Frontiers in Biophysics 2017: Talk and Poster Abstracts

(chronologically ordered)

Clinton Durney (UBC Mathematics)
A Mechanochemical Model of Drosophila Dorsal Closure

Alan Manning (UBC Physics)
A complete picture of nuclear spin relaxation in brain? Microstructures, myelin, and a cow brain from Michigan

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Magnetic Resonance Imaging (MRI) is used extensively to study, diagnose, and manage de-myelinating diseases such as Multiple Sclerosis. Loss of the myelin sheaths surrounding nerve cell axons hinders action potential propagation, causing motor and cognitive impairment. The work presented here is part of an effort to develop accurate in-vivo quantification of myelin using MRI, enabling better detection and understanding of disease pathologies in a non-invasive way.

MRI uses spatially-resolved Nuclear Magnetic Resonance (NMR) signals. Typically, contrast is generated from NMR relaxation properties of 1H nuclei in different tissues. Even though relaxation-weighted MRI has been used clinically for decades, the fundamental mechanisms of 1H NMR relaxation in brain tissue remain poorly understood. The heterogeneous nature of the tissue and the rapid exchange of 1H nuclei among different tissue components complicates the picture.

To completely characterize 1H NMR relaxation in brain tissue, we have employed a suite of NMR experiments on a bovine brain model. Relaxation following a variety of initial magnetization conditions in the different compartments is observed. By using a solid-state NMR spectrometer we are able to track magnetization in both the aqueous components typically observed in MRI, and also the non-aqueous components that are invisible in in-vivo MRI studies.

Our results are well described by a four-pool model that includes two aqueous and two non-aqueous components. The data allow the cross-relaxation between the different components to be determined with unprecedented confidence and show that associating relaxation properties with any one component is misleading.

David Holloway (BCIT Mathematics)
Two-stage patterning dynamics in conifer cotyledon whorl morphogenesis

> Conifers, unlike monocots or dicots, have variable numbers of cotyledons ('seed leaves'), even within the same species. Cotyledons form in a whorl on a dome shaped embryo. That the closely spaced cotyledons are not found outside this ring indicates a radial control on where they can form. Polar transport of the hormone auxin is involved in cotyledon outgrowth, but not in radial positioning or the within-whorl spacing between cotyledons. We developed a two-stage reaction-diffusion model for the spatial patterning, with a radial pattern constraining the higher-frequency cotyledon pattern to a whorl. Growth driven by the second stage generates single whorls across the experimentally observed range of 2 to 11 cotyledons, as well as the circularly symmetric response to auxin transport interference. The model generates the linear relation between cotyledon number and embryo diameter observed experimentally, and the flattening embryo geometry in these stages may contribute to the upward direction of cotyledon growth. This work provides a quantitative framework for the growth and patterning dynamics involved in early conifer embryogenesis and more generally for the morphogenesis of whorls with many primordia.

Lavisha Jindal (SFU Physics)
Plasmid Segregation by the ParA/B protein system

Alexandra Kaspar (SFU Physics)
Energy-Speed-Accuracy Tradeoffs in Driven Stochastic Rotary Machines

> Alexandra Kasper and David Sivak
> Simon Fraser University, Department of Physics
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> Molecular machines are stochastic systems that convert between chemical potential energy and mechanical work. For example, the F1 subunit of ATP synthase couples the rotation of its central crankshaft with the synthesis or hydrolysis of ATP. This machine can reach maximal speeds of hundreds of rotations per second, yet is believed to be capable of nearly 100% efficiency in near-equilibrium conditions. A cycling machine is a non-equilibrium system and therefore must waste some energy in the form of dissipation. We explore the fundamental relationships between the accuracy, speed, and dissipated energy of such driven rotary molecular machines, in a simple model of F1. We find a Pareto frontier governing these tradeoffs and relate it to similar relationships uncovered in distinct biophysical systems.

Joshua Scurll (UBC Mathematics)
StormGraph: A graph-based clustering algorithm for the analysis of super-resolution microscopy data

> With super-resolution microscopy techniques such as Direct Stochastic Optical Reconstruction Microscopy (dSTORM), it is possible to image fluorescently labeled

proteins on a cell membrane with high precision. Often, the extent to which such proteins cluster is biologically meaningful; for example, in B-cells, clustering of the B-cell receptor (BCR) is associated with increased intracellular signaling and B-cell activation, and spontaneous BCR clustering can cause chronic active BCR signaling that results in an aggressive B-cell malignancy. Computational methods are therefore needed to make quantifiable comparisons between the observed clustering in different data sets, such as for different cell types or different experimental conditions.

