Identifying Binding Modes of Two Synthetic Derivatives of Adrenalin to the α_2C -Adrenoceptor by Using Molecular Modeling; Insights into the α_2C -Adrenoceptor Activation

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1 **Abstract:**

2 Although, $\alpha 2C$ adrenergic receptor (AR) mediate a number of physiological functions *in vivo*
3 and has great therapeutic potential, the absence of its crystal structure is a major difficulty in the
4 activation mechanism studies and drug design endeavors. Here, a homology model of $\alpha 2C$ AR has
5 been presented by means of multiple sequence alignment. The used templates were the latest
6 crystal structures of the other ARs (Protein Data Bank IDs: 2R4R, 2RH1, 4GPO, 3P0G, 4BVN and
7 4LDO) that have 38.4% identity with the query.

8 We then conducted docking simulations to understand and analyze the binding of
9 noradrenaline (NOR), and its derivatives, namely arachidonoyl adrenalin (AA-AD) and
10 arachidonoyl noradrenalin (AA-NOR) to the receptor. The existence of H-bonds between the
11 ligands and SER218 residue implies the same binding site of derivatives with respect to the NOR.
12 AA-AD and AA-NOR bind to the receptor with the larger binding affinities.

13 The presence of salt bridge between ARG149 and GLU377 in the free receptor, obtained from
14 molecular dynamics studies proved that the receptor still is in its basal state before binding process
15 take places. The activation process is characterized by increasing in the RMSD values of the
16 backbone receptor in the bound state, increasing the RMSF of the transmembrane involved in the
17 activation process and the disappearance of the ARG149-GLU377 salt bridge.

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19 **Keywords:** Adrenergic receptors, Arachidonoyl adrenalin, Arachidonoyl noradrenalin,
20 Homology Modeling, Molecular Docking, Molecular Dynamics Simulation.

1 **1. Introduction:**

2 G protein-coupled receptors (GPCRs) include the biggest superfamily of membrane receptors
3 in the human body. They are one of the best examples of conformational diversity of biomolecules
4 being coded by 3–4% of the entire genome[1]. Adrenergic receptors (ARs) belong to the amine
5 receptor cluster of the rhodopsin-like family of GPCRs[2] that respond to and mediate effects of
6 endogenous catecholamines adrenaline and noradrenaline. They comprise seven transmembrane
7 (TM1-TM7) helices which is the common feature of all GPCRs. Today, a total of nine AR
8 subtypes, namely $\alpha 1A$, $\alpha 1B$, $\alpha 1D$, $\alpha 2A$, $\alpha 2B$, $\alpha 2C$, $\beta 1$, $\beta 2$, and $\beta 3$, has been identified based on
9 their agonist and antagonist binding characteristics and the way of their gene expression in variant
10 organs[3].

11 The $\alpha 2C$ -AR is involved in responses to stress, locomotion, regulation of the catecholamine
12 release from the adrenal gland[4, 5], and regulating the norepinephrine release from the peripheral
13 sympathetic neurons. As $\alpha 2C$ -AR acts as presynaptic inhibitor[4], it could be considered as
14 important drug target against hypertension, cardiovascular dysfunction, regulating vasoconstriction,
15 opiate withdrawal, alcohol addiction and as adjuvants for anesthesia during surgery[6, 7]. Even
16 though subtype specific drugs for $\beta 1$ and $\alpha 1$ receptors are accessible, but for $\alpha 2$ adrenergic
17 receptors, nonspecific drugs like Clonidine, Medetomidine, and Brimonidine are still in use to the
18 treatment of hypertension, glaucoma, tumor pain, postoperative pain etc.[8]. Thus, the availability
19 of subtype specific ligands would be beneficial for the treatment of diseases related to $\alpha 2$ -receptors.
20 Alternatively, it has been discovered that $\alpha 2C$ -adrenergic agonists, when combined with a
21 cannabinoid receptor agonist, show significant synergism in antinociception in the hot-plate assay
22 and simple additive effect in the tail-flick test[9, 10]. Therefore, the study of agonist/antagonist
23 binding process and activation/deactivation mechanism could lead us to the more effective design
24 of new drugs with higher specificity and less side effects.

