1718-Pos Board B562

Conformational Changes of HIV-1 gp41 Membrane Proximal Ectodomain Region Induced by Broadly Neutralizing Antibodies

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The membrane proximal ectodomain region (MPER) of HIV-1 gp41 is the target of broadly neutralizing antibodies (BNAbs), 4E10, 2F5 and Z13e1. To unravel the molecular basis of BNAbs interference with virus entry, we have characterized BNAb-induced MPER conformational changes by a combination of electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR) and surface plasmon resonance (SPR) techniques. The analyses of MPER revealed a tilted N-terminal α helix connected via a short hinge to a flat C-terminal helical segment. This metastable L-shaped structure is immersed in viral membrane and, therefore, less accessible to immune attack. 4E10, a potent BNAb, was found to largely alter MPER membrane orientation, extracting buried W672 and F673 residues after initial encounter with the surface-embedded MPER. MPER conformational changes induced by other mAbs with various neutralizing potency were also determined. The data suggest that 2F5 and 4E10 HIV BNAbs may block viral fusion by perturbing the orientation of MPER relative to the lipid bilayer, extracting buried tryptophan residues in the process. These findings have important implications for the design of vaccines to elicit effective broadly neutralizing antibodies.

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Spin Label Spin Label Distance Measurements of Aligned Membrane Proteins

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Both Manganin-2 and M2δ transmembrane segment of the nicotinic acetylcholine receptor (AchR M2δ) are 23-amino acid peptides with strikingly different structural topologies. Manganin-2 is an antimicrobial peptide lying on the surface, whereas AchR M2δ forms a membrane-spanning neurotransmitter gated ion-channel. To determine the structural topology of these peptides, the spin label 2, 2, 6, 6-tetramethyl piperidine-1 oxyl-4-amino-4 carboxylic acid (TOAC) was attached at different sites in these peptides via solid phase peptide synthesis. Since the TOAC spin label is rigidly coupled to the peptide backbone, it accurately reports on position, orientation, and dynamics of the peptide backbone. Amino acids Ser-8 and Lys-14 in Manganin-2 and Ile-7 and Gln-13 in AchR M2δ were singly and doubly replaced with the TOAC spin label. These peptides were cleaved, purified by reverse phase HPLC and then inserted into aligned DMPC lipid bilayers to study the spin label-spin label dipolar interaction by continuous wave electron paramagnetic resonance (CW-EPR) spectroscopy. In aligned bilayers at higher temperatures, the anisotropic EPR spectra of the singly labeled peptides reveal unique lineshapes based upon the orientation of the peptides with respect to the membrane and the magnetic field. The EPR spectra of doubly labeled peptides in aligned bilayers contain unique information about the distance and orientation of the two labels. Thus, by using aligned CW-EPR spectroscopic techniques we can predict the structural topology of membrane proteins and measure the distance and orientation between two spin labels.

1720-Pos Board B564

Design of Soluble Transmembrane Helix for Measurement of Water-Membrane Partitioning

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Use of model transmembrane helices and lipid bilayers is a tractable and straightforward approach to obtain thermodynamic information on fundamental processes of membrane protein folding. The insertion process of transmembrane helices from aqueous phase to membranes, the initial step of the folding, is especially difficult to investigate because of insolubility of helices in aqueous phase. We report here a design of a soluble transmembrane helix, (KR)5-AALALAA-AALWLAA-AALALAA-C(NBD)-NH2 (NBD, 7-nitrobenz-2-oxa-1,3-diazole), which consists of the transmembrane region (AALALAA)3, the central guest residue (W), and the N-terminal charged tag (KR)5. Circular dichroism and fluorescence spectroscopy revealed that the peptide dissolved into water as a monomer with the guest residue exposed to the solvent. After addition of large unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, the peptides rapidly partitioned into the vesicles and assumed a transmembrane topology. Thus, the designed transmembrane helix was found to be a useful template for thermodynamic measurement of partitioning of amino acids from water to the hydrophobic core of membrane.

