polarity of the amino acid chain is influential in the induced membrane permeability of these peptides. In addition, these *in-vitro* studies imply the existence of a factor or a combination of factors aside from strictly membrane sterol content that determines the specificity of these antimicrobial peptides for fungal cells *in-vivo*. Importantly, the results of this study imply that the relative spatial position of the amino acids in histatin 5 is critical for the antifungal activity.

1221-Pos Board B113

Visualizing pHLIP Insertion in Plasmamembrane and Endosomal Membrane

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The pH-(Low) Insertion Peptide (pHLIP) has potential as a tumor-targeting drug carrier. At neutral pH, pHLIP has affinity for the surface of a lipid bilayer, whereas under slightly acidic conditions (e.g. pH ~ 6) pHLIP inserts into the membrane, forming a transmembrane helix. Since many solid tumors are more acidic than healthy tissues, pHLIP may be used to translocate chemotherapeutic agents selectively into cancer cells. Knowledge of the exact location of pHLIP insertion in cells can guide the rational design of delivery constructs. We envision two scenarios for pHLIP insertion in cells: First, pHLIP may directly insert into the plasmamembrane; alternatively, cells may internalize pHLIP molecules via endocytosis, and subsequently pHLIP may insert into the endosomal membrane. In this study, several fluorescently self-quenched pHLIP constructs were synthesized to visualize to what extend these two scenarios are occurring in cells at pH 7.3 and 6.2. In these self-quenched pHLIP constructs, a rhodamine dye (TAMRA or Alexa Fluor 568) is attached to a C-terminal Lys residue, with the quencher QSY-9 conjugated to an adjacent Cys via a cleavable disulfide linker (or a stable thio-ether bond). Upon insertion, pHLIP would translocate its C-terminus into the cell cytoplasm, where cleavage of the disulfide linkage and release of the quencher QSY9 can take place. In turn, the pHLIP construct would become more fluorescent.

1222-Pos Board B114

Phospholipase A2 Activity and Substrate Specificity of Bothrops Asper and Crotalus Durissus Cumanensis Snake Venom Collected from the Guajira Region of Colombia

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Phospholipase A2 (PLA2) is a versatile enzyme present in all organisms that hydrolyzes the sn-2 ester bond of phospholipids resulting in the formation of lysophospholipids and free fatty acids. PLA2 found in snake venom is responsible for multiple systemic effects such as neurotoxicity, myotoxicity, and hemolysis. We measure the specificities of PLA2 in whole venom samples towards different phospholipids. This study provides a first look at the biophysical characteristics of venoms isolated from Colombia. Crude venom samples were taken from Bothrops asper and Crotalus durissus cumanensis in the Guajira region of Colombia. The venom was lyophilized until used. Vesicles composed of the non-hydrolyzable 1,2-di-O-octadecyl-sn-glycero-3-phosphocholine (DEthPC) carrying calcein at a self-quenching concentration (50mM) were used as reporter vesicles, and target vesicles (substrate) made from DMPG, DMPC, POPE or sphingomyelin (SM) were used for measuring snake venom specificity. All measurements were performed in a pc1 Fluorometer (ISS, Urbana, IL) between 20°C and 60°C with and without calcium (30 μM). In the presence of calcium both venoms have similar catalytic action with C. durissus being slightly higher than B. asper. In the absence of calcium B. asper showed the highest activity indicating differences in the intrinsic calcium levels in both venoms. Overall B. asper showed the lowest lagtime for all assays. No calcein release was observed for SM. DMPG was the only one that presented activity at all temperatures suggesting that both venoms have strong affinity towards negatively charged membranes. Both snake venoms showed activity in DMPC only in the presence of extrinsic calcium and only at 24°C, where liquid-crystalline/gel phase coexistence is present, suggesting that the presence of membrane defects plays an important role in the initiation of catalysis.

