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GPCRs and G Protein Activation

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1. Introduction

An efficient intercellular communication system is essential to allow the correct functioning of multicellular organisms. This necessitates extracellular messengers (hormones, or neurotransmitters) as well as receptors , that is, proteins capable of recognizing these extracellular messengers and transducing a signal inside the cell. Each cell expresses several different types of receptors: signal transduction is both temporally and spatially integrated in order to generate the appropriate cellular response to each physiological situation.

Hydrophobic ligands are able to penetrate inside the cell: they recognize intracellular receptors that migrate to the nucleus and regulate protein transcription. Hydrophilic ligands, in contrast, are unable to cross the plasma membrane: these extracellular ligands recognize transmembrane receptors that then produce intracellular messengers to affect the target cell function. Several families of transmembrane receptors are known: some are ligand-gated ion channels, and regulate the transmembrane voltage (depolarization or hyperpolarisation) or the intracellular Ca^{++} concentration; others are ligand-activated enzymes: some synthetize cGMP, others phosphorylate specific target proteins upon ligand recognition; and yet other receptors (known as "G Protein Coupled Receptors" or "GPCRs") activate intracellular trimeric G proteins in response to extracellular signals. These receptor-activated G proteins in turn activate enzymes responsible for "second messenger" synthesis (adenylate cyclase $\rightarrow$ cAMP, or phospholipase C $\rightarrow$ Inositol trisphosphate (Inositol(1,4,5)P$_3$ or "IP3") and diacylglycerol), regulate ion channels, or activate other ("small") G proteins.

2. G protein coupled receptors

2.1 A few examples

The human genome contains at least 800 GPCRs, grouped in five main families (Fredriksson *et al.*, 2003). One of the best characterized GPCR, rhodopsin, is responsible for vision in the dark: it captures photons thanks to its prosthetic group (11-cis retinal), and leads to phosphodiesterase activation in retina rod cells. It is extremely abundant in the rod cell disks, comparatively easy to purify, and therefore has been very extensively studied for many years by biochemists. Other GPCRs allow us to taste and smell, control our appetite, fertility, stress, heart rate and breathing, etc. Adrenaline (the stress hormone), histamine (allergic reactions), glucagon (glycemia control), but also taste and odorant receptors, luteotropic and follicular stimulating hormone receptors (ovule and spermatozoid development), etc. recognize GPCRs and induce G protein activation.

2.2 GPCR families

All G protein coupled receptors possess a glycosylated extracellular amino-terminal (N-term) and an intracellular carboxyl-terminal (C-term) domain, separated by 7 transmembrane helices (TM1 to TM7) joined by three intracellular (IC1 to IC3) and three extracellular (EC1 to EC3) loops (Figure 1).
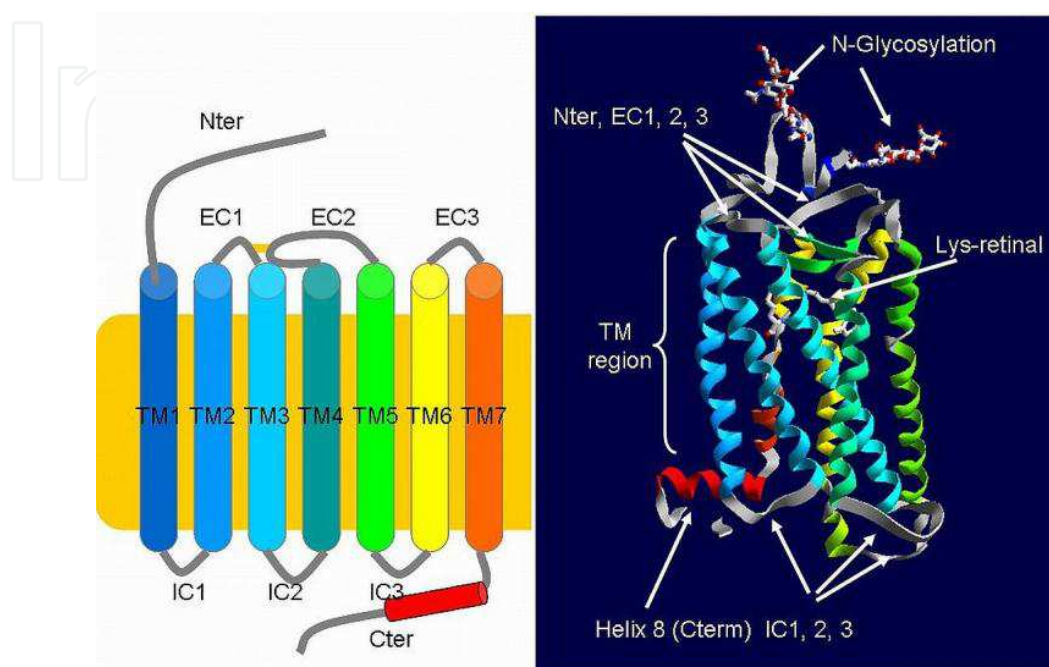

Fig. 1. Ribbon representation of the X-ray structure of rhodopsin. Left: schematic representation of a GPCR, showing the TM helices, intracellular (IC) and extracellular (EC) loops. Right: ribbon representation of the crystal structure of rhodopsin (1GZM). The prosthetic group, retinal, is covalently bound to a lysine side chain in TM7 (sticks).

The first and second EC loops are joined by a conserved disulfide bridge. The C-term region begins by an intracellular α-helix, H8, which lies horizontally on the plasma membrane: it forms an aromatic cluster with a tyrosine side chains from TM7 and interacts with the phospholipid head groups through lysine and arginine side chains. The 7 helices are arranged in a bundle (Figure 1). GPCRs have been identified in animals, yeast, plants. They probably arise from a common ancestor (Fredriksson *et al.*, 2003). Several hundred putative GPCRs have been identified in the human genome where they represent 1-3% of the genes (Fredriksson *et al.*, 2003). The vast majority of GPCRs (including most odorant receptors) share "signature" amino acids with rhodopsin (see below). They have been grouped in "Family A" (Kolakowski, Jr., 1994) or "rhodopsin-like receptor family" (Fredriksson *et al.*, 2003). Other GPCR families do not possess these highly conserved amino acids, but share other signature amino acids. For instance, all "family B" (secretin-receptor like) receptors possess a typical N-terminal "sushi" domain with three conserved disulfide bridges and have very strong sequence homologies in the transmembrane domain. Fifteen of these receptors with a comparatively short N-term domain ("sushi" domain only) are specialized in recognition of peptide hormones and neurotransmitters (glucagon, Growth Hormone Releasing Hormone (GHRH or GRF), parathyroid hormone (PTH) and others).

The majority of family B receptors possess several additional modules (EGF-like, Immunoglobulin-like, etc) before the sushi domain, suggesting that they might function as adhesion proteins (Mizuno and Itoh, 2011). Most of these are "orphan" receptors (that is: their ligand is unknown) and their ability to activate G proteins has not been proven yet. "Family C" receptors recognize amino acids (metabotropic receptors for glutamate and GABA$_B$ receptors for GABA) or calcium ions, through N-terminal "venus flytrap" domains (Jensen *et al.*, 2002; Wellendorph and Brauner-Osborne, 2009). Some of the taste receptors also are GPCRs: sweet and "umami" tasting molecules are recognized by "family C" GPCRs, and the "bitter" taste, by GPCRs that present very little homology with the other GPCRs, and form an additional receptor family (Fredriksson *et al.*, 2003).

2.3 Conserved residues in "family A" receptors

Rhodopsin and related receptors possess a few extremely conserved residues in each TM helix. In the Ballesteros and Weinstein nomenclature, the most conserved amino acid in each TM helix is numbered $X^{h.50}$ (where "h" is the helix number): for instance, $R^{3.50}$ is the most conserved amino acid in TM3; $D^{3.49}$ and $Y^{3.51}$ are the two conserved amino acids immediately preceding and following this arginine.

Some of these very conserved side chains are involved in structural features, like the prolines in helices 5, 6 and 7 that induce kinks in the TM helices. In the different family A receptors crystal structures (rhodopsin but also β-adrenergic, adenosine, histamine H3 receptors), the conserved asparagine of the TM7 $NPxxY(x)_{5-6}F$ motif is part of a hydrogen bond network involving TM1, TM2 ($D^{2.50}$) and TM7, while the tyrosine in this motif constrains TM7 in contact with aromatic side chains in the C term helix 8. Other conserved side chains play a role in the resting and/or active receptor conformation For instance, the arginine of the TM3 "DRY motif" ($E/DR^{3.50}Y$) at the intracellular end of the third transmembrane helix forms in rhodopsin a H bond network with $E^{3.49}$, $E^{6.30}$ and $T^{6.34}$. The "ionic lock" $R^{3.50}$-$E^{6.30}$ stabilizes the resting state: it is broken up in metarhodopsin II (the active rhodopsin conformation). In that structure, $R^{3.50}$ folds back inside the G protein to interact with $Y^{5.58}$, thereby creating an intracellular binding pocket, able to accommodate the G-protein. The ionic lock is less stable in the β-adrenergic receptors compared to rhodopsin, and this is perhaps responsible for their detectable constitutive activity (ability to activate G proteins in the absence of agonist) (Moukhametzianov *et al.*, 2011). $W^{6.48}$ of the $CWxP^{6.50}$ motif is in very close contact with the agonist ligands, and was thought to trip the switch of receptor activation by toggling between different rotamer conformations and thereby affecting the position of neighbouring aromatic side chains. Although this hypothesis is supported by computational mapping (Bhattacharya and Vaidehi, 2010), the toggle is not evident in the metarhodopsin II (Standfuss *et al.*, 2011; Choe *et al.*, 2011) or β$_2$-adrenergic receptor crystal structures (Rasmussen *et al.*, 2011b; Rasmussen *et al.*, 2011a).

3. Trimeric G proteins

3.1 G protein subtypes and GPCR effectors

The G proteins that transduce the signal from GPCRs are heterotrimeric (Gαβγ) proteins. Some of the mRNAs encoding the Gα subunits are subject to alternative splicing so that

sixteen genes encode 23 known Gα proteins: (Birnbaumer, 2007). The Gα proteins are anchored to the plasma membrane by N-terminal myristoylation or palmitoylation. They can be grouped into four families based upon sequence homologies, and each GPCR has a preference for a single Gα or for a single family of Gα subunits. Each Gα subunit regulates one or a few effectors (Birnbaumer, 2007):

- G proteins in the G_s ($G_{s/olf}$) G protein family stimulate adenylate cyclase,
- G proteins in the G_i ($G_{i/o/t/gust/z}$) G protein family inhibit adenylate cyclase and/or regulate ion channels,
- G proteins in the $G_{q/11}$ ($G_{q/11/14/15/16}$) G protein family activate phospholipase C,
- G proteins in the G12/13 G protein family activate "Guanyl nucleotide Exchange Factors" (GEFs) that in turn activate another group of "small" (monomeric) G proteins, the Rho G proteins

The carboxyl-terminal Gα sequence is the major determinant for receptor recognition: exchanging this sequence allows the construction of promiscuous chimeric G proteins that can be used to drive GPCR coupling to a non-physiological effector (Kostenis *et al.*, 2005).

There are five known human Gβ and 12 Gγ genes (Birnbaumer, 2007). Most but not all of the Gβγ and Gα- Gβγ combinations are allowed. All Gγ subunits are C-terminally prenylated (some with geranyl-geranyl, others with farnesyl groups) and carboxymethylated: this helps to anchor the Gβγ subunits to the plasma membrane. The C-terminal sequence determines the nature of the prenyl group (farnesyl or geranyl-geranyl) modifying the Gγ subunit; both the C-terminal sequence and the prenyl group play an active role in the recognition of both rhodopsin and phospholipids (Katadae *et al.*, 2008). Although the literature on this subject is sparse, there is some evidence that other GPCRs also recognize preferentially specific Gβγ subunits (Jian *et al.*, 2001; Kisselev and Downs, 2003; Birnbaumer, 2007). The Gβγ subunits recognize and regulate a growing list of effectors, including ion channels, phospholipase C (PLC), phosphoinositide-3' kinase-γ (PI3Kγ), various adenylate cyclase isoforms, etc. Different PLC isoforms respond differently to different Gβγ isoforms; and the cardiac ATP-inhibited inwardly rectifying K^+ channel (KirATP) is either inhibited or activated by Gβγ depending on the nature of the Gβ subunit (Birnbaumer, 2007).

3.2 G proteins as (inefficient) GTPases

G proteins are (poor) GTPases (Birnbaumer, 2007): they hydrolyze GTP slowly to GDP + inorganic phosphate, then release GDP extremely slowly. The GDP release and the GTP hydrolysis reactions are highly regulated, accompanied by conformation changes, and used as molecular clocks.

