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Biased signaling of G protein coupled receptors (GPCRs): Molecular determinants of GPCR/transducer selectivity and therapeutic potential

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ABSTRACT

G protein coupled receptors (GPCRs) convey signals across membranes via interaction with G proteins. Originally, an individual GPCR was thought to signal through one G protein family, comprising cognate G proteins that mediate canonical receptor signaling. However, several deviations from canonical signaling pathways for GPCRs have been described. It is now clear that GPCRs can engage with multiple G proteins and the line between cognate and non-cognate signaling is increasingly blurred. Furthermore, GPCRs couple to non-G protein transducers, including β-arrestins or other scaffold proteins, to initiate additional signaling cascades.
Receptor/transducer selectivity is dictated by agonist-induced receptor conformations as well as by collateral factors. In particular, ligands stabilize distinct receptor conformations to preferentially activate certain pathways, designated 'biased signaling'. In this regard, receptor sequence alignment and mutagenesis have helped to identify key receptor domains for receptor/transducer specificity. Furthermore, molecular structures of GPCRs bound to different ligands or transducers have provided detailed insights into mechanisms of coupling selectivity. However, receptor dimerization, compartmentalization, and trafficking, receptor-transducer-effector stoichiometry, and ligand residence and exposure times can each affect GPCR coupling. Extrinsic factors including cell type or assay conditions can also influence receptor signaling. Understanding these factors may lead to the development of improved biased ligands with the potential to enhance therapeutic benefit, while minimizing adverse effects. In this review, evidence for ligand-specific GPCR signaling toward different transducers or pathways is elaborated. Furthermore, molecular determinants of biased signaling toward these pathways and relevant examples of the potential clinical benefits and pitfalls of biased ligands are discussed.

Abbreviations: 5-HT_{1B}R, serotonin 1B receptor; 5-HT_{2B}R, serotonin 2B receptor; 5-HT_{2C}R, serotonin 2C receptor; 7TM, seven-transmembrane; A_1R, adenosine A1 receptor; A_2R, adenosine A2 receptor; A_3R, adenosine A3 receptor; AHD, α-helical domain; AC, adenylyl cyclase; AGS, activators of G protein signaling; APJ, apelin receptor; AT_1R, angiotensin II receptor type 1; β_2AR, β2 adrenergic receptor; BRET, bioluminescence resonance energy transfer; cAMP, cyclic adenosine monophosphate; CaSR, calcium-sensing receptor; CB_1R, cannabinoid receptor 1; CB_2R, cannabinoid receptor 2; CHO, Chinese hamster ovary; CNS, central nervous system; CTX, cholera toxin; D_1R, dopamine D1 receptor; D_2R, dopamine D2 receptor; DAG, diacylglycerol; δOR, δ opioid receptor; ECL, extracellular loop; EP_2R, prostaglandin E2 receptor 2; $ERK_{1/2}$, extracellular signal-regulated kinase; FRET, fluorescence resonance energy transfer; FPR_2, formyl peptide receptor 2; GEF, GTP exchange factor; Gi, inhibitory G protein; GnRH, gonadotropin releasing hormone; GLP_1R, glucagon-like peptide 1 receptor; GPCR, G-protein-coupled receptor; GRK, GPCR kinase; Gs, stimulatory G protein; GTP, guanosine 5′-triphosphate; GTPγS, guanosine 5′-O-[γ-thio]-triphosphate; H_1R, histamine H2 receptor; H_4R, histamine H4 receptor; HEK293, human embryonic kidney 293; ICL, intracellular loop; IP_1, inositol monophosphate; IP_3, inositol 1,4,5-triphosphate; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; mAChR, muscarinic acetylcholine receptor; MAPK, mitogen-activated protein kinase; MD, molecular dynamics; mGluR, metabotropic glutamate receptor; NF-κB, Nuclear factor-κB; μOR, μ opioid receptor; κOR, κ opioid receptor; NMR, nuclear magnetic resonance; NT_1R, neurotensin receptor type 1; PGE2, prostaglandin E2; PGI_2, prostaglandin I_2; phosphatidylinositol, (PI); PI_3K, phosphoinositide 3 kinase; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PKD, protein kinase D; PLC, phospholipase C; PTH_1R, parathyroid hormone 1 receptor; PTX, pertussis toxin; RGS, regulator of G protein signaling; RHD, Ras-homology domain; RTK, receptor tyrosine kinase; $S1P_1$R, sphingosine 1-phosphate receptor 1; STAT, signal transducer and activator of transcription; TM, transmembrane; VFD, Venus flytrap domain.

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Contents

1. Introduction

The superfamily of seven transmembrane receptors (7TMR) is characterized by having seven highly conserved transmembrane alpha helices (TM). They are also referred to as G protein-coupled receptors (GPCRs), because they convey signals across biological membranes via interaction with intracellular guanine nucleotide-binding proteins (G proteins). This superfamily comprises approximately 2% of all proteins encoded in the human genome, and is the target of a substantial portion of current pharmaceuticals (Fredriksson, Lagerström, Lundin, & Schiöth, 2003). These receptors are classified according to their ligand binding, structure and physiology. The most frequently used A-F system (A: rhodopsin-like, B: secretin, C: metabotropic glutamate, D: fungal mating pheromone receptors, E: cyclic AMP receptors, and F: frizzled/smoothened receptors) is designed to cover all GPCRs, in both vertebrates and invertebrates. Meanwhile, recent phylogenic studies have revealed that most human GPCRs can be categorized into five major families; Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2, and Secretin, forming the GRAFS classification system (Fredriksson et al., 2003).

G proteins consist of monomeric small G proteins and heterotrimeric (Gαβγ) G proteins, and function as transducers to initiate multiple intracellular signaling pathways. They switch between inactive/active states upon GDP/GTP exchange. While GTP exchange factors (GEF) promote activation, GTP hydrolysis by the GTPase domain, existing in monomeric G proteins and Gα subunit of the heterotrimers, turns them off. The small G proteins are activated by a number of intracellular GEFs (Rojas, Fuentes, Rausell, & Valencia, 2012), whereas the heterotrimers are activated via GPCRs and GEFs such as activators of G protein signaling (AGS) (Marty & Ye, 2010; Siderovski & Willard, 2005). From an initial group of only three members (Gs, Gi and transducin), this family has expanded to approximately 20 α, 6 β and 12 γ subunits (Robishaw & Berlot, 2004). Compared to GPCRs which have endured a larger lineage-specific divergence in gene number and sequence, the Gα proteins remain highly conserved across organisms (Flock et al., 2015). In this regard, development of a common Gα numbering system (CGN) provided significant insights to identify equivalent residues/positions across different Gα subunits, and is used to investigate the common residues involved in selective coupling with the receptor (Flock et al., 2017).

Originally, each GPCR was thought to signal through a single cognate G protein class to initiate "canonical" signaling of the receptor. Thus, a beta-adrenergic receptor was shown to couple to Gs to increase cAMP, but did not couple to Gi or Gq. In the canonical signaling cascade, ligand binding induces structural rearrangement in the receptor, enhancing its interaction with the cognate heterotrimeric G protein. This interaction causes reorganization of the G protein domains and GDP/GTP exchange in the α subunit. This allosteric mechanism is reciprocal; i.e. transducer engagement with the receptor induces conformational reorganization at the ligand binding pocket, resulting in enhanced affinity (DeVree et al., 2016). This process requires all three G protein subunits, and is followed by dissociation of G proteins from the receptor into activated α and βγ subunits, which can then interact with downstream effectors (Park, Scheerer, Hofmann, Choe, & Ernst, 2008).

Several deviations from the canonical pathway have been reported over the years and comprise "non-canonical" signaling. Firstly, G protein signaling is not confined to plasma membrane; rather, they can modulate an array of effector molecules from within endosomal membranes. In this regard, the effector protein content of biological membranes significantly influences the signaling efficiency of GPCRs and G proteins (Hewavitharana & Wedegaertner, 2012). Secondly, GPCRs may form functional dimers that can influence ligand binding, allostery, receptor trafficking, and transducer selectivity (Lane et al., 2014; Ng, Lee, & Chow, 2012). Thirdly, GPCRs are capable of interacting directly with a number of effector proteins in a G protein-independent manner (Walther & Ferguson, 2015). Fourthly, GPCRs from all classes can couple to multiple G proteins (Flock et al., 2017). For example, upon PKA-induced phosphorylation Gs-coupled beta-adrenergic receptors switch to couple to Gi proteins and in turn to β-arrestin recruitment (Daaka, Luttrell, & Lefkowitz, 1997; Luttrell et al., 1999). However, not all GPCRs can couple to all G proteins and in many cases the agonist potency is greater for canonical vs. non-canonical GPCR signaling (Kukkonen, Näsman, & Akerman, 2001). Thus, GPCR/G protein combinations that respond with lower agonist potency or only in some cell types are designated "non-cognate" and mediate non-canonical signaling. Finally, GPCR coupling to β-arrestins, once believed to terminate receptor signal transduction, has been demonstrated to initiate a new set of signaling pathway (Peterson & Luttrell, 2017).

Given the potentially pleiotropic properties of GPCRs to couple to multiple signaling pathways, it is possible to develop ligands to direct signaling toward a select pathway, a concept called 'functional selectivity' or 'biased signaling'. The field has significantly evolved with the development of Förster resonance energy transfer (FRET) or bioluminescence resonance energy transfer (BRET) probes to monitor GPCR/transducer interaction with a temporal resolution of seconds (Masuho et al., 2015; Unen et al., 2016). Improved methods of data analysis have also enabled quantification of bias (Kenakin, 2017; Onaran et al., 2017). In addition, molecular dynamics (MD) studies along with biophysical and structural evidence from recently solved GPCR-G protein complexes from class A (Draper-Joyce et al., 2018; Garcia-Nafria, Nehme, Edwards, & Tate, 2018; Kang et al., 2018; Koehl et al., 2018; Rasmussen et al., 2011) and class B (Liang et al., 2017; Zhang et al., 2017) as well as active/inactive conformations of class C (Geng et al., 2016) receptors have provided significant breakthroughs in our understanding of mechanisms of receptor/transducer selectivity. Furthermore, recent evidence indicates distinct receptor conformations for a number of GPCRs when bound to biased ligands (Kahsai et al., 2011; Liang et al., 2018; Peng et al., 2018), cognate or non-cognate G proteins (Capper & Wacker, 2018; Rose et al., 2014), and β-arrestins (Liu, Horst, Katritch, Stevens, & Wüthrich, 2012). Interestingly, active

Table 1
Non-exhaustive list of GPCRs coupling to non-cognate G proteins.

Receptor	Cognate Gα	Non-cognate Gα	Receptor expression (endogenous or heterologous)	Methods	Ref.
Class A					
5-HT_{1A}	Gi	Gs, Gz, Gq	endogenous/heterologous	GTPγS labeling, second messenger analysis, PTX, Gz antisense oligonucleotide	(Barr, Brass, & Manning, 1997; Malmberg & Strange, 2000; Okada et al., 2004; Serres et al., 2000)
5-HT_{1E}	Gi	Gs	heterologous	second messenger analysis, PTX and CTX	(Adham, Vaysse, Weinshank, & Branchek, 1994)
5-HT_{2A}	Gq/11	Gi/o, G12/13	heterologous	second messenger analysis, PTX treatment, antagonistic peptide constructs	(Kurrasch-Orbaugh, Parrish, Watts, & Nichols, 2003)
5-HT_{2B}	Gq/11	G13	endogenous/heterologous	second messenger analysis	(Manivet et al., 2000)
5-HT_{2C}	Gq/11	Gi_3, Go, G13	endogenous/heterologous	GTPγS labeling, Gα antibody capture or antisense oligonucleotide, second messenger analysis, PTX	(Alberts, Pregenzer, Im, Zaworski, & Gill, 1999; Chen, Baez, & Yu, 1994; Cussac et al., 2002; McGrew, Chang, & Sanders-Bush, 2002; Okada et al., 2004; Price, Weiner, Chang, & Sanders-Bush, 2001)
5-HT_{4A}	Gs	G13	heterologous	GTPγS labeling, co-precipitation, transcriptional activity of SRE, p115RhoGEF-RGS	(Ponimaskin, Profirovic, Vaiskunaite, Richter, & Voyno-Yasenetskaya, 2002)
5-HT_{4B}	Gs	Gi/o	heterologous	GTPγS labeling, second messenger analysis, PTX	(Pindon et al., 2002)
5-HT_7	Gs	Gi, Gq/11, G12	heterologous	GTPγS labeling, second messenger analysis, transcriptional activity of SRE and CREB	(Alberts, Chio, & Im, 2001; Kvachnina et al., 2005; Kvachnina et al., 2009)
Adenosine A_1	Gi	Gi, Gs, Gq	heterologous	GTPγS labeling, second messenger analysis, PTX	(Baltos, Gregory, et al., 2016; Cordeaux et al., 2000; Cordeaux, Ijzerman, & Hill, 2004)
Adenosine A_3	Gi	Gq/11	heterologous	co-precipitation, GTP-AA labeling	(Palmer, Gettys, & Stiles, 1995)
Adrenergic α_2	Gi	Gs	heterologous	second messenger analysis, co-precipitation PTX and CTX	(Eason et al., 1992; Eason, Jacinto, & Liggett, 1994)
Adrenergic β_1	Gs	Gi	heterologous	PTX treatment, second messenger analysis	(Martin et al., 2004)
Adrenergic β_2	Gs/olf	Gi/o, Gq, G15	endogenous/heterologous	GTPγS or GTP-AA labeling, second messenger analysis, receptor/Gα fusion, NanoBRET	(Daaka et al., 1997; Kilts et al., 2000; Masuho, Ostrovskaya, et al., 2015; Wenzel-Seifert & Seifert, 2000)
Angiotensin AT_{1A}	Gq	Gi_3	heterologous	second messenger analysis, BRET	(Hunyady & Catt, 2006; Saulière et al., 2012)
Bradykinin B_2	Gq/11, Gi/o, G14	Gs	endogenous/heterologous	GTPase activity, second messenger analysis, protein capture, FRET, NanoBRET	(Liao & Homcy, 1993; Liebmann et al., 1996; Masuho, Ostrovskaya, et al., 2015; van Unen et al., 2016)
Cannabinoid CB_1	Gi/o	Gs, Gq/11, G16	endogenous/heterologous	second messenger analysis, CRE-luciferase reporter gene, PTX, dominant negative Gq/11, co-precipitation	(Bonhaus et al., 1998; Calandra et al., 1999; Chen et al., 2010; Ho, Current, & Drewett, 2002; McIntosh, Hudson, Yegorova, Jollimore, & Kelly, 2007; Paquette et al., 2007)
Cholecystokinin CCK_1	Gq/11	Gs	heterologous	second messenger analysis	(Wu et al., 1997)
Dopamine D_{1A}	Gs	Gs, Gi, Gq	endogenous	GTPγS or α-32P-GTP labeling, co-precipitation, second messenger analysis	(Jin, Wang, & Friedman, 2001)
Dopamine D_3	Gi	Gs, Gz	heterologous	second messenger analysis, PTX	(Obadiah et al., 1999)
Galanin GAL_2	Gq/11	Gi/o, G12	endogenous/heterologous	GTP-AA labeling, second messenger analysis	(Wittau et al., 2000)
Gonadotropin releasing hormone	Gq/11	Gi, Gs, G14/15	endogenous/heterologous	second messenger analysis, PTX and CTX	(Stanislaus, Ponder, Ji, & Conn, 1998; Ulloa-Aguirre et al., 1998)
Luteinizing hormone	Gs	Gi2	endogenous/heterologous	GTP-AA labeling, second messenger analysis	(Herrlich et al., 1996; Kühn & Gudermann, 1999)
Histamine H_2	Gs	Gi, Gq/11, G14/15	endogenous/heterologous	GTP-AA labeling, second messenger analysis	(Kilts et al., 2000; Kühn, Schmid, Harteneck, Gudermann, & Schultz, 1996)
Melanin concentrating hormone MCH_1	Gi/o	Gq, Gs	heterologous	GTPγS labeling, second messenger analysis, PTX	(Hamamoto et al., 2015; Hamamoto, Mizusawa, Takahashi, & Saito, 2011; Hawes et al., 2000; Pissios, Trombly, Tzameli, & Maratos-Flier, 2003)
Melanocortin MC4	Gs	Gq/11, Gi/o	endogenous/heterologous	GTPγS labeling, second messenger analysis, PTX, CRE reporter gene, Gα knockout mice	(Büch, Heling, Damm, Gudermann, & Breit, 2009; Li et al., 2016)
Melatonin MT_1	Gi2, Gi3	Gq/11	endogenous/heterologous	co-precipitation, second messenger analysis, PTX	(Brydon et al., 1999)
Muscarinic M_1	Gq/11	Gi, Gs,	endogenous/heterologous	GTPγS or GTP-AA labeling, second messenger analysis	(Akam, Challiss, & Nahorski, 2001; Masuho, Ostrovskaya, et al., 2015; Offermanns et al., 1994; Thomas et al., 2008)
Muscarinic M_2	Gi/o	Gi, Gs, Gq/11	heterologous	Second messenger analysis, Gα siRNA, PTX	(Michal, El-Fakahany, & Dolezal, 2007; Mistry, Dowling, & Challiss, 2005)
Muscarinic M_3	Gq/11	Gi, G12, Gs	heterologous	GTPγS or GTP-AA labeling, second messenger analysis, PTX, FRET, NanoBRET	(Akam et al., 2001; Jones, Heilman, & Brann, 1991; Masuho, Ostrovskaya, et al., 2015; Offermanns et al., 1994; Rümenapp et al., 2001; van Unen et al., 2016)
Muscarinic M_4	Gi/o	Gi, Gs	heterologous	second messenger analysis, PTX	(Dittman et al., 1994; Mistry et al., 2005)
Neurokinin NK_1	Gq/11	G13, Gz	heterologous	GTPγS labeling	(Barr et al., 1997)
Neurotensin NTS_1	Gq	Gi	heterologous	GTPγS labeling, second messenger analysis Receptor-Gα fusion, PTX	(Gailly, Najimi, & Hermans, 2000; Grisshammer & Hermans, 2001)

Table 1 (*continued*)

Receptor	Cognate Gα	Non-cognate Gα	Receptor expression (endogenous or heterologous)	Methods	Ref.
Opioid μ	Gi/o	Gs, Gq	endogenous/heterologous	co-precipitation, second messenger analysis	(Chakrabarti & Gintzler, 2007; Chakrabarti, Regec, & Gintzler, 2005; Seyedabadi et al., 2012; Wang & Burns, 2009; Wang, Friedman, et al., 2005)
Oxytocin OT	Gq/11	Gi/o	heterologous	second messenger analysis, PTX	(Favre et al., 2005; Hoare et al., 1999)
Prostanoid IP	Gs	Gi, Gq/11	heterologous	co-precipitation, second messenger analysis, PTX	(Lawler, Miggin, & Kinsella, 2001; Miggin & Kinsella, 2002)
Prostanoid EP_2	Gs	Gq/11	endogenous	second messenger analysis, PTX, Gα siRNA	(Kandola et al., 2014)
Lysophospholipid $S1P_3$ and $S1P_5$	Gi	Gq, G13	heterologous	GTPγS labeling	(Windh et al., 1999)
Proteinase-activated PAR	Gq/11	G13, Gi_2	endogenous/heterologous	GTPγS or 32P-GTP labeling, second messenger analysis, PTX	(Barr et al., 1997; Ogino, Sakamoto, Kinouchi, & Shimizu, 2000; Ogino, Tanaka, & Shimizu, 1996)
Thyrotropin	Gs	Gi/o, Gq/11, G12, G13	endogenous/heterologous	GTP-AA labeling, second messenger analysis, PTX	(Allen, Neumann, & Gershengorn, 2011; Allgeier et al., 1994; Allgeier, Laugwitz, Van Sande, Schultz, & Dumont, 1997; Laugwitz et al., 1996)
Vasopressin V1a	Gq/11	Gi_3	heterologous	GTP-AA labeling, second messenger analysis, Gα antisense	(Abel et al., 2000)
Class B					
Calcitonin	Gs	Gq/11, G14/15/16	heterologous	second messenger analysis, PTX	(Offermanns, Iida-Klein, Segre, & Simon, 1996)
Corticotrophin-releasing hormone	Gs	Gq/11, Gi, Go and Gz	endogenous	GTP-AA labeling	(Grammatopoulos, Randeva, Levine, Kanellopoulou, & Hillhouse, 2001)
Glucagon	Gs	Gi	endogenous	GTP-AA labeling, second messenger analysis	(Kilts et al., 2000)
Glucagon like peptide GLP-1	Gs	Gi/o, Gq/11	heterologous	GTPγS or GTP-AA labeling, second messenger analysis, PTX and CTX	(Bavec, Hällbrink, Langel, & Zorko, 2003; Hällbrink et al., 2001; Montrose-Rafizadeh et al., 1999)
Parathyroid hormone	Gs	Gq/11, G14/15/16 Gi_1	heterologous	GTP-AA labeling, second messenger analysis, PTX	(Offermanns et al., 1996; Schwindinger et al., 1998)
Vasoactive intestinal peptide	Gs	Gi	endogenous	second messenger analysis, Gα antibody capture, βARK-ct, RGS4, G15 knockout mice,	(Luo et al., 1999)
Class C					
Calcium sensing CaSR	Gi/o, Gq/11	G12/13, Gs	endogenous/heterologous	GTPγS or GTP-AA labeling, second messenger analysis, PTX, RGS4, p115RhoGEF-RGS	(Arthur, Collinsworth, Gettys, Quarles, & Raymond, 1997; Huang, Hujer, Wu, & Miller, 2004; Mamillapalli & Wysolmerski, 2010)
Metabotropic glutamate 1a	Gq/11	Gi/o, Gs	heterologous	GTPγS labeling, second messenger analysis, PTX	(Hermans et al., 2000; Selkirk, Price, Nahorski, & Challiss, 2001; Thomsen, 1996)
Metabotropic glutamate 5	Gq/11	Gs	heterologous	second messenger analysis	(Joly et al., 1995)

βARK-ct, β-adrenergic kinase c-terminus; BRET, Bioluminescence resonance energy transfer; CRE, cAMP response element; CREB, CRE binding protein; FRET, Förster resonance energy transfer; GTP-AA, alpha-32P-GTP-gamma-azidoanilido; GTPγS, ^{35}S-GTP-gamma-S; PTX, pertussis toxin; CTX, cholera toxin; RGS, regulator of G protein signaling; SRE, Serum Response Element.

conformations beyond G protein/β-arrestin signaling is also reported (Zhang et al., 2017). In fact, biased ligands can be used to direct the traffic jam in receptor-transducer junction toward a specific road, though we are still at the beginning of the path.

In this review, evidence for ligand-specific GPCR signaling toward different transducers or pathways is elaborated. Furthermore, molecular determinants of biased signaling toward these pathways as well as therapeutics potentials are presented.

2. Differential strength of signal, functional selectivity, and biased signaling

It is well accepted that a particular GPCR agonist can activate multiple signaling pathways through binding to different receptor subtypes. For example, epinephrine can activate Gs, Gi, and Gq through binding to β, $α_2$ and $α_1$ adrenoceptors, respectively. Another level of complexity, however, arises from the capacity of the same receptor to activate multiple pathways. Through evolutionary mapping of GPCR coupling to G proteins, it is estimated that ~85% of receptors, at least once during their evolution, have changed in their Gα selectivity (Flock et al., 2017). Furthermore, GPCRs (of all classes) are capable of coupling to multiple Gα proteins in heterologous or endogenous expression systems (Table 1). In addition, GPCR interaction with β-arrestins has been shown to have active roles in cell signaling (Peterson & Luttrell, 2017).

GPCRs may activate non-cognate pathways, particularly when receptor density or agonist concentration becomes high enough to perturb the fidelity of their interaction, implying 'differential strength of signal' (Tucek, Michal, & Vlachová, 2002). In this case, the rank order of agonist potency is expected to be similar across all pathways (Bonhaus, Chang, Kwan, & Martin, 1998). However, some agonists show greater efficacy and potency to activate one pathway among all downstream repertoire of the same receptor, indicating 'ligand bias'. For instance, the relative order of potency for the pituitary adenylyl cyclase-activating polypeptides (PACAP-27 and -38) in terms of activation of adenylyl cyclase (AC) and phospholipase C (PLC) is reversed. The former is more potent for activation of AC, while the latter displays greater potency for the PLC pathway (Spengler et al., 1993). Likewise, structurally diverse $α_2$ adrenoceptor ($α_2$AR) agonists, catecholamines and phenolamines, display different orders of potency for Gi, Gs or Gq activation (Airriess, Rudling, Midgley, & Evans, 1997). Such ligand-specific divergence in receptor-mediated activation of downstream pathways are also reported for class B (Gesty-Palmer et al., 2009) and class C (Davey et al., 2012) members of GPCR superfamily.

In this regard, ligand bias is defined as a property of the ligand-receptor complex highlighting ligand-specific receptor conformations that induce/select a subset of, but not necessarily all, transducer molecules. Meanwhile, 'functional selectivity' is a broad term characterizing any difference between two ligands in terms of pharmacological effects, and includes differences in pharmacokinetics, molecular target(s), intrinsic efficacy, and target receptor conformation (Violin, Crombie, Soergel, & Lark, 2017). Thus, ligand bias is ultimately demonstrated by biophysical or structural data highlighting a difference between two ligands in terms of receptor conformation, leading to different pharmacological readouts. However, biochemical assays can also be exploited to study ligand bias even in the absence of structural data. In fact, the diversity of pharmacological profile of two ligands may stem from ligand bias (the ability of a ligand to favor signaling to one pathway among all possible receptor pathways), system bias (difference in relative efficacy of receptor for distinct pathways), or observational bias (difference in receptor, transducer or effector expression characteristics of the experimental platform in which the assays are conducted) (van der Westhuizen, Breton, Christopoulos, & Bouvier, 2014).

Since the first description of this concept (Kenakin, 1995), most related studies have focused on characterization of β-arrestin biased ligands (Wisler, Xiao, Thomsen, & Lefkowitz, 2014). However, biased signaling is not limited to the bimodal G protein vs. β-arrestin pathways. Rather, biased ligands may show subtle differences in terms of magnitude or rate of activation of preselected pathways (Fig. 1). Interestingly, endogenous ligands acting at a single receptor may not activate all signaling pathways of the receptor to the same extent at the same time with similar potency, a phenomenon called 'natural bias' (Kohout et al., 2004; Zidar, Violin, Whalen, & Lefkowitz, 2009). Furthermore, post-translational modification of endogenous ligands of a select receptor may create ligands with different bias profiles (Teixeira et al., 2017; Vacchini et al., 2018).

Allosteric modulators may also reshape the pharmacological profile of neutral (demonstrating equal activity in all downstream pathways) and/or biased agonists. In fact, allosteric ligands can 'impose' biased signaling on natural agonists, through 'biased antagonism' of agonist signaling (Kenakin & Miller, 2010). In this regard, a non-linear shift in signaling repertoire of agonist-occupied receptors by allosteric modulators has been reported for cholecystokinin CCK_B (Pommier et al., 1999), tachykinin NK_2 (Maillet et al., 2007), prostanoid DP_2 (Mathiesen et al., 2005), and cytokine receptors such as interleukin 1 receptors (Quiniou et al., 2008). It is also noteworthy that some competitive antagonists can induce biased signaling *per se* independent of the agonist through biased inverse agonism. For instance, atropine acts as a low efficacy partial agonist, an inverse agonist, or a neutral antagonist of muscarinic M_3 receptor (M_3R)-mediated activation of G12, Gq, and $Gi_{1/2}$, respectively (Stewart, Sexton, & Christopoulos, 2010). Likewise, naloxone acts as a pure antagonist for δ opioid receptor (δOR), but induces μ opioid receptor (μOR) -mediated activation of Go and Gz (but not Gi_1, Gi_2, Gi_3 or G15) as well as κ opioid receptor (κOR) -mediated activation of Gi/o (but not G15) proteins (Masuho, Ostrovskaya, et al., 2015). Thus, atropine and naloxone display receptor- and signal-dependent activity as biased agonists, biased inverse agonist, or antagonists. Understanding the signaling bias of receptor ligands is important since they may be used to differentiate beneficial vs. adverse receptor-mediated effects. To further identify on-target vs. off-target effects that are independent of the receptor, specific antagonists or systems in which the receptor is knocked-down, knocked out or absent can be used to block the ligand-induced signal.

3. Quantification of bias

GPCR signal transduction involves multi-step pathways leading to a specific response. Thus, when comparing ligand efficacy across different signaling pathways several confounding factors need to be considered (Fig. 2). To determine true ligand bias, the system and observational biases need to be controlled for. System and observational bias are especially evident for protean agonists, which produce positive agonism in quiescent receptor systems or inverse agonism in constitutively-active systems (Kenakin, 2001). Furthermore, different systems demonstrate different levels of signal amplification. In particular, *Saccharomyces cerevisiae* platforms expressing chimeric mammalian/yeast Gα offer a

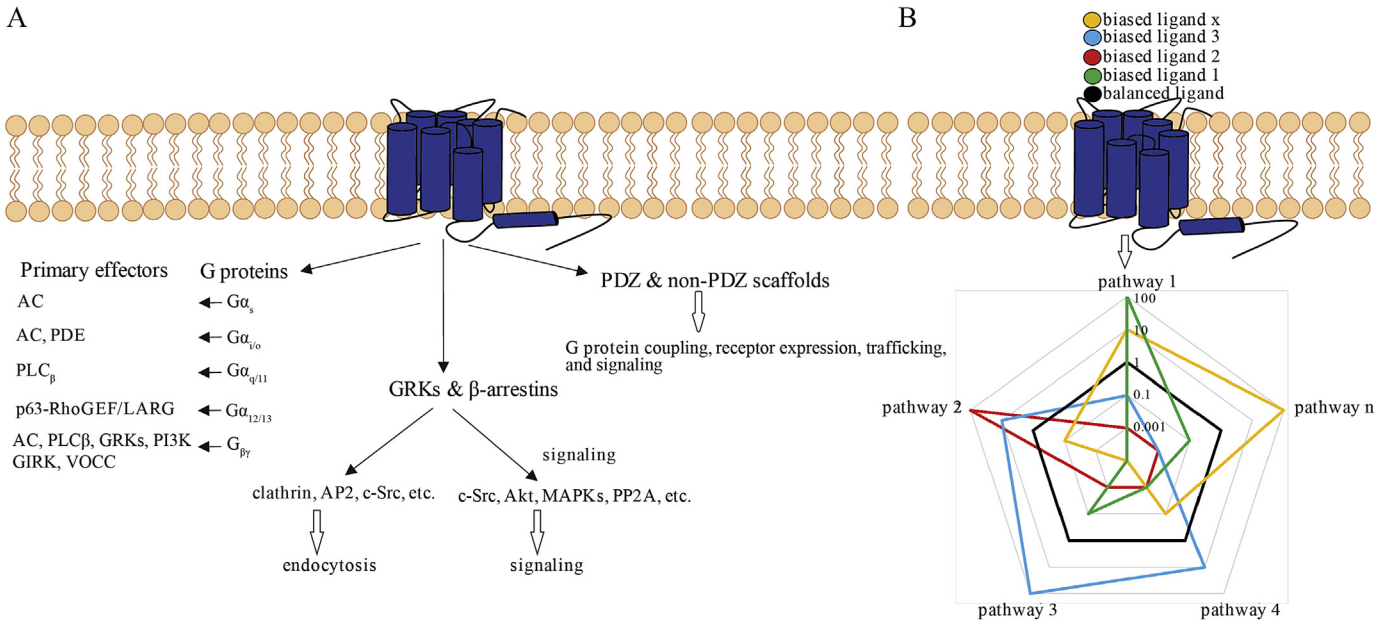

Fig. 1. GPCRs signaling toward multiple pathways. A: GPCRs relay signals through cognate/non-cognate G proteins, β-arrestins, and G protein/β-arrestin-independent pathways. Signaling toward each of these pathways can activate different effector molecules, resulting in different cellular responses. B: Different ligands acting at the same receptor may induce distinct receptor conformations favoring one among all downstream pathways. Signaling is not a matter of YES/NO bimodal system; rather, different ligands may display subtle differences in terms of the magnitude or rate of activation downstream repertoire of a single receptor. Thus, presuming a balanced ligand which activates all pathways with similar efficacy and potency, biased ligands (a-x) induce a different pattern of activation of signaling pathways (1-n) compared to that of the balanced ligand, as described in a radar graph. AC, adenylyl cyclase; AP2, activating protein 2, GIRK, G protein inward rectifying K channel; GRK, GPCR kinase; MAPK, mitogen-activated protein kinase; PED, phosphodiesterase; PLC, phospholipase C; PKD, protein kinase D; PLC, phospholipase C; PP2A, protein phosphatase 2A;

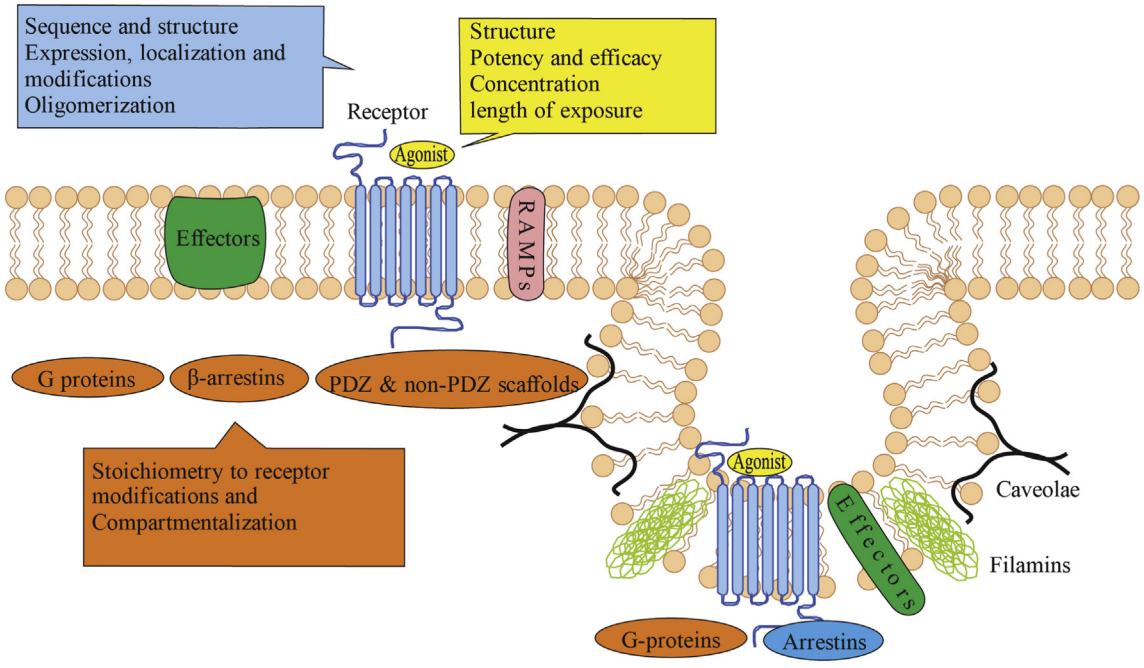

Fig. 2. Schematic presentation of selectivity determinants of receptor/transducer interaction. The interaction of GPCRs to G proteins, β-arrestins or other GIPs depends on several factors in the receptor, G protein, and agonist. Furthermore, GPCR/transducer selectivity is influenced by a number of scaffolds (PDZ or non-PDZ) as well as membrane content and other proteins involved in localization and compartmentalization of interacting molecules. GIPs, GPCR-interacting molecules; RAPMs, receptor-activity-modifying proteins (RAMPs).

simple, fast and inexpensive screen for GPCR/G protein signaling. Because signaling of mammalian receptors can be assayed from one Gα at a time, this system shows good alignment of concentration-response and concentration-occupancy curves, but its low sensitivity is not optimal for low-efficacy ligands (Stewart et al., 2010). For example, the A_1 receptor agonist VCP-189 exhibits bias toward $G\alpha i_2$ in yeast, but in CHO cells it activates both Gi_2 and Go when examined with second messenger or GTPγS assays (Stewart et al., 2009). Also, different cellular pathways may display distinct levels of signal amplification. For example, second messenger levels are intensively amplified by enzymatic catalysis, whereas little amplification occurs for enzyme-based β-arrestin recruitment assays (Rajagopal et al., 2011). In addition, GPCR/G protein coupling can be altered depending on the time of exposure to agonist, and platforms considering the kinetic parameters provide additional information about agonist/GPCR/G protein signaling cascade (Masuho, Martemyanov, & Lambert, 2015; Masuho, Ostrovskaya, et al., 2015).

