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# Making the switch: The role of Gq in driving GRK selectivity at GPCRs

**Parishmita Sarma**, **Shirsha Saha**, **Arun K. Shukla**[*]
Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur 208016, India

## Abstract

Selective engagement of signal transducers such as G proteins and β-arrestins with GPCRs upon stimulation with biased agonists is thought to be due to distinct receptor conformations. Kawakami *et al.* propose an additional mechanism whereby activation of Gq determines GPCR kinase subtype selectivity to the activated angiotensin receptor, leading to distinct binding modalities of β-arrestins and functional outcomes.

G protein–coupled receptors (GPCRs) typically engage two distinct signal transducers upon agonist stimulation: the heterotrimeric guanine nucleotide–binding proteins (G proteins) and β-arrestins. Ligands that trigger preferential engagement of selected signal transducers over others have been developed and characterized for several different GPCRs, and such ligands have been used extensively to decipher the molecular mechanisms underlying the phenomenon of biased agonism, also referred to as functional selectivity (1). Whereas G protein coupling to GPCRs is driven solely by receptor activation, the interaction of β-arrestins requires an additional step of receptor phosphorylation on the intracellular domains, primarily by GPCR kinases (GRKs). Compared to unbiased agonists, biased ligands are generally believed to trigger the engagement of different GRKs to phosphorylate distinct sites on a given receptor, which has been proposed to be a key determinant of the resulting conformations and subsequent functional responses of β-arrestins (2). The differential engagement of GRK subtypes upon stimulation of GPCRs with biased versus unbiased agonists is typically conceived to be determined by distinct receptor conformations that are enriched and stabilized by these ligands. Kawakami *et al.* now propose that Gq activation, rather than receptor conformation per se, may act as a regulatory switch to direct GRK subtype selectivity at the angiotensin II subtype 1 receptor (AT1R) to influence β-arrestin conformation and subsequent functional responses (3).

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There are seven GRK subtypes in humans (GRK1 to GRK7), of which GRK2/3 and GRK5/6 constitute two distinct subfamilies and exhibit cytosolic and plasma membrane localization, respectively. These GRK subtypes are widely expressed in different tissues in the body, and they have been studied extensively in the context of GPCR signaling and regulatory paradigms. To investigate the contribution of specific GRK subtypes to

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[*]Corresponding author. arshukla@iitk.ac.in.

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interactions between AT1R and β-arrestins, Kawakami *et al.* first generated and validated cell lines deficient in GRK2 and GRK3 ( GRK2/3), GRK5 and GRK6 ( GRK5/6), and all four GRKs ( GRK2/3/5/6) through a CRISPR-Cas9–based approach (3). The authors observed that β-arrestin recruitment in response to the natural, unbiased agonist angiotensin II (AngII) required GRK2/3 and GRK5/6 subtypes, with the latter having a relatively minor contribution. On the other hand, β-arrestin recruitment to AT1R upon stimulation with β-arrestin–biased ligands, including TRV027, was dependent primarily on GRK5/6, and the extent of β-arrestin recruitment inversely correlated with the residual ability of the β-arrestin–biased ligands to activate Gq (3). Interestingly, pharmacological inhibition of Gq activity with the chemical inhibitor YM-254890 (YM) resulted in AngII-induced β-arrestin recruitment becoming dependent primarily on GRK5/6, thereby causing a switch in GRK subtype selectivity (3). The combination of AngII with YM also provided an experimental setup analogous to that of stimulation with TRV027, with the exception of the receptor being occupied by AngII instead of TRV027, which enabled the authors to investigate the mechanistic and functional aspects of β-arrestin bias downstream of AT1R.

So, what is the mechanism for this switch in GRK subtype selectivity upon inhibition of Gq activity? The authors used a combination of single-molecule tracking in a cellular context and a set of intermolecular interaction sensors to demonstrate that AT1R, Gq, and GRK5/6 were in close proximity to each other, although not necessarily in a physical complex (3). Upon stimulation with AngII, these proteins separated from each other, and the activation of Gq enabled the recruitment of GRK2/3 to the receptor, presumably through their membrane targeting being facilitated by dissociated Gβγ subunits (Fig. 1A). This resulted in the GRK2/3-W mediated phosphorylation of AT1R, which led to an interaction with β-arrestin and sub-[d] sequent functional outcomes. On the other hand, stimulation with TRV027 or with AngII in the presence of YM did not enable the dissociation of AT1R, $G_q$, and GRK5/6. Rather, under these conditions, their interactions were enhanced, which facilitated receptor phosphorylation solely by GRK5/6 without any substantial involvement of GRK2/3 (Fig. 1B). This pre-assembly or proximity of AT1R, Gq, and GRK5/6 in "immobile domains" is analogous to the "hot spots" in the plasma membrane where activated GPCRs and G proteins accumulate upon agonist stimulation to initiate downstream signaling (4). Together, these studies underscore the emerging phenomenon of localized organization of signaling modules in the plasma membrane to facilitate and precisely regulate the spatiotemporal aspects of GPCR signaling.