Inspired by the success of graph-based clustering algorithms such as PhenoGraph in other research areas, we developed StormGraph, a graph-based clustering algorithm for analyzing Single Molecule Localization Microscopy (SMLM) data such as would be obtained by dSTORM. This talk will present StormGraph, which distinguishes clusters from random background and assigns individual localizations to specific clusters, allowing for a detailed analysis of statistics such as cluster area. The utility of StormGraph will be illustrated on dSTORM data of BCRs imaged on malignant B-cells.

Alastair Jamieson-Lane (UBC Mathematics)
Bi-molecular reaction rates in 2d chemistry

While we may live in a three dimensional universe, chemistry occurring on the surface of a cell membrane can be thought of as taking place in a 2d environment. Unlike 3d, where the collision rate between particles quickly reaches equilibrium, no such equilibrium exists in 2d and over time reaction rates tend towards zero (undermining the very notion of "Reaction Rate Constant"). In this talk I discuss the results of past papers and (hopefully) and a few details of my own - in particular discussing some of the assumptions that past results depend upon, and where there assumptions may fail.

Lisa Zhang (SFU Physics)
Observation of the Markovian Mpemba Effect

A container filled with hot water can sometimes freeze faster than a similar container of warm water placed in the same environment. The origins of this “Mpemba effect” remain controversial, despite many studies. Recently, Lu and Raz (PNAS, 2017) have proposed that an analogous effect can take place in small systems where thermal fluctuations play an important role. Here, I report preliminary observations of this effect in a system consisting of a colloidal particle in water in a controllable potential. Although one cannot define the temperature of such a system when it is not in equilibrium, one can use the probability distribution of its position to define a measure of its distance from equilibrium such that at equilibrium there is a one-to-one mapping with the temperature. Using this measure, we then observe that systems at “hot temperatures” can approach equilibrium faster than systems at “warm temperatures” when placed into carefully chosen energy landscapes. By showing that analogues of the Mpemba effect can be understood quantitatively in small

systems, we can gain insight into applications involving switching between equilibrium states in general.

Jagesh Shah (Harvard Medical School)
How do motile cells measure?

Orientation of motile cells in chemical gradients, or chemotaxis, plays a critical role in the survival of single-celled organisms and the development and immune function of multicellular organisms. While an abundance of molecules modulating chemotaxis have been identified, measuring their dynamical behavior to explain how cells become oriented has been far more challenging. Microfabricated devices, with features the size of single cells, permit quantitative programming of chemical and physical cues in space and time to connect experimental results to computational models. Using these devices we have identified a novel inputs into cell orientation such as hydraulic resistance and memory that belie a complex measurement scheme at play in migrating cells that goes beyond chemical cues and includes physical inputs and past history. We will describe these findings and our current efforts to develop in an integrated quantitative model of how migrating cells measure.

Aidan Brown (SFU Physics)
Allocating dissipation across a molecular machine cycle to maximize flux

Biomolecular machines consume free energy to break symmetry and make directed progress. Nonequilibrium ATP concentrations are the typical free energy source, with one cycle of a molecular machine consuming a certain number of ATP, providing a fixed free energy budget. Since evolution is expected to favour rapid-turnover machines that operate efficiently, we investigate how this free energy budget can be allocated to maximize flux. We find that unconstrained optimization eliminates intermediate metastable states, indicating that flux is enhanced in molecular machines with fewer states. When maintaining a set number of states, we show that - in contrast to previous findings - the flux-maximizing allocation of dissipation is not even. This result is consistent with the coexistence of both `irreversible' and reversible transitions in molecular machine models that successfully describe experimental data, which suggests that in evolved machines different transitions differ significantly in their dissipation.

Luke McAlary (UBC Physics)
Tryptophan-32 of SOD1 is an aggregation modulating residue

Mutations in the cytosolic anti-oxidant enzyme superoxide dismutase-1 (SOD1) are known to cause the fatal neurodegenerative disease amyotrophic lateral sclerosis (ALS). There are currently over 150 mutations that can occur within the SOD1 structure that have the ability to destabilise the native state, leading to pathogenic aggregation and a possible prion-like spread of disease. Interestingly, when human SOD1 variants are overexpressed in mouse models, the endogenous mouse SOD1 does not co-aggregate into the observed cellular inclusions. By comparing sequence

conservation a sequence segment of high non-conservation was identified, where the most drastic change from mouse to human was at position 32, which is tryptophan in humans and serine in mice). Here we make a serine substitution at position 32 in human SOD1 and assess the effects it has on aggregation propensity. We find that a W32S substitution reduces the aggregation propensity of a range of ALS-associated mutants in both recombinant protein and cellular models, but curiously decreases its stability as measured by native mass spectrometry. Providing preformed aggregates as a template for the more unfolded W32S variants, has little effect on their aggregation propensity, indicating a role for W32 in the prion-like spread of pathology in SOD1-associated ALS cases. We propose W32 of SOD1 as a target for therapeutic intervention in ALS.

