Active state structures of G protein-coupled receptors highlight the similarities and differences in the G protein and arrestin coupling interfaces

Byron Carpenter[1] & Christopher G. Tate*

MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Francis Crick Avenue, Cambridge, CB2 0QH, UK

[1]Current address: Warwick Integrative Synthetic Biology Centre, School of Life Sciences, Gibbet Hill Campus, The University of Warwick, Coventry, UK

*For correspondence: cgt@mrc-lmb.cam.ac.uk

Abstract

G protein-coupled receptors (GPCRs) regulate cellular signalling through heterotrimeric G proteins and arrestins in response to an array of extracellular stimuli. Structure determination of GPCRs in an active conformation bound to intracellular signalling proteins has proved to be highly challenging. Nonetheless, three new structures of GPCRs in an active state have been published during the last year, namely the adenosine A_{2A} receptor ($A_{2A}R$) bound to an engineered G protein, opsin bound to visual arrestin and the μ opioid receptor (μOR) bound to a G protein-mimicking nanobody. These structures have provided novel insight into the sequence of events leading to GPCR activation, and have highlighted both similarities and differences in the structure of the interface between GPCRs and different signalling proteins.

Highlights

- Active state structures of a μOR–nanobody complex determined to 2.3 Å resolution.
- The first GPCR–arrestin structure defines the arrestin–opsin interface.
- Structure determined of $A_{2A}R$ coupled to an engineered G protein.
- Inactive, intermediate-active and active structures have now been solved for $A_{2A}R$.
- G protein and arrestin complexes provide insight into GPCR coupling specificity.

Introduction

The most significant development in G protein-coupled receptor (GPCR) structural biology in recent years has been the crystallisation of receptors in an active state, *i.e.* the conformation of the receptor when it is coupled to a cytoplasmic signalling partner such as a G protein or arrestin (Figure 1). This has been possible only by co-crystallisation of the receptor with a binding partner that stabilises the active conformation (Figure 1). However, due to the technical challenges of working with native signalling complexes, a number of novel G protein-mimicking surrogates have been developed to simplify crystallisation of GPCRs in their active state. At present, six different binding partners have been used (Table 1): (1) the C-terminal peptide (GαCT) from the G protein transducin α-subunit [1-7]; (2) single chain camelid antibodies (nanobodies; Nb) raised against specific GPCRs [8-12]; (3) the heterotrimeric G protein G_s, composed of $G\alpha_s$, β_1 and γ_2 subunits, and stabilised by a nanobody (Nb35) [13]; (4) the finger loop peptide from visual arrestin [14]; (5) visual arrestin [15]; (6) an engineered minimal G protein, mini-G_s, composed of a single domain from the $G\alpha_s$ subunit [16]. The first active state structure of a GPCR solved was that of opsin complexed with GαCT, published in 2008 [5]. In 2011 the structure of the β_2 adrenergic receptor (β_2AR) was determined in complex with either a nanobody [10] or heterotrimeric G_s [13]. Since then three additional GPCRs have been determined in their active state, namely the muscarinic acetylcholine receptor M2 [9], μ opioid receptor (μOR) [8], and most recently, the adenosine A_{2A} receptor (A_{2A}R) [16].

This review will focus on structures of GPCRs in their active conformation, therefore, we must start by defining what constitutes the active state. From a structural perspective, the conformational changes that occur within the core of the receptor upon coupling to a cytoplasmic binding partner appear to be the best marker of activation. In particular, there is a characteristic rearrangement of three highly conserved residues, $Tyr^{5.58}$, $Tyr^{7.53}$ from the NPxxY motif and $Arg^{3.50}$ from the DRY motif (superscripts indicate Ballesteros-Weinstein numbering [17]). These residues adopt almost identical positions in all active state structures determined to date, irrespective of the binding partner involved (Figure 1; see References [8] and [18] for detailed comparisons of active state structures). Importantly, the conformational changes of these three residues can be used to differentiate the active G protein-coupled conformation of A_{2A}R [16] from the agonist-bound intermediate-active state [19-21], *i.e.* the conformation of A_{2A}R when it is bound to an agonist, but *not* coupled to a cytoplasmic signalling partner (Figure 2; discussed below). Pharmacological analyses have confirmed that all the different binding partners reproduce the increase in agonist-binding affinity induced by coupling to native signalling proteins [8-11,13,16]. The most notable difference between GPCR complexes involving a G protein or arrestin compared to a peptide or nanobody is the extent to which the intracellular end of helix six (H6) moves away from the transmembrane helical bundle. The magnitude of this movement, which is typically between 8 Å [5] and 17 Å [16], is highly dependent on the

binding partner involved, with the largest displacements observed for G protein complexes, but this does not influence the conformational changes that occur in the core of the receptor. Therefore, the structures solved to date (Table 1) likely represent a range of closely related active conformations, but, critically, the receptors have all undergone the core conformational changes that appear to represent transition into the active state.

Owing to the technical challenges of crystallising active state GPCRs, the frequency of publication of these structures has been relatively low, nonetheless, during the past year three new structures have been reported that have significantly enhanced our knowledge of GPCR activation. First, the high resolution structure of the μOR–Nb39 complex provided insight into activation of μOR and revealed a conserved polar network in the active state [8]. Second, the opsin–arrestin complex structure provided insight into how non-G protein signalling partners interact with GPCRs [15]. Third, publication of an $A_{2A}R$–mini-G_s complex made $A_{2A}R$ the first hormone receptor for which structures have been solved in the active [16] and intermediate-active [19-21] conformations in addition to the inactive state [22-25], *i.e.* the conformation of the receptor when it is bound to an antagonist or inverse agonist. In light of these recent developments, this review will focus on two main topics: (1) The conformational changes associated with activation of $A_{2A}R$; (2) Comparison of the interface between GPCRs and binding partners derived from native signalling proteins.

