

treated with pHLIP-K(rho)C(aph) also showed signs of cytoskeletal immobilization, consistent with the knowledge that phalloidin binds to F-actin and stabilizes the filament against depolymerization. However, the antiproliferative effect was not observed with pHLIP-C(aph). The insertion behavior of both constructs were studied in POPC liposomes using Trp fluorescence: pHLIP-K(rho)C(aph) and pHLIP-C(aph) insert with the same apparent pK of 6.1-6.2, similar to that of pHLIP (without any cargo). However, kinetic experiments suggest that pHLIP-C(aph) inserts much slower than pHLIP-K(rho)C(aph), possibly accounting for its lack of antiproliferative effects in cell assays. In short, our results obtained with pHLIP-K(rho)C(aph) lay the foundation for the development of a new class of anti-tumor agents that would selectively enter and destroy cancer cells while not affecting normal cells. Such pHLIP-mediated delivery of otherwise cell-impermeable agents may enhance the efficacy of treatment, as well as significantly reducing the side effect.

1454-Pos

Membrane Superficial Charge Modification Affects Mitochondrial Permeabilization by Derivatives of the Polycationic Peptide Btm-P1

Victor V. Lemeshko.

Universidad Nacional de Colombia, Medellin, Colombia.

Polycationic peptides demonstrate antimicrobial and anticancer properties. Earlier we designed, on the basis of the protoxin Cry11Bb, a 26-aa polycationic peptide BTM-P1, which demonstrated ionophoric and antimicrobial activities. It could be modified in the future to enhance anticancer action. In this work we found that the reverse peptide, BTM-RP1, has one order of magnitude lower capacity than BTM-P1 to permeabilize rat liver mitochondria. The activity of BTM-RP1 was increased by its modifications with tryptophane attached to its N-terminal (BTM-WRP1) or C-terminal (BTM-RP1W). The similar modifications of BTM-P1 peptide did not increase, or even decreased (BTM-P1W) the peptide activity. All these peptides, designed by us, were synthesized by Gen Script Company (USA) (>90% purity). When 10 μ M cationic fluorescent probe safranin O, but not endogenous NAD(P)H fluorescence, was used as indicator of mitochondrial energization, the inner membrane potential markedly recovered after a decrease caused by each of 3 serial additions of 1 μ M BTM-RP1. We also found that safranin O significantly decreased the rate of mitochondrial swelling induced by BTM-RP1 or by its tryptophane derivatives. These data suggest that the superficial electrical charge of biomembranes, in addition to the trans-membrane potential, significantly affects the membrane permeabilization and selectivity in cell killing by polycationic peptides. We conclude that agents modifying superficial electrical charge of biological membranes could be used to influence the peptide cytotoxicity and selectivity. (Colciencias grant #111840820380 and the National University of Colombia grant #20101007930).

1455-Pos

Structure-Function Investigation of A Novel Dendrimeric and Lipidated Antimicrobial Peptide

Mariano A. Scorciapino^{1,2}, Andrea Ardu¹, Jochen Buerck³, Carla Cannas¹, Mariano Casu¹, Andrea Giuliani⁴, Giovanna Pirri⁴, Andrea C. Rinaldi⁵, Anne S. Ulrich^{3,6}.

¹Dep. of Chemical Sciences - University of Cagliari, Monserrato, Italy,

²CNR/INFN SLACS (Sardinian Laboratory for Computational materials

Science), Monserrato (CA), Italy, ³Institute for Biological Interfaces –

Karlsruhe Institute of Technology, Karlsruhe, Germany, ⁴Research and

Development Unit - Spider Biotech S.r.l., Colletterto Giacosa (TO), Italy,

⁵Dep. of Biomedical Sciences and Technologies – University of Cagliari,

Monserrato, Italy, ⁶Institute of Organic Chemistry – Karlsruhe Institute of

Technology, Karlsruhe, Germany.

Antimicrobial peptides are usually polycationic with high affinity for bacterial membranes. Upon approaching the lipid bilayer, they tend to fold into an amphiphilic structure and bind to the membrane. In order to understand the detailed mode of action of such antimicrobial peptide inside the membrane, and to understand which properties of the peptide and/or lipids are important for selectivity, it is fundamental to examine the peptide structure and its association with lipid bilayers. In this work, first experiments were carried out to assess the thermodynamic and kinetic parameters of a promising novel antibiotic dendrimeric peptide interacting with lipid bilayers. With the goal of enhancing the antimicrobial activity of a particular sequence with the polyvalent framework of a dendrimer, two identical deca-peptides were assembled via a lysine-linker, carrying at the same time an octanoyl-lipid anchor. A highly active compound was obtained, but its structure and mode-of-action remain unexplored. The dendrimer and the linear deca-peptide were studied in parallel, to highlight the relevant properties and differences between dendrimeric structure and simple amino-acid sequence. Experiments were performed with different zwitterionic/negatively-charged lipids mixtures in order to assess the role of lipid

