

Membrane Active Peptides & Toxins I

1209-Pos Board B101

Non-Enzymatic Acyl Transfer from Lipids to Peptides is a General Process

Robert H. Dods, Jackie A. Mosely, John M. Sanderson.

Durham University, Durham, United Kingdom.

Acyl group transfer from glycerophospholipids to melittin has recently been demonstrated using MS, LC-MS and LC-MSⁿ methods (doi: c2ob07113d). This transfer is not mediated by enzyme catalysis, but rather is a consequence of the innate reactivity of the peptide toward lipids. Transfer from phosphatidylcholines (PCs) to melittin has been observed in a range of conditions of salt (from water to physiological concentrations), temperature (20 °C to 37 °C) and peptide to lipid ratio (P:L = 1:100 to 1:5). Lipidated peptides may be detected after 2–3 h, with a half-life for acyl transfer of ~24 h for melittin. Other peptides that have been found to undergo acyl transfer in this manner include magainin II, PGLa, LAK1, LAK3 and penetratin. In mixed membranes (PC + PE, PG, or PS), this intrinsic lipidation exhibits selectivity in respect of lipid type and the acyl chain composition of the individual components. This process is of high relevance for peptide and protein turnover *in vivo*, as well as an important factor to consider when interpreting data accumulated over significant time periods *in vitro*.

1210-Pos Board B102

Dynamics and Mechanism of Membrane-Induced Folding of a Small Beta-Hairpin

Caitlin Davis, James Kindt, R. Brian Dyer.

Emory University, Atlanta, GA, USA.

A complementary approach of experiments and computation were used to characterize the dynamics and mechanism of peptide folding and insertion into the membrane. SVS-1, an 18-residue anti-cancer peptide that is designed to be disordered in solution, but fold into a β -hairpin at an anionic bilayer surface, was used as a model system for protein folding on the membrane. Infrared and fluorescence were used to characterize the folding of the peptide in the membrane. Laser induced temperature-jumps (T-jump) were used to initiate the folding reaction. Computational models of tryptophan-containing SVS-1 mutants were used to guide the choice of targets for fluorescent studies. Molecular dynamic simulations model the folded and unfolded states at the bilayer surface to estimate timescales of binding, folding and insertion. Simulations were used to interpret and guide future experiments.

1211-Pos Board B103

Structure and Energetics of Alamethicin Oligomers

Asif Rahaman, Themis Lazaridis.

The City College of New York, New York, NY, USA.

We have investigated the structure and energetics of alamethicin oligomers in cylindrical pores using molecular dynamics simulations with a modified version of implicit membrane model 1 (IMM1-pore). We have added a new energy term to IMM1-pore to include the free energy cost of hydrophobic area exposure. With this new energy function, we have studied monomer to nonamer in cylindrical pores with radii ranging from 5 to 12 Å. Stable, low energy pores are obtained for the right combination of radius and oligomeric number. The smaller oligomers (trimer to tetramer) formed closed pores for pore radius 6 Å while the larger oligomers formed open pores at their optimal pore size. The octamer in a pore of radius 11 Å gives the lowest effective energy per monomer. The tilt angles of the helices in the pore are 10–25° with respect to the membrane normal. The kink produced by Pro14 makes the pore funnel-like. Voltage seems to have little effect on the structure and stability of the pore.

1212-Pos Board B104

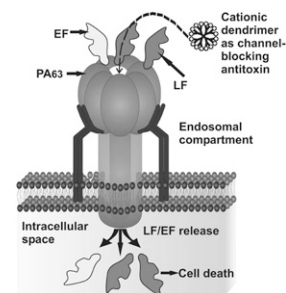
Cationic Dendrimers as a New Group of Channel-Blocking Antitoxins

Nivedita Bondhu, Ekaterina M. Nestorovich.

The Catholic University of America, Washington, DC, USA.