Conformational changes involved in $A_{2A}R$ activation

In 2011 two structures of agonist-bound $A_{2A}R$ were solved [19,21], which showed significant conformational differences compared to either the antagonist or inverse agonist-bound inactive states [23-25] (Figure 2). However, the agonist-bound conformation did not fully resemble the active state of β_2AR [10,13], particularly in regard to the positioning of residues Tyr197$^{5.58}$, Tyr288$^{7.53}$ and Arg102$^{3.50}$, and it was concluded that it most likely represented an intermediate conformation [19]. A similar intermediate-active state has subsequently been observed in structures of the neurotensin receptor (NTSR1) [26,27], but this conformation is *not* observed in agonist-bound structures of β_1AR [28] or β_2AR [29]. The propensity of receptors to occupy the intermediate-active state is probably dependant on the energy landscape of receptor activation, which has been shown by ^{19}F-NMR and DEER spectroscopy to differ significantly between $A_{2A}R$ [30] and β_2AR [29,31]. Two distinct active states of $A_{2A}R$ have indeed been identified in ^{19}F-NMR spectra [30], however, it is not yet clear if the intermediate-active state of $A_{2A}R$ trapped in the crystal structures [19-21] directly correlates to one of these species.

The structure of $A_{2A}R$ bound to mini-G_s completes a series of structures (Figure 2) from the inactive state [22-25], through an intermediate-active conformation [19-21] to the fully active state [16]. The conformational changes associated with the transition from the inactive to intermediate-active state are

distributed throughout the receptor and include a 0.8 Å contraction of the ligand-binding pocket (measured between H5 and H7), bulging of H5 around the NPxxY motif and a combined rotation and outward movement of H6 by 40° and 5 Å, respectively [19,32]. In contrast, the conformational changes associated with the transition from the intermediate-active to active state are confined to the intracellular half of $A_{2A}R$ (Figure 2). The most striking rearrangement is a 14 Å outward movement of the cytoplasmic end of H6 [16], which generates a cavity on the cytoplasmic surface of the receptor that engages the α5 helix of $G\alpha_s$. Rotamer changes in $Tyr197^{5.58}$, $Tyr288^{7.53}$ and $Arg102^{3.50}$ position these side chains to occupy the space generated by the outward movement of H6 (Figure 2). Thus, rearrangement of these residues appears to stabilise the receptor in its active conformation by improving packing in the core of the transmembrane bundle, furthermore, the extended conformation of $Arg102^{3.50}$ forms the upper surface of the cytoplasmic cavity and directly interacts with the G protein. No significant changes were observed in the ligand-binding pocket of $A_{2A}R$ upon G protein coupling, suggesting that the intermediate-active structure may already represent the high affinity agonist-bound state. Thus the modest 10-40 fold increase in agonist-binding affinity observed for either the $A_{2A}R$–G_s [33,34] or $A_{2A}R$–mini-G_s [34] ternary complex could arise by a different mechanism, for example a reduction in conformational dynamics associated with G protein binding.

One of many outstanding questions about the mechanism of GPCR activation is which conformation of the receptor is responsible for G protein recognition? Spectroscopic studies have demonstrated that some receptors partially occupy the active state in the absence of G protein [30,31,35], but it is unclear if the active conformation is actually responsible for recognition of the G protein. Significant conformational changes are observed in the G protein upon receptor binding, which are thought to be coupled to GDP release [13], and it is likely that these rearrangements are driven by simultaneous conformational changes in the receptor. Therefore, it is tempting to speculate that the intermediate-active state crystallised for $A_{2A}R$ [19-21] and NTSR1 [26,27] represents a conformation that is responsible for initial recognition of the G protein, before cooperative conformational changes drive GDP release from Gα and result in formation of the nucleotide-free ternary complex. Intriguingly, recent NMR data on μOR [36] suggest that agonists induce a distinct change in environment of residues at the H1/H8 interface in the absence of a G protein mimetic, but upon binding of the G protein mimetic the largest changes are observed around H6. Although the NMR data cannot define what the change in environment at these positions entails (e.g. a rotamer change, helix movement etc), it is noticeable in the $A_{2A}R$ structures that there is a distinct conformation change at the H1/H8 interface upon agonist binding, but not upon G protein coupling (Figure 2).

Comparison of the interfaces between GPCRs and binding partners derived from native signalling proteins

Crystal structures have now been published for receptors in complex with five binding partners that are derived from native signalling proteins, namely G_s [13], GαCT [1-7], mini-G_s [16], visual arrestin finger loop peptide [14] and visual arrestin [15]. As expected from extensive mutagenesis work on a number of different receptors [37], the major areas of the receptor that interact with the G protein are at the cytoplasmic ends of H3, H5 and H6 and also in cytoplasmic loop 2 (Figure 3). The structural information has now allowed a more detailed analysis of the similarities and differences between the interfaces of different complexes. The $A_{2A}R$–mini-G_s complex [16] aligned well with the β_2AR–G_s structure [13], with only a slight variation in the orientation of G protein binding. β_2AR and $A_{2A}R$ share only ~30% sequence identity, but the majority of residues that form direct interactions are conserved (Figure 3). The positions of 14 interacting residues within the two interfaces are conserved, with 8 of these amino acid residues being identical between $A_{2A}R$ and β_2AR. In contrast, the positions of 6 contacting residues are unique to $A_{2A}R$, with 10 positions that are exclusive to β_2AR. The most significant difference between the complexes is the additional contacts involving the H7-H8 boundary of $A_{2A}R$ (discussed below). The buried surface area of the interface is similar in both complexes, involving approximately 1050 $Å^2$ of $A_{2A}R$ and 1280 $Å^2$ β_2AR, and the number of direct contacts across the two interfaces varies by less than 10%. In most cases, when a contact is lost, due to the divergent amino acid sequence of the receptors, it is compensated for by the formation of an additional contact elsewhere in the interface. The overall density of interactions in the interface is relatively low, which may allow the reorganisation of contacts in response to the divergent amino acid sequence of different receptors (Figure 3). This may explain how a large number of different receptors with low sequence homology are able to couple a common G protein.

