



Opioid receptors: Structural and mechanistic insights into pharmacology and signaling

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ABSTRACT

Opioid receptors are important drug targets for pain management, addiction, and mood disorders. Although substantial research on these important subtypes of G protein-coupled receptors has been conducted over the past two decades to discover ligands with higher specificity and diminished side effects, currently used opioid therapeutics remain suboptimal. Luckily, recent advances in structural biology of opioid receptors provide unprecedented insights into opioid receptor pharmacology and signaling. We review here a few recent studies that have used the crystal structures of opioid receptors as a basis for revealing mechanistic details of signal transduction mediated by these receptors, and for the purpose of drug discovery.

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1. Introduction

Opioid receptors belong to the super-family of G-protein coupled receptors (GPCRs), which are by far the most abundant class of cell-surface receptors, and also the targets of about one-third of approved/marketed drugs (Vorthers and Roth, 2005). Residing in different parts of the body (e.g., brain, spinal cord, digestive tract, etc.), opioid receptors are widely studied due to their crucial role in pain management (Pasternak, 2014), drug abuse/addiction (Kreek et al., 2012), and mood disorders (Lutz and Kieffer, 2013). There are three major subtypes of opioid receptors: δ receptor, μ receptor, and κ receptor. These receptors are activated by endogenous peptides such as endomorphins, enkephalins, and dynorphins, but also by naturally occurring alkaloids and other semi-synthetic and synthetic small-molecule ligands (McCurdy et al., 2003). Although a fourth receptor subtype, i.e., the nociceptin opioid receptor (NOP receptor), is phylogenetically related to δ receptor, μ receptor, and κ receptor, it does not bind the same ligands.

In addition to their still unbeatable analgesic effects, opioid drugs are accompanied by a variety of undesirable side effects, including vomiting, nausea, constipation, tolerance, addiction etc. (Feng et al., 2012). Thus, substantial drug discovery efforts have

been devoted over the years to reduce the disadvantages of these drugs while retaining their therapeutic efficacy. In the absence of high-resolution crystal structures of opioid receptors until 2012, the majority of these efforts used ligand-based strategies, although some also resorted to rudimentary molecular models of the receptors based on relatively distant structural templates. Notwithstanding this substantial amount of work over the course of several years, safe and effective opioid ligands remain the holy grail of the pharmaceutical industry.

The recent advances in membrane protein crystallization (Chun et al., 2012), which enabled the determination of various high-resolution crystal structures of GPCRs, including those of all four opioid receptor subtypes (Fenalti et al., 2014; Granier et al., 2012; Manglik et al., 2012; Thompson et al., 2012; Wu et al., 2012) (see Fig. 1), marked the beginning of a new era in opioid research. By revealing important details of ligand–receptor interactions at the orthosteric binding site (i.e., the site at which endogenous opioid ligands bind), or allosteric sites (e.g., the much anticipated sodium binding site (Fenalti et al., 2014)), these structures evidently offer new opportunities for drug discovery at opioid receptors (Filizola and Devi, 2013). Notably, comparison between the four opioid receptor crystal structures (Filizola and Devi, 2013) reveals common ligand–receptor interactions that may be responsible for the molecular recognition of classical opioid drugs. In contrast, the different ligand–receptor interactions that are mostly located at the extracellular side of the receptor may be responsible for the

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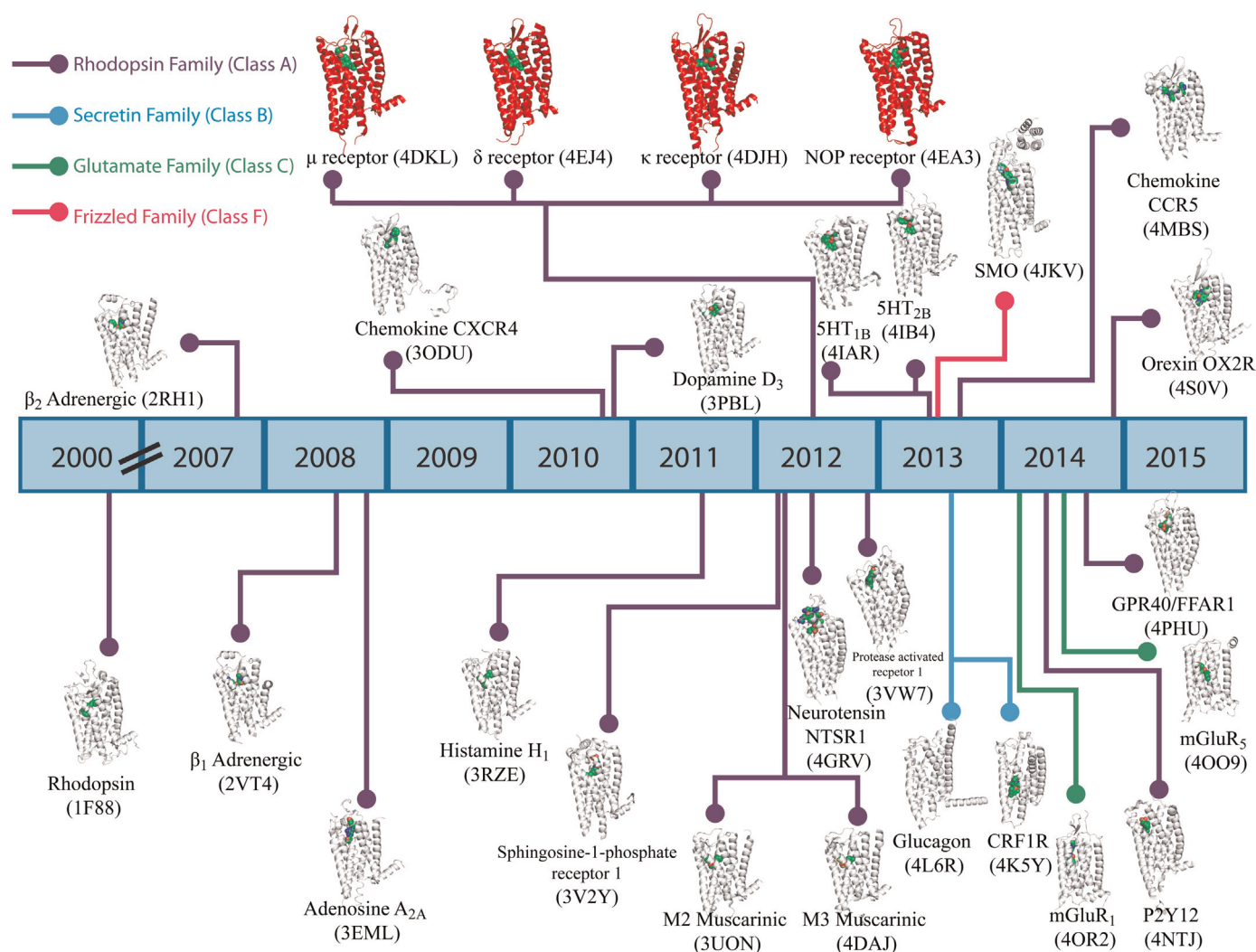


