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cells and that they localize to specific compartments. Based upon our observations, we hypothesize that crowders can influence stem cell differentiation by influencing molecular kinetics.

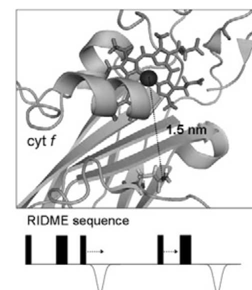
772-Pos Board B572
Volume and Morphological Changes in Red Blood Cells with Pressure Probed by Optical Imaging In-Situ
Silki Arora, Sang Hoon Park, Jennifer Mauser, Debopam Chakrabarti, Alfons Schulte.
Functional properties of living cells depend on the thermodynamic variables temperature and pressure. A unique tool to investigate volume effects on structure and metabolism of the cell is pressure perturbation. We employ a setup that allows visualizing individual live cells at variable pressure in real time. We present measurements of volume changes in red blood cells (RBC) over the pressure range from 0.1 to 200 MPa. Up to a pressure of 35 MPa the size of a healthy erythrocyte remains constant. Over the pressure range from 35 MPa to 200 MPa the lateral diameter decreases linearly and reversibly with a slope of 0.015 micron / MPa, while there are no significant alterations in shape. The RBC deformability is discussed in terms of the cell membrane elasticity and effects of the cytoskeletal network. Our experiments are extended to RBCs infected with the malaria parasite *Plasmodium falciparum*. Here, we observe clear differences in the deformability with pressure and between the compression and decompression curves.

773-Pos Board B573
In Vivo Imaging of Tumor Cell Migration
Christian Weis, Andreas Hess, Tim St. Pierre, Ben Fabry.
The process of metastasis formation involves the migration and 3-D invasion of tumor cells from a primary tumor to distant sites. We propose that the dynamics of the migration and invasion process of magnetically labeled tumor cells can be monitored in animal models over prolonged time periods using magnetic resonance imaging (MRI). Human breast carcinoma cells (MB-MDA-231) were labeled with superparamagnetic Fe_2O_3 iron oxide nanoparticles coated with poly-L-lysine. The particles are readily taken up by cancer cells and stored in intracellular clusters. During cell division, the nanoparticle clusters are divided and split unevenly between daughter cells (mean partitioning fraction 0.85 to 0.15). Nanoparticles are non-toxic, are not degraded by the cell and remain stable for at least 3 weeks. In vitro collagen gel assays show no differences in contractile properties and invasion behavior of magnetically labeled vs. non-labeled tumor cells. MRI of cells suspended in agarose gave a detection limit of the spin-spin-relaxation-rate above the agar background of approximately 70 cells per 1 mm^3. The minimal detection volume of tumor cells in agarose was 25 μl. Detection limit and minimal volume were verified by injecting labeled cancer cells in mice. Spin-spin-relaxation-weighted (T2-weighted) and susceptibility-weighted images show a rapid relaxation behavior and pronounced phase shifts in the vicinity of the injection area compared to control scans. These studies demonstrate the feasibility of the method for long-term observation of cancer cell migration in vivo with MRI.

774-Pos Board B574
Effect of Formalin Fixation on CARS Microscopy of Neural Tissue
Yeon Ho Kim, **Timothy J. O'Leary**, Jeffrey T. Mason.
The cognitive impairments associated with blast-induced mild traumatic brain injury (bmTBI) suggest that exposure to blast may disrupt the connectivity of the fiber tracts that form the neural network of the brain. Coherent anti-Stokes Raman scattering (CARS) microscopy is ideally suited to observe blast-induced structural changes in the myelinated axons of the neural white matter. The sensitivity of the CARS technique to the CH_2 stretching vibrations of the myelin sheath of the axons allows for the label-free imaging of fiber tracts at high spatial resolution with large depth penetration. To date, most CARS studies have been performed on living *in vivo* and *ex vivo* tissues. However, studies of the structural changes associated with bmTBI will require the ability to examine *post mortem* tissue. In this study, we report our findings on the use of CARS microscopy to observe myelin fibers in formalin-fixed mouse and porcine brain tissue. Neural structures including the cerebrum, spinal cord, corpus callosum, and hippocampus were examined. Our findings demonstrate that CARS microscopy can be used to determine fiber orientation and continuity, fiber area percentage, myelin density, and the g-ratio of individual myelin axons in neural tissue fixed in formalin for up to 3 months. In conclusion, we demonstrate that the spectroscopic and morphological artifacts produced by formalin fixation do not interfere with the ability of CARS microscopy to observe and characterize the structure of fiber tracts in formalin-fixed neural tissue.

