BIAS SIGNALLING MODEL and BIAS LİGANDS

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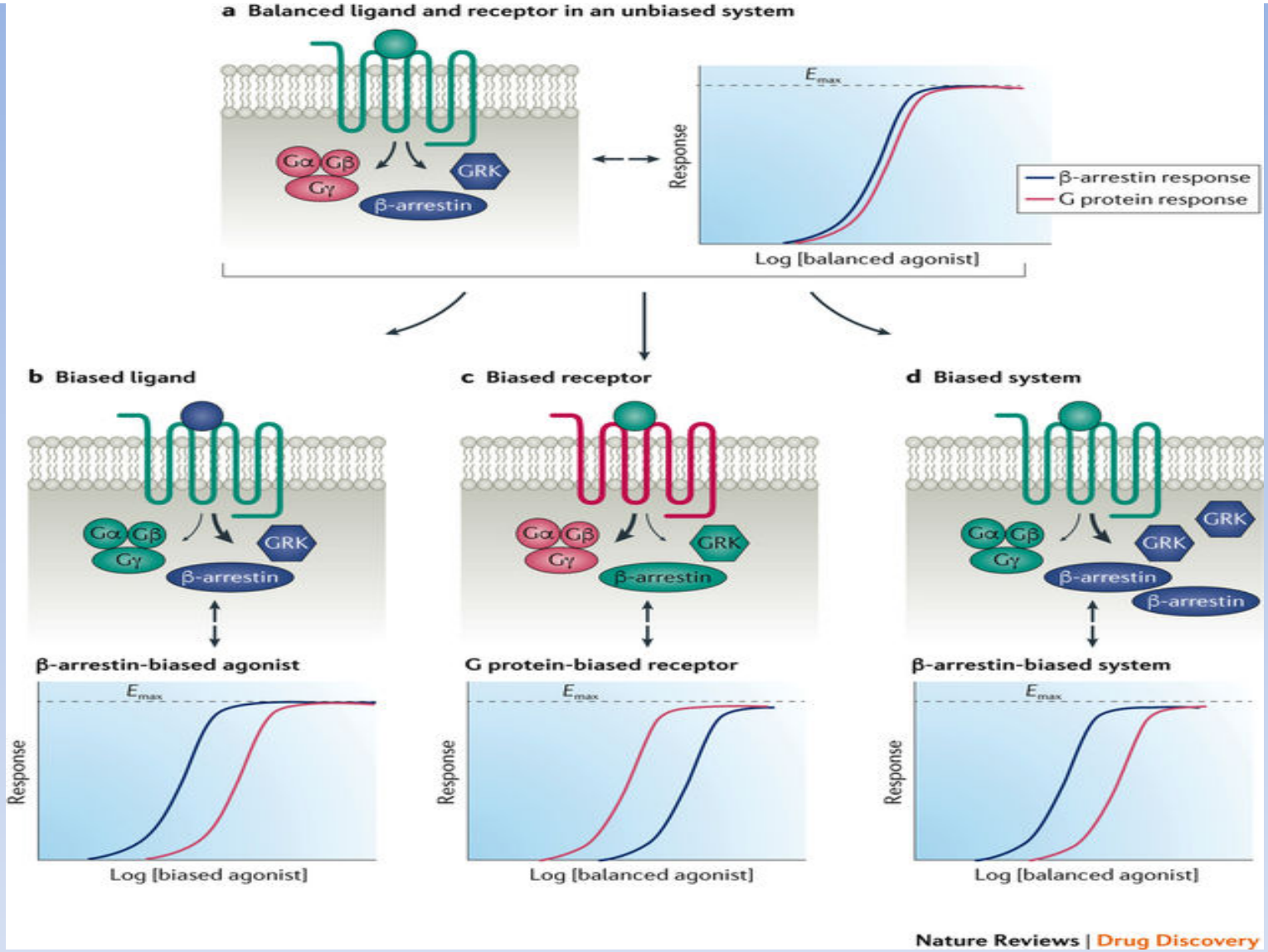
Biased Activation

- **Initially, GPCR signaling was considered to be simply mediated by intracellular G proteins. However, this concept was completely revised after β-arrestin was also found to have the capability to mediate diverse GPCR signaling independently. Namely, when GPCRs are activated, they can activate either the G protein or β-arrestin pathway. It is now appreciated that several GPCR ligands can selectively activate either the G protein pathway or the β-arrestin pathway, which is called biased activation signaling.**

- *Trends Pharmacol Sci. 2018 Jan 30. pii: S0165-6147(18)30024-5. doi: 10.1016/j.tips.2018.01.001. [Epub ahead of print] New Insights into Modes of GPCR Activation.Wang W et al.*

