Published in final edited form as:

Trends Pharmacol Sci. 2013 January ; 34(1): 6–12. doi:10.1016/j.tips.2012.10.002.

Grand Opening of Structure-Guided Design for Novel Opioids

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Abstract

Twelve years after the publication of the first crystal structure of a G protein-coupled receptor (GPCR), experimental crystal structures of the four opioid receptor subtypes have made their entrance into the literature in the most extraordinary way, that is, all at once. Not only do these crystal structures contribute unprecedented molecular details of opioid ligand binding and specificity, but they represent important tools for structure-based approaches to guide the discovery of safer and more efficient opioid therapeutics. We provide here an overview of these latest breakthroughs in structural biology of GPCRs with a focus on the differences and similarities between the four opioid receptor structures, as well as their limitations, in the context of challenges for translating this new knowledge from bench to bedside.

Opioids and their receptors

Opioids such as morphine, codeine, and fentanyl comprise a major class of analgesics used in the clinical management of pain. They exert their effects through activation of three major opioid receptor families [1, 2], the μ -, δ -, and κ -opioid receptors (MOP, DOP, and KOP receptors, respectively, according to a IUPHAR subcommittee recommendation [3]), signaling primarily through interaction with the inhibitory G protein for adenylyl cyclase, $G_{i/o}$ [4]. These receptors were cloned in early 1990s and belong to the superfamily of GPCRs [4]. Not only are they targets for a diverse set of small molecules, from classical morphine-derivatives (also known as morphinans) to a variety of other classical and non-classical opioid ligands, but they can also be activated by peptides [5]. The latter include endogenous opioids such as endorphins, endomorphins, and morphiceptin for the MOP receptor, enkephalins for the DOP receptor, and dynorphins for the KOP receptor. The endogenous nociceptin/orphanin FQ peptide binds selectively to a fourth member of the opioid receptor family, which was discovered much later [6, 7], and is known as NOP or ORL-1 receptor. Despite the high sequence identity between this receptor and the other major opioid receptor subtypes [67% in the transmembrane (TM) region [8]], the NOP receptor does not bind with the same high affinity morphinans and other opioid ligands [6, 7].

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Although they are the best analgesics currently on the market, the effective medical use of opioid ligands is usually obscured by myriad undesirable side effects [4, 5], including nausea, vomiting, constipation, etc. In addition, the prolonged use of opioids often leads to the development of tolerance and/or addiction. The latter, together with a pronounced sense of euphoria, makes them among the most frequently abused drugs in the USA [9]. Although considerable resources have been devoted to the discovery of safer opioids over the past decades, the promise of more effective analgesics has gone largely unfulfilled. There are several reasons for this. First, atomic-level structural information of opioid receptors in particular, and GPCRs in general, has traditionally lagged behind due to technical difficulties in purifying and crystallizing these complex membrane protein systems. Second, similar to the other GPCRs, the biology of opioid receptors is quite complex. In fact, opioid ligands acting at these receptors can activate multiple signaling pathways, most often through either $G_{i/o}$ or arrestins. Preferential activation of a specific signaling pathway by a ligand has been termed in the literature functional selectivity, ligand-directed signaling/receptor trafficking and/or biased agonism (e.g., see [10, 11]). Examples of this complex signaling have recently been reported in the case of all major opioid receptors [12]. For instance, opioids such as morphine and oxymorphone have recently been shown to be effective agonists for G protein coupling but competitive antagonists or partial agonists for arrestin at the DOP and MOP receptors, respectively [13]. Similarly, 6'-guanidinonaltrindole (6'-GNTI), a potent partial agonist at the KOP receptor for G protein activation, functions as an antagonist to block the arrestin recruitment [14]. In contrast, the KOP receptor partial agonist 12-epi-salvA has been recently shown to be an arrestin-biased ligand, being an efficacious activator of β -arrestin-2 mediated signaling pathways [15]. Given that the most addictive opioid ligands appear to promote interactions with $G_{i/o}$ more strongly than with arrestins [13], and that, unlike analgesia, the adverse dysphoric effects induced by opioid receptor activation appear to be arrestin-dependent (at least in the case of KOP [16]), identifying effective biased opioid ligands for one protein or another represents a new direction for developing non-addictive and/or effective analgesics. Selective and specific modulation of opioid receptor signaling and function leading to analgesia without the adverse side effects has also been suggested to depend upon new therapeutic targets resulting from interaction of opioid receptors among themselves or other GPCRs to form dimers and/or higher-order oligomers [17, 18].

