

Membrane pores: from structure and assembly, to medicine and technology

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Biological membranes are essential barriers, defining and protecting living cells. Pore-forming proteins (PFPs) are recognised as important players in infection and immunity, and target membranes by opening up channels through them. They achieve this by converting from a form that is soluble in aqueous solution to one that is inserted into a membrane. Therefore, they must adopt two different structures with one converting into the other during membrane attack. During this conversion, individual PFP subunits usually self-associate into higher-order assemblies with which lipid molecules may be associated.

Over the last 25 years, the biology of membranes and membrane proteins, including pore-forming proteins, have undergone a remarkable maturation. We now understand far better than we did how soluble proteins can convert themselves into membrane-inserted pore-forming assemblies by refolding from one stable topology to another, by mechanisms which differ remarkably in detail.

The aim of the meeting, which has given rise to the papers published in this issue of Philosophical Transactions Series B, was to look at the physical basis of membrane pore formation from both the perspective of the protein making the attack and that of the lipid bilayer being targeted. Our aim was to reset the debate on how pore-forming proteins work, seeking areas of commonality and giving research in the field a new momentum. A second aim was to place investigations of pore-forming proteins in a biomedical context. The sense of the meeting was clearly that these aims were achieved and this was reflected in the final talk of the meeting, which was given by Dame Carol Robinson. Her perspective as a biophysical chemist working with membrane-associated proteins and protein-lipid interactions brought an invaluable oversight to our discussions. In her presentation, Professor Robinson reviewed ways in which native mass spectrometry, in particular, has proved a very powerful way to interrogate protein-protein and protein-lipid complexes from membranes in a manner with excellent complementarity to other techniques: structural, biophysical and computational.

Indeed, a diverse set of researchers with many different perspectives was present at the meeting, enabling discussion of different ways of understanding the biology of pore-forming proteins, their structures and mechanisms of action, and their possible applications. The discussions produced an enriched sense of how PFPs act of benefit both to biomedical scientists working for a better understanding of their basic mechanisms and on ways of addressing them in a clinical context, and also to those looking at the various ways in which they might be used in technological applications such as biosensor technology and DNA sequencing.

This issue of the journal starts with a review of membrane biophysics before moving on to discussion of the ways in which proteins have evolved to target membranes and act within them. It then focuses on the technological application of pores and the design of novel transmembrane channels.

One important concept for understanding how proteins interact with membranes has been the electroporation effect whereby pores are opened in pure lipid bilayers by an application of electrical charge. In the first paper in this volume, Sengel and Wallace discuss the temperature dependence of this phenomenon, using data from droplet interface bilayers. They find evidence in support of the formation of “toroidal pores”, namely the rearrangement of bilayer lipids into a structure resembling the interior of a torus. This is relevant to our understanding of the activities of PFPs, several of which are believed to open up a pore through a similar effect on lipid packing, including members of the membrane attack complex/perforin-cholesterol dependent cytolysin (MACPF/CDC) superfamily.

Before the MACPF/CDC proteins are addressed, a review by Podobnik and colleagues discusses pore formation by a family of proteins which act by a different mechanism. The aerolysin family of PFPs uses small (7-9 subunit) assemblies to target membranes. Aerolysin itself was the first bacterial pore-forming toxin to have its structure determined, in 1994, and although at the time there was a tantalising low-resolution map of the pore state, it is only recently that the mechanism of pore formation by aerolysin and related proteins has been properly understood via the use of structural and computational methods focused on lysenin as well as aerolysin.

MACPF/CDC proteins are an especially interesting group of PFPs because they are so diverse not only in the different ways various members of the family work, but also in the capacity of the same protein to function in different ways in response to the environment. This aspect of MACPF/CDC proteins is addressed by Osborne and colleagues in their discussion of the way in which listeriolysin (the MACPF/CDC protein from *Listeria monocytogenes*) can form pores with both large and small functional sizes. This enables a subtle response of the bacterium to the context in which it finds itself through the use of different sizes of pore at different stages of intracellular infection.

The mechanism of pore formation by the membrane attack complex (MAC) itself is the topic of the review by Bayly-Jones and colleagues, which brings together researchers who have independently been working on the structures of the native MAC and of the homo-oligomeric C9 component which is a model for it. Although the structures of the MAC and poly(C9) were determined by single-particle cryo-electron microscopic reconstruction, the use of cryo-electron tomography has also had a significant impact on PFP research, including on the MAC, and this is discussed by Dunstone and de Marco in their review.

