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Physiological relevance of GPCR oligomerization and its impact on drug discovery

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The potentially large functional and physiological diversity of G-protein coupled receptor (GPCR) dimers has generated a great deal of excitement about the opportunity that dimerization provides for enabling novel drug discovery. The discovery of physiologically relevant GPCR dimers suggests that new drug targets for diseases such as schizophrenia and pre-eclampsia can be developed by targeting dimers. Most of the previous work on GPCR dimers made use of the overexpression of differentially tagged GPCRs in heterologous cell systems. Current emphasis on the development of physiologically relevant cell systems that endogenously express the appropriate combination of GPCR dimers and accessory proteins is leading to dramatic increases in our understanding of GPCR dimers. These and other new tools such as GPCR-specific antibodies will be required to develop GPCR dimer specific drugs. Given that ligands are available for only a small percentage of the large number of potentially druggable GPCRs, the use of GPCR dimers might provide the necessary targets to increase the breadth and depth of receptors available for therapeutic interventions.

G-protein coupled receptors (GPCRs) are seven transmembrane domain (TMD) proteins that mediate the intracellular effects of a large number of different extracellular stimuli that include light, odorants, neurotransmitters and hormones [1]. It is well established that GPCRs are involved in mediating a multitude of physiological and pathophysiological processes [2–4]. Not surprisingly, GPCRs are the primary target for 26.8% of prescription drugs and new drug candidates are continually being developed for selective GPCRs [5–7]. Additionally, GPCRs are also of great importance in other aspects of human biology including drug addiction because many recreational drugs target these receptors [8,9].

GPCRs are associated with a heterotrimeric G-protein that consists of α, β and γ subunits with the G_α subunit bound to GDP [1]. Upon occupancy by the agonist, the receptor conformation is shifted to the active state, causing the heterotrimeric G-protein to dissociate. This results in the displacement of the GDP by GTP on the G_α subunit while the $G_{\beta\gamma}$ subunits remain bound. Both $G_{\beta\gamma}$ and the GTP bound G_α are thus activated and can stimulate or inhibit effector proteins such as adenylyl cyclase, phospholipases and a variety of ion channels [1]. The observed diversity in the responses in terms of magnitude and physiological effects of different GPCR agonists has historically largely been attributed to the large repertoire of available G-protein and effector systems. Although the basic tenets of this model have stood up to vigorous testing, it is increasingly apparent that the model is greatly oversimplified [10–12]. For example, up until a few years ago it was largely thought that the intrinsic GTPase activity of the G_α subunit was sufficient to regulate the G-protein activation/deactivation cycle. Today, it is well known that the regulators of G-protein signaling (RGSs) serve as GTPase activating (or accelerating) proteins (GAPs) and are capable of greatly stimulating the intrinsic GTPase activity of most G_α proteins [13]. Thus, alterations in the levels of RGS proteins will greatly influence GPCR-mediated responses without altering the levels of active receptor.

In addition to RGSs, a multitude of other GPCR interacting proteins (GIPs) have been identified and the functional implications of their interactions are being characterized [14–16]. For example, the brain has been shown to express dozens of different

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dopamine receptor interacting proteins (DRIPs) that have been shown to interact with the dopamine D2 receptor [17]. A variety of terms including signalsome and signaplex have been coined to describe the protein complexes that are now known to be involved in mediating the effects and the regulation of GPCRs [17]. These complexes are involved in all the aspects of GPCR biology including receptor targeting, pharmacology, potency and signaling specificity. For example, proper targeting of the α_{1D} adrenergic receptor requires the scaffolding protein dystrophin [18]. A more widespread example involves the ability of many different GPCRs to signal to different intracellular pathways in both G-protein-dependent and -independent manners [1,12,15]. A single GPCR can activate different G-proteins and associated effector systems or they can activate different intracellular pathways by interacting with and activating other proteins such as β-arrestins [19]. The stimulation of two different effector systems for GPCRs like the β_2-adrenergic receptor (β_2AR) can lead to the concomitant activation of adenylyl cyclase via $G_{\alpha s}$ proteins and the ERK1/2 MAP kinases via the recruitment of β-arrestin. There is clinical significance to such findings because the β-blocker carvedilol antagonizes the $G_{\alpha s}$ pathway while stimulating the β-arrestin pathway [20]. Such pharmacological and functional pleotropism also serves to increase the repertoire of GPCR-mediated responses [10,11].

Increases in our understanding of the effects of the cellular and phenotypic context encountered by endogenously expressed receptors along with a greater appreciation of the allosteric complexity of GPCRs themselves have led to some fairly dramatic changes in the way GPCRs are viewed and studied [4,10,11,21]. As well as posing a great challenge, these new aspects of GPCR biology are also of great interest because there is enormous potential for drug discovery. To take advantage of the knowledge gained, these new paradigms will have to be taken into account when developing strategies to screen for new GPCR drugs and the functional analysis of the newly identified compounds [4,22–24]. Although almost 30% of all prescription drugs target GPCRs, it should be noted that only a very small number of the total repertoire of GPCRs are actually targeted. Of the 323 GPCRs that are estimated to have bona fide possibilities of being drug targets (excluding odorant and taste receptors), only a small number of these (46) are actually used [3,22]. Adapting drug discovery strategies to the new GPCR paradigms might allow this vast potential of untapped GPCRs to be targeted [4,22–25].

Of all the new aspects of GPCR biology, GPCR dimerization (or oligomerization), the ability of these receptors to interact with each other in conjunction with agonist trafficking, is likely to have profound impacts on drug discovery. The ability to form homo- and hetero-dimers involving diverse GPCR subtypes with the same or different ligand has potentially far reaching implications, especially with regard to drug discovery [26,27]. Although this topic has been extensively reviewed in recent years [26,28–34], in this review we examine some recent advances in the field that serve to exemplify some of the successes, pitfalls and practical aspects of the biology of GPCR dimerization.

