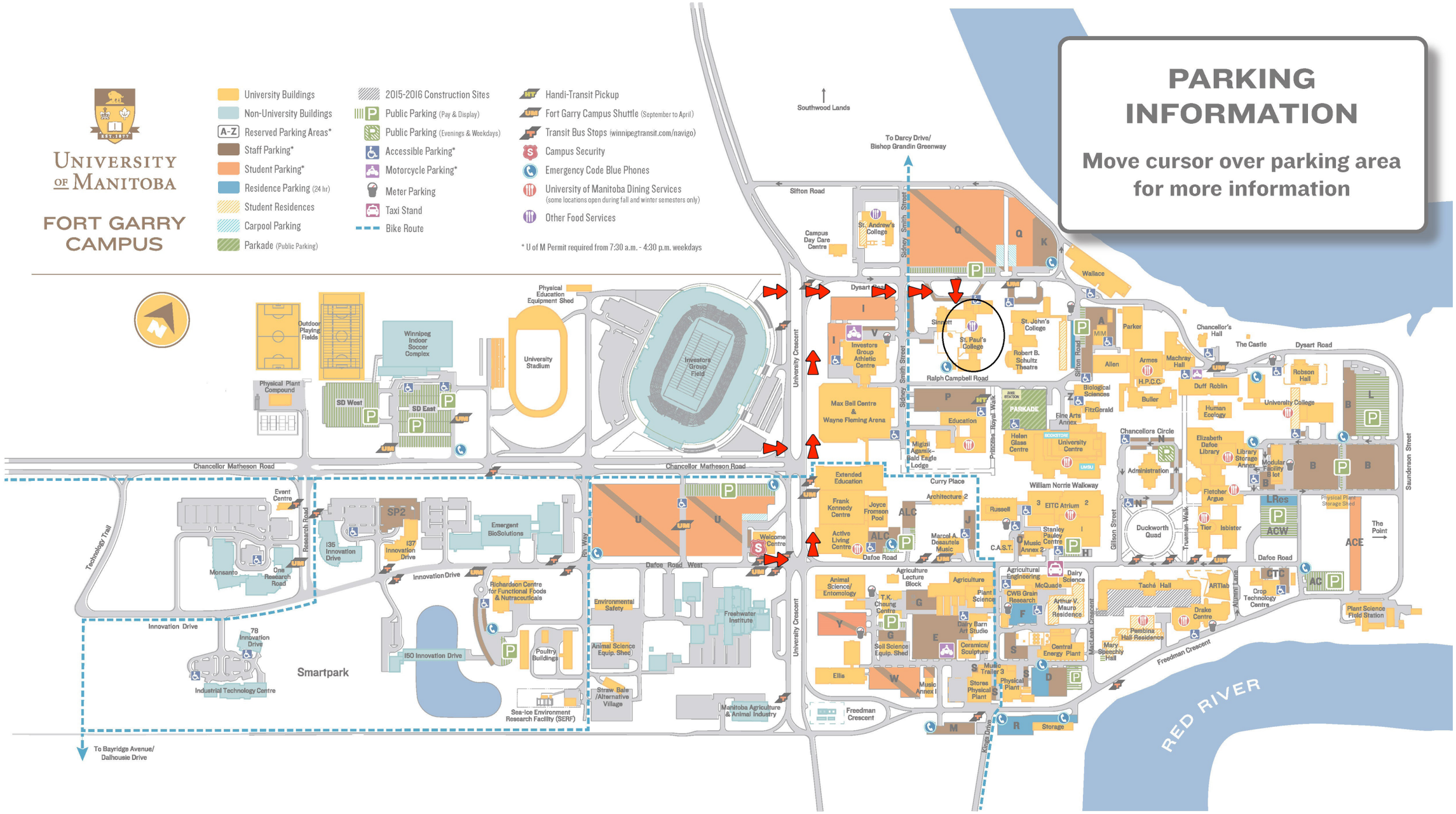
Second Annual Meeting of the Biophysical Society of Canada,

St. Paul's College, University of Manitoba,
Winnipeg, Manitoba

June 1st to June 3rd, 2016

University of Manitoba

Faculty of Science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2nd Annual Meeting of the Biophysical Society of Canada

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Department of Physics, University of Manitoba

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Department of Microbiology, University of Manitoba

Stephanie Portet,
Department of Mathematics, University of Manitoba

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2:40	**Natalie Goto**, University of Ottawa *Investigation of the Functional Role of Conformational Changes in the MinE Regulator of Bacterial Cell Division*
3:00	**Tony Mittermaier**, McGill University *Addition of Negative Charge through Mutation of an Evolutionarily Conserved Tyrosine Residue Induces Global, Concerted Millisecond Timescale Dynamics in the Intrinsically Disordered Carboxyl Terminus of Gamma-tubulin*
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bioSAXS-2000 AUTO: Biological Solution Scattering in the Home Laboratory

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5:40 **Linda Nicholson**, Cornell University
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Novel Light-Driven Anion Transporters of Cyanobacteria – Adding to the Spectrum of Ion Pumps and Sensors

6:20 **Michele Auger**, Université Laval
Effect of pH on the Structure of the Recombinant C-Terminal Domain of Nephila Clavipes Dragline Silk Protein

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NMR: Why Bother?

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Role of the Outermost M4 Transmembrane α-Helix Shaping the Agonist-Induced Response of a Pentameric Ligand-gated Ion Channel

9:40 **Jorg Stetefeld**, University of Manitoba
A Hybrid Method Approach to Unravel Higher-Order Signaling Complexes

10:00 **Gil Prive**, University of Toronto
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10:20 **Alba Guarne**, McMaster University
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11:00 **John Dutcher**, University of Guelph
Structure and Hydration of Highly-Branched, Monodisperse Phytoglycogen Nanoparticles: Nature's Dendrimer

11:40 **Steve Bourgault**, Université du Québec à Montréal
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12:00 **Peter Pawelek**, Concordia University
Studies on the Protein IroB, a Glycosyltransferase that Modifies the Bacterial Siderophore Enterobactin for Circumvention of Mammalian Immune Surveillance

12:20 **Justin MacCallum**, University of Calgary
Protein Folding Guided by Data: Automated Protein Structure Determination Based on Sparse NMR Spectroscopy Data

12:40 **Ksenia Beyrakhova**, University of Saskatchewan
Applying Biophysical Toolbox to Investigate the Role of Bacterial Effectors of Unknown Function

1:00 Conference wrap up and Lunch

Abstracts of Talks

In Presenter Alphabetical Order

Effect of pH on the Structure of the Recombinant C-Terminal Domain of Nephila Clavipes Dragline Silk protein

Martin Gauthier, Jérémie Leclerc, Thierry Lefèvre, Stéphane M. Gagné and Michèle Auger

Université Laval, Québec City, Québec

Spider silk proteins undergo a complex series of molecular events before being converted into an outstanding hierarchically organized fiber. Recent literature has underlined the crucial role of the C-terminal domain in silk protein stability and fiber formation. However, the effect of pH remains to be clarified. We have then developed an efficient purification protocol to obtain stable native-like recombinant MaSp1 C-terminal domain of Nephila clavipes (NCCTD). Its structure was investigated as a function of pH using circular dichroism, fluorescence and solution NMR spectroscopy. The results show that the NCCTD structure is very sensitive to pH and suggest that a molten globule state occurs at pH 5.0 and below. Electronic microscopy images also indicate fiber formation at low pH and coarser globular particles at more basic pH. The results are consistent with a spinning process model where the NCCTD acts as an aggregation nucleus favoring the β-aggregation of the hydrophobic polyalanine repeats upon spinning.

Role of the Outermost M4 Transmembrane α-Helix Shaping the Agonist-Induced Response of a Pentameric Ligand-gated Ion Channel

Camille M. Hénault (1), Sruthi Murlidaran (2), Grace Brannigan (2,3), and John E. Baenziger (1)

(1) University of Ottawa, Ottawa, Ontario, (2) Rutgers University-Camden, Camden, New Jersey

Pentameric ligand-gated ion channels are central to synaptic communication and are implicated in many neurological diseases. Subtle modifications in activity have profound effects on human health. The prototypic member of this family, the nicotinic acetylcholine receptor (nAChR), is exquisitely sensitive to lipids, suggesting that lipids play a role modulating synaptic communication. The outermost transmembrane α-helix in each subunit likely plays a key role in lipid sensing, even though it is peripheral to the structures thought to mediate the conformational changes that link agonist binding to channel gating. In the homo-pentameric prokaryotic homolog, GLIC, the C-terminus of M4 interacts with residues on adjacent transmembrane α-helices, as well as implicated structures located in the extracellular agonist binding domain, thus providing a potential mechanism by which lipid-dependent disruptions in M4 structure alter channel gating. In support of this possible mechanism, disruption of these interactions completely abrogates channel folding and/or function. In contrast, a similar cluster of essential interactions involving M4 that links the agonist binding domain to the transmembrane domain is absent in another prokaryotic homolog, ELIC. We sequentially deleted up to 7 residues in the M4 C-terminus in ELIC with no effect on function. Subsequent deletion of 8 or more residues, however, led to a fast-desensitizing phenotype similar to that of the therapeutically important α7 nAChR. A similar phenotype was also observed with a single Ala mutation of a Pro residue located at the midpoint of M4. This mutation removes a kink in the M4 α-helix and thus alters how M4 interacts with the adjacent α helices, M1 and M3. Through mutagenesis and molecular dynamics simulations, we show how M4 shapes the agonist-induced response.

Applying Biophysical Toolbox to Investigate the Role of Bacterial Effectors of Unknown Function

Ksenia Beyrakhova, Lei Li, Karin van Straaten, Alla Gagarinova, Miroslaw Cygler

University of Saskatchewan, Saskatoon, Saskatchewan

Gram negative pathogenic bacteria such as *Salmonella*, *Shigella*, *Legionella* use syringe-like secretion systems to deliver bacterial virulence factors into the cells. These translocated proteins, called bacterial effectors, facilitate intracellular survival and replication of the pathogen by interference with host cell signaling pathways. A significant effort has been made in the past decade to elucidate the role of effectors in infection, but due to functional redundancy of effectors standard knock out approaches and functional screening techniques fail to identify the mechanism of action of a major part of effectors. Many effectors are unique proteins with novel folds, and uncovering there 3D structure and biochemical properties might advance our understanding of their role in infection cycle. Secreted effector protein kinase LegK2 from pathogenic *Legionella* is known to be crucial for bacterial virulence, alleviating intracellular replication of the pathogen. However, the detailed mechanism of action of this enzyme at the molecular level has not been reported. We used limited proteolysis approach coupled to *in vitro* kinase assays to identify the active kinase domain of LegK2. Phage display and phosphoproteomics methods allowed us to determine substrate specificity of LegK2 and several other bacterial effector protein kinases. The structure of the C-terminal non-kinase domain of LegK2, presumably involved in interactions with host cell proteins, was determined at 1.7 Å resolution. LpiR1 (Lpg0634) is another *Legionella* effector with novel fold and unknown function. The crystal structure of the protein revealed that it consists of two similar domains aligned in an antiparallel fashion. Two conserved regions were mapped on the surface of the protein, one of which harboures a phosphate binding site. To further investigate the possible ligands of LpiR1 we performed Thermofluor Assay screening that showed that divalent cations (Mg^{2+}, Ca^{2+} and especially Mn^{2+}) stabilized the protein. The interaction was confirmed by ITC experiments and the metal binding site was identified with X-ray crystallography. The structural data obtained by biophysical methods can provide a deeper insight into the mechanisms of action of bacterial effectors, as well as give valuable clues on how to approach the functional characterization of proteins with unknown biological activity.

Amyloid Self-Assembly of Natively Unfolded Polypeptides: Investigating Helical Folding by Conformationally Constrained Derivatives

Noé Quittot, Phuong Trang Nguyen and Steve Bourgault
Université du Québec à Montréal, C.P., Succursale Centre-Ville, Montréal, Québec

Amyloid deposition is a hallmark of many diseases, such as the Alzheimer's disease and transmissible spongiform encephalopathies. Numerous amyloidogenic proteins, including the islet amyloid polypeptide (IAPP) associated with type II diabetes and the amyloid β-peptide, are natively unfolded and need to undergo conformational rearrangements allowing the formation of locally ordered structure(s) to initiate self-assembly. Recent studies have indicated that the formation of alpha-helical intermediates accelerates fibrillization, suggesting that these species are on-pathway to amyloid assembly. By designing an IAPP derivative with a restricted conformational ensemble that co-assembles with IAPP, we observed that helical species are off-pathway in homogenous environment and in presence of lipid bilayers or glycosaminoglycans. Moreover, preventing helical folding potentiated membrane perturbation and IAPP pancreatic cytotoxicity, indicating that stabilization of helical motif(s) is a promising strategy to prevent cell degeneration associated with amyloidogenesis. Besides, by designing a novel fluorescent based-assay for peptide self-assembly, we are studying the early steps in peptide amyloidgogenesis in complex biological environment.

Novel Light-Driven Anion Transporters Of Cyanobacteria – Adding To The Spectrum Of Ion Pumps And Sensors

Andrew Harris and Leonid S. Brown
University of Guelph, Guelph, Ontario

Cyanobacteria possess a number of the unique retinal-binding proteins, microbial rhodopsins, which can perform ion transporting and photosensory functions. These proteins can complement photobiological processes performed by other commonly known systems such as chlorophyll-based photosystems, light-collecting phycobilisomes, and various photosensors (such as phytochromes and cryptochromes). Our group has been involved in studies of novel microbial rhodopsins combining biospectroscopy (electronic, vibrational, NMR) and site-directed mutagenesis. Previously, we characterized a cyanobacterial light-driven proton pump from Gloeobacter and a unique cyanobacterial photosensor from Anabaena. Here, we will present our latest results on the new group of light-driven anion pumps from cyanobacteria, as characterized by laser spectroscopy and Raman scattering.

Mutations that Destabilize Phosphatidylserine Decarboxylase 1 Pre- and Post- Autocatalysis

Steven M. Claypool, Oluwaseun B. Ogunbona, Ouma Onguka, Pingdewinde N. Sam, James O. Owusu, Elizabeth Calzada
The John Hopkins School of Medicine, Baltimore, Maryland

Phosphatidylserine decarboxylase 1(Psd1p) is a mitochondrial enzyme that converts phosphatidylserine to phosphatidylethanolamine and is conserved across many species. To become functional, Psd1p undergoes a seronolytic self-processing event known as autocatalysis, which occurs at a conserved LGST motif. Autocatalysis severs the protein into a large membrane anchored β subunit that non-covalently associates with the small α subunit on the intermembrane space side of the inner membrane. In yeast, Psd1p re-directed to the secretory pathway undergoes autocatalysis and is fully functional indicating that Psd1p encodes everything needed for this self-activating proteolytic process. With the goal of identifying novel motifs required for Psd1p autocatalysis and function, we took advantage of a temperature sensitive (ts) PSD1 allele with four mutations in the β subunit. We hypothesized that the temperature sensitivity of Psd1ts is due to a defect in autocatalysis. Consistent with this prediction, Psd1ts displayed a temperature-dependent autocatalytic defect. When shifted to the restrictive temperature, the failure of the Psd1ts precursor to accumulate at steady state reflected a significantly increased rate of degradation. Interestingly, even at the permissive temperature, the interaction between the α and β subunits of Psd1ts is impaired. This result strongly suggests that the four mutations located in the β subunit of the ts allele compromise its ability to adopt a stable final structure. The mutations in Psd1ts cluster near a conserved histidine, His345, which serves as the base of a catalytic triad that includes the nucleophile Ser463 of the LGST motif and the acid, Asp210. This indicates that yeast Psd1p becomes a decarboxylase as a direct result of its initial function as a self-cleaving serine protease. Both in vivo and in isolated mitochondria, the α subunit of Psd1ts was more sensitive to restrictive temperature than its β subunit. On the flip side, Psd1β is unstable in the absence of the 38 amino acids that form the COOH terminus of the Psd1p precursor and which are destined to become the α subunit. Interestingly, the α subunit partially increased the steady state abundance of Psd1β when expressed in trans. Therefore, we propose that pre-autocatalysis the β subunit requires the α subunit for its stability while post autocatalysis, the α subunit depends on the β subunit to preserve its function and prevent its proteolytic degradation.

BioSAXS-2000 AUTO: Biological Solution Scattering in the Home Laboratory

A. Criswell, M. Del Campo, C. Acheson, T. Hendrixson, K. Sasaki
Rigaku Americas Corporation, Auburn Hill, Michigan

Small angle X-ray scattering (SAXS) is a useful technique for extracting structural information from biological samples in solution. However, the X-ray scattering signal from macromolecules is challenging to measure because of low particle concentrations and high background from solvent. As a result, instrument designs for home

laboratories, where X-ray intensities are lower than synchrotrons, require special scrutiny. In particular, collimation design, X-ray source and area detector contribute greatly to improved data quality for home laboratory experiments. Rigaku Oxford Diffraction's BioSAXS-2000 system is a SAXS instrument for the home lab, which combines 2D Kratky collimation with confocal optics to achieve maximum X-ray flux on the sample without the need for data desmearing. The BioSAXS-2000 uses Rigaku's hybrid photon counting detector (HPC), the HyPix-3000. HPCs are ideal for measuring weak scattering from biological solutions because they combine ultra low noise, high dynamic range and direct detection of X-ray photons. In fact, HPCs are the detector of choice for most SAXS beamlines and modern home laboratory SAXS instruments. Results with the BioSAXS-2000 consistently show that this instrument produces high quality SAXS data in minutes. The BioSAXS-2000 includes an AUTO package that features automation tools to achieve unattended SAXS data collection and analysis. The BioSAXS AUTO package consists of a 96-sample Automatic Sample Changer and an Automatic Analysis Pipeline (AAP). The AAP uses the industry standard ATSAS package for automatic data processing and analysis. The BioSAXS-2000, coupled to a Rigaku rotating anode source and a Rigaku HyPix-3000 hybrid pixel counting detector, collects high quality SAXS data in minutes and the AUTO package makes it possible to load a 96-well plate of samples and come back when all data collection and data processing have finished.

