In the two years since the last issue of COSB Membranes, the structural biology of membrane proteins has continued to make seminal contributions to our understanding of this fundamental class of proteins. The editors then pointed to “new kids on the block”, referring at the time to the formidable progress made by cryo-electron microscopy and single particle analysis in deriving structures to high resolution. The electron microscopy revolution is marching on and, on par with X-ray crystallography, is driving the field of membrane protein structural determination. Moreover, exciting innovations in areas such as atomic force microscopy and cryo-electron tomography are being utilised to probe the structure and dynamics of membrane proteins and complexes to a deeper level. The 2016 Membranes issue features contributions from experts in a wide range of methodologies and emerging areas of endeavour, interspersed with structural highlights.

Contributions from X-ray crystallographers include that of Shen and co-workers with the description of an extraordinary structure of a photosystem I (PSI) in a complex with light harvesting complex I (LHCI), a supercomplex totalling over 600 kDa in mass. LHCI harvests light energy and transfer them to the PSI core to initiate charge separation and electron transfer reactions. The complex is of mind-boggling complexity with 16 subunits cooperating with 205 cofactors including 155 Chlorophils, 35 carotenoids, five luteins and four violaxanthins (Vios)], three Fe4S4 clusters, two phylloquinones to form a supremely efficient light capturing and chemical conversion machine essential to life on earth.

Cryo-electron microscopy has become an immensely powerful technique to derive near-atomic models of membrane proteins and protein assemblies. However, as for X-ray crystallography, it requires prior stabilization in detergents and purification, which is not always possible. Much more convenient would be the possibility of imaging directly proteins in their native membrane environment. This is possible using electron tomography but progress has been slow due to the poor resolution achievable until now. However, with the advent of direct electron detector, progress is on the cards. More is hoped for with technical breakthroughs in energy filters and phase plates. In this issue, Gold and Kudryashev present the latest structural characterisation of a pilus biogenesis machinery that produces type IV pili at the surface of bacteria. Unique insights are provided on an assembly system which many researchers (including one of the editors) have failed to extract intact from the membrane.

Intriguingly, AFM has become an important player in investigating membrane proteins and we solicited a contribution to one of the experts in the field, Bart Hoogenboom. He and Saibil imaged the pore forming toxin cytolysin by AFM and exploited clever engineering to not only image the pore in membranes but also the pre-pore to pore transition, providing unique insights into this remarkable transition from a soluble protein to a vast assembly pore embedded in the membrane bilayer.

The membranes surrounding and compartmentalising cells are characterised by an asymmetric distribution of lipids across the twin leaflets of the bilayer. Scramblases randomise the lipid distribution in response to cellular cues such as second messengers. In this issue, Brunner, Schenk and Dutzler describe unprecedented insights into the rapid, passive, process of bilayer scrambling gleaned from the first crystal structure of a lipid scramblase.

Lipid association has been shown to influence the structure, function and dynamics of integral membrane proteins, yet the nature of the association is in most cases poorly characterised.

Precise criteria are likely to differ for each class of membrane protein and a critical step toward understanding has been the capacity to maintain protein-lipid interactions in the gas phase. Within these pages, Landreh, Marty, Gault and Robinson describe new innovations in mass spectrometry that make it possible to identify specific lipid interactions and to differentiate co-purified endogenous lipids from preferentially bound lipids and weakly interacting annular lipids, opening new windows onto protein function.

Receptor signalling is mediated by agonists binding to receptor ectodomains and consequential conformational or molecular changes. The process by which these signals are transmitted across the membrane is less clear-cut. For single pass receptors such as receptor tyrosine kinases, there is evidence that the information transfer is mediated by association into higher order structures within the membrane. Technical challenges in studying either complete receptors or single-pass transmembrane segments have hindered progress in elucidation of mechanism. In a skilfully crafted review, Trenker, Call and Call outline bilayer-based approaches to structure determination of receptor transmembrane complexes and provide perspective on the type of interactions between juxtaposed membrane domains likely to facilitate clustering and regulate signal propagation.

Distinct from cryo-electron microscopy, electron crystallography has particular advantages for structural analysis of membrane proteins. A timely and informative overview by Abe and Fujiyoshi appearing in this issue employs illuminating examples to highlight recent advances in electron crystallographic studies of membrane proteins, describing the importance of the lipid bilayer as more than a simple fluidic “stage” for the membrane protein. The review draws attention to the ways in which the membrane may affect protein structure and/or function and, conversely, how proteins may affect membrane qualities such as curvature. In closing, the authors introduce a conceptual strategy for single particle cryo-electron microscopy analysis of membrane proteins in a lipid environment.

A triumph of recent times has been elucidation of a suite of structures of the ryanodine receptor, at approximately 5,000 residues the largest and most complex of ion channels, in discrete functional states. In this issue, Clarke and Hendrickson discuss how 3-D classification of cryo-electron microscopy images has enabled the architecture and gating mechanism of the ryanodine receptor to be illuminated in unprecedented detail. Amongst the conceptual advances facilitated by these structures is one of ‘priming’ by activating ligands, where ligand binding elicits conformational changes that preface dilation of the mouth of the Ca^{2+}-selective pore.

We are grateful to the contributing authors for the revealing insights into membrane biology and methodology described in their reviews. The advances described presage even greater discoveries as cryo-electron microscopy becomes more widely accessible and a suite of innovations become integrated into the toolkit of the structural biology community.