1 Understanding of proteins function, accurate docking of existing ligands into their binding sites
2 and achievement of rational drug design are succeeded only in the presence of high resolution
3 crystal structure of the target protein. Homology modeling has been considered as suitable method
4 to get the 3D structure of a protein, in the absence of crystal structure. When the sequence identity
5 is less than 30%, which is the case in GPCRs, the reliability of model decreases. The accuracy of
6 homology model is exclusively reliant on the template and could be increases by the multiple
7 sequence comparison and alignment. Several homology models of α subtype of adrenoceptors have
8 been developed based on the crystal structure of bovine rhodopsin[11], human dopamine D3
9 receptor[12] and β 2-adrenergic receptor[13-15] so far. However, in all the resulted models the
10 sequence identity of the target-template did not exceeded than the 30%. Consequently, in this study
11 a new homology model of the α 2C subtype receptor has been presented based on the multiple
12 sequence alignment with the most recent crystal structures of the other adrenergic receptors. The
13 created model, was used to the study the interaction of arachidonoyl adrenalin (AA-AD) and
14 arachidonoyl noradrenalin (AA-NOR) (their chemical structures are shown in Scheme 1) which
15 could be considered as concurrent agonists of both ARs and cannabinoid receptors due to their
16 structures. Molecular docking and molecular dynamics were used to study the binding process,
17 identifying the ligand affinities, driving forces, involved residues, and the activation process.
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Scheme. 1. Chemical structures of AA-AD and AA-NOR

2. Methods

2.1 Homology Modeling

In the absence of crystal structure, the 3D coordinates of $\alpha 2C$ was predicted by homology modeling using primary and secondary structural knowledge. The sequence of Human $\alpha 2C$ (P18825) adrenergic receptor was retrieved from SWISSPROT database (<http://www.uniprot.org>). Bioinformatics Toolkit platform of Max-Planck institute (<http://toolkit.tuebingen.mpg.de/>) has been used to identify the proper template proteins for multiple sequence alignment (MSA), since GPCR proteins share a very low percentage of sequence identity. A total of 6 related structures with the PDB IDs of 2R4R, 2RH1, 4GPO, 3P0G, 4BVN and 4LDO were identified and subjected to MSA. The $\alpha 2C$ and template proteins sequences used as a query and templates, respectively, in the MAFFT program[16] for sequence alignments (SI, Fig S1) to identify conserved regions. The conserved motifs in the ARs such as “DRY” in TM3, “CWxPx” and “D/E” 6.30” in TM6, and “NPxxY” in TM7 were checked to certify that the TM domain alignments were reasonable. The resulted aligned sequences investigated carefully and manually adjusted to avoid insertions and deletions in the transmembrane regions. The final sequence identity was 38.4 %. The 3D model was finally generated using the software Modeller 9v15[17].

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2.2 Molecular Docking

The structure obtained from homology modeling used as initial coordinates of $\alpha 2C$ receptor for molecular docking. The docking studies were conducted with AutoDock 4.2 program package. The ligands were checked for hydrogens and protonation states. Their structures were built using the Gaussview and then were energy minimized by the quantum chemistry package Gaussian 03 using B3LYP functional and 6-31G(d,p) basis set to get the optimized and stable geometry[18].

Binding location were defined on the binding cavity according to the prior experimental and theoretical evidences, by a docking box of 60×60×60 dimensions and a grid point spacing of 0.375Å. The center of grid box was set at the centroid of the active site residues consisting D3.32 (ASP131), S5.42 (SER214) and S5.46 (SER218) residues[19-21]. All docking computations were performed using AutoDock empirical free energy function and the Lamarckian genetic algorithm with local search[22]. During the docking simulations, for ligands, single bonds were free to rotate and non-aromatic rings were allowed to flip. For each docking calculation, a pool of 200 independent conformations generated and assessed with 25,000,000 energy evaluations while the default values were used for all other docking parameters.

