Frontiers In Biophysics

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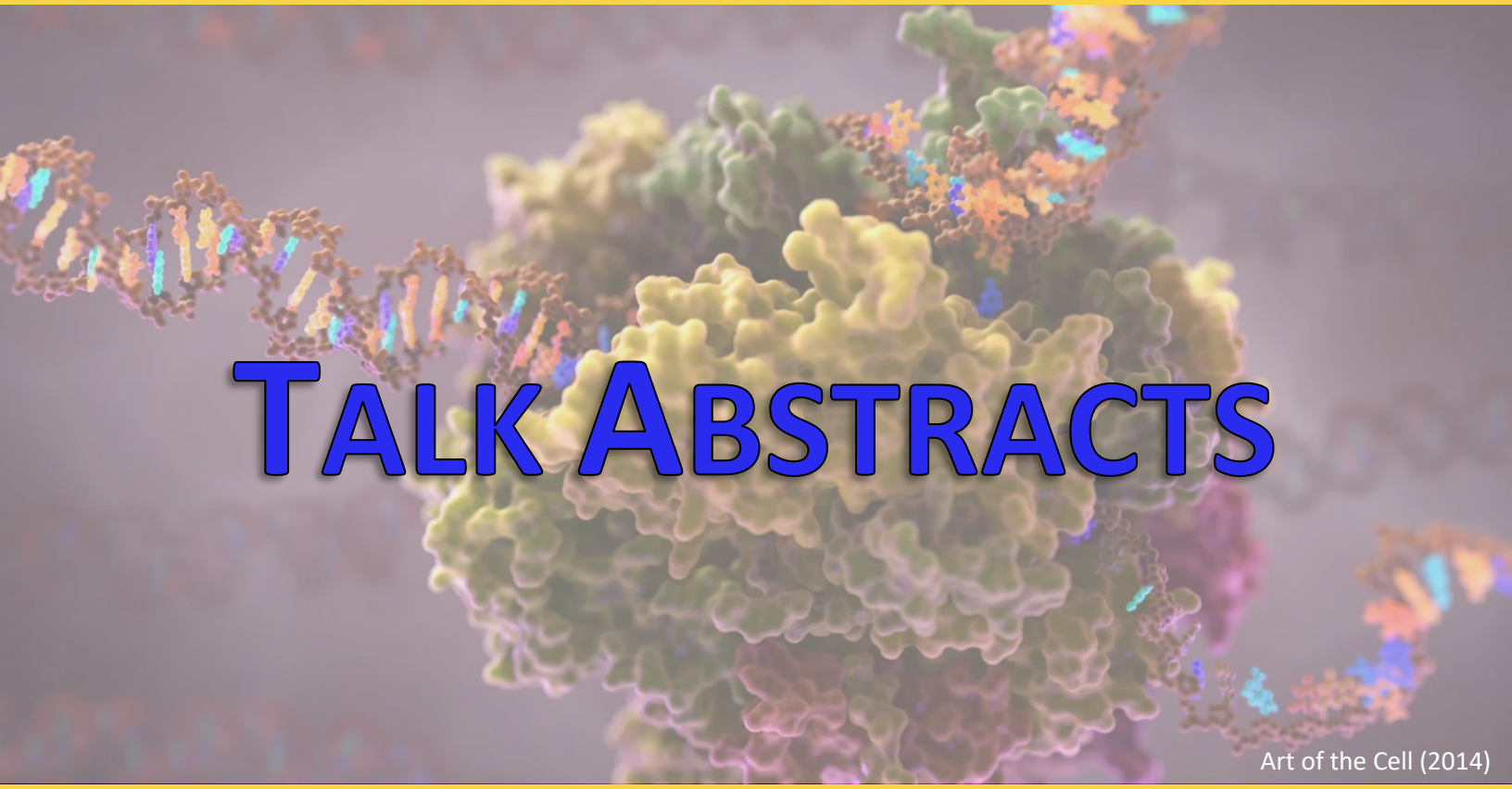
Simon Fraser University – Harbour Centre Vancouver BC



Art of the Cell (2014)

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KEYNOTE

SPEAKER: PALLAV KOSURI *(The Salk Institute, California, USA)*

Origami Movement Microscopy

Mechanical movements of DNA are integral to human biology: Polymerases pry apart the double-stranded helix to transcribe or replicate DNA; chromatin factors bend DNA to restrict or enable access to specific regions of the genome. Collectively, dynamic reactions like these shape the physical organization of our genome and determine the fate of every cell. If we could measure these DNA movements, then we would be able to shed new light on genetic processes and the emerging role of DNA mechanics in gene regulation. However, most DNA movements have never been observed due to the challenge of measuring such minute motions.