Trimeric G proteins are no exception to this rule: GTP binding is necessary to allow transient effectors activation (Oldham and Hamm, 2006; Birnbaumer, 2007). As summarized in Figure 2, the GDP release from trimeric G proteins is accelerated by G Protein Coupled Receptors (GPCRs) that function as "Guanyl nucleotides Exchange Factors" (GEFs): they allow GDP release, and this is rapidly followed by GTP recognition and dissociation of the two G protein subunits. Both subunits can then transiently recognize their respective effectors. The GTP hydrolysis reaction (leading to signal interruption) is accelerated by "Regulators of G

protein Signaling" (RGS) proteins, that function as "GTPase Activator Proteins" (GAPs) and accelerate signal interruption.
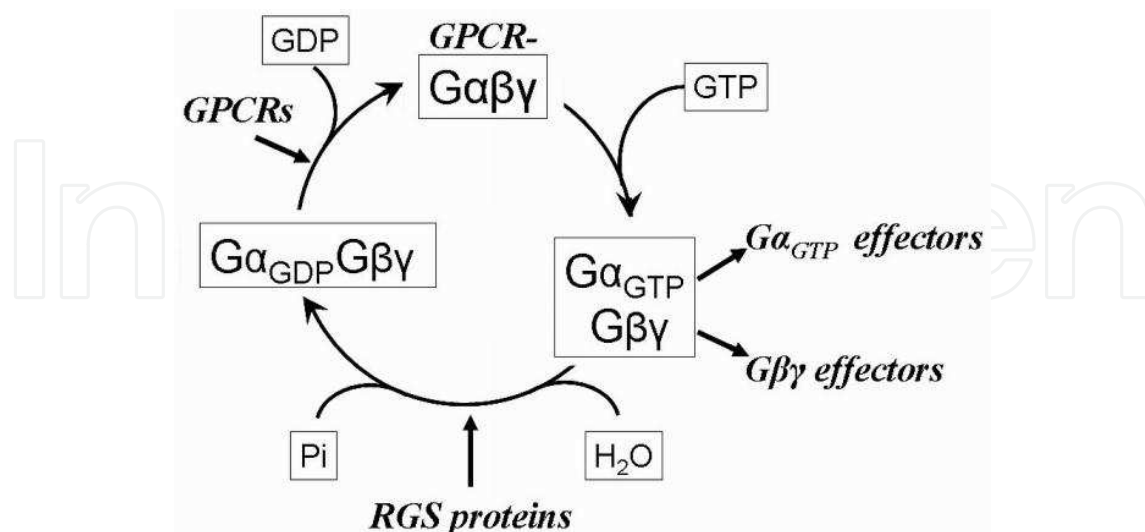

Fig. 2. the G protein activation cycle. In the resting state, the G protein is trimeric ($G\alpha_{GDP}G\beta\gamma$) and occupied by GDP. GPCRs interact with resting (GDP-bound) G proteins, facilitate the GDP release and stabilize an empty G protein conformation. GTP induces the dissociation of the two G protein subunits, $G\alpha_{GTP}$ and $G\beta\gamma$: this allows both subunits to recognize and regulate their respective effectors (enzymes, channels or regulators of G protein signaling). $G\alpha_{GTP}$ hydrolyses GTP to GDP and the $G\alpha_{GDP}$ complex recognizes $G\beta\gamma$ with a very high affinity: the resting trimeric complex reforms spontaneously. GTP hydrolysis can be accelerated by "Regulators of G protein Signaling" (RGS) molecules.

3.3 G protein structures

The G proteins regulated by GPCRs are heterotrimeric (Birnbaumer, 2007). The three polypeptide chains form two independent subunits: the Gα and Gβγ subunits (Figure 3). The Gα protein structure can be divided into two domains held together by mutual interactions with the guanyl nucleotide: a N-terminal "Ras-like domain" (with strong structural homology with the small GTPases, Ras) and a C-terminal α-helical domain (Figure 3). In the agonist-receptor-G protein complex, the guanyl nucleotide has dissociated, and the helical domain "floats away" from the Ras –like domain (Rasmussen *et al.*, 2011b). The Gβ protein "WD repeats" (blue) forms a 7 blades beta-propeller domain, and the Gγ protein (green) wraps around Gβ, one of the two small α-helices forming a coiled-coil with the Gβ protein α-helix (Figure 3). Gβγ forms a stable complex that cannot be dissociated without denaturation but Gα can dissociate from Gβγ upon GTP binding.

As shown in Figure 4, the conformation of three segments ("switch regions") of Gα changes during the GTPase catalytic cycle (Oldham and Hamm, 2006; Rasmussen *et al.*, 2011b). This regulates the interaction of Gα with Gβγ and with its effectors. Switch 2 together with either switch 1 or 3 indeed forms part of the Gα – protein binding interface (Figure 5). It participates to the recognition of Gβγ, but also of effectors and "Regulators or G protein Signaling" (RGS) proteins (Figure 5): the Gα- Gβγ dissociation is essential to allow effectors activation by $G\alpha_{GTP}$.
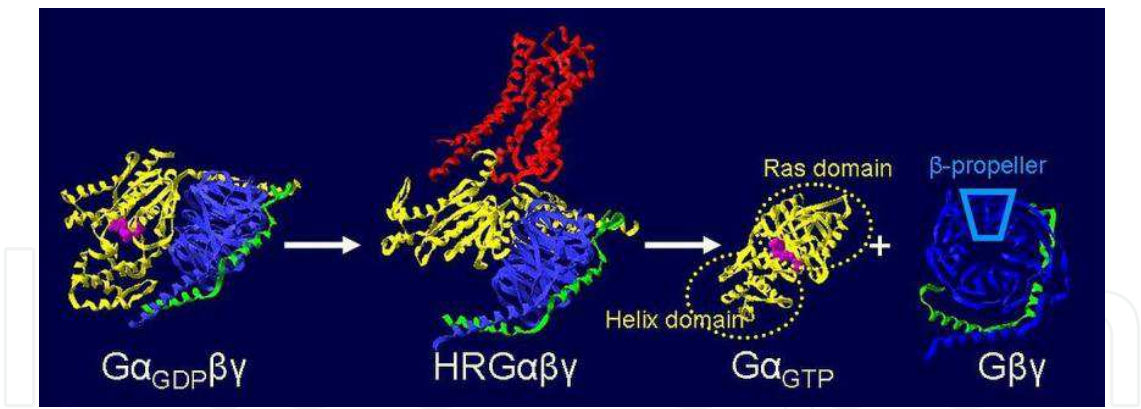
Fig. 3. Ribbon representations of G protein structures. The Gα subunits are presented in yellow, the β subunits in blue and the γ subunits in green and the β2-adrenergic receptor, in red. GDP and GTP are shown in fuchsia as space-filling structures. From left to right: the G_i protein (1GG2), the β2 adrenergic receptor-agonist-$G_s$complex (3SN6), the activated Gα$_{GTP}$ (1GIL) and Gβ1γ2 (1TBG) structures. The latter two structures have been rotated separately by approximately 90° compared to Gα$_{GDP}$βγ, to show the different domains (center right: the ras-like and α-helical domains of the Gα subunit; far right: a β-propeller structure in the Gβ subunit).
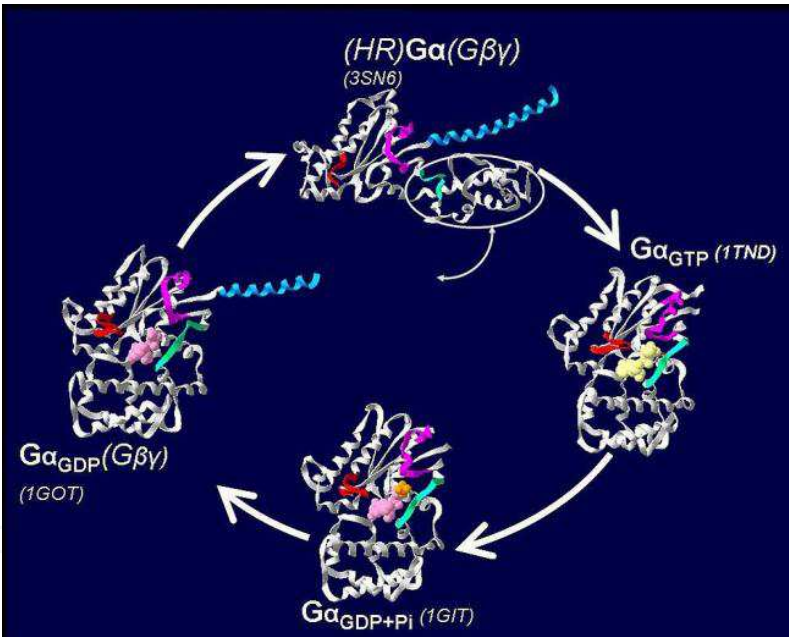

Fig. 4. Effect of guanyl nucleotide binding on the Gα subunit conformation. Ribbon representation of Gα in different crystallized complexes : the Gα subunit only is shown for simplicity; the Gβγ subunit when present would be in front and to the right of the Gα subunit. The N-terminal region, when structurally defined (stabilized by interactions with Gβγ) is represented by a blue ribbon. Three regions change conformation during the GTPase catalytic cycle: "switch 1" is shown in green, "switch 2" in gold, and "switch 3" in red. GDP (pink), GTP (yellow) and phosphate (pink) are shown as space filling structures. Left: the Gα$_t$ subunit in the Gα$_{GDP}$βγ complex (PDB 1GOT); top center: the structure of Gα$_S$ in the ternary complex, HRG (PDB 3SN6), stabilized by a nanobody; right: the structure of the GTP analogue GTPγS-activated Gα$_t$ (PDB 1TND) and bottom center: the structure of Gα$_i$ bound to GDP and inorganic phosphate during GTP hydrolysis (PDB 1GIT).
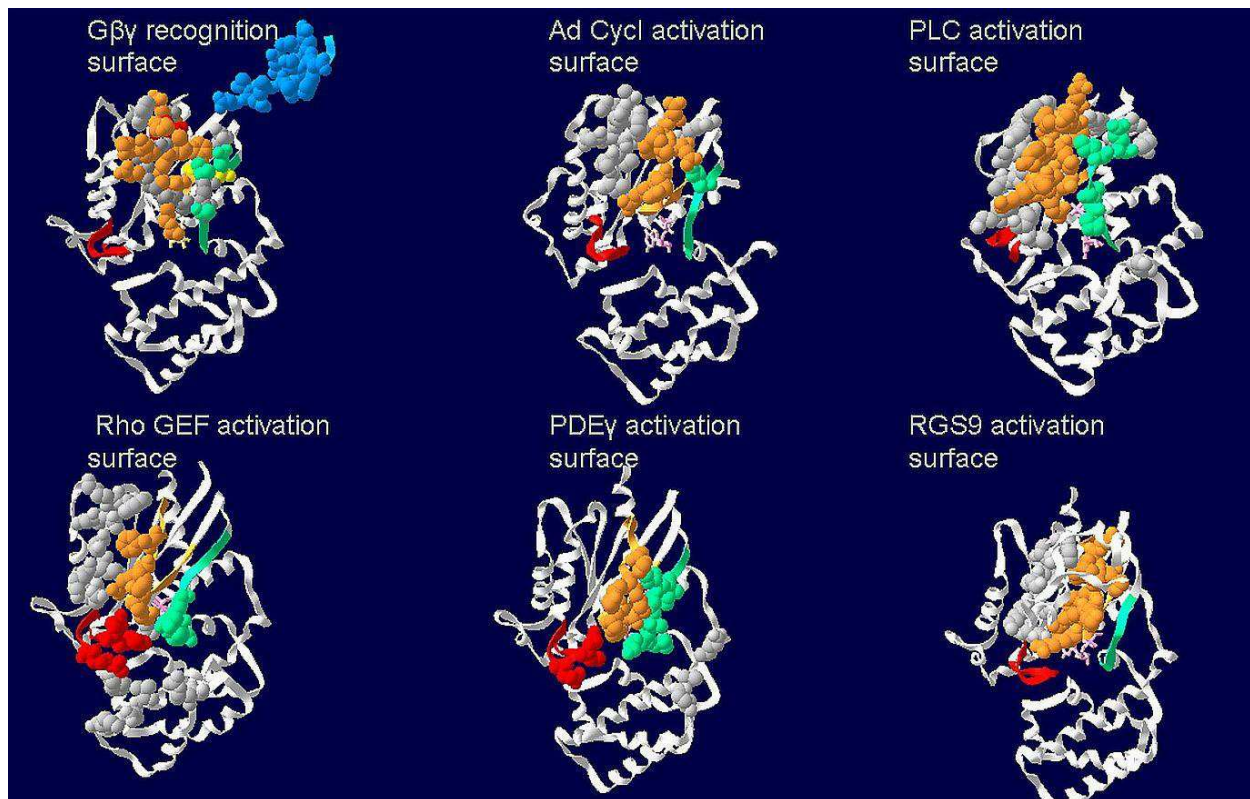
Fig. 5. Ribbon representation of Gα subunits in complex with Gβγ or with their effector and regulator proteins, showing the side chains that belong to the protein binding site. The Gα subunit only is shown for simplicity. The amino acids that belong to the protein binding sites of the different Gα structures are shown as space filling. When structurally defined, the ribbon (and side chains) that belong to the N-terminal helix are shown in blue, those that belong to switch 1 in green, to switch 2 in orange, to switch 3 in red, and to the rest of the protein in light grey. Top left: the GDP-bound transducin-G_i chimera showing the interaction surface with Gβγ (1GOT); top center: GTP*-bound $Gα_S$ showing the interaction surface with adenylate-cyclase (1CUL); top right: GTP-bound $Gα_q$ showing the interaction surface with phospholipase-C (3OHM); bottom left:GTP-bound $Gα_{13}$ showing the interaction surface with the Rho GEF, p115 (1SHZ); bottom center: GTP-bound transducin-G_i chimera showing the interaction surface with the phosphodiesterase inhibitor subunit, PDEγ (1FQJ) and bottom right, GTP-bound transducin-G_i chimera showing the interaction surface with RGS9 in a complex with PDEγ and RGS9 (1FQK).

