Open Pores on Membrane

This report features the work of U-Ser Jeng, Ming-Tao Lee and their co-workers published in Biochim. Biophys. Acta ***1828****, 528 (2013) and of Huey W. Huang, Wei-Chin Hung, Ming-Tao Lee and their co-workers published in Proc. Natl. Acad. Sci. USA* ***110****, 14243 (2013).*

In the past three decades, antimicrobial peptides, composing up to 60 amino acids, have been discovered to serve as a versatile defence against bacteria. They are part of an innate immunity in plants and animals. At a large concentration, these peptides penetrate and form pores on the membrane envelope of bacteria, thus disrupting the internal environment of bacteria and even disintegrating the bacterial membrane. A defence of this kind is immediately effective and is of commercial interest. Antimicrobial peptides have even been proposed for use as antibiotics. While much research focuses on the antimicrobial peptides, less is known about the state of the membrane affected by these peptides at a molecular level. The general idea is that, at a small concentration, these peptides lie on the surface of a membrane; they might form a transient pore briefly and might translocate to the other side of the membrane. At a large concentration, they aggregate and form pores on the membrane. There is a large energy barrier for peptides to penetrate a membrane. How a membrane responds to the presence of antimicrobial peptides so as to open pores is, therefore, an important question. This year, two publications pertained to an understanding how melittin, the major component of bee venom that is a 26-amino acid peptide, affects and alters the membrane structure.

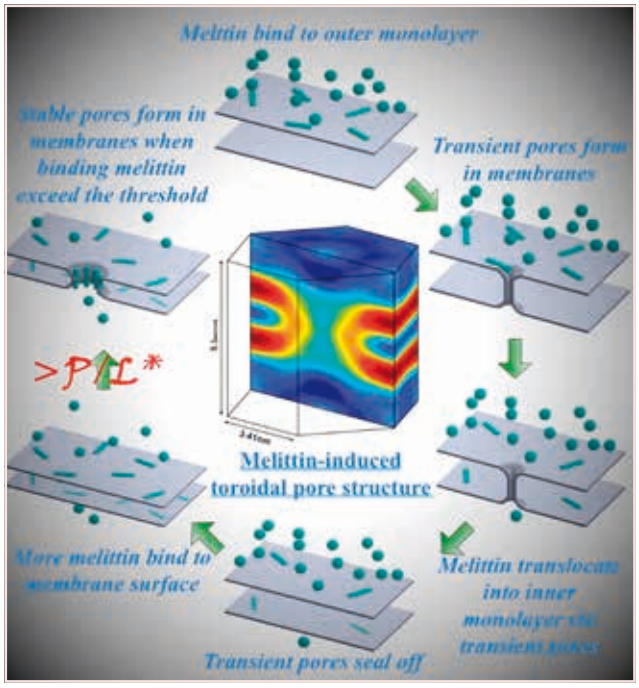

Fig. 1: Scheme diagram of melittin-induced pore formation. Melittin (blue) is a random coil (sphere) in solution and an α-helix (cylinder) on the membrane. Melittin above a critical concentration (P/L*) forms stable pores on a membrane. A reconstructed three-dimensional structure of pores from X-ray diffraction appears in the middle.

For an investigation of the membrane structure, multilamellar membranes (MLM) are commonly used because they generate diffraction with X-rays or neutrons. In reality, a bacteria membrane is a single closed bilayer. A unilamellar vesicle (ULV) would be a superior model to test the effect of antimicrobial peptides on a membrane; it is basically a bubble of a single bilayer lipid in aqueous solution. Small-angle X-ray scattering (SAXS) can then be used to probe the structural alteration of unilamellar vesicles upon binding of an antimicrobial peptide. The groups of Ming-Tao Lee and U-Ser Jeng at NSRRC compared the membrane-thinning effect of multilamellar membranes and a unilamellar vesicle caused by melittin;[1] they tried to address the dynamic equilibrium such that, for free floating ULV, only part of the peptides bind onto the vesicle, whereas most peptides are premixed in the multilamellar membrane.

