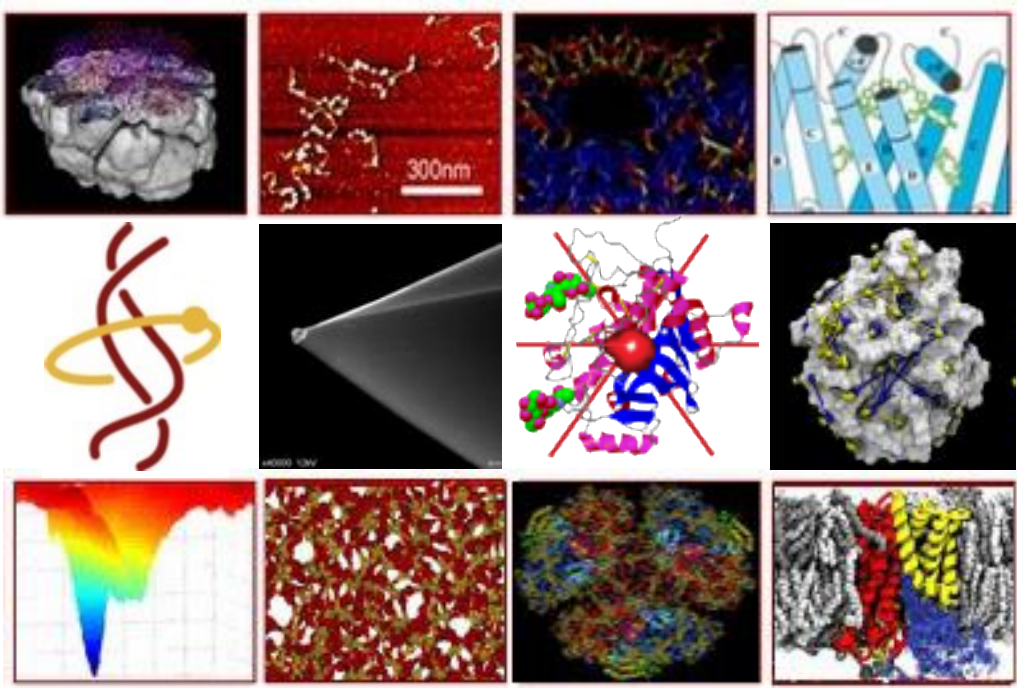
BioPhest



BioPhest 2015 ASU Arizona State University

Saturday May 2, 2015

Tempe, Arizona

ASU & A

ABSTRACTS

Talk Presentations

9:00AM **Sean Seyler** slseyler@asu.edu
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Physics, Arizona State University

Path Similarity Analysis: a method for quantifying macromolecular transition paths

Diverse classes of proteins function through large-scale conformational changes and various sophisticated enhanced sampling methods have been proposed to generate these macromolecular transition paths. Because such paths are curves in a high-dimensional space, they have been difficult to compare quantitatively, a prerequisite to, for instance, assess the quality of different sampling algorithms. We introduce a comprehensive method—"Path Similarity Analysis" (PSA)—that enables us to quantify the similarity between two arbitrary paths and extract the atomic-scale determinants responsible for their differences. PSA employs the Hausdorff or Fréchet metrics—adopted from computational geometry, for quantifying the degree of similarity between piecewise-linear curves—to utilize the complete information in 3N-dimensional trajectories in configuration space. It thus avoids relying on projections into low-dimensional spaces, as used in traditional approaches. We tested PSA on a toy system to examine the effect of path roughness induced by thermal fluctuations and to elucidate the principles of the approach. We used the closed-to-open transition of the enzyme adenylate kinase (AdK) in its substrate-free form as an example to compare a range of path-sampling algorithms. These methods included the molecular dynamics-based dynamic importance sampling (DIMS-MD) method and nine publicly accessible servers, which included elastic network-based and purely geometric approaches such as FRODA. For the AdK transition, the new concept of a Hausdorff-pair map enabled us to extract the molecular structural determinants responsible for differences in transition paths, namely a set of conserved salt bridges whose charge-charge interactions are fully modeled in DIMS-MD but not in FRODA. PSA has the potential to enhance our understanding of path-sampling algorithms, validate them, and ultimately help guide future research toward deeper physical insights into conformational transitions.

9:25AM **Ioanna Zoi** ioannazoi@email.arizona.edu
Chemistry and Biochemistry, University of Arizona

Homologous enzymes have distinct reaction paths

Bacterial enzymes Escherichia coli and Vibrio cholerae 5’ -Methylthioadenosine nucleosidases (MTANs) have different binding affinities for the same transition state analogue. This was surprising as these enzymes share 60% sequence identity, have almost identical active sites and act under the same mechanism. We performed Transition Path Sampling simulations of both enzymes to reveal the atomic details of the catalytic chemical step, to explain the inhibitor affinity differences. Unlike EcMTAN, VcMTAN has multiple distinct transition states, which is an indication

that multiple sets of coordinated protein motions can reach a transition state. We also identified the important residues that participate in each enzyme's reaction coordinate and explained their contribution. Subtle dynamic differences manifest in difference of reaction coordinate and transition state structure and also suggest that MTANs differ from most ribosyl transferases. As experimental approaches report averages regarding reaction coordinate information, this study offers, previously unavailable, detailed knowledge to the explanation of bacterial MTANs catalytic mechanism, and could have a significant impact on pharmaceutical design.

9:50AM **Dmitry Matyushov** dmitrym@asu.edu
Chemistry and Biochemistry; Physics, Arizona State University

Is thermodynamics OK for bioenergetics?

Simple physical mechanisms are behind the flow of energy in all forms of life. Energy comes to living systems through electrons occupying high-energy states, either from food (respiratory chains) or light (photosynthesis). Life's ability to transfer electrons over large distances with nearly zero loss of free energy is puzzling and has not been accomplished so far in synthetic systems. I discuss the emerging understanding of how this energetic efficiency is realized. Ergodicity is often broken in protein-driven reactions and thermodynamic free energies become irrelevant. Breaking the grip of thermodynamics allows for an efficient optimization between the rates of individual electron-transfer steps and the spectrum of relaxation times. Time, it appears, plays as significant role as the free energy in optimizing biology's performance.

10:45AM **Ashini Bolia** abolia@asu.edu
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How hinge flexibility alters the glycan binding affinity of Cyanovirin

Cyanovirin-N (CVN) is a cyanobacterial lectin with potent anti-HIV activity, mediated by binding to Manα(1,2)Manα with high affinity and specificity. These sugar-binding sites are located in two quasi-symmetric domains (A and B) of the CVN protein. The hinge region is an integral part of the high affinity-binding site of CVN and mutations in this hinge region dictate the preferential oligomerization state of CVN. Constructs with the P51G mutation preferentially exists as monomers whereas wild type cyanovirin can form domain swapped dimers in certain conditions. In this study, we investigated whether the P51G mutation affects the shape, flexibility and binding affinity of domain B for dimannose through both computational and experimental strategies. Our computational analysis shows that enhanced flexibility leads to blocking of the binding site by N53 position. Moreover, at elevated temperatures an interaction between N53 and N42 can be established that further hinders access to the binding site. Our study also indicates that the capability of monomeric wild type CVN to resist mechanical perturbations is enhanced compared to constructs in which the hinge region is more flexible. Based on these results, we predicted that binding affinity for dimannose would be more favorable for cyanovirin constructs containing a wild type hinge region, whereas affinity would be impaired in the case of mutants containing P51G. Furthermore, the experimental characterization by isothermal titration calorimetry

(ITC) on a set of cyanovirin mutants confirms this hypothesis that constructs with P51G mutation are consistently inferior binders. Overall, our results suggests the fact that constructs where the hinge region is mostly unchanged from that of wt-CVN may be at an advantage for binding over other multivalent systems.

References:
• Bolia, A.; Woodrum, B. W.; Cereda, A.; Ruben, M. A.; Wang, X.; Ozkan, S. B.; Ghirlanda, G. A flexible docking scheme efficiently captures the energetics of glycan-cyanovirin binding. Biophys J. 2014. 106, 1142-1151.
• Woodrum, B.W.: Maxwell, J.; Bolia, A.; Ozkan, S. B.; Ghirlanda, G. The antiviral lectin cyanovirin-N: probing multivalency and glycan recognition through experimental and computational approaches. Biochem Soc Trans 2013. 41(5): p. 1170-6.

