968-Pos Board B723
Probing the Secondary Structure of Membrane Proteins with the Pulsed EPR ESEEM Technique
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A novel approach is being developed to probe the secondary structure of membrane proteins and peptides qualitatively utilizing the three-pulse Electron Spin Echo Envelope Modulation (ESEEM) pulse sequence. In order to demonstrate the practicality of this biophysical technique, the M2delta subunit of AChR (α-helical), KAGAKI (β-sheet) and VRL8 (310-helix) peptides were incorporated into phospholipids bicelles to probe their secondary structure with ESSEM spectroscopy. Utilizing site-directed spin-labeling (SDSL) coupled with deuterated amino acid labeling of the peptides, the corresponding ESEEM spectra reveal characteristic patterns for α-helix, β-sheet and 310-helical structures. This ESSEM secondary structural approach can be used with different deuterated amino acids and provide pertinent qualitative structural information on membrane proteins in a short period of time (10 minutes) with small amounts of sample (30 μg).

969-Pos Board B724
Site-Directed Spin Labelling of Sulfite Oxidase using Non Natural Amino Acids
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Site-directed spin labeling (SDSL) in combination with EPR spectroscopy has proven to be a valuable tool to investigate structure and dynamics of proteins. Commonly, spin labels are introduced into proteins using cysteine residues. However, this strategy fails if proteins contain functionally important cysteines. A recent proof-of-principle study using T4 Lysozyme as a model system has shown that non-natural p-acetylphenylalanine binding the spin label through a ketoxime ligation can be used for SDSL [1]. While T4 Lysozyme does not contain essential amino acids and is a rather stable protein, the current contribution will present a study on sulfite oxidase, a molybdo-enzyme, which carries an essential cysteine residue binding the Moco-cofactor, using the same spin labeling strategy as in [1].
Within this contribution we will discuss challenges involved in the labeling of a more fragile protein using the less reactive ketoxime ligation in comparison to the disulfide linkage and will explore the information gained from the EPR line shapes of these spin labels in terms of structure determination, using mutations along a helical turn as an exploratory example. In addition, doubly spin labeled proteins were created to determine distances between the spin labels using pulsed electron-electron double resonance (pELDOR/DEER) experiments. The distance distributions extracted from these measurements will be discussed in light of expectations based on simple geometric considerations and will be compared to results of cysteine based distance determinations.
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970-Pos Board B725
High-Resolution Measurement of Distance and Orientation in Myosin: EPR of a Bifunctional Spin Label
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We present a method for obtaining high-resolution information on protein backbone structure and dynamics using electron paramagnetic resonance (EPR) of a bifunctional spin label (BSL) and molecular modeling. Two complementary EPR techniques were employed to measure protein orientation (conventional EPR) and intra-protein distances (dipolar electron-electron resonance, DEER). BSL attaches at Cys positions *i* and *i*+4 on a helix, greatly reducing probe mobility relative to the peptide backbone, compared to monofunctional labels. Accurate modeling of BSL provides the coordinates required to directly relate spectroscopic data to backbone structure (both orientation and distance), and dynamics (rotational motion). In the current work, the motor protein *Dictyostelium* myosin II was used to demonstrate this approach. We measured nucleotide-dependent structural transitions of two key helices within the myosin catalytic domain (CD). Two double-Cys sites were engineered, with one Cys pair located on the relay helix, and the other on a stable helix in the upper 50kD domain. BSL on a construct with one of these pairs was used to measure myosin orientation relative to oriented actin. BSL on a construct with two pairs was used to measure interprobe distances. The effect of ADP binding on both orientation and distance was clearly detected with BSL, but not with a monofunctional label. The significance of this work is twofold: (1) A structural transition in the relay helix upon ADP binding was clearly defined with high resolution. (2) BSL spectra demonstrate superior resolution, compared to monofunctional spin labels, making it possible to directly translate spectroscopic data to protein structure and dynamics. This work was funded by grants from NIH (R01 AR32961, T32 AR07612, P30 AR0507220).

