BioPhest

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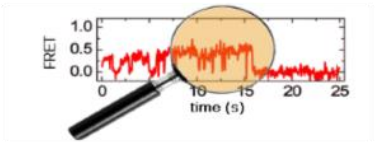

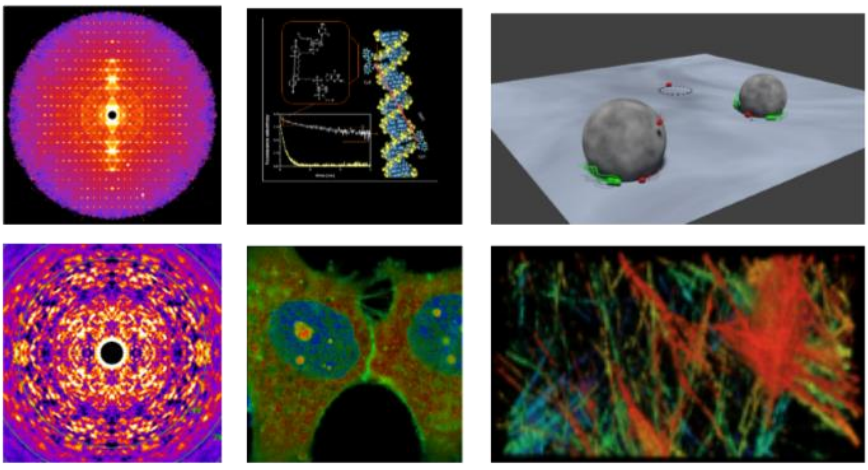

ASU & A

Saturday, April 22, 2017
Tempe, Arizona

ABSTRACTS

Talk Presentations

9:00AM ***Developing a hybrid atomistic-continuum method for simulating large-scale heterogeneous biomolecular systems***

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Biological macromolecules such as proteins, usually immersed in an ionic aqueous environment, are often highly heterogeneous systems whose dynamics can span femtosecond to millisecond timescales and beyond. Explicit solvent molecular dynamics (MD) is necessary to fully capture solute-solvent interactions, though water molecules contribute to most of the computational cost; sufficiently long simulations that can capture relatively slow processes, like large-scale conformational changes, are often infeasible. One approach is to hybridize MD with a comparatively frugal hydrodynamic (HD) model that replaces some (or all) of the solvent. We have been developing a hybrid method—with a view toward biomolecular simulation—that couples the LAMMPS MD engine to a discontinuous-Galerkin-based fluctuating HD solver. Several hydrodynamic test problems carried out on Blue Waters, used to assess the performance of the HD code, are presented. We also report on the current state of development of the prototype hybrid code and discuss remaining challenges to be addressed.

9:20AM ***Dynamical transition in liquids and proteins***

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Dynamical transition is the phenomenon of an abrupt change in the temperature dependence of the mean-square displacement of atoms in glass-formers and proteins. It was first used to explain the drop in the scattering intensity in elastic neutron scattering experiments. Mossbauer spectroscopy also showed a similar change, although not necessarily at the same temperature. The origin of such behavior, whether thermodynamic or dynamic , is still debated. Here we present the results of our study of two different systems using Molecular Dynamics simulations. Our study on glycerol suggests a dynamical origin of the crossover at low temperatures. On the other hand the ergodicity breaking model tends to reproduce the experimental data on cytochrome-c using parameters calculated by MD simulations.

9:40AM ***Dynamic Flexibility Index Sheds Light on Pin1Allostery***

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Allostery, which is regulation from distant sites, plays a major role in biology. Pin1 is a modular protein containing a WW domain and a larger peptidyl prolyl isomarase domain (PPIase) that isomerizes phospho-serine/threonine-proline (pS/T-P) motifs, which are critical for signaling within intrinsically disordered loops of cell cycle proteins. Pin1 utilizes allosteric regulations for its function, and binds (pS/T-P) motifs in both domains. The WW domain serves as a docking module, whereas catalysis solely takes place within the PPIase domain. However, enzymatic activity gets enhanced when WW is in the bound form, highlighting PIN1's allosteric regulation. Previous work using NMR and molecular dynamics analysis has shown that binding induced quenching of fast local motions and strengthening of the interaction between two domains, indicated in particular by decrease in flexibility of catalytic loops. Here we present a novel method, the dynamic flexibility index (DFI) analysis, for characterizing the underlying allosteric communications between two domains. DFI measures the resilience of a given position to the perturbations that occur at different parts of the protein, using linear response theory. This index captures multi-dimensional effects when the protein is displaced out of equilibrium. Moreover, we can also identify the allosteric response in dynamic flexibility based on the perturbation response fluctuation profile of the PPIase domain upon WW binding and distinguish the positions that contribute the most. Finally we also explore the mechanistic link between conformational dynamics and co-evolution to identify mutational positions to alter enzymatic function.

10:30AM ***GPCR Activation Probed by Spectroscopic and Scattering Methods***

Suchithranga M.D.C. Perera, Xiaolin Xu, Andrey V. Struts, Trivikram R. Molugu, Udeep Chawla, Soohyun K. Lee, Rami Musharrafieh, Annie Huang, Thomas A. Knowles, Michael F. Brown
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G-protein–coupled receptors (GPCRs) are the targets of most pharmaceuticals used worldwide. Here we address the multiscale activation mechanism of the Rhodopsin class of GPCRs in proteolipid membranes. Solid-state 2H NMR line-shapes are used to determine the changes in average conformation and orientation of the retinal cofactor for rhodopsin in aligned membranes. Comparison between our NMR and crystal structures informs us how crystal packing forces affect the results. We used X-ray free electron laser (XFEL) technology in a pump-probe, time-resolved wide-angle X-ray scattering (TR-WAXS) experiment to study the early activation events of the visual receptor rhodopsin. On-the-fly data analysis using the OnDA software package revealed the light-triggered “protein quake” in rhodopsin during very early stages of its activation (within 400 fs). Moreover, large-scale protein conformation changes are studied using quasi-elastic neutron scattering and small-angle neutron

