a highly branched, water-soluble polysaccharide produced by plants that is very similar to glycogen, which is an energy storage molecule in animals. The Dutcher laboratory has succeeded in generating monodisperse phytoglycogen nanoparticles (Figure 1), and these particles are attractive candidates for a wide variety of cosmetic, industrial and medical applications. Many of these promising applications arise because of a range of interesting physical properties resulting from the interaction between the particles and water: (1) high solubility; (2) low viscosity and high stability in aqueous dispersions; and (3) a remarkable capacity to sequester and retain water. Our previous rheology measurements have revealed that phytoglycogen particles behave like ideal hard spheres in water, with the zero-shear viscosity diverging at a concentration greater than 25% (w/w). Because of this, aqueous suspensions of phytoglycogen provide an ideal system for detailed testing of theories of colloidal dispersions. To further explore the interaction of the phytoglycogen particles and water, we have performed inelastic and small angle neutron scattering (SANS) measurements at the Spallation Neutron Source at Oak Ridge National Laboratory. Measurements on the Extended Q-Range SANS (EQ-SANS) diffractometer were performed to probe the structure and interaction of the phytoglycogen particles below and above the divergence in the zero-shear viscosity. Measurements were also performed on the BASIS
backscattering spectrometer to study the quasielastic scattering from the particles, yielding information about how the viscosity shifts from a dependence on structural relaxations coupled to bulk water to structural relaxations connected to bound water as the concentration is increased. Preliminary analysis of the comprehensive neutron scattering data set will be presented.

**Figure 1**: Atomic force microscopy (AFM) image of monodisperse polysaccharide nanoparticles. The image was collected in the Dutcher laboratory, University of Guelph.

---

**Polymer Stencil Lift-Off: A Simple and Rapid Method for Patternning Arrays of Single or Stacked Lipid Bilayers Containing Phase-Segregated Domains**

Yujie Zhu, Jose Moran-Mirabal  
*Department of Chemistry and Chemical Biology, McMaster University*

Supported lipid bilayers (SLBs) provide an excellent model system for studying the structural and functional characteristics of biomembranes. Patterning model membranes on solid supports provides powerful and rapid means for reconstituting tissue-like conditions for cell culture, or creating engineered microenvironments with specific functionality for the study of important biological processes, such as cell signaling, ligand–receptor interactions, pathogen attack and enzymatic reactions occurring at the plasma membrane.

In this presentation, polymer stencil lift-off (PSLO) is introduced as a robust technique for patternning SLBs, which allows the faithful pattern transfer of micron-sized lipid domains onto solid surfaces under aqueous conditions. We show that using the PSLO technique patterns containing both homogeneous and phase-segregated SLBs can be produced. Furthermore, methods to prevent SLBs from spreading or deforming after patternning were developed using Bovine Serum Albumin. A two-step incubation method is also shown as a simple and easy technique for patternning two lipid phases, allowing the precise control of the domain size and geometry. Finally, a strategy is investigated to build patterns of stacked lipid bilayers through the electrostatic interaction between the positively and negatively charged lipid mixtures. Both homogeneous and phase-segregated stacked SLB patterns are demonstrated, and are currently being used to further study lipid behaviour.
Specific Ion Effects on the Micellization of a Non-Ionic Surfactant

Hayden Glor, Mazkak Khajehpour

Department of Chemistry, University of Manitoba

Specific ion effects on protein interfaces have been observed for many years. These effects are typically seen in the form of protein precipitation from solution by small, hard ions, while larger ions of more diffuse charge tend to denature and stabilize the unfolded protein in solution. Although specific ionic effects at protein interfaces have been known for some time, little information regarding the mechanisms by which ions interact with proteins and more general aqueous interfaces have been obtained. However, a sizeable quantity of data has been collected on ionic effects at the air-water interface. In this work the effects of different ions on the critical micelle concentration (CMC) of 1,2 Hexanediol, an oil-water interface, have been examined. A linear relationship between the ion concentration and the change in free energy of micelle formation is observed for various ions of both positive and negative charge. This linear relationship, known as the Micellization Free Energy Increment (MFI), was compared to Surface Tension Increments (STI) obtained for specific ions by Marcus. The effect of ions on surface tensions has been explained by the difference of ions partitioning out of the air-water interface and into the bulk solution. Pegram and Record have interpreted this and other ion specific interfacial phenomena in terms of partitioning coefficients. Results from the present study show that correlation between MFI and STI is not always reliable. Rather than following a partitioning model between the interface and bulk solution, the MFI values seem to correlate better with Scaled Particle Theory, in which specific ionic effects are thought to take place in the bulk solution rather than at the interface. Specifically how the ions affect the free energy of cavity formation in solution, which should be minimized for micelle formation.

Two photon fluorescence microscopy for the analysis of trabecular bone architecture

Hemanth Akkiraju¹, Christopher Price², Liyun Wang², Jeff Caplan³, Anja Nohe¹

¹Department of Biological sciences, ² Department of Mechanical Engineering, and ³ Delaware Biotechnology Institute, University of Delaware, Newark, DE, USA

Two photon fluorescence microscopy revolutionized the imaging of biological specimens utilizing its unique capabilities. The three-dimensional (3D) imaging based on nonlinear excitation of the fluorophores brings multiple advantages for imaging skeletal tissue. However, noise generated by the subsurface signal and autofluorescence of the local tissue make imaging of trabecular bone problematic. Imaging of calcified tissue presents a unique challenge to address the
aberrations produced through the noise generated. Also a general practice of immunolabeling of the plasticized bone for antigen stability are to be optimized. We show here for the first time using two-photon fluorescence imaging of trabecular bone and its architecture to properly identify the structural differences and different cell populations lining the trabecular cavity and also the cells embedded in it. Furthermore, we developed a shortened method of immunohistochemistry for plastic embedded bone tissue providing antigen stability for antibody labeling. Two photon fluorescence imaging greatly reduces photo damage and helps image of specimens of uneven planes to submicrometer resolution making this an ideal source for imaging in vivo signaling of trabecular bone. We show here the labelling of multi colored fluorophores measuring Smad and ERK activity in trabecular bone growth in mice that are systemically injected with Bone Morphogenetic Protein 2 (BMP2). We have optimized the conditions for in vivo imaging of bone tissue that is calcified and plasticized. We demonstrate for the first time that two photon fluorescence microscopy of the trabecular bone can be used for understanding the molecular mechanisms which control bone growth and development in vivo.

