basis for helping cells cope and survive ever-changing environments. In this work, we profile the heterogeneity of protein expression across the entire Escherichia coli proteome. Our novel approach integrates live-cell single-molecule microscopy with a high-throughput microfluidic platform to systematically reveal noise properties, localization, and functions in the E. coli proteome.

We have constructed chromosomal fluorescent protein fusions for over 1,000 ORFs from the E. coli genome by an efficient, low-cost conversion of an existing Sequential Peptide Affinity tag library. We have developed a microfluidic platform for high-throughput fluorescence microscopy, coupled with automated imaging analysis, enabling us to record the protein expression of over 100,000 cells per hour, which is sufficient to describe the statistics of about 100 different reporter strains. We have measured the distribution of protein expression across cell populations and determined the noise properties of each gene with single molecule sensitivity as necessary. In addition, we have imaged the localization of proteins to the membrane, cytoplasm, and DNA.

To determine possible factors affecting the noise of specific genes, we correlate our protein expression data with biological markers and other global data sets. We find that a substantial fraction of the proteome is expressed at low copy numbers, in agreement with previous predictions, and these genes are subject to high values of noise. We also observe global properties of protein noise in E. coli and find differences in the scaling between noise and average expression for proteins present at low or high copy numbers. Our data provides the first comprehensive proteomic resource of expression levels and noise with high sensitivity for the model organism E. coli.

EPR Spectroscopy

1575-Pos Board B419

Multifrequency Pulsed EPR investigation of Fe-histidine Interaction of the Uniquely Coordinated [2Fe-2S] Cluster in the Outer Mitochondrial Membrane Protein, MitoNEET

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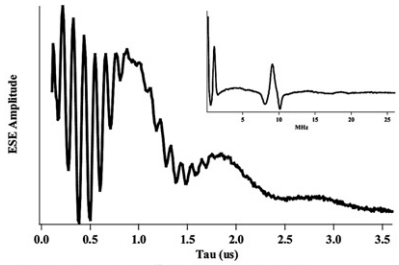
Complimentary pulsed EPR techniques (ESEEM, ENDOR, HYSCORE), over multiple frequencies (X, Ka, Q-bands), were used to characterize bonding interactions of the [2Fe-2S] redox active center of the Outer Mitochondrial Membrane protein, MitoNEET. MitoNEET is the first example of a 3Cys-1His coordinated [2Fe-2S] cluster containing protein. Specifically targeting the uniquely single Fe-histidine interaction, EPR investigations integrated both natural abundance ^{14}N and isotopically labeled ^{15}N protein to determine the hyperfine tensor of a strongly coupled imidazole nitrogen of the bound histidine ligand. 1D-ESEEM experiments in the 31, 35GHz frequency region resulted in deep modulation patterns indicative of being near the ''exact cancellation'' limit and was favorable for a more direct spectral assignment of nuclear quadrupolar transition frequencies. Assignment of His87 as the bound ligand was supported by parallel experiments using H87C mutant. An additional advantage of these higher field experiments allows for greater resolved g-anisotropy and a finer degree of orientation-selected experiments, in progress. These should provide a more accurate description of the [2Fe-2S] ligand bonding interaction important for understanding the electronic structure of this new class of redox active proteins.



2-Pulse ESEEM time domain ^{15}N labeled MitoNEET protein (Inset is Fourier Transform-frequency domain) Data taken at CalEPR Center, UC Davis

1576-Pos Board B420

Comparing the Structural Topology and Dynamic Properties of a Model Peripheral Membrane Peptide Magainin-2 Utilizing X- and Q-Band EPR Spectroscopy

