**1494-Pos Board B224**
**Translocation of Cationic Amphipathic Peptides Across the Membranes of Pure Phospholipid Giant Vesicles**
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The ability of amphipathic polypeptides with substantial net positive charges to translocate across lipid membranes is a fundamental problem in physical biochemistry. These peptides should not passively cross the bilayer nonpolar region, but they do. Here we present a method to measure peptide translocation, and test it on three representative membrane-active peptides. In samples of giant unilamellar vesicles (GUVs) prepared by electroformation, some GUVs enclose inner vesicles. When these GUVs are added to a peptide solution containing a membrane-impermeant fluorescent dye (carboxyfluorescein), the peptide permeabilizes the outer membrane, and dye enters the outer GUV, which then exhibits green fluorescence. The inner vesicles remain dark if the peptide does not cross the outer membrane. But if the peptide translocates, it permeabilizes the inner vesicles as well, which then show fluorescence. We also measure translocation, simultaneously on the same GUV, by the appearance of fluorescently-labeled peptides on the inner vesicle membranes. All three peptides examined are able to translocate, but to different extents. Peptides with smaller Gibbs energy of insertion into the membrane translocate more easily. Further, translocation and influx occur broadly over the same period but with very different kinetics. Translocation across the outer membrane follows approximately an exponential rise, with a characteristic time of 10 minutes. Influx occurs more abruptly. In the outer vesicle, influx happens before most of the translocation. But some peptides cross the membrane before any influx is observed. In the inner vesicles, influx occurs abruptly sometime during peptide translocation across the membrane of the outer vesicle.
(Supported by NIH grant GM072507)

**1495-Pos Board B225**
**Dimer is a Minimal Functional Unit of Influenza a Virus M2 Channel on Living Cells**
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The integral matrix protein M2 of influenza A virus has been proposed to form an acid-activated, proton-selective channel in the viral lipid envelope upon infecting host cells. Although X-ray crystallography and NMR studies using fragment peptides suggested that M2 stably forms a tetrameric channel irrespective of pH, the oligomeric states of the full-length protein on living cells are not clear yet. In the present study, we investigated the oligomerization of the protein in living CHO cells using the coiled-coil labeling method developed in our laboratory and the spectral imaging technique [1]. Contrary to previous models, the protein existed as a dimer at neutral pH, although it formed a tetramer at pH below 5. The proton channel activity was determined with the pH-sensitive dye SNARF-4F. The intracellular proton concentration was exponentially increased when the extracellular pH was reduced. The rate constant per protein for the dimer was larger than that for the tetramer. Alanine-scanning experiments showed that His37 was crucial to the channel activity. The addition of the antiviral drug amantadine hydrochloride inhibited the tetramerization at acidic pH and the activity of the dimeric channel, suggesting that the drug binds to the dimeric form and blocks both the proton conductance and tetramerization instead of clogging the tetrameric pore. Cholesterol removal resulted in a significant decrease in the activity of the dimer. These results indicate that a minimum functional unit of M2 protein is dimer, which forms a complex with cholesterol for its function.
[1] Kawano et al., Anal. Chem. 85, 3454 (2013)

**1496-Pos Board B226**
**Process of Inducing Pores in Membranes by Melittin**
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Melittin is a prototype of the ubiquitous antimicrobial peptides that induce pores in membranes. It is commonly used as a molecular device for membrane permeabilization. Even at concentrations in the nanomolar range, melittin can induce transient pores that allow transmembrane conduction of atomic ions but not leakage of glucose or larger molecules. At micromolar concentrations, melittin induces stable pores allowing transmembrane leakage of molecules up to tens of kDa, corresponding to its antimicrobial activities. Despite extensive studies, aspects of molecular mechanism for pore formation remain unclear. To clarify the mechanism, one must know the states of melittin-bound membrane before and after the process. By correlating experiment of giant unilamellar vesicles with that of peptide-lipid multilayers, we found that melittin bound on the vesicle translocated and redistributed to both sides of the membrane before the formation of stable pores. Furthermore, stable pores are formed only above a critical peptide-to-lipid ratio. The initial states for transient and stable pores are different, which implies different mechanisms at low and high peptide concentrations. To determine the lipidic structure of the pore, the pores in peptide-lipid multilayers were induced to form a lattice and examined by anomalous X-ray diffraction. The electron density distribution of lipid labels shows that the pore is formed by merging of two interfaces through a hole. The molecular property of melittin is such that it adsorbs strongly to the bilayer interface. Pore formation can be viewed as the bilayer adopting a lipid configuration to accommodate its excessive interfacial area.

**1497-Pos Board B227**
**1+1=3? Concerted Action of Membrane Permeabilizers**
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Certain antibiotic peptides are thought to permeabilize membranes of pathogens by effects that are also observed for simple detergents, such as membrane thinning and disordering, asymmetric bilayer expansion, toroidal pore formation, and micellization. Here we test the hypothesis that such peptides act additively with detergents when applied in parallel. Additivity is defined analogously to a fractional inhibitory concentration index (FICI) of unity, and the extent and mechanism of leakage are measured by the fluorescence lifetime-based vesicle leakage assay using calcein-loaded vesicles. Concerted action was tested for the detergent C12EO8 with the detergents octyl glucoside and CHAPS, the antimicrobial peptide magainin 2, and the fungicidal lipopeptides surfactin, fengycin and iturin from Bacillus subtilis QST713, respectively. The results are discussed in terms of an optimum heterogeneity of the system that governs the leakage mechanism, extent of leakage, and additivity of action with another agent. The results are important for understanding the nature of detergent-like actions of peptides as well as for optimizing formulations of such antimicrobials for medical applications or crop protection.

**1498-Pos Board B228**
**Antimicrobial Peptides Piscidin 1 and Piscidin 3 Kink at a Central Glycine to Maximize their Hydrophobic Moments**
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Piscidin 1 (p1) and the less active piscidin 3 (p3) are α-helical, amphipathic, and cationic antimicrobial peptides. They adsorb onto the outer membrane of pathogenic species and induce leakage beyond a threshold peptide concentration. While different mechanisms of membrane disruption have been proposed, an atomic-level description of the events leading to cell death is lacking. Elucidating these mechanisms is critical in the design of new antibiotics. Here, the structures, orientations, and positions of p1 and p3 in two different bacterial membrane mimics (3:1 DMPC/DMPG and 1:1 POPE/POPG) are studied by solid-state NMR spectroscopy, all-atom molecular dynamics (MD) simulations, and neutron diffraction to better understand molecular determinants that contribute to antimicrobial activity. The tilts of the helical axis, τ, are perpendicular to the bilayer normal (83-93°) and the average azimuthal rotation, ρ, corresponds to burial of hydrophobic residues in the bilayer. The refined NMR and MD structures reveal a slight kink at Gly13 of the α-helix. This kink is characterized by a small difference in τ (<10°) and significant difference in ρ (~20°) and maximizes the hydrophobic moment for the α-helix. The depths of insertion are similar in DMPC/DMPG. In contrast, p1 and p3 are 1.0 and 3.0 Å less inserted in POPE/POPG. Insertion of the peptides corresponds to greater thinning of the bilayer and a decrease in the lipid order parameter. Deformation of the DMPC/DMPG bilayer is characterized by uniform membrane thinning around the peptide, while deformation in the POPE/POPG bilayer is characterized by localized thinning near the N-terminus of the peptide. The increase in thinning in DMPC/DMPG relates to a greater activity than in POPE/POPG in dye-leakage assays and may be a precursor to a more disruptive state.