2.3 Molecular Dynamics Simulation

Four systems were built for explicit MD simulation in lipid bilayer: (1) apo- $\alpha 2C$, (2) $\alpha 2C$ -NOR, (3) $\alpha 2C$ -(AA-AD) and (4) $\alpha 2C$ -(AA-NOR). MD simulations were implemented using the GROMACS version 4.5.5 package[23] with Gromos96-43a2 force field. In this regards, for the complex systems, the conformations with the lowest binding energy resulted from the docking simulations were picked as the initial geometry for MD simulations. The topology and other force field parameters of ligands were obtained from the ATB server. The lipid bilayer consisted of a pre-equilibrated layer of 128 molecules of 1-palmitoyl-2oleoyl-sn-glycerophosphocholine (POPC), and

its parameters were obtained from P. Tieleman's site at <http://moose.bio.ucalgary.ca/Download>. The protein in both free and bound state, was introduced carefully into the lipid bilayer using the InflateGro program[24]. The entire lipid bilayer was inflated and then slowly compressed around the protein by using successive energy minimization steps with the steepest descent algorithm to relax the lipid molecules, keeping protein restrained. An area per lipid value of $67 \text{ \AA}^2$ was reached, which is very close to the experimental value of $65 \text{ \AA}^2$ for pure POPC. The entire system was then solvated with a single point charge (SPC) water model and neutralized with 17 Cl^- counter ions.

All systems were then energy minimized using the steepest descent algorithm. A 1 ns position restraining simulation was then carried out to restrain the protein to relieve the close contacts with POPC and water under NVT ensemble conditions, with a V-rescale (modified Berendsen) temperature coupling. This was followed by another 1 ns equilibration under NPT ensemble conditions, before a final production run of 50 ns. The systems were then run under isothermal-isobaric ensemble (NPT) for 50 ns and Nosé-Hoover temperature and Parrinello- Rahman pressure coupling algorithms were used to maintain the temperature and pressure values in 300 K and 1 bar, respectively. Protein-ligands, and lipids-waters (plus ions) coupled separately with a coupling constant of $\tau=0.1$ ps. Semi-isotropic pressure coupling was set with $\tau_p=2$ ps, allowing the bilayer to deform in the x-y plane independently of the z-axis. A time-step of 2 fs was used throughout with periodic boundary conditions.

Long-range electrostatic interactions were evaluated using the particle-mesh Ewald (PME) method. Van der Waal's interactions and Coulomb interactions were cut off at $12 \text{ \AA}$ with updates every five steps. The results were analyzed using the in-built analysis package of GROMACS and SIGMAPLOT. The trajectories were visualized using VMD and all the images were rendered using PyMol.

3. Results and Discussions

1 **3.1 Homology Modeling-** The $\alpha 2C$ receptor belongs to the GPCRs. The common feature of

2 this superfamily is the low sequence identities with high structure similarities of its members. The
3 binding site typically has been located among the seven transmembrane (TM) helices; together with
4 three extracellular and three intracellular loops. Due to the difficulty in the crystallization of
5 GPCRs, homology modeling is a key method for the study of the dynamics, activation, and
6 structure-based drug design in this family.

7 Given that this low sequence identity of $\alpha 2C$ to any of the known other GPCR structures, and
8 consequently the low accuracy of any structural predictions, the multiple sequence comparison has
9 been applied to fortify the accuracy of the predicted structure. The produced model was
10 geometrically refined and energy minimized using conjugate gradient algorithms and short MD
11 simulations, as part of the Modeller protocol to reduce the side chain steric clashes. The loop
12 regions were refined using the in-built “loopmodel” class of Modeller. Figure 1 shows the
13 established homology model of $\alpha 2C$. The three-dimensional structure of binding pocket,
14 surrounded by TM3, TM5, TM6, and TM7, is especially close to the templates due to the high
15 sequence identity. However, there are much more difference in the loops, as the residues in these
16 domains share the lowest conservation with the templates.