GPCR dimers are ubiquitous

Although there was some early evidence that GPCRs might exist as dimers [35], it was not until the discovery that a dimer between the

TABLE 1

Examples of GPCR dimers[a]

Names of GPCRs	Function	Highlight	Clinical relevance	Ref
$GABA_BR1$–$GABA_BR2$	Targeting	Both receptors required for $GABA_BR$ function	$GABA_BR$ agonist Baclofen is an antispasm	[36]
T1R1–T1R3	Targeting	Dimer is required for umami taste	?	[37]
T1R1–T1R2	Targeting	Dimer might be required for sweet taste	?	[37]
EP_1–β_2AR	Decrease potency	Decrease airway smooth muscle relaxation	Asthma?	[38]
KOR–DOR	Altered pharmacology	6′-GNTI is selective for the dimer	Tissue-specific agonist for pain	[40]
KOR–CXCR4	Altered desensitization	Fate of one GPCR can be altered by different GPCR	Pain?	[46]
GnRHR–GnRHR	Dominant negative	ER-trapped GnRHR mutant retains wild-type GnRHR in the ER	Hypogonadotropic hypogonadism	[49]
MT_1–GRP50	Dominant negative	Orphan GPR50 might be a ligand-independent regulator	?	[51]
CB_1–A(2A)R	Coactivation	Adenosine interferes with motor responses to cannabinoid in the rat brain	Parkinson's disease?	[62]
5-HT_{2A}–mGLuR2	Altered signaling	Site of action of hallucinogenic drugs	Schizophrenia?	[63]
AT_1–B_2	Altered potency	Increased dimers observed in patients with pre-eclampsia	Pre-eclampsia hypertension	[64]

[a] See text for more details.

two different GABA receptors, $GABA_BR1$ and $GABA_BR2$, was required to form fully functional GABA receptors and the existence of GPCR dimers was commonly accepted [36]. When expressed alone $GABA_BR1$ is retained in the ER because it requires the coexpression of $GABA_BR2$ to be properly targeted to the cell surface. Furthermore, both subunits seem to have further distinct roles in the complex given that the $GABA_BR1$ seems to be largely responsible for ligand binding while the $GABA_BR2$ is required for G-protein coupling. Further support for the fact that GPCR dimers might in fact be more like a functional synergy of two or more proteins than a simple complex of proteins came from the dimers formed by the family of three taste receptors (T1R1, T1R2 and T1R3) [37]. In addition to being required to get to the cell surface, dimerization of the different family members is required for the response to certain agonists. Thus T1R1 and T1R3 dimers are required for umami taste while T1R1 and T1R2 dimers seem to behave like the well-known sweet receptor. The GABA and T1R receptors are typical of the C family of GPCRs and serve to illustrate the ubiquity of the process of dimerization for the receptors in this family [3].

In many ways, the A or the rhodopsin family of GPCRs is of more general interest because it is the largest family and it contains most of the receptors for well-characterized agonists such as adrenergic, opioid and dopaminergic [3]. A large number of *in vitro* studies have served to catalog the ability of different family members to form homo- and hetero-dimers when overexpressed in cultured mammalian cell systems [30]. Here we will largely focus on hetero-dimers given that these are of more general interest in terms of developing novel drug targets. These types of experiments have been useful in documenting the apparent selectivity in the choice of receptors that can form dimers as well as some of the functions of the different dimers. The latter includes proper targeting of the receptor (biogenesis), altered coupling, altered pharmacology, altered internalization and ligand-independent functions such as the ability of some receptors to function as dominant negatives. Several interesting examples exist for each of these categories and here we will highlight a few examples that are likely to be functionally relevant (Table 1).

Functions of GPCR dimers

The formation of dimers to achieve appropriate targeting of a given receptor is a commonly observed phenomenon in the field [28]. Mutant receptors unable to leave the ER often serve to trap wild-type receptors in the ER and prevent them from reaching the cell surface. In a few cases, the wild-type receptor can rescue an ER-trapped mutant receptor and bring it to the cell surface. Thus many early studies exploited this phenomenon to identify potential GPCR dimers [1]. It should be noted that the use of a GPCR as a chaperon to achieve proper targeting for another GPCR is not limited to GPCRs. Receptor activity-modifying proteins (RAMPS) are probably the most widely studied example of these non-GPCR chaperons [1]. These single TMD proteins are absolutely essential for the targeting of the calcitonin-receptor-like receptor (CRLR) and surprisingly they also have profound effects on receptor pharmacology [16].

There are many reported cases where one of the receptors in a GPCR dimer can serve to alter the coupling or regulate the potency of the other receptor [27]. Using the contraction of airway smooth muscle (ASM) cells as a physiological readout, McGraw *et al.* [38] were able to show that stimulation of the prostaglandin E1 EP_1 receptor in an EP_1–β_2-adrenergic dimer results in a decrease in the ability of β_2-adrenergic-mediated ASM relaxation. This example serves to highlight that there will be tissue-specific effects of some GPCR ligands depending on the dimer that is expressed.

Alterations in the pharmacology of individual receptors in a dimer, such that the dimer has a new pharmacological profile, might be one of the most interesting aspects of dimerization when it comes to drug discovery [31,33] (see Box 1). Drugs targeting opioid receptors have an important effect in alleviating pain but it is well known that the activation of opioid receptors also leads to complications with tolerance, addiction and desensitization in

BOX 1

Development of GPCR dimers as drug targets

Bouvier's group provided one of the earliest studies that directly examined GPCR dimerization [84]. They used sequential immunoprecipitation to demonstrate that the overexpressed differentially tagged β_2-adrenergic receptors (β_2AR) form homo-dimers. This group additionally showed that a peptide corresponding to the sixth transmembrane domain (TMD) portion of the β_2AR could be used to disrupt the dimer and decrease receptor function. Others have shown that the use of TMD-derived peptides could also be used to disrupt dimers for other GPCRs [85,86]. It has recently been shown that a dimer-inhibiting TMD4 peptide of the chemokine receptor CXCR4 shows promise as a therapeutic strategy for several inflammatory conditions [86]. Some drugs like the opioid agonist 6′GNTI originally thought to be specific for the KOR receptor have been shown to have preference for the KOR–DOR opioid receptor dimer [40]. In line with these data, many groups have tried to identify contact sites in GPCR dimers to guide them in developing models that would permit rational drug design. Computer modeling followed by mutagenesis studies has been used with varying degrees of success to identify crucial regions involved in dimerization [87]. The difficulties encountered are not surprising considering that an understanding of the fine structure of GPCRs is still lacking so that even rational drug design of monomeric GPCRs cannot be generically applied [88]. Disulphide trapping and domain swapping experiments have also been used to identify crucial residues involved in dimerization [63,89,90]. Most of these studies point to the importance of the TMDs of family A receptors being involved in dimer formation [30]. The ability to develop models of GPCR dimers would theoretically allow the development of dimeric or bivalent drugs that could interact with both receptors in a dimer and provide potentially new therapeutics [91]. With this in mind, rational strategies to develop novel bivalent ligands for dimeric receptor combinations such as the Mu/Kappa opioid receptors as well as homo-dimeric gonatropin-releasing hormone receptors (GnRHRs) have been reported [92,93]. Although such strategies have had only modest success, they might prove much more fruitful when combined with our increasing knowledge of the structure of GPCR dimers [34,91]. Of increasing importance to our understanding of the pharmacology of GPCRs is the expansion of the concept that GPCRs are highly allosteric proteins [94]. Thus GPCR ligands that bind to sites other than the natural orthosteric agonist binding site can serve to modulate GPCR responses. The specificity of allosteric modulators for GPCR dimers is also a growing interest [31,33,95,96]. Because individual receptors in a dimer appear to cooperate to form the functional GPCR dimer, the potential exists that allosteric modulators for one receptor might affect the function of each dimer partner.

