Kutatási jelentés

Biokémiai vizsgálatok neurofarmakon hatóanyagokkal (BGP-15, Bisoprolol, Bimoclomol)

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Effect of BGP-15 and bimoclomol in the presence of bisoprolol on membranes and beta-adrenergic receptors

1. Introduction

1.1. β Adrenergic Receptor

G-protein-coupled receptors (GPCRs) are a very large protein family that share limited amino acid sequence homology and a similar protein topology: a N-terminal extracellular segment, seven hydrophobic membrane spanning α-helices and a C-terminal cytoplasmic region (Fig. 1). β-adrenergic receptors (β-AR), that respond to adrenergic sympathetic stimulation, mediate increased activity of the human heart, while decreased cardiac activity is mediated by the cholinergic parasympathetic system working through muscarinic acetylcholine GPCRs. There are two forms of the receptor: β1 and β2. The two proteins are rather similar in protein sequence, though the β1-AR is significantly larger due to a longer N-terminal extracellular region and a larger L3 cytoplasmic loop (Fig. 1). These β-AR interact with heterotrimeric guanine nucleotide binding regulatory proteins, usually known as G-proteins. G-proteins consist of an α, β and γ subunit, each represented by several different genes in humans [1].

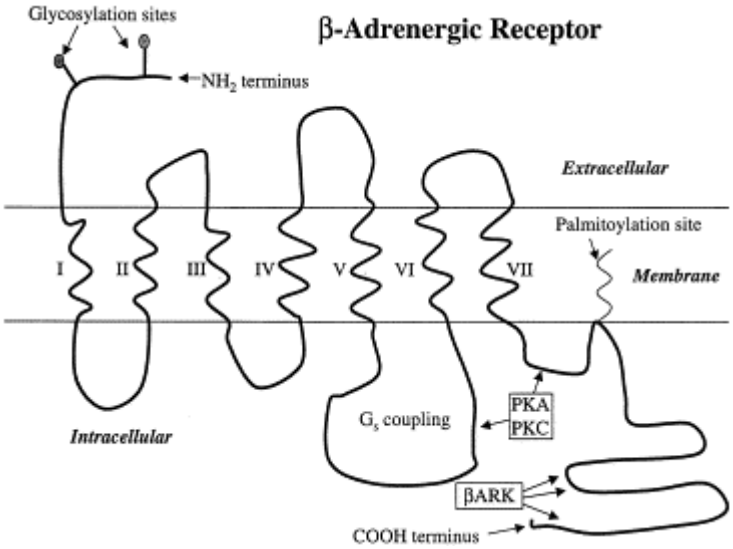

Figure 1 Diagram of the essential features of β -adrenergic receptors .

Protein G-coupled receptors exist in an active and inactive state. Ligands stabilize or induce different receptor states. What differs in the two stages is the conformation of a transmembrane helix (TM6), which opens to allow interaction, at a part of the receptor, with the G-Protein.

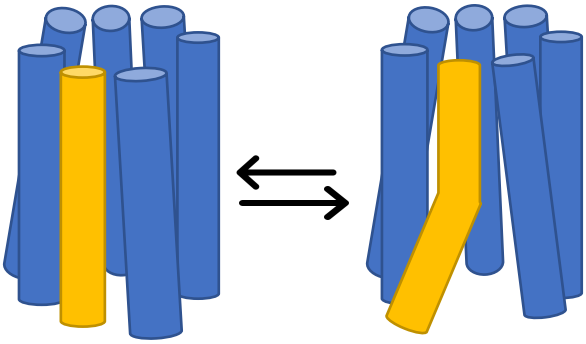

Figure 2 Open and closed ADRB2 receptor conformation. In yellow the change of conformational of TM6 after the activation of the receptor.

The β2 adrenergic receptor (β2AR) activation of Gs, the stimulatory G protein for adenylyl cyclase, has long been a model system for GPCR signaling. The principal interactions between the β2AR and Gs involve the amino- and carboxy-terminal α-helices of Gs, with conformational changes propagating to the nucleotide-binding pocket. The largest conformational changes in the β2AR include a 14 Å outward movement at the cytoplasmic end of transmembrane segment 6 (TM6) and an α-helical extension of the cytoplasmic end of TM5. Figure 2a compares the structures of the agonist-bound receptor in the β2AR–Gs complex and the inactive carazolol-bound β2AR. The largest difference between the inactive and active structures is a 14 Å outward movement of TM6 when measured at the Cα carbon of E268. There is a smaller outward movement and extension of the cytoplasmic end of the TM5 helix by 7 residues. A stretch of 26 amino acids in the third intracellular loop (ICL3) is disordered. Another notable difference between inactive and active structures is the second intracellular loop (ICL2), which forms an extended loop in the inactive β2AR structure and an α-helix in the β2AR–Gs complex. This helix is also observed in the β2AR–Nb80 structure (Fig. 2b); however, it may not be a feature that is unique to the active state, because it is also observed in the inactive structure of the highly homologous avian β1AR[2].

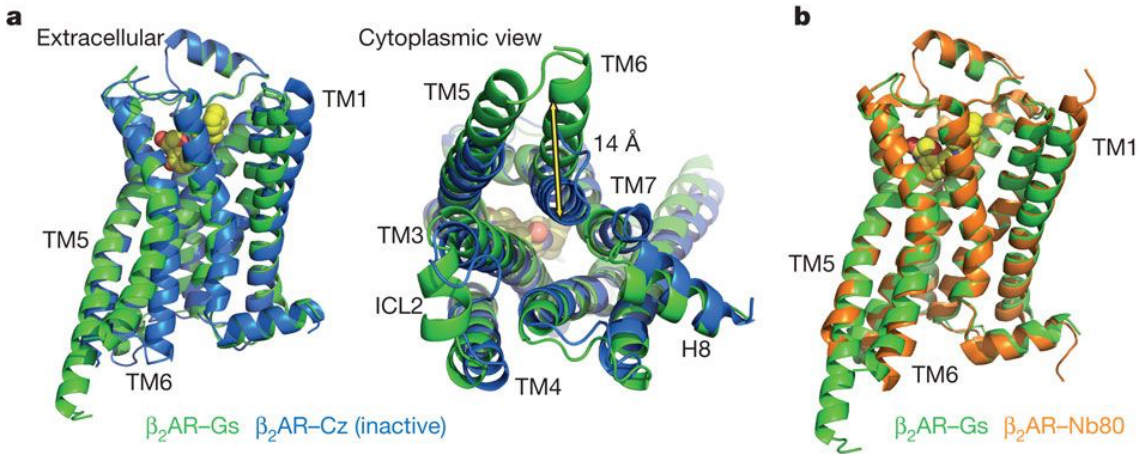

Figure 3 Comparison of active and inactive receptor structures [2]

The table shows the numbering of the β2AR receptor residues. (UniProtKB - P07550 (ADRB2_HUMAN))

Table 1 ADRB2 receptor residue numbering

1–34	Extracellular
35–58	Helical;Name=1
59–71	Cytoplasmic
72–95	Helical;Name=2
96–106	Extracellular
107–129	Helical;Name=3
130–150	Cytoplasmic
151–174	Helical;Name=4
175–196	Extracellular
197–220	Helical;Name=5
221–274	Cytoplasmic
275–298	Helical;Name=6 (GIIMGTFTLCWLPFFIVNIVHVI)
299–305	Extracellular
306–329	Helical;Name=7
330–413	Cytoplasmic

1.2. Bimoclomol and BGP-15

Amidoxime derivatives such as Bimoclomol and BGP-15 are inhibitor of poly-(ADP-ribose)-polymerase and a heat shock proteins (HSP) inducers acting as a protective agent against side effects of other drugs [3] and have a positive effect on various conditions, such as neuronal injury and aging, muscular dystrophy, arthritis and insulin resistance. Clinical studies show BGP-15 activity as a potential antidiabetic drug [4]. BGP-15 and its analogues have also been patented as compounds that ameliorate the tissue regeneration effect of adult stem cells, their survival and adherence, and promote the regulation of adult stems cell differentiation. Amidoximes exist in two tautomers, I and II, and it has been confirmed by IR and NMR spectra and other studies that the former tautomer is more stable [5]. They represent a special group of α-nucleophiles within the series of efficient acyl group acceptors – hydroxamic acids, oximes, and amidoximes. Although they are structurally similar to oximes, their kinetic behavior in reactions with the halides and esters of phosphorus and carboxylic acids differs substantially from kinetic behavior of oximes. In the case of oximes, the acceptor of the acyl group is the oximate anion.

