# Functional Selectivity in GPCR Heterocomplexes

J. González-Maeso[1,2,3] and S.C. Sealfon*[,2,3,4]

*Departments of [1]Psychiatry and [2]Neurology; [3]Friedman Brain Institute and [4]Center for Translational Systems Biology, Mount Sinai School of Medicine, New York, New York, USA*

**Abstract:** G protein-coupled receptors (GPCRs) can couple to more than one signaling pathway. Biophysical studies and pharmacological theory indicate that they exist in different active conformations that differ in their capacity to activate specific signaling pathways. Individual agonists stabilize particular active conformations and thereby can differ in their relative activation of different signaling pathways coupled to the same receptor, a phenomenon referred to as functional selectivity. Many pairs of GPCRs have been shown to interact and form heterocomplexes *in vitro* and *in vivo*. Recent studies implicate these complexes in the responses to some therapeutic drugs and drugs of abuse, and raise the possibility that they may be involved in mediating functional selectivity.

**Keywords:** G protein-coupled receptor (GPCR), agonist trafficking, biased agonism, GPCR heteromerization.

## INTRODUCTION

In the mid-1990's, Terry Kenakin introduced the hypothesis that agonists stabilize different GPCR active structural conformations [1], which may differ in their regulation of the activity of separate receptor-dependent signaling pathways (see also [2,3]). This concept of functional selectivity, (initially referred to as agonist trafficking of receptor signaling [1]), has been validated experimentally using a wide range of pharmacological and biophysical tools.

In the classical model of GPCR signaling (ternary complex model), the receptor exists in equilibrium between two structural conformations: active and inactive [4,5]. Based on this model, a single active state may affect separate cellular responses when stabilized by different ligands only to the extent that the intrinsic efficacy of receptor-G protein coupling of the active state correlates with these selective effects. One example of this is the serotonin 5-$HT_{2A}$ receptor ligands ergotamine and lisuride, which act as agonists or antagonists depending on the experimental paradigm used [6,7].

The ternary complex model does not provide a mechanism that explains how ligands targeting the same GPCR subtype modulate the activity of different cellular signaling pathways with equally high efficacy. Lysergic acid diethylamide (LSD) and lisuride are both 5-$HT_{2A}$ receptor partial agonists for activation of $G_{q/11}$ G proteins [8-11], yet LSD (and not lisuride) also activates $G_{i/o}$ G proteins [8,9] and increases the expression of the immediately early genes *egr-1* and *egr-2* [8,12,13]. This finding is not a consequence of a lower efficacy because lisuride (and not LSD) modulates other 5-$HT_{2A}$ receptor-dependent cellular responses such as the expression of *STY kinase* gene. Findings in several experimental systems show differences in efficacy at certain signaling pathways compared to others that are not consistent with a single active conformation of the receptor. The concept of functional selectivity (also termed agonist trafficking and biased agonism) is now widely accepted and has been reviewed elsewhere [14-16].

Another important area in understanding the mechanisms of receptor signaling that may be related to functional selectivity is the expression and function of GPCR as homo- and hetero-complexes. Several lines of evidence support the involvement of GPCR hetero-complexes in the cellular and physiological responses induced upon receptor activation [17-19]. In this review, we will summarize recent advances in our understanding of the structural conformations stabilized by different ligands, and the potential role of GPCR hetero-complexes in the concept of agonist functional selectivity.

## DIFFERENT AGONISTS INDUCE DISTINCT GPCR STRUCTURAL CONFORMATIONS

Biophysical and mutagenesis studies provide evidence for conformational rearrangement during agonist-induced receptor activation that involve TM3, TM5 and TM6 domains [20]. Using an approach based on fluorescent labeling of purified GPCRs, a series of studies by Kobilka and co-workers demonstrate the agonist-induced clockwise movement of the cytoplasmic part of TM6 toward TM5. The $\beta_2$-adrenergic receptor was purified and labeled with fluorescent maleimide on $Cys265^{6.27}$. The authors examined ligand-dependent changes in fluorescent lifetime ranging from several nanoseconds to a few picoseconds based upon measuring the interaction between the fluorescein and fluorescent quenching reagents located at different structural domains of the receptor [21,22]. They also mutated $Ala271^{6.33}$ and $Ile135^{3.54}$, which are in close proximity to each other and well positioned for fluorescent quenching. They found that when the receptor is bound to a full agonist, the domains involved in G protein coupling are present in two different conformations that can be distinguished from