**Treatment of osteoporotic patient samples using peptide CK2.3 induces bone mineralization**

Miho Maeda, Christopher M. Bowens, Jeremy C. Bonor, Anja G. Nohe  
*Department of Biological Sciences, University of Delaware.*

Osteoporosis is a progressive bone disease that leads to weakened bones susceptible to fractures. About half of all women over the age of 50 will have an osteoporosis related fracture in their lifetime. Furthermore, the mortality rate during the year following an osteoporosis related hip fracture is about 20% in women. Existing treatments for osteoporosis are limited in efficiency and safety, and thus new treatments are needed. Bone morphogenetic protein 2 (BMP2) is considered as a promising therapeutic for osteoporosis. Treatment with BMP2 was found to stimulate osteoblast differentiation and bone formation. However, mesenchymal stem cells (MSCs) stimulated with BMP2 were found to differentiate into osteoblasts as well as adipocytes. The mechanism behind the differentiation is poorly understood. The primary BMP2 signaling pathway that leads to
osteogenesis is through the BMPRI and BMPRII receptors. The release of casein kinase 2 (CK2) from BMPRIa was found to be a molecular switch that directed the differentiation of MSCs into osteoblasts. Three potential phosphorylation sites on BMPRIa by CK2 were identified, and a novel mimetic peptide was made that blocked one of these three sites. Treatments of mouse MSCs with the peptide CK2.3 led to increased mineralization and decreased lipid droplet formations. Osteoblasts from patient femoral heads were isolated and treated with either BMP2 or CK2.3. We found that both treatments led to increased mineralization, but only treatment of CK2.3 led to decreased adipogenesis. Correlation studies between the age of patients and effectiveness of BMP2 found that cells from older patients responded less to BMP2 than cells from younger patients. However, this same trend was not observed between patient age and treatments with CK2.3.

Intracellular Routing in Breast Cancer Cells of Streptavidin-Conjugated Trastuzumab Fab Fragments Linked to Biotinylated Doxorubicin-Functionalized Metal Chelating Polymers

Peng Liu, Hyungjun Cho, Yijie Lu, Sachdev Sidu*, Raymond M. Reilly*, Mitchell A. Winnik*
Department of Chemistry, University of Toronto

We describe the synthesis of metal-chelating polymers (MCPs) having biotin and doxorubicin (Dox) as functional chain ends and diethylenetriaminepentaacetic acid (DTPA) pendant groups as the binding sites for radioactive metal ions. An MCP without the Dox end-group was also synthesized. Trastuzumab Fab (tmFab) fragments covalently linked to streptavidin (SAv) were complexed with MCPs via the strong affinity between biotin and SAv. Then the tmFab-MCP was labeled with radioactive $^{111}$In, forming a radioimmunoconjugate (RIC). tmFab targets human epidermal growth factor receptor-2 (HER2), which is overexpressed in certain human breast cancer cells. $^{111}$In is an Auger electron emitting radionuclide that can cause lethal DNA double strand breaks, but only if they are emitted intracellularly and, particularly, in close proximity to the nucleus. Specific binding of RICs was observed by comparing their binding with HER2-overexpressing SKOV-3 ovarian cancer cells in the presence and absence of excess free tmFab to bind available HER2 receptors. This demonstrated that although the presence of polymer increased nonspecific binding, HER2 targeting ability was retained. Surface plasmon resonance (SPR) experiments with the extracellular domain (ECD) of HER2 showed that incorporation of the MCPs to tmFab had no significant effect on the association or dissociation rate with the HER2 ECD or the dissociation constants ($K_d$). To evaluate the cellular and nuclear uptake of tmFab-SAv-Bi-MCP-Dox, we incubated HER2-overexpressing SK-BR-3 human breast cancer cells with the MCP complexes saturated with cold In$^{3+}$ and
visualized their distribution by confocal fluorescence microscopy by monitoring the fluorescence of Dox. Both radiolabeled complexes showed cell internalization and nuclear localization. We conclude that the MCP with this composition appeared to encourage internalization and nuclear uptake.

A second generation of polymers of similar design are being synthesized with PEG pendant groups to confer ‘stealth’ to the polymer and with protected functional chain end-groups, which will be rendered reactive for direct bioconjugation to intact antibodies.

**Reporte-Assisted Assembly of Gold Nanorod Complexes**

Alexander F. Stewart, Gilbert C. Walker  
*Department of Chemistry, University of Toronto*

The assembly of gold nanorods into larger structures (such as chains) has been well studied by various researchers for applications that would benefit from their nature as Surface-Enhanced Raman Scattering (SERS) probes. Such assembly is often accomplished through facilitating polymeric association between the rod monomers (and establishing SERS hotspots in the inter-rod gaps). Recently, we have conducted investigations into controlling this assembly through phospholipid encapsulation and have achieved a significant level of control regarding the populations of chain species (oligomers of various lengths) resultant from self-assembly. However, there are some disadvantages to this approach, such as the difficulty of reliably integrating reporters into polymeric domains (and keeping them there during assembly) and reducing the distance between the rods, important for optimizing the observed Raman signal and enhancement.