This issue of confounding factors in biased signaling becomes critical given that GPCR signaling in physiological or pathological conditions is not determined solely by the agonist and the receptor. In fact, a variety of contextual factors come to play. For example, the chemokine CCR_7 receptor ligands (CCL19 and CCL21) showed different patterns of β-arrestin recruitment, receptor internalization, and MAPK activation, suggesting ligand bias (Kohout et al., 2004). However, these differences disappear when all pathways are analyzed in the same system (Corbisier, Galès, Huszagh, Parmentier, & Springael, 2015). Given that a single phenomenon can be modulated through different processes in different cells, the clinical efficacy of a given functionally selective ligand must be verified in physiological/pathological contexts that closely resemble the condition in which the drug is to be used. Of particular note, disease states may influence the stoichiometry of signaling molecules as well as other parameters, eventually altering the pharmacological readout of a given agonist (discussed in Sections 6.2 to 6.6). In this regard, the potential of using human induced pluripotent stem cells as screening platforms may provide a sufficiently relevant system that could be scaled for high-throughput analysis (Horvath et al., 2016).

Initially, ligand bias was determined through distinct ligand behaviors in terms of maximal effects (E_{max}) or potencies (EC_{50}; the concentration at which half maximal effect is achieved) for different pathways (Galandrin & Bouvier, 2006). However, these parameters fail to account for confounding factors such as receptor reserve or signal amplification, sometimes leading to erroneous conclusions. This is especially true when a partial agonist is compared to a full agonist, or when bias is evaluated between pathways with different degrees of signal amplification, e.g. second messenger vs. β-arrestin assays (Rajagopal, Rajagopal, & Lefkowitz, 2010). Similarly, equimolar approaches, comparing the responses across different pathways at the same concentration for a single ligand, can be highly system- and assay-dependent (Rajagopal et al., 2011).

The alternative equi-active approach compares concentrations of ligands that induce equal response in two pathways. This is done utilizing the relative intrinsic activity (RA), which can be calculated from maximal effects and potencies of the test and reference ligands, i.e. $RA = (E_{max,test}{}^{\times} EC_{50,reference})/EC_{50,test}{}^{\times} E_{max,reference})$ (Figueroa, Griffin, & Ehlert, 2009). The RA of test and reference ligands are then normalized across pathways to give the bias factor (Rajagopal et al., 2011). The formula is strengthened by using the maximal response (max) expressed as the fraction of maximal capacity of the system, and not as the fraction of response to the most effective ligand in the assay. For instance, if forskolin induces more cAMP production than any other tested receptor agonist, maximal response to a given agonist must be expressed as proportional to the maximal effect of forskolin, and not to that of the full agonist in the assay. When compared to a reference agonist, i.e. $\Delta\log(max/EC_{50}) = \log(max/EC_{50})_{reference} - (max/EC50)_{test}$, ligand bias can be measured as antilog of $\Delta\Delta\log(max/EC_{50})_{j1\text{-}j2}$ values between the pathways (j1 and j2). The reference agonist must be the same so that the data can be used to compare bias in signaling. However for valid comparison, the slope of concentration-response curve must be >0.5 and the maximal response >35% (Kenakin, 2017). This approach provides a more accurate assessment of signaling bias than the equimolar approaches by taking into account both potency and efficacy.

Based on operational models of pharmacological agonism (Black & Leff, 1983), several indices can be derived from concentration-dependence curves for bias analysis. Of particular note is transduction efficacy (τ/K_A), where τ is the total number of receptors (R_T) divided by the coupling efficacy of agonist-occupied receptor to a specific

pathway (K_E), and K_A represents the agonist binding affinity. Given that τ encompasses both the ligand's intrinsic efficacy as well as system-dependent factors such as receptor density and coupling efficiency, the transduction efficacy of the test ligand is first adjusted to that of a reference ligand, i.e. $\Delta\text{Log}\,(\tau/K_A) = [\text{Log}\,(\tau/K_A)_{test} - \text{Log}\,(\tau/K_A)_{reference}]$, and then this normalized coefficient is adjusted across pathways (j1 and j2), i.e. $\Delta\Delta\text{Log}\,(\tau/K_A)_{j1\text{-}j2} = [\Delta\text{Log}\,(\tau/K_A)_{j1} - \Delta\text{Log}\,(\tau/K_A)_{j2}]$. In fact, stimulus bias is demonstrated only when the pattern of activation/inhibition of different pathways does not follow that of the reference compound normalized across different pathways, i.e. $\Delta\Delta\text{Log}\,(\tau/K_A)$ is significantly different from zero (Kenakin, Watson, Muniz-Medina, Christopoulos, & Novick, 2012). For example, comparing the transduction ratios of the M_2 receptor agonist arecoline for $ERK_{1/2}$ activation (log τ/KA: 7.08) and GTPγS binding (log τ/KA: 6.46), one may conclude that arecoline is biased toward the former pathway (Gregory, Hall, Tobin, Sexton, & Christopoulos, 2010). However, normalization for a reference agonist has revealed that arecoline, compared to ACh, has 2.8-fold bias toward GTPγS binding over $ERK_{1/2}$ activation (Gregory, Sexton, Tobin, & Christopoulos, 2012). Alternatively, effective signaling (σ) for both ligand and reference agonists in one pathway is calculated using only τ from the operational models, i.e. $\sigma_{test} = \log(\tau_{test}/\tau_{reference})$, and then is normalized across pathways (j1 and j2), i.e. bias factor $(\beta) = [(\sigma_{j1} - \sigma_{j2})/\sqrt{2}]$ (Rajagopal et al., 2011).

These methods, however, assume that the transduction machinery of a select pathway, as well as the maximum system responsiveness, are equal for all agonists (Gregory et al., 2012) and that the assay condition influences the kinetics of ligand-receptor interaction and signaling processes equally in all experiments (Violin et al., 2017). However, a single event may involve different pathways depending on the receptor or experimental context (Charfi et al., 2014; Rajagopal et al., 2013). While some research groups have found good overall consistency between different analytical methods (Brust, Hayes, Roman, Burris, & Watts, 2015), others have reported significant discrepancies. Of particular note, analysis of a single set of data with seven methods including τ, τ/K_A, E_{max}/EC_{50}, and equi-effective approaches has revealed huge inconsistencies between the resultant bias factors for β_2 adrenoceptor (β_2AR) agonists toward Gs, Gi or β-arrestin pathways. Furthermore, these models demonstrated bias for signaling steps downstream of the same receptor with the same ligand, for which no biased efficacy is theoretically plausible, as well as false positive bias for neutral ligands (Onaran et al., 2017). Therefore, model-independent strategies are proposed based on relative intrinsic activity (RA) from concentration-response curves. In fact, a reference trajectory is drawn based on the normalized response to a reference full agonist in each pathway, on which the RA of all unbiased ligands should theoretically lie and biased agonists display deviations from this line. These methods generate less false positive outcomes, and seem more robust and reliable, but cannot quantify the extent of biased efficacy (Onaran et al., 2017).

4. Revisiting the receptor/transducer interaction

Understanding the molecular mechanism of receptor-mediated transducer activation is a rapidly progressing topic in current pharmacological research. Given that GPCRs respond to a large variety of ligands, conformational reorganization in the ligand binding site is undoubtedly diverse. Upon activation, however, these receptors seem to share conserved receptor domain interaction networks and structural rearrangements at the transducer binding site (Venkatakrishnan et al., 2013). In particular, displacement of TM3, TM6 and TM7 in class A GPCRs allows interaction with subdomains of Gα, mainly carboxy-terminal of α5 helix (Venkatakrishnan et al., 2016). Given that α5 helix as well as α1 helix are in direct contact with GDP, transition from disorder to order in α5 helix upon recruitment to the receptor permits more flexibility to the α1 helix, resulting in GDP release. This would allow displacement of α5 helix and full engagement with the receptor (Dror et al., 2015). It is, however, noteworthy that additional contacts between the receptor and G protein can occur, some of which are involved in selective coupling to a specific G protein (Flock et al., 2017). Moreover, coupling with the transducer seems to be necessary for GPCRs to adopt fully active state (Nygaard et al., 2013). Interestingly, G proteins can form contacts with the non-cognate receptors and can prime the signaling of a given receptor through its cognate pairing (Gupte, Malik, Sommese, Ritt, & Sivaramakrishnan, 2017).

Class B GPCRs are characterized by a large extracellular domain at their N-terminal, which adopts a conserved 3D structure to interact with their peptidergic ligands. Peptide agonist binding causes reorganization of extracellular loop (ECL) 2 and outward movement of extracellular ends of TM6 and TM7 as well as inward movement of extracellular end of TM1. This conformational rearrangement is then translated to a sharp kink in the middle of TM6 and an outward shift of its intracellular half, which accommodates interaction with α5 helix of the Ras-like domain of Gα (Hollenstein et al., 2013; Liang et al., 2017; Zhang, Sun, et al., 2017) for G protein activation.

Class C GPCRs are obligate dimers, characterized by a very large extracellular domain that adopts a conserved 'venus fly trap' (VFT) structure. Although no crystal structure of the full-length protein is available for this family, current evidence indicates a conformational change in VFT domain upon ligand binding that is transmitted to reorganize the TM domains to initiate signaling (Geng et al., 2016; Geng, Bush, Mosyak, Wang, & Fan, 2013). In support of this, cross-linking of TM4 and TM5 in metabotropic glutamate receptor 2 ($mGluR_2$) prevents agonist-mediated activation, while crosslinking of TM5 and TM6 confers constitutive activity (Xue et al., 2015).

Given the capacity of GPCRs from class A, B and C to engage with multiple transducers (Table 1), GPCR activation models should incorporate multiple active states. In this regard, the simple 'two-state' model is limited since it describes only two receptor conformations as active (R^*) and inactive (R), where the signal is determined by the efficacy of ligand toward either of the states. Agonists have higher affinity for R^*, inverse agonists for R, and partial agonists have intermediate affinities; neutral antagonists have equal affinity for both states and do not shift the equilibrium. In contrast, rhodopsin activation in a native-like lipid environment does not follow the simple R/R^* binary conformation; rather, the activated receptor is in equilibrium between multiple conformers (Van Eps et al., 2017), underpinning the fact that receptors can interact with multiple partners.

Given that different transducer molecules tend to have distinct receptor binding sites at the cytosolic plate (Zhang, Han, et al., 2017), the model can be extrapolated to assume 'multiple states', each favoring a specific transducer pathway. In this regard, biased signaling is defined as the capacity of ligands to induce or select distinct active conformations (R^{*1}, R^{*2}, …, R^{*n}), each associated with different functional readouts (Kobilka & Deupi, 2007; Woo, Song, Zhu, & Xiao, 2015). In this model of multiple receptor states, the sum of free energies (α) gained from residual movements due to ligand or transducer binding define the signal efficacy and functional selectivity. Ligand bias is observed when α values of an agonist for two transducer pathways are different (Onaran, Rajagopal, & Costa, 2014). A clear relationship between a distinct receptor structure and coupling to a specific transducer pathway remains to be elucidated. In this regard, the crystal structures of adrenergic β_2AR-Gs (PDB code: 3SN6), adenosine A_1R-Gi_2 (PDB code: 6D9H), opioid μOR-Gi_1 (PDB code: 6DDE), serotonin 5-HT_{1B}-Go (PDB code: 6G79), and rhodopsin-β-arrestin (PDB code: 4ZWJ) complexes from class A as well as glucagon like peptide-1 receptor (GLP_1R)-Gs complexes from class B when bound to endogenous (PDB code: 5VAI) or biased (PDB code: 6B3J) ligands, and inactive/active (PDB codes: 5K5T and 5K5S) states of calcium sensing receptor (CaSR, extracellular domain) from class C along with other biophysical and structural data have provided new insights into structure function relationships at a molecular level (discussed in section 6).

5. GPCR biased signaling pathways

5.1. Biased signaling between G protein and β-arrestin pathways

The arrestin family of adapter molecules, consisting of visual arrestin (arrestin1), cone arrestin (arrestin4), as well as two nonvisual arrestins, β-arrestin1 (arrestin2) and β-arrestin2 (arrestin3) were first identified in receptor desensitization, endocytosis and degradation. In fact, agonist-mediated activation of GPCRs recruits and stimulates GPCR kinases (GRKs), which thereby phosphorylate multiple serine or threonine residues within the C-terminus or third intracellular loop, producing high-affinity arrestin binding sites, and causing steric hindrance of G protein coupling (Benovic et al., 1987). Furthermore, The carboxy-tail of β-arrestin directly interacts with the clathrin-adapter protein-2 (AP-2) complex, promoting receptor endocytosis. The internalized receptor is either recycled to the membrane or subjected to ubiquitination-dependent degradation depending on the stability of receptor-arrestin complexes (Oakley, Laporte, Holt, Caron, & Barak, 2000).

5.1.1. Arrestins take active roles in signal transduction

In addition to receptor desensitization and endocytosis, arrestins were found to mediate cell signaling as scaffold proteins, linking the agonist-occupied β_2AR and c-Src, resulting in mitogenic signaling (Luttrell et al., 1999). Later on, it was shown that β-arrestins can form a 'signalosome' to connect 7TMRs to extracellular signal- regulated kinase $_{1/2}$ ($ERK_{1/2}$), p38 mitogen-activated protein kinas (MAPK), c-jun N-terminal kinase (JNK), tyrosine kinas c-Src, phosphatidylinositol-3 kinase (PI3K)/Akt, Ser/Thr protein phosphatase (PP)2A, phosphodiesterases (PDEs), E3 ubiquitin ligases and deubiquitinases, and nuclear factor-κB (NF-κB) (Peterson & Luttrell, 2017). Furthermore, β-arrestins may relay signals even independently of the GPCR-agonist complex, possibly due to heterologous receptor phosphorylation (Toth et al., 2018). Their realm of activity extends beyond GPCRs, and includes atypical 7TMRs such as frizzled and smoothened receptors, receptor tyrosine kinases (RTKs), cytokine receptors, and ion channels (Lefkowitz, Rajagopal, & Whalen, 2006).

Arrestins contain two beta-sheet sandwiches, namely at the N- and C- domains, the interface of which generates the finger loop (residues G68–S78), the key receptor-binding element, the middle loop (residues Q133–S142), and the C-loop (residues V247–Y254). The structure of inactive arrestin is stabilized by interactions between different regions, including hydrophobic or polar interactions between the N- and C-domains or between C-tail and N-domain. Upon recruitment to the receptor, the phosphorylated tail of the receptor displaces the acidic C-tail, gaining access to several residues of the N-domain, and inducing a conformational rearrangement, resulting in a high-affinity receptor-arrestin complex (Scheerer & Sommer, 2017). Two GPCR/β-arrestin conformations have recently been described. In 'tail conformation', β-arrestin mainly engaged with the phosphorylated C-terminus and induced GPCR internalization and β-arrestin-mediated signaling but failed to interrupt receptor/G protein engagement. In 'core conformation', however, β-arrestin formed additional interactions with the receptor transmembrane core resulting in desensitization of G protein signaling (Cahill et al., 2017). This finding helps distinguish β-arrestin functions, and may explain why in some reports different ligands discriminated between β-arrestin recruitment or signaling, receptor desensitization and internalization (Table 2).

Formation of receptor complexes beyond the traditional ternary complex of agonist/receptor/G protein has also been reported. Class-A and B GPCRs may form megaplexes, in which the receptor simultaneously interacts with G proteins and β-arrestin. These megaplexes are implicated in sustained receptor signaling, where G protein activation occurs from internalized compartments, Gs binds to the receptor transmembrane core and, concurrent with G protein coupling, β-arrestin binds to the receptor C-terminal tail (Thomsen et al., 2016). Likewise, activated parathyroid hormone receptor (PTH_1R) produces a ternary complex including the Gβγ dimer and β-arrestin, resulting in an accelerated rate of Gs activation and prolonged cAMP formation (Wehbi et al., 2013). These studies suggest that a single receptor may signal to multiple transducers simultaneously. However, to date the GPCR-G protein-arrestin supercomplex has been reported only for mutant and chimeric GPCRs, but not for native receptors.

Given the potential of β-arrestins for signal transduction, a number of ligands were identified that differentiate between G protein and β-arrestin pathways (Table 2). For example, the β_2AR inverse agonists for Gs-mediated cAMP synthesis, propranolol and ICI118551, induced β-arrestin-dependent phosphorylation of $ERK_{1/2}$, even in cells with compromised Gs or Gi functioning (Azzi et al., 2003). Furthermore, carvedilol (Galandrin & Bouvier, 2006; Wisler et al., 2007) and SR121463B (Azzi et al., 2003) also had opposite actions on different pathways, antagonizing the Gs/cAMP pathway while promoting β-arrestin-dependent MAPK activation through β_2AR and vasopressin receptor 2 (V_2R), respectively. These examples show that biased ligands can have markedly different signaling properties compared to the endogenous ligand which activates both pathways.

In addition, it was found that MAPK phosphorylation can be triggered via both G protein and β-arrestin pathways (Pierce, Luttrell, & Lefkowitz, 2001). For example, the β_1AR (Galandrin et al., 2008), PTH_1R (Gesty-Palmer et al., 2009) and bombesin/gastrin-releasing peptide receptor (MacKinnon, Waters, Jodrell, Haslett, & Sethi, 2001) activated $ERK_{1/2}$ through G protein-mediated or G protein-independent β-arrestin pathways depending on the agonist. These pathways seem to converge at src protein kinase, which is implicated in $ERK_{1/2}$ phosphorylation by both pathways (Galandrin et al., 2008). Similarly, a parallel study of G protein, β-arrestin and $ERK_{1/2}$ pathways revealed that κOR agonists can display differences in biased activity for the $ERK_{1/2}$ pathway while preserving bias for G protein over β-arrestin activation, underpinning the fact that MAPK activation can occur through either of the pathways (Lovell et al., 2015). While earlier observations suggested differences in kinetics and spatiotemporal parameters for the determination of the source of such converging G-protein and β-arrestin dependent $ERK_{1/2}$ signals (Ahn, Shenoy, Wei, & Lefkowitz, 2004; Gesty-Palmer et al., 2006; Pierce et al., 2001), this could not be extrapolated to other GPCRs (Goupil et al., 2012; Zheng, Loh, & Law, 2008). Recent evidence using genome editing, conditional gene deletion, and small interfering RNAs (siRNAs) has revealed that β-arrestins 1 and 2 as well as receptor internalization are dispensable for β_2AR-mediated $ERK_{1/2}$ phosphorylation, whereas for Gαs and Gβγ-mediated $ERK_{1/2}$ signaling, src, sos, ras, raf, and MEK were required (O'Hayre et al., 2017). Accordingly, selective depletion of G proteins or β-arrestins via clustered regularly interspaced short palindromic repeats (CRISPR)/Cas technology has revealed that β-arrestin recruitment to the receptor can occur in the absence of active G proteins. However, G proteins but not arrestins dictate $ERK_{1/2}$ activation, even with prototypical β-arrestin biased ligands acting on β_2AR or angiotensin A_1 receptors (AT_1R). Although the arrestin scaffold constrains $ERK_{1/2}$ activity and determine signal amplitude and duration, arrestin action is downstream from, but not independent of, G proteins (Grundmann et al., 2018; Wang et al., 2017). This implies that ligands previously characterized as β-arrestin-biased based on indirect assays such as MAPK phosphorylation likely also activate G proteins and need be re-evaluated using direct assays. Furthermore, some ligands may differentiate between β-arrestin isoforms (Pradhan et al., 2016), or induce distinct active conformations in β-arrestin (Zimmerman et al., 2012), which can result in different pharmacological readouts.

5.1.2. Therapeutic potential of bias between G protein or β-arrestin pathways

An array of physiological and pathophysiological roles is suggested for the β-arrestin pathway (Whalen, Rajagopal, & Lefkowitz, 2011). Thus, biased ligands discriminating between β-arrestin and G protein

Table 2
Non-exhaustive list of GPCR ligands showing biased activity and their potential clinical benefits*.

Receptor	Biased activity	Potential clinical benefits	Ref.
Class A			
5-HT_1	Some arylpiperazine derivatives display bias toward G protein activation over β-arrestin recruitment	unknown	(Stroth et al., 2015)
5-HT_2	LSD, bromocriptine and pergolide displayed bias toward Gq/11 activation than calcium release; ergotamine showed strong bias for β-arrestin via 5-HT_{2B} but no bias via 5-HT_{1B}	Gq/11 biased signaling may be associated with hallucinogenic activity	(Cussac et al., 2008; Wacker et al., 2013)
Adenosine A1	VCP746 and capadenoson showed significant bias for cAMP inhibition relative to $ERK_{1/2}$ phosphorylation and calcium mobilization	biased agonists may protect against ischemic injuries without causing bradycardia	(Baltos, Gregory, et al., 2016; Valant et al., 2014)
Adenosine A3	MRS5679 display bias toward survival over $ERK_{1/2}$ pathway compared to IB-MECA	unknown	(Baltos, Paoletta, et al., 2016)
Adrenergic β_1/β_2	bisoprolol and metoprolol activated Gαi/o but not Gαs; Carvedilol was a partial agonist for β-arrestin signaling but an antagonist for Gs signaling; Epinephrine, norepinephrine and isoprenaline activated β-arrestin pathway at much higher doses than that required for Gs-pathway activation; **Salmeterol behaved as an antagonist for β-arrestin pathway but as a full agonist for Gs pathway, **Salmeterol and formoterol compared to epinephrine were biased toward β-arrestin pathway	signaling toward β-arrestin may explain therapeutic benefits of carvedilol in chronic heart failure; Signaling toward Gs may explain longer duration of action of salmeterol	(Carter & Hill, 2005; Malik et al., 2013; Onfroy et al., 2017; Rajagopal et al., 2011; Wisler et al., 2007)
Adrenergic β_3	CL316243 and SR59230A displayed bias toward/away from cAMP accumulation or P38 MAPK phosphorylation	modulation of P38 MAPK activity may produce positive effects in cardiac disorders	(Sato et al., 2007)
Angiotensin AT_{1A}	TRV120027 displayed bias toward β-arrestin and antagonizes angiotensin II mediated G protein signaling; SII activated Gq, Gi, and β-arrestin with different signaling module to angiotensin II	TRV120027 preserved pro-contractile effects of angiotensin and inhibits apoptosis without causing vasoconstriction and cardiac hypertrophy; however, it did not produce a composite clinical benefit in acute heart failure in a phase IIb trial	(Pang et al., 2017; Saulière et al., 2012; Violin et al., 2010)
Apelin APJ	MM07 and CMF-019 displayed significant bias toward G protein pathway over β-arrestin pathway	G protein-biased agonists increase myocardial contractility and vasodilation without detrimental cardiac hypertrophy	(Brame et al., 2015; Read et al., 2016)
Cannabinoid CB_1	WIN-55212 was equally efficacious in Gi and Gs pathways, while anandamide favored Gi over Gs; 2-arachidonoylglycerol and anandamide displayed bias toward Gi/o over β-arrestin, while CP-55940 and tetrahydrocannabinol favored β-arrestin pathway	Gi/o biased activity is correlated with cell viability and may be useful in Huntington's disease	(Bonhaus et al., 1998; Laprairie, Bagher, Kelly, & Denovan-Wright, 2016)
Cannabinoid CB_2	the Gi-biased LY2828360 inhibited cAMP synthesis, and activated $ERK_{1/2}$, but failed to induce arrestin recruitment, IP signaling, or receptor internalization	the Gi biased agonists induced analgesia with less potential for tolerance	(Lin, Dhopeshwarkar, Huibregtse, Mackie, & Hohmann, 2017)
Chemokine CCR	CCR_2 receptor ligand CCL8 activated $G\alpha i_1$ and $G\alpha i_2$ more efficiently than Gα12, and displayed significant bias for $G\alpha o_B$ activation relative to arrestin; The CCR_5 ligand CCL5 showed bias in terms of G protein activation; The CCR_7 ligands CCL19 and CCL27 displayed difference in terms of G protein activation, β-arrestin recruitment, receptor internalization and $ERK_{1/2}$ phosphorylation; The CCR_{10} ligand CCL28 displayed significant bias toward G protein pathway over β-arrestin recruitment	biased ligands may display different effects in immune function or cancer invasion	(Corbisier et al., 2015; Kohout et al., 2004; Rajagopal et al., 2013)
Chemokine CXCR	$CXCR_3$ receptor ligands FAUC1036 and FAUC1104 displayed bias toward G protein and β-arrestin pathways, respectively; the $CXCR_4$ receptor ligand ATI-2341 displayed functional selectivity for Gi and negative bias for G13 or the β-arrestins	biased ligands may display different effects in chemotaxis and receptor internalization	(Milanos et al., 2016; Quoyer et al., 2013)
Dopamine D_1	A-77636 induced profound internalization; dihydrexidine and N-propyl-apomorphine were full agonists for Gs but partial agonists for Golf signaling; PF-8294 and PF-6142 induce Gi-mediated inhibition of cAMP synthesis with impaired β-arrestin recruitment	long-lasting internalization may explain A-77636 inefficiency in parkinsonism; dihydrexidine may be effective in the treatment of negative symptoms of schizophrenia devoid of locomotor activation; G protein biased agonists may show antiparkinsonian properties without desensitization	(Gray et al., 2018; Ryman-Rasmussen et al., 2007; Yano et al., 2018)
Dopamine D_2	***bifeprunox and/or aripiprazole showed bias in terms of $ERK_{1/2}$ phosphorylation, Gα activation, cAMP synthesis, receptor internalization, arachidonic acid release and cellular impedance; S-(−)-3-PPP and p-tyramine activated Gi3 and Go but not Gi_2 and Gi_1; Some ligands differentiated Go_1, Gi_2, and β-arrestin2; FAUC350 was antagonist	the biased ligands showed better efficacy in terms of improving the negative symptoms of schizophrenia; selective antagonism of β-arrestin pathway may provide antipsychotics without extrapyramidal side effects	(Bonifazi et al., 2017; Chen et al., 2012; Gazi, Nickolls, & Strange, 2003; Klein Herenbrink et al., 2016; Moller et al., 2014; Moller et al., 2017; Tschammer et al., 2011; Urban, Vargas, von Zastrow, & Mailman, 2007; Weïwer et al., 2018)

Table 2 (*continued*)

Receptor	Biased activity	Potential clinical benefits	Ref.
	for AC inhibition and partial agonist in $pERK_{1/2}$ pathway; UNC9975, UNC9994, UNC9995 potently induced β-arrestin recruitment without G protein activation; BRD5814 display selective antagonistic activity in β-arrestin pathway.		
Dopamine D_3	some ligands differentiated between Go_A, Gi_2, and β-arrestin2	D_2/D_3 biased ligands preferentially activating Go_A may induce neurite outgrowth and improve negative symptoms of schizophrenia	(Moller et al., 2014)
Endothelin	certain agonists produced distinct profiles of coupling to Gi and Gq/11	β-arrestin signaling may be involved in NF-κB and β-catenin signaling	(Cianfrocca et al., 2014; Rosano et al., 2013; Shraga-Levine & Sokolovsky, 2000)
Formyl peptide FPR_2	$F2Pal_{10}$ was G protein-biased compared to WKYMVM	$F2Pal_{10}$ induced less receptor internalization and neutrophil chemotaxis	(Gabl et al., 2017)
Ghrelin 1A	compared to Ghrelin which induced Gi/Gq activation and β-arrestin recruitment, JMV 3002 and JMV 3018 were partial agonist for Gq activation and failed to induce Gi activation or β-arrestin recruitment	unknown	(Mary et al., 2012)
Gonadotropin releasing hormone	Ant135-25 displayed bias toward Gi and away from Gq activation	Gi-biased ligands may promote pro-apoptotic signaling in peripheral reproductive tumor cells	(Maudsley et al., 2004)
Histamine H1	*trans*-PAT enhanced AC/cAMP but not PLC/IP signaling	*trans*-PAT may offer benefits in neuropsychiatric and neurodegenerative disorders devoid of cardiovascular, respiratory or gastrointestinal side effects	(Moniri, Covington-Strachan, & Booth, 2004)
Histamine H4	very closely related isothiourea derivatives VUF9107, VUF5222, and VUF5223 behaved as neutral antagonist, G protein-biased and β-arrestin-biased agonists, respectively	biased ligands may display benefits in allergic diseases	(Nijmeijer, Vischer, Rosethorne, Charlton, & Leurs, 2012)
Leukotriene OXE	Gue1654 inhibited Gβγ but not Gαi signaling	Gue1654 may become useful in a variety of diseases where Gβγ plays a role	(Blättermann et al., 2012; Smrcka, 2013)
Melanocortin MC_4	agouti-related protein inhibited Gs but induced Gi/o activation	Gi-biased agonists may induce orexigenic effects	(Büch et al., 2009)
Muscarinic M_1	the bitopic ligands AC-42 and 77-LH-28-1 failed to support Gi/o activation; VCP794, 77-LH-28-1 and xanomeline showed bias toward $pERK_{1/2}$ pathway	biased ligands may show pro-cognitive effects in Alzheimer's disease or schizophrenia	(Keov et al., 2013; Keov et al., 2014; Masuho, Ostrovskaya, et al., 2015; Thomas et al., 2008)
Muscarinic M_2	McN-343 activated G15 ~ 10 folds more than Gi; **Ach, CCh, and arecoline showed bias toward $pERK_{1/2}$, whereas 77-LH-28-1, AC-42, McN-A-343 and NDMC showed bias toward G protein pathway; pilocarpine appeared to be balanced agonist for these pathways; **Compared to ACh, arecoline and NDMC showed significant bias toward G protein activation relative to $pERK_{1/2}$ and internalization; pilocarpine, AC-42, 77-LH-28-1, and McN-A-343 failed to induce internalization	biased ligands may show pro-cognitive effects in Alzheimer's disease or schizophrenia	(Gregory et al., 2010; Gregory et al., 2012; Griffin, Figueroa, Liller, & Ehlert, 2007)
Muscarinic M_3	atropine was a low efficacy agonist for coupling to G12, an inverse agonist for Gq, and a pure antagonist for Gi; Pilocarpine was biased toward β-arrestin and $pERK_{1/2}$, but did not induce Ca^{+2} mobilization or insulin secretion	bias away from G12 may provide benefit in a number or immune-related diseases	(Pronin, Wang, & Slepak, 2017; Stewart et al., 2010)
Neuropeptide Y_2	NPY13-36 increased GTPγS binding to Gq and Gi_2 but not to Gi_1 or Gi_3	unknown	(Misra et al., 2004)
Neurotensin NTR_1	ML314 displayed significant bias toward β-arrestin over Gq-mediated Ca^{2+} mobilization	ML314 may be helpful in methamphetamine abuse treatment	(Barak et al., 2016)
Niacin GPR109a	MK-0354 activated Gi pathway but did not induce β-arrestin-mediated MAPK activation	The Gi biased agonists decreased plasma triglycerides devoid of vasodilation and flushing. However, MK-0354 produced only a weak effect on serum lipids compared with niacin	(Boatman et al., 2012; Semple et al., 2008)
Opioid μ	DAMGO, morphine, fentanyl and other agonists induced different pattern of G protein activation, β-arrestin recruitment and receptor internalization; herkinorin, TRV130, and PTI-609, displayed bias toward Gi compared to β-arrestin2/internalization; Naloxone was biased toward Go and Gz	The Gi biased agonists induced analgesia with less potential for tolerance, respiratory suppression and constipation	(Burns & Wang, 2010; Chen et al., 2013; Masuho, Ostrovskaya, et al., 2015; McPherson et al., 2010; Tidgewell et al., 2008; Váradi et al., 2016)
Opioid κ	Nalfurafin and 6'-GNTI display bias toward Gi over β-arrestin pathway; Naloxone activated Gi/o	biased agonists away from β-arrestin may produce less dysphoric effects	(Masuho, Ostrovskaya, et al., 2015; Schattauer, Kuhar, Song, & Chavkin, 2017; Schmid et al., 2013)
Opioid δ	SNC-80 and UFP-512 showed bias toward AC inhibition compared to receptor internalization	unknown	(Charfi et al., 2014)
Opioid N/OFQ	RTI-4229-816 and RTI-4229-856 display bias toward G protein activation away from β-arrestin pathway	The G protein biased agonists may produce analgesia with less potential for tolerance	(Chang et al., 2015)
Oxytocin	the majority of ligands activated Gq, Gi_2, and Gi_3 but not Gi_1, Go_A, and Go_B; DNalOVT and atosiban were entirely biased toward Gi_1 or Gi_3 activation	biased ligands may show different effects in labor induction or neuropsychiatric disorders	(Busnelli et al., 2012)

(*continued on next page*)

Table 2 (*continued*)

Receptor	Biased activity	Potential clinical benefits	Ref.
Prostanoid EP_2	Prostacyclin analogues differentiate between G protein and β-arrestin pathways	G protein-biased analogues may provide neuroprotection devoid of tumorigenesis and angiogenesis	(Ogawa et al., 2016)
Prostanoid FP	PDC113.824 induced a shift of receptor signaling toward Gq/PKC/$ERK_{1/2}$ at the expense of G12/Rho/Rock	PDC113.824 may offer tocolytic properties	(Goupil et al., 2010)
Sphingosine 1-phosphate $S1P_1$	BMS-986104 displayed significant bias for cAMP over $ERK_{1/2}$ and internalization compared to fingolimod	BMS-986104 may produce less bradycardia when used for multiple sclerosis	(Dhar et al., 2016)
Thyrotropin	NCGC00379308 induced β-arrestin1 recruitment but did not activate Gs/cAMP pathway	NCGC00379308 enhanced TSH-induced differentiation of a human pre-osteoblast as a positive allosteric modifier	(Neumann et al., 2018)
Vasopressin V_2	MCF14 was Gs agonist and β-arrestin antagonist; SR121463 was Gs inverse agonist and β-arrestin partial agonist; amphipol was neutral	unknown	(Rahmeh et al., 2012)
Class B			
Glucagon like peptide 1	exendin-4 showed bias for cAMP synthesis relative to $pERK_{1/2}$, and weaker relative bias for $pERK_{1/2}$ over Ca^{+2} mobilization; Oxyntomodulin was weakly biased toward cAMP formation relative to $pERK_{1/2}$, and was poorly coupled to Ca^{+2} mobilization; GLP-1(7–36)- NH2 displayed strong bias toward cAMP synthesis relative to either $pERK_{1/2}$ or Ca^{+2} mobilization, and only weak bias for the $pERK_{1/2}$ relative to Ca^{+2}; EXENDIN-P5 displayed significant bias toward Gs and Gq activation over β-arrestin pathway	EXENDIN-P5 was a weak insulin secretagogue, but more effective in long-term glycemic control; β-arrestin biased ligands may offer anti-apoptotic effects	(Koole, Wootten, Simms, Miller, et al., 2012; Koole, Wootten, Simms, Savage, et al., 2012; Quoyer et al., 2010; Zhang et al., 2015)
Parathyroid hormone	(D-Trp^{12},Tyr^{34})-PTH(7–34) displayed bias toward β-arrestin pathway	β-arrestin biased agonists promoted bone formation without bone resorption	(Gesty-Palmer et al., 2009)
Class C			
Calcium sensing CaSR	Strontium, barium, spermine, tobramycin and neomycin displayed bias in terms of activation of Gi/o, Gq/11 and $ERK_{1/2}$ pathways; AC-265347 and R,R-cacimimetic were biased for $ERK_{1/2}$ and IP_1 accumulation away from ca^{2+} mobilization	Bias toward $ERK_{1/2}$ may help avoid hypocalcaemia and hyperphosphatemia which restricts cinacalcet application in secondary hyperparathyroidism	(Cook et al., 2015; Thomsen et al., 2012; Thomsen, Hvidtfeldt, & Bräuner-Osborne, 2012)
Glutamate $mGlu_1$	Quisqualate and DHPG were G protein biased, whereas glutamate, glutaric acid and succinic acid were β-arrestin biased	β-arrestin-biased ligands may be neuroprotective	(Emery et al., 2012)
Glutamate $mGlu_5$	VU0409551 induced robust Gq-mediated Ca^{2+} mobilization without enhancing NMDA receptor currents	Bias away from NMDA receptor currents may provide antipsychotic effects devoid of excitotoxicity	(Rook et al., 2015)

*the table contains examples of receptors for which a bias ligand is present, and studies experimenting the behavior of neutral or endogenous ligands in systems lacking β-arrestin or G proteins are not included; also, bias is described as reported in the original papers without any attempt to re-analyze data with other analytical methods; **performed with two different analytical methods; ***note a kinetic bias; AC, adenylyl cyclase; $ERK_{1/2}$, extracellular regulated kinas $_{1/2}$; IP, inositol phosphate; MAPK, mitogen activated protein kinase; $pERK_{1/2}$, phosphorylated $ERK_{1/2}$.

pathways may offer potential clinical benefits (Table 2). For instance, adenosine $A_{1A}R$ activation is protective against cardiac or cerebral ischemic-reperfusion injury, possibly through MAPK, Akt/PKB and certain protein kinase (PKC) isoforms. However, clinical trials with $A_{1A}R$ biased agonists have displayed limited success, in part because of suboptimal dosing due to undesirable hemodynamic side effects (Ross, Gibbons, Stone, Kloner, & Alexander, 2005). In this regard, the $A_{1A}R$ bitopic agonists VCP746 and capadenoson, which display bias away from G-protein mediated Ca^{+2} mobilization (Baltos et al., 2016), retained cytoprotective signaling in the absence of bradycardia, providing a rationale for clinical trials with these β-arrestin biased agents (Albrecht-Kupper et al., 2012; Sabbah et al., 2013). Likewise, the angiotensin AT_1R biased agonist TRV120027, which potently directs signaling toward β-arrestin, decreases blood pressure and improves myocardial contractility (Violin et al., 2010). However, in a phase IIb trial (BLAST-AHF), TRV120027 did not produce a composite clinical benefit in acute heart failure compared with placebo (Pang et al., 2017). Meanwhile, adjustment to baseline systolic blood pressure revealed beneficial effects for 1 mg/h dose of TRV120027 on 180-day all-cause death and cardiovascular mortality in patients with high blood pressure, while low blood pressure patients experienced more adverse effects (Cotter et al., 2018). Hence, biased ligands may target more specific types of physiological functions for clinical benefit of specific symptoms.