Does the GRK subtype preference also affect β-arrestin conformations? Kawakami *et al.* used a combination of an intrabody-based conformational sensor (5) and β-arrestin constructs with a shortened finger loop that are incapable of engaging with the transmembrane core of the receptor (6) to investigate β-arrestin conformations upon AT1R activation. Currently, there are three distinct modalities of GPCR: β-arrestin interaction and activation. A relatively stable GPCR:β-arrestin complex may form either through tail engagement, where the receptor and the β-arrestin are associated through the phosphorylated C terminus of the receptor, or as a fully engaged complex that involves an additional core interaction between the receptor and the β-arrestin (7). On the other hand, a transient GPCR:β-arrestin interaction also appears to be sufficient to drive an active-like β-arrestin conformation, even in the absence of a ligand-bound receptor, which has been referred to

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as catalytic activation (8). Kawakami *et al.* demonstrated that stimulation with TRV027 or with AngII and YM led to the formation of a mostly tail-engaged AT1R:β-arrestin complex, whereas stimulation with AngII alone triggered full engagement between AT1R and β-arrestin (Fig. 1). Moreover, Kawakami *et al.* also found that β-arrestin2 appeared to interact with the transmembrane core of the receptor more strongly than did β-arrestin1, a notion that was previously demonstrated with other GPCRs, and it provides a potential mechanism underlying the functional divergence of the β-arrestin isoforms (9).

So, why are these results exciting in the context of biased agonism? Previous studies have proposed that biased and unbiased agonists enrich and stabilize distinct conformations of the receptor, which enables receptor phosphorylation by different GRKs, presumably through preferential engagement of different GRK subtypes. This, in turn, imparts distinct conformational signatures on β-arrestins associated with different functional responses, such as desensitization, endocytosis, and signaling. Kawakami *et al.* (3) now provide an additional mechanism for imparting GRK subtype selectivity that is driven primarily by Gq activity and localized organization of the key players in the plasma membrane. In other words, the presence of Gq, even without substantial activation, affects the availability of the receptor to specific GRK subtypes, ensuring specific β-arrestin conformations and the resulting functional outcomes. Note that the mechanism proposed by Kawakami *et al.* does not necessarily contradict the existing notion, but rather, it can be integrated with the current paradigm to further refine our understanding of the origin of biased signaling. Moreover, these data add to the ever-growing body of evidence of a more intricate interplay between G proteins and β-arrestins than was previously anticipated or appreciated.

This study also raises several interesting questions that require additional investigation. For example, how generalizable is this phenomenon for other GPCRs? The authors suggest that pharmacological inhibition of Gq exerts a similar preference for GRK5/6-mediated β-arrestin recruitment for some other receptors. However, investigating this mechanism for a broader set of GPCRs, including Gs- and Gi-coupled receptors, with pharmacological or genetic inhibition of the corresponding Gα subtypes, may help establish the generality, or lack thereof, of this intriguing phenomenon. Overall, Kawakami *et al.* demonstrate an additional level of regulatory mechanism encoded in the GPCR–G protein–GRK–β-arrestin system that provides inherent control and fine-tuning of receptor-transducer coupling and the resulting functional responses. Together with another study describing GRK knock-out cell lines (10), this study also provides an important toolset for the GPCR community to potentially discover other aspects of GRK-mediated signaling and the regulation of GPCRs.