EPR Spectroscopy

775-Pos Board B575
Distances Between Paramagnetic Metal Centers and Spin Labels in Proteins by Pulsed EPR: The RIDME Method As a New Tool
Sergey Milikisyants, Francesco Scarpelli, Michela Finiguerra, Marcellus Ubbink, **Martina Huber**.



Structure determination in biological systems by electron paramagnetic resonance (EPR) is becoming increasingly popular. Distances in the nm range between spin labels in proteins yield structure restraints[1]. Transition metal-ion centers abound in proteins, but their potential as markers for distance determination is limited by their large g-anisotropies and fast relaxation times.
For many of these centers, the known pulse sequences for e.g. DEER or PELDOR cannot be applied because of excitation bandwidth limitations. The RIDME method[2] circumvents this problem by making use of the spin-lattice relaxation (T_1)-induced spin-flip of the transition-metal ion. Designed to measure distance between such a fast relaxing metal center and a radical, it suffers from a dead time problem. This disadvantage can be avoided by the five-pulse RIDME (5p-RIDME) sequence. An Fe(III)-spin label distance in ths protein cytochrome *f* is determined.[3]

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776-Pos Board B576
The Global Analysis of DEER Data
Eric J. Hustedt.
Previously, global analysis has been successfully used to analyze both continuous wave and saturation transfer EPR data[1-3]. In this work, algorithms have been developed for the global analysis of DEER data. Applications include the analysis of DEER data collected at multiple frequencies or multiple timescales. Analysis of DEER data from the soluble protein CDB3 (MW ≈ 90 kD) has shown that the background DEER signal is not well-fit by an exponential decay due to the large size of the CDB3 dimer. As a result, background correction with an exponential decay prior to analysis results in a poor fit to the data. An algorithm has been developed which explicitly fits the background signal with the radius of the molecule (assuming it is spherical) and the spin concentration as parameters. Using this approach, excellent fits to DEER data can be obtained without prior background correction. Also, DEER data can be globally analyzed to determine changes in the relative populations of components of the distance distribution as a function of experimental conditions. For example, DEER has been previously used to study the structural effects of a proline to arginine mutation at residue 327 of CDB3. Intradimer distances in spin-labelled wild type CDB3 can be fit using a single component distance distribution[4]. The same measurements on P327R CDB3 indicate the mutation induces a second more disordered component in the distance distribution[5]. The global analysis of DEER data collected for multiple spin-labelling sites in both the WT and P327R background is being used to further test this two-component model.
Supported by NIH GM 080513.

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777-Pos Board B577
Nitroxide Spin Label Side Chain Dynamics of Solvent Exposed Sites on Membrane Proteins
Brett M. Kroncke, Justin Kim, Linda M. Columbus.
Understanding the structure and functional dynamics of membrane proteins in their native, hydrophobic environment is key to understanding how proteins function. EPR spectroscopy in combination with site directed spin labeling (SDSL) has the potential to quantify structure and dynamics of proteins of arbitrary weight in their native lipid environment. Several studies have elucidated the structural origins of CW EPR lineshapes of water-soluble proteins; however, CW EPR spectra of nitroxide spin labeled proteins in a detergent/lipid environment have characteristic differences from their water-soluble counterparts. Membrane protein spectra are generally broader and frequently

contain multiple spectral components. In this study, membrane exposed sites on model α-helical and β-barrel proteins LeuT and OmpA, respectively, were investigated using X-ray crystallography, mutational analysis, nitroxide side chain derivatives, and spectral simulations in order to obtain a motional model of the nitroxide. For each crystal structure of spin labeled LeuT, the nitroxide ring of the spin label is resolved and makes contacts with hydrophobic residues on the protein surface. In addition to the crystal structures, the spectral effect of nearest neighbor mutations and the spectral simulations suggest the spin label has a greater affinity for its local protein environment compared to water-soluble proteins. These results for spin labeled sites on LeuT and OmpA begin to explain the observed trends in membrane protein CW EPR spectra.

