molecular blockades studied here, the accuracy inherited is 99.9% to distinguish between terminal base pairs of two DNA hairpins. The pattern recognition software consists of Hidden Markov Model (HMM) feature extraction software, and Support Vector Machine (SVM) classification/clustering software, that is optimized for data acquired on a nanopore channel detection system. The PRI-feedback system is based on a server running a LabWindows protocol that is set up to control the amplifier settings that eject a molecule from the channel if the SVM determined confidence level is not high enough to accept the signal. The control server takes signal information and broadcasts it via TCP/IP to a collection of compute nodes to do the HMM and SVM computations, and decision-making, in a distributed fashion, to stay within the time-frame of the signal acquisition in-process – ending that process if identified as not in the desired subset of events, or once recognized as non-diagnostic in general.

Due to purification limitations and fragmentation of antibodies and some antigens, it is time consuming to acquire the required subset of signals during analysis and for biosensing with a passive, uninformed (or manual), sampling capability. The advantage of PRI data acquisition for an antibody-antigen system is due to the reduction in wasted observation time on eventually rejected "junk" (non-diagnostic) signals. The use of PRI feedback for nanopore channel data collection reduces the amount of time required to acquire data, using fixed objective criteria, and thus, considerably improves the quantity and quality of the data analysis.

2463.13-Pos Using a Small Number of Cryo-EM Particle Images to Build an Icosahedral Density Map at Subnanometer Resolution

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Board B577.13

Several thousand cryo-EM particle images are now typically cited for generating a 3-D icosahedral reconstruction at subnanometer resolution. To achieve the same resolution, theoretically, only a few evenly sampled particles are needed. We developed Multi-Path Simulated Annealing, a Monte Carlo type of optimization algorithm, for globally and simultaneously searching the center and orientation parameters of each particle. This method avoids error propagation and model bias. A consistency criterion was derived to exclude particles in the iterative refinement process. Following this procedure with a new empirical double threshold particle selection method, we are able to pick small number of best quality particles to reconstruct a subnanometer resolution map without any model as a template. Using the best 62 particles of rice dwarf virus, the reconstruction reached 9.6Å resolution at which 4 helices of the inner capsid protein are resolved. Furthermore, with the 284 best particles, the reconstruction is improved to 7.9Å resolution, and 21 of 22 helices and 6 of 7 beta sheets are resolved. The relationship between numbers of particles and the best achievable resolution has also been studied.

This work was supported by NIH grants (R01GM070557 and P41RR02250).

EPR Spectroscopy

2464-Pos Computational Studies Of A Nitroxide Spin Label At Solvent-exposed, Helix Surface Sites In Proteins

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Board B578

We study the conformational dynamics of the spin label R1 (1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl-methanethiosulfonate) at solvent-exposed, helix surface sites in proteins through molecular dynamics (MD) simulations. In addition, we develop a theoretical framework to compute electron spin resonance (ESR) spectra from the simulated trajectories. First, we characterize the behavior of the spin label on the surface of a solvated poly-alanine alpha-helix. This system serves as a model of R1 at an idealized, noninteracting, solvent-exposed site. It allows us to critically assess the chi4/chi5 model, commonly invoked to rationalize the observed ESR spectra in terms of the internal spin label dynamics at such sites. Then, we proceed with simulations of R1 at positions 131 and 72 in T4 Lysozyme. We observe that specific and non-specific interactions of the nitroxide with the neighboring amino acids, rather than putative hydrogen-bonding of the disulfide to the backbone, are responsible for the relative ordering and immobilization of the spin label. This raises questions regarding the extent to which the spectral line shape reports on fluctuations of the protein backbone. To evaluate the relevance of our observations, we calculate ESR spectra at 9, 95 and 170 GHz simulated MD trajectories. The results are in remarkably good agreement with experimental spectra. We conclude that the rigorous interpretation of ESR spectra has to take into consideration the molecular nature of the spin label and its environment.