Fig. 1. Time-line of representative crystal structures of GPCR subtypes. Each representative crystal structure is shown in cartoon representation, with the bound ligand shown as colored spheres. With the exception of opioid receptors, which are colored in red, all the others are depicted in silver. Rhodopsin, Secretin, Glutamate, and Frizzled subfamilies of receptors are indicated with purple, cyan, green, and magenta colors, respectively. PDB IDs of the crystal structures are included in parenthesis after each protein name. Specifically, the reported crystal structures are (in chronological order): rhodopsin (1F88 (Palczewski et al., 2000)), β_2 Adrenergic (2RH1 (Cherezov et al., 2007)), β_1 Adrenergic (2VT4 (Warne et al., 2008)), Adenosine A_{2A} (3EML (Jaakola et al., 2008)), Chemokine CXCR4 (3ODU (Wu et al., 2010)), Dopamine D_3 (3PBL (Chien et al., 2010)), Histamine H_1 (3RZE (Shimamura et al., 2011)), Sphingosine-1-phosphate receptor 1 (3V2Y (Hanson et al., 2012)), M2 Muscarinic (3UON (Haga et al., 2012)), M3 Muscarinic (4DAJ (Kruse et al., 2012)), Neurotensin NTSR1 (4GRV (White et al., 2012)), μ receptor (4DKL (Manglik et al., 2012)), δ receptor (4EJ4 (Granier et al., 2012)), κ receptor (4DJH (Wu et al., 2012)), NOP receptor (4EA3 (Thompson et al., 2012)), Protease activated receptor 1 (3VW7 (Zhang et al., 2012)), 5HT $_{1B}$ (4IAR (Wang et al., 2013a)), 5HT $_{2B}$ (4IB4 (Wacker et al., 2013)), SMO (4JKV (Wang et al., 2013b)), Glucagon (4L6R (Siu et al., 2013)), CRF1R (4K5Y (Hollenstein et al., 2013)), Chemokine CCR5 (4MBS (Tan et al., 2013)), mGluR $_1$ (4OR2 (Wu et al., 2014)), P2Y12 (4NTJ (Zhang et al., 2014)), mGluR $_5$ (4OO9 (Dore et al., 2014)), GPR40/FFAR1 (4PHU (Srivastava et al., 2014)), and Orexin OX2R (4SOV (Yin et al., 2014)).

specificity of the ligands for a given receptor subtype. Among them are the interactions crystallographic ligands of μ receptor and δ receptor form with residues of the transmembrane (TM) helices TM6 and/or TM7, or those that the crystallographic ligands of κ receptor and NOP receptor form with TM2 and TM3 residues.

Additional, important details of opioid receptor binding and signaling were provided by the ultra-high resolution crystal structure of δ receptor (Fenalti et al., 2014), which only recently appeared in the literature. In particular, this structure revealed the presence of an allosteric binding site occupied by sodium, which had been suggested to serve as an allosteric modulator of opioid receptors for quite some time (Pasternak and Snyder, 1975), and was recently found in ultra-high resolution crystal structures of other GPCRs (Katritch et al., 2014; Liu et al., 2012). In all these ultra-high resolution crystal structures, this ion is located near the conserved D2.50 residue, which is about 10 Å below the D3.32 residue that interacts with several orthosteric ligands of GPCRs,

including classical opioid ligands (note that all the residues mentioned in this manuscript are numbered according to the Ballesteros–Weinstein generic numbering scheme (Ballesteros and Weinstein, 1995)).

Although a detailed knowledge of the crystal structures of opioid receptors provides a new dimension for structure-guided drug discovery efforts, the realizations that these receptors are rather dynamic systems and that several opioid ligands can activate multiple signaling pathways add another level of complexity to an already complicated problem. Various cases of so-called functional selectivity or biased agonism, primarily through $G_{i/o}$ or arrestin, have been reported in the literature for all major opioid receptors (e.g., see (Luttrell, 2014; Thompson et al., 2014, 2015; Violin et al., 2014) for recent reviews). This selectivity in opioid receptor signaling and function may be achieved through (i) conformational preferences induced by ligands with different efficacies binding at the orthosteric site and inducing coupling of

an intracellular protein over another, (ii) bias in the downstream functional outcome, (iii) allosteric modulation of the efficacy of orthosteric ligands, and/or (iv) dimerization/oligomerization of opioid receptors among themselves or with other GPCRs (e.g., see (Fujita et al., 2014) for a recent review). Based on recently reported examples (e.g., see (Chen et al., 2013; DeWire et al., 2013; Soergel et al., 2014a, 2014b)), it appears that developing biased opioid ligands for one intracellular signaling pathway or another may provide a more effective route to analgesics with reduced side effects.

We summarize here a few studies that have been inspired by the new structural information available for opioid receptors, and their impact on both a mechanistic understanding of opioid receptor function and rational drug discovery.

2. Structure-based drug design at the orthosteric site using opioid receptor crystal structures

Compared to the shallow pockets of many soluble proteins, the more buried orthosteric binding pockets of GPCRs appear to be particularly well suited to small-molecule docking for the purpose of rational drug discovery. Additionally, chemical libraries that are typically used for virtual screening contain many GPCR binders because of the special attention GPCRs have received from medicinal chemists over the years. Thus, not surprisingly, various structure-based drug design campaigns carried out in recent years using currently available crystal structures of GPCRs have resulted in both the discovery of novel chemical scaffolds, and the optimization of lead candidates (e.g., see (Jacobson, 2013; Kooistra et al., 2014; Shoichet and Kobilka, 2012) for recent reviews).

The new crystal structures of opioid receptors bear no exception. In fact, a few examples have already appeared in the literature in which crystal structures of opioid receptors have successfully been used to provide retrospective models of the binding of known opioid ligands to their receptors (e.g., see (Martinez-Mayorga et al., 2013; Wu et al., 2012)) or to identify novel chemical scaffolds binding at the orthosteric site from virtual screening (e.g., see (Negri et al., 2013)). These examples mostly refer to κ receptor, whose ligands may play a significant role in pain management, anxiety, depression, stress, and psychotic behavior without activating the reward pathway (Vanderah, 2010; Wang et al., 2010).

A number of investigators have used various computational tools, including molecular docking, molecular dynamics (MD), free-energy perturbations, and ab initio calculations (e.g., see (Goldfeld et al., 2015; Leonis et al., 2014; Martinez-Mayorga et al., 2013; Polepally et al., 2014; Vardy et al., 2013; Wu et al., 2012)) to provide a molecular description of both the binding and function of selective κ receptor ligands at the κ receptor crystal structure, in relation to the crystallographic binding pose of the highly selective antagonist JD_{Tic} ((3R)-1,2,3,4-tetrahydro-7-hydroxy-N-[(1S)-1-[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl]-2-methylpropyl]-3-isoquinolinecarboxamide) (Wu et al., 2012). For instance, comparison of the predicted poses of the κ receptor selective morphine-based antagonists nor-binaltorphimine (nor-BNI) and 5'-guanidinonaltrindole (GNTI) with the crystallographic pose of JD_{Tic} emphasized the role of κ receptor residues V2.53 (A in μ and δ receptors), V2.63 (N in μ receptor and K in δ receptor), I6.55 (V in μ and δ receptors), and Y7.35 (W in μ receptor and L in δ receptor) as molecular determinants of the binding selectivity of JD_{Tic} for κ receptor (Wu et al., 2012). In contrast, E6.58 appeared to be the only κ receptor residue that most likely confers κ receptor selectivity to nor-BNI and 5'-GNTI. Docking studies (Vardy et al., 2013) of κ receptor selective agonists representing four different chemotypes, including a peptide (dynorphin A), an arylacetamide (U-69593), a non-nitrogenous

diterpene (salvinorin A), and an octahydroisoquinolinone carboxamide (1xx), inspired functional assays on mutants at eighteen positions of the putative agonist binding site within the κ receptor crystal structure. Analysis of the experimental data indicated differential effects of the mutated residues on binding and function (Vardy et al., 2013). For instance, the D3.32A mutation dramatically decreased the affinity of the basic amine-containing agonists dynorphin A and U-69593, but not that much the affinity of the non-basic compounds 1xx and salvinorin A. Similarly, D3.32N decreased the affinity of dynorphin A and U-69593, but caused an increase in both the affinity and potency of compounds 1xx and salvinorin A. Notably, some mutations (e.g., at Y3.33, M3.36, Y7.43, Y7.35, and K5.39) appeared to affect function more than binding, and were suggested to be directly involved, along with crystallographic water molecules, in signal transduction propagated through specific conformational changes in the receptor (Vardy et al., 2013). The role of the water structure within the κ receptor active site was also emphasized in another recent study motivated by the apparent failure of two established scoring functions (Glide SP and Glide XP) to effectively distinguish between κ receptor binders and decoys (Goldfeld et al., 2015). This observation prompted the development of a new empirical scoring function, WScore, to assess interactions between ligands and water molecules at an active site.