1223-Pos Board B115

Identification and Characterization of the Glycan Binding Site of Vibrio Cholerae Cytolysin

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Vibrio cholerae cytolysin (VCC) is a pore-forming toxin secreted by the Vibrio cholera O1 El Tor strain. VCC is hypothesized to perform an important function by lysing immune cells and thereby protecting the bacteria from host defenses. VCC consists of multiple domains including two possible carbohydrate-binding lectin domains (β-prism and β-trefoil). Recently it has been found that the β-prism domain binds to both mono and oligosaccharides, but interacts with highest affinity to glycans found on eukaryotic cell membranes. Crystal structures exist for VCC in water-soluble and membrane assembled forms, but the precise molecular mechanism by which VCC recognizes carbohydrates is still not well understood. Here we show that the Asp 617 residue in the β-prism domain plays an important role in both monosaccharide and cell surface glycan binding. Site directed mutagenesis of the Asp 617 to alanine resulted in a ~80-fold decrease in monosaccharide binding activity and a ~280-fold decrease in cell-surface glycan binding activity. Furthermore, the Asp 617 mutant displayed a decline in hemolytic activity compared to wild type VCC when incubated with rabbit red blood cells. We also show that both monosaccharides and purified cell surface glycans can compete with VCC and cause a dose dependent lag in the half-life of rabbit blood hemolysis. These results indicate that the VCC interacts with glycans on target cell membranes and Asp 617 is involved in this binding process. Having more insight into the molecular mechanism and residues involved in sugar recognition may enable the development of sugar-based therapies against VCC and other pore-forming toxins.

1224-Pos Board B116

Membrane Association of Diphtheria Toxin T-Domain Characterized by Coarse-Grained and Atomistic Molecular Dynamics Simulations and Experiments

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Acidification of the endosome interior induces a series of conformational changes in diphtheria toxin T-domain ultimately resulting in its membrane insertion and translocation of its N-terminus with the attached catalytic domain into the cytoplasm. Previously, we have used a combination of various computational and experimental approaches to characterize the first step of this complex pathway, namely the formation of the membrane-competent state. Here we extend this study to investigate how this refolded state interacts with surfaces of lipid bilayers in preparation for transmembrane insertion. We present the results of multiple microsecond coarse-grained molecular dynamics simulations of the T-domain performed in the presence of the lipid bilayers of various compositions. The MARTINI force-field was used in our simulations. Binding of the protein to the membrane was improved as the anionic content of the mixed POPG:POPC bilayer increases. We have identified two preferred surfaces on the T-domain structure that are likely to participate in the early stages of membrane binding. The first surface is comprised of residues in the loops of helices TH2-3, TH8-9 and helix TH5. The second preferred surface of contact contains residues of the N-terminal (helices TH1-4) and those located in the loop between TH7-8. The experimental results corroborate these observations. Detailed atomistic MD simulations are presented to estimate free energy of the protein-lipid association to further validate our models. Supported by NIH GM069783

1225-Pos Board B117

Membrane Interactions of the Alzheimer's Disease Aβ42 Peptide and a Soluble Aβ42 Fusion Protein

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The 42-residue amyloid-beta (Aβ42) peptide is a primary constituent of the neuritic plaques diagnostic of Alzheimer's disease, but its exact role in the disease remains unclear. Growing evidence supports that small, soluble oligomers of Aβ42 are responsible for neurodegeneration, perhaps through disruptive membrane interactions. However, the notorious propensity of the peptide to aggregate causes such interactions to be elusive to most studies. We have biosynthetically produced a highly soluble fusion construct, in high-yields (>100 mg/L of culture), from which native human Aβ42 can be cleaved and purified. Perturbations of phospholipid bilayers by the fusion construct are compared to Aβ42 peptide alone using differential scanning calorimetry and by 31P and 2H solid-state NMR measurements. Preliminary structural characterization is performed using circular dichroism and solution NMR on uniformly 15N, 13C-enriched protein.

1226-Pos Board B118

Structure- Function Relationship Studies of Piscidin 1 Bound to Model Tumor Cell Membranes

