oligosaccharide head group for hydrolytic cleavage by the hydrolase, HexA. Mutations in GM2AP or HexA lead to an accumulation of GM2 in the lysosomes, causing lysosomal storage diseases such as Tay Sachs or the AB variant of Sandhoff's disease. Our lab is utilizing site directed spin-labeling (SDSL) with electron paramagnetic resonance (EPR) spectroscopy to probe the conformational changes and the membrane bound orientation of GM2AP. This protein contains eight naturally occurring cysteine (CYS) residues involved in four disulfide bonds. With site directed mutagenesis, a ninth CYS residue is introduced as the reporter site for spin labeling . A series of 10 single CYS mutants have been generated. To validate the EPR results, the mass spectrometry protocol described here was developed to characterize spin-labeled GM2AP samples. For mass spectrometry measurements, either biotin-linked maleimide (BM) or 4-maleimide tempo (4MT) were used to modify and trap available CYS residues in a thioesterbond. The remaining eight native CYS residues, which are disulfide bonded, are then reduced and modified with iodoacetamide. Samples were analyzed by high performance nano-liquid chromatography electrospray ionizaton Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) equipped with a 14.5 T magnet. FT-ICR spectra of enzymatically digested BM-labeled or 4MT-labeled GM2AP were utilized to determine to which CYS residue is modified with the maleimide moeity. Those sites labeled with acetamide are inferred to have been disulfide bonded. The fragment that contains the maleimide moeity tells us, which CYS residue (and how many CYS residues) is accessible for reaction with the spin label for EPR studies.

2220-Pos Board B190

Purification And Reconstitution Of The Connexin43 Carboxyl Terminus Attached To The 4Th Transmembrane Domain In Detergent Micelles

Rosslyn Grosely, Admir Kellezi, Fabien Kieken, Gloria E.O. Borgstahl, Paul L. Sorgen.
UNMC, Omaha, NE, USA.

In recent years, reports have identified that many eukaryotic proteins contain disordered regions spanning greater than 30 consecutive residues in length. In particular, a number of these intrinsically disordered regions occur in the cytoplasmic segments of plasma membrane proteins. These intrinsically disordered regions play important roles in cell signaling events, as they are sites for protein-protein interactions and phosphorylation. Unfortunately, in many crystallographic studies of membrane proteins, these domains are removed because they hinder the crystallization process. Therefore, a purification procedure was developed to enable the biophysical and structural characterization of these intrinsically disordered regions while still associated with the lipid environment. The carboxyl-terminal domain from the gap junction protein connexin43 attached to the 4th transmembrane domain (TM4-Cx43CT) was used as a model system (residues G178-I382). The purification was optimized for structural analysis by nuclear magnetic resonance (NMR) because this method is well suited for small membrane proteins and proteins that lack a well-structured three-dimensional fold. The TM4-Cx43CT was purified to homogeneity with a yield of ~6 mg per liter from C41(DE3) bacterial cells, was reconstituted in the anionic detergent 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)], and circular dichroism and NMR were used to demonstrate that the TM4-Cx43CT was properly folded into a functional conformation by its ability to form a-helical structure and associate with a known binding partner, the c-Src SH3 domain, respectively.

2221-Pos Board B191

A Refinement Protocol to Define the Structure, Topology and Depth of Insertion of Membrane Proteins using Hybrid Solution/Solid-state NMR Restraints

Lei Shi, Nathaniel J. Traaseth, Raffaello Verardi, Gianluigi Veglia.
University of Minnesota, Minneapolis, MN, USA.

To fully describe the folding space and ultimately the biological function of membrane proteins (MPs), it is necessary to determine their interactions with lipid membranes. This intrinsic property of MPs referring to as structural topology is not possible to resolve using x-ray crystallography or solution NMR. Here, we present a hybrid solution and solid-state NMR approach for the simultaneous determination of the structure, topology, and depth of insertion of MPs. Distance and angle restraints obtained from solution NMR of MPs solubilized in detergent micelles are combined with backbone orientational restraints (15N chemical shift anisotropy and 15N-1H dipolar couplings) derived from solid-state NMR in orientated lipid bilayers (PISEMA) into a hybrid objective function. In addition, a supplementary energy term, Ez (insertion depth potential), is used to ensure the correct positioning of helical MPs domains with respect to a virtual membrane. The hybrid objective function is optimized using a two-stage simulated annealing protocol and is implemented into XPLOR-NIH software for general use. To validate the hybrid method, the effects of chemical shift tensor orientations, principal tensor values, and dipolar constant magnitudes on the structural ensemble are determined.
The hybrid method is applied to monomeric and pentameric PLN (phospholamban), a integral MP that regulates sarco(endo)plasmic reticulum Ca-ATPase (SERCA) function in cardiac muscle. The hybrid conformational ensemble defines the structure, topology and depth of insertion of PLN in lipid bilayer simultaneously. This ensemble is compared with solution NMR structures in DPC micelle obtained using conventional solution NMR data (NOEs, J-couplings) and residual dipolar coupling as orientational restraints.
This research was supported by NIH Grants GM64742 and K02HL080081 to G.V. and we also thank University of Minnesota, Supercomputing Institute (MSI) for providing the computing resource.