Daniel Knowles (SFU Molecular Biology and Biochemistry)
A conserved linker in CTP: Phosphocholine cytidylyltransferase transduces activating signals between regulatory and catalytic domains

How can regulatory domains of allosteric enzymes transmit ligand binding signals to physically distinct catalytic domains? To address this question, we are using CTP: Phosphocholine cytidylyltransferase (CCT) as a model. CCT catalyzes the rate-limiting step of phosphatidylcholine (PC) synthesis through the CDP-choline pathway, using a membrane as its allosteric activator. The catalytic and membrane-binding domains are joined by a conserved, amphipathic linker segment that connects to the active site via helix αE. Initial mutagenesis and enzyme activity analysis showed that CCT activation requires the presence of the linker, and it cannot be replaced by a non-specific glycine-rich linker. Our hypothesis is that this linker undergoes a sequence-dependent conformational change upon CCT binding the membrane that triggers a structural change which propagates to the active site. By replacing hydrophobic residues in the linker with tryptophans and monitoring fluorescence in the presence of lipid-phase quenchers, we found that the linker superficially interacts with the membrane upon CCT activation. Currently, we are knocking out large, hydrophobic linker residues and using fluorescence and activity analysis to determine if this linker-membrane interaction is vital for activation. To probe the conformational landscape of the active site and αE helix we performed extensive molecular dynamic simulations of CCT using the crystal structure of the inactive soluble form to provide starting coordinates. The simulations reveal a malleability of the αE helices with a bend that enables direct interaction between the αE helix and a loop containing the key catalytic residue and the substrate CTP. We hypothesize that these interactions facilitate catalysis. As well, this bend would pull the catalytic domain close to the membrane. The linker segment in its membrane-bound form may stabilize the bent αE helix. These analyses will help fill in the gaps that still exist to help generate a CCT starting structure for simulations with CCT docked on a membrane. Uncovering the mechanism of activation used by CCT may help us understand how numerous enzymes that are regulated by reversible membrane binding are activated and more generally, how multidomain enzymes facilitate transfer of signals between domains to efficiently regulate catalysis of vital biological reactions.

Alejandra Herrera Reyes (UBC Mathematics)
Counting individual fluorophores in STORM data

Immune cell activation is believed to be triggered by clustering of membrane receptors. Experimentally this system requires a precise fluorescence labelling, obtained using stochastic optical reconstruction microscopy (STORM). STORM uses photoswitchable fluorophores to achieve resolutions at or below 20nm, with the down side of possibly observing a given fluorophore multiple times in the process. We are developing a mathematical model to estimate the number of fluorophores present in the experiment. We apply a Markov chain model to describe the temporal dynamics, and a Gaussian mixture model for the spatial information. This approach will enhance a microscopy technique that is already widely used in biological applications, and will allow more precise analysis of receptor cluster formation and its effects on immune cell signaling.

Mike Kirkness (SFU Molecular Biology and Biochemistry)
Accessible high-throughput force studies: enzymatic cleavage of collagen

The effect of force on the triple helical structure of collagen is unresolved, with reports of unwinding, no effect and tightening of the triple helix. With collagen as the main structural protein in the human body, it is under a constantly changing load in its native state. Our method combines centrifuge force microscopy (CFM) along with a single molecule method of non-collagenolytic protease cleavage to determine collagen's triple helical structure under force. Using the CFM we are able to probe concurrently hundreds of single molecules under the same conditions. Multiple locations along the collagen's length including the MMP site can be probed by using different proteases such as trypsin and thrombin. We report on our findings of collagen's response to applied force; trypsin cleavage rate of collagen increases with force suggesting an induced melting of the triple helix. Demonstrating the capabilities' of the CFM as an accessible and high throughput instrument excites with many biological interactions possible for future studies.