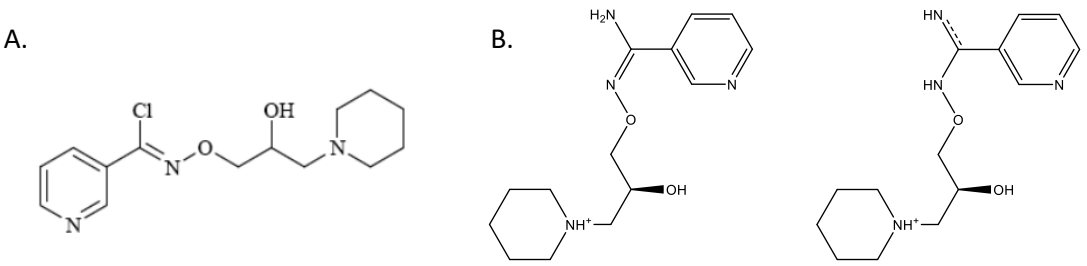

Figure 4 structure of (A.) Bimoclomol and (B.) BGP15 tautomers

1.3. Bisoprolol

Bisoprolol is a cardio-selective β1-adrenergic blocking agent used to treat high blood pressure. It is considered a potent drug with a long-half life that can be used once daily to reduce the need for multiple doses of antihypertensive drugs. Bisoprolol is generally well tolerated, likely due to its β1-adrenergic receptor selectivity and is a useful alternative to non-selective β-blocker drugs in the treatment of hypertension. It is an adrenoceptor blocking agent without significant membrane stabilizing activity or intrinsic sympathomimetic activity in its therapeutic dosage range. This preferential effect is not absolute, however, and at higher dose bisoprolol may also inhibit β2-adrenoceptors, located chiefly in the bronchial and vascular musculature. It may be used alone or in combination with other drugs to manage hypertension and can be useful in patients with chronic obstructive pulmonary disease (COPD) due to its receptor selectivity [6]. Bisoprolol is indicated for the control of symptoms in patients with angina. Studies have shown that bisoprolol can reduce the ischemic burden in patients with angina relative to the CCB nifedipine and reduce angina attacks and improve exercise tolerance relative to isosorbide dinitrate [7].

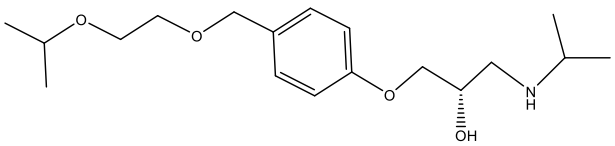

Figure 5 Bisoprolol structure

2. Aim of the work

The examined receptor is the adrenergic β2 receptor (β2AR). First, we focused on the interactions of membrane lipids with the receptor, to verify if the composition of the membrane affects ligand-target binding energy and whether phospholipids can be considered as cofactors that increase the interaction of drugs with the receptor. We then analyzed if the presence of lipids at the α site affects the binding energy of a compound bound at the β site (see Materials and methods for α and β sites definition). Finally, we analyzed what happens if lipids, BGP-15 and bisoprolol are all present on the receptor.

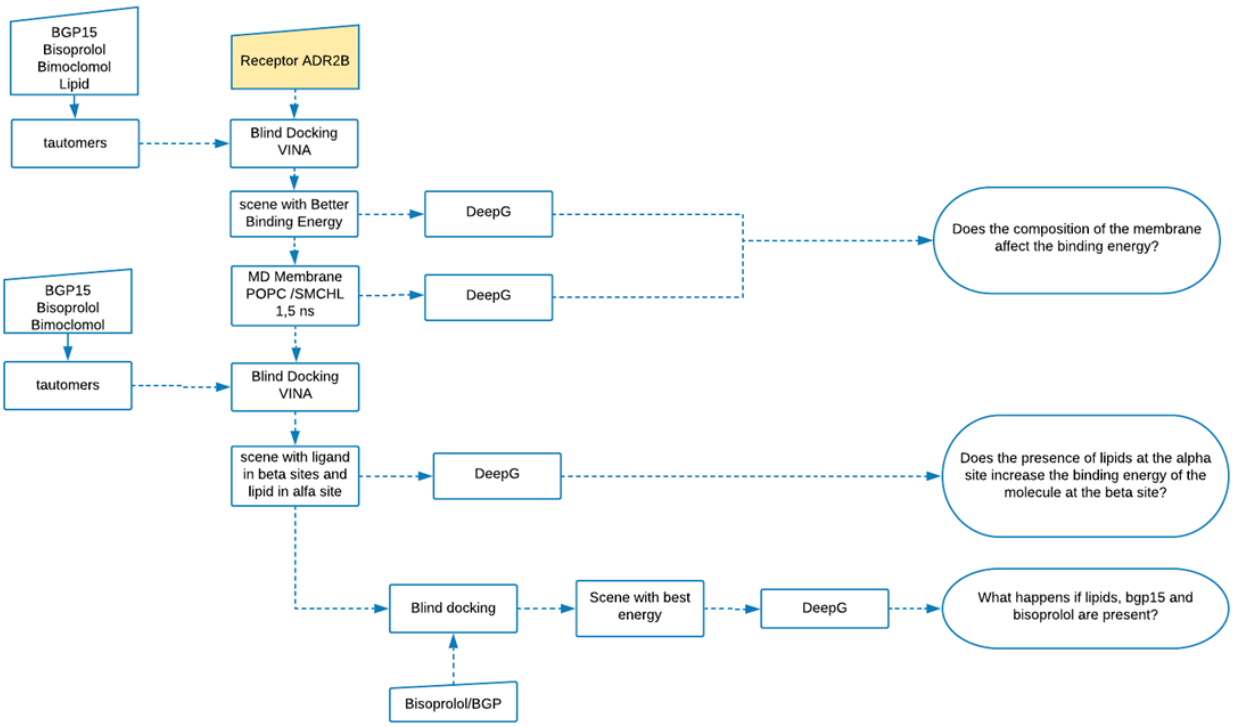

Figure 6 Workflow

3. Materials and methods

All calculations are performed on two crystalline receptor structures: the first is the active form in complex with the G-protein (Protein Data Bank code 3sn6); the second is the inactive form (Protein Data Bank code 4gbr). A first docking study was performed using different membrane lipids (CHOL, DMPC, DMPE, DMPG, DMPS, DOPC, DOPE, DOPG, DOPS, SSM) and some molecules active on the β-adrenergic receptor (BGP-15 tautomers, Bisoprolol, Bimoclomol) as ligands. The docking was performed by setting a simulation cell of 5Å around the receptor and using VINA algorithm, 100 runs and a cluster RMSD of 5 Å. This helped us understand which the main binding sites of the receptor are. The result was the identification of 3 sites that, for simplicity, we named α, β and γ (fig. 7). The binding site α is usually occupied by the α subunit of the G protein, the β site is the binding site for most drugs and the γ site is the receptor area near the TM6.

For each ligand and for each binding site the receptor-ligand complexes with the best binding energies were selected, both in the open and closed form of the receptor. We selected 47 scenes and for each of them we performed a molecular dynamics (MD) simulation of 1.5 ns in Ld (100% POPC) and Lo (SM 60%-CHOL 40%) membranes. AMBER15FB force field under NVT conditions was used. At the end of the MD run, we evaluated the RMSF (root mean square fluctuation) of the TM6 chain residues and on each final scene we calculated the new binding energy, using the DeepG plugin. To understand if membrane phospholipids could have a positive effect on the ligand-receptor binding, a new docking experiment was performed, in which the α site was occupied by a membrane phospholipid and the β site was left free for docking. Finally, we analyzed the behavior of the receptor in the presence of three ligands by running another set of docking experiments, in which the α site is occupied by a membrane lipid, while the β site is occupied by BGP-15 and bisoprolol together.

