Poster Abstracts

Biomechanical Forces of Cell Attachment

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Cell-derived biomechanical forces are essential for cellular proliferation and migration and in order for any of these processes to occur a cell must first attach to its underlying substrate. However, the role cell-substrate stresses, which we call tractions, play in cell attachment is unclear. In this study, single human umbilical vein endothelial cells (HUVECs) were seeded in suspension onto soft, polyacrylamide gels and allowed to attach and spread for approximately one day. Within the first five minutes of imaging, cells begin to immediately attach to the gel surface and tractions could be observed. Tractions steadily increased until 3 hours. Since tractions can be measured directly following the brief settling period before imaging, this work suggests that HUVECs begin attachment and exerting tractions soon after seeding with some cells attaching well before two hours. We also measured cell area and velocity which, was observed to increase and remain unchanged respectively, as cells attached and spread. Understanding endothelial cell attachment and detachment will allow researchers to better determine the link between several disease pathologies that result in endothelial dysfunction, which can lead to detachment and circulation of mature endothelial cells in the bloodstream and is considered to be one of the indicators of the development of various forms of cardiovascular disease. In addition, we believe this work will increase understanding of the role cell-derived biomechanical forces play in cell attachment.

The Effect of Organic Osmolytes on Actin Bundling by Chlamydia trachomatis Tarp

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The actin cytoskeleton is often reorganized by bacterial pathogens to aid pathogen entry into the host cell. *Chlamydia trachomatis* infection requires the translocated actin-recruiting phosphoprotein (Tarp), whose function is to alter the cytoskeleton to assist in internalizing the bacterium. Trimethylamine N-oxide (TMAO) is a natural organic osmolyte which regulates osmotic stress and stabilizes cellular structure. Due to its ability to stabilize proteins in aqueous conditions, we hypothesize that TMAO will aid Tarp in restructuring actin filaments by increasing the bundling efficiency of Tarp. In this study, we use TIRF microscopy and biophysical analysis of the bending persistence length (Lp) of filaments to characterize bundle formation over time and the effects of TMAO and Tarp on the mechanical properties of actin bundles. Our results suggest that moderate concentrations of TMAO increase the bundling efficiency of Tarp while also forming bundles with more rigidity. Defining the effect of TMAO on Tarp-induced bundles is important for further understanding the infectious mechanism of actin cytoskeletal reorganization by *C. trachomatis* on human host cells.

Molecular Basis for Actin Polymerization Kinetics Modulated by Solution Crowding

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Actin polymerization drives cell movement and provides cells with structural integrity. Intracellular environments contain high concentrations of solutes including organic compounds, macromolecules, and proteins. Macromolecular crowding has been shown to affect actin filament stability and bulk polymerization kinetics. However, the molecular mechanism behind how crowding influences individual actin filament assembly is not well understood. In this study, we investigate how crowding modulates filament assembly kinetics using total internal reflection fluorescence (TIRF) microscopy imaging and pyrene fluorescence assays. The elongation rates of individual actin filaments analyzed from TIRF imaging depended on the types of crowding agents (polyethylene glycol, bovine serum albumin, and sucrose) as well as their concentrations. Further, we utilized the all-atom molecular dynamics (MD) simulations to evaluate the effects of crowding on diffusion coefficients of actin monomers during assembly. Taken together, our data suggest that solution crowding can regulate actin assembly kinetics at the molecular level.

Controlled Phase Behavior of the Responsive Polymer Poly (N-isopropylacrylamide) at Variable Temperature and Pressure

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Responsive polymers undergo significant changes in their physical properties (e.g. a reversible demixing transition) in response to external stimuli such as temperature and pressure. Poly(*N*-isopropylacrylamide) (PNIPAM) is water-soluble near physiological temperatures. However, as the temperature is increased, it undergoes a phase separation, and the solution becomes cloudy at the lower critical solution temperature (LCST). At the transition, the polymer chains collapse and are accompanied by hydration changes. This phase transition can also be induced by pressure, where the formation of larger scale structures (mesoglobules) in the two-phase region is observed. Our group studies the temperature-and-pressure-dependent phase behavior of the thermoresponsive polymer, PNIPAM, and its dynamic self-assembly qualities. PNIPAM was dissolved in aqueous solution and securely sealed inside a microcapillary cell. The formation of mesoglobules is investigated using optical microscopic imaging at variable temperature and pressure. Micro-Raman spectroscopic measurements provide information on changes in chemical bonding and hydration across the transition. The formation of mesoglobules is explored along different pathways in the temperature-pressure plane. This research contributes to an understanding of liquid-liquid phase separation in responsive polymers with relevance for the design of smart materials including biosensors, drug delivery systems, and other microscale biomedical implementations.