Gβγ is also capable of activating certain effectors. Its binding site for Gα overlaps in part the Gβγ-effector and Gβγ- regulator binding sites: the dissociation of $Gα_{GTP}$ from Gβγ is essential to allow Gβγ to recognize its effectors (Figure 6).

GTP hydrolysis, rapidly followed by the release of the phosphate ion, modifies the switch regions conformation (Figure 4). The conformation change does not only inhibit the Gα-effector interaction (Figure 5) but also favors Gβγ recognition by $Gα_{GDP}$, thereby also inactivating Gβγ (Figure 6). Agonist-bound receptors interact with both G protein subunits (Figure 3): the formation of the "ternary complex" is an essential step for G protein activation, but the ternary complex Gα subunit is not in the right conformation to activate G protein effectors (Figure 3).
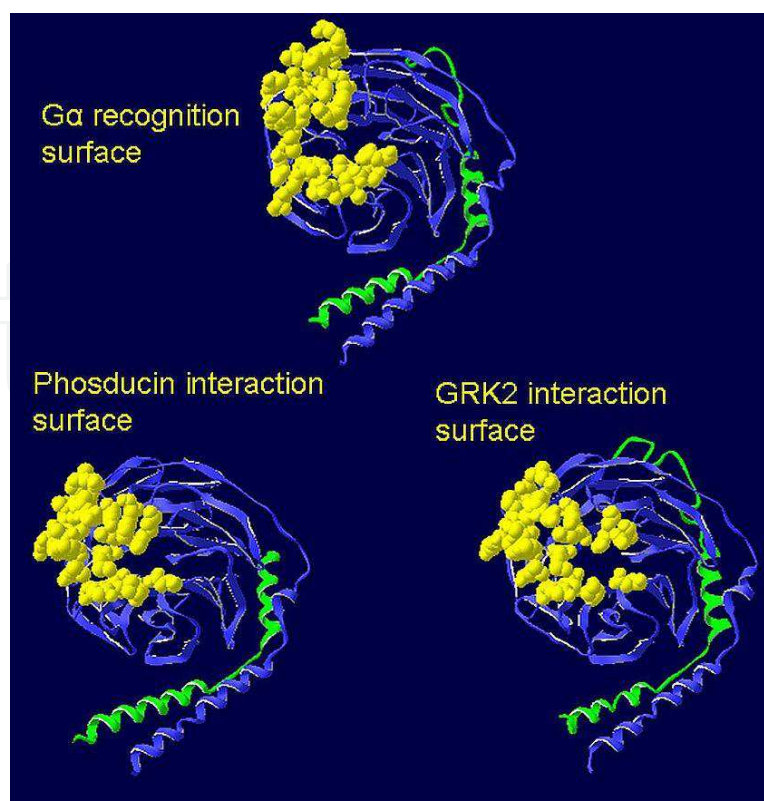
Fig. 6. Ribbon representation of Gβγ showing the interaction surface with Gα or with effectors. Ribbon representation of Gβγ in different crystallized complexes – the Gβ (blue) and Gγ (green) subunits only are shown for simplicity. The amino acids that belong to the Gα recognition surface (PDB ref 2BCG: top), to the phosducin binding site (1GP2: bottom left) or to the kinase, GRK2 binding surface (bottom right : 1OMW) are shown as space filling structures, in yellow.

4. G protein activation kinetics

Rhodopsin and related receptors **catalyze** G protein activation (Hamm, 1998): this means that each receptor sequentially activates several G proteins by facilitating the release of GDP, thereby allowing GTP binding. Rhodopsin does not enter in one of the Enzyme Commission "E.C." subclasses, as it does not catalyze the rupture or formation of covalent bond(s). The equations describing the reaction kinetics are nevertheless identical to those describing "ping pong" (double displacement) enzyme reaction (Waelbroeck *et al.*, 1997; Heck and Hofmann, 2001; Ernst *et al.*, 2007): G protein binding (substrate 1) is followed by GDP release (product 1), and GTP binding (substrate 2) is followed by the release of the activated G protein (product 2).

The kinetics of transducin activation by rhodopsin have been analyzed in detail (Heck and Hofmann, 2001; Ernst *et al.*, 2007). Rhodopsin recognizes transiently the GDP-bound trimeric G protein, transducin, and activates transducin at the diffusion limit (Ernst *et al.*, 2007). The physiological concentrations of the two "substrates" (GDP-bound transducin and GTP) are close to their respective Michaelis constants, K_M. In the case of double displacement reactions, it is unfortunately impossible to the individual rate constants of each reaction from the kinetic data (K_M, V_{max}). The [Substrate]/K_M ratios at physiological concentrations

nevertheless strongly suggest that the concentrations of the four reaction intermediates, Rh*, Rh*-G_{GDP}, Rh*-G and Rh*-G_{GTP} (where Rh* is the light activated rhodopsin) are similar (Roberts and Waelbroeck, 2004): none of the reaction intermediates accumulates. These characteristics are reminiscent of the properties of triose phosphate isomerase and other "kinetically perfect enzymes" (Albery and Knowles, 1976): all the reaction intermediates have very similar free energies at physiological substrate concentrations and the energy barriers separating the different enzyme states are very low, thereby allowing the reaction to proceed at the diffusion limit.

Enzymes accelerate reactions by stabilizing the "transition state", that is, the state with the highest energy along the reaction coordinates. Trimeric G proteins cannot be purified in the absence of guanyl nucleotides: they are unstable when empty. As explained below, agonists stabilize the agonist-receptor-G protein ternary complex (that includes an empty G protein): like enzymes, active GPCRs catalyze G protein activation by decreasing the free energy of the transition state, that is, the empty G protein (Waelbroeck, 1999). GTP recognition by the G protein destabilizes the ternary complex: this induces activated G protein release - and allows the catalytic activation of several G proteins by a single receptor.

5. Ligand binding studies and the ternary complex model

Ligands that induce G protein activation are termed "agonists", and ligands that do not affect the receptor activity, "antagonists". Even at 100% receptor occupancy, some agonists have a larger effect than others on G protein activation: the more effective agonists are called "full agonists" and the less efficient compounds, "partial agonists". More recently, it has been demonstrated that most GPCRs have the ability to activate (inefficiently) their cognate G proteins in the absence of any ligand: this is called "constitutive activity". Compounds that counteract the receptors' constitutive activity are called "inverse agonists".

Agonist binding to GPCRs in the absence of either GDP or GTP facilitates the formation of the ternary complex involving the receptor, an agonist, and an empty G protein (Figure 3) (Lefkowitz *et al.*, 1976; De Lean *et al.*, 1980; Rasmussen *et al.*, 2011b). This is evident from the formation of a high molecular weight "ternary complex" (ligand-receptor-G protein, LRG) with a much higher affinity for agonists compared to isolated receptors. Guanyl nucleotides (GTP, GTP analogues or GDP) destabilize the G protein interaction with agonist-bound receptors by markedly decreasing the G-protein affinity for the receptor. When recognizing the high affinity ternary complex, guanyl nucleotides dramatically increase the agonists' dissociation rate, and decrease the receptor affinity for agonists while increasing their affinity for inverse agonists (see for instance (Lefkowitz *et al.*, 1976; Berrie *et al.*, 1979)); GDP is typically needed in larger concentrations than GTP or GTP analogues.

The effect of GTP on ligand binding can be used as a measure of the relative stability of the ternary complex compared to the binary ligand-receptor complex (Lefkowitz *et al.*, 1976). Full agonists have a higher affinity in the absence of GTP and inverse agonists have a higher affinity in its presence: the effect of GTP on ligand recognition is correlated with the ligands' ability to induce or inhibit G protein activation by the receptor (Lefkowitz *et al.*, 1976; De Lean *et al.*, 1980).

The original ternary complex model (Figure 7: top left) (Lefkowitz *et al.*, 1976) was designed to describe ligand binding to GPCRs. It describes the allosteric interactions between the

ligand (L) and the G protein (G) recognizing different binding sites on the same receptor (R). Guanyl nucleotides were assumed to "prevent" G protein interaction with the receptor. The ternary complex model was later completed to the cubic ternary complex model (Figure 7: bottom right) (Weiss *et al.*, 1996a; Weiss *et al.*, 1996c; Weiss *et al.*, 1996b). Two receptor conformations (R and R*) without and with the ability to activate G proteins respectively are assumed to coexist at equilibrium (R⟷R*) in the absence and presence of ligands or G proteins. Agonists and G proteins favor the active (R*) receptor conformation while inverse agonists stabilize the inactive (R) conformation. As in the ternary complex model, the cubic model describes the binding (as opposed to functional) properties of GPCRs; guanyl nucleotides are assumed to "prevent" the receptor-G protein interaction.
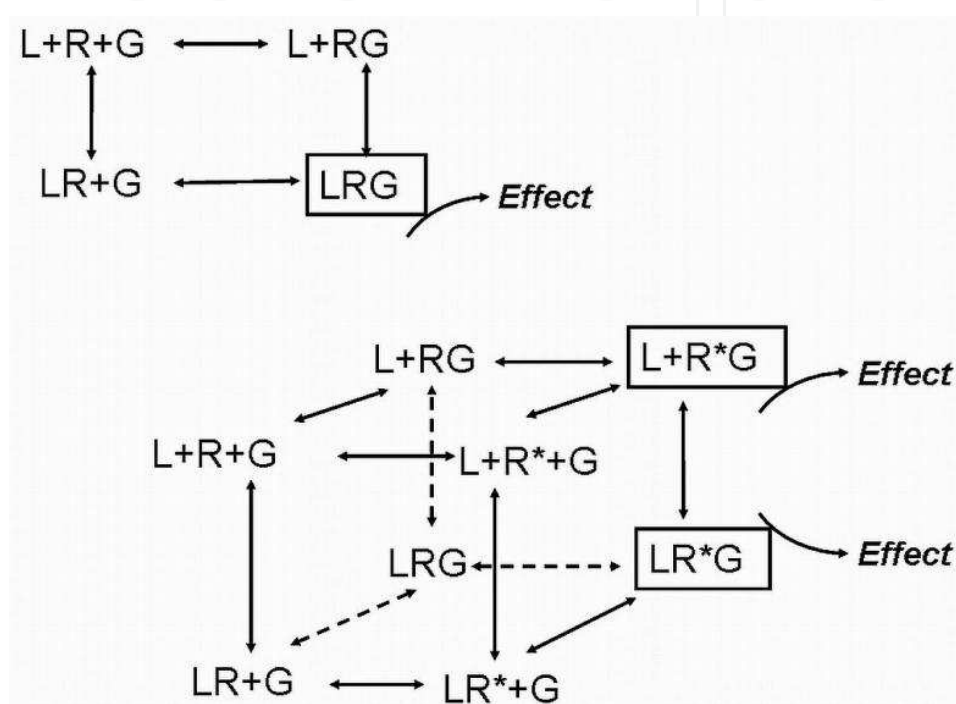

Fig. 7. The ternary complex model. Top left: the ternary complex model assumes that the receptor (R) can interact simultaneously with a ligand (L) and the G protein (G). Agonists facilitate and antagonists inhibit the receptor-G protein interaction; the ternary complex (LRG) is somehow responsible for transduction of the effect. Bottom right: the "cubic ternary complex model" assumes that the receptor can be found in a resting (R) or in an active (R*) conformation. G proteins (G) and agonist ligands stabilize R* while inverse agonists stabilize the R conformation. R*G and LR*G complexes are responsible for the biological effects of the active receptor.

