3085-Pos Board B240
Action of Daptomycin on Membranes
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Daptomycin is a clinically important 13 amino acid lipopeptide antibiotic. Its N-terminus is acylated with n-decanol and its C-terminal 10 amino acids form a ring. It is structurally and size-wise different from other well-known membrane-active antimicrobials. Daptomycin is known to disrupt the cytoplasmic membrane function of Gram-positive bacteria by causing leakage of potassium (and potentially other) ions, leading to the loss of membrane potential and cell death. The critical factor affecting the function of daptomycin is its interaction with negatively charged lipids such as PG in a calcium (Ca^{++}) dependent manner. Based on previous research on cell membranes, daptomycin has been assumed to insert and aggregate in the membrane, and then to alter the membrane curvature. However these details have not been demonstrated by biophysical studies. In our aspirated GUV experiments, we found that with a DOPG-containing GUV and a sufficient concentration of Ca^{++}, daptomycin can extract lipid molecules, and form lipid-peptide aggregations. The lipid-peptide aggregates did not occur if cardiolipin replaced PG, or if other divalent ions, such as Mg^{++}, Ba^{++} replaced Ca^{++}. Similarly, daptomycin did not bind to a GUV when cardiolipin substituted for DOPG or in the absence of Ca^{++}. Daptomycin with Ca^{++} did bind to a pure DOPC GUV, but had no other effects. Furthermore, with the presence of daptomycin, DOPG and Ca^{++}, we found Ca^{++} permeates into the GUV, while a content dye, Texas red dextran, did not leak out. This result suggests that daptomycin and Ca^{++} do not form pores on the membrane of DOPG-contained GUV, but cause leakage of ions. Finally, daptomycin with DOPG and Ca^{++} produces a negative exciton CD couplet centered at the 225 nm absorption peak of Trptophan1 and Kynurenine13, whereas in all other conditions, the exciton CD couplet is positive.

3086-Pos Board B241
Amino Acid Sequence and Membrane Binding for a Series of Closely related Amphipathic Peptides
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We have investigated the dependence of peptide activity on the amino acid sequence for a series of synthetic peptides derived from δ-lysin. δ-Lysin is a 26 amino acid, N-terminally formylated, hemolytic peptide that forms an amphipathic α-helix bound at membrane-water interfaces. A shortened peptide, lysette, was derived from δ-lysin by deletion of the 4 N-terminal amino acid residues. Five variants of lysette were synthesized by altering the amino acid sequence under the constraint that the overall hydrophobic moment be essentially the same for all peptides. Peptide-lipid equilibrium dissociation constants and helicities of peptides bound to zwitterionic lipid vesicles were determined by stopped-flow fluorescence and circular dichroism. We then compared the thermodynamics of peptide binding calculated using the Wimley-White hydrophobicity scale with the experimentally determined free energy of binding. We found a systematic deviation of the experimentally determined dissociation constant and that predicted by the Wimley-White scale. Molecular dynamics simulations suggest two factors that account for the very favorable experimental binding free energy. (1), in all δ-lysin-derived peptides simulated, the initial bilayer contact is made with the polar peptide face, allowing charged residues to establish strong interactions with the bilayer headgroup region early on. (2), if the two aspartate residues contained in the lysette sequences are located at the C-terminus, they remain exposed to water and are, thus, effectively removed from the bilayer headgroup region.

