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Characterizing the Effects of Membrane Fluidity and Lipid Chain Length on the Antimicrobial Activity of Protegrin-1

Oliver S. Shafaat[1], Matthew Chapman[1], Kin Lok Lam[1], Alan J. Waring[2], Robert Lehrer[2], Ka Yee Lee[1].
[1]University of Chicago, Chicago, IL, USA, [2]University of California Los Angeles, Los Angeles, CA, USA.

Antimicrobial peptides are naturally occurring short amphipathic proteins, innate to the immune system and shown to induce selective lytic activity towards microbial pathogens. Protegrin-1 is an 18-residue, cationic, β-sheet antimicrobial peptide stabilized by two disulfide bonds. Concentration-dependent structural transformations of supported lipid bilayer patches as a result of peptide-membrane interactions have been visualized through the use of atomic force microscopy. A three-stage concentration-dependent transformation has been characterized, which begins with edge instability, followed by pore formation and worm-like micelle formation. This suggests that protegrin-1 acts to lower the line-energy at the edge of the bilayer. Membrane and lipid characteristics, including fluidity, charge and acyl chain length, can alter the activity of antimicrobial peptides. To identify the importance of both acyl-chain length and fluidity on the activity of protegrin-1, these two variables were decoupled. When the bilayers are examined at the same relative fluidity levels, they demonstrate the three-stage transformation observed on a fluid control bilayer, in contrast to the structural transformations that were observed in the gel phase bilayers. This suggests that fluidity exhibits a large influence on the transformations that occur as a result of protegrin-1. To examine the importance of acyl-chain length, the activity of antimicrobial peptides was studied using unsaturated bilayers. Our results indicate that the longer chain bilayers are less susceptible to disruption. This could be due to the hydrophobic mismatch between protegrin-1 and the thicker hydrophobic portion of longer chain lipid bilayers . These results highlight the importance of subtle membrane characteristics in the activity of antimicrobial peptides towards bacterial cells. Lipid bilayers with cholesterol are more accurate eukaryotic cell mimics and will allow examination of the selective preference of antimicrobial peptide activity.

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A Systematic Approach Towards Elucidation of the Mode of Action of a Bacterial Thermosensor

Larisa E. Cybulski[1,2], Joost Ballering[1], Jacques P.F. Doux[1], Martijn C. Koorengevel[1], Ben de Kruijff[1], Diego de Mendoza[2], **J. Antoinette Killian**[1].
[1]Institute of Biomembranes and Bijvoetcenter for Biomolecular Research, Utrecht University, Utrecht, Netherlands, [2]Instituto de Biología Celular y Molecular de Rosario-CONICET. Universidad Nacional de Rosario, Rosario, Argentina.

The membrane sensor and signalling protein DesK is involved in detecting temperature changes in the bacterium Bacillus subtillis. At low temperatures it triggers expression of a desaturase, which introduces double bonds into pre-existing phospholipids, thereby regulating membrane fluidity. Recently it was discovered [1] that both sensing and transmission of DesK, which has five transmembrane segments, can be captured into one single chimerical transmembrane segment, the so-called 'minimal sensor'. It was hypothesized that a group of hydrophilic amino acids flanking this transmembrane segment represents the molecular switch responsible for turning on and off the kinase state of DesK. This switch would be regulated by the extent of exposure of this group to the aqueous phase, which in turn would depend on membrane thickness. Here we tested this hypothesis by employing different biophysical approaches, using synthetic peptides corresponding to functional and non-functional mutants of the minimal sensor in artificial model membranes of phosphatidylcholines of varying thickness and at different temperatures. The results of these studies will be reported.

[1] Cybulski et al., manuscript submitted.

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Engineering a Thermosensor To Dissect a Transmembrane Signaling System

Larisa E. Cybulski[1], Ariel Fernández[2], Diego de Mendoza[1].
[1]Instituto de Biología Molecular y Celular de Rosario, Rosario, Argentina, [2]Department of Bioengineering, Rice University, Houston, TX, USA.

The DesK-DesR two-component system regulates the order of membrane lipids in the bacterium Bacillus subtilis by controlling the expression of the des gene coding for the delta 5-acyl-lipid desaturase. In this work, we address the process by which DesK transmembrane segments (TMS) transmit temperature signals across the membrane by engineering the 5 TMS domain of the DesK into a single-TMS chimeric sensor. This so-called Minimal Sensor (MS) fully retains *in vivo* and *in vitro* the sensing input and transmission output of the parental system. Progressive deletions of TM segments revealed that only the first TM segment (TM1) is essential to regulate the kinase activity. Therefore, our engineered MS combines the N-terminal 17-residue portion of TM1 with the C-terminal 14-residue portion of TM5 which is naturally fused to the cytosolic catalytic domain. The MS N-terminus contains three hydrophilic aminoacids near the lipid-water interface creating an instability hot spot. This boundary-sensitive motif controls the sensing and transmission activity. Accordingly, we hypothesize that membrane thickness is the temperature agent that determines the signaling state of the cold sensor by dictating the hydration level of the meta-stable hydrophilic spot. This hypothesis is supported through the study of the signaling behavior of MS variants purposely constructed.