Peptide-based Inhibitors of Amyloid Beta Aggregation and Toxicity

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Neurotoxic aggregates of amyloid beta (Aβ) peptide contribute to the etiology of Alzheimer's disease. $A\beta_{1-42}$ forms oligomeric structures that undergo further aggregation into protofibrils and fibrils. Previous work has shown that seven overlapping fragments of $A\beta_{1-42}$ can disrupt the process of fibril formation, possibly through intercalation into the growing aggregate. The potential neuroprotective properties of these fragments were not directly examined. In this project, $A\beta_{1-42}$ was allowed to aggregate in aqueous buffer for 2, 4, or 24 h in the absence and presence of an Aβ fragment, at 1:2 $A\beta_{1-42}$/fragment molar ratio. Neuronal PC12 cells were then incubated with the samples for 50 h before conducting a cell viability assay. Thioflavin-T fluorescence assays to detect the fibrillar form of $A\beta_{1-42}$ were also conducted. There was an inverse correlation between the time span of $A\beta_{1-42}$ aggregation and cellular toxicity, with the 2 h sample exhibiting the greatest level of toxicity. Aβ fragments displayed varying effects on the inhibition of $A\beta_{1-42}$ fibrillogenesis and suppression of its toxicity. Three fragments completely neutralized the toxicity of the 2 h aggregate, while two others conferred weak cellular protection against $A\beta_{1-42}$ toxicity. One fragment enhanced $A\beta_{1-42}$ toxicity at all stages of aggregation. Every fragment disrupted fibril formation to some extent, but there was no obvious correlation between the inhibition of $A\beta_{1-42}$ aggregation and the inhibition of cellular toxicity. This suggests that fragment intercalation into the growing fibril may block further aggregation but not the toxicity of oligomeric $A\beta_{1-42}$. Fragments that block both $A\beta_{1-42}$ aggregation and toxicity represent potential targets for therapeutic development.

Spectroscopic and biochemical analysis of DNA light-up aptamer complexes with environment-sensitive dyes

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Fluorescent light-up aptamers (FLAPs) are single stranded DNA/RNA molecules that bind and thereby enhance fluorescence of fluorogenic, environment sensitive, dyes. They have found application in sensing and intracellular monitoring of biomolecules. DNA FLAPs are promising for reporting biorecognition events in non-cellular applications. The mechanism behind the ability of FLAPs to turn-on fluorescence of their ligands rely on inhibition of non-radiative relaxation due to sequestering of the dye in a complex within the aptamer stabilized by such interactions as hydrogen-bonding and p-stacking, among others. Many FLAPs have guanine (G) quadruplexes as a structural element forming the dye-binding sites. In this work, we study the structure of DNA FLAPs – dapoxyl binding aptamers DAP1 and DAP-10-42. Via spectroscopic and biochemical techniques, we have confirmed the presence of G-quadruplex domains in both aptamers and suggested guanines involved in their formation. Based on rational mutagenesis of the DAP-10-42 sequence and effect of nucleotide substitutions on the spectroscopic properties of the aptamer-dye complexes, we have proposed location of the dye-binding site of the aptamer in the junction between its G-quadruplex and duplex domains. We have also shortened the sequence of the functionally active DAP1 from 76 to 47 nucleotides to promote utilization of the aptamer in biosensing applications. Understanding of interactions involved in the formation of dye-aptamer complexes would enable full development of the potential of DAPs to serve as label-free signal reporters in bioanalytical assays.

Effect of Molecular Crowders on the Activation of Cholera Toxin by Protein Disulfide Isomerase

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Cholera toxin (CT) is a classic A-B type protein toxin that has an A subunit (A1 + A2) and a pentameric B subunit. The catalytic A1 domain is linked to the A2 domain via a disulfide linkage. CTA1 must be dissociated from the rest of the toxin to cause a cytopathic effect. Protein disulfide isomerase (PDI) can reduce the CTA1/CTA2 disulfide bond, but disassembly of the reduced toxin requires the partial unfolding of PDI that occurs when it binds to CTA1. This unfolding event allows PDI to push CTA1 away from the rest of the toxin.

My research question is whether the efficiency of PDI in disassembling CT would be affected by molecular crowding, where a dense internal cell environment is recreated in vitro by the use of chemical agents such as ficoll. This will give insight on how CT behaves inside a cell. Our hypothesis was that molecular crowding would make CTA1 disassembly more efficient by recreating the tight packing of macromolecules in cells, which provides an extra nudge to enhance toxin disassembly. We then used enzyme-linked immunosorbent assays (ELISAs) and a biochemical assay to determine how molecular crowders affect the binding, reduction, and disassembly of CT by PDI. Our results will bring about a deeper understanding of the cellular events that may affect the course of a cholera infection.

