Neurochem Res (2014) 39:1850–1861
DOI 10.1007/s11064-014-1398-8

OVERVIEW

The Receptor Concept in 3D: From Hypothesis and Metaphor to GPCR–Ligand Structures

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Received: 16 May 2014 / Revised: 21 July 2014 / Accepted: 22 July 2014 / Published online: 8 August 2014
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Abstract The first mentioning of the word "receptor" for the structure with which a bioactive compound should react for obtaining its specific influence on a physiological system goes back to the years around 1900. The receptor concept was adapted from the lock and key theory for the enzyme substrate and blockers interactions. Through the years the concept, in the beginning rather being a metaphor, not a model, was refined and became reality in recent years. Not only the structures of receptors were elucidated, also the receptor machineries were unraveled. Following a brief historical review we will describe how the recent breakthroughs in the experimental determination of G protein-coupled receptor (GPCR) crystal structures can be complemented by computational modeling, medicinal chemistry, biochemical, and molecular pharmacological studies to obtain new insights into the molecular determinants of GPCR–ligand binding and activation. We will furthermore discuss how this information can be used for structure-based discovery of novel GPCR ligands that bind specific (allosteric) binding sites with desired effects on GPCR functional activity.

Keywords G protein-coupled receptor · GPCR medicinal chemistry · Protein–ligand interactions · Histamine receptors · Structural chemogenomics · Protein modeling

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From Receptor Hypothesis to Receptor Binding Metaphor

From the beginning of its existence mankind has needed means to treat afflictions and diseases. A variety of natural products, mainly obtained from plants were used for this purpose. The selection of "medicines" was based on experience, on observations. It took relatively long until this changed and the selection primarily focused on properties of plants: shape, color, taste, etcetera (according to the doctrine of signatures) [1]. Things changed dramatically in the nineteenth century. First synthetic organic chemistry emerged, followed by the milestone work of Crum-Brown and Fraser [2]. The latter realized that it were the properties of compounds (e.g. present in plants), which determined their influence on biological systems. Shortly after these developments overenthusiastic scientists suggested that "soon pharmacopeia would be composed on basis of structure–activity relationships" or "soon doctors will have a series of medicines to influence practically any physiological action". Obviously matters have developed in a rather different manner. A major obstacle was the very poor understanding of the underlying cause and underlying mechanism of the diseases and method of action of any medicine. Around the turn of the twentieth century the *receptor concept* was introduced by scholars like Langley and Ehrlich [3]. Comparisons were made with the lock and key theory for substrates and blockers of enzymes as proposed some 20 years before by the German biochemist Fischer [4].

The defined "receptor" was nothing more than a hypothesis, the lock and key idea being a useful metaphor, which seemed to represent a kind of understanding of the way a medicine reached its activity. Indeed, in the late 1960s the famous Dutch pharmacologist Ariëns (prime