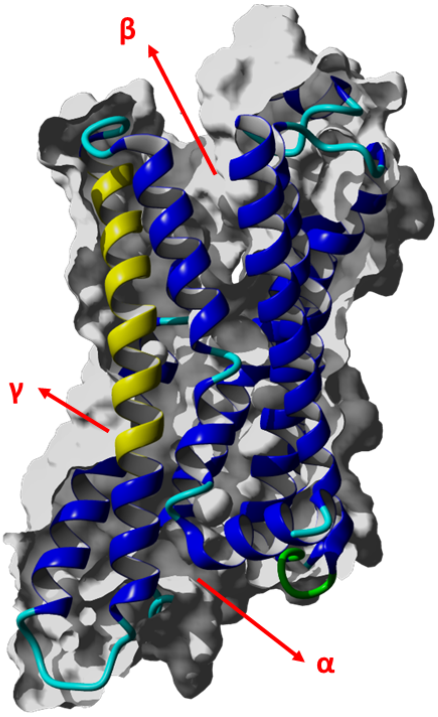

Figure 7 Sites identified by blind docking

4. Results

4.1. Binding Energies with DeepG

We evaluated the binding energy of a series of molecules with the DeepG plugin. The resulting values are shown in the following figure (Fig. 8-10).

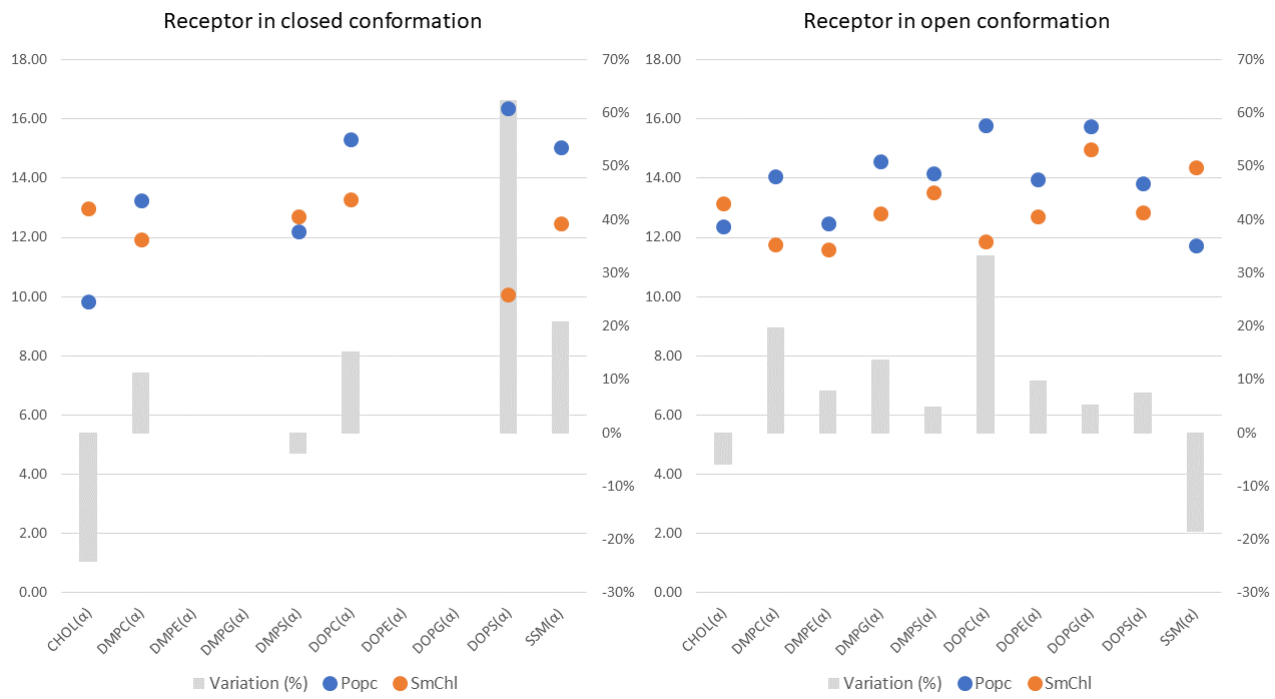

*Figure 8 Binding energies of the ligands in the **alpha site**. on the left the closed form and on the right the open form of the receptor.*

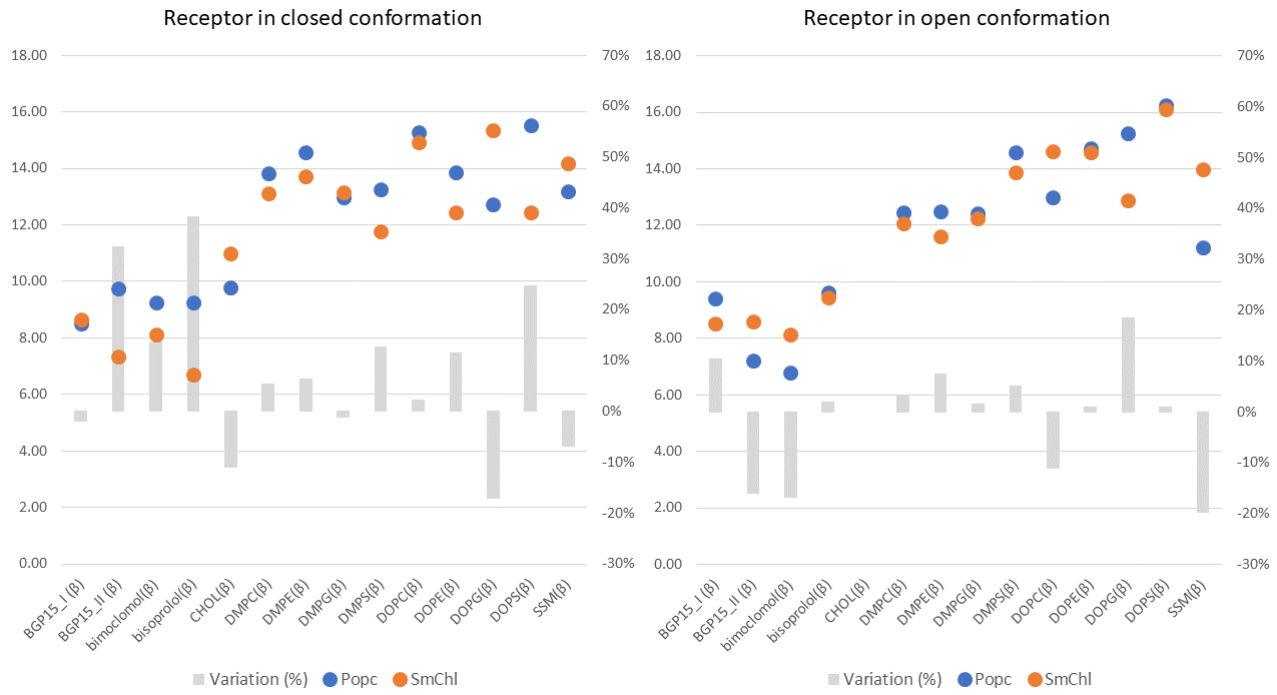

*Figure 9 Binding energies of the ligands in the **beta site**. on the left the closed form and on the right the open form of the receptor.*

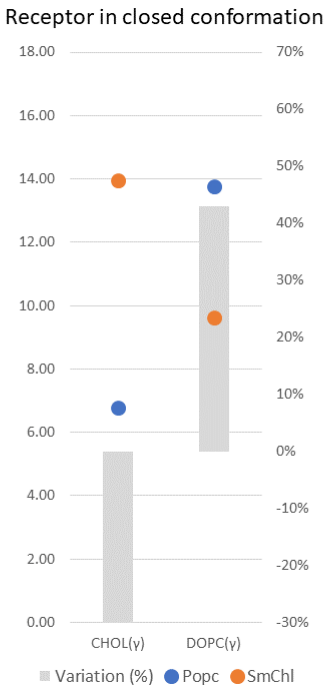

*Figure 10 Binding energies of the ligands in the **gamma site**. Gamma site is present only for the closed form of receptor.*

In fig. 8-10, the binding energies of the ligands are shown for each binding site identified.