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Synergistic Cell Permeabilization by External Electrical Pulses and New Anticancer Peptides Designed on the Basis of the Cry11Bb Protoxin
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Electrical aspects of the membrane permeabilization by various polycationic peptides were studied. The peptides were designed on the basis of synthetic 16-mer and 14-mer fragments of the Cry11Bb protoxin by their conjugation to the cell penetrating hepta-arginine vector trough two glycine residues. Some of these peptides demonstrated selective killing of human leukemia Jurkat cells but not of the normal wild type CHO cells. In this respect, the designed peptides were more active than the known anticancer peptide R7-KLA. The peptides permeabilized the energized mitochondria as well as the red blood cells with relatively high plasma membrane potential generated in the presence of valinomycin. The efficiency of the peptides was remarkably higher in the lower ionic strength media. The capability of the plasma membrane permeabilization by the designed peptides was strongly potentiated by the external high voltage electrical pulses applied to the cell suspension. Similar effect was observed using the planar lipid membrane, demonstrating that the formation of peptide pores in the lipid bilayer is highly increased by an increase in the applied transmembrane potential (minus at the trans-side). The obtained results open the perspective of the local destruction of solid tumors using the combined ''peptide-electrical pulses'' synergistic treatment. Colciencias (Colombia) research grants #111840820380 and #111852128625.

3088-Pos Board B243
Membrane Permeability of Peptides and Drugs
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Amphipathic peptides bind to the outer leaflet of membrane bilayers. Do they translocate across the membrane? The answers are directly related to their functions in the case of cell-penetrating peptides and drugs, or to their mechanisms in the case of AMPs. To our knowledge, few permeability experiments have been performed so far, probably because of the difficulty of detecting the peptides and also because a meaningful test must be conducted at biologically relevant concentrations which are usually low. In the case of pore forming peptides, the test must be performed without pore formation. Here we demonstrate a membrane permeation experiment for a peptide drug NYAD-1 (an HIV-1 inhibitor). There are several factors that make this experiment possible. We used a FITC labeled peptide called NYAD-2 for the peptide detection. We discovered that it is possible to produce GUVs in pH 9, although not in pH 7. We found that the intensity of FITC in pH 9 is 1.7 times higher than in pH 7. By using an aspirated GUV, we measured the relative binding coefficients of NYAD-2 to the GUV in pH 7 and pH 9, and found that the former is 2 to 3 times higher than the latter. With all these provisions, we performed the permeability test by transferring an aspirated GUV produced in pH 9 solution containing Texas red dextran (TRD) MW 625 to a solution of pH 7 containing 2 micro-Mole NYAD-2. We found the concentration of NYAD-2 inside the GUV increased with time while the membrane remained intact and there was no leakage of TRD. We are able to extend this method to test the membrane permeability of other peptides and drugs such as Melittin.

3089-Pos Board B244
Effects of Sequence Length and Composition on Antimicrobial Peptide Action
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Antimicrobial peptides are short, cationic, amphiphilic naturally occurring sequences that have selective antimicrobial properties. The proposed mechanism of action for these peptides is through interaction with and disruption of the bacterial membrane. We have investigated the length and amino acid composition dependence of the antimicrobial peptides ponericin L1 and C18G on antimicrobial activity and membrane binding ability. Truncation of the peptide sequence at different points through synthesis allowed for investigation of length, overall hydrophobicity, and net charge on function. Circular dichroism and fluorescence spectroscopy were used to determine the binding affinity and structure of the peptide in the presence of lipid vesicles. All of the L1 derived peptides exhibited the ability to form alpha helices and bind to the lipid membranes to different degrees. The data suggests that the modified peptide (L1A) and the truncated peptides (L1A-13T, L1A-16T, and L1A-21T) work by forming an alpha helix to permeabilize the bacterial membrane and cause bacteriolysis. Alternatively, the C18G derived truncates exhibited a length threshold in their ability to bind membranes with high affinity and form helical structures. Ongoing experiments are probing membrane topography.

