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# Conformational restriction of G-proteins Coupled Receptors (GPCRs) upon complexation to G-proteins: A putative activation mode of GPCRs?

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ABSTRACT

**GPCRs undergo large conformational changes during their activation. Starting from existing X-ray structures, we used Normal Modes Analyses to study the collective motions of the agonist-bound β2-adrenergic receptor both in its isolated "uncoupled" and G-protein "coupled" conformations. We interestingly observed that the receptor was able to adopt only one major motion in the protein:protein complex. This motion corresponded to an anti-symmetric rotation of both its extra- and intra-cellular parts, with a key role of previously identified highly conserved proline residues. Because this motion was also retrieved when performing NMA on 7 other GPCRs which structures were available, it is strongly suspected to possess a significant biological role, possibly being the "activation mode" of a GPCR when coupled to G-proteins.**


## 1. Introduction

G-protein Coupled Receptors (GPCRs) form a large family of proteins constituted by seven hydrophobic, trans-membrane helical segments (noted TM1 to TM7). Since 2007, many X-ray structures have been solved describing GPCRs in complex with their ligands and/or protein partners. Together, these X-ray structures concluded to a common highly conserved fold and binding crevice for these receptors, despite a low sequence conservation (~25% identity). More surprisingly, co-crystallized ligands of these receptors that include agonists, antagonists and inverse agonists all bind to the same orthosteric binding pocket, without any clear differences in their binding modes and in the resulting conformation of the receptor [1]. This is in contradiction with biophysical data that clearly indicate large conformational re-arrangements of these receptors that are directly dependent on the nature of the bound partner(s) [2]. Recently solved structures of the β2-adrenergic receptor have confirmed that conformational re-arrangements occur upon the complexation of this receptor to intra-cellular G-proteins [3]. The related conformational re-arrangement mostly include a spreading of the trans-membraneous (TM) helices 5 and 6 that permits to the G-protein C-ter helix to penetrate inside the receptor [4]. Because the putative mechanism of GPCRs activation has for a long time been primarily associated to a G-protein recruitment, these re-arrangements have been logically associated to the "inactive" and "active" states of GPCRs. However, these rather reflect a "coupling motion" of these receptors. In agreement, and because several recent studies have argued for a pre-coupling between GPCRs and G-proteins [2,5,6], one can ask what is the real activation mechanism/motion of a GPCR after its complexation to G-proteins.

The recently solved structure of the β2-adrenergic receptor complexed to both an agonist molecule and to the Gs heterotrimeric G-protein [3] therefore appears as a good starting material to decipher such an activation mechanism. Nevertheless, the activation mechanism of these large complexes still requires to be elucidated at the molecular scale. Because the activation mechanism of GPCRs involves different key steps that are thought to occur on highly diverse time-scales from the nanosecond to the millisecond [7] it cannot, or hardly, be addressed by experiments. This activation mechanism includes three main, consecutive steps: (i) ligand binding in the receptor that promotes stabilization of an active conformation [8], (ii) GDP:GTP exchange in the $G_\alpha$ subunit of the G-protein that is the rate limiting step [9]; and (iii) the subsequent dissociation of the G-protein into two membrane-anchored $G_\alpha$:GTP and $G_{\beta\gamma}$ subunits [10]. Both GDP release and dissociation

*Abbreviation:* MRMS, Mass-weighted Root Mean Square

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of the G-protein are known to be triggered by the presence of the receptor [11,12], even if these steps can occur in absence of any ligand in case of constitutive activity.

Molecular modeling appears to be a method of choice to study class A GPCRs as reviewed in recent published papers [13,14]. Moreover, because GPCR undergo large conformational changes, the Normal Modes Analysis (NMA), which is a good tool to predict collective motions, has been used to study GPCRs [15] and G-proteins [16] in their isolated conformations. We have shown that NMA was a powerful technique to study functional motions of these membrane proteins. Moreover, several studies proved that lowest frequencies normal modes are often related to protein functions and permit to study motions occurring on large timescales, as it is the case for GPCRs [17,18].

In this study, we first validated our NMA protocol on the isolated B2AR receptor. Then, we employed the same NMA technique to predict motions that could exist in the GPCR:G-protein complex.

## 2. Materials and methods

### 2.1. Building of initial models

Several isolated β-2 adrenergic receptor (B2AR) (Protein Data Bank (PDB) ids: 2RH1, [19] 3D4S, [20] 3NY8, [21] 3NY9, [21] 3NYA, [21] 3P0G) [22] and the B2AR:Gs-protein complex (PDB id: 3SN6) [3] solved by X-ray crystallography were subjected to Normal Mode Analyses (NMA). Seven other receptors were also tested including the Beta-1 (2VT4), [23] CXCR4 (3ODU), [24] Dopamine (3PBL), [25] Histamine (3RZE), [26] Adenosine (3EML), [27] Sphingosine (3V2W) [28] and Muscarinic (3UON) [29] receptors. The third intracellular loop of all receptors was in each case completed by 6 alanine residues, whereas the lacking N-ter and C-ter regions of receptors were not built to avoid unrealistic folding of loops. Co-crystallized ligands were included in calculations using the CHARMM General Forces Field (CGENFF) [30]. The crystallographic structure of the GPCR:G-protein complex was modified according to other available G-proteins structures. These modifications included the repositioning of the helical domain of $G_\alpha$ at proximity of the ras-like domain as described in the PDB id 1GP2 X-ray structure [31]. Indeed, this domain was rotated by about 180° in the initial X-ray structure, probably resulting from crystallographic artifacts including antibody co-crystallization. This reconstruction, more compatible with recently published data, [32] allowed to compare the Normal Modes (NMs) obtained for the complex to those computed for the isolated G-protein published elsewhere [16].