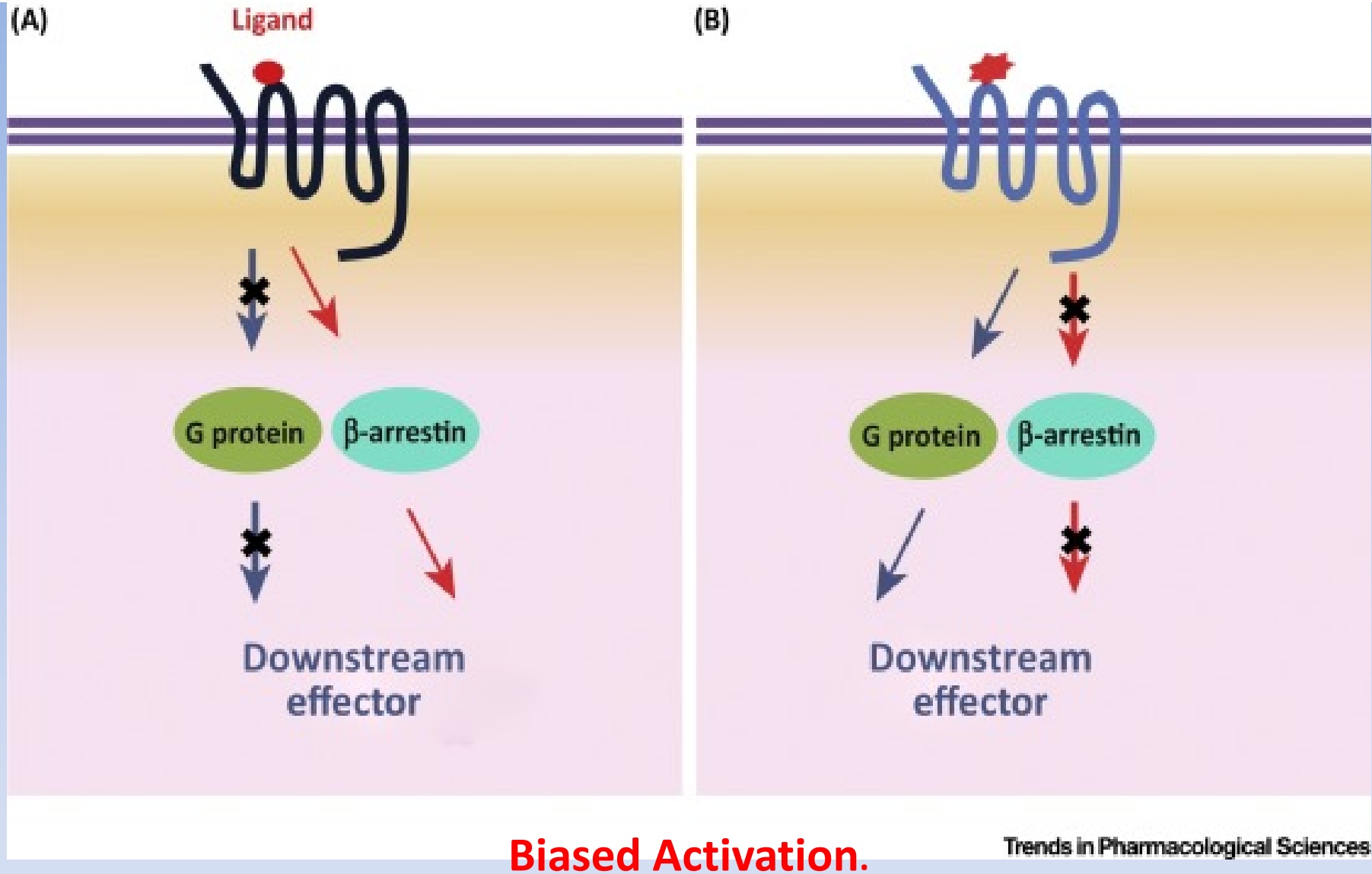
- **G protein-coupled receptors (GPCRs) adopt multiple conformational states that can activate or block distinct intracellular signalling pathways, such as those regulated by heterotrimeric G proteins or β-arrestins.**
- **Different agonists for the same receptor can stabilize distinct GPCR conformational states. Agonists that preferentially activate certain intracellular pathways relative to others are referred to as biased agonists.**
- **Structural studies support a model in which GPCRs act as allosteric microprocessors that integrate diverse extracellular and intracellular stimuli to generate distinct conformations that result in varied intracellular responses.**
- **In addition to biased agonists, biased signalling may be encoded by the receptor ('receptor bias') or by the relative expression levels of transducers ('system bias').**
- **Biased signalling is also observed in other receptor families, such as nuclear hormone receptors and receptor tyrosine kinases.**
- **Recent preclinical and clinical work suggests that by more selectively targeting signalling pathways of interest, biased agonists have the potential to increase clinical efficacy while reducing undesirable side effects.**

- *Nat Rev Drug Discov. 2018 Jan 5. doi: 10.1038/nrd.2017.229. [Epub ahead of print]Biased signalling: from simple switches to allosteric microprocessors. Smith JS*



Biased signalling can be encoded through three general mechanisms.

Mol Cell Endocrinol. 2017 Jul 5;449:28-41. doi: 10.1016/j.mce.2017.01.052. Epub 2017 Feb 4. ***β-arrestin signalling and bias in hormone-responsive GPCRs.*** *Reiter E et al*



Biased Activation.

Two major patterns of based activation: (A) β-arrestin-mediated biased signaling and (B) G protein-mediated biased signaling.