2465-Pos α-Synuclein Fibril Structure Investigated by Continuous Wave and Pulsed EPR Spectroscopy

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Board B579

The misfolding and aggregation of α-synuclein plays an important role in the pathogenesis of Parkinson and other neurodegenerative diseases. Misfolding of α-synuclein appears to involve specific structural conformation that populates certain steps of the misfolding. This includes the oligomeric state and the fibril state. We aim to decipher these structures in order to understand the mechanism of misfolding. Of particular interest have been fibrils inasmuch as they represent the endpoint product. α-Synuclein fibrils contain signifi-

cant amount of β-structure in contrast to the highly dynamic, largely unstructured α-synuclein in solution. Despite intense efforts, the precise structure of α-synuclein fibrils remains unknown. Here we applied continuous wave (cw) and pulsed EPR spectroscopy coupled with site-directed spin-labeling to define the structure of α-synuclein fibrils.

We find that the core region of α-synuclein fibrils is tightly packed and extends from residues 36 to 98. The structure within the core regions is parallel and in-register, with same residues from different polypeptides stacking on top of each other. Only few areas are significantly less ordered. Oxygen accessibility suggests the location of potential β-sheet structures. We have begun to determine intra-molecular distances in order to build more detailed structural models. We generated a series of double-cysteine mutants in the region of potential β-structures (e.g. residues 70 to 80), in which two residues were labeled simultaneously. Distances were obtained from cw and pulsed (4-pulse DEER) EPR measurements. The results are in excellent agreement with predicted distances for extended β-sheet structures, confirming that the tested regions indeed form β-structures. We are presently mapping inter-strand distances, which, together with molecular modeling, should allow us to generate an accurate three-dimensional model of α-synuclein fibril.

2466-Pos The External Stalk of the FOF1-ATPase: Three-Dimensional Structure as Determined by Site-Specific Spin Labeling, ESR and Molecular Modeling

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Board B580

We generated double-cysteine mutants in the soluble domains of the homo-dimeric b2 from E. coli and the hetero-dimeric bb‘ from Synechocystis sp. PCC 6803 ATPsynthase and reacted introduced cysteines with SH-specific spin-labels. ESR spectroscopy and dipolar line broadening of the resulting spectra was used to determine inter-spin distances. Inter-molecular distances of spin-labels attached to the homodimeric E. coli b2 or between residues in b and b’gave information about subunit packing.

Furthermore, we analyzed coiled coil structures found in the protein data bank. Sequence-based predictions of coiled coil domains from these proteins and the subunits b and bb’ were performed. The analyses predicted left-handed coiled coil (LHCC) structures for subunits b2 and bb’ in an about 100 amino acid long region of the b-subunits of both organisms. Other bacterial and eukaryotic b-subunits from photosynthetic organisms also were predicted to contain LHCC in elongated parts of these proteins. Homology models of E. coli and Synechocystis b-dimers were created using molecular modeling techniques. The created models were refined and energy minimized. The MTS-modified cysteinyl residues were modeled using topology and parameter files.

The ESR-experimentally observed inter-and intra-molecular spin distances correlate very well with distances obtained in molecular modeling experiments of LHCC structures for both b- and bb’-dimers. The data strongly support LHCC structures as a low energy structures for the subunit b-dimer of the ATP synthase. Experiments are currently underway to investigate the structure of the E. coli b-dimer in the presence of F1-ATPase. We have generated single cysteine b-subunit mutants of the FoF1-ATPsynthase. Labeling and ESR in the presence of ATP, ADP or trapped in a transition state will show potential conformational changes in the second stalk of the synthase during turnover.

