2061-Pos Single Molecule Kinetics of ENTH Binding to Lipid Membranes

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Board B176

Transient recruitment of proteins to membranes is a fundamental mechanism by which the cell exerts spatial and temporal control over protein localization and interactions. Thus the specificity and kinetics of peripheral proteins' membrane-residence are an attribute of their function and reactivity. While bulk measurements can quantify average properties of these important protein-membrane associations, they are unable to delineate the details of their underlying mechanism. In here, we report direct visualization of protein-membrane interactions of a representative membrane targeting protein module - the Epsin N terminal homology (ENTH) domain. The use of simple model membranes (i.e. a fluid lipid bilayer deposited on a silica) allows precise control over the membrane physio-chemical properties as well as unprecedented details of membrane integrity during the measurements. Single binding events of the ENTH protein to lipid bilayers was imaged by total internal reflection fluorescence microscopy to discriminate bound and unbound protein. Subsequent single fluorophore tracking permits us to build up distributions of residence times. We report ENTH dissociation rate for various membrane compositions.

2062-Pos Investigating And Modeling Possible Mechanisms By Which Healthy Cell Membranes Become Resistant To Hydrolysis By Secretory Phospholipase A2

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Board B177

Secretory phospholipase A2 (sPLA2) behaves differently toward the membranes of healthy cells compared to those of damaged or dying cells. The enzyme catalyzes rapid and sustained hydrolysis of compromised cells consistent with a simple catalytic mechanism. In contrast, when healthy cells are incubated with sPLA2, they become resistant to hydrolytic attack as manifest by three unusual observations: First, hydrolysis is transient and represents only a small fraction of the total membrane phospholipid content. Second, subsequent addition of sPLA2 fails to generate additional product. Third, the apparent potency of the enzyme to cause the membrane to be refractory is much greater than the potency for catalyzing hydrolysis. The mechanism responsible for this resistance has not yet been identified. Using Monte Carlo and direct analytical methods, we have developed a model capable of explaining all three of these observations. The model requires two salient elements: only a small pool of phospholipids in the healthy cell membrane is available for catalysis by sPLA2, and hydrolyzed phospholipids are re-acylated and restored very slowly to the accessible pool. The requirement for initial hydrolysis (as opposed to the simple physical presence of the enzyme as previously thought) was confirmed experimentally. The model also predicts that total substrate, reacylation rate, and the return rate of phospholipids to the membrane should all be constant as enzyme concentration is varied. This prediction was tested by quantitative analysis of hydrolysis time courses at varied enzyme concentrations. Lastly, initial experiments with a fluorescent probe, merocyanine 540 suggest that resistance may also involve physical changes to the membrane beyond the kinetic mechanisms hypothesized in the model.

Interfacial Protein-Lipid Interactions, Peptides

2063-Pos Interaction of Bactenecin with 1,2-dipalmitoyl-sn-glycero-3-Phosphocholine Monolayers at the Air-Water Interface

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We show results of the interaction of the antimicrobial peptide bactenecin and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine monolayers (DPPC) at the air/water interface. We investigated the role of different salt solutions in the subphase on the structure and elastic properties of Bactenecin/DPPC monolayers. Bactenecin interacting with DPPC monolayers change the equilibrium phase LC-LE of DPPC monolayers, depending on the ionic strength and salt type in the subphase. BAM images were obtained in the expansion compression cycles to visualize the Bactenecin-DPPC interaction. AFM images were performed for Bactenecin/DPPC monolayers, where the Bactenecin domains are shown, surrounded by DPPC molecules. We also performed circular dichroism measurements to obtain some information about the peptide conformation in solution and on Langmuir-Blodgett films. Ellipsometry experiments gave us some information of he peptide penetration into DPPC monolayer by measuring the growing of the monolayer size.

2064-Pos The Role of Lipids for the Functional Integrity of Porin

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Board B179

OmpF and OmpG are channel-forming proteins found in the outer membrane of gram negative bacteria. Despite having similar β-barrel structure and thermal stability, OmpG is monomer but OmpF is homo-trimer, which is essential for functioning [1]. We have investigated the temperature-dependent interaction of these two members of porin family with lipid molecules by FTIR spectroscopy. Different reporter groups from the lipid and the protein were used in the analysis of their interaction. These are CH2 and CH3 stretching modes (position and width) from the hydrophobic core of the membrane; the C=O stretching mode from the interfacial region of membrane; the phosphate headgroup of lipids from hydrophilic region of membrane; aromatic side chains like tyrosine from the protein, and secondary structure analysis using the amide I region of the IR spectrum. OmpF porin-lecithin interaction analysis revealed three distinct temperature regions. Between room temperature and 55 °C, both porins form hydrogen-bonding with interfacial region of the bilayer. Between 55 °C and 75 °C, H-bonding is weakened for OmpF and it starts monomerization while OmpG shows no difference in H-bonding properties. Between 75 °C and 95 °C, lipid-monomer interaction breaks down and both porins aggregate. In this study we provide evidence that monomerization of OmpF takes place at higher temperature values, relative to detergent environment, if reconstituted in lipids. We also show that loss of function at 50 °C reported previously [1] is indeed related with loss of H-bonding between the aromatic girdle of porin and the interfacial region of bilayer.

References

1. Sukumaran, S. Spectroscopic investigation of stability, unfolding and refolding of outer membrane protein porin from Paracocus denitrificans. Dissertation zur Erlangung des Doktorgrades der Physik, Johann Wolfgang Goethe Universitaet, Frankfurt am Main. 86 pp.