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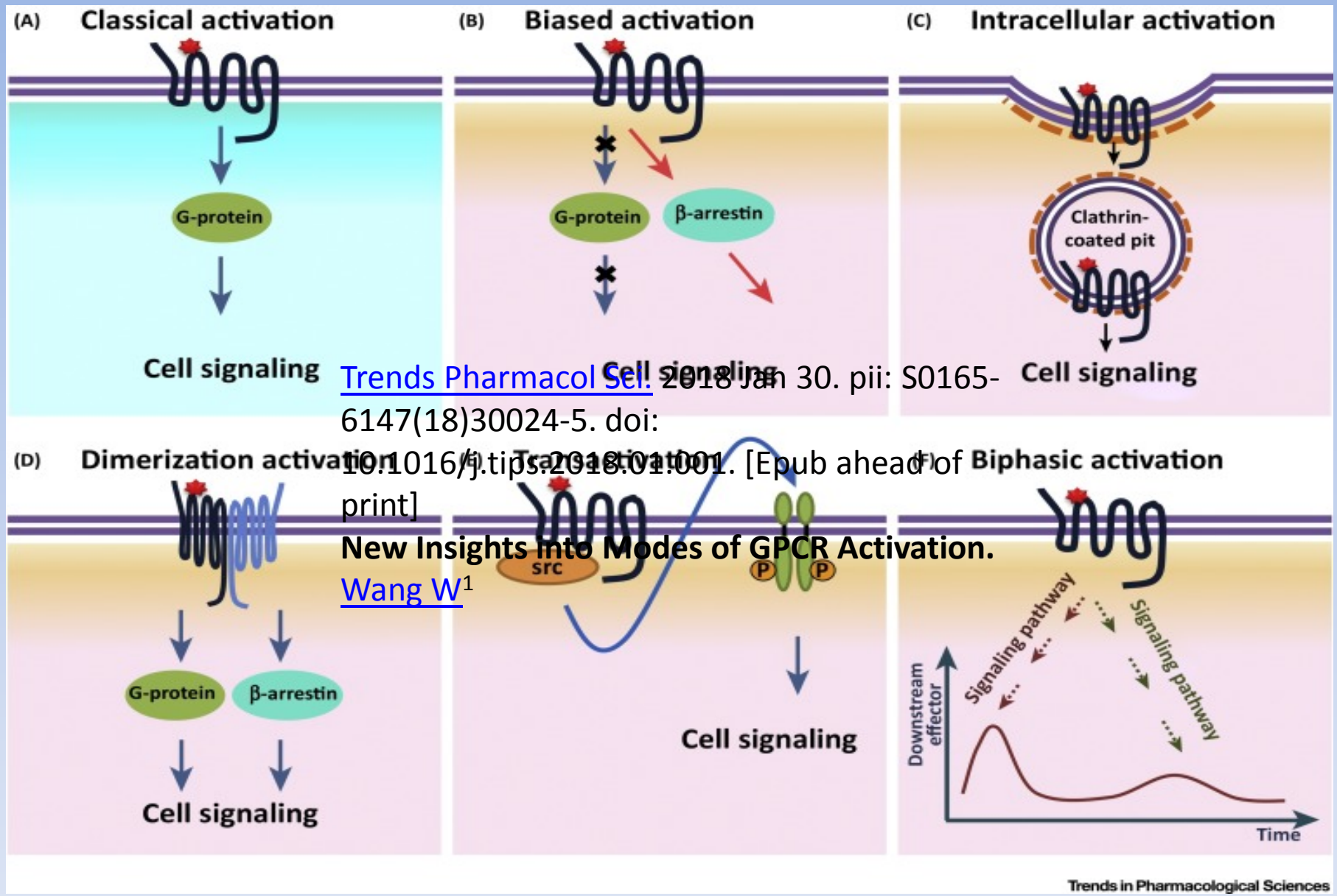
New Insights into Modes of GPCR Activation

Trends Pharmacol Sci. 2018 Jan 30. pii: S0165-6147(18)30024-5. doi: 10.1016/j.tips.2018.01.001. [Epub ahead of print] *Wang W, Qiao Y, Li Z.*

Abstract

In classical G-protein-coupled receptor (GPCR) activation, GPCRs couple to a variety of heterotrimeric G proteins on the membrane and then activate downstream signaling pathways. More recently, GPCRs have been found to couple to different effector proteins, including different G protein subtypes and regulatory proteins, such as arrestins. Some novel modes of GPCR activation have been proposed to explain their complex behaviors. In this review, we summarize the main novel modes of GPCR activation, including biased activation, intracellular activation, dimerization activation, transactivation, and biphasic activation. In addition, we also discuss the relationship among the five modes to show the complex picture of GPCR activation. The complex activation modes regulate precisely GPCR downstream signaling, including physiological and pathological signaling. Thus, there is the potential to develop GPCR precision drugs that target precise GPCR activation modes to accurately strengthen their beneficial functions and block specific pathological processes.



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New Insights into Modes of GPCR Activation.

Wang W[1]

