

# 7. Mark S. P. Sansom

## Membrane Proteins: Structural Bioinformatics and Computational Biochemistry

### Group Members

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### Overview

- **Why membrane proteins?**

Membrane proteins are central to many, if not all, cellular processes. In particular they play key roles in signalling between cells, in transport across cell membranes, and in energy transduction processes. From a post-genomic perspective, their importance can be assessed from the observation that ~25% of genes code for membrane proteins. Thus there are ~8000 membrane proteins encoded within the human genome. From a biomedical perspective, one may note that membrane proteins constitute ~50% of possible targets for novel drugs, ranging from drugs acting on the nervous systems to novel antimicrobial therapies.

- **Why computational methods?**

Despite the importance of membrane proteins, they remain under-explored territory. High resolution structures are known for ~50 membrane proteins, in contrast to ~22,000 structures for water soluble proteins. However, the number of structures of membrane proteins is starting to grow exponentially. Computational methods play an essential role in understanding the relationship between structure and function of membrane proteins. Such studies can be divided into three broad categories:

- a. simulation and theory to aid understanding of the relationship between (static) crystallographic structure and (dynamic) physiological function of membrane proteins;
- b. modelling and simulation to develop accurate models of e.g. human membrane proteins based on the structures of bacterial homologues; and
- c. structural bioinformatics and simulation to aid in prediction of the folds of membrane proteins *ab initio* in an attempt to bridge the sequence-structure gap.

- **Unit Website:** [sccb.bioch.ox.ac.uk](http://sccb.bioch.ox.ac.uk)

### Ion Channels, Receptors & Transporters

- **Overall aim: from physics to physiology**

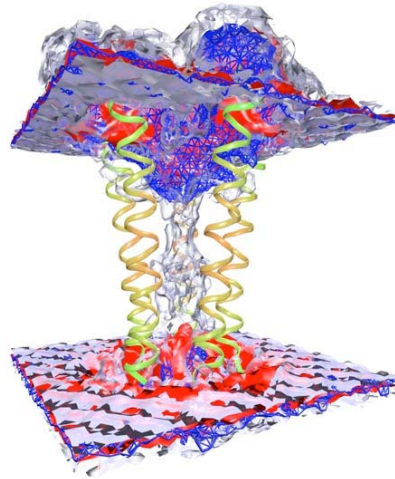
Ion channels play a central role in the physiology of both excitable (e.g. neurones, muscle, heart) and non-excitable (e.g. pancreas, bacteria) cells. They allow rapid (~10<sup>7</sup> ions s<sup>-1</sup>) flux of selected ions through a transmembrane pore. Channels are gated, i.e. regulated, by a number of factors including e.g. transmembrane voltage and/or binding of ligands to extra-membranous domain. The overall aims of computational studies of ion channels in my research group are:

- a. to understand fundamental physical mechanisms of ion channel processes, including permeation, selectivity and gating; and
- b. to relate atomic resolution structures of ion channels to their physiological function, with a longer term aim of prediction of the functional consequences of channel mutations.

- **Which systems?**

Our research embraces a number of channels and related membrane proteins. In particular, the current focus is on:

- a. potassium channels, both voltage gated (Kv) and ligand/protein gated (Kir);
- b. nicotinic acetylcholine receptors (see Fig. 1) and related 'cys-loop' receptor-channels; and
- c. glutamate receptors, with an especial emphasis on gating mechanisms. In addition to ion channels, we have started to investigate a number of transport proteins which although mechanistically more complex than channels, may be addressed by similar computation strategies. Systems currently under investigation include ABC transporters, lactose permease, and AcrAB.



**Figure 1.** Water and ions within the pore of the nicotinic acetylcholine receptor (image courtesy of Oliver Beckstein).

- **Understanding physical processes**

In this area, our research has focussed on the properties of ions and water when confined within transmembrane pores of sub-nanometer dimensions. In particular, we are interested in how the dynamic behaviour of water and ions within pores may differ from that in the bulk state. Such basic information is fundamental to an accurate (and hence predictive) understanding of the relationship between single channel structure and function.

- **From structure to physiological function**

The main research challenge here is to develop conceptual and computational approaches that enable us to bridge several timescales, from sub-ns protein motions that are essential to smoothing the energy landscape for ion permeation, to ms timescale motions involved in channel gating. In this way it should be possible to construct a bridge from atomic resolution structure to single channel (i.e. patch clamp) physiological measurements. This in turn provides a paradigm for a rigorous approach linking structure with function for a range of more complex transport proteins. Potassium channels provide a focus for developing this approach as a wealth of physiological data is available, in addition to a number of (bacterial) structures.

- **From bacterial to human models**

The majority of membrane protein structures are of bacterial homologues of membrane proteins. It is therefore essential to develop accurate models of the corresponding human membrane proteins and to assess the extent to which simulations may be used to relate (model) structure to physiological function. Having achieved some success in this area with K channels we wish to build upon this approach, extending it to a wider range of membrane proteins. A component of this will involve development of a high-throughput pipeline for modelling and simulation of whole families of human membrane proteins based on bacterial homologue structures.