The κ receptor crystal structure was also recently used to rationalize the binding of several different derivatives of the selective non-nitrogenous diterpene κ receptor agonist salvinorin A (Polepally et al., 2014), including 22-thiocyanatosalvinorin A (RB-64) (Wu et al., 2012). A model of the latter was based on previous experimental evidence suggesting that the agonist 2-acetoxy moiety forms an irreversible, covalent bond with the κ receptor residue C7.38. According to this docking hypothesis, the fused tricyclic scaffold of salvinorin A is oriented towards TM2, while the C-2 acetoxy group of the ligand is accommodated in a pocket surrounded by residues D3.32, Y3.33 and M3.36. Different was the case for the recently synthesized series of salvinorin A derivatives containing potentially reactive Michael acceptor functional groups at the C-2 position (Polepally et al., 2014), which cannot form an irreversible bond with C7.38. While the predicted binding was somehow similar to that of salvinorin A, the bulky substitutions at the C-2 position could not be accommodated in the same pocket, and led to a decreased affinity of the ligands for κ receptor. Notably, the polarity introduced by a substitution with a heterocyclic nitrogen atom made the ligand loose affinity for the κ receptor while gaining it at the μ receptor. A possible explanation for this κ/μ receptor selectivity is that the ligand adopts an alternative binding mode in the μ receptor stabilized by an interaction with the non-conserved residue N2.63 (V in κ receptor and K in δ receptor) (Polepally et al., 2014). MD simulations, free energy, and ab initio calculations of the κ receptor crystal structure in complex with salvinorin A or JD_{Tic} showed that the ligands are stabilized within the binding pocket through interactions with different residues (Leonis et al., 2014).

In addition to provide insights into the molecular determinants required for ligand binding to opioid receptors, the high-resolution crystal structures of these receptors can be used in virtual screening campaigns to identify novel compounds. To the best of our knowledge, to date, ours is the only published example of a virtual screening performed using the crystal structure of an opioid receptor that led to the identification of novel ligands. Specifically, using the κ receptor crystal structure, we screened in silico over 4.5 million "lead-like" small molecules from the ZINC database (Negri et al., 2013) at the crystallographic orthosteric site of the receptor. Following a series of criteria, including novelty, interaction with κ receptor residues that are different from μ and δ receptors, and limited flexibility, we purchased 22 small molecules

for experimental testing. Although the used κ receptor crystal structure corresponds to an inactive conformation of the receptor, we identified a selective, κ receptor agonist in this study. As this compound does not resemble the chemotype of a classical opioid ligand, there is every expectation that it may be developed into improved medications. To this end, additional, collaborative studies are ongoing in our lab.

3. Allosteric modulators of the binding and/or signaling efficacy of opioid receptor orthosteric ligands

Limiting the search for novel opioid receptor ligands to the orthosteric site may not be the best strategy to achieve drug selectivity because of the evolutionary pressure orthosteric sites face to accommodate endogenous ligands. Targeting allosteric sites on the receptor, i.e., sites that are topographically different from those recognizing endogenous ligands, and therefore less conserved in their amino acid sequences, represents an attractive alternative to achieve greater selectivity. Allosteric modulators of GPCRs range from small organic molecules or peptides, to ions and lipids (Christopoulos, 2014). They can bind at the receptor extracellular side, inside the TM helical bundle, or even at the intracellular part of the receptor (Shukla, 2014). Based on the positive (i.e., increase), negative (i.e., decrease), or neutral effect they have on the affinity and/or efficacy of the orthosteric ligand, allosteric modulators of GPCRs are termed positive allosteric modulators (PAMs), negative allosteric modulators (NAMs), or silent allosteric modulators (SAMs), respectively. The latter do not have any effect on the affinity and/or efficacy of the orthosteric ligand, yet they share the same allosteric binding pocket of, and therefore can compete with, PAMs or NAMs. Whichever the effect induced by the allosteric modulator, its magnitude and/or direction strongly depend on the orthosteric ligand that is used to probe receptor function, according to a phenomenon that has been termed in the literature ‘probe dependence’ (e.g., see Christopoulos, 2014). This is one of the reasons that make allosteric modulators standout candidates for drug discovery, alongside their possibly improved selectivity, their maintenance of the temporal and spatial characteristics of endogenous signals, their potential for biased signaling, and their possibly reduced on-target overdosing risks given that their effect is limited to ligand cooperativity.

Like other GPCRs (Katritch et al., 2014), allosteric modulators of opioid receptors can be as small as ions. As early as in the 1970s (Pasternak and Snyder, 1975; Pert et al., 1973; Preininger et al., 2013), physiological concentrations of sodium were shown to decrease the binding of agonists, but not antagonists, to the opioid

receptors (e.g., see (Pasternak and Pan, 2013)). Notably, manganese ions restored full agonist binding to μ receptor in the presence of sodium, but did not have any effect on the binding of antagonists (Pasternak et al., 1975). That an ion actually binds to an opioid receptor was demonstrated for the first time unambiguously by the very recent ultra-high resolution crystallographic structure of δ receptor (PDB: 4N6H (Fenalti et al., 2014)). This structure revealed a sodium ion forming a salt bridge with the GPCR family A conserved residue D2.50, as well as additional polar interactions with two water molecules and receptor side chains S3.39 and N3.35. Notably, site-directed mutagenesis and functional studies of mutants of these sodium-coordinating residues revealed that they act as ‘efficacy switches’ at the δ receptor as they either augmented constitutive β -arrestin-mediated signaling or transformed classical δ receptor antagonists (e.g., naltrindole) into potent β -arrestin-biased agonists (Fenalti et al., 2014).

To provide mechanistic detail into the sodium control of opioid receptor binding and signaling, we recently carried out extensive all-atom MD simulations to explore the dynamic nature of sodium binding to the crystal structures of δ , μ , and κ receptors (Shang et al., 2014) in an explicit lipid–water environment. According to these simulations, sodium enters all three opioid receptors exclusively from the extracellular side, and within a few hundred nanoseconds it forms a stable coordination with the residues seen crystallographically (Fenalti et al., 2014), passing through the orthosteric site. Another recent MD study of the μ receptor crystal structure (Yuan et al., 2013) also concluded that sodium enters preferentially from the extracellular side, and it binds to the crystallographic site defined by residue D2.50. In our simulations, the experimentally-observed inhibition of agonist binding by sodium was attributed to the extended hydrogen bond network that puts into communication the orthosteric and allosteric sites, and contributes to the stability of an inactive conformation of the receptor (Shang et al., 2014). Using random accelerated molecular dynamics, we also predicted possible egress pathways of the ion from the receptor, which included both extracellular and intracellular pathways (Shang et al., 2014).

Recent high-throughput screening campaigns using a β -arrestin recruitment assay have successfully identified small-molecule allosteric modulators of μ and δ receptors (Burford et al., 2013, 2014a, 2014b). Further characterization of these compounds using additional functional assays, such as adenylyl cyclase activity inhibition and G protein activation, confirmed these ligands as μ -receptor-selective PAMs. Further studies are ongoing in our lab to predict the energetically preferred binding poses of these ligands at putative allosteric binding pockets within the μ receptor crystallographic structure. Fig. 2 provides a preliminary idea of binding

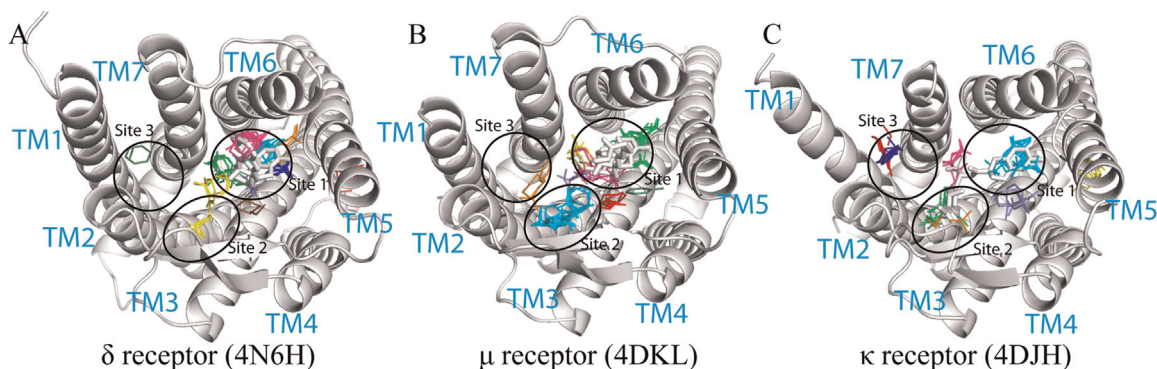


Fig. 2. FTMAP results using δ (A), μ (B), and κ (C) receptor crystal structures. Only chains A of the receptors, excluding non-receptor atoms, were uploaded to the FTMAP web server for fragment docking calculation. Receptors are shown in silver cartoon representation, ligands in the crystal structures are shown in silver sticks, and FTMAP probe clusters are shown as colored lines. The FTMAP probe clusters are colored in the order of decreasing size as follows: green, cyan, magenta, yellow, red, blue, purple, orange, dark green, and chocolate.