Although most MACPF proteins seem to form pores, others do not and the review by Ni and Gilbert discusses in particular such perforin-like proteins with alternative, and currently poorly understood, means of action. A key focus is the astrotactin family of perforin-like proteins, which are highly conserved (53% sequence identity between species with a 485 Ma evolutionary separation) making them “living fossils”, which may provide novel insights into vertebrate evolution, in particular.

Having dealt with examples of proteins that form pores using membrane-inserted β -sheets or β -barrels, examples of proteins which use instead inserted α -helices are addressed, beginning with one of the most striking examples, the bacterial cytolysin ClyA, which undergoes a refolding process reminiscent of Class I fusion proteins to produce an α -helix barrel that spans the target membrane.

Glockshuber and colleagues describe how a series of monomers associate and are triggered to refold cooperatively into a transmembrane pore.

ClyA is an example of a PFP in which lipids do not participate directly in the structure of the pore. Having discussed the MACPF/CDC proteins which use arcs of protein subunits interfacing with lipid components of the target membrane to generate a functional channel, we now turn to α -helix based structures which do the same. Firstly, Caaveiro and colleagues discuss haemolytic actinoporins such as fragaceatoxin C (FraC) that are able to form well-defined (octameric) oligomers that nevertheless incorporate lipid molecules. The inclusion of lipids in PFP assemblies confers heterogeneous and potentially flexible properties on the pores, and the evolution of such modes of assembly suggests they have been of selective advantage.

Examples of such adaptive advantage are then explored for another class of α -helical pore formers, the Bax family of proteins. In a pair of reviews, by Ugarte-Uribe and Garcia-Saéz, and by Uren and colleagues, the mechanisms of the homologous proteins Bak and Bax are extensively and expertly discussed. This pair of papers is another example of a measure of consensus – here, with respect to the role of lipids in pore formation – while maintaining alternative perspectives on exactly how this is achieved.

Willems and colleagues then discuss the application of PFPs in single-molecule nanopore enzymology, namely ways in which we can adapt nanopores as biosensors by using the additional activities conferred by enzymes trapped within them.

By harnessing self-association, PFPs achieve in a spontaneous way a property for which cells have evolved dedicated machinery. Botos and colleagues discuss one example of such cellular machinery, the BAM complex, which is used by bacteria to thread β -barrel proteins into membranes. Their discussion is paired with one that focuses on the insertion of lipopolysaccharide also into the bacterial outer membrane.

Computational methods are of particular value when addressing membrane structure and function and protein-membrane interactions. The effectiveness with which theoretical and experimental data converge on ways of understanding membrane biology was noted during the meeting. Lipkin and Lazaridis discuss such computational approaches to gain an understanding of the interactions of pore-forming peptides with membranes, which (again) suggests a structural role for lipids in pore-forming assemblies. These studies are of relevance for understanding the possible pore-forming activity of β -amyloid in Alzheimer's disease.

The capacity of protein-membrane interactions to be modelled computationally is one reason why *de novo* design has proved to be an effective strategy for the development of membrane proteins with novel properties. Joh and colleagues discuss the development of Rocker, a $\text{Zn}^{2+}/\text{H}^{+}$ antiporter, and experimental data demonstrating its functional properties. In a complementary fashion, Niitsu and colleagues discuss the design and development not of transporter systems but of stable transmembrane barrels, from first principles.

Such approaches to the engineering of functionally useful pore structures in membranes were complemented at the meeting by discussions of DNA-based structures and carbon nanotubes. The nanotube example is represented by the final paper in this volume, by Zhang and colleagues. Such

structures offer both complementary and contrasting properties when compared with biological pores, which are discussed here with respect to their real-time dynamics in membranes: a topic of emerging interest to those who have previously confined their investigations to protein pores, such as listeriolysin, perforin and α -hemolysin.

We greatly enjoyed organising this Royal Society Discussion Meeting and found it to be a most effective way to bring together an international body of expertise in the field of pore-forming proteins. We hope that readers will find this Issue of the Philosophical Transactions Series B an interesting exploration of these fascinating molecules, which reveals both their diversity and common properties.