To address these issues, we have continued to refine the process through efforts towards the elimination of the polystyrene domain and more pronounced integration of reporter molecules in the electromagnetic hotspots. This has been accomplished through the attachment of short, charged species in the place of the previous polymers and the use of a reporter species of opposite charge as a connector between the short species on each monomer. Practically, this has implications regarding the inter-rod gap size (due to the short-connector-short ‘unit’ being much shorter than a polymer of kilodalton size) the speed of assembly (by introducing a direct electrostatic attraction) and the amount of reporter present in the gap (as a result of the reporter itself being the agent of assembly). Such assembled species are similarly suitable for encapsulation (and can be subjected to the same methods of oligomer population control) as those assembled via polymeric association.
Polymer Substrates for Waveguide Evanesence Field Microscopy

Rony Sharon, Silvia Mittler
Department of Biomedical Engineering and Department of Physics and Astronomy, Western University, London, ON

Waveguide Evanescent Field Fluorescence (WEFF) microscopy is an optical evanescent field based technique for studying cell-substratum interactions and enables characterization of cell adhesions as well as distance determination of fluorophores near the surface of the waveguide. A laser light is coupled into a waveguide to create an exponentially decaying evanescence field, which serves as the illumination source to excite fluorophores at the sample surface of the waveguide with a depth of roughly 70-200 nm depending on the particular waveguide.

A key component in the system is an optical device made of a glass substrate, containing a planar waveguide and an optical grating embedded on it for coupling the laser source and allowing interaction of the evanescence field with the sample. Previous work displayed good imaging results but limited cell growth on the all-glass devices.

The objective of this work is to develop a polymer based optical device to replace the existing glass one. Our polymer coated glass devices and biologist’s experience indicated better growth of cells on polymer substrates.

One millstone to be achieved is the development of an optical grating silicon mold by means of laser interference lithography. This will be implemented as a master stamp for hot embossing to imprint the grating pattern into the polymer substrate. Once achieved, we plan on considering different kinds of materials to be employed as waveguides, e.g. to study cell attachment to different surface chemistries.

In addition, a flexible waveguide system will be developed allowing mechanical training of cells (stretching of substrate), which has been described in literature to improve cell attachment to surfaces.

Evidence for electron-transfer among Polyvinylamine bound TEMPO moieties during the oxidation of cellulose membranes

Qiang (Sean) Fu, Robert Pelton
Department of Chemical Engineering, McMaster University

Cellulose can be selectively oxidized by exposure to laccase in the presence of TEMPO. Laccase activates the TEMPO, which then shuttles to cellulose surfaces to induce oxidation of the primary hydroxyl groups. In a variation of this technology we have shown that colloidal
complexes of laccase with polyvinylamine with grafted TEMPO (PVAm-T) also induces cellulose oxidation. This was a surprise because both TEMPO and laccase are immobilized in these microgel complexes – there can be no shuttling of TEMPO moieties. We explained the oxidation activity of the laccase/PVAm-T complex by the electron transfer between the active site of laccase and the cellulose surface via TEMPO to TEMPO electron transfer in the complex. The current work uses electrochemical techniques to give some evidence for this proposed mechanism.

**Decomposition of Surface Enhanced Raman Spectroscopy (SERS) Data using Target Factor Analysis and Band Selection**

Frauke Breitgoff, Christina MacLaughlin, Brandon Gagnon, Gilbert Walker  
*Department of Chemistry, University of Toronto*

Determining changes in dye spectra when placed in different environments - such as a cell - is crucial when measuring spectra *in vivo*. Target Factor Analysis was applied to SERS data to obtain dye spectra the way they are present in their respective environments. The datasets consisted of SERS measurements of dyes adsorbed on gold nanoparticles. The measurements were taken on dyes in mixtures/aggregates and after uptake in chronic lymphocytic leukemia (CLL) cells. SERS is a valuable tool in immunophenotyping to detect several surface proteins at once, enabling the specific distinction between types of cancer cells. Spectral changes give insight into interactions of the SERS dyes and enable more exact determination of the concentrations of the single dyes.

In order to detect the dyes in cells with a flow cytometer – as is done in clinical settings – only a few spectral bands need be chosen. The criterion for band selection was its ability to yield an optimal prediction of the dye concentration. The best method for this band selection was found to be the Genetic Algorithm. The error of the predicted concentrations was determined for different numbers of filters and signal-to-noise ratios.

**Distribution of amino acid heterodoublets in native proteins: Dependence on chain length, residue polarity, & location**

Gustavo Arteca and Chunhang Gong  
*Département de Chimie et Biochimie, Laurentian University, Sudbury, Ontario P3E 2C6*

Oligomeric repeats in protein chains (also known as ‘amino acid multiplets’) are relevant to several biophysical phenomena. For example, homomultiplets comprising 4 to 6 residues are associated with protein misfolding, aggregation, and genetic disorders; mid-size heteromultiplets are involved in substrate recognition and thus play an
important role in enzymatic reactions. In this communication, we discuss the distribution of heterodoublets. Even though these multiplets are too short for biological function, their distribution provides an insight in what constitutes a realistic primary sequence, something which is invaluable to improve modeling techniques for predicting native structure and protein folding/unfolding transitions.

From an ensemble of ca. 8,000 nonredundant structures deposited in the Protein Data Bank, we first establish which homodoublets deviate from a random distribution consistent with the experimental mean frequencies for amino acids. Although several low-frequency amino acids exhibit a nearly-random distribution of doublets, others show deviations that depend on protein length, location in secondary structure, and residue polarity. Here, we show that these deviations persist in the case of many heterodoublets; we even find instances of nonrandom behaviour that depend on sequence direction. For example, the -VL-pair (leucine preceded by valine) has a larger global probability than the –LV-pair; for alanine and glycine, the -GA-doublet is less likely than –AG-. Strong asymmetries are also found for pairs involving glutamate (E) and lysine (K). In terms of chain length and location, our results indicate a switch in heterodoublet ij/ji-relative probabilities in α-helices for proteins with 200 to 250 residues. Interestingly, this region of chain lengths is also known for changes in scaling law for mean size and accessible surface area. We also show that strong asymmetries occur at the ‘frontiers regions’ where α–helices and β–strands articulate with coils and hairpins.