scattering (SANS) reveal a volumetric expansion of the protein during the receptor activation. The protein environment within the membrane plays a key role. Notably removal of water by osmolytes yields striking changes in the rhodopsin activation in native lipid membranes. The lipid-protein interactions entail a flexible surface model (FSM) which illuminates the role of soft matter (membrane lipids plus water) in signaling. Frustration of the monolayer spontaneous curvature of the lipids is counterbalanced by the solvation energy of the proteolipid interface. Protein conformational changes take place within the curvature stress field of the lipid bilayer allowing energetic coupling to the membrane. Our combined biophysical approach gives us an overall description of how GPCR activation occurs within lipid membranes resulting in their key signaling functions. S.M.D.C. Perera et al. (2016) J. Phys. Chem. Lett. 7, 4230-4235.

10:50AM ***Combining Nanodiscs with Native Mass Spectrometry to Study the Biophysics of Protein-Lipid Interactions***

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Membrane proteins play critical biochemical roles. However, their poor solubility makes analysis challenging, and it is especially difficult to study the biophysics of interactions between membrane proteins and their surrounding lipid environment. Noncovalent or native mass spectrometry (MS) has proven to be a powerful tool for detecting lipids bound directly to membrane proteins, but conventional approaches using detergents for solubilization are only able to resolve a small number of bound lipid species. Nanodiscs, which are discoidal lipid bilayers encircled by two amphipathic membrane scaffold proteins (MSP), offer an attractive alternative to detergent micelles due to their relative monodispersity. I will present the development of Nanodiscs as a vehicle for native MS, from early work with simple systems to large membrane protein oligomers. Initial spectra of Nanodiscs without embedded membrane proteins demonstrated that they could be maintained intact in the mass spectrometer. Combining breakthroughs in instrumentation and data analysis has allowed us to count the number of lipids bound to membrane protein oligomers within the Nanodisc. From this, we discovered that membrane proteins ejected from Nanodiscs retain a large number of lipids bound in several distinct states. The largest distributions measured by MS agreed with the stoichiometry of the lipid annular belt predicted by molecular dynamics simulations. Looking ahead, the techniques developed on bacterial membrane proteins are now being applied to more complicated mammalian membrane protein transporters. We are moving beyond homogenous lipid systems by using mixed-lipid Nanodiscs for studying lipid stoichiometry and composition. These experiments demonstrate the unique ability of native MS with Nanodiscs to measure a large number of protein-lipid interactions and explore the biophysics of interactions at the interface between membrane proteins and local lipid environment.

11:10AM ***Biofunctionalized-phospholipid nanoshell immobilized microarrays and the application for bacterial toxin detection***

Phuong-Diem Nguyen, Jinyan Wang, Malithi Fonseka, Scott Saavedra, Xuemin Wang and Craig A. Aspinwall
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A biofunctionalized-phospholipid nanoshells (PPN) microarray was developed for the research of toxin-receptor binding. Highly versatile-membrane architecture of PPN allowed the reconstitution of pathogen receptors and the modification of sulfonate derivatives for covalent immobilization of PPN onto PEG-based microarray. The PPN microarray technique will provide an important tool for multiplexed toxin detection and for lipid membrane-based research opportunity.

11:30AM ***Fluctuations as a source of information in fluorescence spectroscopy: from protein oligomerization to excited-state dynamics.***

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The use of fluorescent probes in the biologically-related disciplines is widespread and continues to grow. In addition to visualization, fluorescent probes with properties that are sensitive to the physical and chemical properties of the environment are often used to characterize biological systems at the nanoscale. We are mainly interested in two aspects of biological fluorescence: 1) the development and applications of fluorescence-based techniques to investigate biopolymer conformational dynamics in a variety of timescales and 2) the study of the photophysical properties of fluorescent probes in biomolecular environments. The latter is becoming increasingly important to interpret and design experiments that rely on the observation of single molecules (e.g. super-resolution microscopy), where the individual transitions between 'bright' and 'dark' states contribute to fluctuations in the signal in a variety of timescales. In the context of biophysical research, the analysis of fluorescence fluctuations can be used to gain useful dynamic information about the system. Examples include Brownian diffusion, conformational fluctuations in biomolecules, binding equilibria, and aggregation. In this presentation I will illustrate applications of these and other spectroscopic approaches to problems ranging from protein oligomerization to excited-state dynamics.

2:30PM ***EGF-free activation of ERK in epithelial cells by localized AC stimuli through customizable microelectrode chip***

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The MAPK/ERK pathway is a chain of proteins that communicates a signal from a cell membrane receptor to the DNA in the nucleus. It plays an essential role in cell migration, proliferation and differentiation. Here we report for the first time the ERK activation in non-electrogenic human epithelial MCF-10A cells triggered by highly localized AC electric field stimuli. The MCF-10A cells were cultured on a glass cover slip substrate with microelectrodes patterned by top-down lithography in a customized chamber under controlled environment. Fluorescence labeled ERK translocation reporter (ERKTR) expressed in individual cells was continuously monitored as a sequence of pulses was delivered to a selected group of cells. We have observed reproducible ERK activation within a time frame of 5-20 minutes by 1.5 V 50 kHz monopolar pulses, or 1 V 50 kHz bipolar pulses. The timing for initiation and oscillations observed in the ERK activities indicated that both a positive feedback and a negative feedback circuits were involved in the process. Additional tracking of EKAR3 reporter during activation and u0126 inhibitor tests demonstrated interesting difference from the EGF-activated ERK. In addition, preliminary analysis of cell response time showed a dependence on the distance to the electrodes. Our findings may provide important insights to the activation of ERK signaling pathway and how the electric field network is coupled with the biochemical network inside the cell, which can lead to potential applications in wound healing and cancer treatments.