2467-Pos Improved Distance Determination from Dipolar Broadening of EPR Spectra

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Board B581

Double Electron Electron Resonance (DEER) has become very popular to determine interspin distances of 20–50 Å, but is generally not suitable for much closer distances. Below ∼20 Å, the dipolar interaction is sufficient to broaden the CW EPR spectrum. In 2001, we showed[1] that the deconvolution of the dipolar broadening[2] can be used to determine interspin distance distributions in double spin-labeled proteins at room temperature as long as the interspin vector varies slowly, a condition easily met in larger proteins or by increasing solvent viscosity[1]. However, the deconvolution analysis has been a complex process in the past, requiering significant experience and operator intervention. Here we present an new set of software tools written in LabVIEW to facilitate and automate the determination of distance distributions from dipolar broadening using Tikhonov regularization and nonlinear optimization.

References

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2468-Pos Identifying Conformational Exchange in Proteins with Site-Directed Spin Labeling

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Board B582

Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA 90095 Recent evidence suggests that proteins can exist in a manifold of conformational substates in equilibrium, and that some of these substates play important roles in protein function. Therefore, there is great interest in identifying sequence regions within proteins which are in conformational exchange. Site-directed spin labeling (SDSL) combined with EPR spectroscopy appears to be a

promising tool for this purpose. In SDSL, a paramagnetic side chain (R1) is introduced in a site specific manner into the protein of interest and the EPR spectrum of the spin labeled protein reflects the local environment around the nitroxide probe. Multiple components in the EPR spectrum can reflect conformational substates of the protein, or multiple rotameric states of the R1 side chain itself. Osmolytes have profound effects on conformational equilibria of proteins, but rotameric equilibria of R1 are found to be insensitive to osmotic perturbation. Thus, osmolytes provide a means to differentiate the two possibilities. Using this strategy, conformational exchange has been identified in the important α2 helix (switch II) of Gα (GDP) but not in the activated Gα (GTP) state. These and other results suggest that switch II exhibits conformational polymorphism in its GDP bound state, but is transformed to a unique conformation upon nucleotide exchange.

2469-Pos Gd(III)-Nitroxide Pairs for Structural Studies of Membrane Proteins: Long-range Distance Rulers and Side-chain Orientations

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Board B583

Distance measurements using site-directed spin labeling (SDSL) and EPR are based on detecting magnetic interactions of a nitroxide spin-label with another paramagnetic center. The second center could be another nitroxide label or a paramagnetic metal ion. Previously, distance-dependent relaxation effects of Cu2+ (1) and Gd3+ (2) on nitroxides were measured with X-band EPR. We are interested in extending the well-established method of SDSL EPR to high magnetic field experiments in order to fully utilize advantages of HF EPR. Here we report on experiments to investigate magnetic interactions between Gd3+ ion positioned at the surface of the phospholipid bilayer and a nitroxide label attached to the transmembrane WALP peptide with X-band and W-band (95 GHz) EPR. Slow (as compared with other paramagnetic metal ions) electronic relaxation of Gd3+ at magnetic fields above 3 T and the highest possible for an ion electronic spin state (S=7/2) results in easily observable relaxation enhancement effects for the nitroxide labels. We also demonstrate the feasibility of manipulating the nitroxide-Gd3+ interactions by changing the magnetic field of the experiment: the electronic relaxation of Gd3+ slows with the field increase. We also show that for nitroxide-labeled phospholipid bilayer the relaxation enhancement is anisotropic allowing for deriving the label orientation with respect to the bilayer surface. The latter measurements are attainable to the excellent angular resolution of HF EPR.

T.I.S. acknowledges support from NSF grant MCB-0451510. B.D was supported by Undergraduate Research Fellowship from NCSU and NSF REU Fellowship.