pockets within the crystal structures of the major δ , μ , and κ receptor subtypes as assessed by FTMAP (Brenke et al., 2009), an efficient Fast Fourier Transform correlation approach that allows to sample billions of probe positions on a protein, and to predict 'hot spots' or putative binding pockets for small molecules based on the common location of several probe clusters. Specifically, 8–10 probe clusters are identified by FTMAP for the different receptor subtypes we studied, ranging from 1 to 28 probes per cluster. Notably, the majority of probe clusters nicely overlaps with the crystallographic ligands, and at least three main pockets are identified by FTMAP in the extracellular region of the receptors. These correspond to the orthosteric site of morphinans surrounded by helices TM3, TM5, TM6, and TM7 (site 1 in Fig. 2), its extension between TM2 and TM3 as revealed by the JDTic-bound κ receptor crystal structure (site 2 in Fig. 2), and a third pocket delimited by TM1, TM2, and TM7 (site 3 in Fig. 2). The latter two sites are likely to correspond to allosteric sites for some ligands, or may even represent pockets for metastable states along the ligand binding pathway.

4. Functional selectivity at opioid receptors

The concept of functional selectivity or biased GPCR signaling (e.g., see (Shonberg et al., 2014; Violin et al., 2014) for recent reviews) has changed the traditional two-state model of receptor activation in that multiple conformations are assumed to be differentially stabilized by ligands with different efficacy as a prerequisite to activating G protein-dependent or independent (e.g., through β -arrestin) signaling pathways, thus resulting in beneficial or adverse effects. This appears to be also the case for opioid receptors (e.g., see (Raehal and Bohn, 2014; Raehal et al., 2011; Thompson et al., 2015; Williams et al., 2013)), where morphine was the first ligand to be shown (a) to function as a weak internalizer notwithstanding its efficacy in mediating G protein activation (Keith et al., 1996; Sternini et al., 1996), and (b) to exert enhanced analgesia and reduced respiratory depression and constipation in a β -arrestin2 knockout mouse (Raehal et al., 2005). These observations prompted additional studies to identify functionally selective opioid receptor ligands, and to understand which signaling pathway(s) regulated their beneficial and adverse effects. For instance, recent evidence suggests that while κ receptor agonists may in principle be developed into effective analgesics without a high abuse potential, their often associated dysphoria (Land et al., 2009), a side effect that has been suggested to be related to the activation of the arrestin pathway (Chavkin, 2011), limits their clinical development. Taken together, these results suggest that biased opioid ligands towards the G-protein pathway and away from the β -arrestin pathway may hold greater potential as future opioid receptor therapeutics (Raehal and Bohn, 2014). Thus, data from studies that attempt to quantify biased agonism at opioid receptors (e.g. see (Frolich et al., 2011; McPherson et al., 2010; Molinari et al., 2010; Rivero et al., 2012; White et al., 2014; Zhou et al., 2013)), especially in the context of tissues, organs, and the whole animal (Zhou and Bohn, 2014), are in high demand (Thompson et al., 2015).

Among the most recently characterized chemical scaffolds with a range of signaling bias *in vitro* are several κ receptor agonists (e.g., see (White et al., 2014; Zhou et al., 2013)). These compounds range from endogenous peptides and diterpene scaffolds, which are all G-protein biased, to arylacetamide compounds which are both G-protein and β -arrestin biased ligands (White et al., 2014), to triazole and isoquinoline analogs, which preferentially activate the G protein pathway with minimal effects on β 2-arrestin recruitment (Zhou et al., 2013). It will be of great interest to see whether simple information from docking of these compounds to

the crystal structure of κ receptor is sufficient to identify the key molecular determinants that are responsible for functional selectivity at the G-protein or β -arrestin signaling pathway. A complete understanding of the conformational changes in the receptor induced by the two sets of differently biased ligands may be necessary to elucidate functional selectivity at opioid receptors.

That conformational plasticity may be an important contributor of functional selectivity is not surprising. Like other GPCRs, opioid receptors are intrinsically flexible molecules, and the mechanistic properties of their crystal structures have been recently investigated by MD simulations at the sub-microsecond and microsecond time scales (e.g., see (Fossepre et al., 2014; Shang et al., 2014; Shim et al., 2013)). Simulations of the ligand-free form of μ receptor in a simplified membrane model (Fossepre et al., 2014) drew attention to both the plasticity of the receptor binding site and correlated motions between the intra- and extra-cellular regions of the receptor notwithstanding a certain rigidity of the central part of the receptor. Additional MD simulations of the μ receptor crystal structure in complex with three agonists, three antagonists, and a partial agonist, and on the constitutively active T6.34K mutant (Shim et al., 2013) showed a differential involvement of D3.32 in direct interaction with the basic nitrogen of agonists compared to antagonists. These simulations also drew attention to differences in the conformational properties of the intracellular region of the receptor when bound to agonists or antagonists. Further studies are necessary to understand how biased agonists affect the conformational properties of the receptor, and what conformation(s) they preferentially stabilize.

Notwithstanding the number of known biased agonists of opioid receptors and the interest in understanding their effect on the conformational plasticity of the receptor, there is no indication for the majority of these compounds that they may result in improved therapeutics. The G protein-biased μ receptor ligand TRV130 [(3-methoxythiophen-2-yl)methyl]({2-[(9R)-9-(pyridin-2-yl)-6-oxaspiro[4.5]decan-9-yl]ethyl}amine) (Chen et al., 2013; DeWire et al., 2013) represents an exception as it was recently demonstrated to be an improved analgesic with reduced side effects (i.e., respiratory suppression and nausea) compared to morphine (Soergel et al., 2014a, 2014b) as it entered Phase II of drug development. Given its potential clinical relevance, a thorough understanding of the molecular basis of the TRV-130 action is highly desirable, and the availability of the μ receptor crystal structure offers an unprecedented opportunity to advance knowledge in that direction.

5. Opioid receptor dimerization

Like for other GPCRs, the possibility that opioid receptors form physiologically relevant dimers/oligomers has been suggested more than once (e.g., see (Ferre et al., 2014) for a recent review), notwithstanding the still active fierce debate that exists on the topic of oligomerization of family A GPCRs (e.g., see Bouvier and Hebert, 2014a, b; Lambert and Javitch, 2014a, b). The several recent crystal structures that show interacting parallel receptors in the crystal unit cell (Fig. 3) have been used as an argument in favor of GPCR dimerization, although the possibility exists that these are crystallographic artifacts and/or they do not necessarily represent physiologically relevant interfaces.