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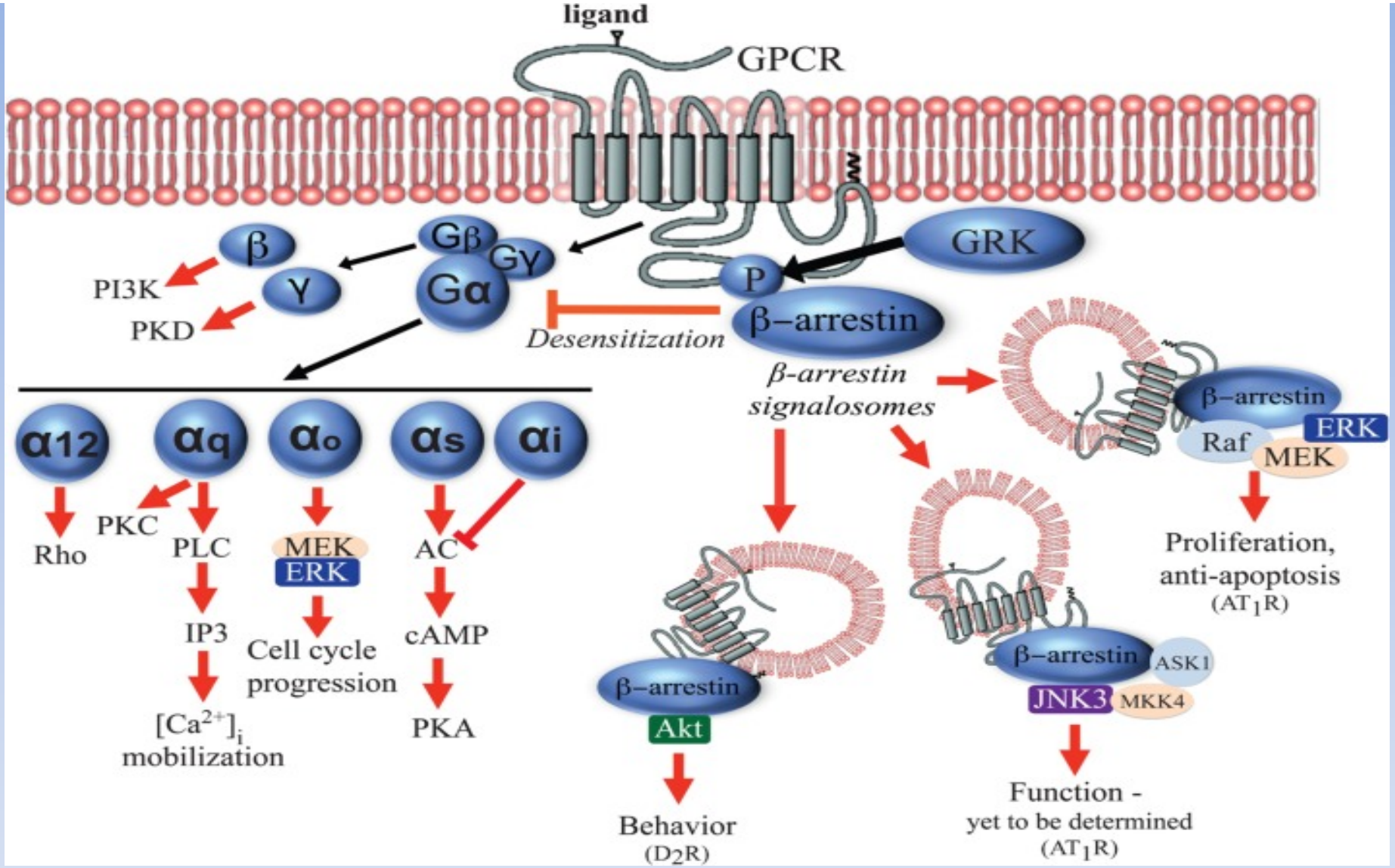
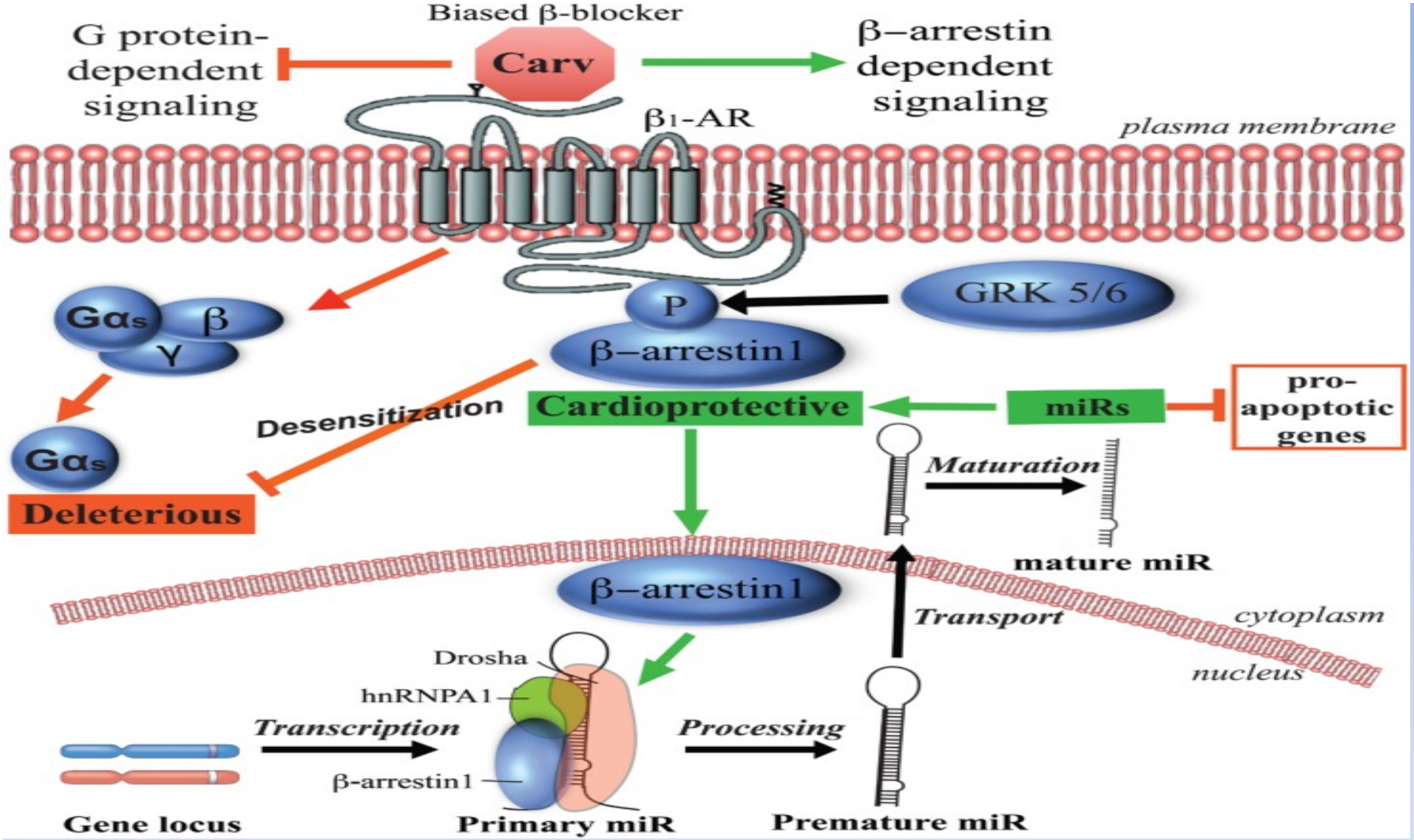