The past few months have offered real breakthroughs in the opioid receptor field through the release of high-resolution crystal structures of all the existing opioid receptor subtypes. These structures correspond to Protein Data Bank (PDB) identification codes 4DKL for the MOP receptor [19], 4EJ4 for the DOP receptor [20], 4DJH for the KOP receptor [21], and 4EA3 for the NOP receptor [22]. Notably, they have provided the first high-resolution molecular insight into the selective binding of classical (e.g., morphinan derivatives) and non-classical opioid ligands to their receptors. In this review, we will draw attention to the differences and similarities between the different opioid receptor crystal structures, as well as other available GPCR crystal structures, and discuss their implications and limitations in the structure-based development of safer painkillers and anti-addiction medications.

A common molecular architecture

The newly available crystal structures of mouse MOP [19], mouse DOP [20], human KOP [21], and human NOP [22] receptors at 2.8 Å, 3.4 Å, 2.9 Å, and 3.0 Å resolution, respectively, represent important milestones in our understanding of the opioid receptor function. Figure 1 illustrates an overlap of the typical GPCR seven-pass TM helix folds of the 4 opioid receptor crystal structures. These TM helices are connected by three intracellular and three extracellular loops (ILs and ELs, respectively). As expected by the high sequence identity among the TM regions of these receptors (from a minimum of 67%

for the NOP receptor compared to the other opioid receptors to a maximum of 76% between the MOP and DOP receptors [8]; see sequence alignment in Figure 2), these structures share a very similar structural fold, even in the less conserved loop regions. This is especially evident in the characteristic β -hairpin structure of the EL2, which is, interestingly, also present in the crystal structure of the chemokine receptor CXCR4 [23], another GPCR that binds peptides.

As shown in Figure 1, the largest difference between the opioid receptor crystal structures is found in the extracellular half of TM1, which appears to be much straighter in the KOP receptor crystal structure compared to those of DOP, MOP, and NOP receptors. Notably, the KOP receptor TM1 also differs from the equivalent portion of the CXCR4 crystal structure [23]. However, different conformations of TM1 have been observed even within the same crystal as seen in the case of turkey β 1-adrenergic receptor, suggesting that different crystallization conditions or crystal contacts might be responsible for these structural deviations [24]. Particularly different is also the EL3 link between TM6 and TM7 (see Figure 1), which exhibits low sequence conservation (see alignment in Figure 2) and high temperature factors in all opioid receptor structures. Notably, this loop has not been resolved in the KOP receptor.

Crystal contacts or physiological interfaces?

As for the majority of available GPCR crystal structures, the four opioid receptor structures correspond to engineered receptor forms in which the T4 lysozyme (or T4L for MOP, DOP, and KOP receptors) or the thermostabilized apocytochrome b₅₆₂RIL (or BRIL for the NOP receptor) have been introduced to stabilize the receptor in a specific conformation that is amenable to crystallization. Specifically, T4L replaces the third intracellular loop in the MOP, DOP, or KOP receptor constructs used for crystallography, whereas BRIL replaces most of the N-terminal domain in the NOP receptor construct. In all of these receptor constructs, the poorly ordered N- and C-terminal domains have been removed.