2222-Pos Board B192

Solution and Solid-State NMR Analysis of Phosphorylated and Pseudo-Phosphorylated Phospholamban

Raffaello Verardi, Nathaniel J. Traaseth, Martin Gustavsson, Kim H. Ha, Gianluigi Veglia.
University of Minnesota, Minneapolis, MN, USA.

Phospholamban (PLN) is a 52 residue transmembrane protein that regulates the Ca2+ ATPase (SERCA) of muscle cells. At low μM Ca2+ concentration, PLN binds to SERCA and decreases the rate of uptake of Ca2+ into the sarcoplasmic reticulum (SR) thereby promoting muscle relaxation. This inhibitory activity is reversed by phosphorylation of PLN at Serine-16 or Threonine-17 in response to ß-adrenergic stimulation. Phosphorylation of PLN is associated with increased cardiac contractility of muscle cells due to the larger Ca2+ load into the SR.
PLN has become important as a therapeutic target in fighting heart failure, a complex disease associated with impaired cardiac contractility. It has been shown that delivering of phospho-mimiking PLN (Ser-16 → Glu substitution) to failing mice cardiomyocytes significantly improves contractility.
Although functional effects of PLN phosphorylation have been extensively studied, the mechanistic details of how phosphorylated and pseudo-phosphorylated (S16E) PLN interacts with SERCA to reverse inhibition are still unclear. In here we present data on the structural characterization of Ser-16 phosphorylated and S16E monomeric and pentameric phospholamban (AFA-PLN and WT-PLN) in the presence and absence of SERCA as probed by solution and solid-state NMR spectroscopy in detergent micelles and oriented lipid bilayers. For solid-state NMR, SERCA and PLN were reconstituted in planar lipid bilayers and uniaxially aligned on glass plates. Residue-specific information as well as topology of PLN monomer and pentamer was determined by PISEMA experiments.

2223-Pos Board B193

Hybrid Solution and Solid-State NMR Analysis of SERCA/Phospholamban Interactions in lipid membranes: From Structural Dynamics to Function

Gianluigi Veglia, Nathaniel J. Traaseth, Raffaello Verardi, Lei Shi, Kim Ha.
University of Minnesota, Minneapolis, MN, USA.

Phospholamban (PLN) is a sarcoplasmic reticulum (SR) integral membrane protein that regulates calcium translocation in cardiac muscle. Upon interaction with SERCA (the SR calcium ATPase). PLN decreases the rate of calcium uptake, reducing the apparent affinity of the enzyme for Ca^{2+} ions. This process is reversed by adrenergic stimulation of protein kinase A, which phosphorylates PLN at Ser16, re-starting the muscle contraction cycle. Here, we present the hybrid solution and solid-state NMR structural analysis of PLN in its pentameric (storage), monomeric (active), and SERCA-bound forms in lipid membranes. This knowledge about the structural dynamics PLN under these different stages is used to steer the extent of PLN control on SERCA activity. These findings lay the groundwork for the rational design of PLN loss-of-function mutants to be used in gene therapy.

2224-Pos Board B194

Topology of Phospholamban when Bound to Ca2 ± ATPase by Solid-State NMR

Nathaniel Traaseth, Raffaello Verardi, Lei Shi, Gianluigi Veglia.
University of Minnesota, Minneapolis, MN, USA.

Calcium cycling in muscle cells drives the relaxation and contraction of both skeletal and heart tissue. The sarcoplasmic reticulum (SR) Ca^{2+}-ATPase is central in the relaxation of the heart, accounting for ~70% of calcium sequestration. Phospholamban (PLN) is a small integral membrane protein regulator of Ca^{2+}-ATPase. Its inhibition of the enzyme is shown in calcium dependence ATPase activity curves, resulting in decreased ATPase affinity for Ca^{2+}. While there have been several successful attempts to gain structural knowledge of the complex between PLN and Ca^{2+}-ATPase, no high-resolution structure exists.