To meet this challenge, we are developing Origami Movement Microscopy (OMM), a new technology that enables direct observation of previously invisible DNA movements. Our technology works by structurally amplifying DNA movements with the help of lever-arm-like DNA origami devices. We have designed a set of such devices where each device amplifies a specific mode of DNA movement and makes this movement visible in a standard fluorescence microscope. Using our new approach, we can now observe protein-DNA movements at a resolution of single base pairs, revealing the mechanics of these reactions in rich detail. We envision OMM to become a standard method in our pursuit to illuminate the largely unexplored universe of protein-DNA interaction dynamics.

SESSION 1

SPEAKER: MATTEO FERRARESSO *(University of British Columbia)*

Thermodynamic Limits of Cytoskeletal Gel Contraction

Polymer gels can be used to model the cytoskeleton and specifically cytoskeletal contraction. Two main types of molecular motors modulate the gel microstructure to enact contraction: dynamic crosslinking (DC) motors and chain shortening (CS) motors. This study builds on previously developed DC models for contraction and introduces a new model for CS. We find that both DC and CS motor models yield similar contraction mechanics and energetic costs for contraction. The coupled thermodynamic framework of neo-Hookean polymer chains and Flory-Rehner solvent-chain mixing energies yield a region of energetically unfavourable contractions. Smaller contractions tend to be unfavourable as the energetic cost due to solvent outflow is greater than the energetic budget provided by a small strain energy increase associated with motor activation. Whether or not a contraction can proceed spontaneously is affected by initial gel structure, such as crosslink density, and external environmental conditions, such as the hydrostatic pressure and chemical potential of the solvent. We predict the spontaneity boundary based on initial chain density and molecular motor concentration using the newly introduced CS model that decouples chain density from the contraction mechanism. We find the predicted non-spontaneity region matches that of cytoskeletal contraction experiments from literature.

SPEAKER: JIACHENG ZUO *(University of British Columbia)*

Single-Molecule Force Spectroscopy Studies of Missense Titin Mutations That Are Likely Causing Cardiomyopathy

The giant muscle protein titin plays important roles in heart function. Mutations in titin have emerged as a major cause of familial cardiomyopathy. Missense mutations have been identified in cardiomyopathy patients; however, it is challenging to distinguish disease-causing mutations from benign ones. Given the importance of titin mechanics in heart function, it is critically important to elucidate the mechano-phenotypes of cardiomyopathy-causing mutations found in the elastic I-band part of cardiac titin. Using single-molecule atomic force microscopy (AFM) and equilibrium chemical denaturation, we investigated the mechanical and thermodynamic effects of two missense mutations, R57C-I94 and S22P-I84, found in the elastic I-band part of cardiac titin that were predicted to be likely causing cardiomyopathy by bioinformatics analysis. Our AFM results showed that mutation R57C had a significant destabilization effect on the I94 module. R57C reduced the mechanical unfolding force of I94 by ~30-40 pN, accelerated the unfolding kinetics, and decelerated the folding. These effects collectively increased the unfolding propensity of I94, likely resulting in altered titin elasticity. In comparison, S22P led to only modest destabilization of I84, with a decrease in unfolding force by ~10 pN. It is unlikely that such a modest destabilization would lead to a change in titin elasticity. These results will serve as the first step toward elucidating mechano-phenotypes of cardiomyopathy-causing mutations in the elastic I-band.

