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Review

Membrane protein structure determination — The next generation ☆,☆☆

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ABSTRACT

The field of Membrane Protein Structural Biology has grown significantly since its first landmark in 1985 with the first three-dimensional atomic resolution structure of a membrane protein. Nearly twenty-six years later, the crystal structure of the beta2 adrenergic receptor in complex with G protein has contributed to another landmark in the field leading to the 2012 Nobel Prize in Chemistry. At present, more than 350 unique membrane protein structures solved by X-ray crystallography (http://blanco.biomol.uci.edu/mpstruc/exp/list, Stephen White Lab at UC Irvine) are available in the Protein Data Bank. The advent of genomics and proteomics initiatives combined with high-throughput technologies, such as automation, miniaturization, integration and third-generation synchrotrons, has enhanced membrane protein structure determination rate. X-ray crystallography is still the only method capable of providing detailed information on how ligands, cofactors, and ions interact with proteins, and is therefore a powerful tool in biochemistry and drug discovery. Yet the growth of membrane protein crystals suitable for X-ray diffraction studies amazingly remains a fine art and a major bottleneck in the field. It is often necessary to apply as many innovative approaches as possible. In this review we draw attention to the latest methods and strategies for the production of suitable crystals for membrane protein structure determination. In addition we also highlight the impact that third-generation synchrotron radiation has made in the field, summarizing the latest strategies used at synchrotron beamlines for screening and data collection from such demanding crystals. This article is part of a Special Issue entitled: Structural and biophysical characterisation of membrane protein-ligand binding.

Contents

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1. Introduction

Membrane proteins play a vital role in many critical biological processes. Nearly 30% of proteins in eukaryotic cells are known to be membrane proteins [1]. Mutations or improper folding of these proteins is associated with many known diseases such heart disease, cystic fibrosis, depression, obesity, cancer and many others. Currently approximately 60% of available drugs target membrane proteins of which G protein-coupled receptors (GPCRs) and ion channels constitute the largest groups [2,3]. Although most of the drugs commercially available have emerged through conventional drug discovery methods such as high-throughput screening (HTS), computational methods and functional assays, it is the structural information provided by the three-dimensional (3D) atomic structures that discloses details regarding the binding mode of such proteins. This information is critical in the rational design of better drugs with improved selectivity and pharmaceutical properties [4–7]. Since X-ray crystallography has been the only tool capable of delivering detailed empirical information on protein structures at atomic level, its use in drug discovery programs became popular and well established.

The first membrane protein structure solved by X-ray crystallography was reported in 1985 [8]. Since then more than 300 unique membrane protein structures have been solved using the same method (http://blanco.biomol.uci.edu/mpstruc/exp/list, Stephen White Lab at UC Irvine). Many high-resolution 3D structures of integral membrane proteins have proven to be fundamental for a better understanding of many biological processes [9–11]. Most recently the crystal structure of the beta2 adrenergic receptor in complex with the G protein was solved by Kobilka's group [12]. This structure has made an enormous contribution not only to biology but also to drug discovery by revealing the mechanism of action of GPCRs at the molecular level. However in spite of recent successes, the path to a high-resolution structure of a membrane protein still involves several bottlenecks including poor expression, limited extraction success, low purification yields and paucity of well-ordered 3D crystals (Fig. 1). Yet, the field of membrane protein structural biology is in a "log" phase. In recent years much effort has been put toward innovative developments to overcome the numerous obstacles associated with X-ray structure determination of membrane proteins. For instance much progress has been made regarding: (i) overexpression of recombinant membrane proteins in different expression hosts [13–18]; (ii) development of new detergents and lipids for more efficient solubilization and crystallization [19–22]; (iii) improvement in protein stability through mutations, deletions, engineering of fusion partners and monoclonal antibodies, to promote diffraction quality crystals [23–27]; (iv) developments in automation, miniaturization and integration which have contributed to the increasing number of initial crystallization conditions and crystal optimization strategies [28]; and (v) in synchrotron radiation and beamline developments [29]. This article provides an overview of the most recent advances regarding the growth of membrane protein crystals and how to best assess crystal quality-diffraction in a high-throughput fashion using synchrotron radiation.

2. Overview of detergents

Detergents play a vital role in membrane protein structure determination. They are essential during the processes of solubilization, purification and crystallization. Once the protein of interest has been expressed at the required levels, it is usually necessary to extract it from its membrane environment. The biological membrane is a complex mosaic lipid bilayer in which membrane proteins interact

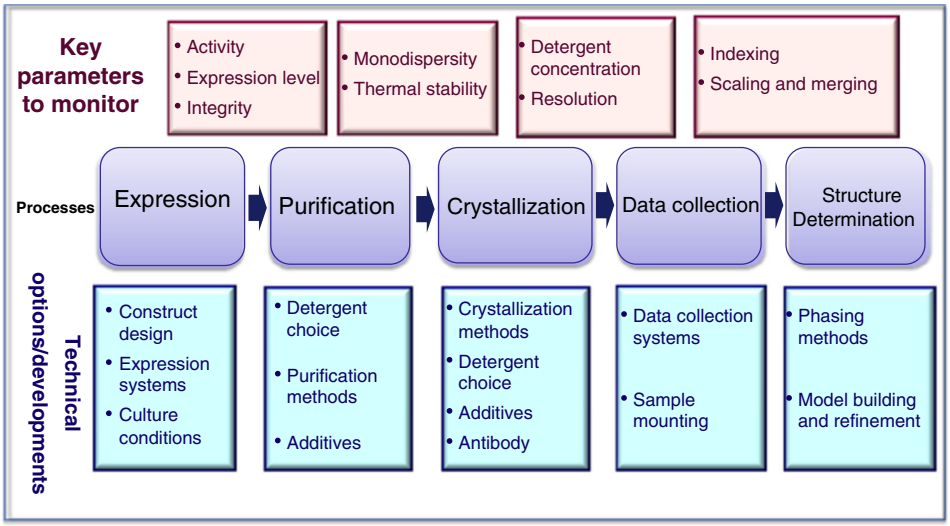

Fig. 1. Bottlenecks in membrane protein structure determination. Picture courtesy of Prof. So Iwata.