At the alpha binding site (fig.8), ligands have greater energy when the receptor is present in the Ld membrane (POPC), except for sphingomyelin and cholesterol which have better binding affinity in the SM-CHL membrane. The ligand with the best binding energy is DOPS (16.35 kcal/mol) for the closed form of the receptor, while for the open form it is DOPC (15.79 Kcal/mol).

In the beta binding site (fig.9), most ligands have a higher energy when the receptor is present in the Ld membrane (POPC). In the closed conformation of the receptor and in the Ld membrane (POPC), the ligands with the best binding energy are BGP15 (I), CHOL, DOPG, and SSM.In the open receptor conformation and Lo membrane (SSM-CHOL), the ligands with better binding energy are BGP15(II), Bimoclomol and SSM.

The ligands in the gamma site are present only when the receptor is in closed form, and in particular the only two ligands are CHOL and DOPC. The first has a preference for binding the membrane Lo, while the second for Ld.

4.2. Presence of an ionic bridge between R175 and E107

The inactive form is further stabilized by the presence of an ionic bridge between Arg and Glu of TM3. The presence of this interaction reduces the space available for a ligand to enter.

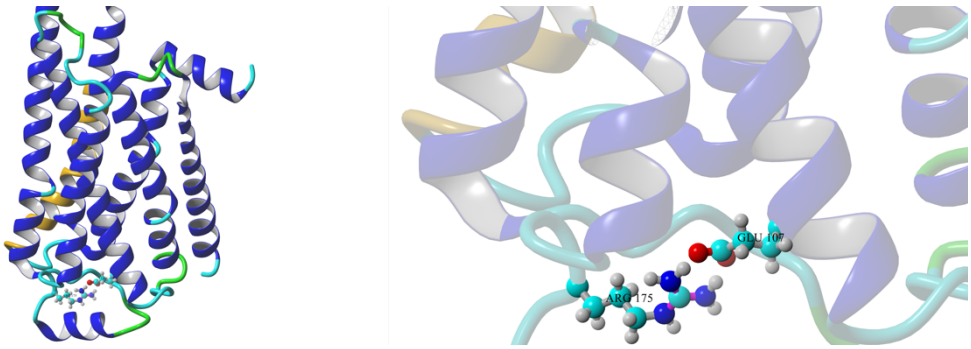

Figure 5 – The closed form is stabilized by the presence of an ionic bridge between Arg175 and Glu107

4.3. RMSF TM6 chain

As mentioned above, the receptor coexists in the open and closed form, constantly changing its conformation. This conformational change is at the expense of the TM6 that moves about 14A to allow the entry of protein G (Figure 2). The presence of binding agents may affect the mobility of the chain. We calculated the RMSF (root mean square fluctuation) of the chains at the end of an MD of 1.5 ns.