Two of the five available opioid receptor crystal structures (Fenalti et al., 2014; Granier et al., 2012; Manglik et al., 2012; Thompson et al., 2012; Wu et al., 2012), specifically the structures of μ (Manglik et al., 2012) and κ (Wu et al., 2012) receptors, also reveal parallel arrangements of interacting receptors. As shown in Fig. 3, these correspond to two different interfaces in the case of μ receptor, one of which is also seen in the κ receptor crystal

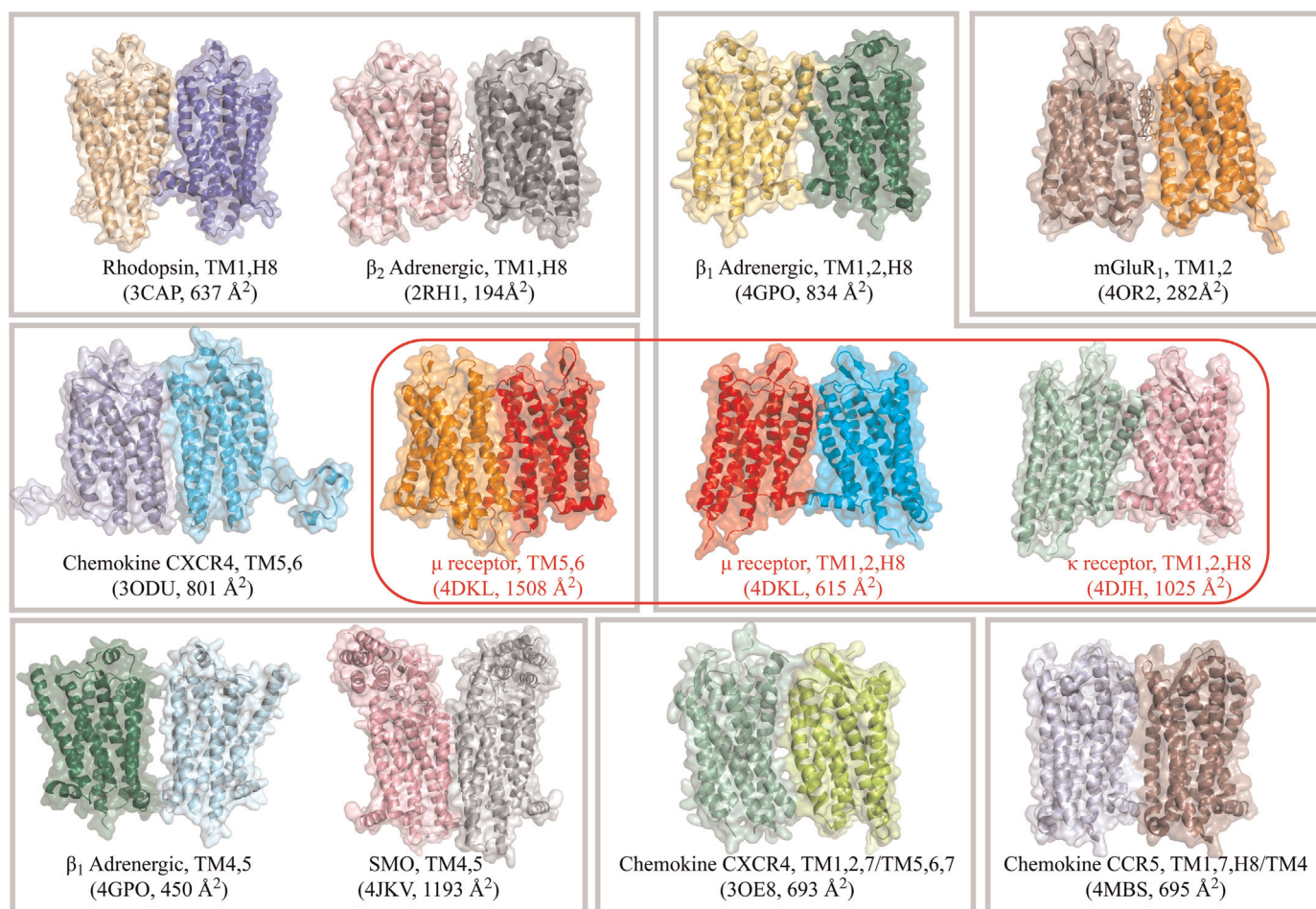


Fig. 3. Putative dimer interfaces inferred by existing GPCR crystal structures. Protein name and interface type are reported under each dimeric structure. PDB ID and interface area are included in parenthesis. Dimers are boxed (gray color) according to interface type. Opioid receptors are indicated by a red rounded rectangle, as well as red text. The interface area between protomers was calculated using the PISA web server (Krissinel and Henrick, 2007). All non-receptor atoms were excluded from this calculation.

structure. Specifically, the interface that is unique to the μ receptor (herein called the TM5,6/TM5,6 interface) is made of symmetric interactions between TM5 and TM6 helices. The other interface that is common to both μ and κ receptors (herein called the TM1,2, H8/TM1,2,H8 interface), although slightly different between the two, involves TM1, TM2, and helix 8 (H8). Notably, biochemical experiments suggested the involvement of TM1 (He et al., 2011), as well as TM4/TM5 (Johnston et al., 2011), or the carboxyl tail and the intracellular loop 3 in the dimerization of opioid receptors (O'Dowd et al., 2012).

To investigate the relative stability of the μ and κ receptor crystallographic interfaces in an explicit lipid–water environment, and in the absence of the T4L replacing the intracellular loop 3 in the crystal structure, we recently carried out umbrella sampling MD simulations of coarse-grained representations of the opioid receptor crystallographic dimers (Johnston and Filizola, 2014). We found that these interfaces are indeed viable dimeric configurations in an explicit membrane environment, as they appear to be in general, quite stable from a thermodynamic view point. Whether or not these interfaces are more kinetically favorable than others, and would occur in vivo remains to be assessed. To facilitate experimental testing, our studies provide specific testable hypotheses of residues that, if mutated, might either increase or decrease the strength of opioid receptor dimerization (Johnston and Filizola, 2014).

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References

- Ballesteros, J., Weinstein, H., 1995. Integrated Methods for the Construction of Three-dimensional Models and Computational Probing of Structure-function Relations in G Protein-coupled Receptors. Elsevier, pp. 366–428.
- Bouvier, M., Hebert, T.E., 2014a. CrossTalk proposal: Weighing the evidence for Class A GPCR dimers, the evidence favours dimers. *J. Physiol.* 592, 2439–2441.
- Bouvier, M., Hebert, T.E., 2014b. Rebuttal from Michel Bouvier and Terence E. Hebert. *J. Physiol.* 592, 2447.
- Brenke, R., Kozakov, D., Chuang, G.Y., Beglov, D., Hall, D., Landon, M.R., Mattos, C., Vajda, S., 2009. Fragment-based identification of druggable 'hot spots' of proteins using Fourier domain correlation techniques. *Bioinformatics* 25, 621–627.
- Burford, N.T., Clark, M.J., Wehrman, T.S., Gerritz, S.W., Banks, M., O'Connell, J., Traynor, J.R., Alt, A., 2013. Discovery of positive allosteric modulators and silent allosteric modulators of the mu-opioid receptor. *Proc. Natl. Acad. Sci. USA* 110, 10830–10835.
- Burford, N.T., Traynor, J.R., Alt, A., 2014a. Positive allosteric modulators of the mu-opioid receptor: a novel approach for future pain medications. *Br. J. Pharmacol.*