In some cases, however, favorable outcomes have been associated with increased G protein-mediated signaling. Of particular note, μOR agonist TRV130 (oliceridine) displayed significant bias toward Gi activation and was devoid of β-arrestin recruitment (Chen et al., 2013). Interestingly, in a phase II trial TRV130 produced comparable or greater pain relief than morphine in patients following bunionectomy (Viscusi et al., 2016) or abdominoplasty (Singla et al., 2017), while having fewer side effects (nausea, vomiting and respiratory dysfunction). Similarly, the Gi-biased agonists of the GPR109a receptor show promise to reduce plasma triglycerides without inducing vasodilation or flushing (Semple et al., 2008). Meanwhile, the Gi-biased ligand MK-0354 produced only a weak effect on serum lipids compared with niacin, indicating that beneficial effects of niacin may be independent of GPR109a receptor (Boatman et al., 2012). These examples suggest that the greater potential specificity of a biased ligand, while minimizing adverse or off-target effects, may also be its "Achilles heel" to limit its effectiveness to only certain subsets of patients. Thus, the biased agonist approach may be optimal when a clear signaling process is implicated, while in other cases such as mental illness, targeting a diversity of GPCRs may be more effective (Roth, Sheffler, & Kroeze, 2004).

While biased agonists may offer better therapeutic responses devoid of unfavorable effects (Table 2), it is usually difficult to attribute a particular clinical response to either G protein or β-arrestin mediated pathways, given that a single physiological phenomenon may be regulated by multiple pathways. For example, the seemingly analgesic (decrease of lactic acid-induced stretching) and antipruritus (decreased of serotonin-induced scratching) properties of the G protein-biased κOR agonist, nalfurafine, may reflect nonselective reduction in motivated behavior rather than actual decrease of pain and itch (Lazenka et al., 2018). In some cases, bias in G-protein rather than effector signaling is a better predictor of outcome. For example, bias toward GTPγS-binding was found to be a better predictive factor than cAMP assays for greater analgesic activity and reduced potential for respiratory suppression with μOR agonists (Váradi et al., 2016). In other cases, both G protein and β-arrestin pathways have been implicated, such as for chemokine receptor ($CCXR_3$)-mediated chemotaxis (Fong et al., 2002; Milanos et al., 2016). Similarly, while blockade of dopamine D_{2L} receptor ($D_{2L}R$) interaction with β-arrestin2 was initially reported as a common property of clinically effective antipsychotics (Masri et al., 2008), it was subsequently discovered that both G protein and β-arrestin -biased ligands of D_2R demonstrate antipsychotic properties (Allen et al., 2011; Moller et al., 2017). Recently, a G protein-biased D_2R agonist, BRD5814, with antagonistic properties in β-arrestin pathway has shown significant antipsychotic activity devoid of extrapyramidal side effects in animal models (Weïwer et al., 2018). Thus although biased ligands appear to target more specific physiological processes, it is not always clear whether G-protein, β-arrestin, or both pathways mediate this specificity. This underpins the necessity for more research to elucidate the relationship between a specific pathway and a physiological or pathological event.

5.2. Biased signaling between G protein families

Canonical G protein signaling is dictated by the G protein α subunits, which are divided into four families according to their sequence homology and function. The stimulatory G protein family includes Gαs and Gαolf which are positively coupled to AC, and stimulate cAMP synthesis (Sunahara, Dessauer, Whisnant, Kleuss, & Gilman, 1997). Gαs is also shown to modulate a number of other effector molecules such as tubulin and Src family kinases (Wettschureck & Offermanns, 2005). The pertussis toxin (PTX) sensitive inhibitory G proteins (Gαi1, Gαi2, Gαi3, Gαo1 and Gαo2) as well as the PTX-insensitive Gz inhibit AC (Birnbaumer, 2007). Furthermore, some isoforms of this family can also relay signals to tubulin, Src, ras1 GTPase activating protein, K^+ inward rectifier (GIRK) channels or voltage operated Ca^{2+} channels (VOCC) (Kinoshita et al., 2001; Ma, Huang, Ali, Lowry, & Huang, 2000; Peleg, Varon, Ivanina, Dessauer, & Dascal, 2002; Roychowdhury, Panda, Wilson, & Rasenick, 1999). Other members of this family, transducin (Gαt) and gustducin (Gαg), modulated phosphodiesterase (PDE) activity and cyclic nucleotide-gated channels in specific sensory neurons (Wettschureck & Offermanns, 2005). The Gq/11 family (Gαq, Gα11, Gα14, Gα16, and its murine homologue Gα15) activate PLCβ, which in turn hydrolyzes phosphatidylinositol (4,5)-bisphosphate (PIP2) into inositol triphosphate (IP3) and diacylglycerol (DAG), resulting in PKC activation and Ca^{2+} mobilization (Rhee, 2001). The Gα12/13 primarily activate Rho family GTPases (Rho, Rac, and Cdc42) and regulate cytoskeletal polymerization and gene expression (Kozasa, Hajicek, Chow, & Suzuki, 2011). Once believed to only act as a negative regulator and/or membrane anchor for Gα subunits, the Gβγ dimer has been shown to interact with an array of effector molecules, including AC, PLC, GRKs, PI3K, and ion channels (Dupré, Robitaille, Rebois, & Hébert, 2009).

5.2.1. GPCRs of all classes can couple to multiple G protein families

The selectivity of GPCRs for distinct G proteins is supported by several experimental approaches including second messenger analysis, receptor/transducer cross-linking and co-precipitation (Hu et al., 2010; Wang, Friedman, Olmstead, & Burns, 2005), agonist-induced incorporation of radiolabeled GTP analogues (Milligan, 2003), chimeric mammalian/yeast Gα (Stewart et al., 2010), and energy transfer approaches. The latter approach has seen several developments containing energy transfer between the receptor/Gα, receptor/Gβ, receptor/Gγ (Gales et al., 2005) as well as chimeric probes consisted of truncated receptor/Gα C-termini (Malik et al., 2013), and energy transfer between Gα/Gγ (van Unen et al., 2016), Gα/Gβ (Janetopoulos, Jin, & Devreotes, 2001) or Gβγ/GRK3 (Masuho, Ostrovskaya, et al., 2015). Likewise, several approaches are employed to distinguish the activity of Gα and Gβγ subunits. In this regard, cholera toxin (CTX) (Eason, Kurose, Holt, Raymond, & Liggett, 1992), PTX (Locht, Coutte, & Mielcarek, 2011), regulator of G protein signaling (RGS)-4 (Hains, Siderovski, & Harden, 2004) and the RGS homology domain of p115RhoGEF (Kozasa et al., 1998) have been used for functional studies of Gs, Gi/Go/Gz, G11/Gq and G12/G13 proteins, respectively. Furthermore, the C-terminus of βAR kinase (BARK-ct or GRK2-ct) (Ghahremani, Cheng, Lembo, & Albert, 1999; Koch, Hawes, Inglese, Luttrell, & Lefkowitz, 1994) as well as small molecules such as gallein (Bonacci et al., 2006) can inhibit signaling from Gβγ subunit. Also, transfection of PTX-insensitive Gi/Go protein mutants as well as expression of Gβγ-insensitive AC subtypes (Dittman et al., 1994) or selective knockdown using siRNA, antisense oligonucleotides, or CRISPR/Cas9 technology (Alvarez-Curto et al., 2016) are used to discriminate signaling from a specific transducer.

However, the results of different biosensor approaches are not always consistent in assessing G protein selectivity. For instance, β_2AR failed to cause subunit dissociation in Gi_1, and only slightly dissociated Gi_2 and Gi_3 subunits (van Unen et al., 2016), nor did it couple to Gi_1, Gi_2 and Gi_3 in experiments using Gβγ/GRK3 BRET sensors (Masuho, Ostrovskaya, et al., 2015). In contrast, truncated receptor/Gα sensors revealed an increase in FRET ratio between β_2AR and both Gs and Gi, depending on the ligands employed (Malik et al., 2013). These incongruous findings may be explained by the fact that a negative response in GTPγS or RET does not always imply absence of interaction, given that low sensitivity of the GTPγS assay or loss of energy transfer in RET stemming from inappropriate orientation of donor/acceptor molecules may result in false negative outcomes (Marullo & Bouvier, 2007; Milligan, 2003). Furthermore, receptor-mediated activation of G proteins does not necessarily result in subunit dissociation and may occur through conformational rearrangement (Bai, Jiang, Cai, & Chen, 2014; Gales et al., 2006). Another drawback of these interaction assays is the reliance on tagging with protein tags that are sometimes larger than the targeted subunits, and may interfere with normal interactions. Thus, variations in receptor-G protein selectivity may stem from the system or assay conditions and need to be carefully considered in order to demonstrate the true GPCR functional selectivity or ligand bias.

Given the pleiotropic nature of GPCR coupling to G proteins (Table 1), biased agonists can also stabilize distinct receptor conformations favoring one among several G protein families (Table 2). For instance, SB242084 is a $5\text{-}HT_{2C}R$ inverse agonist for the Gi/phospholipase A2/arachidonic acid pathway but an agonist for the Gq/PLC/inositol phosphate pathway (De Deurwaerdere, Navailles, Berg, Clarke, & Spampinato, 2004). Of particular note, allosteric or bitopic agonists, that link orthosteric and allosteric pharmacophores, can also distinguish downstream G protein-mediated pathways (Lane, Sexton, & Christopoulos, 2013). For example, muscarinic M_1R bitopic (AC-42 and 77-LH-28-1) and orthosteric (OXO-M, arecoline and pilocarpine) agonists induce GTPγS binding to Gq/11 and Gs, while only the latter enhanced nucleotide binding to $Gi_{1/2}$ (Thomas, Mistry, Langmead, Wood, & Challiss, 2008). Similarly, the prostanoid FP receptor allosteric agonist PDC113.824 inhibited basal, prostaglandin F2α- or lipopolysaccharide-induced parturition due to a shift of receptor signaling toward Gq/$PKC/ERK_{1/2}$ and away from the G12/Rho/Rock pathway (Goupil et al., 2010). Intriguingly, even subtle differences in ligand structure can result in shift of preference toward one G protein pathway over another. For

instance, stereoisomers of the β_2AR agonist fenoterol demonstrated significant differences in bias for activation of Gs and Gi (Woo et al., 2009).

Biased agonists not only distinguish between G protein/β-arrestin pathways or paralogue G proteins members, but they may also discriminate between orthologous G proteins. In this regard, the D_2R ligand S-(−)-3-PPP was a partial agonist in terms of GTPγS binding to $G\alpha o_A$, but was an antagonist/inverse agonist for $G\alpha i_1$, $G\alpha i_2$ and $G\alpha i_3$ activation (Lane, Powney, Wise, Rees, & Milligan, 2007). Similarly, the neuropeptide Y_2 receptor agonist $NPY_{13\text{-}36}$ as well as cannabinoid CB_1 receptor (CB_1R) agonists desacetyllevonantradol and R-methanandamide discriminated between Gi family subtypes (Misra, Murthy, Zhou, & Grider, 2004; Mukhopadhyay & Howlett, 2005).

Given that Gβγ subunits have distinct effectors from Gα (Birnbaumer, 2007; Dupré et al., 2009), and that several combinations of αβγ heterotrimers are possible (Albert & Robillard, 2002), coupling to a distinct αβγ trimer may activate specific signaling pathways (Schwindinger et al., 2003). In addition, a strong body of evidence indicates signaling specificity among Gβγ subtypes, as well as distinct roles for different Gβ or Gγ subunits (Dupré et al., 2009; Yim et al., 2019). However, little is known about which G protein αβγ assemblies actually exist *in vivo* or about how GPCRs discriminate between different αβγ combinations (Hillenbrand, Schori, Schöppe, & Plückthun, 2015). In this regard, biased ligands have been identified that direct signaling to either Gα or Gβγ subunits. For example, the leukotriene OXE receptor (OXER) is a Gi-coupled receptor controlling a number of immune cell responses including chemotaxis (Grant, Rokach, & Powell, 2009). The OXER antagonist Gue1654 selectively inhibited agonist-stimulated Gβγ but not Gαi signaling. In more detail, Gue1654 or Gβγ inhibitors antagonized OXER-induced PTX-sensitive Ca^{+2} responses and GIRK opening to inhibit chemotaxis. In contrast, these treatments did not influence Gαi-mediated inhibition of cAMP synthesis (Blättermann et al., 2012). This indicates that Gβγ not only couples to its own effectors, but may also be the main transducer for some receptor-mediated events (Konya et al., 2014; Surve, Lehmann, & Smrcka, 2014). This finding opens up a new window for biased ligands as therapeutic agents to selectively activate/inhibit Gα or Gβγ signaling or even differentiate between distinct Gβγ subunits downstream of the same receptor. However, the structural basis for selective Gβγ signaling over Gα signaling remains unclear.

5.2.2. Therapeutic potential of bias between G protein families

Functional selectivity of ligands across G protein families may offer potential clinical benefits (Table 2). For example, biased ligands shifting vasopressin V_1 and gastrin-releasing peptide (GRP) receptors away from Gq/11 and toward Gi/$ERK_{1/2}$ inhibit the growth of small-cell lung cancer cells (MacKinnon et al., 2005). However, more research is required to precisely determine the consequences of signal direction toward a given pathway. In this regard, β_2AR antagonist, ICI-118551, actively reduces myocyte contraction in the failing heart through Gi-biased signaling (Gong et al., 2002), whereas Gs signaling increased chamber contractility. This suggests potential application for Gs-biased ligands in heart failure (Woo, Song, Xiao, & Zhu, 2015), but enhanced β_2AR coupling to Gs and/or inhibition of Gi coupling resulted in ventricular arrhythmia in experimental models of heart failure (Wang et al., 2015). Conversely, a β-arrestin-biased pepducin improved cardiomyocyte contractility, yet the Gi/ β-arrestin biased β_2AR agonist carvedilol failed to induce cardiomyocyte contractility (Carr et al., 2014; Carr III et al., 2016). Thus, mapping biased signaling to physiology is not straightforward. Similarly, melanocortin receptor 4 (MC_4R) agonist melanotan II produced its anorectic effects through coupling to Gq/11 and its adverse cardiovascular effects through Gs coupling, suggesting potential therapeutic benefit in obesity for Gq/11-biased ligands (Li et al., 2016). However, several phenotypes of mutant MC_4R with loss of function, increased Gs signaling, increased Gi/o signaling or no alteration of function have been isolated from obese patients (Hinney et al., 2003). Thus, a direct correlation between heart failure or obesity and βAR or MC_4R coupling to certain G proteins is premature, and further studies of these pathways *in vivo*, are required.

Ligand-mediated discrimination of orthologue G proteins may also offer potential clinical benefits. For instance, oxytocin activates Gq and all Gi/o family members, whereas DNalOVT and atosiban are entirely biased toward Gi_1 or Gi_3 activation, and do not activate Gq or Gi_1, nor do they induce β-arrestin recruitment to the oxytocin receptor (OTR) (Busnelli et al., 2012). Given that OTR signaling toward Gi inhibits cell proliferation (Reversi et al., 2005) and migration (Zhong, Boseman, Millena, & Khan, 2010), Gi-biased ligands acting at this receptor such as atosiban may produce beneficial effects for tumor regression and metastasis (Busnelli et al., 2012). In the same manner, dihydrexidine and N-propyl-apomorphine are full agonists for D_1R-mediated Gs activation but partial agonists for Golf activation. Since Golf and Gs are predominant in the striatum and in the cortex, respectively, Gs/Golf biased D_1R ligands provide the opportunity for the treatment of cortex- or striatum-specific neuropsychiatric dysfunction. In particular, dihydrexidine may be useful for the treatment of cognitive deficits without adverse locomotor effects (Yano et al., 2018). It is, however, noteworthy that different receptors may require distinct G protein subunits to elicit a single phenomenon. For instance, D_{2L}R required Go for inhibition of prolactin synthesis, whereas 5-HT_{1A}R and somatostatin receptors required Gi_2 and Gi_3, and the effect of muscarinic receptors was resistant to depletion of any Gi/Go protein. Also, these receptors exhibited distinct G protein requirements for inhibition of lactotroph DNA synthesis (Albert, 2002).

5.3. Biased signaling beyond G protein/β-arrestin pathway

Biased signaling is not limited to G protein or β-arrestin, but can affect downstream signaling pathways. In fact, some ligands may display pronounced bias toward/away from one pathway, i.e. they activate or fail to activate one pathway. In the majority of cases, however, biased ligands do not display a bimodal ON/OFF signaling phenotype (Table 2). Rather, they may induce subtle differences in potency, magnitude or rate of activation of one among all signaling repertoire (Fig. 1). Also, a biased agonist may not be restricted to one among several downstream pathways activated by the endogenous ligand. Indeed, biased ligands can sometimes induce new signaling pathways not observed with the endogenous agonist. For example, the angiotensin AT_{1A}R ligand SII ($^1Sar^4$ Ile^8 Ile-angiotensin II), once characterized as a biased agonist toward β-arrestin pathway (Violin et al., 2010), was later discovered to also activate Gi and Gq (Saulière et al., 2012). Moreover, fingerprinting of angiotensin II, SII and TRV120027 revealed signaling profiles in common to all three, as well as downstream effector molecules unique to each peptide (Santos et al., 2015).

Furthermore, bias toward G protein or β-arrestin pathways does not necessarily correlate with bias toward the cognate downstream pathway (internalization, cAMP, $ERK_{1/2}$, etc.), i.e. a ligand displaying bias toward β-arrestin does not necessarily demonstrate bias toward $ERK_{1/2}$, given that these pathways may receive signals from multiple sources (Fig. 1). This indicates that a receptor's functional selectivity to multiple signaling outputs needs to be tested in order to identify ligand bias for activation of different pathways. As an example, GLP-1 analogues containing β-amino acid residues were initially described as β-arrestin biased ligands (Hager, Johnson, Wootten, Sexton, & Gellman, 2016). However, further investigation in a different cell line revealed biased activity beyond β-arrestin recruitment, to include calcium mobilization and $ERK_{1/2}$ phosphorylation (Hager, Clydesdale, Gellman, Sexton, & Wootten, 2017). Therefore, some investigators have decided to study the bias profile in terms of specific pharmacological readouts in a more global manner which cannot necessarily be attributed to either G protein or β-arrestin pathways. In this regard, Namkung et al., have developed pathway-selective BRET biosensor to monitor the activation of effector molecules such as PKC, PLC, p63RhoGEF, and Rho, and investigated the correlation between bias profile of different ligands for these

pathways and upstream transducers (Namkung et al., 2018). Such biosensors are very useful for precise delineation of ligand-receptor behaviors as well as the involvement of a specific pathway in the pathophysiology of a given clinical event. Given that G proteins and β-arrestins are not the only molecules that directly interact with the receptor, ligand-specific receptor conformations beyond these pathways can be presumed.

Many GPCRs contain a PDZ (PSD-95/Dlg/ZO-1)-binding domain at the C-terminus which can interact with PDZ domain-containing proteins (Dunn & Ferguson, 2015). Interaction with these proteins opens a new window of effector molecules beyond G protein or β-arrestins. For instance, κOR interact with sodium-hydrogen exchanger regulatory factor-1 (NHERF-1)/Ezrin-radixin-moesin-binding phosphoprotein-50 (EBP50) to stimulate Na^+/H^+ exchange independent of the G proteins (Huang et al., 2004). Similarly, other PDZ-scaffolds can interact with the C-terminus of GPCRs, and connect them to a variety of effector molecules (kinases or phospholipases) or ion channels (Table 3). Also, these scaffolds alter receptor-G protein selectivity, receptor internalization and desensitization (Walther & Ferguson, 2015). Importantly, selective expression of GPCR effectors and interacting proteins, like PDZ domain proteins, in different cell types or tissues can result in cell-specific signaling readouts of a given ligand (discussed in Sections 6.2 to 6.6).

Non-PDZ scaffold proteins such as A kinase anchoring proteins (AKAPs), RGS proteins and other scaffolds can also interact with the cytoplasmic domains (ICL3 and C-terminus) of the receptor (Table 3), and assist in activation of kinases, phosphatases, IP3 receptors and a variety of other cytosolic or nuclear elements (Maurice et al., 2011). Scaffolding proteins provide a docking site for interacting molecules, holding them in close proximity with the appropriate orientation to ensure the selectivity of interaction as well as spatiotemporal control of signaling processes. For example, AKAP-bound PKA can cause a Gs/Gi switch in β_2AR signaling through receptor phosphorylation, whereas β-arrestin holds PDE-4D5 in the vicinity of receptor and reverses the coupling of the receptor to Gi via cAMP degradation and inactivation of PKA (Baillie & Houslay, 2005). Similarly, Filamin A binding to different GPCRs plays a central role in receptor localization and functional selectivity toward cAMP, $ERK_{1/2}$ and serum response element (Maurice et al., 2011). Interestingly, a high affinity binding site at the C-terminus of filamin A is found for naloxone and naltrexone, disruption of which increased non-cognate μOR/Gs coupling (Wang & Burns, 2009; Wang, Frankfurt, & Burns, 2008). Hence, filamin A binding compounds such as PTI-609, are capable of binding to opioid receptors, and are under development as analgesics with less potential for tolerance than other opioids (Burns & Wang, 2010).

Given the plethora of GPCR interacting proteins (GIPs) (Table 3), a huge diversity of receptor conformations favoring binding to each of these molecules is presumable. Alternatively, these molecules can alter receptor-G protein selectivity through lateral allostery (Kenakin, 2017). Interestingly, the crystal structure of the angiotensin AT_2R revealed a non-canonical position for helix-8 (Fig. 3), in which it stabilized the active conformation, but simultaneously prevented receptor interaction with G proteins and β-arrestin (Zhang, Han, et al., 2017). However, further research is required for precise delineation of molecular mechanisms of receptor interaction with each of these scaffolds.

6. Molecular determinants of transducer selectivity and biased signaling

6.1. Receptor conformations and biased signaling

Several residues at both the receptor and transducer are involved in selective signaling, but no single obligatory structural element or sequence can be generalized for all GPCRs (Moreira, 2014). For the determination of residues that are critical in selective coupling, the majority of studies have focused on conserved residues at the receptor/G protein interface. Nonetheless, residues at the binding pocket ought not to be neglected. While residues at the GPCR-transducer interface directly influence coupling selectivity, residues at the ligand binding pocket can alter the bias fingerprint of certain agonists and alter the equilibrium of receptor conformational states. Interestingly, naturally occurring genetic polymorphisms can significantly influence GPCR signaling toward different pathways (Hauser et al., 2018). This gene-induced shift in GPCR signaling may negate the binding or signaling biased ligands toward their preferred signaling pathway. Failure to detect confounding genetic parameters *in vivo* may result in significant adverse effects to the patient or may perturb the results of clinical trials for the discovery of therapeutic biased ligands.

6.1.1. Selectivity barcodes

Recent research has provided significant breakthroughs for generalization of mechanisms of receptor-transducer selectivity. Specifically, analysis of structural data from β_2AR-Gs (PDB code: 3SN6), Adenosine A_{2A}R-mini Gαs (PDB code: 5G53), and RhR-Gαt C-terminal (PDB codes: 2X72, 3DQB, 3PQR, 4A4M) complexes as well as sequence alignments within GPCR families and within orthologue/paralogue G protein families has identified the conserved residues at the interface, which are more likely to contribute to selective coupling, i.e. 'selectivity barcodes'. In this way, notable interactions between ICL3, TM5, ICL2, DRY motif,

Table 3
Non-exhaustive list of GPCR interacting proteins and their effects on receptor function.

PDZ proteins		
NHERFs	G protein coupling, AC/cAMP signaling, PLCβ/IP3/Ca^{2+} signaling, MAPK activation, β-arrestin-2 recruitment, receptor expression, desensitization and trafficking	(Broadbent et al., 2017)
PIST (CAL, GOPC)	MAPK activation, receptor expression and trafficking	(Bauch, Koliwer, Buck, Honck, & Kreienkamp, 2014; Zhang et al., 2008)
GIPC	G protein coupling, MAPK activation	(Hu et al., 2003; Jeanneteau, Diaz, Sokoloff, & Griffon, 2004)
SAP-97	cAMP signaling, IP_3 signaling, MAPK activation, receptor phosphorylation	(Hu et al., 2003; Jeanneteau et al., 2004)
MAGI-3	MAPK activation	(Zhang, Wang, Sun, Hall, & Yun, 2007)
Sorting Nexin27	Receptor recycling	(Nakagawa & Asahi, 2013)
Spinophilin	Ca^{2+} signaling, MAPK signaling, receptor expression, phosphorylation and trafficking	(Charlton et al., 2008; Wang et al., 2004; Wang et al., 2005)
MUPP-1	G protein coupling, AC/cAMP signaling	(Guillaume et al., 2008)
PICK1	cAMP signaling, receptor expression	(Katsushima et al., 2013)
Non-PDZ proteins		
14-3-3	cAMP signaling, Ras/Raf signaling, receptor expression, desensitization, and trafficking	(Cohen, Nechamen, & Dias, 2004; Okamoto & Shikano, 2011; Wang & Limbird, 2007)
AKAPs	cAMP signaling, receptor phosphorylation and trafficking	(Appert-Collin, Baisamy, & Diviani, 2006)
Filamin A	G protein coupling, receptor trafficking	(Wang et al., 2008; Wang & Burns, 2009).
Jak2	STAT phosphorylation	(Ali, Sayeski, & Bernstein, 2000)
RAMPs	G protein coupling, receptor expression and trafficking	(Hay & Pioszak, 2016)
RGSs	AC/cAMP signaling, PLCβ/IP_3/Ca^{2+} signaling, MAPK activation, receptor trafficking	(Woodard, Jardín, Berna-Erro, Salido, & Rosado, 2015)

AC, adenylyl cyclase; AKAP, A kinase anchor proteins; CAL/PIST/GOPC, Golgi-associated PDZ and coiled-coil motif-containing protein; IP_3, inositol 1,4,5-trisphosphate; Jak, Janus kinase; MAGI, membrane-associated guanylate kinase, WW, and PDZ domain-containing proteins; MAPK, mitogen activated protein kinase; MUPP-1 multiple PDZ domain protein; NHERF, Na^+/H^+ exchanger regulatory factor; PLC, phospholipase C; RGS, regulator of G protein signaling; SAP97, synapse-associated protein 97; STAT, signal transducers and activators of transcription.

and TM6 from the receptor with α4, α5, αN helices, and segment 1/3 from the G protein alpha subunit were mapped. Although selectivity signatures are largely divergent for the receptor groups, a subset of closely related receptors that engage a given Gα family share a distinct set of signatures, that is absent in those members of the same family that are incapable of coupling to that G protein (Flock et al., 2015; Flock et al., 2017). Comparison of data from this database (http://gpcrdb.org/signprot/ginterface) with structural data from two class B GPCRs that were solved subsequently, human calcitonin receptor (CTR)-Gs (5UZ7) and rabbit GLP_1R-Gs (5VAI) complexes, indicates receptor interfaces with some common key Gα residues as well as a number of different receptor contacts in these structures (Furness & Sexton, 2017). This suggests potential sites to probe whether such differences are involved in receptor engagement with non-cognate transducers, given that β_2AR promiscuously interacts with Gαs and Gαi, while CTR and GLP_1R interact with Gαs, Gαq and Gαi. Also, transducer selectivity seems to be mediated by mechanisms beyond a specific epitope or a conserved linear sequence (discussed in Section 6.1.6). Nonetheless, comparison of the crystal structures of GPCRs in complex with different G proteins or β-arrestin as well as with biased or unbiased ligands have provided significant insights to understanding the mechanisms of transducer selectivity.

6.1.2. Conformation of ECLs

Comparison of the structures of ergotamine-bound $5\text{-}HT_{1B}$ and $5\text{-}HT_{2B}$ receptors, where ergotamine behaves as unbiased or β-arrestin-biased ligand respectively, has revealed an extra helical turn in ECL2 of the $5\text{-}HT_{2B}$ receptor. This allows ergotamine to contact with several residues in TM5, TM6 and TM7 of $5\text{-}HT_{2B}$, and is possibly involved in β-arrestin-dependent signaling (Wacker et al., 2013). Furthermore, mutagenesis of the ECL2 in class B GLP_1 (Koole et al., 2012; Koole et al., 2012) as well as class A muscarinic M_1 (Keov et al., 2014) and M_2 (Gregory et al., 2010; Gregory et al., 2012) receptors has revealed a critical role for this segment in terms of signal transduction in a ligand- and pathway- dependent manner. While some residues were of global importance for receptor signaling and bias direction, other residues influenced explicitly the binding and/or signaling of a distinct agonist in a specific pathway. In this regard, comparison of the cryo-electron microscopy structures of class B GLP_1R when bound to endogenous GLP-1 (Zhang, Sun, et al., 2017) or G protein-biased ligand exendin-P5 (Liang et al., 2018) revealed key structural differences in the conformation of ECL3 and the proximal TM1 segment. Also, class C $mGlu_1R$ mutants having different residues in the ligand binding domain displayed biased signaling explicitly toward G protein or β-arrestin pathways (Emery et al., 2012).

6.1.3. Conformation of ICLs

Study of GPCR-G protein complexes has revealed that the αN helix and αN-β1 junction/loop of Gα interacts with ICL2 of the receptor. Therefore, agonist-induced alteration of receptor conformation transmits from ICL2 to αN helix, disrupting the P-loop interaction with the nucleotide's β-phosphate, and resulting in GDP dissociation (Mahoney & Sunahara, 2016). This may explain why a difference in ICL2 can result in a change of G protein selectivity (Table 4). The importance of this loop for selective coupling is also noted in members of class A (Erlenbach et al., 2001; Hamamoto, Kobayashi, & Saito, 2015) as well as class B

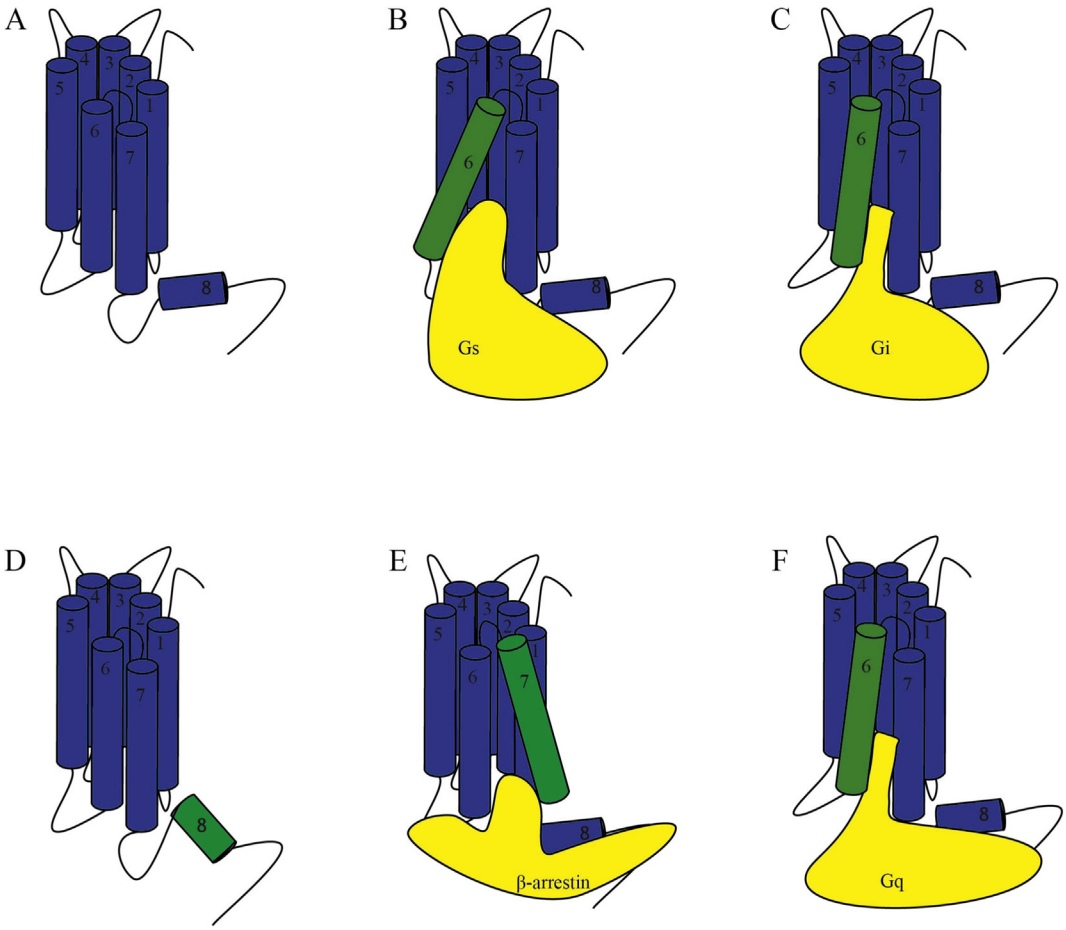

Fig. 3. Distinct GPCR conformations in complex with different transducers. Schematic representation of an inactive GPCR (A), GPCR-Gs complex (B), GPCR-Gi complex (C), GPCR-Gq (F), GPCR-β-arrestin (E), and active states beyond G protein and β-arrestin pathways (D). Note that the position of TM6, TM7 and helix 8 differ when the receptor is bound to different transducers.

Table 4
examples of receptor chimeras for the evaluation of coupling selectivity.

Receptor	Intervention	Effect	Ref.
Adrenergic α_2	exchange of TM5, TM6 and ICL3 with β2AR	gain of AC stimulation	(Kobilka et al., 1988)
Adrenergic α_{2C10}	exchange of ICL3N with 5-HT_{1A}	loss of AC stimulation	(Eason & Liggett, 1995)
Adrenergic α_{2A}	exchange of ICL2 with 5-HT_{1A} exchange of ICL2 with β2AR	loss of AC stimulation suppression of AC inhibition	(Eason & Liggett, 1996)
Adrenergic β_2	exchange of ICL3N with α_{1B}	gain of PI hydrolysis	(Cotecchia, Ostrowski, Kjelsberg, Caron, & Lefkowitz, 1992)
Angiotensin AT_{1A}	exchange of ICL3C with β2AR exchange of ICL3C with AT_2	gain of AC activation, reduction of PI hydrolysis and Ca^{2+} mobilization loss of AC stimulation, PI hydrolysis and Ca^{2+} mobilization	(Conchon, Barrault, Miserey, Corvol, & Clauser, 1997)
Calcitonin CT (porcine)	replacement of ICL1 with human CT_1	gain of AC stimulation, loss of PI hydrolysis	(Nussenzveig et al., 1994)
Cholecystokinin CCK_2	replacement of 5 residues in ICL1 with CCK_1	gain of PI hydrolysis, AC stimulation	(Wu et al., 1997)
Muscarinic M_2	exchange of the whole ICL3 or ICL3N with M_3	M_2 gained PI hydrolysis; M_3 lost PI hydrolysis	(Wess, Brann, & Bonner, 1989)
Prostanoid $EP_{3\beta}$	exchange of ICL2 or ICL2N with EP_2	$EP_{3\beta}$ gained AC stimulation, EP_2 lost AC stimulation	(Sugimoto et al., 2004).
Cannabinoid CB_2	exchange of ICL2, ICL3 and CT with CB_1	gain of PLC activation	(Ho et al., 2002)
Cannabinoid CB_1	replacement of ICL1 and ICL2 with CB_2	loss of AC activation	(Calandra et al., 1999)
Vasopressin V_{1A}	exchange of ICL3N with V_2	gain of AC stimulation	(Erlenbach & Wess, 1998)
Dopamine D1	exchange of helix 8 with D2	Lower β-arrestin-mediated internalization without significant effect on Gs/Gi selectivity	(Yang et al., 2019)

AC: adenylyl cyclase, ICL: intracellular loop, ICLN: ICL N-terminus, ICLC: ICL C-terminus, PI: phosphatidylinositol, PLC: phospholipase C.