The only structural information available for binding of non-G_s-coupled receptors to a G protein-derived partner comes from the opsin–GαCT structures (Table 1) [1-7]. GαCT binds opsin in a significantly different orientation compared to G_s binding to either β_2AR or $A_{2A}R$ (GαCT is rotated by ~30° compared to G_s), however the conformational changes in the core of opsin are similar to other active state GPCRs. It is plausible that transducin does indeed bind opsin in a different conformation to that observed for the G_s-coupled receptors. However, it is also possible that the isolated GαCT peptide is incapable of stabilising H6 of the receptor in its fully extended conformation, which could potentially explain the different binding orientation observed in the crystal structures. A similar situation is observed for the opsin–arrestin complex, where the binding orientation of an arrestin-derived peptide [14] differs significantly from the whole arrestin molecule [15]. Thus, at present, the molecular determinants of specificity between different classes of GPCRs and G proteins remain unclear.

The overall architecture of the opsin–arrestin structure [15] is similar to that of the β_2AR–G_s [13] and $A_{2A}R$–mini-G_s [16] complexes. Arrestin forms a larger interface with the receptor than a G protein, which

involves contacts with intracellular loop 1 (ICL1) that are not observed in the G protein complexes (Figure 3). Both arrestin and G_s bind with a high degree of shape complementarity to a cavity in the cytoplasmic surface of the receptor created by the outward movement of H6. Recent negative stain electron microscopy data demonstrate that receptor–arrestin complexes in which arrestin is bound only to the phosphorylated C-terminus of the receptor are still capable of binding and activating G proteins [38]. Therefore, competitive binding between the arrestin and G protein to a common cytoplasmic binding pocket on the receptor appears to be critical for terminating G protein signalling. Despite the fact that arrestin and G_s interact with common regions of the receptors, namely the intracellular ends of H3, H5 and H6, and ICL2, the organisation of the interfaces differs significantly. For example, the finger loop of arrestin, forms a helical segment analogous to the α5 helix of $G\alpha_s$, which fits into the cytoplasmic cavity of the receptor [14], however, the finger loop helix is rotated by ~45° compared to the α5 helix of $G\alpha_s$ [15] (Figure 3). One of the most interesting regions of the interface is the H7-H8 boundary, which forms direct contacts with the finger loop of arrestin (Figure 3), and has been implicated in potentiating signalling by arrestin-biased ligands [39,40]. No contacts involving this region are observed in the β_2AR–G_s complex [13], but, in contrast, four residues from the H7-H8 boundary of A_{2A}R form direct contacts with mini-G_s [16] (Figure 3). Therefore, it now appears that this region could play a wider role in the coupling specificity of both arrestins and G proteins. A unique feature of the opsin–arrestin complex is the central role played by electrostatic interactions [15,41,42]. Two β-arrestins are potentially responsible for the desensitisation of ~800 human GPCRs, so they need to be far more promiscuous than G proteins. Complementation between negatively charged finger loop residues and positively charged regions on the cytoplasmic surface of the receptor may represent a simple mechanism that has evolved to facilitate arrestin binding to a large number of GPCRs with low sequence homology [15,43].

Future perspectives

GPCR activation [44] and the subsequent activation of heterotrimeric G proteins [45] are both highly conserved mechanisms. However, despite the similarities between the active-state structures of opsin, β_2AR, M2, μOR and A_{2A}R [8-10,13,16], there are also differences, which are probably a reflection of their different amino acid sequences, the different kinetics of activation, their coupling to different binding partners and, ultimately, their different roles in human physiology. Atomic resolution structures are a necessary prerequisite to understand the molecular mechanism of receptor function and, more specifically, structures are required of a given receptor in a minimum of four different states, the inactive state bound to an inverse agonist, the receptor bound to an agonist, the agonist–receptor–G protein complex and the agonist–receptor–arrestin complex. Using the same agonist for each of the activated states will avoid confounding ligand-specific changes with receptor conformational changes. GPCR complexes remain

difficult targets for structural studies, and the development of new technologies, such as nanobodies [46-48] and mini G proteins [34], is key to ensuring the continued elucidation of new structures.

More structures of receptor–G protein and receptor–arrestin complexes will allow us to address key questions with respect to the specificity of coupling. What determines whether a receptor couples to either G_s, G_i or G_q, or perhaps to more than one different G protein? How can only 2 arrestins couple to hundreds of different GPCRs? How do the affinities of G protein/arrestin coupling vary between different GPCRs and how does this impact what happens in a cell? A recent study that tested the ability of specific GPCRs to couple to 14 different Gα subunits found widely different patterns of coupling in 4 different GPCRs, both in terms of specificity, kinetics and maximal responses [49]. It remains to be seen whether the pattern of activation differs when there is competition between different G proteins coupling to the same receptor, something that may be expected in cells that typically express multiple different G proteins. How the conformational dynamics of the GPCRs impacts upon the coupling processes within the cell are also only understood poorly. Spectroscopic studies [30,31,35] are providing evidence of multiple conformational states in the absence of ligands, with specific states becoming more populated in the presence of inverse agonists, agonists and agonists plus a G protein mimetic. A key objective will be to correlate these spectroscopic data with crystallographic structures of different conformational states to produce a concerted model of activation for individual receptors. In this regard, differences in the agonist bound structures of β_1AR [28] and β_2AR [29] compared to A_{2A}R [19-21] indicate a very different energy landscape of activation between receptors. New structures of other agonist-bound receptors will no doubt expand the repertoire of energy landscapes further, whilst maintaining the evolutionary conserved mechanism of receptor–G protein coupling.

Acknowledgements

This work was supported by the Medical Research Council (MC_U105197215), the ERC (EMPSI 339995) and by Heptares Therapeutics Ltd. We thank R. Grisshammer, A.G.W. Leslie and M.M. Babu for constructive comments on the manuscript.