11:10AM **Sara Vaiana** sara.vaiana@asu.edu
Physics, Arizona State University

Evidence for internal friction in disordered proteins of the Ct family

The rate at which Intrinsically Disordered Proteins (IDPs) undergo large conformational changes in solution is expected to substantially affect their function. The roughness in the energy landscape of IDPs can significantly slow down this diffusional dynamics, giving rise to internal friction. Both experiments and simulations of unfolded states of proteins show that compact states exhibit greater internal friction than denaturant-expanded states. Yet it is unclear to what extent, and in which way, changes in a protein sequence may affect the internal friction. Using a nanosecond laser-pump spectroscopy technique we quantify the rate at which two ends of a polypeptide chain come into contact, while also quantifying the relative end-to-end distance (or compactness) of the chain. We compare these values for different IDPs of the Calcitonin peptide (Ct) family. Our previous studies showed that these IDPs populate highly compact states in near native conditions and become more expanded at increasing denaturant concentrations. We find that, under conditions in which the end-to-end distances are the same, certain sequences exhibit significantly slower contact formation rates than others, indicating greater internal friction. As expected, such differences are apparent in water but not in denaturant. We experimentally investigate the possible causes for the observed sequence-dependence.

11:35AM **Zahra BahramiDizicheh** zbahrami@asu.edu
Chemistry and Biochemistry, Arizona State University

Conversion of a b Type Cytochrome to a c Type: Unnatural Amino Acid Incorporation into Cytochrome b562

The sequence of a b-type cytochrome has been modified to introduce an unnatural amino acid, p-azido phenylalanine, using orthogonal aminoacyl-tRNA synthetase/tRNAAUC pairs that have been evolved to genetically encode the unnatural amino acid in response to the amber stop codon in Escherichia coli. We used cytochrome b562 (Cyt b562), a 106-residue monomeric heme protein, for which the X-ray structure is available. Two residues, which are close to the propionate group of heme, have been selected for artificial amino acid

incorporation, Glu4 and Glu8. Protein purification via HPLC, SDS gel electrophoresis, and mass spectroscopy have confirmed the purification of the modified Cyt b562 harboring the artificial amino acid. Furthermore, the introduced amino acid is able to covalently attach to chemically modified heme-cofactor via Staudinger ligation, as confirmed by MALDI-TOF mass spectrometry and HPLC. The covalent linkage to the heme and its ligation to two axial residues would increase the heme stability in its reduced form and impact its refolding ability. Future work will characterize the biophysical properties of the cyt cb562, specifically the ability of covalent modification to impact refolding stability.

2:00 PM **Jinghui Luo** jluo16@asu.edu
Chemistry and Biochemistry, Arizona State University

Deterministic absolute negative mobility induced for micron-sized particles in a microfluidic device

While efficient separation techniques in the micron- and submicron range are in demand for various analytical challenges related to nanotechnology and microbiology, current methods are not sufficient to distinguish species with similar density or surface properties. Here, we demonstrate a novel size-based separation method for µm-particles which is based on dielectrophoresis (DEP) in combination with a periodic electric field applied to a nonlinear post array in a tailored microfluidic device. We induce a non-intuitive migration of microparticles opposite to the direction of the average applied force, while smaller particles migrate normally under the same conditions. This observation is characteristic to absolute negative mobility (ANM). Due to the combination with DEP trapping which scales with the particle size, this approach is termed deterministic ANM (dANM) contrasting previously reported ANM based on random Brownian motion. dANM was investigated in both numerical simulations and experiments with µm-sized polystyrene beads and mice liver mitochondria. Excellent agreement between the model and experiment was observed. The resulting migration velocities were at least two orders of magnitude larger in the dANM approach compared to previous reports with similar sized particles for which ANM occurred. Moreover, the selectivity of dANM could potentially be applied in nanotechnological applications, organelle sub-population studies or the fractionation of protein nanocrystals.

2:25 PM **Joshua Sadar** jsadar@asu.edu
Physics, Arizona State University

Preparation of a sub-10 nm fluidic system with self-aligned nanogap electrodes for biomolecule characterization

Nanopore sensors, an emerging third-generation DNA sequencing technique with rapid speed, single-base sensitivity and long read lengths, exemplify a new strategy in the characterization of biomolecules. In such designs, the dimension of the sensor matches precisely with a single target molecule, so that the presence and/or motion of the molecule inside the sensor can generate measurable time-dependent electrical read-out signals containing significant local structural information. The capability of single-molecule level and label-free detection of sequence and post-transcription modification of DNA and protein molecules promise a new paradigm in both fundamental studies and biomedical applications in personal medicine. However,

existing techniques face great challenges such as the scalability and reproducibility of fabrication, lack of control of translocation, and low specificity in read-out signals. Here we propose a new framework of preparing a sub-10 nm fluidic system with the additional integration of a pair of embedded nanogap electrodes in a self-aligned manner. We will introduce our impedance-based feedback control system for the electrochemical deposition of metal on pre-defined nanoscale electrodes within a confined space to construct sub-10 nm nanopores with gate electrodes. Preliminary results of the nanogap will be presented and the mechanism of the feedback control process will be discussed. Our design can provide a promising platform for the scalable preparation of single-molecule characterization devices with active translocation control and additional readout mechanisms, including recognition tunneling signals and surface enhanced Raman spectrum.

2:50 PM **Bryant Doss** bldoss@asu.edu
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Physics, Arizona State University

AFM indentation reveals actomyosin-based stiffening of metastatic cancer cells during invasion into collagen I matrices

Atomic force microscopy (AFM) is a widely used technique to directly probe the mechanical response of mammalian cells to external forces to determine their elasticity. To date, the majority of AFM-based studies are limited to cells that are adhered to flat substrates, however these measurements lose information regarding cell mechanobiology in a physiologically relevant 3D microenvironment. We have performed combined AFM indentation and confocal fluorescence measurements on MDA-MB-231 metastatic breast cancer cells which have either partially or fully invaded into polymerized bovine collagen I matrices. In order to interpret the raw data from the experiments to determine the cells' elastic modulus, we have developed numerous analytical and simulation techniques. A sphero-conical tip geometry to represent an AFM probe with a spherical cap transitioning to a cone is derived and applied to analyze deep indentations into the cell-collagen layer. For partially invaded cells, a generalized bonded two-layered elastic half-space model is numerically solved to assist with decoupling the mechanical response of the collagen matrix from the cell. For fully embedded cells, finite element analysis is used to simulate an AFM indentation to extract their elastic moduli. Using these techniques, we demonstrate that the elastic modulus of MDA-MB-231 cells significantly increases by ~80% as they invade into collagen compared to cells on glass and cells on top of collagen. Inhibiting ROCK decreases the rigidity of cells on a surface as well as the magnitude of stiffening during invasion into collagen. These results corroborate recent actomyosin-based rounded cell motility models in 3D and demonstrate the ability of AFM to study cell mechanics in tissue-like environments.

3:45 PM **Soohyun K. Lee** keivin7shlee@email.arizona.edu
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Applications of Solid-State NMR Spectroscopy in Membrane Biophysics

Solid-state NMR (ssNMR) is a powerful tool to study the structure and dynamics of biomembranes. We implement ssNMR in two different ways: using oriented

samples (OS) and with magic-angle spinning (MAS) to the external magnetic field. Solid-state 2H NMR spectroscopy of perdeuterated membrane lipids yields average structures in relation to equilibrium properties. Additionally 2H NMR relaxation methods provide the time scales of the molecular motions over a wide frequency range, covering fast segmental motions to slow collective dynamics [1]. For random dispersions of phospholipids, residual quadrupolar couplings (RQCs) are obtained by de-Paking the powder-type 2H NMR spectra [2]. The RQC values give nearly complete profiles of the order parameters SCD(i) versus acyl segment position (i). One can then apply a statistical mean torque model to calculate the bilayer structure in terms of volumetric bilayer thickness DC and average area per lipid <A> [3]. Alternatively lipid segmental order parameters are measured using separated local-field (SLF) 13C NMR methods. Application to ternary mixtures of α-synuclein, cholesterol, and sphingomyelin explains the bilayer thinning effect by α-synuclein in raft-like membranes [4]. Furthermore, we measure 2H longitudinal (R1Z and R1Q) and transverse (R2QE and R2¬CPMG) relaxation rates as a function of external variables to interpret the molecular motions with regards to local and collective dynamics. Our current projects include investigations of the role of osmotic stress on collective interactions of membrane lipids and magnetic field-dependent relaxation studies. These studies provide time and length scales of the interactions that are crucial for validating force fields for molecular dynamics (MD) simulations of membrane constituents.