2065-Pos Modulation of Lipid Domains in Mixed Model Systems through Binding of Annexin A5 and the C2 Protein

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Board B180

The control of lipid domain formation in biological membranes has received limited consideration. This mechanism is quantitatively investigated using Monte Carlo computer simulations of a simple model system. The simulations are based on the hypothesis that thermodynamic coupling between lipid-lipid, protein-lipid, and protein-protein interactions can lead to domain formation. Monte Carlo simulations are performed on a simple model system composed of phosphatidylcholine (PC), and phosphatidylserine (PS) mixtures, as well as mixtures of PC, PS and Cholesterol (Chol), which has been indicated to preferentially interact with the negatively charged PS lipids. Domain formation induced by the addition of the calcium-dependent phospholipid binding protein, Annexin A5 (A5) is investigated. Monte Carlo calculations for models containing PS/PC lipids have indicated that the addition of A5 does not induce lipid domain formation. The addition of Chol to PS/PC systems was found to induce lipid demixing in the absence and presence of A5. Incorporation of a preferential protein-protein interaction to PS/PC and PS/PC/Chol systems was found to further increase lipid demixing for all mixtures. Similar simulations were performed for the C2 protein motif and results from the two proteins were compared.

This research was supported in part by NIH Grant GM064443.

2066-Pos Structure Activity Relationships of Aurein 1.2, Citropin 1.1 and Maculatin 1, Antimicrobial Peptides From the Skin Secretions of Australian Tree Frogs

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Board B181

Aurein 1.2, Citropin 1.1 and Maculatin 1.1 are members of three structurally related families of antimicrobial peptides produced in the skin secretions of several species of Australian tree frogs. Studies have shown that these peptides are unstructured in aqueous solution but readily fold into fairly hydrophobic amphipathic α-helices when dissolved in organic solvents or incorporated into lipid membranes. Although, the variations in the antimicrobial activities of these peptides and in their capacities to interact with and perturb the structure and organization of lipid membranes can be largely rationalized on the basis of variations in their sizes and net charges carried on their polar surfaces, we find that many aspects of the biological and overall membrane disruptive activities of these peptides cannot be explained within such concepts. Our data suggest that the antimicrobial and other membrane disruptive activities of these peptides are also strongly influenced by the helical-forming propensities of these peptides and by their capacity for self-association in aqueous media, factors which also affect their capacity to partition into lipid membranes

2067-Pos The Interaction Of Alpha-hemolysin With Specific Microdomains At Sublytic Concentration

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Board B182

α-Hemolysin (HlyA) is a pore forming toxin secreted by pathogenic strains of *Escherichia coli.* The toxin is synthesized as a protoxin, pro-HlyA, which is matured in the cytosol to the active form by acylation at two internal lysines. This toxin with a wide target cell specificity is considered to be the prototype of a family of toxins called RTX (repeat in toxin), a series of protein toxins that share genetic and structural features.

In this report, we studied the interaction of HlyA with lipid membranes using as experimental system sheep erythrocytes, one of the most studied target cell for this type of studies. A variety of pathogens, toxins and acylated proteins interact with microdomains enriched in cholesterol and sphingolipids (lipid rafts), therefore we studied the association of HlyA with these microdomains on sheep erythrocytes. Our experiments in functional properties of the toxin such as oligomerization and hemolytic activity studied by FRET and light scattering, respectively, using cholesterol- depleted erythrocytes indicate the possible interaction of the toxin with these microdomains. We studied the effect of the toxin on the membrane fluidity using Laurdan Generalized Polarization (GP) images taken in a two-photon Fluorescence Microscope. After incubation of the erythrocytes with sublytic concentration of the toxin, the GP images showed an increase in the GP values of the membrane, interpreted as a decrease of membrane fluidity when the toxin is bound, and a decrease in the size of the erythrocytes. On the other side removal of cholesterol by methyl-β-cyclodextrin (CD) showed an increase in the GP value indication of an increase in rigidity of the membrane. These results suggest that at sublytic concentration, the interaction of HlyA with the sheep erythrocytes membrane involves microdomains enriched in cholesterol and sphingolipids.

2068-Pos Interactions Of Hal18 With Model Bacterial Membranes

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Halocidin is a heterodimeric peptide consisting of two α-helical subunits Hal15 and Hal18 and was isolated from hemocytes, *Halocynthia aurantium.* Hal18 has antibacterial properties against *Bacillus subtilis* (15 μM) and *Escherichia coli* (100 μM), however, the mechanism of action has not yet fully been elucidated. Here, Langmuir Blodgettry was used to investigate the surface properties of Hal18 and its interaction with phospholipids. Hal18 was able to produce stable monolayers, which were comparable to other α-helical peptides predicted to orientate parallel to the surface of the interface. Constant area assays showed that Hal18 was surface active (4 μM) inducing surface pressure changes >30 mN m^1 characteristic of membrane interactive peptides. The peptide induced stable surface pressure changes in monolayers that were mimetic of *B. subtilis* membranes (*circa* 7 mN m^{-1}) and *E. coli* membrane-mimics (*circa* 4 mN m^{-1}). Hal18 inserts readily into zwitterionic DOPE and anionic DOPG monolayers inducing surface pressure changes *circa* 8 mN m^{-1}, providing evidence that interaction is not headgroup specific. Thermodynamic analysis of compression isotherms showed that the presence of Hal18 destabilise *B. subtilis* membranes ($G_{Mix} > 0$), which is in contrast to it effect on *E. coli* lipid extract implying the differential antimicrobial activity may be driven by lipid packing.