Aspirin Increases the Solubility of Cholesterol in DMPC Membranes

Richard J. Alsop, Matthew A. Barrett, Songbo Zheng, Hannah Dies, Maikel C. Rheinstädter
Department of Physics and Astronomy, McMaster University

The lipid membrane is the most important biological interface, and the fluidity of the membrane is a key property. Cholesterol is a well-known mediator of membrane fluidity. Using X-ray and neutron diffraction we have recently shown that cholesterol induces lateral membrane organization at physiological concentrations. Incorporation of cholesterol into the membrane leads to immiscible cholesterol plaques at high concentration (>37.5mol%), and creates transient ordered structures, known as rafts or domains, at physiological concentrations of ~30mol% [1,2]. Non-steroidal anti-inflammatory drugs such as Aspirin or Ibuprofen are amphiphilic molecules, which may influence membrane structure and dynamics. However, a direct interplay between Aspirin and cholesterol has never been investigated. We show that Aspirin partitions in lipid bilayers and increases bilayer fluidity [3]. As shown in the Figure, additional peaks
in the diffraction profile at cholesterol concentrations of more than 40mol% are indicative of the formation of cholesterol plaques. We observed that the addition of Aspirin leads to dissolve cholesterol plaques and to a more fluid bilayer structure, as indicated by the decrease in peak intensity. We present direct experimental evidence for an interaction between Aspirin and cholesterol on the level of the cell membrane.


Energetics of the interaction of an antimicrobial peptide with phospholipid membranes

Mostafa Nategholeslam*, Bruno Tomberli† and Chris G. Gray*

* Department of Physics, University of Guelph, Guelph, ON, Canada
† Department of Physics and Astronomy, Capilano University, North Vancouver, BC, Canada

Antibiotic resistance is becoming a pressing problem globally. It is estimated that 70% of bacteria are resistant to at least one antibiotic in clinical use today. There is thus an urgent need for introduction of new, effective antibiotic drugs for clinical use. HHC-36 (KRWWKWWRR) is a computationally discovered cationic antimicrobial peptide, whose great antimicrobial activity against several strains of multi-drug resistant bacteria has been established through in vivo and in vitro experiments, while it has exhibited minimal hemolytic activity at medical doses. We report the results of all-atom molecular dynamics simulations as well as isothermal titration calorimetry experiments performed to quantify the energetics of the interaction of HHC-36 with three different phospholipid membranes, mimicking mammalian and bacterial cytoplasmic membranes. Simulation and experimental results both confirm the selectivity of the peptide, and also provide enthalpy-entropy decomposition of the peptide-membrane binding. We then discuss why adsorption to charged membranes (mimicking bacterial cell walls) is entropically favourable, based on the change in the ordering of the membrane hydration layer upon peptide adsorption, and also the change in the structure of the peptide in the vicinity of the charged membranes studied.
Interactions of Complexes of Macromolecules in Charged Aqueous Nanodroplets

Falana Sheriff, Styliani Constas
Department of Chemistry, The University of Western Ontario

Macromolecular complex–solvent interactions are ubiquitous in nature, especially in biological systems with proteins and nucleic acids. Studies of these interactions in both bulk and droplet environments are critical for understanding the practical applications such as aerosol chemistry and electrospray ionization (ESI) experiments, in which charged aqueous nanodroplets, often containing biological macromolecules, are important intermediates. Two distinct cases were examined by using molecular dynamics simulations: (1) two PEG64 polymers, a hydrophobic case, and (2) one turn of dsDNA, a hydrophilic case, both in aqueous nanodroplets. It was found that the two PEG64 polymers share the droplet surface and transiently encapsulate Na\(^+\) ions at the droplet-vacuum interface. In contrast, the dsDNA remains solvated within the droplet and some double helix interactions breakdown.

Solvation of a Polyethylene Glycol Chain in Aqueous Nanodroplets

Myong In Oh, Styliani Constas
Department of Chemistry, The University of Western Ontario

Conformational changes of a polyethylene glycol chain with 64 monomers (PEG64) solvated in aqueous nanodroplets of various sizes were studied using constant-temperature molecular dynamics simulations. Structural properties such as the radius of gyration and the end-to-end distance were used to analyze conformational transitions of the polymer. It was revealed that the most probable radius of gyration and end-to-end distance of PEG64 and the degrees of fluctuations increase as the size of the droplet increases. Also it was observed that PEG64 moves out to the surface of the droplet, although it is widely known as a hydrophilic synthetic polymer. It was found that the hydrophobicity of a PEG chain in a finite system may be affected by the number of monomers and the presence of positive charges (sodium ions) in the droplet. It was interesting to observe that a PEG chain may be solvated even after severe Coulomb explosion in a highly charged aqueous droplet beyond the Rayleigh limit.
Escape from adaptive conflicts in protein evolution: bi-stability, mutational robustness, and gene duplication

Tobias Sikosek, Erich Bornberg-Bauer, Hue Sun Chan
Department of Biochemistry, University of Toronto

Many organisms live under complex and changing environmental conditions, while having a limited number of proteins to deal with these conditions. Multi-functionality, as exhibited by many functionally promiscuous enzymes, has been hypothesised as an advantageous compromise whenever the same protein is under selection to conserve an existing function while adapting towards a new function (adaptive conflict). A stage of multi-functionality may or may not be followed by gene duplication and divergence. We use simple biophysical models to explain the basic principles behind the multi-functionality of proteins that can fold into more than one stable structure (using structure formation as a proxy for functionality). Our model predicts that proteins evolving under selection for two alternative structures can follow gradients of stability shift from the formation of only one stable structure towards an equilibrium state between two stable structures (bi-stability), each providing an independent function. Population dynamics simulations show that weak conflicting selection pressures may be sufficient to direct protein evolution towards bi-stability. Our results also suggest that models of protein evolution may underestimate evolvability if they do not account for bi-stability. However, while bi-stable proteins provide many more mutational connections to other protein structure phenotypes in genotype space, they are also less stable. This shows the inherent conflict between conservation of structure (by maximising stability), and adaptation towards new structures (which requires some destabilisation). Bi-stable proteins may provide the necessary compromise.