Both ternary complex models have had a tremendous impact on our vision of GPCR function: the agonist-receptor-G protein complex is more and more often considered as "**the** active receptor". It should be remembered, however, that the ternary complex is certainly not "biologically active": it accumulates only under conditions where the G protein is unable to activate its' effectors (in the absence of GTP), and the G protein conformation in the β-adrenergic receptor-G protein complex (Figure 3) is not compatible with G_S-adenylate cyclase interaction (Figure 5)! The ternary complex model was designed to describe ligand binding to the receptors, as opposed to effectors activation.

6. "Resting" and "active" receptor structures

When rhodopsin is illuminated, its conformation passes through a number of intermediates (bathorhodopsin ($t_{1/2}$ 50ns), lumirhodopsin ($t_{1/2}$ 50μs), followed by metarhodopsin I and II) before releasing the all-trans retinal. Metarhodopsin II is biologically active: it catalyses G protein (transducin) activation.

The three dimensional structure of rhodopsin and several "family A" GPCRs has been elucidated by X-ray crystallography in the absence and presence of antagonists, agonists, G protein surrogates, or of the trimeric-G_s protein (Choe *et al.*, 2011; Lebon *et al.*, 2011; Rasmussen *et al.*, 2011a; Rasmussen *et al.*, 2011b; Rosenbaum *et al.*, 2011; Standfuss *et al.*, 2011; Warne *et al.*, 2011; Xu *et al.*, 2011). A conserved ionic bond between rhodopsin arginine 135 ($R^{3.50}$) (in the conserved E/DRY motif at the intracellular end of TM3) and glutamate 247 ($E^{6.30}$), at the end of the third intracellular loop-TM6 junction tethers rhodopsin TM3 to TM6. In antagonist-bound β2-adrenergic receptors this hydrogen bond between the conserved arginine and glutamate is not visible in the crystal and β2-adrenergic receptors are known to activate slightly their cognate G_S G protein even in the absence of agonist; the ionic bridge is less stable than in rhodopsin (Moukhametzianov *et al.*, 2011).
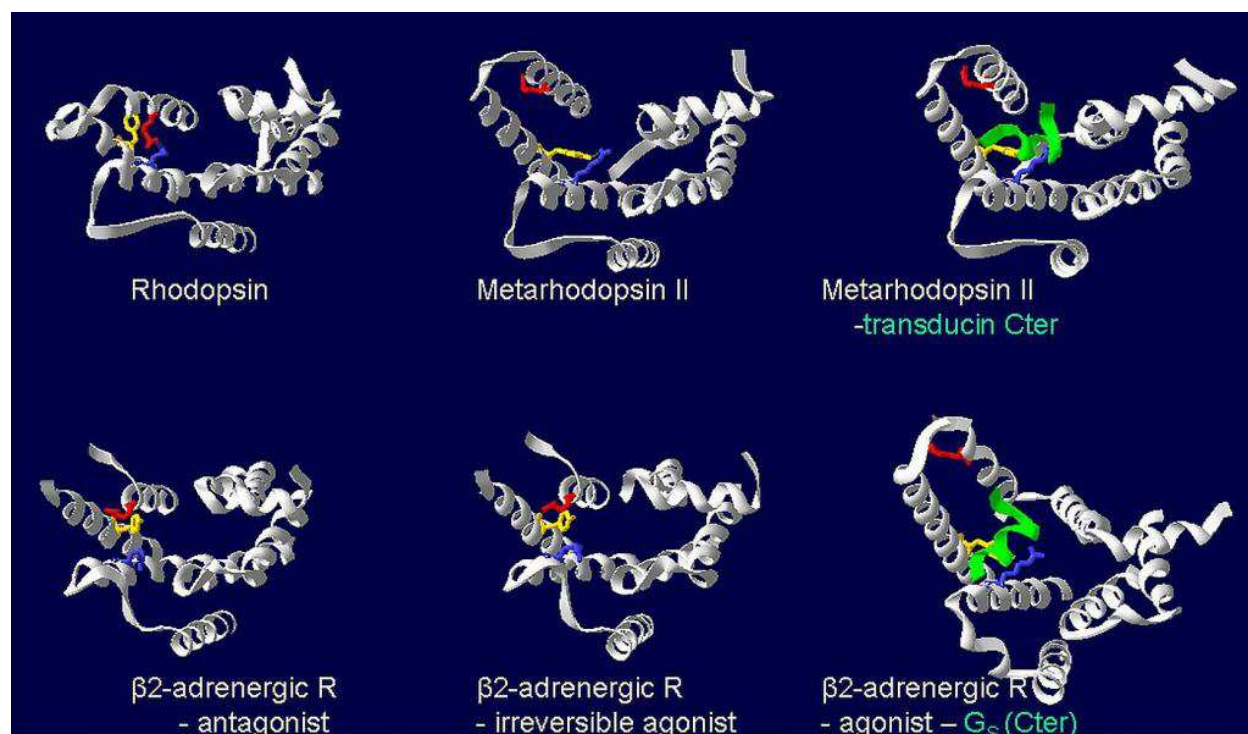

Fig. 8. Resting and activated GPCR conformations. Ribbon representation of the IC and TM regions of rhodopsin (1GZM), metarhodopsin II (3PXO), metarhodopsin II – Gt C-term complex (3PQR), antagonist-bound β2-adrenergic receptor (2RH1), agonist-bound β2-adrenergic receptor (3PDS) and agonist-G_S (Cterm) bound β2-adrenergic receptor (3SN6), seen from the cytosol. The conserved TM3 arginine ($R^{3.50}$), TM5 tyrosine ($Y^{5.58}$) and TM6 glutamate ($E^{6.30}$) side chains are shown in red, yellow and blue respectively. The C-terminal transducin peptide (top right) and the C-terminal region of Gα$_S$ (bottom right) are shown as green ribbons.

In contrast with (dark adapted) rhodopsin, a large intracellular binding pocket is present between the TM helices of metarhodopsin II (Choe *et al.*, 2011): the distance between the conserved arginine ($R^{3.50}$) at the intracellular end of TM3 (E/DRY motif) and the conserved glutamate at the junction between the third intracellular loop and TM6 ($E^{6.30}$) increases from less than 3.3Å in rhodopsin (PDB 1GZM), bathorhodopsin (PDB 2G87) and lumirhodopsin (PDB 2HPY) to >15 Å in opsin (PDB 3CAP, 3DBQ) and metarhodopsin II (PDB 3PQR, 3PXO) (Figure 8). Arginine $R^{3.50}$ forms in opsin and metarhodopsin II a strong ion-dipole interaction with the conserved tyrosine, $Y^{5.58}$ in TM5. The G-protein binding pocket is created by the rotation of TM 5 and 6. It is large enough to accommodate the C-terminal helix of the transducin Gα subunit or of $Gα_S$, at almost 40° from the membrane surface (Park *et al.*, 2008; Scheerer *et al.*, 2008; Choe *et al.*, 2011) (Figure 8): this movement forces the opening of the GDP binding pocket and release GDP from the G protein (Rasmussen *et al.*, 2011b).

The sixth transmembrane helix (TM6) of the crystallized β1-, β2-adrenergic and adenosine A_{2A} receptors remains very close to TM3 even in the presence of agonists (Rasmussen *et al.*, 2011a; Rosenbaum *et al.*, 2011; Warne *et al.*, 2011); and the conserved E/DRY motif arginine folds towards the cytoplasm, in the direction of the conserved TM6 glutamate: the G protein binding pocket is unavailable (Figure 8). An open, "metarhodopsin II-like" structure is achieved by β2-adrenergic receptors only in the presence of a G protein surrogate or of G_S (Rasmussen *et al.*, 2011a; Rosenbaum *et al.*, 2011; Rasmussen *et al.*, 2011b): TM5 and TM6 rotate away from TM3, and the arginine side chain $R^{3.50}$ toggles away from the IC loop $E^{6.30}$ towards the conserved tyrosine, $Y^{5.38}$.

6.1 Is the "active receptor" mobile or rigid?

It is well known that crystallization rigidifies proteins and can lead to selection of an unusual conformation stabilized by "within the crystal" (non physiological) protein-protein interactions. For instance, opsin and metarhodopsin II conserve the same "opened" conformation in the crystal in the absence and presence of a transducin surrogate (Figure 8) (Altenbach *et al.*, 2008). Nevertheless, opsin – in contrast to metarhopsin II – has a very low ability to activate transducin: it is thus clear that crystallization of opsin "selected" a protein conformation with a low probability in intact membranes, stabilized through its contacts with other opsin molecules in the crystal.

X-ray diffraction studies have a tremendous impact on our perception of protein structure: they enhance the impression that proteins are rigid molecules with a well defined, stable conformation. In addition, the activity of most allosteric enzymes can explained in terms of two conformations with very different enzyme activities, stabilized by allosteric enhancers and inhibitors, respectively (Monod *et al.*, 1965). This is not an absolute rule, however: some enzymes change markedly in conformation upon substrate binding and dissociation – a phenomenon known as "induced fit" (Ma and Nussinov, 2010) and this can play an important role in enzyme regulation (Heredia *et al.*, 2006; Molnes *et al.*, 2011).

As suggested above, the ternary complex model had a tremendous impact on the way we understand GPCR activation, to the extent that the agonist-receptor-(empty) G protein complex is now described as "the" (one and only?) active receptor conformation – despite the fact that the Gα subunit conformation in the ternary complex (Figure 3) is not compatible with effectors activation. This interpretation was further supported by the initial computational mapping of conformational energy landscape of the β2-adrenergic receptor:

preliminary results (Bhattacharya and Vaidehi, 2010) indeed suggested that full agonists-bound receptors switch spontaneously to a more stable, active conformation very similar to metarhodopsin II. Detailed computational mapping in the presence of water and lipid molecules of rhodopsin (Provasi and Filizola, 2010) and of the agonist-bound β2-adrenergic receptor (Niesen *et al.*, 2011) however indicate that both proteins are flexible and able to sample a large number of conformations. In the case of the β2-adrenergic receptor, reversible "shearing" movements of TM5-6 relative to TM 1-4 and 7 and "breathing" movements (opening and closing of the ligand binding pocket) have been predicted.

In the case of traditional receptors (including the β2-adrenergic receptor), GTP has a tremendous effect on agonists' recognition: agonists have a significantly lower affinity and much greater dissociation rate in the presence of GTP. This suggests that the predominant receptor conformation under "functional" conditions (in the presence of GTP) is different from the ternary complex conformation (that accumulates in the absence of GTP). I should like to suggest that most GPCRs are able to recruit G proteins while in the "closed" (low agonist affinity) conformation (Hu *et al.*, 2010), open a G protein binding pocket and force GDP release to achieve the "high affinity" (ternary complex) conformation, then return to the "closed" conformation upon GTP recognition and activated G protein release. Agonists do not only facilitate the transition between the "closed" and "opened" conformations described by X-ray diffraction, but also decrease the free energy difference between the ternary complex and uncoupled receptors, thereby stabilizing the empty G protein conformation and facilitating GDP release, GTP binding. G protein dissociation from the receptor is then necessary to complete G protein activation.

6.2 Partial GPCR activation: Agonist efficacy

Some compounds seem less efficient than others to activate G protein coupled receptors: the rate of G protein activation by agonist-bound receptors varies depending on the ligand. Two explanations are usually put forward to account for this very common observation: partial agonists might stabilize the same "active" receptor conformation as full agonists but to a lesser extent; alternatively, they might stabilize an alternative receptor conformation, not quite as appropriate as the conformation induced by full agonists for G protein activation. These two explanations are non-exclusive and both explanations might in fact be correct at least where β2-adrenergic agonists are concerned (Bhattacharya and Vaidehi, 2010). Indeed, while dopamine was predicted to stabilize (less efficiently) the same "opened" receptor conformation as norepinephrine, salbutamol was predicted to stabilize a slightly different, less opened, receptor conformation. Yet a third explanation has been suggested for muscarinic receptors: agonists dissociate from muscarinic receptors with a rate constant comparable to the G protein exchange reaction rate. The efficacy of agonists activating M_3 muscarinic receptors was correlated with their dissociation rate constant, suggesting that the G protein activation reaction can be aborted prematurely if the agonist dissociates too early in the reaction cycle (Sykes *et al.*, 2009).

7. Do GPCRs function as monomers or dimers?

$GABA_B$ receptors (a "family C" GPCR) function as obligate dimers (Jones *et al.*, 1998; White *et al.*, 2002). One of the two subunits is trapped intracellularly by an endoplasmic reticulum retention signal; the second forms non-functional homodimers. Upon coexpression,

formation of a heterodimer is driven by dimerization of the N-terminal region, and by formation of a coiled coil by the C-terminal regions α-helices. This masks the E.R. retention signal of the first subunit, and allows the expression of the functional heterodimer at the plasma membrane, (Jones *et al.*, 1998; White *et al.*, 2002). Likewise, all other family C receptors form heterodimers.