778-Pos Board B578

Utilizing Electron Spin Echo Envelope Modulation (ESEEM) Spectroscopy to Probe the Structure of Membrane Proteins

Daniel J. Mayo, Andy Zhou, Indra Dev Sahu, Robert M. McCarrick, Parker J. Walton, Adam C. Ring, Kaylee R. Troxel, Aaron T. Coey, Jaclyn M. Hawn, Abdul-Hamid Emwas, Gary A. Lorigan.

New approaches are needed to more efficiently probe the structural properties of membrane proteins. A new approach has been developed to probe the structural properties of membrane peptides and proteins using the pulsed Electron Paramagnetic Resonance (EPR) technique of Electron Spin Echo Envelope Modulation (ESEEM). This technique can measure short-range distances between a nitroxide spin label and a 2H nucleus out to approximately 8Å. For this study a model membrane peptide M2δ, was constructed by solid phase peptide synthesis and inserted into a DMPC/DHPC bicelle membrane. We report for the first time, the direct detection of 2H modulation between a 2H-labeled d8 Val residue and a nitroxide spin label three and four residues away that is characteristic of an alpha-helical secondary structure. Simulations of the ESEEM data reveal a distance of approximately 6.4 +/- 0.5Å that agrees well with molecular modeling studies. ESEEM spectra in this work yielded high-quality data in less than an hour with as little as 35μg of protein sample.

779-Pos Board B579

Structural Studies on the Conformation of Human KCNEL1 Membrane Protein via Electron Paramagnetic Resonance Spectroscopy

Indra D. Sahu, Aaron T. Coey, Kaylee R. Troxel, Thusitha S. Gunasekera, Jaclyn M. Hawn, Robert M. McCarrick, Congbao Kang, Richard Welch, Carlos G. Vanoye, Charles R. Sanders, Gary A. Lorigan.

Multi-frequency CW-EPR, Electron Spin Echo Envelope Modulation (ESEEM), and Double Electron Electron Resonance (DEER) coupled with site-directed spin labeling (SDSL), molecular dynamics modeling, and rigorous data analysis can be used to report both qualitative and quantitative information about structure and dynamics of a complex biological system. The short range distances can be measured between isotopically coupled nuclear spins and nitroxide electronic spin labels up to a distance of about 8Å using ESEEM and long range distances of 20–70Å between two nitroxide electronic spin labels using DEER. The transmembrane domain (TMD) of KCNE1 membrane protein plays a key role in the modulation of voltage gated channel activity. In order to describe the conformation of TMD of KCNE1, cysteine mutants were generated along the TMD and extracellular region of KCNE1 and further modified by MTSL nitroxide spin labels. The purified proteins were reconstituted into model membranes: Fos-Choline, LMPG micelles and POPC/POPG bilayer vesicles. CW-EPR experiments were performed on the mutants at X and Q-bands in the rigid limit and motional regime. A simultaneous multi-frequency EPR data analysis was employed to obtain the dynamic behavior of spin labels along the protein sequence. The isotropic motion of spin probe was found to decrease towards the interior region of the TMD of the protein and reaches a minimum at the G60C position indicating that the motion of the probe is hindered by the nearby overlapped hydrophobic residues and membrane environment. Additional structural information was revealed by performing ESEEM experiments on i+1 to i+5 sites, where i represents the deuterium position V502H on the TMD, and DEER was on sites V47C-I66C and V50C-S68C. The distances extracted from ESEEM and DEER are in good agreement with NAMD/ VMD and MMM modeling results.

780-Pos Board B580

Probing the Structural and Dynamic Properties of KCNE1 Using Site-Directed Spin Labeling EPR Spectroscopy

Kaylee R. Troxel, Indra D. Sahu, Aaron T. Coey, Thusitha S. Gunasekera, Charles R. Sanders, Gary A. Lorigan.

KCNE1 (minK), a membrane protein known to modulate the function of the KCNQ1 voltage-gated potassium ion channel in the human heart, has been successfully overexpressed, purified into detergent micelles, labeled, and incorporated into POPC:POPG lipid bilayers. CW-EPR and Power Saturation EPR Spectroscopy has been used to examine the structural properties of KCNE1 in lipid bilayer vesicles, as opposed to detergent micelles. We are also currently making efforts to develop a technique which will allow quick determination of the local secondary structure of membrane proteins such as KCNE1 using ESEEM spectroscopy. It is important that these studies be conducted in lipid bilayers as opposed to micelles to better model the native membrane environment.

