One important feature that differentiates membrane proteins from soluble ones is topology. This is the specific *entanglement* between the membrane protein and the lipid bilayer. Probing this specific orientation of the protein has been best achieved using oriented solid-state NMR experiments such as PISEMA, SAMPI4, and HIMSELF. These experiments correlate an anisotropic ^{15}N (or ^{13}C) chemical shift with a ^{1}H-^{15}N (or ^{1}H-^{13}C) dipolar coupling, allowing for the resolution of backbone restraints with respect to the lipid bilayer normal. To investigate whether topology plays a role in this membrane protein complex, we reconstituted PLN (^{15}N labeled) in the presence and absence of Ca^{2+}-ATPase (purified from rabbit skeletal muscle) into oriented lipid bilayers. Our results show unambiguously and with high reproducibility that PLN's topology is substantially altered upon binding the ATPase. Specifically, we see that the membrane embedded helix (residues 23-52) changes its tilt angle with respect to the bilayer normal from ~23° in the absence of the enzyme to ~40° in its presence. This substantial change in topology might be a necessary attribute of regulation by PLN and potentially a central difference between an active and inactive PLN bound state to Ca^{2+}-ATPase.

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A Solid-State NMR Study on the Structure and Dynamics of the Myristoylated N-Terminus of the Human Guanylate-Cyclase Activating Protein-2

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Guanylate cyclase-activating protein-2 (GCAP-2) is a retinal Ca^{2+} sensor protein. It plays a central role in shaping the photoreceptor light response and in light adaptation through the Ca^{2+}-dependent regulation of the transmembrane retinal guanylate cyclase (GC). GCAP-2 is N-terminally myristoylated and the full activation of the GC requires this lipid modification. The structural and functional role of the N-terminus and particularly of the myristoyl moiety is currently not well understood. In particular, detailed structural information on the myristoylated N-terminus in the presence of membranes was not available. Therefore, we studied the structure and the dynamics of a 19 amino acid peptide representing the myristoylated N-terminus of GCAP-2 bound to lipid membranes (DMPC liposomes) by solid-state NMR.^{13}C isotropic chemical shifts measured in CP HETCOR experiments revealed a random coiled secondary structure of the peptide. Order parameters for Cα, Cβ, and side chain carbon atoms obtained from DIPSHIFT experiments are relatively low suggesting high mobility of the membrane associated peptide. Static ^{2}H solid-state NMR measurements show that the myristoyl moiety is fully incorporated into the lipid membrane. The order parameters of the myristoyl moiety and the DMPC lipid chains are quite similar. Further, dynamical parameters (obtained from ^{2}H NMR relaxation rates) of the peptide's myristic acid chain are also comparable to those of the lipid chains of the host matrix. Therefore, the myristoyl moiety of the N-terminal peptide of GCAP-2 fills a similar conformational space as the surrounding phospholipid chains. However, we did not find a specific hint for a membrane interaction of the amino acids adjacent to the lipid modification.

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A Molecular Gearbox: The Mechanical And Regulatory Complexity Of The Vacuolar ATPase Revealed

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Vacuolar H^{+}-ATPases (V-ATPases) are ATP-driven rotary molecular motors that function as transmembrane proton pumps in all eukaryotic cells. Central roles of V-ATPases in cell physiology include: energising secondary active transport, maintaining acidity in intracellular compartments, and pumping acid out of the cell. Loss of V-ATPase function is associated with forms of kidney disease and inherited deafness; excess or unregulated activity is implicated in diseases such as osteoporosis and in tumour metastasis or multidrug resistance. The V-ATPase is a large ~1 MDa complex of about 30 subunits of at least 13 types. It has a basic architecture comprising 2 domains, with the soluble V_1 domain responsible for ATP hydrolysis and the integral membrane V_0 domain responsible for proton translocation. We have generated the first 3D reconstruction of a native V-ATPase using cryo-electron microscopy of single particles of the pump from tobacco hornworm (*Manduca sexta*). The derived density map, with resolution at 17 Å, reveals a complex network of interactions that both drive and regulate the V-ATPase. In particular, 3 peripheral stators are present, which are linked via a horizontal collar of density that circles 250° around the inter-domain region. In contrast, the related but simpler F_1F_0 ATPase has only 1 stator responsible for connecting the ATP hydrolysing and proton translocation domains. A fourth central stalk forms the V-ATPase axel and is attached to the V_0 membrane domain, but makes minimal contact with the V_1 ATP-hydrolysing region. The definition of the reconstruction is such that previously characterised subunits crystal structures can be accurately fitted into the density map. This provides insight into the organisation of key components directly involved in regulation of activity.