[1] A. Leftin et al. (2015) eMagRes 3, 199-214. [2] J. J. Kinuun et al. (2013) J. Chem. Ed. 93, 123-128. [3] J. J. Kinnun et al. (2015) BBA 1848, 246-259. [4] A. Leftin et al. (2013) JMB 425, 2973–2987.

4:10 PM **Michael Pitman** mikecpitman@email.arizona.edu
Chemistry and Biochemistry, University of Arizona

Dual Catalysis Role of Rhodopsin in Signal Transduction: A New Model for GPCR Signaling

We propose an extension to the classical activation role of the prototypical GPCR rhodopsin to include an additional role in signal amplification. This new role follows from recent thermodynamic results establishing reversible internal hydration in rhodopsin active substate equilibria. It completes a unified mechanistic picture drawing together decades of findings. The new model presents rhodopsin as playing an active role in signal amplification by supporting accelerated turnover of G-protein activation through state-dependent affinity switching. Rhodopsin catalyzes binding of G-protein with GDP bound, but once GDP is exchanged with GTP, rhodopsin catalyzes G-protein release. This dual role accelerates turnover of G-protein supported exchange of GDP for GTP, and helps to explain kinetics measurements indicating in vivo G-protein exchange rates that are higher than the diffusion limit. We build a multi-scale mechanistic picture of rhodopsin function that accounts for (a) how the lipid influences on activation couple to reversible hydration and volume changes, (b) how osmolyte size effects explains a hydration paradox and 18OH labeling studies, (c) how hydration provides a physical basis for entropic activation in the forward direction from Meta-I to Meta-II, (d) how hydration states underlie the dynamic pKa shifts of both the Schiff base and Glu134, and finally (e) how coupling of the two-proton switches with reversible hydration synergistically accelerates cyclic turnover in the G-protein supported exchange of

GDP for GTP. The GDP-GTP exchange is the known rate limiting step in G-protein signal transduction. Against this background, we argue that the dual role of rhodopsin acting as both detector and amplifier in GPCR signal transduction may likely exemplify function across a broader class of GPCRs. We further argue how a hydration-centric mechanism would have been accessible and operative throughout the evolution of GPCRs. It is potentially an ancient feature upon which selection pressures have acted within the class A GPCRs based on the fundamental physical properties of membrane-water interfaces

4:35 PM **Parthasarathi Rath** prath@asu.edu
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Chemistry and Biochemistry, University of Arizona

Biophysical studies of the Sensing Domain of the human Transient Receptor Potential Melastatin - 8 (hTRPM8) ion channel

The TRPM8 ion channel is so called as the cold receptor in humans and is involved in pain sensation, regulation of thermogenesis and also plays a significant role in carcinogenesis. This non-selective cation channel is activated at cooling temperatures and in presence of menthol. Moreover, TRPM8 is modulated by various stimuli such as temperature, voltage, chemical ligands and with accessory membrane proteins. Hence TRPM8 possesses important medical implications and thereby immense interests going on at number of pharmaceutical companies at preclinical and early phase clinical trials of TRPM8 modulators. Basically the Sensing Domain (transmembrane helix-1 to helix-4) and the Pore Domain (transmembrane helix-5 and helix-6) forms the central part of the ion channel with a tetrameric architecture and also experimentally shown as this part interacts with various modulators by electrophysiology measurements. However the molecular mechanism of these interactions is poorly understood. Here we present the over-expression, purification of the hTRPM8 Sensing Domain and the transmembrane domain (helix 1 to helix 6) in a stable form in various artificial hydrophobic environments and also biophysically characterized using NMR and CD spectroscopy. Furthermore, the effect of menthol on the change in conformation of the Sensing Domain was studied by point mutation analysis. These studies paved the way for the structure determination of this ion channel at atomic level by joint methods such as NMR spectroscopy, cryo electron microscopy and also by x-ray crystallography.

Poster Presentations

Bahige Abdallah bobdallah@asu.edu
Poster **#1**

Chemistry and Biochemistry, Arizona State University

Microfluidic sorting of protein nanocrystals by size for XFEL diffraction
BG Abdallah, NA Zatsepin, S Roy-Chowdhury, J Coe, CE Conrad, K Dörner, RG Sierra, HP Stevenson, F Camacho-Alanis, TD Grant, G Nelson, DR James, G Calero, RM Wachter, JCH Spence, U Weierstall, P Fromme, A Ros

The advent and application of the X-ray free-electron laser (XFEL) has uncovered the structures of proteins that could not previously be solved using traditional crystallography. While this new technology is powerful, optimization of the process is still needed to improve data quality and analysis efficiency. One area is sample heterogeneity, where variations in crystal size (among other factors) lead to the requirement of large data sets (and thus 10-100 mg of protein) for determining accurate structure factors. To decrease sample dispersity, we developed a high-throughput microfluidic sorter operating on the principle of dielectrophoresis, whereby polydisperse particles can be transported into various fluid streams for size fractionation. Using this microsorter, we isolated several milliliters of photosystem I nanocrystal fractions ranging from 200 to 600 nm in size as characterized by dynamic light scattering, nanoparticle tracking, and electron microscopy. Sorted nanocrystals were delivered in a liquid jet via the gas dynamic virtual nozzle into the path of the XFEL at the Linac Coherent Light Source where we obtained diffraction to ~ 4 Å resolution, indicating that the small crystals were not damaged by the sorting process. Using simulations, we show that narrow crystal size distributions significantly improve merged data quality in serial crystallography. From this proof-of-concept work, we can expect that the automated size-sorting of protein crystals will become an important step for sample production by reducing the volume of data needed for a high quality final structure.

Nethmi Ariyasinghe nariyasi@asu.edu
Poster **#2**
Physics, Arizona State University

AFM nanoindentation and fluorescence microscopy reveal changes in nuclear mechanics during chromatin decondensation
BL Doss, N Ariyasinghe, S Senapati, S Lindsay, R Ros

Cancer cell have a more aberrant chromatin structure than their normal cell counterparts. This may play a large role in the transformation from a normal cell to a cancerous cell. It has been shown that chromatin structure contributes to nuclear mechanics and resistance to deformation. Using atomic force microscope based nanoindentation and fluorescence imaging, four cell lines from two tissue types (CP-D and EPC2 as esophageal cancer and normal cells, and RKO and FHC as colon cancer and normal cells) were characterized. Preliminary results demonstrate that Trichostatin-A, a histone deacetylase inhibitor (HDACi), which relaxes chromatin structure in the cell nucleus, induced a significant nuclear softening in the cancer cells but not in normal cells. Morphological changes were detected in all cell lines except RKO for cell nuclei diameter; however, the RKO cells exhibit altered eccentricity in the presence of Trichostatin-A.

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Physics, Arizona State University

Study of ion transport in sodium-proton antiporter proteins by molecular dynamic simulations

DL Dotson, C Lee, D Drew, AC Cameron, O Beckstein

Sodium/proton antiporters are membrane proteins that are vital for cell homeostasis but the mechanistic details of their transport mechanism remain unclear, in particular, how Na+ and protons bind to the transporter. We recently solved X-ray crystal structures for two such antiporters (NhaA and NapA) in two different conformations of the transport cycle. All-atom molecular dynamics (MD) simulations (for a total simulated time >10 μs), indicate that sodium binding is dependent on the charge states of two conserved aspartate residues. A conserved lysine forms a previously unidentified salt bridge with one of the asparates. Under simulated physiological pH the presence of a Na+ ion disrupts and breaks the salt bridge in NhaA. The calculations support our novel hypothesis that the conserved lysine in these antiporter binds protons in a sodium-dependent manner and thus acts as part of the transport machinery. In conjunction with simulations of the conformational transition we propose a new mechanistic model of ion binding for the CPA2 class of antiporters within the larger framework of the alternating access mechanism of transmembrane transport.