2069-Pos Interaction of Novel Antimicrobial Peptide Mimics with Model Plasma and Bacterial Membranes

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Board B184

Non-natural mimics of antimicrobial peptides are excellent candidates for anti-infectious agents due to their stability towards enzymatic degradation and broad adjustability of physicochemical properties. Arylamide and acyllysine oligomers have demonstrated capability to be fine-tuned to high antimicrobial activity and negligible toxicity towards human cells. In the present work we examine interactions of both types of oligomers with model lipid monolayers using the liquid surface X-ray scattering techniques of X-ray reflectivity and grazing incidence X-ray diffraction. Lipid monolayer formed at the air-liquid interface models an approximate environment where antimicrobial peptides approach the outer leaflet of a target cell membrane. Simplified model of an outer leaflet of a bacterial membrane was represented either with DPPG or Lipid A. Due to the ability of cholesterol to promote insertion of conventional antimicrobial peptides, such as MSI-78, into zwitterionic lipid membranes mammalian cell membrane was mimicked with Cholesterol/DPPC mixture. The peptides were subsequently injected into the aqueous subphase and allowed to interact with the lipid layer. In addition to X-ray experiments, the lipid phase morphology before and after peptide mimics insertion for each lipid film was visualized by epifluorescence microscopy. Insertion of both types of peptide mimic into anionic but not zwitterionic lipid monolayers strongly supports the activity trends observed in previously reported antimicrobial and hemolytic assays.

2070-Pos Effect of PIP2 Binding on the Membrane Docking Geometry of the PKCalpha C2 Domain: An EPR Site-directed Spin-labeling

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Board B185

Ca^{2+}-dependent membrane targeting of C2 domains is a crucial function required for driving membrane localization of critical signaling proteins during many cellular processes. The C2 domain of PKCα specifically targets this enzyme to the inner leaflet of the cell plasma membrane during intracellular Ca^{2+} signaling. This exquisite membrane specificity arises from synergistic recognition of the specific target phospholipids, phosphatidylserine (PS) and phosphatidylinositol-4,5-bisphosphate (PIP_2), which are known to be abundant at the inner leaflet of the cell plasma membrane. Here, we use site-directed spin-labeling combined with EPR power saturation and depth parameter methods to determine the Ca^{2+}-dependent, membrane docking geometry and depth of penetration of the PKCα-C2 domain docked to membranes containing its physiological targeting phospholipids, PS and PIP_2. We selected 14 sites, mostly localized to the known Ca^{2+}-binding loops and PIP_2 binding site of the C2 domain, and performed cysteine mutagenesis to generate a spin-labeled library. Of these 14 sites, 10 functional positions were used in EPR power saturation experiments where we measured the depth parameters of each position bound to physiological membrane mimics containing PS, in the absence and presence of PIP_2. Using this information we modeled the docking geometry and penetration depth of the PKCα-C2 domain in two bound states. The results from this study reveal a PIP_2 triggered change in the membrane docking geometry that tilts the C2 domain relative to the membrane surface, moving the β-sandwich core from a nearly parallel docking geometry toward a more perpendicular docking geometry. This structural change at the membrane docking face, mediated by PIP_2, likely contributes to an enhanced bound state stability, thus providing structural insight to the PIP_2 specificity mechanism of the Ca^{2+}-dependent membrane targeting reaction.

2071-Pos Screening for Stable Pore-Forming Peptides Designed to Mimic β-barrel Proteins

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Board B186

There are three main mechanisms for pore-forming peptides to disrupt lipid bilayers. In the carpet model, peptides orient parallel to the cell surface and distort the lipid bilayer. The stable toroidal pore model describes a continuous bend of the bilayer with inserted peptides. The focus of this work is the barrel-stave model, when inserted peptides' residues align either out into the lipid bilayer or into the hydrophilic core without a continuous bend of the bilayer. We have synthesized a combinatorial beta-strand peptide library based on beta-barrel protein statistics. Peptides are either 10 or 12 residues long with a net charge of +2. To test for peptides that form stable pores, our lab has further developed a known quenching assay using large unilamellar vesicles (LUVs). These vesicles contain 1 mol% NBD fluorophore-attached to phospholipids in both inner and outer leaflets of the bilayer. Because the quencher, dithionite, cannot penetrate membranes, only outer leaflets lose their fluorescence. In a high throughput screen, peptides and vesicles are incubated overnight in a 96-well plate format at a peptide-to-lipid ratio of 1:200. LUVs without pore-formers show 60% quenching after one hour of added dithionite, consistent with external NBD-lipids. Vesicles with stable pore-formers show close to 100% quenching as a result of structural access for dithionite to the inner leaflets. Because NBD vesicles can be fully quenched by peptides that cause flip-flop, additional steps will be taken to confirm that the selected peptides are stable pore-formers. These include incubating carpet-model peptides as negative controls and testing pore-formers with leakage assays. Selected peptides will be characterized with regard to mechanism of pore formation, pore properties, secondary structure, as well as biological activity using antimicrobial, hemolysis and cytotoxicity assays.

2072-Pos A Synthetic Variant of Cecropin A is Significantly More Active Toward Pure POPC Membranes

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Board B187

Cecropin A (CecA) is a 37-residue antimicrobial, amphipathic peptide, which has a net charge of +7 at neutral pH. In a recent study [Gregory et al. (2007) Biophys J, in press] the mechanism of CecA was examined in detail. CecA causes efflux of carboxyfluorescein (CF) lipid vesicles of POPC/POPG with compositions of 50:50, 70:30, and 80:20. However, it is essentially inactive against membranes composed of 100 % POPC. A new peptide was designed closely based on the amino acid sequence of CecA. This variant peptide is also composed of 37 residues, but with minimal sequence variation from CecA, and has a net charge of +1 at neutral pH. In order to determine the activity of the designed peptide toward lipid vesicles, efflux kinetics experiments were preformed using synthetic lipid vesicles composed of 100% POPC and 50:50 POPC:POPG. The kinetics of dye release were used to measure peptide activity. The binding affinity of the designed peptide to synthetic POPC membranes was also measured. The variant peptide is significantly more active than CecA toward pure POPC. The reasons for this change in activity are discussed.

The research was supported in part by NIH Grant GM072507.