SPEAKER: TIANYU DUAN *(University of British Columbia)*

Protein Hydrogels with Reversibly Patterned Multidimensional Fluorescent Images for Information Storage

Fluorescent polymeric hydrogels are promising building blocks for constructing soft and wet information storage media that are desirable in developing flexible electronics for various applications. Hydrogels based on engineered proteins have attracted considerable interests. However, their potential utility as soft and wet information storage media has remained largely unexplored. Here we report the engineering of a protein-based hydrogel that can serve as a media for reversible information storage. Using the light-controlled association and dissociation of LOVTRAP, which consists of the light-responsive protein LOV2 and its designed protein binding partner ZDark1 (zdk1), we developed a novel strategy that enabled us to readily decorate/release fluorescent proteins onto/from a blank protein hydrogel slate in a light-controlled and spatially defined fashion, leading to the generation of fluorescent patterns that can be reversibly written and erased. Such patterns can be used readily to store information, resulting in protein hydrogel-based soft and wet information storage media, such as bar codes and quick response (QR) codes. To increase the information storage capacity, we successfully developed greyscale patterning of protein hydrogels based on LOVTRAP to generate pseudo colored multi-dimensional fluorescence images. The information written on these protein hydrogels is stable and can be erased, representing a novel reversible information storage approach in soft and wet materials. Our results open a new avenue towards developing next-generation protein-based smart materials for information storage and anti-counterfeit applications.

SPEAKER: DANE MARIJAN *(Simon Fraser University)*

Intrinsic Protein Structural Properties Regulate Physiological Amyloid Aggregation

All cells must respond to changing conditions if they are to survive. One of the ways mammalian cells react to harsh stimuli is by forming physiological, RNA-seeded amyloid bodies (A-bodies) within their nuclei (Audas et al. 2016). Amyloids are highly organized, very degradation resistant protein structures commonly associated with debilitating diseases such as Alzheimer's and Parkinson's, earning a reputation of an irreversible, pathological protein state. Amyloid bodies, however, quickly disassemble upon stimulus termination, highlighting that amyloid aggregation does not always generate toxic structures. The proteomes of A-bodies induced by different stressors vary significantly, and their constituent proteins can be sequestered by one or more stressors (Marijan et al. 2019). This would suggest that cells use this pathway to selectively immobilize and inactivate only a particular subset of proteins under a given condition, thereby providing a tailored response. However, the molecular mechanisms that determine whether a protein is targeted to A-bodies during a particular stimulus have so far been largely unknown. Here, by using two highly related proteins as a model system, we have identified critical structural elements that regulate their heat shock specific amyloid aggregation. Our data shows that selectively modifying distinct, stabilizing structural pockets can either induce or restrict the A-body targeting of these proteins. We propose a model where A-body targeting during heat shock is regulated by the intrinsic structural stability of proteins, acting as thermal switches that expose or conceal their own A-body targeting motifs. Furthering understanding of the mechanisms of stimulus specific amyloid aggregation and its consequences on cell function will advance fundamental knowledge of how cells interact with their surroundings, both in physiological and pathological settings, and clue into how these pathways can be dysregulated.

SPEAKER: SHAWN HSUEH *(University of British Columbia)*

First Principles Calculation of Protein-Protein Dimer Affinities of ALS-Associated SOD1 Mutations

Cu,Zn superoxide dismutase (SOD1) is a 32 kDa homodimer that converts toxic oxygen radicals in neurons to less harmful species. The dimerization of SOD1 is essential to the stability of the protein. Monomerization increases the likelihood of SOD1 misfolding into conformations associated with aggregation, cellular toxicity, and neuronal death in familial amyotrophic lateral sclerosis (fALS). The ubiquity of disease-associated mutations throughout the primary sequence of SOD1 suggests an important role of physicochemical processes, including monomerization of SOD1, in the pathology of the disease. Herein, we use a first-principles statistical mechanics method to systematically calculate the free energy of dimer binding for SOD1 using molecular dynamics, which involves sequentially computing conformational, orientational, and separation distance contributions to the binding free energy. We consider the effects of two ALS-associated mutations in SOD1 protein on dimer stability, A4V and D101N, as well as the role of metal binding and disulfide bond formation. We find that the penalty for dimer formation arising from the conformational entropy of disordered loops in SOD1 is significantly larger than that for other protein–protein interactions previously considered. In the case of the disulfide-reduced protein, this leads to a bound complex whose formation is energetically disfavored. Somewhat surprisingly, the loop free energy penalty upon dimerization is still significant for the holoprotein, despite the increased structural order induced by the bound metal cations. This resulted in a surprisingly modest increase in dimer binding free energy of only about 1.5 kcal/mol upon metalation of the protein, suggesting that the most significant stabilizing effects of metalation are on folding stability rather than dimer binding stability. The mutant A4V has an unstable dimer due to weakened monomer-monomer interactions, which are manifested in the calculation by a separation free energy surface with a lower barrier. The mutant D101N has a stable dimer partially due to an unusually rigid β-barrel in the free monomer. D101N also exhibits anticooperativity in loop folding upon dimerization. These computational calculations are, to our knowledge, the most quantitatively accurate calculations of dimer binding stability in SOD1 to date.