*Address correspondence to this author at the Departments of Center for Translational Systems Biology, Mount Sinai School of Medicine, New York, New York, USA; Tel: (212) 241-7075; Fax: (212) 987-7635;
E-mail: stuart.sealfon@mssm.edu

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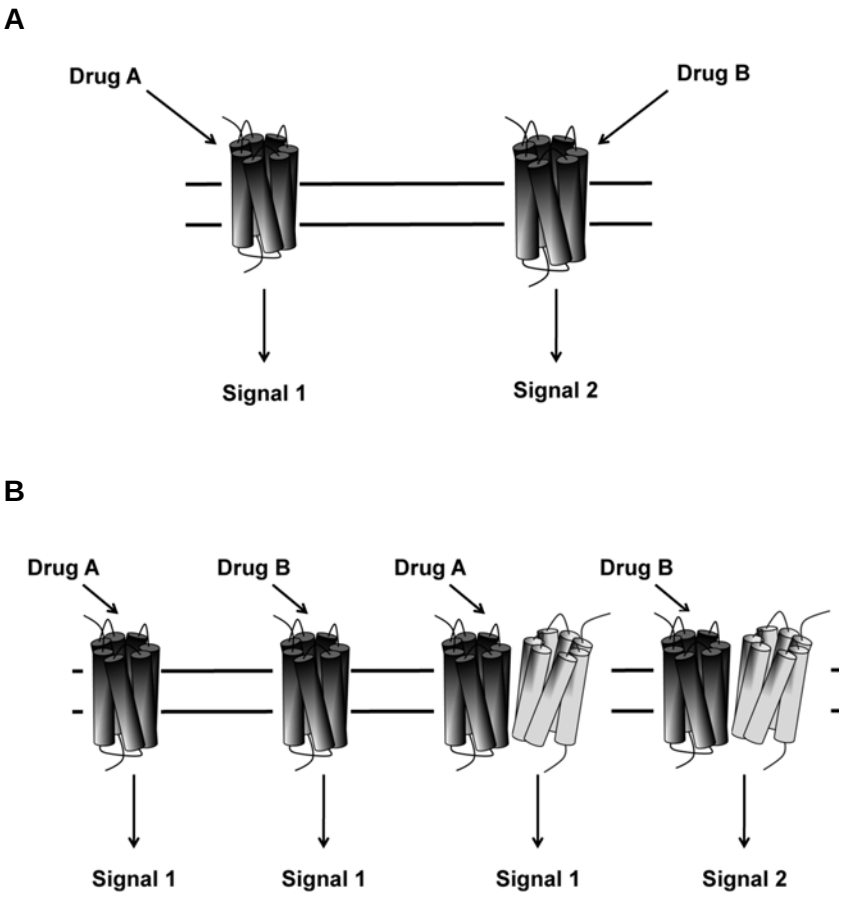


**Fig. (1).** Schematic model of agonist functional selectivity in GPCR heterocomplexes. **A,** Agonists stabilize different active conformations and modulate two different patterns of cellular signaling pathways. **B,** Crosstalk between the components of a GPCR heterocomplex is necessary to translate the agonist-dependent active conformations into different cellular responses.

those stabilized by partial agonists [23]. It was suggested that these two structural states might be part of a larger number of conformations differentially stabilized by ligands.