### 2.2. Generation of low-energy conformations along the normal modes vectors

Normal Mode Analyses (NMA) were performed with the CHARMM software [33] and the CHARMM27 [34] forces field, excluding CMAP [35] parameters. The energy of each initial structure was first minimized in vacuo by combining Steepest-Descent and Adopted Based Newton–Raphson algorithms to reach a low energy gradient of $10^{-5}$ kcal mol Å$^{-2}$. NMA were computed with the DIMB module as implemented in CHARMM [36]. The first 20 lowest frequencies NMs of each initial structure were then used as constraints to generate low-energy conformers along the normal mode directions. At this step, the CMAP corrections were turned on again. This method is described in detail and was validated on different biological systems elsewhere [16,37,38].

61 conformers were generated for each of the initial structures with Mass Weighted Root Mean Square (MRMS) ranging from −3 to +3 Å with a step of 0.1 Å. A negative value of MRMS applies to displacements along the negative direction of the vector (see Ref. [37] for a graphical representation of the MRMS). During the minimization protocol, the cut-off for non-bonded interactions was set to 10 Å, with a switching function applied between 8 and 10 Å. Minimization was performed in two successive stages. In a first stage a force constant of 1000 kcal mol$^{-1}$ Å$^{-2}$ was applied during 2000 and 10000 steps of SD and Conjugate Gradient algorithms, respectively. In a second stage, the force constant was increased to 20000 kcal mol$^{-1}$ Å$^{-2}$ for 5000 additional step of conjugate gradient to push the system exactly to the desired MRMS value along the vector. For both stages, the minimization process was stopped when the energy gradient get lower than $10^{-2}$ kcal mol$^{-1}$ Å$^{-2}$. Translational and rotational force constants were set, respectively to 1000 and $10^{-5}$ kcal mol$^{-1}$ Å$^{-2}$.

## 3. Results and discussion

### 3.1. Validation of the Normal Modes Analysis protocol on the isolated β-2 adrenergic receptor

Lowest frequencies Normal Modes (NMs) were computed for six different X-ray structures describing the isolated B2AR in the Protein Data Bank (PDB ids: 2RH1, 3D4S, 3NYA, 3NY8, 3NY9, 3P0G). This receptor can be considered as a perfect case study as it has been crystallized in both its uncoupled (2RH1, 3D4S, 3NYA, 3NY8, 3NY9) or coupled (3P0G) states with different effectors including agonist (3P0G), inverse agonists (2RH1, 3D4S, 3NY8 and 3NY9) or antagonist (3NYA). The "coupling motion" described by the transition between the 2RH1 and 3P0G structures was the unique significant collective motion that was observed among these structures. In previous published studies, this motion was associated to the activation of GPCRs. After computation of the Normal Modes of each of the upper mentioned ligand:receptor complexes, low-energy conformations were generated along each of their twenty lowest frequencies NMs. The resulting motions identified along these modes were then quantitatively compared to the "coupling motion" using the same method we described previously [16]. Briefly, this method consists to compute correlation coefficients between all pairs of generated motions, assuming that values greater than 0.5 (mean + 2 std) depict highly related motions in the Cartesian space. Interestingly, it was concluded that at least one of the computed lowest frequencies NMs of each B2AR structure was able to reproduce the 2RH1:3P0G conformational transition. To convince, a Root Mean Square Deviation computation is shown in Fig. SI1 and showed that the transition is effective. This analysis definitively validated our protocol and confirmed the ability of our approach in identifying GPCR realistic motions, including the "coupling motion" described by B2AR crystallographic structures.

### 3.2. The isolated receptor adopts a large set of different conformations...

A systematic pair by pair comparison of all the motions described by the twenty lowest frequencies NMs computed for the isolated B2AR (PDB id: 3P0G) was performed. Interestingly, using a correlation coefficient threshold of 0.5, we observed that the isolated receptor can adopt a large set of different motions (see Fig. 1A). Indeed, only three groups of related modes were formed by (i) the modes 11, 13, 14, 21, 23, 25; (ii) the modes 19, 20, 24 and (iii) the modes 17 and 18. All the other modes described unique motions with correlation coefficients <0.5 with any other mode. Highly similar results were obtained when performing the same analysis on motions computed for others B2AR X-ray structures, not only the 3P0G structure (data not shown).