Frontiers in Biophysics 2010

Graduate student talks:

Presenter	Time	Title/Abstract
Harris Huang Grad Student MBB, SFU	**3:30-3:47**	**Mechanism of silencing of the catalytic domain by the regulatory membrane lipid-binding domain of an amphitropic protein, cytidylyltransferase** CTP: phosphocholine cytidylyltransferase (CT) is an auto-regulated, homodimeric enzyme that catalyzes the rate-limiting step in the synthesis of phosphatidylcholine. Although it is known that its lipid binding M domain plays a critical role in the auto-inhibition, the mechanism has not been established. To approach this problem, contact sites between various sub-regions of M domains and other CT domains have been identified via photo-cross-linking and mass-spectrometry. Specifically, weak, but lipid-sensitive contacts with the active site were found. As revealed by anisotropy measurements, this interaction may foster rigidity via introduction of structure into otherwise flexible M domains. These results support an emerging model in which auto-inhibition of CT is achieved through a collection of transient inhibitory contacts that may require flexibility. These interactions would be replaced with protein-lipid interaction upon activation.
Jennifer Morrison Grad Student Mathematics, UBC	**3:47-4:04**	**Deciphering multi-state lateral mobility in single particle trajectories with a hidden Markov model** Single particle tracking is a powerful technique often used in the study of dynamic mechanisms on the cell surface such as binding, confinement and trafficking. A potential problem in the analysis of single particle trajectories is to account for transitions between modes of mobility. I will present a new method based on a hidden Markov model that detects transient periods of drift diffusion or reduced mobility within single trajectories due to transient associations with other biomolecules. We analyzed single particle trajectories of two critical T cell immune receptors, CD45 and LFA-1, with this method.
David K. Jones Grad Student Biomedical Physiology & Kinesiology, SFU	**4:04-4:21**	**pH Modulation of the Cardiac Voltage-Gated Sodium Channel, Nav1.5** We sought to characterize the effects of low pH on the cardiac-specific voltage-gated sodium channel, $Na_V1.5$. $Na_V1.5$ was expressed in *Xenopus* oocytes and currents were recorded using the cut-open voltage clamp technique with extracellular solution titrated to either pH 7.4 or 6.0. pH 6.0 reversibly depolarized the voltage dependence of activation and inactivation, increased window current, inhibited use dependent current reduction, as well as accelerated recovery from and slowed the onset of fast inactivation. These data suggest that lowering extracellular pH destabilizes the fast-inactivated state of $Na_V1.5$ channels. This could act as an arrhythmogenic trigger during cardiac ischemia.
Jun Allard Grad Student, Mathematics, UBC	**4:21-4:38**	**Spontaneous organization of cortical microtubule arrays in plants; modeling from molecular to cellular scales** Microtubules on the two-dimensional cortex of plant cells must form parallel yet dispersed arrays for proper cell elongation. Organization has been hypothesized to arise from microtubule collisions, which can result in two phenomena called entrainment ("zippering") and induced catastrophe. Here we present (1) a cell-scale model of microtubule organization and (2) a molecular-scale model for microtubule collisions. Our results address the emergence or order, tuning of the dominant direction, mutant systems mor1-1 and clasp-1, and observed differences between different plants, including Arabidopsis and Tobacco.
BREAK (10 Minutes)		
Saeed Saberi Grad Student, Physics, SFU	**4:48-5:05**	**Chromosome driven spatial patterning of proteins in bacteria** Asymmetric cell division is the process in which the daughter cell differs from the mother cell. The bacteria, Caulobacter Crescentus undergo such a process producing motile daughter cells from immotile mother cells. Asymmetric cell division in these bacteria involves differentially positioning proteins between the mother and future daughter cell at the dividing cell's poles. It has been found that the polymering protein PopZ plays a major role in marking and capturing other proteins at the poles. We have developed a simple model for PopZ localization driven solely by attractive polymerizing interactions and self-avoidance with the bacterial chromosome. By changing either the volume fraction of PopZ or the chromosome we can reproduce the varied localization patterns that PopZ shows in experiment.

Qing Peng Grad Student Chemistry, UBC	**5:05-5:22**	**Direct observation of the tug-of-war during the folding of a mutually exclusive protein** By inserting a guest protein I27w34f into a host protein GL5, we engineered a novel mutually-exclusive protein GL5/I27w34f. From GL5/I27w34f, we observed the first kinetic evidence that the guest and host domains engage in a folding 'tug-of-war', in which the host domain folds rapidly and then unfolds automatically driven by the folding of the guest domain. Our results indicate that protein folding can generate sufficient mechanical strain to unravel the host protein, and highlight important roles played by the intricate coupling between folding kinetics, thermodynamic stability and mechanical strain in the folding of multi-domain proteins.
