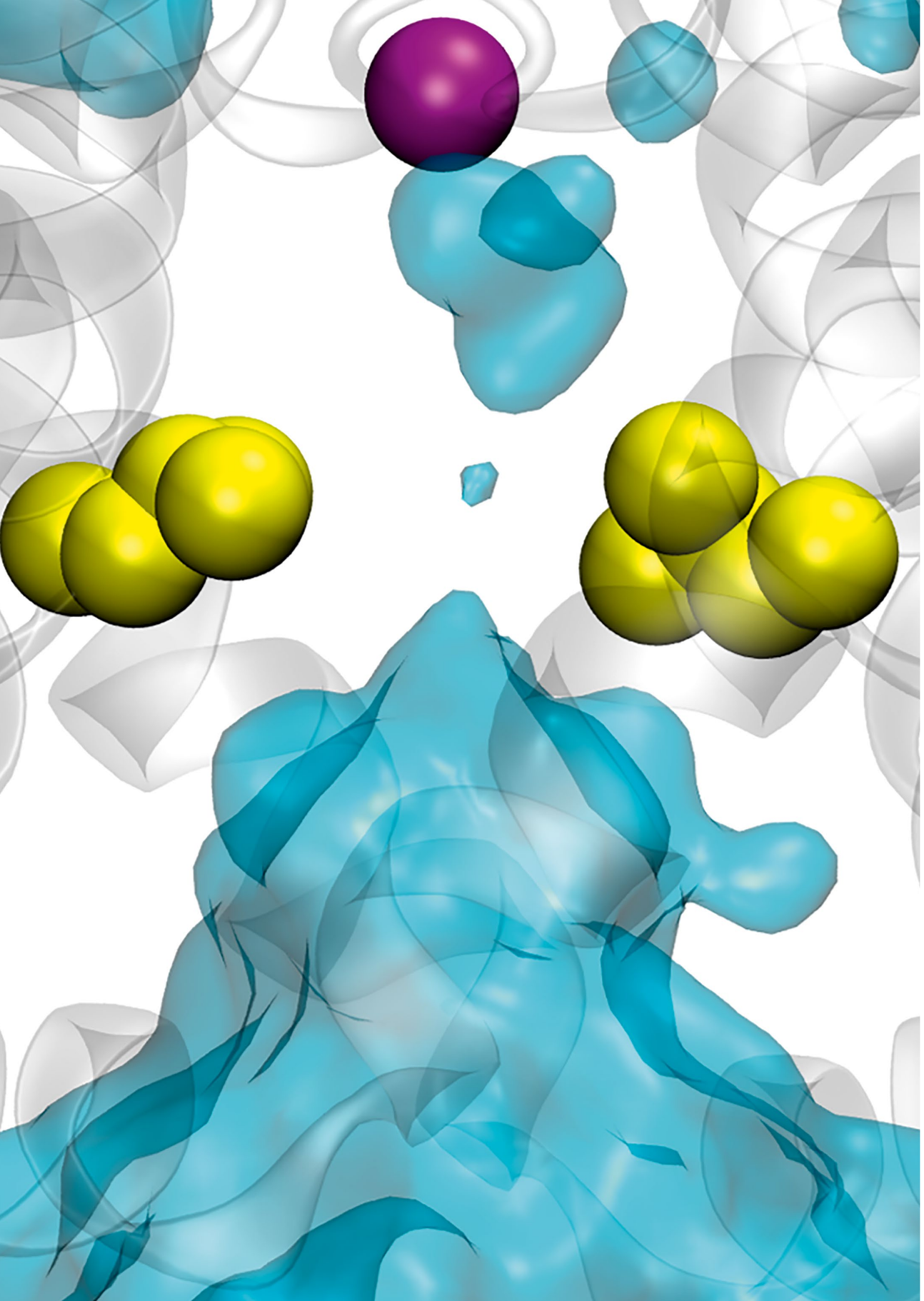

From ion channel structure to function: computational tools to annotate membrane protein structures

Professor Stephen Tucker and **Professor Mark Sansom** are leading researchers in the field of membrane biophysics. One of their current joint research projects focuses on the development of an easy-to-use method that exploits the unusual behaviour of water in order to identify and characterise hydrophobic gates in novel channel structures. This research, performed at the University of Oxford, offers new insight into the regulation and function of ion channels. The ultimate goal of this project is to provide an automated web-accessible tool to assist researchers in annotating the many new ion channel structures that are now beginning to emerge.