Fig. 1. 3D structure of $\alpha 2C$ based on multiple sequence alignment predicted by Modeller, after energy minimization (sequence identity: 38.4%, PDB IDs of templates: 2R4R, 2RH1, 4GPO, 3P0G, 4BVN and 4LDO).

The quality of the model was assessed with different criteria. The PROCHECK [25] showed 98.5% of amino acids in the favored and additional allowed regions [A, B, L, a, b, l, and p regions in Fig. 2], 0.8% in allowed [$\sim a$, $\sim b$, $\sim l$, and $\sim p$ regions in Fig. 2], and 0.7% in disallowed region [XX region in Fig. 2] as shown in the Ramachandran map (Figure 2). G-factors provide a measure of how unusual, or out-of-the-ordinary a property is. This factor ranks the values above -0.5 as acceptable models in homology modeling. In this study, the average score of G-factors for the dihedral angles and main-chain covalent forces were found to be 0.05 and -0.24 , respectively. The GA341 score[26], a fold assessment composite score derived by a genetic algorithm uses to overall fold quality evaluation of the created model by Modeller. A reliable model has a probability of the correct fold when this score is larger than 95%. The calculated GA341 factor of the presented model found to be 99.8%, represents the correct fold in the predicted structure.

Fig. 2. Stereo-chemical property analysis of the predicted model of $\alpha 2C$. Analysis of the Ramachandran plot (obtained from PROCHECK) confirms the stereo-chemical quality of the model, with 98.5% of the residues falling within the “favored and additional allowed regions”.

In sum, since the active pocket is located in the upper half of the TMs, the differences of the loops do not affect the study on the binding mode of ligands. Also, the quality evaluation of the created model showed the structure properly modeled and refined, therefore, it can be used for the accurate molecular docking and dynamics studies.

3.2 Molecular Docking—NOR, AA-AD and AA-NOR have been docked to the homology structure of the $\alpha 2C$ receptor to understand the putative binding pocket and the residues involved in the bonding and nonbonding interactions. The final selection of the binding poses was based on the Autodock scoring function. The scoring function takes into account hydrogen bonds, hydrophobic, van der Waal's, coulomb and polar interactions in the binding pocket. We also repeated the docking calculations with the simulated apo protein. The results showed the incorrect prediction of the binding site and affinities (SI, Fig S3, Table S1), as the apo protein in the selected force field goes toward the inactive state (it would be discussed in the next section, comprehensively). The docking studies of the ligands on the homology modeled structure of receptor revealed that all three ligands bind to the $\alpha 2C$ receptor in roughly the same site surrounded by TM3, TM5, TM6 and TM7, which has been proved as agonist active site of the ARs, previously[20, 27]. The analysis of binding site residues indicated that three compounds is surrounded by the following hydrophobic and hydrophilic residues: 127, 131 and 132 of TM3, 210, 214, 215 and 218 of TM5, 392 and 402 of TM6 and 423, 424 and 427 of TM7 (Table. 1).

Table. 1. Involved amino acid residues, the free binding energy and the related binding constant for the binding of catecholamines with $\alpha 2C$ receptor.

