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Preface

Structural and biophysical characterisation of membrane protein–ligand binding

A detailed understanding of membrane protein structure and function remains of profound importance on many fronts, not least of which includes drug discovery. In a post-genomic era it is now well established that a sizeable 20–30% of eukaryotic, eubacterial and archaean open reading frames encode integral membrane proteins. Furthermore, membrane proteins constitute approximately 49% of current therapeutic targets in higher organisms, providing evidence of their strong potential as targets for new drugs of the future. A detailed understanding of how agonist and antagonist ligands interact and bind with membrane protein drug targets is important for the development and refinement of new and novel therapeutics. Although commercially-available drugs targeting membrane proteins have so far been generated mainly through conventional means (high throughput screening, functional assays etc.), the availability of structure-based and/or biophysical techniques for investigating membrane protein–ligand interactions reveals the details of how ligands and potential new drugs bind. The purpose of this Special Issue is to provide a review of some biophysical techniques available for studying membrane protein interactions with ligands and drugs. It also reviews some of the latest developments in methods and approaches for structure elucidation, particularly focusing on optimisation of membrane protein crystal growth for structure determination using X-ray crystallography, one of the pre-requisites for structure-based investigations of ligand binding. Utilisation of the important techniques of solid- and solution-state NMR for structural studies of membrane proteins has already been described recently in another Special Issue in this journal, and therefore the technique has not been included here; the reader is therefore referred to the *BBA Biomembranes* Special Issue 'NMR Structural Studies on Membrane Proteins' 1768 (2007) issue 12.

In the lead article, Dr. Richard Ward and Professor Graeme Milligan describe how resonance energy transfer and fluorescent labelling techniques have been applied to ligand identification and binding studies of G protein-coupled receptors (GPCRs). They also describe how the identification of GPCR ligands using these techniques has aided progress in structural determinations of these proteins, as successful crystal trials have usually been performed in the presence of receptor ligands to promote receptor stabilisation. A description of how widely fluorescence spectroscopy techniques have been used for studies of GPCR proteins is presented by Dr. Rajashri Sridharan and colleagues. They present an overview of the extensive array of fluorescent ligands that have previously been developed and how studies with such ligands have contributed to our understanding of the diversity of receptor conformations adopted through binding by different ligands. The use of circular dichroism (CD) spectroscopy for investigating membrane protein–ligand interactions, and the particular advantages of using synchrotron radiation CD for studies of purified membrane proteins available in only limited supplies are aspects addressed in the chapter by Dr. Giuliano Siligardi, Dr. Rohanah Hussain and colleagues. Use of synchrotron radiation to generate CD spectra has enabled increased accuracy in secondary structural information due to the extended spectral range that can be reliably acquired in the far-UV region. Also described is the recent collaborative work between my own laboratory and the Diamond Light Source, which demonstrated for the first time the suitability of CD spectroscopy for obtaining quantitative binding data for ligand binding to a membrane protein, in this case a bacterial membrane histidine protein kinase. This work also revealed the conditions necessary for ensuring the stability of purified proteins prior to obtaining spectra and therefore this sensitive technique has subsequently proved useful in structural determination efforts as a screening technique to monitor sample stability prior to crystallisation trials. Dr. Simon Patching discusses surface plasmon resonance (SPR) spectroscopy, a rapidly developing technique in the field of membrane protein–ligand interactions. A wide range of protein examples are described, including representatives from several protein families, and including GPCRs, a range of human and bacterial receptors, an ABC transporter and α-haemolysin. The chapter also focuses on the potential of the method for future drug discovery. Dr. Allison Whited and Professor Paul Park discuss atomic force microscopy (AFM), including the basic principles of the technique and how it has evolved for visualising single membrane protein–ligand complexes and conformational changes elicited through ligand binding. They also describe how AFM can be used to characterise interactions through dissociation of membrane protein–ligand complexes by force. The well-established technique of isothermal titration calorimetry (ITC) is described in detail by Professor Krishna Rajarathnam and Dr. Jörg Rösgen. Its application for studies of membrane protein–ligand interactions is emerging; fewer examples of membrane protein–ligand studies are currently available. However, the authors provide a comprehensive description of conditions that have proved successful so far in ITC studies of membrane proteins. They describe the effects of detergent choice and considerations of protein/ligand concentrations that have proved successful during ITC measurements. They go on to describe several examples of ligand binding studies involving mammalian proteins, including chemokine binding to the human GPCR CCR5 and agonist and antagonist binding to a human ion channel, as well as a host of bacterial examples. Finally, Dr. Isabel Moraes and colleagues discuss structure determination, focusing particularly on recent developments in methods and strategies for membrane protein crystal production (including choice of detergents, rational design of crystallisation screens based on knowledge of membrane proteins already successfully crystallised and use of crystal dehydration techniques). They also describe ways in which synchrotron radiation and free electron lasers have recently been applied to enable data collection from small fragile crystals of membrane proteins.

A striking feature that emerges from many of these articles in this Special Issue is the rapidity with which new applications of some

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well-established biophysical techniques are emerging for investigations of membrane proteins. Not only are these advances occurring at a rapid rate, but some provide the possibility of improved or modified approaches for structure determination, for example, by identifying improved conditions for promoting successful growth of protein crystals. In turn, of course, the reverse is also true; it is well established that structural details at atomic resolution provide a valuable source of data for detailed ligand binding studies. What is clear is how inter-linked and inter-reliant the structural and biophysical methodologies are for progress; it seems clear that future advances in the field of membrane proteins are served well by continued utilisation and progress in all the techniques described in this Special Issue—and others too. These approaches go hand in hand, and the future looks rosy. My thanks to all the contributing authors of this Special Issue for their hard work and excellent contributions.

Dr. Phillips-Jones is an expert in the field of bacterial two-component signal transduction systems, with a specific interest in the mechanisms by which environmental ligands initiate phosphorylation-based signalling in the membrane sensor kinase components of these regulatory systems. She spent 16 years at the Astbury Centre for Structural Molecular Biology, University of Leeds (UK) where she was Senior Lecturer and in 2012 joined the School of Pharmacy & Biomedical Sciences, University of Central Lancashire (UK) where she is using purified intact membrane sensor kinases in *in vitro* activity assays, SRCD spectroscopy and other biophysical approaches to identify and characterise interacting ligands and inhibitors.

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