971-Pos Board B726
Dynamic and Contrasting Information by Oriented-Sample Solid-State NMR Spectroscopy of Membrane Proteins
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Oriented-sample NMR (OS NMR) has emerged as a powerful technique for structure determination of membrane proteins in their native-like lipid environment. We have developed a model relating OS NMR lineshapes to uniaxial ordering (mosaic spread) and rotational diffusion of the protein within the membrane. The model is exemplified by 15N NMR spectra of Pf1 coat protein in both magnetically aligned phage and reconstituted in oriented bicelles. In the case of Pf1 phage, the lineshapes are dominated by static uniaxial disorder; whereas fast rotational diffusion of the protein is responsible for the motional line narrowing in perpendicularly oriented bicelles. From the analysis of 15N NMR linewidths, rotational diffusion coefficient can be estimated. Since the value of the diffusion coefficient is ultimately related to the overall protein size, information about oligomerization states is potentially obtainable. Second, the use of various membrane-embedded radicals allows one to both dramatically speed up data acquisition, on the one hand, and obtain contrasting information for membrane-embedded proteins, on the other. While membrane-bound paramagnetic species drastically affect the T1Z relaxation times (at 2:1 molar ratio relative to the protein), the transverse T2 relaxation is only slightly affected. 5-DOXYL stearic acid, TEMPOL, and CAT-1 radicals exhibit different partitioning within the membrane, and result in differential paramagnetic effect on the spectral peaks arising from different residues of Pf1 protein in bicelles. This allows one to obtain contrasting information about the location of the residues relative to the membrane. As was shown by EPR, TEMPOL partitions itself equally in and out of the membrane, and almost uniformly affects all residues within the bilayer. By contrast, 5-DOXYL stearic acid affects mostly the residues below the interfacial region, while CAT-1 affects the residues located within the polar head groups.

972-Pos Board B727
Structure and Function of Bacterial Biofilms by Solid-State NMR
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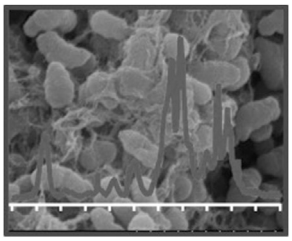
Biofilms are multi-cellular communities formed by bacteria, and they consist of bacteria encased within a non-crystalline extracellular matrix (ECM) of proteins, polysaccharides, and small molecules. Biofilm formation provides increased protection of bacteria from antibiotics and host defenses. New quantitative approaches are needed to define composition and architecture of biofilms. We have utilized solid-state NMR to quantitatively define the composition of the wild-type uropathogenic E. coli UTI89 extracellular matrix. Recently, we have extended the same methodology to elucidate changes in biofilm composition due to inhibitory small molecules and to define the composition of Vibrio cholerae biofilm.



973-Pos Board B728
NMR Structural Studies of Antimicrobial Peptides as In-Plane Helix of Membrane Proteins
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Antibiotics from organic compounds are a long established part of our defense against bacterial pathogens. Their wide use has led to pathogens' increased

drug resistance and the need to find novel classes of antimicrobial peptides as alternatives to antibiotics. Lactophoricin (LPcin), a cationic amphipathic peptide consists of 23-mer peptide, corresponds to the carboxy terminal 113-135 region of component-3 of proteose-peptone. LPcin is a good candidate as a peptide antibiotic because it has an antibacterial activity but no hemolytic activity. Three different analogs of LPcin, LPcin-yk2 which has mutant amino acids, LPcin-yk1 and LPcin-yk3 that has shorter mutant amino acids are recently developed by using peptide engineering in our laboratory. These three LPcin analogs show better antibioitic activities than wild-type LPcin and no toxicity at all. In order to understand the structural correlation between LPcin analogs structure and antimicrobial activity under the membrane environments, we tried to express and purify as large as amounts of LPcin and three different LPcin analogs. We finally optimized and succeed to overexpress in the form of fusion protein in *Escherichia coli* and purified with biophysical techniques like Ni-affinity chromatography, dialysis, entrifuge, chemical cleavage, and reversed-phase semiprep HPLC. In here, we will present the optimizing processes for high-yield expression and purification and solution NMR spectra and solid state NMR spectra for antimicrobial mechanisms.

974-Pos Board B729

NMR Analyses of the Structure and Dynamics of Klebsiella Pneumoniae OMPA Domains and Full Length Protein

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The transmembrane domain of kpOmpA possesses four long extracellular loops which exhibit substantial sequence variability throughout OmpA homologues in *Enterobacteria*. These loops are responsible for the immunological properties of the protein, such as cellular and humoral recognitions. Using liquid state NMR we have determined the 3D structure of kpOmpA in DHPC micelles (M. Renault et al., *J. Mol. Biol.* 2009). In a micellar environment, a complex dynamical behavior has been observed: a rigid barrel core, ms motion at the micellar-water interface, and sub-ns motion within the loops. Using solid state NMR relaxation and proteolysis experiments, we have demonstrated the persistence of this complex motional behavior in *E. coli* polar lipid bilayers (I. Iordanov et al., Biochim. Biophys. Acta, 2012). Using single molecule force spectroscopy (with D. Muller and A. Engel) we have shown that kpOmpA is able to unfold and refold reversibly its β-barrel core (P. Bosshart et al., *Structure* 2012).