2073-Pos Interaction of Perfringolysin O with Membranes is Affected by Sterol and Membrane Structure

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Board B188

Perfringolysin O (PFO) is a cholesterol-dependent pore-forming cytolysin used to detect the location of cholesterol-rich regions in cells. To understand the molecular basis of PFO behavior and

membrane interaction, we first studied their dependence upon sterol structure using fluorescence spectroscopy methods. The interaction of PFO with sterols (and sterol derivatives) was affected by double bond position in the sterol rings, sterol side chain structure and sterol polar group structure. The ability of a sterol to participate in formation of ordered membranes domains (lipid rafts) was not critical for interaction with PFO. Interestingly, experiments using the Y181A (pre-pore) mutant demonstrated that sterol binding strength and specificity was not affected by whether or not PFO forms a transmembrane beta-barrel. Interaction with sterol-containing membranes was enhanced by incorporation of ceramide and both saturated and unsaturated fatty alcohols, which have been shown to displace cholesterol from ordered domains. These molecules by themselves, however, were not sufficient to substitute for sterol in the membrane. Membrane sterol depletion was carried out with methyl-beta-cyclodextrin, causing a loss in domain 4 association with sterol, even though PFO remained membrane-bound in its oligomeric state. Similar experiments with the Y181A mutant caused a nearly complete loss of membrane interaction. Combined, these observations are consistent with a model in which the strength and specificity of sterol interaction arises from both sterol interactions with domain 4 and sterol chemical activity within membranes. This places important constraints on the origin of PFO affinity for rafts.

2074-Pos Effect of Linker on Transmembrane Peptide-Anchored Lactoferricins in Neutral and Anionic Lipids

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Board B189

Electrostatic interactions between positively charged amino acids and negatively charged membrane lipids in eukaryotes (15% of cytoplasmic lipids) and bacteria (20% of outer membrane) are important to membrane protein structure and function. To study electrostatic interactions between the membrane proximal domain of transmembrane proteins and anionic lipids, a peptide has been designed with the cationic, amphipathic lactoferricin peptide (**LfB**) anchored to the N-terminus of a model α-helical transmembrane peptide ("**LfB**-AA-TM:" **RRW<u>A</u>WR**-AA-$(LA)_7$KKA-NH_2). The central glutamine of **LfB** was replaced with alanine (**<u>A</u>**) for selective deuteration. The transmembrane domain contains a hydrophobic (Leu-Ala) repeat that spans the membrane, with two lysine anchors at the C-terminus. An Ala-Ala spacer separates the LfB and TM domains. Solid state ^{2}H and ^{31}P NMR spectra from oriented samples of **LfB**-AA-TM in mixed neutral:anionic lipids (DMPC:DMPG, 3:1) indicate that the **LfB** domain is well oriented and the lipids are predominantly bilayer. The observed quadrupolar splitting for the deuterated Ala in the **LfB** domain (22 kHz) suggests a motionally restricted environment, most likely at the bilayer interface. Circular dichroism spectra of **LfB**-AA-TM indicates a higher alpha-helical content in TFE (86%) than in DMPC or DMPC:DMPG (28%) vesicles. The tryptophan fluorescence spectrum of **LfB-AA-TM** in DMPC:DMPG vesicles exhibits increased intensity and is slightly blue-shifted (λ_{max} 341 nm) compared to DMPC (λ_{max} 343 nm), confirming a more hydrophobic environment in the presence of anionic lipids. To examine the influence of a longer, helix-breaking Gly-Pro-Gly-Gly spacer between the transmembrane and juxtamembrane domains, a new peptide has been designed ("**LfB**-GPGG-TM:" **RRWQWR**-GPGG-$(L\underline{A})_7$KKA). This peptide offers opportunities for investigating the importance of the spacer for peptide structure and peptide/lipid interactions, using spectroscopic methods that have proven useful for **LfB**-AA-TM. Results and comparisons in neutral and anionic lipids will be reported.

2075-Pos How Does the Amino Acid Sequence Affect the Activity of Amphipathic Peptides?

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Board B190

The influence of the amino acid sequence on the activity of amphipathic peptides was investigated using a series of synthetic peptides. A truncated version of δ-lysin, a cytotoxic peptide from *S. aureus*, served as a template for three synthetic peptides. In each of the sequence variants, the amino acid sequence of the template was shuffled in a way that each variant had the same hydrophobic moment and net charge of +2 at neutral pH. This ensured that the only factor altered was the sequence of the amino acids. The interaction of the original truncated sequence and the variants with bilayer membranes were tested using liposomes composed of diacylphophatidylcholine (POPC) and diacylphosphatidylglycerol (POPG) membranes. All peptides were investigated with respect to their ability to bind to and release dye from lipid vesicles. Binding to the vesicles and kinetics of dye efflux were found to be significantly different for all peptides. The significance of the results will be discussed.

The research was supported in part by NIH Grant GM072507.

2076-Pos Specificity Of Antimicrobial Lipopeptides For Bacterial Membranes: Insights From Molecular Dynamics Simulations

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Board B191

Antimicrobial peptides are a critical part of the innate immune system, and have long been considered promising drug candidates. However, their application in clinical settings have been inhibited by the cost of synthesizing large amounts of protein, and by their vulnerability to degradation by peptidases. Recently, the Shai group

has presented results using a new class of compounds, synthetic lipopeptides containing only 4 residues attached to a fatty acid chain (Makovitzki et al, **PNAS** (2006), *103*, 15997–16002). The presence of lysine side chains encourages specificity to more anionic bacterial membranes relative to mostly zwitterionic mammalian membranes, the use of at least 1 D-amino acid protects the lipopeptide from peptidases, and the acyl chain guarantees that the compound is sufficiently hydrophobic to bind to membranes. Here we present a series of molecular dynamics simulations designed to elucidate the molecular-level mechanism of specific binding to bacterial membranes. Specifically, we examine the lipid-peptide interactions for two different lipopeptides (only one of which binds specifically to bacterial membranes) bound to two different membrane compositions (one designed to mimic bacteria, the other mammalian cells).