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Chemistry and Biochemistry, University of Arizona

Biological Physics in the Brown Laboratory

TR Molugu, SMDC Perera, U Chawla, X Xu, SK Lee, R Musharrafieh, AV Struts, MF Brown

Knowledge of both structure and dynamics is crucial to understanding biomembrane function. Nuclear magnetic resonance (NMR) methods give powerful tools for providing such information. We utilize solid-state NMR methods such as order parameter analysis and relaxation methods to study membrane proteins, lipids, and their interactions with cholesterol in raft-like lipid mixtures. Currently we apply solid-state NMR spectroscopy to study proteins involved in neuroscience and G-protein–coupled receptors (GPCRs), as well as an amino acid transport protein in plant chloroplasts. Our structural work involves organic synthesis for isotopic labeling of the ligands for integral membrane proteins. Choosing rhodopsin as a model GPCR, we determined the solid-state NMR structure of the retinal ligand of rhodopsin, and the conformational changes triggered by light [1]. We also investigate rhodopsin photoactivation in nanocrystals using the short intense X- ray pulses of the free-electron laser at the Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory. For membrane lipids, our magnetic field-dependent NMR relaxation rates are crucial for validating force fields for molecular dynamics (MD) simulations of membrane constituents. We are also investigating collective interactions of membrane lipids, including the role of osmotic stress using NMR relaxation [2]. Our solid-state NMR approach has recently been extended through separated-local field 13C NMR spectroscopy [3]. We have extended the standard fluid mosaic model by discovering how membrane lipids govern the conformational energetics

of proteins by the innovation of a flexible surface model (FSM). Our focus is on how curvature stress and membrane surface electrostatic potential govern the light activation of rhodopsin. Currently our research team is conducting MD simulations to discover how local motions of the retinal cofactor of rhodopsin yield catalytic activation of transducin. We are testing the relations between hydration, binding affinity, and catalysis of transducin as a basis for understanding visual signaling. By combining solid-state NMR studies with MD simulations, we can better understand the receptor activation process [4]. [1] A. V. Struts et al. (2011) NSMB 18, 392-394. [2] J. J. Kinnun et al. (2015) BBA 1848, 246-259. [3] A. Leftin et al. (2013) JMB 425, 2973-2987 [4] B. Mertz et al. (2012) BBA 1818, 241-251.

Brandon Butler brandon.mac.butler@gmail.com
Poster **#5**
Physics, Arizona State University

Protein dynamics elucidates phenotypic effects of polymorphisms on residues coupled to hot spots at protein interfaces
B Butler, A Kumar, S Kumar, SB Ozkan

Biological processes are facilitated largely by protein-protein interactions. At any instant, myriads of interacting proteins form complexes to perform their specific function. Thus, non-synonymous single nucleotide variants (nsSNVs) on interface sites can have detrimental consequences for protein function. In a previous study, we investigated the conformational dynamics of interface sites on 333 complexes using a site-specific structural dynamic flexibility metric (dfi). The analysis revealed that interface sites have lower dfi as compared to those at non-interfaces. Moreover, interface sites with deleterious nsSNVs were found to have significantly lower dfi than those with neutral nsSNVs, which relates structural dynamics to functional importance. Overall, certain interface sites were shown to be critical for functionally related dynamics (low dfi) and, therefore, mutations at those sites have a high propensity to be deleterious. In a new study, we considered a small fraction of interface residues known as "hot spots", which account for a large portion of the total binding free energy. Not only are hot spot residues critical for function, but residues that are dynamically coupled to them may also be important for function. Using a new dynamic measure, functional-dfi (f-dfi), which considers hot spots and estimates their allosteric impact on other residues when nsSNVs are present, we conducted an analysis on the same set of 333 complexes. Our new metric, f-dfi, suggests that dynamic-based metrics that account for allosteric impacts can be crucial in assessing phenotypes of residues that are not at first glance obvious as being functionally important, but are critical residues since they are coupled to functionally critical positions such as hot spots.

Paul Campitelli paul.campitelli@gmail.com
Poster **#6**
Physics, Arizona State University

Physics Based Structure Refinement using Geometric Unfolding and Hierarchically Restrained Replica Exchange Molecular Dynamics
P Campitelli, A Kumar, SB Ozkan, MF Thorpe

We have developed a new physics based approach to the protein refinement problem by mimicking the mechanism of chaperons that rehabilitate proteins. The template structure is unfolded by selectively pulling on different portions of the protein using the geometric based technique FRODA, and refolding the protein using hierarchically restrained replica exchange molecular dynamics. FRODA unfolding is used to create a diverse set of topologies for surveying near the native like structures from a template. The unfolding trajectories are then used to find energetic restraints to enforce contacts and dihedral restraints. An REMD simulation is performed for the entire ensemble using consensus and reservoir techniques, which allow multiple structural candidates to "swap" into the replica cascade at the highest temperature replica and the most favorable folds to propagate to the lowest temperature replica. The restraints are added in a hierarchical fashion where local contacts are restrained first followed by the addition of non-local restraints to narrow the conformational search toward the native state. After REMD structures are clustered, refined structures are selected based on the highest populated cluster, RMSD and DFIRE score.

Udeep Chawla udchawla@email.arizona.edu
Poster **#7**
Chemistry and Biochemistry, University of Arizona

Role of Electrostatics in Rhodopsin Activation
U Chawla, W Zheng, L Kuang, SMDC Perera, H Liang, MF Brown

G-protein–coupled receptors (GPCRs) are integral membrane proteins that are extremely important in pharmaceuticals and constitute about 50% of known drug targets. Rhodopsin is a canonical GPCR that is responsible for vision in dim light. The retinal chromophore upon photoillumination changes from an 11-cis to all-trans conformation. A series of protein conformational change is followed by deprotonation of the Schiff base, establishing an equilibrium between inactive Metarhodopsin-I (Meta-I) and active Metarhodopsin-II (Meta-II) states [1,2]. The question we are addressing here is: how does the membrane surface potential influence rhodopsin activation? We hypothesize that the membrane properties are important in rhodopsin function and that the membrane surface potential affects its activation. To test our hypothesis, we constructed membrane lipid recombinants and polymerosomes by using a charge-induced directed (CIDR) reconstituted mechanism. In the CIDR mechanism the negatively charged protein, above its isoelectric point, spontaneously recombines with the positively charged membranes and polymerosomes [3]. We used synchrotron small-angle X-ray scattering (SAXS) to determine the assembly of the recombinants. Using SAXS, we show that n-dodecyl-β-D-maltoside solubilized bovine rhodopsin is reconstituted spontaneously to form 2-D proteomembrane arrays, which in turn are coupled along the transmembrane direction to form a 3-D multilamellar structure. The lamellar periodicity is about 5.7 nm, which matches closely the transmembrane dimension of rhodopsin. Using UV-visible spectroscopy we found that the positive membrane surface charge and positively charged polymerosomes favor the activated Metarhodopsin-II state. The positively charged membranes and polymerosomes stabilize protonated Glu134 by electrostatic interactions, analogous to the interaction formed by Glu134 and Arg135 of the ERY motif in the MetaIIH+ substate . Our study gives insight in the role of electrostatics in rhodopsin and will be applied to other GPCRs. [1] M. F. Brown (1997) Curr. Top. Membr. 44, 285-

356 [2] A. V. Struts et al. (2015) Meth. Mol. Biol. 1271, 133-158. [3] L. J. Kuang et al. (2014) ACS Nano 8, 537-545.

Wayne Christenson wayne.christenson@asu.edu
Poster **#8**
Physics, Arizona State University

Single Cell Force Spectroscopy and Total Internal Reflection Fluorescent Microscopy Techniques for Studying Cell Adhesion
W Christenson, I Yermolenko, T Ugarova, R Ros

Single cell force spectroscopy (SCFS) is an increasingly useful tool for studying cellular adhesion to an extracellular environment. We present a method for quantifying specific integrin-ligand interactions using atomic force microscopy (AFM) based SCFS experiments. SCFS data from HEK 293 cells expressing the Mac-1 integrin (HEK Mac-1) and wild-type HEK 293 (HEK WT) cells on surfaces with varying concentrations of fibrinogen were analyzed to identify specific "rupture events." Rupture events were classified into 2 types based on the force-load leading up to the rupture. High force load ruptures imply an activated integrin interaction while low force load ruptures imply the formation of a non-specific membrane tether. 41% of all rupture events were found to have a high force load in the HEK Mac-1 data on 0.6µg/ml fibrinogen surfaces compared to only 9% of rupture events having a high force load for HEK WT data on the same surface concentration of fibrinogen. The high force load events in the HEK Mac -1 data showed a median rupture force of 55pN, and 29pN for HEK WT cells. This analysis demonstrates the ability to quantify specific integrin-ligand interactions within SCFS data. On substrates with high concentrations of fibrinogen the median rupture force of high force-load events is 30pN, and high force-load events account for 27% of all rupture events. We developed a method based on the combination of total internal reflection fluorescence microscopy, single cell manipulation with an AFM and microcontact printing to demonstrate that the HEK Mac-1 cells are capable of pulling fibrinogen molecules out of the high concentration fibrinogen matrix indicating that the fibrinogen-fibrinogen bonds are weaker than the integrin-fibrinogen bonds. This reduced stability in high concentration fibrinogen matrices can account for the smaller rupture forces in the SCFS data.