PTH_1R (Iida-Klein et al., 1997) and class C $mGlu_2R$ and $mGlu_4R$ (Havlickova et al., 2003). Of particular note, chimeric CB_1 receptors containing ICL2 of the CB_2R displayed a switch from Gs to Gi (Chen et al., 2010). In more detail, crystal structure of the μOR-Gi_1 complex demonstrates key ionic interactions between $D177^{ICL2}$ [class A numbering system (Ballesteros & Weinstein, 1995)] and especially $R179^{ICL2}$ with αN or α5 helices of Gα. Given that most Gi-coupled receptors contain a basic residue (arginine or lysine) at R179 position compared to varied equivalents in Gs-coupled receptors, and that mutations at this site display loss of function, a role for this residue is suggested for Gi coupling (Koehl et al., 2018). Conversely, the bulky aromatic $F139^{ICL2}$ of β_2AR forms a deep hydrophobic pocket with Gs, whereas the equivalent residue in adenosine A_1R ($L113^{ICL2}$) does not contribute much to its interaction with Gi_2 (Draper-Joyce et al., 2018). Given that mutation of equivalent residues of $F139^{ICL2}$ to alanine or hydrophilic amino acids significantly alters G protein selectivity in a number of GPCRs (Arora, Sakai, & Catt, 1995; Berg, Dunlop, Sanchez, Silva, & Clarke, 2008; Chen et al., 2010; Moro, Lameh, Högger, & Sadée, 1993; Sugimoto et al., 2004; Wacker et al., 2008), a role for this residue is suggested for Gs coupling. This indicates the possibility of signal direction toward Gs or Gi proteins based on the residues at these regions. Moreover, mutation of the highly conserved E/DRY^{ICL2} motif of angiotensin $AT_{1A}R$ (DRY/AAY) generated receptors that do not couple to G proteins but display β-arrestin-mediated signaling (Wei et al., 2003).

Receptor chimeras indicate a critical role for ICL3 in transducer selectivity (Table 4). In addition, the complete ICL3 of the GLP_1R, a member of class B GPCRs, activated both CTX- and PTX-sensitive G proteins, whereas its N-terminal segment only activated Gs and its C-terminal half exclusively activated Gi (Hällbrink et al., 2001). Of particular note, amino acids at the C-terminus of ICL3 differ significantly in Gs-coupled receptors from those in Gi- or Gq- coupled receptors. For instance, β adrenergic and dopamine D_1R have the sequence ALKTL in this region. In the majority of Gi-coupled receptors, however, this region begins with a nonpolar/uncharged amino acid (alanine, valine, or phenylalanine) followed by a threonine. Interestingly, the corresponding amino acids in the 5-$HT_{1A}R$ are shuffled, and this region begins with threonine followed by valine; mutations at these sites (T343A/V344E) resulted in a shift from AC inhibition toward AC stimulation (Malmberg & Strange, 2000). Also, inversion of $L341^{ICL3}$ and $A342^{ICL3}$ reduced CB_1R-mediated activation of $G\alpha i_1$, and caused a shift in preference toward Gs (Abadji, Lucas-Lenard, Chin, & Kendall, 1999; Ulfers et al., 2002). In this regard, ICL3 of μOR forms similar hydrophobic interactions with Gi_1 compared to β_2AR-Gs complex as well as unique polar interactions with β6 sheet of Gα which are absent in the latter. These polar interactions along with mutational assays suggests a difference between Gi and Gs –coupled receptors in terms of nucleotide exchange (Koehl et al., 2018).

6.1.4. Conformation of TM helices

Evolutionary trace analysis of GPCRs has revealed critical residues for transducer activation. In particular, β_2AR mutant ($T68F^{2.39}$, $Y132G^{3.51}$, $Y219A^{5.58}$) does not couple to G proteins but displays β-arrestin-mediated signaling (Shenoy et al., 2006). Likewise, structural (Ring et al., 2013), mutagenesis (Ambrosio, Molinari, Cotecchia, & Costa, 2000) and NMR (Isogai et al., 2016) studies indicate a fundamental role for $S^{5.42}$, $S^{5.43}$ and $S^{5.46}$ in β_1AR and β_2AR-mediated G protein activation. These residues were also found to be critical for functional selectivity of D_{2L} receptors (Fowler, Bhattacharya, Urban, Vaidehi, & Mailman, 2012). In this regard, indole-piperazine derivatives of aripiprazole displayed comparable activity toward both Gi/o and β-arrestin2 pathways via dopamine D_2R. Introduction of a N-methyl group to the aripiprazole structure significantly precluded hydrogen bonding interaction with $S^{5.42}$ and promoted hydrophobic interactions with ECL2, resulting in remarkable loss of activity toward G protein activation while preserving β-arrestin2 recruitment (McCorvy et al., 2018).

Conformational changes in β_2AR or β_1AR detected by quantitative mass spectrometry, site-specific NMR spectroscopy, or X-ray crystallography have revealed distinct patterns of reactivity even between functionally similar ligands. Despite the overall similarity of their conformations, β-arrestin-competent ligands interacted with additional residues at ECL2, TM2, TM3 and TM7 helices (Kahsai et al., 2011; Kofuku et al., 2012; Warne, Edwards, Leslie, & Tate, 2012). While β_2AR full agonists in the G protein pathway caused a large shift in TM6 and TM7, β-arrestin biased ligands altered only the TM7 helix (Liu, Horst, et al., 2012). Similar patterns of TM6 and TM7 conformations were also detected when comparing G protein-biased ligands with β-arrestin-biased or unbiased ligands acting at vasopressin V_2R (Rahmeh et al., 2012) and ghrelin GHS_{1A} receptors (Mary et al., 2012). Moreover, comparison of the crystal structure of 5-$HT_{2C}R$ bound to ergotamine or ritanserin

highlighted multiple active states, where a "toggle switch" $W^{6.48}$ and a "trigger motif" $P^{5.50}$-$I^{3.40}$-$F^{6.44}$ were essential for Gαq activation without a significant role in β-arrestin recruitment (Peng et al., 2018). This indicates the difference in TM6 and TM7 mobilization as a potential signature for the differentiation of receptor conformations that prefer either β-arrestin or G protein as the primary transducer, at least in class A GPCRs (Fig. 3).

Comparison of the structures of β_2AR-Gs complex (Rasmussen et al., 2011) with the recently solved adenosine A_1R-Gi_2 complex (Draper-Joyce et al., 2018), reveals that these receptors engage with their transducers in a different orientation. Despite their overall similarity of conformations more interactions between α5 helix (mainly residues GH5.8 to GH5.20, CGN system) and TM3 and TM5 were noted in the β_2AR-Gs complex. Conversely, five C-terminal residues in α5 helix of Gi_2 demonstrate stronger interactions with TM2, TM3, TM5–TM7 and helix 8 of the A_1R. Particularly, additional salt bridges ($D351^{GH5.22}$ and $K294^{8.49}$) or Van der Waals contacts ($C352^{GH5.23}$ and $G353^{GH5.24}$ with $R291^{7.56}$ and $I292^{8.47}$) between the G protein and the receptor are formed in A_1R-Gi_2 complex. As such, α5 helix is rotated and translated toward TM7 and away from TM6, resulting in a smaller TM6 outward shift compared with the β_2AR-Gs (10.5 Å vs. 14 Å) (Draper-Joyce et al., 2018). Interestingly, a similar conformation of TM6 is noted in recently solved rhodopsin-Gi_1 complex (Kang et al., 2018), μOR-Gi_1 complex (Koehl et al., 2018), and 5-HT_{1B}R-Go complex (Garcia-Nafria et al., 2018). Also, the position of TM6 in these receptors is almost identical to that of metarhodopsin II, activate state of rhodopsin (RhR*), in complex with C-terminus of Gαt (GαtCT), which is consistent with previous findings that five C-terminal residues in α5 helix confers selectivity to receptor-transducer interaction (Stewart et al., 2009). Furthermore, upon binding to the G protein, Gs-coupled calcitonin CTR (Liang et al., 2017) and GLP_1R (Zhang, Sun, et al., 2017) receptors from class B GPCRs display even larger TM6 outward movements than that observed in β_2AR-Gs complex. Collectively, these findings suggest the positioning of TM6 as a fingerprint for Gi vs Gs selectivity (Fig. 3). However, this conformational signature may be due to differences in receptor activation regardless of G protein selectivity or because of peptides or mutations that are required for protein crystallization.

Interestingly, MD simulations indicate two distinct TM6 positions in β_2AR when bound to Gi or Gs. Given that β_2AR engages promiscuously with Gs and Gi, and that bovine rhodopsin engages only with Gt, a member of the Gi family, comparison of the structures of β_2AR-Gs complex (Rasmussen et al., 2011) with RhR*-GαtCT (Choe et al., 2011) is performed to discover the mechanisms of receptor/transducer selectivity. Specifically, the C-termini of both Gαs and Gαt bind to the $R^{3.50}$ in the E(D)RY motif. Because the reverse turn in GαsCT is bulkier than that of Gαi-CT or GαtCT, cation−π interaction between $R^{3.50}$ and $Y391^{GH5.23}$ in the β_2AR-Gs complex demands a 5-6 Å larger outward movement of TM6 compared to the hydrogen bridge between the $R^{3.50}$ and $C^{GH5.23}$ in RhR*-GαtCT complex (Kang et al., 2018; Rose et al., 2014). In fact, a broad range of tilts in TM6 of β_2AR is noted in the absence of cytoplasmic partners with two peak distances of 28 Å and 23 Å from TM2. Large tilts of TM6 were absent in RhR*, which does not engage Gs, thus can be interpreted as crucial for the Gs coupling state. The latter 23 Å peak, however, characterizes another population of β_2AR with conformations similar to that observed in RhR*-GαtCT complex, capable of interaction with Gi family (Elgeti et al., 2013; Rose et al., 2014).

Thus, designer ligands capable of biased signaling between G protein and β-arrestin pathways or distinguishing across G protein families may be identified based on their capacity to interact with residues at these segments. For example, FAUC350 was designed based on its interaction with $H393^{6.55}$ in TM6 of dopamine D_{2L} receptors, and demonstrated antagonistic or partial-agonistic properties for cAMP and $ERK_{1/2}$ pathways, respectively (Tschammer, Bollinger, Kenakin, & Gmeiner, 2011). In contrast, the ortho CF3 moiety in BRD5814 seems to prevent the rotation of $H393^{6.55}$, resulting in stabilization of a G protein-preferred conformation (Weïwer et al., 2018). In the same manner, amino acid differences at 7.35 position between μOR ($W^{7.35}$) and κOR ($Y^{7.35}$) could explain why IBNtxA robustly induced β-arrestin recruitment to κOR but not to μOR (Che et al., 2018). Furthermore, DAMGO displayed more activity toward β-arrestin2 activation at μOR $W320A^{7.35}$ mutant, but lost its efficacy in this pathway at $Y328F^{7.43}$. Conversely, endomorphin-1 induced β-arrestin recruitment to the μOR at Y328F mutant, but failed to do so at W320A mutant (Hothersall et al., 2017). Similarly, agonist-induced conformational re-arrangement around $W422^{7.35}$, between the allosteric and orthosteric binding pockets in muscarinic M_2R, as well as ligand-receptor interactions at $Y308^{7.35}$ of β_2 adrenoceptors are proposed as the mechanism by which chemically diverse agonists induce dual Gs and Gi signaling or display bias toward either of the G proteins (Bock et al., 2012; Woo et al., 2014).

6.1.5. Conformation of Carboxy-terminus

Given the lack of significant interactions between the C termini of β_2AR (Rasmussen et al., 2011) and μOR (Koehl et al., 2018) in complex with their nucleotide-free G proteins in contrast to the importance of this region in selective engagement of muscarinic M_3R with Gq (Qin, Dong, Wu, & Lambert, 2011), a role for helix 8 is speculated in signaling toward Gq (Fig. 3). Furthermore, the crystal structure of the angiotensin AT_2R revealed a non-canonical orientation for helix-8 (Fig. 3), in which it stabilized the active conformation, but simultaneously prevented receptor interaction with G proteins and β-arrestin, indicating active states beyond G protein and β-arrestin pathways (Zhang, Han, et al., 2017). In this regard, exchange of helix 8 in dopamine D_1R (Gs-coupled) with D_2R (Gi-coupled) did not alter their G protein selectivity. Meanwhile, D_1R containing the D_2R helix 8 or more hydrophobic residues at this segment demonstrated enhanced Gs signaling due to lower β-arrestin-mediated desensitization (Yang et al., 2019).

6.1.6. Pocket complementarity

The position of TM6, TM7, and helix 8 can be used to distinguish active receptor conformations having different affinities for G proteins, β-arrestins, or other potential transducers (Fig. 3). However, these differences are provided from a limited number of receptors and ligands, and may not be extrapolated to other GPCRs. Furthermore, an array of other structural alterations is reported for coupling to specific transducer pathways. For instance, an outward movement of TM2 was a common feature of adenosine A_3R ligands displaying bias toward cell survival, possibly through β-arrestin pathway (Baltos et al., 2016). Also, receptors differing only in ICL_1 demonstrated distinct affinities for Gs and Gq (Arora, Krsmanovic, Mores, O'Farrell, & Catt, 1998; Nussenzveig, Thaw, & Gershengorn, 1994; Wu et al., 1997; Zhang, Yang, & Tiberi, 2015). Thus, much more structural data is required to precisely delineate the relationship between the conformational state of a receptor and its preference for a specific transducer.

Given the failure of predicting the transducer of interest for a given GPCR based solely on primary amino acid sequence, other parameters need to be considered. In this regard, a common structural feature among recently elucidated GPCR-Gi/o complexes, especially 5-HT_{1B}R-Go, is markedly small receptor-transducer interface with considerable plasticity compared to the Gs-coupled β_2AR or adenosine A_2R conformations, highlighting again that transducer selectivity is not necessarily determined through conserved residues in a specific epitope or sequence motif. Rather, an ensemble of conformational rearrangements on the cytoplasmic plate provides 'pocket complementarity' and may mediate selective interaction with a given transducer (Capper & Wacker, 2018). In fact, a regional 3D structure enabling interaction between certain domains of the interacting molecules confers selectivity to receptor-transducer interaction. In the majority of cases, these 3D structures involve multiple residues from different receptor domains. As an example, a naturally occurring mutation R680G in class C CaSR seems to disrupt the salt bridge between R680 in TM3 and E767 in ECL2, and selectively enhances β-arrestin-mediated MAPK activation

without altering G protein mediated Ca^{2+} or cAMP response (Gorvin et al., 2018). Moreover, GLP_1R in complex with unbiased or biased ligands demonstrated a six-degree of difference in the angle of α5 helix of Gαs engagement with the receptor as well as different levels of flexibility in the intracellular end of TM5 and ICL3 and different rates of conformational rearrangements, all of which can modulate the magnitude or kinetics of transducer activation. Furthermore, selectivity can be determined at an intermediate step in the formation of the receptor-transducer complex, and these 'initial encounter' complexes may display different energy levels, ultimately determining whether or not a given receptor engages with a specific transducer (Koehl et al., 2018).

6.2. Stoichiometry of signaling molecules and biased signaling

GPCR function involves an array of signaling molecules beside the receptor and transducer. A growing number of GPCR-interacting proteins (GIPs) interact with receptors along their life cycle to assist proper folding, localization and signaling (Maurice et al., 2011), and in some cases influence ligand signaling. Receptor activity-modifying proteins (RAMPs) are prototypical GIPs involved in receptor trafficking to the cell surface and formation of the ligand binding pocket. Interestingly, co-expression of human calcitonin receptors with RAMP3, but not RAMP1 or RAMP2, reversed the rank order of potency of calcitonin and amylin for change in cell transmittance (Armour, Foord, Kenakin, & Chen, 1999), suggesting a role for these proteins in biased signaling. In this regard, the molecular chaperone Ric-8 is required for Gα14, Gα15, and Gαolf to produce functional G protein complexes (Masuho, Ostrovskaya, et al., 2015), and AGS3 produces a signaling complex with $G\alpha i_1$ and the cell surface receptor in an agonist dependent manner (Oner et al., 2010). Also, RGS8 and AGS1 change the fingerprint of muscarinic M_3R agonists for activation of different G proteins (Masuho, Ostrovskaya, et al., 2015).

Given the marked disparities in mRNA and/or protein levels of signaling molecules involved in GPCR function in different human cell lines (Atwood, Lopez, Wager-Miller, Mackie, & Straiker, 2011; Geiger, Wehner, Schaab, Cox, & Mann, 2012), these changes may influence the pharmacological profile of ligands. In this regard, cell type dependent GPCR coupling to exclusively cognate or to multiple non-cognate G proteins has been reported for class A (Peters & Scott, 2009), class B (Schwindinger et al., 1998) and class C (Hermans & Challiss, 2001) GPCRs. In some cases, non-cognate G proteins are activated only at high levels of receptor expression without significant change in the rank order of potency of ligands, indicating 'differential strength of signal' (Allen, Neumann, & Gershengorn, 2011; Cussac, Newman-Tancredi, Duqueyroix, Pasteau, & Millan, 2002; Michal, Lysíková, & Tucek, 2001). In other cases, however, a change in the expression of receptor and transducer or their stoichiometry induced non-linear changes in the potency and/or efficacy of agonists toward different pathways, indicating 'biased signaling' (Cordeaux et al., 2000; Deng, Sun, & Fang, 2013; Sato, Horinouchi, Hutchinson, Evans, & Summers, 2007; Watson et al., 2000). Thus, in addition to distinct receptor conformations, ligand-specific alterations in the expression of signaling molecules may also cause biased signaling. For example, chronic opioid treatment produces non-linear effects on G protein expression levels (especially $G\alpha i_3$ and Gα12) as well as GTPγS binding and cAMP synthesis, depending not only on the receptor subtype (μ, κ or δ) but also on the ligand tested (Xu et al., 2008). In this regard, the disparate relative activities of CT_2R agonists in cell backgrounds differing only in Gαs levels is attributed to stabilization of different active states (R*) having diverse relative affinities for Gαs (Watson et al., 2000). Likewise, the levels of Gα expression can reform the G protein fingerprint of β_2AR biased ligands towards different effector molecules, possibly through a change in membrane partitioning of the Gα and adrenoceptor (Onfroy et al., 2017). Furthermore, cell type specific expression of effector molecules (Federman, Conklin, Schrader, Reed, & Bourne, 1992), cell-cycle dependent expression of regulatory proteins (Abel, Wittau, Wieland, Schultz, & Kalkbrenner, 2000), or GPCR competition for available transducers (Jarrahian, Watts, & Barker, 2004) have been shown to alter receptor/transducer selectivity.

Collectively, the stoichiometry of signaling molecules may impose bias to neutral agonists or change the pattern of bias for functionally selective ligands. This can occur through a change in compartmentalization, in the assembly of signalosomes, or simply by competition for available partners. Thus, the selection of a physiologically relevant GPCR expression level, cell system and signaling pathways are crucial to distinguish system bias from ligand bias, and to enable better translation of experimental data to clinically meaningful events. Selecting the appropriate assay conditions becomes critical given that the expression of GPCRs and signaling proteins can significantly change in varying physiological or pathological conditions, such as in circadian rhythms (Doi et al., 2016), immune cell development and function (Chang et al., 2007), pregnancy (Stilley et al., 2016), cancer (Yajima et al., 2012), Parkinson's disease (Corvol et al., 2004) or cardiac disorders (Onfroy et al., 2017). In this regard, switching from uterine relaxation to prolabor contraction involves a change in follicle stimulating hormone (FSH) and prostanoid EP_2 receptor signaling from the Gαs/cAMP to the Gαq/IP3 pathway due to alterations in receptor density (Kandola et al., 2014; Stilley et al., 2016). Also, the clinical efficacy of β-blockers (metoprolol and bisoprolol) in heart failure may be supported by their biased signaling being independent of βAR/Gα stoichiometry (Onfroy et al., 2017).

6.3. Dimerization and biased signaling

GPCR dimerization or oligomerization is the simplest assembly of interacting molecules, which has been shown to influence ligand binding, allostery, receptor trafficking, and transducer selectivity (Lane et al., 2014; Ng et al., 2012). The most striking example is the class C GABA-B receptor, which requires heterodimer formation for its function with R1 subunit required for ligand binding and R2 subunit for trafficking to the plasma membrane (Bettler, Kaupmann, Mosbacher, & Gassmann, 2004). For other receptor classes, dimerization can affect signaling bias. For example, monomeric thyrotropin receptors (TSHR) interacted with Gs, whereas the receptor homodimer requiring two bound TSH molecules can signal through the Gq/IP_3 pathway (Allen, Neumann, & Gershengorn, 2011). Similarly, heterodimerization of $5\text{-}HT_{2A}R/mGlu_2R$ (Fribourg et al., 2011), orexin receptor 1 (OX_1R)/CB_1R (Hilairet, Bouaboula, Carriere, Le Fur, & Casellas, 2003), and μOR/δOR (George et al., 2000) modifies the efficacy and/or potency of ligands for different signaling pathways.

Of particular note, asymmetric oligomers, consisted of a GPCR/cognate G protein in complex with another GPCR/non-cognate G protein, can enhance signaling from the cognate transducer, a process called 'GPCR priming'. In this regard, the presence of non-cognate Gq potentiated AC stimulation through β_2AR and D_1R. Reciprocally, Gs enhanced inositol phosphate formation through Gq-coupled vasopressin V_{1A} receptor, but not α_1-AR, highlighting the fact that GPCR priming is receptor specific (Gupte et al., 2017). However, evidence for dimerization of class A and B GPCRs remains controversial. For instance, initial reports indicated D_2R dimerization with D_1R as a mechanism for SKF83959-mediated Gq signaling from the heterodimer, otherwise having no efficacy for Gs, Gi or Gq pathways through either of the monomers (Rashid et al., 2007). Meanwhile, analysis of receptor/transducer expression and localization along with behavioral studies have indicated the absence of Gq/11 engagement with D_1R and D_2R homomers or heteromers as well as absence of D_1R/D_2R complexes even in neurons with active promoters of both receptors, and possible explanation of SKF83959-mediated behaviours via mechanisms other than receptor dimerization (Frederick et al., 2015).

6.4. Compartmentalization and biased signaling

Given the complex nature of physiological membranes, containing many different types of lipids, a single receptor may produce different responses to the same agonist when located in a different environment. In fact, membrane composition and fluidity influences receptor trafficking, compartmentalization and even structure, all of which can influence transducer selectivity resulting in different pharmacological profile (Escribá, Wedegaertner, Goñi, & Vögler, 2007; Liu et al., 2012). This becomes critical given that lipid content of membranes changes in a number of metabolic disorders (Desai & Miller, 2018). In this regard, long-term stimulation of CB_1R (Paquette, Wang, Bakshi, & Olmstead, 2007) and μOR (Wang & Burns, 2009) decreased their engagement with Gi/o but enhanced interaction with Gs, possibly due to a change in receptor compartmentalization (Chakrabarti, Chang, Liu, & Gintzler, 2016; Halls et al., 2016). Furthermore, an inverse correlation between the efficacy of agonists to induce caveolin-dependent receptor endocytosis and activation of non-cognate pathways has been reported (Wang et al., 2010; Zhao, Loh, & Law, 2006).

Ligands acting at the same receptor but causing different patterns of compartmentalization, as well as other molecules influencing receptor compartmentalization, can be of potential clinical importance. For example, CB_1R ligands causing short endocytic 'dwell times', the time receptor clusters with β-arrestins into clathrin pits before endocytosis, elicited little or no β-arrestin signaling, while those stimulating prolonged dwell times elicited strong signaling (Flores-Otero et al., 2014). Given the role of β-arrestin in tolerance, longer analgesic efficacy is predicted for ligands inducing short dwell time.

6.5. Receptor trafficking, ligand residence/exposure time and biased signaling

Post-transcriptional or post-translational modifications can influence receptor trafficking, function and transducer selectivity. The earliest evidence suggesting this was from studies of the peptide RRSSKFCLKEHKALK, corresponding to the ICL3-C terminus of β_2AR. This peptide activates Gs at nanomolar concentrations *in vitro* and weakly activates Gi. Whereas, PKA-mediated phosphorylation at the conserved RRXS site dramatically reduced Gs activation, but enhanced Gi activation (Okamoto et al., 1991). Phosphorylation-dependent switch of coupling to non-cognate G protein pathways has also been reported for many GPCRs including adrenergic β_1 and β_2 (Martin, Whalen, Zamah, Pierce, & Lefkowitz, 2004; Zamah, Delahunty, Luttrell, & Lefkowitz, 2002), prostanoid IP receptor (Miggin & Kinsella, 2002), μOR and δOR (Kramer, Andria, Esposito, & Simon, 2000; Zhang, Zhao, Qiu, Loh, & Law, 2009), CB_1R (Paquette et al., 2007), and glutamate $mGluR_5$ (Dupont, Loomekandja Lokenye, & Challiss, 2011). Likewise, palmitoylation (Doi, Sugimoto, Arimoto, Hiroaki, & Fujiyoshi, 1999; Okamoto et al., 1997), prenylation (O'Meara & Kinsella, 2005), or alternative splicing (Nussenzveig et al., 1994) can also enable signaling from non-cognate G proteins. Furthermore, post-translational modifications of the transducer may also enable them to interact with the non-cognate receptors (Ammer & Schulz, 1997; Chakrabarti & Gintzler, 2007; Seyedabadi et al., 2012). In the same line, S-nitrosylation (Daaka, 2012; Hayashi et al., 2018) or S-glutathionylation (Gandhirajan et al., 2016) of either GPCRs and/or their associated effector molecules alter receptor signaling and may play important roles in pathology-mediated alterations in signaling bias or in therapeutic response to ligands acting at these receptor sites.

Different cells may express different levels of signaling molecules involved in receptor trafficking such as PKA, GRK, β-arrestin, and so forth. Cell-type specific phosphorylation and/or internalization has been reported for a number of GPCRs (Daigle, Kwok, & Mackie, 2008; Koch et al., 2005; Liu, Bee, & Schonbrunn, 2009; Torrecilla et al., 2007). Interestingly, both morphine and herkinorin induce weak or no β-arrestin recruitment and μOR internalization. Overexpression of GRK2, however, enabled morphine to stimulate β-arrestin translocation to the μOR and its internalization, but did not influence the herokinorin response (Groer et al., 2007). Furthermore, recruitment of GRK2 but not GRK5 to the α_2-AR/$G\alpha i_1\beta_1\gamma_2$ complex induced conformational rearrangement, resulting in a change in functional properties of the signaling unit independent of kinase activity (Breton, Lagace, & Bouvier, 2010). Also, GRK2 contains an RGS homology domain through which it sequesters Gq/11 and inhibits PLC-β activation (Carman et al., 1999). Given that distinct roles have been attributed to different GRK subtypes (desensitization to GRK2 and 3 and signalsome response to GRK5 and 6 (Reiter & Lefkowitz, 2006)), cell-type specific expression of these kinases may impose system bias to a neutral agonist, or change the pattern of bias for a functionally selective ligand. However, such specification of GRK duties may not be extrapolated to all receptors in all systems (Gaudreau, Le Gouill, Venne, Stankova, & Rola-Pleszczynski, 2002). Furthermore, given the dynamic nature of receptor trafficking, GPCR signaling of a given ligand may change in the same cell over time. For instance, immune cells fine-tune the expression of GRK and arrestins based on the severity of inflamation. Altered expression of GRK2 and/or GRK6 is also reported in inflammatory disorders (Vroon, Heijnen, & Kavelaars, 2006), hypertension and maladaptive fibrotic remodeling (Eckhart, Ozaki, Tevaearai, Rockman, & Koch, 2002; Zhu et al., 2012). One might, therefore, expect changes in GPCR signaling concommitant with altered GRK levels.

Additional insights into the mechanisms of bias arise from the fact that individual ligands may induce distinct patterns of receptor phosphorylation, i.e. 'phosphorylation barcode' (Jones & Hinkle, 2008; Zidar et al., 2009). Ligand-specific activation of downstream pathways may result in distinct patterns of receptor phosphorylation, resulting in a shift toward different transducers. For example, isoproterenol and carvedilol induced different patterns of β_2AR phosphorylation by GRK2 and GRK6, respectively (Nobles et al., 2011). These changes correlate with distinct patterns of β-arrestin recruitment and MAPK phosphorylation (Ren et al., 2005). Likewise, the CCR7 receptor endogenous ligands, CCL19 and CCL21, both caused GRK6/β-arrestin2-dependent phosphorylation of $ERK_{1/2}$. However, only the former induced additional phosphorylation of the receptor by GRK3, leading to a more robust β-arrestin2 recruitment, redistribution of the receptor into endocytic vesicles and desensitization (Zidar et al., 2009). This indicates that the reciprocal signaling between the receptor and cytosolic machinery plays a key role in functional selectivity.

Given that the natural agonist may activate only a subset of the signaling repertoire in a time-dependent manner via the same receptor, exogenous ligands may also engage signaling pathways with different rates and amplitudes (Lane, May, Parton, Sexton, & Christopoulos, 2017). In this regard, muscarinic M_3R, β_2AR and bradykinin receptor 2 (B_2R) discriminate between different G protein families, depending on the agonist exposure time (Masuho, Ostrovskaya, et al., 2015). Furthermore, the pattern of bias may change over time or even reverse for some agonists. For example, the dopamine D_2R biased agonist bifeprunox was more potent for Go_B activation than for Gi-mediated AC inhibition at early time points, while it displayed more potency toward AC inhibition at later time points (Klein Herenbrink et al., 2016). Interestingly, the discrepancies between earlier reports regarding the presence or absence of biased activity for aripiprazole toward AC/$ERK_{1/2}$ pathways (Szabo, Klein Herenbrink, Christopoulos, Lane, & Capuano, 2014; Tschammer et al., 2011) could be explained by differences in time points or temperatures at which the bias was measured (Klein Herenbrink et al., 2016).

Drug-target residence time may also influence the signaling paradigm of GPCRs (Guo, Hillger, IJzerman, & Heitman, 2014). For example, the slow receptor association/dissociation rate of ergot derivatives at $5HT_{2B}R$ was associated with lower potency in calcium assays relative to inositol phosphate assays, even when normalized to system or observational bias. Ligand potencies for $ERK_{1/2}$ phosphorylation were also highly time-dependent (Unett et al., 2013). This can also be clinical

importance, given that the binding kinetics of D_2R ligands is associated with motoric side effects of the antipsychotics (Sykes et al., 2017).

6.6. Ionic strength and transducer selectivity

The ionic content of the extracellular medium produces a significant effect on GPCR function and biased signaling. Of particular note, a conserved Na^+ pocket in the middle of 7TM bundle is found in class A GPCRs, which collapses upon receptor activation. Therefore, Na^+ acts as universal allosteric modulator for several class A GPCRs (Katritch et al., 2014). Furthermore, given the existence of both acidic and basic residues in GPCRs sequence, these residues can be protonated in media with pH values that differ from the isoelectric pH of protein (Vickery, Machtens, & Zachariae, 2016). This becomes clinically important given that the ionic strength as well as the pH of tissues can change in physiological or diseases states. This indicates that the pharmacological profile of a given agonist ultimately must be verified in a model which resembles the physiological medium in which it is to be used.

7. Conclusion

The functional selectivity of GPCRs opens a new horizon for pathway-selective drug discovery (Fig. 1). Some biased ligands have shown promise in clinical trials for cardiovascular disorders or management of pain. Moreover, some drugs already on the market may be found to show biased properties. However, three major milestones remain to be addressed for further progress in targeting, designing and validating biased ligands for clinical treatment.

Firstly, there is a need for more structural and conformational data with respect to biased ligands. Recent findings indicate distinct GPCR structures for β-arrestin vs. G protein, or Gs vs. Gi complexes. Although biased ligands tend to recognize distinct receptor conformations compared to that of balanced agonists, much more structural data is required to precisely differentiate these conformations and for extrapolation to other GPCR families and ultimately for rational, structure-based drug design.

Secondly, the role of specific signaling pathways in the pathophysiology of most human disorders has remained ill-defined. Given the polygenic nature of most diseases, it is often difficult to attribute a single signaling pathway to the pathophysiology of a specific disease. Hence, from a clinical perspective, screening for newly designed compounds showing bias on preselected pathways is useful only once preclinical evidence shows the benefit of biased ligands in the selected signaling pathways. Thus, developing ligands to bias signaling toward a specific pathway is one step, but may not be useful unless there is evidence that the pathways being targeted are clinically meaningful. With this aim in mind, the inhibitors of signaling pathways (e.g., protein kinase inhibitors, coupling inhibitors, etc.) could be first used to assess the role of different signaling pathways in disease state. Furthermore, pre-existing compounds with divergent pharmacological profile of on-target efficacy or adverse effects can be examined to decrypt the missing links in this puzzle.

The third milestone is development of appropriate screening platforms to identify ligand bias and minimize system and/or observational bias. An array of pharmacological and structural factors can influence signaling toward/away from a specific pathway (Fig. 2). Of particular note, the level of expression or localization of GPCRs, transducers, effectors, regulatory molecules, and so forth vary in different cells at different times, and may even change in different physiological or pathophysiological conditions. In this regard, advancement in analytical methods as well as well-controlled experimental platforms for robust differentiation of what bias stems from is very helpful. Furthermore, given that a single phenomenon can be modulated through different processes in different cells, the clinical efficacy of a given functionally selective ligand must be verified in physiological/pathological contexts that closely resemble the condition in which the drug is to be used.

Taken together, by directing GPCR signaling towards specific pathways, biased ligands have potential therapeutic benefits, especially in conditions with a well-understood pathophysiology. However, biased ligands may fail clinically due to a failure to demonstrate the same signaling readout in patient tissues as seen *in vitro*, or due to disease complexities such as multifaceted pathophysiology or genetic variation in trial subjects. Care in assay conditions, ligand verification, and patient and disease selection will be necessary to achieve successful biased ligand therapies.

Author contribution

Wrote or contributed to the writing of the manuscript: Seyedabadi, Ghahremani, Albert.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Abadji, V., Lucas-Lenard, J. M., Chin, C., & Kendall, D. A. (1999). Involvement of the carboxyl terminus of the third intracellular loop of the cannabinoid CB1 receptor in constitutive activation of Gs. *Journal of Neurochemistry 72*(5), 2032–2038 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10217281.

Abel, A., Wittau, N., Wieland, T., Schultz, G., & Kalkbrenner, F. (2000). Cell cycle-dependent coupling of the vasopressin V1a receptor to different G proteins. *The Journal of Biological Chemistry 275*(42), 32543–32551. https://doi.org/10.1074/jbc.M002171200.

Adham, N., Vaysse, P. J., Weinshank, R. L., & Branchek, T. A. (1994). The cloned human 5-HT1E receptor couples to inhibition and activation of adenylyl cyclase via two distinct pathways in transfected BS-C-1 cells. *Neuropharmacology 33*(3–4), 403–410. https://doi.org/10.1016/0028-3908(94)90070-1.

Ahn, S., Shenoy, S. K., Wei, H., & Lefkowitz, R. J. (2004). Differential kinetic and spatial patterns of beta-arrestin and G protein-mediated ERK activation by the angiotensin II receptor. *The Journal of Biological Chemistry 279*(34), 35518–35525. https://doi.org/10.1074/jbc.M405878200.

Airriess, C. N., Rudling, J. E., Midgley, J. M., & Evans, P. D. (1997). Selective inhibition of adenylyl cyclase by octopamine via a human cloned alpha 2A-adrenoceptor. *British Journal of Pharmacology 122*(2), 191–198. https://doi.org/10.1038/sj.bjp.0701348.

Akam, E. C., Challiss, R. A., & Nahorski, S. R. (2001). G(q/11) and G(i/o) activation profiles in CHO cells expressing human muscarinic acetylcholine receptors: dependence on agonist as well as receptor-subtype. *British Journal of Pharmacology 132*(4), 950–958. https://doi.org/10.1038/sj.bjp.0703892.

Albert, P. R. (2002). G protein preferences for dopamine D2 inhibition of prolactin secretion and DNA synthesis in GH4 pituitary cells. *Molecular Endocrinology (Baltimore, Md.) 16*(8), 1903–1911. https://doi.org/10.1210/me.2001-0329.

Albert, P. R., & Robillard, L. (2002). G protein specificity: traffic direction required. *Cellular Signalling 14*(5), 407–418. https://doi.org/10.1016/S0898-6568(01)00259-5.

Alberts, G. L., Chio, C. L., & Im, W. B. (2001). Allosteric modulation of the human 5-HT(7A) receptor by lipidic amphipathic compounds. *Molecular Pharmacology 60*(6), 1349–1355 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11723242.

Alberts, G. L., Pregenzer, J. F., Im, W. B., Zaworski, P. G., & Gill, G. S. (1999). Agonist-induced GTPgamma35S binding mediated by human 5-HT(2C) receptors expressed in human embryonic kidney 293 cells. *European Journal of Pharmacology 383*(3), 311–319 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10594325.

Albrecht-Kupper, B. E., Leineweber, K., Nell, P. G., Albrecht-Küpper, B. E., Leineweber, K., & Nell, P. G. (2012). Partial adenosine A1 receptor agonists for cardiovascular therapies. *Purinergic Signalling 8*(Suppl. 1), 91–99. https://doi.org/10.1007/s11302-011-9274-3.