Rhodopsin, the best known "family A" GPCR, forms quasi crystalline arrays in rod cells disk membranes: this led to the suggestion that not only family C receptors but all GPCRs might function as homo- or heterodimers. Non-radiative energy transfer between two fluorophores ("FRET") or from a luminescent protein to a fluorophore ("BRET") can be easily demonstrated if the "donor" and "acceptor" molecules are close enough (typically less than 50 Å from each other). Chimeric constructs including "donor" and "acceptor" proteins (luciferase, fluorescent proteins from jellyfish, etc.) and the protein of interest can be built by molecular biology techniques; alternatively, the donor and acceptor fluorophores can be tagged chemically to the protein of interest, or to an antibody raised against this protein. BRET and FRET have been used to demonstrate not only protein-protein interaction, but also conformational changes of a single protein (by tagging for instance the N- and C-terminal of the protein of interest). In analogy with "family C" receptors, the vast majority of family A and several family B receptors have been shown by BRET or FRET experiments to either dimerize or oligomerize. This idea raised a lot of interest, because the potential consequences of dimerization are so multiple and important (Milligan, 2009; Milligan, 2010; Birdsall, 2010):

- Dimerization is essential for "family C" receptor expression at the plasma membrane (Temussi, 2009)) and might play a role in several other systems;
- Dimerization affects the "pharmacology" of some receptors. For instance: the sweet taste is sensed by a T1R2-T1R3 heterodimer, while "umami" is detected by a T1R1-T1R3 heterodimer (Temussi, 2009) (NB. The bitter taste is sensed by a non-family C GPCR, the T2R receptor; and salt and acid are recognized by "ligand gated channels" receptors (Temussi, 2009)). Likewise, dimerization of some GPCRs (i.e. dopamine, opiate or taste receptors) has been shown to alter their pharmacological properties - suggesting that their interaction is stable enough to affect the receptor conformation (Milligan, 2010; Milligan, 2009). Negative cooperativity has been observed between agonists binding to TSH and chemokine receptor dimers (Springael *et al.*, 2005; Urizar *et al.*, 2005)): binding of one agonist ligand to the dimer decreased the affinity of the second agonist by increasing its dissociation rate.
- Dimerization may have important functional consequences: a single agonist is sufficient for activation of G_S by the TSH receptor, but (low affinity) double occupancy of the dimer is necessary to support the activation of $G_{q/11}$ proteins and of phospholipase C (see below).
- Most if not all GPCRs do not only interact with G proteins, but also with other associated proteins, often in an agonist-modulated manner (Magalhaes *et al.*, 2011). Two receptors rather than one might be necessary to form optimal interactions with the receptor-associated proteins; alternatively, interaction of one subunit in the dimer with an associated protein might hinder or prevent the recognition of the second receptor subunit by steric hindrance, leading to "half of the sites reactivity".
- Several of the receptor-associated proteins act as scaffolds, recruiting in their turn other proteins in the vicinity of the receptor and of each other (Magalhaes *et al.*, 2011).

Dimerization of the receptors might be necessary to bring together some of the different accessory proteins recruited by each monomer.

It is unfortunately necessary to reassess the presence and consequences of dimerization for each receptor of interest: no generalization can be made in this respect. Indeed:

- Monomeric rhodopsin and β-adrenergic receptors, isolated and reconstituted in high density lipoprotein particles, function normally (Whorton *et al.*, 2007; Whorton *et al.*, 2008); and the receptor associated proteins arrestins, like GRKs, are able to recognize one receptor per protein (Hanson *et al.*, 2007; Bayburt *et al.*, 2011).
- Muscarinic M1 receptors dimerize only transiently: monomeric and dimeric forms are present at comparable concentrations in the plasma membrane at equilibrium and dimers dissociate rapidly ((Hern et al., 2010); see also (Johnston et al., 2011)). Cross-talk between two or more receptors is likely to necessitate strong interactions between the different monomers.
- Muscarinic M3 receptors (McMillin *et al.*, 2011) (and perhaps other receptors: (Johnston *et al.*, 2011)) are able to use several dimerization interfaces: this might explain why so many different dimerization interfaces have been observed when studying different receptors. This does not support the hypothesis that proteins like arrestin, G proteins or receptor kinases **need** a dimer for receptor recognition: the relative position of the two receptor monomers would be very important in that case.

8. Receptor promiscuity and biased signaling

GPCR "promiscuity" is defined as the ability of a given receptor to activate several different effectors (for review: (Hermans, 2003). While most receptors can probably induce parallel signaling by the $G\alpha_{GTP}$ and $G\beta\gamma$ subunits (see above), some are capable of activating different G proteins; and some use both G protein dependent and G protein independent signaling pathways.

"Biased signaling" refers to the observation that when receptors two or more signaling pathways, a few agonists preferentially use only one of the signaling pathways available to the other agonists – an observation that suggests that the activated receptor takes different conformations, depending on the agonist occupying its binding site.

8.1 Activation of several G protein subtypes by the same receptors

Each cell expresses several G proteins, belonging or not to the same family: all these G proteins will compete for recognition of each activated GPCR. Most G_i-coupled receptors activate several G_i isoforms with variable efficiency; some G_i and G_S coupled receptors activate in addition $G_{q/11}$ G proteins - less efficiently, or only at much higher agonist concentrations... Does this reflect a lower (but measurable) affinity of the non-cognate G protein, or less efficient activation?

GPCRs catalyze G protein activation: they should be considered like honorary enzymes. If several substrates compete for transformation by the same enzyme, the proportion of substrates transformed by the enzyme per minute, at steady state, is proportional to their relative substrate concentration over specificity constant ratios, $[S]/K_S$:

$$\frac{v^A}{v^B} = \frac{[A]/K_S^A}{[B]/K_S^B} \qquad (1)$$

(where A and B represent the two substrates (G proteins), respectively, and K_S^A and K_S^B are

their respective specificity constants : $K_S = \dfrac{k_{cat}}{K_M}$).

The equation is extremely similar to the equation describing the competition of several ligands for the same receptor: the proportion of receptor occupied by each ligand ([RA] and [RB]) is proportional to their relative ligand concentration over dissociation constant ratios:

$$\frac{[RA]}{[RB]} = \frac{[A]/K_D^A}{[B]/K_D^B} \quad (2)$$

The meaning of "K_D" and "K_S" is however very different: the dissociation constant, K_D = $1/K_{affinity}$, is a concentration. It measures the ligand concentration necessary to occupy, at equilibrium and in the absence of competitors, 50% of the receptors. The specificity constant K_S, in contrast is a bimolecular reaction rate constant and measured in $M^{-1}sec^{-1}$. It measures the rate of formation of the "productive complex", ES† in the absence of alternative substrates of inhibitors.

Multiple G protein signaling has more often been observed in transfected systems, where it depends on the receptor expression level (for review: (Hermans, 2003)). Transiently expressed α2 adrenergic receptors inhibit adenylate cyclase at low agonist concentrations but activate the enzyme at high agonist concentrations (Fraser et al., 1989). Adenylate cyclase inhibition but not activation is prevented by Gi protein inactivation by pertussis toxin (Fraser et al., 1989): these results indicate that α2 adrenergic receptors are capable of activating both Gi and GS. The equations above predict that the relative activation rate of "Gi" and "GS" is proportional to their relative concentrations. Activation of GS by α2 adrenergic receptors is observed only at very high agonist concentrations: this suggests that, at very high agonist concentrations, Gi becomes unable to compete for receptor activation: in contrast with Gi-GDP, the activated Gi-GTP complex is probably unable to recognize agonist-bound α2 adrenergic receptors (Waelbroeck, 2001).

A few GPCRs are capable of activating several G proteins in physiological settings: the G protein specificity is not always "absolute". Although this is unusual in Family A, some G protein coupled receptors can be expressed as related isoforms due to alternative splicing of RNA expressed from a single gene or to RNA editing: this may lead to receptor isoforms with different abilities to activate G proteins (Hermans, 2003; Bresson-Bepoldin et al., 1998). Alternatively, post-translational modifications such as phosphorylation of the receptor may alter its G protein specificity: β2-adrenergic receptors activate G_S proteins, leading to adenylate cyclase and protein kinase A stimulation, then – after phosphorylation by protein kinase A – activate G_i proteins (Zamah et al., 2002). The TSH receptor is able to activate G proteins from all four families (Allgeier et al., 1997; Laugwitz et al., 1996). Its binding properties are compatible with the hypothesis that it forms a stable dimer, and that occupancy of the dimer by one TSH molecule decreases the affinity of the second binding

site ("negative cooperativity" (Urizar *et al.*, 2005)). While signaling through G_S is induced at very low TSH concentrations, low affinity occupancy of two binding sites per dimer appears to be necessary to drive receptor activation of G_i (Allen *et al.*, 2011).

8.2 GPCR phosphorylation and desensitization

Activated rhodopsin (metarhodopsin II) activates the rhodopsin kinase (GRK1), which in turn phosphorylates preferentially the activated rhodopsin (Premont and Gainetdinov, 2007). Both activation to metarhodopsin II and phosphorylation synergistically increase the rhodopsin affinity for an adaptor protein, arrestin. This protein competitively inhibits transducin recognition by steric hindrance – resulting in rhodopsin desensitization: light activated rhodopsin becomes unable to activate transducin and signaling is "arrested".

Likewise, ligand-activated GPCRs recognize and activate "GRKs" (G protein coupled Receptor Kinases), that in turn preferentially phosphorylate activated GPCRs (Premont and Gainetdinov, 2007; Huang and Tesmer, 2011). Most GPCRs are, in addition, targets for "second messenger activated kinases": they possess consensus sequences for protein kinase A that is activated in response to the increased cAMP, or for protein kinase C, activated by the phospholipase C signaling pathway (cytosolic Ca2+ and diacylglycerol). Receptor phosphorylation by these kinases will lead to "heterodesensitization", since a given agonist can induce the desensitization of receptors it does not activate.

Mammalian cells express seven GRKs: two of them (GRK1 and 7) are found only in rod and cone cells in the retina; GRK4 is found mainly in the testes and to a lesser extent in some brain regions and in the kidney, and the last four (GRK2, 3, 5 and 6) are ubiquitous (Yang and Xia, 2006). They can be subdivided in three subgroups: GRK1 and 7; GRK2 and 3, and GRK 4, 5 and 6. The C-terminal region of GRK2 and 3 is longer than in other GRKs and possesses a "Pleckstrin Homology" (PH) domain: these two GRKs are cytosolic and recruited by Gβγ in response to G protein activation (Yang and Xia, 2006). In contrast, GRK 1 and 7 are C-terminal farnesylated, GRK4 and 6 are palmitoylated on C-terminal cysteines and GRK4-6 have a highly conserved binding site for phosphatidyl inositol 4-phosphate: the PH domain and post-translational modifications facilitate the permanent localization of these GRKs at the plasma membrane (Yang and Xia, 2006). The N-terminal region of all GRKs is similar and important for receptor recognition – GRKs are highly specific in their receptor preference (Yang and Xia, 2006).

Three dimensional structures for at least one representative of the three GRK families have been determined by X-ray diffraction (Figure 9). In most structures, the N-terminal region (that is essential for receptor recognition) is undefined, and the active cleft is too "open" for substrate recognition, suggesting that the kinases usually crystallize in the resting conformation. Very recently, GRK6 was crystallized in a form very likely resembling its' active conformation, with a relatively "closed" active cleft (Figure 10): this structure probably resembles the active GRK (Boguth et al., 2010). It is characterized by a well defined extended N-terminal α-helix, that could easily be fitted – superimposed on the G protein C-terminal α-helix (Boguth et al., 2010) - in the intracellular pocket formed in the metarhodopsin II structure (Figure 10). This would bring the active cleft in close proximity to the receptors IC3 and Cter – the two regions that are phosphorylated by GRKs.
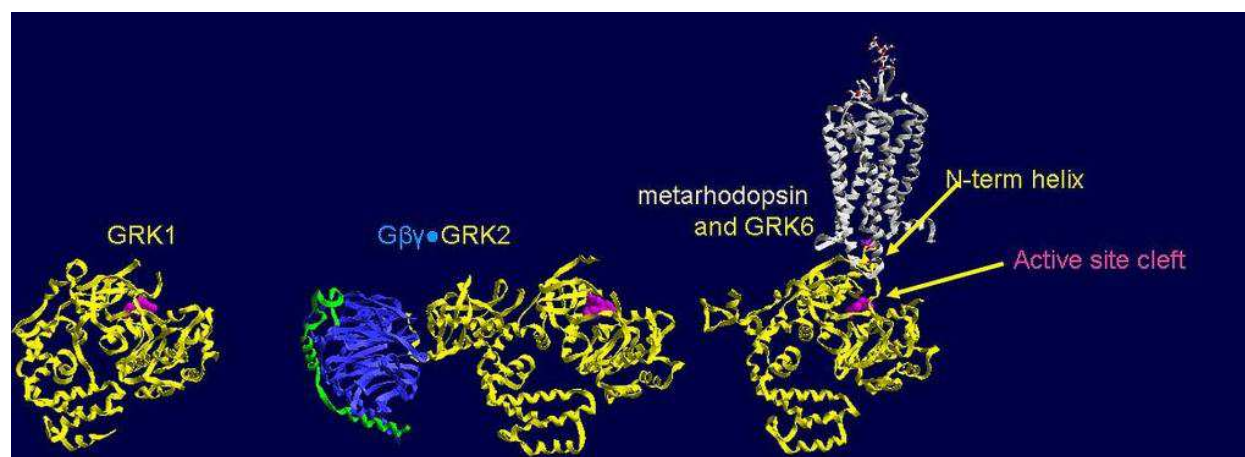
Fig. 9. Representative X-ray structures of GRKs from the three families. Ribbon structure of GRK1 (left: 3C4W), of the GRK2-Gβγ complex (center: 3KRW), and of the presumed "active conformation" of GRK6 (right: 3DQB) superimposed on the transducin C-term peptide in close apposition with the metarhodopsin II structure (3NYN), to form a hypothetical active GRK-receptor complex (according to (Boguth *et al.*, 2010)). The GRKs are shown by a yellow ribbon, co-crystallized ATP or ATP analogues in pink to identify the active site; Gβ and Gγ is shown in blue and green, respectively, and metarhodopsin II, in light grey.