3090-Pos Board B245
Interaction of Magainin 2 with Gangliosides as a Target for Human Cell Binding
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It is important to understand how AMPs interact with human cells for development of antimicrobial agents of systemic use or anticancer drugs. However, litte is known about the mechanisms by which AMPs bind to them and exert cytotoxicity. Negatively charged gangliosides on cell surface are a potential target for cell binding. [1] In this study, we investigated the interaction of MG2 (F5W-magainin 2) with gangliosides in detail. MG2 was colocalized with gangliosides on HeLa cells, indicating that gangliosides act as a receptor for MG2. Physicochemical studies using liposomes revealed that MG2 interacts with monosialoganglioside GM1 differently from the typical bacterial anionic lipid phosphatidylglycerol (PG). MG2 bound to GM1 more strongly than to PG, and the bindig isotherm for GM1 could be analyzed by the Langmuir equation assuming the charge neutralization. This makes a contrast to the binding of AMPs

to PG-containing bilayers, which has been described by the electrostatic attraction and surface partitioning model. [2] FRET experiments supported the clustering of GM1, but not PG, by MG2. Quenching data suggested that MG2 is bound to the sugar region of GM1. The bound peptide assumed a helical structure and induced the leakage of calcein and the coupled flip-flop of lipids, indicating the peptide also forms a toroidal pore in GM1-containing vesicles. However, the membrane permeabilization activity against GM1-containing membranes was weaker than that against PG-doped liposomes in accordance with the trapping of the peptide in the sugar region. These data shed light on AMP-human cell interaction.

References.

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3091-Pos Board B246

Characterization of Antimicrobial Peptide Insertion in Tethered Bilayer Lipid Membranes by Pulse Amperometry and Linear Sweep Voltammetry Methods

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We describe new techniques to study the insertion of pore forming antimicrobial peptides (AMPs) into tethered bilayer lipid membranes (tBLMs). A consequence of tethering a membrane to a gold surface is that electrical contact to the PBS bathing solution is intrinsically capacitive, preventing the direct application of a steady-state voltage across the bilayer. However, by using pulsed waveforms, defined potentials may be expressed across the membrane for tens to hundreds of milliseconds, and the resulting I-V plots provide valuable data about AMP insertion rates and voltage dependence (Fig 1).

Using this technique in the presence of PGLa, we demonstrate how AMP insertion into zwitterionic and negatively charged lipid membranes can be rapidly measured and compared. To better understand the voltage dependence of AMP insertion into tBLMs, ramped potentials can also be applied which can determine the potential thresholds of peptide insertion and pore formation.

Advantages of using tBLMs:

- tBLMs are more robust and longer lasting than black lipid membranes, or micropipette patches;
- Easier, quicker sample preparation than conventional voltage clamp experiments;
- Physiologically relevant AMP concentrations can be used (cf. NMR).

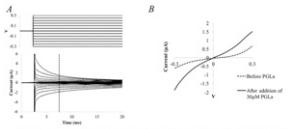

Fig 1. A Voltage clamp data measured from a tethered lipid bilayer containing 40% negatively charged POPG lipids in the presence of 30μM PGLa peptide. The dashed line is the time period at which currents were measured and plotted in B, which is an I-V plot of before and after addition of PGLa.

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All-Atom Molecular Dynamic Simulations of Piscidin 1 and Piscidin 3 in Lipid Bilayers

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Piscidin 1 (p1) and the less active piscidin 3 (p3) are α-helical, amphipathic, and cationic antimicrobial peptides (AMPs). They adsorb onto the anionic outer membrane of pathogenic species and induce leakage beyond a threshold peptide concentration. While different mechanisms of membrane disruption have been proposed, an atomic-level description of the events leading to cell death is lacking and elucidating these mechanisms aid in the antibiotic design. Here, p1 and p3 in three different lipid bilayers (3:1 DMPC:DMPG, 1:1 POPE:POPG, and 4:1 POPC:Cholesterol) are studied by solid-state NMR spectroscopy and all-atom molecular dynamics (MD) simulations to identify factors that differentiate the structure, orientation, and depth of insertion of piscidin. 15N-H dipolar coupling calculated for the membrane-bound peptides are in good agreement with values measured by NMR. The tilts of the peptides in the bilayer (τ) determined by fitting dipolar waves to the coupling data agree with those of the NMR-derived and MD-average structures when deviation from an ideal α-helix is included; when ideal values are assumed for the dipolar wave fitting, deviations range from 4° to 6°. While the instantaneous tilt fluctuates approximately ± 10° in the simulation, averaging over a time series of structures yields the same tilt as one for an average structure (as might be obtained from NMR-based structure determination). This is because $\tau \approx 90$. For transmembrane helices ($\tau \approx 0$), the average over a MD time series may be significantly different from a single averaged structure. Correlation between τ and the depth of insertion for p1 shows that as the peptide becomes more buried, the peptide tilts to bury the C-terminus. Moreover, the depth of insertion is 0.5 to 1.5Å greater for p3 than p1, which likely reduces the activity of p3.