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Membrane-Associated Folding and Unfolding

Alexander G. Karabadzhak[1], Dhammika Weerakkody[1], Mak S. Thakur[1], Gregory O. Andreev[2], Donald M. Engelman[3], Oleg A. Andreev[1], Yana K. Reshetnyak[1].
[1]University of Rhode Island, Kingston, RI, USA, [2]University of California San Diego, La Jolla, CA, USA, [3]Yale University, New Haven, CT, USA.

We are studying the molecular events that occur when a peptide inserts across a membrane or exits from it. Using pH jumps to trigger insertion/exit of the pHLIP (pH Low Insertion Peptide) to enable kinetic analysis, we show that insertion occurs in several steps, with rapid (0.1 sec) interfacial helix formation followed by a much slower (100 sec) insertion pathway to form a transmembrane helix. The reverse process of unfolding and peptide exit from the bilayer core, which can be induced by a rapid pH jump from acidic to basic, proceeds much faster than folding/insertion and through different intermediate states. In the exit pathway, the helix-coil transition is initiated while the polypeptide is still inside the membrane. We also designed two pHLIP-variants where Asp and Glu residues were removed from the C-terminus, which inserts across the membrane. The variants preserve the same pH-dependent properties of pHLIP peptide interaction with the membrane, but insertion occurs 10-30 times faster than in the case of the parent pHLIP peptide. A kinetic model of peptide-membrane insertion/folding and exit/unfolding will be discussed. The work was in part supported by grant from the National Institutes of Health, National Cancer Institute RO1 133890 to OAA, DME, YRK.

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Enhanced Uptake of Integral Membrane Proteins by Cubic Nanoparticles

Charlotte E. Conn[1], Connie Darmanin[2], Xavier Mulet[1], Minoo Moghaddam[3], Jose Varghese[2], Calum J. Drummond[1].
[1]CSIRO Molecular & Health Technologies, Clayton South, Australia, [2]CSIRO Molecular & Health Technologies, Parkville, Australia. [3]CSIRO Molecular & Health Technologies, North Ryde, Australia.

Self-assembled lipidic cubic phases are attracting increasing interest as biocompatible carriers of large biomolecules including proteins, peptides, DNA and drugs.[1] Their unique structure of interpenetrating but unconnected water channels divided by a lipid bilayer can accommodate hydrophobic, hydrophilic and amphiphilic moieties without significant structural perturbation. This has led to their use in a diverse range of applications including the delivery of drugs and other active agents, as biomimetic crystallization media for membrane proteins, as the basis of biofuel cells, and as biosensors.[2]

Lipidic cubic phases can be emulsified into diluted non-viscous aqueous dispersions consisting of cubic nanoparticles, offering significant advantages for many of the applications listed above.[3] Here we have incorporated an integral membrane protein and important neurological drug target within cubic nanoparticles. We have characterised the structural effect on the micro- and meso-scale properties of the nanoparticles. In addition we have shown that protein loading can be significantly enhanced by doping the cubic nanoparticles with a second amphiphile which chemically binds to the integral membrane protein.

[1]Yaghmur A, Glatter O. Advances in Colloid and Interface Science 2009; 147-148; 333-342.
[2]Nazaruk E, Bilewicz R, Lindblom G, Lindholm-Sethson B. Anal Bioanal Chem 2008; 391; 1569-1578.
[3]Spicer PT. Current Opinion in Colloid & Interface Science 2005; 10; 274 - 279.

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Real-Time Detection of Apolipoprotein A-I's Lipidation State by Fluorescence Resonance Energy Transfer

Giorgio Cavigiolio, Ethan G. Geier, Michael N. Oda.
Children's Hospital Oakland Research Institute, Oakland, CA, USA.

Apolipoprotein A-I (apoA-I), the main protein component of high density lipoprotein (HDL), is the principal facilitator of cholesterol efflux from cholesterol-laden macrophages. Lipid-free apoA-I is the preferred substrate over lipid-associated apoA-I for cholesterol mobilization by the membrane transporter ATP binding cassette A1, which is responsible for more than 60% of cellular cholesterol efflux from cholesterol-laden macrophages. However, more