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Probing the structural and dynamic properties of membrane proteins poses a very difficult problem due to their hydrophobic amino-acid composition and lipid environment they are associated with. To unravel this dilemma lipid membrane mimics have been used to establish a medium by which membrane proteins can be studied. Magnetically aligned phospholipid bilayers (bicelles) coupled with magnetic resonance spectroscopy can be used to extract pertinent information related to their structural topology. This information can be obtained by aligning the samples with respect to the static magnetic field and measuring the corresponding anisotropic spectral parameters. Our lab uses both solid-state NMR and spin-label EPR spectroscopy to study membrane proteins. EPR spectroscopy offers unique advantages over NMR spectroscopy due to a higher sensitivity and a different frequency domain for probing dynamics. These facts have led us to perform EPR spectroscopic alignment studies on the surface peptide magainin-2, which has been shown to exhibit antimicrobial activity by pore formation in two different frequency domains X-Band (9 GHz) and Q-Band (34 GHz). New and unique EPR lineshapes were obtained which not only elegantly contrast integral and peripheral peptide topologies, but also have implications for further elucidating antimicrobial dynamics and their corresponding mechanisms.

1577-Pos Board B421

Crystalline Spin-Labeled Hemoglobin as a Model to Compare Distances Measured by DEER Spectroscopy and X-Ray Crystallography

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We have recently crystallized and resolved the structure of spin-labeled hemoglobin, while simultaneously using double electron-electron resonance (DEER) spectroscopy to measure inter-spin-label distances within these crystals. Previously, no spin-labeled protein has been analyzed by both DEER-spectroscopy and X-ray crystallography to determine whether the two techniques are in good agreement. Human hemoglobin (Hb) is a useful model system for this comparison, as it readily crystallizes and reacts specifically with the maleimide-TEMPO spin-label (MSL) at Cys93, found within the β subunits of the $\alpha_2\beta_2$ hemoglobin tetramer. For our experiments, we have generated two crystal populations. The first consisted entirely of paramagnetic, EPR-active MSL-Hb, which was used in our X-ray crystallography experiments. The second population contained a low concentration of MSL-Hb in a large excess of hemoglobin labeled with an EPR-silent MSL analog, which assured that distances measured by DEER spectroscopy would not be altered by dipolar interactions between spin-labels of adjacent Hb tetramers. Our results show that both techniques yield similar inter-spin distance measurements, provided that certain precautions are taken to avoid EPR artifacts. We have varied DEER acquisition parameters, such as the dipolar evolution time and acquisition temperature, and analysis methods that affect the accuracy and precision of distance distributions observed by DEER spectroscopy, as compared to those obtained by X-ray crystallography. These results provide the most rigorous analysis to date of the reliability of EPR-based distance measurements. This work was supported by NIH grants (GM27906, AR32961, AG26160, RR22362, GM08700).

1578-Pos Board B422

Structure of the cdb3-ankD34 Complex from Site Directed Spin Labeling Studies

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The spectin-based membrane skeleton is responsible for the remarkable mechanical stability and the unique viscoelastisity of the erythrocyte membrane, which are both essential for the survival of red blood cells in the circulatory system. One of the major junctional sites that links the membrane skeleton to the plasma membrane is a protein complex formed by the cytoplasmic domain of band3 (cdb3) and ankyrinR. In this study, site directed spin labeling (SDSL) has been utilized to investigate the global structure of the complex formed between cdb3 and ankD34 (ankyrin repeats 13-24 of full length ankyrinR). We first characterized physicochemical properties of the complex using gel permeation chromatography and sucrose-gradient sedimentation and determined the stoichiometry of the complex to be one cdb3 dimer bound to two ankD34s *in vitro*. For a series of surface sites residing on the binding interface of cdb3, spin label

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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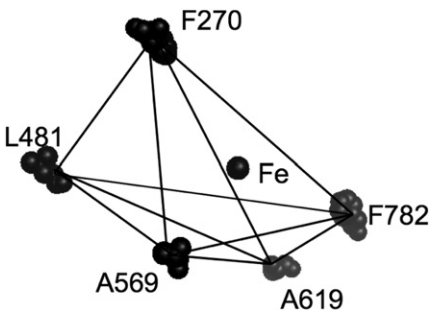
How fatty acid substrates and inhibitors enter and reside in the internal cavity of lipoxygenases is still unresolved. A medium-resolution, solution structure of lipoxygenase is obtained by positioning spin labels on a grid of five sites near the protein surface, all 20-30 Å distant from iron. Cysteine 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