R1 side chain mobility and solvent accessibility were scanned using cw-EPR and the power saturation technique. Both parameters show significant changes at multiple sites which are widely scattered over the peripheral domain of cdb3 indicating that the binding interface involves multiple discontinuous patches rather than the single binding motif revealed by previous mutation deletion studies. The global structure of the complex has been investigated by determining multiple inter-molecular distance constraints using DEER (double electron electron resonance) between selected sites on the peripheral domain of cdb3 and surface sites on the backbone region of ankD34. The measured distances are not consistent with the previous docking model reported in the literature (Michaely et al., *EMBO J.* **21**(23):6387-96, 2002). The EPR and DEER data are now being utilized in concert with molecular modeling approaches to construct a new structural model for the cdb3●ankD34 complex. Supported by: NIH P01 GM080513.

1579-Pos Board B423

Structural Dynamics Of Myosin'S Light Chain Domain In A Pre-power Stroke Conformation

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Cross-linking the two most reactive Cys of the myosin catalytic domain (CD) (SH1 and SH2) inhibits force production and ATP hydrolysis and locks myosin in a weak actin-binding conformation. Recent work using a bifunctional spin label (BSL) to crosslink SH1 and SH2 has shown that the CD is immobilized and orientationally disordered, suggesting that cross-linking traps myosin in a an intermediate state that primes the myosin head to generate force (Thompson et al., BJ, in press). In the present study, we measured light chain domain (LCD) structural dynamics in muscle fibers as a function of SH crosslinking. If the CD really is orientationally disordered by crosslinking and the two domains are structurally coupled, some of this disorder should be propagated to the LCD. To measure LCD structural dynamics, we used site-directed spin labeling to label chicken gizzard regulatory light chain (RLC), and then exchanged this labeled RLC for the native RLC in rabbit psoas fibers. Prior to crosslinking, EPR spectra acquired with the fiber axis parallel and perpendicular to the external field were very different, but cross-linking decreases this difference, indicating increased disorder. Saturation transfer EPR on these fibers showed that the heads remained immobile and thus attached to actin. These results support our hypothesis that SH1-SH2 crosslinking traps an actomyosin complex, possibly the first force-generating state in the power stroke, in which the CD is highly disordered and LCD is partially disordered, indicating a partially flexible linkage. A secondary goal of this research is to improve the technology for EPR on muscle fibers by developing a novel high-sensitivity EPR resonator for analysis of spin-label mobility, orientation, and force on labeled fibers.

1580-Pos Board B424

Interactions of Lipoxygenase with Paramagnetic Substrate Analogs

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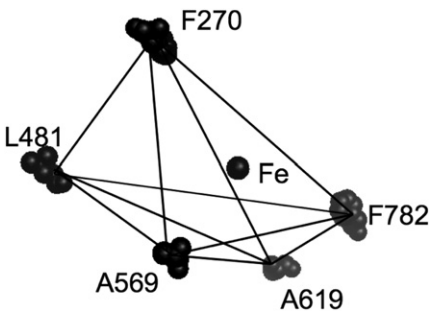
How fatty acid substrates and inhibitors enter and reside in the internal cavity of lipoxygenases is still unresolved. A medium-resolution, solution structure of lipoxygenase is obtained by positioning spin labels on a grid of five sites near the protein surface, all 20-30 Å distant from iron. Cysteine substitutions, for subsequent spin labeling, are made on a background of soybean lipoxygenase in which serine replaces four native cysteines. Dynamics and solvent exposure of the spin labeled side chains are characterized by solution electron paramagnetic resonance (EPR) spectra in the presence and absence of viscogens and fatty acid substrate analogs. Additionally, more detailed structural information is obtained from site directed spin labeling of helix-2 to examine how binding of inhibitors influences the conformation of this critical helix. Examples of changes in separation of outer hyperfine extrema in the EPR spectra when 30% sucrose is added include the following. For helix-2: spin labeled mutants T259C (change=0.17mT); K260C (mobile); F270C (0.11mT); and for grid points: L480C (0.16mT); L541C (0.16mT); and A569C (mobile). This study precedes determining the location of paramagnetic substrate analogs by examining dipolar-determined distances between the protein labeled sites and spin labeled substrate analogs.



1581-Pos Board B425

Solute Effects on Spin Label Mobility for Aqueous Exposed Sites on HIV-1 Protease

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We investigated the effects of four solutes on the protein rotational correlation time, the local dynamics of the protein backbone, and the mobility of labels covalently attached to multiple aqueous exposed sites in the HIV-1 protease (HIV-1 PR) using X-band continuous wave (CW) and pulsed electron paramagnetic resonance and fluorescence anisotropy. The HIV-1 PR contains two Beta-hairpin flaps that sit over the active site and are believed to undergo a large conformational change to allow the substrate access to the active site. The solutes used in this study were glycerol, sucrose, PEG3000 and Ficoll400 providing for a comparison between hydrophilic solutes (sucrose and Ficoll) and solutes that can interact with hydrophobic patches on the protein (glycerol and PEG3000) as well as a comparison between small viscogens and macromolecular crowders. The CW-EPR line shapes for four commonly used nitroxide spin labels attached to the Beta-hairpin flap region of HIV-1 protease were found to have to largest change in the presence of glycerol and PEG3000 whereas sucrose and glycerol have the larger affects on the steady-state fluorescence anisotropy. Comparison of the CW line shapes and distance distribution profiles from the pulsed EPR experiments for HIV-1 protease in the absence and presence of inhibitor reveals that the presence of solutes affects the line shape by perturbing the spin label mobility via preferential interactions between the solutes and the spin label.