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Poster **#9**
Chemistry and Biochemistry, Arizona State University

Exchange Mechanisms in Polymeric Micelles
B Donaphon, S Tang, F Raymo, M Levitus.

The following project is in collaboration with Dr. Francisco Raymo of the University of Miami who has been studying the biofunctionality and physical properties of polymeric micelles. When amphiphilic polymers are exposed to aqueous solvent, the polymers can form micelles and encapsulate hydrophobic cargo. In one of Dr. Raymo's studies, micelles containing anthracene dyes were mixed with micelles containing BODIPY dyes. When the resulting micelle mixture was illuminated with an excitation wavelength appropriate for anthracene, enhanced BODIPY fluorescence was observed. These observations suggested an

energy transfer between anthracene and BODIPY. Since this energy transfer is distance dependent, the energy transfer could only occur if the micelles exchanged cargo and brought the dyes close together. There were two proposed exchange mechanisms: 1) dye leakage out of the micellar core into the aqueous medium 2) during collisions, the micelles exchange polymeric components and cargo. In order to answer this question, various polymer and BODIPY dye samples were provided by Dr. Raymo's Research Group. After the micelle batches were prepared, these micelle batches were then sequentially diluted to the suspected critical micelle concentration. Fluorescence Correlation Spectroscopy (FCS) was next performed on the micelle batches and their serial dilutions to monitor the potential release of dye. However, the majority of the autocorrelation curves fit well with one component implying only encapsulated dye. In addition, the BODIPY dye is not very soluble in aqueous medium. In light of these measurements, the second exchange mechanism appears to be more likely.

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Poster **#10** http://smallerthings.org/
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MDSynthesis: a Python package to enable data-driven molecular dynamics research
DL Dotson, O Beckstein

Studies using molecular dynamics simulations can routinely generate terabytes of data, perhaps spread over hundreds of individual trajectories. Oftentimes these trajectories are not just repeats, but instead sample a wide range of different starting configurations, forcefield parameters, protein conformations, mutations, protonation states, etc., which can make data management difficult. Furthermore, because of their size and cost to calculate, it is often necessary to store intermediate data for collective variables of interest. This adds to the complexity of managing data, and serves as a barrier to answering scientific questions. To address this problem, our lab has developed MDSynthesis, a Python package that handles the boring and time-consuming logistics of intermediate data storage and retrieval. MDSynthesis features container objects that use the robust MDAnalysis library for dissecting the details of individual trajectories, and they store their states persistently to disk on-the-fly. These containers are memory efficient and built for aggregation, including convenience methods for quickly combining and comparing datasets across hundreds of simulations in arbitrary ways. Use of these containers make data-driven exploration possible with MD data, and they allow the user to more easily write analysis code that works across all variants of simulations they are running. The package is actively developed and freely available under the GNU General Public License from https://github.com/Becksteinlab/MDSynthesis.

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Poster **#11**
Chemistry and Biochemistry, University of Arizona

The Mechanism of Hydride Transfer Enzymes from Quantum and Classical Transition Path Sampling
MW Dzierlenga, D Antoniou, SD Schwartz

The mechanisms involved in enzymatic hydride transfer are not completely understood due to the difficulty in determining the participation of protein dynamics and the unclear role of quantum effects, especially hydrogen tunneling. In this study, we use transition path sampling (TPS) with normal mode centroid molecular dynamics (CMD) to calculate the barrier to hydride transfer in yeast alcohol dehydrogenase (YADH) and lactate dehydrogenase (LDH). This method corrects two problematic approximations used in computational free energy barrier calculations: imperfect guesses of the reaction coordinate and the classical propagation of particles in the reactive region. Calculation of the work applied to the hydride during the reaction allows for observation of the change in barrier height due to inclusion of quantum effects. In YADH, the average barrier height was 1.25 kcal/mol without quantum effects and 1.05 kcal/mol with quantum propagation of the transferring particle. In LDH, the average barrier height was 7.77 kcal/mol with classical propagation and 3.61 kcal/mol with quantum effects. The change in barrier height in YADH is indicative of a zero-point energy contribution, and is evidence that catalysis occurs via a protein compression which mediates a near barrier-less hydride transfer. While the average barrier height in LDH does not necessarily require tunneling as a mechanism, examination of the large range in barrier height shows that tunneling is playing a role in some but not all trajectories. The methods described provide insight into enzymatic reactions from an atomistic perspective that is otherwise unobtainable.

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Poster **#12**
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Biphasic Droplet-Based Sample Introduction for Protein Crystallography with an X-Ray Free Electron Laser
A Echelmeier, G Nelson, BG Abdallah, U Weierstall, JCH Spence, A Ros

As a result of new technology with the X-Ray Free Electron Laser (XFEL), determining the structures of proteins which are not accessible with traditional x-ray crystallography techniques has become possible. Growing crystals is however still cumbersome and time consuming for most protein complexes with important biological function, and with current methods of continuous aqueous sample introduction into the XFEL, large amounts of precious crystals are wasted. To reduce the amount of crystals injected into an XFEL, we propose generating controlled droplets in a microfluidic cross channel that can be coupled to current injector technology. By introducing an aqueous phase of protein crystals into a hydrophobic carrier phase at the channel intersection, droplets can be generated. The frequency at which these droplets are generated, as well as this size of the droplets, can be varied with parameters such as surface tension and viscosity of the two phases as well as the pressures applied to the aqueous and hydrophobic liquid streams. Adjusting these parameters can lead to a frequency of droplet generation matching the frequency of the XFEL, and when synchronized, hit rates can be increased requiring fewer crystals needed to determine a structure. We demonstrate our efforts in generating droplets with a fluorescent dye and with a suspension of photosystem I (PSI) crystals. The droplets have been coupled to a capillary, which can further be coupled to the gas dynamic virtual nozzle injector for XFEL analysis. Droplet generation frequencies of about 20 Hz with droplet volumes of about 8-10 nL have been achieved for suspensions containing PSI crystals. We are further

developing droplet generation conditions matching the current 120 Hz frequency of the XFEL laser and future XFEL instrumentation to provide an efficient method for sample introduction for crystallography with XFELs.

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Poster **#13**
Physics, Arizona State University

Dynamic AFM Nanoindentation on Cancer Cells
KR Eliato, BL Doss, R Ros

Cells undergo significant mechanical changes as they become cancerous. Atomic Forced Microscopy (AFM) is a powerful tool to measure and quantify the mechanical properties of living cells and many other bio-systems. Cells possess elastic and viscous properties, therefore, we investigate viscoelasticity of cell by applied a sine wave force on the sample and measured the response. Using the Hertz model, applied force and indentation amplitudes and phase lag we obtain shear storage (G') and shear loss (G") moduli as well as loss tangent (G"/G'). Shear storage (G') modulus shows the elastic properties of a sample, it is related to Young's modulus by $E = 2(1 + \nu)G'$, where ν is the Poisson ratio. Shear loss modulus (G") shows the viscosity of the sample. In a purely elastic material G" and in a purely viscose material G' are zero. Loss tangent (G"/G') provides model-independent information about a viscoelastic system. When loss tangent is less than one (more than one) the system is solid-like (liquid-like). In this work we measured the loss tangent, shear storage and loss moduli of metastatic colon cells (RKO) and breast cells (MDA-MB-231).

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Functional Characterization of Transient Receptor Potential Ion Channel Modulatory Proteins
JK Hilton, T Salehpour, CVM Helsell, MS Waris, WD Van Horn

Temperature is a basic environmental factor that affects organism viability; therefore, the ability to sense temperature is necessary to maintain life. In mammals, thermosensation is accomplished by a class of integral membrane ion channels belonging to the Transient Receptor Potential (TRP) family. These channels are activated across a range of temperatures and modulated by diverse stimuli, including membrane potential, pH, lipids, and small molecules. Precisely how TRP channels integrate these stimuli at the molecular level is currently not well understood. Recently, a novel two-span membrane protein called PIRT was shown to regulate the activity of two thermosensitive TRP channels: TRPV1, which is sensitive to heat, and TRPM8, a cold sensor. Using electrophysiology experiments under a variety of conditions, we have begun to functionally characterize the modulatory effect of PIRT on TRPM8. Studies using mouse and human genes have revealed that modulation of TRPM8 by PIRT is species dependent; human PIRT attenuates TRPM8 currents whereas mouse PIRT potentiates them. Additional studies using TRP channel chimeras have identified the region of TRP channels that

is important for modulation by PIRT. These experiments yield key insights into the molecular mechanisms underlying the regulation of TRP ion channel activity.