Furthermore, bi-stable proteins may provide an additional advantage after gene duplication, because they provide excellent starting points for subfunctionalisation (functional divergence driven by adaptation and/or genotype space entropy), as consistent with the "Escape from Adaptive Conflict" model. The potential for increased evolvability due to bi-stable proteins is thus two-fold by allowing adaptation before and after gene duplication.
Towards Multiplexed Ultrabright J-Aggregate – Nanoparticle SERS

Brandon P. Gagnon, Christopher M. Walters, Gilbert C. Walker
Department of Chemistry, University of Toronto

Raman active J-aggregate – nanoparticles hybrids as cell surface protein labels stand as an alternative to fluorescence labeling due to the potential for a higher number of unique labels to be analyzed simultaneously. There is a need to increase the library of available and unique J-aggregate Raman labels to allow for highly specific multiplexing. This work employs use of positively charged J-aggregates in place of negatively charged J-aggregates (Zamecnik et al., 2013) to increase the library of J-aggregate nanoparticle hybrids. These positively charged J-aggregates combine with gold nanoparticles to create unique and enhanced Raman spectra. The labeled particles have been successfully encapsulated with both lipid bilayers and in a silicon coating, with no decrease in signal intensity. The encapsulated particles can then be functionalized with antibodies for cell marker targeting. These particles have potential to be used in both Raman based mapping of cell surface for cancer marker abundance determination, and for flow cytometric measurements of cells.


Solvation of Poly(ethylene glycol) in Aqueous Nanodroplets

Sepideh Soltani, Styliani Constas
Department of Physics and Astronomy, and Department of Chemistry, The University of Western Ontario

Desolvation mechanisms of macromolecules from nanodroplets that contain excess charge may determine the charge state of a macromolecule in aerosols produced by electrospray ionization (ESI) methods. Polyethylene glycol (PEG) is a polyether compound which has many applications from industrial manufacturing to medicine. Solvation mechanisms of macromolecules (PEG) in different size of droplet has been studied in this poster. PEG has similar behavior to protein in solution so the important question about PEG is hydrophilicity of it in small size of waters. This simulation is more interesting when it is as a reference in comparison with PEG in water with ions. Computer modeling has been used to demonstrate the effect of size of droplet on solvations of PEG in water droplet. Gromacs package has been used with OPLS-all force field to model interaction between atoms explicitly.
Polycation-π interactions are a likely driving force for molecular recognition by an intrinsically disordered oncoprotein family

Jianhui Song, Sheung Chun Ng, Peter Tompa, Kevin A. W. Lee, Hue Sun Chan  
Department of Biochemistry, University of Toronto

Protein-protein interactions involving intrinsically disordered proteins (IDPs) commonly entail target-induced disorder to order transitions; but some IDPs can remain disordered in the bound state. This phenomenon, coined "fuzziness", is often characterized by IDP polyvalency, sequence-insensitivity and a dynamic ensemble of disordered bound-state conformations. Specific biophysical models for fuzzy complexes are mostly lacking at present. The transcriptional activation domain of the Ewing's Sarcoma oncoprotein family (EAD) is an IDP that exhibits many features of fuzziness, with multiple EAD aromatic side chains driving molecular recognition. Recognizing the prevalent role of cation π interactions at various protein-protein interfaces, we hypothesized that EAD-target binding involves polycation-π contacts between a disordered EAD and basic residues on the target. We evaluated the polycation-π hypothesis via functional and theoretical interrogation of EAD variants. The experimental effects of a range of EAD sequence variations, including aromatic number, aromatic density and charge perturbations, all support the cation-π model because these experimental trends are all well captured by a coarse-grained EAD chain model as well as a corresponding analytical model based upon interaction between EAD aromatics and surface cations of a generic globular target. EAD-target binding, in the context of pathological Ewing's Sarcoma oncoproteins, is thus seen to be driven by a balance between EAD conformational entropy and favorable EAD-target cation-π contacts.

Minimum Nanoindentation Methods and Their Application to Biomaterials

Chuan Xu, Erika F Merschrod S  
Department of Chemistry, Memorial University of Newfoundland

Nanoindentation is an effective method to investigate biomaterial mechanical properties. Different models are developed for specific applications. When the object under study is a nanostructured biomaterial, nanoindentation with minimum indentation force and depth is desirable. This can not only minimize the substrate effect but also prevent
sample damage. Minimum sample damage is especially important when researchers need to maintain the viability and/or integrity of the samples (e.g. virus) during the test. Regarding the minimum indentation force and depth, two inevitable questions are: what is the smallest force applicable, and how does one achieve the minimum indentation force.

These two questions are answered by investigating the jump-to-contact phenomenon during the nanoindentation process. Without applying any external mechanical force the tip jumps into contact with the sample surface when the cantilever spring constant is exceeded by the gradient of the indenter-sample attraction. Since no external load is applied to the indenter, the indentation force only contains the capillary force which can not be avoided. Hence the minimum indentation is achieved and justified.

This method is then applied to the study of the nanomechanical properties of the type I collagen segment-long-spacing crystallites (SLS). The results are consistent with literature values.

Hierarchical, Self-Similar Structure in Native Squid Pen

Fei-Chi Yang, Robert Peters, Hannah Dies, and Maikel C. Rheinstädter
Department of Physics and Astronomy, McMaster University

Proteins and chitin form the elementary building blocks of many biomaterials. How these molecules assemble into larger, macroscopic structures with very different properties is the fundamental question we are trying to answer [1].

The structure of native squid pen was investigated in two different species on different length scales. By combining microscopy, atomic force microscopy (AFM), and X-ray diffraction, the experiments probed length scales from millimetres down to nanometres. The pens showed a hierarchical, self-similar structure in the optical experiments with fibres of different size oriented along the long axis of the pen. The fibre-like structure was reproduced on the nanoscale in AFM measurements and fibres with diameters of 500 μm, 100 μm, 10 μm, 2 μm and 0.2 μm were observed. Their molecular structure was determined using X-ray diffraction. In squid pen, the chitin molecules are known to form nano-crystallites of monoclinic lattice symmetry surrounded by a protein layer, resulting in β-chitin nano-fibrils. Signals corresponding to the α-coil protein phase and β-chitin were observed in the X-ray experiments and their orientations with respect to the fibre axis were determined in-situ.
We find that the molecular structure is highly anisotropic with ~90% of the α-coils and β–chitin crystallites oriented along the fibre-axis indicating a strong correlation between macroscale structure and molecular orientation. The nano-fibrils were found to have a diameter of ~15 nm such that ~100 nano-fibrils are needed to form a micro-fibre.