patients chronically administered with opioid agonists [8]. The opioid receptors provide one of the more compelling examples that illustrate the potential that dimerization has in increasing our ability to develop highly specific drugs. The κ-opioid receptor (KOR) has been shown to dimerize with the δ-opioid receptor (DOR) [39]. The pharmacology of this dimer was also found to differ from either of the monomers such that the agonist, 6′-guanidinonaltrindole (6′-GNTI), which was previously thought to be a KOR selective analgesic agonist, has been shown to be selective for the dimer. Furthermore, the effect of 6′-GNTI is tissue selective because analgesia was observed when it was injected in the spinal cord of the mouse but not in the brain [40]. These and other studies suggest that highly selective drugs having fewer side effects can be developed by targeting GPCR dimers expressed in tissue-specific manners [41]. There is evidence that several other drugs that were originally believed to be agonists for a single GPCR including antiparkinsonian and antipsychotic dopamine ligands [42,43] might well be specific for GPCR dimers. Finally, there are other drugs that are thought to have therapeutic value in part because they cross-bind several different receptors [22]. Some of the off-target effects of these so-called 'dirty drugs' may be because of their ability to bind GPCR dimers.

Homologous desensitization of a GPCR is largely because of agonist-mediated molecular changes on the receptor that can lead to its internalization [44]. The rate and extent of internalization is very much receptor and context dependent [45]. Some receptors internalize rapidly while others appear to avoid internalization altogether. Although receptor internalization was once thought to be exclusively involved in terminating signal responses, it is now well known that internalized GPCRs might also be involved in activating new signaling cascades [12]. Heterologous desensitization by contrast involves the process by which the agonist-mediated activation of one receptor leads to the inactivation (and often the internalization) of a different receptor. Receptor-mediated activation of a protein kinase (such as PKA) that can phosphorylate the unoccupied receptor is the common mechanism evoked to explain this phenomenon. Numerous studies, most of which are on the basis of receptor overexpression, have demonstrated that the rates of desensitization and/or internalization of a given receptor can be greatly influenced by coexpressing another receptor, as observed for KOR and the chemokine CXCR4 receptor [32,46]. Many of these cross-desensitizations, including the CXCR4-mediated decrease in KOR responsiveness, are also observed *in vivo* [46]. Despite these findings and the fact that appropriate controls were performed that show receptor specificity in the process, there remains a great deal of scepticism as to whether dimer formation or some other mechanism is involved in the observed effects of coexpressed receptors on internalization [32,34].

Coexpression of a mutant GPCR receptor (i.e. unable to bind agonist, to leave the ER or to activate the G-protein) with a wild-type homologous or heterologous receptor will often result in blocking the function of the wild-type receptor [47]. This type of dominant negative effect can be observed in yeast and it might also be responsible for certain forms of genetically dominant diseases associated with defective GPCRs [48]. For example, in hypogonadotropic hypogonadism a mutant allele expressing an ER-trapped gonadotropin-releasing hormone receptor (GnRHR) can serve as a dominant negative and block the biogenesis of the receptor that is expressed by the wild-type allele [49]. The development of cell permeable receptor specific chaperon-like drugs is likely to be an effective therapeutic avenue for these types of syndromes [50]. The use of dominant negative GPCRs has also been shown to be part of the normal repertoire that is used to regulate receptor signaling [47]. For example, alternative splicing of numerous GPCR-encoding genes, including the dopamine D3 and the calcitonin receptors produces truncated proteins that dimerize with their cognate receptor and serve as dominant negative regulators [26]. Other examples include the identification of some orphan receptors, like GPR50, that are capable of inhibiting the heterologous melatonin MT_1 receptor [51]. Using an immortalized human endothelial cell line that endogeneously expresses both receptors, it was shown that siRNA-mediated downregulation of GPR50 expression by 60% was sufficient to increase the level of pharmacologically detectable MT_1 receptors and to increase its signaling potency. There is somewhere in the order of 140 orphan receptors identified in the human genome and it has been postulated that no actual ligands might exist for many of these orphan receptors and instead they would dimerize with, and regulate in a ligand-independent manner, the signaling response of other GPCRs [30,51]. These and other studies suggest that siRNA might be used against certain GPCRs to modulate responses for therapeutic purposes [47].

Criticism of the ubiquity of dimers

One of the rallying cries from some of the proponents of GPCR dimerization is centered around the concept that GPCRs are born, function and die as dimers (or oligomers) [29]. The evidence for this concept is very strong in many cases especially for family C receptors, including $GABA_BRs$. For family A receptors, a good deal of the support for this concept is on the basis of experiments that involve co-overexpression of differentially tagged GPCRs in cultured mammalian cells. Methodologically, the interactions between receptors can be detected using sequential immunoprecipitation (i.e. HA, c-myc tags) or by resonance energy transfer (RET) techniques (i.e. luciferase, different fluorescent proteins (YFP) tags) such as fluorescence resonance energy transfer (FRET) or bioluminescence resonance energy transfer (BRET) [27,30,34]. Many modifications of these techniques have been developed, some of which are rather sophisticated and allow for precise monitoring of GPCR–GPCR interaction under different conditions such as the real time examination of their interactions [52]. Although no one now refutes the existence of GPCR dimers on the basis of the cumulated data available, there is nevertheless debate within the field regarding the interpretation of GPCR dimerization data [53,54]. The majority of this criticism is on the basis of the overinterpretation and over-reliance on methodologies that have some potential shortcomings. One major criticism is in regard to the fact that most studies rely on the use of transfected cells that overexpress tagged GPCRs [34,53]. The fact that these proteins are endogenously expressed at very low levels coupled to the lack of high-quality antibodies to GPCRs, makes it such that their overexpression is in most cases required to monitor the receptor [25]. Overexpressed GPCRs represent a nonphysiological increase in the levels of the receptor, so it is not hard to imagine that under such conditions there would not be a certain