surface charge. In particular, monolayer intercalation was investigated with microtensiometry. Fluorescence spectroscopy was applied to study thermodynamics and kinetics of the binding process. Circular dichroism, multidimensional liquid-state NMR, and solid-state NMR of oriented samples allowed to obtain first information on the 3D structure of the peptide both in the free and membrane-bound state. Transmission electron microscopy images showed the formation of highly intriguing aggregates with, to our knowledge, a previously unreported kind of branched three-dimensional morphology.

1456-Pos

Effects of Bacillus Lipopeptides on Lipid Membrane Structure and Dynamics

Mozhgan Nazari, Mustafa Kurdi, Hiren Patel, Heiko Heerklotz.

UofT, Toronto, ON, Canada.

Bacillus subtilis strain QST713 produces a unique combination of lipopeptides from the surfactin (SF), fengycin (FE) and iturin (IT) families. The fungicidal activity of this peptide mix is used by a biopesticide for crop protection and believed to be based on the permeabilization of target membranes by the peptides. To shed light on the activity, selectivity and synergisms of the peptides, we have studied their membrane binding and the subsequent effects on the structure and dynamics of the membrane. We measured the time-resolved fluorescence and fluorescence anisotropy of intrinsic tyrosine and hydrophobic dyes (e.g., DPH), time-resolved dipolar relaxation of Laurdan, interaction thermodynamics by ITC, and size and zeta potential of vesicles by DLS. The results are compared with the effects of synthetic surfactants and provide valuable information about the molecular background of the very unusual leakage and lysis behaviour of the lipopeptides.

1457-Pos

Rapid Binding and Transmembrane Diffusion of Pepducins in Phospholipid Bilayers

Kellen B. Fontanini¹, Jay Janz², Richard Looby², James A. Hamilton¹.

¹Boston University School of Medicine, Boston, MA, USA, ²Ascent

Therapeutics, Cambridge, MA, USA.

Pepducins are GPCR-targeted lipopeptides designed to anchor in the cell membrane lipid bilayer and modulate the receptor/G protein signal transduction pathway via an allosteric mechanism. It is thus presumed that pepducins cross the plasma membrane by some mechanism, possibly passive diffusion. The goal of this research is to study the biophysical transport properties of pepducins in model membranes. We utilized fluorescent probes that measure the binding (fluorescein phosphatidylethanolamine - FPE) and diffusion (pH probe - pyranine) of charged ligands across the lipid bilayer of large unilamellar vesicles (LUV) comprised of egg-phosphatidylcholine. We tested pepducins with a palmitate or myristate linked to the N-terminal of the peptide sequence (KKSRALF). The GPCR target for these pepducins is the protease activator receptor 1 (PAR1). Addition of pepducins (0.16-5.0 mol%) to LUVs labeled in the outer leaflet with FPE or containing entrapped pyranine produced a fast (<2s) and dose-dependent increase in the fluorescence of both probes. The fast response of FPE, resulting from the insertion of positive charges (lysine and arginines residues) into the outer leaflet, demonstrated rapid partitioning into the membrane. The increase in pyranine fluorescence indicated alkalization of the intravesicular compartment, probably due to protonation of the lysine residues. In order for this to be detected, the pepducin must cross the membrane. The peptide alone (not acylated) did not cause any change in the fluorescence of either FPE or pyranine. These data are consistent with favorable partitioning of pepducins into the membrane and rapid passive diffusion to the sites of their action at the cytosolic leaflet of the plasma membrane.

1458-Pos

Nanostructure Determines Antifungal Activity of De Novo Designed pH Dependent Histidine Containing Ultra-Short Lipopeptides

Christopher J. Arnusch¹, H. Bauke Albada², Rob M.J. Liskamp²,

Yecheil Shai¹.

¹Weizmann Institute of Science, Rehovot, Israel, ²University of Utrecht,

Utrecht, Netherlands.