**Nanomechanical Response of Bacterial Cells to Cationic Antimicrobial Peptides**

Shun Lu, Grant Walters, Richard Parg, John R. Dutcher

*Department of Physics, University of Guelph*

The effectiveness of antimicrobial compounds can be easily screened, however their mechanism of action is much more difficult to determine. Many compounds act by compromising the mechanical integrity of the bacterial cell envelope, and our study introduces an atomic force microscopy (AFM)-based creep deformation technique to evaluate changes in the time-dependent mechanical properties of *Pseudomonas aeruginosa* PAO1 bacterial cells upon exposure to two different but structurally related antimicrobial peptides: polymyxin B and polymyxin B nonapeptide [1]. We observed a distinctive signature for the loss of integrity of the bacterial cell envelope following exposure to the peptides. Measurements performed before and after exposure, as well as time-resolved measurements and those performed at different concentrations, revealed large changes to the viscoelastic parameters that are consistent with differences in the membrane permeabilizing effects of the peptides. The AFM creep deformation measurement provides new, unique insight into the kinetics and mechanism of action of antimicrobial peptides on bacteria.


**Aqueous based process of fabricating nanostructured block copolymer films as effective marine antifouling coatings**

Kris S. Kim, Drew MacNeil, Nikhil Gunari and Gilbert C. Walker

*Department of Chemistry, University of Toronto*

Although effective, most known marine antifouling paints have been banned due to their toxicities, most notably tributyltin oxide and, more recently copper has become regulated. The search for ecofriendly alternatives has sparked resurgence in the development of antifouling surfaces and alternative materials without heavy metals are desired. For
example, amphiphilic materials have shown promising antifouling properties (C.M. Grozea et al., 2009). By tuning materials of different composition to exhibit various surface morphologies, a variety of nanostructured surfaces can be fabricated, further enhancing antifouling abilities.

In this paper we report an aqueous based process of fabricating nanostructured poly( styrene-block-2 vinyl pyridine-block-ethylene oxide) (PS-b-P2VP-b-PEO) triblock copolymer films as effective antifouling coatings. With surface probe microscopy techniques, adhesion forces, topographical images and mechanical properties of nanostructured PS-b-P2VP-b-PEO films were collected. Over 80% of adhesion forces measured between BSA-coated probes and these films reported to be less than 20pN, which was significantly lower than the average adhesion force of 23nN measured against an uncoated surface. Additional adhesion forces were measured on PS-b-P2VP-b-PEO films at various dwell times, resulting in maximum adhesion forces of 41pN after 10s of interaction time. Ulva linza settlement analysis showed 6±3 (zoospore/mm²) on PS-b-P2VP-b-PEO coated surfaces after 45 min, in contrast to 370±40 (zoospore/mm²) on an untreated surface, further demonstrating the significant ability of nanostructured PS-b-P2VP-b-PEO films to resist adsorption.

Preparation and Surface Modification of NaLnF₄ NPs as Potential Elemental Tags for Mass Cytometry

Lemuel Tong, Guangyao Zhao, Pengpeng Cao, Elsa Lu, Mark Nitz*, Mitchell A. Winnik*
Department of Chemistry, University of Toronto

Mass cytometry is a novel technique that combines cell-by-cell injection with inductively coupled plasma mass spectrometry and time-of-flight detection. This technique allows simultaneous high-throughput measurement of more than 30 different biomarkers. Quantitative multiplexed cell assays based on mass cytometry requires metal containing
tags such as the current state-of-the-art metal-chelating polymers. Since the limit of detection depends on the number of lanthanides (Ln) per tag, we plan to increase the sensitivity with Ln nanoparticles (NPs). In this work we prepared monodispersed oleate-capped NaLnF4 (Ln = Sm to Ho) NPs with an average diameter between 10 and 20 nm. To satisfy the requirements of cell labeling experiments, the surface of the NaLnF4 NPs has to be modified to render them colloidal stable in aqueous buffer media. So far two different surface modification strategies have been developed in our group. One is ligand exchange with polyethylene glycol (PEG) based ligands such as PEG-lysine-tetraphosphonate and PEG-poly(amidoamine)-tetraphosphonate. The multidentate anchoring groups of PEG-tetraphosphonate ligands improve the colloidal stability of NaLnF4 NPs in competitive phosphate-containing buffers that are commonly used in cell incubation. The PEG ligands also provide protein repellency, thus reducing the non-specific binding of NPs to cells. The second strategy is to encapsulate the NPs with a uniform silica shell. The surface of the silica shell can further react with functional silanes to introduce reactive groups such as amines, carboxylates and maleimides. These functional groups not only provide colloidal stability for silica coated NPs via electrostatic repulsion, but can also be used to for conjugating bioaffinity agents for targeting cells. In the future, we plan to attach antibodies and peptides onto the surface of the PEG-tetraphosphonate-capped or silica-coated NaLnF4 NPs and evaluate their performance as cell-targeting elemental tags using mass cytometry.

**Design and Synthesis of Potential Theranostic Metal-Chelating Polymers Chelating Cu\(^{64}\) Radioisotope with Reactive End-Group for Bioconjugation for PET Imaging and Treatment of Pancreatic Cancer**

Hyungjun Cho\(^1\), Amanda J. Boyle\(^1\), Peng Liu\(^1\), Yijie Lu\(^1\), Raymond M. Reilly*\(^{1,5}\), Mitchell A. Winnik*\(^{1,5}\)

\(^{1}\)Department of Chemistry and \(^{5}\)Department of Pharmaceutical Sciences, University of Toronto

