Preface

The past 5–10 years have brought major developments in our understanding of G protein-coupled receptors (GPCRs), one of the most important families of therapeutic drug targets. Drugs acting directly on GPCRs still represent nearly 30% of the most commonly prescribed medicines, including many of the new chemical entities currently being approved.

Perhaps the most stunning advance in the GPCR field has been the deluge of crystal structures of receptor 7TM domains. Starting with only a single structure (rhodopsin) in 2000, research over the past decade has provided many new structures (e.g., β_1 and β_2 adrenergic, A_{2a} adenosine, D_3 dopamine, and CXCR4 chemokine receptors) with both agonist- and antagonist-bound versions represented. Further, with the availability of several new technical approaches to the crystallization of GPCRs, many new structures should be appearing in rapid order. The developments that have facilitated these structural advances, as well as the meaning and implications of this new knowledge, are outlined in the first chapter of this volume by Congreve and colleagues.

The field of orphan GPCRs has also provided a rich area for pharmaceutical discovery. While the numerical pace of deorphanization may have decreased from its heyday, important new information is emerging. Three examples are represented in this volume. The hydroxycarboxylic acid receptors (e.g., nicotinic acid receptor), free fatty acid receptors, and GPR55 which responds to several lipid ligands are all revealing new information of potential importance in physiology, pathophysiology, and therapeutics.

A number of new or newly exploited pharmacological concepts have also advanced rapidly in recent years. The identification and understanding of allosteric modulators of GPCRs has made a number of challenging receptors pharmacologically accessible. This includes both "standard"

In this chapter, we review recent technology developments, the new structures available, and their application to drug discovery.

II. Technology Developments Enabling GPCR Structure Determination

Until recently, progress in obtaining structures of GPCRs was very slow, with 7 years elapsing between the solving of the structure of bovine rhodopsin (Palczewski et al., 2000) and the solutions of the β_2-adrenergic receptor (β_2AR) (Cherezov et al., 2007; Rasmussen et al., 2007). There have been a number of technical difficulties which are now starting to be overcome, thereby enabling the recent increase in GPCR structures. First, large quantities of purified functional protein are required for structural studies. The low level of expression of GPCRs and their instability outside the membrane environment when solubilized with detergent are still major problem areas. For most structural programs, at least 200 mg of protein may be required to identify and refine the crystallization conditions (Kobilka & Schertler, 2008). However, even when sufficient protein has been produced, this has often failed to produce diffraction quality crystals, most likely due to the structural instability and conformational heterogeneity of GPCRs. A number of alternative approaches have recently been developed to overcome these issues.

A. Optimizing Expression, Purification, and Stability

GPCRs generally express at much lower levels (<1 mg/L) than soluble proteins (>10 mg/L). A large number of expression systems have been evaluated in attempts to increase expression with the majority of success coming from bacterial, yeast, insect, or mammalian cell expression. Although total yield of protein is important, further considerations include whether the protein is correctly folded, the homogeneity of the protein (e.g., with respect to posttranslational modifications) as well as the cost and scalability of the process. Although bacterial expression systems are cost-effective and straightforward to use, bacteria are unable to carry out the correct posttranslational modifications, such as glycosylation which may be required for correctly folded GPCRs. The reductive environment in bacteria may also affect the correct formation of disulphide bonds within the tertiary structure. A number of techniques have been developed which facilitate the correct insertion and folding of GPCRs in bacteria, such as expression of GPCRs as fusion proteins. These approaches have allowed protein production for a number of GPCRs (such as the neurotensin receptor and the adenosine A_{2A} receptor) to be achieved (Grisshammer et al., 1993; Weiss & Grisshammer, 2002). An alternative approach is to refold receptors after purification from