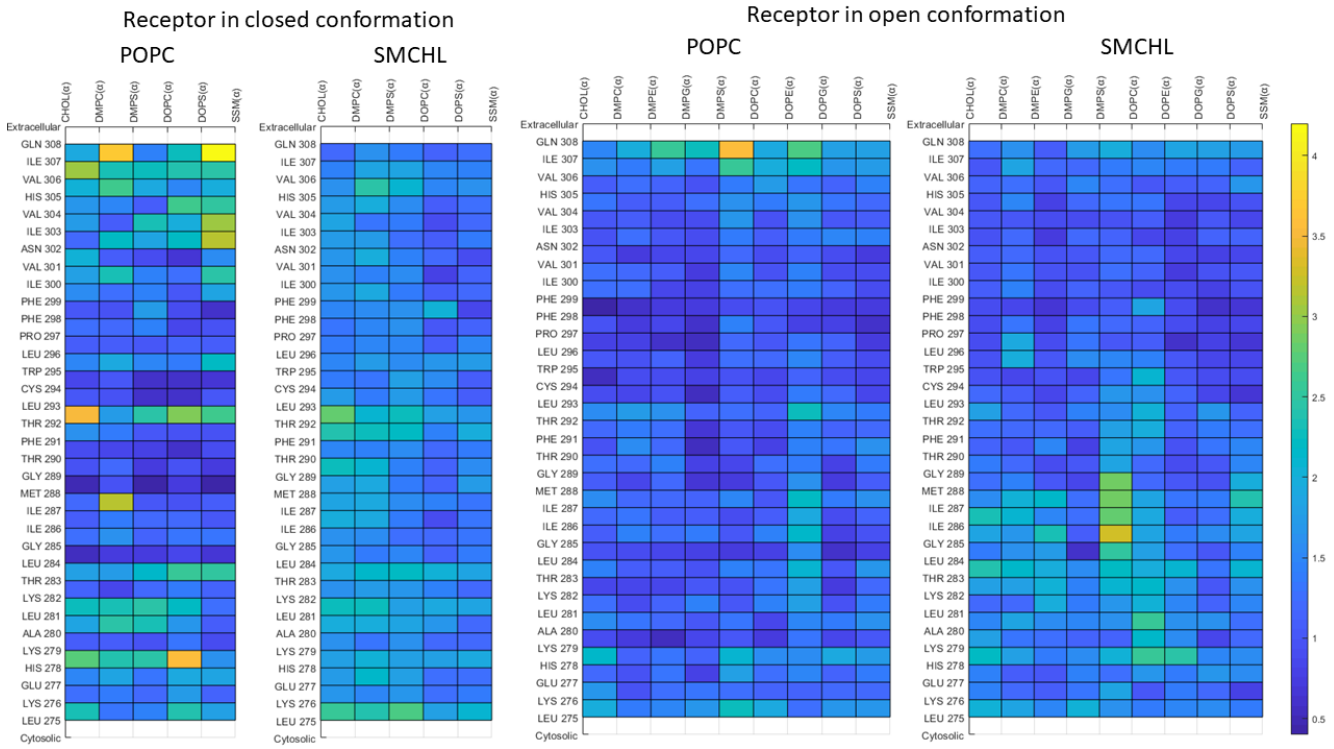

Figure 11 TM6 chain RMSF calculated when the alpha site is occupied by a ligand

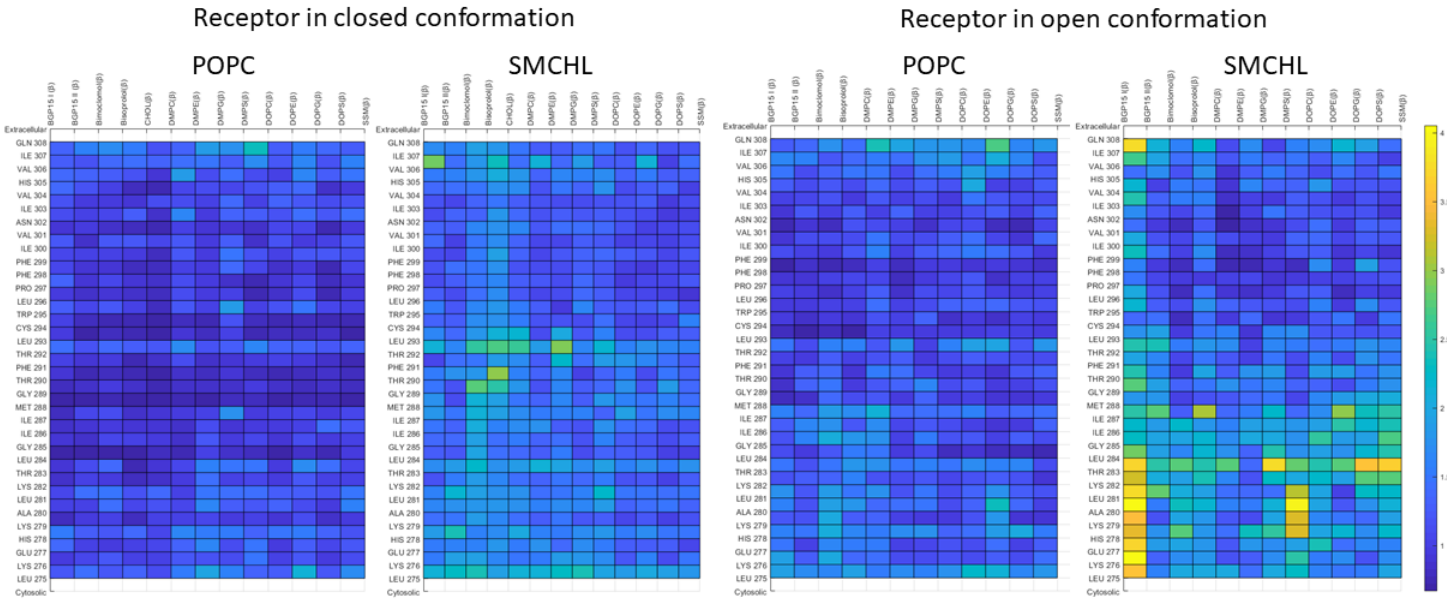

Figure 12 TM6 chain RMSF calculated when the beta site is occupied by a ligand

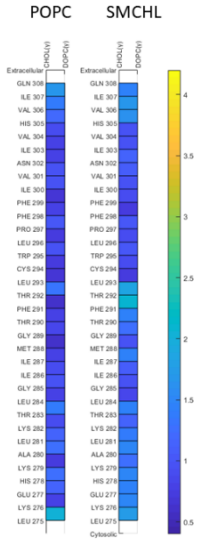

Figure 13 TM6 chain RMSF calculated when the gamma site is occupied by a ligand

Figures 11-13 show how the presence of a ligand in the different binding sites influences the movement of the TM6 chain. We can notice how, in correspondence of the residue LEU 293, the TM6 chain seems to be divided into two blocks: a more mobile region (the cytoplasmic part) and a less mobile one (the extracellular part).

The greatest flexibility can be seen when the receptor is present in SM-CHOL membrane. Particular is the behavior of the BGP15(I), in the beta binding pocket, which causes the greater movement of the TM6, comparable only to the DMPS.

In fact, DMPS is the only ligand, present in both the alpha and beta sites, that causes a significant variation in flexibility.

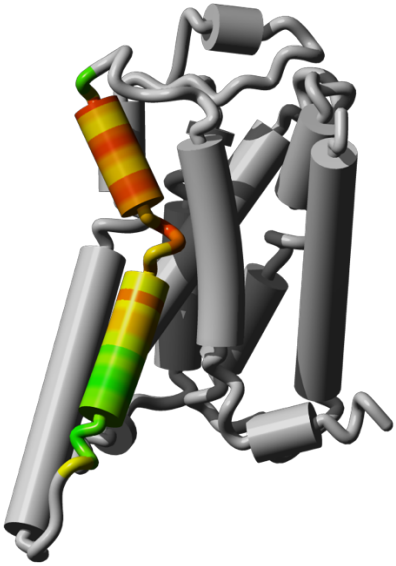

Figure 14 Structure of the receptor with TM6 colored by RMSF. In green the most mobile residues, in red the least mobile ones.

4.4. Double Binding

To understand whether membrane phospholipids can be considered as spontaneous cofactors for binding extracellular molecules to the receptor, a second docking experiment was performed, where the α binding pocket is occupied by different types of membrane phospholipids and the β site is occupied by BGP-15 (both tautomers), Bisoprolol or Bimoclomol.

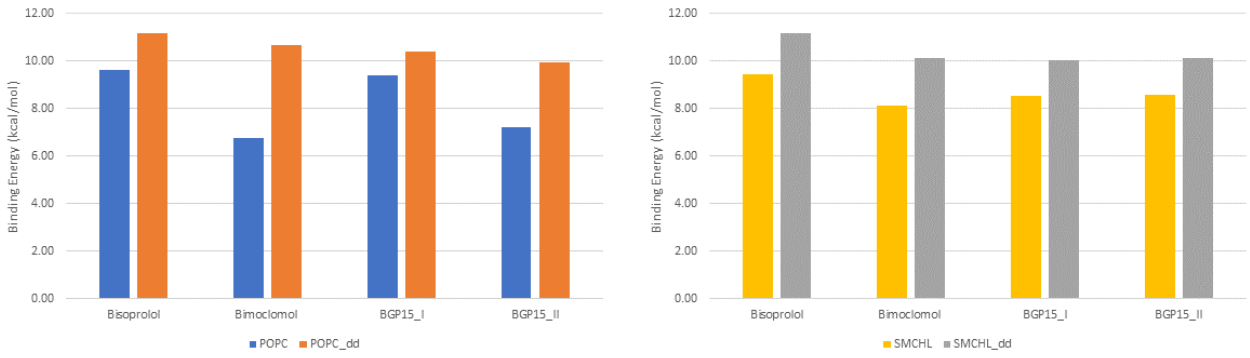

Figure 15 The graph shows the binding energy of the tautomers BGP-15 I and II, Bisoprolol, Bimoclomol in the open receptor conformation (PDB 3sn6). Left: the membrane Ld receptor (POPC). In blue the binding energies of the ligand alone are shown, in orange when the alpha site is occupied by a lipid. Right: the receptor in membrane Lo (SM-CHOL). In yellow are shown the binding energies of the ligand alone, in gray when the alpha site is occupied by a lipid.

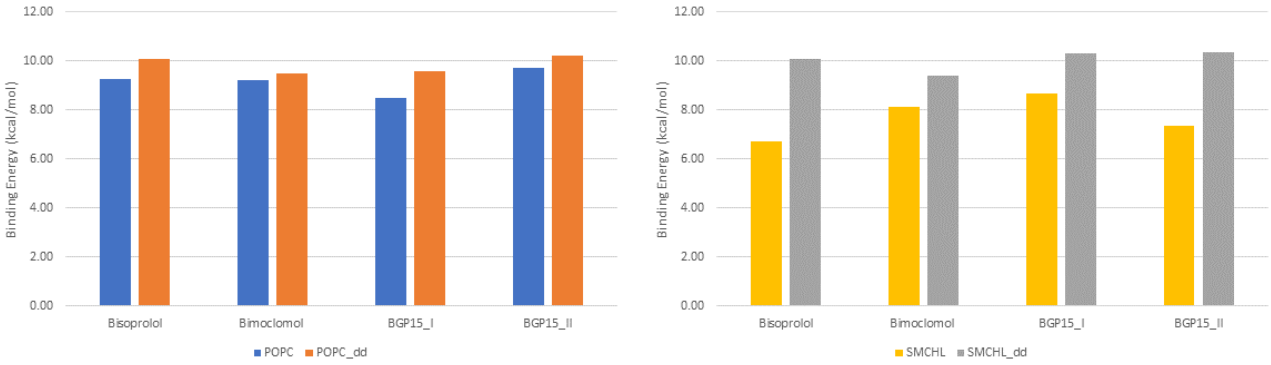

Figure 16 The graph shows the binding energy of the tautomers BGP-15 I and II, Bisoprolol, Bimoclomol in the closed receptor conformation (PDB 4gbr). Left: the membrane Ld receptor (POPC). In blue the binding energies of the ligand alone are shown, in orange when the alpha site is occupied by a lipid. Right: the receptor in membrane Lo (SM-CHOL). In yellow are shown the binding energies of the ligand alone, in gray when the alpha site is occupied by a lipid.

The graphs (fig.10-11) show that there is an improvement in the binding affinity of molecules at the beta site, when the alpha site is occupied by a membrane lipid. The phospholipids with the highest improvement are CHOL, DMPS and DOPS. The highest increase is observed for BGP15 (tautomer II) in the POPC membrane and for bisoprolol in the SM-CHOL membrane.

Bisoprolol increases its binding energy from 9.61 kcal/mol to 11.18 kcal/mol and bimoclomol from 6.77 kcal/mol to 10.65 kcal/mol. BGP15 tautomers have higher binding energy when cholesterol is present at the site of the α receptor in the POPC membrane. When the receptor is present in the SM-CHOL membrane, the DOPG in the α site shows the highest increase.

Table 3 shows the binding energies of the ligands, showing which lipid has the strongest synergistic effect with the ligands at the β site.

Table 3 – Binding energies in the β site upon occupation of the α site.