From the preliminary results, molecular crowders increased PDI's ability to bind to CTA1 and did not prevent PDI from cleaving the CTA1/CTA2 disulfide bond. Based off the disassembly results, molecular crowders reduced PDI's ability to displace CTA1 from the rest of the toxin. This contradicts our original hypothesis. Our new hypothesis is that crowders block PDI unfolding, which is required for CT disassembly. Biophysical experiments using Fourier Transform Infrared Spectroscopy will test this prediction in future work.

The Fate of the CdtA Subunit from the *Haemophilus Ducreyi* Cytolethal Distending Toxin

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Cytolethal distending toxin (CDT) is a virulence factor produced by many Gram-negative bacteria, including *Haemophilus ducreyi*, the causative agent of genital cancroid. CDT is a heterotrimeric toxin consisting of a cell-binding domain (CdtA + CdtC) and a catalytic domain (CdtB) that has DNase activity. After binding to the host plasma membrane, CDT undergoes endocytosis and travels to the endoplasmic reticulum (ER). Only then does CdtB move into the nucleus, causing DNA damage that induces cell-cycle arrest and apoptosis. The previous CDT trafficking model suggested that CdtA remains on the plasma membrane while the CdtB/CdtC heterodimer is transported inside the cell. This model is based on experiments that were unable to detect CdtA inside the host cell. Here, we examine this model and demonstrate that CDT is internalized as an intact holotoxin. Furthermore, the acidification of the endosomes induces CdtA release from the CdtB/CdtC heterodimer. Using a cell-based ELISA, we report that CdtA facilitates CDT binding to the plasma membrane and demonstrate that nearly the entire pool of surface-bound toxin is internalized from the plasma membrane within 20 minutes. As determined by Western blot, all of internalized CdtA and most of internalized CdtB and CdtC are rapidly degraded in the lysosomes. Acidic conditions found in the early endosomes (pH 6.0-6.3) led to a loss of protein structure in CdtA and its release from the CDT holotoxin as determined by circular dichroism and surface plasmon resonance, respectively. These results demonstrate that CDT is internalized as an intact holotoxin, with the acidic environment of endosomes triggering the separation of CdtA from the CdtB/CdtC heterodimer.

Structure of Unmodified and Pyroglutamylated Amyloid Beta Peptides in Lipid Membranes

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Alzheimer's disease (AD) is a neurodegenerative disorder that causes brain atrophy, neuronal and synaptic loss, cognitive decline, and death. A major histopathological trait of AD is extracellular deposition of amyloid-beta (Aβ) peptide in brain parenchyma and vasculature, but the most toxic species are the intracellular oligomeric forms of Aβ. Aβ occurs in different lengths; the most abundant and toxic forms are 40- and 42-residue peptides, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$. Pyroglutamylated Aβ species (pEAβ) are formed naturally by enzymatic action and are hypertoxic. An established mechanism of Aβ cytotoxicity is disruption of cellular ionic homeostasis by modulation of Ca^{2+}-permeable ion channels in plasma, endoplasmic reticulum, or mitochondrial membranes, leading to dysregulation of neurotransmission and other neuronal functions. Aβ has also been documented to directly form pores in cell membranes, but visualization and structural/functional characterization of Aβ pores have yet to be achieved. Here, we report structural characterization of four Aβ species, i.e., $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}40}$, $pEA\beta_{3\text{-}42}$, and $pEA\beta_{3\text{-}40}$, reconstituted in supported lipid membranes by polarized Fourier transform infrared (FTIR) spectroscopy. Our data indicate that all four peptides in lipid membranes contain β-sheet, a-helix, b-turn and irregular structures. $A\beta_{1\text{-}42}$ assumes the highest b-sheet fraction (44%), followed by $pEA\beta_{3\text{-}42}$, $pEA\beta_{3\text{-}40}$, and $A\beta_{1\text{-}40}$. The tilt angle of β-strands relative to the membrane normal was between 30 and 38 degrees. Overall, data suggest formation of b-barrel-like membrane pores by $A\beta_{1\text{-}42}$ and $pEA\beta_{3\text{-}42}$, which may exert their cytotoxic effect by causing membrane leakage and loss of cellular ionic balance.