2077-Pos Membrane Thinning Effect of Curcumin

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Board B192

For an introduction of cucumin, please see another poster "Interaction of Curcumin with Lipid Bilayers: GUV and ITC experiments." Curcumin apparently interacts with lipid bilayers, but it is not clear how. A recent paper reports that it lengthens the gramicidin channel lifetime in DOPC bilayers, but does not affect the channel conductance. This is a good indication that it interacts with the bilayer rather than directly with gramicidin itself. Yet the capacitance measurement reported no change in membrane thickness. Here we undertake a membrane thickness measurement of DOPC-curcumin mixtures. The mixtures produced well aligned multilayers and excellent diffraction patterns. The results clearly show significant membrane thinning. But interestingly the degree of thinning is a nonlinear function of the curcumin concentration. This is contrary to membrane-active amphipathic peptides which all showed a linear membrane thinning effect, proportional to the peptide concentration until pore formation. The comparison with the membrane thinning effect of amphipathic peptides helps us understand the mode of interaction of curcumin. Curcumin binds to lipid bilayers in two possible states. In the S state, curcumin binds to the interface where it has an effect of expanding the membrane area therefore thins the hydrocarbon region. In the I state, curcumin inserts into the hydrocarbon region where it expands the membrane area but has no thinning effect. However the energy of the S state includes the energy cost of membrane thinning that is proportional to the square of thinning, therefore proportional to the square of the number of curcumin in the S state. That is why as the curcumin concentration increases, more curcumin molecules shift from the S state to the I state. This model explains the non-linear thinning data. It also explains the responses of GUVs exposed to curcumin reported in another poster.

2078-Pos Molecular interactions of lactoferricin peptides

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Board B193

Peptides derived from bovine lactoferricin (LfB-25), a 25-residue peptide of sequence FKCRRWQWRMKKLGAPSITCVRRAF-NH_2 released from the N-terminal region of bovine lactoferrin, exhibit antimicrobial and anti-angiogenic properties (Mader, et al. *Amer. J. Path.* 2006 *169*:1753). The fragment LfB-6 (**RRWQWR-NH_2**) retains significant antimicrobial activity (Tomita et al. 1994. *Acta Paediatr Jpn.* 36:585). LfB peptides selectively target negatively charged bacterial membranes, initially through electrostatic interactions, after which the amphipathic tryptophan (Trp) indole rings interact at the membrane-aqueous interface. LfB-6 peptides have been modified by Trp methylation and N-acylation to enhance their membrane interactive properties. Peptide/lipid interactions of Cn-**RRWQMeWR-NH_2**, where n ranges from 2 to 12 carbons, were monitored by solid state NMR in oriented bilayers of mixed neutral:anionic lipids (DMPC:DMPG, 3:1). ^{2}H-NMR spectra indicate that LfB peptides acylated up to n = 6 align well. ^{31}P-NMR spectra reveal that the lipids remain predominantly in a bilayer configuration, although the phosphate head group is perturbed by the peptides. By contrast LfB-6 acylated with a 12-carbon chain induces a nonbilayer, isotropic phase. The ^{2}H NMR spectra also suggest that the MeTrp is located in a motionally restricted environment. Antimicrobial assays against *E. coli* demonstrate a correlation between acyl chain length and activity. The minimal bacteriocidal concentration of C12-LfB-6 (6.25 μg/mL) is 90 times greater than that of the non-acylated peptide. The complexation of LfB-25 with heparin-like structures on the surface of endothelial cells has been shown to inhibit receptor-stimulated angiogenesis. We are using isothermal titration calorimetry to characterize the binding interactions between LfB-25 and heparin analogues.

2079-Pos Investigation of "Half-WALP" Peptides in Presence of Model Membrane Lipids using ^{2}H NMR Spectroscopy

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Board B194

Protein anchoring residues such as tryptophan facilitate presence and positioning of membrane spanning peptides within lipid bilayers. The WALP model has proven to be a versatile model system enabling elucidation of various characteristics of membrane spanning peptides. WALP-like peptides consist of an alpha helical Leu-Ala core transmembrane segment flanked on both ends by anchoring residues such as tryptophan. To further study the membrane inter-

active properties of tryptophan, we are investigating the behavior of "half-WALP" peptides. These peptides, acetyl-$(AL)_n$WWG-ethanolamide, can be synthesized containing deuterium-labeled alanines for solid-state ^{2}H NMR spectroscopy. In our experiments, the length of the hydrophobic "core" varies, with n ranging from 3 to 8. A half-WALP containing eleven total residues (n=4) is potentially capable of spanning a monolayer or one leaflet of a lipid bilayer. For this peptide, ^{2}H NMR spectra of samples containing oriented bilayers of DMPC or DLPC indicate slow motion on the NMR timescale, probably due to aggregation. This potential aggregation may be attributed to the short length of the (Leu-Ala) stretch and/or to the absence of a second anchoring region. It is of interest to investigate these features and to characterize members of the "half-WALP" family using not only ^{2}H NMR spectroscopy, but also vesicle leakage studies and ^{31}P-NMR to monitor the lipids. By varying the bilayer lipid composition, the relationship between the chemical design of the "half-WALP" peptides and the hydrophobic length of the host bilayer will be addressed.