Binary pore-forming bacterial protein toxins, including anthrax, are among Nature's most potent biological weapons. *Bacillus anthracis*, the causative agent of anthrax, secretes three polypeptides: protective antigen (PA), lethal factor (LF), and edema factor (EF), which interact at the surface of mammalian cells to form toxic complexes. LF and EF are enzymes that target substrates within the cytosol; PA provides a heptameric pore to facilitate LF and EF transport into the cytosol. In the present study, we evaluate a series of positively charged poly(amido amine) (PAMAM) dendrimers with the goal of identifying potent inhibitors of anthrax toxin. We show that all tested cationic dendrimers block

PA₆₃ channels. However, size of the dendrimers increases with the generation number, which limits ability of the large multi-charged dendrimer to enter the channels. We identify optimal size and charge characteristics of the blockers testing the G0 - G8 generations of PAMAM dendrimers against the single PA₆₃ using the high-resolution single-channel measurements in planar bilayer membranes. Besides, we investigate the kinetics of the PAMAM dendrimers - pore binding/dissociation reactions at different voltages and salt concentrations.



1213-Pos Board B105

Curvature Driven Membrane Organisation Revealed by Single Molecule Imaging in Droplet Interface Bilayers

Oliver K. Castell, Brid Cronin, Mark I. Wallace.

University of Oxford, Oxford, United Kingdom.

Membrane curvature plays a crucial role in lipid and protein sorting within the cell membrane [1] giving rise to spontaneous self-organisation and localisation of complex biological macromolecules. Such spatial organisation in response to, and in generating, membrane curvature contributes to many important biological processes such as neurotransmission, trafficking and cell division [2]. Despite this importance, the majority of *in-vitro* studies are performed in planar or pseudo-planar (GUV) bilayer systems. As such, there is a need for novel strategies to more closely mimic the curvature of cellular membranes, whilst allowing for correlated measurements of protein function.

Here, we create Droplet Interface Bilayers (DIBs) [3] on an underlying curved substrate, resulting in the morphology of the underlying substrate being reflected in the curvature of the bilayer. Total Internal Reflection Fluorescence (TIRF) imaging reveals the correlation of lipid domain formation at sites of intrinsic curvature and the preferential localisation and pore formation by antimicrobial peptides at regions of positive curvature. This technique provides a new method for dictating the curvature of artificial bilayers, enabling single molecule studies on the role of curvature in membrane organisation and function.

1. Ramamurthi, K. S. et al. *Science* 2009 **323**, 1354.

2. McMahon, H. T. et al. *Nature* 2005 **438**, 590.

3. Thompson, J. R. et al. *Nano Letters* 2007 **7**, 3875.

1214-Pos Board B106

Action of Arginine and Tryptophan Containing Antimicrobial Peptides on Supported Lipid Bilayers

Gillian Vanderlee¹, Zhigang Liu², Neville R. Kallenbach², Christopher M. Yip¹.

¹University of Toronto, Toronto, ON, Canada, ²New York University, New York, NY, USA.

Direct observation and quantitative insights of the dynamics of interactions between membranes and antimicrobial peptides greatly aid in understanding the mechanisms by which they exert their action. We previously developed a correlated total-internal reflectance fluorescence-atomic force microscopy (TIRF-AFM) platform that enabled direct determination of local order within the membrane [Oreopoulos and Yip, 2009]. We report here, the results of combinatorial studies using this platform into the activity of arginine and tryptophan containing antimicrobial peptides on supported lipid bilayers of varying compositions. These studies provide direct evidence of membrane destruction and insight into the mechanisms by which they act. These studies portend to the usefulness of other correlated approaches, such as coupled ATR-IR-AFM, to directly observe and quantify interactions between membranes and membrane active molecules.

1215-Pos Board B107

NMR Studies of Antimicrobial Peptides Interacting with Intact Bacteria

Taslina Akter, Michael Morrow, Valerie Booth.

Memorial University, St John S, NL, Canada.

Title: NMR studies of antimicrobial peptides interacting with intact bacteria. Antimicrobial peptides (AMPs), are molecules naturally found in the immune system, and protect the host from bacteria, viruses, protozoa and other pathogens. AMPs are considered to be a potential alternative to conventional antibiotics due to the general mechanism of AMP action, which involves membrane disruption. AMP-membrane interactions are commonly studied using solid state NMR with model membranes. A number of components of the bacterial cell envelope, including membrane proteins, peptidoglycan layer, and lipopolysaccharide may well modify the interaction of the AMP with the bilayer. Thus,