2470-Pos EPR Methods for Probing Secondary Structure

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Board B584

The vision system is a model system for studying G-protein coupled receptor (GPCR) signaling, which is marked by a series of protein/protein interactions and often coupled with conformational changes within the proteins. The GPCR rhodopsin is activated by a photon of light, which in turn activates the G-protein transducin. This activated transducin initiates a signaling cascade that eventually leads to an optic nerve impulse. In order to prevent a continuous signal, and to regenerate a fresh rhodopsin, this signal must be terminated. This is accomplished by phosphorylation of rhodopsin by rhodopsin kinase, followed by the binding of the arrestin protein. Arrestin binding prevents further interactions between rhodopsin and transducin, thus halting the signal. Because these protein/protein interactions are often accompanied by large conformational changes, a flexible region within arrestin might serve as an important element in its ability to form protein complexes. Visual arrestin was crystallized as a conformational heterodimer, showing heterogeneity in three regions. Our previously published data confirm that one of these regions (residues 70–75) is important in rhodopsin binding. Here we use site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy to explore the solution structure of another of the heterogeneous regions (residues 155–163). To do this, we have attached a spin label individually at each of these residues and used multiple EPR techniques to characterize the solvent accessibility. This will allow us to determine whether this region exists in a helical conformation, a loop conformation, or a dynamic equilibrium between the two states.

2471-Pos Structural Dynamics of an N-terminal Histone tail that Mechanically Couples ATP Hydrolysis to Nucleosome Movement by a Chromatin Remodeling Enzyme

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Board B585

ATP-dependent chromatin remodeling enzymes catalyze transitions between accessible and repressive chromatin structures. In higher eukaryotes, silenced loci have characteristic chromatin structures consisting of regularly spaced nucleosomes. These structures are speculated to promote silencing by promoting higher order chromatin folding and facilitating the cooperative spreading of repressive proteins. The chromatin remodeling enzyme ACF moves nucleosomes to generate equal DNA spacing between them and is required for silencing in vivo. However, little is known about how ACF converts ATP hydrolysis into force capable of disrupting DNA-

histone interactions. Intriguingly, the basic patch of amino acids on the unstructured N-terminal tail of histone H4, which becomes inaccessible in compacted chromatin, stimulates the activity of ACF. We find the H4 tail has two effects on Snf2H activity:

1. The tail participates in substrate assisted catalysis to enhance ATP hydrolysis, and
2. the tail promotes coupling of ATP hydrolysis to remodeling of the nucleosome.

The observation that ACF co-opts the H4 tail, part of the substrate, to promote coupling presents a novel opportunity to define the catalytic steps, which have not previously been traceable. We hypothesize that if the H4 tail promotes mechano-chemical coupling, it should adopt different functionally relevant conformations as a function of the enzyme's nucleotide state. We have developed spectroscopic methods to characterize tail dynamics during the remodeling reaction. By labeling the H4 tail with environmentally sensitive dyes and spin probes for electron paramagnetic spin spectroscopy (EPR), we are looking at conformational changes the enzyme induces in the H4 tail when trapped in different intermediate steps in the ATP hydrolysis cycle. By determining how the H4 tail contributes to ACF activity, we hope to gain insight into the basic mechano-chemical cycle of ACF.

2472-Pos Biophysical Characterization of CPX-351, A Liposomal Formulation Co-encapsulating Cytarabine and Daunorubicin at a Fixed Synergistic Ratio

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Board B586

CPX-351, a liposomal formulation containing the antineoplastic drugs cytarabine and daunorubicin at a synergistic 5:1 molar ratio, is currently in Phase 1 clinical trials for treatment of advanced hematologic malignancies. Key spectral and physical properties of CPX-351 were determined using a range of biophysical techniques. Detailed spectroscopic characterization delineated extensive intra-liposomal drug-excipient interactions, particularly between the copper gluconate/triethanolamine buffer system and daunorubicin. The combination of NMR and EPR data determined that nearly all of the cytarabine and daunorubicin interact with the buffer system. The EPR spectrum of CPX-351 exhibited resonances from both cytarabine- and daunorubicin-copper complexes with the parallel region dominated by the former and the perpendicular region characterized by a broad featureless resonance from the latter. The UV-Vis spectrum of daunorubicin shifts from 480 to 525 nm upon liposome encapsulation due to copper-drug coordination. Despite the high concentration of both drugs inside the liposomes, electron microscopy did not exhibit any morphological features that could be attributed to precipitated or highly aggregated drug(s). Instead, the micrographs revealed a consistent spherical internal structure within the liposome, subsequently shown to consist of a phospholipid bilayer using fluorescence labeling and chemical quenching of the external lipid leaflet. Formation of the second lamellae was caused by the exposure of the liposomes to high external buffer osmolality during the cytarabine loading process. The presence of the internal vesicle did not appear to affect subsequent daunorubicin loading. Drug encapsulation conditions required for formation of bilamellar liposomes were identified, such that generation of this feature could be predicted and controlled. The physicochemical features described here provide a basic understanding of the physical state of the drugs and excipients inside the liposomes that are associated with the functional performance of CPX-351.