2:50 PM ***Dynamic Constriction Insulator-Based Dielectrophoresis for Particle Manipulation***

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Insulator-based dielectrophoresis (iDEP) has been utilized for the manipulation of particles, cells, and even organelles. Devices employing insulating post arrays, constrictions, or other geometrical features for separation, pre-concentration, and fractionation are limited by predetermined scales of achievable DEP force dependent on the device geometry. While dielectrophoretic forces can generally be increased by applying larger electric potentials, analytes may deteriorate and degrade when exposed to high DC potentials. Here, we propose to circumvent these limitations by using a tunable insulator constriction to induce DEP forces for manipulating biological particles. We present a dynamic constriction realized with a microvalve and integrated in a microfluidic device. This facilitates precise control of dielectrophoretic forces acting on nano- and microparticles. The microvalve serves as the fundamental active component by deflecting a thin elastomer membrane with pneumatic pressure. The controlled deflection of this membrane creates a dynamic constriction, which allows tuning of the electric field non-

uniformity and consequent dielectrophoretic forces near the deflection region. We further characterize the tunable device through numerical modeling to reveal the changes in dielectrophoretic force for varying degrees of deflection. The electric field gradient around the deflected membrane becomes inhomogeneous and can be increased by the amount of pneumatic deflection. With this dynamic iDEP constriction, we believe that controllable dielectrophoresis will overcome the limitations of conventional iDEP and eventually improve our ability for processes such as separation, purification, and trapping of biological particles (eg. DNA, cells, and cell organelles) via dielectrophoresis.

3:10 PM ***A novel method for accurately counting and localizing in time photobleaching steps***

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Photobleach step counting is one of the prime methods used to determine stoichiometry of protein complexes and molecular machines. However, due to noise that increases with the number of fluorophores to be detected, accurate counting has been limited to 20-30 steps maximum, even using the best algorithms available. We report the development of a new Bayesian method for counting and localizing photobleach steps in time. Our method can successfully detect hundreds of photobleach steps even for signal-to-noise ratios 10 times lower than currently possible.

4:00 PM ***Properties of Chromatin Extracted by Salt Fractionation from a Cancerous and Non-cancerous Esophageal Cell Line***

Nethmi Ariyasinghe[1,2], Subhadip Senapati[3], JongOne Im[3], Jin Park[3], Peiming Zhang[3], Stuart Lindsay,[1,2,3,4] and Robert Ros[1,2]
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While cancer is mostly viewed as a genetic disease and characterized by genetic markers and expression of mutant proteins, there is considerable evidence that there is more to cancer than somatic mutations. For example, the first signature looked for by a pathologist is a grossly aberrant cell nucleus. Chromatin compaction and structure play a major role in the overall nuclear structure. We compared chromatin compaction and structure for two esophageal cell lines, EPC2 (non-cancerous) and CP-D (cancerous) by using a combination of salt fractionation, DNA quantification by spectroscopy, and atomic force microscopy. Further, we studied the connection between gene expression and chromatin condensation for the two cell lines using DNA sequencing of salt fractionated samples. We found significant differences for DNA amounts in the different salt fractions and chromatin morphology for the cancerous and non-cancerous cell lines, as well as variations in the nucleosome

partitioning. We anticipate that our results will help to get insights into the mechanisms of cell phenotype changes from normal to cancerous.

4:20 PM ***Biophysical Studies of the Medicinally Important NAMPT Protein***

Udeep Chawla[1], Trivikram R. Molugu[1], Nipuna Weerasinghe[1], Radu C. Oita[2], Ting Wang[2], Michael F. Brown[1,3], Joe G. N. Garcia[2].
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[3]Department of Physics, University of Arizona.

Nicotinamide phosphoribosyltransferase (NAMPT) is a medicinally important protein having pleiotropic functions in human cells. The intracellular NAMPT (iNAMPT) is involved in catalyzing the rate-limiting step in NAD biosynthesis pathway, whereas extracellular NAMPT (eNAMPT) is known to function as a cytokine and is involved in regulation of obesity, cardiovascular diseases, and cancer. Extensive studies (e.g., using X-ray crystallography) have been carried out for iNAMPT protein; however, the mechanism of eNAMPT function is not known. The question we are addressing here is, what are the structural changes involved in eNAMPT activation and function? Interestingly, there is no amino acid sequence difference known between the iNAMPT and eNAMPT proteins. We hypothesize that there are structural changes involved in the eNAMPT protein that regulate protein function. To test our hypothesis, we purified the recombinant protein in bacteria and characterized it using circular dichroism (CD) spectroscopy, dynamic light scattering (DLS) techniques, and activity assays. Furthermore, we plan to use solution NMR spectroscopy to study how the structural state of the protein is involved in regulating protein function. We propose a thermodynamic equilibrium exists between eNAMPT monomers (inactive form), and higher order oligomers (active form). A switch from the monomeric state to the oligomeric state results in activation of the protein. Our studies will give insight to the function of eNAMPT protein, which is extremely important in developing new drugs against obesity, cardiovascular diseases, and cancer.