- Burford, N.T., Wehrman, T., Bassoni, D., O'Connell, J., Banks, M., Zhang, L., Alt, A., 2014b. Identification of selective agonists and positive allosteric modulators for mu- and delta-opioid receptors from a single high-throughput screen. *J. Biomol. Screen.*
- Chavkin, C., 2011. The therapeutic potential of kappa-opioids for treatment of pain and addiction. *Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol.* 36, 369–370.
- Chen, X.T., Pitis, P., Liu, G., Yuan, C., Gotchev, D., Cowan, C.L., Rominger, D.H., Koblish, M., Dewire, S.M., Crombie, A.L., Violin, J.D., Yamashita, D.S., 2013. Structure-activity relationships and discovery of a G protein biased mu opioid receptor ligand, [(3-methoxythiophen-2-yl)methyl]([2-[(9 R)-9-(pyridin-2-yl)-6-ox-aspiro-[4.5]decan-9-yl]ethyl)amine (TRV130), for the treatment of acute severe pain. *J. Med. Chem.* 56, 8019–8031.
- Cherezov, V., Rosenbaum, D.M., Hanson, M.A., Rasmussen, S.G., Thian, F.S., Kobilka, T.S., Choi, H.J., Kuhn, P., Weis, W.I., Kobilka, B.K., Stevens, R.C., 2007. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* 318, 1258–1265.
- Chien, E.Y., Liu, W., Zhao, Q., Katritch, V., Han, G.W., Hanson, M.A., Shi, L., Newman, A.H., Javitch, J.A., Cherezov, V., Stevens, R.C., 2010. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science* 330, 1091–1095.
- Christopoulos, A., 2014. Advances in G protein-coupled receptor allostery: from function to structure. *Mol. Pharmacol.* 86, 463–478.
- Chun, E., Thompson, A.A., Liu, W., Roth, C.B., Griffith, M.T., Katritch, V., Kunken, J., Xu, F., Cherezov, V., Hanson, M.A., Stevens, R.C., 2012. Fusion partner toolchest for the stabilization and crystallization of G protein-coupled receptors. *Structure* 20, 967–976.
- DeWire, S.M., Yamashita, D.S., Rominger, D.H., Liu, G., Cowan, C.L., Graczyk, T.M., Chen, X.T., Pitis, P.M., Gotchev, D., Yuan, C., Koblish, M., Lark, M.W., Violin, J.D., 2013. A G protein-biased ligand at the mu-opioid receptor is potently analgesic with reduced gastrointestinal and respiratory dysfunction compared with morphine. *J. Pharmacol. Exp. Ther.* 344, 708–717.
- Dore, A.S., Okrasa, K., Patel, J.C., Serrano-Vega, M., Bennett, K., Cooke, R.M., Errey, J. C., Jazayeri, A., Khan, S., Tehan, B., Weir, M., Wiggan, G.R., Marshall, F.H., 2014. Structure of class C GPCR metabotropic glutamate receptor 5 transmembrane domain. *Nature* 511, 557–562.
- Fenalti, G., Giguere, P.M., Katritch, V., Huang, X.P., Thompson, A.A., Cherezov, V., Roth, B.L., Stevens, R.C., 2014. Molecular control of delta-opioid receptor signalling. *Nature* 506, 191–196.
- Feng, Y., He, X., Yang, Y., Chao, D., Lazarus, L.H., Xia, Y., 2012. Current research on opioid receptor function. *Curr. Drug Targets* 13, 230–246.
- Ferre, S., Casado, V., Devi, L.A., Filizola, M., Jockers, R., Lohse, M.J., Milligan, G., Pin, J. P., Guitart, X., 2014. G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. *Pharmacol. Rev.* 66, 413–434.
- Filizola, M., Devi, L.A., 2013. Grand opening of structure-guided design for novel opioids. *Trends Pharmacol. Sci.* 34, 6–12.
- Fossepre, M., Leherte, L., Laaksonen, A., Vercauteren, D.P., 2014. On the modularity of the intrinsic flexibility of the micro opioid receptor: a computational study. *PLoS one* 9, e115856.
- Frolich, N., Dees, C., Paetz, C., Ren, X., Lohse, M.J., Nikolaev, V.O., Zenk, M.H., 2011. Distinct pharmacological properties of morphine metabolites at G(i)-protein and beta-arrestin signaling pathways activated by the human mu-opioid receptor. *Biochem. Pharmacol.* 81, 1248–1254.
- Fujita, W., Gomes, I., Devi, L.A., 2014. Revolution in GPCR signalling: opioid receptor heteromers as novel therapeutic targets: IUPHAR review 10. *Br. J. Pharmacol.* 171, 4155–4176.
- Goldfeld, D.A., Murphy, R., Kim, B., Wang, L., Beuming, T., Abel, R., Friesner, R.A., 2015. Docking and free energy perturbation studies of ligand binding in the kappa opioid receptor. *J. Phys. Chem. B* 119, 824–835.
- Granier, S., Manglik, A., Kruse, A.C., Kobilka, T.S., Thian, F.S., Weis, W.I., Kobilka, B.K., 2012. Structure of the delta-opioid receptor bound to naltrindole. *Nature* 485, 400–404.
- Haga, K., Kruse, A.C., Asada, H., Yurugi-Kobayashi, T., Shiroishi, M., Zhang, C., Weis, W.I., Okada, T., Kobilka, B.K., Haga, T., Kobayashi, T., 2012. Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist. *Nature* 482, 547–551.
- Hanson, M.A., Roth, C.B., Jo, E., Griffith, M.T., Scott, F.L., Reinhart, G., Desale, H., Clemons, B., Cahalan, S.M., Schuerer, S.C., Sanna, M.G., Han, G.W., Kuhn, P., Rosen, H., Stevens, R.C., 2012. Crystal structure of a lipid G protein-coupled receptor. *Science* 335, 851–855.
- He, S.Q., Zhang, Z.N., Guan, J.S., Liu, H.R., Zhao, B., Wang, H.B., Li, Q., Yang, H., Luo, J., Li, Z.Y., Wang, Q., Lu, Y.J., Bao, L., Zhang, X., 2011. Facilitation of mu-opioid receptor activity by preventing delta-opioid receptor-mediated codegradation. *Neuron* 69, 120–131.
- Hollenstein, K., Kean, J., Bortolato, A., Cheng, R.K., Dore, A.S., Jazayeri, A., Cooke, R. M., Weir, M., Marshall, F.H., 2013. Structure of class B GPCR corticotropin-releasing factor receptor 1. *Nature* 499, 438–443.
- Jaakola, V.P., Griffith, M.T., Hanson, M.A., Cherezov, V., Chien, E.Y., Lane, J.R., Ijzerman, A.P., Stevens, R.C., 2008. The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science* 322, 1211–1217.
- Jacobson, K.A., 2013. Structure-based approaches to ligands for G-protein-coupled adenosine and P2Y receptors, from small molecules to nanoconjugates. *J. Med. Chem.* 56, 3749–3767.
- Johnston, J.M., Aburi, M., Provasi, D., Bortolato, A., Urizar, E., Lambert, N.A., Javitch, J. A., Filizola, M., 2011. Making structural sense of dimerization interfaces of delta opioid receptor homodimers. *Biochemistry* 50, 1682–1690.
- Johnston, J.M., Filizola, M., 2014. Differential stability of the crystallographic interfaces of mu- and kappa-opioid receptors. *PLoS one* 9, e90694.