Ali, M. S., Sayeski, P. P., & Bernstein, K. E. (2000). Jak2 acts as both a STAT1 kinase and as a molecular bridge linking STAT1 to the angiotensin II AT1 receptor. *The Journal of Biological Chemistry 275*(20), 15586–15593. https://doi.org/10.1074/jbc.M908931199.

Allen, J. A., Yost, J. M., Setola, V., Chen, X., Sassano, M. F., Chen, M., ... Jin, J. (2011). Discovery of β-arrestin-biased dopamine D2 ligands for probing signal transduction pathways essential for antipsychotic efficacy. *Proceedings of the National Academy of Sciences of the United States of America 108*(45), 18488–18493. https://doi.org/10.1073/pnas.1104807108.

Allen, M. D., Neumann, S., & Gershengorn, M. C. (2011). Occupancy of both sites on the thyrotropin (TSH) receptor dimer is necessary for phosphoinositide signaling.

FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 25(10), 3687–3694. https://doi.org/10.1096/fj.11-188961.

Allgeier, A., Laugwitz, K. L., Van Sande, J., Schultz, G., & Dumont, J. E. (1997). Multiple G-protein coupling of the dog thyrotropin receptor. *Molecular and Cellular Endocrinology 127*(1), 81–90. https://doi.org/10.1016/S0303-7207(96)03996-2.

Allgeier, A., Offermanns, S., Van Sande, J., Spicher, K., Schultz, G., & Dumont, J. E. (1994). The human thyrotropin receptor activates G-proteins Gs and Gq/11. *The Journal of Biological Chemistry 269*(19), 13733–13735 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8188646.

Alvarez-Curto, E., Inoue, A., Jenkins, L., Raihan, S. Z., Prihandoko, R., Tobin, A. B., & Milligan, G. (2016). Targeted Elimination of G Proteins and Arrestins Defines Their Specific Contributions to Both Intensity and Duration of G Protein-coupled Receptor Signaling. *The Journal of Biological Chemistry 291*(53), 27147–27159. https://doi.org/10.1074/jbc.M116.754887.

Ambrosio, C., Molinari, P., Cotecchia, S., & Costa, T. (2000). Catechol-binding serines of beta(2)-adrenergic receptors control the equilibrium between active and inactive receptor states. *Molecular Pharmacology 57*(1), 198–210.

Ammer, H., & Schulz, R. (1997). Enhanced stimulatory adenylyl cyclase signaling during opioid dependence is associated with a reduction in palmitoylated Gs alpha. *Molecular Pharmacology 52*(6), 993–999 Retrieved from http://molpharm.aspetjournals.org/content/52/6/993.abstract.

Appert-Collin, A., Baisamy, L., & Diviani, D. (2006). Regulation of g protein-coupled receptor signaling by a-kinase anchoring proteins. *Journal of Receptor and Signal Transduction Research 26*(5–6), 631–646. https://doi.org/10.1080/10799890600923211.

Armour, S. L., Foord, S., Kenakin, T., & Chen, W. J. (1999). Pharmacological characterization of receptor-activity-modifying proteins (RAMPs) and the human calcitonin receptor. *Journal of Pharmacological and Toxicological Methods 42*(4), 217–224.

Arora, K. K., Krsmanovic, L. Z., Mores, N., O'Farrell, H., & Catt, K. J. (1998). Mediation of cyclic AMP signaling by the first intracellular loop of the gonadotropin-releasing hormone receptor. *The Journal of Biological Chemistry 273*(40), 25581–25586. https://doi.org/10.1074/jbc.273.40.25581.

Arora, K. K., Sakai, A., & Catt, K. J. (1995). Effects of second intracellular loop mutations on signal transduction and internalization of the gonadotropin-releasing hormone receptor. *The Journal of Biological Chemistry 270*(39), 22820–22826 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7559413.

Arthur, J. M., Collinsworth, G. P., Gettys, T. W., Quarles, L. D., & Raymond, J. R. (1997). Specific coupling of a cation-sensing receptor to G protein alpha-subunits in MDCK cells. *The American Journal of Physiology 273*(1), F129–F135. https://doi.org/10.1152/ajprenal.1997.273.1.F129 Pt 2.

Atwood, B. K., Lopez, J., Wager-Miller, J., Mackie, K., & Straiker, A. (2011). Expression of G protein-coupled receptors and related proteins in HEK293, AtT20, BV2, and N18 cell lines as revealed by microarray analysis. *BMC Genomics 12*, 14. https://doi.org/10.1186/1471-2164-12-14.

Azzi, M., Charest, P. G., Angers, S., Rousseau, G., Kohout, T., Bouvier, M., & Pineyro, G. (2003). Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proceedings of the National Academy of Sciences of the United States of America 100*(20), 11406–11411. https://doi.org/10.1073/pnas.1936664100.

Bai, B., Jiang, Y., Cai, X., & Chen, J. (2014). Dynamics of apelin receptor/G protein coupling in living cells. *Experimental Cell Research*. https://doi.org/10.1016/j.yexcr.2014.08.035.

Baillie, G. S., & Houslay, M. D. (2005). Arrestin times for compartmentalised cAMP signalling and phosphodiesterase-4 enzymes. *Current Opinion in Cell Biology 17*(2), 129–134. https://doi.org/10.1016/j.ceb.2005.01.003.

Ballesteros, J. A., & Weinstein, H. (1995). Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors. In S. C. B. T. M. in N. Sealfon (Ed.), *Receptor Molecular Biology. Vol. 25*. (pp. 366–428). Academic Press. https://doi.org/10.1016/S1043-9471(05)80049-7.

Baltos, J. A., Gregory, K. J., White, P. J., Sexton, P. M., Christopoulos, A., & May, L. T. (2016). Quantification of adenosine A(1) receptor biased agonism: Implications for drug discovery. *Biochemical Pharmacology 99*, 101–112. https://doi.org/10.1016/j.bcp.2015.11.013.

Baltos, J. A., Paoletta, S., Nguyen, A. T. N., Gregory, K. J., Tosh, D. K., Christopoulos, A., ... May, L. T. (2016). Structure-Activity Analysis of Biased Agonism at the Human Adenosine A3 Receptor. *Molecular Pharmacology 90*(1), 12–22. https://doi.org/10.1124/mol.116.103283.

Barak, L. S., Bai, Y., Peterson, S., Evron, T., Urs, N. M., Peddibhotla, S., ... Caron, M. G. (2016). ML314: A Biased Neurotensin Receptor Ligand for Methamphetamine Abuse. *ACS Chemical Biology 11*(7), 1880–1890. https://doi.org/10.1021/acschembio.6b00291.

Barr, A. J., Brass, L. F., & Manning, D. R. (1997). Reconstitution of receptors and GTP-binding regulatory proteins (G proteins) in Sf9 cells. A direct evaluation of selectivity in receptor.G protein coupling. *The Journal of Biological Chemistry 272*(4), 2223–2229. https://doi.org/10.1074/jbc.272.4.2223.

Bauch, C., Koliwer, J., Buck, F., Honck, H. H., & Kreienkamp, H. J. (2014). Subcellular sorting of the G-protein coupled mouse somatostatin receptor 5 by a network of PDZ-domain containing proteins. *PLoS One 9*(2), e88529. https://doi.org/10.1371/journal.pone.0088529.

Bavec, A., Hällbrink, M., Langel, U., & Zorko, M. (2003). Different role of intracellular loops of glucagon-like peptide-1 receptor in G-protein coupling. *Regulatory Peptides 111*(1–3), 137–144. https://doi.org/10.1016/S0167-0115(02)00282-3.

Benovic, J. L., Kuhn, H., Weyand, I., Codina, J., Caron, M. G., & Lefkowitz, R. J. (1987). Functional desensitization of the isolated beta-adrenergic receptor by the beta-adrenergic receptor kinase: potential role of an analog of the retinal protein arrestin (48-kDa protein). *Proceedings of the National Academy of Sciences of the United States of America 84*(24), 8879–8882.

Berg, K. A., Dunlop, J., Sanchez, T., Silva, M., & Clarke, W. P. (2008). A conservative, single-amino acid substitution in the second cytoplasmic domain of the human Serotonin2C receptor alters both ligand-dependent and -independent receptor signaling. *The Journal of Pharmacology and Experimental Therapeutics 324*(3), 1084–1092. https://doi.org/10.1124/jpet.107.131524.

Bettler, B., Kaupmann, K., Mosbacher, J., & Gassmann, M. (2004). Molecular structure and physiological functions of GABA(B) receptors. *Physiological Reviews 84*(3), 835–867. https://doi.org/10.1152/physrev.00036.2003.

Birnbaumer, L. (2007). Expansion of signal transduction by G proteins. The second 15 years or so: from 3 to 16 alpha subunits plus betagamma dimers. *Biochimica et Biophysica Acta 1768*(4), 772–793. https://doi.org/10.1016/j.bbamem.2006.12.002.

Black, J. W., & Leff, P. (1983). Operational models of pharmacological agonism. *Proceedings of the Royal Society of London, Series B: Biological Sciences 220*(1219), 141–162.

Blättermann, S., Peters, L., Ottersbach, P. A., Bock, A., Konya, V., Weaver, C. D., ... Kostenis, E. (2012). A biased ligand for OXE-R uncouples Gα and Gβγ signaling within a heterotrimer. *Nature Chemical Biology 8*(7), 631–638. https://doi.org/10.1038/nchembio.962.

Boatman, P. D., Lauring, B., Schrader, T. O., Kasem, M., Johnson, B. R., Skinner, P., ... Richman, J. G. (2012). (1aR,5aR)1a,3,5,5a-Tetrahydro-1H-2,3-diaza-cyclopropa[a]pentalene-4-carboxylic acid (MK-1903): a potent GPR109a agonist that lowers free fatty acids in humans. *Journal of Medicinal Chemistry 55*(8), 3644–3666. https://doi.org/10.1021/jm2010964.

Bock, A., Merten, N., Schrage, R., Dallanoce, C., Bätz, J., Klöckner, J., ... Mohr, K. (2012). The allosteric vestibule of a seven transmembrane helical receptor controls G-protein coupling. *Nature Communications 3*, 1044 Retrieved from https://doi.org/10.1038/ncomms2028.

Bonacci, T. M., Mathews, J. L., Yuan, C., Lehmann, D. M., Malik, S., Wu, D., ... Smrcka, A. V. (2006). Differential targeting of Gbetagamma-subunit signaling with small molecules. *Science (New York, N.Y.) 312*(5772), 443–446. https://doi.org/10.1126/science.1120378.

Bonhaus, D. W., Chang, L. K., Kwan, J., & Martin, G. R. (1998). Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. *The Journal of Pharmacology and Experimental Therapeutics 287*(3), 884–888 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9864268.

Bonifazi, A., Yano, H., Ellenberger, M. P., Muller, L., Kumar, V., Zou, M. F., ... Newman, A. H. (2017). Novel Bivalent Ligands Based on the Sumanirole Pharmacophore Reveal Dopamine D2 Receptor (D2R) Biased Agonism. *Journal of Medicinal Chemistry 60*(7), 2890–2907. https://doi.org/10.1021/acs.jmedchem.6b01875.

Brame, A. L., Maguire, J. J., Yang, P., Dyson, A., Torella, R., Cheriyan, J., ... Davenport, A. P. (2015). Design, characterization, and first-in-human study of the vascular actions of a novel biased apelin receptor agonist. *Hypertension (Dallas, Tex.:1979) 65*(4), 834–840. https://doi.org/10.1161/HYPERTENSIONAHA.114.05099.

Breton, B., Lagace, M., & Bouvier, M. (2010). Combining resonance energy transfer methods reveals a complex between the alpha2A-adrenergic receptor, Galphai1beta1gamma2, and GRK2. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 24*(12), 4733–4743. https://doi.org/10.1096/fj.10-164061.

Broadbent, D., Ahmadzai, M. M., Kammala, A. K., Yang, C., Occhiuto, C., Das, R., & Subramanian, H. (2017). Roles of NHERF Family of PDZ-Binding Proteins in Regulating GPCR Functions. *Advances in Immunology 136*, 353–385. https://doi.org/10.1016/bs.ai.2017.05.008.

Brust, T. F., Hayes, M. P., Roman, D. L., Burris, K. D., & Watts, V. J. (2015). Bias analyses of preclinical and clinical D2 dopamine ligands: studies with immediate and complex signaling pathways. *The Journal of Pharmacology and Experimental Therapeutics 352*(3), 480–493. https://doi.org/10.1124/jpet.114.220293.

Brydon, L., Roka, F., Petit, L., de Coppet, P., Tissot, M., Barrett, P., ... Jockers, R. (1999). Dual signaling of human Mel1a melatonin receptors via G(i2), G(i3), and G(q/11) proteins. *Molecular Endocrinology (Baltimore, Md.) 13*(12), 2025–2038. https://doi.org/10.1210/mend.13.12.0390.

Büch, T. R. H., Heling, D., Damm, E., Gudermann, T., & Breit, A. (2009). Pertussis toxin-sensitive signaling of melanocortin-4 receptors in hypothalamic GT1-7 cells defines agouti-related protein as a biased agonist. *Journal of Biological Chemistry 284*(39), 26411–26420. https://doi.org/10.1074/jbc.M109.039339.

Burns, L. H., & Wang, H. y. (2010). PTI-609: a novel analgesic that binds filamin A to control opioid signaling. *Recent Patents on CNS Drug Discovery 5*(3), 210–220 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20726836.

Busnelli, M., Saulière, A., Manning, M., Bouvier, M., Galés, C., & Chini, B. (2012). Functional selective oxytocin-derived agonists discriminate between individual G protein family subtypes. *The Journal of Biological Chemistry 287*(6), 3617–3629. https://doi.org/10.1074/jbc.M111.277178.

Cahill, T. J., Thomsen, A. R. B., Tarrasch, J. T., Plouffe, B., Nguyen, A. H., Yang, F., ... Lefkowitz, R. J. (2017). Distinct conformations of GPCR-β-arrestin complexes mediate desensitization, signaling, and endocytosis. *Proceedings of the National Academy of Sciences of the United States of America 114*(10), 2562–2567. https://doi.org/10.1073/pnas.1701529114.

Calandra, B., Portier, M., Kernéis, A., Delpech, M., Carillon, C., Le Fur, G., ... Shire, D. (1999). Dual intracellular signaling pathways mediated by the human cannabinoid CB1 receptor. *European Journal of Pharmacology 374*(3), 445–455 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10422789.

Capper, M. J., & Wacker, D. (2018). How the ubiquitous GPCR receptor family selectively activates signalling pathways. *Nature 558*(7711), 529–530. https://doi.org/10.1038/d41586-018-05503-4.

Carman, C. V., Parent, J. L., Day, P. W., Pronin, A. N., Sternweis, P. M., Wedegaertner, P. B., ... Kozasa, T. (1999). Selective regulation of Galpha(q/11) by an RGS domain in the G

protein-coupled receptor kinase, GRK2. *The Journal of Biological Chemistry 274*(48), 34483–34492.
Carr, R., III, Schilling, J., Song, J., Carter, R. L., Du, Y., Yoo, S. M., ... Benovic, J. L. (2016). beta-arrestin-biased signaling through the beta2-adrenergic receptor promotes cardiomyocyte contraction. *Proceedings of the National Academy of Sciences of the United States of America 113*(28), E4107–E4116. https://doi.org/10.1073/pnas.1606267113.
Carr, R., Du, Y., Quoyer, J., Panettieri, R. A., Janz, J. M., Bouvier, M., ... Benovic, J. L. (2014). Development and characterization of pepducins as Gs-biased allosteric agonists. *The Journal of Biological Chemistry 289*(52), 35668–35684. https://doi.org/10.1074/jbc.M114.618819.
Carter, A. A., & Hill, S. J. (2005). Characterization of isoprenaline- and salmeterol-stimulated interactions between beta2-adrenoceptors and beta-arrestin 2 using beta-galactosidase complementation in C2C12 cells. *The Journal of Pharmacology and Experimental Therapeutics 315*(2), 839–848. https://doi.org/10.1124/jpet.105.088914.
Chakrabarti, S., Chang, A., Liu, N. J., & Gintzler, A. R. (2016). Chronic opioid treatment augments caveolin-1 scaffolding: relevance to stimulatory μ-opioid receptor adenylyl cyclase signaling. *Journal of Neurochemistry 139*(5), 737–747. https://doi.org/10.1111/jnc.13852.
Chakrabarti, S., & Gintzler, A. R. (2007). Phosphorylation of Galphas influences its association with the micro-opioid receptor and is modulated by long-term morphine exposure. *Molecular Pharmacology 72*(3), 753–760. https://doi.org/10.1124/mol.107.036145.
Chakrabarti, S., Regec, A., & Gintzler, A. R. (2005). Biochemical demonstration of mu-opioid receptor association with Gsalpha: enhancement following morphine exposure. *Brain Research. Molecular Brain Research 135*(1–2), 217–224. https://doi.org/10.1016/j.molbrainres.2004.12.016.
Chang, G. W., Davies, J. Q., Stacey, M., Yona, S., Bowdish, D. M. E., Hamann, J., ... Lin, H. H. (2007). CD312, the human adhesion-GPCR EMR2, is differentially expressed during differentiation, maturation, and activation of myeloid cells. *Biochemical and Biophysical Research Communications 353*(1), 133–138. https://doi.org/10.1016/j.bbrc.2006.11.148.
Chang, S. D., Mascarella, S. W., Spangler, S. M., Gurevich, V. V., Navarro, H. A., Carroll, F. I., & Bruchas, M. R. (2015). Quantitative Signaling and Structure-Activity Analyses Demonstrate Functional Selectivity at the Nociceptin/Orphanin FQ Opioid Receptor. *Molecular Pharmacology 88*(3), 502–511. https://doi.org/10.1124/mol.115.099150.
Charfi, I., Nagi, K., Mnie-Filali, O., Thibault, D., Balboni, G., Schiller, P. W., ... Pineyro, G. (2014). Ligand- and cell-dependent determinants of internalization and cAMP modulation by delta opioid receptor (DOR) agonists. *Cellular and Molecular Life Sciences: CMLS 71*(8), 1529–1546. https://doi.org/10.1007/s00018-013-1461-7.
Charlton, J. J., Allen, P. B., Psifogeorgou, K., Chakravarty, S., Gomes, I., Neve, R. L., ... Zachariou, V. (2008). Multiple actions of spinophilin regulate mu opioid receptor function. *Neuron 58*(2), 238–247. https://doi.org/10.1016/j.neuron.2008.02.006.
Che, T., Majumdar, S., Zaidi, S. A., Ondachi, P., McCorvy, J. D., Wang, S., ... Roth, B. L. (2018). Structure of the Nanobody-Stabilized Active State of the Kappa Opioid Receptor. *Cell 172*(1–2), 55–67.e15. https://doi.org/10.1016/j.cell.2017.12.011.
Chen, X., Sassano, M. F., Zheng, L., Setola, V., Chen, M., Bai, X., ... Jin, J. (2012). Structure−Functional Selectivity Relationship Studies of β-Arrestin- Biased Dopamine D 2 Receptor Agonists. *Journal of Medicinal Chemistry 55*, 7141–7153. https://doi.org/10.1021/jm300603y.
Chen, X. P., Yang, W., Fan, Y., Luo, J. S., Hong, K., Wang, Z., ... Zhou, N. M. (2010). Structural determinants in the second intracellular loop of the human cannabinoid CB1 receptor mediate selective coupling to G(s) and G(i). *British Journal of Pharmacology 161*(8), 1817–1834. https://doi.org/10.1111/j.1476-5381.2010.01006.x.
Chen, X. T., Pitis, P., Liu, G., Yuan, C., Gotchev, D., Cowan, C. L., ... Yamashita, D. S. (2013). Structure-activity relationships and discovery of a G protein biased μ opioid receptor ligand, [(3-methoxythiophen-2-yl)methyl]({2-[(9R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl]ethyl})amine (TRV130), for the treatment of acute severe pain. *Journal of Medicinal Chemistry 56*(20), 8019–8031. https://doi.org/10.1021/jm4010829.
Chen, Y., Baez, M., & Yu, L. (1994). Functional coupling of the 5-HT2C serotonin receptor to G proteins in Xenopus oocytes. *Neuroscience Letters 179*(1–2), 100–102 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7531308.
Choe, H. W., Kim, Y. J., Park, J. H., Morizumi, T., Pai, E. F., Krauss, N., ... Ernst, O. P. (2011). Crystal structure of metarhodopsin II. *Nature 471*(7340), 651–655. https://doi.org/10.1038/nature09789.
Cianfrocca, R., Tocci, P., Semprucci, E., Spinella, F., Di Castro, V., Bagnato, A., & Rosano, L. (2014). beta-Arrestin 1 is required for endothelin-1-induced NF-kappaB activation in ovarian cancer cells. *Life Sciences 118*(2), 179–184. https://doi.org/10.1016/j.lfs.2014.01.078.
Cohen, B. D., Nechamen, C. A., & Dias, J. A. (2004). Human follitropin receptor (FSHR) interacts with the adapter protein 14-3-3tau. *Molecular and Cellular Endocrinology 220*(1–2), 1–7. https://doi.org/10.1016/j.mce.2004.04.012.
Conchon, S., Barrault, M. B., Miserey, S., Corvol, P., & Clauser, E. (1997). The C-terminal third intracellular loop of the rat AT1A angiotensin receptor plays a key role in G protein coupling specificity and transduction of the mitogenic signal. *The Journal of Biological Chemistry 272*(41), 25566–25572 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9325274.
Cook, A. E., Mistry, S. N., Gregory, K. J., Furness, S. G. B., Sexton, P. M., Scammells, P. J., ... Leach, K. (2015). Biased allosteric modulation at the CaS receptor engendered by structurally diverse calcimimetics. *British Journal of Pharmacology 172*(1), 185–200. https://doi.org/10.1111/bph.12937.
Corbisier, J., Galès, C., Huszagh, A., Parmentier, M., & Springael, J. Y. (2015). Biased signaling at chemokine receptors. *The Journal of Biological Chemistry 290*(15), 9542–9554. https://doi.org/10.1074/jbc.M114.596098.
Cordeaux, Y., Briddon, S. J., Megson, A. E., McDonnell, J., Dickenson, J. M., & Hill, S. J. (2000). Influence of receptor number on functional responses elicited by agonists acting at the human adenosine A(1) receptor: evidence for signaling pathway-dependent changes in agonist potency and relative intrinsic activity. *Molecular Pharmacology 58*(5), 1075–1084 (doi:0026-895X/00/051075).
Cordeaux, Y., Ijzerman, A. P., & Hill, S. J. (2004). Coupling of the human A1 adenosine receptor to different heterotrimeric G proteins: evidence for agonist-specific G protein activation. *British Journal of Pharmacology 143*(6), 705–714. https://doi.org/10.1038/sj.bjp.0705925.
Corvol, J. C., Muriel, M. P., Valjent, E., Feger, J., Hanoun, N., Girault, J. A., ... Herve, D. (2004). Persistent increase in olfactory type G-protein alpha subunit levels may underlie D1 receptor functional hypersensitivity in Parkinson disease. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 24*(31), 7007–7014. https://doi.org/10.1523/JNEUROSCI.0676-04.2004.
Cotecchia, S., Ostrowski, J., Kjelsberg, M. A., Caron, M. G., & Lefkowitz, R. J. (1992). Discrete amino acid sequences of the alpha 1-adrenergic receptor determine the selectivity of coupling to phosphatidylinositol hydrolysis. *The Journal of Biological Chemistry 267*(3), 1633–1639 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1309789.
Cotter, G., Davison, B. A., Butler, J., Collins, S. P., Ezekowitz, J. A., Felker, G. M., ... Pang, P. S. (2018). Relationship between baseline systolic blood pressure and long-term outcomes in acute heart failure patients treated with TRV027: an exploratory subgroup analysis of BLAST-AHF. *Clinical Research in Cardiology : Official Journal of the German Cardiac Society 107*(2), 170–181. https://doi.org/10.1007/s00392-017-1168-0.
Cussac, D., Boutet-Robinet, E., Ailhaud, M. C., Newman-Tancredi, A., Martel, J. C., Danty, N., & Rauly-Lestienne, I. (2008). Agonist-directed trafficking of signalling at serotonin 5-HT2A, 5-HT2B and 5-HT2C-VSV receptors mediated Gq/11 activation and calcium mobilisation in CHO cells. *European Journal of Pharmacology 594*(1–3), 32–38. https://doi.org/10.1016/j.ejphar.2008.07.040.
Cussac, D., Newman-Tancredi, A., Duqueyroix, D., Pasteau, V., & Millan, M. J. (2002). Differential activation of Gq/11 and Gi(3) proteins at 5-hydroxytryptamine(2C) receptors revealed by antibody capture assays: influence of receptor reserve and relationship to agonist-directed trafficking. *Molecular Pharmacology 62*(3), 578–589. https://doi.org/10.1124/mol.62.3.578.
Daaka, Y. (2012). S-nitrosylation-regulated GPCR signaling. *Biochimica et Biophysica Acta 1820*(6), 743–751. https://doi.org/10.1016/j.bbagen.2011.03.007.
Daaka, Y., Luttrell, L. M., & Lefkowitz, R. J. (1997). Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature 390*(6655), 88–91. https://doi.org/10.1038/36362.
Daigle, T. L., Kwok, M. L., & Mackie, K. (2008). Regulation of CB1 cannabinoid receptor internalization by a promiscuous phosphorylation-dependent mechanism. *Journal of Neurochemistry 106*(1), 70–82. https://doi.org/10.1111/j.1471-4159.2008.05336.x.
Davey, A. E., Leach, K., Valant, C., Conigrave, A. D., Sexton, P. M., & Christopoulos, A. (2012). Positive and negative allosteric modulators promote biased signaling at the calcium-sensing receptor. *Endocrinology 153*(3), 1232–1241. https://doi.org/10.1210/en.2011-1426.
De Deurwaerdere, P., Navailles, S., Berg, K. A., Clarke, W. P., & Spampinato, U. (2004). Constitutive activity of the serotonin2C receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 24*(13), 3235–3241. https://doi.org/10.1523/JNEUROSCI.0112-04.2004.
Deng, H., Sun, H., & Fang, Y. (2013). Label-free cell phenotypic assessment of the biased agonism and efficacy of agonists at the endogenous muscarinic M3 receptors. *Journal of Pharmacological and Toxicological Methods 68*(3), 323–333. https://doi.org/10.1016/j.vascn.2013.07.005.
Desai, A. J., & Miller, L. J. (2018). Changes in the plasma membrane in metabolic disease: impact of the membrane environment on G protein-coupled receptor structure and function. *British Journal of Pharmacology 175*(21), 4009–4025. https://doi.org/10.1111/bph.13943.
DeVree, B. T., Mahoney, J. P., Velez-Ruiz, G. A., Rasmussen, S. G. F., Kuszak, A. J., Edwald, E., ... Sunahara, R. K. (2016). Allosteric coupling from G protein to the agonist-binding pocket in GPCRs. *Nature 535*(7610), 182–186. https://doi.org/10.1038/nature18324.
Dhar, T. G. M., Xiao, H. Y., Xie, J., Lehman-McKeeman, L. D., Wu, D. R., Dabros, M., ... Dyckman, A. J. (2016). Identification and Preclinical Pharmacology of BMS-986104: A Differentiated S1P1 Receptor Modulator in Clinical Trials. *ACS Medicinal Chemistry Letters 7*(3), 283–288. https://doi.org/10.1021/acsmedchemlett.5b00448.
Dittman, A. H., Weber, J. P., Hinds, T. R., Choi, E. J., Migeon, J. C., Nathanson, N. M., & Storm, D. R. (1994). A novel mechanism for coupling of m4 muscarinic acetylcholine receptors to calmodulin-sensitive adenylyl cyclases: crossover from G protein-coupled inhibition to stimulation. *Biochemistry 33*(4), 943–951. https://doi.org/10.1021/bi00170a013.
Doi, M., Murai, I., Kunisue, S., Setsu, G., Uchio, N., Tanaka, R., ... Okamura, H. (2016). Gpr176 is a Gz-linked orphan G-protein-coupled receptor that sets the pace of circadian behaviour. *Nature Communications 7*, 10583. https://doi.org/10.1038/ncomms10583.
Doi, T., Sugimoto, H., Arimoto, I., Hiroaki, Y., & Fujiyoshi, Y. (1999). Interactions of endothelin receptor subtypes A and B with Gi, Go, and Gq in reconstituted phospholipid vesicles. *Biochemistry 38*(10), 3090–3099. https://doi.org/10.1021/bi981919m.
Draper-Joyce, C. J., Khoshouei, M., Thal, D. M., Liang, Y. L., Nguyen, A. T. N., Furness, S. G. B., ... Christopoulos, A. (2018). Structure of the adenosine-bound human adenosine A1 receptor-Gi complex. *Nature 558*(7711), 559–563. https://doi.org/10.1038/s41586-018-0236-6.
Dror, R. O., Mildorf, T. J., Hilger, D., Manglik, A., Borhani, D. W., Arlow, D. H., ... Shaw, D. E. (2015). Structural basis for nucleotide exchange in heterotrimeric G proteins. *Science (New York, N.Y.) 348*(6241), 1361–1365. https://doi.org/10.1126/science.aaa5264.
Dunn, H. A., & Ferguson, S. S. G. (2015). PDZ Protein Regulation of G Protein-Coupled Receptor Trafficking and Signaling Pathways. *Molecular Pharmacology 88*(4), 624–639. https://doi.org/10.1124/mol.115.098509.

Dupont, G., Loomekandja Lokenye, E. F., & Challiss, R. A. J. (2011). A model for Ca2+ oscillations stimulated by the type 5 metabotropic glutamate receptor: An unusual mechanism based on repetitive, reversible phosphorylation of the receptor. *Biochimie 93*(12), 2132–2138. https://doi.org/10.1016/j.biochi.2011.09.010.

Dupré, D. J., Robitaille, M., Rebois, R. V., & Hébert, T. E. (2009). The role of Gbetagamma subunits in the organization, assembly, and function of GPCR signaling complexes. *Annual Review of Pharmacology and Toxicology 49*, 31–56. https://doi.org/10.1146/annurev-pharmtox-061008-103038.

Eason, M. G., Jacinto, M. T., & Liggett, S. B. (1994). Contribution of ligand structure to activation of alpha 2-adrenergic receptor subtype coupling to Gs. *Molecular Pharmacology 45*(4), 696–702 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7910371.

Eason, M. G., Kurose, H., Holt, B. D., Raymond, J. R., & Liggett, S. B. (1992). Simultaneous coupling of alpha 2-adrenergic receptors to two G-proteins with opposing effects. Subtype-selective coupling of alpha 2C10, alpha 2C4, and alpha 2C2 adrenergic receptors to Gi and Gs. *The Journal of Biological Chemistry 267*(22), 15795–15801 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1322406.

Eason, M. G., & Liggett, S. B. (1995). Identification of a Gs coupling domain in the amino terminus of the third intracellular loop of the alpha 2A-adrenergic receptor. Evidence for distinct structural determinants that confer Gs versus Gi coupling. *The Journal of Biological Chemistry 270*(42), 24753–24760 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7559592.

Eason, M. G., & Liggett, S. B. (1996). Chimeric mutagenesis of putative G-protein coupling domains of the alpha2A-adrenergic receptor. Localization of two redundant and fully competent gi coupling domains. *The Journal of Biological Chemistry 271*(22), 12826–12832 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8662784.

Eckhart, A. D., Ozaki, T., Tevaearai, H., Rockman, H. A., & Koch, W. J. (2002). Vascular-targeted overexpression of G protein-coupled receptor kinase-2 in transgenic mice attenuates beta-adrenergic receptor signaling and increases resting blood pressure. *Molecular Pharmacology 61*(4), 749–758.

Elgeti, M., Rose, A. S., Bartl, F. J., Hildebrand, P. W., Hofmann, K. p., & Heck, M. (2013). Precision vs flexibility in GPCR signaling. *Journal of the American Chemical Society 135*(33), 12305–12312. https://doi.org/10.1021/ja405133k.

Emery, A. C., DiRaddo, J. O., Miller, E., Hathaway, H. A., Pshenichkin, S., Takoudjou, G. R., ... Wroblewski, J. T. (2012). Ligand bias at metabotropic glutamate 1a receptors: molecular determinants that distinguish β-arrestin-mediated from G protein-mediated signaling. *Molecular Pharmacology 82*(2), 291–301. https://doi.org/10.1124/mol.112.078444.

Erlenbach, I., Kostenis, E., Schmidt, C., Serradeil-Le Gal, C., Raufaste, D., Dumont, M. E., ... Wess, J. (2001). Single amino acid substitutions and deletions that alter the G protein coupling properties of the V2 vasopressin receptor identified in yeast by receptor random mutagenesis. *The Journal of Biological Chemistry 276*(31), 29382–29392. https://doi.org/10.1074/jbc.M103203200.

Erlenbach, I., & Wess, J. (1998). Molecular basis of V2 vasopressin receptor/Gs coupling selectivity. *The Journal of Biological Chemistry 273*(41), 26549–26558 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9756892.

Escribá, P. V., Wedegaertner, P. B., Goñi, F. M., & Vögler, O. (2007). Lipid-protein interactions in GPCR-associated signaling. *Biochimica et Biophysica Acta 1768*(4), 836–852. https://doi.org/10.1016/j.bbamem.2006.09.001.

Favre, N., Fanelli, F., Missotten, M., Nichols, A., Wilson, J., di Tiani, M., … Scheer, A. (2005). The DRY motif as a molecular switch of the human oxytocin receptor. Biochemistry, 44(30), 9990–10008. doi:https://doi.org/10.1021/bi0509853.

Federman, A. D., Conklin, B. R., Schrader, K. A., Reed, R. R., & Bourne, H. R. (1992). Hormonal stimulation of adenylyl cyclase through Gi-protein beta gamma subunits. *Nature 356*(6365), 159–161. https://doi.org/10.1038/356159a0.

Figueroa, K. W., Griffin, M. T., & Ehlert, F. J. (2009). Selectivity of agonists for the active state of M1 to M4 muscarinic receptor subtypes. *Journal of Pharmacology and Experimental Therapeutics 328*(1) 331 LP-342. Retrieved from http://jpet.aspetjournals.org/content/328/1/331.abstract.

Flock, T., Hauser, A. S., Lund, N., Gloriam, D. E., Balaji, S., & Babu, M. M. (2017). Selectivity determinants of GPCR-G-protein binding. *Nature 545*(7654), 317–322. https://doi.org/10.1038/nature22070.

Flock, T., Ravarani, C. N. J., Sun, D., Venkatakrishnan, A. J., Kayikci, M., Tate, C. G., ... Babu, M. M. (2015). Universal allosteric mechanism for Gα activation by GPCRs. *Nature 524*(7564), 173–179. https://doi.org/10.1038/nature14663.

Flores-Otero, J., Ahn, K. H., Delgado-Peraza, F., Mackie, K., Kendall, D. A., & Yudowski, G. A. (2014). Ligand-specific endocytic dwell times control functional selectivity of the cannabinoid receptor 1. *Nature Communications 5*, 4589. https://doi.org/10.1038/ncomms5589.

Fong, A. M., Premont, R. T., Richardson, R. M., Yu, Y. R. A., Lefkowitz, R. J., & Patel, D. D. (2002). Defective lymphocyte chemotaxis in beta-arrestin2- and GRK6-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America 99*(11), 7478–7483. https://doi.org/10.1073/pnas.112198299.

Fowler, J. C., Bhattacharya, S., Urban, J. D., Vaidehi, N., & Mailman, R. B. (2012). Receptor conformations involved in dopamine D(2L) receptor functional selectivity induced by selected transmembrane-5 serine mutations. *Molecular Pharmacology 81*(6), 820–831. https://doi.org/10.1124/mol.111.075457.

Frederick, A. L., Yano, H., Trifilieff, P., Vishwasrao, H. D., Biezonski, D., Meszaros, J., ... Javitch, J. A. (2015). Evidence against dopamine D1/D2 receptor heteromers. *Molecular Psychiatry 20*(11), 1373–1385. https://doi.org/10.1038/mp.2014.166.

Fredriksson, R., Lagerström, M. C., Lundin, L. G., & Schiöth, H. B. (2003). The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Molecular Pharmacology 63*(6), 1256–1272. https://doi.org/10.1124/mol.63.6.1256.

Fribourg, M., Moreno, J. L., Holloway, T., Provasi, D., Baki, L., Mahajan, R., ... Logothetis, D. E. (2011). Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. *Cell 147*(5), 1011–1023. https://doi.org/10.1016/j.cell.2011.09.055.

Furness, S. G., & Sexton, P. M. (2017). Coding GPCR-G protein specificity. *Cell Research 27*(10), 1193–1194. https://doi.org/10.1038/cr.2017.92.

