during contraction. The exchanger is regulated by binding of Ca^{2+} to the intracellular domain. This domain is composed of an α-catenin-like domain (CLD) that connects two structurally homologous Ca^{2+} binding domains (CBD1 and CBD2) to the transmembrane domain of the exchanger. NMR and X-ray crystallographic studies have provided structures for the isolated CBD1 and CBD2 domains and have suggested how Ca^{2+} binding alters their structures and motional dynamics. It remains unknown how Ca^{2+} binding to the intact Ca^{2+} sensor signals the transmembrane domain to regulate exchanger activity. We have used site directed spin labeling to address this question. Conventional EPR experiments have shown that: 1) residues in, or near, the Ca^{2+} binding loops of CBD1 and CBD2 show decreased mobility upon Ca^{2+} binding; and 2) residues in the β-sandwich regions are insensitive to Ca^{2+} binding. Double Electron Electron Resonance (DEER) measurements on doubly labeled constructs revealed that: 1) the structure of the β-sandwich domains of CBD1 and CBD2 are not altered upon Ca^{2+} binding; 2) CBD1 and CBD2 do not lie lengthwise antiparallel in close proximity but rather residues in the distal ends that connect to the CLD are greater than 60 Å apart; and 3) residues nearer to the apex of the Ca^{2+} sensor are in close enough proximity to be measured by DEER and these distances are sensitive to Ca^{2+} binding. These studies support recent SAXS studies by Hilge et al. (PNAS 106:14333-8, 2009) and provide additional insight into a structural rearrangement of the intact Ca^{2+} sensor that may be involved in regulation of Na^{+}/Ca^{2+} exchange.

2065-Pos
Structure of the CDB3 - ankD34 Complex from Site - Directed Spin - Labeling Studies
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The association between the cytoplasmic domain of band 3 (CDB3) and ankyrinR forms a critical link between the lipid bilayer of the erythrocyte membrane and its underlying spectrin cytoskeleton. This interaction is responsible for the remarkable mechanical stability of the erythrocyte membrane that is essential for the durability of the erythrocyte. While the structures of CDB3 [1] and ankD34 (repeats 13-24 from full length ankyrinR) [2] have been determined by X-ray crystallography, the structure of the CDB3-ankD34 complex has not been established. Using distance constraints from site-directed spin labeling (SDSL) and DEER spectroscopy, we propose a new structural model of CDB3-ankD34 complex modeled assuming rigid-body docking between the two proteins combined with rigorous modeling of the spin label. Unexpectedly, the new model generated by Rosetta docking calculations and filtered through multiple DEER distance constraints shows features which are quite different from the previously proposed docking model. The binding interface of CDB3 is widely scattered over its peripheral surface but the β6-β7 hairpin loop makes no direct contact with ankD34. Second, the binding interface of ankD34 resides on the opposite side of β-hairpin loops from the concave groove. The validity of our current model is also supported by a series of SDSL and cross-linking experiments where the binding interface of ankD34 was mapped by the model-guided scanning of a series of surface sites on ankD34. Supported by NIH P01 GM080513.
[1] D. Zhang et al., *Blood*, 96, 2925 (2000)
[2] P. Michaely et al., *EMBO J.*, 21, 6387 (2002)

2066-Pos
Structural Origins of Nitroxide EPR Spectra in a β-Barrel Membrane Protein
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Site-directed spin labeling is a powerful tool for studying structure and dynamics in proteins, due to its ability to bypass several fundamental limitations suffered by methodologies such as NMR and x-ray crystallography. The utility of this technique, however, hinges on our ability to reliably interpret EPR lineshapes of spin-labeled proteins, so that spectral features may be unambiguously associated with their structural origins. In the present work, X-ray crystallography has been combined with mutagenesis and a quantitative analysis of EPR spectra to examine for the first time the origins of spectra from a β-barrel membrane protein, BtuB. The hydrocarbon-exposed residue T156C was spin-labeled and gave rise to a two-component EPR spectrum, corresponding to two conformers of the spin-labeled side chain. Quantitative lineshape analysis revealed a dominant population of highly (spatially) ordered yet mobile nitroxide, and a second population of weakly ordered yet immobile nitroxide. EPR spectra show that single mutations to nearest-neighbor residues affect the ordering and or equilibrium of label rotamers, however these changes are small in each case. In the 2.6Å crystal structure of spin-labeled BtuB, the likely source of weak pairwise interaction with nearest-neighbors is attributed to the extent of barrel curvature, β-strand twist, and direction of strand tilt. It is postulated that residues Q158, L160 (periplasmic loop), V166, and L168 (hydrogen-bonded neighbor) may cooperatively stabilize the nitroxide spin label by forming a hydrophobic pocket. This approach is being applied to an additional hydrocarbon-exposed site on BtuB which exhibits a different degree of strand tilt and twist.