SESSION 2

SPEAKER: SINA FALAKIAN *(Simon Fraser University)*

Inference of the DNA Replication Kinetics in Human Genomes

In almost all organisms, genetic information is stored in DNA molecules. Replication of DNA is nature's way of copying information and is an essential part of life. In the human genome, this process occurs at a rate of roughly 100,000 bases per second inside a nucleus having a diameter of about 1 µm, which is remarkable. To understand this process, experimental techniques have been developed since the 1980s, and they are still developing. The kinetics of the replication process in bacteria and yeast have been fairly well understood using the experiments. But the widely used experimental techniques have not been able to provide enough data to understand the kinetics of this process in humans. In this talk, I will briefly review how the replication process works and how experimental techniques have been used to infer this process. Then we will see how a new experimental technique called Optical Replication Mapping (ORM) might be a good candidate for understanding the replication process in the human genome, and I will discuss how we are going to use stochastic modelling of experimental data to shed light on this phenomenon.

SPEAKER: JANNIK EHRICH *(Simon Fraser University)*

Ratchets, Ratchets Everywhere! How Information Can Fuel Molecular Machines and Why You Should Care

Thermal fluctuations and large energy inputs drive molecular machinery away from thermal equilibrium. Quantifying the energy requirements of molecular machinery necessitates a stochastic theory of nonequilibrium thermodynamics. Interestingly, such a stochastic description also elucidates how information is used to rectify fluctuations and build micron-sized devices that apparently violate the second law of thermodynamics. In this talk, I make a case for considering information flows alongside energy flows in the study of molecular machines and show how information plays an important role in the simplest molecular motor model.

SPEAKER: MOHAMMAD-REZA ROKHFOROUZ *(University of British Columbia)*

Brownian Dynamics Simulation of Nanoparticle Transport Through Mucus

Successful transmucosal delivery promises great therapeutic opportunities for the treatment of various diseases such as cystic fibrosis and chronic obstructive pulmonary disease (COPD). Despite promising results in preclinical stages, nanoparticle (NP) for drug delivery still remains insufficiently explored, thereby limiting effective clinical translation. Using Brownian dynamics simulations, we investigated NP-mucus interactions under the influences of steric, electrostatic, and hydrogen bond interactions. The mucus is modeled as a cubic periodic box comprised of straight, rigid, infinitely long rods. To obtain statistically reliable diffusion coefficients, we considered one thousand non-interacting spherical particles over at least 106 steps. The results revealed that both the electrostatic repulsion and the H-bond attraction, if acting alone, will hinder diffusivity. But one factor can mitigate the effect of the other to raise the diffusivity. For stronger electrostatic repulsion, the diffusivity will eventually fall again because of too much repulsive forces. Our results may provide new insights into rational design for mucus-penetrating nanoparticles.

SPEAKER: MATTHEW LEIGHTON *(Simon Fraser University)*

Dynamic and Thermodynamic Bounds for Collective Motor-Driven Transport

Molecular motors work collectively to transport cargo within cells, with anywhere from one to several hundred motors towing a single cargo. For a broad class of collective-transport systems, we use tools from stochastic thermodynamics to derive a new lower bound for the entropy production rate which is tighter than the second law. This implies new bounds on the velocity, efficiency, and precision of general transport systems and a set of analytic Pareto frontiers for identical motors. In a specific model, we identify conditions for saturation of these Pareto frontiers.

SESSION 3

SPEAKER: MIRANDA LOUWERSE *(Simon Fraser University)*

Multidimensional Minimum-Work Control of a 2D Ising Model

A system's conformation can be manipulated by dynamic variation of control parameters, for example by changing magnetic fields applied to spins in an Ising model. When the driving is performed in finite time, the system is driven out of equilibrium and excess work (work above the free-energy change) is done on the system which is dissipated as heat. Here, we study protocols that drive a 2D ferromagnetic Ising model to flip from the all-down to all-up configuration. A linear-response approximation to excess work is employed, which identifies minimum-work protocols as geodesics of a generalized friction metric in control-parameter space between protocol endpoints. The generalized friction quantifies the system's resistance to changes in control parameters during finite-time driving.