- **Receptors: gating mechanisms**

Neurotransmitter receptors provide a focus for studying the gating of ion channels. Two aspects of gating are currently being investigated:

- a. the fundamental properties of hydrophobic gates, in terms of delineation of the effectiveness of a gate in terms of its radius, hydrophobicity and flexibility;
- b. mechanisms of triggering conformational transitions upon ligand binding, focussing on ligand-induced changes in conformation and dynamics in glutamate receptors and related proteins.

- **Transporters: greater complexity, which systems**

A number of transporters (e.g. ABC transporters, lactose permease, AcrAB) appear to operate via a mechanism whereby access to (i.e. gating) of a central pore is coupled to solute translocation. The conformational changes underlying this are relatively slow (~1 ms) compared to the timescales of conventional molecular dynamics simulations (~10 ns). A number of more advanced simulation techniques, ranging from steered simulations to coarse-grained simulation models, will be required to address the mechanisms of these transporters.

## **Bionanoscience of Pores**

- **Theoretical bionanoscience: an overview**

Ion channels and related membrane proteins create nanopores in biological membranes. Therefore theoretical and computational studies of such proteins provide insights into the design principles of nanopores in general. One aspect of current research is to explore the interface between membrane protein biology and nanoscience, with a longer term view of aiding the development of membrane protein based nanotechnologies, such as receptor and channel based biosensors.

- **Physical properties: from nanopore models to biosensors**

Simulation and theoretical studies are being used to understand the physical behaviour of water and electrolytes within nanopores (see above). In collaboration with colleagues undertaking experimental studies (H. Bayley, Chemistry, Oxford) my group is exploring how changes in the pore dimensions and the chemical nature of the pore lining alter the behaviour of water and ions within nanopores.

- **Nanomechanical properties via simulation**

In order for membrane proteins to be exploited in nanotechnological applications we require an improved understanding of their nanomechanical properties. Simulations are being used to explore the behaviour of proteins under nanomechanical stress (e.g. compression in simulated AFM experiments, in collaboration with J. Davis, Chemistry, Oxford), and under non-native conditions (e.g. in non-bilayer environments). These studies will lead to a better understanding of the robustness of selected membrane proteins to changes in their physical environment.

## **Bacterial Outer Membrane Proteins**

- **Importance of outer membrane proteins**

Bacterial outer membrane proteins (OMPs) have a diversity of roles (pores, transporters, enzymes) in the biology of Gram negative bacteria. They are potential targets for novel anti-microbial drugs and vaccines. A substantial number of crystallographic structures, and some from NMR, are known for OMPs and so they provide an opportunity for comparative simulation studies of membrane protein conformational dynamics in relation to both function and stability.

- **OMP conformational dynamics: dynamics, function, environment**

The structures of a number of smaller OMPs (OmpA, OmpX, PagP) have been determined both by protein crystallography and by NMR of mixed protein/detergent micelles. Extended MD simulations are being used to explore how the conformational dynamics of these proteins changes between the experimentally relevant environments (crystal or micelle see fig 2) and the biological environment (membrane). These studies are important in aiding extrapolation from experimentally determined structures to biological function.

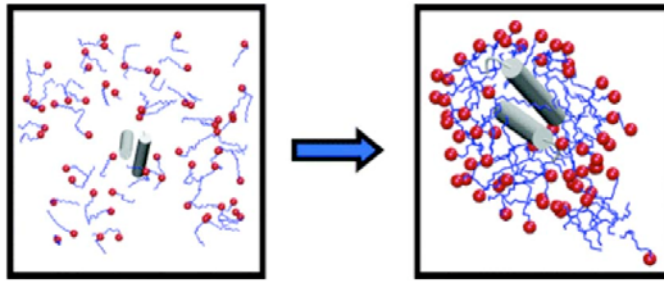


Figure 2. Self-assembly of a protein-detergent micelle by 'brute force' simulation (image courtesy of Peter Bond).

- **Towards a database of OMP simulations**

The OMPs provide an opportunity for large scale comparative studies of conformational dynamics within a rather disparate family of membrane proteins. To this end, high quality (multi-nanosecond) simulations are being performed on all OMPs for which structures have been determined (currently ~25 OMPs). Comparative analyses will exploit the BioSimGRID technology (see below) and will initially focus on e.g. protein dynamics vs. secondary structures, and on lipid/protein interactions.

- **Towards a virtual outer membrane: from atomistic to mesoscale modelling**

The complexities of transport across bacterial membranes make the outer membrane transport systems a viable test case for a systems approach to biomolecular simulation. However, to address emergent phenomena on larger length and time scales than is possible via 'brute force' atomistic simulations, some degree of coarse-graining will be necessary in order to develop suitable mesoscale models. Very large scale outer membrane outer membrane simulations are being performed (using the facilities at HPCx). These will be analysed with a view to parameterisation of mesoscale models.