1582-Pos Board B426

Biochemical and Biophysical Characterization of the Dysfunctional E506Q MsbA Protein

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MsbA is a 65kDa ABC transporter found in the inner membrane of Gram-negative bacteria. The ABC transporter superfamily is one of the largest known and is responsible for the transport of a variety of substrates from lipids to antibiotics. ABC transporter dysfunction is involved in a range of human pathologies from cystic fibrosis to Stargardt's macular dystrophy. MsbA functions as a homodimer comprised of two nucleotide binding domains and two transmembrane domains and transports lipid A across the inner membrane of Gram-negative bacteria. MsbA is an essential protein in *E. coli* and its deletion or dysfunction results in the accumulation of lipid A in the inner membrane causing membrane instability and cell death. The E506Q mutation in the nucleotide binding domain is dysfunctional in MsbA and is a well-known mutation within the ABC transporter superfamily. To characterize this dysfunctional mutation for the first time in MsbA, *in vivo* growth assays, *in vitro* ATPase activity assays, and EPR spectroscopy studies throughout the ATP hydrolysis cycle were conducted. The E506Q mutation was paired with nine different reporter residues, each in or near a key nucleotide binding domain motif. Each pair has been characterized by site-directed spin labeling, ATPase assays, and an *in vivo* growth assay and compared to the reporter residues alone. To identify the stage in the ATP hydrolysis cycle in which the E506Q mutation is dysfunctional, the local tertiary interactions before, during, and after ATP hydrolysis were monitored by EPR spectroscopy at each stage of the ATP hydrolysis cycle, using ATP, ADP, and ATP and vanadate. With the ATPase activity, growth rate, and EPR spectroscopy compared between the reporters alone and paired with the E506Q mutation, the functional step at which the E506Q mutation dysfunctions has been identified in MsbA.

1583-Pos Board B427

Calculation of the EPR Spectrum of a Small Nitroxide from Molecular Dynamics Simulations

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Algorithms have been developed for calculating the continuous wave electron paramagnetic resonance (EPR) spectrum of a nitroxide spin label from a free-induction decay (FID) derived from a molecular dynamics (MD) simulation. These algorithms have been successfully used to simulate the EPR spectra of two spin-labeled mutants of T4 lysozyme, T4L F153R1 and T4L K65R1

[DeSensi et al., *Biophys. J.* **94**, 3798-3809 (2008)]. In this previous work, MD simulations were performed using the AMBER suite of programs, the all-atom AMBER99 force field, the particle mesh Ewald (PME) method for treating long-range electrostatic interactions, and the SPC/E water model. In order to test the effect of the water model used in the AMBER-based molecular dynamics simulations, calculations for a small nitroxide [3-hydroxymethyl-(1-oxy-2,2,5,5-tetramethylpyrroline) or 3MeOHSL] are being performed using a variety of different water models (SPC/E, TIP3P, TIP4P). The EPR spectra calculated from these molecular dynamics simulations of 3MeOHSL will be compared to experimental data.

1584-Pos Board B428

Simulation of Slow Motion EPR Spectra with a General Hindering Potential Expanded in Spherical Harmonics

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Electron paramagnetic resonance (EPR) spectra are simulated by solving the stochastic Liouville equation (SLE) of motion with the incorporation of a hindering potential that restricts the Brownian rotational diffusion of the spin-label. Such a potential has been expanded in spherical harmonic functions in the past, under the assumptions of cylindrical and inversion symmetries appropriate for the description of liquid crystals and other ordered systems. In this work, the theory is formulated to allow for a general potential with no symmetry restrictions. This extends the utility of EPR as a structural tool, by facilitating its connection with molecular dynamics (MD),since the spectral simulation incorporates a more realistic representation of the complicated topology around the spin-label that is found in labeled biomolecules.