Molecule	Involved residues	ΔG° , (kJ/mol)	K_a (M^{-1})
NOR	ASP131, TYR191, VAL132, LEU204, ASP206, SER218, PHE399, TYR402, PHE423, TYR427	-25.11	2.51×10^4
AA-AD	TYR127, ASP131, GLY203, LEU204, TYR210, ASP206, SER214, Cys215, SER218, PHE399, TYR402, PHE423	-42.89	3.26×10^7
AA-NOR	SER108, VAL132, GLY203, LEU204, SER214, SER218, PHE399, TYR402, PHE423, TRP424	-44.94	7.48×10^7

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2 Negatively charged ASP in TM3, corresponding to the D3.32 (ASP131), and polar SER

3 residues S5.42 (SER214) and S5.46 (SER218) in TM5 have been found to be responsible for the

4 polar interactions with ligands through salt bridge and hydrogen bonds, respectively[28]. Docking

5 of NOR to the receptor predicted two H-bonds with SER218 and no any salt bridge with ASP131

6 (Fig. 3a) which could be as a result of this fact that the receptor still is in its basal state. The final

7 conformation of NOR had a score of -25.11 kJ/mol. The experimental value for the binding of the

8 NOR to the β ARs has been reported to be -34.70 kJ/mol[29]. Also, it has been proved that the

9 binding affinity of the endogenous AR agonists (like NOR) to the β subtypes is higher than their

10 affinity to the α ARs. Respect to the agreement of the predicted binding energy of NOR with the

11 relevant experimental evidences, and the correct assessment of H-bond interactions in docking

12 calculations, it could be concluded that the presented homology model of the receptor is reliable

13 and can be used for the further studies.

14 Molecular docking studies showed that AA-AD and AA-NOR bind to the active site of NOR (Fig.

15 3b and 3c), with -42.89 and -44.94 kJ/mol energies, respectively. The higher score represents the

16 higher binding affinity of the derivatives that could be caused by the hydrophobic interactions of

17 arachidonic side chain with amino acid residues in the active site.

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Fig. 3. The best docking pose of a) NOR, b) AA-AD, and c) AA-NOR at the $\alpha 2C$ receptor, simulated by Autodock. Docking results predicted two, one and two H-bonds with SER 218 in the binding of NOR, AA-AD and AA-NOR to the receptor, respectively.

3.3 Molecular Dynamics Simulation- The binding modes of ligands, the binding energy and the involved residues in the interaction with the ligand could be evaluated by docking method. However, in order to investigate the activation process, confirm the main binding mode, the effect of ligands in the activated conformation of the receptor, and the time evaluation of the binding process, the molecular dynamic (MD) simulations should be performed. All simulations were carried out for 50 ns after implanting the protein in a POPC bilayer and solvating the entire system with explicit water. The N-terminal and C-terminal of the modeled structure were removed to reduce the cost of the computations (SI, Fig S2).

To assess the stability of the trajectory for both free and bound receptor, the root mean square deviation (RMSD) of C_{α} atoms of the protein was calculated from the initial structure (homology model), as function of time. Concerning the Fig. 4, it looks that in all systems, after about 7000 ps, the RMSD equilibrated and then oscillated around the mean value, which indicated the reliability of the selected force field and the sufficiency of the applied timescale for investigation of the binding

mode. However, the smooth and continuous increase in the RMSD after that could be attributed to the large size of the systems, the length of simulations and the homology modelling. The mean RMSD values of receptor backbone atoms were 0.24 ± 0.02 , 0.33 ± 0.05 , 0.26 ± 0.03 and 0.32 ± 0.04 nm for free and bound receptor with NOR, AA-AD and AA-NOR, respectively. The higher RMSD value of bound receptor represents the un-stabilizing effect of the ligands on the receptor during the binding process. The RMSD results, plus the observation of the geometry in the last frame of the simulation revealed the maintenance of the TM helices and protein orientation within the lipid bilayer during the simulation.

Fig. 4. RMSD plots of $\alpha 2C$ receptor in free and bound states with NOR, AA-AD and AA-NOR. The higher RMSD value of bound receptor represents the un-stabilizing effects of the ligands on the receptor during the binding process.