				POPC	SM-CHL
Receptor		**Lipid in α**	**Ligand in β**	**Binding Energy (kcal/mol)**	
open	3sn6	CHOL	Bisoprolol	not binding	11,15
open	3sn6	DMPC	Bisoprolol	9,27	9,95
open	3sn6	DMPE	Bisoprolol	8,18	9,94
open	3sn6	DMPG	Bisoprolol	not binding	9,19
open	3sn6	DMPS	Bisoprolol	8,67	10,03
open	3sn6	DOPC	Bisoprolol	not binding	not binding
open	3sn6	DOPE	Bisoprolol	9,12	9,27
open	3sn6	DOPG	Bisoprolol	10,32	10,06
open	3sn6	DOPS	Bisoprolol	11,18	9,13
open	3sn6	SSM	Bisoprolol	10,16	9,70
closed	4gbr	CHOL	Bisoprolol	8,93	9,82
closed	4gbr	DMPC	Bisoprolol	8,98	not binding
closed	4gbr	DMPS	Bisoprolol	10,07	10,09
closed	4gbr	DOPC	Bisoprolol	9,03	10,02
closed	4gbr	DOPS	Bisoprolol	8,65	8,40
closed	4gbr	SSM	Bisoprolol	8,17	not binding
open	3sn6	CHOL	Bimoclomol	8,64	9,07
open	3sn6	DMPC	Bimoclomol	8,33	9,95
open	3sn6	DMPE	Bimoclomol	9,32	not binding
open	3sn6	DMPG	Bimoclomol	9,10	9,80
open	3sn6	DMPS	Bimoclomol	10,65	8,27
open	3sn6	DOPC	Bimoclomol	not binding	9,66
open	3sn6	DOPE	Bimoclomol	not binding	not binding
open	3sn6	DOPG	Bimoclomol	9,05	9,65
open	3sn6	DOPS	Bimoclomol	9,40	7,50
open	3sn6	SSM	Bimoclomol	9,86	10,13
closed	4gbr	CHOL	Bimoclomol	8,39	8,52
closed	4gbr	DMPC	Bimoclomol	8,93	not binding
closed	4gbr	DMPS	Bimoclomol	8,95	9,11
closed	4gbr	DOPC	Bimoclomol	9,50	9,38
closed	4gbr	DOPS	Bimoclomol	7,93	8,98
closed	4gbr	SSM	Bimoclomol	8,34	not binding
open	3sn6	CHOL	BGP15_I	10,41	8,92
open	3sn6	DMPC	BGP15_I	7,56	9,56
open	3sn6	DMPE	BGP15_I	9,53	8,97

open	3sn6	DMPG	BGP15_I	9,34	7,63
open	3sn6	DMPS	BGP15_I	8,59	9,61
open	3sn6	DOPC	BGP15_I	8,70	9,12
open	3sn6	DOPE	BGP15_I	9,50	9,89
open	3sn6	DOPG	BGP15_I	9,00	10,03
open	3sn6	DOPS	BGP15_I	9,19	9,08
open	3sn6	SSM	BGP15_I	9,45	9,72
closed	4gbr	CHOL	BGP15_I	8,98	7,67
closed	4gbr	DMPC	BGP15_I	9,04	not binding
closed	4gbr	DMPS	BGP15_I	9,45	9,88
closed	4gbr	DOPC	BGP15_I	9,57	10,30
closed	4gbr	DOPS	BGP15_I	7,76	8,64
closed	4gbr	SSM	BGP15_I	8,88	9,19
open	3sn6	CHOL	BGP15_II	9,95	8,89
open	3sn6	DMPC	BGP15_II	8,61	10,03
open	3sn6	DMPE	BGP15_II	9,00	8,01
open	3sn6	DMPG	BGP15_II	9,56	8,97
open	3sn6	DMPS	BGP15_II	9,95	9,60
open	3sn6	DOPC	BGP15_II	6,60	8,99
open	3sn6	DOPE	BGP15_II	9,19	not binding
open	3sn6	DOPG	BGP15_II	9,37	10,12
open	3sn6	DOPS	BGP15_II	9,05	9,45
open	3sn6	SSM	BGP15_II	8,54	9,21
closed	4gbr	CHOL	BGP15_II	8,42	9,12
closed	4gbr	DMPC	BGP15_II	8,86	not binding
closed	4gbr	DMPS	BGP15_II	9,28	9,27
closed	4gbr	DOPC	BGP15_II	10,24	10,36
closed	4gbr	DOPS	BGP15_II	9,02	8,08
closed	4gbr	SSM	BGP15_II	8,85	9,46

4.5. Triple Docking

As we have seen in previous Double Docking experiments, membrane lipids have a spontaneous cofactor action for the binding of bisoprolol and BGP-15 on the adrenergic beta receptor. It is to be examined whether the presence of both ligands (BGP15 and bisoprolol) has a synergistic effect. For this reason, a docking experiment was performed with both ligands present in the beta site and the membrane lipids in the α site. The binding energy was calculated using the DeepG plugin.

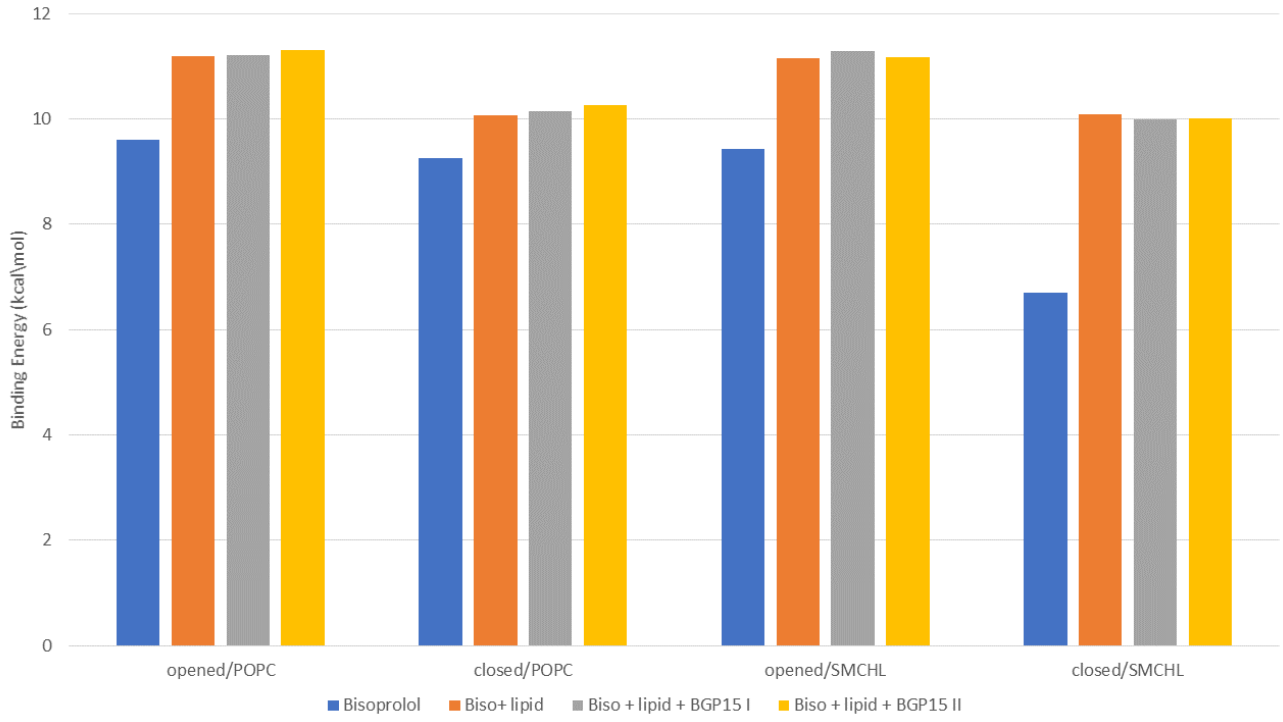

Figure 17 Binding energy of bisoprolol. Comparison of different docking experiments.

Figure 17 shows the binding energy of bisoprolol in the presence of the lipid and the third ligand (BGP15, both tautomers).

There is no improvement in the interaction between ligand and receptor. This is probably due to the already high affinity of bisoprolol for the binding site, such that BGP15 does not entail any change in binding energy.