amount of nonphysiological interactions [55]. These issues are now largely being addressed and there are efforts to control expression levels by using low concentrations of DNA for transfection, as well as by using inducible promoters, to obtain cell lines that express endogenous-like levels of tagged GPCR proteins [55]. Meyer *et al.* [55] addressed this issue by using FRET of fluorescent-labeled neurokinin-1 receptors (NK1R) in HEK293 cells expressing physiological levels and supra-physiological levels of the receptor. They found that NK1R could be found to interact with each other only when overexpressed at high levels. In addition, evidence was presented that the interaction was because of the accumulation of NK1R in cholesterol-rich microdomains and thus the observed positive FRET responses might represent nonspecific receptor interactions. It should nevertheless be noted that in yeast, where physiological levels of the tagged receptors can be studied, all the available evidence strongly suggests that the adage regarding the birth and death of GPCRs as dimers is true at least as it pertains to the *STE2*-encoded GPCR [48] (see Box 2). RET methodologies also come under scrutiny [34] because some critics suggest that RET signals might be seen if GPCRs are in close enough proximity without actually touching each other [53]. Other criticisms, including some physicochemical considerations, suggest that many GPCR dimers might only exist transiently like many other interacting proteins while some studies suggest that some dimers might be formed in response to or during agonist binding [32,54].

One particularly appealing and compelling piece of evidence for higher order GPCR structures is the images published on cryoelectron microscopy of rhodopsin in outer trod segments. They show the receptors in tight groups that suggest a complex quaternary structure [56]. Models, supported by this and a variety of other data, have been put forward to suggest that dimeric forms of rhodopsin and many other GPCRs would be the functional signaling that serves as the activator of a heterotrimeric G-proteins. Biochemical evidence has previously demonstrated that GPCRs were functionally monomeric proteins and a recent review of these studies suggests that the data still hold true today [57]. More recent studies using reconstituted GPCRs show that a variety of these receptors, including rhodopsin, β_2-adrenergic and neurotensin receptors, are capable of activating G-proteins as monomers [58–60].

Taking everything together, it is argued that a more modest interpretation of the available data, and avoiding 'sweeping generalizations', would allow one to conclude that the immense diversity of GPCR structure and function will also apply to the dimerization of GPCRs [53].

BOX 2

Yeast as a genetic system to characterize mammalian GPCR dimers

The process of mating in the yeast *Saccharomyces cerevisiae* is largely mediated by GPCRs [97]. Using powerful genetic approaches, a great deal of information regarding the process of signaling from the α-factor binding GPCR (Ste2p) has been uncovered in yeast including the first demonstration that the $G_{\beta\gamma}$ subunit can mediate the signaling responses of GPCRs, as well as the identification of regions within the endogenous α-factor receptor that are responsible for its dimerization [48,97]. Yeast strains have been developed that have allowed for the functional expression of a multitude of different mammalian GPCRs [98]. This has been exploited for several purposes including the identification of functional important residues in different GPCRs and the identification of agonists capable of activating orphan GPCRs [99,100]. Of interest here, the analysis of dimerization of the mammalian C5a GPCR, including the identification of regions within the receptor that are likely to be involved in mediating this interaction, has also been carried out in yeast [48]. Finally it has recently been demonstrated that the interaction of the ligand bound GPCRs for the two different sex pheromones (Ste2p for α-factor and Ste3p for a-factor) appears to be a necessary step for the fusion of the two haploid cells that occurs during mating [101]. The latter study raises the possibility of using a mating assay to quickly identify mammalian GPCRs that can interact with each other and to develop possible high-throughput assays to identify crucial residues and develop drugs capable of blocking dimerization. Genetic modifications including the removal of the endogenously expressed Ste2p receptor have served to increase the number of mammalian GPCRs that can be expressed in yeast. Many of these heterologously expressed receptor can now be shown to couple to the endogenous pheromone response signaling pathway [98]. Nevertheless, there are still several mammalian GPCRs that cannot be functionally expressed in yeast [102]. It seems probable that the removal of the Ste2p receptor increased the signaling responses from heterologous mammalian GPCRs because the endogenous receptor can interfere with mammalian receptor signaling. This interference is likely to be because of, at least in part, to the yeast receptor dimerizing with the heterologously expressed mammalian GPCR. In this light, it seems probable that some mammalian GPCRs might not function in yeast because of interference from the other yeast GPCR (*GPR1*) [97]. Further modification of the available yeast strains to include the removal of *GPR1* should serve to increase the repertoire of mammalian GPCRs and GPCR dimers that can be studied in yeast. It is worth noting that GPCRs are also actively being studied in several other genetically amenable model systems including fruit fly, slime mold and the worm *C. elegans* [103–105]. These organisms express multiple GPCRs and will probably serve to shed light on the importance of dimerization in the process of GPCR signaling in metazoans.