SAXS results clearly show the membrane thinning effect up to 2 Å by the peptide melittin. The second hump of SAXS profiles is sensitive to the local bilayer structure. The more the peptides bind, the thinner the membrane. The same effect can be observed from the distance between the two electron density maxima of the phospholipid headgroup regions (PtP). SAXS shows also an asymmetric profile of

two maxima, indicating that peptides bind unequally on the outer and inner surfaces. This effect is explicable by the amount of peptide available inside and outside the vesicle. It might also reflect an asymmetric environment of lipids on each side of the membrane, increasing the disorder on the inner leaflet of the membrane.

Based on the model of melittin/MLM interaction, the binding ratio of melittin to free floating ULV was deduced at a small concentration, thus establishing a quantitative correlation between the bound peptides on ULV and free peptides in solution. This condition makes possible a qualitative comparison of data between melittin/ULV and melittin/MLM. This work used beamlines **BL13A1** and **BL23A1** at the TLS.

The groups of Huey W. Huang, Wei-Chin Hung and Ming-Tao Lee continued to investigate the melittin-bound membrane structure at a molecular level.[2] Even though the crystal structure of melittin has been solved and a model for pore formation is proposed, the current model fails to take into account the membrane dynamics.

These scientists first tried to establish a correlation between melittin-bound ULV and melittin-bound MLM before melittin began to penetrate a membrane. Under a fluorescent microscope, ULV expanded linearly about 2.8 - 4.5 % (average 3.4 %) upon melittin binding before the leaking of the content in the ULV. For melittin/MLM, the membrane also became thinner linearly about 3.3 % until the melittin began to penetrate the membrane, measured by oriented circular dichroism. The surface expansion of ULV and the membrane thinning of MLM are about the same, closely correlated with each other.

Once the correlation is established, these scientists literally grew crystals of melittin pores on a multilamellar membrane. Melittin forms stable pores on both ULV and MLM and moves freely in a fully hydrated multilamellar membrane. By dehydration, melittin/MLM eventually proceeds through a phase transition to a rhombohedral crystalline lattice (*R* phase). Instead of seeking melittin, they labeled the headgroup of phospholipid with bromide (Br) and developed a method of multiple anomalous dispersion to reconstruct the structure of the lipid bilayer. The result shows that melittin causes the toroidal pore to form at positions at which the top and bottom layers of lipids bend and merge through the pore, in contrast with the barrel-stave model in which two layers of lipid are separated. Such a structure of a toroidal pore

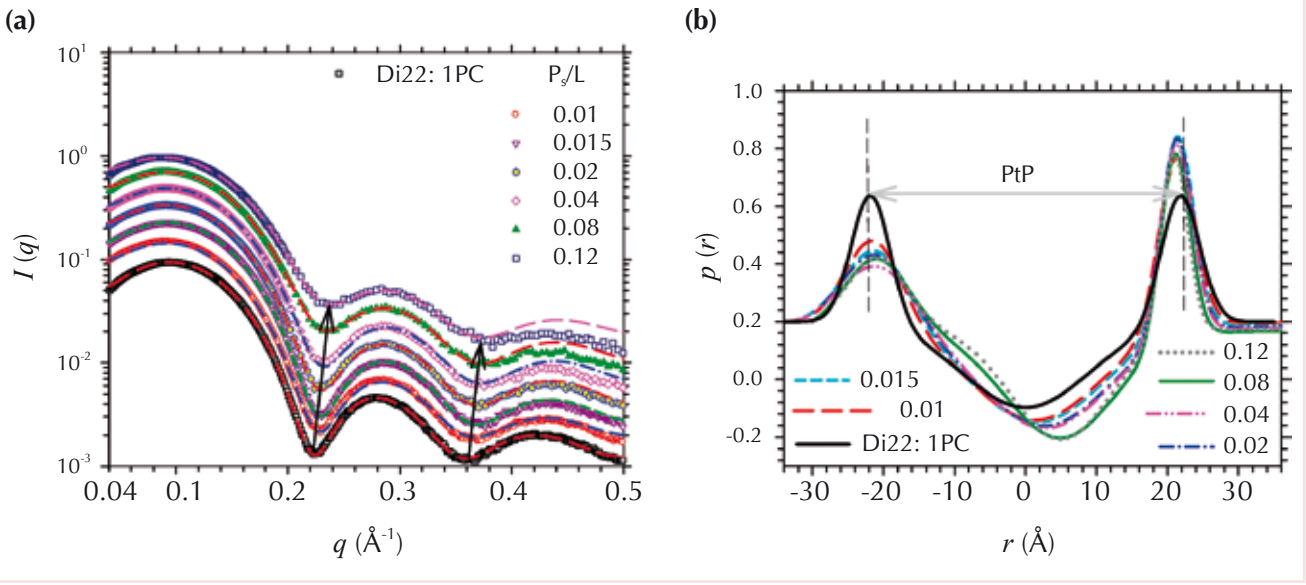

Fig. 2: (a) SAXS data show a shift of membrane thinning along with the concentration (P_s/L) of peptide to lipid. (b) Calculated distance (PtP) of the phosphate to phosphate headgroup of a lipid bilayer also indicates the thinning effect of peptide melittin. (Reproduced from Ref. 1)