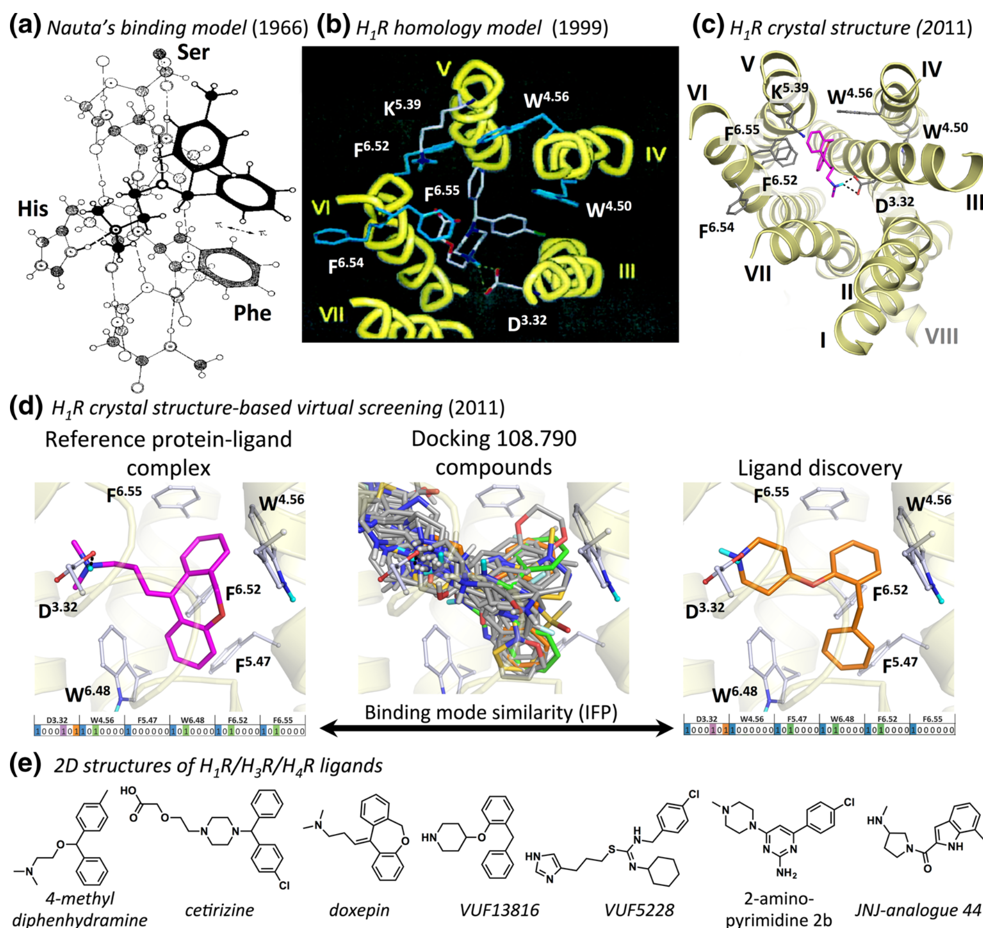

Fig. 1 Structural investigations of the histamine H_1 receptor (H_1R) from 1966 until now. **a** Nauta's binding mode proposal of 4-methyldiphenhydramine [7]. **b** The proposed binding mode of cetirizine in a bacteriorhodopsin-based guinea pig H_1R homology model [13]. **c** A cartoon depiction of the H_1R crystal structure with doxepin (sticks, magenta carbon atoms) [15]. **d** Virtual screening for novel fragment-like H_1R ligands based on the doxepin-bound H_1R crystal structure. A combined scoring approach was applied in which both PLANTS and molecular interaction fingerprint scoring (IFP). IFP evaluates the binding mode similarity of a docked compound with respect to a reference compound (in this case the co-crystallized doxepin) by encoding the interactions of the docked compound with the binding pocket residues into an interaction fingerprint and comparing this to the fingerprint of the reference compound. This lead to the identification of 19 novel inverse agonists, the structure of the highest affinity hit, VUF13816 (Ki = 6 nM), is depicted [45]. **e** 2D structures of the histamine ligands depicted in **a**–**d** and Fig. 2a–d

author of two milestone volumes of "Molecular Pharmacology" [5, 6]), sighed "when I am talking about receptors I am talking about something I know nothing about". There was not an accepted understanding about the chemical structure of any receptor at all. Though another Dutch scientist, Nauta had at that time—and most likely the first one to do so—proposed that a "receptor might be protein in a helix shape"; in this proposal interactions between the ligand and the protein should consist of polar and π–π interactions, together with hydrogen bonds (Fig. 1a) [7]. In the early days ligand–receptor interactions had been considered as being irreversible, but in the meantime it had become clear that they rather were reversible in most cases.

Ariëns, not knowing what the chemical structure of a receptor was, has been a very powerful engine for the transfer of pharmacology from an in vivo science only into an in vitro and more importantly into a molecular science. He and his team introduced many in vitro models to investigate the effect of selected compounds on organs; these models lead eventually to the detection of subtypes of receptors as present on the several tissues. The Ariëns team developed a simple mathematical model for the receptor-ligand interactions, thereby defining dissociation and association constants, parameters like pD_2 (the negative logarithm of the dissociation constant) and pA_2 (the negative logarithm of the concentration of the antagonist that makes it needed to double the concentration of the agonist to reach the same effect), partial agonism, and intrinsic activity (α) [5, 6]. Interactions between agonists and antagonists at a given receptor were considered to be either

of a competitive or a non-competitive nature [5, 6]. The impact of the change of pharmacology into a molecular science has been enormous. From now on the biological effect of compounds could be expressed in real molecular properties. Especially the parameter for agonistic activity of a ligand became not only easily accessible but also very useful to study structure–activity relationships.

From Ligand-Based to Receptor-Based Structure–Activity Relationships

Also, in the 1960s, Hansch introduced the technology for quantitative structure–activity relationships (QSAR) [8]. This approach came in reach when computers allowed the determination of the mathematical relationship between one parameter (in this case the biological activity) and several others (in this case physicochemical properties of a series of congeneric compounds). As it had happened towards the end of the twentieth century it was thought that by QSAR approaches (biological) activities of yet not synthesized compounds could be predicted. Again the high hopes were not justified, but the reason could likely have been foreseen this time. For determining a so-called QSAR formula the assumption is that all compounds under study should interfere with the target—in our case the receptor—in exactly the same way; the QSAR is based on the thermodynamics of the ligand–target interaction. It is likely the fact that the metaphor of the lock and key for ligand–target interferences was used as a model for these interactions, implying that congeneric derivatives "reacted" in the same way with the target, whereas it is nothing more than a metaphor indeed. There was a big need to find out more about the chemical nature of receptors and the mechanisms that lead to receptor activation or blockade.