- Katritch, V., Fenalti, G., Abola, E.E., Roth, B.L., Cherezov, V., Stevens, R.C., 2014. Allosteric signaling in class A GPCR signaling. *Trends Biochem. Sci.* 39, 233–244.
- Keith, D.E., Murray, S.R., Zaki, P.A., Chu, P.C., Lissin, D.V., Kang, L., Evans, C.J., von Zastrow, M., 1996. Morphine activates opioid receptors without causing their rapid internalization. *J. Biol. Chem.* 271, 19021–19024.
- Kooistra, A.J., Leurs, R., de Esch, I.J., de Graaf, C., 2014. From three-dimensional GPCR structure to rational ligand discovery. *Adv. Exp. Med. Biol.* 796, 129–157.
- Kreek, M.J., Levran, O., Reed, B., Schlussman, S.D., Zhou, Y., Butelman, E.R., 2012. Opiate addiction and cocaine addiction: underlying molecular neurobiology and genetics. *The Journal of Clin. Investig.* 122, 3387–3393.
- Krissinel, E., Henrick, K., 2007. Inference of macromolecular assemblies from crystalline state. *J. Mol. Biol.* 372, 774–797.
- Kruse, A.C., Hu, J., Pan, A.C., Arlow, D.H., Rosenbaum, D.M., Rosemond, E., Green, H. F., Liu, T., Chae, P.S., Dror, R.O., Shaw, D.E., Weis, W.I., Wess, J., Kobilka, B.K., 2012. Structure and dynamics of the M3 muscarinic acetylcholine receptor. *Nature* 482, 552–556.
- Lambert, N.A., Javitch, J.A., 2014a. CrossTalk opposing view: weighing the evidence for class A GPCR dimers, the jury is still out. *J. Physiol.* 592, 2443–2445.
- Lambert, N.A., Javitch, J.A., 2014b. Rebuttal from Nevin A. Lambert and Jonathan A. Javitch. *J. Physiol.* 592, 2449.
- Land, B.B., Bruchas, M.R., Schattauer, S., Giardino, W.J., Aita, M., Messinger, D., Hnasko, T.S., Palmiter, R.D., Chavkin, C., 2009. Activation of the kappa opioid receptor in the dorsal raphe nucleus mediates the aversive effects of stress and reinstates drug seeking. *Proc. Natl. Acad. Sci. USA* 106, 19168–19173.
- Leonis, G., Avramopoulos, A., Salmas, R.E., Durdagi, S., Yurtsever, M., Papadopoulos, M.G., 2014. Elucidation of conformational states, dynamics, and mechanism of binding in human kappa-opioid receptor complexes. *J. Chem. Inf. Model.* 54, 2294–2308.
- Liu, W., Chun, E., Thompson, A.A., Chubukov, P., Xu, F., Katritch, V., Han, G.W., Roth, C.B., Heitman, L.H., AP, I.J., Cherezov, V., Stevens, R.C., 2012. Structural basis for allosteric regulation of GPCRs by sodium ions. *Science* 337, 232–236.
- Luttrell, L.M., 2014. Minireview: More than just a hammer: ligand “bias” and pharmaceutical discovery. *Mol. Endocrinol.* 28, 281–294.
- Lutz, P.E., Kieffer, B.L., 2013. Opioid receptors: distinct roles in mood disorders. *Trends Neurosci.* 36, 195–206.
- Manglik, A., Kruse, A.C., Kobilka, T.S., Thian, F.S., Mathiesen, J.M., Sunahara, R.K., Pardo, L., Weis, W.I., Kobilka, B.K., Granier, S., 2012. Crystal structure of the mu-opioid receptor bound to a morphinan antagonist. *Nature* 485, 321–326.
- Martinez-Mayorga, K., Byler, K.G., Yongye, A.B., Giulianotti, M.A., Dooley, C.T., Houghten, R.A., 2013. Ligand/kappa-opioid receptor interactions: insights from the X-ray crystal structure. *Eur. J. Med. Chem.* 66, 114–121.
- McCurdy, C.R., Prisinzano, T.E., Abraham, D.J., 2003. Opioid Receptor Ligands, Burger's Medicinal Chemistry and Drug Discovery. John Wiley & Sons, Inc.
- McPherson, J., Rivero, G., Baptist, M., Llorente, J., Al-Sabah, S., Krasel, C., Dewey, W. L., Bailey, C.P., Rosethorne, E.M., Charlton, S.J., Henderson, G., Kelly, E., 2010. Mu-opioid receptors: correlation of agonist efficacy for signalling with ability to activate internalization. *Mol. Pharmacol.* 78, 756–766.
- Molinari, P., Vezzi, V., Sbraccia, M., Gro, C., Riitano, D., Ambrosio, C., Casella, I., Costa, T., 2010. Morphine-like opiates selectively antagonize receptor-arrestin interactions. *The Journal of biological chemistry* 285, 12522–12535.
- Negri, A., Rives, M.L., Caspers, M.J., Prisinzano, T.E., Javitch, J.A., Filizola, M., 2013. Discovery of a novel selective kappa-opioid receptor agonist using crystal structure-based virtual screening. *J. Chem. Inf. Model.* 53, 521–526.
- O'Dowd, B.F., Ji, X., O'Dowd, P.B., Nguyen, T., George, S.R., 2012. Disruption of the mu-delta opioid receptor heteromer. *Biochem. Biophys. Res. Commun.* 422, 556–560.
- Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Le Trong, I., Teller, D.C., Okada, T., Stenkamp, R.E., Yamamoto, M., Miyano, M., 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* 289, 739–745.
- Pasternak, G.W., 2014. Opiate pharmacology and relief of pain. *J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol.* 32, 1655–1661.
- Pasternak, G.W., Pan, Y.X., 2013. Mu opioids and their receptors: evolution of a concept. *Pharmacol. Rev.* 65, 1257–1317.
- Pasternak, G.W., Snowman, A.M., Snyder, S.H., 1975. Selective enhancement of [3 H] opiate agonist binding by divalent cations. *Mol. Pharmacol.* 11, 735–744.
- Pasternak, G.W., Snyder, S.H., 1975. Identification of novel high affinity opiate receptor binding in rat brain. *Nature* 253, 563–565.
- Pert, C.B., Pasternak, G., Snyder, S.H., 1973. Opiate agonists and antagonists discriminated by receptor binding in brain. *Science* 182, 1359–1361.
- Polepally, P.R., Huben, K., Vardy, E., Setola, V., Mosier, P.D., Roth, B.L., Zjawiony, J.K., 2014. Michael acceptor approach to the design of new salvinorin A-based high affinity ligands for the kappa-opioid receptor. *Eur. J. Med. Chem.* 85, 818–829.
- Preinerling, A.M., Meiler, J., Hamm, H.E., 2013. Conformational flexibility and structural dynamics in GPCR-mediated G protein activation: a perspective. *J. Mol. Biol.* 425, 2288–2298.
- Raeal, K.M., Bohn, L.M., 2014. Handbook of Experimental Pharmacology. 219; , pp. 427–443.
- Raeal, K.M., Schmid, C.L., Groer, C.E., Bohn, L.M., 2011. Functional selectivity at the mu-opioid receptor: implications for understanding opioid analgesia and tolerance. *Pharmacol. Rev.* 63, 1001–1019.
- Raeal, K.M., Walker, J.K., Bohn, L.M., 2005. Morphine side effects in beta-arrestin 2 knockout mice. *J. Pharmacol. Exp. Ther.* 314, 1195–1201.
- Rivero, G., Llorente, J., McPherson, J., Cooke, A., Mundell, S.J., McArdle, C.A.,