Structure and Hydration of Highly-Branched, Monodisperse Phytoglycogen Nanoparticles: Nature's Dendrimer

J Atkinson (1), M Grossutti (1), JD Nickels (2), J Katsaras (2) and JR Dutcher(1)

(1) University of Guelph, Guelph, Ontario, (2) Oak Ridge National Laboratory, Oak Ridge, Tennessee

Phytoglycogen is a highly branched polysaccharide, produced in the form of monodisperse nanoparticles by some varieties of plants. It is used by the plants to store glucose – a role similar to that of glycogen in animals. These nanoparticles offer a variety of properties that are unusual for nanomaterials: they are natural, non-toxic, water soluble, stable and highly monodisperse. These properties point to a wide variety of potential applications from cosmetics to drug delivery, yet these applications need to be enabled by a deeper understanding of the unique structure of the particles and their interaction with water. We have used a variety of techniques to characterize the structure and hydration of the nanoparticles. Small angle and quasi-elastic neutron scattering reveals that the nanoparticles have uniform density and are highly hydrated, with each nanoparticle containing between 250% and 285% of its mass in water. Surface-sensitive infrared absorption (ATR-FTIR) measurements on phytoglycogen films suggest that the high degree of branching in phytoglycogen leads to a well-defined "network" structure of the hydration water within the particles that is considerably more ordered than in unbranched, linear polysaccharides such as hyaluronic acid and chitosan. These studies, together with complementary microscopy, rheology and computational results, provide new insights that are key to fully understanding and exploiting these materials in new technologies and therapies, and suggest new applications as novel nanoprobes in biological research.

Content of Plasmalogen Lipids Markedly Decreases in Barth Syndrome

Richard Epand

McMaster University, Hamilton, Ontario

Barth Syndrome, with a major symptom of dilated cardiomyopathy, is caused by mutations in the enzyme tafazzin that catalyzes acylchain exchange between phospholipids and lysophospholipids. In a tafazzin-deficient heart, the acyl chain composition of cardiolipin, an essential component of cardiac lipids, becomes heterogeneous unlike in a normal heart with a single dominant species. In addition, cardiolipin decreases and monolysocardiolipin appears. In this work we have compared the lipid composition of hearts obtained from normal mice with that of hearts obtained from tafazzin knock-down mice. An extensive series of 31P NMR experiments using a cryoprobe on the biological materials with different solution conditions enabled us to determine detailed compositions of phospholipids in heart tissue. In addition to confirming that hearts obtained from tafazzin knock-down mice had less cardiolipin and detectable amounts of monolysocardiolipin, we found a decrease in plasmenylcholine. While the decrease in both cardiolipin and plasmenylcholine were large, both ~35%, the decrease in plasmenylcholine in terms of changes in the total lipid content was drastic, ~ 11% (32.4→21.5%); while the decrease in cardiolipin was only 2.3% (6.3→4.0%). In order to obtain an accurate and stable value for the amount of plasmalogen from highly resolved NMR resonances, it was required to have both a chelating agent EDTA and an antioxidant BHT present. Different tissues of the same mammalian species vary with regard to the relative amounts of the two principle forms of plasmalogen: plasmenylcholine and plasmenylethanolamine. Heart contains mostly plasmenylcholine, while lymphoblasts have principally plasmenylethanolamine. As a system directly related to the disease, we also studied human lymphoblasts derived from individuals with Barth Syndrome vs. normal controls. In this case we observed a marked decrease in the plasmenylethanolamine in mitochondria from individuals with Barth Syndrome.

Investigation of the Functional Role of Conformational Changes in the MinE Regulator of Bacterial Cell Division

Saud Ayed, Laura McLeod and Natalie K. Goto

University of Ottawa, Ottawa, Ontario

Bacterial cell division requires correct placement of the division septum at the mid-cell position in a process that is determined by three Min proteins; MinC, MinD and MinE. Together these proteins self-organize into membrane-localized zones that grow and decay between the two cell poles with a timing that is critical for normal division. It

is thought that this timing is determined by MinD-catalyzed ATP hydrolysis stimulated by an interaction between MinD and the MinE anti-MinCD domain. This domain is buried inside the dimer interface of the 6-β stranded MinE structure, and must somehow be liberated from this state to interact with MinD, giving rise to a 4-β stranded 'open' structure. In this work we investigate the molecular cues that trigger this high-barrier conformational transition between 6- and 4-stranded closed and open states. We show that direct interactions between MinE and lipid membranes induce a change in structure to a state resembling the open conformation. This mutant induced higher maximal rates of ATP hydrolysis by MinD than wild-type MinE, suggesting that the requirement for MinE conformational change normally slows down the reaction cycle. However, this increased activity was also seen in MinE mutants designed to be deficient in lipid binding for both open and closed structures, which indicates that membrane binding may also have an inhibitory effect on stimulation of MinD-catalyzed ATP hydrolysis. More surprising is the implication that MinE-membrane interactions are not required for the conformational transition. Instead, we provide evidence that interactions between MinD and an anti-MinCD domain residue exposed in the 6-stranded structure is required to trigger the conformational change in the absence of lipid-binding functionality. Overall, this data suggests that MinE has two modes of interaction with MinD; one that facilitates the conformational transition when the membrane is not accessible, and one that can be stimulated by membrane interactions.

The Role of Cardiolipin in Energy Metabolism

Vaishnavi Raja, Wenjia Lou, Yiran Li, Vinay Patil, and Miriam L. Greenberg
Wayne State University

Cardiolipin (CL) is a unique mitochondrial phospholipid that regulates many cellular functions and signaling pathways, both inside and outside of the mitochondria. The significance of CL in human health is apparent from clinical findings that perturbation of CL metabolism leads to the life-threatening disorder known as Barth syndrome. The ubiquitous association of CL with energy transducing membranes is consistent with the role of this lipid in bioenergetics. In fact, CL synthesis and mitochondrial bioenergetics are inter-dependent, as CL synthesis is both required for and stimulated by oxidative phosphorylation. Our recent studies indicate that CL plays a key role in mitochondrial metabolism. In yeast, the loss of CL causes perturbation of iron-sulfur biogenesis, resulting in decreased activity of TCA cycle enzymes and amino acid deficiencies. The CL-deficient mutant crd1Δ is synthetically lethal with mutants in pyruvate dehydrogenase (PDH), which catalyzes the conversion of pyruvate to acetyl-CoA. Consistent with this, crd1Δ cells have decreased levels of acetyl-CoA. We speculate that defects in energy metabolism may be physiological modifiers that account for the wide disparity of clinical phenotypes observed in Barth syndrome. We gratefully acknowledge support from the National Institutes of Health (HL117880) and from the Barth Syndrome Foundation, Barth Syndrome Foundation of Canada, and Association Barth France.

Taking the Temperature of Rotating Magnetotactic Bacteria

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The possible contribution of non-thermal stochastic noise to the rotational diffusion of bacteria has recently come into focus, because of its possible influence on cells' foraging strategies. Magnetotactic bacteria, who possess a magnetic moment and whose orientation can be linked to a magnetic potential energy, provide an appealing system to explore cell rotational behaviors. We have measured the effective temperature of these cells in two separate ways. We find that their distribution of orientations follows a Boltzman-like distribution with an effective temperature Teff ~ 900 K, while their rotational diffusion coefficient corresponds an effective temperature Teff ~ 1000 K This system is therefore strongly influenced by non-thermal biological noise, yet following something akin to a fluctuation-dissipation principle.

Novel Interactions Between FHA and BRCT Domains

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Forkhead-associated (FHA) and BRCA-1 C-terminal (BRCT) domains are ubiquitous phosphoepitope-binding modules present in many proteins of the DNA damage checkpoint response. The replication checkpoint preserves the genomic integrity of the cell by stabilizing stalled forks, boosting DNA repair enzyme levels, and pausing the cell cycle. Rad53 (the yeast ortholog of the tumor suppressor Chk2) is an effector kinase with integral roles in the replication checkpoint. In contrast to other FHA domain-containing proteins, Rad53 has two FHA domains (FHA1 and FHA2) that mediate independent interactions of Rad53 with upstream and downstream branches of the checkpoint. One of the binding partners of FHA1 is Dbf4, the regulatory subunit of the initiator kinase Cdc7. The association of Rad53 and Dbf4 mediates the Rad53-dependent phosphorylation of Dbf4 and the consequent inhibition of Cdc7, thus preventing late origin firing. The region of Dbf4 that interacts with Rad53 includes a single BRCT domain. BRCT domains function primarily as phosphoepitope recognition modules but can also mediate protein-DNA and protein-protein interactions. The latter are rare, and only a few have been studied at a molecular level. We have used a combination of biochemistry, X-ray crystallography, NMR and yeast genetics to characterize the interaction between the FHA1 domain of Rad53 and the BRCT domain of Dbf4. We have found that the Rad53-Dbf4 interaction is phosphorylation-independent and involves a novel interface on the FHA1

domain. Mutations within this surface result in hypersensitivity to genotoxic stress. Importantly, this surface is not conserved in the FHA2 domain of Rad53, suggesting that the FHA domains of Rad53 gain specificity by engaging additional interaction interfaces beyond their phosphoepitope-binding site. Our results suggest that FHA domains function as logic gates to increase their specificity.

Loss of Cardiolipin Stimulates Glucose Uptake Into and Across Human Blood Brain Barrier Endothelial Cells

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Microvessel endothelial cells form part of the blood-brain barrier, a restrictively permeable interface that allows transport of only specific compounds into the brain. Cardiolipin is a mitochondrial phospholipid required for function of the electron transport chain and ATP generation. We examined the role of cardiolipin in maintaining mitochondrial function necessary to support barrier properties of brain microvessel endothelial cells. Knockdown of the terminal enzyme of cardiolipin synthesis, cardiolipin synthase, in hCMEC/D3 cells resulted in decreased cellular cardiolipin levels compared to controls. The reduction in cardiolipin resulted in decreased mitochondrial spare respiratory capacity, increased pyruvate kinase activity and increased 2-deoxy-[3H]glucose uptake and glucose transporter-1 expression in hCMEC/D3 cells compared to controls. The mechanism for the increase in glucose uptake was an increase in adenosine-5'-monophosphate kinase and protein kinase B activity. Although reduction in cardiolipin did not affect permeability of fluorescent dextran across confluent hCMEC/D3 monolayers grown on Transwell® inserts, 2-deoxy-[3H]glucose transport across these monolayers was increased compared to controls. The data indicate that hCMEC/D3 cellular energy status is, in part, dependent on mitochondrial oxidative phosphorylation and that loss of cardiolipin increases glucose transport into and across hCMEC/D3 monolayers.

Art is the Elimination of the Unnecessary: the (fat)al Role of Cardiolipins

Valerian E. Kagan

University of Pittsburgh, Pittsburg, Pennsylvania

Mitochondria have retained a number of the ancestral bacterial attributes including retention of the bioenergetic molecular machinery for making ATP, a bacterial type genome (albeit diminutive) and the conservation of the ancient prokaryotic phospholipid cardiolipin (CL). In the multicellular eukaryotic organisms, mitochondria have evolved to do so much more than their prokaryotic ancestors. The role of mitochondria in regulating cellular energy balance, oxidant production, mitochondrial biogenesis, mitophagy, cell death pathway(s) and innate immunity, now define their central role in sensing metabolic and invasive perturbations that are essential for controlling cellular stability and homeostasis. CL has a role in all of these functions. Over almost 75 years since its discovery, remarkable progress in understanding of the roles that CLs and their metabolites play in mitochondrial functions has been attained. One of the latest achievements is the discovery of multiple signaling functions of CLs not only within mitochondria but also in cells and the entire body. Known unknowns of CL signaling will be the subject of the lecture.

Lateral Membrane Organization in Model Systems and Live Bacteria

John Katsaras

Oak Ridge National Laboratory, Oak Ridge, Tennese

Biomembranes are the active boundary between cells and their surroundings. They are sophisticated and dynamic machines that perform a diverse array of functions, including selective transport, localization, communication and recognition, to name a few. It is also widely accepted that the plasma membrane is laterally heterogeneous, containing nanoscopic regions enriched in certain types of lipids – these lipids have different physical properties from those that surround them. In biology, these functional lipid domains are commonly referred to as "rafts", and are thought to participate in a range of membrane processes including viral entry and exit from cells. Yet, despite their central role in biology, lipid rafts have yet to be observed in the membrane of a living system. So why is it the case that these structures have eluded detection? In recent years, we have used small angle neutron scattering (SANS) and neutron spin echo to study nanoscopic lipid domains in model membrane systems and more recently, in the Gram-positive bacterium Bacillus subtilis. Data from these studies will be presented, including the "visualization" of the membrane in live bacteria by SANS.

NMR: Why Bother?

Lewis E. Kay

University of Toronto, Toronto, Ontario

With the ever-evolving development of new biophysical tools and increasingly powerful techniques for biomolecular structural studies it is reasonable to contemplate the role of solution based NMR spectroscopy in going forward. In this talk I will describe a number of studies on molecular machines from my laboratory, emphasizing the unique role that NMR can play in providing quantitative descriptions of molecular dynamics and how such motion relates to function. The complementarity of NMR to other structural techniques is such that as they continue to advance so to will the utility of NMR. If anything NMR is far more valuable today than it was even a decade ago. This will be emphasized through a number of examples.

Protein-lipid Interaction for Amphipathic Alpha-Helix Bundle Proteins

Edgar Kooijman

Kent State University, Kent, Ohio

Abstract:Lipid droplets are organelles that contribute to various cellular functions which are vital for life. Aside from acting as a neutral lipid storage depot, they are also involved in building new membranes, synthesis of steroid hormones and cell signaling. Many aspects of LD structure and function are not yet well understood. One feature of LD binding proteins is the presence of amphipathic alpha-helix bundles. To shed light on the function of this structural motif we investigate the interaction of perilipin 3, a member of the PAT family of LD binding proteins, and three N-terminal truncation mutants with lipid monolayers. We find that the C-terminal amphipathic alpha-helix bundle of perilipin 3 has different insertion behavior from that of the longer truncation mutants and the full length protein. Inclusion of N-terminal sequences with the C-terminus decreases the ability of the protein construct to insert in lipid monolayers. Anionic lipids, coupled to negative spontaneous curvature, facilitates protein interaction and insertion. The C-terminus shows strong preference for lipids with more saturated fatty acids. This work sheds light on the LD binding properties and function of the different domains of perilipin proteins.

Structural Studies of the Krüppel-like Family of Transcription Factors

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Phenotypic switching of vascular smooth muscle cells (VSMCs) is critical to the development of several cardiovascular diseases, which are the largest cause of death worldwide. Transcriptional regulation of VSMC phenotypic switching is not fully understood but does involve the Krüppel-like Factor (KLF) family of transcription factors. Two of these transcription factors, KLF4 and KLF15, play central and opposing roles in VSMC regulation, with KLF4 being proinflammatory and KLF15 anti-inflammatory. Both KLF4 and KLF15 interact with the histone acetyltransferase homologues CBP/p300 in order to regulate phenotypic switching. In particular, KLF4 and KLF15 both contain acidic transactivation domains (TADs) that are thought to mediate their interactions with the TAZ2 domain of CBP/p300. We have characterized the interactions of KLF4 and KLF15 with the TAZ2 domain of CBP/p300 using a combination of NMR spectroscopy and isothermal titration calorimetry. Both KLF TADs are disordered in solution and in the case of KLF15, undergo a disordered to helical transition upon binding to TAZ2. These results support a model in which KLF4 and KLF15 compete for limited amounts of CBP/p300 to mediate the proinflammatory response of VSMCs.

Molecular Mechanism of Alzheimer's Disease

Zoya Leonenko

Waterloo Institute for Nanotechnology, University of Waterloo, Ontario

Alzheimer's disease (AD) is a neurodegenerative disease characterized by dementia and memory loss for which no cure or prevention is available. Amyloid toxicity is a result of the non-specific interaction of toxic amyloid oligomers with the plasma membrane. We studied amyloid aggregation and interaction of amyloid beta (1-42) peptide with lipid membrane using atomic force microscopy (AFM), Kelvin probe force microscopy and surface Plasmon resonance (SPR). Using AFM-based atomic force spectroscopy (AFS) we measured the binging forces between two single amyloid peptide molecules. Using AFM imaging we showed that oligomer and fibril formation is affected by surfaces, presence of metals and inhibitors. We demonstrated that lipid membrane plays an active role in amyloid binding and toxicity. Effect of lipid composition, surface charge and presence of cholesterol or melatonin are discussed. We discovered that membrane cholesterol creates nanoscale electrostatic domains which induce preferential binding of amyloid peptide, while membrane melatonin reduces amyloid-membrane interactions. Using AFS we showed that metals affect binding forces and inhibitors prevent amyloid-amyloid binding on a single molecule level, the first step which leads to the formation of toxic amyloid oligomers. These findings contribute to better understanding of the molecular mechanisms of Alzheimer's disease and aid to the developments of novel strategies for cure and prevention of AD.