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Poster **#15**
Physics, Arizona State University

3D free-standing probes for targeted tissue electrical stimulation and intracellular measurement
X Jiao, Q Qing

Research work on the electronic-biological interface has significantly advanced the fundamental study of cells and cell networks, and has created powerful tools for medical research and patient care. For example, microelectrodes and multi-electrodes arrays have been used for extracellular recording and stimulation. Additionally, the standard patch-clamp micropipette is widely used for intracellular recording, which is critical to understanding fundamental behavior of cells and cell networks. However, high resolution targeted stimulation and intracellular recording with high biocompatibility remain a central challenge. We overcome this barrier by fabricating a free-standinng probe on which micro-electrodes can bend up from surface and pierce through the first few cell layers and cell membranes after being inserted into tissue as the biodegradable sacrificial layer underneath dissolves. These probes can be manipulated to target specific cell regions or tissue parts while minimizing probe insertion damage to the targeted cell region.

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Poster **#16**
Physics, Arizona State University

New strategy for fabricating ultra-small implantable probes with bend-up micro-electrodes
X Jiao, Q Qing

Implantable electrodes have been widely used for biomedical research and therapy. The biocompatibility of existing probe techniques is heavily hindered due to mismatch in their sizes and mechanical properties with live cells and tissues. Different materials, geometry and strategies have been proposed to solve these challenges, but it is still a difficult task to reconcile the request for a structure that is small but strong so that the probe can be implanted by surgery with minimal damage to the tissue, while still being able to keep the recording units mechanically flexible and geometrically closer to the surrounding active cells. Here we propose a new strategy to address this challenge by integrating bend-up micro-electrodes on a supporting ultra-small silicon shaft, which features a biologically friendly sacrificial layer which allows the formation of such structure in situ only after the surgery. We will show our preliminary results and characterization of these probes.

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Towards an automated pipeline for solvation free energy calculations

I Kenney, BI Iorga, O Beckstein

Solvation free energy calculations from molecular dynamics simulations prove to be a time consuming task. Solvation free energies are used to determine solvent effects on molecules of interest, such as the logP of drug-like molecules. We developed MDPOW, an automated pipeline to compute solvation free energies with molecular dynamics simulations. MDPOW is implemented using Python and Gromacs. In order to optimize the simulation protocol, we examined the effects of varying parameters on solvation free energies in the context of parameterizing MD force fields. We employed benzene as a simple test case with an known experimental hydration free energy. We varied the parameters of the MD hydration free energy perturbation (FEP) calculations, such as temperature and pressure coupling algorithms, sampling time of the FEP windows, dispersion correction, initial conditions. We also employed two different schemes to calculate the free energy, namely thermodynamic integration (TI) and the Bennett acceptance ratio (BAR) method. The Coulomb component of the hydration free energy was generally insensitive to variations in parameters. The Lennard-Jones component, however, can vary by many kJ/mol and is particularly sensitive to the temperature coupling algorithm and inconsistent initial conditions when simulations are performed in the NVT ensemble. Differences between BAR and TI are generally small. Our current ``best-practice" protocol thus consists of NPT simulations using a stochastic integrator and Parrinello-Rahman pressure coupling, separate decoupling of Coulomb and Lennard-Jones interactions (with soft cores), and 20 ns simulation per FEP window for a total of 470 ns MD simulation per hydration free energy calculation.

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Poster **#18**
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Biophysical Characterization of Human TRPV1 Sensing Domain
M Kim, W Van Horn

Transient receptor potential ion channels (TRP channels) are membrane proteins that are responsible for sensing, mainly nociception. Among the many groups of TRP channels, subfamily Vanilloid member 1 (TRPV1) was first discovered as a capsaicin receptor, and later cloned in 1997. TRPV1 is known to be modulated by various chemical and physical stimuli such as temperature, capsaicin, pH, endogenous lipid molecules and small proteins. Interestingly, TRPV1 is also known as a thermoTRPs, however, the mechanism of thermosensation still remains elusive. TRPV1 consists of a sensing domain (SD) and a pore domain, and our hypothesis is that this sensing domain of TRPV1 is responsible for thermosensitivity, and we are using some biophysical techniques such as nuclear magnetic resonance spectroscopy (NMR) and far-uv circular dichroism (CD) to probe this. Our temperature titration with both NMR and far-UV circular dichroism demonstrated that TRPV1-SD undergoes reversible conformational changes as a function of temperature. We have also performed paramagnetic relaxation enhancement (PRE), and we probed the dynamics of TRPV1-SD. Our data strongly suggest that TRPV1-SD is involved with thermosensation. This can be further investigated by performing differential scattering calorimetry (DSC) and the protein dynamics can be studied by Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion NMR.

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Computational Characterization of collagen-based Extracellular Matrix
L Long, CA Rucksack Jones, D Lin, Y Jiao, B Sun
A model of extracellular matrix (ECM) of collagen fibers has been built, in which cells could communicate with distant partners via fiber-mediated long-range-transmitted stress states. The ECM is modeled as a spring-like fiber network derived from skeletonized confocal microscopy data. Different local and global perturbations have been performed on the network, each followed by an optimized global Monte-Carlo (MC) energy minimization leading to the deformed network in response to the perturbations. In the optimization, a highly efficient local energy update procedure is employed and force-directed MC moves are used, which results in a convergence to the energy minimum state 20 times faster than the commonly used random displacement trial moves in MC. Further analysis and visualization of the distribution and correlation of the resulting force network reveal that local perturbations can give rise to global impacts: the force chains formed with a linear extent much further than the characteristic length scale associated with the perturbation sites and average fiber length. This behavior provides a strong evidence for our hypothesis of fiber-mediated long-range force transmission in ECM networks and the resulted possibility of long-range cell-cell mechanical signaling.

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Poster **#20**
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Effect of Hydration on Membrane Dynamics Studied by Solid-State 2H NMR Relaxation
TR Molugu, SK Lee, C Job, MF Brown

Investigations of model membranes aim to understand the atomistic interactions that can explain bulk membrane lipid properties in relation to key biological functions [1]. Solid-state 2H nuclear magnetic resonance (NMR) spectroscopy uniquely provides such information by probing structure and dynamics of membranes [2,3]. Here we examine the effect of water on the liquid-crystalline properties of amphiphilic membrane lipids using NMR relaxometry. We performed NMR longitudinal (R1Z), transverse quadrupolar-echo decay (R2QE), and quadrupolar Carr-Purcell-Meiboom-Gill (QCPMG) relaxation (R2CP) experiments on DMPC-d54 bilayers, to study membrane lipid dynamics over time scales ranging from 10–9s to 10–3s. The plots of R1Z rates versus squared segmental order parameters (SCD2) follow an empirical square-law behavior showing the emergence of collective lipid dynamics [4]. Such a functional behavior characterizes 3-D order-director fluctuations due to the onset of membrane elasticity over atomistic dimensions [4]. The transverse relaxation rates also show similar results at low hydration. Yet at high hydration, a further enhancement versus the functional square-law plot is evident for segments deeper in the bilayer. Additional contributions from slower dynamics involving water-mediated membrane deformation are evident over mesoscopic length scales on the order of bilayer thickness. The slow dynamics at high hydration must be a consequence of

modulation of elastic properties of lipid bilayer. Analysis of the QCPMG frequency dispersions as function of hydration and temperature reveals quantitative information on viscoelastic properties of the liquid-crystalline media. Similar studies in the presence of proteins and peptides give insights into optimized lipid hydration for biomembrane function. [1] A. Leftin et al. (2014) BJ 107, 2274-2286. [2] K. J. Mallikarjuniah et al. (2011) BJ 100, 98-107. [3] J. J. Kinnun et al. (2015) BBA 1848, 246-259 [4] M. F. Brown et al. (2002) JACS 124, 8471-8484.