Strategies to identify physiologically relevant dimers

There are many real life examples in which the stimulation of one type of GPCR leads to the modulation of a different GPCR [10]. These types of functional interactions are often due to crosstalk without direct physical interaction between the receptors. Examples include the oxytocin-mediated release of prostaglandin receptor agonist in parturition and the modulation of neurotransmitter GPCR release by other GPCR agonists such as cannabinoids [9,61]. To investigate the possibility that the observed GPCR crosstalk might be because of dimerization several different strategies can be employed [27,34]. First, it would be wise to make sure that the two receptors investigated are actually expressed in a single cell type (at least at the mRNA level) so that the GPCR dimer being investigated is physiologically relevant [32]. A surprising number of studies have been carried out in which different GPCR pairs that are very unlikely to ever meet *in vivo* are overexpressed. Although a variety of different strategies have been employed, it nevertheless remains difficult to conclusively demonstrate that GPCRs are dimerizing *in vivo*. Many have suggested that the gold standard remains coimmunoprecipitation of the native receptor from an actual tissue or cell [30,31]. There still remain issues associated with coimmunoprecipitation of the multimembrane spanning domain proteins

because the receptors might only be interacting with each other *in vitro* as the cellular extracts are being prepared. A variety of controls including immunoprecipitation of each receptor from tissues that express only one of the receptor pairs would go a long way toward confirming the existence of the bona fide dimer *in vivo*. Physiological studies can then be carried out to determine the role of the dimer and the possibility that this receptor dimer can be used as a candidate drug target. Examples of such studies include the involvement of canabinoid/adenosine A2 dimers in canabinoid-induced motor effects in the striatum [62], the interaction of serotonin 2A (5-HT_{2A}) and glutamate 2 (mGLuR2) receptors as mediators of hallucinogenic drugs [63] and angiotensin II AT_1 and bradykinin B_2 receptor dimers in pre-eclampsia [64]. An alternative strategy to confirm dimerization would be to generate animal models in which differentially tagged versions of the two receptors would be knocked into the genome to replace the wild-type version of the gene. Thus RET analysis could be performed under conditions of wild-type levels of expression and in primary cells derived from a variety of different tissues.

Paradigm shift from molecular-based targets to pathway-based *in vivo* systems

Once a candidate GPCR dimer has been selected, modification to existing drug screening strategies would need to be put in place. Historically, early drugs for GPCRs were often designed on the basis of the structure of the native agonists and further developed by monitoring physiological effects in an isolated tissue (i.e. blood vessel) or whole animal [65]. This was before the advent of molecular cloning and actually required no, or very little, knowledge of the structure of the receptor. The identification and cloning of genes encoding GPCRs ushered in a new era of drug development [66]. The ability to overexpress a desired GPCR-encoding cDNA in a heterologous cell line such as CHO or HEK [67] in conjunction with the development of automated assays to monitor the activation of the different receptors [68] brought about the ability to carry out screens using large chemical libraries [7]. With the probable identification of all human genes encoding GPCRs, the number of potential molecular-based GPCR targets now seem limitless especially when it is considered that drugs actually exist for a very few of these receptors [3]. Such high-throughput screens (HTSs) were successful at identifying *in vitro* ligands for several known and orphan receptors [7]. Subsequent molecular modeling allows for the development of these primary hits into higher affinity ligands for the target. In spite of the successes of this strategy, and notwithstanding the fact that investment into drug discovery is ever increasing, the ability to develop new drugs has been decreasing in recent years. It has been estimated that 6% or less of preclinical stage candidate drugs pass on to market [69]. This decreased success can be attributed to several factors including the depletion of the so-called easy drug targets that are pharmacologically and physiologically easy to monitor, as well as the fact that drugs developed using *in vitro* assays often lack *in vivo* efficacy are toxic or give rise to unwanted side effects [69]. Although the development of novel screening systems will increase the cost of drug discovery, given the diminishing returns, it might be worthwhile to place more resources on developing *in vivo* like HTS technologies. The proposed changes are not trivial, but several promising avenues for the development of such systems have been described [70–75]. Many of these new systems involve a change in philosophy away from single molecular-based targets toward pathway-based systems. These approaches have more resemblance to the strategies used by biotechnologists that endeavor to alter the metabolism of microbes to get them to produce desired biological products [66,69,76–78].

Instead of overexpressing two different GPCRs in a heterologous cell line, a new strategy could consist of the identification of a suitable *in vivo* cell system that endogenously expresses both receptors. Care would be taken to use cells derived from a tissue of interest and that the appropriate response to the stimulation of the receptors is maintained upon culturing of the cells. These could be primary cells like the platelet cells that were used to show that patients with pre-eclampsia contained higher levels of angiotensin II AT_1 and bradykinin B_2 receptor dimers [64]. Although primary cells are commonly used for toxicology assessments, the difficulties associated with their propagation and culture might limit their use to secondary screens or to screen a panel of already available agonists [71,74]. For example, existing agonists for monomeric GPCRs might be screened for their ability to activate a GPCR dimer of interest in cultured primary cells. Given that GPCR dimers, like monomers, are highly allosteric molecules, agonists that alter the behavior of one receptor will probably alter the structure of both GPCRs in the dimer. Thus, though the opioid agonist 6′-GNTI was originally thought to be a KOR-specific agonist, it was later found to have very little affinity for opioid receptor monomers but instead it had high affinity for KOR/DOR dimers [40]. As mentioned above, altered pharmacological responses are also observed in several other dimers including the AngII AT_1 receptor, which shows a fivefold increase in signaling responses when it is dimerized to the bradykinin B_2 receptor [64]. Thus these types of screens using available GPCR ligands would probably serve to uncover several physiologically relevant GPCR dimer ligands [79]. These will probably prove to be invaluable tools that are desperately needed in the pursuit of the structure and function of GPCR dimers [80].

Alternative cells that have been suggested include the use of existing lines that closely mimic endogenous cells or immortalized primary or adult progenitor cells [74,81]. For example, an immortalized human endothelial cerebral cell (hCMEC/D3) was found to express the melatonin MT_1 and the GPR50 orphan receptors endogenously and was used to show that GPR50 dimerizes with and inhibits signaling from MT_1 [82]. The siRNA-mediated knockdown of MT_1 was used to confirm this effect. In addition to siRNA, the use of primary cells derived from gene knockout (KO) animals can also be effective in assessing the importance of both receptors in the dimer.

The use of pluripotent embryonic stem (ES) cell lines, which are derived from the inner cell mass of blastocysts of *in vitro* fertilized human or other mammalian eggs, probably holds the greatest potential for the development of novel screening systems [70–72]. These cells can be propagated *in vitro* and made to differentiate into a wide range of different cell types including many that express multiple GPCRs such as cardiomyocytes, endothelial and smooth muscle cells [70]. Although generating, culturing and using these cells are theoretically rather straightforward [70], a large number of technical hurdles largely impede the widespread use of these cells. The ability to consistently generate terminally differentiated cells with identical phenotypes and

obtaining cell populations that are phenotypically homogeneous are some of the associated problems [72]. In one recent study, it was found that dopaminergic neuronal cells derived from mouse ES cells were found to be heterogeneous because they also contained a relatively high percentage of other cellular subtypes [83]. Of advantage is the ability to generate ES cells from genetically altered, or other, mice strains that serve as models for different diseases. The promise exists that compounds identified in screens using more *in vivo* like cells are more likely to elicit similar responses in *in vivo* systems.