Michael Irvine (UBC Mathematics)
Conservation of pattern as a tool for inference on spatial snapshots in ecological data

As climate change and other anthropogenic factors increase the uncertainty of vegetation ecosystem persistence, the ability to rapidly assess their dynamics is paramount. Vegetation and sessile communities form a variety of striking regular spatial patterns such as stripes, spots and labyrinths, that have been used as indicators of ecosystem current state, through qualitative analysis of simple models. Here we describe a new method for rigorous quantitative estimation of biological parameters from a single spatial snapshot. We formulate a synthetic likelihood through consideration of the expected change in the correlation structure of the spatial pattern. This then allows Bayesian inference to be performed on the model parameters, which includes providing parameter uncertainty. The method was validated against simulated data and then applied to real data in the form of aerial

photographs of seagrass banding. The inferred parameters were found to be able to reproduce similar patterns to those observed and able to detect strength of spatial competition, competition-induced mortality and the local range of reproduction. This technique points to a way of performing rapid inference of spatial competition and ecological stability from a single spatial snapshots of sessile communities.

Samara Pillay (Mathematical Institute, University of Oxford)
Multiscale Modeling of Angiogenesis

Angiogenesis is the process by which new blood vessels develop from existing vasculature. We use an agent-based approach to model the behavior of individual endothelial cells during angiogenesis. We incorporate crowding effects through volume exclusion, motility of cells through biased random walks, and include birth and death-like processes to represent sprout and loop formation. We use the transition probabilities associated with the discrete model and a mean-field approximation to systematically derive a series of partial differential equation (PDE) systems representing collective cell behavior that vary in complexity depending on the extent of volume exclusion incorporated on the microscale. This general framework generates non-linear PDEs that impose physically realistic density restrictions, and are structurally different from existing phenomenologically-developed linear "snail-trail" models, which implicitly view cells as point particles. By comparing solutions to our continuum models and a well-known snail-trail model to discrete simulation results, we determine how microscale cell behavior manifests at the macroscale. In general, we find that linear snail-trail models perform well when exclusion effects are diminished at the macroscale; in other cases, non-linear models should be used. We also classify continuum model performance in terms of descriptive and predictive power based on network morphology statistics extracted from the agent-based model, which is useful for drug development strategies based on PDE models.

Elham Abouei (UBC Physics)
Azimuthal enface image registration for correction of motion artifacts in rotary-pullback 2D and 3D image modalities

We present a new method for the correction of motion artifacts present in vivo 2D and 3D endoscopic images produced by rotary-pullback catheters. This algorithm can correct for cardiac/breathing-based motion artifacts and catheter based motion artifacts such as non-uniform rotational distortion (NURD). This method assumes that slowly varying linear structures exist along the direction of the pullback. The method also assumes that continuous angular mismatch corrections, corresponding to motion artifacts, can be estimated from slowly varying structures that can be seen in enface images. The method is based on dynamic serial warping using a cost matrix measuring similarities between structures in adjacent regions in en face image can be used to align the pullback data. We optimize and demonstrate the suitability of this method using a NURD phantom and in vivo endoscopic pulmonary optical coherence

tomography and autofluorescence images. En face images are presented for evaluation of the method which show enhancement in image quality.

Alaa Al-Shaer (SFU Physics)
Probing the Structural and Mechanical Properties of Molecular Collagen

Beverlie Baquir (UBC Microbiology and Immunology)
Exploring the Role of Cellular Reprogramming During Sepsis

Sepsis is an assemblage of symptoms resulting in a dysregulated host immune response to severe infection and causes over 5 million deaths annually. It has been reported that early identification and diagnosis has the potential to improve patient outcomes and survival. Since sepsis pathogenesis is poorly understood, there is a great need to define and identify the factors that are at play, especially during early sepsis. We have performed a meta-analysis of the transcriptional responses from more than 600 sepsis patients and have discovered a proclivity towards a cellular reprogramming (CR) gene expression signature. Very early sepsis patients showed strong gene expression signatures reflecting CR and immunosuppression, indicating a potential new major immune perturbation during disease progression. This reprogrammed state is predictive of the development of subsequently-diagnosed severe sepsis and organ failure in sepsis. Thus, while a mixed pro- and anti-inflammatory state in early sepsis has been suggested, it is worth noting that CR is a form of cellular amnesia rather than an anti-inflammatory state per se. Ultimately, we want to uncover the mechanisms underlying cellular reprogramming. Macrophages possess the plasticity to change functional states under the appropriate stimuli. We have shown that reprogrammed macrophages have a hampered ability to produce pro-inflammatory cytokines in response to microbial signatures such as lipopolysaccharide and in fact, adopt a M2-like differentiation state. Macrophages might therefore be the major cell population driving CR in pre-sepsis patients and the induction of endotoxin tolerance (ETT), a type of CR in macrophages derived from human induced pluripotent stem (iPS) cells are highly compatible with the CRISPR/Cas9 gene editing technique. Therefore I hypothesize that monocytes/macrophages are the major cell population driving cellular reprogramming in sepsis patients and that reprogrammed iPS-derived macrophages can be used to mimic and study cells expressing the CR expression pattern associated with early sepsis.