2067-Pos
Characterization of the L511P and D512G Mutations in the MsbA Lipid Flippase
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MsbA is a 65kDa lipid flippase found in the inner membrane of Gram-negative bacteria such as E. coli and S. typhimurium. As a member of the ABC transporter superfamily, MsbA contains two nucleotide binding domains and two transmembrane domains, one from each of its two monomers. ABC transporters transport a diverse group of substrates from lipids to antibiotics and their dysfunction contributes to a number of human pathologies including cystic fibrosis. As an essential protein in E. coli, the deletion or dysfunction of MsbA results in the toxic accumulation of lipid A in the inner membrane resulting in membrane instability and cell death. The L511P and D512G mutations have been previously identified through mutational analysis as dysfunctional nucleotide binding domain mutations specific to MsbA and were suggested to have a lower affinity for ATP. To further understand the cause of dysfunction in these point mutations, in vivo growth assays, in vitro ATPase activity assays, DEER and CW EPR spectroscopy studies throughout the ATP hydrolysis cycle were conducted. L511P and D512G were each paired with nine different reporter residues, each in or near an important conserved nucleotide binding domain motif and compared to the reporter residues alone. To identify the stage in the ATP hydrolysis cycle in which the L511P and D512G mutations are dysfunctional, the local tertiary interactions before, during, and after ATP hydrolysis were monitored by EPR spectroscopy at each stage of the ATP hydrolysis cycle.

2068-Pos
Free Radical Generation and Electron Flux in Mitochondrial Fe-S Centers During Cardiac Injury; Changes with Mitochondrial Protective Drug Ranolazine
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Some TCA cycle enzymes, like aconitase, are more susceptible to ischemia reperfusion (IR) injury. Ranolazine (RAN) is cardioprotective against IR injury. It is a late Na^{+} current blocker that may also limit lipid peroxidation and complex I activity. It is unknown if RAN alters the redox state of Fe-S clusters or free radical generation (FRG) to underlie its protection. Here we examined how IR injury affects FRG and Fe-S clusters of aconitase and succinate dehydrogenase, using electron paramagnetic resonance (EPR), and if RAN alters these effects. Guinea pig hearts (n 8) were isolated and perfused with Krebs Ringer buffer and exposed to: a) control, b) 30 min global ischemia, c) 10 μM RAN for 10 min just before ischemia, or d) ischemia and 10 min reperfusion. Hearts were immediately ground in liquid N_2 and packed into EPR tubes. We examined changes in signal intensity in liquid He (10°K) of assigned g 2.016 (aconitase 3Fe-4S), g 1.93 (succinate dehydrogenase 2Fe-2S), g 2.006 (free radical), and g 6.0 (Fe group of cytochrome c). Versus time control (100%), the signal for aconitase Fe-S at the end of ischemia was 46%, suggesting oxidative damage; this was partially restored by 10 min reperfusion to 91% and after I+RAN treatment to 55% of control. Signal intensity for succinate dehydrogenase was unaltered by IR or RAN+IR. The presumptive ubisemiquinone radical signal increased 19% after ischemia, suggesting increased FRG, but only by 4% at 10 min reperfusion. I+RAN treatment decreased the signal by 19%. The signal for cytochrome c (g 6.0) increased 730% after IR, but was only 81% after I+RAN. These data suggest that RAN treatment partially restores electron flow through some Fe-S centers and reduces FRG, which may partially underlie its cardioprotective effects.

2069-Pos
Structural Analysis of the Membrane Docking Geometry of PI(3,4,5)P3-Specific GRP1-PH Domain Via Site-Directed Spin Labeling
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Peripheral membrane binding proteins play critical roles in dynamic cell signaling processes that occur at membrane surfaces. Many of these signaling proteins contain membrane targeting domains that act to mediate signal dependent membrane localization for proper enzyme function. Phosphoinositide-specific