In conclusion, in spite of the challenges associated with GPCR dimers, it is clear that they will continue to attract an inordinate amount of attention because they are likely to lead to novel insights into GPCR biology and pathophysiology.

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References

1 Luttrell, L.M. (2008) Reviews in molecular biology and biotechnology: transmembrane signaling by g protein-coupled receptors. *Mol. Biotechnol.* 39, 239–264

2 Thompson, M.D. *et al.* (2008) Pharmacogenomics of G protein-coupled receptor signaling: insights from health and disease. *Methods Mol. Biol.* 448, 77–107

3 Lagerstrom, M.C. and Schioth, H.B. (2008) Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat. Rev. Drug Discov.* 7, 339–357

4 Gilchrist, A. (2008) A perspective on more effective GPCR targeted drug discovery efforts. *Curr. Opin. Drug Discov.* 3, 375–389

5 Crouch, M.F. and Osmond, R.I. (2008) New strategies in drug discovery for GPCRs: high throughput detection of cellular ERK phosphorylation. *Comb. Chem. High Throughput Screen* 11, 344–356

6 Overington, J.P. *et al.* (2006) How many drug targets are there? *Nat. Rev. Drug Discov.* 5, 993–996

7 Xiao, S.H. *et al.* (2008) High throughput screening for orphan and liganded GPCRs. *Comb. Chem. High Throughput Screen* 11, 195–215

8 Christie, M.J. (2008) Cellular neuroadaptations to chronic opioids: tolerance, withdrawal and addiction. *Br. J. Pharmacol.* 154, 384–396

9 Lopez-Moreno, J.A. *et al.* (2008) The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction. *Addict. Biol.* 13, 160–187

10 Nelson, C.P. and Challiss, R.A. (2007) Phenotypic pharmacology: the influence of cellular environment on G protein-coupled receptor antagonist and inverse agonist pharmacology. *Biochem. Pharmacol.* 73, 737–751

11 Urban, J.D. *et al.* (2007) Functional selectivity and classical concepts of quantitative pharmacology. *J. Pharmacol. Exp. Ther.* 320, 1–13

12 Lefkowitz, R.J. (2007) Seven transmembrane receptors: something old, something new. *Acta Physiol. (Oxf.)* 190, 9–19

13 Jean-Baptiste, G. *et al.* (2006) Regulatory mechanisms involved in modulating RGS function. *Cell Mol. Life Sci.* 63, 1969–1985

14 Bockaert, J. *et al.* (2004) GPCR–GIP networks: a first step in the discovery of new therapeutic drugs? *Curr. Opin. Drug. Discov. Dev.* 7, 649–657

15 Delcourt, N. *et al.* (2007) GPCR-jacking: from a new route in RTK signalling to a new concept in GPCR activation. *Trends Pharmacol. Sci.* 28, 602–607

16 Sexton, P.M. *et al.* (2006) Complexing receptor pharmacology: modulation of family B G protein-coupled receptor function by RAMPs. *Ann. N. Y. Acad. Sci.* 1070, 90–104

17 Kabbani, N. and Levenson, R. (2007) A proteomic approach to receptor signaling: molecular mechanisms and therapeutic implications derived from discovery of the dopamine D2 receptor signalplex. *Eur. J. Pharmacol.* 572, 83–93

18 Lyssand, J.S. *et al.* (2008) Blood pressure is regulated by an alpha 1D-adrenergic receptor/dystrophin signalosome. *J. Biol. Chem.* 283, 18792–18800

19 DeWire, S.M. *et al.* (2007) Beta-arrestins and cell signaling. *Annu. Rev. Physiol.* 69, 483–510

20 Wisler, J.W. *et al.* (2007) A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. *Proc. Natl. Acad. Sci. U. S. A.* 104, 16657–16662

21 Ahmad, S. and Dray, A. (2004) Novel G protein-coupled receptors as pain targets. *Curr. Opin. Investig. Drugs* 5, 67–70

22 Schlyer, S. and Horuk, R. (2006) I want a new drug: G-protein-coupled receptors in drug development. *Drug Discov. Today* 11, 481–493

23 Kenakin, T. (2008) Functional selectivity in GPCR modulator screening. *Comb. Chem. High Throughput Screen* 11, 337–343

24 Kenakin, T. (2008) Functional assays as prismatic views of drug activity: relevance to new drug discovery. *J. Recept. Signal Transduct. Res.* 28, 109–125

25 Fredholm, B.B. *et al.* (2007) G-protein-coupled receptors: an update. *Acta. Physiol. (Oxf.)* 190, 3–7

26 Dalrymple, M.B. *et al.* (2008) G protein-coupled receptor dimers: functional consequences, disease states and drug targets. *Pharmacol. Ther.* 118, 359–371

27 Kent, T. *et al.* (2007) G-protein-coupled receptor heterodimerization: assay technologies to clinical significance. *Curr. Opin. Drug Discov. Dev.* 10, 580–589

28 Minneman, K.P. (2007) Heterodimerization and surface localization of G protein coupled receptors. *Biochem. Pharmacol.* 73, 1043–1050

29 Pin, J.P. *et al.* (2007) International Union of Basic and Clinical Pharmacology. LXVII. Recommendations for the recognition and nomenclature of G protein-coupled receptor heteromultimers. *Pharmacol. Rev.* 59, 5–13

30 Satake, H. and Sakai, T. (2008) Recent advances and perceptions in studies of heterodimerization between G protein-coupled receptors. *Protein Pept. Lett.* 15, 300–308

31 Springael, J.Y. *et al.* (2007) Allosteric properties of G protein-coupled receptor oligomers. *Pharmacol. Ther.* 115, 410–418

32 Milligan, G. (2008) A day in the life of a G protein-coupled receptor: the contribution to function of G protein-coupled receptor dimerization. *Br. J. Pharmacol.* 153, S216–229

33 Franco, R. *et al.* (2008) G-protein-coupled receptor heteromers: function and ligand pharmacology. *Br. J. Pharmacol.* 153, S90–98