Zarrin Basharat (Fatima Jinnah Women University & UBC Molecular Biology and Biochemistry)
Deciphering bacterial mechanism of azo dye degradation through computational biology approach

Bashe Bashe (SFU Molecular Biology and Biochemistry)
Determination of Lipid Phase Behaviour in Drug Delivery Systems via Small Angle X-Ray Scattering

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

Dhananjay Bhaskar & Cole Zmurchok (UBC Mathematics)
A model for Rho GTPase dynamics in epithelial monolayers

Rho family GTPases are proteins that regulate a cell's ability to form protrusions and to contract. As cells change shape, migrate individually or collectively, they experience forces from their environment and neighbours. These forces are translated into chemical signals that regulate GTPase signalling, thus providing a feedback mechanism. We develop and analyze a nonlinear ODE model for Rho GTPase signalling to explore the interplay between cell mechanics and cell signalling. We discover that feedback between Rho signalling and cell mechanics can lead to a variety of dynamics and we simulate Rho activity using a 2-D Cellular Potts Model that mimics an epithelial monolayer. In a large tissue, we observe travelling waves of contraction and collective oscillation of cells mediated by intercellular adhesion.

Darren Christy (UBC Medicine)
CNS-Derived Extracellular Vesicles are Heterogeneous and Tissue Specific

Extracellular vesicles (EVs) are secreted by myriad cells in culture and unicellular organisms, and their identification in mammalian biofluids suggests that vesicle release is occurring at the organism level as well. We applied a protocol for primary

neuronal cell culture and modified it for the collection and purification of EVs from frozen whole neural tissues. By flow cytometry we have identified predominate cell types contributing EVs to neuronal tissue and that the contribution of EVs is tissue dependent for select cell types. When we compared the vesicle populations for 3 month old NTg and SOD1-G93A (ALS model) murine neuronal tissues we found no significant difference suggesting there is no disease correlated change to neuronal vesicle populations. The study sheds light on the cell types responsible for releasing EVs and that the underlying EV phenotype is tissue dependent.

Matt Courtemanche (SFU Physics)
Detecting interhemispheric transfer using magneoencephalography

Functional magnetic resonance imaging studies have detected interhemispheric communication in the corpus callosum by observing correlated blood oxygenation fluctuations. The results suggest that it may be possible to image neurological function in connective white matter. The aim of this research was to detect the neuroelectrodynamics from the same activity using magnetoencephalography. Fourteen right-handed volunteer participants were imaged using fMRI and MEG while performing a visuo-motor interhemispheric transfer task. The participants were presented with word and face stimuli in either the left or right visual hemifield field. The participants were then asked to classify the stimulus as word, pseudo-word, face, or scrambled face by pressing buttons in the left and right hand. Motor interhemispheric transfer was induced by changing the response hand. The stimuli were presented in blocks with periods of rest in between.
The MEG data were collected using a 151-channel CTF system at 1200 Hz with a 300 Hz anti-aliasing filter. Continuous head monitoring was utilized in order to assess participant movement. Using SPM8, the participants' T1-weighted MR images were co-registered with the individual's digitized head shape and segmented. A linearly-constrained-minimum-variance beamformer over a 1-300 Hz bandwidth was used to compute neuromagnetic activity over a voxelized brain volume. As an initial exploration of the data, a contrast was constructed between active and rest blocks using a general linear model approach. The resulting contrasts showed distributed cortical activity consistent with word, face and motor tasks. Additionally, potential activity across the corpus callosum was observed at the individual level, although the location along the tract varied in some cases between participants. These preliminary results suggest that it may be possible to use MEG to image function across the corpus callosum. However, further investigation is needed to eliminate signal leakage and other confounds as well as account for possible source model influences before definitive results can be reported.

Lisa Craig (SFU Molecular Biology and Biochemistry)
Structures of Type IV pili from Pseudomonas aeruginosa, Neisseria gonorrhoeae and Neisseria meningitidis at sub-nanometer resolution