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Determining Peptide Hydration in a Lipid Bilayer Using Isotopically Labeled Peptides

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The hydration properties of proteins/peptides in the presence of a phospholipids bilayer is of considerable interest, as membrane associated proteins play a significant role in, among other things, cell function. Specific isotope-labeling combined with Fourier-transform- infrared (FTIR) spectroscopy can provide molecular level information on protein/peptide structure and dynamics, specifically backbone conformation and local differences in backbone-solvent hydrogen-bonding.(1) Previously, this labeling technique has been used to investigate residue level peptide backbone conformation as well as the peptide backbone desolvation properties of the fluorinated solvent 2,2,2-trifluoroethanol.(1) In this work we will discuss the results of FTIR studies on KL4, KLLLLKLLLLKLLLLKLLLLK, in the presence of a lipid bilayer. KL4 is a 21-residue peptide, used as a lung surfactant protein B replacement, which has shown considerable promise in the treatment of respiratory distress syndrome. This peptide is a great model because of the recently published NMR data on the secondary structure of KL4 as it binds to lipid bilayers(2) and the differential partitioning of the peptide into lipid bilayers of varying degrees of saturation.(3) The differential partitioning and slight structural change of KL4 can be utilized to test the effectiveness of 13C-labeled peptides, in conjunction with FTIR, as a tool for determining peptide hydration in a lipid bilayer.

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1722-Pos Board B566

Structure of the Transmembrane Dimer of gp55-P of the Spleen Focus Forming Virus and its Interaction with the Erythropoietin Receptor

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Gp55-P is a dimeric membrane protein with a single transmembrane helix that is coded by the *env* gene of the polycythemic strain of the spleen focus forming virus. Gp55-P activates the erythropoietin (Epo) receptor through specific transmembrane helix interactions, leading to Epo-independent growth of erythroid progenitors and eventually promoting erythroleukemia. We describe the use of magic angle spinning deuterium NMR to establish the structure of the transmembrane dimer of gp55-P in model membranes. Comparison of the deuterium lineshapes of leucines in the center (Leu396-399) and at the ends (Leu385, Leu407) of the transmembrane sequence shows that gp55-P has a right-handed crossing angle with Leu399 packed in the dimer interface. We extend these NMR studies in two directions. First, deuterium NMR results are presented on Met390 and its interaction with the Epo receptor. Mutation of Met390 to isoleucine (the amino acid at position 390 in gp55-A) eliminates the ability of gp55-P to activate human (Leu238Ser) and mouse Epo receptors. In addition, the M390L mutation was reported to induce anemia, rather than polycythemia. Second, we present 2D dipolar assisted rotational resonance (DARR) NMR measurements of specific helix contacts in the SxxSG sequence that mediate dimerization. We discuss the implications of the structure of the gp55-P transmembrane dimer for activation of the Epo receptor.

1723-Pos Board B567

Making Membrane Protein LspA Samples, and Its Uniformly Aligned Full Length Investigated by Solid State NMR

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Here is a novel procedure for making a membrane protein sample, Lipoprotein Signal Peptidase (LspA) for structural studies by solid state NMR. LspA is expressed in E.Coli, purified and refolded with detergent on a Ni-NTA affinitive column and uniformly aligned in lipid bilayer on glass slides. Final concentrations of reconstituted LspA of up to 35mg/mL have been achieved. Reconstitution of LspA in detergent micelles was monitored by CD and solution NMR HSQC. The aligned LspA in lipid bilayer was monitored by solid state

NMR to indicate the degree of alignment. Based on these results, ssNMR spectrum will be obtained to show resonance patterns known as PISA wheel for the transmembrane domains of LspA.