Gabl, M., Holdfeldt, A., Sundqvist, M., Lomei, J., Dahlgren, C., & Forsman, H. (2017). FPR2 signaling without beta-arrestin recruitment alters the functional repertoire of neutrophils. *Biochemical Pharmacology 145*, 114–122. https://doi.org/10.1016/j.bcp.2017.08.018.

Gailly, P., Najimi, M., & Hermans, E. (2000). Evidence for the dual coupling of the rat neurotensin receptor with pertussis toxin-sensitive and insensitive G-proteins. *FEBS Letters 483*(2–3), 109–113 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11042263.

Galandrin, S., & Bouvier, M. (2006). Distinct signaling profiles of beta1 and beta2 adrenergic receptor ligands toward adenylyl cyclase and mitogen-activated protein kinase reveals the pluridimensionality of efficacy. *Molecular Pharmacology 70*(5), 1575–1584. https://doi.org/10.1124/mol.106.026716.

Galandrin, S., Oligny-Longpré, G., Bonin, H., Ogawa, K., Galés, C., & Bouvier, M. (2008). Conformational rearrangements and signaling cascades involved in ligand-biased mitogen-activated protein kinase signaling through the beta1-adrenergic receptor. *Molecular Pharmacology 74*(1), 162–172. https://doi.org/10.1124/mol.107.043893.

Gales, C., Rebois, R. V., Hogue, M., Trieu, P., Breit, A., Hebert, T. E., & Bouvier, M. (2005). Real-time monitoring of receptor and G-protein interactions in living cells. *Nature Methods 2*(3), 177–184. https://doi.org/10.1038/nmeth743.

Gales, C., Van Durm, J. J. J., Schaak, S., Pontier, S. M., Percherancier, Y., Audet, M., ... Bouvier, M. (2006). Probing the activation-promoted structural rearrangements in preassembled receptor-G protein complexes. *Nature Structural & Molecular Biology 13*(9), 778–786. https://doi.org/10.1038/nsmb1134.

Gandhirajan, R. K., Jain, M., Walla, B., Johnsen, M., Bartram, M. P., Huynh Anh, M., ... Schermer, B. (2016). Cysteine S-Glutathionylation Promotes Stability and Activation of the Hippo Downstream Effector Transcriptional Co-activator with PDZ-binding Motif (TAZ). *The Journal of Biological Chemistry 291*(22), 11596–11607. https://doi.org/10.1074/jbc.M115.712539.

Garcia-Nafria, J., Nehme, R., Edwards, P. C., & Tate, C. G. (2018). Cryo-EM structure of the serotonin 5-HT1B receptor coupled to heterotrimeric Go. *Nature 558*(7711), 620–623. https://doi.org/10.1038/s41586-018-0241-9.

Gaudreau, R., Le Gouill, C., Venne, M. H., Stankova, J., & Rola-Pleszczynski, M. (2002). Threonine 308 within a putative casein kinase 2 site of the cytoplasmic tail of leukotriene B(4) receptor (BLT1) is crucial for ligand-induced, G-protein-coupled receptor-specific kinase 6-mediated desensitization. *The Journal of Biological Chemistry 277*(35), 31567–31576. https://doi.org/10.1074/jbc.M202723200.

Gazi, L., Nickolls, S. A., & Strange, P. G. (2003). Functional coupling of the human dopamine D2 receptor with G alpha i1, G alpha i2, G alpha i3 and G alpha o G proteins: evidence for agonist regulation of G protein selectivity. *British Journal of Pharmacology 138*(5), 775–786. https://doi.org/10.1038/sj.bjp.0705116.

Geiger, T., Wehner, A., Schaab, C., Cox, J., & Mann, M. (2012). Comparative proteomic analysis of eleven common cell lines reveals ubiquitous but varying expression of most proteins. *Molecular & Cellular Proteomics 11*(3). https://doi.org/10.1074/mcp.M111.014050 M111.014050.

Geng, Y., Bush, M., Mosyak, L., Wang, F., & Fan, Q. R. (2013). Structural mechanism of ligand activation in human GABA(B) receptor. *Nature 504*(7479), 254–259. https://doi.org/10.1038/nature12725.

Geng, Y., Mosyak, L., Kurinov, I., Zuo, H., Sturchler, E., Cheng, T. C., ... Fan, Q. R. (2016). Structural mechanism of ligand activation in human calcium-sensing receptor. *ELife 5*. https://doi.org/10.7554/eLife.13662.

George, S. R., Fan, T., Xie, Z., Tse, R., Tam, V., Varghese, G., & O'Dowd, B. F. (2000). Oligomerization of mu- and delta-opioid receptors. Generation of novel functional properties. *The Journal of Biological Chemistry 275*(34), 26128–26135. https://doi.org/10.1074/jbc.M000345200.

Gesty-Palmer, D., Chen, M., Reiter, E., Ahn, S., Nelson, C. D., Wang, S., ... Lefkowitz, R. J. (2006). Distinct β-Arrestin- and G Protein-dependent Pathways for Parathyroid Hormone Receptor-stimulated ERK1/2 Activation. *Journal of Biological Chemistry 281*(16), 10856–10864. https://doi.org/10.1074/jbc.M513380200.

Gesty-Palmer, D., Flannery, P., Yuan, L., Corsino, L., Spurney, R., Lefkowitz, R. J., & Luttrell, L. M. (2009). A β-Arrestin–Biased Agonist of the Parathyroid Hormone Receptor (PTH1R) Promotes Bone Formation Independent of G Protein Activation. *Science Translational Medicine 1*(1) 1ra1 LP-1ra1. Retrieved from http://stm.sciencemag.org/content/1/1/1ra1.abstract.

Ghahremani, M. H., Cheng, P., Lembo, P. M., & Albert, P. R. (1999). Distinct roles for Galphai2, Galphai3, and Gbeta gamma in modulation offorskolin- or Gs-mediated cAMP accumulation and calcium mobilization by dopamine D2S receptors. *The Journal of Biological Chemistry 274*(14), 9238–9245 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10092597.

Gong, H., Sun, H., Koch, W. J., Rau, T., Eschenhagen, T., Ravens, U., ... Harding, S. E. (2002). Specific beta(2)AR blocker ICI 118,551 actively decreases contraction through a G (i)-coupled form of the beta(2)AR in myocytes from failing human heart. *Circulation 105*(21), 2497–2503 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12034656.

Gorvin, C. M., Babinsky, V. N., Malinauskas, T., Nissen, P. H., Schou, A. J., Hanyaloglu, A. C., ... Thakker, R. V. (2018). A calcium-sensing receptor mutation causing hypocalcemia disrupts a transmembrane salt bridge to activate beta-arrestin-biased signaling. *Science Signaling 11*(518). https://doi.org/10.1126/scisignal.aan3714.

Goupil, E., Tassy, D., Bourguet, C., Quiniou, C., Wisehart, V., Petrin, D., ... Laporte, S. A. (2010). A novel biased allosteric compound inhibitor of parturition selectively impedes the prostaglandin F2alpha-mediated Rho/ROCK signaling pathway. *The Journal of Biological Chemistry 285*(33), 25624–25636. https://doi.org/10.1074/jbc.M110.115196.

Goupil, E., Wisehart, V., Khoury, E., Zimmerman, B., Jaffal, S., Hebert, T. E., & Laporte, S. A. (2012). Biasing the prostaglandin F2alpha receptor responses toward EGFR-dependent transactivation of MAPK. *Molecular Endocrinology (Baltimore, Md.) 26*(7), 1189–1202. https://doi.org/10.1210/me.2011-1245.

Grammatopoulos, D. K., Randeva, H. S., Levine, M. A., Kanellopoulou, K. A., & Hillhouse, E. W. (2001). Rat cerebral cortex corticotropin-releasing hormone receptors: evidence for receptor coupling to multiple G-proteins. *Journal of Neurochemistry 76*(2), 509–519 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11208914.

Grant, G. E., Rokach, J., & Powell, W. S. (2009). 5-Oxo-ETE and the OXE receptor. *Prostaglandins & Other Lipid Mediators 89*(3–4), 98–104. https://doi.org/10.1016/j.prostaglandins.2009.05.002.

Gray, D. L., Allen, J. A., Mente, S., O'Connor, R. E., DeMarco, G. J., Efremov, I., ... Ehlers, M. D. (2018). Impaired beta-arrestin recruitment and reduced desensitization by non-catechol agonists of the D1 dopamine receptor. *Nature Communications 9*(1), 674. https://doi.org/10.1038/s41467-017-02776-7.

Gregory, K. J., Hall, N. E., Tobin, A. B., Sexton, P. M., & Christopoulos, A. (2010). Identification of orthosteric and allosteric site mutations in M 2 muscarinic acetylcholine receptors that contribute to ligand-selective signaling bias. *Journal of Biological Chemistry 285*(10), 7459–7474. https://doi.org/10.1074/jbc.M109.094011.

Gregory, K. J., Sexton, P. M., Tobin, A. B., & Christopoulos, A. (2012). Stimulus bias provides evidence for conformational constraints in the structure of a G protein-coupled receptor. *Journal of Biological Chemistry 287*(44), 37066–37077. https://doi.org/10.1074/jbc.M112.408534.

Griffin, M. T., Figueroa, K. W., Liller, S., & Ehlert, F. J. (2007). Estimation of agonist activity at G protein-coupled receptors: analysis of M2 muscarinic receptor signaling through Gi/o,Gs, and G15. *The Journal of Pharmacology and Experimental Therapeutics 321*(3), 1193–1207. https://doi.org/10.1124/jpet.107.120857.

Grisshammer, R., & Hermans, E. (2001). Functional coupling with Galpha(q) and Galpha (i1) protein subunits promotes high-affinity agonist binding to the neurotensin receptor NTS-1 expressed in Escherichia coli. *FEBS Letters 493*(2–3), 101–105 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11287004.

Groer, C. E., Tidgewell, K., Moyer, R. A., Harding, W. W., Rothman, R. B., Prisinzano, T. E., & Bohn, L. M. (2007). An opioid agonist that does not induce mu-opioid receptor–arrestin interactions or receptor internalization. *Molecular Pharmacology 71*(2), 549–557. https://doi.org/10.1124/mol.106.028258.

Grundmann, M., Merten, N., Malfacini, D., Inoue, A., Preis, P., Simon, K., ... Kostenis, E. (2018). Lack of beta-arrestin signaling in the absence of active G proteins. *Nature Communications 9*(1), 1–7. https://doi.org/10.1038/s41467-017-02661-3.

Guillaume, J. L., Daulat, A. M., Maurice, P., Levoye, A., Migaud, M., Brydon, L., ... Jockers, R. (2008). The PDZ protein mupp1 promotes Gi coupling and signaling of the Mt1 melatonin receptor. *The Journal of Biological Chemistry 283*(24), 16762–16771. https://doi.org/10.1074/jbc.M802069200.

Guo, D., Hillger, J. M., IJzerman, A. P., & Heitman, L. H. (2014). Drug-target residence time–a case for G protein-coupled receptors. *Medicinal Research Reviews 34*(4), 856–892. https://doi.org/10.1002/med.21307.

Gupte, T. M., Malik, R. U., Sommese, R. F., Ritt, M., & Sivaramakrishnan, S. (2017). Priming GPCR signaling through the synergistic effect of two G proteins. *Proceedings of the National Academy of Sciences of the United States of America 114*(14), 3756–3761. https://doi.org/10.1073/pnas.1617232114.

Hager, M. V., Clydesdale, L., Gellman, S. H., Sexton, P. M., & Wootten, D. (2017). Characterization of signal bias at the GLP-1 receptor induced by backbone modification of GLP-1. *Biochemical Pharmacology 136*, 99–108. https://doi.org/10.1016/j.bcp.2017.03.018.

Hager, M. V., Johnson, L. M., Wootten, D., Sexton, P. M., & Gellman, S. H. (2016). β-Arrestin-Biased Agonists of the GLP-1 Receptor from β-Amino Acid Residue Incorporation into GLP-1 Analogues. *Journal of the American Chemical Society 138*(45), 14970–14979. https://doi.org/10.1021/jacs.6b08323.

Hains, M. D., Siderovski, D. P., & Harden, T. K. (2004). Application of RGS box proteins to evaluate G-protein selectivity in receptor-promoted signaling. *Methods in Enzymology 389*, 71–88. https://doi.org/10.1016/S0076-6879(04)89005-0.

Hällbrink, M., Holmqvist, T., Olsson, M., Ostenson, C. G., Efendic, S., & Langel, U. (2001). Different domains in the third intracellular loop of the GLP-1 receptor are responsible for Galpha(s) and Galpha(i)/Galpha(o) activation. *Biochimica et Biophysica Acta 1546*(1), 79–86. https://doi.org/10.1016/S0167-4838(00)00270-3.

Halls, M. L., Yeatman, H. R., Nowell, C. J., Thompson, G. L., Gondin, A. B., Civciristov, S., ... Canals, M. (2016). Plasma membrane localization of the μ-opioid receptor controls spatiotemporal signaling. *Science Signaling 9*(414), ra16. https://doi.org/10.1126/scisignal.aac9177.

Hamamoto, A., Kobayashi, Y., & Saito, Y. (2015). Identification of amino acids that are selectively involved in Gi/o activation by rat melanin-concentrating hormone receptor 1. *Cellular Signalling 27*(4), 818–827. https://doi.org/10.1016/j.cellsig.2015.01.008.

Hamamoto, A., Mizusawa, K., Takahashi, A., & Saito, Y. (2011). Signalling pathway of goldfish melanin-concentrating hormone receptors 1 and 2. *Regulatory Peptides 169*(1–3), 6–12. https://doi.org/10.1016/j.regpep.2011.04.001.

Hauser, A. S., Chavali, S., Masuho, I., Jahn, L. J., Martemyanov, K. A., Gloriam, D. E., & Babu, M. M. (2018). Pharmacogenomics of GPCR Drug Targets. *Cell 172*(1–2), 41–54.e19. https://doi.org/10.1016/j.cell.2017.11.033.

Havlickova, M., Blahos, J., Brabet, I., Liu, J., Hruskova, B., Prézeau, L., & Pin, J. P. (2003). The second intracellular loop of metabotropic glutamate receptors recognizes C termini of G-protein alpha-subunits. *The Journal of Biological Chemistry 278*(37), 35063–35070. https://doi.org/10.1074/jbc.M306555200.

Hawes, B. E., Kil, E., Green, B., O'Neill, K., Fried, S., & Graziano, M. P. (2000). The melanin-concentrating hormone receptor couples to multiple G proteins to activate diverse intracellular signaling pathways. *Endocrinology 141*(12), 4524–4532. https://doi.org/10.1210/endo.141.12.7833.

Hay, D. L., & Pioszak, A. A. (2016). Receptor Activity-Modifying Proteins (RAMPs): New Insights and Roles. *Annual Review of Pharmacology and Toxicology 56*, 469–487. https://doi.org/10.1146/annurev-pharmtox-010715-103120.

Hayashi, H., Hess, D. T., Zhang, R., Sugi, K., Gao, H., Tan, B. L., ... Stamler, J. S. (2018). S-Nitrosylation of beta-Arrestins Biases Receptor Signaling and Confers Ligand Independence. *Molecular Cell 70*(3), 473–487.e6. https://doi.org/10.1016/j.molcel.2018.03.034.

Hermans, E., & Challiss, R. A. (2001). Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors. *The Biochemical Journal 359*, 465–484 Pt 3.

Hermans, E., Saunders, R., Selkirk, J. V., Mistry, R., Nahorski, S. R., & Challiss, R. A. (2000). Complex involvement of pertussis toxin-sensitive G proteins in the regulation of type 1alpha metabotropic glutamate receptor signaling in baby hamster kidney cells. *Molecular Pharmacology 58*(2), 352–360 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10908303.

Herrlich, A., Kühn, B., Grosse, R., Schmid, A., Schultz, G., & Gudermann, T. (1996). Involvement of Gs and Gi proteins in dual coupling of the luteinizing hormone receptor to adenylyl cyclase and phospholipase C. *The Journal of Biological Chemistry 271*(28), 16764–16772 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8663226.

Hewavitharana, T., & Wedegaertner, P. B. (2012). Non-canonical signaling and localizations of heterotrimeric G proteins. *Cellular Signalling 24*(1), 25–34. https://doi.org/10.1016/j.cellsig.2011.08.014.

Hilairet, S., Bouaboula, M., Carriere, D., Le Fur, G., & Casellas, P. (2003). Hypersensitization of the Orexin 1 receptor by the CB1 receptor: evidence for cross-talk blocked by the specific CB1 antagonist, SR141716. *The Journal of Biological Chemistry 278*(26), 23731–23737. https://doi.org/10.1074/jbc.M212369200.

Hillenbrand, M., Schori, C., Schöppe, J., & Plückthun, A. (2015). Comprehensive analysis of heterotrimeric G-protein complex diversity and their interactions with GPCRs in solution. *Proceedings of the National Academy of Sciences of the United States of America 112*(11), E1181–E1190. https://doi.org/10.1073/pnas.1417573112.

Hinney, A., Hohmann, S., Geller, F., Vogel, C., Hess, C., Wermter, A. K., ... Hebebrand, J. (2003). Melanocortin-4 Receptor Gene: Case-Control Study and Transmission Disequilibrium Test Confirm that Functionally Relevant Mutations Are Compatible with a Major Gene Effect for Extreme Obesity. *The Journal of Clinical Endocrinology & Metabolism 88*(9), 4258–4267 Retrieved from https://doi.org/10.1210/jc.2003-030233.

Ho, B. Y., Current, L., & Drewett, J. G. (2002). Role of intracellular loops of cannabinoid CB (1) receptor in functional interaction with G(alpha16). *FEBS Letters 522*(1–3), 130–134 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12095632.

Hoare, S., Copland, J. A., Strakova, Z., Ives, K., Jeng, Y. J., Hellmich, M. R., & Soloff, M. S. (1999). The proximal portion of the COOH terminus of the oxytocin receptor is required for coupling to g(q), but not g(i). Independent mechanisms for elevating intracellular calcium concentrations from intracellular stores. *The Journal of Biological Chemistry 274*(40), 28682–28689. https://doi.org/10.1074/jbc.274.40.28682.

Hollenstein, K., Kean, J., Bortolato, A., Cheng, R. K. Y., Dore, A. S., Jazayeri, A., ... Marshall, F. H. (2013). Structure of class B GPCR corticotropin-releasing factor receptor 1. *Nature 499*(7459), 438–443. https://doi.org/10.1038/nature12357.

Horvath, P., Aulner, N., Bickle, M., Davies, A. M., Del Nery, E., Ebner, D., ... Carragher, N. O. (2016, November). Screening out irrelevant cell-based models of disease. *Nature Reviews. Drug Discovery. England.*. https://doi.org/10.1038/nrd.2016.175.

Hothersall, J. D., Torella, R., Humphreys, S., Hooley, M., Brown, A., McMurray, G., & Nickolls, S. A. (2017). Residues W320 and Y328 within the binding site of the μ-opioid receptor influence opiate ligand bias. *Neuropharmacology 118*, 46–58. https://doi.org/10.1016/j.neuropharm.2017.03.007.

Hu, J., Wang, Y., Zhang, X., Lloyd, J. R., Li, J. H., Karpiak, J., ... Wess, J. (2010). Structural basis of G protein-coupled receptor-G protein interactions. *Nature Chemical Biology 6*(7), 541–548. https://doi.org/10.1038/nchembio.385.

Hu, L. A., Chen, W., Martin, N. P., Whalen, E. J., Premont, R. T., & Lefkowitz, R. J. (2003). GIPC interacts with the beta1-adrenergic receptor and regulates beta1-adrenergic receptor-mediated ERK activation. *The Journal of Biological Chemistry 278*(28), 26295–26301. https://doi.org/10.1074/jbc.M212352200.

Huang, C., Hujer, K. M., Wu, Z., & Miller, R. T. (2004). The Ca2+−sensing receptor couples to Galpha12/13 to activate phospholipase D in Madin-Darby canine kidney cells. *American Journal of Physiology. Cell Physiology 286*(1), C22–C30. https://doi.org/10.1152/ajpcell.00229.2003.

Huang, P., Steplock, D., Weinman, E. J., Hall, R. A., Ding, Z., Li, J., ... Liu-Chen, L. Y. (2004). kappa Opioid receptor interacts with Na(+)/H(+)-exchanger regulatory factor-1/Ezrin-radixin-moesin-binding phosphoprotein-50 (NHERF-1/EBP50) to stimulate Na(+)/H(+) exchange independent of G(i)/G(o) proteins. *The Journal of Biological Chemistry 279*(24), 25002–25009. https://doi.org/10.1074/jbc.M313366200.

Hunyady, L., & Catt, K. J. (2006). Pleiotropic AT1 receptor signaling pathways mediating physiological and pathogenic actions of angiotensin II. *Molecular Endocrinology (Baltimore, Md.) 20*(5), 953–970. https://doi.org/10.1210/me.2004-0536.

Iida-Klein, A., Guo, J., Takemura, M., Drake, M. T., Potts, J. T., Abou-Samra, A., ... Segre, G. V. (1997). Mutations in the second cytoplasmic loop of the rat parathyroid hormone (PTH)/PTH-related protein receptor result in selective loss of PTH-stimulated phospholipase C activity. *The Journal of Biological Chemistry 272*(11), 6882–6889. https://doi.org/10.1074/jbc.272.11.6882.

Isogai, S., Deupi, X., Opitz, C., Heydenreich, F. M., Tsai, C. J., Brueckner, F., ... Grzesiek, S. (2016). Backbone NMR reveals allosteric signal transduction networks in the beta1-adrenergic receptor. *Nature 530*(7589), 237–241. https://doi.org/10.1038/nature16577.

Janetopoulos, C., Jin, T., & Devreotes, P. (2001). Receptor-mediated activation of heterotrimeric G-proteins in living cells. *Science (New York, N.Y.) 291*(5512), 2408–2411. https://doi.org/10.1126/science.1055835.

Jarrahian, A., Watts, V. J., & Barker, E. L. (2004). D2 dopamine receptors modulate Galpha-subunit coupling of the CB1 cannabinoid receptor. *The Journal of Pharmacology and Experimental Therapeutics 308*(3), 880–886. https://doi.org/10.1124/jpet.103.057620.

Jeanneteau, F., Diaz, J., Sokoloff, P., & Griffon, N. (2004). Interactions of GIPC with dopamine D2, D3 but not D4 receptors define a novel mode of regulation of G protein-coupled receptors. *Molecular Biology of the Cell 15*(2), 696–705. https://doi.org/10.1091/mbc.e03-05-0293.

Jin, L. Q., Wang, H. y., & Friedman, E. (2001). Stimulated D(1) dopamine receptors couple to multiple Galpha proteins in different brain regions. *Journal of Neurochemistry 78*(5), 981–990 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11553672.

Joly, C., Gomeza, J., Brabet, I., Curry, K., Bockaert, J., & Pin, J. P. (1995). Molecular, functional, and pharmacological characterization of the metabotropic glutamate receptor type 5 splice variants: comparison with mGluR1. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 15*(5), 3970–3981 Pt 2.

Jones, B. W., & Hinkle, P. M. (2008). Arrestin binds to different phosphorylated regions of the thyrotropin-releasing hormone receptor with distinct functional consequences. *Molecular Pharmacology 74*(1), 195–202. https://doi.org/10.1124/mol.108.045948.

Jones, S. V., Heilman, C. J., & Brann, M. R. (1991). Functional responses of cloned muscarinic receptors expressed in CHO-K1 cells. *Molecular Pharmacology 40*(2), 242–247 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1652053.

Kahsai, A. W., Xiao, K., Rajagopal, S., Ahn, S., Shukla, A. K., Sun, J., ... Lefkowitz, R. J. (2011). Multiple ligand-specific conformations of the beta2-adrenergic receptor. *Nature Chemical Biology 7*(10), 692–700. https://doi.org/10.1038/nchembio.634.

Kandola, M. K., Sykes, L., Lee, Y. S., Johnson, M. R., Hanyaloglu, A. C., & Bennett, P. R. (2014). EP2 receptor activates dual G protein signaling pathways that mediate contrasting proinflammatory and relaxatory responses in term pregnant human myometrium. *Endocrinology 155*(2), 605–617. https://doi.org/10.1210/en.2013-1761.

Kang, Y., Kuybeda, O., de Waal, P. W., Mukherjee, S., Van Eps, N., Dutka, P., ... Xu, H. E. (2018). Cryo-EM structure of human rhodopsin bound to an inhibitory G protein. *Nature 558*(7711), 553–558. https://doi.org/10.1038/s41586-018-0215-y.

Katritch, V., Fenalti, G., Abola, E. E., Roth, B. L., Cherezov, V., & Stevens, R. C. (2014). Allosteric sodium in class A GPCR signaling. *Trends in Biochemical Sciences 39*(5), 233–244. https://doi.org/10.1016/j.tibs.2014.03.002.

Katsushima, Y., Sato, T., Yamada, C., Ito, M., Suzuki, Y., Ogawa, E., ... Yanagisawa, T. (2013). Interaction of PICK1 with C-terminus of growth hormone-releasing hormone receptor (GHRHR) modulates trafficking and signal transduction of human GHRHR. *Journal of Pharmacological Sciences 122*(3), 193–204.

Kenakin, T. (1995). Agonist-receptor efficacy. II. Agonist trafficking of receptor signals. *Trends in Pharmacological Sciences 16*(7), 232–238. https://doi.org/10.1016/S0165-6147(00)89032-X.

Kenakin, T. (2001). Inverse, protean, and ligand-selective agonism: matters of receptor conformation. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 15*(3), 598–611. https://doi.org/10.1096/fj.00-0438rev.

Kenakin, T. (2017). A Scale of Agonism and Allosteric Modulation for Assessment of Selectivity, Bias, and Receptor Mutation. *Molecular Pharmacology 92*(4), 414–424. https://doi.org/10.1124/mol.117.108787.

Kenakin, T., & Miller, L. J. (2010). Seven Transmembrane Receptors as Shapeshifting Proteins: The Impact of Allosteric Modulation and Functional Selectivity on New Drug Discovery. *Pharmacological Reviews 62*(2), 265–304. https://doi.org/10.1124/pr.108.000992.

Kenakin, T., Watson, C., Muniz-Medina, V., Christopoulos, A., & Novick, S. (2012). A simple method for quantifying functional selectivity and agonist bias. *ACS Chemical Neuroscience 3*(3), 193–203. https://doi.org/10.1021/cn200111m.

Keov, P., López, L., Devine, S. M., Valant, C., Lane, J. R., Scammells, P. J., ... Christopoulos, A. (2014). Molecular mechanisms of bitopic ligand engagement with the M1 muscarinic acetylcholine receptor. *The Journal of Biological Chemistry 289*(34), 23817–23837. https://doi.org/10.1074/jbc.M114.582874.

Keov, P., Valant, C., Devine, S. M., Lane, J. R., Scammells, P. J., Sexton, P. M., & Christopoulos, A. (2013). Reverse Engineering of the Selective Agonist TBPB Unveils Both Orthosteric and Allosteric Modes of Action at the M1 Muscarinic Acetylcholine Receptor. *Molecular Pharmacology 84*(3), 425–437. https://doi.org/10.1124/mol.113.087320.

Kilts, J. D., Gerhardt, M. A., Richardson, M. D., Sreeram, G., Mackensen, G. B., Grocott, H. P., ... Kwatra, M. M. (2000). Beta(2)-adrenergic and several other G protein-coupled receptors in human atrial membranes activate both G(s) and G(i). *Circulation Research 87*(8), 705–709 Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11029407.

Kinoshita, M., Nukada, T., Asano, T., Mori, Y., Akaike, A., Satoh, M., & Kaneko, S. (2001). Binding of G alpha(o) N terminus is responsible for the voltage-resistant inhibition of alpha(1A) (P/Q-type, Ca(v)2.1) Ca(2+) channels. *The Journal of Biological Chemistry 276*(31), 28731–28738. https://doi.org/10.1074/jbc.M104806200.

Klein Herenbrink, C., Sykes, D. A., Donthamsetti, P., Canals, M., Coudrat, T., Shonberg, J., ... Lane, J. R. (2016). The role of kinetic context in apparent biased agonism at GPCRs. *Nature Communications 7*, 10842. https://doi.org/10.1038/ncomms10842.

Kobilka, B. K., & Deupi, X. (2007). Conformational complexity of G-protein-coupled receptors. *Trends in Pharmacological Sciences 28*(8), 397–406. https://doi.org/10.1016/j.tips.2007.06.003.

Kobilka, B. K., Kobilka, T. S., Daniel, K., Regan, J. W., Caron, M. G., & Lefkowitz, R. J. (1988). Chimeric alpha 2-,beta 2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. *Science (New York, N.Y.) 240*(4857), 1310–1316 Retrieved from http://science.sciencemag.org/content/240/4857/1310.abstract.

Koch, T., Widera, A., Bartzsch, K., Schulz, S., Brandenburg, L. O., Wundrack, N., ... Höllt, V. (2005). Receptor endocytosis counteracts the development of opioid tolerance. *Molecular Pharmacology 67*(1), 280–287. https://doi.org/10.1124/mol.104.004994.

Koch, W. J., Hawes, B. E., Inglese, J., Luttrell, L. M., & Lefkowitz, R. J. (1994). Cellular expression of the carboxyl terminus of a G protein-coupled receptor kinase attenuates G beta gamma-mediated signaling. *The Journal of Biological Chemistry 269*(8), 6193–6197.

Koehl, A., Hu, H., Maeda, S., Zhang, Y., Qu, Q., Paggi, J. M., ... Kobilka, B. K. (2018). Structure of the micro-opioid receptor-Gi protein complex. *Nature 558*(7711), 547–552. https://doi.org/10.1038/s41586-018-0219-7.

Kofuku, Y., Ueda, T., Okude, J., Shiraishi, Y., Kondo, K., Maeda, M., ... Shimada, I. (2012). Efficacy of the β_2-adrenergic receptor is determined by conformational equilibrium in the transmembrane region. *Nature Communications 3*, 1045. https://doi.org/10.1038/ncomms2046.

Kohout, T. A., Nicholas, S. L., Perry, S. J., Reinhart, G., Junger, S., & Struthers, R. S. (2004). *Differential desensitization, receptor phosphorylation, β -arrestin recruitment, and ERK1 / 2 activation by the two endogenous ligands for the CC chemokine receptor 7 *279 (22). (pp. 23214–23222), 23214–23222. https://doi.org/10.1074/jbc.M402125200.*

Konya, V., Blättermann, S., Jandl, K., Platzer, W., Ottersbach, P. A., Marsche, G., ... Heinemann, A. (2014). A biased non-Gαi OXE-R antagonist demonstrates that Gαi protein subunit is not directly involved in neutrophil, eosinophil, and monocyte activation by 5-oxo-ETE. *Journal of Immunology (Baltimore, Md. : 1950) 192*(10), 4774–4782. https://doi.org/10.4049/jimmunol.1302013.

Koole, C., Wootten, D., Simms, J., Miller, L. J., Christopoulos, A., & Sexton, P. M. (2012). Second extracellular loop of human glucagon-like peptide-1 receptor (GLP-1R) has a critical role in GLP-1 peptide binding and receptor activation. *The Journal of Biological Chemistry 287*(6), 3642–3658. https://doi.org/10.1074/jbc.M111.309328.

Koole, C., Wootten, D., Simms, J., Savage, E. E., Miller, L. J., Christopoulos, A., & Sexton, P. M. (2012). Second extracellular loop of human glucagon-like peptide-1 receptor (GLP-1R) differentially regulates orthosteric but not allosteric agonist binding and function. *The Journal of Biological Chemistry 287*(6), 3659–3673. https://doi.org/10.1074/jbc.M111.309369.

Kozasa, T., Hajicek, N., Chow, C. R., & Suzuki, N. (2011). Signalling mechanisms of RhoGTPase regulation by the heterotrimeric G proteins G12 and G13. *Journal of Biochemistry 150*(4), 357–369. https://doi.org/10.1093/jb/mvr105.

Kozasa, T., Jiang, X., Hart, M. J., Sternweis, P. C. M. C. M., Singer, W. D., Gilman, A. G., ... Sternweis, P. C. M. C. M. (1998). p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13. *Science (New York, N.Y.) 280*(5372), 2109–2111 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9641915.

Kramer, H. K., Andria, M. L., Esposito, D. H., & Simon, E. J. (2000). Tyrosine phosphorylation of the delta-opioid receptor. Evidence for its role in mitogen-activated protein kinase activation and receptor internalization*. *Biochemical Pharmacology 60*(6), 781–792 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10930532.

Kühn, B., & Gudermann, T. (1999). The luteinizing hormone receptor activates phospholipase C via preferential coupling to Gi2. *Biochemistry 38*(38), 12490–12498 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10493819.

Kühn, B., Schmid, A., Harteneck, C., Gudermann, T., & Schultz, G. (1996). G proteins of the Gq family couple the H2 histamine receptor to phospholipase C. *Molecular Endocrinology (Baltimore, Md.) 10*(12), 1697–1707. https://doi.org/10.1210/mend.10.12.8961278.

Kukkonen, J. P., Näsman, J., & Akerman, K. E. (2001). Modelling of promiscuous receptor-Gi/Gs-protein coupling and effector response. *Trends in Pharmacological Sciences 22*(12), 616–622. https://doi.org/10.1016/S0165-6147(00)01864-2.

Kurrasch-Orbaugh, D. M., Parrish, J. C., Watts, V. J., & Nichols, D. E. (2003). A complex signaling cascade links the serotonin2A receptor to phospholipase A2 activation: the involvement of MAP kinases. *Journal of Neurochemistry 86*(4), 980–991 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12887695.

Kvachnina, E., Dumuis, A., Wlodarczyk, J., Renner, U., Cochet, M., Richter, D. W., & Ponimaskin, E. G. (2009). Constitutive Gs-mediated, but not G12-mediated, activity of the 5-hydroxytryptamine 5-HT7(a) receptor is modulated by the palmitoylation of its C-terminal domain. *Biochimica et Biophysica Acta 1793*(11), 1646–1655. https://doi.org/10.1016/j.bbamcr.2009.08.008.

Kvachnina, E., Liu, G., Dityatev, A., Renner, U., Dumuis, A., Richter, D. W., ... Ponimaskin, E. G. (2005). 5-HT7 receptor is coupled to G alpha subunits of heterotrimeric G12-protein to regulate gene transcription and neuronal morphology. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 25*(34), 7821–7830. https://doi.org/10.1523/JNEUROSCI.1790-05.2005.

Lane, J. R., Donthamsetti, P., Shonberg, J., Draper-Joyce, C. J., Dentry, S., Michino, M., ... Christopoulos, A. (2014). A new mechanism of allostery in a G protein-coupled receptor dimer. *Nature Chemical Biology 10*(9), 745–752. https://doi.org/10.1038/nchembio.1593.

Lane, J. R., May, L. T., Parton, R. G., Sexton, P. M., & Christopoulos, A. (2017). A kinetic view of GPCR allostery and biased agonism. *Nature Chemical Biology 13*(9), 929–937. https://doi.org/10.1038/nchembio.2431.

Lane, J. R., Powney, B., Wise, A., Rees, S., & Milligan, G. (2007). Protean agonism at the dopamine D2 receptor: (S)-3-(3-hydroxyphenyl)-N-propylpiperidine is an agonist for activation of Go1 but an antagonist/inverse agonist for Gi1,Gi2, and Gi3. *Molecular Pharmacology 71*(5), 1349–1359. https://doi.org/10.1124/mol.106.032722.2000.

Lane, J. R., Sexton, P. M., & Christopoulos, A. (2013). Bridging the gap: bitopic ligands of G-protein-coupled receptors. *Trends in Pharmacological Sciences 34*(1), 59–66. https://doi.org/10.1016/j.tips.2012.10.003.

Laprairie, R. B., Bagher, A. M., Kelly, M. E. M., & Denovan-Wright, E. M. (2016). Biased type 1 cannabinoid receptor signaling influences neuronal viability in a cell culture model of huntington disease. *Molecular Pharmacology 89*(3), 364–375. https://doi.org/10.1124/mol.115.101980.

Laugwitz, K. L., Allgeier, A., Offermanns, S., Spicher, K., Van Sande, J., Dumont, J. E., & Schultz, G. (1996). The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G protein families. *Proceedings of the National Academy of Sciences of the United States of America 93*(1), 116–120 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC40189/.

Lawler, O. A., Miggin, S. M., & Kinsella, B. T. (2001). Protein kinase A-mediated phosphorylation of serine 357 of the mouse prostacyclin receptor regulates its coupling to G (s)-, to G(i)-, and to G(q)-coupled effector signaling. *The Journal of Biological Chemistry 276*(36), 33596–33607. https://doi.org/10.1074/jbc.M104434200.

Lazenka, M. L., Moerke, M. J., Townsend, E. A., Freeman, K. B., Carroll, F. I., & Negus, S. S. (2018). Dissociable effects of the kappa opioid receptor agonist nalfurafine on pain/itch-stimulated and pain/itch-depressed behaviors in male rats. *Psychopharmacology 235*(1), 203–213. https://doi.org/10.1007/s00213-017-4758-7.