\(^{5}\)Toronto General Research Institute, University Health Network, Toronto

Pancreatic cancer is the fourth leading cause of death from cancer. The 5-year survival of detected cases of pancreatic cancer at early and advanced stages are 22% and 2%, respectively, and currently available chemotherapies yields disappointing results. Our goal is to develop a theranostic (the fusion of therapy and diagnostics) treatment for pancreatic cancer to minimize the time between diagnosis and treatment, and to allow for observation of the effects of the treatment within the body. We want to create radioimmunoconjugates (RICs) consisting of a cancer-targeting antibody, and metal-chelating polymers (MCPs) labelled with medically relevant radioisotopes. MCP will act as carriers for multiple radioactive \(^{64}\)Cu ions, a positron emitter for positron emission tomography (PET) imaging and a \(\beta\)-emitter that at high enough doses can be used for radioimmunotherapy. MCPs allow one to obtain antibody conjugates with much higher specific activity than attaching one or two chelators directly to antibodies. 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) is a chelator that binds strongly to \(\text{Cu}^{2+}\) ions, and polyethylene glycol (PEG) can provide “stealth” so
that the RIC is not recognized as a foreign object and intercepted by the mononuclear phagocyte system on its way to the tumour target. We have synthesized MCPs with ca. 13 NOTA pendant groups and ca. 13 PEG pendant groups. In order to test how effective the pendant PEG groups are at preventing liver uptake, two MCPs were synthesized; one with PEG length of $D_{PN}=1$ and another with PEG length of $D_{PN}=12$. These MCPs were radiolabeled and the \textit{in vivo} distribution is currently being studied in BALB/c mice. In the future, MCPs with NOTA chelators will be labeled with $^{64}\text{Cu}$ and conjugated to antibodies targeting pancreatic cancer to form RICs and evaluated as potential theranostic agents.

**Stochastic and Spatial Effects in the Origin and Stability of RNA Replicators**

Christopher Huynh, Paul Higgs  
\textit{Department of Physics and Astronomy, McMaster University}

According to the RNA World Hypothesis, life began with sequences of RNA (or some similar biopolymer) that had the ability to replicate themselves. RNA is a strong candidate because it holds genetic information, can evolve through mutations, catalyzes certain reactions (such as replication of other RNA molecules), and can be formed spontaneously in the early world. Currently the best RNA replicators grown in laboratory settings are limited by factors such as template length and accuracy. Using a theoretical approach, we wish to better understand the impact of factors such as catalyzer rate, diffusion rate, parasites, and deleterious mutations.

The “Two’s Company” model that we study has three types of molecules: the replicator $X$, the complementary template $W$, and other non-functional RNA molecules, $Y$, which act as parasites. Replication occurs when a molecule interacts with $X$ at a rate $k$. Molecules can hop from one site to another in the lattice models at a rate $h$, with up to three molecules per site. A fraction $f$ of the randomly generated molecules are $W$ or $X$, and the remainder are $Y$. When the catalysts reach significant concentration, life is said to occur in the model.

By analyzing this model with well-mixed differential equations, mean-field equations, and spatial simulations, we see that life can occur in our model even as $f$ approaches zero as we would expect in a realistic model, in part due to local concentration fluctuations and spatial correlations. We also see that there is an ideal $h$ that is most conducive to life occurring. Finally, there is a substantial region of parameter space in which life survives in the presence of parasites.
Informatic Prediction of Microbial Secondary Metabolomes

Michael Skinnider, Nathan A. Magarvey
Departments of Biochemistry and Biomedical Science/Chemistry and Chemical Biology, McMaster University

The analysis of natural product biosynthesis gene clusters has matured dramatically since the identification of the erythromycin synthase. The elucidation of the modular strategies employed by nature in the synthesis of nonribosomal peptides and polyketides have defined an opportunity to leverage a wealth of genomic data in search of new chemistries. PRISM is a computational tool to rapidly predict microbial secondary metabolomes on the basis of genomic data. PRISM's algorithm implements recent theoretical advances in genomics to create a library of predicted chemical structures representing the organism's secondary metabolome, and infer which of these metabolites represent novel natural products. Moreover, the Magarvey Lab's GNP platform can interface with PRISM results to conduct mass fragmentation analysis of bacterial extracts, and thereby determine which predicted products are actually produced by the organism. As a rapid tool to selectively identify novel chemistries by chemo- and bioinformatic analysis that is accessible to non-specialists, PRISM represents an important new tool for the discovery of new chemical architectures.

A phylogenetic model to predict the patterns of presence and absence of genes in bacterial genomes and estimate the frequency of horizontal gene transfer

Alireza Zamani, Paul Higgs
Department of Physics and Astronomy, McMaster University

For a group of related bacterial genomes, the core genome is the set of genes present in all the individual genomes and the pangenome is the set of genes present in at least one of the genomes. Typically, a relatively small fraction of genes is in the core, and many other genes are only found in only one or a small number of genomes. This indicates that there is a wide range of time scales of genome evolution, with rapid insertion and deletion of some genes and long-term retention of others. Here, we study the full set of the genes in a group of 40 complete genomes of Cyanobacteria. Genes are clustered using sequence similarity measures, and for each cluster we obtain the pattern of presence and absence of the gene across the 40 species. We use evolutionary models of gene insertion and deletion to calculate the likelihood of each of the observed patterns, and we used likelihood-based model selection criteria to fit the data. One important case we consider is the infinitely many genes model (IMG) in which each gene can only originate once but can be deleted multiple times. In contrast, the finitely many genes model (FMG) allows more than one insertion of the same type of gene in
different genomes, which would be the case if there were horizontal gene transfer. We find that a model that includes several slowly-evolving IMG classes and one rapidly-evolving FMG class usually fits the data well. This allows us to predict which genes have a presence-absence pattern that is best explained by horizontal transfer. The model explains why there are a large number of genes that follow a typical treelike pattern of vertical inheritance, despite the presence of a significant minority of genes that undergo horizontal transfer.

Reduced dimensional stochastic simulation of biochemical systems

Midhun K S¹, Marc R Roussel*¹
¹Department of Applied Mathematics, University of Waterloo, ON
²Department of Chemistry & Biochemistry, University of Lethbridge, AB

A chemical system’s dynamics depends on many different timescales. The evolution of the system is hard to solve due to the stiffness caused by wide range of timescales. In addition, we need to spend a huge computational cost to study the system. Mathematical representation of the system, which is the chemical master equation is hard to solve because of the stiffness caused by the multiple, wide range of timescales. Timescales, which are very fast in the system, is hard to observe experimentally and have less interest to study. A reduced model can be achieved by eliminating these fast modes, which gives the solution in a slow manifold. In our work we produced an exact reduction by separating the timescale into slow and fast modes. By a steady state assumption, we could transform the system into reduced system with only slow timescale of interest. By this reduction we attain a reduced master equation of smaller size, which is non-stiff.