- Rosethorne, L.M., Charlton, S.J., Krasel, C., Bailey, C.P., Henderson, G., Kelly, E., 2012. Endomorphin-2: a biased agonist at the mu-opioid receptor. *Mol. Pharmacol.* 82, 178–188.
- Shang, Y., LeRouzic, V., Schneider, S., Bisignano, P., Pasternak, G.W., Filizola, M., 2014. Mechanistic insights into the allosteric modulation of opioid receptors by sodium ions. *Biochemistry* 53, 5140–5149.
- Shim, J., Coop, A., MacKerell Jr., A.D., 2013. Molecular details of the activation of the mu opioid receptor. *J. Phys. Chem. B* 117, 7907–7917.
- Shimamura, T., Shiroishi, M., Weyand, S., Tsujimoto, H., Winter, G., Katritch, V., Abagyan, R., Cherezov, V., Liu, W., Han, G.W., Kobayashi, T., Stevens, R.C., Iwata, S., 2011. Structure of the human histamine H1 receptor complex with doxepin. *Nature* 475, 65–70.
- Shoichet, B.K., Kobilka, B.K., 2012. Structure-based drug screening for G-protein-coupled receptors. *Trends Pharmacol. Sci.* 33, 268–272.
- Shonberg, J., Lopez, L., Scammells, P.J., Christopoulos, A., Capuano, B., Lane, J.R., 2014. Biased agonism at G protein-coupled receptors: the promise and the challenges—A medicinal chemistry perspective. *Med. Res. Rev.*
- Shukla, A.K., 2014. Biasing GPCR signaling from inside. *Sci. Signal.* 7, 3.
- Siu, F.Y., He, M., de Graaf, C., Han, G.W., Yang, D., Zhang, Z., Zhou, C., Xu, Q., Wacker, D., Joseph, J.S., Liu, W., Lau, J., Cherezov, V., Katritch, V., Wang, M.W., Stevens, R.C., 2013. Structure of the human glucagon class B G-protein-coupled receptor. *Nature* 499, 444–449.
- Soergel, D.G., Ann Subach, R., Sadler, B., Connell, J., Marion, A.S., Cowan, C.L., Violin, J.D., Lark, M.W., 2014a. First clinical experience with TRV130: pharmacokinetics and pharmacodynamics in healthy volunteers. *J. Clin. Pharmacol.* 54, 351–357.
- Soergel, D.G., Subach, R.A., Burnham, N., Lark, M.W., James, I.E., Sadler, B.M., Skobieranda, F., Violin, J.D., Webster, L.R., 2014b. Biased agonism of the mu-opioid receptor by TRV130 increases analgesia and reduces on-target adverse effects versus morphine: a randomized, double-blind, placebo-controlled, crossover study in healthy volunteers. *Pain* 155, 1829–1835.
- Srivastava, A., Yano, J., Hirozane, Y., Kefala, G., Gruswitz, F., Snell, G., Lane, W., Ivetac, A., Aertgeerts, K., Nguyen, J., Jennings, A., Okada, K., 2014. High-resolution structure of the human GPR40 receptor bound to allosteric agonist TAK-875. *Nature* 513, 124–127.
- Sternini, C., Spann, M., Anton, B., Keith Jr., D.E., Bunnett, N.W., von Zastrow, M., Evans, C., Brecha, N.C., 1996. Agonist-selective endocytosis of mu opioid receptor by neurons in vivo. *Proc. Natl. Acad. Sci. USA* 93, 9241–9246.
- Tan, Q., Zhu, Y., Li, J., Chen, Z., Han, G.W., Kufareva, I., Li, T., Ma, L., Fenalti, G., Zhang, W., Xie, X., Yang, H., Jiang, H., Cherezov, V., Liu, H., Stevens, R.C., Zhao, Q., Wu, B., 2013. Structure of the CCR5 chemokine receptor-HIV entry inhibitor maraviroc complex. *Science* 341, 1387–1390.
- Thompson, A.A., Liu, W., Chun, E., Katritch, V., Wu, H., Vardy, E., Huang, X.P., Trappella, C., Guerrini, R., Calo, G., Roth, B.L., Cherezov, V., Stevens, R.C., 2012. Structure of the nociceptin/orphanin FQ receptor in complex with a peptide mimetic. *Nature* 485, 395–399.
- Thompson, G.L., Canals, M., Poole, D.P., 2014. Biological redundancy of endogenous GPCR ligands in the gut and the potential for endogenous functional selectivity. *Front. Pharmacol.* 5, 262.
- Thompson, G.L., Kelly, E., Christopoulos, A., Canals, M., 2015. Novel GPCR paradigms at the mu-opioid receptor. *Br. J. Pharmacol.* 172, 287–296.
- Vanderah, T.W., 2010. Delta and kappa opioid receptors as suitable drug targets for pain. *Clin. J. Pain* 26 (Suppl. 10), S10–S15.
- Vardy, E., Mosier, P.D., Frankowski, K.J., Wu, H., Katritch, V., Westkaemper, R.B., Aube, J., Stevens, R.C., Roth, B.L., 2013. Chemotype-selective modes of action of kappa-opioid receptor agonists. *J. Biol. Chem.* 288, 34470–34483.
- Violin, J.D., Crombie, A.L., Soergel, D.G., Lark, M.W., 2014. Biased ligands at G-protein-coupled receptors: promise and progress. *Trends Pharmacol. Sci.* 35, 308–316.
- Vortherms, T.A., Roth, B.L., 2005. Receptorome screening for CNS drug discovery. *IDrugs: Investig. Drugs J.* 8, 491–496.
- Wacker, D., Wang, C., Katritch, V., Han, G.W., Huang, X.P., Vardy, E., McCorvy, J.D., Jiang, Y., Chu, M., Siu, F.Y., Liu, W., Xu, H.E., Cherezov, V., Roth, B.L., Stevens, R.C., 2013. Structural features for functional selectivity at serotonin receptors. *Science* 340, 615–619.
- Wang, C., Jiang, Y., Ma, J., Wu, H., Wacker, D., Katritch, V., Han, G.W., Liu, W., Huang, X.P., Vardy, E., McCorvy, J.D., Gao, X., Zhou, X.E., Melcher, K., Zhang, C., Bai, F., Yang, H., Yang, L., Jiang, H., Roth, B.L., Cherezov, V., Stevens, R.C., Xu, H.E., 2013a. Structural basis for molecular recognition at serotonin receptors. *Science* 340, 610–614.
- Wang, C., Wu, H., Katritch, V., Han, G.W., Huang, X.P., Liu, W., Siu, F.Y., Roth, B.L., Cherezov, V., Stevens, R.C., 2013b. Structure of the human smoothened receptor bound to an antitumour agent. *Nature* 497, 338–343.
- Wang, Y.H., Sun, J.F., Tao, Y.M., Chi, Z.Q., Liu, J.G., 2010. The role of kappa-opioid receptor activation in mediating antinociception and addiction. *Acta Pharmacol. Sin.* 31, 1065–1070.
- Warne, T., Serrano-Vega, M.J., Baker, J.G., Moukhametzianov, R., Edwards, P.C., Henderson, R., Leslie, A.G., Tate, C.G., Schertler, G.F., 2008. Structure of a beta1-adrenergic G-protein-coupled receptor. *Nature* 454, 486–491.
- White, J.F., Noinaj, N., Shibata, Y., Love, J., Kloss, B., Xu, F., Gvozdenovic-Jeremic, J., Shah, P., Shiloach, J., Tate, C.G., Grisshammer, R., 2012. Structure of the agonist-bound neurotensin receptor. *Nature* 490, 508–513.
- White, K.L., Scepton, A.P., Rives, M.L., Bikbulatov, R.V., Polepally, P.R., Brown, P.J., Kenakin, T., Javitch, J.A., Zjawiony, J.K., Roth, B.L., 2014. Identification of novel functionally selective kappa-opioid receptor scaffolds. *Mol. Pharmacol.* 85, 83–90.
- Williams, J.T., Ingram, S.L., Henderson, G., Chavkin, C., von Zastrow, M., Schulz, S., Koch, T., Evans, C.J., Christie, M.J., 2013. Regulation of mu-opioid receptors: desensitization, phosphorylation, internalization, and tolerance. *Pharmacol. Rev.* 65, 223–254.
- Wu, B., Chien, E.Y., Mol, C.D., Fenalti, G., Liu, W., Katritch, V., Abagyan, R., Brooun, A., Wells, P., Bi, F.C., Hamel, D.J., Kuhn, P., Handel, T.M., Cherezov, V., Stevens, R.C., 2010. Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists. *Science* 330, 1066–1071.
- Wu, H., Wacker, D., Mileni, M., Katritch, V., Han, G.W., Vardy, E., Liu, W., Thompson, A.A., Huang, X.P., Carroll, F.I., Mascarella, S.W., Westkaemper, R.B., Mosier, P.D., Roth, B.L., Cherezov, V., Stevens, R.C., 2012. Structure of the human kappa-opioid receptor in complex with JDTic. *Nature* 485, 327–332.
- Wu, H., Wang, C., Gregory, K.J., Han, G.W., Cho, H.P., Xia, Y., Niswender, C.M., Katritch, V., Meiler, J., Cherezov, V., Conn, P.J., Stevens, R.C., 2014. Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. *Science* 344, 58–64.
- Yin, J., Mobarec, J.C., Kolb, P., Rosenbaum, D.M., 2014. Crystal structure of the human OX orexin receptor bound to the insomnia drug suvorexant. *Nature*.
- Yuan, S., Vogel, H., Filipek, S., 2013. The role of water and sodium ions in the activation of the mu-opioid receptor. *Angew. Chem. Int. Ed. Engl.* 52, 10112–10115.
- Zhang, C., Srinivasan, Y., Arlow, D.H., Fung, J.J., Palmer, D., Zheng, Y., Green, H.F., Pandey, A., Dror, R.O., Shaw, D.E., Weis, W.I., Coughlin, S.R., Kobilka, B.K., 2012. High-resolution crystal structure of human protease-activated receptor 1. *Nature* 492, 387–392.
- Zhang, K., Zhang, J., Gao, Z.G., Zhang, D., Zhu, L., Han, G.W., Moss, S.M., Paoletta, S., Kiselev, E., Lu, W., Fenalti, G., Zhang, W., Muller, C.E., Yang, H., Jiang, H., Cherezov, V., Katritch, V., Jacobson, K.A., Stevens, R.C., Wu, B., Zhao, Q., 2014. Structure of the human P2Y12 receptor in complex with an antithrombotic drug. *Nature* 509, 115–118.
- Zhou, L., Bohn, L.M., 2014. Functional selectivity of GPCR signaling in animals. *Curr. Opin. Cell Biol.* 27, 102–108.
- Zhou, L., Lovell, K.M., Frankowski, K.J., Slauson, S.R., Phillips, A.M., Streicher, J.M., Stahl, E., Schmid, C.L., Hodder, P., Madoux, F., Cameron, M.D., Prisinzano, T.E., Aube, J., Bohn, L.M., 2013. Development of functionally selective, small molecule agonists at kappa opioid receptors. *J. Biol. Chem.* 288, 36703–36716.