34 Szidonya, L. *et al.* (2008) Dimerization and oligomerization of G-protein-coupled receptors: debated structures with established and emerging functions. *J. Endocrinol.* 196, 435–453

35 Zeng, F. and Wess, J. (2000) Molecular aspects of muscarinic receptor dimerization. *Neuropsychopharmacology* 23, S19–31

36 Brauner-Osborne, H. *et al.* (2007) Structure, pharmacology and therapeutic prospects of family C G-protein coupled receptors. *Curr. Drug Targets* 8, 169–184

37 Palmer, R.K. (2007) The pharmacology and signaling of bitter, sweet, and umami taste sensing. *Mol. Interv.* 7, 87–98

38 McGraw, D.W. *et al.* (2006) Airway smooth muscle prostaglandin-EP1 receptors directly modulate beta2-adrenergic receptors within a unique heterodimeric complex. *J. Clin. Invest.* 116, 1400–1409

39 Jordan, B.A. and Devi, L.A. (1999) G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* 399, 697–700

40 Waldhoer, M. *et al.* (2005) A heterodimer-selective agonist shows in vivo relevance of G protein-coupled receptor dimmers. *Proc. Natl. Acad. Sci. U. S. A.* 102, 9050–9055

41 Parker, S.L. *et al.* (2008) The neuropeptide Y (NPY) Y2 receptors are largely dimeric in the kidney, but monomeric in the forebrain. *J. Recept. Signal Transduct. Res.* 28, 245–263

42 Maggio, R. *et al.* (2003) Potent activation of dopamine D3/D2 heterodimers by the antiparkinsonian agents, S32504, pramipexole and ropinirole. *J. Neurochem.* 87, 631–641

43 Natesan, S. *et al.* (2008) The antipsychotic potential of l-stepholidine-a naturally occurring dopamine receptor D(1) agonist and D(2) antagonist. *Psychopharmacology (Berl.)* 199, 275–289

44 Hanyaloglu, A.C. and von Zastrow, M. (2008) Regulation of GPCRs by endocytic membrane trafficking and its potential implications. *Annu. Rev. Pharmacol. Toxicol.* 48, 537–568

45 Tsao, P. and von Zastrow, M. (2000) Downregulation of G protein-coupled receptors. *Curr. Opin. Neurobiol.* 10, 365–369

46 Finley, M.J. *et al.* (2008) Bi-directional heterologous desensitization between the major HIV-1 co-receptor CXCR4 and the kappa-opioid receptor. *J. Neuroimmunol.* 197, 114–123

47 Pello, O.M. *et al.* (2008) Ligand stabilization of CXCR4/delta-opioid receptor heterodimers reveals a mechanism for immune response regulation. *Eur. J. Immunol.* 38, 537–549

48 Overton, M.C. *et al.* (2005) Oligomerization of G-protein-coupled receptors: lessons from the yeast *Saccharomyces cerevisiae*. *Eukaryot. Cell* 4, 1963–1970

49 Leanos-Miranda, A. *et al.* (2005) In vitro coexpression and pharmacological rescue of mutant gonadotropin-releasing hormone receptors causing hypogonadotropic hypogonadism in humans expressing compound heterozygous alleles. *J. Clin. Endocrinol. Metab.* 90, 3001–3008

50 Bernier, V. *et al.* (2004) Pharmacological chaperones: potential treatment for conformational diseases. *Trends Endocrinol. Metab.* 15, 222–228

51 Levoye, A. *et al.* (2006) Do orphan G-protein-coupled receptors have ligand-independent functions? New insights from receptor heterodimers. *EMBO Rep.* 7, 1094–1098

52 Maurel, D. *et al.* (2008) Cell-surface protein–protein interaction analysis with time-resolved FRET and snap-tag technologies: application to GPCR oligomerization. *Nat. Methods* 5, 561–567

53 Gurevich, V.V. and Gurevich, E.V. (2008) GPCR monomers and oligomers: it takes all kinds. *Trends Neurosci.* 31, 74–81

54 Gurevich, V.V. and Gurevich, E.V. (2008) How and why do GPCRs dimerize? *Trends Pharmacol. Sci.* 29, 234–240

55 Meyer, B.H. *et al.* (2006) FRET imaging reveals that functional neurokinin-1 receptors are monomeric and reside in membrane microdomains of live cells. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2138–2143

56 Palczewski, K. (2006) G protein-coupled receptor rhodopsin. *Annu. Rev. Biochem.* 75, 743–767

57 Chabre, M. and le Maire, M. (2005) Monomeric G-protein-coupled receptor as a functional unit. *Biochemistry* 44, 9395–9403

58 White, J.F. *et al.* (2007) Dimerization of the class A G protein-coupled neurotensin receptor NTS1 alters G protein interaction. *Proc. Natl. Acad. Sci. U. S. A.* 104, 12199–12204

59 Whorton, M.R. *et al.* (2007) A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. *Proc. Natl. Acad. Sci. U. S. A.* 104, 7682–7687

60 Whorton, M.R. *et al.* (2008) Efficient coupling of transducin to monomeric rhodopsin in a phospholipid bilayer. *J. Biol. Chem.* 283, 4387–4394

61 Blanks, A.M. and Thornton, S. (2003) The role of oxytocin in parturition. *BJOG* 110, 46–51

62 Carriba, P. *et al.* (2007) Striatal adenosine A2A and cannabinoid CB1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. *Neuropsychopharmacology* 32, 2249–2259

63 Gonzalez-Maeso, J. *et al.* (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* 452, 93–97

64 AbdAlla, S. *et al.* (2001) Increased AT(1) receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness. *Nat. Med.* 7, 1003–1009

65 Triggle, D.J. (2007) Drug discovery and delivery in the 21st century. *Med. Princ. Pract.* 16, 1–14

66 Hellerstein, M.K. (2008) A critique of the molecular target-based drug discovery paradigm based on principles of metabolic control: advantages of pathway-based discovery. *Metab. Eng.* 10, 1–9

67 Eglen, R.M. *et al.* (2008) The use of immortalized cell lines in GPCR screening: the good, bad and ugly. *Comb. Chem. High Throughput Screen* 11, 560–565

68 Siehler, S. (2008) Cell-based assays in GPCR drug discovery. *Biotechnol. J.* 3, 471–483

69 Pache, R.A. *et al.* (2008) Towards a molecular characterisation of pathological pathways. *FEBS Lett.* 582, 1259–1265

