Probing Structural Properties of KCNE1 Membrane Protein: A Site-Directed Spin Labeling EPR Study

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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 when facilitating 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, I42, 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 more 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 lipodisqs. 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-denaturating 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 Föster 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 Cμ atoms of amino acid residues within 9 Å distance. Analysis of network centrality parameters such as degree, closeness, and betweeness 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 ($\Delta\psi$) 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 $\Delta\psi$- 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; Gα has GTP binding and hydrolysis activity and Gβ/Gγinteract