Table 1					
Active state structures of GPCRs					
GPCR	Binding partner	Agonist	Resolution (Å)	PDB code	Reference
$A_{2A}R$	Mini-G_s	NECA	3.4	5G53	[16]
β_2AR	Nb80	BI-167107	3.5	3P0G	[10]
	G_s–Nb35	BI-167107	3.2	3SN6	[13]
	Nb6B9	BI-167107	2.8	4LDE	[11]
	Nb6B9	HBI	3.1	4LDL	[11]
	Nb6B9	Adrenaline	3.2	4LDO	[11]
	Nb6B9	FAUC37	3.3	4QKX	[12]
M2	Nb9-8	Iperoxo	3.5	4MQS	[9]
	Nb9-8	Iperoxo, LY2119620[a]	3.7	4MQT	[9]
μOR	Nb39	BU72	2.1	5C1M	[8]
Opsin	GαCT peptide	All-*trans*-retinal	3.2	3DQB	[5]
	GαCT peptide	All-*trans*-retinal	3.0	2X72	[7]
	GαCT peptide	All-*trans*-retinal	2.9	3PQR	[2]
	GαCT peptide	All-*trans*-retinal	3.3	4A4M	[3]
	GαCT peptide	No ligand[b]	2.7	4J4Q	[4]
	GαCT peptide	Mixed *cis*-retinals	2.9	4BEY	[6]
	GαCT peptide	No ligand[b]	2.3	4X1H	[1]
	Arrestin peptide	No ligand[b]	2.8	4PXF	[14]
	Arrestin	No ligand	3.3	4ZWJ	[15]

[a] Positive allosteric modulator.
[b] The receptor contains a detergent molecule in its orthosteric ligand binding pocket.

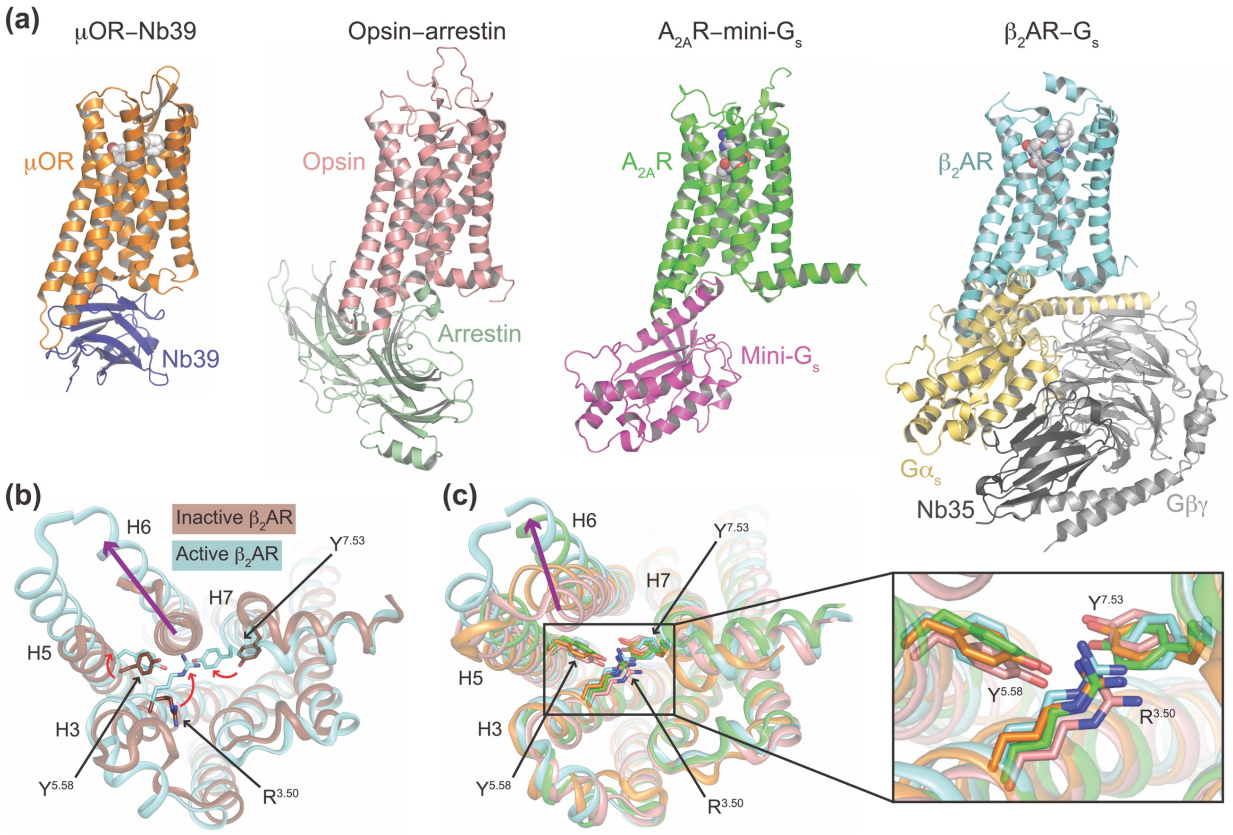

Figure 1. Structural features of active-state GPCRs. **(a)** Comparison of the structures of μOR (orange) in complex with nanobody Nb39 (PDB ID 5C1M [9]), opsin (pink) coupled to arrestin (PDB ID 4ZWJ [15]) and $A_{2A}R$ (green) coupled to mini-G_s (PDB ID 5G53 [16]), which were all determined during the past year, with the archetypal active-state structure of β_2AR (cyan) coupled to heterotrimeric G_s (PDB ID 3SN6 [13]). **(b)** Cytoplasmic view of an alignment between β_2AR in its inactive antagonist-bound state (brown; PDB ID 2RH1 [50]) and the active G_s bound conformation (cyan; PDB ID 3SN6 [13]). The 14 Å outward movement of H6 that is induced by G protein binding is indicated by a purple arrow. Rearrangements of three highly conserved residues ($Y^{5.58}$, $Y^{7.53}$ and $R^{3.50}$; shown as sticks) within the core of the receptor are indicated by red arrows. **(c)** Intracellular view of an alignment between the four active-state GPCRs in **(a)**. Sidechains of residues $Y^{5.58}$, $Y^{7.53}$ and $R^{3.50}$ are shown as sticks; binding partners have been omitted for clarity. The outward movement of H6, which varies between 10 and 17 Å in these structures, is indicated by a purple arrow. An expanded view of residues $Y^{5.58}$, $Y^{7.53}$ and $R^{3.50}$ (shown as sticks), demonstrates that they adopt highly conserved positions in all active active-state GPCR structures.

