GPCRs

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Arrestin-Bound Rhodopsin: A Molecular Structure and its Impact on the Development of Biased GPCR Ligands

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G-protein-coupled receptors (GPCRs) represent a major class of integral membrane proteins and share a common topology consisting of seven transmembrane helices. GPCRs are responsible for signal transduction from the extracellular space to the cytosol and are able to recognize a plethora of stimuli ranging from photons to low-molecular-weight molecules and even complex proteins.[1] The high percentage (more than 30%) of currently prescribed drugs that target GPCRs illustrates their key role in modulating physiological processes. Originally driven by ligand-based discovery, the availability of high-resolution GPCR crystal structures has enabled medicinal chemists to focus on structure-based drug design in their quest for novel GPCR ligands.[2] The first structures of antagonist-bound GPCRs have paved the way to the development of new ligands devoid of intrinsic activities, whereas agonist-bound states have been crystallized by taking advantage of covalent ligands.[3] To solve crystal structures of fully activated GPCRs, the formation of stable ternary complexes of agonist, receptor, and a G protein or G-protein-mimetic nanobody (single heavy chain antibody derived from camelids) is required.[4]

GPCR-mediated signalling via heterotrimeric G proteins leads to the modulation of intracellular enzymes and ion channels. In turn, these molecular effectors alter the level of second messenger molecules (e.g., cAMP, Ca^{2+}), which ultimately results in the cellular response. Interestingly, interactions of GPCRs with intracellular signal transducers are not limited to G proteins (Figure 1). In fact, GPCRs are able to engage arrestins in a process that is greatly facilitated by phosphorylation. While these adaptor proteins were initially found to control the desensitization and internalization of GPCRs, it has become clear that arrestins are also able to transduce GPCR-promoted signaling, and they are thus of considerable importance for physiological and pathophysiological processes.[5] For example, β-arrestins are responsible for tolerance caused by the activation of μ-opioid receptors.[6] Gaining molecular control of the GPCR–arrestin interaction with tailor-made ligands thus represents an exciting challenge for medicinal chemists. However, structure-based drug design has been hampered by missing structural information on the GPCR–arrestin complex.

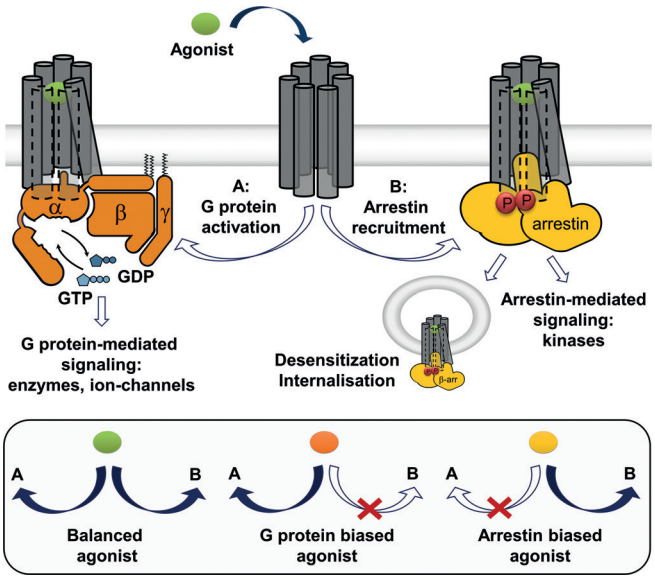

Figure 1. Overview of GPCR-mediated signaling. Activation of the transmembrane receptor can lead to the activation of G proteins and/or receptor phosphorylation followed by arrestin recruitment. The inset depicts possible modalities of biased signaling.

Huge efforts and well thought out experiments from various groups around world were necessary to come up with the first GPCR–arrestin cocrystal structure. Very recently, Xu and colleagues were able to report a breakthrough in obtaining diffractable crystals of human rhodopsin, a prototypical GPCR responsible for the perception of light, in complex with murine visual arrestin.[7]

To solve the structure, the authors had to overcome a whole string of difficulties arising from the transient nature of the interaction between GPCRs and arrestins. First, a constitutively active rhodopsin mutant (E113Q, M257Y) was used to ensure an active-like receptor conformation during the purification process, even in the absence of the tethered agonist, all-*trans* retinal. Second, murine visual arrestin was mutated at three positions (L374A, V375A, F376A) to obtain a preactivated conformation, thereby

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avoiding the need for receptor phosphorylation. Even though these mutations considerably enhanced the rhodopsin–arrestin interaction, it was still necessary to create a fusion protein of the receptor and effector, connected to each other by a 15 amino acid spacer. Moreover, fusion with T4 lysozyme was used to facilitate crystallization. Since crystals remained small (5–15 μm), diffraction data was collected by taking advantage of serial femtosecond crystallography with X-ray free-electron lasers (XFEL). This emerging technology allows the collection of crystal diffraction patterns from single XFEL pulses of less than 50 femtoseconds duration while causing minimal radiation damage to the crystals.[7]