resembles the fusion of secretory vesicles. The size of the barrel-stave pore is fixed because it is framed with the peptide assembly, but the toroidal pore shrinks during dehydration because of loss of water around the pore. This condition is indeed the case of a melittin/MLM pore, of which the diameter decreased to 0.7 nm. Neutron scattering showed the melittin pores in the fully hydrated lipid bilayer to have a diameter of 4.4 nm. This work used beamlines **BL13A1** and **BL23A1** in at the TLS.

The use of SAXS and membrane diffraction provides unique ways to observe membrane structures directly at a molecular level. Many other peptides and drugs interact with a membrane. These techniques will improve our understanding of the membrane structure and dynamics.

References

1. C.-J. Su, S.-S. Wu, U. Jeng, M.-T. Lee, A.-C. Su, K.-F. Liao, W.-Y. Lin, Y.-S. Huang, and C.-Y. Chen, Biochim. Biophys. Acta **1828**, 528 (2013).
2. M.-T. Lee, T.-L. Sun, W.-C. Hung, and H. Huang, Proc. Natl. Acad. Sci. USA **110**, 14243 (2013).

Protein-Protein Interaction

*This report features the work of Gennaro De Libero and his co-workers published in Nat. Immunol. **14**, 908 (2013) and of Jayaraman Sivaraman and his co-workers published in Nat. Commun. **4**, 2546 (2013).*

Most proteins do not act alone; they interact with each other to promote various activities in cells. Such interactions can typically be investigated structurally with protein X-ray crystallography. This year, two studies are selected to illustrate the use of synchrotron radiation in investigating such protein-protein interactions.

Antigens that trigger an immune response of T-cells must be presented by other cells, such as macrophages; this process distinguishes invaders from the self. Antigens are generally presented by major histocompatibility complex (MHC) or CD1 molecule. Gennaro De Libero led a group of scientists in Switzerland and Singapore to find and to study the antigen-presenting molecule (APM) for the human T-cell antigen receptor (TCR) containing a variable region 9 in the γ-chain and a variable region 2 in the δ-chain (Vγ9Vδ2), which senses phosphorylated prenyl metabolites.[1] The antigen-presenting molecule for Vγ9Vδ2 is neither MHC nor CD1, and was unclear before their work, but the evidence indicates the existence of an APM dedicated for phosphorylated antigens, such as isopentenyl-pyrophosphate (IPP) or (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP).

These scientists first developed a method to avoid a complication caused by the autopresentation phenomenon of human T-cells, and pinpointed the gene encoding the APM on chromosome 6. Using micro-array to analyze the expression profile of cell lines that efficiently present IPP and HMBPP, they narrowed the regions to 81 genes. After some analy-

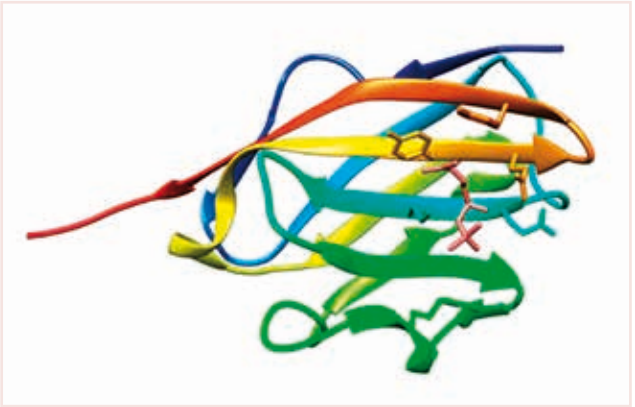

Fig. 1: Structure of BTN3A1 in complex with IPP (color in salmon). Residues interacting with IPP have sidechains presented in sticks.