bilateral. In addition, uniformly oriented samples for solid state NMR spectra of 15N isotopically labeled LspA, have been prepared in synthetic lipid bilayers providing initial structural insights.

267-Pos Board B22
Structural Studies on the N2A-IS Region of Titin
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Sarcomeres, the basic units of striated muscle cells, are built of a highly ordered filament system which includes myosin, actin, and titin. Titin is the largest known protein with ~34800 amino acids which governs muscles some of its elastic properties [1]. Titin is also involved in several signaling pathways via interactions with a number of proteins. Its interaction with MARPs proteins is very important as these interactions are involved in stretch signal in muscle [2]. The MARP proteins, specifically MARP2 is present in sarcomere of skeletal muscles and binds to N2A region of titin [3]. The N2A region consists of four Ig domains I-80 to I-83 and intervening/unique sequence ‘IS’ between Ig domains I-80 and I-81. The structure of N2A region is currently unknown but critical for stress sensing. MARP2 binds to the N2A-IS region in a relaxed state and is dissociated when a muscle is overstretched [2]. This binding is also disrupted in case of the murine mdme deletion [4] which deletes part of the I-83 Ig domain and part of the PEVK region. We are characterizing the N2A-IS region using a combination of CD, FRET and solution state NMR as well as in silico methods. The in silico analysis showed that N2A-IS region consists of α-helices and some of the regions are disorder. These results were also confirmed by CD experiments, which confirmed the α-helical nature of this region. Ongoing solution state NMR studies on U-15N and U-13C labelled N2A-IS constructs will provide high-resolution structural information on this region.


268-Pos Board B23
Single Molecule FRET Characterization of Structural Changes in Antibodies Induced by Enzymatic Deglycosylation
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The deglycosylation of antibodies has been suggested as a potential treatment for some autoimmune disorders as this leads to a diminished immune response. The reduction in immune response is thought to arise from weakened binding of the fragment crystallizable (Fc) region of immunoglobulin G (IgG) antibodies with effector molecules as a result of a conformational change in IgG region. The nature of this structural alteration is uncertain due to conflicting results obtained from x-ray crystallography and small-angle x-ray scattering studies. To further examine the impact of deglycosylation on the structure of the IgG region, we have examined both glycosylated and enzymatically deglycosylated IgG antibodies using single molecule Förster Resonance Energy Transfer (FRET). The FRET efficiency histograms obtained from studying freely-diffusing, dye-labeled antibodies suggest that the flexibility of the IgG region increases upon deglycosylation.

269-Pos Board B24
Single-Molecule and Ensemble Structural Study of the AAA+ Atpase P97 Taihyung Lee1, MinJin Kang2, Eli Chapman, Ashok A. Deniz.1
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p97/Cdc48/VPQ is a highly conserved, essential member of the class of AAA+ ATPases present in both archaea and the eukaryotic cytosol. Like many of the AAA+ proteins, the functional state of p97 is a hexameric ring-shaped structure. However, the interplay between monomer folding and assembly of the quaternary structure of p97, as well as other members of this class, has remained elusive. Here, we present a study of the structural changes of p97 during its folding and assembly, utilizing biophysical techniques including single-molecule Förster Resonance Energy Transfer (smFRET). p97 is large for using standard techniques to dual label with dyes for FRET studies. Therefore, this work aims to gain a deeper understanding about critical structural aspects of p97, while exploring advanced labeling methods that extend the scope of fluorescence studies to larger and more complex protein systems.

270-Pos Board B25
Structure Refinement of the Transmembrane Domain (TMD) of KCNE1 Protein using Deer Spectroscopy
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KCNE1 is a single trans-membrane protein of the KCNE family that modulates the function of voltage gated potassium channels, including KCNQ1. Hereditary mutations in KCNE1 have been linked to diseases such as long QT syndrome (LQTS), atrial fibrillation, sudden death syndrome, and deafness. However, the structure of its physiologically relevant native membrane bound state is unknown. The transmembrane domain of KCNE1 plays a key role in mediating the physical association with KCNQ1, modulating KCNQ1 gating kinetics, and modulating KCNQ1 pore conductance. This mechanism is still not fully understood. Previous solution NMR study indicated that KCNE1 possesses a curvilinear transmembrane domain in LMPG micelles. In this study, we used Double electron electron resonance spectroscopy coupled with site-directed spin labeling and simulated annealing molecular dynamics to report the structure of the TMD of KCNE1 in proteoliposomes. High quality DEER data consisting of similar distance distribution in micelles, proteoliposomes and lipid nanoparticles suggest a curved helical TMD of KCNE1 in proteoliposomes similar to that in micelles. Additionally, we employed DEER distance restraints obtained at 8 double spin labeling sites in simulated annealing molecular dynamics and predicted the structure of the TMD of KCNE1 in vesicles which supports the previous micelle structure. This study will provide useful information about how TMD functions in channel activation during E1/Q1 interactions in diseases causing mutations.