Raheleh Salari Grad Student, Computing Science, SFU	**5:22-5:39**	**A partition function algorithm for interacting nucleic acid strands** Regulatory non-coding RNAs (ncRNAs) play an important role in gene regulation. Such ncRNAs usually bind to their target mRNA to regulate the translation of corresponding genes. The specificity of these interactions depends on the stability of intermolecular and intramolecular base pairing. In order to predict base-pairing probability of any two bases in interacting nucleic acids, it is necessary to compute the interaction partition function over the whole ensemble. I present a dynamic programming algorithm that computes the partition function over (almost) all physically possible joint secondary structures formed by two interacting RNAs. Then I summarize the results of our algorithm in computing (1) the melting temperature for interacting RNA pairs studied in the literature and (2) the equilibrium concentration for several variants of the OxyS-fhlA complex.

Posters:

Presenter	Title/Abstract
Gerhard Blab Postdoc Physics, SFU	**Building an Artificial Molecular Motor: The "Tumbleweed"** In order to move directionally along a track, biological molecular motors must coordinate many processes, such as fuel capture, fuel hydrolysis, associated conformational changes, binding and unbinding from a track, and centre-of-mass movement. A better understanding of the interdependencies between these processes, which take place over a wide range of different time scales, would help elucidate the general operational principles of molecular motors. Artificial molecular motors present a unique opportunity to study this because motor structure and function are known a priori. Here we describe use of a Master Equation approach, integrated with input from Langevin and molecular dynamics modelling, to stochastically model a molecular motor across many time scales. We apply this approach to a specific concept for an artificial protein motor, the Tumbleweed.
Yi Cao Grad Student Chemistry, UBC	**A Force Spectroscopy-based Protein-ligand interaction Assay** Binding of small molecules are crucial to the function and folding of many proteins inside cell. Thus it is of fundamental importance to measure the binding affinity of small molecule ligands to proteins and reveal the binding mechanism. Here we report a force spectroscopy based single-molecule binding assay that is capable of determining the binding affinity as well as the binding mechanism of ligands to proteins at the single-molecule level. This assay is based on the difference in the mechanical stability of the given protein upon ligand binding. We anticipate that it will find unique applications in life sciences.
Clara Chan, Undergraduate student, Physics,SFU **Linda Dekker** Visitor graduate student, Physics, SFU	**Collagen Production and Preparation for Transmission Electron Microscopy Imaging** Collagen is the most abundant structural protein in the human body and breakdown in the stability of this protein can result in a variety of connective tissue diseases. Therefore, identification of the mechanical properties of collagen at the molecular level may help to describe the mechanisms that regulate its stability. Our aim is to study the flexibility of collagen molecules and fibrils using images obtained by transmission electron microscopy (TEM). Production of wild-type procollagen II is achieved using a recombinant mammalian expression system that secretes procollagen, which we then purify by ion exchange chromatography. Here we present TEM images of collagen and the results of our purification scheme.
Michael S. Dahabieh Grad Student Chemistry, SFU	**Interrogating the Secondary and Tertiary structures of the West Nile Virus 5' Untranslated Region** Flaviviruses including Yellow Fever and West Nile (WNV) affect millions worldwide and have reemerged in North America. The WNV RNA genome is flanked by untranslated regions (UTRs) at the 5' and 3' ends, which orchestrate viral replication. High-resolution images of UTRs throughout replication are required to understand and target Flaviviruses. We have begun probing the tertiary structure and folding dynamics of these UTRs using Selective 2'Hydroxyl Acylation analyzed by Primer Extension (SHAPE). This technique maps secondary and tertiary interactions based on 2'Hydroxyl reactivity towards an electrophile. Capillary electrophoresis mapping SHAPE-reactive nucleotides, and structural models of WNV 5'UTR will be presented.

Melissa Dennis Grad Student MBB, SFU	**A nuclear localization signal sequence acts as a secondary membrane binding motif to distinguish the membrane binding affinities of two CCT isoforms** CTP: phosphocholine cytidylyltransferase (CCT) is the key regulatory enzyme in the biosynthesis if phosphatidylcholine, the major phospholipid component of most eukaryotic biomembranes. CCT alternates between an active membrane bound form and an inactive soluble form. Despite having very similar amphipathic α-helical membrane binding domains, the CCTα isoform elicits a stronger binding response to lipids than CCTβ. Using biophysical and molecular techniques, we show that the amino-terminal polybasic nuclear localization signal exclusively in CCTα is a novel secondary membrane binding motif which is largely responsible for the differential response between CCT isoforms to anionic membranes *in vitro* and *in vivo*.