1585-Pos Board B429

Interaction of Antimalarial Drugs with DMPC Model Membranes

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Primaquine Diphosphate (PQD) and Chloroquine Diphosphate (CQD) are potent therapeutic agents used in the treatment of malaria. The investigation of drug-lipid interactions is pivotal for understanding their biological activity. Electron Spin Resonance (ESR) and Differential Scanning Calorimetry (DSC) were used to investigate the effects of drug binding on the lipid phase transition and acyl chain dynamics of model membranes made up of 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC) phospholipids. Labels located at different positions along the lipid chain were used to monitor different membrane regions. ESR results indicated that PQD is more effective in changing the membrane structure than CQD. PQD is effective in perturbing the whole chain of DMPC vesicles, whereas the effect of CQD is more pronounced near the polar headgroup region. Furthermore, the results showed a slight decrease of the membrane packing in DMPC gel phase for both drugs. However, PQD causes a slight increase of the lipid packing close to the membrane center, suggesting a deeper insertion of this molecule into DMPC bilayers. DSC thermograms revealed that PQD interacts with DMPC decreasing the main transition temperature (T_M) by ca. 2°C and completely abolishing its pre-transition. On the other hand, CQD effects are mainly noticed as a decrease in the cooperativity of the main transition. Because of its lipophilic character, PQD penetrates into the bilayer hydrocarbon region causing considerable disorganization. The higher polarity of CQD is probably related with its low membrane permeability. These results shed light on the molecular mechanism of drug-lipid interaction, which may be useful for the development of lipid drug delivery systems of antimalarial drugs. Acknowledgments: FAPESP, CNPq.

1586-Pos Board B430

Development of polymer-coated paramagnetic Implants for biomedical oximetry Applications

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There are a variety of methods available for measuring and mapping of oxygen concentration in biological tissues. The particulate-based, electron paramagnetic resonance (EPR) oximetry is a powerful technology that allows non-invasive and repetitive monitoring of oxygenation in tissues. Lithium naphthalocyanine (LiNc) forms highly stable paramagnetic crystals which can be used for high resolution oximetry in tissues. To show efficient biocompatibility, long-term *in vivo* stability and responsiveness of LiNc oximetry probes, as well as for making the probes surgically implantable/ retrievable, we coated LiNc microcrystals with Teflon AF 2400 (TAF) polymeric materials. EPR linewidths of polymer-coated LiNc probes under anoxic conditions as well as at varying partial pressures of oxygen (pO_2) did not show appreciable change relative to uncoated LiNc particulates, yet the linewidth of coated LiNc crystals was linearly dependent on varying pO_2. The coated implantable probes responded to changes in pO_2 quickly and reproducibly, enabling dynamic measurements of oxygenation in real time. The implants were unaffected by biological oxidoreductants. The oxygen sensitivity and stability of the coated LiNc was demonstrated *in vivo* in mice for more than two months. Thus, new TAF polymer-coated LiNc crystals are potential candidates for future *in vivo* studies including clinical trials for oxygen measurements in pathophysiological tissues.

Vibrational Spectroscopy

1587-Pos Board B431

Monitoring of mechanically induced transitions in biology using Raman tweezers

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Biological systems function within a delicate balance between mechanical forces and chemical reactions. Some well-known examples are the stretching and twisting of DNA during its protein binding actions and protein conformational changes in response to biochemical processes. Indeed, most biological systems could act as mechanochemical transducers since many aspects of chemical binding and enzymatic processes involve changes in structure dimensions. In this work, optical tweezers are used to controllably apply forces to biological systems and the resulting chemical and structural changes are monitored using Raman spectroscopy. The forces are applied in vivo and simulate stretching and compression effects that are normally experienced by the matter.

The first result presented will be spectroscopic evidence of a transition between the oxygenation and deoxygenation states of hemoglobin that is induced through stretching of a red blood cell (1). The applied force mimics that which the cell undergoes mechanically as it passes through vessels and smaller capillaries. The transition is due to hemoglobin-membrane and hemoglobin neighbor-neighbor interactions that are enhanced upon stretching. The latter, lesser known effect is further studied by modeling the electrostatic binding of two of the protein structures using molecular dynamics methods.

This technique is also applied to study DNA stretching. Results will be presented that indicate conformational changes in the DNA structure that are evidenced through changes in its Raman spectrum, upon stretching. The typically low Raman scattering cross section of DNA is countered with the incooperation of silver colloids that enhance the scattered fields. The utilization of surface-enhanced Raman scattering (SERS) allows fast acquisition of spectra during the DNA intermediate stretched states which aides in elucidating its conformational change pathways.

1. S. Rao, et. al., Biophys. J., in press.

1588-Pos Board B432

Pressure-induced Conformational Changes in Poly-peptides and Protein Solutions Probed with Micro-Raman Spectroscopy

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Functional properties of proteins and cells are affected by an elevated pressure environment. Combining Raman microscopy with a micro-capillary high pressure cell enables structural sensitive studies of small amounts of biological material using vibrational signatures. The cell contains less than 50 nano-liter of sample, and Raman spectra can be acquired from atmospheric pressure to 4 kBar. The resolution of the setup is evaluated by measuring the Raman spectrum of standard solutions. We investigate pressure effects of the Raman spectrum of poly(L-glutamic acid) and proteins in solution. Spontaneous Raman spectra of poly(L-glutamic acid) in D2O buffer (pH5.4) solution were measured at variable pressure. A shift of the amide I band in poly(L-glutamic acid) to lower frequency with pressure may suggest significant change in secondary structure towards a-helical conformation.