The next big leap forward came from biochemistry, the discipline from which the receptor concept originated. The role of secondary messengers became clear (c-AMP and IP_3). The primary structure of receptors could be established, including receptor subtypes. Things went extremely fast; dimers, heteromers, point mutations, chimeric receptors, receptor up- and down-regulation, constitutive activity, reversed agonism, etcetera [9, 10]. Parallel to the exciting progress in this field coming from biochemistry, especially from cell biology, the exponential increase in computing power contributed much to the understanding of ligand-receptor interactions. Molecular modeling technology took over from classical QSAR approaches. It became very clear that closely related compounds, showing the same pharmacological effect do not necessarily interfere with the same target in the same molecular way. The time was ripe for the change from metaphor to model; compound design came within reach.

However, a next step had to be taken. The question of the conformation of receptor molecules in their natural environment had still not been solved. The first most important finding was the elucidation of the electron cryomicroscopy structure [11] (and later X-ray structure [12]) of bacteriorhodopsin, which became the standard template for the structurally closely related GPCRs. The first steps towards molecular modeling on basis of the structure of the target had become feasible, as will be exemplified in the next paragraphs for the GPCR family of histamine receptors that play important roles in allergy, acid secretion, inflammation, and CNS disorders.

From Customized GPCR Homology Models into a New Era of GPCR Structural Biology

Although bacteriorhodopsin has a low sequence similarity with GPCRs, the shared heptahelical fold allowed for low-resolution homology modeling. By combining these homology models with experimental data the structural understanding of ligand-GPCR binding grew. This was, for example, the approach in a study investigating the binding mode of second-generation antihistamines in the histamine H_1 receptor (H_1R) [13]. A bacteriorhodopsin-based homology model (Fig. 1b) was used in combination with docking studies, a ligand-based pharmacophore [14], and site-directed mutagenesis studies. This led to the first experimentally supported binding-mode hypotheses for second-generation antihistamines [13]. In this study the positively charged $K^{5.39}$ residue was identified as an anchor for the carboxylate-moiety of acrivastine and levocetirizine (Fig. 1b), which still is key in the accepted binding-mode hypothesis for these antihistamines (which was later confirmed by the H_1R crystal structure, Fig. 1c) [15]. Subsequently the structure of bovine rhodopsin (the first crystallized GPCR) was elucidated in 2000 [16]. This allowed for more accurate homology modeling, but still the average sequence similarity of many GPCRs with bovine rhodopsin was low [17].

New Structural Insights into GPCR Ligand Binding Mode Diversity

From that point in time it took over 7 years before the first druggable GPCR was crystalized, namely the β_2-adrenoceptor. A large array of techniques [18] including thermostabilizing mutants, insertion of T4-lysozyme/cytochrome b562/rubredoxin, addition of nanobodies and covalently-bound ligands have yielded 110 GPCR crystal structures to this date, comprising 25 different GPCRs in four GPCR classes (class A, B, C, and frizzled). These crystal structures have given unique insights into the structural mechanism of ligand binding. Moreover, they

show that the location for ligand binding is not as conserved and static as assumed during the introduction of the key and lock metaphor.

Several unique ligand-binding sites have been revealed that differ between GPCRs, but also multiple binding sites within a single GPCR have been identified. The most frequently observed binding site is the so-called major pocket (between TM 3, 5, 6, and 7), which is the orthosteric binding site for many class A GPCRs, as exemplified by the binding mode of doxepin in H_1R [15] (3RZE, Figs. 1c, 2i) and epinephrine [19] and carazolol [20] in β_2R (2RH1 and 4DLO, Fig. 2h). Opposite to this pocket is the minor pocket (between TM 2, 3, and 7) that was (for the first time) found to be occupied in the CXCR4 crystalized with the small molecule IT1t [21] (3ODU, Fig. 2f). Larger ligands have also been co-crystalized since then that occupy both this major and minor pocket, e.g. antiretroviral drug maraviroc in CCR5 [22] (4MBS, Fig. 2d) and beta-blocker carvedilol in β_1-adrenoceptor [23] (not shown). Some allosteric modulators have been shown to bind higher up in the GPCRs (between the extracellular loops), as shown for the muscarinic M_2 receptor in an X-ray structure with both an agonist (iperoxo) in the major pocket and a positive allosteric modulator (PAM), LY2119620, in the loop region [24] (4MQT, Fig. 2b). For multiple GPCRs also dualsteric/bitopic/bivalent ligands have been developed that target multiple pockets (like maraviroc). Ergotamine is such a dualsteric agonist for the 5-hydroxytryptamine family, targeting both the loop-region and the major pocket, and has been crystallized in the 5-HT_{2B} and 5-HT_{1B} [25] receptor (4IAR, Fig. 2c). Instead of binding small molecules, many GPCRs are also known protein/peptide-binders and therefore have a large open pocket to accommodate these large(r) ligands. So far two receptors have been crystalized in combination with a large peptide ligand: the NTS_1 receptor with a part of neurotensin [26] (4BUO, Fig. 2a) and CXCR4 with peptide-antagonist CVX15 [21]. More recently also non-class A GPCRs have been crystalized. From class F (frizzled) the SMO receptor has been crystalized, once in combination with cyclopamine [27] (not shown) that also binds in the loop region, and therefore has extensive contacts with the extracellular loops, but also with the elongated TM6 (compared to class A). The first class B GPCRs that were crystalized are the glucagon receptor [28] and the CRF_1 receptor [29]. Although no density for a ligand could be found in the glucagon receptor, in combination with extensive site-specific mutagenesis a high-resolution model of glucagon bound to its native receptor could be created. In the CRF_1 receptor an antagonist, CP-376395, was co-crystallized and was found to bind in an unusual deep binding pocket (within the cytoplasmic half) between TM 3, 5, and 6 (4K5Y, Fig. 2j) [30]. The $mGlu_1$ receptor [31], the first crystalized class C GPCR, was crystalized in combination with a negative allosteric modulator (NAM) binding in the major pocket (not shown). Also the $mGlu_5$ receptor [32] was crystallized with a NAM (mavoglurant) binding in the major pocket, however, mavoglurant extends downward into the ion-binding site (not shown). Other unique observations for class A GPCRs are the binding mode of antagonist AZD1283 and agonist 2MeSADP in the $P2Y_{12}$ receptor [33] that binds perpendicular to other major pocket binders and has contacts with TM4 (4NTJ, Fig. 2e), and also a conserved ion-binding site that was found to be present in several high-resolution X-ray structures (A_{2A} receptor [34], β_1-adrenoceptor [35], δ (opioid) receptor [36], and PAR1 [37]). This ion binding site between TM1, 2, and 7 (4BVN, Fig. 2g) is tightly interacting with a water network that was shown to influence the activation of GPCRs [34–36, 38]. Moreover, the residues lining this ion-binding site are relatively conserved and it is therefore expected to be present in multiple GPCRs [36, 38].