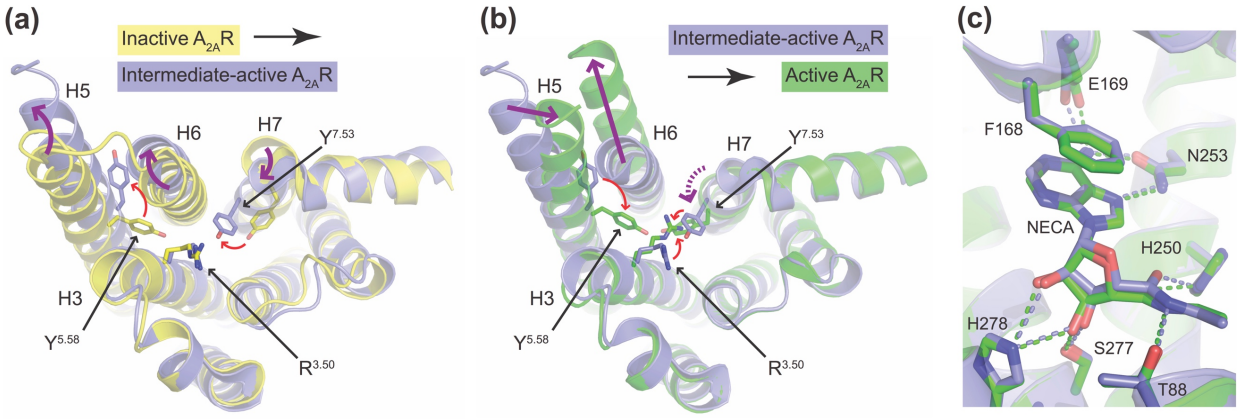

Figure 2. Conformational changes involved in $A_{2A}R$ activation. **(a)** Cytoplasmic view showing the transition of $A_{2A}R$ from the inactive antagonist-bound state (yellow; PDB ID 3REY [23]) to the intermediate-active agonist-bound state (blue; PDB ID 2YDV [19]). The outward movements of H5 and H6 and the inward movement of H7 are coupled to significant helix rotations shown as curved purple arrows. Side chain conformation changes in residues $Y^{5.58}$ and $Y^{7.53}$(shown as sticks) are highlighted by red arrows. **(b)** Cytoplasmic view showing the transition of $A_{2A}R$ from the intermediate-active agonist-bound state (blue; PDB ID 2YDV [19]) to the active mini G protein-bound state (green; PDB ID 5G53 [16]). The linear movement of H5 and H6 is shown as straight purple arrows. The rotation within H7, which is not coupled to any significant lateral movement is highlighted by a dashed purple arrow. Side chain conformation changes in residues $Y^{5.58}$, $Y^{7.53}$ and $R^{3.50}$ (shown as sticks) are highlighted by red arrows. **(c)** Comparison of the ligand-binding pocket of $A_{2A}R$ in the intermediate-active (blue; PDB ID 2YDV [19]) and active (green; PDB ID 5G53 [16]) state. No significant differences are observed in the orientation of the agonist (NECA), or the residues with which it forms direct contacts [16]. Direct polar contacts between $A_{2A}R$ and NECA are shown a dashed lines and are coloured to match the receptor.