Squeezing New Information Out of DNA

Sabrina Leslie

McGill University, Montréal, Québec

Our goal is to understand the physical principles that determine the structure and dynamics of DNA; principles that are central to researching essential information regarding storage and replication functions which govern life. This goal has motivated the development of single-molecule methods that allow us to visualize spatiotemporal dynamics of biopolymers. Existing methods often face challenges in gently templating, chemically modifying, and visualizing delicate DNA polymers and protein-DNA complexes in nanofluidic environments. Here, we present a new high-throughput platform for gently loading and reacting delicate biopolymers and complexes into sub-50 nm nanostructures, which we apply to long-DNA mapping as well as to direct visualization of DNA interactions and dynamics. Our single-molecule manipulation and visualization platform uses the principle of "Convex Lens-induced Confinement" (CLiC). In CLiC, we can continuously adjust the height of a nanofluidic imaging chamber to gently and dynamically unravel long DNA polymers into embedded open-face nanostructures from above (Berard et al, PNAS 2014). Dynamic confinement of the DNA generates tiny entropic forces that cause the DNA to unravel,

ideal for genomic analysis. Recently, we have integrated controlled, in-situ chemistry procedures within the CLiC nanofluidic device, allowing us to first template DNA polymers in embedded nanostructures, and subsequently introduce reagent molecules into the nanofluidic chamber with exquisite temporal control, such as proteins. In one series of experiments, we directly visualize DNA condensation along the nanogrooves in response to inserting reagent molecules including crowding agents, proteins and surfactants. In a complementary series of experiments, we visualize topology-mediated strand-invasion using DNA topoisomers of known winding number, which have been trapped in pits. Studying topology-mediated unwinding of model unwinding sites allows us to tackle mechanistic questions about DNA replication. We directly visualize kinetic invasion and binding in unwinding sites, by specific probes, as a function of the topoisomer supercoiling and temperature. Overall, we present a novel, sensitive, high-throughput platform for a wide range of genomic and biochemical analyses which were previously out of reach and which enable new biophysical studies.

Label-Free & Dye-free Characterization of Protein Stability and Aggregation using nanoDSF

Dinorah Leyva

NanoTemper Technologies, South San Francisco, California

We will introduce the Prometheus NT.48 instrument and its novel nanoDSF technology, which allows for parallel high-precision characterization of stability and aggregation parameters of biologicals. nanoDSF is an advanced Differential Scanning Fluorimetry technology that detects the smallest changes in the fluorescence of tryptophan present in virtually all proteins. The fluorescence of tryptophans in a protein is strongly dependent on its close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion. The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. This yields an ultra-high resolution unfolding curves which allow for detection of even minute unfolding signals. Furthermore, since no secondary reporter fluorophores are required as in conventional DSF, protein solutions can be analyzed independent of buffer compositions, and over a concentration range of 200 mg/ml down to 5 µg/ml. Therefore, nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in membrane protein research, protein engineering, formulation development and quality control.

Protein Folding Guided by Data: Automated Protein Structure Determination Based on Sparse NMR Spectroscopy Data

Justin L. MacCallum

University of Calgary, Calgary, Alberta

Automated methods can help accelerate the process of structure determination by NMR by reducing the amount of human-guided model building that is required. But NMR of large proteins typically requires perdeuteration and site-specific labeling, which poses substantial challenges to existing automated approaches due to the sparsity and ambiguity of the resulting datasets. We have developed an approach to structure determination from sparse, ambiguous, and noisy data sets called Modeling Employing Limited Data. MELD works by a synergistic combination of experimental data with physics-based models. At the simplest level, one can think of MELD as protein folding guided by data. The data serves to focus modeling on "interesting" regions of conformational space, while the physical modeling is powerful enough to infer the correct interpretation of the experimental data. I will show several successful examples where we have applied MELD to sparse NMR data, both from solution and solid-state experiments.

The Orderphobic Effect: A New Paradigm for Membrane Mediated Assembly, Reorganization and Dynamics

Kranthi. K. Mandadapu

University of California, Berkeley, California

The cause of formation of nanoscopic domains in cell membranes is a controlling factor in biological processes such as cell signaling and membrane fusion. In this talk, we present a mechanism for a generic, powerful force of assembly and mobility of transmembrane proteins in lipid bilayers. This force is a pre-transition effect mediated by the first-order phase transition between ordered (i.e. gel) and disordered (i.e. fluid) phases in the membrane. Using large-scale molecular simulations, we show how certain proteins nucleate and stabilize microscopic order–disorder interfaces, which lead to forces of assembly. Analogous to the modern theories of the hydrophobic effect, we refer to this phenomenon as the 'orderphobic effect'. The strength and range of the forces arising from this effect are significantly larger than those that could arise from membrane elasticity. Finally, we discuss the implications of the orderphobic effect for cell membranes, in particular to lipid-rafts, which consist of ordered domains floating in otherwise disordered membranes.

Towards a Treatment for Tay-Sachs and Sandhoff Disease: Construction of a Hybrid β-Hexosaminidase Subunit Capable of Forming Stable Homodimers that Hydrolyze GM2 Ganglioside in Vivo

Brian L Mark (1), Michael B Tropak (2), Sayuri Yonekawa (2), Subha Karumuthil-Melethil (3), Patrick Thompson (4), Karlaina JL. Osmon (4), Evan Woodley (4), Katalina Ong (4), Warren Wakarchuk (5), Steven J Gray (3) Jagdeep S Walia (4) and Don Mahuran (2)

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Tay-Sachs and Sandhoff disease result from loss-of-function mutations in the genes HEXA and HEXB, respectively, which code for the α- or β-subunits of β-hexosaminidase A (HexA). HexA is responsible for hydrolyzing GM2 ganglioside, an acidic glycolipid that accumulates to pathological levels in the brain and peripheral nervous systems of Tay-Sachs and Sandhoff patients. A major impediment to developing a gene therapy based approach to correct for loss of HexA activity is the need to synthesize both subunits within neurons of the central nervous system. To address this issue, critical features of the α- and β-subunits of HexA were combined into a single hybrid μ-subunit that contains the α-subunit active site, the stable β-subunit interface and unique areas in each subunit needed to interact with GM2AP, a protein cofactor that extracts GM2 ganglioside from lysosome membranes and delivers it the enzyme. To facilitate intracellular analysis and the purification of the μ-homodimer (HexM), CRISPR-based genome editing was used to disrupt the HEXA and HEXB genes in a Human Embryonic Kidney 293 cell line stably expressing the μ-subunit. In association with GM2AP, HexM was shown to hydrolyze a fluorescent GM2 ganglioside derivative both in cellulo and in vitro. Importantly, since the μ-subunit is encoded by half the amount of DNA needed to encode HEXA and HEXB, the μ-subunit open reading frame was compact enough to be package into self complementary adeno-associated virus (scAAV) for gene transfer studies in both Tay-Sachs and Sandhoff mouse models. The gene delivery trials demonstrated that HexM expression reduces brain GM2 ganglioside levels and improves motor neuron function and longevity.

"Touring the Protein Folding Landscape: the view depends on how and where you look"

Susan Marqusee

University of California, Berkeley, California

Understanding the structural and dynamic information encoded in the primary sequence of a protein is one of the most fundamental challenges in modern biology. The amino acid sequence of a protein encodes more than the native three-dimensional structure; it encodes the entire energy landscape – an ensemble of conformations whose energetics and dynamics are finely tuned for folding, binding and activity. Small variations in the sequence and environment modulate this landscape and can have effects that range from undetectable to pathological. I will present our recent results probing these sequence and environmental effects using a combination of single-molecule and ensemble-based studies. I will address a fundamental question in protein folding of whether proteins fold through one or multiple trajectories. While most experiments indicate a single pathway, simulations suggest that proteins can fold through many parallel pathways. By using a combination of chemical denaturant, mechanical force and site-directed mutations, we have resolved this apparent contradiction. We can detect the presence of multiple unfolding pathways in a simple, two-state folding protein; the dominant pathway can be altered by small changes in the sequence or environment. I will explore the implications of this result for 1) protein folding in complex environments, such as in the cell, or on the ribosome, and 2) the suggestion that evolution can modulate both the rates of folding and the specific pathway.

Mapping the Folding Free Energy Surface of Superoxide Dismutase: Insights into the Mechanisms of Folding and Aggregation and a Strategy to Discover Small Molecule Therapeutics for Amyotrophic Lateral Sclerosis.

C. Robert Matthews

University of Massachusetts Medical School, Worcester, Massachusetts

Cu,Zn superoxide dismutase (SOD1) plays a key role in mitigating the damaging effects of superoxide on DNA, RNA and proteins. Unfortunately, missense mutations at dozen of positions in human SOD1 are known to cause amyotrophic lateral sclerosis (ALS), an invariably fatal and untreatable motor neuron disease. A combination of biophysical techniques has been harnessed to map the folding free energy surface of this dimeric beta-barrel protein, with the goal of understanding the partitioning between the productive folding pathway and deleterious aggregation. Surprisingly, the combined results point to the unfolded state of SOD1 as the primary source of nucleation for soluble oligomers and aggregates associated with cellular toxicity. Particular attention has been drawn to the C-terminal segment of SOD1 that we have found to spontaneously collapse in solution and others have found to be protected against protease digestion in aggregates isolated from motor neurons. The folding free energy surface also motivated a strategy for the design of a high throughput screen for small molecules that selectively bind to the native dimeric form. By reducing the population of higher energy states, we anticipate that we will be able to retard the formation of oligomers and aggregates and, thereby, ameliorate the effects of ALS-inducing mutations in SOD1.

Molecular Determinants of Kinetic and Thermodynamic Stability in Designed and Natural Proteins

Elizabeth Meiering

University of Waterloo, Waterloo, Ontario

A relatively small number of protein folds with internal structural symmetry account for a large proportion of proteins observed in nature. One of these folds, the commonly observed β -trefoil superfold, has a 3-fold pseudo-symmetric structure, and naturally accommodates a great diversity of functional binding sites. Using consensus, modular and computational approaches, we designed Threefoil, a β-trefoil comprised of 3 identical sequence repeats. This design, obtained on the first attempt, produced a very well folded protein with multivalent carbohydrate binding function. While Threefoil has a typical, moderate thermodynamic stability, its kinetics of unfolding and folding are orders of magnitude slower than those of most natural proteins. Furthermore, Threefoil is extremely stable against thermal and chemical denaturation as well as proteolytic degradation. We find that the kinetic stability of ThreeFoil and many other proteins can be modelled and predicted using absolute contact order (ACO) and long range order (LRO), as well as coarse-grained simulations. Extensive data from proteomic screens and other experiments reveal that a high ACO/LRO is a general feature of proteins with strong resistances to denaturation and degradation. Experiments to engineer increased thermodynamic stability of ThreeFoil will be presented, and the prospects for controlling kinetic and thermodynamic stability by design will be considered.

Addition of Negative Charge through Mutation of an Evolutionarily Conserved Tyrosine Residue
Inhomogeneous Magnetization Transfer Explained. Is it Misnamed?

Alan P. Manning, Kimberley Chang, Alex MacKay, Carl A. Michal

The University of British Columbia, Vancouver, British Columbia

Recently, a new magnetization-transfer (MT) based MRI technique was introduced that claims to give myelin-specific contrast in in-vivo brain imaging. The technique involves the measurement of MT difference signals following prepulses applied off resonance with a positive frequency offset, a negative offset, or both simultaneously. With identical rf power applied in all three cases, myelin produces a larger MT difference signal in the third case while other tissues show (nearly) identical MT signals in all three. The original explanation of the physical mechanism underlying this contrast was based upon a claim of inhomogeneous broadening in the myelin lipids. A subsequent paper provides an explanation based upon Provotorov theory, but any relationship to the original explanation is not made clear. Here we provide a detailed explanation of the physics underlying the "ihMT" signals observed in multilameller bilayer systems. In our formulation, no inhomogenous broadening is required, and the origin of the ihMT effect can be clearly understood based upon a simple two-proton, spin-1 system. Provotorov theory allows these ideas to be applied to more complicated spin systems. Our application of Provotorov theory allows analytical expressions predicting the behaviour to be calculated. Inhomogeneous magnetization transfer appears to be a misnomer, as the underlying physics is unrelated to inhomogeneous broadening. We recommend dipolar MT as a more suitable and descriptive name.

Induces Global, Concerted Millisecond Timescale Dynamics in the Intrinsically Disordered Carboxyl Terminus of Gamma-tubulin

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Tubulins are an ancient family of eukaryotic proteins characterized by an amino terminal globular domain and disordered carboxyl terminus which plays an important role in modulating the behaviour of microtubules in living cells. These C-terminal regions contain multiple acidic residues and their overall charges are modulated in vivo by post-translational modifications, eg phosphorylation, yet the underlying mechanism is not known. We used NMR and MD Simulations to study the dynamics of two 39-residue polypeptides corresponding to the carboxyl terminus of yeast γ-tubulin. One polypeptide composed the wild-type amino acid sequence while the second contained a Y>D mutation at Y11 In the polypeptide (Y445 In the full protein). This mutation introduces additional negative charge at a site that is phosphorylated in vivo and produces a phenotype with perturbed microtubule function. NMR relaxation measurements show that the Y11D mutation produces dramatic changes in the millisecond-timescale motions of the entire polypeptide This observation is supported by microsecond timescale molecular dynamics simulations of mutant (Y11D) and wild-type polypeptides, in which the former, but not the latter, molecule undergoes transitions between globally compact and extended states. We hypothesize that the charge distribution Within the γ-tubulin carboxyl terminus controls its sampling of the local environment at the microtubule minus end and could modulate its interactions with effector proteins.

Mechanical Pathways of Cell Polarization and Motility Initiation

Alex Mogilner

New York University, New York City, New York

Animal cells move using polarized dynamic actomyosin network adhering to the surface. While mechanics of motility based on actin protrusion at the front and myosin contraction at the rear are understood fairly well, explanation of spontaneous polarization remained elusive. I will present simulations of a 2D model of viscous contractile actin-myosin network with free boundary which, coupled with experimental data, suggests that a positive feedback between myosin aggregation and actin flow and a negative feedback between flow and stick-slip adhesion is the key to understanding self-polarization of fish epithelial keratocytes. The model predicts, and

experiment confirms that upregulating myosin accelerates the polarization. On the other hand, epithelial IAR-2 cells self-polarize faster if myosin is inhibited. In that case, combined experiment and theory point out that competition of protruding and contracting actin networks for a common actin pool, coupled with cell movement, is the key to the self-polarization. I will discuss implications of these findings for design principles of cellular self-organization.

A Phosphorylation-Regulated Cis-Trans Molecular Switch in the Amyloid Precursor Protein Cytoplasmic Tail

Linda Nicholson
Cornell University, Ithica, New York

Alzheimer's disease (AD) is a major health care crisis, with a rising toll that has profound economic and social implications. The major scientific hurdles associated with preventing and curing AD lie in deciphering the molecular mechanisms that lead to the production of the neurotoxic amyloid-β peptide (Aβ), derived through specific proteolytic processing of the amyloid precursor protein (APP). Our earlier NMR and cell biology work suggested a model in which the cis prolyl isomer of phosphorylated Thr668, located in the APP cytoplasmic tail, is a pathological conformation that promotes Aβ generation. We have developed cis-locked small molecules to mimic this conformation, have tested these compounds in distinct H4 neuroglioma cell models of AD, and have found that selected cis-locked compounds decrease amyloidogenic processing of APP without inhibiting BACE1 catalytic activity. These findings suggest an important role of the cis conformation in amyloidogenic APP processing, and provide a foundation for development of novel therapeutic strategies to combat Alzheimer's disease.

Studies on the Protein IroB, a Glycosyltransferase that Modifies the Bacterial Siderophore Enterobactin for Circumvention of Mammalian Immune Surveillance.

Peter D. Pawelek
Concordia University, Montréal, Québec

Due to its insolubility at physiological pH under aerobic conditions, ferric iron (Fe3+) is largely inaccessible to bacteria that require it for key metabolic processes, including respiration and nucleic acid biosynthesis. To overcome the challenge of obtaining scarce extracellular Fe3+, bacteria have evolved a high-affinity acquisition strategy employing small-molecule chelators known as siderophores. Escherichia coli and Salmonella synthesizes and secretes the catecholate siderophore enterobactin, which has extremely high affinity for Fe3+. The C-glycosyltransferase IroB catalyzes formation of a C-C bond between the glucose moiety of the UDP-glucose and enterobactin co-substrate to form mono-glycosylated enterobactin (MGE). Additional rounds of glycosylation result in the formation of di- and tri-glycosylated enterobactin (DGE/TGE). Glycosylation of enterobactin has been reported to prevent its binding to the mammalian innate immune system protein Lipocalin 2, allowing IroB-harboring pathogens (e.g., UPEC, Salmonella) to continue to acquire iron in a mammalian host. Through the use of computational modeling combined with biophysical and enzymological approaches, we have identified a number of *E. coli* IroB residues required for binding the donor/acceptor co-substrates: UDP-glucose and enterobactin. To further investigate such residues, we have recently developed a novel enzymatic assay that can be more rapidly performed in comparison with the traditional HPLC/MS-based assay. The novel assay produces outcomes that agree well with the HPLC-based approach, and can be adapted for high-throughput purposes.

Lipid-Protein Interactions and Supramolecular Protein Assemblies in the Pulmonary Surfactant System

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The respiratory function in the mammalian lung is critically dependent on the stabilization of the alveolar surface by a highly cohesive multilayered lipid-protein film. This assembly is simultaneously responsible of maintaining very low surface tension at the respiratory air-water interface, avoiding its colonization by pathogens and permitting an efficient gas exchange. Key roles in these activities are played by highly conserved surfactant-associated proteins, and particularly, by surfactant protein SP-B, whose lack is incompatible with air-breathing and life. Recent experiments have revealed that SP-B, a extremely hydrophobic polypeptide of only 79 amino acids, assembles as a ring-shapped high order oligomer that sustain membrane-membrane contacts in surfactant structures. These membrane contacts ensure the cohesivity and mechanical resistance of surfactant films under the demanding conditions imposed by compression-expansion breathing cycles. At the same time, SP-B-promoted connections permit rapid diffusion of surface active lipids from the epithelium to the interface and a efficient oxygen transport. Characterization of the structure and lipid-protein interactions involving SP-B machinery by electron microscopy, atomic force microscopy and molecular dynamics is opening a whole new perspective on the structure-function determinants in pulmonary surfactant and on the strategies to produce efficient clinical surfactant preparations to treat respiratory pathologies.