4:40 PM ***Structure of the homodimeric reaction center***

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In the focus of photosynthetic reactions is the process of converting light energy into chemical energy this process is maintained by protein pigment complexes which are called reaction centers(RC). The importance of these RC's can be seen as fundamental for any photosynthetic process. The first ever solved membrane protein structure(1984) was of the bacterial reaction center which led to the thesis that the oxygen evolving photosystem II is in its reaction center structurally closely related. In the year 2001 the first structure of photosystem II and the high resolution structure of photosystem I validated this hypothesis. The reaction center of H. modesticaldum takes a special place in between the other known structures. The H.

modesticaldum reaction center is not an ancestor of any known reaction center. From the chemical nature of the Chlorophylls(three different types) as its orientation in the core of the reaction center as its adjacent integrated Chlorophyll based antenna system it is in size and composition unique. Distances between Chlorophylls in the reaction center P800 compared to P700 and to the terminal acceptor FX are different to photosystem I. In the 2.2 Å resolution structure of H. modesticaldum reaction center the farnesyl side chains can be assigned from electron density as the nature of the over 50 Chlorophylls in the homodimer. (Funding support ongoing by DOE grant DE-SC0010575)

Poster Presentations

Poster #1
Theory and Electrochemistry of Cytochrome c

Morteza M. Waskasi[1], Salman S. Seyedi[2] and Dmitry V. Matyushov[1,2]
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Extensive simulations of cytochrome c in solution are performed to address the apparent contradiction between large reorganization energies of protein electron transfer typically reported by atomistic simulations and much smaller values produced by protein electrochemistry. We show that the effective reorganization energy composed of half the Stokes shift (characterizing the medium polarization in response to electron transfer) and the variance reorganization energy (characterizing the breadth of electrostatic fluctuations) is responsible for the activation barrier of electrode electron transfer. This effective reorganization energy is much smaller than the two factors contributing to it and is fully consistent with electrochemical measurements. Calculations in the range of temperatures between 280 and 360 K combine long classical molecular dynamics simulations with quantum calculation of the protein active site. The results are consistent with the Arrhenius plots of reaction rates and cyclic voltammetry of cytochrome c immobilized on self-assembled monolayers. The small value of the effective reorganization energy provides a general mechanism of lowering activation barriers for biological electron transport.

Poster #2
Hydrodynamic Hunters

Hossein Jashnsaz, Mohammed Al Juboori, Corey Weistuch, Nicholas Miller, Tyler Nguyen, Viktoria Meyerhoff, Bryan McCoy, Stephanie Perkins, Ross Wallgren, Bruce D. Ray, Konstantinos Tsekouras Gregory G. Anderson, and Steve Pressé
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Antibiotic-resistant bacteria present a serious rising concern and demands for a new effective treatment are higher than ever. To fight these, one promising candidate is the predatory bacterium, Bdellovibrio bacteriovorus (BV) that has recently successfully been used in vivo in antibacterial therapy. However, despite over 50 years since its discovery and multiple biochemical and genetic studies into the hunting strategy of BV, it is still unclear whether BV chemically detects its prey or collides with it at random. Nonetheless, we identify a novel, hydrodynamic, mechanism by which BV locates its prey bacteria. Here we will present our theoretical and experimental approaches addressing this problem.

Poster #3
Plastic-glass core-shell nanoparticles as selective sensors for radiolabeled biomolecules

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Small thiols such as cysteine play key roles in biological function. Abnormal concentrations of cysteine are observed in patients with diseases such as Alzheimer's, Parkinson's, cystinuria, and cystinosis. Sensors with high cell-permeability and low interference are needed for real-time measurement of intracellular thiols and disulfides since the ratio provides insight into the redox network inside cells. Radioisotopes are used in bioanalytical measurements as they provide the lowest perturbation on analyte properties, such as binding kinetics. 35S isotope has higher decay energy than 3H and the resulting beta particles travel a longer distance (ca. 300 μm in water), making 35S a better tracer for sensitive detection of sulfur-containing analytes. In this research, polystyrene-silica core-shell nanoparticles doped with reporter fluorophores were fabricated for scintillation proximity assay (SPA). SPA works based on the conversion of energy released from bound radiolabeled analyte to detectable visible light. Thiol-functionalized nanoparticles were successfully utilized to quantify cysteine at sub-nM concentration. The signal enhancement by specific binding of 35S-cysteine to these nanoparticles was one order of magnitude in a separation free assay. The extent of binding depends on the ratio of cysteine to cystine which is a function of pH, oxidation state of the compounds, and the presence of thiol-reactive agents. We applied nanoSPA sensors to measure thiols and disulfides, by protecting the thiols and reducing disulfides to more thiols. We also indirectly measured thiol-reactive agents such as HNO, which shows promising pharmaceutical properties.

Poster #4
Adaptive backbone docking

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We introduce a new approach called Adaptive Backbone Docking (Adaptive BP-Dock). This method combines perturbation response scanning (PRS) with Rosetta's flexible ligand procedure1. We first apply a perturbation on the binding site residues and obtain a new conformation based on the residue response fluctuation. Using RosettaLigand we dock ligand/peptide to the new conformation. We repeat this step several times. Adaptive BP-Dock results showed better correlations with experimental binding data compared to other docking protocols. The results suggest our docking approach can capture binding induced conformational changes by simultaneous sampling of protein and ligand/peptide conformations. Adaptive BP-Dock can advance fast and robust docking of novel targets and drug discovery.