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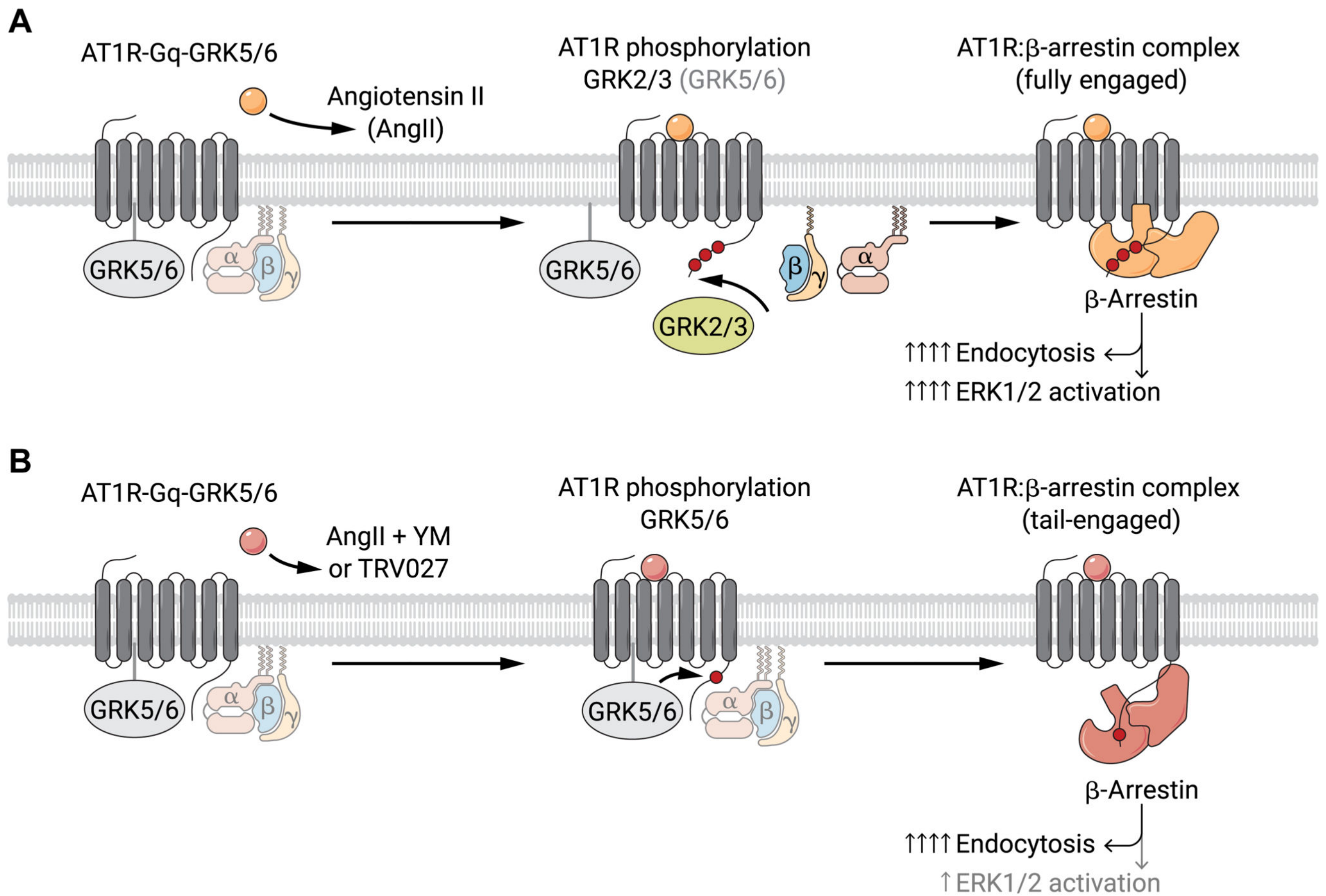


**Figure 1. Scheme depicting the interplay between Gq and GRK5/6 in biased signaling by AT1R.**
(**A**) AngII leads to the separation of the components of the AT1R-Gq-GRK5/6 module that is presumably assembled at the plasma membrane, resulting in GRK2/3 recruitment and phosphorylation of the receptor. Subsequent binding of β-arrestins leads to the formation of a fully engaged AT1R:β-arrestin complex, which promotes receptor endocytosis and activation of signaling by extracellular signal–regulated kinase 1 and 2 (ERK1/2). (**B**) Stimulation of AT1R with either TRV027 (a (β-arrestin-biased ligand) or AngII in the presence of the Gq inhibitor YM-254890 (AngII + YM) does not favor the separation of GRK5/6 from the AT1R-Gq-GRK assembly and therefore facilitates receptor phosphorylation by GRK5/6 without the involvement of GRK2/3. This results in the formation of a tail-engaged AT1R:β-arrestin complex, which efficiently promotes receptor endocytosis but leads to comparatively less ERK1/2 activation than is observed with a fully engaged complex.