Gramicidin A (gA) channels make an ideal system to test molecular dynamics (MD) of membrane proteins (and ion permeation). In addition to being one of the most highly studied membrane ''proteins'', gA channels are tiny, relatively speaking, allowing for long MD runs and calculations of potential of mean force in tractable time. The structure of gA has been determined by solid-state Nuclear Magnetic Resonance (NMR) and solution-state NMR. The structures are in overall agreement, but differ slightly in backbone pitch and in a few amino acid (AA) side chains orientations. Most of these differences have been understood using MD simulations of gA channels in planar bilayers (Allen et al., *J Am Chem Soc.* 125:9868-77, 2003). Because the AA backbone lines the pore and tryptophan side chains are in close proximity to the permeating ion, the average structure and extent of fluctuations of all atoms in the peptide will greatly influence ion permeation. This raises the question of how well molecular mechanical force fields used in potential of mean force studies of ion permeation can reproduce experimental backbone and side chain structure and dynamics. To examine this we measured the gA channel backbone dynamics using solution state ^{15}N-NMR on gA dimers in sodium dodecyl sulfate (SDS) micelles, in parallel with fully atomistic MD simulations on a gA dimer within an explicit SDS micelle. The methods enable us to examine the robustness of the MD simulations done under different conditions (different tryptophan force fields, with/without CMAP corrections), as well as their ability to predict the NMR observables.

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Assessment of Merocyanine Subpopulations in DPPC Vesicles using Ansitropy and Lifetime Measurements

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The purpose of this study was to further investigate the properties of the fluorescent probe merocyanine 540, which has been used frequently for over two decades to assess membrane phase properties under various conditions. Differences in emission spectrum shape at temperatures above and below the thermotropic phase transition of model membranes have been hypothesized to represent changes in the position and orientation of the probe in the bilayer. This hypothesis suggests specific predictions concerning probe mobility in the membrane as a function of temperature and emission wavelength. We tested the hypothesis using measurements of steady state anisotropy and fluorescence lifetimes in dipalmitoylphosphatidylcholine vesicles. Below the lipid phase transition temperature, steady state anisotropy decreased by 0.2 units across the emission spectrum from short to long wavelength. In contrast, anisotropy was more stable as a function of emission wavelength when measured above the transition temperature. Fluorescence lifetimes showed minimal wavelength dependence at either temperature. Anisotropy experiments were repeated at a variety of probe-to-lipid ratios to assess the role of probe aggregation on the observations. The data supported previous findings from measurements of the quantum yield of merocyanine 540: in the gel phase, two separate populations of the probe (monomers and dimers) fluoresce. The monomers, which emit at short wavelengths, are oriented perpendicular to the bilayer surface, and are limited in mobility by neighboring phospholipids. The dimmers, which emit at long wavelengths, are oriented parallel to the bilayer surface, and are localized in a membrane region where motion is less restricted, perhaps in the region between the membrane leaflets. At higher temperatures, only monomers fluoresce but exhibit higher mobility due to the lower order of the membrane phase.

Membrane Active Peptides I

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High Throughput Methods for Discovering Membrane Active Peptides

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Rational design and engineering of membrane active peptides remains a largely unsatisfied goal. We have hypothesized that this us due, in part, to the fact that some membrane activities, such as permeabilization, and cell penetrating ability are not dependent on specific amino acid sequences or specific three-dimensional peptide structures. Instead they depend on interfacial activity; the ability of a molecule to partition into in the membrane-water interface and to strain the packing and organization of lipids. We are testing that idea by taking a novel approach to biomolecular engineering and design of membrane-active peptides. Several rational combinatorial peptide libraries containing 10-16,000 members have been screened for water soluble members that either permeabilize phospholipid membranes or translocate without permeabilization. Stringent, two-phase, high-throughput screens were used to identify dozens of unique peptides that had potent membrane permeabilizing activity or cell penetrating activity, but were also highly water soluble. These rare and uniquely active peptides did not always share a particular sequence motif, peptide length or net charge, but always share common compositional features, secondary structure and core hydrophobicity. We suggest that they function by common mechanisms that depends mostly on interfacial activity. We demonstrate here that composition-space peptide libraries coupled with function-based high-throughput screens can lead to the discovery of diverse, soluble, and highly potent interfacially active peptides.