Poster #5
Single-walled carbon nanotubes probed with insulator-based dielectrophoresis

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Electrical and optical applications of single-walled carbon nanotubes (SWNT) require suitable selection and purification methods due to the little controlled synthesis processes yielding SWNTs in large length dispersity (several tens of nanometers up to centimeters) and resulting in heterogeneous electrical and optical properties. Dielectrophoresis (DEP) is capable of manipulating nanoparticles based on their dielectric properties and the microfluidic channel geometry; as such, it is a suitable technique for separating SWNTs. Here, we present the first study of an insulator based dielectrophoresis (iDEP) manipulation of SWNTs coated with sodium deoxycholate (NaDOC) and wrapped with ssDNA (dT30). The DEP behavior of SWNTs was studied using a polydimethylsiloxane (PDMS) microfluidic device in a low conductivity HEPES buffer at physiological pH (7-7.2). SWNTs were imaged during dielectrophoretic manipulation with fluorescence microscopy based on their unique IR emission. We demonstrate SWNT trapping at low frequency (0-1000 Hz) alternating current (AC) electric fields with applied potentials not exceeding 1000 V. It was found that the trapping behavior of SWNTs is not frequency dependent over the range tested. Interestingly, suspended SWNTs varied between positive (pDEP) and negative (nDEP) dielectrophoresis, which could be attributed to their Zeta potential. Further, we found that well-suspended SWNTs demonstrated pDEP with longer sonication time and nDEP with shorter sonication time. AC iDEP possesses a high potential as a separation technique for SWNTs, and this study is fundamental for future applications of low frequency AC iDEP for technological applications and sample preparation of SWNTs.

Poster #6
Microfluidic Mixing Methods for Serial Crystallography

Austin Echelmeier, J. Coe, I. Ishigami, G. Brehm, U. Weierstall, P. Fromme, R. Kirian, J. C. H. Spence, S.-R. Yeh, D. Rousseau, A. Ros
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X-ray crystallography enables protein structure determination. With the advent of serial femtosecond crystallography (SFX), protein nano- and microcrystals, previously unable to be studied by traditional crystallography, yield diffraction patterns before they are destroyed when probed with an X-ray free electron laser (XFEL). In SFX with XFELs, protein crystals are continuously injected into the path of the XFEL, and the resulting diffraction patterns are merged to create an electron density map of the protein. While understanding a native structure is important, visualizing structural changes as a protein undergoes a reaction has notable implications for elucidating reaction mechanisms, intermediates, and drug design. The first study of this time-resolved (TR-) SFX investigated photoactive proteins by exciting them with a laser and, after a set delay time, probing the

photoexcited crystals with the XFEL. Many proteins are not photoactive, instead react to chemical changes such as decreased pH or bound ligands. These conformational changes can occur on the millisecond to second time scale; thus, fast mixing is key to TR-SFX. To accomplish fast mixing, we have developed a 3D printed microfluidic mixing device that utilizes hydrodynamic focusing to mix a substrate solution with a crystal suspension within a few milliseconds or faster. The reaction time points probed by the XFEL are controlled based on the liquid flow rates and the distance between the mixer and the laser. This mixer was employed for mixing an oxygenated aqueous solution with a suspension of cytochrome c oxidase crystals and achieved delay times on the second time scale.

Poster #7
Proteins with knots

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Under 2% of protein structures deposited in the Protein Data Bank display a knot. The function of the knots remains mostly unknown but it is accepted that they contribute to the enhancement of thermodynamic stability. The presentation will survey experimental and theoretical methods used in the studies of proteins with knots. The main focus will be the issue of how knots form during the folding process. It is demonstrated that nascent conditions in the ribosome enhance the probability to form knots.

Poster #8
Chloroplast Membrane Protein Structure And Functional Dynamics By Nmr Spectroscopy

Trivikram R. Molugu[1], James D. Zook[2], Petra Fromme[2] and Michael F. Brown[1, 3]
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The chloroplast outer envelope membrane protein OEP16 provides a channel for selective diffusion of amino acids across the outer membrane of chloroplasts, which is essential for the function of the chloroplast in photosynthesis. Here we aim to establish a comprehensive understanding of the three-dimensional structure, dynamics, and function of OEP16 by an approach combining both solution and solid-state nuclear magnetic resonance (NMR) spectroscopic methods. The broad significance is to discover how OEP16 can selectively bind and transport specific amino acids across the outer membrane of the chloroplast in the process of photosynthesis. Multi-dimensional high-resolution solution NMR spectroscopy has revealed the secondary structure and showed a four-transmembrane helical structure for OEP16 in detergent micelles [1]. Moreover, 15N relaxation studies indicate a monomeric state in SDS detergent micelles [1]. Additional solution NMR experiments will provide the structure of the OEP16 in detergent micelles as well

as bicelles [2]. Magic-angle spinning (MAS) and oriented-sample (OS) solid-state NMR experiments will determine the high-resolution structure for OEP16 in a lipid environment [2]. Emphasis will be placed on elucidating the transmembrane helical topology and functional oligomeric state. The NMR relaxation studies will shed light on functional dynamics over a wide range of time scales, and will address whether internal protein dynamics explain the difficulties with crystallization. Ligand binding studies will investigate the function of OEP16 as an amino acid transporter and elucidate the substrate specificity. These results will be combined with functional studies on transport phenomena by single-channel conductivity studies. We thus plan to explain the differences in the function of the leaf versus seed isoforms of OEP16 transporter, which can guide possible future crop engineering involving drought-tolerant species in response to global warming.
[1] J.D Zook et al. PLoS ONE 8, e78116 (2013).
[2] T.R. Molugu et al. Chem. Rev. in press (2017).

Poster #9
Survey of Ion Coordination Geometries of Structures in the Protein Data Bank

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According to some estimates, between 30% and 40% of all proteins depend on interactions with ions to perform their function. Ions can be a part of an active site in an enzyme, part of a substrate, or play a structural role. In order to understand the molecular mechanisms in all of these cases, it is important to have an accurate description of the interactions of these ions with amino acid side chains, the protein backbone, water molecules, and cofactors. An automated analysis method was developed to analyze cations contained in crystal structures in the RCSB Protein Data Bank (PDB). The ion coordination geometries of the most prevalent monovalent cations in the PDB (sodium, potassium, lithium, and thallium) were analyzed from the radial distribution functions of oxygen atoms around ions. Monovalent cations coordinate oxygen atoms within about a 6 Å radius, with a clear first "hydration shell," similar to the first hydration shell in bulk water, and a secondary shell also typically visible. However, oxygen atoms are not the only atoms that can be coordinated by cations, and coordinating atoms for anions are much less well characterized than those for cations. We therefore analyzed all atoms within 6 Å of the cations as well as chloride anions using distance and force-field derived partial charges as criteria to identify atoms that are likely a part of the coordination shells. The code is written in Python and is freely available under the GNU Public License v3 at https://github.com/Becksteinlab/PDB_Ion_Survey.