Figure 3 illustrates the different crystal contacts involving the TM domains of the four opioid receptor structures. As evident from the location of T4L and BRIL in these figures, receptors adopt either parallel or anti-parallel orientations in the crystals. Specifically, as shown in Figure 3A and 3D, the DOP and the NOP receptors only crystallized with anti-parallel arrangements of the receptors. By contrast, the MOP receptor (see Figure 3B) exhibits exclusively parallel arrangements in its crystals, characterized by two different interfaces, specifically a very compact one involving TM5 and TM6 with 28 residues involved in the interaction, and a less compact interface involving TM1, TM2, and H8. Notably, the latter is also present in the KOP receptor crystals (see Figure 3C), which also feature an anti-parallel arrangement. The TM5/TM6 interface was also found in five independent crystal structures of the chemokine receptor CXCR4 [23]. In all of these structures, as well as in that of the MOP receptor, contacts between the T4L domains on each protomer account for a portion of the buried surface area across the inter-protomer interface [19, 23]. In the CXCR4 crystal structures, this portion is more extended than in the MOP receptor crystal structure. Although it is tempting to speculate that the parallel arrangements found in MOP and KOP receptors are indicative of physiologically relevant dimerization interfaces, it should be kept in mind that these are the results of crystallogenesis, and as such, they might depend upon the crystallization conditions and/or the different T4L interactions in the crystal.

The existence of opioid receptor dimers or higher-order oligomers with unique pharmacology and signaling properties has been postulated based on the results of several pharmacological and biochemical studies [17, 18]. Prior to the opioid receptor crystal

structures, TM1, TM4, and TM5 had been suggested most often to play a role in the interaction between opioid receptors. Indeed, a combination of computational and biochemical studies of the DOP receptor suggested the involvement of TM4 and/or TM5 at the dimerization interface, albeit with differing association propensities [25]. Several hypotheses of opioid receptor dimerization disruption have also been put forward in the recent literature. For instance, a TAT-fused peptide composed of the MOP TM1 was suggested to disrupt a possible interaction between MOP and DOP in the mouse spinal cord, with a consequent augmentation of morphine analgesia, and reduction of the antinociceptive tolerance to morphine [26]. Recent studies have also reported that substitution of residues in the cytoplasmic carboxyl tail of the DOP receptor or the IL3 region of both MOP and DOP prevent heteromer formation [27]. Notably, the existence of endogenous MOP-DOP or DOP-KOP heteromers with unique pharmacological and signaling properties (e.g., see [28, 29] for recent published evidence) have recently been supported by the use of antibodies that appear to recognize co-expressed receptors but not the individual proteins [30, 31]. Given the different hypotheses of interfaces of opioid receptor dimerization, including those inspired by the recent receptor crystal structures, it is clear that additional studies are needed to understand how endogenous opioid receptors interact in their natural membrane environment.

Molecular determinants of ligand recognition and selectivity

Current opioid receptor crystal structures have advanced our understanding of the way antagonists bind within the bundle of the receptor's TM helices stabilized in an inactive conformation. Specifically, the MOP receptor has been crystallized covalently bound to the irreversible morphinan β -funaltrexamine [32] through an amino acid residue that had originally been suggested by mutagenesis studies [33]. By contrast, DOP, KOP, and NOP receptor crystal structures have been determined in the presence of non-covalent ligands, specifically: naltrindole [34], JD_Tic [35], and the peptide-mimetic compound-24 [36], respectively. The chemical structures of these 4 antagonists are illustrated in Figure 4.

The receptor binding pockets accommodating these opioid ligands are solvent-exposed and similar in shape. Figure 4 shows an overlay of the ligands in a representative opioid receptor crystal structure (central panel) along with schema of the interaction modes of β -funaltrexamine (left panel), naltrindole (right panel), JD_Tic (upper panel), and compound-24 (lower panel), in the MOP, DOP, KOP, and NOP receptor crystal structures, respectively. Residues in this figure and throughout the text are numbered according to the amino acid sequences of mouse MOP, mouse DOP, human KOP, and human NOP receptors, as well as the corresponding Ballesteros-Weinstein numbering scheme [37]. In this 2-number scheme, the first one indicates the TM helix number, whereas the second one is relative to the most highly conserved residue in each TM helix indicated by the number 50. Notably, the opioid receptor binding pockets resemble that of CXCR4 [23], suggesting yet another commonality among GPCRs that bind both peptides and small molecules. As shown in the central panel of Figure 4, ligands occupy a common region delimited by helices TM3, TM5, TM6, and TM7 in the binding pockets of MOP, DOP, KOP, and NOP receptors. Notwithstanding the slight distortion in the binding mode of β -funaltrexamine (owing to its covalent tether to the K5.39 of the MOP receptor), binding of morphinan ligands β -funaltrexamine and naltrindole is mostly confined to the TM3-TM5-TM6-TM7 section of the binding pockets of MOP and DOP receptors, respectively. By contrast, binding of ligands JD_Tic and compound-24 to the KOP and NOP receptors, respectively, extends to a region of the binding pocket defined by TM2, TM7, and TM3.

