**1718-Pos Board B562**

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

**Likai Song**[1,2], Zhen-Yu J. Sun[2], Kate Coleman[1], Kyoung Joon Oh[3], Gerhard Wagner[2], Ellis Reinherz[1,2], Mikyung Kim[1,2].
[1]Dana-Farber Cancer Institute, Boston, MA, USA, [2]Harvard Medical School, Boston, MA, USA, [3]Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA.

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 $\alpha$ 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**

**Harishchandra Ghimire**[1], Johnson J. Inbaraj[1], Daniel J. Mayo[1], Monica Benedikt[1], Eric Hustedt[2], Gary A. Lorigan[1].
[1]Miami University, Oxford, OH, USA, [2]Vanderbilt University, Nashville, TN, USA.

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.

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

**Yoshiaki Yano**, Norie Shimai, Katsumi Matsuzaki.
Kyoto University, Kyoto, Japan.

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**

**Kalani J. Seu**[1], Joanna R. Long[2], Sean M. Decatur[1].
[1]Oberlin College Department of Chemistry & Biochemistry, Oberlin, OH, USA, [2]Department of Biochemistry and Molecular Biology and McKnight Brain Institute, Gainesville, FL, USA.

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

**Miki Itaya**[1], Ian C. Brett[1], Wei Liu[1], Stefan N. Constantinescu[2], Steven O. Smith[1].
[1]Stony Brook University, Stony Brook, NY, USA, [2]Université de Louvain, Brussels, Belgium.

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.

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

**Da Qun Ni**[1,2], Hua Jun Qin[1,2], Mukesh Sharma[1,2], Timothy A. Cross[1,2].
[1]National High Magnetic Field Laboratory, Tallahassee, FL, USA, [2]Florida State University, Tallahassee, FL, USA.

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