Ion channels are pore-forming membrane protein complexes whose primary function is to facilitate the movement of ions across biological membranes. In other words, ion channels create a pathway for charged ions to pass through cell membranes.

ION CHANNELS AND THEIR ROLE IN CELL MEMBRANES

To understand the importance of ion channels, one needs to consider that virtually every cell in the body is electrically active, but unlike the electronic currents that power our daily lives, 'biological electricity' involves the movement of charged ions such as Na+, K+ and Cl- into and out of cells. It is the ability of these electrical signals to move rapidly over long distances between cells and within cells that underlies how nerves communicate with each other, how our muscles are controlled and also how our brains work.

In fact, almost every physiological process in the body is controlled or influenced in some way by biological electricity. However, cell membranes are lipid bilayers, normally impermeable to charged ions. Therefore, without these ion channels acting as pathways for ions across the membrane, biological electricity would not exist, and neither would we.

Nevertheless, these channels do not form continuously open, unregulated pores across the membrane because one of their most important functions is to be able to open and close (i.e., switch on and off, just like normal electrical switches) in response to specific signals. This process of opening and closing is known as 'gating', and gating stimuli can include other electrical signals detected as a change in the voltage across the membrane ('voltage gating'), mechanical signals ('mechano gating') or the binding of some specific molecule to the ion channel itself ('ligand gating'). Another important feature of ion channels is their 'selectivity' or ability to distinguish between different ions – this means that most ion channels will only allow specific ions to pass through their pores.

Research has shifted its attention to understanding and visualising transmembrane pathways, but also into identifying any barriers that exist within ion channel pores

VISUALISING ION CHANNELS

The fundamental role of ion channels is, therefore, self-evident, and research has shifted its attention to understanding and visualising their transmembrane pathways, and identifying any barriers or 'gates' that might exist within these pathways. In fact, controlling these barriers would allow the modification of cellular electrical signals by deciding when to switch them on and off. But why would this be important to researchers? The answer is simple – almost 50% of current drug targets are thought to reside within cell membranes and many of these targets are ion channels. Therefore, it comes as no surprise that great effort has been put into the development of new techniques to visualise their respective 3D structure in exquisite detail, furthering our understanding of their molecular mechanism at an atomic level, as well as understanding their role in physiology and disease.

THE CHALLENGE OF HYDROPHOBIC GATING

Recent findings building on theoretical work over the past decade, now suggest that the greasiness or 'hydrophobicity' of the central pore of an ion channel has a profound impact on the ability of ions to move through it, in some cases even preventing movement completely in a process known as 'hydrophobic gating'. This is because normally the ions on either side of a membrane are dissolved in water, but when water enters the narrow confines of a sub-nanometre hydrophobic pore (the typical size of an ion channel pore), then it can behave in very different ways to water in 'bulk' solution. In some cases, even if the pore looks wides enough to be open, if it is greasy or hydrophobic enough then

it can repel water and dry out to form a hydrophobic gate thereby switching off the electrical current. In fact, research has indicated that biological ion channels may also exploit hydrophobic gating to regulate ion flow within their pores. Therefore, the physical dimensions of a pore cannot always be considered as a reliable indicator of the whether an ion channel is open or closed.

Recent technological advancements now allow us to directly visualise the 3D structure of many new ion channels. However, it is often difficult to predict whether these channels are open or closed. Therefore, there is a need to develop innovative tools that can accurately predict whether these pores will allow ions to pass through them. Several methods have been developed in the past to analyse ion flow but these are computationally demanding. This is where the work of Prof Tucker, group leader in the Biological Physics group, Department of Physics at the University of Oxford, comes in.