2080-Pos Interaction of Curcumin with Lipid Bilayers: GUV and ITC experiments

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In a recent paper, curcumin was shown to affect the lifetime of the gramicidin channel in DOPC bilayer without changing its conductance. This implies that curcumin affects the gramicidin channel by modifying the lipid bilayer, rather than directly interacts with gramicidin. But a measurement of the bilayer capacitance showed no change of bilayer thickness. Curcumin is also known to have solubility and stability problem. Thus despite the immense literature on curcumin, there is still a lack of characterization of curcumin interaction with lipid bilayers. To make up for this deficiency, we investigate the solubility and stability of curcumin by spectroscopy, its partition coefficient to lipid bilayers by isothermal titration calorimetry (ITC) and its effect on the lipid bilayers by observing the responses of individual giant unilamellar vesicles (GUVs). Curcumin is completely soluble in a solution at pH$>$7, but it is unstable – it deteriorates faster in higher pH or when exposed to the light. On the other hand, curcumin is not completely soluble in a solution at pH 7, yet it exhibits long time stability thus suitable for experimental study. We performed ITC and GUV experiments on curcumin at pH$\leq$7. It appears from ITC that only the portion of completely dissolved curcumin will efficiently bind to the lipid bilayer membrane. Using the micropipette aspiration method, a GUV was produced in 200mM sucrose solution and transferred to an isotonic solution containing curcumin. It appears that curcumin bound to the membrane has an effect of expanding the membrane surface. Using a diffusion-binding model, the cross section of curcumin bound to the membrane can be estimated.

2081-Pos Organization of Gramicidin in Lipid

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Board B196

The organization of Gramicidin embedded in model lipid membranes was investigated by measuring the Gramicidin-Gramicidin correlation using grazing incidence small angle X-ray scattering on aligned substrate supported membranes. In this scattering geometry the interacting peptides cause a diffuse correlation peak in the scattering intensity, which can be measured to give information on the most probable nearest neighbor distance between the peptides in the membrane plane. We find that this nearest neighbor distance increases when changing the membrane lipids from dilauroylphosphocholine (DLPC) to ditridecanoylphosphocholine (DTPC) to dimyristoylphosphocholine (DMPC) i.e. with increasing chain length of the lipids. A similar increase in the nearest neighbor distance is observed as the hydration level of the membrane is reduced. In both cases obviously the membrane mediated forces between the peptides become increasingly repulsive. We will discuss the energetics of these forces. We also show that the peptides start to interact and order across neighboring bilayers at low membrane hydration levels, as signified by higher order reflections in the measured scattering intensities along the direction of the membrane normal.

2082-Pos The Conformation of Secondary Structures of Cell-Penetrating Peptide in Lipid

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Board B197

The prevailing theories suggest that cell-penetrating peptides (CPP) primarily bind to heparan sulfate proteoglycans anchored to cell surface. Nevertheless CPPs do interact with lipid bilayers, and we found that penetratin in particular adopts different secondary structures depending on the lipids. This provides a laboratory for studying the energetics of peptide conformations in lipid bilayers. We use OCD to characterize the different secondary structures. By mixing penetratin with different lipids, we measured the secondary structure as a function of lipid compostion, peptide/lipid molar ratio and surface charge density. In DOPC (di18:1PC) the peptide adopts mainly the alpha-helix conformation, while in DMPC (di14:0 PC) it shows mainly the beta-sheet conformation. Interestingly, in the hybrid lipid 18:1/14:0 PC, the peptide exhibits a conversion from alpha-helix to beta-sheet with increasing peptide-lipd molar ratio. Similar phenomena were found in POPC (16:0/18:1PC). In a series of mixtures of PC/PG, we found that increasing the charge density (PG density) promoted the degree of beta-sheet conformation. Hence, lipid with long and unsaturated carbon chain prefers alpha conformation. Low charge density and low peptide concentration would also favor alpha helix conformation. The opposite conditions favor beta-sheet conformation.

2083-Pos Interactions of Wild Type Buforin II with Physiologically Relevant Lipid Membranes

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Board B198

Buforin II is a potent 21 amino acid antimicrobial peptide derived from the naturally occurring buforin I. Like other antimicrobial peptides, buforin II targets a wide variety of organisms, including gram-negative and gram-positive bacteria and fungi. While many of the antimicrobial peptides studied derive their antimicrobial properties from their ability to disrupt the lipid membrane and thereby induce cell death, buforin II enters the target cell without membrane disruption. Due to this, buforin II and similar antimicrobial peptides have received increasing attention as possible vectors for antibiotic drug delivery. Previous work has considered the interactions of buforin II with membranes consisting of phosphatidylcholine and phosphatidylglycerol. This current study expands on these past results by focusing on the interactions of buforin II with varying compositions of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, and phosphatidylserine. Studying these lipid combinations allows us to consider how the peptide interacts with more physiologically relevant lipid bilayers; bacterial cells are primarily composed of phosphatidylglycerol and phosphatidylethanolamine whereas membranes composed of mainly phosphatidylcholine and phosphatidylserine are characteristic of eukaryotic cells. These studies have utilized fluorescence spectroscopy and circular dichroism measurements to determine the membrane affinity and secondary structure of buforin II. We have also employed molecular dynamics simulations to gain additional insight into these peptide-lipid interactions. By analyzing these interactions and comparing them to interactions from cell lipid extracts, we hope to further elucidate the mechanism by which buforin II interacts with and enters cells.