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Piscidins, which are antimicrobial peptides (AMPs) produced in the mast cells of hybrid striped bass, are found in immune-related organs as well as non-immune tissues, including neuronal cell bodies. While they are unstructured in water, they form amphipathic, cationic and alpha-helical structure in the presence of lipid bilayers at physiological pH. The biologically most active piscidin, piscidin 1 (P1), has broad spectrum antimicrobial activity against various bacteria, fungi, and viruses. Recently, it was found to be selective against HT1080 and HeLa cells as compared to healthy cells. This research investigates the factors that imparts P1 this selectivity against cancer cells. We have been particularly interested in special chemical properties of cancer cells, such as their low extracellular pH, presence of phosphatidylserine (PS) in the outer membrane leaflet, and elevated cholesterol level. We hypothesize that the positive charges on the histidine residues of P1 at the low extracellular pH of cancer cells allow P1 to align parallel to the surface and disrupt the membrane via the carpet-like mechanism of action.
Lipid membranes have been made using 2:2:1 phosphatidylcholine/phoshatidylserine/cholesterol to mimic tumor cells. Circular dichroism experiments were performed using large unilamellar vesicles to investigate the effect of pH, cholesterol, and lipid charge on the peptide's helical content and therefore affinity for lipid bilayers. Using 2H and 15N solid-state NMR, we have investigated the fluidity of the lipid bilayers and bilayer orientation of P1, respectively. These results are contrasted with those obtained on healthy mammalian cell mimics. The principles learned from these studies could help design peptides with enhanced activity against cancer cells.

1227-Pos Board B119
Effect of Membrane Cholesterol on the Structure of Alzheimer's Amyloid β Peptide in Lipid Bilayers
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A hallmark feature of Alzheimer's disease is formation of extracellular plaques of the amyloid β (Aβ) peptide. There is evidence that the Aβ peptide inserts into the membranes of surrounding neuronal cells, causing membrane permeabilization or rupture. The level of cholesterol in membranes has been shown to affect the extent and toxicity of Aβ plaques, as well as the mode of membrane insertion of Aβ and its structure. Here we analyze the effect of cholesterol on the secondary structure of the 40-residue Aβ peptide in artificial lipid bilayers. Multilayers composed of 0.3 mol fraction of 1-palmitoyl-2-oleoyl-phosphatidylglycerol, (0.7 - X_{chol}) mol fraction of 1-palmitoyl-2-oleoyl-phosphatidylcholine, and X_{chol} mol fraction of cholesterol were deposited on a germanium plate, with X_{chol} = 0, 0.05, 0.1, 0.2 and 0.4, containing Aβ at a 1:15 peptide/lipid molar ratio. Fourier transform infrared spectra were collected on lipid-peptide samples under three conditions, i) dry multilayers, ii) humidified with D_2O vapors, and iii) in the presence of a bulk D_2O-based buffer. In all cases, the amide I bands of the peptide indicated comparable fractions of α-helical and β-sheet structures. Increase of X_{chol} from 0 to 0.2 resulted in a significant decrease in the β-sheet structure and a slight increase in α-helix content. Further increase in X_{chol} to 0.4 reversed the β-sheet content and increased the α-helical structure; the latter effect was much stronger in hydrated samples. Considering that cholesterol at $X_{chol} > 0.2$ causes a fluid-disordered to fluid-ordered transition in membranes, the observed structural effects may reflect either conformational changes in membrane-bound Aβ peptide or a squeezing-out effect. These possibilities, as well as the effect of cholesterol on membrane permeabilization will elucidate the role of cholesterol in Aβ structure and function.

1228-Pos Board B120
Antihypertensive Antagonists of L-Type Calcium Channel Exert Enhancement of Alzheimer's Aβ Peptides Effect on Cells
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The Alzheimer's Aβ peptides are shown to increase the cytosolic calcium concentrations of cells in culture. Such an alteration may cause a variety of secondary effects, leading to cell degeneration and reduced cell culture growth. Cytosolic calcium concentration can be increased by the formation of cation-selective ion channels by the Aβ peptides. This property is well documented and has been extensively studied both in artificial membrane systems as well as in living cells. Here we show that L-type calcium channel antagonists, commonly used in the treatment of hypertension and ischemic heart disease, intensify the capacity of Aβ peptides to increase cytosolic calcium concentrations and consequently enhance the cytotoxicity of the Aβ peptides. Calcium imaging experiments were conducted with the fluorescent indicator Fura-2 to measure at different periods of times the levels of cytosolic calcium during exposure to Aβ peptides and to the L-type calcium channel antagonists. Two different 1,4-dihydropyridines (nifedipine, and nitrendipine), and Diltiazem, a structurally unrelated Ca^{2+}-channel antagonist belonging to the phenylalkylamine class were used and found to exert significant excitatory effects on the toxicity of Aβ. The reduced toxicity observed in solutions of combinations of Aβ40 and Aβ42 is also reversed by L-type calcium channel antagonists. The toxicity of Aβ peptides was weighed by quantifying the growth of cultures of PC12 cells using the colorimetric assay XTT and by measuring the release of LDH to the media. The enhancement of the Aβ peptides toxicity by L-type calcium channel antagonists can be constrained by specific Aβ ion channel blockers suggesting the mediation of the ion channel property of Aβ peptides. The relevance of our finding derives from the widespread clinical use of L-type calcium channel antagonists in the treatment of hypertension and angina pectoris.