271-Pos Board B26
Structural Studies of the Docking Complex between Cytochrome P450 (CYP101) and Putidaredoxin (PDX) by Double Electron-Electron Resonance (DEER)
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P450cam (CYP101) is an archetype of the cytochrome P450 superfamily of heme mono-oxygenases. This enzyme hydroxylates camphor to 5-exo-hydroxy-camphor following two distinct electron transfers from its unique [2Fe-2S] redox partner, Pdx (Putidaredoxin). Other ferredoxin-type proteins, such as adrenodoxin, are only able transfer the first electron and lack the features of the complex of P450/Pdx required for the second electron transfer. This specificity of the protein electron donor suggests that geometry of the protein-protein complex may play a critical role in the electron transfer process. Moreover, we have observed in previous studies that P450cam shifts from the closed conformation upon binding camphor, and that oxidized but not reduced Pdx induces a shift of substrate-bound P450cam from the closed to the open conformation. The above results lead us to pursue structural information about the solution complex of P450cam and Pdx in different redox and coordination states. Several combinations of site-directed spin labeling (SDSL) sites were made with single methanethiosulfonate (MTSL) spin-labels on P450 and Pdx. The inter-spin label distance constraints in the P450/Pdx complex were measured by Double Electron Electron Resonance (DEER or PELDOR). While these studies show that the solution complex between P450cam and Pdx is consistent with that reported in recent crystallographic studies, the studies also show little difference in the docking conformation of P450cam(oxidized)-Pdx(oxidized) and P450cam(reduced)-Pdx(reduced). We will also report DEER studies of the conformational states of P450cam bound to CN- and NO. These results will help clarify the structural factors responsible for the role of Pdx as a structural effector of P450cam function.

272-Pos Board B27
Probing and Characterizing Distinct Conformational States Populated by Influenza M2 Protein
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The Influenza A virus poses a large public health threat to populations worldwide. One of the proteins on its viral coat, M2 - a homotetrameric membrane
protein, was originally thought to function only as a proton channel. Recently, M2 has been shown to aid in viral budding and interact with cholesterol, which facilitates budding. In order to investigate the interaction of M2 with cholesterol, a construct containing residues 22-60, named M2TMC, was reconstituted into various detergent micelles and lipid bilayers with varying amounts of cholesterol, and studied using EPR spectroscopy. Specifically, four sites of the peptide were studied using CW EPR, 142, L46, I51 and F55. This study showed that the relative populations of the two distinct conformations change depending on the amount of cholesterol present in the lipid bilayer. Supplementary to CW EPR, DEER was performed on three sites, F48, I51 and F55, to measure inter-spin-label distances. Specifically, this study showed that as the amount of cholesterol in the lipid bilayer increased, a higher population of the peptides populated an "immobile" conformation. By fitting these multicomponent CW EPR spectra, exact changes in the relative population of the two states could be quantified. Regarding membrane topology, it was shown that this immobile conformation has spin-labeled that are further away from the membrane surface. DEER studies further showed that each conformation had distinct inter-spin-label distances.

273-Pos Board B28
Probing Structural Properties of KCNE1 Membrane Protein: A Site-Directed Spin Labeling EPR Study
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KCNE1 is a single-transmembrane protein found in heart that modulates the activity of the KCNQ1 voltage-gated potassium channel. KCNE1 is very important for proper cardiac function. However, the structure of KCNE1 in a native membrane environment is not completely understood. Site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy has emerged as a well-established method to study the structural properties of membrane proteins. In this study we have used continuous wave EPR and pulsed EPR techniques in combination with the SMA-Lipodisq nanoparticles based sample preparation to obtain more accurate and precise EPR data to answer pertinent structural questions on KCNE1. CW-EPR and DEER data were collected on several single and double spin labeling sites on KCNE1 in micelles, proteoliposomes and lipodisq. We obtained a significant improvement in the quality of EPR measurements in lipodisq samples compared to proteoliposomes. The structural and dynamic properties of KCNE1 will be discussed.