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Role of the Lipid Bilayer on Transmembrane Protein Function: From Rhodopsin To Influenza Am2 Channel

R Musharrafieh, U Chawla, B Mertz, E Ritter, F Bart, J Wang, MF Brown

Photoactivation of rhodopsin, the light sensing G-protein–coupled receptor (GPCR), involves the isomerization of 11-cis retinal to all-trans that leads to the inactive Meta-I conformation, followed by an ensemble of structurally definable Meta-II substates that result in visual perception [1]. Rhodopsin consists of seven transmembrane helices that are affected by the lipid bilayer during the conformational changes associated with activation. Intrinsic membrane curvature along with lipid-protein hydrophobic interactions lead to elastic coupling of lipids and membrane proteins as described by the flexible surface model (FSM). The FSM predicts that protein conformational substates are dependent on the environment via the lipid bilayer composition [2]. Therefore the effects of temperature and pH on conformational substates were analyzed for rhodopsin reconstituted in lipid vesicles using UV-visible and FTIR spectroscopy. Mixed-chain POPC membranes backshift rhodopsin towards Meta-I, whereas rhodopsin in DOPC favors the active Meta-IIa substate. Analysis of the wavelength-dependent distribution of pKa and alkaline endpoints as estimated from FTIR difference spectra reveals an ensemble of substates for each lipid bilayer-rhodopsin system indicative of an ensemble-activated mechanism. Our results are in agreement with the FSM, in which lipids having a negative monolayer curvature (DOPC) favor the active Meta-II state, while lipids with zero spontaneous curvature (POPC) favor the inactive Meta-I state [3]. The fundamental insight gained from lipid-protein interactions for rhodopsin can be expanded to the influenza A matrix protein 2 ion channel (AM2), a protein of pharmacological interest. The lipid bilayer environment is known to alter AM2 structure and activity [4]. The concept of ensemble activation of rhodopsin substates together with the FSM can be used to characterize the structural and malfunctioning changes in AM2 caused by the lipid membrane environment. [1] A.V. Struts (2011) PNAS 108, 8263-8268. [2] M.F. Brown (2012) Biochemistry 51, 9782-9795. [3] E. Zaitseva (2010) JACS 132, 815-4821. [4] H-X. Zhou (2013) Protein Science 22, 381-394.

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Breast cancer cell invasion in a highly organized three dimensional (3D) hydrogel based tumor model

N Peela, FS Sam, W Christenson, AW Watson, R Ros, G Mouneimne, M Nikkhah

Cancer cell invasion is a highly orchestrated and complex biological process, which is influenced by a plethora of biophysical and biochemical signals. Despite its significance, the mechanistic understanding of cellular invasion within the tumor microenvironment has been largely limited due to the lack of physiologically relevant 3D tumor models. Microfabrication of organized cellular constructs offers numerous applications in tracking cell-cell and cell-matrix interactions as well as in anti-cancer drug screening. In this regard, the majority of the reported 3D tumor models lack specific organization and therefore cannot effectively isolate individual effects of microenvironmental cues on cellular invasion. In this project, by building upon our expertise in microscale tissue engineering and cancer biology, we aim to develop a highly organized 3D tumor model using gelatin methacrylate (GelMA) hydrogel, and to study breast cancer cell invasion in response to various microenviromental cues (e.g. cancer associated fibroblasts (CAFs), and matrix stiffness).

Breast cancer MDA-MB-231 cells and normal mammary MCF10A cells were encapsulated in GelMA hydrogel patterned in the form of highly organized circular constructs (500μm diameter, 100 μm height) using photolithography techniques. Subsequently, pure GelMA was patterned in between the constructs and crosslinked for a significantly lower time compared to the circular tumor region (6s vs. 14 s). This process created a microenvironment with high-stiffness circular constructs (representative of tumors) surrounded by a lower-stiffness stroma. After days 1, 3, and 5 of culture, cancer cell invasion was characterized through quantifying the number of cells that disseminated from the tumor construct and migrated towards the surrounding stroma.

Atomic Force Microscope (AFM) based nanoindentation measurements confirmed significantly higher stiffness within the micropatterned circular constructs (747.8 ± 89.6 Pa) compared to the surrounding stroma (313.3 ± 37.5 Pa) resembling a distinct physiological condition. Preliminary studies demonstrated high cellular viability (84.36 ± 5.96%) without any significant difference between the MCF10A and MDA-MB-231 tumor models. Furthermore, MDA-MB-231 cells proliferated quickly within the model at an average rate of 32.19% compared to the MCF10A cells which did not exhibit any statistically significant increase in cell count. The highly metastatic MDA-MB-231 cells displayed invasive behavior from day 1, and by day 5, 12.87 ± 1.85% of total cells invaded the surrounding stroma; on the other hand, MCF10A cells remained confined within the central circular region, where only 1.08 ± 0.24% of the cells invaded into the surrounding stroma by day 5. Our findings demonstrate a promising approach to create highly organized tumor models to mechanistically study cancer cell invasion within a 3D microenvironment. This platform has applications in high-throughput drug screening as well as in creating tumor models which model complex cell-cell/cell-matrix interactions such as the effects of CAFs signaling on cellular invasion.

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Poster **#23**
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Neutron Scattering Reveals Conformational Flexibility in Rhodopsin Photoactivation

SMDC Perera, U Chawla, U Shrestha, D Bhowmik, AV Struts, S Qian, XQ Chu, MF Brown

G-protein-coupled receptors (GPCRs) are the target of more than 40% of the molecular drugs. The structure and function of GPCRs are crucial for the development of improved pharmaceuticals. Rhodopsin is the GPCR responsible for vision under dim light conditions. Light activation of rhodopsin effects the isomerization of the 11-cis retinal chromophore to the all-trans conformation, which eventually leads to an equilibrium between inactive Meta-I and active Meta-II states [1]. The protein conformational changes accompanying this chromophore isomerization is not clearly understood. Here we investigate the structure and dynamics associated with the rhodopsin photoactivation using techniques such as small-angle neutron scattering (SANS), and quasi-elastic neutron scattering (QENS). In SANS experiments the intensity of the neutrons scattered by a sample is recorded as a function of the scattering angle. It reveals the global structural information such as the size and shape of the scatterers. In QENS experiments, relaxation times associated with the dynamics such as translational diffusion and molecular reorientation are studied. Rhodopsin was purified in CHAPS which transiently traps inactive Meta-I, and dodecylmaltoside (DDM) which promotes active Meta-II upon photoactivation [2]. SANS experiments conducted on rhodopsin-detergent complexes with contrast variation enabled us to probe the structural changes occur in the protein upon photo-illumination. We discovered that activation of rhodopsin is favored in more relaxed protein environments such as the rhodopsin-DDM complex, in contrast to more compact environments such as the rhodopsin-CHAPS complex. The QENS experiments revealed the differences in the flexibility of the protein in the dark state and ligand free apo-protein, opsin. Our neutron experiments delineate the plasticity of the protein structure that is important for the GPCR activation, which is consistent with an ensemble activation mechanism. [1] A. V. Struts et al. (2011) PNAS 108, 8263-8268 [2] A. V. Struts et al. (2015) Meth. Mol. Biol. 1271, 133-159.

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Neutron Scattering Reveals Conformational Flexibility in Rhodopsin Photoactivation
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The objective of this project is to study dynamics of interface of β clamp in solution. β clamp is a ring shaped homo dimeric protein. It plays a significant role in increasing processivity during DNA replication in E.coli. In presence of gamma complex bound with ATP, known as clamp loader, β clamp is opened up and encircles DNA strand in it and holds DNA polymerase III which is the principal enzyme for replication. It also prevents dissociation of DNA Pol III from template strand to increase efficiency of replication.
The goals of our project first, to study the dynamics of the interface subunits of the beta clamp. Secondly, to find out the specific charged amino acids interactions in the interface which are responsible for the stability of the β clamp.
We have collaboration with Prof. Linda Bloom, University of Florida . She prepared samples for us. She mutated wild type amino acids with cysteine residues and incorporated TMR dye in those positions. In this way she prepared single labeled at interface (I305C), double labeled at interface (R103 C, I305C) and

another double labeled at internal side chain(S181C, S356C) . Techniques we used to study characteristics of the protein samples are FCS (fluorescence correlation spectroscopy) and TCSPC (Time correlated single photon counting). In FCS (Fluorescence correlation Spectroscopy) Single labeled sample shows diffusion only curve. For double labeled sample at interface shows dynamic behavior in micro second scale beside only diffusion. According to our hypothesis we expected sample having mutation at internal side chain should not show any dynamic behavior so we can use this sample as a negative control to study the dynamics of the interface. But in FCS study, we found different results that contradicted our hypothesis that prompted us to make a new negative control by labeling DNA with TMR-Maleimide dye because we know DNA is more rigid in nature than protein. With this negative control we can study dynamic nature of protein samples meticulously in microsecond scale.