Lefkowitz, R. J., Rajagopal, K., & Whalen, E. J. (2006). New roles for beta-arrestins in cell signaling: not just for seven-transmembrane receptors. *Molecular Cell 24*(5), 643–652. https://doi.org/10.1016/j.molcel.2006.11.007.

Li, Y. Q., Shrestha, Y., Pandey, M., Chen, M., Kablan, A., Gavrilova, O., ... Weinstein, L. S. (2016). G(q/11)α and G(s)α mediate distinct physiological responses to central melanocortins. *The Journal of Clinical Investigation 126*(1), 40–49. https://doi.org/10.1172/JCI76348.

Liang, Y. L., Khoshouei, M., Glukhova, A., Furness, S. G. B., Zhao, P., Clydesdale, L., ... Wootten, D. (2018). Phase-plate cryo-EM structure of a biased agonist-bound human GLP-1 receptor-Gs complex. *Nature 555*(7694), 121–125. https://doi.org/10.1038/nature25773.

Liang, Y. L., Khoshouei, M., Radjainia, M., Zhang, Y., Glukhova, A., Tarrasch, J., ... Sexton, P. M. (2017). Phase-plate cryo-EM structure of a class B GPCR-G-protein complex. *Nature 546*(7656), 118–123. https://doi.org/10.1038/nature22327.

Liao, J. K., & Homcy, C. J. (1993). The G proteins of the G alpha i and G alpha q family couple the bradykinin receptor to the release of endothelium-derived relaxing factor. *The Journal of Clinical Investigation 92*(5), 2168–2172. https://doi.org/10.1172/JCI116818.

Liebmann, C., Graness, A., Ludwig, B., Adomeit, A., Boehmer, A., Boehmer, F. D., ... Wetzker, R. (1996). Dual bradykinin B2 receptor signalling in A431 human epidermoid carcinoma cells: activation of protein kinase C is counteracted by a GS-mediated stimulation of the cyclic AMP pathway. *The Biochemical Journal 313*, 109–118 *Pt 1*(1. Retrieved from http://www.biochemj.org/content/313/1/109.abstract.

Lin, X., Dhopeshwarkar, A. S., Huibregtse, M., Mackie, K., & Hohmann, A. G. (2017). The slowly signaling G protein-biased CB2 cannabinoid receptor agonist LY2828360 suppresses neuropathic pain with sustained efficacy and attenuates morphine tolerance and dependence. *Molecular Pharmacology 93*(2). https://doi.org/10.1124/mol.117.109355 mol.117.109355.

Liu, J. J., Horst, R., Katritch, V., Stevens, R. C., & Wüthrich, K. (2012). Biased signaling pathways in β2-adrenergic receptor characterized by 19F-NMR, Science (New York, N.Y.), 335(6072), 1106–1110. doi: https://doi.org/10.1126/science.1215802.

Liu, Q., Bee, M. S., & Schonbrunn, A. (2009). Site Specificity of Agonist and Second Messenger-Activated Kinases for Somatostatin Receptor Subtype 2A (Sst2A) Phosphorylation. *Molecular Pharmacology 76*(1), 68 LP–80 Retrieved from http://molpharm.aspetjournals.org/content/76/1/68.abstract.

Liu, R., Wang, D., Shi, Q., Fu, Q., Hizon, S., & Xiang, Y. K. (2012). Palmitoylation regulates intracellular trafficking of β2 adrenergic receptor/arrestin/phosphodiesterase 4D complexes in cardiomyocytes. *PLoS One 7*(8), e42658. https://doi.org/10.1371/journal.pone.0042658.

Locht, C., Coutte, L., & Mielcarek, N. (2011). The ins and outs of pertussis toxin. *The FEBS Journal 278*(23), 4668–4682. https://doi.org/10.1111/j.1742-4658.2011.08237.x.

Lovell, K. M., Frankowski, K. J., Stahl, E. L., Slauson, S. R., Yoo, E., Prisinzano, T. E., ... Bohn, L. M. (2015). Structure-activity relationship studies of functionally selective kappa opioid receptor agonists that modulate ERK 1/2 phosphorylation while preserving G protein over betaarrestin2 signaling bias. *ACS Chemical Neuroscience 6*(8), 1411–1419. https://doi.org/10.1021/acschemneuro.5b00092.

Luo, X., Zeng, W., Xu, X., Popov, S., Davignon, I., Wilkie, T. M., ... Muallem, S. (1999). Alternate coupling of receptors to Gs and Gi in pancreatic and submandibular gland cells. *The Journal of Biological Chemistry 274*(25), 17684–17690 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10364208.

Luttrell, L. M., Ferguson, S. S., Daaka, Y., Miller, W. E., Maudsley, S., Della Rocca, G. J., ... Lefkowitz, R. J. (1999). Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science (New York, N.Y.) 283*(5402), 655–661.

Ma, Y. C., Huang, J., Ali, S., Lowry, W., & Huang, X. Y. (2000). Src tyrosine kinase is a novel direct effector of G proteins. *Cell 102*(5), 635–646. https://doi.org/10.1016/S0092-8674(00)00086-6.

MacKinnon, A. C., Tufail-Hanif, U., Lucas, C. D., Jodrell, D., Haslett, C., & Sethi, T. (2005). Expression of V1A and GRP receptors leads to cellular transformation and increased sensitivity to substance-P analogue-induced growth inhibition. *British Journal of Cancer 92*(3), 522–531. https://doi.org/10.1038/sj.bjc.6602366.

MacKinnon, A. C., Waters, C., Jodrell, D., Haslett, C., & Sethi, T. (2001). Bombesin and substance P analogues differentially regulate G-protein coupling to the bombesin receptor. Direct evidence for biased agonism. *The Journal of Biological Chemistry 276*(30), 28083–28091. https://doi.org/10.1074/jbc.M009772200.

Mahoney, J. P., & Sunahara, R. K. (2016). Mechanistic insights into GPCR-G protein interactions. *Current Opinion in Structural Biology 41*, 247–254. https://doi.org/10.1016/j.sbi.2016.11.005.

Maillet, E. L., Pellegrini, N., Valant, C., Bucher, B., Hibert, M., Bourguignon, J. J., & Galzi, J. L. (2007). A novel, conformation-specific allosteric inhibitor of the tachykinin NK2 receptor (NK2R) with functionally selective properties. *The FASEB Journal 21*(9), 2124–2134. https://doi.org/10.1096/fj.06-7683com.

Malik, R. U., Ritt, M., DeVree, B. T., Neubig, R. R., Sunahara, R. K., & Sivaramakrishnan, S. (2013). Detection of G protein-selective G protein-coupled receptor (GPCR) conformations in live cells. *The Journal of Biological Chemistry 288*(24), 17167–17178. https://doi.org/10.1074/jbc.M113.464065.

Malmberg, A., & Strange, P. G. (2000). Site-directed mutations in the third intracellular loop of the serotonin 5-HT(1A) receptor alter G protein coupling from G(i) to G (s) in a ligand-dependent manner. *Journal of Neurochemistry 75*(3), 1283–1293 Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10936212.

Mamillapalli, R., & Wysolmerski, J. (2010). The calcium-sensing receptor couples to Galpha(s) and regulates PTHrP and ACTH secretion in pituitary cells. *The Journal of Endocrinology 204*(3), 287–297. https://doi.org/10.1677/JOE-09-0183.

Manivet, P., Mouillet-Richard, S., Callebert, J., Nebigil, C. G., Maroteaux, L., Hosoda, S., ... Launay, J. M. (2000). PDZ-dependent activation of nitric-oxide synthases by the serotonin 2B receptor. *The Journal of Biological Chemistry 275*(13), 9324–9331 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10734074.

Martin, N. P., Whalen, E. J., Zamah, M. A., Pierce, K. L., & Lefkowitz, R. J. (2004). PKA-mediated phosphorylation of the beta1-adrenergic receptor promotes Gs/Gi switching. *Cellular Signalling 16*(12), 1397–1403. https://doi.org/10.1016/j.cellsig.2004.05.002.

Marty, C., & Ye, R. D. (2010). Heterotrimeric G protein signaling outside the realm of seven transmembrane domain receptors. *Molecular Pharmacology 78*(1), 12–18. https://doi.org/10.1124/mol.110.063453.

Marullo, S., & Bouvier, M. (2007). Resonance energy transfer approaches in molecular pharmacology and beyond. *Trends in Pharmacological Sciences 28*(8), 362–365. https://doi.org/10.1016/j.tips.2007.06.007.

Mary, S., Damian, M., Louet, M., Floquet, N., Fehrentz, J. A., Marie, J., ... Banères, J. L. (2012). Ligands and signaling proteins govern the conformational landscape explored by a G protein-coupled receptor. *Proceedings of the National Academy of Sciences of the United States of America 109*(21), 8304–8309. https://doi.org/10.1073/pnas.1119881109.

Masri, B., Salahpour, A., Didriksen, M., Ghisi, V., Beaulieu, J. M., Gainetdinov, R. R., & Caron, M. G. (2008). Antagonism of dopamine D2 receptor/beta-arrestin 2 interaction is a common property of clinically effective antipsychotics. *Proceedings of the National Academy of Sciences of the United States of America 105*(36), 13656–13661. https://doi.org/10.1073/pnas.0803522105.

Masuho, I., Martemyanov, K. A., & Lambert, N. A. (2015). Monitoring G protein activation in cells with BRET. *Methods in Molecular Biology (Clifton, N.J.) 1335*, 107–113. https://doi.org/10.1007/978-1-4939-2914-6_8.

Masuho, I., Ostrovskaya, O., Kramer, G. M., Jones, C. D., Xie, K., & Martemyanov, K. A. (2015). Distinct profiles of functional discrimination among G proteins determine the actions of G protein-coupled receptors. *Science Signaling 8*(405), ra123. https://doi.org/10.1126/scisignal.aab4068.

Mathiesen, J. M., Ulven, T., Martini, L., Gerlach, L. O., Heinemann, A., & Kostenis, E. (2005). Identification of Indole Derivatives Exclusively Interfering with a G Protein-Independent Signaling Pathway of the Prostaglandin D2 Receptor CRTH2. *Molecular Pharmacology 68*(2), 393 LP–402 Retrieved from http://molpharm.aspetjournals.org/content/68/2/393.abstract.

Maudsley, S., Davidson, L., Pawson, A. J., Chan, R., López de Maturana, R., & Millar, R. P. (2004). Gonadotropin-releasing hormone (GnRH) antagonists promote proapoptotic signaling in peripheral reproductive tumor cells by activating a Galphai-coupling state of the type I GnRH receptor. *Cancer Research 64*(20), 7533–7544. https://doi.org/10.1158/0008-5472.CAN-04-1360.

Maurice, P., Guillaume, J. L., Benleulmi-Chaachoua, A., Daulat, A. M., Kamal, M., & Jockers, R. (2011). GPCR-interacting proteins, major players of GPCR function. *Advances in Pharmacology (San Diego, Calif.) 62*, 349–380. https://doi.org/10.1016/B978-0-12-385952-5.00001-4.

McCorvy, J. D., Butler, K. V., Kelly, B., Rechsteiner, K., Karpiak, J., Betz, R. M., ... Roth, B. L. (2018). Structure-inspired design of beta-arrestin-biased ligands for aminergic GPCRs. *Nature Chemical Biology 14*(2), 126–134. https://doi.org/10.1038/nchembio.2527.

McGrew, L., Chang, M. S. S., & Sanders-Bush, E. (2002). Phospholipase D activation by endogenous 5-hydroxytryptamine 2C receptors is mediated by Galpha13 and pertussis toxin-insensitive Gbetagamma subunits. *Molecular Pharmacology 62*(6), 1339–1343 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12435801.

McIntosh, B. T., Hudson, B., Yegorova, S., Jollimore, C. A. B., & Kelly, M. E. M. (2007). Agonist-dependent cannabinoid receptor signalling in human trabecular meshwork cells. *British Journal of Pharmacology 152*(7), 1111–1120. https://doi.org/10.1038/sj.bjp.0707495.

McPherson, J., Rivero, G., Baptist, M., Llorente, J., Al-Sabah, S., Krasel, C., ... Kelly, E. (2010). μ-opioid receptors: correlation of agonist efficacy for signalling with ability to activate internalization. *Molecular Pharmacology 78*(4), 756–766. https://doi.org/10.1124/mol.110.066613.

Michal, P., El-Fakahany, E. E., & Dolezal, V. (2007). Muscarinic M2 receptors directly activate Gq/11 and Gs G-proteins. *The Journal of Pharmacology and Experimental Therapeutics 320*(2), 607–614. https://doi.org/10.1124/jpet.106.114314.

Michal, P., Lysíková, M., & Tucek, S. (2001). Dual effects of muscarinic M(2) acetylcholine receptors on the synthesis of cyclic AMP in CHO cells: dependence on time, receptor density and receptor agonists. *British Journal of Pharmacology 132*(6), 1217–1228. https://doi.org/10.1038/sj.bjp.0703931.

Miggin, S. M., & Kinsella, B. T. (2002). Investigation of the mechanisms of G protein: effector coupling by the human and mouse prostacyclin receptors. Identification of critical species-dependent differences. *The Journal of Biological Chemistry 277*(30), 27053–27064. https://doi.org/10.1074/jbc.M203353200.

Milanos, L., Brox, R., Frank, T., Poklukar, G., Palmisano, R., Waibel, R., ... Tschammer, N. (2016). Discovery and Characterization of Biased Allosteric Agonists of the Chemokine Receptor CXCR3. *Journal of Medicinal Chemistry 59*(5), 2222–2243. https://doi.org/10.1021/acs.jmedchem.5b01965.

Milligan, G. (2003). Principles: extending the utility of [35S]GTP gamma S binding assays. *Trends in Pharmacological Sciences 24*(2), 87–90. https://doi.org/10.1016/S0165-6147(02)00027-5.

Misra, S., Murthy, K. S., Zhou, H., & Grider, J. R. (2004). Coexpression of Y 1, Y 2, and Y 4 Receptors in Smooth Muscle Coupled to Distinct Signaling Pathways. *Pharmacology 311*(3), 1154–1162. https://doi.org/10.1124/jpet.104.071415.Berglund.

Mistry, R., Dowling, M. R., & Challiss, R. A. J. (2005). An investigation of whether agonist-selective receptor conformations occur with respect to M2 and M4 muscarinic acetylcholine receptor signalling via Gi/o and Gs proteins. *British Journal of Pharmacology 144*(4), 566–575. https://doi.org/10.1038/sj.bjp.0706090.

Moller, D., Banerjee, A., Uzuneser, T. C., Skultety, M., Huth, T., Plouffe, B., ... Gmeiner, P. (2017). Discovery of G Protein-Biased Dopaminergics with a Pyrazolo[1,5-a]pyridine Substructure. *Journal of Medicinal Chemistry 60*(7), 2908–2929. https://doi.org/10.1021/acs.jmedchem.6b01857.

Moller, D., Kling, R. C., Skultety, M., Leuner, K., Hubner, H., & Gmeiner, P. (2014). Functionally selective dopamine D(2), D(3) receptor partial agonists. *Journal of Medicinal Chemistry 57*(11), 4861–4875. https://doi.org/10.1021/jm5004039.

Moniri, N. H., Covington-Strachan, D., & Booth, R. G. (2004). Ligand-directed functional heterogeneity of histamine H1 receptors: novel dual-function ligands selectively activate and block H1-mediated phospholipase C and adenylyl cyclase signaling. *The Journal of Pharmacology and Experimental Therapeutics 311*(1), 274–281. https://doi.org/10.1124/jpet.104.070086.

Montrose-Rafizadeh, C., Avdonin, P., Garant, M. J., Rodgers, B. D., Kole, S., Yang, H., ... Bernier, M. (1999). Pancreatic glucagon-like peptide-1 receptor couples to multiple G proteins and activates mitogen-activated protein kinase pathways in Chinese hamster ovary cells. *Endocrinology 140*(3), 1132–1140. https://doi.org/10.1210/endo.140.3.6550.

Moreira, I. S. (2014). Structural features of the G-protein/GPCR interactions. *Biochimica et Biophysica Acta 1840*(1), 16–33. https://doi.org/10.1016/j.bbagen.2013.08.027.

Moro, O., Lameh, J., Högger, P., & Sadée, W. (1993). Hydrophobic amino acid in the i2 loop plays a key role in receptor-G protein coupling. *The Journal of Biological Chemistry 268*(30), 22273–22276 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8226735.

Mukhopadhyay, S., & Howlett, A. C. (2005). Chemically distinct ligands promote differential CB1 cannabinoid receptor-Gi protein interactions. *Molecular Pharmacology 67*(6), 2016–2024. https://doi.org/10.1124/mol.104.003558.

Nakagawa, T., & Asahi, M. (2013). beta1-adrenergic receptor recycles via a membranous organelle, recycling endosome, by binding with sorting nexin27. *The Journal of Membrane Biology 246*(7), 571–579. https://doi.org/10.1007/s00232-013-9571-6.

Namkung, Y., LeGouill, C., Kumar, S., Cao, Y., Teixeira, L. B., Lukasheva, V., ... Laporte, S. A. (2018). Functional selectivity profiling of the angiotensin II type 1 receptor using pathway-wide BRET signaling sensors. *Science Signaling 11*(559), eaat1631. https://doi.org/10.1126/scisignal.aat1631.

Neumann, S., Eliseeva, E., Boutin, A., Barnaeva, E., Ferrer, M., Southall, N., ... Gershengorn, M. C. (2018). Discovery of a Positive Allosteric Modulator of the Thyrotropin Receptor: Potentiation of Thyrotropin-Mediated Preosteoblast Differentiation In Vitro. *The Journal of Pharmacology and Experimental Therapeutics 364*(1), 38–45. https://doi.org/10.1124/jpet.117.244095.

Ng, S. Y. L., Lee, L. T. O., & Chow, B. K. C. (2012). Receptor oligomerization: from early evidence to current understanding in class B GPCRs. *Frontiers in Endocrinology 3*(JAN), 175. https://doi.org/10.3389/fendo.2012.00175.

Nijmeijer, S., Vischer, H. F., Rosethorne, E. M., Charlton, S. J., & Leurs, R. (2012). Analysis of multiple histamine H_4 receptor compound classes uncovers Gαi protein- and β-arrestin2-biased ligands. *Molecular Pharmacology 82*(6), 1174–1182. https://doi.org/10.1124/mol.112.080911.

Nobles, K. N., Xiao, K., Ahn, S., Shukla, A. K., Lam, C. M., Rajagopal, S., ... Lefkowitz, R. J. (2011). Distinct phosphorylation sites on the beta(2)-adrenergic receptor establish a barcode that encodes differential functions of beta-arrestin. *Science Signaling 4*(185), ra51. https://doi.org/10.1126/scisignal.2001707.

Nussenzveig, D. R., Thaw, C. N., & Gershengorn, M. C. (1994). Inhibition of inositol phosphate second messenger formation by intracellular loop one of a human calcitonin receptor. Expression and mutational analysis of synthetic receptor genes. *The Journal of Biological Chemistry 269*(45), 28123–28129 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7961748.

Nygaard, R., Zou, Y., Dror, R. O., Mildorf, T. J., Arlow, D. H., Manglik, A., ... Kobilka, B. K. (2013). The dynamic process of β(2)-adrenergic receptor activation. *Cell 152*(3), 532–542. https://doi.org/10.1016/j.cell.2013.01.008.

Oakley, R. H., Laporte, S. A., Holt, J. A., Caron, M. G., & Barak, L. S. (2000). Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors. *The Journal of Biological Chemistry 275*(22), 17201–17210. https://doi.org/10.1074/jbc.M910348199.

Obadiah, J., Avidor-Reiss, T., Fishburn, C. S., Carmon, S., Bayewitch, M. L., Vogel, Z., ... Levavi-Sivan, B. (1999). Adenylyl cyclase interaction with the D2 dopamine receptor family; differential coupling to Gi, Gz, and Gs. *Cellular and Molecular Neurobiology 19*(5), 653–664 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10384262.

Offermanns, S., Iida-Klein, A., Segre, G. V., & Simon, M. I. (1996). G alpha q family members couple parathyroid hormone (PTH)/PTH-related peptide and calcitonin receptors to phospholipase C in COS-7 cells. *Molecular Endocrinology (Baltimore, Md.) 10*(5), 566–574. https://doi.org/10.1210/mend.10.5.8732687.

Offermanns, S., Wieland, T., Homann, D., Sandmann, J., Bombien, E., Spicher, K., ... Jakobs, K. H. (1994). Transfected muscarinic acetylcholine receptors selectively couple to Gi-type G proteins and Gq/11. *Molecular Pharmacology 45*(5), 890–898 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8190105.

Ogawa, S., Watanabe, T., Sugimoto, I., Moriyuki, K., Goto, Y., Yamane, S., ... Maruyama, T. (2016). Discovery of G Protein-Biased EP2 Receptor Agonists. *ACS Medicinal Chemistry Letters 7*(3), 306–311. https://doi.org/10.1021/acsmedchemlett.5b00455.

Ogino, Y., Sakamoto, Y., Kinouchi, T., & Shimizu, N. (2000). Thrombin stimulates pertussis toxin-sensitive and -insensitive GTPase activities and ADP-ribosylation of G(i) in human neuroblastoma SH-EP. *Pharmacology 61*(1), 11–13 doi:28374.

Ogino, Y., Tanaka, K., & Shimizu, N. (1996). Direct evidence for two distinct G proteins coupling with thrombin receptors in human neuroblastoma SH-EP cells. *European Journal of Pharmacology 316*(1), 105–109 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8982657.

O'Hayre, M., Eichel, K., Avino, S., Zhao, X., Steffen, D. J., Feng, X., ... Gutkind, J. S. (2017). Genetic evidence that β-arrestins are dispensable for the initiation of β2-adrenergic receptor signaling to ERK. *Science Signaling 10*(484). https://doi.org/10.1126/scisignal.aal3395.

Okada, M., Goldman, D., Linnoila, M., Iwata, N., Ozaki, N., & Northup, J. K. (2004). Comparison of G-protein selectivity of human 5-HT2C and 5-HT1A receptors. *Annals of the New York Academy of Sciences 1025*, 570–577. https://doi.org/10.1196/annals.1316.070.

Okamoto, T., Murayama, Y., Hayashi, Y., Inagaki, M., Ogata, E., & Nishimoto, I. (1991). Identification of a Gs activator region of the beta 2-adrenergic receptor that is autoregulated via protein kinase A-dependent phosphorylation. *Cell 67*(4), 723–730. https://doi.org/10.1016/0092-8674(91)90067-9.

Okamoto, Y., Ninomiya, H., Tanioka, M., Sakamoto, A., Miwa, S., & Masaki, T. (1997). Palmitoylation of human endothelinB. Its critical role in G protein coupling and a differential requirement for the cytoplasmic tail by G protein subtypes. *The Journal of Biological Chemistry 272*(34), 21589–21596 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9261180.

Okamoto, Y., & Shikano, S. (2011). Phosphorylation-dependent C-terminal binding of 14-3-3 proteins promotes cell surface expression of HIV co-receptor GPR15. *The Journal of Biological Chemistry 286*(9), 7171–7181. https://doi.org/10.1074/jbc.M110.199695.

O'Meara, S. J., & Kinsella, B. T. (2005). The effect of the farnesyl protein transferase inhibitor SCH66336 on isoprenylation and signalling by the prostacyclin receptor. *The Biochemical Journal 386*, 177–189. https://doi.org/10.1042/BJ20041290 Pt 1.

Onaran, H. O., Ambrosio, C., Ugur, O., Madaras Koncz, E., Gro, M. C., Vezzi, V., ... Costa, T. (2017). Systematic errors in detecting biased agonism: Analysis of current methods and development of a new model-free approach. *Scientific Reports 7*, 44247. https://doi.org/10.1038/srep44247.

Onaran, H. O., Rajagopal, S., & Costa, T. (2014). What is biased efficacy? Defining the relationship between intrinsic efficacy and free energy coupling. *Trends in Pharmacological Sciences 35*(12), 639–647. https://doi.org/10.1016/j.tips.2014.09.010.

Oner, S. S., An, N., Vural, A., Breton, B., Bouvier, M., Blumer, J. B., & Lanier, S. M. (2010). Regulation of the AGS3.G{alpha}i signaling complex by a seven-transmembrane span receptor. *The Journal of Biological Chemistry 285*(44), 33949–33958. https://doi.org/10.1074/jbc.M110.138073.

Onfroy, L., Galandrin, S., Pontier, S. M., Seguelas, M. H., N'Guyen, D., Sénard, J. M., & Galés, C. (2017). G protein stoichiometry dictates biased agonism through distinct receptor-G protein partitioning. *Scientific Reports 7*, 7885. https://doi.org/10.1038/s41598-017-07392-5.

Palmer, T. M., Gettys, T. W., & Stiles, G. L. (1995). Differential interaction with and regulation of multiple G-proteins by the rat A3 adenosine receptor. *The Journal of Biological Chemistry 270*(28), 16895–16902 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7622506.

Pang, P. S., Butler, J., Collins, S. P., Cotter, G., Davison, B. A., Ezekowitz, J. A., ... Felker, G. M. (2017). Biased ligand of the angiotensin II type 1 receptor in patients with acute heart failure: a randomized, double-blind, placebo-controlled, phase IIB, dose ranging trial (BLAST-AHF). *European Heart Journal 38*(30), 2364–2373. https://doi.org/10.1093/eurheartj/ehx196.

Paquette, J. J., Wang, H. Y., Bakshi, K., & Olmstead, M. C. (2007). Cannabinoid-induced tolerance is associated with a CB1 receptor G protein coupling switch that is prevented by ultra-low dose rimonabant. *Behavioural Pharmacology 18*(8), 767–776. https://doi.org/10.1097/FBP.0b013e3282f15890.

Park, J. H., Scheerer, P., Hofmann, K. P., Choe, H. W., & Ernst, O. P. (2008). Crystal structure of the ligand-free G-protein-coupled receptor opsin. *Nature 454*(7201), 183–187. https://doi.org/10.1038/nature07063.

Peleg, S., Varon, D., Ivanina, T., Dessauer, C. W., & Dascal, N. (2002). G(alpha)(i) controls the gating of the G protein-activated K(+) channel, GIRK. *Neuron 33*(1), 87–99 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11779482.

Peng, Y., McCorvy, J. D., Harpsoe, K., Lansu, K., Yuan, S., Popov, P., ... Liu, Z. J. (2018). 5-HT2C Receptor Structures Reveal the Structural Basis of GPCR Polypharmacology. *Cell 172*(4), 719–730.e14. https://doi.org/10.1016/j.cell.2018.01.001.

Peters, M. F., & Scott, C. W. (2009). Evaluating cellular impedance assays for detection of GPCR pleiotropic signaling and functional selectivity. *Journal of Biomolecular Screening 14*(3), 246–255. https://doi.org/10.1177/1087057108330115.

Peterson, Y. K., & Luttrell, L. M. (2017). The Diverse Roles of Arrestin Scaffolds in G Protein–Coupled Receptor Signaling. *Pharmacological Reviews 69*(3), 256 LP–297. https://doi.org/10.1124/pr.116.013367.

Pierce, K. L., Luttrell, L. M., & Lefkowitz, R. J. (2001). New mechanisms in heptahelical receptor signaling to mitogen activated protein kinase cascades. *Oncogene 20*(13), 1532–1539. https://doi.org/10.1038/sj.onc.1204184.

Pindon, A., van Hecke, G., van Gompel, P., Lesage, A. S., Leysen, J. E., & Jurzak, M. (2002). Differences in signal transduction of two 5-HT4 receptor splice variants: compound specificity and dual coupling with Galphas- and Galphai/o-proteins. *Molecular Pharmacology 61*(1), 85–96 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11752209.

Pissios, P., Trombly, D. J., Tzameli, I., & Maratos-Flier, E. (2003). Melanin-concentrating hormone receptor 1 activates extracellular signal-regulated kinase and synergizes with G(s)-coupled pathways. *Endocrinology 144*(8), 3514–3523. https://doi.org/10.1210/en.2002-0004.

Pommier, B., Da Nascimento, S., Dumont, S., Bellier, B., Million, E., Garbay, C., ... Noble, F. (1999). The cholecystokininB receptor is coupled to two effector pathways through pertussis toxin-sensitive and -insensitive G proteins. *Journal of Neurochemistry 73*(1), 281–288.

Ponimaskin, E. G., Profirovic, J., Vaiskunaite, R., Richter, D. W., & Voyno-Yasenetskaya, T. A. (2002). 5-Hydroxytryptamine 4(a) receptor is coupled to the Galpha subunit of heterotrimeric G13 protein. *The Journal of Biological Chemistry 277*(23), 20812–20819. https://doi.org/10.1074/jbc.M112216200.

Pradhan, A. A., Perroy, J., Walwyn, W. M., Smith, M. L., Vicente-Sanchez, A., Segura, L., ... Evans, C. J. (2016). Agonist-Specific Recruitment of Arrestin Isoforms Differentially Modify Delta Opioid Receptor Function. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 36*(12), 3541–3551. https://doi.org/10.1523/JNEUROSCI.4124-15.2016.

Price, R. D., Weiner, D. M., Chang, M. S., & Sanders-Bush, E. (2001). RNA editing of the human serotonin 5-HT2C receptor alters receptor-mediated activation of G13 protein. *The Journal of Biological Chemistry 276*(48), 44663–44668. https://doi.org/10.1074/jbc.M106745200.

Pronin, A. N., Wang, Q., & Slepak, V. Z. (2017). Teaching an Old Drug New Tricks: Agonism, Antagonism, and Biased Signaling of Pilocarpine through M3 Muscarinic Acetylcholine Receptor. *Molecular Pharmacology 92*(5), 601–612. https://doi.org/10.1124/mol.117.109678.

Qin, K., Dong, C., Wu, G., & Lambert, N. A. (2011). Inactive-state preassembly of G(q)-coupled receptors and G(q) heterotrimers. *Nature Chemical Biology 7*(10), 740–747. https://doi.org/10.1038/nchembio.642.

Quiniou, C., Sapieha, P., Lahaie, I., Hou, X., Brault, S., Beauchamp, M., ... Chemtob, S. (2008). Development of a Novel Noncompetitive Antagonist of IL-1 Receptor. *The Journal of Immunology 180*(10), 6977 LP–6987 Retrieved from http://www.jimmunol.org/content/180/10/6977.abstract.

Quoyer, J., Janz, J. M., Luo, J., Ren, Y., Armando, S., Lukashova, V., ... Bouvier, M. (2013). Pepducin targeting the C-X-C chemokine receptor type 4 acts as a biased agonist favoring activation of the inhibitory G protein. *Proceedings of the National Academy of Sciences of the United States of America 110*(52), E5088–E5097. https://doi.org/10.1073/pnas.1312515110.

Quoyer, J., Longuet, C., Broca, C., Linck, N., Costes, S., Varin, E., ... Dalle, S. (2010). GLP-1 mediates antiapoptotic effect by phosphorylating Bad through a beta-arrestin 1-mediated ERK1/2 activation in pancreatic beta-cells. *The Journal of Biological Chemistry 285*(3), 1989–2002. https://doi.org/10.1074/jbc.M109.067207.

Rahmeh, R., Damian, M., Cottet, M., Orcel, H., Mendre, C., Durroux, T., ... Granier, S. (2012). Structural insights into biased G protein-coupled receptor signaling revealed by fluorescence spectroscopy. *Proceedings of the National Academy of Sciences 109*(17), 6733–6738. https://doi.org/10.1073/pnas.1201093109.

Rajagopal, S., Ahn, S., Rominger, D. H., Gowen-MacDonald, W., Lam, C. M., DeWire, S. M., ... Lefkowitz, R. J. (2011). Quantifying Ligand Bias at Seven-Transmembrane Receptors. *Molecular Pharmacology 80*(3), 367–377. https://doi.org/10.1124/mol.111.072801.

Rajagopal, S., Bassoni, D. L., Campbell, J. J., Gerard, N. P., Gerard, C., & Wehrman, T. S. (2013). Biased agonism as a mechanism for differential signaling by chemokine receptors. *The Journal of Biological Chemistry 288*(49), 35039–35048. https://doi.org/10.1074/jbc.M113.479113.

Rajagopal, S., Rajagopal, K., & Lefkowitz, R. J. (2010). Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nature Reviews. Drug Discovery 9*(5), 373–386. https://doi.org/10.1038/nrd3024.

Rashid, A. J., So, C. H., Kong, M. M. C., Furtak, T., El-Ghundi, M., Cheng, R., ... George, S. R. (2007). D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proceedings of the National Academy of Sciences of the United States of America 104*(2), 654–659. https://doi.org/10.1073/pnas.0604049104.

Rasmussen, S. G. F., DeVree, B. T., Zou, Y., Kruse, A. C., Chung, K. Y., Kobilka, T. S., ... Kobilka, B. K. (2011). Crystal structure of the β2 adrenergic receptor-Gs protein complex. *Nature 477*(7366), 549–555. https://doi.org/10.1038/nature10361.

Read, C., Fitzpatrick, C. M., Yang, P., Kuc, R. E., Maguire, J. J., Glen, R. C., ... Davenport, A. P. (2016). Cardiac action of the first G protein biased small molecule apelin agonist. *Biochemical Pharmacology 116*, 63–72. https://doi.org/10.1016/j.bcp.2016.07.018.

Reiter, E., & Lefkowitz, R. J. (2006). GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. *Trends in Endocrinology and Metabolism: TEM 17*(4), 159–165. https://doi.org/10.1016/j.tem.2006.03.008.

Ren, X. R., Reiter, E., Ahn, S., Kim, J., Chen, W., & Lefkowitz, R. J. (2005). Different G protein-coupled receptor kinases govern G protein and beta-arrestin-mediated signaling of V2 vasopressin receptor. *Proceedings of the National Academy of Sciences of the United States of America 102*(5), 1448–1453. https://doi.org/10.1073/pnas.0409534102.

Reversi, A., Rimoldi, V., Marrocco, T., Cassoni, P., Bussolati, G., Parenti, M., & Chini, B. (2005). The oxytocin receptor antagonist atosiban inhibits cell growth via a "biased agonist" mechanism. *The Journal of Biological Chemistry 280*(16), 16311–16318. https://doi.org/10.1074/jbc.M409945200.

Rhee, S. G. (2001). Regulation of phosphoinositide-specific phospholipase C. *Annual Review of Biochemistry 70*, 281–312. https://doi.org/10.1146/annurev.biochem.70.1.281.

Ring, A. M., Manglik, A., Kruse, A. C., Enos, M. D., Weis, W. I., Garcia, K. C., & Kobilka, B. K. (2013). Adrenaline-activated structure of beta2-adrenoceptor stabilized by an engineered nanobody. *Nature 502*(7472), 575–579. https://doi.org/10.1038/nature12572.

Robishaw, J. D., & Berlot, C. H. (2004). Translating G protein subunit diversity into functional specificity. *Current Opinion in Cell Biology 16*(2), 206–209. https://doi.org/10.1016/j.ceb.2004.02.007.

Rojas, A. M., Fuentes, G., Rausell, A., & Valencia, A. (2012). The Ras protein superfamily: evolutionary tree and role of conserved amino acids. *The Journal of Cell Biology 196*(2), 189–201. https://doi.org/10.1083/jcb.201103008.

Rook, J. M., Xiang, Z., Lv, X., Ghoshal, A., Dickerson, J. W., Bridges, T. M., ... Conn, P. J. (2015). Biased mGlu5-Positive Allosteric Modulators Provide In Vivo Efficacy without Potentiating mGlu5 Modulation of NMDAR Currents. *Neuron 86*(4), 1029–1040. https://doi.org/10.1016/j.neuron.2015.03.063.

Rosano, L., Cianfrocca, R., Tocci, P., Spinella, F., Di Castro, V., Spadaro, F., ... Bagnato, A. (2013). beta-arrestin-1 is a nuclear transcriptional regulator of endothelin-1-induced beta-catenin signaling. *Oncogene 32*(42), 5066–5077. https://doi.org/10.1038/onc.2012.527.

Rose, A. S., Elgeti, M., Zachariae, U., Grubmüller, H., Hofmann, K. P., Scheerer, P., & Hildebrand, P. W. (2014). Position of transmembrane helix 6 determines receptor G protein coupling specificity. *Journal of the American Chemical Society 136*(32), 11244–11247. https://doi.org/10.1021/ja5055109.

Ross, A. M., Gibbons, R. J., Stone, G. W., Kloner, R. A., & Alexander, R. W. (2005). A randomized, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD-II). *Journal of the American College of Cardiology 45*(11), 1775–1780. https://doi.org/10.1016/j.jacc.2005.02.061.

Roth, B. L., Sheffler, D. J., & Kroeze, W. K. (2004). Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nature Reviews. Drug Discovery 3*(4), 353–359. https://doi.org/10.1038/nrd1346.