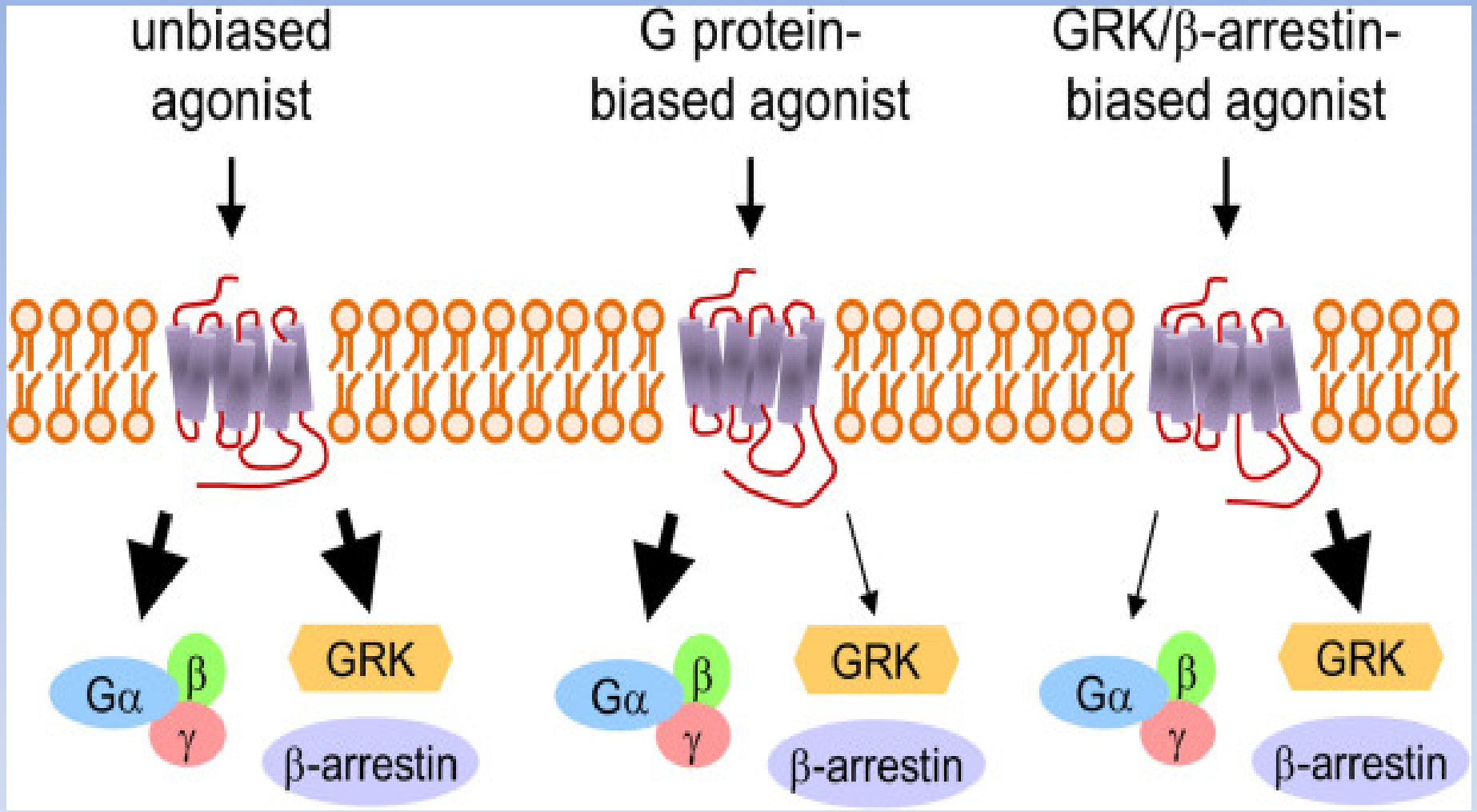
Examples of G protein- and β-arrestin-mediated downstream signaling pathways on GPCRs. Upon agonist binding to GPCRs, both G proteins ($G\alpha_{12}$, $G\alpha_{q/11}$, $G\alpha_{i/o}$, $G\alpha_{s}$, Gβ and Gγ subunits) and β-arrestin

Biomol Ther (Seoul). 2017 25(1): 12–25. Bologna Z. Et al.



Carvedilol-mediated β-arrestin biased signaling on β_1-adrenergic receptors in cardiomyocytes and hearts.

Carvedilol selectively stimulates GRK5/6- and β-arrestin-dependent cardioprotective signaling without activating deleterious G protein signaling. Carvedilol-mediated GRK5/6 phosphorylation of β_1-adrenergic receptors leads to β-arrestin1's translocation into nucleus where β-arrestin1 interacts with a subset of primary miRs and components of the Drosha microprocessor complex. This results in an increased level of a subset of miRs, which act as cardioprotective miRs by repressing pro-apoptotic genes in cardiomyocytes and hearts. Biomol Ther (Seoul). 2017 25(1): 12–25. Bologna Z. et al



GRKs are involved in cellular signaling that is independent of G protein activation.

Biased agonist activates either G protein signaling or GRK/β-arrestin-dependent signaling. Each agonist promotes distinct conformational changes of GPCRs. Unbiased agonists activate both G protein signaling and GRK/β-arrestin-dependent signaling, whereas biased agonists activate either G protein- or GRK/β-arrestin-dependent signaling as shown in bold arrows. Physiological responses mediated by GRK/β-arrestin-dependent signaling are believed to be distinct from those by G protein activation.

Watari K et al.J Mol Signal. 2014; 9: 1.