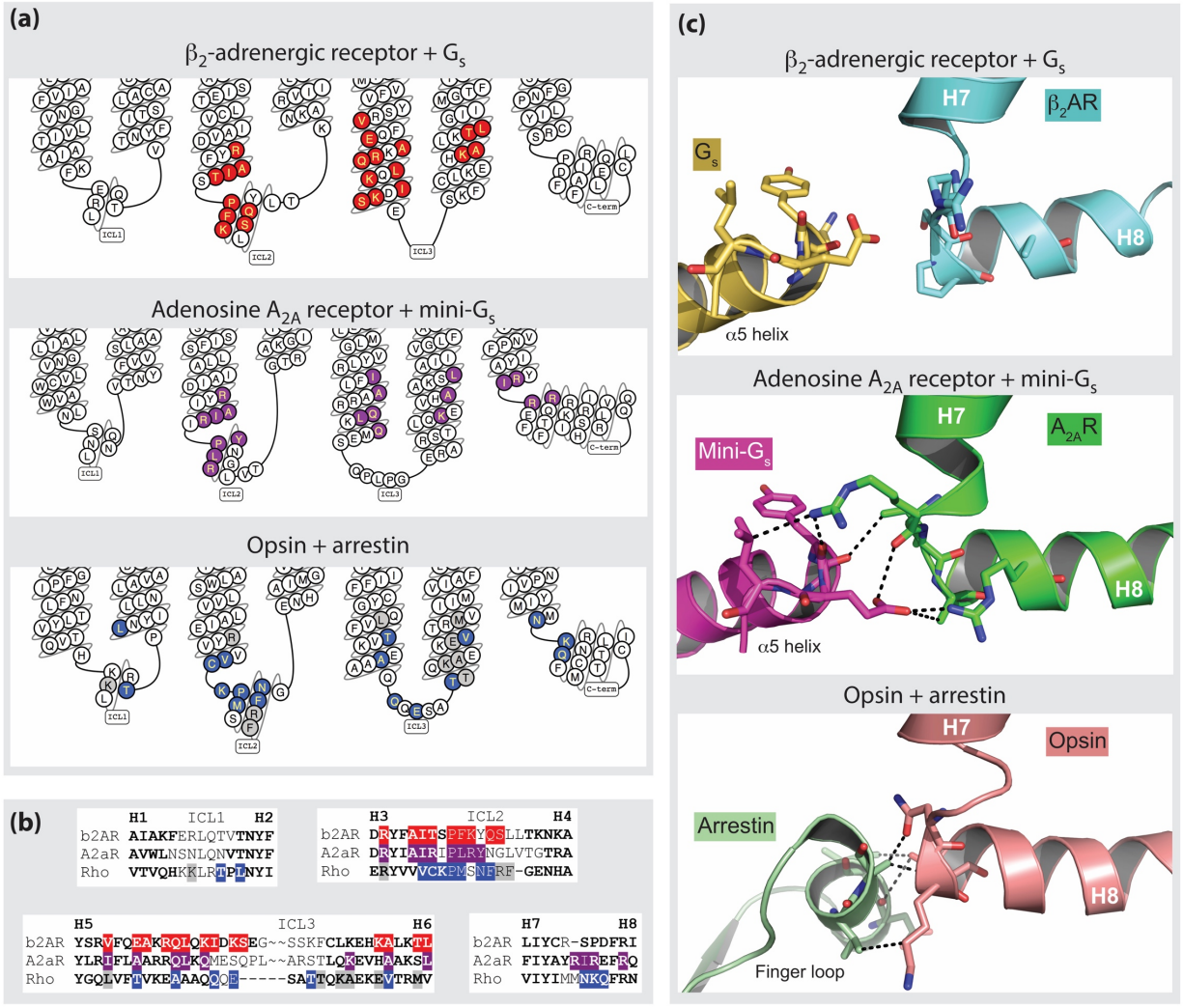

Figure 3. Comparison of the interfaces between GPCRs and binding partners derived from native signalling proteins. (a) The intracellular halves of β_2AR, A_{2A}R and opsin are depicted as snake plots, with amino acid residues that are within 3.9 Å of the binding partner in either red, purple or blue, respectively. In the opsin–arrestin crystal, there were 4 opsin–arrestin complexes in the asymmetric unit; on the snake plot, residues coloured blue make contact to arrestin in either 3 or 4 of the complexes, whereas those coloured in grey occur in only 1 or 2 of the complexes. The figures were made using www.gpcrdb.org [51]. **(b)** Structural alignment of β_2AR, A_{2A}R and opsin amino acid sequences with residues within 3.9 Å of the binding partner highlighted as in **(a)**. The alignments were based on data in www.gpcrdb.org [51], but were adapted to remove gaps; '~~' represent non-conserved amino acid residues removed from the alignment of ICL3 for reasons of clarity, and '–' represent gaps in the alignment. **(c)** Divergent interactions of the H7-H8 boundary in coupling to cytoplasmic signalling proteins. No interactions are observed between the H7-H8 boundary

of β_2AR (cyan) and G_s (gold) in the β_2AR–G_s complex (PDB ID 3SN6 [13]), however, interactions are present between this region of A_{2A}R (green) and the α5 helix of mini-G_s (magenta) in the A_{2A}R–mini-G_s complex (PDB ID 5G53 [16]). Extensive interactions are also observed between the H7-H8 boundary of opsin (pink) and the finger loop of arrestin (light green) in the opsin–arrestin complex (PDB ID 4ZWJ [15]). Residues that form direct contacts are shown as sticks, and direct interactions are shown as dashed lines (only the closest contact to each residue is shown and all are less than 3.9 Å).

References and recommended reading

•1. Blankenship E, Vahedi-Faridi A, Lodowski DT: **The High-Resolution Structure of Activated Opsin Reveals a Conserved Solvent Network in the Transmembrane Region Essential for Activation**. *Structure* 2015, **23**:2358-2364.

• This high resolution structure of opsin in its active state, in complex with GαCT, revealed the most extensive network of ordered water molecules observed in a GPCR structure to date. This work adds to a growing body of evidence suggesting that polar networks play an integral role in GPCR activation (see also reference [8]).

2. Choe HW, Kim YJ, Park JH, Morizumi T, Pai EF, Krauss N, Hofmann KP, Scheerer P, Ernst OP: **Crystal structure of metarhodopsin II**. *Nature* 2011, **471**:651-655.
3. Deupi X, Edwards P, Singhal A, Nickle B, Oprian D, Schertler G, Standfuss J: **Stabilized G protein binding site in the structure of constitutively active metarhodopsin-II**. *Proc Natl Acad Sci U S A* 2012, **109**:119-124.
4. Park JH, Morizumi T, Li Y, Hong JE, Pai EF, Hofmann KP, Choe HW, Ernst OP: **Opsin, a structural model for olfactory receptors?** *Angew Chem Int Ed Engl* 2013, **52**:11021-11024.
5. Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, Hofmann KP, Ernst OP: **Crystal structure of opsin in its G-protein-interacting conformation**. *Nature* 2008, **455**:497-502.
6. Singhal A, Ostermaier MK, Vishnivetskiy SA, Panneels V, Homan KT, Tesmer JJ, Veprintsev D, Deupi X, Gurevich VV, Schertler GF, et al.: **Insights into congenital stationary night blindness based on the structure of G90D rhodopsin**. *EMBO Rep* 2013, **14**:520-526.
7. Standfuss J, Edwards PC, D'Antona A, Fransen M, Xie G, Oprian DD, Schertler GF: **The structural basis of agonist-induced activation in constitutively active rhodopsin**. *Nature* 2011, **471**:656-660.
••8. Huang W, Manglik A, Venkatakrishnan AJ, Laeremans T, Feinberg EN, Sanborn AL, Kato HE, Livingston KE, Thorsen TS, Kling RC, et al.: **Structural insights into micro-opioid receptor activation**. *Nature* 2015, **524**:315-321.

•• The 2.1 Å resolution structure of μOR in its active state, in complex with a nanobody and high affinity agonist. This structure revealed that activation of μOR results in more subtle changes in the organisation of the ligand-binding pocket compared to other receptors such as β_2AR. The high resolution nature of this structure also revealed novel details about rearrangements in a network of ordered water molecules during the transition from the inactive to active state.

9. Kruse AC, Ring AM, Manglik A, Hu J, Hu K, Eitel K, Hubner H, Pardon E, Valant C, Sexton PM, et al.: **Activation and allosteric modulation of a muscarinic acetylcholine receptor**. *Nature* 2013, **504**:101-106.
10. Rasmussen SG, Choi HJ, Fung JJ, Pardon E, Casarosa P, Chae PS, Devree BT, Rosenbaum DM, Thian FS, Kobilka TS, et al.: **Structure of a nanobody-stabilized active state of the beta(2) adrenoceptor**. *Nature* 2011, **469**:175-180.
11. Ring AM, Manglik A, Kruse AC, Enos MD, Weis WI, Garcia KC, Kobilka BK: **Adrenaline-activated structure of beta2-adrenoceptor stabilized by an engineered nanobody**. *Nature* 2013, **502**:575-579.
12. Weichert D, Kruse AC, Manglik A, Hiller C, Zhang C, Hubner H, Kobilka BK, Gmeiner P: **Covalent agonists for studying G protein-coupled receptor activation**. *Proc Natl Acad Sci U S A* 2014, **111**:10744-10748.

13. Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, et al.: **Crystal structure of the beta2 adrenergic receptor-Gs protein complex**. *Nature* 2011, **477**:549-555.