Recent crystal structures have provided further insight into the active and inactive conformations of certain GPCRs, including the stabilized active state of the $\beta_2$-adrenergic receptor [24] and the irreversible agonist-$\beta_2$-adrenergic receptor complex [25]. These findings demonstrate an 11 Å outward movement of TM6 and inward movement of TM3 and TM5 as the largest change induced by full agonists at the $\beta_2$-adrenergic receptor (see also [26-29] for additional crystal structures). Within the ligand binding pocket, agonists alter packing interactions involving TM3, TM5, TM6 and TM7, which results in a rotation of TM6 and an outward movement of the cytoplasmic half of TM6 together with other changes. Based on these findings and using an NMR spectroscopy approach, further investigation also demonstrated correlations between distinct extracellular surface conformations of the $\beta_2$-adrenergic receptor and the cytoplasmic conformations differentially affected by full agonists, partial agonists, neutral antagonists and inverse agonists [30]. Studies with crystal structures of the $\beta_1$-adrenergic receptor bound to full and partial agonists showed differences in the interactions of full and partial agonists with specific transmembrane side chains. Two conserved residues in TM5 (Ser211$^{5.42}$ and Ser215$^{5.46}$) form hydrogen bonds with full agonists [31]. However, partial agonists from hydrogen bonds only with Ser211$^{5.42}$, and not with Ser215$^{5.46}$ [31]. Of interest, a similar mechanism has been proposed using an intramolecular fluorescence resonance energy transfer (FRET) approach with the donor fluorophore fluorescein arsenical helix binder (FlAsH) attached to the cytoplasmic end of TM6 and the acceptor Alexa 568 attached to Cys265$^{6.27}$ [32]. These findings demonstrate that structurally different ligands induce specific changes in intramolecular FRET signal, which supports the existence of ligand-specific receptor conformations.

The hypothesis of functional selectivity is further supported by studies *in vivo* in murine models. Some of these examples include the μ- and δ-opioid receptors (MOR and DOR). A large number of experimental results converge toward the idea that lateral domains enriched in sphingomyelin and cholesterol exist in biological membranes [33]. These nanosized domains, called functional lipid rafts, have been suggested to take part in various dynamic cellular processes such as signal transduction, membrane trafficking and modulation of the activity of membrane proteins [34]. It has been shown that, in the absence of agonist, the MOR is located within the lipid raft domains. The MOR agonist etorphine, but not the MOR agonist morphine, induces the

translocation of the MOR from lipid raft to non-raft domains [35]. The molecular mechanism is based upon the different abilities of these two MOR agonists to modulate either $G\alpha_{i2}$-dependent or β-arrestin-dependent signaling [35].

DORs have been shown to play a role in chronic, but not in acute, pain. Recent studies investigated analgesic tolerance of two DOR agonists with similar pharmacological profiles and analgesic effects, but high (SNC80) and low (ARM390) potencies of DOR internalization [36]. The authors found that, in mouse models, chronic treatment with SNC80 resulted in severe downregulation of the DOR, but the expression of DOR at the plasma membrane was not affected by chronic treatment with ARM390. Chronic SNC80 and ARM390 both produce analgesic cross-tolerance, but the tolerance induced by chronic ARM390 is dependent on a different mechanism that requires adaptive responses at the level of voltage-dependent $Ca^{2+}$ channels in dorsal root ganglia [36].

All these findings support the significance of functional selectivity using biophysical assays *in vitro* and as well as murine biochemical and behavioral models. However, a new explanation of functional selectivity has recently been proposed with which different ligands induce unique cellular responses through a mechanism that requires crosstalk between the components of a GPCR heterocomplex.

## GPCR HETEROCOMPLEXES AND THEIR ROLE IN FUNCTIONAL SELECTIVITY.

GPCRs were thought to function as monomers in which one GPCR molecule was able to couple to and activate a single G protein. In the last several years, however, several lines of evidence support the hypothesis that GPCRs are expressed as homo- and hetero-dimers, or even higher order oligomers (see [19] for review). It has been only in the last few years that functional selectivity and crosstalk between the components of a GPCR heterocomplex has begun to emerge as a novel target for selective drug design. Among these, we will focus especially on the serotonin-glutamate 5-$HT_{2A}$-mGlu2 and the dopamine D1-D2 receptor heterocomplexes.