One of the characteristics of the inactive state of GPCRs is the presence of the ionic-lock salt bridge between the highly conserved D(E)R^{3.50}Y motif in TM3 and an E/D^{6.30} residue in TM6[28] (Ballesteros-Weinstein numbering system is in superscripts[30]). These residues correspond to the ARG149 (D3.50) and GLU377 (E6.30) in the $\alpha 2C$ receptor. This ionic lock has been observed in

1 the last step of the apo-protein simulation and presented in Fig. 5. The estimated mean value of the
 2 ARG149-GLU377 distance during the MD simulation was 3.8 Å. However, the highest and lowest
 3 values of this distance were 6.9 Å and 3.6 Å, which refer to the first (homology model structure)
 4 and last steps of simulation, respectively. This finding is consistent with the inactive state of ligand-
 5 free basal-state of ARs, therefore, it may be concluded that in the apo-protein simulation,
 6 configuration of the receptor goes toward the inactive state in the applied force field.

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 8 **Fig. 5.** The ionic lock between ARG149 and GLU377 in the last step of simulation of the α 2C
 9 receptor in ligand-free state. The mean value of the ARG149-GLU377 distance has been estimated
 10 3.8 Å during the MD simulation.

11
 12 In the first step of the bound protein studies, the ligand's RMSD during the binding process has
 13 been calculated and depicted in the Fig. 6. With respect to this figure, the RMSD value for all
 14 ligands converged and equilibrated after 12 ns, and then fluctuated around the mean value of 0.07,
 15 0.26 and 0.21 Å for NOR, AA-AD and AA-NOR, respectively. As all the ligands have the same
 16 binding site and molecular charge, the less RMSD value of the NOR could be related to its smaller
 17 molecular size and lower flexibility.

Fig. 6. The RMSD values of ligands in the binding site of $\alpha 2C$ receptor with respect to its starting structure, obtained from docking simulation. The smaller RMSD value of the NOR could be attributed to its smaller molecular size and lower flexibility.

Because the binding mode prediction of Autodock is based on rigid docking, the docking model excludes conformational changes caused by binding interactions. However, the conformational changes of the key residues in the binding pocket, during the binding is reasonable. In order to depth investigation of the fluctuations and local motilities in the protein during the binding and activation processes, the root mean square fluctuations (RMSF) of free and bound receptor with NOR, were calculated. The results have been presented in Fig. 7. The conformational changes of TM3, TM5, TM6 and TM7 helices that are in close contact to the binding pocket of the bound receptor, are clearly observed in this figure. The higher value of the RMSF of the bound receptor in these region implies the movement of the involved helices during the binding of NOR, and the opening of the binding pocket to translocation of the ligand. This fact is in agreement with the increase of the backbone RMSD value in the presence of the ligands and proposes the conformational changes of the protein from the inactive state to the active state.

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Fig. 7. RMSF of $\alpha 2C$ receptor residues respect to their time-averaged positions for free and bound states with NOR. RMSF value of the bound receptor has remarkably increased in the helices in close contact to the binding pocket, namely TM3, TM5, TM6 and TM7.

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The existence of salt bridge between the ARG149 and GLU377 (basal-state characteristic) was examined in the bound receptor in the next step. The MD simulation results showed the lack of such ionic lock salt bridge in the bound receptor. ARG149–GLU377 distance in the bound $\alpha 2C$ with NOR, AA-AD and AA-NOR, obtained 11.5, 5.6 and 7.4 Å, respectively. Hence, the disappearance of this bridge may be considered as the initial step of the receptor activation and subsequent opening of the hydrophobic binding pocket.