Antimicrobial peptides are an essential part of the innate immune system of most living things and the understanding of the biophysical properties and the different mechanisms of action are crucial for the de-novo development of simple and effective analogs. More specifically, antimicrobial lipopeptides have been gaining increased attention because of the pressure for new antimicrobial agents against resistant pathogens. The addition of a lipophilic fatty acid has proven to be an effective method to increase the association of a peptide with the membrane, thus increasing the biological activity of certain peptide sequences. Previously, we reported that linear ultrashort cationic lipopeptides even as short as 4 amino acids have potent antimicrobial and antifungal properties. We described the minimum peptide length, and fatty acid length

necessary for activity. Also, innate-immunity-like peptides were described that contained multiple histidine residues. Although these peptides consisted of 12 to 15 amino acids, these were less toxic to the host, and were lytic to numerous pathogens and cancer cells at slightly acidic environments. Here we report the design of an ultrashort histidine containing peptide whose antifungal activity could be significantly increased in a covalent trimeric form. Low micromolar activity was observed for *Aspergillus fumigatus* and *Cryptococcus neoformans* but not *Candida albicans*. Using transmission electron microscopy, we observed that this trimeric ultrashort histidine containing peptide formed distinct and differing nanostructures at pH 5 and 7, which could explain the activity differences. Since various organs or areas of the human body have a slightly acidic pH environment such as tumors, gastric lumen and lung-lining fluids in cystic fibrosis and asthma, understanding the importance of nanostructure-activity relationships of these pH dependent ultrashort peptides could lead to improvements in the delivery and administration of the peptides.

1459-Pos

Spectroscopic Studies of the Interaction of Native and TOAC-Labeled Peptide Hormones with Model Membranes: Angiotensin II

Nélida Marín¹, Erick Poletti², Clóvis Ryuichi Nakaie², Shirley Schreier¹.

¹University of São Paulo, São Paulo, Brazil, ²Federal University of São Paulo, São Paulo, Brazil.

The peptide hormone angiotensin II (DRVYIHPF, AII) plays an important role in the renin-angiotensin-aldosterone system. AII derivatives containing the paramagnetic amino acid 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) replacing residues 1 (TOAC¹-AII) and 3 (TOAC³-AII) were synthesized and their conformational properties, as well as those of the native peptide, were examined in the presence of model membranes - micelles of 1-palmitoyl-2-hydroxy-phosphatidylcholine (LPC) and 1:1 mol:mol LPC: 1-palmitoyl-2-hydroxy-phosphatidylglycerol (LPG) and large unilamellar vesicles (LUV) of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and 1:1 mol:mol POPC:1-palmitoyl-2-oleoyl phosphatidylglycerol (POPG). Experiments were conducted at pH 4.0, 7.0, and 10.0 to evaluate the effect of peptide charge on peptide-membrane interaction. Fluorescence spectra showed that the peptides bound to negatively charged micelles to a much larger extent than to zwitterionic micelles. CD spectra of AII and TOAC¹-AII showed acquisition of secondary structure upon binding to LPC:LPG micelles at pH 4.0; the changes occurred to a lesser extent at the higher pHs. In the case of TOAC³-AII, binding had a small effect on peptide conformation since the TOAC ring imposes a more constrained conformation already in solution. In the case of bilayers, the peptides interacted only with POPC:POPG LUV, especially at pH 4.0. Line broadening of EPR spectra of the labeled peptides also provided evidence for interaction of the labeled peptides with negatively charged micelles and bilayers. In several cases, two-component spectra were obtained, one due to the peptides in solution and the other to the bilayer-bound population, allowing for the calculation of partition coefficients. The rigidity of the TOAC-labeled analogue is very likely responsible for its inability to acquire the correct receptor-bound conformation, leading to loss of biological activity. These data show that spectroscopic studies can provide relevant information regarding peptide-membrane interaction.

1460-Pos

Unraveling the Molecular Basis of the Selectivity of the HIV-1 Fusion Inhibitor Sifuvirtide Towards Phosphatidylcholine-Rich Rigid Membranes

Henri G. Franquelim, A. Salomé Veiga, Nuno C. Santos, Miguel A.R.B. Castanho.

Instituto de Medicina Molecular, Lisbon, Portugal.

Sifuvirtide, a 36 amino acid anionic peptide, is a novel HIV-1 fusion inhibitor with improved antiretroviral activity. The selective ability of this peptide to interact with lipid bilayers has already been identified (Franquelim et al, *J Am Chem Soc* 2008, **130**, 6215-23) and the aim of this work is to evaluate the interaction of sifuvirtide with several biomembrane model systems, retrieving details of its mode of action at the membrane level. Since this peptide has aromatic residues, fluorescence spectroscopy techniques were mostly used. The interaction was assessed by partition and fluorescence quenching experiments. Results showed no significant interaction with large unilamellar vesicles composed by sphingomyelin and ceramide. In contrast, sifuvirtide presented selectivity towards vesicles composed by phosphatidylcholines (PC) in the gel phase, in opposition to fluid phase PC vesicles. The interaction of this peptide with gel phase PC (zwitterionic) membranes ($K_p = 1.2 \times 10^2$) is dependent on the ionic strength, which indicates the mediation of electrostatic interactions at an interfacial level. The effects of sifuvirtide on the lipid membranes' structural properties were further evaluated using dipole potential membrane probes, zeta-potential, dynamic light scattering and atomic force microscopy measurements. The results show that sifuvirtide does not cause a noticeable effect on