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Towards an atomic model of the Hepatitis C virus p7

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The hepatitis C virus (HCV) p7 protein forms ion channels which are essential for the assembly and secretion of infectious virions, making it an important therapeutic target. Electron microscopy experiments suggest that p7 oligomers may co-exist as hexamers and heptamers. Electrophysiology experiments show that Cu^{2+} has an inhibitory effect on the p7 ion channel and that the amino acid responsible for this inhibition is one histidine in each monomer. These results coupled with the p7 sequence data suggest that the N-terminal helix of p7 lines the transmembrane pore and that this histidine is pore-lining. We combine these results with a previously described hexameric model of the pore derived from transmission electron microscopy and random conical tilt reconstruction to generate improved models of the p7 pore. We discuss the improved models in relation to mutagenesis and potential inhibitor interactions.

2228-Pos Board B198

Structural and Functional Studies of M2 Proton Channel from Influenza A Virus

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M2 protein of influenza A virus forms a homo-tetrameric proton channel involved in modifying virion and trans-Golgi pH for virus infection and inhibited by drugs Amantadine and Rimantadine. The aim of this research is to study the correlation of structure and function of M2 proton channel and mechanism of inhibition by influenza drugs. In order to obtain high resolution structural information in native bilayer like environment, we have expressed and purified intact M2 protein and two peptides M2(22-46) and M2(22-62) as a maltose binding fusion protein from E. Coli. membrane and reconstituted in a DMPC:DMPG(4:1) and DOPC:DOPE(4:1) lipid bilayer. We have applied a range of NMR approaches to study the structure of M2 protein and truncated peptides in apo state as well as complexed with amantadine reconstituted in lipid bilayer and uniformly aligned with respect to external magnetic field. Multidimensional Solid State NMR experiments performed on uniform 15N labeled and amino acid specific labeled protein suggest that helical tilt angle of transmembrane domain in intact M2 protein is smaller compared to that of isolated peptides M2(22-46) and M2(22-62) and oligomeric state of the channel is stabilized due to interactions of amphipathic helices. Using the proteoliposome fusion assay of channel activity, we found that M2 (22-62) (1 mg peptide/8 mg DMPC + 2 mg DMPG/ml H2O, diluted to 3% in the cis chamber) forms active channels in planar lipid bilayers (4 POPE: 1 POPC: 1 POPS: 2 cholesterol in decane). With asymmetric KCl solutions (1 M, pH 8 cis, 0.1 M, pH 6 trans), the time-averaged membrane current was >10-fold higher using proteoliposomes than with protein-free liposomes (N>8) for Vm = ±100 mV. Similar experiments with M2 (22-46) yielded no channel activity (N>80).

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EPR Spectroscopy of the C-terminal Domain of the M2 Protein from Influenza A Virus

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The M2 protein from influenza A is a pH-activated proton channel that plays an essential role in the viral life cycle and serves as a drug target. Using spin labeling EPR spectroscopy we studied a 38-residue M2 peptide spanning the transmembrane region and its C-terminal extension. We obtained residue-specific environmental parameters under both high and low pH conditions for eleven consecutive C-terminal sites. We have also collected data in the presence of the antiviral drugs amantadine and rimantadine. The C-terminal region forms a membrane surface helix at both high and low pH although the arrangement of the monomers within the tetramer changes with pH and drug binding.