Recent advances involve: a) characterizing the structure of its C-terminal domain and its interaction with the peptido-glycane; b) analyzing ssNMR spectra of N-terminal membrane domain in liposomes using MAS at 1 GHz and 60 kHz spinning frequency (with G. Pintacuda); c) comparing the NMR spectra of the various domains and the full length protein in solution, in liposomes and in intact cell envelopes using cellular solid state NMR as established in (M. Renault et al., PNAS 2012).

975-Pos Board B730

High-Resolution NMR Spectroscopy Reveals Structure of Lipoprotein flpp3

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Characterization of membrane proteins continues to be an enormous challenge in the field of structural biology compared to their soluble counterparts. Membrane proteins require the presence of a detergent micelle to protect the hydrophobic-exposed residues that are, under native conditions, inserted into a membrane. This provides challenging hurdles for expression, purification, and structural determination. However, for some lipoproteins and in the case of flpp3 from Francisella tularensis, the hydrophilic domain of the protein can be expressed and purified separately from the transmembrane domain. Flpp3 contains a single transmembrane helix at the N-terminus and therefore this technique allows for structural investigation of the majority of the protein without the necessity of a detergent micelle. In this study we report the results of expression, purification, biophysical characterization and NMR analysis of the soluble domain of flpp3 (flpp3-Sol). Sufficient quantities of pure flpp3-Sol are obtained via recombinant expression in *E. coli* cells followed by purification via Ni-NTA chromatography and gel filtration chromatography. Monodispersity and global secondary structure is investigated by dynamic light scattering (DLS) and circular dichroism (CD) respectively. NMR studies report local secondary structure via secondary chemical shifts and are used to generate a structure-model using CS-ROSETTA. Structures generated from NMR data are compared to structures in the Protein Data Bank using the Dali server and provide insight into possible protein function. The structure of flpp3-Sol is similar to the C-terminal domain of the lipoprotein bamC from *E coli*, a protein important for the insertion of beta-barrel proteins into the outer membrane of cells via the BAM complex; and as of yet unidentified in *F tularensis*.

Advances in Single-Molecule Spectroscopy I

976-Pos Board B731

A Sequential Monte Carlo Method for Identifying Motion Parameters from Particle Tracking Trajectories

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Optimal estimation of diffusion coefficients from single- and multiple-particle tracking data has undergone several breakthroughs in the past few years. Prior to these breakthroughs, the most common method for extracting motion parameters from trajectories was by fitting a curve to the mean square displacement [1]; it has been known for quite some time, however, that this a possibly inaccurate and unreliable method due to the presence of noise in the trajectory [2]. As a result, recent efforts by A. J. Berglund and X. Michalet have provided an optimal framework for trajectories corrupted by motion blur and Gaussian white noise [3]. These methods typically require that localization be performed prior to the estimation of motion parameters rather than in conjunction. These two problems, however, are dependent on each other. In this work, we present a Sequential Monte Carlo approach that utilizes the Expectation Maximization algorithm to simultaneously localize particle positions and estimate motion parameters from raw data (e.g. image sequences). The method provides a clear accuracy-vs-complexity trade-off and can be parallelized for greater efficiency. We demonstrate its effectiveness by detailing its use on arbitrary point spread functions, such as the double helix, and motion models, such as those driven by Markov jump processes.

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977-Pos Board B732

A Highly Specific Gold Nanoprobe for Live-Cell Single-Molecule Imaging in Confined Environments: Intracellular Tracking and Long-Term Single Integrin Tracking in Adhesion Sites

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Single molecule tracking in live cells is the ultimate tool to study subcellular protein dynamics, but it is often limited by the probe size and photostability. Due to these issues, long-term tracking of proteins in confined and crowded environments, such as adhesion sites, synaptic clefts or intracellular spaces, remains challenging. We present a novel optical probe consisting of 5-nm gold nanoparticles functionalized with a small fragment of camelid antibodies that recognize widely used GFPs with a very high affinity (1). These small gold nanoparticles can be detected and tracked using photothermal imaging for arbitrarily long periods of time (1-2). Surface and intracellular GFP-proteins can effectively be labeled even in very crowded environments such as adhesion sites and cytoskeletal structures both in vitro and in live cell cultures. Comparison with performances obtained by superresolution methods such as PALM and STED are presented for single integrin tracking in and out adhesion sites (3). These nanobody-coated gold nanoparticles are single molecule probes with unparalleled capabilities; small size, perfect photostability, high specificity, and versatility afforded by combination with the vast existing library of GFP-tagged proteins.

References:

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