Figure 5 shows surface views of a representative opioid receptor crystal structure with residues colored according to sequence conservation. As shown by a blue-colored surface in

the middle of the TM bundle, which coincides with the bottom part of the binding pocket, this region is highly conserved, as it is the cytoplasmic side of the receptor. Although the former suggests the presence of common molecular determinants for the recognition of the portion of opioid ligands that may be responsible for their efficacy (traditionally referred to as the 'message' part of opioid ligands), the latter denotes similarities among the opioid receptors due to their binding the same G-protein and arrestin subtypes. The fully conserved interacting residues in MOP, DOP, KOP, and NOP receptors that are within 4 Å of any atom in the ligands (see Figure 4 and Table 1) are: D3.32, Y3.33, M3.36, W6.48, and Y7.43. Notably, residue Y7.43 acquires a slightly different orientation in the KOP binding pocket compared to MOP, DOP, and NOP receptors, which seems to be necessary to accommodate the KOP receptor ligand JD_{Tic}. Additional interacting residues that are conserved in three of the opioid receptors are listed in Table 1. Although no crystallographic water molecules could be seen in the DOP receptor structure owing to its low resolution compared to the MOP, KOP, and NOP receptor structures, it is tempting to speculate on the presence of two conserved water molecules that could form a hydrogen bonding network between the ligands and the H6.52 residue of MOP, DOP, and KOP receptors. As reported above, this residue is a glutamine in the NOP receptor.

Figure 5 also shows that the majority of divergent residues, possibly conferring subtype specificity, are either located in the upper part of the binding pocket or in the portion of the binding pocket delimited by TM2, TM3, and TM7. The location of the majority of these residues in the upper part of the binding pocket has led to the suggestion [38] that these might form a selectivity filter [39], which happens to resemble the allosteric binding region identified in the crystal structures of muscarinic M2 and M3 receptors [40, 41]. The ligand chemical moieties responsible for opiate selectivity (the so-called 'address' portion of the ligand) are shown to interact with some of these divergent residues of the receptors in Figure 4. For instance, L7.35 represents a selectivity determinant for naltrindole because it corresponds to sterically incompatible residues, specifically a tryptophan and a tyrosine, in the MOR and KOR receptors, respectively. In the NOP receptor, there is also a leucine at this position, but it is far away (>7 Å) from the NOP receptor ligand compound-24. Additional interacting residues that may be responsible for selectivity are listed in Table 1.

New opportunities for discovering novel opioid ligands

The newly released crystal structures suggest that, although the chemical moieties responsible for the opioid ligand efficacy interact similarly within the seven TM helical bundle, two different regions of the binding pocket (the upper region mostly defined by TM5, TM6, and TM7 and the region defined by TM2 and TM7) are involved in interactions with the chemical moieties responsible for the opioid selectivity. This observation is extremely valuable for structure-based compound optimization and screening, as it offers a unique opportunity to either optimize existing opioid ligands or discover novel molecules that, by occupying different sites simultaneously, may become highly potent and/or selective for specific opioid receptor subtypes. However, it should be kept in mind that the available crystal structures may provide a limited understanding of ligand selectivity given the small chemical variability among the crystallized ligands and the current absence of agonist and peptide-bound structures of the receptors. Another possibly novel way for developing new opioid ligands is to use dimeric interfaces as potential new targets. If the interfaces revealed by crystallography or other methods (e.g., see [25, 26, 42, 43]) are physiologically relevant, then it would be very helpful to use them to discover dimerization-destabilizing compounds. Even if the latter will not result in new drugs, the discovery of chemical probes that can help understand the potential role of oligomerization in GPCR function would be invaluable.