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Charge patterning, salt screening and denaturant expansion in the CGRP neuropeptide
S Sizemore, S Cope, A Soranno, S Vaiana

Calcitonin gene related peptide (CGRP) is a neuropeptide of the calcitonin peptide family, which acts as a vasodilator and is involved in the transmission of pain signals in the nervous system upon binding to the correspondent receptor. It also triggers migraine attacks, and is a major therapeutic target for the prevention of migraines. In physiological conditions, CGRP is intrinsically disordered and, therefore, the binding to its receptor (or to drugs) will depend strongly on the structural and dynamical properties of the disordered unbound state. Such properties can be affected in vivo by changes in salt concentration and pH. However, while some information is available on CGRP's sampling of local secondary structural elements, very little is known about its long-range ("tertiary") structural and dynamical properties. Detecting such properties is challenging because CGRP has a low molecular weight and samples many different conformations on very fast time scales. We use a nanosecond laser-pump spectroscopy technique, based on tryptophan triplet quenching, which allows probing the end-to-end distance and the rate of end-to-end contact formation in IDPs. This provides similar information to FRET, but without the use of prosthetic dyes. Our data show that CGRP populates compact states in buffer, which are extremely sensitive to pH and salt concentration. We find that a change from pH 8 to pH 3 can induce a significant expansion of conformations due to the modulation of charge interactions, with a dramatic change of the corresponding salt screening effects. This suggests a key role of specific charged residues in CGRP. In addition, we find a "denaturant expansion" effect that depends on the nature of the denaturant. The observations can be rationalized in terms of polymer models where the polyelectrolyte/polyampholyte nature of the peptide is taken into account.

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Engineering of regulated stochastic cell fate determination
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Both microbes and multicellular organisms actively regulate their cell fate determination to cope with changing environments or to ensure proper development. Here, we use synthetic biology approaches to engineer bistable gene networks to demonstrate that stochastic and permanent cell fate determination can be achieved through initializing gene regulatory networks (GRNs) at the boundary between dynamic attractors. We realize this experimentally by linking a synthetic GRN to a natural output of galactose metabolism regulation in yeast. Combining mathematical modeling and flow cytometry, we show that our engineered systems are bistable and that inherent gene expression stochasticity does not induce spontaneous state transitioning at steady state. Mathematical analysis predicts that stochastic cell fate determination in this case can only be realized when gene expression fluctuation occurs on or near attractor basin boundaries (the points of instability). Guided by numerical simulations, experiments are designed and performed with quantitatively diverse gene networks to test model predictions, which are verified by both flow cytometry and single-cell microscopy. By interfacing rationally designed synthetic GRNs with background gene regulation mechanisms, this work investigates intricate properties of networks that illuminate possible regulatory mechanisms for cell differentiation and development that can be initiated from points of instability.

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Poster **#27**
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Label-free Silicon nanowire field effect transistor for impedance sensing of molecules
Y Wang

Impedance biosensors are promising electrical biosensors due to low cost, ease of miniaturization and label-free operation. Recent investigations on impedance measurement yield a wealth of information about different molecular motion and relaxation process, utilizing a wide frequency range from 10uHz to 1THz, which typically measure the impedance between two large chemically modified electrodes as target molecules bind to the surface. Our question is: can we scale this method down to single molecule level by matching the size of the whole device with that of the target molecules, which falls in the 10-100 nm length scale, and integrating the amplifier directly within such nano-electrodes? Here we will show our prototype devices based on silicon nanowire field-effect transistors (SiNW FETs) with a paired-gate structure. And we will focus on the high frequency AC impedance characterization of these devices. We hope that our results can give some indication of how low copy number biomolecules can be enriched and detected in physiological environments.
Key words: Impedance biosensor, Label-free, Silicon nanowire field effect transistor, Electrochemical impedance spectroscopy.

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Poster **#28**
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Tip Induced Nanophotonic Effects on Single Fluorophores
A Ward

Progress in nanosciences and life sciences is closely related to developments of high resolution imaging techniques. During the last decades the invention and improvement of scanning probe microscopy techniques like atomic force microscopy (AFM) have opened new views onto nanoscale materials. On the other side, optical microscopy has been pushed beyond the diffraction limit and the observation of single molecules has evolved into a standard technology. Here we present experiments with a combined AFM/fluorescence lifetime setup to evaluate the feasibility of using such a combination for contact mode AFM topographic and super-resolution fluorescence imaging. We show distinct modulation of the fluorescence intensity and lifetime due to AFM probes of different materials in contact mode. We have used AFM probes of three different materials for combined scans in contact mode. For all tested materials, namely silicon, silicon nitride, and quartz tips, we see a distinct dark spot in the emission from single Atto655, Alexa647 and Alexa488 molecules on a glass surface. For silicon probes, we found spot sizes as small as 5nm (FWHM)

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Poster **#29**
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Quantifying Solvent Dynamics With Kinetic Networks
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Explicit solvent biomolecular simulations often contain thousands of water molecules. These molecules exhibit highly complex kinetics both nearby the protein and within its interior. The large number of particles present a significant challenge when attempting to distinguish biologically or physically relevant water trajectories or components of trajectories. We present a method which projects the molecular dynamics (MD) water trajectory data onto a discrete subspace whose elements are regions of space where a large time averaged density relative to bulk is observed ('hydration sites'). Potentially important water kinetics are more apparent visually and can be more easily identified quantitatively. Moreover, the statistics of this kinetic network can be used to build stochastic models to probe dynamical water behavior. We apply this technique to quantify the permeation of water through the water-transporting aquaporin Aqp1 and the benzyl-hydantoin transporter Mhp1, which other studies suggest have sizable water permeability. Our analysis clearly identified water pathways through both proteins. By taking into account the kinetic rates between hydration sites and total permeation rates through each protein, dominant and secondary pathways could be distinguished. MD simulations of Mhp1 transporter in different conformations in its transport cycle were analyzed and revealed that some conformations were more water permeable than others. The kinetic analysis is implemented as a Python package ("hop") based on the MDAnalysis library https://mdanalysis.github.io/.

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Poster **#30**
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Computations and Experiments Provide a Molecular Basis for Calcium Dysregulation
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We now present the newest revision of our cardiac thin filament (CTF) model which includes explicit solvation. The previous version of model consisted of the troponin complex (cTn), two coiled-coil dimers of tropomyosin (Tm), and 29 actin units. The model was developed to continue our study of genetic mutations in the CTF proteins which are linked to familial hypertrophic cardiomyopathies. Binding of calcium to the cTnC subunit causes subtle conformational changes to propagate through the cTnC to the cTnI subunit which then detaches from actin. Conformational changes propagate through to the cTnT subunit, which allows Tm to move into the open position along actin, leading to muscle contraction. Calcium disassociation allows for the reverse to occur, which results in muscle relaxation. The inclusion of explicit TIP3 water solvation allows for the model to get better individual local solvent to protein interactions; which are important when observing the N-lobe calcium binding pocket of the cTnC. We are able to compare in silico and in vitro experimental results to better understand the physiological alterations from mutations, such as the R92L/W of the cTnT, on the calcium binding affinity compared to the wild type. We found that the cTnI E32 interacts with the calcium and the mutations alter the rate of release.

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Transducin activation by Rhodopsin investigated with solid-state 2H NMR spectroscopy
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X-ray structures of active-state rhodopsin (Meta-II) and the cognate G-protein transducin are available, yet the transducin activation mechanism by rhodopsin is still unknown. Here we investigate how the presence of the all-trans retinal agonist yields substantial differences in activation of transducin compared to opsin. Solid-state NMR experiments are currently underway with a selectively 2H-labeled C-terminal peptide of transducin to study its interaction with the rhodopsin transmembrane helices [1]. Our hypothesis is that association and dissociation cycles of transducin depend on the local dynamics of the peptide and involve the rhodopsin hydration. Experimental line shapes and relaxation rates show that motions of specific methyl groups are restricted even for dry peptides with loose packing. Temperature dependences of the T1Z and T1Q relaxation times were similar for all three positions of the high-affinity peptide. Differences in relaxation times for opsin-bound peptides are due to interactions with the receptor (or water in the transducin binding pocket). Solid state 2H NMR experiments tell us not only how rhodopsin activates transducin, but also the general mechanisms whereby GPCRs activate their cognate G-proteins. Our studies open the door to investigating both the structure and dynamics of the peptide agonist bound to rhodopsin in a natural membrane lipid environment. [1] A.V. Struts et al. (2011) PNAS 108, 8263.

[2] X. Xu et al. (2014) Encycl. Mag. Res. 3, 275–286. [3] B. Mertz et al. (2012) BBA 1818, 241–251. [4] A.V. Struts et al. (2007) JMB 372, 50–66.