The Mechanism of Acid Sphingomyelinase at Membrane Surfaces

Gil Privé and Zi-Jian Xiong

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Sphingomyelin (SM) is a major component of most eukaryotic membranes where it has roles in determining the physical properties of membranes and also serves a pool for the bioactive lipid ceramide. This lipid is in constant turnover, and the degradation of SM by the enzyme acid sphingomyelinase (ASM) in late endosomes/lysosomes is important for membrane homeostasis and normal cell functioning. We have determined the crystal structure of ASM in order to better understand how ASM carries out the breakdown of SM at lysosomal membrane surfaces. The protein consists of an N-terminal saposin-like domain, a connector domain and a calcineurin-like catalytic domain, and the structure reveals how these three regions work together to bring the sphingomyelin headgroup to the catalytic center at the membrane surface for the hydrolysis of the phosphoester bond.

Cardiolipin and the Assembly of Mitochondrial Membranes

Michael Schlame

New York University, New York City, New York

Cardiolipin (CL) is a specific mitochondrial phospholipid that has a high affinity for proteins and that stabilizes the assembly of supercomplexes involved in oxidative phosphorylation. The physical basis of this effect lies in the unique shape of CL and its charge state, which together produce a tendency to cluster and a tendency to form strong non-covalent interactions with proteins. We found that the fatty acids of CL play a crucial role in the assembly process and that the sequestration of CL in protein complexes is critical to protect it from degradation. In Barth syndrome, where the fatty acid composition of CL is altered, supercomplex assembly is impaired and CL is rapidly degraded. Treatments that induce supercomplex assembly decrease the turnover of CL whereas dissociation of supercomplexes has the opposite effect. Our data suggest that cardiolipin is uniquely protected from normal lipid turnover by its association with proteins, but in Barth syndrome, where this association is compromised, cardiolipin becomes unstable.

A Hybrid Method Approach to Unravel Higher-Order Signaling Complexes

Jorg Stetefeld

University of Manitoba, Winnipeg, Manitoba

Multicomponent Ligand-Receptor complexes are higher-order signaling assemblies for transmission of receptor activation information to cellular responses. A molecular understanding of these highly complex signaling pathways will shed light into essential key processes such as proximity driven cascade activation, signal-to-noise behavior, signal amplification as well as temporal and spatial control of signal transduction. The Stetefeld Laboratory performs a Hybrid-Method approach – combining biophysical techniques in combination with integrated structural biology techniques- to unravel complex formation. In this presentation, key examples, including netrin-1 driven dependence receptor activation will be discussed.

Conjugation of Derivatives of Aurein 2.2 and HPG Yields Interesting Antimicrobials

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

University of British Columbia, Vancouver, British Columbia

With the advent of bacterial resistance, it has become increasingly important to find substitutes to conventional antibiotics. Antimicrobial peptides (AMPs) are considered to be viable alternatives, because they are broad spectrum and give rise to very limited resistance effects. The aurein peptides are a family of AMPs involved in the first line of defense of the frog Litoria aurea. They are quite active against a range of Gram positive bacteria, but have associated problems such as toxicity, short circulation half-life (t1/2), and rapid kidney clearance. To circumvent such challenges, we have recently demonstrated ways to conjugate aurein AMPs to the polymer hyperbranched polyglycerol (HPG) to alter residence time and biodistribution in the body, without loss in activity. Results on more recent work, involving peptides derived from aurein 2.2, as well as polymers of different molecular weights, will be presented.

Sample Preparation and Handling Methods for Maximizing Data Quality in Protein Crystallography

Robert E. Thorne, Benjamin Apker, David Closs, and Robert Newman

MiTeGen, LLC, Ithaca, NY

Advances in synchrotron X-ray sources, beam line optics and detectors, and in scaling and refinement software for combining data collected from large numbers of crystals are transforming crystallographic study of proteins and other biomolecules. At the same time, they have highlighted limitations imposed by current methods of sample preparation, including challenges in placing large numbers of small crystals in the X-ray beam, excessive background X-ray scatter, crystal nonisomorphism due to variations in crystallization conditions, unintentional dehydration, and cryocooling, and in dealing with membrane protein crystals grown using increasingly popular lipidic cubic phase-based methods. This talk will review technologies for crystallization, in situ X-ray inspection, serial microcrystallography, and high throughput cryocooling being developed by MiTeGen and our collaborators, and discuss experimental best practices to maximize data quality and throughput.

Mapping the Conformational Landscapes of Proteins by Variable Temperature X-ray Crystallography

Robert E. Thorne (1), Daniel Keedy (2), Matthew Warkentin (1), and James Fraser (2)

(1)Cornell University, Ithaca, NY 14853, (2)University of California, San Francisco, CA 94143

In most areas of science, temperature is a key experimental variable. Yet for the last 20 years, protein crystallography has been fixated on one temperature: 100 K. In the entire history of protein crystallography, only a handful of studies have probed structure in arguably the most interesting region — between the protein-solvent glass transition near 200 K and ~ 260 K — largely due to the rapid formation of ice at these temperatures. Moreover, computational methods developed in the last decade for mining information in electron density maps at levels previously considered as noise are revealing just how much information about room/biological temperature structure and function is lost on cooling to T=100 K. Detailed study of ice formation in aqueous solutions and in protein crystals has yielded general methods for collecting high quality crystallographic data at all temperatures between 180 K and 300 K in crystals with supercooled liquid solvent, often without use of penetrating cryoprotectants. Application of these methods to the proteins urease and cyclophilin A has revealed how both main chain and side chain conformations evolve with temperature. Multiconfomer models show that minority side chain conformations typically "freeze out" with decreasing temperature between 300 K and 180 K, and that they do so heterogeneously, ruling out one model for the protein-solvent glass transition. Variable temperature crystallography, with its all-atom, site-resolved mapping of conformational heterogeneity, provides an excellent complement to NMR studies in understanding protein energy landscapes and the relation between protein structure and dynamics.

Correlated Imaging and Force Mapping Studies on Model Membranes and Living Cells

Shan Zou

Measurement Science and Standards, National Research Council Canada

Using combined advanced microscopy and spectroscopy techniques in biophysical chemistry enables the understanding of cellular constituent structures and processes. Integrated atomic force microscopy (AFM) and fluorescence imaging on the same microscope platform provide high sensitivity and spatial resolution that facilitate functional and molecular level characterization of biological processes. This multimodal approach allows imaging individual molecules and probing interactions of molecular assemblies by AFM imaging and force spectroscopy, with the advantages of fluorescence microscopy for the selective and specific identification of labelled molecules in bacteria and mammalian cancer cells. As an example, integrated imaging and force mapping reveal a direct correlation of the structures with their mechanical stability in phase-segregated model membrane systems, mimicking "rafts" within cell membranes. The influence of different cholesterol levels (5-40%) on the morphology and nanomechanical stability of phase-segregated lipid bilayers was further explored as a function of loading rate using a self-developed analysis toolset. A couple of examples using AFM indentation measurements, correlated with optical imaging in systematically evaluating drug mediated responses of living cells will be discussed.

Abstracts of Posters

In Presenter Alphabetical Order

Group A

Poster#1: Parameterization of Cysteine Palmitoyl, Cysteine Farnesyl, Cysteine GeranylGeranyl and Glycine Myrsitol for the Martini Forcefield

Yoav Atsmon-Raz and Peter D. Tieleman
University of Calgary, Calgary, Alberta

Peripheral membrane proteins such as the Ras and Src superfamilies go through various post-translational modifications that extend specific amino acids with fatty acid tails. A modified protein can then bind to a biological membrane by inserting the fatty acid tail into the bilayer. In our work we have used all-atom simulations in the CHARMM36 force field to parameterize four of the more common post-translational modifications into the martini force field – palmitoylated cysteine, farnesylated cysteine, geranylated cysteine and myrsitoylated glycine. Our process has been designed to reproduce the all-atom bonded parameters and the water-octanol partitioning free energies of these amino acids which we have successfully achieved in the martini force field.

Poster #2: Curvature-Induced Lipid Sorting in Plasma Membrane Tethers

Svetlana Baoukina, Helgi I. Ingolfsson, Siewert J. Marrink and D. Peter Tieleman
University of Calgary, Calgary, Alberta

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

Poster#3: Bax-Dependent Mitochondrial Outer Membrane (MOM) Permeabilization: An Investigation of the Role of MOM Phospholipid Asymmetry

J.C. Bozelli Jr., R. M. Epand
McMaster University, Hamilton, Ontario

Biological membranes are assembled as a functional lipid bilayer, which presents transverse asymmetry in the lipid composition of the outer and inner monolayers. Although lipid asymmetry has been known for several decades, still very little is understood of why cells and organelles spend energy to maintain lipid asymmetry. In order to evaluate the role of lipid asymmetry in a specific biological process, this work employs a cell-free system where a direct comparison between an asymmetric and a symmetric model membrane could be made.The biological process of interest is Bax-dependent mitochondrial outer membrane permeabilization (MOMP), which is considered the point where cell is committed to die via the mitochondrial-pathway of apoptosis. Since, it is well known that the mitochondrial outer membrane (MOM) bears lipid asymmetry; asymmetric and symmetric liposomes were prepared with a lipid composition mimicking MOM. While symmetric liposomes were prepared by conventional lipid film hydration methodology, asymmetric liposomes were prepared employing the methyl-β-cyclodextrin catalyzed exchange. Contrary to the well-used physical properties to evaluate asymmetry in liposomes, in this work a precise measurement of the composition of each leaflet of the asymmetric bilayer was evaluated by the use of head groups-specific probes. The role of phospholipid asymmetry on Bax activity was evaluated by comparing leakage of aqueous contents of asymmetric and symmetric liposomes. In the bottom-up approach to understand physiological processes at the membrane level *in vitro*, the employment of asymmetric model membranes is a sophisticated way to more faithfully mimic them.

Poster #4: Controlled Uptake of Negatively Charged Lipids into the Outer Leaflet of Liposomes

Sebastian Fiedler (1), Marie Markones (2), Carina Zorzin (2), Louma Kalie (2), Heiko Heerklotz (2,3)
(1)Lelsie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada (2)Institute for Pharmaceutical Sciences, University of Freiburg, Freiburg, Germany (3)BIOSS Centre for Biological Signalling Studies, Freiburg, Germany

The presence of negatively charged lipids in the outer leaflets of cellular membranes is a key feature of bacterial membranes. Eukaryotic cell membranes do not exhibit negatively charged lipids on their extracellular membrane sides under normal circumstances. However, the accumulation of phosphatidylserine on the extracellular membrane side attracts macrophages and is part of the events that induce programmed cell death through apoptosis. Recently, a cyclodextrin-based lipid exchange assay has been introduced that transports phospholipids from the outer leaflet of donor liposomes to that of acceptor liposomes, this way preparing liposomes of asymmetric lipid composition. In an effort to master and further develop the cyclodextrin method, we utilize the zeta potential of mixed POPG/POPC liposomes of symmetric and asymmetric composition to quantify the incorporation of

negatively charged POPG into POPC liposomes. Furthermore, we determine the equilibrium constants of POPG/POPC-cyclodextrin complex formation by ITC. This allows us to adjust cyclodextrin and POPG concentrations to various levels of cyclodextrin's maximum lipid binding capacity. By doing so, we precisely control the amount of POPG that is transported into the outer leaflet of the POPC vesicles. Notably, our approach does not aim at a complete replacement of POPC by POPG in the outer lipid layer but seeks the incorporation of negatively charged lipids at physiologically relevant contents. This allows for more realistic model membranes that carry negative charges exclusively in their outer leaflets to study, for example, processes that involve bacterial membranes or apoptosis in eukaryotic cellular membranes.

Poster #5: Evaluating Binding Affinities of the 16 'Priority' Polycyclic Aromatic Hydrocarbons to the Right-Handed Coiled Coil Nanotube

Olga Francisco, Matthew MacDougall, Mazdak Khajehpour, Gregg Tomy and Jörg Stetefeld
University of Manitoba, Winnipeg, Manitoba

Right-handed coiled coil (RHCC) is a surface (S-) layer protein isolated from *Staphylothermus marinus*, an extremophile archaebacterium that is found in a deep ocean hydrothermal vents. Coming from severe environment RHCC considered to be a unique protein that can function under harsh conditions such as high or low temperatures, significant changes of pH, salinity and pressure. The presence of four large inner-channel hydrophobic cavities (330-380Å) has been shown to facilitate binding a variety of ligands, including Polycyclic Aromatic Hydrocarbons (PAHs). PAHs are known as persistent contaminants present in our environment (air, water, sediments) that pose a high risk to human health as well as aquatic life. As a result, 16 different PAHs are designated as a priority pollutants by the US Environmental Protection Agency (EPA). Due to PAHs wide distribution, it is of a great importance to monitor the levels of these compounds in the aquatic environments. Therefore there is an urgent need to develop an environmental monitoring tool that can accurately predict PAHs concentrations in waters. To this end, we have established fluorescence based protocol to characterize the binding affinities of individual priority PAHs to the RHCC. Our results show that all 16 priority PAHs are bound to the hydrophobic interior of the protein. Additionally, minimum equilibration times for binding were determined. Our data provides a platform for developing RHCC as a PAHs monitoring device with its future potential to be used during remediation process in the case of oil spills.

Poster #6: The External Gate of the Serotonin Transporter Requires a Basic/Acidic Amino Acid Pair for Amphetamine Translocation and the Induction of Substrate Efflux

Natalie R. Sealover (1), Bruce Felts (2)* Charles P. Kuntz (1), Rachel E. Jarrard (1), Gregory H. Hockerman (1), Eric L. Barker (1) and L. Keith Henry (2)*
(1) Purdue University College of Pharmacy, West Lafayette, Indiana and
(2) University ofNorth Dakota School of Medicine and Health Sciences, Grand Forks, North Dakota

The substituted amphetamine, 3,4-methylenedioxy-methamphetamine (MDMA, ecstasy), is a widely used drug of abuse that induces non-exocytotic release of serotonin, dopamine, and norepinephrine through their cognate transporters as well as blocking the reuptake of MDMA by the same transporters. The resulting dramatic increase in the synaptic levels of these neurotransmitters leads to psychotropic, stimulant, and entactogenic effects. The mechanism by which amphetamines drive reverse transport of the monoamines remains largely enigmatic, however, promising outcomes for the therapeutic utility of MDMA for post-traumatic stress disorder and the long-time use of the dopaminergic and noradrenergic-directed amphetamines in treatment of attention-deficit hyperactivity disorder and narcolepsy increases the importance of understanding this phenomenon. Previously, we identified functional differences between the human and *Drosophila melanogaster* serotonin transporters (hSERT and dSERT, respectively) revealing that MDMA is an effective substrate for hSERT but not dSERT even though serotonin is a potent substrate for both transporters. Chimeric dSERT/hSERT transporters revealed that the molecular components necessary for recognition of MDMA as a substrate was linked to regions of the protein flanking transmembrane domains (TM) V through IX. Here, we performed species-scanning mutagenesis of hSERT, dSERT and *C. elegans* SERT (ceSERT) along with biochemical and electrophysiological analysis and identified a single amino acid in TM10 (Glu394, hSERT; Asn484, dSERT, Asp517, ceSERT) that is primarily responsible for the differences in MDMA recognition. Our findings reveal that an acidic residue is necessary at this position for MDMA recognition as a substrate and serotonin releaser.

Poster #7: Tryptophan Mutations Effect the Stability of the Thioredoxin-like, Core-fold of the SCO Protein

Bruce C. Hill, Diann Andrews and Shina Hussain
Queen's University, Kingston, Onatario

Synthesis of Cytochrome c Oxidase, or SCO, protein family members aid in the assembly of the CuA center of cytochrome c oxidase. The core structure of SCO is a thioredoxin-fold augmented by two loops that house the copper binding ligands. SCO has been proposed to act as a copper delivery factor in the assembly of CuA. Cu(II) binding to SCO shifts its melting temperature by more than 20 oC in the absence of any large scale structural changes. We have made a series of mutations of SCO to probe the foundations of its inherent stability. The protein has two tryptophan residues (W36 and W101) located in its structural core and at different distances from the functional metal binding site. When W36 is changed to alanine the melting temperature shifts to 32 oC from 53 oC seen for wild-type SCO. Copper binds to SCO W36A and stabilizes the protein resulting in a melting

temperature of 64 oC. Copper binding SCO W36A induces a measureable change in the secondary structure complement relative to apo-SCO W36A. Replacing F42, which is closer to the putative copper binding site (i.e., 5-8 Å), with tryptophan stabilizes the protein and shifts the melting temperature of apo-SCO F42W to 60 oC. The F42W mutant still binds copper, but copper binding causes little change to the melting temperature. Analysis of these mutations has been done using an empirical force field as implemented by Fold-X to calculate the stability change introduced by single mutations. These values are compared to experimentally determined values obtained by isothermal denaturation induced by urea. Some double mutants such as W36A/W101A seem to be severely unstable, have low yields on purification, exhibit an unfolded form in vitro and are unable to function in assembly of CuA. Another double mutant (W36A/F42W) has reduced stability (TM ≈ 38 oC), but is still able to bind copper in vitro and can function in vivo to assemble CuA when cells are cultured below its melting temperature. The fine balance between folded and disordered states of SCO, and its functional status are exhibited in these mutant forms.