1724-Pos Board B568

Oriented Synchrotron Radiation Circular Dichroism and Linear Dichroism Spectroscopy of Peptides in Model Membranes

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The orientation of membrane-associated alpha-helical peptides was investigated using novel methodologies of oriented Synchrotron Radiation Circular Dichroism (SRCD) and linear dichroism (SRLD) spectroscopies. Because of its enhanced signal-to-noise and a detector geometry that minimised optical artefacts associated with conventional CD studies of membrane suspensions, SRCD enabled the measurement of oriented CD spectra. To accomplish this a specially-designed sample cell holder was produced which would maintain constant humidity in hydrated film samples. Distinct spectra were obtained for peptides oriented parallel or normal to the direction of the beam, corresponding to the parallel and perpendicular pi to pi* and n to pi* electronic transitions.

To provide similar information for peptides associated with lipid vesicles, SRLD was used to examine suspensions of vesicles in a coquette flow-cell. SRCD studies of the samples in the same couette enabled interpretation of the information.

The model systems used in this study were peptides of the KALP family with a number of different phospholipids. In TFE solution and as well in lipid vesicle suspensions, KALP produced CD spectrum typical of an alpha helix in an isotropic solution, whilst in oriented samples different spectra associated with the different directional transitions of the peptide bonds were found for peptides oriented transmembrane or parallel to the membrane surface. The alignments of the peptides under the different conditions were compared with the results obtained by 15N solid state NMR of the peptide in oriented lipid multilayers. Thus, these new approaches to examining peptides in membranes can provide information that is complementary to the secondary structural information present in conventional CD spectra.

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1725-Pos Board B569

Spectral Characterization Of Het-C2, A Glycolipid-transfer Protein

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Het-C2 is a small 23 kDa protein, isolated from the fungi Podospora anserina, homologous to mammalian GLTP, and capable of transferring glycospingolipids in vitro. The crystal structure of Het-C2 is unknown, but molecular models suggest conservation of the GLTP-fold. Here, the locations of the Trp residues in Het-C2 have been investigated to gain further insights into their function. Sequence homology shows one of Het-C2's two Trp residues aligned with GLTP Trp96 which resides in the sugar headgroup liganding site. The other Het-C2 Trp did not align with either of GLTP's other two Trp residues. The Trp fluorescence spectrum of native Het-C2 exhibited an emission maximum at 355nm which red shifted 2nm upon denaturation with 8M urea, indicating Trp localization to a more polar environment. Acrylamide and KI quenched >90% of the average Trp fluorescence confirming that Het-C2 Trp residues are not buried in hydrophobic environments but reside in exposed polar regions. The linearity of Stern-Volmer plots for native Het-C2 and urea-denatured (8M) Het-C2 suggested dynamic quenching at physiological pH and ionic strength. The Stern-Volmer constants were higher for native protein than denatured protein. Upon interaction with probe-sonicated POPC vesicles, the Trp emission maximum blue shifted (~2nm) and decreased in intensity (~13.5%). Including glycolipid in the vesicles slightly enhanced the blue shift (~3nm) and significantly decreased Trp intensity (~21%). Far-UV-CD of Het-C2 showed secondary structure dominated by alpha-helices and with a highly cooperative, thermally induced melting transition near 43°C. Near-UV-CD indicated the induced optical activity of Trp/Tyr residues was unaffected by interaction with vesicles lacking or containing glycolipid. The results are analyzed and discussed within the context of the known locations and functions of human GLTP's three Trp residues. [Support: NIH/NIGMS GM45928 & GM34847, NIH/NCI CA121493, The Hormel & Mayo Foundations]

1726-Pos Board B570

Aquaporin-4 Dynamics and Determinants of Assembly in Orthogonal Arrays Revealed by Single-Molecule Fluorescence Imaging

