Bilayers at the ILL

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Using suspended bilayers as a novel bacterial membrane mimetic for investigating mechanosensitive ion channels with neutron reflectivity

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In Gram negative bacteria, the inner membrane is suspended from a thin peptidoglycan layer and contains embedded membrane proteins. This composite layer controls the shape of the cell and the flux of materials into and out of the cell. Mechanosensitive ion channels act as safety valves, protecting bacteria from osmotic shock, by opening when the membrane is subject to a stress. In addition to osmotic shock, this stress can be triggered by insertion of amphipathic molecules. We are investigating whether these alternative triggers could make mechanosensitive ion channels an Achilles heel to bacteria. Specifically we are using neutron reflectivity and small angle scattering to investigate whether the insertion of the antimicrobial molecules lyso-PC and pexiganan (an amphipathic antimicrobial peptide) into POPC/POPG bilayers triggers prolonged opening of mechanosensitive ion channels of large conductance (MscL). Such channel opening could explain the antimicrobial behaviour of these molecules, without the requirement for them to induce pore formation in the membrane. In addition to using solid-supported, tethered POPC/POPG bilayers, we have developed a novel mimetic in which the POPC/POPG bilayer is suspended beneath a DODAB monolayer at the air/water interface. In this approach the bilayer is formed by vesicle rupture of either liposomes or proteoliposomes. In the latter case, the ion channels are incorporated into the proteoliposomes by cell free protein expression. In addition to mimicking the manner in which the inner cell membrane is suspended from a peptidoglycan layer in a bacterial cell, the approach offers some advantages over solid-supported mimetics such as floating bilayers. The implementation of this approach, the characterization of the resulting lipid-only and protein-containing lipid bilayers using neutron reflectivity, as well as what has been learned about the way these layers repsond to challenge by lyso-PC and pexiganan, will be described.

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