Poster #8: Form and Location of the 'Chanzyme' TRPM7 in Proliferating or Non-Proliferating Liver Cells

Adenike Ogunrinde, Mustafa Ahmadzai and Bruce Ceredwyn Hill
Queen's University, Kingston, Ontario

Transient Receptor Potential Melastatin 7 is an integral membrane protein comprising a non-specific cation channel, a cytosolic C-terminal kinase and a functionally undefined cytosolic N-terminal domain. TRPM7 is 'ubiquitously' expressed, playing vital roles in cell survival, prolifer ation and differentiation. Proliferating cells generally exhibit larger TRPM7 currents than non-dividing, terminally differentiated cells. Channel properties are well described and are thought to be responsible for the Mg^{2+}/Ca^{2+} influx supporting cell biological functions. The channel exhibits strong rectification in the presence of extracellular or cytosolic Mg^{2+}. Kinase functionality is less well understood other than that channel properties are unaltered between wild type (WT) and kinase-inactive mutants. Recent work shows that the kinase domain can be cleaved from the channel *in vivo* [Krapivinsky et al. Cell. 157 (2014) 1061]. The ectopically expressed kinase domain localizes to the nucleoplasm and the kinase-deleted channel (ΔK) exhibits a possible length-dependent range of activities relative to WT. Here we addressed the role of the kinase domain in regulating channel activation. We confirmed the nuclear expression of ectopically expressed murine TRPM7 kinase (residues 1511-1863) and a similar localization of endogenous TRPM7 in proliferating liver cells (WIF-B) suggesting that these cells express the cleaved kinase. Comparison of immune-reactive TRPM7 with nuclear pSTAT3, pHistone3 and laminA/C supports the nucleoplasmic localization of TRPM7 kinase in WIF-B cells. Conversely, non-dividing hepatocytes are labelled in the nuclear envelop indicating that TRPM7 is mainly intact. Fractional currents at break-in in HEK293 cells are significantly lower in ΔK (0.18±0.08) compared with WT (0.47±0.12) expressors. Mean activation time constants do not differ significantly whereas maximum currents are 2.5 times lower in ΔK versus WT cells. Endogenous currents in WIF-B cells reflect a hybrid of these properties. We conclude that a complex dynamic exists between subcellular compartmentalization, proteolysis and the cellular role(s) of TRPM7.

Poster #9: High-Resolution Structure of a DNA G-Quadruplex With Cytosine Bulge

Markus Meier, Aniel Moya, Natalie Krahn, Trushar R. Patel, Matt McDougal, Evan Booy, Sean McKenna and Jörg Stetefeld
University of Manitoba, Winnipeg, Manitoba

G-quadruplexes are four-stranded nucleic acid structures acting as genetic switches in promoter regions and other regulatory sites or playing important biological functions in e.g. telomere structure and telomere replication, genetic recombination or nuclear organization. Four tracts of guanylates (RNA) or deoxyguanylates (DNA) assemble into parallel or antiparallel arrangements whereby each tract supplies one guanine to form a planar tetrad. These G-quartets are stabilized by Hoogsteen hydrogen bonding. Three or more consecutive G-quartets stack on top of each other. The stacks are stabilized by monovalent or divalent cations that occupy the central channel between the tetrads. In unimolecular G-quadruplexes, the consecutive G-tracts are interspersed by loops that can range from one nucleotide to several hundred nucleotides. The G-tracts themselves can also be interrupted by nucleotides, forming a bulge. We obtained two high resolution X-ray crystal structures with different crystal symmetries of a unique parallel DNA G-quadruplex that contains a single cytosine bulge, providing insight in how such an interrupting nucleotide can be accommodated into the compact quadruplex structure. We further provide a biophysical characterization of the G-quadruplex using analytical ultracentrifugation, dynamic light scattering and circular dichroism. The presence of bulges alters the tetraplex surface which has implications on G-quadruplex recognition by other macromolecules. A few G-quadruplex structures with bulges have been previously reported by other groups and should be expected to occur quite frequently. Since the penalty for interrupting the G-tracts appears to be small, it should be possible to functionalize G-quadruplexes by introducing artificial or modified nucleotides at such positions or use them as scaffolds for DNA origami.

Poster #10: Folding up BC200: the dynamic structure of a cytoplasmic RNA

G.Pauline Padilla-Meier, Evan Buoy, Markus Meier, Trushar Patel, Jörg Stetefeld and Sean McKenna
University of Manitoba, Winnipeg, Manitoba

BC200 (also Brain Cytoplasmic RNA 1) is a 200 nucleotide non-coding RNA that is highly enriched in synaptic neurons. Although mainly found in dendritic cells, high levels of BC200 have been detected in various cancer cell types. The function of BC200 has not been fully elucidated, however it has been shown to associate with

other RNA and proteins to form complexes in the cytoplasm. Moreover, Booy and colleagues (2016) showed that specific regions of BC200 are binding sites for an RNA quadruplex helicase (RHAU; RNA helicase associated with AU rich elements) and unwound RNA quadruplexes, suggesting a possible function for BC200 as a stabilizer of unwound quadruplex conformation. Our current work is focused on elucidating the structure of BC200 and its different truncations to gain insight into the structural features required for function. Self-association and structure of BC200 constructs were measured by dynamic light scattering, analytical ultracentrifugation, small angle x-ray scattering, with the ultimate goal of elucidating high resolution structure using x-ray crystallography.

Group B

Poster #11: The Movement of Fungal mS917 Intron Encoded Homing Endonucleases into Different Genes

Iman Bilto, TuhinGuha,Alvan Wai and Georg Hausner
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Group I introns encode mobility-facilitating proteins called homing endonucleases (HE). Most fungal mtDNA HEs belong to the LAGLIDADG (LAG) or the GIY-YIG HE families; names are based on conserved amino acid motifs. Mobility is also referred to as homing and it is initiated by the intron encoded endonuclease generating a double-stranded cut at a specific target (cleavage) site in the intron minus allele. The double-stranded break is repaired by the "host genome's" double-stranded break DNA repair system using the intron plus allele as a template for repairing the break. Among the fungi group I introns can potentially be transmitted between individuals by out-crossing and anastomosis. HEs are of great interest as they have applications in biotechnology as DNA cutting enzymes and as genome editing tools. In some species of the Ascomycota a group ID1 intron encoding a double motif LAG ORF is located within the mtDNA*rns* gene at position S917. This intron is unique as it occurs in two versions, either as a single ID1 intron or as a nested intron where the internal ORF containing ID1 intron is inserted within an external ID1 intron. Comparative sequence and phylogenetic analyses have shown that homologs of this intron/HE combination are inserted in different fungi in the *rns, rnl, cox3, nad5* and *nad6* genes. These composite elements therefore offer an opportunity to address the following questions: 1. are these HEs active and what are their target sites; and 2. how did members of this family of HEGs invade so many different genes (i.e. adapt to recognize and cut different target sites)? This is also an opportunity to study how HEs and their amino acid sequences evolve in order to recognized new target sequences; something that is frequently attempted in laboratories in order to engineer HEs with new target site specificities. The first phase of this work involved data mining for additional members of the mS917 family of intron/HEG combination (seven sequences have been identified so far), and the overexpression of codon optimized versions of these HE ORFs in *E. coli* in order to characterize the HE proteins and their potential cleavage sites.

Poster #12: Modeling Mitochondrial Complex IV Bioenergetics

Chris Cadonic (1, 2); Ella Thomson (2);Wanda Snow (2); Subir K Roy Chowdhury (2); Danielle McAllister (2); Paul Fernyhough (1, 2); Jason Fiege (1); Stephanie Portet (1); Benedict Albensi (1,2)
(1)University of Manitoba, Winnipeg, Manitoba (2) Division of Neurodegenerative Disorders, St. Boniface Hospital Research, Winnipeg, Manitoba

In this project, a computational model of mitochondrial function, with a focus on modeling complex IV of the mitochondrial electron transport chain, has been developed from oxygen concentration data, which represents mitochondrial bioenergetic activity. This data was acquired using the Seahorse XF24 Analyzer (Seahorse Biosciences) and the Oroboros Oxygraph o2k analyzer (Oroboros Instruments). Measurements in the XF24 Analyzer were conducted on embryonic-cultured cortical neurons from CD1 mice, while measurements in the Oxygraph o2k were conducted on isolated mitochondrial sample from CD1 mice. To modulate the activity of the mitochondria, specific dysfunctions were introduced by injecting the inhibiting reagents oligomycin, rotenone, and *antimycin A*, and the uncoupling reagent *carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP)*, during experimental measurements. Based on the biological mechanism of mitochondrial activity, a deterministic model was developed using biochemical kinetic modeling techniques. This model was calibrated using the optimization genetic algorithm Ferret by fitting real-time oxygen data. Model parameters were also analyzed using sensitivity analysis. The model was coded in MATLAB R2015a (Mathworks) along with a graphical user interface, allowing for *in silico* simulation of mitochondrial activity. Thus the model can be used as a supplemental research tool for investigating mitochondrial bioenergetics, even without knowledge of MATLAB scripting. With a working model that has been fit to biological data, the future aim is to now apply this model to disease-state datasets and expand the model to describe the entire electron transport chain.

Poster #13: Distribution of C_{60} fullerenes inside asymmetric lipid bicelles

Y. Cheriavskyi (1), S. Yesylevskyy (2)
(1)University of Calgary, Calgary, Canada (2) Institute of Physics, National Academy of Science of Ukraine, Kiev, Ukraine

Coarse-grained molecular dynamics simulations were used to investigate role of local curvature of bilayer in the process of penetration and distribution of fullerenes inside curved asymmetric DOPC/DOPS bicelle. To distinguish effects guided by asymmetricity of lipid composition in the different leaflets of the membrane, we compared our data with data collected from the simulation of symmetric DOPC bicelle. We analyzed dynamics of clusterization and evolution of the fullerene aggregates inside the bicelle. Comparison of the mean and Gaussian curvature of

DOPC/DOPS bicelle with and without fullerenes shows that fullerenes do not significantly change both mean and Gaussian curvatures of the membrane, but increase ordering of the lipid tails which are in direct contact with fullerenes. Also we studied influence of the mean and Gaussian curvatures on the distribution of fullerenes inside the bicelle. Our data shows that distribution of fullerenes in asymmetric DOPC/DOPS bicelle only slightly influenced by local mean curvature.

Poster #14: The Regulation of Defence Responses in Canola in Response to the Biocontrol Agent Pseudomonas Chlororaphis PA23 Revealed by RNA-seq

Kelly Duke, Mark Belmonte, Dilantha Fernando and Teresa de Kievit
University of Manitoba, Winnipeg, Manitoba

The necrotrophic fungus Sclerotinia sclerotiorum is a persistent pathogen of canola because of its wide host range and persistence in the environment. Pseudomonas chlororaphis PA23 is an effective fungicidal agent which prevents S.sclerotiorum growth in both in vitro and greenhouse assays. Compounds excreted by PA23 which contribute to its efficacy against S.sclerotiorum include phenazines, pyrrolnitrin, proteases, lipases and siderophores. However, a biocontrol system consists of not only antibiosis between the pathogen and biocontrol agent, but also interactions between these organisms and the host organism. Many studies have shown evidence of nonpathogenic organisms eliciting a systemic response in plants which boosts their defenses to react stronger and more quickly to future threats. This "priming" of plant defenses involves short- and long-term cellular changes. While the mechanisms behind PA23's antifungal interaction with the pathogen in this biocontrol system are well understood, it is not known whether PA23 has any effect on its canola host. "Priming" responses can involve many complex changes in the host plant, including but not limited to the synthesis of reactive oxygen species and the induction systemic defenses such as induced systemic resistance (ISR) and systemic acquired resistance (SAR). These systemic responses can involve the production of many different signaling molecules. The objective of this study is to identify PA23's role in mediating defence responses to S. sclerotiorum through gene expression changes and physical manifestations of these changes. The efficacy of PA23 as a protective agent against S.sclerotiorum infection was first tested, and biocontrol treatment prior to pathogen exposure had significantly lower levels of lesion formations from potentially infectious petals (4% versus 58%, respectively). RNA sequencing revealed that treatment with PA23 prior to pathogen exposure functioned mainly in modulating defence responses, as shown by comparing differentially expressed gene counts among treatments. Staining of leaf tissue to reveal the presence of reactive oxygen species showed a similar trend. While PA23 alone causing little ROS production, significant reduction of these compounds compared to the pathogen only treatment was observed when leaves were treated with PA23 prior to exposure. PA23 also caused changes in plant chloroplasts, which contained more thylakoids and plastoglobules than the water control or pathogen-treated leaves. These PA23-treated leaves also had higher levels of chlorophyll b. We therefore hypothesize that PA23 mainly functions to modulate plant response to S. sclerotiorum infection, but also induces changes in leaf chloroplasts.

POSTER #15: Biophysical Characterization of Interaction Between dsRNA-Binding Protein Kinase and its Inhibitor Adenovirus Virus-Associated (VA) RNA I

Edis Dzananovic (1), Trushar R. Patel (2), Astha (3), Grzegorz Chojnowski (3), Janusz M. Bujnicki (3,4), Sean A. McKenna (1)

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The mammalian innate immune system provides a first line of defence against microbial pathogens and also serves to activate an antigen-specific acquired immune program. Key components of innate immunity are the interferons (IFNs), a family of related cytokines. The IFNs exert their effects through the stimulation of numerous genes, one of which is the double-stranded RNA-dependent Ser/Thr protein kinase (PKR), a key antiviral protein found in human cells. After binding to viral dsRNA, PKR is autophosphorylated and in turn phosphorylates the alpha-subunit of eukaryotic translation initiation factor 2, causing attenuation of cellular and viral protein synthesis. Certain viruses specifically transcribe dsRNA that bind to PKR and thereby prevent its autophosphorylation. We are investigating one such inhibitor, the adenovirus virus-associated (VA) RNA I. VA_I RNA contains 3 stem-loop regions; apical PKR-biding stem-loop, central inhibitory stem-loop, and terminal region that is shown to not affect binding or inhibition of PKR. We are interested in studying specific features of inhibitors that enables them to bind to PKR and inhibit its function. We have created mutant versions of VAI RNA and tested whether these mutations impact binding and/or inhibition of PKR. We have also taken advantage of in-house small angle scattering instrumentation to obtain low-resolution structural data on PKR-dsRNA complexes. SAXS envelopes and raw data were then used as constraints for computational tertiary RNA structure prediction. Taken together, we propose that a specific pseudoknot RNA fold is required for efficient inhibition of PKR.

POSTER #16: Estimation of Intracellular Ion Concentrations and Cytoplasm Conductivity for CHO cells

A. Fazelkhah, G. E. Bridges, D. J. Thomson
University of Manitoba, Winnipeg, Manitoba

Cells are dynamic electronic materials that change electronic state in response to environmental pressures. There have been many demonstrations that dielectric changes occur in cells that are coincident with important

physiological changes such as during programmed cell death (apoptosis). The change in cell physiological state is accompanied with the change in cell dielectric properties. In our group, we use DEP cytometer to detect apoptosis in Chinese Hamster Ovary (CHO) cells through the change in conductivity due to change in ion concentrations and followed their transition from a viable to non-viable state. There are few existing models which can link physiological state of the cells to its dielectric properties. Therefore, to have a better understanding of experimental data and its relationship with physiological state of the cell, in this work we used theoretical calculations, combined with existing measured cellular parameters to estimate intracellular ion concentrations for CHO cells and used these values to estimate the conductivity of the cytoplasm. The estimated cytoplasm conductivity based on the applied theory is in good agreement with the experimental values measured using a dieletrophoretic (DEP) cytometer. The estimated cytoplasm conductivity changes from 0.419 S/m to 0.087-0.13 S/m range as the viable cells undergo apoptosis which follows our experimental results. The measured cytoplasm conductivity for viable and non-viable CHO cells is about 0.42 S/m and 0.09 S/m, respectively.

POSTER #17: Investigation of the C-Terminal Region of VDAC

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The voltage dependent anion-selective channel (VDAC) is a pore forming protein that allows for the transfer of ions and small molecules, most notably ATP/ADP, across the mitochondrial outer membrane. VDAC forms a transmembrane anti-parallel β-barrel consisting of 19 β-strands. This is unusual as all other known transmembrane β-barrel proteins contain an even number of strands. Here we examine the VDAC from the fungus Neurospora crassa to determine the function of the C-Terminal (19th) β strand. Both full length *N. crassa* VDAC (wt-VDAC) as well as a C-terminally truncated VDAC (ΔC VDAC), which lacks the 19th β-strand, were produced in *E. coli* where they formed inclusion bodies. The VDACs were then purified and refolded in a detergent solution. Through the use of analytical ultra-centrifugation and dynamic light scattering, full-length VDAC was found to be monomeric (MW 30 kDa) in detergent solution and monodisperse. However ΔC-VDAC was found to form a mixture of dimers and monomers in solution. This suggests a role for 19th β-strand in inhibiting the dimerization of VDAC molecules.

Poster #18: Beyond the Lateral Gate - an Expanded Role for TM Helix 2 and 5 in the Rhomboid Catalytic Cycle

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The rhomboid family of intramembrane serine proteases has a unique ability to catalyze proteolysis below the surface of the cell membrane that is required in a diverse array of biological processes, including parasitic host cell invasion and growth factor signalling. High-resolution structures of the *E. coli* GlpG rhomboid indicate that the active site is sequestered away from the membrane environment by two transmembrane α-helices (TM2, TM5). It has been proposed that these helices act as a lateral gate for substrate entry, potentially representing a key control point for proteolysis. Here, we assessed the effect of several point mutations designed to destabilize the gate and increase gate dynamics. In general, these alterations enhanced the activity of the catalytic transmembrane domain core of GlpG (TMD) against a model transmembrane substrate in dodecylphosphocholine, in line with previous observations made using full-length GlpG in both detergents and phospholipid membranes. However, when the activity of these samples was tested against a water-soluble model substrate, this enhancement was not retained for some of these mutants, in one case actually reducing activity relative to wild-type TMD. This suggests that, in addition to substrate gating, proposed gate residues are also required for key interactions that stabilize the catalytic core structure. In agreement with this hypothesis, solution-state NMR and circular dichroism studies on these loss-of-function mutants suggest a disruption to both the structure and dynamics of rhomboid protease beyond the gate region. Taken together, these results describe an expanded role for the gate region and substrate binding in proteolysis.