2473-Pos Structural Changes of Amyloid Fibrils Due to Annexin A5 Interaction

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Board B587

The hallmark feature of diseases such as Alzheimer disease, Parkinson disease and type II diabetes mellitus is a pathological accumulation of a stable amyloidogenic protein that results in the formation of fibrillar deposits. Amyloid fibril formation is a multi-step process starting with monomeric protein that forms into oligomeric intermediates and ultimately forms fibrils. Although the end stage of amyloid disease progression is the accumulation of fibrils within these deposits, there has been increasing evidence that non-fibrillar oligomers are the predominant toxic species. It is thought that these amyloid oligomers misfold and cause cell death by perturbing cellular membranes. Modification of this misfolding pathway can help prevent the formation of toxic species that cause cell death. We made the discovery that annexin A5 has the ability to alter the misfolding of amyloid-β, α-synuclein and IAPP. According to electron paramagnetic resonance (EPR) spectroscopy we found that the addition of annexin A5 reduces the spin-spin interaction normally evident in parallel, in-register fibrils. Electron microscopy images show significantly reduced fibrils and increased amounts of aggregated protein when annexin A5 was added to amyloid proteins. Thioflavin assays showed a loss of the fibrillar form of amyloid proteins when they were grown in the presence of annexin A5. Additionally, we show that annexin A5 is expressed in human islet cells and exogenous annexin A5 reduces IAPP-induced apoptosis. Thus, our data suggests an interaction between amyloid proteins and annexin A5 that disrupts fibril formation and decreases cellular toxicity. This information can perhaps pave way to new therapeutic interventions for amyloid diseases.

2474-Pos The Release Of NO Detected By EPR From The Delocalized Intermediate Formed During The Reaction Of Nitrite With DeoxyHb

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Board B588

Recent studies support a role for nitrite reduction to NO in red blood cells (RBCs) in providing a pool of NO that is available to initiate the signalling pathway of vasodilation. However, in RBCs NO rapidly reacts with oxyhemoglobin ($Hb^{(II)}O_2$) to form methemoglobin ($Hb^{(III)}$) and nitrate and with deoxyhemoglobin ($Hb^{(II)}$) to form heme-nitrosylated hemoglobin ($Hb^{(II)}NO$). To explain this phenomenon, the formation of a metastable intermediate that retains NO bioactivity without reacting with hemoglobin was postulated. The presence of this intermediate has now been demonstrated by comparing nitrite consumption with the formation of the endproducts in the reaction. To explain the release of NO from the intermediate we have compared the release of NO from the Fe(II) nitrosonium complex (present as part of the delocalized intermediate) and the autoxidation of oxyhemoglobin , which releases superoxide into the heme pocket by a nucleophiloic attack of the distal histidine on the heme iron. Incubation of the nitrite intermediate at $-20\,^{\circ}C$ has, thus, been shown to release NO into the heme pocket. At elevated temperatures this trapped NO will be released from hemoglobin. Coupling this release with hemoglobin membrane interactions can release NO to the vasculature in the same way that superoxide is released from RBCs.