Benjamin Downing Grad Student, Physics, SFU	**Probing the Mechanical Properties of Single Fibrillar Proteins with Optical Tweezers** Structural proteins play vital roles in many human tissues, roles to which their mechanical properties are of direct relevance. Optical tweezers allow us to quantitatively probe these properties at the single molecule level, potentially revealing a wealth of information on how such proteins fulfil their physiological functions. We have worked toward applying this technique, in which micron-sized beads chemically linked to the protein are manipulated by focused laser beams, to structural proteins, particularly elastin. We discuss experimental challenges presented by elastin's relatively short contour length and unusual biochemical properties, along with our efforts to overcome them.
Michel Gauthier Postdoc Physics, SFU	**How replication defects, fork blocks, and repair affect DNA replication kinetics** DNA replication is usually modeled using a series of replication origins at which a pairs of replication forks are initiated. After initiation, these forks duplicate DNA bi-directionally from the initiation site until they eventually coalesce with another fork. Unfortunately, defects along the DNA can slow, or even stall, replication forks. We propose a new model to calculate the time to replicate a genome as a function of the initiation rate and fork speed that includes a density of DNA defects. Our model uses coupled master equations to calculate the average fraction of replicated DNA and the fork densities as a function of time.
Iman Hajirasouliha Grad Student, Computing Science, SFU	**Detection of locus and content of novel sequence insertions using paired-end next-generation sequencing technology** We present a computational framework to discover the /locus/ and /content/ of novel sequence insertions using the NGS platforms. We test our methods with the high-coverage short-insert sequence library generated from the genome of a Yoruba individual (NA18507) sequenced by Illumina (Bentley et al. 2008). We validate the content of the predicted novel sequence insertions by using arrayCGH and sequenced fosmid clones generated from the genome of the same individual (Kidd et al. 2008). Our method can be used to characterize the DNA sequences missing from the reference assembly to obtain a better picture of the human genome diversity.
Fereydoun Hormozdiari Grad Student Computing Science, SFU	**Personalized copy-number and segmental duplication Maps using Next-Generation sequencing Technologies** Here we present a set of computational methods we developed to study human copy number variation using the next generation sequencing technologies. We first developed a tool to efficiently map micro reads to all possible locations in the human genome. We then used our tool to map reads generated by traditional capillary sequencing (Venter), 454 FLX (Watson), Illumina 1G Analyzer from CEPH trio samples as part of the 1000 Genomes Project (NA12878, NA12891, and NA12892), a Chinese (Yuan Huang), and an African sample (NA18507) to build maps of duplications and deletions larger than 20Kb, and then validated through array CGH. Our initial analyses suggest that the detection of SNPs, and structural and copy number variants, using short read sequences is feasible, fast, and reliable.
Paul Jaschke Grad Student, Microbiology and Immunology, UBC	**Kinetics and Energetics of Electron Transfer Reactions in a Photosynthetic Bacterial Reaction Center Assembled with Zinc Bacteriochlorophylls** We studied electron-transfer (ET) properties of the photosynthetic reaction center of a *Rhodobacter sphaeroides* mutant that synthesizes an unusual form of chlorophyll called zinc-bacteriochlorophyll. This so-called zinc-reaction center exhibits ET rates similar to wild-type despite having very different chlorophylls1. Thus, protein-cofactor interactions involving electron sharing between the metal of zinc-bacteriochlorophyll and the protein, play an important role in adjusting the energies of the cofactors to form an efficient ET system that is analogous to wild-type but with novel composition, similar to photosystem I in plants.
Nicole Jinn Undergrad Student, Mathematics, UBC	**Diffusion with a State Dependent Diffusion Coefficient** Diffusion is a net transport of molecules from a region of higher concentration to one of lower concentration by random molecular motion. The setting of this project begins with a rectangular, spatially homogeneous domain in two dimensions. Then the domain is split into panels of equal size that alternate between two diffusion coefficients. A large spacing gives a diffusion coefficient twice that of the smaller spacing. We are simulating movement of one particle diffusing through two different homogeneous media. Two approaches at hand are contrary (theoretical) predictions of the motion of the diffusing particle in a solid.