Most GRKs are probably able to regulate GPCR signal transduction by phosphorylation-independent mechanisms. All GRKs have a "Regulator of G protein Signaling (RGS) homology" (RH) domain, and GRK2 and 3 have been shown to specifically interact through this domain with $G\alpha_q$ family members, thereby blocking their interaction with their effector, phospholipase C (see Figure 5). At least some GRKs are able to compete with G protein recognition by the activated receptor and/or compete with effector proteins for Gβγ recognition (Yang and Xia, 2006). By phosphorylating the receptor, they also increase markedly the receptor affinity for "arrestin" molecules that compete with G proteins for receptor recognition, facilitate receptor internalization in endosomes, and may serve as "scaffold", allowing "G protein independent signaling" (see below) (Premont and Gainetdinov, 2007; Huang and Tesmer, 2011).

Since GRKs and G proteins compete for the same (active) receptor conformation, the sequence of receptor recognition is important: GPCRs should recognize first the G proteins, then GRKs. "Sequential" recognition of two ligands is easily explained under the assumption that they have different *dissociation* rate constants (Motulsky and Mahan, 1984): the ligand with the faster dissociation rate constant will occupy the receptor rapidly, then progressively give place to the ligand with the slower dissociation rate constant (see Figure 10).

The most important factor under non equilibrium conditions is the relative **dissociation** (not association) rate constant of the two ligands. This might seem counterintuitive, but can easily be explained. Let us first examine the case of two ligands with different affinities due to different association rate constants. The lower affinity ligand will be needed in larger concentrations to significantly occupy the receptors at equilibrium: its lower association rate constant is then automatically compensated by the larger ligand concentration used. (The association rate is equal to $k_{on}[L]$, where k_{on} is the association rate constant and [L], the

ligand concentration). In contrast, if the two ligands have different affinities because of different dissociation rate constant: the larger dissociation rate constant of the low affinity ligand cannot be compensated by the larger ligand concentrations used to occupy the receptor at equilibrium: the dissociation rate, $k_{off}[LR]$ does not depend on the free ligand concentration. In order for the G protein, GRKs (and arrestin) to recognize sequentially the receptors, it is therefore necessary and sufficient that they have a different dissociation rate constants from the receptor. This is not a problem, as a very rapid G protein dissociation from the receptor is also necessary to allow receptor recycling and efficient catalytic activation of the G proteins...
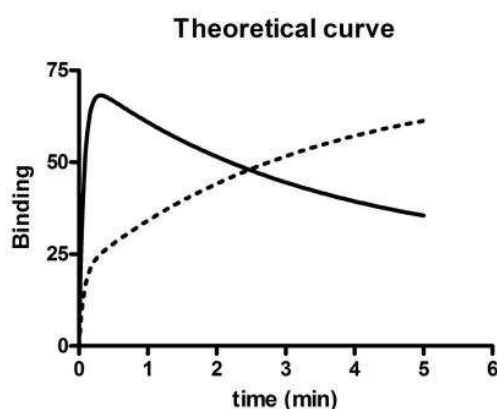

Fig. 10. Competitive binding of two ligands to the same receptor as a function of time. Ligand A (full line) has a $k_{on}=10^8 M^{-1} min^{-1}$, $k_{off} = 1$ min^{-1} and is present at a concentration of 100 nM (10 K_D); it will occupy 24% of the receptors at equilibrium in the presence of ligand B. Ligand B (hatched line), has the same $k_{on} = 10^8 min^{-1} M^{-1}$, a lower dissociation rate ($k_{off} = .0.1$ min^{-1}) and is present at a concentration of 30 nM (30 K_D): it will occupy 73% of the receptors at equilibrium, in the presence of ligand A.

8.3 Arrestin recognition by GPCRs

All mammalian cells express at least one of the four "arrestins": rod and cone cells from the visual system express arrestins 1 and 4, respectively; arrestins 2 and 3 (also known as β-arrestin 1 and 2) are ubiquitously expressed. These proteins recognize and are activated by multi-phosphorylated, activated GPCRs: arrestin (arrestin 1) is specific for rhodopsin, arrestin 4, for the iodopsins, and arrestins 2 and 3 recognize most if not all GPCRs. Phosphorylation and receptor activation synergistically enhance rhodopsin-arrestin interactions: light activated rhodopsin and resting but phosphorylated rhodopsin have a 10-100 fold lower affinity for arrestin, and rhodopsin does not detectably interact with arrestin 1. β-arrestin binding to "traditional" GPCRs is affected more by phosphorylation than by agonist binding (Gurevich *et al.*, 1995).

All known arrestin 3D structures are rather similar to visual arrestin (Figure 11). They can be subdivided into two concave β-sheet domains held together by a hinge region, an ionic bridge network between two arginine and three aspartate side chains (center of the structure on Figure 11), and by interactions between the C-term tail, the first N-term β strand and the α helix (left of Figure 11).
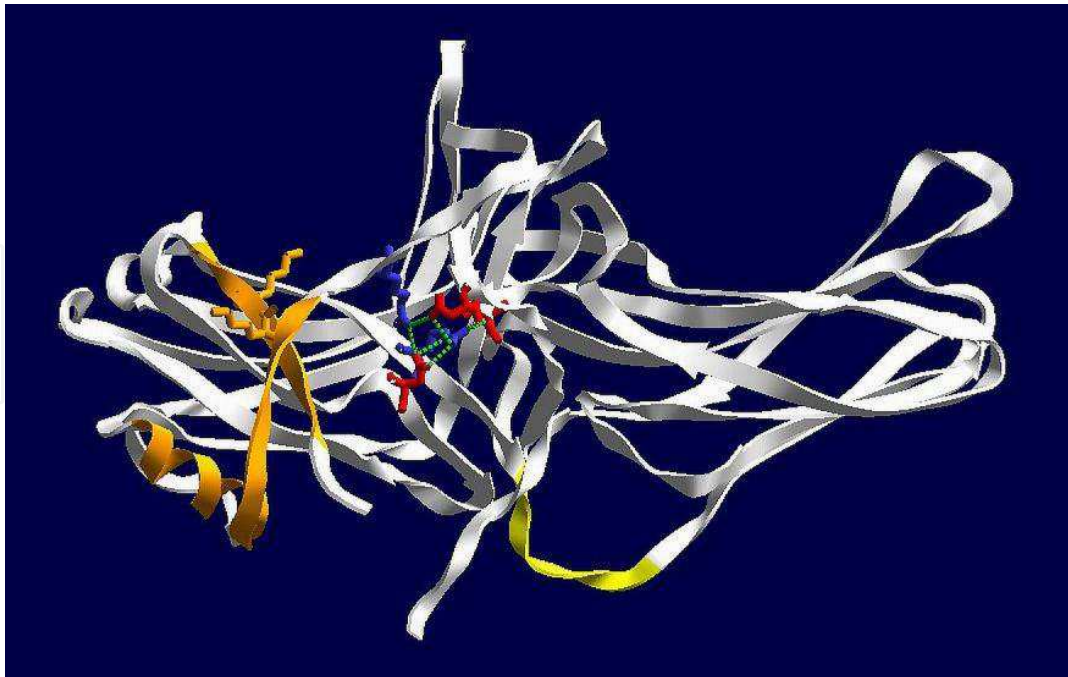
Fig. 11. The visual arrestin 1 crystal structure 1CF1. The N-terminal lobe is shown on the left, and the C-terminal lobe on the right. They are joined by a single random coil stretch (yellow) but held together through ionic interactions between buried arginine (blue) and aspartate (red) side chains, and by close contacts of the first N-terminal β-sheet stretch and α-helix with a C-terminal β-sheet stretch (orange). The two lysine side chains that are important for preferential phosphorylated>non-phosphorylated (light activated) rhodopsin recognition are shown in orange (left).

Each of the two arrestin domains is large enough to interact with a rhodopsin monomer (Figure 12). Even though visual arrestin forms a one to one complex with rhodopsin both in vitro (Bayburt *et al.*, 2011) and in vivo (Hanson *et al.*, 2007), several side chains covering both domains are implicated in rhodopsin recognition or rhodopsin – GPCR discrimination (Bayburt *et al.*, 2011; Vishnivetskiy *et al.*, 2011; Skegro *et al.*, 2007) (Figure 12). This indicates that arrestin undergoes a significant conformation change when it recognizes the phosphorylated receptors. This is confirmed by the observation that the arrestin sensitivity to proteolytic degradation increases upon GPCR recognition, and that the intramolecular BRET between the N- and C-terminal region of a luciferase–arrestin–Yellow Fluorescent Protein (YFP) construct is markedly affected by arrestin recognition of agonist-bound receptors (Shukla *et al.*, 2008).

At least two rhodopsin Ser/Thr must be phosphorylated to allow arrestin interaction with metarhodopsin; three phosphates support stronger arrestin binding, and heavier phosphorylation promotes arrestin binding , in addition, to neighbouring dark (inactive) rhodopsin and to phospho-opsin, two unpreferred rhodopsin forms (Vishnivetskiy *et al.*, 2007). Likewise, β-arrestin recognition increases mainly in response to multi-phosphorylation of the GPCRs C-terminal or IC3 sequence rather than in response to agonist binding (Gurevich *et al.*, 1995; Oakley *et al.*, 2000). "Phosphoserine/phosphothreonine rich" patches are necessary for stable, high affinity arrestin recognition (Oakley *et al.*, 2001). GPCRs that present patches of phosphorylated Ser/Thr residues (angiotensin II type 1A,

neurotensin 1, vasopressin V2, thyrotropin-releasing hormone and substance P receptors) have a high affinity and do not discriminate the arrestin 1, 2 and 3 isoforms; they are rapidly internalized and recycle inefficiently or not at all. In contrast, β_2-adrenergic, μ opioid, endothelin type A, dopamine D_{1A}, and α_{1b} adrenergic receptors (with separate phosphorylated Ser/Thr residues) have a low affinity for β-arrestin 2 (arrestin 3), an even lower affinity for β-arrestin 1 (arrestin 2) and do not detectably recruit arrestin 1. Upon internalization, these receptors are rapidly dephosphorylated and recycled to the plasma membrane (Oakley *et al.*, 2000).
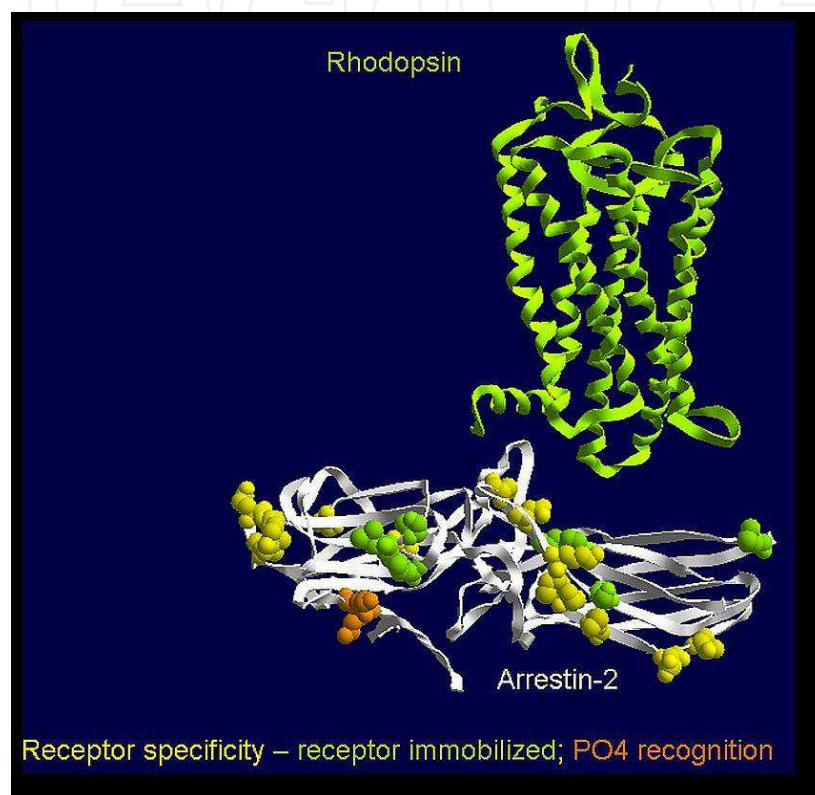

Fig. 12. Arrestin 3 (β-arrestin 2), close to opsin structure. Grey ribbon: arrestin 3 ribbon structure structure (1JSY), showing some of the side chains that are immobilized upon dark phosphorhodopsin recognition (light green), involved in the discrimination of light activated phosphorhodopsin from carbachol-activated phosphorylated M2 muscarinic receptor (yellow) or necessary for recognition of the phosphoserine/threonines (tan) (Vishnivetskiy *et al.*, 2011). Green ribbon: opsin structure 3CAP is shown for size comparison.