MOLECULAR DYNAMICS SIMULATIONS

In collaboration with Prof Mark Sansom and his team in the Department of Biochemistry at Oxford, Prof Tucker has recently developed powerful computational methods that can accurately and efficiently model the behaviour of water within these nanometre-sized pores, allowing for distinct predictions of potential hydrophobic barriers. In fact, their method of implementing 'Molecular Dynamics' simulations can be practically considered as a 'computational microscope' to visualise the behaviour of water in a pore, and thus predict its conductive status.

The development of this new computational tool is based on the assumption that the ability of a pore to become hydrated represents a reliable indicator of permeability. Therefore, various experimental approaches, including direct changes to the 'wettability' of the channel, have allowed Profs Tucker and Sansom and their team to analyse several new structures. These include the identification of a hydrophobic barrier deep within the inner pore of a K2P potassium channel and the existence of a hydrophobic gate within the narrow neck of Bestrophin chloride channel. The underlying aim of this current project is to develop analytical and visualisation tools that will be fast, easy to use, freely available and capable of integrating with existing analytical pipelines such as MemPROTMD (http://memprotmd.bioch.ox.ac.uk/).

Overall, these 'channel annotation' simulations of water developed by Profs Tucker and Sansom can be used as a proxy to accurately predict hydrophobic gates in novel channel structures, thus providing major new insights into the way channels are regulated. The team expects to have a version of this tool ready for distribution and beta-testing by the end of the year, thus satisfying the rapidly growing demand for such tools within the membrane protein structural biology community.

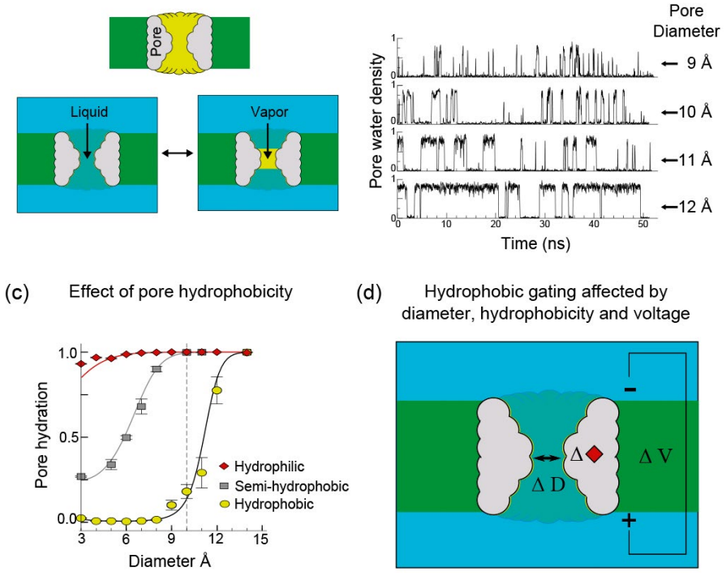

Figure 1: Principles of hydrophobic gating
(a) Cartoon representation of a cross-section through a model hydrophobic nanopore. Hydrophobic surfaces are shown in yellow, the membrane in green. In solution, these nanopores can switch stochastically between both wet and dry states via liquid-vapour transitions within the pore. The dewetted vapour state presents an effective barrier to water and ion permeation. (b) These oscillations occur on the nanosecond timescale, and the stability of the wetted state is highly dependent upon pore diameter. (c) The probability of the pore being in the liquid or wetted state is not only dependent upon diameter, but also the hydrophobicity of atoms lining the pore. This was shown by progressively adding hydrophilic atoms to a model nanopore. A fully hydrophilic pore remains fully occupied by water. However, a hydrophobic pore starts dewetting below 14 Å and becomes completely dewetted below ~8–10 Å. Semi-hydrophobic pores also exhibit similar dewetting below ~ 10 Å (dotted vertical line). (d) The process of hydrophobic gating has now been shown to be influenced by pore diameter, hydrophobicity and also changes in transmembrane voltage. This figure was first published by Elsevier under CC BY 3.0 licence: https://creativecommons.org/licenses/by/3.0/