2084-Pos Determining the Role of Proline in the Membrane Interactions of Buforin II

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Board B199

Antimicrobial peptides are a promising alternative to conventional therapeutic agents due to their selective toxicity towards bacterial cells, fast killing abilities, broad antimicrobial range, and their relative insusceptibility to bacterial resistance. Buforin II (BF2) is a twenty-one amino acid polycationic antimicrobial peptide derived from the naturally occurring peptide buforin I, isolated from the stomach of the Asian toad *Bufo bufo gargarizans*. Previous research suggests BF2 targets bacteria by translocating into cells and binding to nucleic acids without causing membrane permeabilization. BF2's mechanism of action and potency against a broad spectrum of bacteria make it a potential tool for drug delivery and cellular transfection, as well as an antimicrobial agent. The sole proline residue of BF2 (P11) has been shown to form a disruption in the peptide's α-helical structure and to be important to translocation of buforin across lipid membranes. Interestingly, proline residues are also though to play an important role in some other cell penetrating peptides. In order to improve our understanding of the role proline residues play in translocation, we have investigated a series of BF2 variants that alter the position of its proline by one residue or one helical turn. Using circular dichroism, we found that these proline mutations have distinct effects on the secondary structure of the peptide when bound to lipid membranes, although fluorescence spectroscopy measurements showed that the variants have a similar membrane affinity. We have also measured the translocation ability of the variants into lipid vesicles using an ELISA-based assay with biotin-labeled peptides. In order to gain molecular-level insight into the role of proline on BF2·membrane interactions, we performed molecular dynamics simulations of BF2 variants with explicit lipid membranes.

2085-Pos Mechanisms of Melittin-Induced Pore Formation

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Board B200

Interactions between Melittin, an amphiphilic peptide found in bee venom, and 1, 2-Dioleoyl-sn-Glycero-3-phosphocholine (DOPC) bilayer in the process of pore formation are investigated by coarse-grained molecular simulations. A mechanism of melittin-induced pores at various peptide-lipid ratios is being tested against several existing models.

2086-Pos Domain Organization of Commd1 and Targeting to the Membrane Via Specific Interactions with Ptdins(4,5)p2

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Board B201

Copper Metabolism Murr1 Domain 1 (COMMD1) is a 21-kDa protein with roles in copper export from the liver, Na-transport, and NF-kappaB signaling. COMMD1 binds to several structurally unrelated proteins including the copper-transporting ATPase ATP7B, presumably regulating its targeting, as well as the integral membrane epithelial sodium channel ENaC (through δ-ENaC), which is negatively regulated by the presence of COMMD1. COMMD1 has been shown to suppress NF-kappaB signaling, alluding to a significantly plieotropic function. The biochemical basis of COMMD1 function is unknown. To gain insight into the biochemical properties of human COMMD1 we generated and experimentally tested an ab initio molecular model for this protein.

The studies revealed two structurally distinct domains connected by a proteolytically sensitive linker. The generated recombinant C-terminal domain forms stable dimers and higher order oligomers, which are characteristic of the full-length COMMD1, while the N-terminal domain appears monomeric. The asymmetric distribution of charges at the surface of COMMD1 suggested the possibility of significant electrostatic interactions with charged proteins and/or lipids. Fractionation of lysates from polarized hepatocytes confirmed the presence of endogenous COMMD1 in microsomal membranes as well as in cytosol. The interaction of COMMD1 with lipids does not require other proteins, as demonstrated by binding to lipid-spotted arrays and equilibrium binding to liposomes. COMMD1 specifically binds phosphatidylinositol PtdIns (4,5)P2, an important regulatory molecule, and does not bind structural lipids; phosphorylation of the phosphatidylinositol head group at position 4 is essential for COMMD1 binding. Both domains of COMMD1 appear to contribute to the binding site, since only full-length protein, and not individual domains, binds to PtdIns(4,5)P2. Recruitment to PtdIns(4,5)P2-containing liposomes stabilizes the COMMD1 dimer. Our results suggest mechanism through which soluble COMMD1 may function at cell membranes as a novel phosphatidylinositol-binding protein.

2087-Pos Molecular Recognition At Membrane Interfaces: Blocking By HIV-1 Neutralizing Antibodies Of Membrane-active Peptides

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Board B202

The conserved sequence preTM, preceding the transmembrane anchor of Human Immunodeficiency virus type-1 gp41 glycoprotein subunit, is accessible to the broadly neutralizing 4E10 and 2F5 antibodies and, therefore, constitutes a potential target for vaccine design. Our earlier analyses indicated that the two major envelope raft-lipids, cholesterol and sphingomyelin, may regulate the surface dynamics and membrane-restructuring capacity of a peptide representing this region. Recently reported structural data are compatible with preTM insertion into the viral external membrane monolayer in the gp41 pre-fusion state. Our studies demonstrate that the broadly neutralizing 4E10 and 2F5 antibodies are able to specifically block the membrane-restructuring activity of peptide mimics inserted into membranes. Peptide recognition and restructuring blocking occurs modulated by the lipids existing in the viral envelope as well as by the amino-terminal ampipathic-at-interface preTM sequence (AIS). Our experimental data support that membrane-bound epitope recognition by 4E10 and 2F5 might interfere with the formation of fusion-competent complexes, even at the low spike densities existing in the HIV-1 membrane.

2088-Pos Metal Effects on the Membrane Interactions of Amyloid Beta Peptides

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Board B203

Aβ(1–42) peptide, found as aggregated species in Alzheimer's disease brain, is linked to the onset of dementia. We report the results of ^{31}P and ^{2}H solid-state NMR studies of model membranes with Aβ peptides and the effect of metal ions (Cu^{2+} and Zn^{2+}), which are found concentrated in amyloid plaques. The effects on the lipid bilayer and the peptide structure are different for membrane incorporated or associated peptides. Copper ions alone destabilise the lipid bilayer and induce formation of smaller vesicles, but not when Aβ (1–42) is associated with the bilayer membrane. Aβ (25–35), a fragment from the C-terminal end of Aβ (1–42), which lacks the metal coordinating sites found in the full length peptide, is neurotoxic to cortical cortex cell cultures. Addition of metal ions has little effect on membrane bilayers with Aβ (25–35) peptides. ^{31}P magic angle spinning NMR data show that Aβ (1–42) and Aβ (1–42)-Cu^{2+} complexes interact at the surface of anionic phospholipid membranes. Incorporated peptides, however, appear to disrupt the membrane more severely than associated peptides. Solid-state ^{13}C NMR was used to compare structural changes of Aβ (1–42) to those of Aβ (25–35) in model membrane systems of anionic phospholipids and cholesterol. The Aβ peptides appeared to have an increase in β-sheet structure at the C-terminus when added to phospholipid liposomes. The inclusion of Cu^{2+} also influenced the observed chemical shift of residues from the C-terminal half, providing structural clues for the lipid-associated Aβ/metal complex. The results point to the complex pathway(s) for toxicity of the full-length peptide and indicate structural differences between the amyloid fibril and the metal-complexed forms.

