Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Reprogramming G protein coupled receptor structure and function

D Keri[1] and P Barth[1,2,3,4]

The prominence of G protein-coupled receptors (GPCRs) in human physiology and disease has resulted in their intense study in various fields of research ranging from neuroscience to structural biology. With over 800 members in the human genome and their involvement in a myriad of diseases, GPCRs are the single largest family of drug targets, and an ever-present interest exists in further drug discovery and structural characterization efforts. However, low GPCR expression and stability outside the natural lipid environments have challenged these efforts. *In vivo* functional studies of GPCR signaling are complicated not only by the need for specific spatiotemporal activation, but also by downstream effector promiscuity. In this review, we summarize the present and emerging GPCR engineering methods that have been employed to overcome the challenges involved in receptor characterization, and to better understand the functional role of these receptors.

Addresses
[1] Swiss Federal Institute of Technology (EPFL), Interfaculty Institute of Bioengineering, 1015 Lausanne, Switzerland
[2] Ludwig Institute for Cancer Research Lausanne Branch, 1066 Lausanne, Switzerland
[3] Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[4] Department of Pharmacology and Chemical Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Corresponding author: Barth, P. (patrick.barth@epfl.ch)

Current Opinion in Structural Biology 2018, **51**:187–194

This review comes from a themed issue on **Engineering and design**

Edited by **Giovanna Ghirlanda** and **Ivan Korendovych**

https://doi.org/10.1016/j.sbi.2018.07.008

0959-440X/© 2018 Elsevier Ltd. All rights reserved.

Introduction

G-protein coupled receptors (GPCRs) constitute the largest family of signaling membrane receptors. They are involved in a wide diversity of cellular and physiological processes, including immune responses, vision, neuronal communication and behavior [1]. GPCRs are also associated with severe diseases and represent the target of close to 40% of marketed drugs [2]. GPCRs function as sophisticated allosteric machines. They respond to diverse extracellular stimuli in the form of light, small molecules, peptides, lipids and proteins by transmitting the signal across the membrane and activating a number of intracellular signaling pathways [3]. High conformational flexibility is a hallmark of GPCRs which allow them to sense diverse stimuli and couple to different signaling pathways [4], but represents a challenge for structure characterization which often require conformationally stable proteins. Hence, initial GPCR engineering efforts have focused on developing approaches to identify thermostabilized receptor variants for accelerating X-ray structure determination and rational drug design (Figure 1). In parallel, methods have also been established to create GPCR variants that can be controlled by external cues for better studying cellular signaling. Lastly, computational approaches have recently emerged to rationally design GPCR functions, and pave the road for the design of novel biosensors that should prove useful in cell engineering applications (Figure 1). Below, we first describe empirical, experimentally-driven approaches and then outline recent computational techniques for engineering GPCR structure and function.

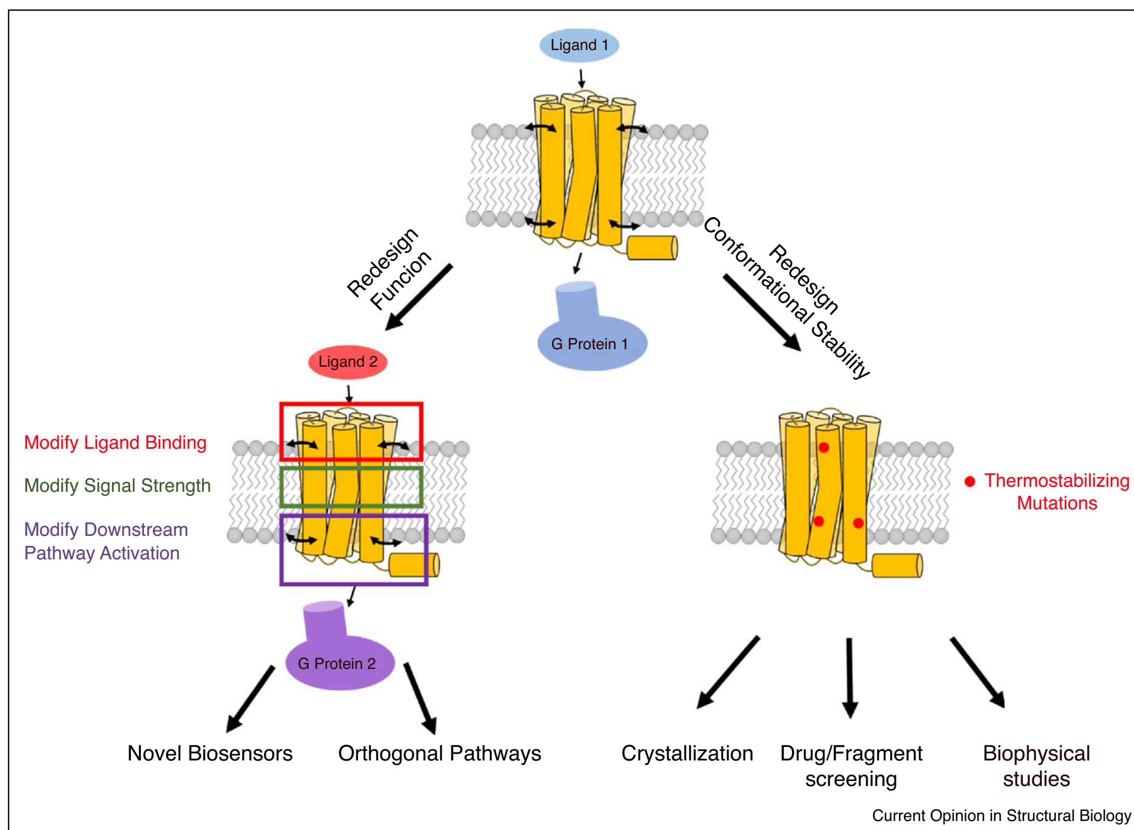
Empirical experimentally driven design of GPCRs