lipid bilayer structure. Altogether, one can conclude that sifuvirtide presents a specific affinity towards rigid PC membranes (in agreement with the adsorption model previously proposed), and the interaction is mediated by electrostatic factors, not affecting the membrane architecture. Because saturated PC lipids are found in high concentration in lipid rafts, but mainly in the viral envelope, the efficacy of sifuvirtide may be related to its screening ability towards those regions, allowing an increased concentration of this peptide drug near the fusion site.

1461-Pos

Fusion Peptide of Gp41 Self Associates in the Model Membrane and then Interacts with its Trans-Membrane Domain

Hirak Chakraborty¹, David G. Klapper², Barry R. Lentz¹.

¹Biochemistry and Biophysics Department & Molecular and Cellular Biophysics Program, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, ²Microbiology & Immunology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

We have examined the peptide structure and membrane packing when the fusion peptide (FP) of gp41 was added either in the membrane alone or in the membrane containing gp41 the trans-membrane domain (TMD). Circular Dichroism (CD) measurements showed that FP is mostly in a beta sheet conformation independent of FP concentration. TMD has ~ 30% helical, ~25% beta sheet and the complex of TMD and FP has less alpha helix than the TMD itself, which indicates the TMD loosens its alpha helical structure upon interacting with FP. DPH and TMA-DPH fluorescence anisotropy revealed that FP alone increased the interior packing of the membrane, but FP in the presence of TMD increased the interior packing at lower concentrations of FP and then decreased it at higher concentrations. FP alone increased membrane surface packing at lower concentrations but increased it at higher concentrations. In the presence of TMD, FP addition decreased surface packing cooperatively. From the lifetime of TMA-DPH in H₂O and D₂O we documented water penetration into the membrane. FP alone increases water penetration slightly whereas FP in presence of TMD significantly increased water penetration into the interface region of the membrane in a cooperative fashion. The fluorescence lifetime of C6NBDPC revealed that FP alone fills more space than the FP in the presence of TMD. In summary, our results clearly demonstrate that gp41 FP forms a complex with the gp41 TMD to alter both TMD structure and membrane structure. It remains to be seen whether this complex promotes membrane fusion. Supported by NIGMS grant 32707 to BRL.

1462-Pos

HIV Fusion Peptides Significantly Soften Lipid Bilayers

Pavlo Shchelokovskyy¹, Stephanie Tristram-Nagle², Reinhard Lipowsky¹, Rumiana Dimova¹.

¹Max Plank Institute of Colloids and Interfaces, Potsdam, Germany,

²Carnegie Mellon University, Pittsburgh, PA, USA.

The fusion peptide (FP) of the human immunodeficiency virus (HIV) is found on N-terminus of the viral envelope glycoprotein gp41 and is believed to play an important role in the virus entry process. In order to understand the immediate effect of this peptide on the cell membrane we have studied the influence of the synthetic fusion peptide residue FP-23 on the mechanical properties of model lipid bilayers. For this purpose, giant unilamellar vesicles (GUV) were prepared by electroformation from the unsaturated lipid dioleoylphosphatidylcholine mixed in various ratios with the fusion peptide. The bending stiffness of the vesicles was measured with two different methods: fluctuation analysis and aspiration with micropipettes. The data obtained from both of these approaches show that the bending stiffness of the membrane decreases gradually with increasing the concentration of the fusion peptide in the bilayer. Even low concentrations of only a few mol % FP-23 are sufficient to decrease the bending stiffness of the lipid bilayer by more than a factor of two. This observation is in agreement with previous results obtained with X-ray scattering on stacked lipid layers; see Tristram-Nagle and Nagle, *Biophys. J.* 93: 2048 (2007). Ongoing research is carried out to investigate the effect of FP-23 on the spontaneous fusion of GUVs.

1463-Pos

Augmentation of Single Channel Water Permeability by Modification of Membrane Anchoring

Florian Zocher¹, Tanya Polupanowa², Danila Boytsov¹, Guillem Portella³, Bert de Groot⁴, Ulf Diederichsen², Peter Pohl¹.

¹Universitaet Linz, Linz, Austria, ²Universitaet Göttingen, Göttingen,

Germany, ³Institute for Research in Biomedicine, Barcelona, Spain,

⁴Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

Water transport through very narrow channels occurs according to the single file mechanism. While entering the channel, every water molecule loses most of its neighbouring water molecules. The energetic costs are thought to be