Apollos C. Kim MBB equipment technician, SFU	**Biochemical analysis of catalytic reactions executed by *Escherichia coli* signal peptide peptidase A ($SppA_{EC}$)** The amino-terminal signal peptides are critical for secreted or membrane proteins to be successfully threaded through the membrane. During the translocation, the signal peptides are first cleaved by signal peptidase and then further processed by signal peptide hydrolases. In *E. coli*, $SppA_{EC}$ has been identified to be involved in the processing of the remnant signal peptides. We recently reported its first three-dimensional structure and have undertaken its functional characterization. Structure-based screening of substrates has led us to develop an effective assay to investigate its substrate specificity and catalytic efficiency. Here, we report the progress in our biochemical characterization of $SppA_{EC}$.
Suzana Kovacic Researcher Physics, SFU	**Understanding the Principles of Molecular Motors: Building a Protease Lawnmower** Given the importance of biological motors in a variety of biological processes, it is desirable to develop an understanding of their operational principles. Here, we describe a concept for a protein-based synthetic motor which couples enzymatic substrate cleavage with Brownian motion to produce biased motion on a symmetric linear track. The motor is based on a quantum dot hub that is attached to proteases while the substrate is composed of peptides tethered to a linear DNA track. The protease motor acts as a "lawnmower" which "mows" the substrate lawn inducing track asymmetry and biasing the direction of diffusion.
Cindy Li Undergraduate student MBB, SFU	**Linking collagen to beads for single-molecule stretching experiments** We are interested in the mechanical properties of collagen, an extracellular matrix protein that structurally supports cells and is found in connective tissues. Our goals are to develop methods to link collagen and its precursor form, procollagen, to beads and to stretch the proteins using optical tweezers. We have tested the linking of wildtype procollagen to antibody-derivatized beads using immunoprecipitation. Alternatively, we have utilized site-directed mutagenesis to engineer unique tags into collagen to allow covalent and non-covalent attachment to the beads. Mutant collagen was then expressed and producing using a eukaryotic expression system, followed by protein purification from media.
Sara Sadeghi Grad Student, Physics, SFU	**Length-dependent force characteristics of coiled coils** Coiled-coil consist of two or more α-helices that form a superhelical structure due to packing of the hydrophobic residues that pattern each helix. A recent continuum model [1] showed that the correspondence between the chirality of the pack to that of the underlying hydrophobic pattern comes about because of the internal deformation energy associated with each helix in forming the superhelix. We have developed a coarse-grained atomistic model for coiled coils that includes the competition between the hydrophobic energy that drives folding and the cost due to deforming each helix. Using this model we investigate the mechanical properties of coiled coils by applying transverse and parallel load. Our results are consistent with experimental results [2]. [1] PRL, 100, 038105(2008) [2] PRL 96, 118102 (2006)
Sara Sadeghi Grad Student, Physics, SFU	**Conceptual Models for a Synthetic Bipedal Stepping Motor** Biomolecular nanomotors have provided the inspiration for the design and construction of artificial nanoscale motors and machines based on several types of molecule including DNA. However, no synthetic nano-motors have yet been constructed from building blocks of protein-based material even though biomotors themselves are proteins. In this context we present the results of numerical simulations for a bipedal motor with two connected peptide legs and with some of the properties of the tumbleweed motor. This motor walks on a one-dimensional track of periodically arranged binding sites
Laleh Samii Grad Student, Physics, SFU	**Biased motion and molecular motor properties of molecular spiders** Molecular spiders are synthetic molecular motors featuring multiple legs that each can interact with a substrate through binding and cleavage. Experimental studies suggest the motion of the spider in a matrix is biased towards uncleaved substrates. We investigate the origin of biased motion and molecular motor properties of bipedal spiders using Monte Carlo simulations. We find that substrate cleavage and spider detachment from the track are both contributing mechanisms to population bias. We investigate the contributions of stepping mechanism; hand-over-hand (HOH) and inchworm (IW) to speed, randomness parameter, processivity, coupling and efficiency, and comment on how these molecular motor properties can be altered by changing experimentally tunable kinetic parameters. In order to investigate the effect of number of legs in the above parameters, we have also done simulations for four-legged spiders on a 1D track.
Marjan Shayegan Grad Student Chemistry, SFU	**Application of optical tweezers to passive microrheology of collagen solutions** How the structure and interactions of proteins in solution correlate with response to deformation is not known. Passive microrheology techniques, which probe local mechanical properties, are well suited to addressing this question. One experimental approach to PMR uses optical tweezers, which trap and probe μm-sized particles, located within the material. In this study, we have probed the power spectral density of fluctuations of 1-μm-diameter microspheres optically trapped in acidic solutions of varying concentration of collagen type I (0, 0.5, and 1 mg/ml). The results show evidence that at higher concentration of collagen the solutions develop elasticity due to the chemical or physical interactions between molecules.