Over the years, a number of experimental approaches have been developed to create GPCR variants for facilitating structural and functional studies. A first line of investigations has focused on modifying and stabilizing receptors to make them more amenable to structural determination and biophysical studies, including drug discovery efforts. A second line of approaches aimed at better understanding the role of GPCRs in neuronal, cellular signaling, and behavior. In each case, the methods similarly involved random or systematic mutagenesis, or a grafting approach to reach the desired molecular properties. The methods used are described below, and highlighted in Figure 2.

Structural characterization

Up until 2007, the only GPCR with a solved three-dimensional structure was rhodopsin [5,6]. Due to the low endogenous expression of GPCRs and their inherent instability outside biological membranes, new techniques were necessary to enable their crystallization. Today, over 50 unique GPCRs [7] have been crystallized, thanks to, in no small part, to various GPCR engineering methods. Successful receptor stabilization could be achieved by conformationally stabilizing the flexible intracellular loop 3 (ICL3) by antibody fragments recognizing the receptor, or by replacing the ICL3 entirely with different soluble

Figure 1



Potential applications of GPCR engineering. A wildtype receptor (top) can be engineered to: Left, create novel receptor functions to respond to different ligands, to transmit ligand-induced signals with different strengths, or to activate a novel effector protein. Right, another route is to modify the wildtype receptor's stability in either the active or inactive state, to generate receptors with higher thermostabilities, which then can be used in other applications. Small curved arrows on GPCRs represent conformational flexibility. Note its absence on the thermostabilized receptor.

proteins promoting crystal packing such as T4L or BRIL. Conformational thermostabilization was also achieved via scanning mutagenesis, although in many cases a combination of ICL3 insertion and mutagenesis were used [8,9]. Systematic mutagenesis work has been carried out, demonstrating the thermostabilization of receptors by mainly replacing leucines to alanines and alanines to leucines (though other mutations also work), which locked the receptor into a specific conformation, so-called Stabilized Receptors (StaRs) [10]. This technique has proven successful in the generation of antagonist, partial agonist, and agonist-bound structures [11,12,13•].

Various directed evolution techniques such as CHESS [14] and SaBRE [15••] have also been applied to GPCRs to screen and select for stabilizing mutations. These techniques rely on the detection of highly expressed mutants using fluorescently labeled ligands and flow cytometry. The increase in expression and ligand binding is thought to be linked to increase in properly folded receptors and increased thermostability [16]. Given the time and effort required to find suitable thermostabilizing mutations and the low success rate of scanning mutagenesis, directed evolution offers a faster route to a more thermostable receptor. Typically, the process to discovering suitable mutations has a hit rate of less than 10% and hundreds of mutations are tested. In contrast, 2–3 rounds of CHESS can result in a thermostable receptor with a significantly higher level of expression than the wildtype receptor.

Biophysical studies

Although surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR) are commonly used techniques in drug discovery, their application to GPCRs has been limited due to receptor instability and low expression [13•]. There has been some success in applying these methods to the wild-type B2 receptor [17], however the generation of StaRs provides a solution to this obstacle. Unlike wild-type receptors, StaRs exhibit wild-type-like binding affinity only to the class of drug (inverse agonist, antagonist or agonist) which was used during StaR generation, due to conformational selection, with a reduced affinity for other classes. While this may bias drug

Download English Version:

https://daneshyari.com/en/article/8319361

Download Persian Version:

https://daneshyari.com/article/8319361

Daneshyari.com