Simulations of a sphere-dimer nanomotor in an oscillating chemical environment

Bryan Robertson, Raymond Kapral
Department of Chemistry, University of Toronto

The role of the environment is one important aspect to consider in studying the dynamics of synthetic nanomotors. In this study, a sphere dimer motor is simulated in a medium in which the concentrations of the key species involved in producing the nanomotor’s directed motion undergo oscillations over time. The simulations are performed at the mesoscopic scale using a hybrid molecular dynamics – reactive multi-particle collision dynamics (MD-RMPC) scheme. In
this approach, the dimer-dimer and dimer-solvent interactions are calculated every MD timestep and the solvent-solvent interactions are considered in a separate collision step. Additionally, the chemical reactions are performed using a birth-death stochastic process at the collision step. Results show that the presence of the sphere dimer motor strongly influences the local oscillatory behaviour of the chemical species, and that the effect is less pronounced at far distances. The sphere dimer motor also exhibits directed motion, but the oscillatory behaviour of the chemical species has little effect on the nanomotor velocity. Overall, the chemical kinetics of the medium was influenced by the presence of the nanomotor, and such interactions may become important in media with spatio-temporal patterns.

**Derivative-free methods for stochastic models of biochemical kinetics**

Monjur Morshed, Silvana Ilie
*Department of Applied Mathematics, University of Waterloo ** Department of Mathematics, Ryerson University

Biochemical systems have important practical applications, in particular to understanding critical intra-cellular processes. In the cellular environment, many biological processes are inherently stochastic. Then, stochastic models are required for an accurate description of the system dynamics. An important stochastic model of biochemical kinetics is the Chemical Langevin Equation. In this work, we provide a numerical method for approximating the solution of the Chemical Langevin Equation, namely the derivative-free Milstein scheme. The proposed strategy has the advantage that it does not require the calculation of exact derivatives; therefore it may be used for an automatic simulation of the numerical solution of the Chemical Langevin Equation. In addition, we propose an adaptive time-stepping scheme for the derivative-free Milstein scheme. The tests on several models of practical interest show the accuracy of the derivative-free Milstein scheme and the advantages of adaptive methods over the fixed step size ones.

**Membrane Leakage and Antimicrobial Action of Polymers and Surfactants**

Sara G. Hovakeemian¹, Runhui Liu², Samuel H. Gellman², Heiko Heerklotz¹
¹Dept. of Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada. ²Dept. of Chemistry, University of Wisconsin, Madison, WI, USA.

Surfactants and nylon-type polymers have been found to induce leakage of lipid membranes as detected by dye efflux from liposomes. They also show inhibitory or cytotoxic activity against living cells. We aim at better understanding the
quantitative correlation between these two activities. We hypothesize that this correlation depends crucially on the mechanism of membrane leakage. The graded mechanism involves partial efflux of entrapped dye from all vesicles, suggesting that it is based on frequent yet very small and short-lived defects distributed over all liposomes. Partial efflux by the “all-or-none” mechanism means that some liposomes leaked out all entrapped dye whereas others remained fully intact. This scenario implies the existence of distinct pores that develop in some of the liposomes and remain there during the incubation time of the experiment. The experiments are done using the fluorescence lifetime-based leakage assay.

Cellular Mechanisms of Force Generation in Antibody-Mediated Phagocytosis

Maria Goiko1, John R. de Bruyn1, Bryan Heit2
Department of Physics and Astronomy1 & Schulich School of Medicine and Dentistry2, University of Western Ontario

During antibody-mediated phagocytosis Fc receptors in the membrane of the phagocyte are engaged by an antibody-opsonized pathogen, which is subsequently internalized into an intracellular compartment for degradation. This internalization is thought to require actin polymerization to extend the cell membrane around the pathogen and contractile myosins to pull this structure into the cell. How these forces are generated and applied is, however, unclear, since Fc receptors are not tethered to the cytoskeleton. A recent re-evaluation of membrane structure suggests that it is compartmentalized into corrals bordered by fences of actin. New evidence showing that Fc receptor diffusion is confined to sub-micron regions of the membrane supports this model. Based on these data, we hypothesize that Fc receptor confinement and actin polymerization are required for Fc receptor activation and the generation of cellular forces. Using traction force microscopy (TFM) and total internal reflection fluorescence (TIRF) microscopy, we will characterize Fc receptor diffusion, forces, and force-generating mechanisms.

TFM can be used to determine a spatial map of the cellular forces in phagocytosis by measuring the displacements of fluorescently-labeled beads embedded in an antibody-coated elastic substrate, while that substrate is engaged by a cell. Combined with TIRF, single-particle tracking can be employed to assess Fc receptor mobility and local changes in cytoskeletal structure during phagocytosis. To perform TIRF on TFM substrates, we must generate specialized substrates with a high refractive index and well-characterized thickness and elasticity. TIRF/TFM will first be used to simultaneously measure forces in undisturbed phagocytosis. We will then selectively recruit actin-modifying proteins to the portion of the cell in contact with the TFM substrate, by photo-recruiting into the TIRF field fusion proteins based on the reversible photodimerization of Cry2PHR/CIBN. These experiments will enable us to elucidate the underlying mechanisms of force generation in phagocytosis at the sub-cellular level.
CBP would not be possible without a little help from our friends:

Department of Physics
Department of Cell and Systems Biology
Leslie Dan Faculty of Pharmacy
Department of Chemical and Physical Sciences (UTM)
Vice-Principal Research Office (UTM)
Vice-Dean Graduate Office (UTM)
University of Toronto Mississauga Association of Graduate Students (UTMAGS)
University of Toronto Chem Club

Thank you for your generosity and support.