Poster #19: Interaction of the Biocontrol Agent Pseudomonas Chlororaphis Strain PA23 with the Grazing Predator A. Castellanii

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The gram negative bacterium *Pseudomonas chlororaphis* PA23 is a biocontrol agent that is able to protect canola against the pathogenic fungus *Sclerotinia Sclerotiorum*. A number of metabolites contribute to fungal antagonism including pyrrolnitrin (PRN), phenazine (PHZ), hydrogen cyanide (HCN) and degradative enzymes. Beyond pathogen suppression, the success of a biocontrol agent is dependent upon its ability to persist in the environment and to resist the threat of grazing predators, including protozoa. The focus of the current study was to investigate whether PA23 is able to resist predation by the protozoan predator *Acanthamoeba castellanii* and to define the role of antifungal compounds in the bacterial-protozoan interaction. This interaction was explored using a combination of co-cultivation assays, viable counts, and laser confocal microscopy. Our findings revealed that the wild type PA23 and two mutants with elevated antifungal activity, namely *rpoS* and *phz* mutants, were able to resist and kill the predator over the course of experiment. In comparison, *A. castellanii* propagated on a *gacS* mutant, and two quorum-sensing deficient strains, all three of which are devoid of antifungal activity, were not killed and

actually increased in number by 3 fold. We conclude that PA23 can effectively avoid predation by *A. castellanii* through the production of biocontrol compounds, which bodes well for the persistence of this biocontrol bacterium in the environment.

Poster #20: Simulating Crystallization of Amyloid-Beta Fragments In Silico Using An Atomic-Contact Weighted Force Field

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Through genetic mutation or the effects of aging, cellular proteins and peptides can aggregate into insoluble fibril plaques leading to a host of ailments, including Alzheimer's Disease (AD) and its hallmark amyloid-beta (Aβ) peptide. Recent experiments by the Tycko group [1,2] have demonstrated Aβ fibril polymorphism in the AD Iowa mutant and between fibrils grown from post-mortem autopsies of patients with AD and Lewy Body Disease (LBD). Unfortunately, current experimental techniques can only show us the end result of complex association processes, and can do very little to provide insight into the mechanisms contributing to this polymorphism; requiring theoretical modelling to fill the gap. Simulating full length (Aβ) is computationally expensive for large systems and few experimental polymorphic structures are known, requiring an alternative means to study polymorphism. Luckily, recent work from the Eisenberg group [3-4] has observed polymorphism within a variety of microcrystals generated from relatively short Aβ fragments, and demonstrated that these microcrystals are can be used as representative models of full length fibrils, making them ideal candidates for modelling studies. In our poster, we present an all atom discontinuous molecular dynamics (DMD) model which has demonstrated the ability to aggregate peptides of one such microcrystal from the random monomer state into stable crystal bilayers. This is a knowledge-based force field, based upon the likelihood of inter-peptide atomic contacts from the Aβ fragment microcrystal's experimental structure. To our knowledge this is one of the first studies exploring the polymorphic association of these microcrystals in this manner. The simulations generated crystal bilayers with a 4.8Å intra-β-sheet peptide spacing, characteristic of the original crystal structure and Aβ plaques. A clear phase transition is observed between solvated and aggregated structures, and numerous oligomers of varying size and conformations are observed before assembling into a more stable bilayer structure. The results presented here are the first step in the development of a more general polymorphic force field for Aβ which will provide knowledge about the nature of polymorphism and the oligomeric states of Aβ fragments.

Poster #21: "Controlling a DNA Chopper": Group II Introns as Attenuators for Homing Endonuclease Expression

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A nested intron (twintron) interrupts the mitochondrial DNA (mtDNA) small ribosomal subunit gene (*rns*) of the fungus *Chaetomium thermophilum* (DSM 1495). This nested intron is composed of an external group I intron encoding a potential open reading frame (ORF) and an internal group II A intron that interrupts the ORF. The objective of this study is to examine if splicing of the internal group II intron allows for the expression of a functional homing endonuclease (HEase). The study also examined if group II introns could serve as potential "switches", where the expression of the HEase (*in vivo*) within *Escherichia coli* can be regulated by manipulating the splicing efficiency of the internal group II intron by varying the concentration of magnesium chloride ($MgCl_2$) in the growth media. A codon optimized (intronless) version of the HEase sequence (histidine tagged) was overexpressed in E.coli and purified with a nickel affinity column. In vitro cleavage assays showed that the HEase cleaves a substrate sequence that essentially is a "twintron-less" version of the C. thermophilum rns gene. Two expression constructs were designed where the HEase ORF was interrupted either by a group II A or a group II B introns. Upon induction and expression of the HEase ORF in *E. coli*, RT-PCR followed by sequence analysis of the cDNA showed that MgCl2 stimulated splicing of the internal group II introns, hence allowed for the expression of the HEase. In addition, an in vivo endonuclease assay was established where two compatible plasmids (HEase construct and substrate construct) were maintained in *E. coli* BL21 (λDE3) based on antibiotic selection strategy. Cells grown in the presence of external $MgCl_2$ (5 mM) resulted in efficient splicing of the internal group II introns and religation of the ORF exons. The expressed HEase cleaved the target site in the substrate plasmid resulting in the loss of the resistance marker. Cells grown in the absence of $MgCl_2$ failed to splice out the internal group II thus yielding no functional HEase thereby resulting in the maintenance of the substrate plasmid hence the survival of colonies on the selection plates. The addition of $CoCl_2$ to the media however discouraged the expression of the HEase, presumable $CoCl_2$ blocked the import of $MgCl_2$ and thus acted as an antagonist to splicing. These results will be used to develop and potentially optimize an "on/off switch" which can be applied to regulating HEase expression within *E. coli*. This work suggests that group II intron sequence can be added to other HEase ORFs and this will lead to the development of HEases that can be controlled with regards to timing of DNA cutting activity thus potentially minimizing their toxicity to cells and allowing for better precision genome engineering.

Poster #22: Exploring the Dynamic Structure and Function of E. coli Glycerol Facilitator

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The transport of molecules across cell membranes is important in many areas of biology; there is a particular interest in drug development and design, as about 60% of all drug targets are integral membrane proteins. Membrane proteins (MP) can act as channels for passive transport or use energy in order to actively transport molecules across the membrane. Understanding the atomic structure and dynamics of integral membrane transport proteins may yield insights into the mechanisms by which these molecules control important cell processes. Glycerol facilitator (GF) is an *E. coli* inner membrane protein that allows for passive diffusion of glycerol across the membrane. GF is a helix bundle protein that forms a homotetramer in the membrane (1) however, not much is known about its dynamics except for short timescale molecular dynamics simulations (2) and a hydrogen-deuterium exchange study (3). We present a system for the inexpensive preparation of large amounts of uniform and site-specific 15N-isotope-labelled GF for dynamics measurements by NMR spectroscopy based on the work of Studier (5). The secondary structure and oligomeric state of GF is monitored by circular dichroism spectropolarimetry, dynamic light scattering, size exclusion chromatography multi-angle light scattering and sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. NMR spectra of the monomeric protein dissolved in SDS will be presented and provide insights into a partially unfolded membrane protein.

Poster #23: Probing the Conformational Dynamics of the Active Site of an OTU Deubiquitinase Enzyme

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Enzymes are fundamental to cell function. Despite this, exactly how enzymes work remains unknown. Enzymes exist as 3D folded structures determined by their amino acid sequence. These structures are necessary for function. Solving these structures using methods such as X-ray crystallography provides great insight into protein function at the molecular level. However, these methods only provide structural detail about a single stable conformation. In reality, enzymes change their 3D conformation frequently during the course of a reaction, thus the structure of a single conformation will provide incomplete information. To completely understand how enzymes function, we must understand their dynamics – their changes in conformation over time. To study enzyme dynamics, we have used the catalytic domain of Otu1, a deubiquitinase from yeast. Deubiquitinases are enzymes that catalyze the breakdown of ubiquitin, and play an important role in regulating cell functions including immune response and signal transduction [1]. Our goal is to completely understand the conformational changes that occur during the Otu1 reaction cycle. First we must examine the dynamics of the free enzyme. The crystal structure of Otu1 contains a loop of amino acids that covers the active site where catalysis takes place [2]. We hypothesize this loop must open up to allow the active site to access substrate. In our model, Otu1 exists in equilibrium between an open-loop conformation and a closed-loop conformation. We have employed a series of ligand-binding experiments using stopped-flow fluorescence spectroscopy to examine the dynamics of the free enzyme, and have determined a loop opening rate of about 100 s^{-1}.

Poster #24: The Structural and Enzymatic Characterization of Purified Human Diacylglycerol Kinase Epsilon

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We have previously reported the first purification and characterization of the arachidonoyl-specific diacylglycerol kinase epsilon (DGKε). We have purified human DGKε and a truncated form lacking the first 40 residues (DGKεΔ40) to near homogeneity using Nickel-affinity chromatography. Enzyme activity measurements showed that both purified constructs retained their substrate acyl chain specificity and have a specific activity comparable to N-terminally FLAG epitope tagged forms of these proteins expressed in Cos-7 cells. We observed that purified DGKε is highly unstable and enzymatic activity is rapidly lost even upon storage at 4°C. Furthermore, freeze thawing is particularly damaging to the enzymatic activity of DGKε and consequently enzyme stored at -80°C is not viable for studies of enzymatic activity. We have previously shown that storage in 50% glycerol stabilizes DGKε significantly at 4°C, -80°C and during freeze thawing. We have since modified the purification of this unstable enzyme to include glycerol as well as a reducing agent leading to a dramatic improvement of enzymatic activity and structural stability. Secondary structure analyses using circular dichroism reveal that DGKε contains significant amounts of both α-helical and β-structure, in agreement with predictive algorithms. Circular dichroism reveals a gradual and irreversible loss of secondary structure on heating to 100°C with only slight stabilization upon adding dioleoyl-phosphatidylcholine (DOPC). In contrast, the addition of glycerol dramatically improves the thermal stability of DGKε and DGKεΔ40. In the presence of 50% glycerol, DGKε and DGKεΔ40 lose ~40% of their secondary structure when heated to 100°C. Addition of glycerol makes the thermal unfolding ~80% reversible, in contrast to the irreversibility of the transition in the absence of glycerol. Interestingly, atomic force microscopy shows that DGKεΔ40 forms smaller and fewer aggregates on mica and on supported lipid bilayers (SLBs) of DOPC compared to DGKε, despite both forms displaying similar enzymatic properties. Aggregation on SLBs can be reduced by the addition of the preferred DAG substrate; 1-stearoyl-2-arachidonoyl glycerol. In contrast, less preferred substrates such as 1,2-dioleoyl glycerol and 1,2-dilinoleoyl glycerol had less effect. This study provides the first successful purification of DGKε and a partial characterization of its enzymatic and

conformational properties. This purification procedure and detailed characterization of DGKε has facilitated initial studies on incorporating the active enzyme into liposomes of various lipid compositions and will soon lead to the development of a liposome based activity assay.

Poster #25: The Transition of Disorder to Order of HMGA2a with Replication Fork DNA

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Stem cell research is becoming an attractive field for treatment of aging diseases such as Alzheimers and diabetes or even cancer. High-motility group AT-hook 2 (HMGA2) is a human protein that has been found to be highly expressed in embryonic stem cells due to its role in regulation of gene expression and cell differentiation. If this protein becomes overexpressed in adult cells, tumours begin to form due to uncontrolled cell growth and the ability of HGMA2 to stabilize stalled replication forks. The presence of the three AT hooks on HMGA2 are not only for binding to DNA, but this protein exhibits properties of base excision repair which allows cells to become resistant to chemotherapy treatments invoked to damage DNA. The goal of this research is to understand the interaction that HMGA2 has with DNA, specifically replication forks. Biophysical studies including dynamic light scattering, circular dichroism, analytical ultra centrifugation, nuclear magnetic resonance, and small angle x-ray scattering were used to structurally characterize recombinantly produced HMGA2a alone and in complex with replication fork DNA. These results show the unstructured nature of HMGA2a which allows it to bind various forms of DNA at which point it takes on a more structured nature.

Poster #26: Coarse-Grained Model of Fragments of Amyloid-Beta *Aβ* peptides

Apichart Linhananta and Robert Girardin

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Amyloid-beta (*Aβ*) peptides are 36 to 43 amino acid residues, implicated by the amyloid cascade hypothesis as one of the cause of Alzheimer's disease (AD). In the brain, *Aβ* forms small peptide aggregates, called oligomers, leading to *β*- sheet fibrils that, with time, forms the 3D amyloid plaque that is the hallmark of AD. The structure of fibrils consists of parallel *β* and/or anti-parallel *β*- sheets wound in a wide array of complex three-dimensional structures. Many researchers believe that it is the structural polymorphism of *Aβ* fibrils is the key to understanding the origin of AD. Unfortunately, the structures of the fibrils are very difficult to resolve. An alternative approach is by Eisenberg et al., who examine 5 to 8 amino-acid segments of the 40-residue $A\beta_{1\text{-}40}$ and 42-residue $A\beta_{1\text{-}42}$, to obtain 8 distinct classes of 3D structures. These fragments of *Aβ* display similar polymorphism as observed in full-length *Aβ*. This paper presents our effort in building a coarse-grained model of the formation of micro-crystal fibril of fragments of *Aβ*. For computational efficiency, an amino acid is represented as a spherical bead, located at the center of its C_α position. This allows the study of systems of up to 1000 peptides. In the spirit of *Gō* models, the intra-peptide dihedral and van der Waals interactions, as well as inter-peptide interactions are biased to the experimental crystal structures. Two C_α beads on different peptides can also interact by a Mercedes-Benz-type hydrogen bond, used by Yap, Fawzi and Head-Gordon, in their coarse-grained model of proteins. The hydrogen bonds stabilize the *β*-sheet structures of the fibrils. We will present the results of MD simulation of the eight amino acid residues $A\beta_{35\text{-}42}$, which can adopt both parallel and anti-parallel *β*- sheet conformations.

Poster #27: Holographic Optical Tweezers for Microrheology Measurements

Victoria Loosemore and Nancy R. Forde

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Collagen, the most abundant protein in the human body, assembles into an extra-cellular fibrillar gel, which has both viscous and elastic properties. We can determine the properties of collagen gels by using optical tweezers to hold a micron-sized bead within the sample. Measurement of the bead's thermally induced motion enables the determination of the frequency-dependent viscoelasticity. Rather than just probing response at a single location, holographic optical tweezers create multiple, independent traps, permitting simultaneous tracking of multiple embedded beads and characterization of their correlated motion. By using this technique in a collagen gel, we will be able determine local and through-space viscoelastic properties, which vary at different locations during its formation. Implications of this research lie in the fields of health and biomaterials. The initial aim of our work is to devise and validate protocols for using holographic optical tweezers to measure local and through-space viscoelasticity. Rather than using laser deflection to track particle motion, we are implementing the use of a high-speed camera and image analysis to track the simultaneous motion of multiple beads. This approach provides nm-scale resolution of particle position at sampling rates of 2.5 kHz. We discuss differences arising from the two position detection techniques, such as blur and a lower sampling frequency of the camera. We show how, upon proper consideration, these return identical results for optical trap stiffness calibration. This work paves the way towards the use of holographic optical traps for microrheology assays.

Poster #28: The Effects of Antitumor Lipid Drugs on the Fuidity of Biomimetic Liposomes Composed of Phosphatidylcholine, Sphingomyelin and Cholesterol

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Synthetic anti-tumour lipids are a class of drugs derived from lysophosphatidylcholine that are both cytotoxic and cytostatic and exert these effects preferentially in tumour cells. While lysophosphatidylcholine is metabolized in vivo, structural differences in the drugs confer enzymatic resistance, increasing stability. While the mode of action has not been fully elucidated, it is believed that these drugs accumulate preferentially in rigid lipid domains leading to breakdown of these membrane structures. These domains are important sites for signal transduction and membrane trafficking and their disruption impacts cellular functions at various levels. Lipids traditionally associated with these domains include sphingolipids and cholesterol, as opposed to phosphatidylcholine predominant found in the bulk membrane. The preference of these drugs for cancer cells is not understood. In this study biomimetic liposomes comprised of varying amounts of phosphatidylcholine, sphingomyelin and cholesterol, were generated which included the fluorescent dye Laurdan. This dye is sensitive to solvent polarity, and exposure to water will red shift the emission spectra. Spectral analysis is used to determine changes in membrane fluidity. Fluorescence spectra were obtained both without drug, and after successive titrations of either lysophosphatidylcholine, or one of the synthetic anticancer drugs edelfosine, miltefosine or perifosine. Results have shown that with varying cholesterol levels in the liposomes all drugs have comparable effects, but show a much more dramatic fluidization of membranes at lower sterol. In contrast the drugs have widely differing effects in liposomes containing sphingomyelin, and the drug impact heavily depended on sphingomyelin levels.