Arrestins change conformation upon receptor recognition (Shukla *et al.*, 2008) and behave as receptor-dependent "scaffold proteins" bringing together a number of other proteins (for review: (Premont and Gainetdinov, 2007; DeFea, 2011)). Some of their binding sites are shown in Figure 13.

Several β-arrestin scaffolds have been identified: this protein can recruit either MAP kinase partners, PI3Kinase or Akt, phosphodiesterase of actin assembly proteins scaffolds when bound to activated, phosphorylated receptors (DeFea, 2011). The different binding sites are very close (Figure 13): only some well-defined complexes can be formed or dissociated in

response to agonist-receptor recognition by arrestin. The factors determining which complex is formed in response to a given receptor are still elusive.
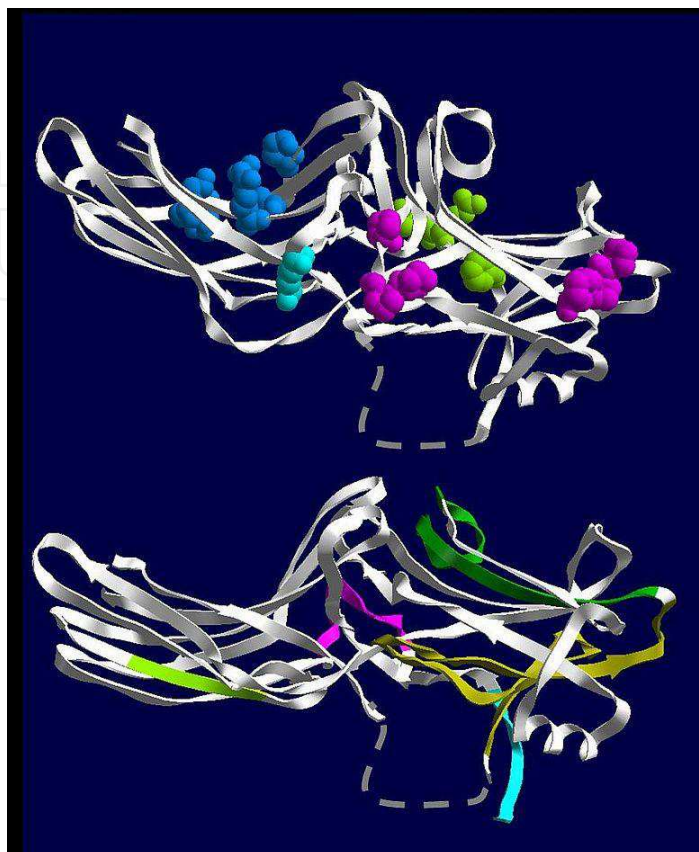

Fig. 13. Scaffolding sites on arrestin. Top: 3 arrestin 3 structure 3P2D The Proline-rich regions that allow SH3 domains recognition are shown in fuchsia, the PIP$_2$ binding site, in blue and side chains essential for β-adaptin binding, in green. The clathrin recognition site LφxφE is in the hatched (unstructured) region. R307, that is essential for cRaf1 recognition, is shown in light blue. Bottom : arrestin 2 structure (1JSY) showing the partially overlapping Ask1 and MEK binding regions (kaki), the MKK4 (dark green), PDED5 (light green), ERK2 (fuchsia) binding sites, and the partially overlapping Akt and clathrin binding sites (light blue) (according to (DeFea, 2011)).

9. References

Albery WJ and Knowles JR (1976) Free-Energy Profile of the Reaction Catalyzed by Triosephosphate Isomerase. *Biochemistry* 15: 5627-5631.

Allen MD, Neumann S and Gershengorn MC (2011) Occupancy of Both Sites on the Thyrotropin (TSH) Receptor Dimer Is Necessary for Phosphoinositide Signaling. *FASEB J.*

Allgeier A, Laugwitz KL, Van Sande J, Schultz G and Dumont JE (1997) Multiple G-Protein Coupling of the Dog Thyrotropin Receptor. *Mol Cell Endocrinol* 127: 81-90.

Altenbach C, Kusnetzow AK, Ernst OP, Hofmann KP and Hubbell WL (2008) High-Resolution Distance Mapping in Rhodopsin Reveals the Pattern of Helix Movement Due to Activation. *Proc Natl Acad Sci U S A* 105: 7439-7444.

Bayburt TH, Vishnivetskiy SA, McLean MA, Morizumi T, Huang CC, Tesmer JJ, Ernst OP, Sligar SG and Gurevich VV (2011) Monomeric Rhodopsin Is Sufficient for Normal Rhodopsin Kinase (GRK1) Phosphorylation and Arrestin-1 Binding. *J Biol Chem* 286: 1420-1428.

Berrie CP, Birdsall NJ, Burgen AS and Hulme EC (1979) Guanine Nucleotides Modulate Muscarinic Receptor Binding in the Heart. *Biochem Biophys Res Commun* 87: 1000-1005.

Bhattacharya S and Vaidehi N (2010) Computational Mapping of the Conformational Transitions in Agonist Selective Pathways of a G-Protein Coupled Receptor. *J Am Chem Soc* 132: 5205-5214.

Birdsall NJ (2010) Class A GPCR Heterodimers: Evidence From Binding Studies. *Trends Pharmacol Sci* 31: 499-508.

Birnbaumer L (2007) Expansion of Signal Transduction by G Proteins. The Second 15 Years or So: From 3 to 16 Alpha Subunits Plus Betagamma Dimers. *Biochim Biophys Acta* 1768: 772-793.

Boguth CA, Singh P, Huang CC and Tesmer JJ (2010) Molecular Basis for Activation of G Protein-Coupled Receptor Kinases. *EMBO J* 29: 3249-3259.

Bresson-Bepoldin L, Jacquot MC, Schlegel W and Rawlings SR (1998) Multiple Splice Variants of the Pituitary Adenylate Cyclase-Activating Polypeptide Type 1 Receptor Detected by RT-PCR in Single Rat Pituitary Cells. *J Mol Endocrinol* 21: 109-120.

Choe HW, Kim YJ, Park JH, Morizumi T, Pai EF, Krauss N, Hofmann KP, Scheerer P and Ernst OP (2011) Crystal Structure of Metarhodopsin II. *Nature* 471: 651-655.

De Lean A, Stadel JM and Lefkowitz RJ (1980) A Ternary Complex Model Explains the Agonist-Specific Binding Properties of the Adenylate Cyclase-Coupled Beta-Adrenergic Receptor. *J Biol Chem* 255: 7108-7117.

DeFea KA (2011) Beta-Arrestins As Regulators of Signal Termination and Transduction: How Do They Determine What to Scaffold? *Cell Signal* 23: 621-629.

Ernst OP, Gramse V, Kolbe M, Hofmann KP and Heck M (2007) Monomeric G Protein-Coupled Receptor Rhodopsin in Solution Activates Its G Protein Transducin at the Diffusion Limit. *Proc Natl Acad Sci U S A* 104: 10859-10864.

Fraser CM, Arakawa S, McCombie WR and Venter JC (1989) Cloning, Sequence Analysis, and Permanent Expression of a Human Alpha 2-Adrenergic Receptor in Chinese Hamster Ovary Cells. Evidence for Independent Pathways of Receptor Coupling to Adenylate Cyclase Attenuation and Activation. *J Biol Chem* 264: 11754-11761.

Fredriksson R, Lagerstrom MC, Lundin LG and Schioth HB (2003) The G-Protein-Coupled Receptors in the Human Genome Form Five Main Families. Phylogenetic Analysis, Paralogon Groups, and Fingerprints. *Mol Pharmacol* 63: 1256-1272.

Gurevich VV, Dion SB, Onorato JJ, Ptasienski J, Kim CM, Sterne-Marr R, Hosey MM and Benovic JL (1995) Arrestin Interactions With G Protein-Coupled Receptors. Direct Binding Studies of Wild Type and Mutant Arrestins With Rhodopsin, Beta 2-Adrenergic, and M2 Muscarinic Cholinergic Receptors. *J Biol Chem* 270: 720-731.

Hamm HE (1998) The Many Faces of G Protein Signaling. *J Biol Chem* 273: 669-672.

Hanson SM, Gurevich EV, Vishnivetskiy SA, Ahmed MR, Song X and Gurevich VV (2007) Each Rhodopsin Molecule Binds Its Own Arrestin. *Proc Natl Acad Sci U S A* 104: 3125-3128.

Heck M and Hofmann KP (2001) Maximal Rate and Nucleotide Dependence of Rhodopsin-Catalyzed Transducin Activation: Initial Rate Analysis Based on a Double Displacement Mechanism. *J Biol Chem* 276: 10000-10009.

Heredia VV, Thomson J, Nettleton D and Sun S (2006) Glucose-Induced Conformational Changes in Glucokinase Mediate Allosteric Regulation: Transient Kinetic Analysis. *Biochemistry* 45: 7553-7562.

Hermans E (2003) Biochemical and Pharmacological Control of the Multiplicity of Coupling at G-Protein-Coupled Receptors. *Pharmacology & Therapeutics* 99: 25-44.

Hern JA, Baig AH, Mashanov GI, Birdsall B, Corrie JE, Lazareno S, Molloy JE and Birdsall NJ (2010) Formation and Dissociation of M1 Muscarinic Receptor Dimers Seen by Total Internal Reflection Fluorescence Imaging of Single Molecules. *Proc Natl Acad Sci U S A* 107: 2693-2698.

Hu J, Wang Y, Zhang X, Lloyd JR, Li JH, Karpiak J, Costanzi S and Wess J (2010) Structural Basis of G Protein-Coupled Receptor-G Protein Interactions. *Nat Chem Biol* 6: 541-548.

Huang CC and Tesmer JJ (2011) Recognition in the Face of Diversity: Interactions of Heterotrimeric G Proteins and G Protein-Coupled Receptor (GPCR) Kinases With Activated GPCRs. *J Biol Chem* 286: 7715-7721.

Jensen AA, Greenwood JR and Brauner-Osborne H (2002) The Dance of the Clams: Twists and Turns in the Family C GPCR Homodimer. *Trends Pharmacol Sci* 23: 491-493.

Jian X, Clark WA, Kowalak J, Markey SP, Simonds WF and Northup JK (2001) Gbetagamma Affinity for Bovine Rhodopsin Is Determined by the Carboxyl-Terminal Sequences of the Gamma Subunit. *J Biol Chem* 276: 48518-48525.

Johnston JM, Aburi M, Provasi D, Bortolato A, Urizar E, Lambert NA, Javitch JA and Filizola M (2011) Making Structural Sense of Dimerization Interfaces of Delta Opioid Receptor Homodimers. *Biochemistry* 50: 1682-1690.

Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, Tang C, Shen Q, Salon JA, Morse K, Laz T, Smith KE, Nagarathnam D, Noble SA, Branchek TA and Gerald C (1998) GABA(B) Receptors Function As a Heteromeric Assembly of the Subunits GABA(B)R1 and GABA(B)R2. *Nature* 396: 674-679.

Katadae M, Hagiwara K, Wada A, Ito M, Umeda M, Casey PJ and Fukada Y (2008) Interacting Targets of the Farnesyl of Transducin Gamma-Subunit. *Biochemistry* 47: 8424-8433.

Kisselev OG and Downs MA (2003) Rhodopsin Controls a Conformational Switch on the Transducin Gamma Subunit. *Structure* 11: 367-373.

Kolakowski LF, Jr. (1994) GCRDb: a G-Protein-Coupled Receptor Database. *Receptors Channels* 2: 1-7.