274-Pos Board B29
The Conformations of the DrkN SH3 Domain Studied by Single Molecule Fluorescence Spectroscopy
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SH3 domains are highly involved in signal transduction and cellular localization. The N-terminal SH3 domain of Drosophila adaptor protein Drk is found to be marginally stable, exchanging between folded and unfolded states under non-denaturing conditions. The high unfolded state population makes DrkN SH3 a useful model system to study the physical polymeric properties of disordered protein states and to advance the understanding of the mechanism of protein folding.

Single-molecule techniques have the unique capability to resolve populations of protein conformations and also the rates of exchange dynamics among them. Here, single-molecule Förster resonance energy transfer (smFRET) is performed in order to study the conformational distribution and dynamics of the DrkN SH3 domain, using fluorophores attached to two cysteines mutated at the N- and the C-termini of the 61amino acid chain. Freely-diffusing proteins in diluted solutions give rise to fluorescence bursts which can be quantitatively characterized. Multiparameter fluorescence analysis reveals two populations with different end-to-end distances, attributed to the folded and unfolded states coexisting under normal conditions. Conformational populations and internal chain dynamics are measured in both physiological and non-physiological conditions in order to understand the role of solvent-protein interactions for the structural stability. Fluorescence correlation spectroscopy (FCS) and FRET-FCS are applied to investigate local chain dynamics and the inter-conversion kinetics between the ordered and disordered conformations of DrkN SH3 in different solvent conditions.

Protein Conformation

275-Pos Board B30
Modeling Proteins as Residue Interaction Networks to Understand Structure-Function Relationship
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Proteins participate in various cellular processes in their monomeric and oligomeric states. Their structural stability and interaction capability are important for their efficient functioning in oligomeric state. Identification of residues important for those interactions remains a problem. Here, we model proteins as network with amino acid residues being the nodes of the network and proximity (both bonded and non-bonded interactions) between residues are the edges. Network studies of proteins have shown that active sites and functionally important residues in monomeric proteins can be identified. In the current work we use Network Analysis (NA) for identifying important residues in our interacting protein database, FLIPdb. We adapted a coarse grained approach to construct network by connecting Cβ atoms of amino acid residues within 9 Å distance. Analysis of network centrality parameters such as degree, closeness, and betweenness indicate that the network characteristics of residues involved in quaternary interactions (particularly those functionally-linked) are differentiable from the network characteristics of other residues.

276-Pos Board B31
Withdrawn
This abstract has been withdrawn.

277-Pos Board B32
The Protein Translocase Activity of Anthrax Toxin Protective Antigen is Stereoselective
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Translocase channels deliver proteins across membranes within living cells. Many classes of these translocases have narrow pores that require substrate unfolding. Additionally, transport is known to be highly directional. These energy intensive processes of course require the consumption of an energy gradient. The conformation of the translocating chain is likely critical to these mechanisms; however, these areas are largely unexplored due in large part to the lack of appropriate biophysical models. Presently, we use anthrax toxin as a tractable model to study translocation. The toxin is comprised of a channel, called protective antigen (PA), which translocates its two substrates, lethal and edema factors (LF and EF, 90kDa each), across a host cell’s endosomal membrane. Using planer lipid bilayer electrophysiology, we have demonstrated the translocation of full length LF, EF, and the N-terminal domain of LF (LFN : LF residues 1-263) via the PA oligomer. Translocation is driven by a membrane potential (Δψ) or proton gradient (ΔpH), albeit the latter is likely more physiological. Here we report single-channel and ensemble studies of translocation using long synthetic peptides designed in a manner that only their stereochemistry is varied. Specifically, we have taken the first 50 residues of LF and constructed peptides with all L-amino acids, all D-amino acids, and mixtures of D and L amino acids. The Δψ- or ΔpH-dependent translocation of these different peptides reveals that the peptides with uniform stereochemistry translocate more rapidly than those with mixed stereochemistry. These data are consistent with the model that the substrate does not solely translocate in an extended conformation, but rather the substrate also forms more-compact helical states only accessible to substrates with uniform stereochemistry.

278-Pos Board B33
Structural Studies on the A. Thaliana Heterotrimeric G-Protein: Understanding the Mechanism of α Subunit
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The heterotrimer G protein consists of the alpha (Gα), beta (Gβ) and gamma (Gγ) subunits; Gz has GTP binding and hydrolysis activity and Gβ/Gγ interact...