- **Multiple functions of G protein-coupled receptor kinases.**

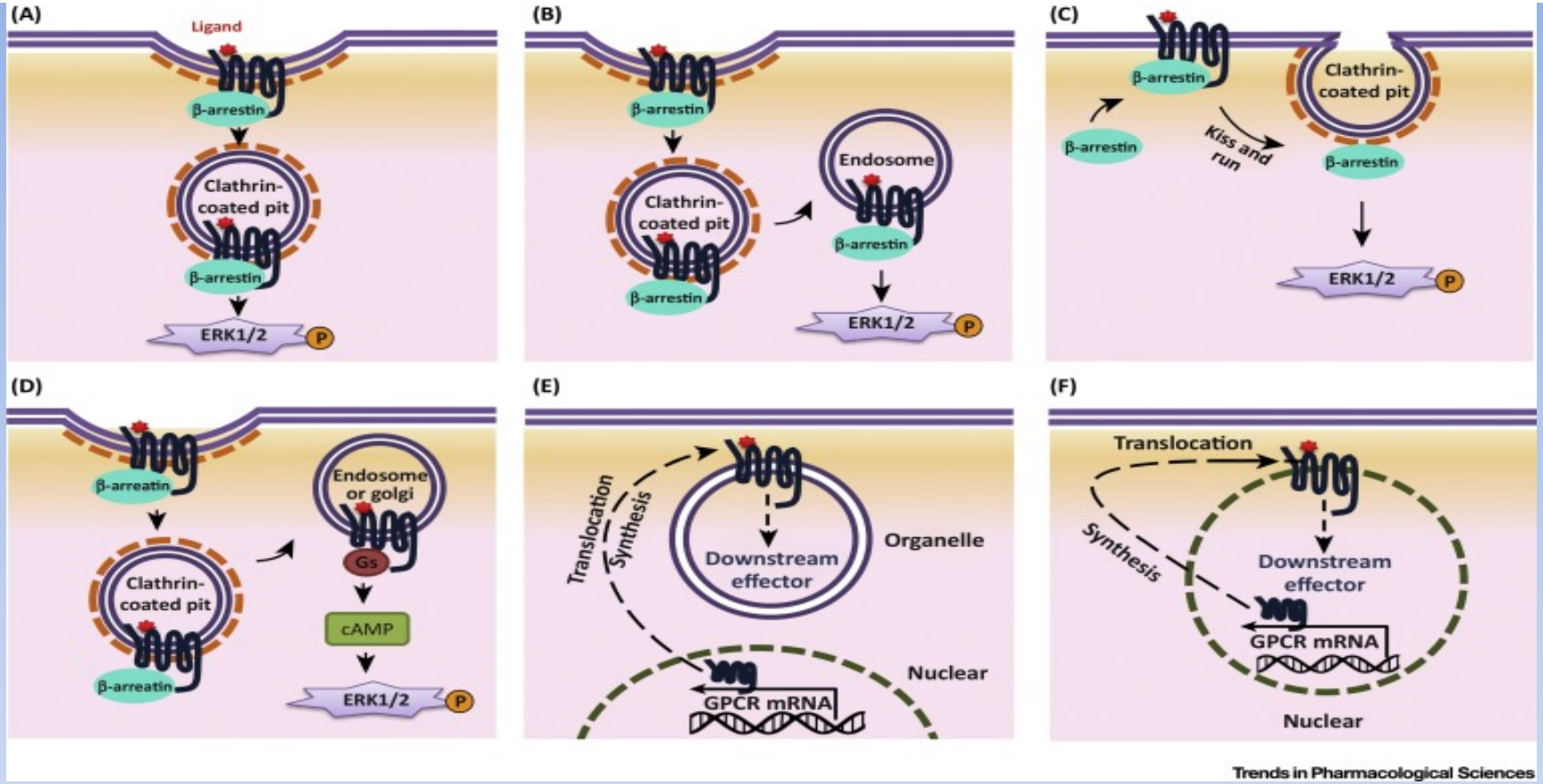
- J Mol Signal. 2014 Mar 6;9(1):1. doi: 10.1186/1750-2187-9-1. Watari K, Nakaya M, Kurose H[1].

- **Abstract**
- **Desensitization is a physiological feedback mechanism that blocks detrimental effects of persistent stimulation. G protein-coupled receptor kinase 2 (GRK2) was originally identified as the kinase that mediates G protein-coupled receptor (GPCR) desensitization. Subsequent studies revealed that GRK is a family composed of seven isoforms (GRK1-GRK7). Each GRK shows a differential expression pattern. GRK1, GRK4, and GRK7 are expressed in limited tissues. In contrast, GRK2, GRK3, GRK5, and GRK6 are ubiquitously expressed throughout the body. The roles of GRKs in GPCR desensitization are well established. When GPCRs are activated by their agonists, GRKs phosphorylate serine/threonine residues in the intracellular loops and the carboxyl-termini of GPCRs. Phosphorylation promotes translocation of β-arrestins to the receptors and inhibits further G protein activation by interrupting receptor-G protein coupling. The binding of β-arrestins to the receptors also helps to promote receptor internalization by clathrin-coated pits. Thus, the GRK-catalyzed phosphorylation and subsequent binding of β-arrestin to GPCRs are believed to be the common mechanism of GPCR desensitization and internalization. Recent studies have revealed that GRKs are also involved in the β-arrestin-mediated signaling pathway. The GRK-mediated phosphorylation of the receptors plays opposite roles in conventional G protein- and β-arrestin-mediated signaling. The GRK-catalyzed phosphorylation of the receptors results in decreased G protein-mediated signaling, but it is necessary for β-arrestin-mediated signaling. Agonists that selectively activate GRK/β-arrestin-dependent signaling without affecting G protein signaling are known as β-arrestin-biased agonists. Biased agonists are expected to have potential therapeutic benefits for various diseases due to their selective activation of favorable physiological responses or avoidance of the side effects of drugs. Furthermore, GRKs are recognized as signaling mediators that are independent of either G protein- or β-arrestin-mediated pathways. GRKs can phosphorylate non-GPCR substrates, and this is found to be involved in various physiological responses, such as cell motility, development, and inflammation. In addition to these effects, our group revealed that GRK6 expressed in macrophages mediates the removal of apoptotic cells (engulfment) in a kinase activity-dependent manner. These studies revealed that GRKs block excess stimulus and also induce cellular responses. Here, we summarized the involvement of GRKs in β-arrestin-mediated and G protein-independent signaling pathways.**

Biased G Protein-Coupled Receptor Signaling: New Player in Modulating Physiology and Pathology.