1229-Pos Board B121
Chronic Cytosolic Calcium Changes Induced by Alzheimer's Peptides Aβ Allows for Sorting of Aβ-Resistant Cell Subpopulations
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The Alzheimer's Aβ peptides interaction with the plasma membrane has been shown to result in a chronic increase in the cytosolic calcium concentrations of cells in culture. Such alteration may cause a variety of secondary effects which may lead to cell degeneration and reduced cell culture growth. We have proposed that the alteration in the cytosolic calcium concentrations induced by Aβ is initiated by early formation of Aβ ion channels in the plasma membrane. To study the Aβ-induced cytosolic calcium change dynamics we monitored the level of cytosolic calcium from hundreds of individual cells in culture exposed to Aβ for prolonged period of time. Because of the continuous cytotoxic effect of Aβ on cells, the number of viable cells in the cultures varied with time of exposure. Histograms based on the individual intracellular calcium levels showed that while most cells in the culture remain unaffected, a defined subpopulation of cells show increasingly higher than normal cytosolic calcium levels. After days of continuous exposure to Aβ cells more sensitive to Aβ died, and consequently the averaged cytosolic calcium for remaining cells in the whole culture approached control values. Aβ ion channel blockers prevented the induced cytosolic calcium changes and preserved cell viability, confirming the participation of Aβ ion channels. Confocal microscopic analysis using fluorescent annexin V on cells loaded with fura 2 showed that those cells that remained after prolonged exposure to Aβ did not display the proposed extracellular Aβ receptor phosphatidyl serine. Further addition of Aβ to this sorted cell subpopulation did not induce cytosolic calcium changes and cells continued to grow and divide. Application of this procedure allows for sorting out Aβ resistant subpopulation of cells.

1230-Pos Board B122
Membrane Destabilization by Alzheimer's Amyloid β Peptide
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Alzheimer's Amyloid-β peptide (Aβ) is a 39-42 amino acid fragment of the amyloid precursor protein. Accumulation of the neurotoxic Aβ in the brains of Alzheimer's patients forms detrimental plaques. Another mechanism of neuronal damage is membrane destabilization by insertion of the peptide into brain cell membranes and disruption of ionic homeostasis. The molecular mechanism of membrane permeabilization by Aβ remains largely uncharacterized. Experiments with live cells and lipid membranes have identified ion channels formed by Aβ. The channel formed by an 11 amino acid residue fragment, Aβ25-35, has been modeled by Molecular Dynamics simulations as an 8-stranded β-barrel, but this structure has not been supported by direct experimental data. The objective of this work was to study the mechanism of membrane permeabilization by Aβ using biophysical techniques. Unilamellar lipid vesicles were prepared in a buffer containing 30 mM $CaCl_2$, followed by removal of calcium from the external medium via a desalting column and addition of 0.1 mM Quin-2, a fluorescent calcium indicator. Before peptide addition, the vesicle sample showed little fluorescence because of spatial sequestration of calcium and Quin-2. Addition of submicromolar concentration of the Aβ25-35 peptide resulted in gradual increase in Quin-2 fluorescence, indicating formation of Ca^{2+}-permeable pores in vesicle membranes. Addition of higher concentrations of the peptide resulted in instantaneous increase in fluorescence, comparable to the effect of detergents like Triton X-100, indicating rupture of vesicle membranes. Thus, the data suggest membrane pore formation at low peptide concentrations and membrane disruption at higher concentrations. These data are consistent with a mechanism of detergent-like action of the Aβ peptide;