Hallucinogenic drugs of abuse such as LSD, mescaline and psilocybin all have in common a high affinity for serotonin 5-$HT_{2A}$ receptors [6,15]. However, not all the drugs that activate the 5-$HT_{2A}$ receptor have psychotic properties. For instance, drugs such as lisuride and ergotamine bind to and activate the 5-$HT_{2A}$ receptor, yet they are not psychoactive and are indeed used as therapeutic drugs in the treatment of Parkinson's disease [37] and migraine [38], respectively. The 5-$HT_{2A}$ receptor modulates the activity of different signaling pathways when activated by chemically distinct ligands. Hallucinogenic drugs (DOI, DOM, DOB, mescaline, psilocin and LSD) induce the expression of *c-fos* and *egr-2* genes in mouse somatosensory cortex, an effect that required both $G_{q/11}$- and $G_{i/o}$-dependent signaling mechanisms [8,12]. However, non-hallucinogenic drugs (R-lisuride, S-lisuride, and ergotamine), although they induce a 5-$HT_{2A}$-dependent and $G_{q/11}$-dependent expression of *c-fos*, the expression of *egr-2* was absent—a cellular response that correlated with the behavioral response head-twitch induced in murine models [8,12]. A similar pattern of $G_{q/11}$ versus $G_i$ G protein coupling downstream the 5-$HT_{2A}$ receptor was found with DOI, LSD and lisuride in CHO cells [9,10]. It has also been demonstrated that DOI and the serotonin precursor L-5-hydroxytryptophan (5-HTP) induces head-twitch through different signaling mechanisms [39,40]. 5-HTP induces head-twitch by a mechanism that requires β-arrestin-2, whereas DOI induces a β-arrestin-2-independent head-twitch behavior. These findings all suggest functional selectivity of hallucinogenic and non-hallucinogenic 5-$HT_{2A}$ agonists. However, they do not unravel the mechanism through which LSD-like drugs activate $G_{q/11}$- and $G_{i/o}$ G proteins, whereas non-hallucinogenic 5-$HT_{2A}$ agonists do not. Physiologically, it appears that activation of metabotropic glutamate 2 receptor (mGlu2) modulates the cellular and behavioral responses that require 5-$HT_{2A}$ receptor function [41,42]. It was recently reported that 5-$HT_{2A}$ and mGlu2 are co-expressed in cortical pyramidal neurons, and that they form a GPCR heterocomplex in human frontal cortex [43] and in tissue cultures [43,44]. The functional significance of this serotonin-glutamate heterocomplex has been demonstrated using [$^{35}$S]GTPγS binding assays followed by immunoprecipitation with anti-$G\alpha_{q/11}$ or anti-$G\alpha_{i1,2,3}$ antibodies. It was shown that the hallucinogenic 5-$HT_{2A}$ agonist DOI activates $G_{q/11}$ and $G_i$ G proteins only when the 5-$HT_{2A}$ receptor is expressed as a receptor heterocomplex with the mGlu2 receptor [43]. The behavioral significance of the 5-$HT_{2A}$-mGlu2 receptor heterocomplex has been recently demonstrated with the use of head-twitch behavior, which is a mouse behavior model of hallucinogenic action [8,12]. Thus, hallucinogenic 5-$HT_{2A}$ receptor agonists do not induce head-twitch behavior in mGlu2 knockout mice [45]. Since expression of the components of the 5-$HT_{2A}$-mGlu2 receptor heterocomplex has been shown to be dysregulated in postmortem human brain of untreated schizophrenic subjects [43], these findings suggest that the glutamate-serotonin receptor complex might be responsible for some of the psychotic symptoms in schizophrenia.