On the contrary, the BGP15 decreases its energy when bisoprolol is added (fig.18-19). The reason for this is that bisoprolol has a greater affinity with the receptor. Consequently, in the presence of both ligands, the preferred bond is with bisoprolol. This explains the decrease in the binding energy of BGP15 and the increased binding energy of Bisoprolol.

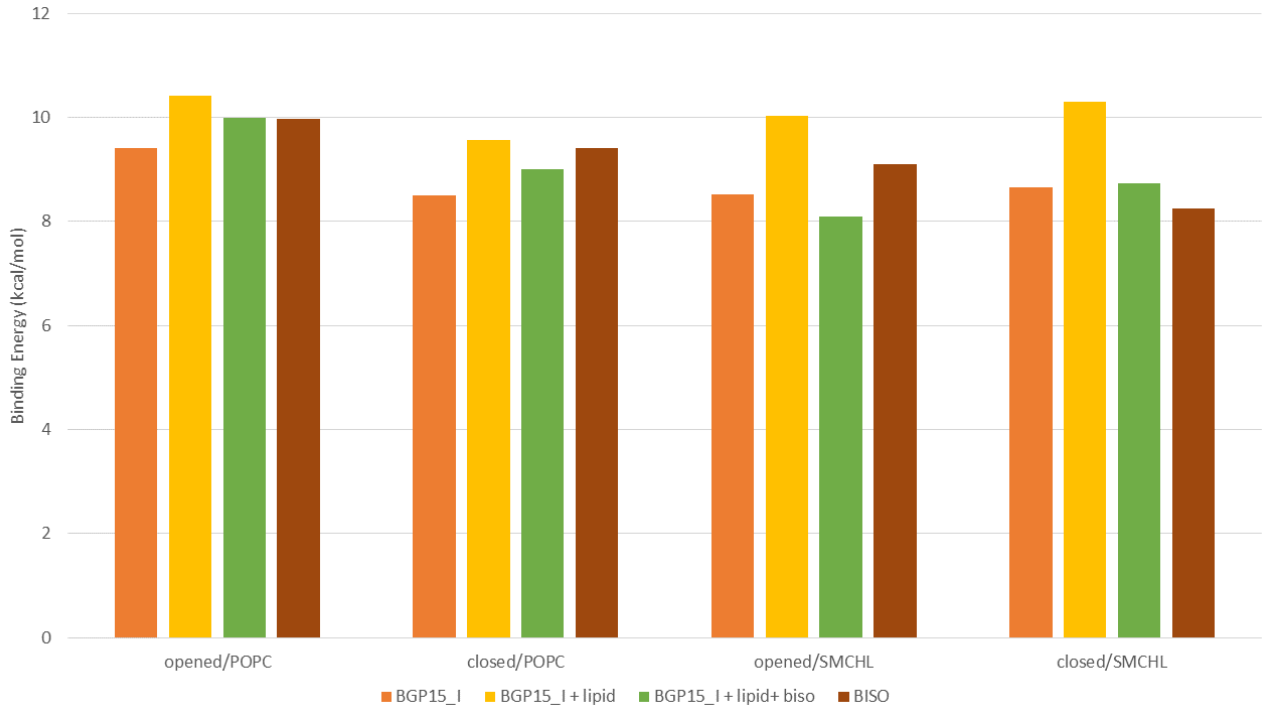

Figure 18 Binding energy of BGP15 (tautomer I). Comparison of different docking experiments.

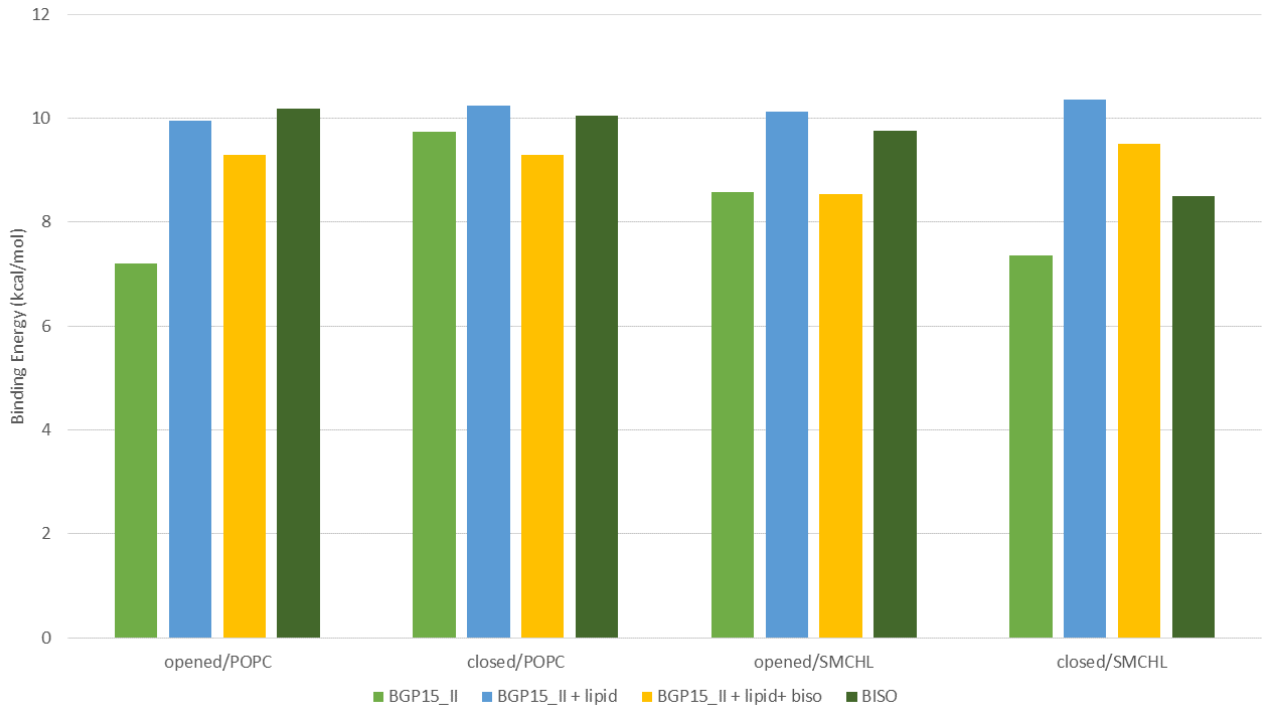

Figure 19 Binding energy of BGP15 (tautomer II). Comparison of different docking experiments.

The only exception is in the closed form of the receptor in the SM-CHOL membrane. The energy of bisoprolol is lower than that of BGP15 because the beta binding pocket closes after binding BGP15. As a result, bisoprolol is not able to fully enter the beta site, as we can see in Figure 20.

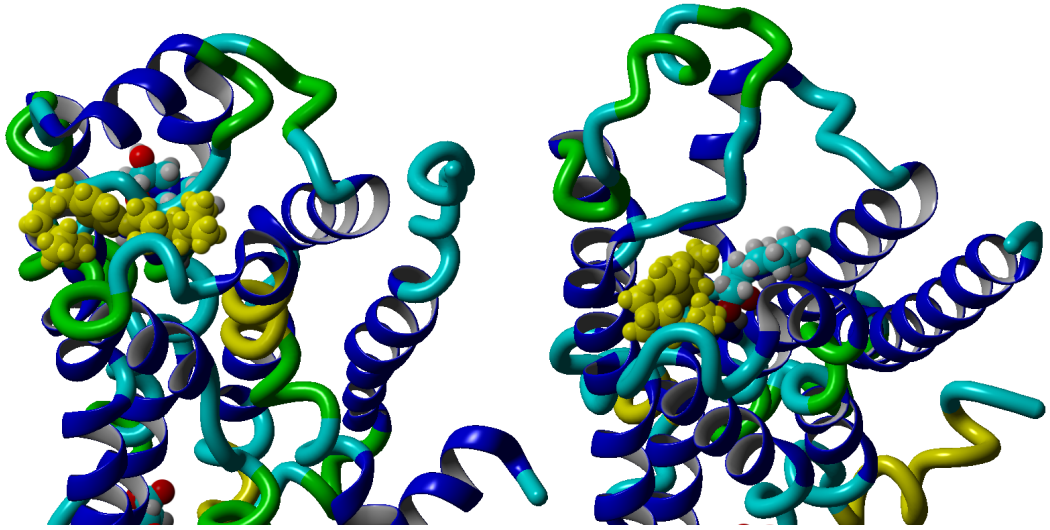

Figure 20 On the left the SMCHL membrane receptor in the closed conformation, on the right in the open form. bisoprolol is highlighted in yellow.