781-Pos Board B581

The Structural, Dynamic, and Functional Changes in the KCNE1 Membrane Protein Between Detergent Micelles and Lipid Bilayers

Aaron T. Coey, Indra D. Sahu, Kaylee R. Troxel, Thusitha S. Gunasekera, Congbao Kang, Richard Welch, Carlos G. Vanoye, Gary A. Lorigan.

Structural studies of membrane proteins in natural lipid bilayer environments are often difficult to perform because the nature of the samples produced is not conducive for the collection of reliable data. In this study KCNE1 (minK), an integral membrane protein found in the human heart, was over expressed and purified into SDS detergent micelles.. After obtaining the pure protein in micelles, we were able to successfully insert it into lipid bilayer vesicles composed of POPC and POPG using various dialysis techniques. Proper insertion of the protein into vesicles was confirmed through CD spectroscopy, CW EPR (X-Band), and Power Saturation EPR. Functional studies performed in Xenopus oocytes expressing KCNQ1 showed KCNE1 maintained its activity when removed from a micelle environment and placed in a lipid bilayer. We discuss the importance and merits of studying membrane proteins in a more native lipid bilayer environment over detergent micelles.

782-Pos Board B582

Structure and Function of Synaptotagmin 1 C2 Domains as Determined by Site-Directed Spin Labeling

Dawn Z. Herrick, Weiwei Kuo, Jeffrey F. Ellena, David S. Cafiso.

Synaptotagmin 1 (syt1) is a synaptic vesicle protein believed to act as the Ca2+ 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 Ca2+ ions and bind anionic phospholipids. Syt1 also interacts with the neuronal SNARE proteins, which may play a role in the fusion process. We are characterizing the structure of syt1 both in its aqueous and membrane bound states and bound to the soluble core SNARE complex. Double cysteine mutations were engineered into a water soluble fragment of syt1 C2A-C2B and derivatized with the methanethiosulfonate spin label. Four-pulse DEER was used to obtain distance measurements between C2A and C2B in solution, with membranes, and bound to the soluble SNARE complex. The DEER-derived distances were used as restraints in a simulated annealing routine. The predominant structure is one where the C2 domains are separated by about 40 Angstroms and are oriented anti-parallel so that their Ca2+-binding loops are positioned in opposite directions. Broad distance distributions are obtained by DEER, and indicate structural heterogeneity which may be the result of the flexible linker segment joining the two domains. This structural arrangement does not change when the protein is bound to membranes or the soluble SNARE complex. Furthermore, C2A-C2B is shown to bridge bilayers, which is mediated by multiple contacts of the positive charged regions of the C2B domain and the anti-parallel orientation of C2A and C2B. The result suggests that one role for syt1 in fusion is to bridge across the vesicle and plasma membrane surfaces in a Ca2+-dependent manner. The work was supported by NIGMS grant GM 72694.

783-Pos Board B583

In the Arms of EcoRI - probing the Binding Specificity of the Restriction Endonuclease Using Electron Spin Resonance

Jessica Sarver, Katherine Stone, Jacque Townsend, Paul Sapienza, Linda Jen-Jacobson, Sunil Saxena.

Pulsed electron spin resonance (ESR) was used to probe the binding specificity of EcoRI, a restriction endonuclease that binds to and cleaves a six base pair sequence of DNA. EcoRI binds to the specific sequence GAATTC with an affinity that is 50,000-90,000-fold greater than that of a miscognate site that differs by only one base pair. Low binding affinity is also exhibited at non-specific binding sites which differ from the specific sequence by two or more base pairs. Distance measurements were performed on several spin labeled EcoRI mutants when bound to specific, miscognate, and non-specific sequences of DNA using Double Electron-Electron Resonance. These distances demonstrated that on average the arms of EcoRI, thought to play a major role in binding specificity, are similarly positioned. Additionally, noncognate (miscognate and non-specific) complexes demonstrated broader distance distributions indicating that the flexibility of the arms is greater in these complexes. Room temperature continuous wave (CW) experiments were also performed on the EcoRI mutant complexes