•14. Szczepek M, Beyriere F, Hofmann KP, Elgeti M, Kazmin R, Rose A, Bartl FJ, von Stetten D, Heck M, Sommer ME, et al.: **Crystal structure of a common GPCR-binding interface for G protein and arrestin**. *Nat Commun* 2014, **5**:4801.

• This structure of opsin bound to an arrestin-derived peptide provided the first insight into the potential organisation of the opsin–arrestin interface.

••15. Kang Y, Zhou XE, Gao X, He Y, Liu W, Ishchenko A, Barty A, White TA, Yefanov O, Han GW, et al.: **Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser**. *Nature* 2015, **523**:561-567.

•• This is the first structure of a GPCR–arrestin complex. The structure was solved by femtosecond X-ray laser crystallography, highlighting the usefulness of this technique in the structure determination of challenging proteins. The structure revealed the molecular organisation of the opsin–arrestin interface and provides novel insight into the molecular basis for biased signalling.

••16. Carpenter B, Nehme R, Warne T, Leslie AG, Tate CG: **Structure of the adenosine A(2A) receptor bound to an engineered G protein**. *Nature* 2016, **536**:104-107.

•• The 3.4 Å resolution structure of human $A_{2A}R$ in its active state, in complex with mini-G_s and a high affinity agonist. This is the first structure of $A_{2A}R$ in its active state and revealed a common mechanism for coupling of different GPCRs to G_s. The structure also highlighted subtle differences in the organisation of the GPCR–G protein interface compared to the β_2AR–G_s complex.

17. Ballesteros JA, Weinstein H: **Integrated methods for the construction of three dimensional models and computational probing of structure function relations in G protein-coupled receptors**. In *Methods in Neurosciences.* Edited by Sealfon SC, Conn PM: Academic Press; 1995:366-428. vol 25.]

•18. Venkatakrishnan AJ, Deupi X, Lebon G, Heydenreich FM, Flock T, Miljus T, Balaji S, Bouvier M, Veprintsev DB, Tate CG, et al.: **Diverse activation pathways in class A GPCRs converge near the G-protein-coupling region**. *Nature* 2016, **536**:484-487.

• A comprehensive analysis of 27 GPCR structures highlights the diversity of mechanisms for the activation of the receptor by agonists in the extracellular half of the receptor and the conserved conformational changes this elicits in the intracellular half of the receptor.

19. Lebon G, Warne T, Edwards PC, Bennett K, Langmead CJ, Leslie AG, Tate CG: **Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation**. *Nature* 2011, **474**:521-525.

20. Lebon G, Edwards PC, Leslie AG, Tate CG: **Molecular Determinants of CGS21680 Binding to the Human Adenosine A2A Receptor**. *Mol Pharmacol* 2015, **87**:907-915.

21. Xu F, Wu H, Katritch V, Han GW, Jacobson KA, Gao ZG, Cherezov V, Stevens RC: **Structure of an agonist-bound human A2A adenosine receptor**. *Science* 2011, **332**:322-327.

22. Congreve M, Andrews SP, Dore AS, Hollenstein K, Hurrell E, Langmead CJ, Mason JS, Ng IW, Tehan B, Zhukov A, et al.: **Discovery of 1,2,4-triazine derivatives as adenosine A(2A) antagonists using structure based drug design**. *J Med Chem* 2012, **55**:1898-1903.

23. Dore AS, Robertson N, Errey JC, Ng I, Hollenstein K, Tehan B, Hurrell E, Bennett K, Congreve M, Magnani F, et al.: **Structure of the adenosine A(2A) receptor in complex with ZM241385 and the xanthines XAC and caffeine**. *Structure* 2011, **19**:1283-1293.
24. Hino T, Arakawa T, Iwanari H, Yurugi-Kobayashi T, Ikeda-Suno C, Nakada-Nakura Y, Kusano-Arai O, Weyand S, Shimamura T, Nomura N, et al.: **G-protein-coupled receptor inactivation by an allosteric inverse-agonist antibody**. *Nature* 2012, **482**:237-240.
25. Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, Ijzerman AP, Stevens RC: **The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist**. *Science* 2008, **322**:1211-1217.
•26. Krumm BE, White JF, Shah P, Grisshammer R: **Structural prerequisites for G-protein activation by the neurotensin receptor**. *Nat Commun* 2015, **6**:7895.

• This paper reports the structure of minimally thermostabilised neurotensin receptor mutants in their intermediate-active conformation, and delineates the effect of thermostabilising mutations on G protein coupling.

27. White JF, Noinaj N, Shibata Y, Love J, Kloss B, Xu F, Gvozdenovic-Jeremic J, Shah P, Shiloach J, Tate CG, et al.: **Structure of the agonist-bound neurotensin receptor**. *Nature* 2012, **490**:508-513.
28. Warne T, Moukhametzianov R, Baker JG, Nehme R, Edwards PC, Leslie AG, Schertler GF, Tate CG: **The structural basis for agonist and partial agonist action on a beta(1)-adrenergic receptor**. *Nature* 2011, **469**:241-244.
29. Rosenbaum DM, Zhang C, Lyons JA, Holl R, Aragao D, Arlow DH, Rasmussen SG, Choi HJ, Devree BT, Sunahara RK, et al.: **Structure and function of an irreversible agonist-beta(2) adrenoceptor complex**. *Nature* 2011, **469**:236-240.
••30. Ye L, Van Eps N, Zimmer M, Ernst OP, Prosser RS: **Activation of the A2A adenosine G-protein-coupled receptor by conformational selection**. *Nature* 2016, **533**:265-268.

•• ^{19}F-NMR spectroscopy was used to identify four conformational states, including two active states, of $A_{2A}R$ that exist in equilibrium in the absence of ligand. Partial agonists and full agonists increased the population of different active states, indicating that partial agonism is achieved through selection of a distinct conformational state.

••31. Manglik A, Kim TH, Masureel M, Altenbach C, Yang Z, Hilger D, Lerch MT, Kobilka TS, Thian FS, Hubbell WL, et al.: **Structural Insights into the Dynamic Process of beta2-Adrenergic Receptor Signaling**. *Cell* 2015, **161**:1101-1111.