Poster #10

Tilted post array for sorting λ-DNA using insulator-based dielectrophoresis (iDEP)

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Sequencing of the entire human genome was a significant milestone in genome research. Various Next Generation Sequencing (NGS) technologies have been developed that promise fast, low-cost sequencing analysis. Recently, nanopore technologies have emerged as 4th generation NGS technology. In contrast to past NGS approaches, it achieves real-time and single-molecule sequencing using artificial or biological nanopores without additional DNA amplification and synthesis. Nanopore sequencing requires large DNA (> 10 kbp); thus, DNA sample preparation must be tailored for this size range. However, achieving efficient size-based fractionation of DNA still has considerable challenges. Dielectrophoresis (DEP) is a powerful tool for microscale particle transport within an inhomogeneous electric field. The polyelectrolyte DNA exhibits an interfacial charge and surrounding counterion cloud inducing a dipole moment in an electric field, and this allows to manipulate DNA molecules in electric field gradients in a size selective manner. Therefore, the DEP method satisfies the need for size specific fractionation of DNA for NGS technologies. We propose to induce DNA DEP sorting in continuous-flow microfluidic devices based on DEP. This approach offers significant advantages for sample preparation of 4th generation NGS technology in terms of low sample consumption and short processing times. We have designed and fabricated a continuous-flow microfluidic sorting device including a tilted post array to study DEP characteristics of λ-DNA (48.5 kbp). This work is demonstrated under alternating current (AC) insulator-based dielectrophoresis ranging from 500 to 1430 V/cm with frequencies of 100 and 1000 Hz. The injected stream of λ-DNA deflects along the tilted post array at 100 Hz, and the deflection can be varied based on the magnitude of the applied AC potential. This behavior is associated with the DEP force generated at the post array. Thus, these results based on DEP present a high possibility for effective sorting of relatively long-chain DNA molecules for 4th generation NGS sample preparation.

Poster #11

Scalable fabrication framework of implantable thin-film probes to reconcile rigidity for accuracy and flexibility for biocompatibility with biodegradable sacrificial layers

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Implantable electrodes have been the central unit of implanted sensors and electronics for biomedical research, diagnostics and therapy. However, it remains a rather difficult task to reconcile the request for scaling down the size of probe, maintaining the mechanical strength required for accurate implantation surgery,

while having the core functional structure small and flexible enough for better interfacing with cells. Here we propose a new design and fabrication procedures of ultra-small implantable probes with inorganic biologically degradable sacrificial layers which allow in-situ formation/release of 3D flexible thin-film device structures accurately in deep tissue with minimal lesion.

Poster #12
Retinal Orientation and Mobility of GPCR Rhodopsin

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Rhodopsin is the G-protein-coupled receptor (GPCR) responsible for scotopic vision in vertebrates. Although published X-ray structures are similar, the conformation and orientation of the retinal chromophore of active rhodopsin are not established unambiguously. Under debate is a proposed flip of all-trans retinal in the active state, due to a change of the roll angle of the β-ionone ring, as well as the central part of the polyene chain that includes the C9-methyl group [1]. Here we applied solid-state 2H NMR spectroscopy to study the structure and orientation of the retinylidene ligand in the active state of rhodopsin in aligned lipid membranes [2]. Rhodopsin was regenerated with retinal 2H-labeled at the C5-, C9-, or C13-methyl groups, recombined into phospholipid membranes, and then trapped in the light-actived state [3]. By theoretically fitting the solid-state 2H NMR spectra, we determined the orientations of the 2H-labeled methyl groups to the local membrane normal. The 2H NMR data together with the electronic transition dipole moment from linear dichroism data provided the orientational restraints for calculating retinal structures using a three-plane model. We discovered two structures without any steric clashes in the binding pocket of the active rhodopsin. One of the two structures has a very similar conformation to retinal in the flipped X-ray structures [1]. The other structure has the same position of the β-ionone ring, but the polyene chain and β-ionone ring are not flipped, and the chromophore orientation is similar to the dark state. Finally we discuss the implications of our findings for understanding of the rhodopsin activation mechanism.

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Poster #13
Can double-stranded DNA function as the simplest and fastest biomolecular rotary motor?

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We present a bio-inspired nanoscale rotary motor that reproduces some of the dynamic properties of biological rotary motors with 10 kHz rotation speeds. Here, we derive a fluid mechanics model from Navier-Stokes equations to support our hypothesis that double stranded DNA helix (dsDNA) will undergo rotary motion through a nanopore as a result of its conformation. Remarkably, our calculation shows that the disarmingly-simple motor will exhibit a 10 kHz rotational speed at a voltage of 1 mV applied across the pore. We are planning to validate this prediction using single molecule techniques. In our experiment, the rotary motor will consist of dsDNA, labeled with gold nanoparticles, traversing into a nanopore with diameter of 2–5 nm under electrophoresis. The proposed experimental validation may reveal that dsDNA can, in fact, be used as the rotor for the simplest and fastest biomolecular motor, and may have implications for nanopore-based DNA sequencing.