3093-Pos Board B248

Interactions of Two Amphipathic Cell-Penetrating Peptides with Complex Model Membranes: Insights from Molecular Dynamics Simulations

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Cell-penetrating peptides (CPP) can permeate cellular membranes and are therefore attractive vectors for gene therapy and drug delivery. However their uptake mechanisms are still poorly understood [1]. Peptides classified as CPP are usually enriched in basic residues and thus positively charged but except from this shared characteristic display a wide range of physico-chemical properties. A unifying interpretation of experimental results is rendered all the more difficult by the great diversity in experimental setups; nevertheless there is evidence that some CPP can be internalized by both endocytic and direct translocation pathways. In particular, peptide concentration and amphipathicity have been shown to be important for membrane disruption and passive permeation. Membrane lipid composition and electrostatic properties also appear to play a crucial role in the peptides activity.

Here we apply multi-scale molecular dynamics simulations to gain molecular level insights into the interactions of both a primary amphipathic CPP (Transportan) and a secondary amphipathic CPP (Penetratin) with model membranes. Coarse-grained (CG) simulations were performed to investigate the behaviour of the peptides over several microseconds in large, asymmetric bilayers with complex, biologically relevant lipid compositions. The influence of peptide secondary structure was also explored. The CG simulations highlighted different lipid bilayer perturbations by the two peptides. Membrane permeation was investigated further using more detailed simulation methods (both CG simulations with polarisable water model and atomistic representations).

[1] A. Ziegler, Thermodynamic studies and binding mechanisms of cell-penetrating peptides with lipids and glycosaminoglycans, Adv. Drug Del. Rev. 60 (2008) 580-597.

3094-Pos Board B249

High Resolution Structures and Structure-Function Relationships in Histidine-Rich Antimicrobial Peptides from Cod

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Two paralogous antimicrobial peptide (AMP) sequences, Gad-1 and Gad-2, were previously identified from an Atlantic cod (Gadus morhua) expressed sequence tag database. Both peptides are rich in histidine, suggesting that their activity might be pH dependent. Indeed, minimal inhibitory concentration (MIC) assays with Gram-negative bacteria demonstrate that the activity of Gad-2 is substantially higher at pH 5 than it is at pH 7, whereas the activity of Gad-1 at pH 5 is similar to its activity at pH 7. The paralogues also appear to differ from each other in their level of activity against Gram-negative bacteria, with Gad-1 exhibiting more activity than Gad-2, even at pH 5. Clues to the origin of the different pH dependencies of the activity of the two peptides, as well as the difference in activity of Gad-1 and Gad-2 at pH 5, were provided by circular dichroism (CD) studies and high resolution NMR structures in sodium dodecyl sulfate (SDS) micelles at pH 5. Gad-1 takes on a helical configuration from residue 4 to 21. Gad-2 also appears to be predominantly helical but has a kink at the junction between H10 and H11. This deformation in the helix is likely due to the electrostatic repulsion between histidine sidechains at pH 5.

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Membrane Perturbing Effects of Antimicrobial Peptides: A Systematic Spectroscopic Analysis

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Antimicrobial peptides (AMPs) exhibit a strong activity against a wide range of microorganisms, mainly by perturbing the permeability of bacterial membranes through the formation of pores. However, AMPs effects on membrane properties probably extend beyond pore-formation. We performed a systematic