In this work, we explore features of the generalized friction and minimum-work protocols when multiple control parameters are used to drive the system, comparing with driving using a single control parameter. We find a significant decrease in excess work during multidimensional protocols and analyze aspects that improve their efficiency relative to one-dimensional control. The multidimensional protocol avoids high-friction regions of control-parameter space and flattens the energy landscape (which decorrelates spins), speeding up the rate-limiting spin-inversion reaction and keeping the system close to equilibrium for a wide range of protocol duration. We discuss how these factors reduce excess work, leading to general principles of multidimensional minimum-work control that could be investigated in other systems.

SPEAKER: STEVEN BLABER *(Simon Fraser University)*

Efficient Two-Dimensional Control of Barrier Crossing

Modern advances in single-molecule biophysics make possible the precise spatial and temporal control of biological systems. Despite the relative freedom of control, experiments and simulations rarely exploit the possibility of optimized control protocols, and the ones that do are generally limited to optimization of a single control parameter. We design minimum-dissipation protocols for harmonic trapping potentials under two-dimensional control (of both trap center and stiffness), for driven barrier crossing. This greater control allows specification of both the time-dependent mean and variance of the position distribution, and results in qualitatively distinct designed protocols. For any duration, the designed protocols significantly improve performance in terms of both dissipation and flux compared to naive and one-dimensional control.

SPEAKER: GUOJUN CHEN *(University of British Columbia)*

Templated Folding of the RTX Domain of the Bacterial Toxin Adenylate Cyclase Revealed by Single Molecule Force Spectroscopy

The bacterial toxin adenylate cyclase (CyaA) is the key virulence factor of whooping cough. Upon secretion from the bacterial cytosol, CyaA binds extracellular Ca2+ and folds into a functional form that can invade host cells. The RTX domain, which is located at the C-terminus of CyaA and contains five blocks of tandemly arranged RTX repeats (RTX-i to RTX-v), plays important roles in ensuring the efficient secretion of CyaA toxin, and mediating CyaA toxin activity and pathogen virulence. It has been shown that the C-terminal capping structure of RTX-v is critical for the folding of the whole RTX domain. However, it remains unknown how the folding signal transmits within the RTX domain from RTX-v to RTX-i. Here we used single molecule optical tweezers to investigate the folding mechanism of RTX-iv and its influence by the folding of RTX-v. Our results revealed that RTX-iv alone is intrinsically disordered even in the presence of 10 mM Ca2+, but folds into a Ca2+-loaded β-roll structure in the presence of a folded RTX-v. By directly monitoring the folding trajectories of RTX-iv-v, we showed that the folding of RTX-iv is strictly conditional upon the folding of the whole RTX-v. Our results demonstrate that the folding of RTX-iv is templated by the folding of RTX-v. This templated folding effect not only allows RTX-iv to fold rapidly, but also leads to a significant mutual stabilization effect between RTX-iv and RTX-v. This templated folding provides a possible molecular mechanism allowing for the transmitting of the folding signal along the chain of the RTX domain.

SPEAKER: ERIC JONES *(Simon Fraser University)*

How Do Organisms Acquire Their Gut Microbiomes?

Observational studies reveal substantial variability in microbiome composition across individuals. While some of this variability can be explained by external factors like environmental, dietary, and genetic differences between individuals, here we show that the process of microbiome assembly is inherently stochastic for the model organism Drosophila melanogaster. Individuals are constantly exposed to microbial organisms that may or may not colonize their gut microbiome, and this contributes a baseline level of microbiome variability even among organisms that are identically reared, housed, and fed. In germ-free flies fed known combinations of bacterial species, we find that some species colonize more frequently than others even when fed identically. Incorporating context-dependent interactions substantially improves our ability to explain the observed variability in colonization outcomes. Stochastic, context-dependent microbiome assembly underlies clinical therapies like fecal microbiota transplantation and probiotic administration, and is relevant for the design of synthetic fecal transplants and dosing regimes.