Electrokinetic Brownian Dynamics of Current Blockade of a dsDNA through a Nanopore*

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The Brownian Dynamics (BD) simulation technique has proven to be a valuable tool for investigating the intricate process of long double-stranded DNA (dsDNA) translocation through nanopores, offering insights into the physical behavior of such systems [1-3]. In this study, we extend the BD approach to incorporate the movements of co-ions and counter-ions alongside a short-chain dsDNA, decorated with oligo-flap markers, through a nanopore. This allows for the direct calculation of current blockade (CB) from electrokinetic Brownian dynamics (EKBD) simulations, while also enabling comparison to a simpler but computationally efficient volumetric CB model. By evaluating both CB data sets, we tune the adjustable parameters of the volumetric CB model, resulting in highly accurate CB data for a very long dsDNA strand (~Mbp) at a significantly reduced computational cost. Additionally, we validate our findings by comparing the Peclet number obtained from simulations with experimental data. Furthermore, we investigate the influence of mono- and divalent ion concentration on the noise levels in CB and translocation speed, with potential implications for improving the signal-to-noise ratio of nanopore CB data.

Gelsolin-Mediated Actin Filament Severing and Conformational Changes by Various pH

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The actin cytoskeleton plays a key role in controlling various cellular processes including muscle contraction and cell signaling. Numerous actin binding proteins (ABPs) regulate the assembly/disassembly dynamics and mechanics of the actin cytoskeleton. Gelsolin is a calcium-regulated ABP that nucleates, severs and caps actin filaments. Previous studies have shown that both pH and free calcium ions increase the rates of actin filament elongation and severing by gelsolin. However, the effects of pH alone on gelsolin-mediated actin assembly kinetics and severing are not well understood. We hypothesize that at lower pH, the formed gelsolin-actin complex will have greater severing efficiency compared to neutral pH due to conformational changes in both gelsolin and actin. In this study, we investigate gelsolin-mediated filament severing activities and assembly/disassembly kinetics at various pH conditions utilizing total internal reflection fluorescence microscopy and pyrene fluorescence assays. We demonstrate that gelsolin-mediated filament severing rates and filament assembly/disassembly kinetics were increased at low pH. Furthermore, we used atomic force microscopy (AFM) to visualize conformational changes of gelsolin-induced filaments at various pH conditions. This work will allow us to understand the gelsolin-mediated actin regulatory mechanism in living cells.

Distinct Pore-Forming Conformation of Amyloid Beta Peptide Aβ1–42 in Membrane Environments

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The extracellular accumulation of fibrillar assemblies of amyloid beta (Aβ) peptides in patients' brains is a hallmark of Alzheimer's disease. The interactions of lipid membrane and Ab peptides are known to further modulate the assembly and cytotoxicity of Ab peptides. However, there is no consensus regarding the effect of their interactions.

In this work, we reconstituted $A\beta_{1\text{-}42}$ peptides in lipid bilayers emulating various important components (POPC: POPG: Cholesterol = 6 : 3 : 1) in the cell membrane and applied solid state NMR (ssNMR) to characterize the resulting structure. Our ssNMR results found that $A\beta_{1\text{-}42}$ peptides adopt a distinct conformation from those observed previously, due to the presence of lipids. The charge-residue populated N-terminus forms two short pieces of b-strands, with considerable flexibility. The C-terminus comprises two long pieces of b-strands with a short one at the end of the peptide. The observed non-sequential contacts indicate that the peptide assumes an extended parallel b-sheet format. The relative position between the lipid acyl chain and $A\beta_{1\text{-}42}$ peptides was measured by heteronuclear correlation experiments, which suggests that the peptide is inserted deep into the lipid bilayer. Based on our NMR restraints, molecular dynamics simulations were performed to establish a high-resolution model. It indicates that $A\beta_{1\text{-}42}$ aggregate to form a pore-like structure in the bilayer lipids, which can disrupt the membrane integrity and may lead to ion leakage.

High-resolution, High-contrast Imaging via Deep-learning Based Two-step Image Restoration

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Widefield volumetric imaging is essential for studying live cells and tissues, typically done by serially z-scanning, deconvolution, and obtaining a maximum/average intensity projection. Despite its high resolution and high contrast, this approach has a low throughput and high photodamage due to multiple z-scanning of each field-of-view (FOV). To increase the throughput of a microscope, its PSF can be axially elongated using a low numerical aperture (NA) objective lens. However, a lower NA results in degrading the lateral resolution. To overcome these limitations, here we present a two-step deep learning framework that transforms recorded data under a low light-dose condition with a low NA objective (low SNR & low resolution) to average intensity projection (AIP) deconvolved images (high SNR & high resolution). Our network architecture combines an encode-decoder network (UNet) and a residual channel attention network (RCAN) first to improve the SNR of a noisy image and second to enhance its contrast and resolution. This two-step image restoration approach will be a powerful tool in long-term live-cell imaging and fast high-throughput imaging.