5. Conclusion

Molecular simulations have suggested that the active state of the receptor is stable only in the presence of a bond with a stabilizing molecule. The energy differences between the two open states and the energy of an intermediate state related to the rupture of an interaction between Arg-Glu on TM3 suggest interconversion times of more than tens of microseconds and this observation precludes the extended study using all-atom MD. It was chosen to perform numerous dynamics of 5 ns at a geometry corresponding to local energy minima.

In this study we investigated the specific interactions of membrane lipids and some ligands with the adrenergic β receptor and we evaluated how and whether membrane lipids could play the dual role of controlling the physical state of the membrane (MPS) and spontaneous cofactors.

We have verified that the activity of the receptor is controlled by at least two allosteric sites, called α and β. The α site is the binding region of the Gα subunit and is itself an allosteric site. In fact, the binding of a molecule at the beta site is favoured by the concomitant binding of some negatively

charged and neutral lipids at the alpha site. Negative charged lipids can be inserted into the α pocket and the arginine bridge in TM6.

We have observed that anionic lipids are superior to zwitterionic lipids in coordination with Arg and can therefore accelerate receptor deactivation, in line with available experimental data.

MD calculations have shown that the physical state of the membrane (MPS) influences the mobility of TM6, demonstrating that membrane lipids have a dual role: regulating the receptor open-close clock and interacting directly with one of the allosteric sites. In addition, we have verified how the inclusion of certain exogenous molecules such as bisoprolol and BGP15 has a combined effect with lipids. The double docking experiments showed a synergistic effect between the ligands at the β site and the lipid or anion at the α site.

Bisoprolol and BGP15 are only able to bind to the β-allosteric site and show a preference for the receptor inserted into an Ld membrane.

Interesting is the behaviour of the two tautomers of BGP15, which have an opposite behaviour: the tautomer (I) binds better when the receptor is present in a Lo membrane (SM-CHL), the BGP15 (II) has a better affinity when the receptor is present in an Ld membrane (POPC). The best binder is Bisoprolols a higher in particular when assisted by cholesterol.

The Triple docking experiment, it was evaluated whether the addition of a second exogenous molecule could increase or decrease the affinity of the ligand already bound to the receptor. We have shown that by adding BGP15 (both tautomers) to the receptor bound to bisoprolol, there is no change in the binding energy. On the contrary, when bisoprolol is added to the already bound BGP15, there is a decrease in the binding energy of the BGP15. Figure 21 shows the bisoprolol occupies the same binding pocket of Noradrenaline (crystallographic data PDB 7BU6) in the transmembrane protein portion of β1 receptor. The bisoprolol is a good binder as confirmed by the binding energy calculated of 10.39 kcal/mol.

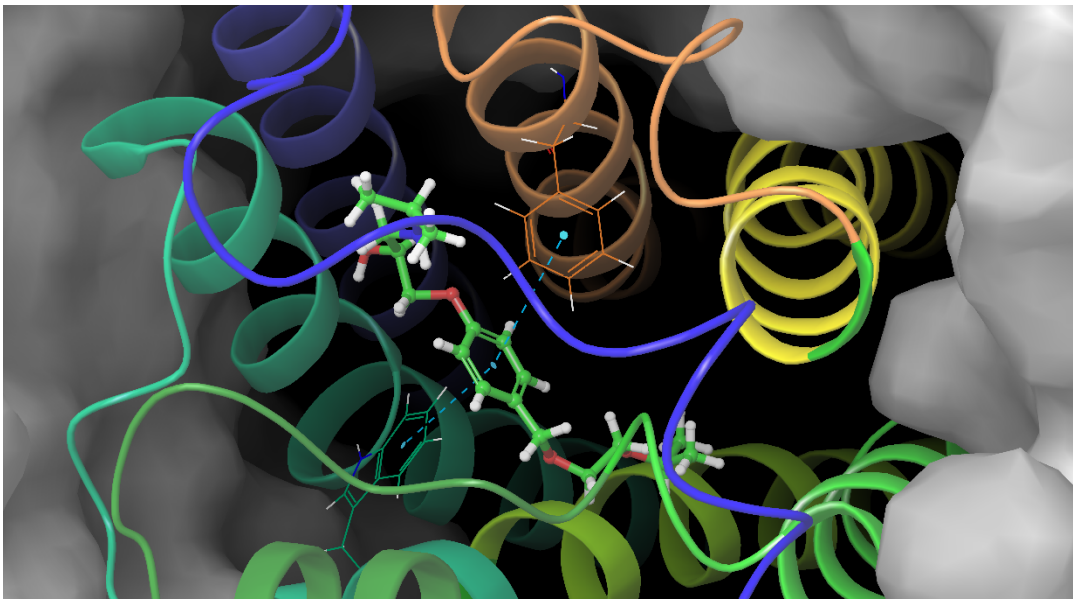

Figure 21. The binding site view from above of the ADRB1/Bisoprolol complex. Dashed light blue lines highlight interactions between bisoprolol ligand and residues Trp134 and Phe 359. The complex was immersed in POPC membrane represented by a grey surface.

From the formation energy calculation, we selected the BGP-15 tautomer to be used in subsequent calculations and observed (in the figure 2) that in the ADRB1/bisoprolol/BGP-15 complex, the BGP-15 molecule is positioned in the vicinity of bisoprolol at the interface between the membrane and the extracellular aqueous solution. Metadynamics studies indicate that the presence of BGP-15 blocks bisoprolol at the binding site.

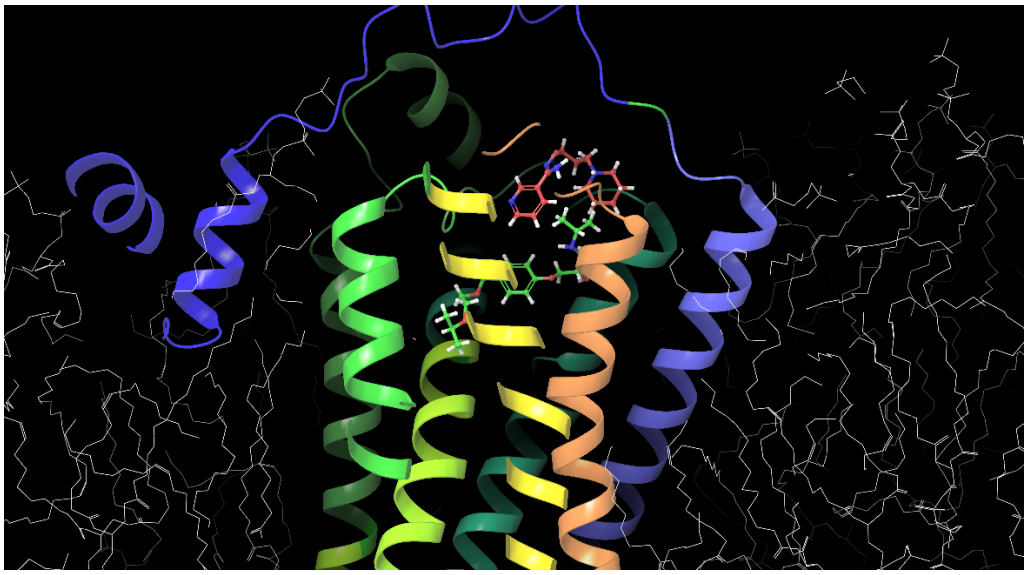

Figure 22. The docked pose of ADRB1/Bisoprolol with BGP-15. The BGP-15 is close to the bisoprolol in the protein portion in the interface between membrane and water. The membrane is schematized with grey line.

This action could have as a biological effect an increase in the blocking activity of the ADRB1 receptor in a specific conformation by bisoprolol. Therefore, BGP-15 act as an antagonist enhancer.

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