70 Sartipy, P. *et al.* (2007) The application of human embryonic stem cell technologies to drug discovery. *Drug Discov. Today* 12, 688–699

71 Eglen, R.M. *et al.* (2008) An overview of drug screening using primary and embryonic stem cells. *Comb. Chem. High Throughput Screen* 11, 566–572

72 Pouton, C.W. and Haynes, J.M. (2007) Embryonic stem cells as a source of models for drug discovery. *Nat. Rev. Drug Discov.* 6, 605–616

73 Cezar, G.G. (2007) Can human embryonic stem cells contribute to the discovery of safer and more effective drugs? *Curr. Opin. Chem. Biol.* 11, 405–409

74 Dragunow, M. (2008) The adult human brain in preclinical drug development. *Nat. Rev. Drug Discov.* 7, 659–666

75 Desbordes, S.C. *et al.* (2008) High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. *Cell Stem. Cell* 2, 602–612

76 Cezar, G.G. *et al.* (2007) Identification of small molecules from human embryonic stem cells using metabolomics. *Stem. Cells Dev.* 16, 869–882

77 Watterson, S. *et al.* (2008) Logic models of pathway biology. *Drug Discov. Today* 13, 447–456

78 Hellerstein, M.K. (2008) Exploiting complexity and the robustness of network architecture for drug discovery. *J. Pharmacol. Exp. Ther.* 325, 1–9

79 Siehler, S. *et al.* (2008) Pharmacological profile of somatostatin and cortistatin receptors. *Mol. Cell Endocrinol.* 286, 26–34

80 Milligan, G. (2006) G-protein-coupled receptor heterodimers: pharmacology, function and relevance to drug discovery. *Drug Discov. Today* 11, 541–549

81 Roy, N.S. *et al.* (2007) Retrovirally mediated telomerase immortalization of neural progenitor cells. *Nat. Protoc.* 2, 2815–2825

82 Levoye, A. *et al.* (2006) The orphan GPR50 receptor specifically inhibits MT1 melatonin receptor function through heterodimerization. *EMBO J.* 25, 3012–3023

83 Raye, W.S. *et al.* (2007) Heterogeneous population of dopaminergic neurons derived from mouse embryonic stem cells: preliminary phenotyping based on receptor expression and function. *Eur. J. Neurosci.* 25, 1961–1970

84 Hebert, T.E. *et al.* (1996) A peptide derived from a beta2-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. *J. Biol. Chem.* 271, 16384–16392

85 Harikumar, K.G. *et al.* (2007) Transmembrane segment IV contributes a functionally important interface for oligomerization of the Class II G protein-coupled secretin receptor. *J. Biol. Chem.* 282, 30363–30372

86 Wang, J. and Norcross, M. (2008) Dimerization of chemokine receptors in living cells: key to receptor function and novel targets for therapy. *Drug Discov. Today* 13, 625–632

87 Taylor, M.S. *et al.* (2008) Mutations affecting the oligomerization interface of G-protein-coupled receptors revealed by a novel de novo protein design framework. *Biophys. J.* 94, 2470–2481

88 Yeagle, P.L. and Albert, A.D. (2007) G-protein coupled receptor structure. *Biochim. Biophys. Acta* 1768, 808–824

89 Klco, J.M. *et al.* (2003) C5a receptor oligomerization. I. Disulfide trapping reveals oligomers and potential contact surfaces in a G protein-coupled receptor. *J. Biol. Chem.* 278, 35345–35353

90 Mancia, F. *et al.* (2008) Ligand sensitivity in dimeric associations of the serotonin 5HT2c receptor. *EMBO Rep.* 9, 363–369

91 Zhang, A. *et al.* (2007) Receptor dimerization – rationale for the design of bivalent ligands. *Curr. Top. Med. Chem.* 7, 343–345

92 Bonger, K.M. *et al.* (2008) Synthesis and evaluation of homodimeric GnRHR antagonists having a rigid bis-propargylated benzene core. *Bioorg. Med. Chem.* 16, 3744–3758

93 Matthews, J.L. *et al.* (2008) In vivo characterization of (−)(−)MCL-144 and (+)(−)MCL-193: isomeric, bivalent ligands with Mu/Kappa agonist properties. *Neurochem. Res.* 10.1007/s11064-008-9752-3

94 Raddatz, R. *et al.* (2007) Allosteric approaches to the targeting of G-protein-coupled receptors for novel drug discovery: a critical assessment. *Biochem. Pharmacol.* 74, 383–391

95 Milligan, G. and Smith, N.J. (2007) Allosteric modulation of heterodimeric G-protein-coupled receptors. *Trends Pharmacol. Sci.* 28, 615–620

96 Casado, V. *et al.* (2007) Old and new ways to calculate the affinity of agonists and antagonists interacting with G-protein-coupled monomeric and dimeric receptors: the receptor-dimer cooperativity index. *Pharmacol. Ther.* 116, 343–354

97 Versele, M. *et al.* (2001) Sex and sugar in yeast: two distinct GPCR systems. *EMBO Rep.* 2, 574–579

98 Ladds, G. *et al.* (2005) Functional analysis of heterologous GPCR signalling pathways in yeast. *Trends Biotechnol.* 23, 367–373

99 Overton, H.A. *et al.* (2006) Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. *Cell Metab.* 3, 167–175

100 Li, B. *et al.* (2007) Rapid identification of functionally critical amino acids in a G protein-coupled receptor. *Nat. Methods* 4, 169–174

101 Shi, C. *et al.* (2007) A role for a complex between activated G protein-coupled receptors in yeast cellular mating. *Proc. Natl. Acad. Sci. U. S. A.* 104, 5395–5400

102 Kong, J.L. *et al.* (2002) Inhibition of somatostatin receptor 5-signaling by mammalian regulators of G-protein signaling (RGS) in yeast. *Biochim. Biophys. Acta* 1542, 95–105

103 Husson, S.J. *et al.* (2007) Neuropeptidergic signaling in the nematode *Caenorhabditis elegans*. *Prog. Neurobiol.* 82, 33–55

104 Schioth, H.B. *et al.* (2007) Mining the gene repertoire and ESTs for G protein-coupled receptors with evolutionary perspective. *Acta. Physiol. (Oxf.)* 190, 21–31

105 Wicher, D. *et al.* (2008) Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. *Nature* 452, 1007–1011