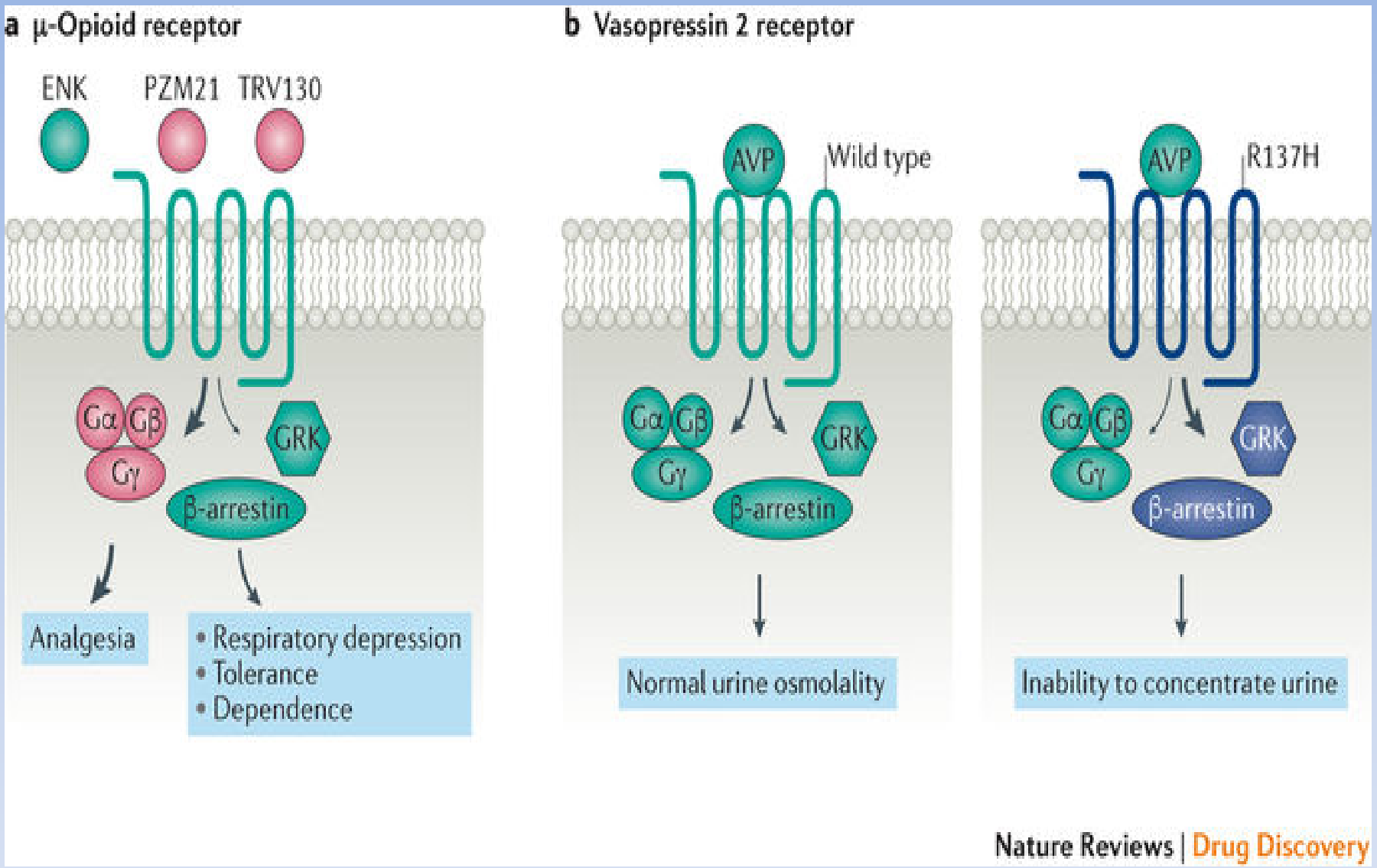
- Biomol Ther (Seoul). 2017 Jan 1;25(1):12-25. doi: 10.4062/biomolther.2016.165. Bologna Z, Teoh JP, Bayoumi AS, Tang Y, Kim IM.

- **Abstract**
- **G protein-coupled receptors (GPCRs) are a family of cell-surface proteins that play critical roles in regulating a variety of pathophysiological processes and thus are targeted by almost a third of currently available therapeutics. It was originally thought that GPCRs convert extracellular stimuli into intracellular signals through activating G proteins, whereas β-arrestins have important roles in internalization and desensitization of the receptor. Over the past decade, several novel functional aspects of β-arrestins in regulating GPCR signaling have been discovered. These previously unanticipated roles of β-arrestins to act as signal transducers and mediators of G protein-independent signaling have led to the concept of biased agonism. Biased GPCR ligands are able to engage with their target receptors in a manner that preferentially activates only G protein- or β-arrestin-mediated downstream signaling. This offers the potential for next generation drugs with high selectivity to therapeutically relevant GPCR signaling pathways. In this review, we provide a summary of the recent studies highlighting G protein- or β-arrestin-biased GPCR signaling and the effects of biased ligands on disease pathogenesis and regulation.**