The 'Molecular Dynamics Simulations' developed by Profs Tucker and Sansom and their team can be used as a proxy to accurately predict hydrophobic gates in novel channel structures

Q&A

Your research is highly innovative and could revolutionise how we 'see' ion channels. What are the future objectives for the team, especially when it comes to developing analytical methods for visualising hydrophobic gating?
At one level, we expect that our new tool will continue to be a 'work in progress'. In other words, we expect that it will continue to be updated as our understanding of these different processes becomes clearer. In particular, there are many different groups around the world who are continuously improving the computational models that define the behaviour of water. We are therefore designing our platform to be able to incorporate new developments in this field as and when they appear.

What are the technological conditions that will allow us to directly visualise the behaviour of water in such nanometre-sized pores?
Water is an incredibly complex fluid and very difficult to visualise directly. It is both highly mobile and also very small, therefore it becomes almost invisible in many of the techniques used to determine protein structures. However, we not only want to see it, but we also want to understand how it moves and behaves within channel pores. At the moment, the major way this is done is to produce atomic scale models of the protein with water and ions and to simulate their behaviour using molecular dynamics.

How easy and accessible will this beta version of the platform be for researchers?
We are aiming to produce several versions of this tool. One will be more like an 'app' that even the most inexperienced of users should be able to use, either by downloading it onto their desktop computer, or through a web interface. The other version will be almost identical, but one where more expert users can play with conditions themselves. We also expect that the tool will become part of the current MemProtMD pipeline which automatically analyses all new ion channel and membrane protein structures as they are released into the PDB database. But we expect that some of the more exciting developments will come from just allowing people to play with the tool themselves.

What is the most important new structure that you have analysed, which can aid drug research?
In our initial study published in the journal *Structure* we analysed new structures of the 5-HT3 receptor which is the target of potent drugs that alleviate the nausea and vomiting associated with chemotherapy, and which is also involved in various psychiatric disorders. In the same study, we also examined three different structures of the Glycine Receptor which is very important for transmission of nerve signals between cells and which is found to be defective in many neurological diseases. However, this technique can be applied to any ion channel protein from any organism, and so we hope that it will have many broad applications from looking at how drugs affect ion channels in the human brain to how drugs might be used to treat diseases caused by bacteria or fungi.

Let us imagine that we are, ultimately, capable of controlling ion channels and their behaviour. How important is that for the scientific community, and what are the prospective advantages and dangers of implementing such research in clinical trials?
Ion channels either directly control, or indirectly influence, almost every single process in the human body, from the way our brains work to the way our muscles move and our kidneys function. Therefore being able to influence ion channel behaviour presents immeasurable opportunities to influence human health and disease. However, because ion channels are everywhere and can often be involved in controlling several different pathways, one of the greatest challenges in current drug development is the ability to selectively target the channel you are interested in without affecting others.

Detail

RESEARCH OBJECTIVES

Prof Tucker's research focuses on understanding the intimate relationship between ion channel structure and function. For many years he has worked in close collaboration with Prof Sansom whose research employs computational techniques to explore the structure and function of ion channels and related membrane proteins.

FUNDING

Biotechnology and Biological Sciences Research Council (BBSRC)

BIO

Prof Tucker received his undergraduate and graduate degrees in Biochemistry from the University of Oxford. He then held a succession of Research Fellowships from the Wellcome Trust and the Royal Society before becoming a Professor of Biophysics in 2015. Prof Sansom also studied Biochemistry in Oxford where he is now the Head of Department and also the David Phillips Professor of Molecular Biophysics.

CONTACT

Prof Stephen J Tucker
Clarendon Laboratory
Department of Physics
University of Oxford
Oxford
OX1 3PU
UK

E: stephen.tucker@physics.ox.ac.uk
W: https://biophysics.physics.ox.ac.uk/tucker/

Prof Mark S P Sansom
Department of Biochemistry
University of Oxford
Oxford
OX1 3QU
UK

E: mark.sansom@bioch.ox.ac.uk
W: http://www.bioch.ox.ac.uk/