2475-Pos Characterization of Dysfunctional MsbA Mutants Using SDSL EPR Spectroscopy

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Board B589

MsbA is classified as an ATP-binding cassette (ABC) transporter. The ABC transporter superfamily is among the largest known and transports a diverse group of substrates including lipids, antibiotics, sugars, and amino acids. There are a number of human pathologies in which dysfunctional ABC transporters play a crucial role. MsbA is a 65 kDa inner membrane protein comprised of two nucleotide binding domains and two transmembrane helical domains and functions as a homodimer to transport lipid A across the inner membranes of Gram-negative bacteria such as E. coli. MsbA is an essential bacterial protein and its deletion or dysfunction causes the accumulation of lipid A within the inner membrane, eventuating membrane instability and cell death. Using electron paramagnetic resonance (EPR) spectroscopy, previously identified dysfunctional point mutations were paired with spin-labeled cysteines to act a reporter groups. These reporter groups were comprised of residues from the LSGGQ, H-, Walker A, Walker B, and the Q-loop motifs. Each reporter group residue has been studied by site-directed spin labeling, serving as an appropriate background with which to compare the results of the dysfunctional mutant reporter pairs. The interactions of the mutated protein at each point during hydrolysis, using ATP, ADP, and ATP with vanadate, have been investigated to discern the stage of inactivation of the point mutations by observing the local tertiary interactions before, after, and during ATP hydrolysis. This aims to assess the role of the dysfunctional mutations in ATP hydrolysis and lipid A transport. ATPase activity assays were carried out to assess the ATPase activity of each MsbA mutant. The EPR spectroscopy data, when compared to the ATPase activity data, provides an indicator of dysfunction in the ATP hydrolysis and lipid A transport process.

Supported by: NIH GM 070642.

2476-Pos Domain Domain Orientation in p85 subunit of Phosphatidylinositol 3-Kinase Determined by Pulsed EPR and Modeling

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Board B590

Pulsed electron paramagnetic resonance (EPR) spectroscopy, in combination with molecular modeling were utilized to determine the domain-domain orientation in the p85 subunit of Phosphatidylinositol 3-kinase (PI3K). PI3K are regulators of proliferation, cell growth, and apoptosis in mammalian cells. PI3K functions as a dimer consisting of subunits p85, a regulatory switch, and p110, which contains the catalytic sites. Oncogenic mutations in both monomers have been identified, however, overall conformation and the regulatory mechanism are unclear. It is proposed that the nSH2 domain of p85 adopts different orientations with respect to iSH2 domain in the oncogenic mutants relative to the wild type, which impacts the activity of the catalytic subunit. In this study, we determined nSH2-iSH2 inter-domains distances in p85ni construct using site directed spin labeling (SDSL) and double electron-electron resonance (DEER). The spin label side chain conformation were modeled on p85ni via Monte Carlo simulations, and the domain-domain orientation was predicted via molecular dynamics incorporating the experimental distances as constraints.

2477-Pos The Influence of Membrane Lipid Composition on the Mode of Action of Cecropin-melittin Antimicrobial Peptides

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Board B591

Cecropin-Melittin (CM) hybrid peptides belong to a class of linear, amphipathic antimicrobial peptides (AMPs) that are good candidates for development as novel antibiotics. These peptides form α-helices in the presence of membranes and bind co-operatively to induce membrane lysis. Although it is known that CM peptides can disrupt membranes and cause leakage of internal solutes, their mode of action and the basis of their specificity for bacterial membranes is not clear. Here we use EPR spin labeling methods and fluorescence spectroscopy to study the interaction of CM15 (a 15-residue CM peptide) with membranes whose compositions mimic the bacterial inner membrane (IM-LUV) or the RBC membrane (RBC-LUV). While the RBC membranes contain high levels of zwitterionic lipids and cholesterol, bacterial membranes are devoid of cholesterol and carry a high content of anionic lipids (~30 mol %). Both the binding of CM15 and the release of entrapped solutes were significantly decreased for RBC-LUV as compared to IM-LUV. These differences were due primarily to electrostatic surface interactions, since increasing the concentration of anionic lipids in cholesterol-containing membranes restored binding and lysis. Our results indicate a strong dependence of lipid composition on CM15 binding and membrane lysis.