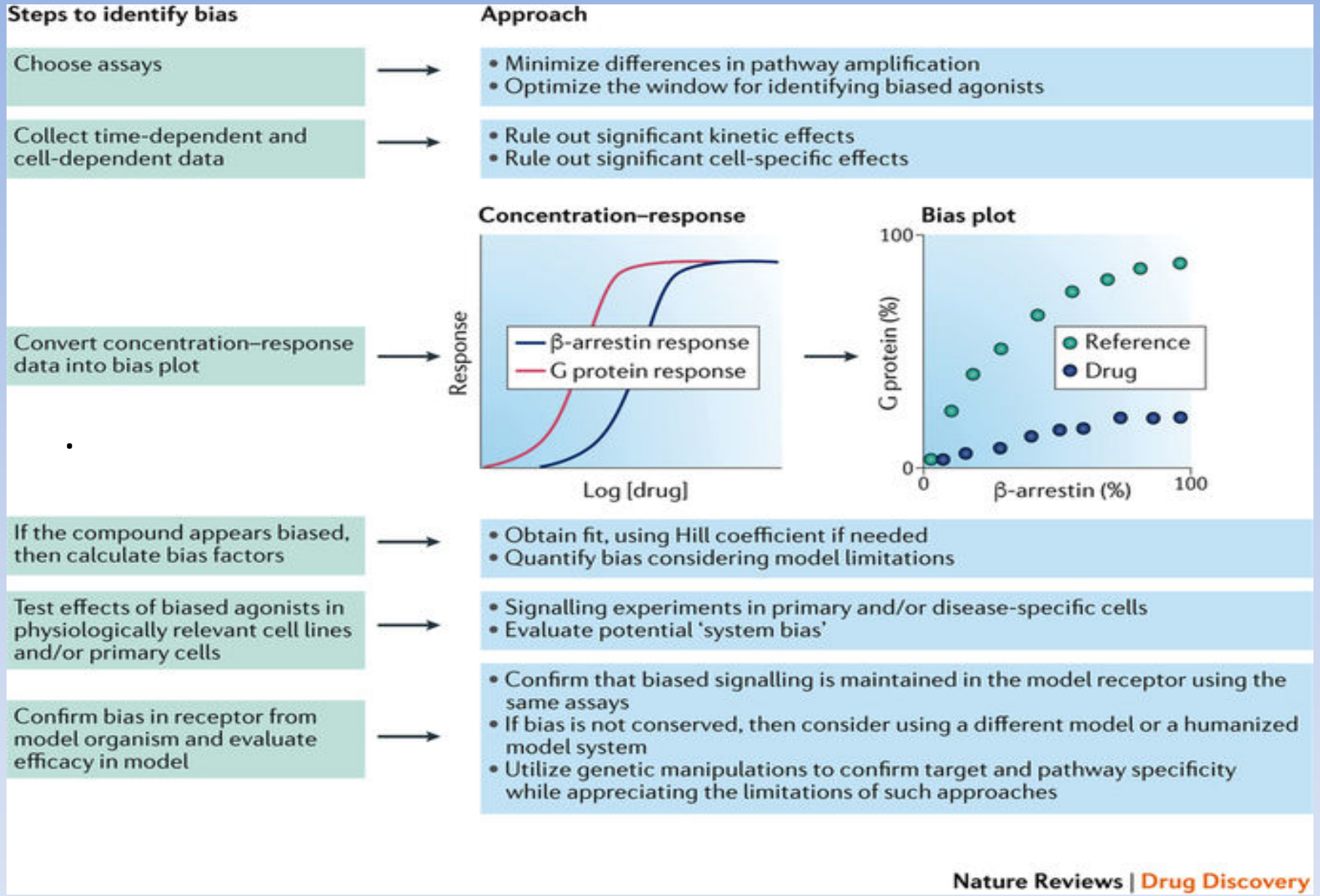
Intracellular Activation. Four major patterns of internalization-dependent intracellular activation: (A) Class A receptors interact with β-arrestin and the complexes are targeted to clathrin-coated pits for subsequent ERK1/2 activation near the membrane. The process is transient and is followed by rapid recycling of the receptors back to the plasma membrane. (B) Class B receptors interact robustly with β-arrestin and the complexes are targeted to endosomes for subsequent G-protein-coupled receptor(GPCR) degradation and ERK1/2 activation. (C) β-arrestin is internalized without forming a complex with the β1-adrenergic receptor (β1-AR). β-arrestin briefly 'kisses' β1-AR, locates to clathrin-coated structures at the plasma membrane, and then activates ERK1/2. (D) Internalization-dependent activation is mediated by G protein in the cytoplasm. After internalization, GPCRs target the endosome or Golgi. The receptors then recruit the Gs protein, resulting in cAMP accumulation that induces ERK1/2 activation in the endosome or Golgi. Two patterns of internalization-independent intracellular activation are proposed: GPCRs can reside on either the organelle (E) or nuclear membrane (F) and then initiate internal signaling *in situ*, which is independent of receptor internalization from the plasma membrane.

Trends Pharmacol Sci. 2018 Jan 30. pii: S0165-6147(18)30024-5. doi: 10.1016/j.tips.2018.01.001. [Epub ahead of print] ***New Insights into Modes of GPCR Activation.*** *Wang W*



Biased signalling can be encoded through three general mechanisms.

Nat Rev Drug Discov. 2018 Jan 5. doi: 10.1038/nrd.2017.229. [Epub ahead of print] Biased signalling: from simple switches to allosteric microprocessors. Smith JS, Lefkowitz RJ, Rajagopal S.



General approach to characterizing biased ligands

Nat Rev Drug Discov. 2018 Jan 5. doi: 10.1038/nrd.2017.229. [Epub ahead of print] **Biased signalling: from simple switches to allosteric microprocessors.** Smith JS, Lefkowitz RJ, Rajagopal S

- **The paradigm of biased agonism, that different ligands can generate discrete receptor conformations that lead to distinct biological processes, is supported by numerous structure–function and pharmacological studies. These studies suggest that GPCRs act as allosteric microprocessors as opposed to binary 'switches'. Basic and translational studies conducted within the past 5 years have led to the explosion of promising compounds with putative biased signalling and demonstrate that the therapeutic potential for biased GPCR ligands is profound. The discovery of alternative GPCR signalling pathways, such as those mediated by β-arrestin, warrants the application of drug screening techniques beyond technologies that focus solely on proximal signalling responses mediated by G proteins. In addition, screening methods that are unable to identify allosteric ligands are likely to overlook potentially useful drugs. It is imperative to note the limitations of screening assays, especially when identifying potentially biased ligands. Bias plots, combined with quantification methods based on intrinsic relative activity, functional affinity and/or the operational model, are reasonable depending on the context and physiology of the system of interest. The available preclinical data suggest that selectively targeting G protein, β-arrestin or other non-canonical signalling pathways, depending on the physiological response desired, could improve current GPCR-based therapies through increased efficacy and reduced side-effect profiles. The true therapeutic potential will not be realized until more biased ligands are tested in preclinical and clinical trials. Given the substantial costs of late-phase drug discovery, accurately quantifying the relative signalling properties of biased agonists early in the drug discovery process and their effects in suitable preclinical models of disease is necessary. Beyond their potential therapeutic superiority, biased ligands can also be employed as tool compounds which, when combined with advances in signalling pathway analysis, can be used to dissect fundamental biological processes. Such use of biased ligands as tools will help to advance our basic understanding of intracellular signalling.**

Nat Rev Drug Discov. 2018 Jan 5. doi: 10.1038/nrd.2017.229. [Epub ahead of print] **Biased signalling: from simple switches to allosteric microprocessors.** Smith JS, Lefkowitz RJ, Rajagopal S.