The neurotransmitter dopamine has been shown to play key role in regulating brain functions involved locomotion, cognition, reward, and emotion [46]. Alterations in the dopaminergic system have been implicated in a number of neuropsychiatric disorders such as schizophrenia, drug abuse, and Parkinson's disease [47]. The dopamine receptors are divided into two broad classes: D1-like and D2-like. The D1-like receptors include D1 and D5, while the D2-like are D2, D3 and D4 [48]. The major signaling pathway of the D1-like receptors is stimulation of adenylate cyclase (AC) via activation of $G_s$ proteins, whereas activation of D2-like receptors inhibits AC through coupling to $G_{i/o}$ proteins. It has been shown that D1 and D2 receptor subtypes co-localize in several regions of the basal ganglia, including nucleus accumbens, globus pallidus and caudate putamen [49]. Several approaches have shown that D1 and D2 receptors form a GPCR heterocomplex *in vitro* and in the basal ganglia (see [50] for review). Among these, co-immunoprecipitation [51] and FRET in primary striatal neurons [52], and in both striatum and nucleus accumbens [49]. More importantly, there is a functional crosstalk between the components of the D1-D2 heterocomplex, and co-application of the D1 receptor

agonist SKF83959 and the D2 receptor agonist quinpirole induced a concentration-dependent rise in calcium in HEK293 cells. This effect was dependent upon $G_{q/11}$ activation of PLC and production of $IP_3$, and was independent of AC activity. The D1-D2 receptor heterocomplex shows a different pharmacological profile compared to that of D1 or D2 homo-complexes. Thus, SKF83959 activates the heteromer by binding to both D1 receptor and a different structural conformation of the D2 receptor that depends on the D1-D2 receptor heterocomplex, which leads to activation of $G_{q/11}$ protein-dependent signaling pathways [52,53]. The potential implications of the D1-D2 receptor heterocomplex in neuropsychiatric disorders have been further supported with preclinical findings in rodents and pharmacological assays in postmortem human brain of schizophrenic subjects. The fraction of high-affinity binding sites of SKF83959 displacing [$^3$H]raclopride was increased in globus pallidus of schizophrenic subjects [49]. It has also been shown that disruption of the D1-D2 receptor heterocomplex in mouse prefrontal cortex induces antidepressant-like effects [54]. The authors found that co-immunoprecipitation of D1 receptor by the D2 receptor-specific antibody was significantly increased in postmortem human brain of subjects with major depression compared to controls. Glutathione-S-transferase (GST) fusion proteins have a range of applications since their introduction as tools for synthesis of recombinant proteins in bacteria [55]. The authors prepared various GST fusion proteins containing different regions of D1 and D2 receptors [54]. Interestingly, they found that the region of Met257-Glu271 of the D2 receptor ($D2_{IL3\text{-}29\text{-}2}$) can pull down the D1 receptor. Using a similar approach, they concluded that D1 receptor interacts with D2 receptor through the D1 C-terminal domain. More importantly, local administration of the peptide $D2_{IL3\text{-}29\text{-}2}$ into mouse frontal cortex (but not hippocampus or nucleus accumbens) exerts antidepressant-like effects in the forced swim test without affecting basal locomotor activity [54]. Antidepressant treatments require weeks of months to produce a therapeutic response [56]. These findings with the peptide $D2_{IL3\text{-}29\text{-}2}$ may provide a new approach to accelerate and improve the treatment of major depression and mood disorders.

## CONCLUSIONS AND FUTURE DIRECTIONS

In general, there is evidence to support the existence of GPCR hetero-dimers and heterocomplexes in tissue cultures and most importantly in native tissue preparations. What remains to be established is the physiological and/or behavioral significance of GPCR heterocomplexes *in vivo* in whole animal models and their possible therapeutic implications. Similarly, although the notion of ligand-selective GPCR conformations and its physiological relevance is widely accepted, the effect of agonist functional selectivity and biased agonism on the stability and functional crosstalk between the components of GPCR heterocomplexes remains are active area of exploration. A better understanding of the effects of drugs on the structure, pharmacology and dynamics of GPCR heterocomplexes may provide the basis for the rational design of novel compounds with therapeutic potential that modulate the activity of the signaling pathways that are only affected by GPCR heterocomplex function.

## CONFLICT OF INTEREST

None declared.

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