The Type IV pili are hair-like filaments displayed on the surfaces of many bacteria. These pili are retractile, a feature that contributes to their diverse array of functions,

which include adhesion, bacterial aggregation, twitching motility, DNA uptake and secretion. As such, they are critical virulence factors. Type IV pili are several microns in length and only 6-9 nm in diameter, yet have remarkable tensile strength, flexibility and extensibility. They are polymers of the major pilin subunit, but minor pilins contribute to pilus assembly and retraction and are thought to be incorporated into the pili in low levels. Here we report cryo-electron microscopy reconstructions of Type IV pili (T4P) from three important human pathogens, Pseudomonas aeruginosa and Neisseria gonorrhoeae and Neisseria meningitidis, at ~ 8 Å, 6 Å and 5 Å resolution, respectively. The Pseudomonas and Neisseria structures reveal distinct arrangements of the major pilin globular domains on the pilus surfaces, which impart different helical parameters, but similar packing of the conserved N-terminal α-helices, α1, in the filament core. In contrast to the continuous α-helix seen in the x-ray crystal structures of the major pilin subunits from these pathogens, in all cases α1 in the pilus filaments has a melted segment located between conserved helix-breaking residues Gly14 and Pro22. Using mutagenesis we show that Pro22 of the N. meningitidis major pilin is critical for pilus assembly, as are Thr2 and Glu5, which are positioned to hydrogen bond in the hydrophobic filament core. We propose that α1 melting is a consequence of packing of the pilin subunits into the pilus, and contributes to the biophysical properties of these pili. We further report the x-ray crystal structure of the N. meningitidis minor pilin PilV, which we model into the pilus reconstruction. These structures provide a framework for understanding T4P assembly, function and biophysical properties.

Navi Garcha (SFU Molecular Biology and Biochemistry)
Localization and Assembly of the Vibrio cholerae Type IV Pilus Secretin Channel

Type 4 pili (T4P) are filamentous structures found on the surfaces of many Gram-negative bacteria, including Vibrio cholerae. The V. cholerae T4P are the toxin-coregulated pili (TCP), which mediate bacterial aggregation and exoprotein secretion, critical functions in colonization of the human intestine to cause the diarrheal disease cholera. TCP assemble at the inner membrane (IM), grow through a multiprotein conduit in the periplasm and through a secretin channel in the outer membrane (OM). The multimeric secretin channel is formed by secretin subunits, which are transported across the IM by the Sec apparatus, and in most T4P systems are transported to the OM with the help of a lipoprotein co-chaperone. In the V. cholerae TCP the secretin subunit itself, TcpC, is a lipoprotein, and its putative co-chaperone, TcpQ, is non-lipidated. Here we use mutagenesis, cellular fractionation and functional assays to investigate secretin channel assembly in V. cholerae. We show that lipidation of TcpC is essential for localization and stability of its N-terminal periplasmic domains as the Cys1Ser variant is highly unstable. The full-length TcpC Cys1Ser variant localizes to the OM but is unable to rescue pilus assembly in a tcpC V. cholerae mutant. The putative co-chaperone TcpQ is not required for TcpC stability or membrane localization, negating its role as a chaperone, but is required for formation of a functional pilus assembly apparatus. When TcpQ is expressed in a ΔtcpQ strain, pilus assembly is restored, suggesting that TcpQ is crucial for completing the pilus assembly conduit. Our results lead to a

model whereby TcpC Cys1 is lipidated at the IM by the LOL machinery and lipidated TcpC is transported to the OM in complex with TcpQ. TcpC inserts into the OM at two points: via its C-terminal portion, which forms β-barrel channel with other TcpC C-terminal domains, and via lipid moieties in its N-terminal domain, which interacts with TcpQ to link the OM channel to periplasmic pilus conduit.

Ismail Khater (SFU Computer Science)
Molecular Level Quantification of Cav1 Clusters in Super-Resolution Imaging Data

The spatial resolution of contemporary fluorescence microscopy is limited by the diffraction of light to ~200-250 nm. Super-resolution microscopy has broken the diffraction barrier, enabling biologists to visualize molecular structures with minimally invasive techniques at a resolution approaching that of electron microscopy (EM). Of the various super-resolution approaches, the best resolution is obtained using single molecule localization microscopy (SMLM), based on the repeated activation (blinking) of small numbers of discrete fluorophores (using, for instance, PALM, STORM or GSD). The precise localization of the blinks is determined using a Gaussian fit of the point-spread function (PSF) and provides ~20 nm X-Y (lateral) resolution and, with the addition of an astigmatic cylindrical lens into the light path, ~25-50 nm Z (axial) resolution .

Quantitative approaches to analyzing the large super-resolution datasets generated by SMLM are limited. We developed a computational pipeline and applied it to analyze SMLM 3D point clouds of the caveolar coat protein, caveolin-1 (Cav1), in prostate cancer cells differentially expressing PTRF/CAVIN1, also required for caveolae formation. Our pipeline includes an iterative blink merging algorithm to remove high degree (strongly-interacting) points, followed by a denoising module that compares Cav1 network properties with randomly generated networks in order to retain a sub-network of geometric structures (or clusters/blobs). Then, machine-learning based classification is used to determine the best threshold to construct the networks while statistical analysis is used to find the significant network features that are both used in clusters extraction. Unsupervised machine-learning is used to analyze the clusters by extracting 28 features describing the size, shape, topology and network measures of ~80,000 blobs. We identified small S1 scaffolds corresponding to SDS-resistant Cav1 oligomers, as yet undescribed intermediate hemi-spherical S2 scaffolds and, only in PTRF/CAVIN1-expressing cells, larger spherical, hollow caveolae.