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Aquaporin-4 (AQP4) water channels exist in two predominant isoforms in cell plasma membranes. The long N-terminus 'M1' form exists as dispersed tetramers, while the short N-terminus 'M23' form assembles in large supermolecular structures known as orthogonal arrays of particles (OAPs) that are visible in freeze-fracture electron microscopy. We investigated the determinants and dynamics of AQP4 assembly in OAPs by visualizing fluorescently labeled AQP4 isoforms and mutants in living cell membranes using quantum dot single particle tracking and total internal reflection fluorescence microscopy. In several transfected cell types, including primary astrocyte cultures, AQP4-M1 diffused freely with diffusion coefficient ~5 x 10^{-10} cm^2/s, covering ~5 μm in 5 min, while AQP4-M23 was relatively immobile, moving only ~0.4 μm in 5 min. Biophysical analysis of short-range AQP4-M23 diffusion within OAPs indicated a spring-like confining potential with a restoring force of ~6.5 pN/μm. Analysis of AQP4 deletion mutants revealed progressive prevention of OAP formation by addition of 3-7 residues at the AQP4-M23 N-terminus, with polyalanines as disruptive as native AQP4 fragments. OAPs disappeared upon downstream deletions of AQP4-M23, which, from analysis of point mutants, involves hydrophobic interactions at residues Val24, Ala25 and Phe26. OAP formation could also be prevented by disrupting secondary structure through the introduction of proline residues at sites downstream from the hydrophobic N-terminus. AQP1, an AQP4 homolog that does not form OAPs, was able to form OAPs upon replacement of its N-terminal domain with that of AQP4-M23. Our results indicate that OAP formation by AQP4-M23 is stabilized by a hydrophobic homo-tetrameric interaction involving a structured N-terminus domain near the cytoplasmic membrane interface. Absence of OAPs in AQP4-M1 results from non-selective blocking of this interaction by seven residues just upstream from Met23.

1727-Pos Board B571

Physical Properties of the Zona Pellucida

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The zona pellucida (ZP) is the extracellular coat that surrounds the mammalian oocyte. It forms a spherical shell of remarkably uniform thickness (5-10 μm in eutherian mammals) composed of three glycosylated proteins (ZP1, ZP2 and ZP3).

Penetration of this shell by spermatozoa plays a crucial role in mammalian fertilization and any inability of spermatozoa to penetrate the ZP inevitably leads to infertility. The purpose of this work is to shed light to the three-dimensional structure of the ZP, its construction and its properties as a polymer.

By means of the Atomic Force Microscopy (force-distance curves) we have determined for the first time several ZP structural properties (elasticity, plasticity, adhesion, etc.) during the ovulatory, periovulatory and fertilized phase.

Moreover information about single polymers interactions has been obtained by means detachment experiments (pull-off curves).

1728-Pos Board B572

Characterization of the Cx32/hDlg Complex and its Role in Tumor Suppression

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Gap junctions provide an intercellular pathway for the propagation of signals, which are necessary for cellular differentiation and development, metabolic homeostasis, and in excitable tissue, electrical coupling. This exchange of electrical and molecular signals allows individual cell events to synchronize into the functional response of an entire organ. Previous studies indicate that the disruption of gap junctional intercellular communication (GJIC) leads to a loss of growth control that contributes to the development of human cancer. The most direct evidence supporting the role of GJIC in transformation involved the introduction of the gap junction protein connexin32 (Cx32) into communication-deficient human tumors and tumor cell lines. Cx32 resulted in restoration of GJIC in liver epithelial and glioma cells leading to an inhibition of tumorigenicity. In this study, we are focusing on the intermolecular interactions that define the Cx32 structure when associated with the tumor suppressor protein human Discs Large (hDlg). Previously, we have shown that Cx32 interacts with the C-terminal half of hDlg, and in the absence of this interaction, hDlg aberrantly localizes to the nucleus. Using the yeast two-hybrid and HA Tag IP/Co-IP assays, we are defining the minimal region of hDlg required for the Cx32/hDlg interaction. Additionally, studies have reported that Cx32 can also interact with another MAGUK family member, Zonula Occludens-1 (ZO-1). ZO-1 interacts with other connexins via its PDZ2 domain; therefore,