Poster #14
Detergent-Free Formation of Nanodiscs

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Given the importance of membrane proteins in cellular communication pathways and disease states, we need an efficient model for studying the function of these proteins in a physiological state. Membrane proteins have traditionally been shackled to the use of detergents, which may disrupt their native structure and interactions. Nanodiscs offer an avenue to study these proteins in a nanoscale lipid bilayer, but detergent is critical for nanodiscs formation. To preserve native interactions, we are developing detergent-free approaches for nanodisc formation. Utilizing peptides that mimic the conventional ApoAI scaffold, we formed nanodiscs directly from bacterial membrane extracts without the use of detergents. Future work will exploit this approach to characterize the protein and lipid interactome of a variety of membrane protein targets.

Poster#15
One-pot synthesis of upconverting nanoparticles

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Up-converting nanocrystals (UCNCs) are man-made materials that absorb two or more photons of infrared light and emit in the visible range. Since they are excited in the infrared, the excitation light can penetrate deeper and does not produce background fluorescence in biological matrices. In addition, these nanoparticles are very robust, do not photobleach, and have very large Anti-Stoke shifts and narrow emission bands. These particles are of interest for areas such as multimodal imaging and solar energy. In addition, the upconversion emission dependence with temperature allows for robust ratiometric temperature sensing in micro-and nanoliter volumes. We have developed a one-pot microwave solvothermal synthesis method that yields particles with fairly monodisperse particles, with mean size ranging from 5 to 15 nm, depending on the aging time in the microwave reactor (5 to 150 minutes). The synthesis solvent enables high microwave power absorbance, high synthesis temperature (300 C), ultrasmall particle size, and nanocrystals that are readily dispersible in alcohol/water mixtures. The evolution with the aging time in the microwave reactor of the UCNC luminescence, luminescence lifetime, morphology, and crystal structure have been characterized.

Poster #16
Improve capillary electrophoretic analysis using upgraded laser-induced fluorescence and isotachophoretic pre-concentration

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Capillary electrophoresis (CE) is an important analytical separation method that has found many applications in biology. Laser-induced fluorescence (LIF) is an extremely sensitive method used for capillary electrophoresis detection. In our lab, we developed an upgraded LIF-CE system that used a laser expander to better focus laser on the capillary and provided easier optical alignment. A permanent coating method was adopted to increase CE analytical stability and reproducibility. In order to further improve the detection limit of CE, we are applying isotachophoresis as a pre-concentration prior to CE analysis.

Poster #17

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

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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. We will present results of controlled and reversible deposition in a 10-nm space to produce a nano-fluidic channel with finely-tunable nanogap control electrodes. 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.

Poster #18

Simulating the conformational transitions of the transmembrane symporter Mhp1

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The function of many proteins depends on large-scale conformational changes. Because these conformational transitions are rare events, it is very difficult to investigate them with equilibrium molecular dynamics (MD) simulations, which have otherwise become an important tool to study the molecular mechanisms of macromolecular systems. A variety of techniques --- such as the Dynamic IMportance Sampling (DIMS) [1] method and various elastic network-based approaches have been developed to overcome timescale limitations and produce physically plausible trajectories between putative metastable states. We sought to characterize a number of different path generating and sampling methods, including

DIMS with and without an implicit membrane model, by producing multi-directional trajectories of the transmembrane nucleobase symporter Mhp1[2]. All trajectories were compared to one another using Root-Mean-Square Distances (RMSDs), structural order-parameters and Path Similarity Analysis (PSA)[3]. In particular, PSA showed that while trajectory generating methods were broadly similar, paths from each method were also clearly distinguishable.

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Poster #19

Detection of Slow Degrees of Freedom in Absolute Binding Free energy Calculations of Sodium Ions to the Sodium/Proton Antiporter NapA

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Sodium/Proton antiporters are integral membrane proteins that function as secondary active transporters by using the free energy in the transmembrane proton gradient to move sodium ions against their electrochemical gradient out of the cell. The detailed molecular mechanism of this fundamental physiological process is not well understood quantitatively. Our previous work showed that the prototypical Na+/H+ antiporter NapA undergoes a large conformational change between an inward and an outward facing conformation [1, 2] and that Na+ ions bind at a conserved aspartate residue in both NapA [1, 2] and the related NhaA transporter [3]. However, we do not know how the conformational changes as well changes in protonation states affect binding of Na+ ions. In order to quantitatively assess Na+ binding we performed alchemical free energy simulations to calculate the absolute ion binding free energies, depending on conformation and protonation states. Such calculations require hundreds to independent simulations that are performed along the "legs" of a thermodynamic cycle during which various ion-environment interaction components are changed in a rigorous manner. The data that are collected and then recombined using either the thermodynamic integration or the multistate Bennett acceptance ratio method, yielding the free energy of transferring the ion from bulk solution to the binding site. However, these demanding calculations are difficult to converge. In particular, the strong Coulomb interaction between ion and charged aspartate residues appears problematic. We hypothesize that the convergence problem for the Coulomb legs can be attributed to slow degrees of freedom, namely slow exchanges between different rotamers of the

aspartate side chains closest to the Na+ ion. We quantify the time scale in these slow motions in the molecular dynamic simulations and evaluate the binding free energy conditional on the different states of the slow degrees of freedom. Preliminary results indicate an improvement in convergence and hint a way to improve binding free energy calculations.

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Poster #20

3D Tissue Model with Reversible Tunable Stiffness

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Cells can sense and respond to the properties of their environment. For example, it is well accepted that the stiffness of the surrounding matrix dictates the phenotype or differentiation of a cell. During cancer progression, the mechanical properties of the extracellular matrix changes dramatically, but little is known about the dynamic interplay of matrix mechanics and cell behavior in 3D. We developed a novel hydrogel based on gelatin modified with single-stranded DNA handles, allowing us to tune the stiffness reversible in the physiologically relevant range by using DNA-based crosslinkers. In this poster, we present the underlying concepts of the hydrogel design, the compatibility of the novel gels for 3D cell culture and quantifications of the Young's moduli. We anticipate that the novel tunable 3D tissue model will allow us to get insights into the molecular mechanisms and underlying processes of cancer progression and stem cell differentiation.