The advances in the elucidation of GPCR structures in the paste decade have been tremendous and show a high diversity of ligand binding modes (Fig. 2). Interestingly, the H_1R crystal structure [15] (Fig. 1c) shows that the antihistamine-receptor interaction model of Nauta [7] (Fig. 1a) correctly captured important determinants of H_1R ligand binding, and confirms the previously proposed H_1R-antihistamine binding orientations based on protein homology modeling and mutation studies (Fig. 1b) [13, 14]. These interaction models feature: (1) an essential hydrogen-bond between the amine group of the ligand and a polar H_1R residue (a His residue in the Nauta model, $Asp^{3.32}$ in both homology model and X-ray structure), (2) aromatic π–π stacking between the ligand and several aromatic residues in TM helices 4, 5 and 6; and (3) an anionic interaction site above the orthosteric H_1R binding pocket [39].

Molecular Determinants of (Selective) GPCR Ligand Binding

Apart from the insights directly obtained from the GPCR X-rays, the new crystal structures can be complemented with experimental data and computational modeling to construct and validate higher resolution homology models that can be used to gain more insight in GPCRs that have not (yet) been crystallized, as was for example recently demonstrated for histamine H_3 and H_4 receptors [40, 41]. The integration of experimental ligand SAR and receptor mutagenesis data with ligand-based and protein–ligand based computer models allowed for the elucidation and experimental validation of the binding modes of different histamine H_4 receptor ligand chemotypes (Fig. 3c) [40] and the identification of molecular determinants of