Aaron Van Slyke Grad Student Biomedical Physiology & Kinesiology, SFU	**Flexibility of the S4-S5 Linker is Required for Normal Gating of the hERG Cardiac Potassium Channel** Activation and deactivation of hERG voltage-gated potassium channels are slow, due to rate-limiting voltage sensor movement. Mutations within the S4-S5 linker altered these processes. Substitution of G546 with residues possessing different physico-chemical properties all resulted in a hyperpolarizing-shift of the voltage-dependence of activation. Mutations at G546 also affected deactivation, although different substitutions had different effects. Of note, G546V deactivation was slow and voltage-independent due to altered voltage sensor movement. These results suggest that hERG channel activation is associated with S4-S5 linker flexibility, and that hERG channel deactivation is the result of at least two reconfigurations of the voltage sensor.
Charles Stevens Grad Student MBB, SFU	**Multidisciplinary structural analysis of the /E. coli/ chaperone protein DmsD** In Bacteria, the Twin arginine translocase (TAT) exports fully folded protein complexes across the plasma membrane. In order to coordinate the assembly and targeting of substrate proteins, a specific molecular chaperone is required. The best studied of these chaperones is DmsD, which assists in the biogenesis of DMSO reductase, Here we present the X-ray crystal structure of E. coli DmsD to 2.0Å resolution, and molecular dynamics simulation of DmsD in complex with an /ab initio /generated signal peptide. This interaction is being analyzed by NMR spectroscopy. This multidisciplinary approach will elucidate the structural basis of the function of DmsD.
Nessy Tania Postdoc Mathematics, UBC	**A Mathematical Model of Cell Motility Regulation by the Cofilin Pathway** Recent experiments on carcinoma cells show that cofilin plays a crucial role in initiating cell movement. Upon activation, cofilin severs actin filaments and generates new fast-growing barbed-ends. We have built a differential equation model of the cofilin regulatory pathway and its response to stimulation by epidermal growth factor. Results from our model captures the early dynamics of barbed end generation observed experimentally. Interestingly, we find that increasing cofilin inactivation via LIM kinase promotes barbed-end production. We show that this effect can be explained in terms of the steady-state level of the different cofilin forms.
Ben Vanderlei Postdoc Mathematical Biology, UBC	**A mechanical model of cell polarization and movement** Cell motility is a complex process related to many biological and medical phenomena. I will present an introduction to the problem, including the physical forces relevant to cell motility, and some of the biological mechanisms through which these forces are generated. The proposed model includes descriptions of the actin network, plasma membrane, cytosol flow, and a simplified model of the biochemistry responsible for the polarization of the cell. The plasma membrane is modeled as an elastic structure immersed in the cytoplasm. The biochemistry signalling is described using a system of bistable reaction diffusion equations. Preliminary results will also be presented.
Yury Y. Villin Grad Student Biomedical Physiology & Kinesiology, SFU	**Differential ph-dependent regulation of NaV channels** Voltage-gated sodium channels play a crucial role in neuronal and muscle excitability. Using whole-cell recordings we studied effects of low extracellular pH on the biophysical properties of neuronal (NaV1.2) and skeletal muscle (NaV1.4) sodium channels. Low pH had little effect on NaV1.4. In contrast, fast inactivation in NaV1.2 was destabilized at pH 6.0, show by a two-state Eyring model. Slow and cumulative inactivation in NaV1.2 were enhanced at pH 6.0. Our data suggest that pH differentially regulates NaV1.2 and NaV1.4. Such differential regulation reflects unique physiological roles and tissue-specific distributions of NaV1.2 vs. NaV1.4.
Lin (Leon) Wang Grad Student Chemistry, SFU	**The Effect of Flow on Microfluidic DNA Microarray Analysis** Microfluidics has been widely applied to DNA microarray technology at both probe immobilization and sample hybridization stages. A better understanding of DNA conformation and concentration variations in microflows is thus in demand. In this work, the effect of microchannel aspect ratios on the immobilization of oligonucleotides to glass substrate was studied. Moreover, the solid-phase hybridization behaviors of both oligonucleotides and long PCR products inside microchannels were investigated at different microchannel aspect ratios, buffer viscosities as well as flow speeds.
Scott Yang Grad Student Physics, SFU	**Building budding yeast's replication program with stochastic initiators** Largely on the basis of naive interpretation of microarray-chip experiments, people have concluded that DNA replication in budding yeast is a nearly deterministic process, in which the position and activation time of each origin of replication is pre-determined. We introduce a mathematical model for replication in yeast and show that the microarray data actually imply a picture of replication where the timing of origin activation is highly stochastic. We then propose a biological mechanism for building a "temporal replication program" from stochastic initiators.