Kostenis E, Waelbroeck M and Milligan G (2005) Techniques: Promiscuous Galpha Proteins in Basic Research and Drug Discovery. *Trends Pharmacol Sci* 26: 595-602.

Laugwitz KL, Allgeier A, Offermanns S, Spicher K, Van Sande J, Dumont JE and Schultz G (1996) The Human Thyrotropin Receptor: a Heptahelical Receptor Capable of Stimulating Members of All Four G Protein Families. *Proc Natl Acad Sci U S A* 93: 116-120.

Lebon G, Warne T, Edwards PC, Bennett K, Langmead CJ, Leslie AG and Tate CG (2011) Agonist-Bound Adenosine A2A Receptor Structures Reveal Common Features of GPCR Activation. *Nature* 474: 521-525.

Lefkowitz RJ, Mullikin D and Caron MG (1976) Regulation of Beta-Adrenergic Receptors by Guanyl-5'-Yl Imidodiphosphate and Other Purine Nucleotides. *J Biol Chem* 251: 4686-4692.

Ma B and Nussinov R (2010) Enzyme Dynamics Point to Stepwise Conformational Selection in Catalysis. *Curr Opin Chem Biol* 14: 652-659.

Magalhaes AC, Dunn H and Ferguson SS (2011) Regulation of G Protein-Coupled Receptor Activity, Trafficking and Localization by GPCR-Interacting Proteins. *Br J Pharmacol*.

McMillin SM, Heusel M, Liu T, Costanzi S and Wess J (2011) Structural Basis of M3 Muscarinic Receptor Dimer/Oligomer Formation. *J Biol Chem* 286: 28584-28598.

Milligan G (2009) G Protein-Coupled Receptor Hetero-Dimerization: Contribution to Pharmacology and Function. *Br J Pharmacol* 158: 5-14.

Milligan G (2010) The Role of Dimerisation in the Cellular Trafficking of G-Protein-Coupled Receptors. *Curr Opin Pharmacol* 10: 23-29.

Mizuno N and Itoh H (2011) Signal Transduction Mediated Through Adhesion-GPCRs. *Adv Exp Med Biol* 706: 157-166.

Molnes J, Teigen K, Aukrust I, Bjorkhaug L, Sovik O, Flatmark T and Njolstad PR (2011) Binding of ATP at the Active Site of Human Pancreatic Glucokinase--Nucleotide-Induced Conformational Changes With Possible Implications for Its Kinetic Cooperativity. *FEBS J* 278: 2372-2386.

Monod J, Wyman J And Changeux JP (1965) On the Nature of Allosteric Transitions: a Plausible Model. *J Mol Biol* 12: 88-118.

Motulsky HJ and Mahan LC (1984) The Kinetics of Competitive Radioligand Binding Predicted by the Law of Mass Action. *Mol Pharmacol* 25: 1-9.

Moukhametzianov R, Warne T, Edwards PC, Serrano-Vega MJ, Leslie AG, Tate CG and Schertler GF (2011) Two Distinct Conformations of Helix 6 Observed in Antagonist-Bound Structures of a Beta1-Adrenergic Receptor. *Proc Natl Acad Sci U S A* 108: 8228-8232.

Niesen MJ, Bhattacharya S and Vaidehi N (2011) The Role of Conformational Ensembles in Ligand Recognition in G-Protein Coupled Receptors. *J Am Chem Soc*.

Oakley RH, Laporte SA, Holt JA, Barak LS and Caron MG (2001) Molecular Determinants Underlying the Formation of Stable Intracellular G Protein-Coupled Receptor-Beta-Arrestin Complexes After Receptor Endocytosis*. *J Biol Chem* 276: 19452-19460.

Oakley RH, Laporte SA, Holt JA, Caron MG and Barak LS (2000) Differential Affinities of Visual Arrestin, Beta Arrestin1, and Beta Arrestin2 for G Protein-Coupled

Receptors Delineate Two Major Classes of Receptors. *J Biol Chem* 275: 17201-17210.

Oldham WM and Hamm HE (2006) Structural Basis of Function in Heterotrimeric G Proteins. *Q Rev Biophys* 39: 117-166.

Park JH, Scheerer P, Hofmann KP, Choe HW and Ernst OP (2008) Crystal Structure of the Ligand-Free G-Protein-Coupled Receptor Opsin. *Nature* 454: 183-187.

Premont RT and Gainetdinov RR (2007) Physiological Roles of G Protein-Coupled Receptor Kinases and Arrestins. *Annu Rev Physiol* 69: 511-534.

Provasi D and Filizola M (2010) Putative Active States of a Prototypic G-Protein-Coupled Receptor From Biased Molecular Dynamics. *Biophys J* 98: 2347-2355.

Rasmussen SG, Choi HJ, Fung JJ, Pardon E, Casarosa P, Chae PS, Devree BT, Rosenbaum DM, Thian FS, Kobilka TS, Schnapp A, Konetzki I, Sunahara RK, Gellman SH, Pautsch A, Steyaert J, Weis WI and Kobilka BK (2011a) Structure of a Nanobody-Stabilized Active State of the Beta(2) Adrenoceptor. *Nature* 469: 175-180.

Rasmussen SG, Devree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK and Kobilka BK (2011b) Crystal Structure of the Beta(2) Adrenergic Receptor-Gs Protein Complex. *Nature*.

Roberts DJ and Waelbroeck M (2004) G Protein Activation by G Protein Coupled Receptors: Ternary Complex Formation or Catalyzed Reaction? *Biochem Pharmacol* 68: 799-806.

Rosenbaum DM, Zhang C, Lyons JA, Holl R, Aragao D, Arlow DH, Rasmussen SG, Choi HJ, Devree BT, Sunahara RK, Chae PS, Gellman SH, Dror RO, Shaw DE, Weis WI, Caffrey M, Gmeiner P and Kobilka BK (2011) Structure and Function of an Irreversible Agonist-Beta(2) Adrenoceptor Complex. *Nature* 469: 236-240.

Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, Hofmann KP and Ernst OP (2008) Crystal Structure of Opsin in Its G-Protein-Interacting Conformation. *Nature* 455: 497-502.

Shukla AK, Violin JD, Whalen EJ, Gesty-Palmer D, Shenoy SK and Lefkowitz RJ (2008) Distinct Conformational Changes in Beta-Arrestin Report Biased Agonism at Seven-Transmembrane Receptors. *Proc Natl Acad Sci U S A* 105: 9988-9993.

Skegro D, Pulvermuller A, Krafft B, Granzin J, Hofmann KP, Buldt G and Schlesinger R (2007) N-Terminal and C-Terminal Domains of Arrestin Both Contribute in Binding to Rhodopsin. *Photochem Photobiol* 83: 385-392.

Springael JY, Urizar E and Parmentier M (2005) Dimerization of Chemokine Receptors and Its Functional Consequences. *Cytokine Growth Factor Rev* 16: 611-623.

Standfuss J, Edwards PC, D'Antona A, Fransen M, Xie G, Oprian DD and Schertler GF (2011) The Structural Basis of Agonist-Induced Activation in Constitutively Active Rhodopsin. *Nature* 471: 656-660.

Sykes DA, Dowling MR and Charlton SJ (2009) Exploring the Mechanism of Agonist Efficacy: a Relationship Between Efficacy and Agonist Dissociation Rate at the Muscarinic M3 Receptor. *Mol Pharmacol* 76: 543-551.

Temussi PA (2009) Sweet, Bitter and Umami Receptors: a Complex Relationship. *Trends Biochem Sci* 34: 296-302.

Urizar E, Montanelli L, Loy T, Bonomi M, Swillens S, Gales C, Bouvier M, Smits G, Vassart G and Costagliola S (2005) Glycoprotein Hormone Receptors: Link Between Receptor Homodimerization and Negative Cooperativity. *EMBO J* 24: 1954-1964.

Vishnivetskiy SA, Gimenez LE, Francis DJ, Hanson SM, Hubbell WL, Klug CS and Gurevich VV (2011) Few Residues Within an Extensive Binding Interface Drive Receptor Interaction and Determine the Specificity of Arrestin Proteins. *J Biol Chem* 286: 24288-24299.

Vishnivetskiy SA, Raman D, Wei J, Kennedy MJ, Hurley JB and Gurevich VV (2007) Regulation of Arrestin Binding by Rhodopsin Phosphorylation Level. *J Biol Chem* 282: 32075-32083.

Waelbroeck M (1999) Kinetics Versus Equilibrium: the Importance of GTP in GPCR Activation. *Trends Pharmacol Sci* 20: 477-481.

Waelbroeck M (2001) Activation of Guanosine 5'-[Gamma-(35)S]Thio-Triphosphate Binding Through M(1) Muscarinic Receptors in Transfected Chinese Hamster Ovary Cell Membranes; 1. Mathematical Analysis of Catalytic G Protein Activation. *Mol Pharmacol* 59: 875-885.

Waelbroeck M, Boufrahi L and Swillens S (1997) Seven Helix Receptors Are Enzymes Catalysing G Protein Activation. What Is the Agonist Kact? *J Theor Biol* 187: 15-37.

Warne T, Moukhametzianov R, Baker JG, Nehme R, Edwards PC, Leslie AG, Schertler GF and Tate CG (2011) The Structural Basis for Agonist and Partial Agonist Action on a Beta(1)-Adrenergic Receptor. *Nature* 469: 241-244.

Weiss JM, Morgan PH, Lutz MW and Kenakin TP (1996a) The Cubic Ternary Complex Receptor-Occupancy Model. I. Model Description. *J Theor Biol* 178: 151-167.

Weiss JM, Morgan PH, Lutz MW and Kenakin TP (1996b) The Cubic Ternary Complex Receptor-Occupancy Model. II.Understanding Apparent Affinity. *J Theor Biol* 178: 169-182.

Weiss JM, Morgan PH, Lutz MW and Kenakin TP (1996c) The Cubic Ternary Complex Receptor-Occupancy Model. III. Resurrecting Efficacy. *J Theor Biol* 181: 381-397.

Wellendorph P and Brauner-Osborne H (2009) Molecular Basis for Amino Acid Sensing by Family C G-Protein-Coupled Receptors. *Br J Pharmacol* 156: 869-884.

White JH, Wise A and Marshall FH (2002) Heterodimerization of Gamma-Aminobutyric Acid B Receptor Subunits As Revealed by the Yeast Two-Hybrid System. *Methods* 27: 301-310.

Whorton MR, Bokoch MP, Rasmussen SG, Huang B, Zare RN, Kobilka B and Sunahara RK (2007) A Monomeric G Protein-Coupled Receptor Isolated in a High-Density Lipoprotein Particle Efficiently Activates Its G Protein. *Proc Natl Acad Sci U S A* 104: 7682-7687.

Whorton MR, Jastrzebska B, Park PS, Fotiadis D, Engel A, Palczewski K and Sunahara RK (2008) Efficient Coupling of Transducin to Monomeric Rhodopsin in a Phospholipid Bilayer. *J Biol Chem* 283: 4387-4394.

Xu F, Wu H, Katritch V, Han GW, Jacobson KA, Gao ZG, Cherezov V and Stevens RC (2011) Structure of an Agonist-Bound Human A2A Adenosine Receptor. *Science* 332: 322-327.

Yang W and Xia SH (2006) Mechanisms of Regulation and Function of G-Protein-Coupled Receptor Kinases. *World J Gastroenterol* 12: 7753-7757.

Zamah AM, Delahunty M, Luttrell LM and Lefkowitz RJ (2002) Protein Kinase A-Mediated Phosphorylation of the Beta 2-Adrenergic Receptor Regulates Its Coupling to Gs and Gi. Demonstration in a Reconstituted System. *J Biol Chem* 277: 31249-31256.
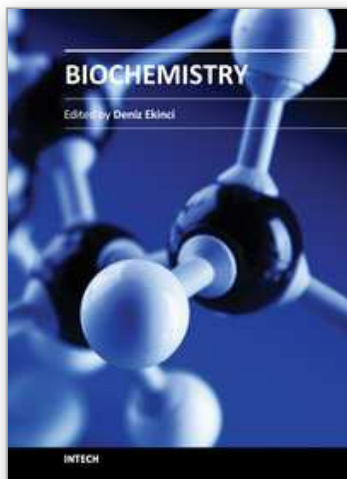
Biochemistry

Edited by Prof. Deniz Ekinci

Over the recent years, biochemistry has become responsible for explaining living processes such that many scientists in the life sciences from agronomy to medicine are engaged in biochemical research. This book contains an overview focusing on the research area of proteins, enzymes, cellular mechanisms and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in biochemistry. Particular emphasis is devoted to both theoretical and experimental aspect of modern biochemistry. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in biochemistry, molecular biology and associated areas. The book is written by international scientists with expertise in protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the book will enhance the knowledge of scientists in the complexities of some biochemical approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of biochemistry.

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