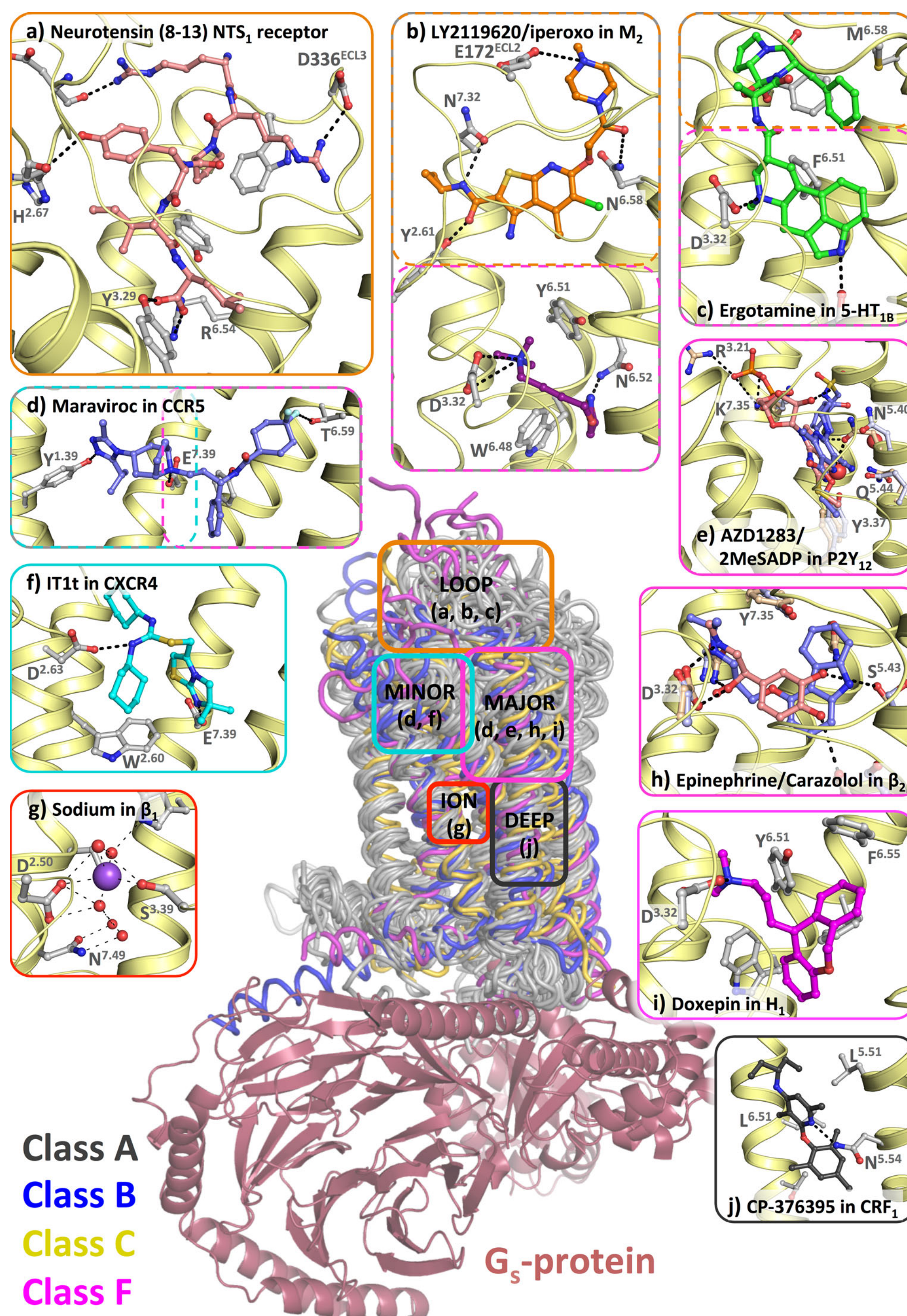

Fig. 2 Overlay of GPCR crystal structures and comparison of different GPCR–ligand binding modes. **a** Neurotensin-peptide (salmon) bound to the NTS_1 receptor (PDB-code 4BUO [26]). **b** PAM LY2119620 (*orange*) and agonist iperoxo (*purple*) bound to muscarinic M_2 receptor (PDB-code 4MQT [24]). **c** Ergotamine (*green*) bound to 5-HT_{1B} (PDB-code 4IAR [25]). **d** Maraviroc (*blue*) bound to CCR5 (PDB-code 4MBS [22]). **e** Antagonist AZD1283 (*slate*) and agonist 2MeSADP (*salmon*) bound to $P2Y_{12}$ receptor with the ribbon of the agonist structure shown (PDB-codes 4PXZ [69], 4NTJ [33]). **f** IT1t (*cyan*) in CXCR4 (PDB-code 3ODU [21]). **g** A sodium ion (*purple*) in the β_1-adrenoceptor (PDB-code 4BVN [35]). **h** Doxepin (*magenta*) bound to the histamine H_1 receptor (PDB-code 3RZE [15]). **i** Carazolol (*slate*) and epinephrine (*salmon*) with the ribbon of the active-state epinephrine structure shown (PDB-codes 2RH1 [20], 4DLO [19]). **j** CP-376395 (*dark gray*) bound to the CRF_1 receptor (PDB-code 4K5Y [29]). The ribbon overlay of all crystallized GPCRs also shows the G_s-protein coupled to the β_2-adrenoceptor (PDB-code 3SN6 [58]). For selected residues the B&W numbers [104] are indicated in *gray* (for class B the translated B&W numbering is used as previously proposed, i.e., the translated B&W positions 5.51, 5.54, and 6.51 correspond to positions 5.47b, 5.50b, 6.46b of the class B Wootten numbering scheme, respectively [30, 105]). In **e**, **h** only the interactions of the agonist are indicated (Color figure online)