Poster #29: Understanding the Assembly of Tripartite Resistance-Nodulation-Division (RND) Efflux Pumps in Gram-negative Bacteria

Manu Singh and Ayush Kumar
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Multidrug resistance efflux pumps Resistance-Nodulation-Division (RND) family are the major determinant of intrinsic antibiotic resistance in Gram-negative bacteria. RND pumps form a tripartite channel across the Gram-negative cell envelope facilitating the efflux of ligands (e.g. antimicrobials) into the external medium. This tripartite complex is composed of outer-membrane protein (OMP; present in the outer membrane), membrane-fusion protein (MFP; present in the periplasm), and RND protein (present in the inner membrane). The mechanisms that govern the interaction of the three components of RND complex are not very well known. Particularly, it is not clear if the formation of complex is substrate-induced or constitutive. Using novel genetic tools and Pseudomonas aeruginosa as a model, we are trying to elucidate these mechanisms. In this study, we characterize the interactions of MexJK and MexXY RND pumps with their respective OMPs. Both these pumps have been shown to interact with two different OMPs and therefore serve as an excellent model to study whether the assembly of the complex is substrate-driven or constitutive. We have created artificial inducible operons that encode different combinations of each member of the tripartite complex (*mexJK-oprM, mexJK-opmH, mexXY-oprM*, and *mexXY-oprA*). These operons have been inserted in single copy in an efflux-sensitized mutant of P. aeruginosa. Quantitative RT-PCR, Western blot, and antimicrobial susceptibility assays were used to confirm the expression of genes as well the functionality of the complex. Pull down assays in the absence or presence of substrates are being carried out to elucidate if the assembly of the complex is substrate-induced or constitutive. Knowledge generated from this work will help in therapeutic efforts directed towards the inhibition of the assembly of the RND complex in Gram-negative bacteria.

Poster #30: Structural Insight into Netrin-4 Mediated Basement Membrane Assembly

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Composed of five members in humans, the netrin family of proteins exhibit diverse biological functions and a wide range of protein interacting partners. Unlike the other netrins, Netrin-4 is absent in the central and peripheral nervous systems; instead, it is present in the basement membrane of a number of other tissues. Netrin-4 has been shown to prevent the association of the Laminin trimer that is essential for proper basement membrane assembly. We demonstrate that Netrin-4 knockout mice have altered morphology in the basement membrane of the lung and kidneys, and lowered resistance to lung tumour metastasis. In addition, using Xray Crystallography, Small Angle Xray Scattering (SAXS), Dynamic Light Scattering (DLS), and Site Directed Mutagenesis, we have characterized the interaction of Netrin-4 with its binding partner, laminin-γ1.

Poster #31: Validating Putative Quadruplex Binding RNA Helicase Proteins

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Four stranded structures of oligonucleotides (quadruplex) are abundant in the human genome and have been shown to be dynamically regulated during cell cycle progression. Accumulating evidence suggests that their dysregulation can alter gene expression and affect genetic and epigenetic stability, resulting in various disease states. Multiple proteins have been shown to unwind DNA quadruplex but relatively few have been reported

to possess RNA quadruplex unwinding activity. We have performed pull-down assays with biotinylated RNA quadruplex in HEK293T cell lysates to identify potential RNA quadruplex binding proteins by mass spectrometry. This experiment identified several known quadruplex binding proteins, including the well characterized RNA quadruplex resolving enzyme DHX36, and many more proteins that have not yet been shown to interact with quadruplex. From this list of potential quadruplex binding proteins we selected RNA helicase proteins which we could purify recombinantly from HEK293T cells and further validated their binding affinity and selectivity for quadruplex using electrophoretic mobility shift assays (EMSA) and microscale thermophoresis (MST). We then created truncation mutants to refine the interaction site to a size amenable for future structural studies. Finally, we performed quadruplex unwinding assays using two fragments (quadruplex single stranded trap) of the human telomerase RNA which have previously been shown to toggle between quadruplex and the active P1 duplex forms in the presence of DHX36 and ATP. Together these assays have demonstrated the potential of a new RNA helicase protein to interact with and modify the structure of a physiologically relevant RNA quadruplex.

Poster #32: The Role of Tafazzin and MLCL AT-1 in Mitochondrial Function

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Cardiolipin (CL) is a major mitochondrial polyglycerophospholipid comprised of a glycerol backbone and four acyl chains. In the inner mitochondrial membrane, where the majority of CL is found, it plays a major role in regulating mitochondrial bioenergetics. Tafazzin (TAZ) is a transacylase that transfers fatty acids between phospholipids and lysophospholipids and is a key enzyme required for CL metabolism. Barth Syndrome (BTHS) is a rare X-linked genetic disease caused by impaired TAZ production resulting in mitochondrial dysfunction that leads to patients exhibiting symptoms such as cardiomyopathies and neutropenia. Monolysocardiolipin Acyltransferase-1 (MLCL AT-1) is a mitochondrial enzyme that has been shown to utilize acyl-Coenzyme A as a substrate to incorporate fatty acids onto CL. However, the precise role of MLCL AT-1 in CL metabolism is currently unknown. Our main objectives for this project were to 1. Determine if a relationship exists between TAZ and MLCL AT-1 expression and 2. Determine if MLCL AT-1 expression in BTHS cells leads to improvement in mitochondrial function. In BTHS lymphoblasts, MLCL AT-1 mRNA expression was significantly reduced compared to healthy age-matched control lymphoblasts. Knockdown of TAZ in healthy lymphoblasts resulted in an increase in MLCL AT-1 mRNA expression compared to controls. Expression of MLCL AT-1 in BTHS resulted in various improvements in mitochondrial function including reduced superoxide production. The results indicate that expression of MLCL AT-1 has the potential to improve the mitochondrial phenotype observed in BTHS cells. (Supported by HSFC, Barth Syndrome Foundation of Canada, Research Manitoba and Children's Hospital Research Institute of Manitoba).

Poster #33: Investigating Membrane Damage by Fragaceatoxin C on Model Membranes

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Actinoporins are produced by sea anemones.They are excellent models of eukaryotic α pore forming toxins (PFTs). To date, two models have been proposed to explain the mechanism of pore-formation of actinoporins: the toroidal pore and the h ybrid pore. Both mechanisms propose several steps in common in the pore formation process: binding of monomeric protein to the membrane, followed by oligomerization and insertion of the N-terminal α-helix into the lipid bilayer. One of the main differences between be toroidal pore model suggests the monomeric structure as a key structural element in the formation of fragaceatoxin C pores in membranes; while the hybrid pore model proposes a prepore structure formed by addition of dimeric units. Here we focus on the relevance of the dimeric intermediate as a consensus point between both models. We performed atomistic molecular dynamics simulations to determine whether the first thirty N-terminal residues are able to detach from the core of the protein in its monomeric and dimeric forms. These results would suggest the mechanism of N-terminal insertion in the membrane.

Poster #34: Ion Specificity and pH-Dependence Determinants in the Vc-NhaP2 Cation-Proton Antiporter from Vibrio cholerae

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The alanine scanning mutagenesis was carried out on a set of conserved amino acids that are presumably involved in cation binding/translocation by the K+(Na+)/H+ antiporter Vc-NhaP2 from Vibrio cholerae. Residues L154, E155, I156, E157, S158, G158, N161, D162, D273, L287, L289, R315, G341, R343, G344, S376 and T383 were substituted by alanine, and the activity of mutant variants of Vc-NhaP2 was assayed in everted sub-bacterial vesicles. Obtained mutations caused 3 major types of effect: (a) Diminished antiport activity, (b) Change in the pH profile of activity, and (c) Substrate selectivity change. Of note, the G159A have showed dramatic changes in substrate specificity, "unlocking" the ability of Vc-NhaP2 to exchange Li+ for H+. In addition, the G159A variant of Vc-NhaP2 had Na+ but not K+ as a preferred substrate alkali cation, while the wild type antiporter mediated predominantly K+/H+ exchange with concomitant Na+/H+ antiport activity. To the best of our knowledge, a

single point mutation with such drastic effects has never been reported before in any cation-proton exchanger. Another noteworthy mutation was E155A. In this case, the pH regulation of activity was totally lost, resulting in nearly equal K+/H+ and Na+/H+ antiport activities over the wide pH range (6.5 to 9.5). The L289A substitution converted Vc-NhaP2 into an exclusive K+/H+ antiporter whose activity was gradually increasing from pH 7.0 to 9.5. The obtained results support the "ligand shading" hypothesis suggested to explain peculiar ion selectivity in NhaP-type antiporters.

Poster #35: The Anaerobic Regulator ANR Plays an Essential Role in Pseudomonas Chlororaphis Strain PA23 Biocontrol

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Pseudomonas chlororaphis strain PA23 is able to protect canola from sclerotinia stem rot caused by the phytopathogen *Sclerotinia sclerotiorum* in both green house and field studies. A number of biocontrol compounds including antibiotics and degradative enzymes are responsible for fungal antagonism by PA23. Multiple regulatory elements oversee production of these secondary metabolites, including the Gac-Rsm regulatory cascade, the PhzI/PhzR QS system, and the stationary phase sigma factor RpoS. In the present study, we characterized the role of the anaerobic regulator ANR (anaerobic regulator of arginine deiminase and nitrate reductase) in expression of PA23 metabolites. An *anr* mutant was generated that showed a complete loss of fungal antagonism, establishing that ANR is essential for PA23 biocontrol. PA23 produces antibiotics, such as pyrrolnitrin (PRN) and phenazines (PHZs) that contribute to biocontrol and we discovered that both antibiotics were markedly reduced in an *anr* mutant. In PA23, both PHZ and PRN are under quorum sensing (QS) control; thus, we were interested in determining whether the absence of *anr* affects QS. Qualitative autoinducer (AI) assay for the Δ*anr* strain showed a lack of QS signal production. In addition, we found that an *anr* mutant exhibited a complete loss of extracellular protease activity and produced very little HCN compared to the wild type. The production of PRN, PHZ, HCN and AI molecules as well as AF and protease activity were restored to that of the wild type when *anr* was provided in trans. qRT-PCR analysis was conducted to compare expression of *phzA, prnA, hcnA, phzI, phzR, rpoS* and *anr* in PA23 versus the Δ*anr* strain. In the *anr* mutant, all of the aforementioned genes showed reduced expression indicating that ANR positively regulates these biosynthetic and regulatory genes in PA23, consistent with our phenotypic assays. Additionally we found that ANR and QS are subject to cross-regulation, with ANR positively regulating phzI/phzR and PhzR negatively regulating *anr*. Moreover, we discovered that expressing *anr* in trans partially complements the QS-deficient phenotype with respect to expression of several biocontrol genes and exoproducts. Although ANR is generally assumed to be an anaerobic regulator, it was essential for PA23 secondary metabolite production under aerobic conditions. Protein sequence alignment with FNR from E. coli revealed amino acid differences in close proximity to the N-terminal cysteine residues, which might affect the O_2 sensitivity of the PA23 ANR, as suggested previously. Future studies are required to understand how ANR functions at different O_2 levels and how this impacts PA23 growth, metabolism and ultimately biocontrol under a variety of different conditions.

Poster #36: Site-Specific Asparagine Deamidation Accelerates Islet Amyloid Polypeptide Self-Assembly

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Asparagine deamidation is a spontaneous non-enzymatic post-translational modification resulting in the conversion of a asparagine to a mixture of aspartic acid (Asp) and iso-aspartic acid (iso-Asp). In alkaline condition, Asp/isoAsp is deprotonated and could alter to the protein net charge. Otherwise, this chemical conversion is known to influence the structure and the stability of protein and can ultimately lead to protein misfolding and aggregation, two hallmark events in diverse amyloid related diseases including Alzheimer's disease and diabetes mellitus type 2 (DM-2). The aim of this study was to examine the effects of site-specific asparagine deamidation on the amyloidogenecity and the cytotoxicity of the peptide Islet Amyloid Polypeptide (IAPP). IAPP is a natively disordered 37-residue peptidic hormone that is co-expressed and co-secreted with insulin by pancreatic islet beta-cells. Its deposition as insoluble amyloid aggregates correlates proportionally to the progression of DM-2. The kinetics of IAPP amyloid formation and its post-translationally deamidated analogs at specific asparagine position were evaluated by a combination of Thioflavin T (ThT) fluorescence assay and transmission electron miscroscopy (TEM). We observed that site-specific asparagine deamidation to aspartic acid accelerates amyloid formation, whereas asparagine deamidation to isoaspatic acid perseveres aggregation. The process of fibrillogenesis in absence or in presence of deamidation modifications was also monitored by circular dichroism (CD) spectroscopy and we observed that the conformational transition disordered-to-beta-sheet associated with IAPP/IAPPs deamidated assembly was modulated. Using rat pancreatic beta-cells, we observed that deamidation reaction does not prevent IAPP-induced toxicity. These results show that site-specific asparagine deamidation could potentially play a key role in the etiology of DM-2 by promoting IAPP amyloidogenic cascade and/or by stabilizing the resulting amyloid fibrils.

Poster #37: CFTR Lipid-Protein Interactions: an Approach by Coarse-Grained Molecular Dynamics Simulation

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The cystic fibrosis transmembrane conductance regulator (CFTR) protein belongs to the ATP-binding cassette (ABC) superfamily. As any other ABC transporter, CFTR binds and hydrolyzes ATP at the cytosolic nucleotide binding domains (NBDs), triggering conformational changes in the transmembrane domains. However, CFTR is also an atypical ABC transporter, as the ATP-induced NBD dimerization is coupled with the opening of a channel for Cl- ions; thus an alternating access mechanism proposed for other ABC members cannot be readily applied to CFTR. Mutations in CFTR are responsible for cystic fibrosis (CF), where defective CFTR gating and folding, and its retention in the endoplasmic reticulum result in abnormal Na+, Cl– and water transport across epithelia. Experimental structural data for CFTR are limited to the crystal structure of the monomeric cytosolic domains (1Q12, 4A82). Recently, we proposed models of CFTR in open and closed states (Corradi et al., J. Biol. Chem. 2015). Here, we apply coarse-grained (CG) molecular dynamics (MD) simulations to investigate the lipid-environment of CFTR as a function of different conformational states of its gating cycle, as very little is known about CFTR lipid-protein interactions. We use a membrane model that consists of more than 60 lipid types, asymmetrically distributed between lower and upper leaflet (Ingólfsson et al., J. Chem. Amer. Soc. 2014). Simulation systems were set up for the open and the closed state model. Each system contains four molecules of the same protein and more than 6000 lipid molecules. Simulations were carried out for 20 μs. We present an overview of the lipid-protein interactions as well as the main lipid distribution around the protein.

Poster #38: Live Fluorescence Microscopy as a Tool to Study Morphogen Dynamics During Drosophila Melanogaster Embryonic Development

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In embryos, cell differentiation occurs via the formation of spatial gradients of molecules called morphogens, which control the expression of a number of target genes determining cell identity. A common model system to study morphogens is the Bicoid gradient, which determines anterio-posterior (AP) patterning in Drosophila melanogaster, or fruit fly. We are esp ecially interested in understanding how a noisy morphogen input can give a precise output of its target, and accomplish robustness during embryonic development. Here, we aim to apply novel methods in both fly genetics (to label the nascent mRNA of target genes) and fluorescence imaging (to detect the fluctuations in signal caused by the periodic creation of new mRNA at transcription sites) in order to measure the rate of transcription of the Bicoid target gene, hunchback, in each nucleus along the AP axis. Systematic measurements will allow us to determine which factors influence this transcription rate (e.g. morphogen diffusion rate, morphogen/target concentration, and polymerase activity at the target promoter), especially in the border region where there is a switch between expression and no expression. Given the rapidity of establishment of a precise transcriptional response, our hypothesis is that this response at the border relies on a memorization process, allowing nuclei to recall Bicoid concentration from one cycle to the next, by keeping track of the Hunchback promoter transcriptional status across mitosis. Future experiments aiming to challenge this hypothesis, using novel methods in live embryonic imaging are discussed.

Poster #39: A Study of Electroporation Induced Changes in Internal Conductivity of Cells

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Increasing the permeability of a biological cell membrane using intense pulsed electric fields has found numerous biological and medical applications such as electrogenetherapy, electrochemotherapy, and irreversible electroporation of cancerous cells. The phenomenon, electroporation, facilitates cells' uptake of impermeable materials by making pathways in the cell membrane through which ions and polar molecules can pass and enter the cytoplasm. Besides the conventional methods of studying electroporation such as dye exclusion techniques, fluorescent cytometry, and patch-clamp techniques, dielectric based methods are attracting interest as a new label-free and non-invasive modality to investigate the phenomenon. The dielectric properties of a cell exposed to electroporating pulses are affected during and after electroporation due to changes in the membrane structure and ions/molecules transport through the created pores. Therefore, electroporation and its subsequent physiological effects on a cell can be studied by monitoring changes in the cell dielectric properties. We employ a microfluidic device to electroporate a single biological cell using a high intensity pulsed electric field (PEF) and simultaneously study induced changes in its dielectric properties by dielectrophoresis. The microfluidic chip consists of sensing and actuating electrodes embedded in its microfluidic channel to detect single cells, electroporate them, and apply a DEP force to them. Using this system we perform a study on changes in the cytoplasm conductivity of single cells a few seconds after exposure to a PEF and their relationship to the pulse intensity.

Poster #40: How DNA do the Twist: Visualizing Supercoil-Induced Site-Unwinding and Site-Invasion in DNA Loops

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In living prokaryotic cells, DNA experiences constant torsional strain due to its supercoiled state. On the one hand, this topological property of DNA leads to dense storage of genetic code. On the other hand, DNA supercoiling is conjectured to play a key role in mechanically regulating the local unwinding of sites of DNA transcription, replication, and repair. While our understanding of the effects of supercoiling on these essential cellular processes has been developing for decades, the kinetics of local DNA unwinding and DNA strand-invasion have remained largely unaddressable with existing microscopic techniques. Advances in our methodologies have been required to visualize weak and slow interactions between unwinding sites on untethered supercoiled DNA and DNA oligonucleotide probes that can invade unwinding sites. In this work, we demonstrate a new method and assay for visualizing supercoiling-induced site-unwinding, and consequent site-invasion by small molecules, which overcomes these challenges. Our approach uses Convex Lens-induced Confinement (CLiC) microscopy to trap and visualize Cy3B-conjugated probe DNA molecules and plasmid DNA in micron-sized pits etched into a glass coverslip. We demonstrate trapping of DNA molecules ranging from several-kB plasmids to few-bp oligonucleotides in pits and we watch their dynamics and interactions over several minutes. The pits are much larger than the trapped molecules, allowing them to explore accessible conformations. As a model for supercoiled DNA, we use a DNA plasmid with a known unwinding site, prepared in different topoisomer states. We use a suite of short DNA oligonucleotides, which are complementary to different target sequences within the unwinding site, as a model for small molecules invading unwinding sites. In this work, we study how supercoiling, applied temperature and solution conditions, as well as oligonucleotide sequence and length influence site-unwinding and site-invasion. Beyond this study, the flexibility of our microscopy assay opens the door to performing new measurements of weak and slow molecular interactions in a wide range of biophysical, biochemical, and biotechnological contexts.