Roychowdhury, S., Panda, D., Wilson, L., & Rasenick, M. M. (1999). G protein alpha subunits activate tubulin GTPase and modulate microtubule polymerization dynamics. *The Journal of Biological Chemistry 274*(19), 13485–13490 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10224115.

Rümenapp, U., Asmus, M., Schablowski, H., Woznicki, M., Han, L., Jakobs, K. H., ... Schmidt, M. (2001). The M3 muscarinic acetylcholine receptor expressed in HEK-293 cells signals to phospholipase D via G12 but not Gq-type G proteins: regulators of G proteins as tools to dissect pertussis toxin-resistant G proteins in receptor-effector coupling. *The Journal of Biological Chemistry 276*(4), 2474–2479. https://doi.org/10.1074/jbc.M004957200.

Ryman-Rasmussen, J. P., Griffith, A., Oloff, S., Vaidehi, N., Brown, J. T., Goddard, W. A., III, & Mailman, R. B. (2007). Functional selectivity of dopamine D1 receptor agonists in regulating the fate of internalized receptors. *Neuropharmacology 52*(2), 562–575. https://doi.org/10.1016/j.neuropharm.2006.08.028.

Sabbah, H. N., Gupta, R. C., Kohli, S., Wang, M., Rastogi, S., Zhang, K., ... Albrecht-Küpper, B. E. (2013). Chronic therapy with a partial adenosine a1 receptor agonist, improves left ventricular function and remodeling in dogs with advanced heart failure. *Circulation. Heart Failure 6*(3), 563–571. https://doi.org/10.1161/CIRCHEARTFAILURE.112.000208.

Santos, G. A., Duarte, D. A., Parreiras-E-Silva, L. T., Teixeira, F. R., Silva-Rocha, R., Oliveira, E. B., ... Costa-Neto, C. M. (2015). Comparative analyses of downstream signal transduction targets modulated after activation of the AT1 receptor by two β-arrestin-biased agonists. *Frontiers in Pharmacology 6*, 131. https://doi.org/10.3389/fphar.2015.00131.

Sato, M., Horinouchi, T., Hutchinson, D. S., Evans, B. a., & Summers, R. J. (2007). Ligand-directed signaling at the beta 3 -adrenoceptor produced by 2S-2-propanol oxalate (SR59230A) relative to receptor agonists. *Molecular Pharmacology 72*(5), 1359–1368. https://doi.org/10.1124/mol.107.035337.SR59230A.

Saulière, A., Bellot, M., Paris, H., Denis, C., Finana, F., Hansen, J. T., ... Galés, C. (2012). Deciphering biased-agonism complexity reveals a new active AT1 receptor entity. *Nature Chemical Biology 8*, 622 Retrieved from https://doi.org/10.1038/nchembio.961.

Schattauer, S. S., Kuhar, J. R., Song, A., & Chavkin, C. (2017). Nalfurafine is a G-protein biased agonist having significantly greater bias at the human than rodent form of the kappa opioid receptor. *Cellular Signalling 32*, 59–65. https://doi.org/10.1016/j.cellsig.2017.01.016.

Scheerer, P., & Sommer, M. E. (2017). Structural mechanism of arrestin activation. *Current Opinion in Structural Biology 45*, 160–169. https://doi.org/10.1016/j.sbi.2017.05.001.

Schmid, C. L., Streicher, J. M., Groer, C. E., Munro, T. A., Zhou, L., & Bohn, L. M. (2013). Functional selectivity of 6′-guanidinonaltrindole (6′-GNTI) at kappa-opioid receptors in striatal neurons. *The Journal of Biological Chemistry 288*(31), 22387–22398. https://doi.org/10.1074/jbc.M113.476234.

Schwindinger, W. F., Betz, K. S., Giger, K. E., Sabol, A., Bronson, S. K., & Robishaw, J. D. (2003). Loss of G protein gamma 7 alters behavior and reduces striatal alpha(olf) level and cAMP production. *The Journal of Biological Chemistry 278*(8), 6575–6579. https://doi.org/10.1074/jbc.M211132200.

Schwindinger, W. F., Fredericks, J., Watkins, L., Robinson, H., Bathon, J. M., Pines, M., ... Levine, M. A. (1998). Coupling of the PTH/PTHrP receptor to multiple G-proteins. Direct demonstration of receptor activation of Gs, Gq/11, and Gi(1) by [alpha-32P]GTP-gamma-azidoanilide photoaffinity labeling. *Endocrine 8*(2), 201–209. https://doi.org/10.1385/ENDO:8:2:201.

Selkirk, J. V., Price, G. W., Nahorski, S. R., & Challiss, R. A. (2001). Cell type-specific differences in the coupling of recombinant mGlu1alpha receptors to endogenous G protein sub-populations. *Neuropharmacology 40*(5), 645–656 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11311892.

Semple, G., Skinner, P. J., Gharbaoui, T., Shin, Y. j., Jung, J. k., Cherrier, M. C., ... Richman, J. G. (2008). Nicotinic Acid Receptor, G-Protein Coupled Receptor 109a, with Antilipolytic but No Vasodilatory Activity in Mice. *Journal of Medicinal Chemistry 51*(16), 5101–5108.

Serres, F., Li, Q., Garcia, F., Raap, D. K., Battaglia, G., Muma, N. A., & Van de Kar, L. D. (2000). Evidence that G(z)-proteins couple to hypothalamic 5-HT(1A) receptors in vivo. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 20*(9), 3095–3103 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10777773.

Seyedabadi, M., Ostad, S. N., Albert, P. R., Dehpour, A. R., Rahimian, R., Ghazi-Khansari, M., & Ghahremani, M. H. (2012). Ser/ Thr residues at α3/β5 loop of Gαs are important in morphine-induced adenylyl cyclase sensitization but not mitogen-activated protein kinase phosphorylation. *The FEBS Journal 279*(4), 650–660. https://doi.org/10.1111/j.1742-4658.2011.08459.x.

Shenoy, S. K., Drake, M. T., Nelson, C. D., Houtz, D. A., Xiao, K., Madabushi, S., ... Lefkowitz, R. J. (2006). beta-arrestin-dependent, G protein-independent ERK1/2 activation by the beta2 adrenergic receptor. *The Journal of Biological Chemistry 281*(2), 1261–1273. https://doi.org/10.1074/jbc.M506576200.

Shraga-Levine, Z., & Sokolovsky, M. (2000). Functional coupling of G proteins to endothelin receptors is ligand and receptor subtype specific. *Cellular and Molecular*

Neurobiology 20(3), 305–317 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10789830.

Siderovski, D. P., & Willard, F. S. (2005). The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. *International Journal of Biological Sciences 1*(2), 51–66 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15951850.

Singla, N., Minkowitz, H. S., Soergel, D. G., Burt, D. A., Subach, R. A., Salamea, M. Y., ... Skobieranda, F. (2017). A randomized, Phase IIb study investigating oliceridine (TRV130), a novel micro-receptor G-protein pathway selective (mu-GPS) modulator, for the management of moderate to severe acute pain following abdominoplasty. *Journal of Pain Research 10*, 2413–2424. https://doi.org/10.2147/JPR.S137952.

Smrcka, A. V. (2013). Molecular targeting of Gα and Gβγ subunits: a potential approach for cancer therapeutics. *Trends in Pharmacological Sciences 34*(5), 290–298. https://doi.org/10.1016/j.tips.2013.02.006.

Spengler, D., Waeber, C., Pantaloni, C., Holsboer, F., Bockaert, J., Seeburg, P. H., & Journot, L. (1993). Differential signal transduction by five splice variants of the PACAP receptor. *Nature 365*(6442), 170–175. https://doi.org/10.1038/365170a0.

Stanislaus, D., Ponder, S., Ji, T. H., & Conn, P. M. (1998). Gonadotropin-releasing hormone receptor couples to multiple G proteins in rat gonadotrophs and in GGH3 cells: evidence from palmitoylation and overexpression of G proteins. *Biology of Reproduction 59*(3), 579–586 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9716556.

Stewart, G. D., Sexton, P. M., & Christopoulos, A. (2010). Detection of novel functional selectivity at M3 muscarinic acetylcholine receptors using a Saccharomyces cerevisiae platform. *ACS Chemical Biology 5*(4), 365–375. https://doi.org/10.1021/cb900276p.

Stewart, G. D., Valant, C., Dowell, S. J., Mijaljica, D., Devenish, R. J., Scammells, P. J., ... Christopoulos, A. (2009). Determination of adenosine A1 receptor agonist and antagonist pharmacology using Saccharomyces cerevisiae: implications for ligand screening and functional selectivity. *The Journal of Pharmacology and Experimental Therapeutics 331*(1), 277–286. https://doi.org/10.1124/jpet.109.158667.

Stilley, J. A. W., Guan, R., Santillan, D. A., Mitchell, B. F., Lamping, K. G., & Segaloff, D. L. (2016). Differential Regulation of Human and Mouse Myometrial Contractile Activity by FSH as a Function of FSH Receptor Density. *Biology of Reproduction 95*(2), 36. https://doi.org/10.1095/biolreprod.116.141648.

Stroth, N., Niso, M., Colabufo, N. A., Perrone, R., Svenningsson, P., Lacivita, E., & Leopoldo, M. (2015). Arylpiperazine agonists of the serotonin 5-HT1A receptor preferentially activate cAMP signaling versus recruitment of beta-arrestin-2. *Bioorganic & Medicinal Chemistry 23*(15), 4824–4830. https://doi.org/10.1016/j.bmc.2015.05.042.

Sugimoto, Y., Nakato, T., Kita, A., Takahashi, Y., Hatae, N., Tabata, H., ... Ichikawa, A. (2004). A cluster of aromatic amino acids in the i2 loop plays a key role for Gs coupling in prostaglandin EP2 and EP3 receptors. *The Journal of Biological Chemistry 279*(12), 11016–11026. https://doi.org/10.1074/jbc.M307404200.

Sunahara, R. K., Dessauer, C. W., Whisnant, R. E., Kleuss, C., & Gilman, A. G. (1997). Interaction of Gsalpha with the cytosolic domains of mammalian adenylyl cyclase. *The Journal of Biological Chemistry 272*(35), 22265–22271.

Surve, C. R., Lehmann, D., & Smrcka, A. V. (2014). A chemical biology approach demonstrates G protein betagamma subunits are sufficient to mediate directional neutrophil chemotaxis. *The Journal of Biological Chemistry 289*(25), 17791–17801. https://doi.org/10.1074/jbc.M114.576827.

Sykes, D. A., Moore, H., Stott, L., Holliday, N., Javitch, J. A., Lane, J. R., & Charlton, S. J. (2017). Extrapyramidal side effects of antipsychotics are linked to their association kinetics at dopamine D2 receptors. *Nature Communications 8*(1), 763. https://doi.org/10.1038/s41467-017-00716-z.

Szabo, M., Klein Herenbrink, C., Christopoulos, A., Lane, J. R., & Capuano, B. (2014). Structure-activity relationships of privileged structures leads to the discovery of novel biased ligands at the dopamine D2 receptor. *Journal of Medicinal Chemistry*. https://doi.org/10.1021/jm500457x (May).

Teixeira, L. B., Parreiras-E-Silva, L. T., Bruder-Nascimento, T., Duarte, D. A., Simoes, S. C., Costa, R. M., ... Costa-Neto, C. M. (2017). Ang-(1-7) is an endogenous beta-arrestin-biased agonist of the AT1 receptor with protective action in cardiac hypertrophy. *Scientific Reports 7*(1), 11903. https://doi.org/10.1038/s41598-017-12074-3.

Thomas, R. L., Mistry, R., Langmead, C. J., Wood, M. D., & Challiss, R. A. J. (2008). G protein coupling and signaling pathway activation by m1 muscarinic acetylcholine receptor orthosteric and allosteric agonists. *The Journal of Pharmacology and Experimental Therapeutics 327*(2), 365–374. https://doi.org/10.1124/jpet.108.141788.

Thomsen, A. R. B., Hvidtfeldt, M., & Bräuner-Osborne, H. (2012). Biased agonism of the calcium-sensing receptor. *Cell Calcium 51*(2), 107–116. https://doi.org/10.1016/j.ceca.2011.11.009.

Thomsen, A. R. B., Plouffe, B., Cahill, T. J., Shukla, A. K., Tarrasch, J. T., Dosey, A. M., ... Lefkowitz, R. J. (2016). GPCR-G Protein-β-Arrestin Super-Complex Mediates Sustained G Protein Signaling. *Cell 166*(4), 907–919. https://doi.org/10.1016/j.cell.2016.07.004.

Thomsen, A. R. B., Worm, J., Jacobsen, S. E., Stahlhut, M., Latta, M., & Brauner-Osborne, H. (2012). Strontium is a biased agonist of the calcium-sensing receptor in rat medullary thyroid carcinoma 6-23 cells. *The Journal of Pharmacology and Experimental Therapeutics 343*(3), 638–649. https://doi.org/10.1124/jpet.112.197210.

Thomsen, C. (1996). Metabotropic glutamate receptor subtype 1A activates adenylate cyclase when expressed in baby hamster kidney cells. *Progress in Neuro-Psychopharmacology & Biological Psychiatry 20*(4), 709–726.

Tidgewell, K., Groer, C. E., Harding, W. W., Lozama, A., Schmidt, M., Marquam, A., ... Prisinzano, T. E. (2008). Herkinorin analogues with differential beta-arrestin-2 interactions. *Journal of Medicinal Chemistry 51*(8), 2421–2431. https://doi.org/10.1021/jm701162g.

Torrecilla, I., Spragg, E. J., Poulin, B., McWilliams, P. J., Mistry, S. C., Blaukat, A., & Tobin, A. B. (2007). Phosphorylation and regulation of a G protein–coupled receptor by protein kinase CK2. *The Journal of Cell Biology 177*(1), 127 LP–137 Retrieved from http://jcb.rupress.org/content/177/1/127.abstract.

Toth, A. D., Prokop, S., Gyombolai, P., Varnai, P., Balla, A., Gurevich, V. V., ... Turu, G. (2018). Heterologous phosphorylation-induced formation of a stability lock permits regulation of inactive receptors by beta-arrestins. *The Journal of Biological Chemistry 293*(3), 876–892. https://doi.org/10.1074/jbc.M117.813139.

Tschammer, N., Bollinger, S., Kenakin, T., & Gmeiner, P. (2011). Histidine 6.55 is a major determinant of ligand-biased signaling in dopamine D2L receptor. *Molecular Pharmacology 79*(3), 575–585. https://doi.org/10.1124/mol.110.068106.

Tucek, S., Michal, P., & Vlachová, V. (2002). Modelling the consequences of receptor-G-protein promiscuity. *Trends in Pharmacological Sciences 23*(4), 171–176. https://doi.org/10.1016/S0165-6147(00)01996-9.

Ulfers, A. L., McMurry, J. L., Miller, A., Wang, L., Kendall, D. a., & Mierke, D. F. (2002). Cannabinoid receptor-G protein interactions: G(alphai1)-bound structures of IC3 and a mutant with altered G protein specificity. *Protein Science: A Publication of the Protein Society 11*(10), 2526–2531. https://doi.org/10.1110/ps.0218402.

Ulloa-Aguirre, A., Stanislaus, D., Arora, V., Väänänen, J., Brothers, S., Janovick, J. A., & Conn, P. M. (1998). The third intracellular loop of the rat gonadotropin-releasing hormone receptor couples the receptor to Gs- and G(q/11)-mediated signal transduction pathways: evidence from loop fragment transfection in GGH3 cells. *Endocrinology 139*(5), 2472–2478. https://doi.org/10.1210/endo.139.5.6022.

van Unen, J., Stumpf, A. D., Schmid, B., Reinhard, N. R., Hordijk, P. L., Hoffmann, C., ... Goedhart, J. (2016). A New Generation of FRET Sensors for Robust Measurement of Gαi1, Gαi2 and Gαi3 Activation Kinetics in Single Cells. *PLoS One 11*(1), e0146789. https://doi.org/10.1371/journal.pone.0146789.

Unett, D. J., Gatlin, J., Anthony, T. L., Buzard, D. J., Chang, S., Chen, C., ... Gaidarov, I. (2013). Kinetics of 5-HT2B receptor signaling: profound agonist-dependent effects on signaling onset and duration. *The Journal of Pharmacology and Experimental Therapeutics 347*(3), 645–659. https://doi.org/10.1124/jpet.113.207670.

Urban, J. D., Vargas, G. A., von Zastrow, M., & Mailman, R. B. (2007). Aripiprazole has Functionally Selective Actions at Dopamine D2 Receptor-Mediated Signaling Pathways. *Neuropsychopharmacology 32*(1), 67–77. https://doi.org/10.1038/sj.npp.1301071.

Vacchini, A., Mortier, A., Proost, P., Locati, M., Metzemaekers, M., & Borroni, E. M. (2018). Differential Effects of Posttranslational Modifications of CXCL8/Interleukin-8 on CXCR1 and CXCR2 Internalization and Signaling Properties. *International Journal of Molecular Sciences 19*(12). https://doi.org/10.3390/ijms19123768.

Valant, C., May, L. T., Aurelio, L., Chuo, C. H., White, P. J., Baltos, J. A., ... Christopoulos, A. (2014). Separation of on-target efficacy from adverse effects through rational design of a bitopic adenosine receptor agonist. *Proceedings of the National Academy of Sciences 111*(12), 4614–4619. https://doi.org/10.1073/pnas.1320962111.

Van Eps, N., Caro, L. N., Morizumi, T., Kusnetzow, A. K., Szczepek, M., Hofmann, K. P., ... Hubbell, W. L. (2017). Conformational equilibria of light-activated rhodopsin in nanodiscs. *Proceedings of the National Academy of Sciences 114*(16), E3268 LP–E3275 Retrieved from http://www.pnas.org/content/114/16/E3268.abstract.

Váradi, A., Marrone, G. F., Palmer, T. C., Narayan, A., Szabó, M. R., Le Rouzic, V., ... Majumdar, S. (2016). Mitragynine/Corynantheidine Pseudoindoxyls As Opioid Analgesics with Mu Agonism and Delta Antagonism, Which Do Not Recruit β-Arrestin-2. *Journal of Medicinal Chemistry 59*(18), 8381–8397. https://doi.org/10.1021/acs.jmedchem.6b00748.

Venkatakrishnan, A. J., Deupi, X., Lebon, G., Heydenreich, F. M., Flock, T., Miljus, T., ... Babu, M. M. (2016). Diverse activation pathways in class A GPCRs converge near the G-protein-coupling region. *Nature 536*(7617), 484–487. https://doi.org/10.1038/nature19107.

Venkatakrishnan, A. J., Deupi, X., Lebon, G., Tate, C. G., Schertler, G. F., & Babu, M. M. (2013). Molecular signatures of G-protein-coupled receptors. *Nature 494*(7436), 185–194. https://doi.org/10.1038/nature11896.

Vickery, O. N., Machtens, J. P., & Zachariae, U. (2016). Membrane potentials regulating GPCRs: insights from experiments and molecular dynamics simulations. *Current Opinion in Pharmacology 30*, 44–50. https://doi.org/10.1016/j.coph.2016.06.011.

Violin, J. D., Crombie, A. L., Soergel, D. G., & Lark, M. W. (2017). Biased ligands at G-protein-coupled receptors: promise and progress. *Trends in Pharmacological Sciences 35*(7), 308–316. https://doi.org/10.1016/j.tips.2014.04.007.

Violin, J. D., DeWire, S. M., Yamashita, D., Rominger, D. H., Nguyen, L., Schiller, K., ... Lark, M. W. (2010). Selectively engaging β-arrestins at the angiotensin II type 1 receptor reduces blood pressure and increases cardiac performance. *The Journal of Pharmacology and Experimental Therapeutics 335*(3), 572–579. https://doi.org/10.1124/jpet.110.173005.

Viscusi, E. R., Webster, L., Kuss, M., Daniels, S., Bolognese, J. A., Zuckerman, S., ... Skobieranda, F. (2016). A randomized, phase 2 study investigating TRV130, a biased ligand of the mu-opioid receptor, for the intravenous treatment of acute pain. *Pain 157*(1), 264–272. https://doi.org/10.1097/j.pain.0000000000000363.

Vroon, A., Heijnen, C. J., & Kavelaars, A. (2006). GRKs and arrestins: regulators of migration and inflammation. *Journal of Leukocyte Biology 80*(6), 1214–1221. https://doi.org/10.1189/jlb.0606373.

Wacker, D., Wang, C., Katritch, V., Han, G. W., Huang, X. P., Vardy, E., ... Stevens, R. C. (2013). Structural features for functional selectivity at serotonin receptors. *Science (New York, N.Y.) 340*(6132), 615–619. https://doi.org/10.1126/science.1232808.

Wacker, J. L., Feller, D. B., Tang, X. B., Defino, M. C., Namkung, Y., Lyssand, J. S., ... Hague, C. (2008). Disease-causing mutation in GPR54 reveals the importance of the second intracellular loop for class A G-protein-coupled receptor function. *The Journal of Biological Chemistry 283*(45), 31068–31078. https://doi.org/10.1074/jbc.M805251200.

Walther, C., & Ferguson, S. S. G. (2015). Minireview: Role of intracellular scaffolding proteins in the regulation of endocrine G protein-coupled receptor signaling. *Molecular Endocrinology (Baltimore, Md.) 29*(6), 814–830. https://doi.org/10.1210/me.2015-1091.

Wang, H. Y., & Burns, L. H. (2009). Naloxone's pentapeptide binding site on filamin A blocks Mu opioid receptor-Gs coupling and CREB activation of acute morphine. *PLoS One 4*(1), e4282. https://doi.org/10.1371/journal.pone.0004282.

Wang, H. Y., Frankfurt, M., & Burns, L. H. (2008). High-affinity naloxone binding to filamin a prevents mu opioid receptor-Gs coupling underlying opioid tolerance and dependence. *PLoS One 3*(2), e1554. https://doi.org/10.1371/journal.pone.0001554.

Wang, H. Y., Friedman, E., Olmstead, M. C., & Burns, L. H. (2005). Ultra-low-dose naloxone suppresses opioid tolerance, dependence and associated changes in mu opioid receptor-G protein coupling and Gbetagamma signaling. *Neuroscience 135*(1), 247–261. https://doi.org/10.1016/j.neuroscience.2005.06.003.

Wang, J., Hanada, K., Staus, D. P., Makara, M. A., Dahal, G. R., Chen, Q., ... Rockman, H. A. (2017). Gαi is required for carvedilol-induced β1 adrenergic receptor β-arrestin biased signaling. *Nature Communications 8*(1), 1706. https://doi.org/10.1038/s41467-017-01855-z.

Wang, Q., & Limbird, L. E. (2007). Regulation of alpha2AR trafficking and signaling by interacting proteins. *Biochemical Pharmacology 73*(8), 1135–1145. https://doi.org/10.1016/j.bcp.2006.12.024.

Wang, Q., Zhao, J., Brady, A. E., Feng, J., Allen, P. B., Lefkowitz, R. J., ... Limbird, L. E. (2004). Spinophilin blocks arrestin actions in vitro and in vivo at G protein-coupled receptors. *Science (New York, N.Y.) 304*(5679), 1940–1944. https://doi.org/10.1126/science.1098274.

Wang, X., Zeng, W., Soyombo, A. A., Tang, W., Ross, E. M., Barnes, A. P., ... Muallem, S. (2005). Spinophilin regulates Ca2+ signalling by binding the N-terminal domain of RGS2 and the third intracellular loop of G-protein-coupled receptors. *Nature Cell Biology 7*(4), 405–411. https://doi.org/10.1038/ncb1237.

Wang, Y. h., Sun, J. f., Tao, Y. m., Xu, X. j., Chi, Z. q., & Liu, J. g. (2010). Paradoxical relationship between RAVE (relative activity versus endocytosis) values of several opioid receptor agonists and their liability to cause dependence. *Acta Pharmacologica Sinica 31*(4), 393–398. https://doi.org/10.1038/aps.2010.19.

Wang, Y., Yuan, J., Qian, Z., Zhang, X., Chen, Y., Hou, X., & Zou, J. (2015). β2 adrenergic receptor activation governs cardiac repolarization and arrhythmogenesis in a guinea pig model of heart failure. *Scientific Reports 5*, 7681. https://doi.org/10.1038/srep07681.

Warne, T., Edwards, P. C., Leslie, A. G. W., & Tate, C. G. (2012). Crystal structures of a stabilized beta1-adrenoceptor bound to the biased agonists bucindolol and carvedilol. *Structure (London, England : 1993) 20*(5), 841–849. https://doi.org/10.1016/j.str.2012.03.014.

Watson, C., Chen, G., Irving, P., Way, J., Chen, W. J., & Kenakin, T. (2000). The use of stimulus-biased assay systems to detect agonist-specific receptor active states: implications for the trafficking of receptor stimulus by agonists. *Molecular Pharmacology 58*(6), 1230–1238. https://doi.org/10.1124/mol.58.6.1230.

Wehbi, V. L., Stevenson, H. P., Feinstein, T. N., Calero, G., Romero, G., & Vilardaga, J. P. (2013). Noncanonical GPCR signaling arising from a PTH receptor-arrestin-Gβγ complex. *Proceedings of the National Academy of Sciences of the United States of America 110*(4), 1530–1535. https://doi.org/10.1073/pnas.1205756110.

Wei, H., Ahn, S., Shenoy, S. K., Karnik, S. S., Hunyady, L., Luttrell, L. M., & Lefkowitz, R. J. (2003). Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proceedings of the National Academy of Sciences of the United States of America 100*(19), 10782–10787. https://doi.org/10.1073/pnas.1834556100.

Weïwer, M., Xu, Q., Gale, J. P., Lewis, M., Campbell, A. J., Schroeder, F. A., ... Holson, E. B. (2018). Functionally Biased D2R Antagonists: Targeting the β-Arrestin Pathway to Improve Antipsychotic Treatment. *ACS Chemical Biology 13*(4), 1038–1047. https://doi.org/10.1021/acschembio.8b00168.

Wenzel-Seifert, K., & Seifert, R. (2000). Molecular analysis of beta(2)-adrenoceptor coupling to G(s)-, G(i)-, and G(q)-proteins. *Molecular Pharmacology 58*(5), 954–966 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11040042.

Wess, J., Brann, M. R., & Bonner, T. I. (1989). Identification of a small intracellular region of the muscarinic m3 receptor as a determinant of selective coupling to PI turnover. *FEBS Letters 258*(1), 133–136 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2556294.

van der Westhuizen, E. T., Breton, B., Christopoulos, A., & Bouvier, M. (2014). Quantification of ligand bias for clinically relevant β2-adrenergic receptor ligands: implications for drug taxonomy. *Molecular Pharmacology 85*(3), 492–509. https://doi.org/10.1124/mol.113.088880.

Wettschureck, N., & Offermanns, S. (2005). Mammalian G proteins and their cell type specific functions. *Physiological Reviews 85*(4), 1159–1204. https://doi.org/10.1152/physrev.00003.2005.

Whalen, E. J., Rajagopal, S., & Lefkowitz, R. J. (2011). Therapeutic potential of β-arrestin- and G protein-biased agonists. *Trends in Molecular Medicine 17*(3), 126–139. https://doi.org/10.1016/j.molmed.2010.11.004.

Windh, R. T., Lee, M. J., Hla, T., An, S., Barr, A. J., & Manning, D. R. (1999). Differential coupling of the sphingosine 1-phosphate receptors Edg-1, Edg-3, and H218/Edg-5 to the G(i), G(q), and G(12) families of heterotrimeric G proteins. *The Journal of Biological Chemistry 274*(39), 27351–27358. https://doi.org/10.1074/jbc.274.39.27351.

Wisler, J. W., DeWire, S. M., Whalen, E. J., Violin, J. D., Drake, M. T., Ahn, S., ... Lefkowitz, R. J. (2007). A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. *Proceedings of the National Academy of Sciences of the United States of America 104*(42), 16657–16662. https://doi.org/10.1073/pnas.0707936104.

Wisler, J. W., Xiao, K., Thomsen, A. R. B., & Lefkowitz, R. J. (2014). Recent developments in biased agonism. *Current Opinion in Cell Biology 27*(1), 18–24. https://doi.org/10.1016/j.ceb.2013.10.008.

Wittau, N., Grosse, R., Kalkbrenner, F., Gohla, A., Schultz, G., & Gudermann, T. (2000). The galanin receptor type 2 initiates multiple signaling pathways in small cell lung cancer cells by coupling to G(q), G(i) and G(12) proteins. *Oncogene 19*(37), 4199–4209. https://doi.org/10.1038/sj.onc.1203777.

Woo, A. Y. H., Jozwiak, K., Toll, L., Tanga, M. J., Kozocas, J. A., Jimenez, L., ... Xiao, R. P. (2014). Tyrosine 308 is necessary for ligand-directed Gs protein-biased signaling of β2-adrenoceptor. *The Journal of Biological Chemistry 289*(28), 19351–19363. https://doi.org/10.1074/jbc.M114.558882.

Woo, A. Y. H., Song, Y., Xiao, R. P., & Zhu, W. (2015). Biased beta2-adrenoceptor signalling in heart failure: pathophysiology and drug discovery. *British Journal of Pharmacology 172*(23), 5444–5456. https://doi.org/10.1111/bph.12965.

Woo, A. Y. H., Song, Y., Zhu, W., & Xiao, R. P. (2015). Advances in receptor conformation research: the quest for functionally selective conformations focusing on the beta2-adrenoceptor. *British Journal of Pharmacology 172*(23), 5477–5488. https://doi.org/10.1111/bph.13049.

Woo, A. Y. H., Wang, T. B., Zeng, X., Zhu, W., Abernethy, D. R., Wainer, I. W., & Xiao, R. P. (2009). Stereochemistry of an agonist determines coupling preference of beta2-adrenoceptor to different G proteins in cardiomyocytes. *Molecular Pharmacology 75*(1), 158–165. https://doi.org/10.1124/mol.108.051078.

Woodard, G. E., Jardín, I., Berna-Erro, A., Salido, G. M., & Rosado, J. A. (2015). Regulators of G-protein-signaling proteins: negative modulators of G-protein-coupled receptor signaling. *International Review of Cell and Molecular Biology 317*, 97–183. https://doi.org/10.1016/bs.ircmb.2015.02.001.

Wu, V., Yang, M., McRoberts, J. A., Ren, J., Seensalu, R., Zeng, N., ... Walsh, J. H. (1997). First intracellular loop of the human cholecystokinin-A receptor is essential for cyclic AMP signaling in transfected HEK-293 cells. *The Journal of Biological Chemistry 272*(14), 9037–9042. https://doi.org/10.1074/jbc.272.14.9037.

Xu, H., Wang, X., Partilla, J. S., Bishop-Mathis, K., Benaderet, T. S., Dersch, C. M., ... Rothman, R. B. (2008). Differential effects of opioid agonists on G protein expression in CHO cells expressing cloned human opioid receptors. *Brain Research Bulletin 77*(1), 49–54. https://doi.org/10.1016/j.brainresbull.2008.05.003.

Xue, L., Rovira, X., Scholler, P., Zhao, H., Liu, J., Pin, J. P., & Rondard, P. (2015). Major ligand-induced rearrangement of the heptahelical domain interface in a GPCR dimer. *Nature Chemical Biology 11*(2), 134–140. https://doi.org/10.1038/nchembio.1711.

Yajima, I., Kumasaka, M. Y., Naito, Y., Yoshikawa, T., Takahashi, H., Funasaka, Y., ... Kato, M. (2012). Reduced GNG2 expression levels in mouse malignant melanomas and human melanoma cell lines. *American Journal of Cancer Research 2*(3), 322–329.

Yang, H. S., Sun, N., Zhao, X., Kim, H. R., Park, H. J., Kim, K. M., & Chung, K. Y. (2019). Role of Helix 8 in Dopamine Receptor Signaling. *Biomolecules & Therapeutics*. https://doi.org/10.4062/biomolther.2019.026.

Yano, H., Cai, N. S., Xu, M., Verma, R. K., Rea, W., Hoffman, A. F., ... Ferré, S. (2018). Gs- versus Golf-dependent functional selectivity mediated by the dopamine D1 receptor. *Nature Communications 9*(1), 486. https://doi.org/10.1038/s41467-017-02606-w.

Yim, Y. Y., Betke, K. M., McDonald, W. H., Gilsbach, R., Chen, Y., Hyde, K., ... Hamm, H. (2019). The in vivo specificity of synaptic Gbeta and Ggamma subunits to the alpha2a adrenergic receptor at CNS synapses. *Scientific Reports 9*(1), 1718. https://doi.org/10.1038/s41598-018-37222-1.

Zamah, A. M., Delahunty, M., Luttrell, L. M., & Lefkowitz, R. J. (2002). Protein kinase A-mediated phosphorylation of the beta 2-adrenergic receptor regulates its coupling to Gs and Gi. Demonstration in a reconstituted system. *The Journal of Biological Chemistry 277*(34), 31249–31256. https://doi.org/10.1074/jbc.M202753200.

Zhang, B., Yang, X., & Tiberi, M. (2015). Functional importance of two conserved residues in intracellular loop 1 and transmembrane region 2 of Family A GPCRs: insights from ligand binding and signal transduction responses of D1 and D5 dopaminergic receptor mutants. *Cellular Signalling 27*(10), 2014–2025. https://doi.org/10.1016/j.cellsig.2015.07.006.

Zhang, H., Han, G. W., Batyuk, A., Ishchenko, A., White, K. L., Patel, N., ... Cherezov, V. (2017). Structural basis for selectivity and diversity in angiotensin II receptors. *Nature 544*(7650), 327–332. https://doi.org/10.1038/nature22035.

Zhang, H., Sturchler, E., Zhu, J., Nieto, A., Cistrone, P. A., Xie, J., ... A, R. (2015). Autocrine selection of a GLP-1R G-protein biased agonist with potent antidiabetic effects. *Nature Communications 6*, 8918. https://doi.org/10.1038/ncomms9918.

Zhang, H., Wang, D., Sun, H., Hall, R. A., & Yun, C. C. (2007). MAGI-3 regulates LPA-induced activation of Erk and RhoA. *Cellular Signalling 19*(2), 261–268. https://doi.org/10.1016/j.cellsig.2006.06.008.

Zhang, J., Cheng, S., Xiong, Y., Ma, Y., Luo, D., Jeromin, A., ... He, J. (2008). A novel association of mGluR1a with the PDZ scaffold protein CAL modulates receptor activity. *FEBS Letters 582*(30), 4117–4124. https://doi.org/10.1016/j.febslet.2008.10.054.

Zhang, L., Zhao, H., Qiu, Y., Loh, H. H., & Law, P. Y. (2009). Src phosphorylation of micro-receptor is responsible for the receptor switching from an inhibitory to a stimulatory signal. *The Journal of Biological Chemistry 284*(4), 1990–2000. https://doi.org/10.1074/jbc.M807971200.

Zhang, Y., Sun, B., Feng, D., Hu, H., Chu, M., Qu, Q., ... Skiniotis, G. (2017). Cryo-EM structure of the activated GLP-1 receptor in complex with a G protein. *Nature 546*(7657), 248–253. https://doi.org/10.1038/nature22394.

Zhao, H., Loh, H. H., & Law, P. Y. (2006). Adenylyl cyclase superactivation induced by long-term treatment with opioid agonist is dependent on receptor localized within lipid rafts and is independent of receptor internalization. *Molecular Pharmacology 69*(4), 1421–1432. https://doi.org/10.1124/mol.105.020024.

Zheng, H., Loh, H. H., & Law, P. Y. (2008). Beta-arrestin-dependent mu-opioid receptor-activated extracellular signal-regulated kinases (ERKs) Translocate to Nucleus in Contrast to G protein-dependent ERK activation. *Molecular Pharmacology 73*(1), 178–190. https://doi.org/10.1124/mol.107.039842.

Zhong, M., Boseman, M. L., Millena, A. C., & Khan, S. A. (2010). Oxytocin Induces the Migration of Prostate Cancer Cells: Involvement of the Gi-Coupled Signaling Pathway.

Molecular Cancer Research 8(8), 1164–1172. https://doi.org/10.1158/1541-7786.MCR-09-0329.

Zhu, W., Petrashevskaya, N., Ren, S., Zhao, A., Chakir, K., Gao, E., ... Xiao, R. P. (2012). Gi-biased β2AR signaling links GRK2 upregulation to heart failure. *Circulation Research 110*(2), 265–274. https://doi.org/10.1161/CIRCRESAHA.111.253260.

Zidar, D. A., Violin, J. D., Whalen, E. J., & Lefkowitz, R. J. (2009). Selective engagement of G protein coupled receptor kinases (GRKs) encodes distinct functions of biased ligands. *Proceedings of the National Academy of Sciences 106*(24), 9649–9654. https://doi.org/10.1073/pnas.0904361106.

Zimmerman, B., Beautrait, A., Aguila, B., Charles, R., Escher, E., Claing, A., ... A, S. (2012). Differential beta-arrestin-dependent conformational signaling and cellular responses revealed by angiotensin analogs. *Science Signaling 5*(221), ra33. https://doi.org/10.1126/scisignal.2002522.