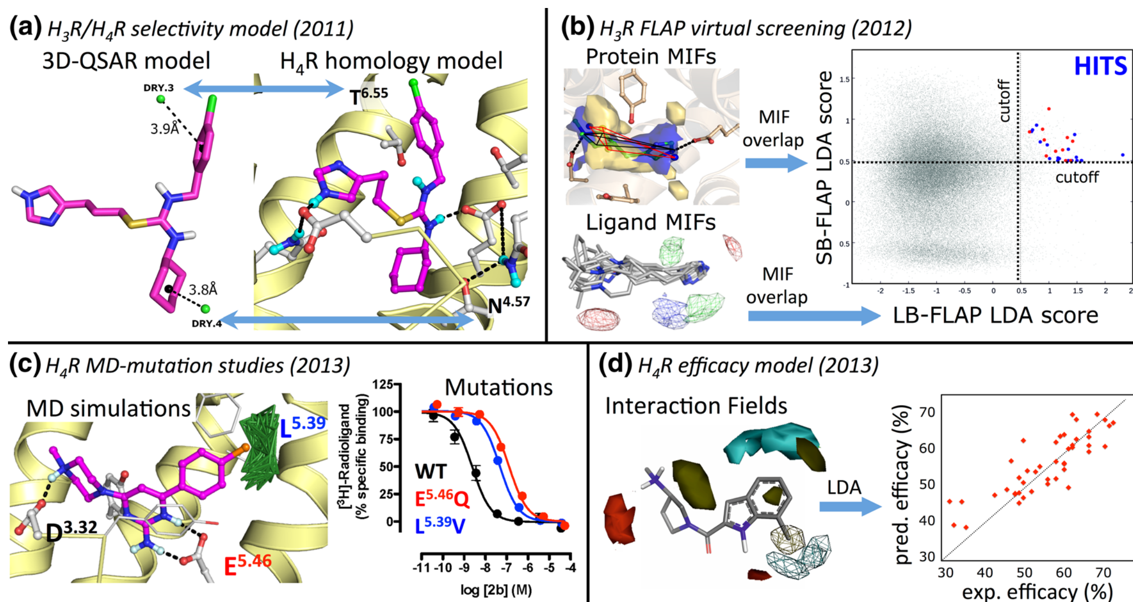

Fig. 3 Combined ligand-based and structure-based approaches elucidate the structural determinants of H_3 and H_4 receptor ligand binding and/or signaling. **a** A 3D-QSAR model capturing the selectivity in affinity for the H_3/H_4 receptor for a series of clobenpropit-analogues obtained by analysis of molecular interaction fields (MIFs). Two hydrophobic hotspots (DRY.3 and DRY.4) that were identified as selectivity determinants were subsequently mapped onto a homology model that lead to the identification and experimental validation of selectivity inducing residues in the binding pocket [41]. **b** FLAP software was used to build ligand-based (LB) and structure-based (SB) models through linear discriminant analysis (LDA) of MIF fingerprints based on a library of true active and true inactive fragment-like molecules. The resulting FLAP models were used to screen a series of 156 090 fragment-like compounds and lead to the identification of 18 new H_3R ligands [54]. **c** Systematic comparison of different modeling templates, protonation states and binding modes of the ligands through application of docking and MD simulations combined with SAR studies and site-directed mutagenesis studies lead to the elucidation of the binding mode of H_4R ligands with different scaffolds [40]. **d** In vitro experiments identified that 47 out of a series of 48 JNJ-7777120 analogues were β-arrestin2-biased agonists. Subsequently, a ligand-based FLAP analysis was performed in order to gain more insight in the structure–activity relationship (based on the β-arrestin2 signaling) of the 48 compounds. The resulting FLAP model was combined with a homology modeling and used to identify receptor regions that are important for biased H_4R signaling [78]. 2D structures of the depicted ligands are shown in Fig. 1e

histamine H_3/H_4 receptor selectivity (Fig. 3a) [41]. Systematic consideration of different H_4R homology modeling templates ($β_2$-adrenoceptor and H_1R crystal structures), ligand binding poses, and ligand protonation states in combination with docking and MD simulations enabled the prediction of subtle differences in H_4R ligand SAR and ligand-specific mutation effects (Fig. 3c) [40]. H_3/H_4 selectivity hotspots identified by ligand-based 3D-QSAR studies were linked to H_4R specific residues in H_4R homology models (Fig. 3a) [41]. Subsequent mutagenesis studies confirmed the role of these residues in the H_4R binding pocket that determine H_3/H_4 selectivity and validated the predicted ligand binding modes [41].

The increasing number of aminergic GPCR crystal structures (Fig. 2) now for the first time allows the integration of (fragment-like) ligand affinity data, receptor mutagenesis studies, and amino acid sequence analyses to high-resolution structural chemogenomics analyses of aminergic GPCR–ligand interactions [18, 39, 42]. Such structure- and fragment-based chemogenomics analyses enable a more accurate description and prediction of the molecular and structural determinants of ligand affinity and selectivity in different binding regions of (aminergic) GPCRs [39, 42], and may ultimately be used to support the design of ligands with desired polypharmacological profiles [43].

Structure-Based Discovery of Novel GPCR Ligands

The new GPCR crystal structures can not only improve the understanding of ligand-binding and receptor activation mechanisms, but can also facilitate the discovery of novel GPCR ligands [44], as was for example demonstrated in structure-based virtual screening studies against the histamine H_1 receptor crystal structure (Fig. 1d) [45]. After docking 108,790 basic, fragment-like compounds in the H_1R crystal structure the resulting predicted binding modes were scored using a combination of a “classical” energy-based scoring function (ChemPLP using PLANTS [46]) and interaction fingerprint [47] (IFP) scoring method. The IFP of doxepin in the crystal structure was used as a reference in comparison to the IFPs of the docked compounds.

Based on a retrospective validation cutoffs were selected for the energy-based scores as well as the IFP-similarity and subsequently 26 compounds were selected after visual inspection. Experimental validation learned that 19 out of the 26 selected compounds were novel fragment-like inverse agonists. Structure-based virtual screening [18] against other GPCR crystal structures have enabled the discovery of new ligands for the β_2-adrenoceptor [48, 49], CXCR4 [50], D_3 receptor [51], and A_{2A} receptor [52, 53].