Last but not the least, the structural characterization of the cytoplasmic side of the opioid receptors, which corresponds incidentally to the G protein or arrestin binding pockets, could guide the identification of additional small molecules that can modulate the opioid receptor function.

Concluding remarks

Recent opioid receptor crystal structures provide unprecedented molecular details of opioid ligand binding and specificity. Notwithstanding the tremendous potential of current structure-guided approaches to designing novel compounds acting at the opioid receptors, there are several limitations one has to consider that can still hamper full success in drug discovery. For instance, there is still much to be learned about opioid receptor structure and dynamics before one can fully understand the molecular mechanisms underlying opioid function. One problem is that the available opioid receptor crystal structures are limited to inactive forms of the receptors stabilized by high-affinity ligands during expression and crystallogenesis. More structures of different states of the receptors, ideally in complex with G protein, arrestin, or other regulatory accessory proteins such as GPCR kinases are needed but might not be easy to obtain. Future efforts include characterization of opioid receptor conformations with signaling and/or regulatory proteins, as well as understanding the potential role of oligomerization and protein dynamics in the receptor function.

It is well known that GPCRs can adopt multiple conformations [44, 45], and opioid receptors are not an exception. It is also known that while some opioid agonists (e.g., morphine [13], oxymorphone [13], 6'-GNTI [14]) can stimulate G protein signaling but not arrestin-mediated signaling, others (e.g., 12-epi-salvA) are arrestin-biased ligands [15]. Structural characterization of active conformations for the G-protein and the β -arrestin signaling pathways is therefore the immediate future challenge researchers working in the opioid receptor field will have to face. Not only have recent spectroscopic studies shown that ligands with different efficacies stabilize different receptor conformations [44, 45], but active conformations for the G-protein and the β -arrestin signaling pathways appear to be different. Specifically, it has been suggested that β -arrestin active states require the rearrangement of TM7 and H8, whereas the conformational changes associated with G protein activation mostly require the movement of TM6 [46].

Although additional crystal structures are highly desirable, understanding how specific ligands induce or stabilize specific conformations will most likely require different methods, either experimental or computational, than crystallography, because the composition of the lipid bilayer might play a key role in this understanding. Elucidation of the dynamic character of GPCRs and the way opioid receptors are activated by endogenous peptides and/or small molecule ligands will then be necessary to develop more effective drugs.

Acknowledgments

We wish to thank Dr. Davide Provasi for providing the python script that allowed us to generate Figure 5. The authors' work on opioid receptors is supported by NIH grants DA026434 and DA034049 (to MF), and DA008863 and DA019521 (to LAD).

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Figure 1. Vertical (left), extracellular (top right), and intracellular (bottom right) views of the overlap of the 4 opioid receptor crystal structures that are currently available
 The MOP, DOP, KOP, and NOP receptor crystal structures corresponding to PDB identification codes 4DKL, 4EJ4, 4DJH and 4EA3 are shown in red, cyan, magenta, and green, respectively.

Figure 2. Sequence alignment of the 4 crystallized opioid receptors

The TM helices and loop regions are indicated and labeled accordingly. The small arrows indicate the most conserved residue in each TM helix, which is assigned a number 50 in the Ballesteros-Weinstein numbering scheme. Identical residues in all 4 receptors are shown in blue. Identical residues in the MOP, DOP, and KOP but unique to the NOP receptors are shown in cyan. Divergent residues in all 4 opioid receptors are shown in red. Divergent residues in the MOP, DOP, and KOP, but not in NOP receptors are shown in brown. Unique residues to either MOP, DOP, or KOP receptors are shown in orange. Missing residues in crystal structures are highlighted in grey. N- and C-termini are not included in the alignment.