The clusters/blobs were further divided into subclusters/modules by extracting the communities of every cluster of Cav1 blinks. The Newman community detection method is employed for subclusters extraction. Spectral community detection algorithm that is based on the eigenvectors decomposition of the characteristic matrix of every cluster's network is used to find the subclusters. The subclusters were studied to find the relationship between the different groups by finding the one-to-one matching across the learned groups of clusters and their subclusters. Multi-threshold modularity analysis suggests that interaction of four S1 Cav1 scaffolds forms larger,

curved S2 scaffolds and that S1 dimers group together, in the presence of PTRF/CAVIN1, to form the caveolae coat.

Emma Lathouwers (SFU Physics)
The thermodynamics of living things: energy and information transmission in soft, noisy matter

Living things are extremely complex, yet they manage to operate precisely and reproducibly even while working with floppy soft-matter materials like proteins. Organisms, cells, indeed individual molecular machines all rely on the transmission of energy and information for their functioning. How do the complicated structures and dynamics of proteins reliably communicate energy? How does a protein `know' precisely about spatially distant changes communicated through such pliable, noisy material? Through numerical investigation of simple model systems, we explore the governing thermodynamic principles that determine how energy and information flow through strongly coupled soft-matter systems, and discuss generalizations.

Aaron Lyons (SFU Physics)
Probing the Structural and Mechanical Properties of Molecular Collagen

As the primary load- and tension-bearing protein in vertebrates, the physical properties of collagen are of significant biomedical interest. By virtue of its high aspect ratio, the flexibility of the collagen monomer – a 300 kDa triple helical structure, 300 nm in length and 1.5 nm in diameter – can be described using the worm-like chain model of polymer physics. To address the variability in flexibility of collagen reported in the literature, which spans nearly an order of magnitude, we employ atomic force microscopy (AFM), circular dichroism (CD) spectroscopy and fluorescence correlation spectroscopy (FCS) to study the structural and mechanical properties of the collagen monomer. This multimodal approach demonstrates the modulation of collagen mechanics by solvent conditions, and provides insight into the wide range of flexibilities reported in the literature.

Joanne Mercer (SFU Physics)
Investigating the Phase Behaviour of a Model Lipid Nanoparticle System with DLinKC2-DMA/Distearoylphosphatidylserine and the Addition of Cholesterol

Lipid nanoparticles (LNPs) are a favoured delivery device for short pieces of RNA (siRNA) used in gene silencing. Whilst LNPs can easily undergo endocytosis into targeted cells, the actual release of siRNA into the cytoplasm has an efficiency of only 1%. Understanding the molecular interactions between both LNP and endocytic components is essential for improving LNP efficiency. The release trigger for LNPs is an ionisable cationic lipid, which becomes protonated in an acidifying endosome. The hypothesis is that the protonated cationic lipid will electrostatically interact with anionic lipids in the endosomal membrane, inducing the formation of the inverted hexagonal (HII) phase and releasing siRNA into the cytoplasm. To study this, a model system comprised of the cationic lipid XTC2 and an anionic lipid DSPS-d70

was investigated using 2H NMR spectroscopy. Experiments were conducted at an acidic pH (pH 4.7) to establish full protonation of XTC2 and observe the resulting interaction with DSPS-d70. Using a temperature range from 5-40°C, we observed a phase transition from gel to HII with a transition temperature (TH) of ~17°C. We then introduced 10 mol% cholesterol (found in LNPs) which reduced the TH and increased order in the HII phase. Small angle X-ray scattering (SAXS) experiments will allow identification of phase coexistence within each system, and enable measurement of lattice spacings. Experimental results are being fed into computational simulations at the University of Calgary to develop a comprehensive model of LNP interactions for improved efficiency in the future.

Shawan Narayan (UBC Physics)
Investigating the behaviour of a square quadrupole system

PhET's simulation - Charges and Fields - allows a platform to perform an experiment to investigate the relationship between the distance from four point charges arranged in a square and its electric field and corresponding electric potential (University of Colorado, 2017). The distance and electric field are measured using online tools available in the simulation. By collecting several data points around the quadrupole system, the data is interpreted and plotted to analyze the relationship between the arrangement of the charges and the distance away from the system. The best fit line reveals the behaviour of the system. The relationship between the electric field and the distance of the system follows Coulomb's law and also contains quadrupole interactions for certain distances. It is clear that the system's electric potential and the distance are inversely proportional; meaning that the electric field is also inversely proportional to the distance away from the system.