Ligand-based and structure-based virtual screening approaches can also be combined, as recently for example demonstrated in H_3 histamine receptor 3D-QSAR studies. Ligand-based and protein-based molecules fingerprint models (FLAP) from molecular interactions fields were trained to discriminate known histamine H_3 receptor ligands from true inactive fragment-like compounds [42], and were successfully applied to identify new fragment-like H_3R ligands from a chemical library of fragment-like compounds (Fig. 3b) [54]. The growing amount of GPCR crystal structures improves our understanding of ligand binding and together with advances in computational chemistry can lead to efficient identification of novel GPCR ligands.

From Ligand Binding Mode to Receptor Signaling

The structural advances have also improved our understanding of the activation mechanism of GPCRs [55]. This has lead to the replacement of the classical two-state model with a model in which multiple transition states are possible [56, 57]. The crystal structure of a G_s-protein coupled to the β_2-adrenoceptor showed a large outward movement of the intracellular half of TM 6 and an extension and small outward movement of TM 5 [58]. Moreover, this crystal structure uncovered a major movement of the α–helical domain of $G\alpha_s$ relative to its Ras-domain [58]. Crystal structures of (pre-)activated β-arrestin highlighted the movement of the finger, middle and lariat loop and a relative rotation of the individual lobes upon activation [59, 60]. Moreover, the structure of β-arrestin in combination with the phosphorylated C-tail of the V_2 receptor (V_2R) revealed a hydrogen-bonding network between the phosphorylated residues of C-tail of V_2R and β-arrestin [60]. Recently also low-resolution models of β-arrestin bound to a chimeric GPCR have been published, which were constructed based on single-particle negative-stain electron microscopy density maps [61]. These models support a biphasic mechanism [62] in which β-arrestin is first recruited by the GPCR via its C-tail, after which the finger loop of β-arrestin is inserted into the intracellular core of the GPCR. Crystal structures of GPCR kinases (GRKs) have been solved [63], but structures of GRK-GPCR complexes have not yet been solved.

Despite the improvements, structure-based prediction of the functional efficacy of GPCR ligands remains challenging [47, 64] and requires multiple GPCR structures bound to ligands of different functional classes (partial/full agonist, antagonist, inverse agonist) [65]. The 31 β-adrenoceptors (β_1R/β_2R) structures, covering multiple receptor activation states in combination with 19 ligands with different functional effects, show how subtle differences in the binding pocket can accommodate ligands with different functional activities (Fig. 2h) [47, 64], and give insights into the molecular mechanisms of G-protein binding (Fig. 2) [58] and activation [56]. Structure-based virtual screening studies against agonist-bound β-adrenoceptor crystal structures (Fig. 2h) [47, 64], agonist-customized models [64], and different agonist-bound MD simulation snapshots indeed enables the selective identification of agonists (over antagonists and inverse agonists). Comparison of the inactive antagonist/inverse agonist bound and the (active/active-like) agonist bound crystal structures of the β_2-adrenoceptor (Fig. 2h) [19, 58, 66], rhodopsin [67], adenosine A_{2A} receptor [68], $P2Y_{12}$ (Fig. 2e) [69], and muscarinic M_2 receptor (Fig. 2b) [24] shows that activation of the muscarinic M_2 receptor and $P2Y_{12}$ are associated with larger structural changes in the orthosteric ligand-binding site compared to the relatively small changes observed for β_2-adrenoceptor, rhodopsin, and A_{2A} receptor. These new structural insights into GPCR activation suggest that a detailed understanding of the GPCR *specific* ligand-binding modes [70–72] and conformational changes [73–75] associated with (specific) signaling pathways are required for the development of selective structure-based virtual screening strategies for agonists over antagonists (or vice versa) or ultimately even for *biased* ligands.

In the absence of such crystal structural information (which is still the case for most GPCRs), experimentally guided (e.g. mutagenesis studies) protein modeling can be used to: (1) predict ligand-stabilized conformational changes in the ligand binding site (for example the construction of the agonist-bound β_2-adrenoceptor based on the antagonist bound crystal structure [64]), and/or (2) the molecular mechanisms of signal transduction between the ligand binding site and the intracellular site (as was for example shown for histamine H_1 [76] and H_4 [77] receptors). Alternatively, ligand-based computational models can be trained using experimental ligand functional efficacy data, as was for example recently demonstrated in 3D-QSAR modeling studies to predict β-arrestin2 recruitment efficacies of a series of histamine H_4 receptor ligands (Fig. 3d) [78]. Interestingly, the only H_4R ligand that was *not* β-arrestin2 biased (but displayed an equal preference

for the Gαi and β-arrestin2 pathway) was an outlier of the 3D-QSAR model [78].