Figure 3. Crystal contacts involving the TM domains of the 4 opioid receptor structures
 As evident from the location of T4L (white cartoon) and BRIL (grey cartoon) in these figures, receptors adopt either parallel or anti-parallel orientations in the crystals. (A) Anti-parallel arrangement of the DOP receptor; (B) parallel arrangement of the MOP receptor with TM5 and TM6 or TM1, TM2, and H8 at the interface. (C) Parallel (interface TM1, TM2, and H8) and anti-parallel arrangement of the KOP receptor; (D) Anti-parallel arrangement of the NOP receptor.

Figure 4. An overlay of the crystallized opioid ligands in a representative opioid receptor crystal structure along with schema of their interaction modes in each opioid receptor crystal structure. The central panel shows an overlay of β -funaltrexamine (red), naltrindole (cyan), JDtic (magenta), and compound-24 (green) in the MOP receptor crystal structure, which is partially shown in a grey cartoon representation. Interaction schema for β -funaltrexamine, naltrindole, JDtic, and compound-24 in the MOP, DOP, KOP, and NOP receptor crystal structures are shown in the left, right, upper, and lower panels, respectively. Residues within 4 Å of any atom in the ligands are numbered according to the amino acid sequences of mouse MOP, mouse DOP, human KOP, and human NOP, as well as the corresponding Ballesteros-Weinstein numbering scheme. Identical residues in all 4 receptors are shown in blue. Identical residues in the MOP, DOP, and KOP but unique to the NOP receptors are shown in cyan. Divergent residues in all 4 opioid receptors are shown in red. Divergent residues in the MOP, DOP, and KOP, but not in NOP receptors are shown in brown. Unique residues to either MOP, DOP, or KOP receptors are shown in orange.

Figure 5. Surface views of a representative opioid receptor crystal structures with residues colored according to sequence conservation

Left and upper right panels indicate horizontal views (extracellular and cytoplasmic, respectively) of a representative opioid receptor crystal structure. Lower panels refer to vertical views of the same receptor. Identical residues in all 4 receptors are shown in blue. Identical residues in the MOP, DOP, and KOP but unique to NOP receptors are shown in cyan. Divergent residues in all 4 opioid receptors are shown in red. Divergent residues in MOP, DOP, and KOP, but not in NOP receptors are shown in brown. Unique residues to either MOP, DOP, or KOP receptors are shown in orange.

Table 1

Residues within a 4 Å distance from ligands in the opioid receptor crystal structures.

Interacting Residues*	MOP	DOP	KOP	NOP
A/A/V/V 2.53	–	–	+	–
T/T/T/T 2.56	–	–	+	–
Q/Q/Q/Q 2.60	–	–	+	+
N/K/V/D 2.63	–	–	+	+
V/V/V/V 3.28	–	–	+	+
I/L/L/I 3.29	–	–	+	+
D/D/D/D 3.32	+	+	+	+
Y/Y/Y/Y 3.33	+	+	+	+
M/M/M/M 3.36	+	+	+	+
F/F/F/F 3.37	–	–	–	+
E/D/D/G 5.35	+	–	–	–
K/K/K/A 5.39	+	+	+	–
V/V/V/I 5.42	+	+	+	+
A/A/A/S 5.46	–	–	–	+
W/W/W/W 6.48	+	+	+	+
I/I/V 6.51	+	+	+	+
H/H/H/Q 6.52	+	+	+	+
V/V/L/V 6.55	+	+	+	+
K/W/E/Q 6.58	+	+	–	–
W/L/Y/L 7.35	+	+	+	–
I/I/T 7.39	+	+	+	+
G/G/G/G 7.42	–	–	+	–
Y/Y/Y/Y 7.43	+	+	+	+
W/W/W124/W(ELI)	–	–	+	–

* Amino acid residues in the far left column refer to MOP/DOP/KOP/NOP receptors. Receptor residues are indicated with a '+' or '–' sign depending on whether or not they are within a 4 Å distance from ligands. Identical residues in all 4 receptors are shown in blue. Identical residues in the MOP, DOP, and KOP but unique to NOP receptors are shown in cyan. Divergent residues in all 4 opioid receptors are shown in red. Divergent residues in MOP, DOP, and KOP, but not in NOP receptors are shown in brown. Unique residues to either MOP, DOP, or KOP receptors are shown in orange.