Supported by NIH grant GM068829

2478-Pos Structural Basis for the Allosteric Regulation of the Ribonucleotide Reductase from L. leichmannii

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Board B592

Ribonucleoside triphosphate reductase is the enzyme responsible for catalyzing the conversion of the four canonical ribonucleotides to the corresponding 5′-deoxyribonucleotides (dNTPs). Binding of dNTPs to an allosteric site is required for enzyme activity, and is known to regulate substrate specificity, but the structural changes responsible for these effects have yet to be determined. Four loops border the allosteric site, and it is proposed that changes in the position and/or the structural dynamics of these loops could mediate allosteric regulation. We have used site-directed spin labeling and EPR spectroscopy to study the structural dynamics of these loops in the presence of each of the dNTPs and in the absence of dNTPs. Our results show that these loops are intrinsically well-ordered, despite the absence of secondary structural elements. Furthermore, binding of dNTPs to the allosteric site results in only small changes in the observed motion of the spin labels attached to these loops. These changes are observed only at positions very close to the dNTP-binding site. Therefore, they likely represent restriction of spin label side chain motion due to steric interactions with the dNTPs. There is no evidence of a large-scale order-disorder transition. This suggests that these loops mediate allosteric regulation by a change in their position relative to the catalytic domain and the active site.

2479-Pos Structure of the Human Erythrocyte CDB3 Variant G130R from Site Directed Spin Labeling Studies and Double Electron-Electron Resonance

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Board B593

Hereditary spherocytosis (HS) is a congenital disorder of the red blood cell membrane that leads to spherically shaped erythrocytes and hemolytic anemia. Defects in erythrocyte membrane cytoskeletal proteins, including the spectrin α- and β-chains, ankyrin, protein 4.2 and band 3, can account for the molecular pathophysiology of many cases of HS. Three individual point mutations in cdb3 including E40K, P327R, and G130R have each been shown to lead to a reduction in protein 4.2 binding to the membrane and to HS. The P327R mutation causes subtle changes in the positioning of a subpopulation of helix 10 at the dimer interface of cdb3 but no detectable global structural changes (Zhang et al., *Biochemistry 46*, 10248–10257, 2007). In the current studies, electron paramagnetic resonance (EPR) and pulsed double electron electron resonance (DEER) spectroscopies have been employed to examine the structure of the cdb3 G130R variant. It is demonstrated that the G130R point mutation does not alter the global structure of the cdb3 dimer, but it does disrupt the packing of surface α-helix 2 comprised of residues 128–141. Studies are underway to determine the consequences of this structural change on the binding of ankyrin and protein 4.2 to the G130R variant.

Supported by NIH R37 HL034737.

2480-Pos Aqueous and Membrane Structure of Synaptotagmin I Using Pulse EPR Spectroscopy

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Board B594

Synaptotagmin I (sytI) is a synaptic vesicle protein believed to act as the Ca^{2+} sensor for neuronal exocytosis. It consists of one N-terminal transmembrane helical segment and two C2 domains (C2A and C2B) that are connected by a short, flexible linker. The calcium binding loops of each C2 domain coordinate Ca^{2+} ions and bind anionic phospholipids. Furthermore, sytI is thought to promote fusion in the Ca^{2+}-bound state, although it is unclear to what extent this is caused by the phospholipid binding of the C2 domains. We are characterizing the structure of sytI both in its aqueous and membrane bound states. The orientation and depth of penetration of Ca^{2+}-bound sytI C2A-C2B to 3:1 POPC:POPS membranes have been determined (Herrick et al., Biochem., 2006,*45*, 9668), however the relative spatial arrangement of the two domains is unknown. There have been reports that sytI's tandem C2A and C2B domains interact

and change conformation in solution in the presence of Ca^{2+}. To test this prediction and determine the configuration of the two domains, double cysteine mutations were engineered into a water soluble fragment of sytI C2A-C2B and derivatized with the methanethiosulfonate spin label. Four-pulse DEER (Pannier et al., J. Mag. Res., 2000, 142, 331) was used to obtain distance measurements between tandem C2A and C2B in solution and with membranes. The data obtained thus far indicate that there are no direct interactions between the two domains, and that Ca^{2+} does not significantly alter the relative orientation of the domains in solution. Models are being generated by means of simulated annealing for both solution and membrane-bound forms of sytI, using DEER-derived distance constraints and depth constraints from EPR power saturation.