Alternative (Allosteric) Ligand Binding Sites

Allosteric modulators can alter orthosteric ligand affinity and/or efficacy, potentially with higher receptor selectivity due to lower sequence similarity between allosteric sites of different receptor subtypes (compared to the conserved orthosteric pocket) [39, 79, 80]. The TM binding sites of several crystal structures of class A (chemokine receptors CXCR4 [21] and CCR5 [22], Fig. 2d, f), class B (glucagon [28] and CRF_1 [29] receptors, Fig. 2j) and class C ($mGlu_1$ [31] and $mGlu_5$ [32] receptors) GPCRs represent allosteric binding pockets that overlap with/are adjacent to (class A [75, 81] and class B [30]) or are located far away from (class C [31, 32]) the orthosteric binding sites of the corresponding receptors. Recent GPCR crystal structures furthermore show that ligands cannot only target the TM binding site (Fig. 2) [75, 81], but can also interact with (allosteric) binding sites in the extracellular loop region (e.g. class A NTS_1 receptor [26], class F SMO receptor [27], class A muscarinic M_2 receptor [24], Fig. 2a, b), or deep in the TM domain below the "classical" TM binding site (class B CRF_1 receptor [29], Fig. 2j). In the recent muscarinic M_2 receptor crystal structure an orthosteric agonist (iperoxo) and a positive allosteric modulator (LY2119620) are bound simultaneously (Fig. 2b), providing new insights into the molecular mechanism of allosteric modulation and activation of GPCRs [24]. Most virtual screening and structure-based ligand design studies have focused on the TM binding sites [82, 83], but these alternative ligand binding sites identified in GPCR crystal structures, as well as the intracellular G protein binding site (Fig. 2) [58] and GPCR dimer interfaces [84, 85] provide novel sites to target with small molecules to regulate GPCR function [86, 87]. Moreover, the simultaneous consideration of GPCR–ligand interactions in both orthosteric and allosteric pockets in molecular dynamics simulations [88] and structure-based virtual screening studies [87]. In silico discovery of (fragment-like) ligands in multiple distinct binding sites offer opportunities for the structure-based design of bitopic ligands [89, 90] that target both orthosteric and allosteric binding sites (e.g. by fragment linking, merging, or growing [91]).

From Receptor Structure (Dynamics) to Rational Optimization of Ligand Binding (Kinetics)

GPCR crystal structures and homology models have been successfully used to identify new ligands [44], and extensively used to guide and/or explain SAR and site-directed mutagenesis studies (e.g. Figs. 1, 3), and rational structure-based ligand optimization [44, 83] has now become feasible for more and more GPCRs (as for example illustrated for the A_{2A} [92–94]). Moreover, the new GPCR crystal structures in combination with molecular dynamics simulations give insights into receptor flexibility and (potential) ligand access and exit channels [95–97]. The association and dissociation pathways revealed by computer simulations in combination with experimental studies (e.g. mutagenesis data and/or biophysical measurements [93, 98, 99]) can ultimately be used to relate ligand structure to kinetic properties, thereby changing the focus from solely affinity-based optimizations to optimization of kinetic properties (as for example illustrated for the histamine H_4 receptor [100]). Furthermore consideration of water molecules in GPCR binding sites that can mediate, facilitate, or hamper receptor-ligand (un)binding (dynamics) may be required to improve the resolution of GPCR–ligand interaction predictions [101]. Computational and biophysical assessment of the thermodynamics of water interaction networks allow the identification of energetically favorable water molecules that may be targeted and/or unfavorable ("unhappy") water molecules that can be displaced in structure-based ligand optimization studies [93, 102] to improve GPCR–ligand binding affinity and kinetics [93, 103].

Conclusion

As a working model, an educational model, a metaphor, and a hypothesis the lock and key theory was and still is very valuable. The recent breakthroughs in the elucidation of GPCR structures illustrate how structural biology, molecular pharmacology, medicinal chemistry, and computational modeling methods can help to identify the different molecular keys that fit and trigger or block one or more of the unique locks each receptor has. GPCR crystal structures display a large diversity of GPCR–ligand binding modes and GPCR–ligand specific conformational changes associated with different receptor activation states. The investigation and ultimately the prediction of the molecular determinants and dynamics of GPCR–ligand binding (kinetics) and receptor activation therefore require the combination of static crystal structural information with experimental and computational studies. After the progress from receptor theory, metaphor, to three-dimensional structural view of GPCR–ligand interactions, integrated GPCR research approaches can enable the steps towards structure-based discovery and optimization of novel ligands that bind specific (allosteric) binding sites with desired effects on GPCR functional activity.

Acknowledgments With this contribution to this special issue, the Division Medicinal Chemistry of the VU University Amsterdam honors Professor Povl Krogsgaard Larsen. The authors would like to thank all members of the Medicinal Chemistry group of the VU University Amsterdam who have contributed to the histamine receptor research described in this article. A.J.K. and C.d.G. participate in the European Cooperation in Science and Technology Action CM1207 [GPCR–Ligand Interactions, Structures, and Transmembrane Signalling: A European Research Network (GLISTEN)].

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