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Non-membranolytic, Translocating Peptides Selected From A Peptide Library

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Translocation is a defining characteristic of cell-penetrating peptides and antimicrobial peptides that act intracellularly. Found in nature, these membrane-active peptides are being redesigned as therapeutic agents of drug delivery, gene-therapy, and anticancer activity. Such peptides can only be loosely categorized as small, cationic peptides, and even with that broad definition there are outliers. They are best defined by their function to translocate across membranes where they exert their action. Here we have used a function-based approach to isolate 12 translocating peptides from a 10,000+ member peptide library of rational design, using a high-throughput screen for monitoring the non-membranolytic translocation of peptides across lipid bilayers. The 12 residue framework of the library, designed with translocation in mind, is a series of 9 combinatorial sites followed by a C-terminal alpha-1-chymotrypsin cleavage site, integral to the screen. The resulting residue in each combinatorial site is one of 2 - 4 variable amino acids, with a hydrophobic or cationic residue available in each position. The sequences of the translocating peptides from the screen have no specific motif, but similarities do arise in overall compositional features, hydrophobicity, and general deficiency of an ordered structure. The continued trend in a lack of convergence regarding a structure-function relationship supports function based screening of peptide libraries as the best way to arrive at de novo membrane-active peptides with specific functions of interest.

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PG-1 Orientation in Lipid Bilayers: Insights from Molecular Dynamics Simulations and Calculations of Potentials of Mean Force as a Function of Its Tilt Angle

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Antimicrobial peptides, the so-called host defense peptides (usually 12 to 50 amino acids long), exist in all living organisms and play a key role in host defense and innate immune response. Protegrin-1 (PG-1) is one of such peptides and has the β-hairpin conformations in aqueous solution and membrane environments because of inter-strand disulfide bonds. The oligomer states of PG-1 largely depend on membrane types. PG-1 inserts spontaneously and exists as a monomer in a DLPC membrane. In POPC, the minimum structural unit of PG-1 appears to be a dimer that exists in the membrane. To investigate the PG-1 orientation in lipid bilayers, we have performed comparative molecular dynamics simulations of PG-1 monomer in DLPC and POPC membranes. We have also calculated the potentials of mean force (PMF) of PG-1 monomer (with two different rotation angles) in DLPC and POPC membranes as a function of its tilt angle using the β-hairpin restraint potential that we have recently developed. In this work, we will present the simulation results and the calculated PMFs, along with the comparison of calculated solid-state NMR properties with available experimental data.

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Structure And Dynamics Of Phospholamban In The Context Of SERCA

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Sacroplasmic reticulum Ca^{+2}-ATPase (SERCA) plays an essential role in transporting Ca^{2+} ions from the cytosol to the lumen during contraction of cardiac cells. Phospholamban (PLB) is a membrane-bound peptide involved in SERCA regulation. Unphosphorylated PLB inhibits SERCA while phosphorylation of PLB relieves the inhibitory effect. It has been proposed that phosphorylation of phospholamban causes a structural change in SERCA in a switching mechanism between the two main conformations of SERCA, E2 and E1. Detailed structural information of membrane-bound PLB, especially in the context of SERCA is lacking. Molecular dynamics simulations of PLB with and without SERCA are presented to provide atomistic information about its structure and dynamics as a function of phosphorylation, binding to SERCA, and E2-E1 conformational switch in SERCA.

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Antimicrobial Lipopeptides In Anionic And Zwitterionic Membranes Investigated By Molecules Dynamics Simulations

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