Karlton Scheu (SFU Physics)
Investigating a biological specificity conundrum: the role of dynamics in transcription factor binding

A biological specificity conundrum challenges our understanding of the DNA-binding mechanisms underlying many transcription factor families. With the increasing number of genome-wide DNA-protein interaction studies, it has become evident that static X-ray crystallographic studies of isolated DNA-binding domains with consensus DNA oligonucleotides do not fully explain the different in vivo specificities exhibited by members of transcription factor families. We hypothesize that, despite sharing a structurally conserved DNA-binding domain, the dynamic properties of different transcription factor family members modulate their individual DNA-binding specificities; to the extent that a more dynamic protein is less inclined to bind its cognate DNA sequence and will more readily bind alternative sequences. To test this hypothesis, we have constructed a library of mutants of ETV6, and ETS transcription factor member, which exhibit altered levels of plasticity. Then utilizing an unbiased Bind-N-Seq approach we will examine how the dynamic properties of ETV6 affect its relative affinity for DNAs ranging from those with a consensus ETS binding site to those with completely "non-specific" sequences.

Reza Siavashi (SFU Physics)
Gel/Liquid-ordered Phase Coexistence in Bilayers Containing Palmitoyl Sphingomyelin, Palmitoyl Ceramide and Cholesterol

Elucidation of the molecular mechanisms of apoptosis have significant therapeutic benefits since it reveals how cell death can be modulated. One of the unknown aspects of apoptosis is its effects on the phase behavior of the plasma membrane (PM). Due to apoptotic signaling, enzymatic activity of sphingomyelinase (SMase) converts SM to ceramides (Cer) in the PM. It has been suggested that SMs are localized in domains within the PM. Accumulations of Cer in the vicinity of SM induce gel phase in the SM:Cer vesicles. On the other hand, cholesterol (Chol) is one of the major components of the PM and it forms liquid-ordered (lo) phase in SM:Chol bilayers. Therefore, SM:Chol:Cer interactions seem to be crucial in determining the cell's fate. In this study, we are interested in determining the physical effects of Cer accumulation in SM/Chol vesicles to give an insight on the phase behaviour of the PM during apoptosis. Using 2H-NMR, we have characterized the phase behavior of palmitoyl-SM (PSM) and palmitoyl-Cer (PCer) in their ternary mixtures with Chol. According to our results, there is lo-gel phase coexistence in the PSM:Chol:PCer bilayers with the molar ratios of (7:3:1), (7:3:2) and (7:3:3) at room and physiological temperatures. These results are consistent with the combined fluorescent microscopy data using NAP and DiIC18 as the fluorescence dyes for the (7:3:3) mixture (Busto J V, et al. Lamellar gel (Lβ) phases of ternary lipid composition containing ceramide and cholesterol. Biophys J. 2014).

Martin Zuckermann (SFU Physics)
Transforming a Protein Nano-walker into a Nano-motor by Feedback

Biological protein nano-motors are vital to the survival and function of cells. The object of our research is to design synthetic protein motors which mimic such biological nano-motors. To this purpose we have conceived a concept for a protein nano-walker dubbed "Synthetic Kinesin Inspired Protein (SKIP)", so called because it is inspired by kinesin's use of restricted diffusional search for forward head binding. The SKIP model is composed of three ligated coiled coils and four ligand-gated repressor proteins; its two types of repressor each bind to specific sequences patterned symmetrically along a linear double-stranded DNA track. SKIP is designed to undergo biased directional motion along this track in the presence of a temporally symmetric ligand pulse sequence. Thus, in this design, directionality is achieved by persistence rather than asymmetry. Furthermore this symmetry allows SKIP to reverse either through the influence of a sufficiently large rearward force or by the choice of specific track termini. Here we explore the role of feedback in enhancing SKIP's performance. In the first approach, we alter the direction of the applied force when SKIP reverses in order to maximize the work being done by SKIP as it walks along the track (Zuckermann et al., New J. Phys. 2015). In an alternative approach, we alter the duration of the ligand pulses, switching to a new potential binding state as soon as forward binding is detected. This scheme allows us to maximize output power. Finally we evaluate the information cost and the benefits of feedback in both

scenarios. The properties of this new construct demonstrate that the SKIP walker can indeed be engineered by feedback to become a directional and processive nano-motor.