The work was supported by NIGMS grant GM 72694.

2481-Pos The Mechanism of Ribonucleotide Triphosphate Reductase Reaction, as Studied with Rapid Freeze-Quench Electron Paramagnetic Resonance and Electron Nuclear Double Resonance

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Board B595

Ribonucleotide reductases convert ribonucleotides to their corresponding deoxyribonucleotides. Ribonucleotide tripshosphate reductase (RTPR) from *L. leichmanii* is an adenosylcobalamin-dependent allosterically regulated monomeric enzyme that relies on the radical generation for catalysis. The catalytically relevant intermediates are all spin $^1/_2$ species, which makes the enzyme amenable to study with electron paramagnetic resonance (EPR). We capture the intermediates using rapid freeze-quench (RFQ). This technique arrests the catalytic cycle at predetermined timepoints and delivers samples in the form of ice powder. Samples quenched on the order of seconds have been studied, and substrate radical has been detected and characterized by high-frequency EPR and Electron-Nuclear Double Resonance. To obtain samples at faster quench times, a commercially available RFQ apparatus has been transformed in our lab: to facilitate sample collection, the sample is sprayed onto liquid nitrogen-cooled copper wheels; to enhance contact of the solutions, a 250 lines per inch mixing grid is being used; consistent freezing of the solution at the copper surface is ensured through the use of precision blade scrapers. The performance of the mixer/RFQ/EPR sequence has been tested and a dead time of 15 +/− 5 msec has been demonstrated.

2482-Pos Novel Fluorescent Spin Traps

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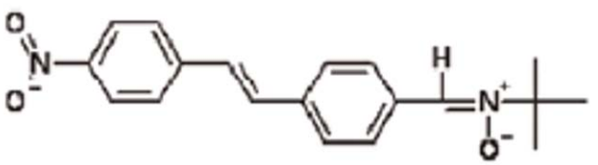
Board B596

A fluorescent nitrone composed of a nitrostilbene moiety and the t-butyl-nitrone has been synthesized. Upon addition of short-lived radicals (ROS) a relatively stable nitroxide is formed which quenches the fluorescence. Simultaneously, the fluorescence maximum is shifted to shorter wavelength due to the shorter conjugated system. Hence, by means of confocal laser microscopy the formation of ROS may be followed with subcellular resolution and their nature eventually even be determined by EPR spectroscopy.



Single Molecule Biophysics - I

2483-Pos Single-Molecule Protein Folding Studies on Barnase

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Board B597

The mechanistic details of protein folding reactions are complex and often involve numerous intermediates through which a protein must pass before reaching its final folded state. Single molecule methods allow the direct resolution of such intermediates from more stable states such as the completely folded or unfolded populations. Here we present single molecule FRET studies conducted under a variety of equilibrium conditions on Barnase, a protein which has such an intermediate (1). Using a capillary-based continuous-flow single molecule mixing device (2), we plan to resolve the major protein folding phases of Barnase under non-equilibrium folding conditions using single molecule FRET as our reaction coordinate. A comparison between the equilibrium and non-equilibrium data sets will reveal the degree to which protein folding mechanisms can change under these widely different experimental conditions.

References

1. Dalby PA, Oliveberg,M, Fersht AR. 1998. "Movement of the Intermediate and Rate Determining Transition State of Barnase on the Energy Landscape with Changing Temperature". Biochemistry *37*:4674–4679
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2484-Pos Dynamics of Single Annexin V Molecules on Supported Lipid Bilayers

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