Poster #41: Lipid-Protein Interactions of G-Protein Coupled Receptors

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G Protein - Coupled Receptors (GPCRs) are a large family of membrane proteins involved in a large array of biochemical processes. Recent studies show that in order to explain the activity of GPCRs, their lipid environment has to be taken into account. Most notably, the interaction of GPCRs with cholesterol molecules has been the center of focus of several studies conducted in the past few years. To understand this lipid-protein dynamics, we have carried out large-scale molecular dynamics simulations, using the MARTINI coarse grained model, of several biologically important GPCRs (including the human chemokine (CXCR1) receptor involved in breast cancer, and the delta opioid receptor important in pain sensing). Our simulation setup consists of four copies of proteins embedded in a 40nm x 40nm complex lipid bilayer, composed of more than 60 lipid types and simulated for 30 microseconds. Here, we present our results on the lipid organization at different distances from each protein copy, and show their relative lipid enrichment. In addition, we present our results on the lipid distribution and the interactions of cholesterol with GPCRs. We believe that the simulation setup design we have employed and the long time scales we have achieved offer a unique perspective on the lipid-protein interaction dynamics of GPCRs.

Poster #42: Global Transcriptomic Analysis Reveals Decreased Biocontrol Gene Expression in a Pseudomonas Chlororaphis PA23 ptrA Mutant

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Pseudomonas chlororaphis strain PA23 inhibits several fungal pathogens in both greenhouse and field studies. We have discovered a LysR-type transcriptional regulator (LTTR) called PtrA (Pseudomonas transcriptional regulator A) that is essential for Sclerotinia sclerotiorum antifungal activity. P. chlororaphis PA23 produces the antibiotics phenazine 1-carboxylic acid, 2-hydroxyphenazine, pyrrolnitrin and several additional products that contribute to biocontrol, all of which are markedly reduced in a ptrA mutant. In greenhouse studies with S. sclerotiorum-challenged canola, the incidence of stem rot and leaf infection was significantly increased in plants inoculated with the ptrA-mutant compared to PA23. Thus this LTTR plays an important role in the ability of PA23 to protect canola from the pathogenic effects of S. sclerotiorum. Complementation studies with the ptrA mutant revealed that plasmid-borne ptrA was able to restore the wild-type phenotype. Moreover, providing gacS, but not gacA in trans leads to partial complementation of antifungal activity, protease activity as well as phenazine and pyrrolnitrin production. Thus, there seems to be a regulatory link between PtrA and the sensor kinase GacS. Using qRT-PCR to monitor expression of gacA, gacS, phzA, phzI, phzR, rsmA, rsmE, rsmX, rsmZ, prnA, psrA, rpoS and scd, we observed a decrease in gacA expression and an increase in rsmE expression in the ptrA mutant compared to the wild type. GacA and RsmE are responsible for activation and repression of biocontrol factors, respectively. RNA-seq analysis indicated that the expression of biocontrol-related genes phzA, phzI, phzR, prnA, rpoS, as well as the chitinase-encoding gene is decreased in the ptrA mutant. The observed changes in gene expression are consistent with the ptrA phenotype. At present, it is not known whether the effects of PtrA on these genes is direct or indirect. Collectively our results indicate that PtrA is an essential regulator of PA23 biocontrol that is

linked to GacS. These findings add to the increasingly complex cascade overseeing expression of antifungal compounds in strain PA23.

Poster #43: Mitochondrial Membrane Properties in Different VDAC Mutants

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Mitochondria are the primary ATP producing organelles in most Eukaryotic cells. The mitochondrion is bound by an outer and inner membrane. Voltage dependent anion-selective channel (VDAC) is the most prominent protein in the mitochondrial outer membrane (MOM). This pore forming protein is composed of 19 β-strands and a flexible N-terminal alpha helix. VDAC works as a passage through the MOM to join the intermembrane space and cytosol to transport different metabolites and Ca2+, hence maintaining mitochondrial functions and cellular homeostasis. In the model organism Neurospora crassa, a VDAC-less strain has a dysfunctional electron transport chain and slow growth rate. Similar phenotypes have been observed in an N-terminal truncated VDAC mutant. To understand the observed deficiencies in the mutant strains mitochondrial membranes have been analyzed. Whole mitochondrial membrane fluidity was not changed for VDAC-less and mitochondria containing N-terminally mutated VDAC. However, membrane composition analysis of those two mutants revealed less ergosterol and more saturated fatty acids than wild-type. Moreover, VDAC-less mitochondrial membrane was more sensitive compared to wild-type mitochondrial membrane when exposed to different types of detergents. Whereas, N-terminal truncated VDAC mitochondria have similar detergent-resistance compared to wild-type mitochondria. Thus the absence of VDAC in mitochondria influences mitochondrial membrane composition and physical properties.

Poster #44: Phase Behaviour of Palmitoyl Sphingomyelin in Model Membranes containing Palmitoyl Ceramide and Cholesterol

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Membrane domains like lipid rafts and ceramide-enricheddomains have been postulated to play important roles in several biological processes like cell signaling and metabolic regulation.Palmitoyl Sphingomyelin (PSM) is the most abundant lipid in the outer leaflet of biological membranes[1], and also an important lipid regarding the generation of micro-domain phase separation. For instance, lipid rafts are rich inSphingomyelin (SM) and Cholesterol (Chol), and the interaction between SM and Chol induces the generation of the liquid-ordered (l¬o¬) phase. Moreover, under stress, SM hydrolysis by Sphingomyelinase (SMase) leads to SM conversion to Ceramide (Cer),making the SM and Chol rich domains a potential target for the action of SMase. It is known thatCerincorporation into SM membranes induces highly ordered lamellar gel phase (Lβ) phase[2]–[4]. Therefore, there has been a growing interest in the interaction of the three lipids together in ternary lipid mixtures. Recently, it has been proposed that model membranes formed by the ternary mixture of PSM: PCer: Chol with the lipid molar ratio of 70: 30: 30 respectively, would result in the formation of homogeneous stable gel phase[5]–[7]. However, there is a conflict between the fluorescence microscopy data from two probes, NAP and DiIC18. NAP-stainedternary mixture at the aforementioned lipid ratio at room temperature showed phase coexistence of lo and Lβphases while DiIC18 shows a homogeneous Lβ phase. We have implemented 2H-NMR experiments to address this discrepancy.The phase behavior of ternary mixtures of PSM: PCer: Chol as determinedfrom analyzing 2H-NMR spectra as a function of temperatureis presented andcompared to the phase behavior of pure PSM-d31, and the binary mixture of PSM-d31: Chol (70: 30).

Poster #45: Understanding the Hydrophobic Effect using -Cyclodextrin as a Model System

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Cyclodextrins (CD) are cyclic oligosaccharides consisting of 6, 7, or 8 -1,4-linked D-glucose units with a truncated cone structure. Three polymers of CD exist: hexamer (-CD), heptamer (-CD), and octamer (-CD). It has previously been demonstrated that cyclodextrins are useful models for exploring guest-host chemistry as they have hydrophobic centers while retaining moderate to high solubilities in aqueous mediums. Guest-host chemistry examines weak, non-covalent intermolecular interactions such as electrostatic, van der Waals, hydrogen bonds, and hydrophobic interactions to address scientific questions. We hypothesize that as salt concentration increases, the hydrophobicity of the cavity is increased thereby facilitating faster binding of the probe 2,6 Anilinonaphthalene-6-Sulfonic Acid (2,6 ANS). From steady-state fluorescence experiments, sodium chloride appears to increase fluorescence which can be related to an increase in 2,6 ANS binding to the cavity of CD. With temperature-jump fluorescence spectroscopy data we were able to determine that sodium chloride is involved in the final step of the mechanism—drawing water out of the sugar cavity. We believe the increase in ligand binding is due to a decrease in the activity of water. Our results indicate that 31 6 water molecules are disrupted for every molecule of 2,6 ANS that binds to the cavity of CD. Therefore, water molecules surrounding cyclodextrin and located inside the cavity are drawn towards the area of lower activity. This supports the idea that NaCl is lowering the activity of water. Once the hydrophobic lining of the cavity is exposed, 2,6 ANS can establish energetically favorable, hydrophobic interactions rather than maintain contact with the surrounding waters—lowering the energy of the system. In summary, sodium chloride effectuates ligand binding by decreasing the activity of water thereby enhancing hydrophobic interactions.

Poster #46: Dynamics Study of HIV-1 Transactivator of Transcription by NMR

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The type 1 Human Immunodeficiency Virus (HIV-1) transactivator of transcription (Tat) is a small RNA-binding protein essential for viral gene expression and replication. It has also been shown to bind to a large number of human proteins and to have an impact on many different cellular activities. Our study used NMR spectroscopy and hydrogen exchange chemistry to measure backbone dynamics of full-length Tat protein. The NMR spectra indicate that the protein is intrinsically disordered. 15N NMR relaxation parameters have been measured and analyzed by spectral density mapping to give information about the protein on the picosecond to nanosecond time scale. The results indicate that the protein exists in an extended disordered conformational ensemble. The model of a disordered backbone is further supported by NMR relaxation dispersion which detects no conformational exchange for any of the residues on the millisecond time scale. NMR chemical shift differences from random coil values suggest that some segments of the protein have a modest propensity to fold; comparison to X-ray diffraction structures of Tat complexes indicates that some segments of the protein function through an induced-fit mechanism whereas other segments likely operate by conformational selection. Hydrogen exchange measurement by NMR shows higher exchange rates compared with predicted values for a disordered protein. This is explained by the high net charge on the protein that enhances base-catalyzed hydrogen exchange. Our study provides a deeper understanding of the Tat protein conformational ensemble and forms a foundation for future studies of the conformational changes of the protein in complexes.

Poster #47: Structural and Biophysical Characterization of Prohibitin

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Prohibitin (PHB) is a multimeric protein found on lipid rafts of the eukaryotic inner mitochondrial membrane. PHB has been shown to be a major intracellular signalling mediator in various cellular and overall biological processes such as iron homeostasis, immunology of mammals and more. Many of these processes are associated with the diseases that affect the general population ranging from cancer to obesity. Since no exact structure of PHB is known, such a determination will provide insight towards its function and furthermore the role it plays within the cell as well as the entire organism. X-Ray Diffraction is the desired method for structure determination therefore preliminary data pertaining to the protein's behaviour in various solvent conditions will facilitate protein crystallization. This study will include biophysical and structural results of PHB from dynamic light scattering, analytical ultracentrifugation and small angle X-ray scattering.

Poster #48: Structural Insights into Inhibitors Designed to Block the Glycoside Hydrolase NagZ and Suppress β-Lactam Resistance in Pseudomonas Aeruginosa

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AmpC β-lactamase overproduction is a major cause of β-lactam antibiotic resistance in clinically important Gram-negative bacteria like Pseudomonas aeruginosa and enterobacteria, and is linked to peptidoglycan (PG) recycling. The exo-N-acetyl-β-glucosaminidase NagZ removes the GlcNAc sugar from PG recycling metabolites to yield 1,6-anhydromuramoyl-peptide, a ligand that converts the transcriptional regulator AmpR into an activator of AmpC gene expression. NagZ plays a pivotal role in regulating AmpC β-lactamase expression in P. aeruginosa, making it a promising target for small molecule inhibitors designed to suppress β-lactam-resistance. This study explores the potential of two different small molecule inhibitors of NagZ by structurally characterizing their binding interactions within the enzyme active site: MM-156, a 2-N-acyl substituted azepane (seven-membered iminoalditol), and ethyl-butyl-PUGNAc, a derivative of the potent glycosidase inhibitor PUGNAc. The X-ray structure of a NagZ homologue from Burkholderia cenocepacia in complex with MM-156 demonstrates that MM-156 selectively blocks NagZ activity by mimicking the stereochemistry of the GlcNAc sugar that NagZ removes. The crystal structure of NagZ bound to ethyl-butyl-PUGNAc reveals how addition of a bulky substituent on the 2-acetamido group on PUGNAc imparts selectivity for NagZ over functionally related human glycosidases. Interestingly, the phenyl-carbamate moiety on ethyl-butyl-PUGNAc was found to displace a loop in NagZ that contains the general acid/base catalytic residue of the enzyme. Displacement of the loop by the inhibitor yields an incomplete active site, which may make it more difficult for resistance mutations to arise against the inhibitor. Interestingly, unlike ethyl-butyl-PUGNAc, MM-156 allows the catalytic loop to remain close to the active site as it does not contain a substituent at the site of the phenyl-carbamate moiety. Overall, the results of this study provide new insights into NagZ inhibitor development to suppress AmpC β-lactamase production in P. aeruginosa.

Poster #49: The in Silico Characterization of the Fungal Mitochondrial Ribosomal Protein S3

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Besides the usual core genes within the filamentous Ascomycota mitogenomes, one can encounter genes encoding for the ribosomal protein S3 (rps3). This gene is unique as it is present as an intron-encoded version or it can be a freestanding gene. In addition this ribosomal protein can be variable in size ranging from 200 to 1000 (plus) amino

acids. Research on the intron encoded version (from within the rnl gene) of Neurospora crassa has shown that the rps3 gene is expressed and the protein is incorporated within the small subunit of the ribosome. In addition, in Schizosaccharomyces pombe, the mtDNA-encoded version of this protein is associated with a mutator phenotype presumably implicating it in DNA repair, suggesting RPS3 could be a moonlighting protein. A survey of mtDNA rps3 genes across the Ascomycota was performed in order to assess the relationship between the intron encoded version and those that are free standing. Phylogenetic analysis suggests that these versions are orthologous they do not appear to be paralogs or analogs. Utilizing comparative sequence analysis of mitochondrial fungal RPS3 sequences, in combination with in silico structural analysis (Phyre2 and MISTIC), regions that are conserved were defined and the locations of the more variable segments within the protein were identified.

Poster #50: Mechanism of Immunoglobulin Light Chain Self-Assembly: Contributions of Tyrosine and Histidine Chemical Modifications

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Light chain amyloidosis (AL) is the most common form of systemic amyloidosis, which originates from plasma cell over proliferation. This lethal disease is primarily characterized by an overproduction of immunoglobulin light chains (LC) and followed by pathological deposition of amyloid fibrils in the extracellular space of vital organs causing organ dysfunction. Non-enzymatic post-translational modifications (PTMs) can profoundly affect protein properties and have been shown to contribute to the pathogenesis of several protein misfolding diseases. However, few is known about PTMs effects on LC amyloidogenicity. Here, we investigated the impact of oxidative PTMs, particularly carbonylation by hydroxynonenal (HNE), oxidation and nitration, on the structure, thermodynamic stability and aggregation of Wil, a LC variable domain of the λ6 germline. In order to achieve this, we initially identified the residues that are prone to oxidative chemical modifications by LC-MS/MS analysis performed after pepsin digestion. Subsequently, we noted that HNE-carbonylation at specific His residues and nitration of precise Tyr side chains modulate Wil propensity to self-assemble and to form ThT-positive fibrillar aggregates. Nitration appears to accelerate the formation of aggregates with low cross-β-sheets quaternary structure. This effect has been associated with a decrease in thermodynamic stability. In contrast, HNE-conjugation on specific His imidazole group did not affect the structural stability although it altered the conformational conversion driving the aggregation process. No effect on LC Wil aggregation and structural stability has been noted for oxidation Wil PTMs. Thus, both the thermodynamic stability and the physicochemical and structural properties have to be considered concomitantly when evaluating the amyloidogenic propensity of a LC variable domain in the context of AL.

Poster #51: The Intron Landscape for the mtDNA Fungal nad5 Gene.

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Although fungal mitochondrial genomes share a core set of genes they are quite variable in size (18 to >200 kb) in part due to intergenic regions and the presence of introns. Fungal mitochondrial introns can be referred to as self-splicing introns commonly referred to as group I and group II introns. These introns are different from the nuclear spliceosomal introns as group I and II introns are ribozymes and they encode proteins that assist in the mobility of group I and II introns. Group I intron typically encode homing endonculeases and group II intron encode reverse transcriptases. These introns are referred to as mobile introns as they can move from intron containing alleles to cognate alleles that lack introns. In this study the NADH dehydrogenase subunit 5 gene (*nad5*) has been examined for members of the Ascomycota and Basidiomycota with regards to the presence of introns. In silico analysis was performed on these sequences with regards to identifying introns, intron/exon junctions, intron open reading frames and the data have been summarized in order to generate an "intron landscape" for the nad5 gene. Intron landscapes are a resource to those involved in annotating mitochondrial genomes and in bioprospecting for endonucleases and reverse transcriptases which have applications in biotechnology. However, intron landscapes also offer an opportunity to examine if there is a biased pattern to the insertion of mobile introns. Mobile introns are probably "parasitic DNAs" that have to balance their own survival against minimizing their impact on the host genome. This sets up a rather complex scenario and the long term objective of this work is to gain a better understanding of the "evolutionary dynamics" of mobile introns and their impact on fungal mtDNA.

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