## Structural Bioinformatics & Prediction

- **Structural bioinformatics of transmembrane helices**

The increase in number of membrane protein structures enables structural bioinformatics studies in order to discover general principles of membrane protein structure. Current studies are focussed on transmembrane (TM) helices for which ~400 structures are available, and include:

- analysis of accuracy of methods for prediction of TM helices and genome-wide application of optimised consensus prediction methods
- analysis of environmental (bilayer vs. non-bilayer) propensities of residue distributions and of residue-residue interactions between TM helices
- combined structural bioinformatics and simulation studies of distortions and flexibility in TM helices (e.g. due to proline residues)

- **Membrane protein/lipid interactions**

A combined database and comparative simulations approach is being used to probe the nature of interactions between membrane proteins and lipid molecules. A particular focus is on how amphipathic aromatic and basic residues help to 'lock' membrane proteins into their preferred transbilayer orientation.

- **Towards *ab initio* prediction of TM helix packing by simulation**

A number of simulation approaches (implicit solvent models, micelle simulations, replica exchange) are being combined to develop MD simulations as a 'brute force' approach to predicting TM helix packing as a component of *ab initio* fold prediction for (simple) membrane proteins.

## Technologies

- **Biomolecular simulation databases and GRID computing**

We are heading a UK consortium to develop a prototype distributed database for biomolecular simulation data (BioSimGRID: see [www.biosimgrid.org](http://www.biosimgrid.org)). This project will exploit the developing UK GRID/e-science infrastructure, and involves collaboration between 6 UK biomolecular simulation laboratories and 2 computer science departments.

- **Multi-level simulations and HPC**

We are heading a newly established collaboration between 4 UK simulation laboratories and 2 computer science departments to implement multiscale (from QM/MM through to mesoscale) simulations of membrane bound enzymes.

- **Integrative databases for membrane proteins**

A number of membrane protein databases (membrane protein structure, lipid/protein interactions, ABC transporters, glutamate receptors etc.) are being developed in my laboratory. The intention is to use e-science technology to integrate these to provide a single resource for membrane protein structural bioinformatics and simulation data.

## **Biomolecular Computational Systems Biology**

- **Modelling of complex transport and signalling systems**

There is a pressing need to develop methodologies that enable vertical integration between biomolecular and higher-level 'systems' descriptions of membrane proteins and their biological roles. A project is being initiated to explore different algorithmic approaches to bridging the gap between biomolecular and more conventional systems level descriptions of membrane biology. The initial applications will be to prokaryotic transport and signalling, and these are somewhat more data-rich. However, the technologies to be developed will also be applicable to integrated approaches to ion channels and related transport proteins in the context of a computational systems biology approach to cardiac function (work in collaboration with D. Noble, P. Hunter et al., Physiology, Oxford and Univ. of Auckland).

## **Recent Publications**

1. Bond, P.J., Cuthbertson, J.M., Deol, S.S. and Sansom, M.S.P. (2004) MD simulations of spontaneous membrane protein/detergent micelle formation. *J. Amer. Chem. Soc.* 126: 15948-15949
2. Deol, S.S., Bond, P.J., Domene, C. and Sansom, M.S.P. (2004) Lipid-protein interactions of integral membrane proteins: a comparative simulation study *Biophys. J.* 87:3737-3749
3. Campbell, J.D., Deol, S.S., Ashcroft, F.M., Kerr, I.D. and Sansom, M.S.P. (2004) Nucleotide dependent conformational changes in HisP: molecular dynamics simulations of an ABC transporter nucleotide binding domain. *Biophys. J.* 87:3703-3715 Tai, K., Murdock, S., Wu, B., Ng, M.H., Johnston, S., Fangohr, H., Cox, S.J., Jeffreys, P., Essex,
4. J.W. and Sansom, M.S.P. (2004) BioSimGrid: towards a worldwide repository for biomolecular simulations *Org. Biomol. Chem.* 2: 3219-3221
5. Beckstein, O., Tai, K. and Sansom, M.S.P. (2004) Not ions alone: barriers to ion permeation in nanopores and channels. *J. Amer. Chem. Soc.* 126:14694-14695
6. Baaden, M. and Sansom, M.S.P. (2004) OmpT: molecular dynamics simulations of an outer membrane enzyme. *Biophys. J.* 87: 2942-2953
7. Domene, C., Bond, P.J., Deol, S.S. and Sansom, M.S.P. (2003) Lipid-protein interactions and the membrane/water interfacial region. *J. Amer. Chem. Soc.* 125:14966-14967.
8. Faraldo-Gómez, J.D., Smith, G.R. and Sansom, M.S.P. (2003) Molecular dynamics simulations of the bacterial outer membrane protein FhuA: a comparative study of the ferrichrome-free and bound states. *Biophys. J.* 85:1-15
9. Bond, P. and Sansom, M.S.P. (2003) Membrane protein dynamics vs. environment: simulations of OmpA in a micelle and in a bilayer *J. Mol. Biol.* 329:1035-1053
10. Beckstein, O. and Sansom, M.S.P. (2003) Liquid-vapor oscillations of water in hydrophobic nanopores. *Proc. Natl. Acad. Sci. USA* 100:7063-7068