•• ^{19}F-NMR and DEER spectroscopy were used to study β_2AR activation. In the absence of ligand β_2AR exists in equilibrium between two inactive states, agonist binding shifts the equilibrium towards an active conformation, but complete occupation of the active state requires coupling to a G protein or a functional mimetic thereof. The energy landscape of β_2AR activation appears quite different to that of $A_{2A}R$.

32. Lebon G, Warne T, Tate CG: **Agonist-bound structures of G protein-coupled receptors**. *Curr Opin Struct Biol* 2012, **22**:482-490.
33. Murphree LJ, Marshall MA, Rieger JM, MacDonald TL, Linden J: **Human A(2A) adenosine receptors: high-affinity agonist binding to receptor-G protein complexes containing Gbeta(4)**. *Mol Pharmacol* 2002, **61**:455-462.
••34. Carpenter B, Tate CG: **Engineering a minimal G protein to facilitate crystallisation of G protein-coupled receptors in their active conformation**. *Protein Eng Des Sel* 2016, **29**:583-594.

•• This paper describes the design of an engineered G protein (mini-G_s) that is composed of a single domain from $G\alpha_s$. Mini-G_s is a small, stable protein that mimics the pharmacological response induced in GPCRs by heterotrimeric G_s. Mini-G_s is a useful surrogate for heterotrimeric G proteins for the characterisation and crystallisation of GPCRs in their active conformation. The work also provided novel insight into the mechanism of allosteric activation of G proteins by GPCRs.

35. Nygaard R, Zou Y, Dror RO, Mildorf TJ, Arlow DH, Manglik A, Pan AC, Liu CW, Fung JJ, Bokoch MP, et al.: **The dynamic process of beta(2)-adrenergic receptor activation**. *Cell* 2013, **152**:532-542.
36. Sounier R, Mas C, Steyaert J, Laeremans T, Manglik A, Huang W, Kobilka BK, Demene H, Granier S: **Propagation of conformational changes during mu-opioid receptor activation**. *Nature* 2015, **524**:375-378.
37. Wess J: **Molecular basis of receptor/G-protein-coupling selectivity**. *Pharmacol Ther* 1998, **80**:231-264.
38. Thomsen AR, Plouffe B, Cahill TJ, 3rd, Shukla AK, Tarrasch JT, Dosey AM, Kahsai AW, Strachan RT, Pani B, Mahoney JP, et al.: **GPCR-G Protein-beta-Arrestin Super-Complex Mediates Sustained G Protein Signaling**. *Cell* 2016, **166**:907-919.
39. Liu JJ, Horst R, Katritch V, Stevens RC, Wuthrich K: **Biased signaling pathways in beta2-adrenergic receptor characterized by 19F-NMR**. *Science* 2012, **335**:1106-1110.
40. Wacker D, Wang C, Katritch V, Han GW, Huang XP, Vardy E, McCorvy JD, Jiang Y, Chu M, Siu FY, et al.: **Structural features for functional selectivity at serotonin receptors**. *Science* 2013, **340**:615-619.
41. Gurevich VV, Benovic JL: **Visual arrestin interaction with rhodopsin. Sequential multisite binding ensures strict selectivity toward light-activated phosphorylated rhodopsin**. *J Biol Chem* 1993, **268**:11628-11638.
42. Ostermaier MK, Peterhans C, Jaussi R, Deupi X, Standfuss J: **Functional map of arrestin-1 at single amino acid resolution**. *Proc Natl Acad Sci U S A* 2014, **111**:1825-1830.
43. Kang Y, Gao X, Zhou XE, He Y, Melcher K, Xu HE: **A structural snapshot of the rhodopsin-arrestin complex**. *FEBS J* 2016, **283**:816-821.
44. Venkatakrishnan AJ, Deupi X, Lebon G, Tate CG, Schertler GF, Babu MM: **Molecular signatures of G-protein-coupled receptors**. *Nature* 2013, **494**:185-194.
•45. Flock T, Ravarani CN, Sun D, Venkatakrishnan AJ, Kayikci M, Tate CG, Veprintsev DB, Babu MM: **Universal allosteric mechanism for Galpha activation by GPCRs**. *Nature* 2015, **524**:173-179.

• A comprehensive analysis of all G proteins highlights the conserved pathway of activation within the Gα subunit. A unified nomenclature for all residues in G proteins is also presented, which will simplify comparison of functional residues between distantly related proteins.

46. Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, Bendahman N, Hamers R: **Naturally occurring antibodies devoid of light chains**. *Nature* 1993, **363**:446-448.
•47. Pardon E, Laeremans T, Triest S, Rasmussen SG, Wohlkonig A, Ruf A, Muyldermans S, Hol WG, Kobilka BK, Steyaert J: **A general protocol for the generation of Nanobodies for structural biology**. *Nat Protoc* 2014, **9**:674-693.

• Nanobodies have proved to be one of the best tools for the crystallisation of GPCRs in their active conformation. This paper provides detailed protocols for the selection of nanobodies for structural biology applications.

48. Steyaert J, Kobilka BK: **Nanobody stabilization of G protein-coupled receptor conformational states**. *Curr Opin Struct Biol* 2011, **21**:567-572.

••49. Masuho I, Ostrovskaya O, Kramer GM, Jones CD, Xie K, Martemyanov KA: **Distinct profiles of functional discrimination among G proteins determine the actions of G protein-coupled receptors**. *Sci Signal* 2015, **8**:ra123.

•• This is a fascinating and comprehensive study of the differential coupling of 14 different G proteins to four different GPCRs in the cellular context. Both the kinetics and the magnitude of the responses varied and were important in determining efficacy and potency of coupling.

50. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, et al.: **High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor**. *Science* 2007, **318**:1258-1265.

•51. Munk C, Isberg V, Mordalski S, Harpsoe K, Rataj K, Hauser AS, Kolb P, Bojarski AJ, Vriend G, Gloriam DE: **GPCRdb: the G protein-coupled receptor database - an introduction**. *Br J Pharmacol* 2016, **173**:2195-2207.

• The database GPCRdb is a fantastic resource for everyone working on GPCRs and it has just got even better, with new tools for structure-based sequence alignments and data presentation.