Poster #21
Protein phosphatase-1 holoenzyme regulates cell cycle transition

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Phosphorylation is the key post translational modification that regulates the progression of the cell cycle. Research in the last decade has focused on a variety of kinases that phosphorylate protein substrates and mark various stages of the cell cycle. However, the role of phosphatases that counteract the action of these kinases and how they maintain the order and dynamics of the cell cycle is not well understood. Protein phosphatase 1 (PP1) is a ser/thr phosphatase that is crucial for the progression of the various stages of the cell cycle. The specificity and localization of the substrate that is dephosphorylated by PP1 depends on its interaction with regulatory proteins. Misregulation of the cell cycle is often associated with cancer and understanding the precise role of PP1 in this process is important in order to establish PP1 as potential drug target. To meet this need, we are investigating the interaction of PP1 with one of its regulators that governs the metaphase-anaphase transition using an integrated approach of NMR spectroscopy, X-ray crystallography and biochemistry. Novel Aurora B phosphorylation sites have been identified and their role in both holoenzyme assembly and recruitment to microtubules have been determined using NMR spectroscopy. Together, these studies are revealing the molecular basis of PP1 binding and the counteracting effect of Aurora B Kinase phosphorylation in a PP1 regulator that orchestrates metaphase-anaphase transition.

Poster #22
Water Mediates Elastic Deformations In Biological Membranes

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Lipid membranes are excellent examples of biological soft matter [1]. Many functions of biomembranes involve collective phenomena with motional timescales spanning several decades (10-12 s to s). For liquid-crystalline membranes atomistic interactions often explain bulk material properties in relation to key biological functions. Solid-state 2H NMR spectroscopy provides such information by simultaneously probing structure and dynamics [2]. Here we examine the effect of hydration on the liquid-crystalline properties of membranes using NMR relaxation methods. We performed 2H NMR longitudinal (R1Z) and transverse quadrupolar-echo decay (R2QE) experiments on DMPC-d54 bilayers, to study membrane-lipid dynamics. Plots of the R1Z rates versus squared segmental order parameters (SCD2) follow an empirical square-law showing the emergence of collective lipid dynamics [3]. Such a functional behavior characterizes 3-D order-director fluctuations due to the onset of membrane elasticity over mesoscopic dimensions

[3]. The R2QE rates also showed similar results. At high hydration there is an R2QE enhancement of the functional square-law for the 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. Such membrane deformations are also evident from bilayer structural parameters calculated using a statistical mean-torque model [4]. In addition, the square-law confinement must be due to water penetration into the hydrophobic interior of the bilayer. The slow dynamics at high hydration must be a consequence of modulation of membrane elastic properties. The QCPMG frequency dispersions provide quantitative viscoelastic properties of the liquid-crystalline membranes. Such studies on model membranes give insights into lipid rafts and membrane compositions relevant for biomembrane functions.

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Poster #23
Influence of Lipid Bi-layer on G-protein coupled receptor activation

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G-protein-coupled receptors (GPCRs) are essential components of cellular signaling mechanisms, and also comprise over thirty percent of pharmaceutical targets worldwide. Our goal is to investigate the role of the lipid bilayer in the activation mechanism of GPCRs using rhodopsin, a key protein involved in vision, as the model GPCR. Previous studies have shown the essential role that spontaneous membrane curvature, caused by an imbalance of the lateral forces between lipid headgroups and acyl chains, plays in mechanically favoring the transition of rhodopsin to its elongated active state, metarhodopsin II. Building upon this knowledge, we hypothesize that as the quantity of receptors within a bilayer increases with respect to the amount of lipid, the increased crowding of rhodopsin will inhibit the ability of the membrane intrinsic curvature energy to drive the protein's conformational changes upon activation. Mechanistically, greater crowding of the proteins should yield a competition between the elastic curvature energy of the lipid bilayer and the hydrophobic matching of the bilayer acyl chains to the protein intramembranous surface. We are testing our hypothesis by recombining varying proportions of dark state rhodopsin with artificial membranes of varying lipid compositions. UV-Visible spectroscopy is then used to measure the time-dependent change in active-state protein following exposure to light. These findings should present an alteration to the current protein-centric model of biochemistry by revealing roles that soft matter, in the form of lipid bilayers, plays in mechanisms of biological signaling.

Poster #24
Biophysical Inference with Bayesian Non-Parametric Methods

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Biophysical measurements, such as those obtained in single molecule experiments, are commonly analyzed by means of Bayesian statistics. Despite their popularity, traditional Bayesian methods can lead to miss fittings mainly because they require the number of different states the biomolecules attain to be pre-specified and fixed. Here, I present methods that lift this requirement and therefore avoid overfitting. These methods utilize novel concepts from non-parametric statistics and machine learning that allow inference without assuming a pre-specified or fixed number of molecular states. This characteristic makes them ideal for the analysis of experimental data.

Poster #25
Morphological and mechanical properties of Salmonella Typhimurium correlate with antimicrobial resistance

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Atomic force microscopy (AFM) offers significant potential for studying the structures and mechanical properties of nanoscale bacteria cell walls. According to a recent study, a PhoP mutant of Salmonella Typhimurium bacteria is more susceptible to cationic antimicrobial peptides (CAMPs) in comparison to wild type Salmonella Typhimurium. The mechanism behind this phenomenon could be alterations of the surface properties of the bacteria. We used AFM to characterize the nanoscale morphology and elasticity of the two bacteria strains and found significant differences in the root mean square (RMS) roughness and Young's moduli between the mutant and wild type bacteria. The interplay of nanoscale surface properties and CAMP resistance could lead to new insights into the underlying mechanism.