2089-Pos Alpha Defensins Structure and Lipid Interactions

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Board B204

Interaction of proteins with membranes plays a central role in several important biological processes such as communication between the cells, membrane trafficking, signal transduction and viral infection. Structural characterization of protein-membrane interactions is key to understanding such diverse phenomena. Certain components of the innate immune system are known to target microbial membranes. Defensins are a family of antimicrobial peptides that exert potent activities against a broad spectrum of targets, including gram-positive and gram-negative bacteria, fungi, spirochetes, mycobacterium, protozoans, and enveloped viruses. The details of mode of action of these peptides are unclear. It is proposed that they disrupt the membranes of target pathogen. The

initial interaction of the defensins with the microbial membrane is thought to occur via non-specific interactions based on surfaces charges and amphipathicity. However it is not understood how these peptides are able to selectively target the membranes of the pathogens and ultimately kill them. To date structures of alpha defensins have been reported for human neutrophils, rabbit kidney and mouse intestinal epithelium. We report the first NMR structure of an alpha defensin from myeloid cells of rhesus macaque. It adopts the canonical alpha defensin fold with the core structure made of three-stranded beta sheets stabilized by three intra-molecular disulfide bridges. However there are differences in the defensin electrostatic surface that could manifest a distinct flexibility and mode of action. Results from the studies of interaction of defensins with bicelles (bilayer containing micelles) will be presented and the possible role of each of the residues involved in its interaction with membranes will be discussed.

Interfacial Protein-Lipid Interactions, Surfactants

2090-Pos Palmitoylation Promotes Stabilization of Interdigitated-Like Phospholipid Phases by the N-terminal Segment of Pulmonary Surfactant Protein SP-C

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Board B205

SP-C is the smallest pulmonary surfactant protein and is required for the formation and stability of surface-active films at the air-liquid interface in the lung. The protein consists of a transmembrane hydrophobic α-helix and a cationic N-terminal segment that contains palmitoylated cysteines. In the present work, the lipid/protein interactions of palmitoylated and non-palmitoylated versions of synthetic peptides designed to mimic the N-terminal segment of SP-C have been analyzed and compared by electron spin resonance (ESR) spectroscopy.

Both palmitoylated and non-palmitoylated peptides decrease the mobility of phosphatidylcholine (5- PCSL) and phosphatidylglycerol (5-PGSL) spin probes in dipalmitoylphosphatidylcholine (DPPC) or dipalmitoylphosphatidylglycerol (DPPG) bilayers. Both peptides have a greater effect at temperatures below than above the main gel-to-liquid-crystalline phase transition, although the palmitoylated peptide induced greater lipid immobilisation than does the non-palmitoylated form.

The effect of SP-C N-terminal peptides on the chain flexibility gradient of DPPC and DPPG bilayers at temperatures below the main gel-to-liquid-crystalline phase transition, registered by spin probes at different positions in the *sn*-2 acyl chains, is consistent with the existence of a peptide-promoted interdigitated phase. The palmitoylated peptide, but not the non-palmitoylated version, is able to stably segregate interdigitated and non-interdigitated populations of phospholipids in DPPC bilayers at 37°C. This feature suggests that the palmitoylated N-terminal segment takes part in and stabilizes ordered domains such as those containing interdigitated lipids. We propose that palmitoylation may be important to promote and facilitate association of SP-C and SP-C-containing membranes with ordered lipid structures like those potentially existing in highly compressed states of the interfacial surfactant film.

2091-Pos Polyelectrolyte Mediated Competitive Adsorption Between Lung Surfactant and Serum Proteins

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Board B206

The adsorption of lung surfactant (LS) to an air-liquid interface is strongly inhibited by the competitive adsorption of surface active serum proteins and is likely the explanation of LS inactivation in Acute Respiratory Distress Syndrome (ARDS). Utilizing a cycling Langmuir trough as an in vitro model to approximate LS/serum protein behavior at alveolar interface, we show that LS adsorption to the interface is restored by the addition of cationic polyelectrolytes such as chitosan, suggesting a promising therapy for ARDS. Fluorescence microscopy images show distinct changes in interfacial morphology between serum protein and LS-covered regions offering a visual confirmation of LS adsorption to the interface. Freeze fracture transmission electron microscopy (FFTEM) images of model LS vesicles show untreated vesicles are ~50 nm in diameter and uniformly distributed throughout the solution while treatment with a low concentration of chitosan (0.05 mg/mL) causes aggregation of the vesicles.

This competitive adsorption of serum proteins to the alveolar air-liquid interface can be modeled as an energy barrier to LS adsorption and can be analyzed using a variation of the classical Smolukowski description of colloidal stability. The serum proteins generate both a steric and electrostatic barrier to LS adsorption due to net repulsion between the negatively charged surfactant aggregates and serum proteins. Chitosan binds to and neutralizes the negatively charged LS and serum protein surfaces, lowering the electrostatic barrier to LS adsorption.

2092-Pos Mechanisms of Surfactant Membrane Inactivation by C-Reactive Protein

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