Most strikingly, the overall architecture of the complex reveals an asymmetric arrangement. While the N-terminal domain and the central crest region of arrestin are involved in receptor recognition, the C-terminal arrestin "wing" points towards the cell membrane, thereby not providing any receptor contacts. Although alanine mutation within this area has revealed this region to be important for arrestin recognition,[8] the exact mechanism remains to be determined. Moreover, the overall arrestin geometry shows a 20° rotation of the N- and C-terminal domains relative to each other compared to the inactive state,[7] a finding that is in excellent agreement with previously described (pre)activated arrestin crystal structures.[9]

Compared to its basal state, rhodopsin also undergoes several changes upon interaction with arrestin. Some of them, like the 10 Å outward movement of transmembrane helix 6 (TM6) and the elongation of TM5, have been previously identified as prototypical signs of GPCR activation. Moreover, these findings are in agreement with the structure of metarhodopsin II in complex with a C-terminal peptide derived from the G protein transducin (GαCT).[10] However, the TM6 outward movement observed for rhodopsin remains less pronounced compared to that of the β2-adrenoreceptor in complex with the GαS protein.[4a] Other changes include the appearance of a short helix within intracellular loop 2 (ICL2) and movements of intracellular helix 8 (H8) close to the receptor C terminus.

The crystal structure gave insight into the network of intermolecular interactions between rhodopsin and arrestin. Validation of these contact regions was provided by DEER spectroscopy, HD exchange, and disulfide crosslinking experiments. In agreement with previous studies,[9] interacting residues of the arrestin molecule were found to be located within the loops connecting the β-sheet sandwiches (finger loop, middle loop, back loop, and C-loop) and one adjacent β-strand (Figure 2). Contact sites at rhodopsin involve four distinct regions of the cytosolic surface of the receptor. Extensive interactions were mainly promoted by ICL2, the C-terminal part of TM7, the N-terminal part of H8, and the lower parts of TM5 and TM6, as well as their connection by ICL3. Unfortunately, sufficient electron densities for the contact region between the (phosphorylated) receptor C terminus and the N-terminal β-strand of arrestin were missing (residues 327–348 not resolved). However, results from biochemical experiments and molecular modeling strongly suggest the presence of this crucial interaction. Moreover, the authors provide further evidence for the electrostatic nature

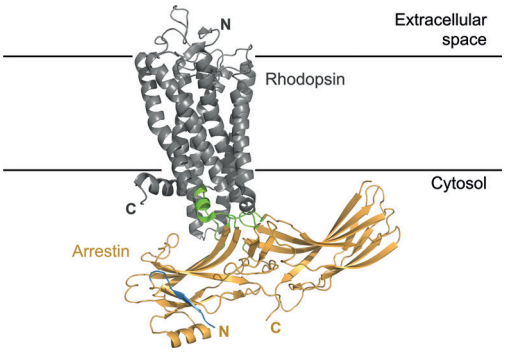

Figure 2. Crystal structure of human rhodopsin in complex with visual arrestin. Key interacting regions on the arrestin are highlighted in green. The putative interaction site with the receptor C terminus (not resolved) is displayed in blue. PDB ID: 4ZWJ, Molecule A, Ref. [7]

of the interaction between arrestin and rhodopsin, thus underlining the previously proposed sequential engagement of arrestin with the receptor C terminus, accompanied by arrestin preactivation and subsequent interaction with the receptor core.[7,9b]

Altogether, the study by Kang et al. represents the pinnacle of considerable efforts in GPCR–arrestin structural biology. However, owing to its comparatively high stability and its tethered ligand, rhodopsin represents a special type of GPCR. It is therefore tempting to speculate on how a ternary complex of a therapeutically relevant GPCR together with its ligand and a β-arrestin molecule would look. In this context, the complex of the β2-adrenoreceptor with β-arrestin-1 and a stabilizing antibody fragment determined by Shukla et al. represents a promising first approach, although it did not provide sufficient quality for X-ray crystallography.[9c]

Understanding how receptors control the recruitment of effectors (e.g. arrestins) and further direct subsequent signaling events is of particular interest for taking advantage of the phenomenon of biased agonism. Biased agonists preferentially orient the signaling of a GPCR to a subset of the available signal transducers (Figure 1).[5a] In part, functional selectivity may be controlled by ligand-specific phosphorylation by GPCR kinases, which would serve as a "signaling bar code".[5b] Thus, crystal structures of differentially phosphorylated GPCR–arrestin complexes bound to balanced and biased agonists are eagerly awaited. Nevertheless, this first rhodopsin–arrestin complex constitutes an important step towards the understanding of biased GPCR signaling. Furthermore, the crystal structure may serve as a valuable template for homology modeling and long-time molecular dynamics simulations of therapeutically relevant GPCRs, thereby paving the way for the rational design of functionally selective drugs.

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