As mentioned, one of the features of the active state of the ARs is the presence of the salt bridge between the catechol group of the agonists and D3.32 (ASP) residue of the receptor. Molecular docking simulations (Fig. 3) did not predict any salt bridge between these two groups. Distance distribution of ASP131 oxygen and amine hydrogen of the catechol group of the ligands has been shown in the Fig. 8. As shown in this figure, this distance decreased as the simulation time increased, and receded from the predicted value from docking, for all three ligands. The mean value of the bridge length in $\alpha 2C$ -NOR, $\alpha 2C$ -(AA-AD) and $\alpha 2C$ -(AA-NOR) complexes were 3.7, 1.9

1 and 3.4 Å, respectively. The orientation of the ligands in the binding site and the formation of salt
2 bridge in the last step of simulation, have been presented in the Fig. 9. As this bridge is one of the
3 most important feature of active ARs, it could be concluded that the AA-AD and AA-NOR are
4 agonists of the $\alpha 2C$, as like as NOR.

Fig. 8. Distance distribution of ASP131 and catechol group of the ligands during the 50 ns simulation. The mean length value of this bridge in $\alpha 2C$ -NOR, $\alpha 2C$ -(AA-AD) and $\alpha 2C$ -(AA-NOR) complexes were 3.7, 1.9 and 3.4 Å, respectively.

Fig. 9. The ligand orientation in the binding site and the formation of salt bridge in the last step of simulation for the binding of a) NOR, b) AA-AD, and c) AA-NOR to the $\alpha 2C$ receptor.

The presence of H-bonds between receptor and ligands were calculated every 100 ps using the g_hbond module of GROMACS. It was found that the NOR formed three H-bonds with SER214 and SER 218 in the initial steps of the simulation. The H-bond with SER218 vanished after 20 ns. However, the loss of this H bond was compensated by the formation of the other H bonds between the NOR and ASP206 and TYR191 residues which were significantly preserved in the remaining simulation time. For the AA-AD and AA-NOR compounds, H-bond with SER218 disappeared in the 12 and 15 ns of simulation, respectively, and instead, some H-bonds with ASP206 and TYR405, in AA-AD, and ASP206, TYR210 and CYS135, in AA-NOR, were formed and conserved in the rest of the simulation time. These results imply the importance of SER214 and ASP206 in the binding and activation processes.

The results of molecular dynamics simulation revealed the closing of the binding pocket in the basal state, due to the formation of the salt bridge between the ARG and GLU. Although, agonist binding to the pocket induces the conformational changes, disappearance of the salt bridge, movement of TM3, TM5, TM6 and TM7 helices and subsequent opening of the ligand binding-pocket. It was concluded that the electrostatic interactions and H-bonds play an important role in the agonist binding process. Loss of the ARG–GLU salt bridge and the formation of the ionic lock between the ligand and ASP131, have been observed in both investigated derivatives. Hence, the both arachidonic based compounds may be considered as the α 2C agonists. Future studies such as drug design cases would help to further verification of the model, and identification of the mechanism.

4. Conclusions

We built the α 2C model based on reported GPCR crystal structures. The model constructed from the β -AR structures in various states and environments. The predicted structure of receptor was found to be trustworthy with about 98.5% of the residues falling within the favored and

1 additional allowed regions of the Ramachandran plot. The model quality also, was further refined
2 and established through MD simulations. The simulations proved the stability of receptor structure
3 in an explicit lipid bilayer environment over the period of the performed simulations, and the
4 conservation of helical conformations in parallel orientation with lipid bilayer.

5 Molecular dockings of the NOR, AA-AD and AA-NOR ligands with the receptor model were
6 carried out to find the ligand-receptor complex that was most consistent with the known
7 experimental data. The results predicted the same binding site for all three ligands to the $\alpha 2C$
8 receptor which was surrounded by TM3, TM5, TM6 and TM7 helices. The analysis of binding site
9 residues indicated that three compounds is enclosed by the following hydrophobic and hydrophilic
10 residues: 127, 131 and 132 of TM3, 210, 214, 215 and 218 of TM5, 392 and 402 of TM6 and 423,
11 424 and 427 of TM7. Binding energy of NOR to the receptor was found to be -25.11 kJ/mol which
12 was in good agreement with the experimental data, and further validation of the built model. The
13 higher binding affinity of the AA-AD and AA-NOR to the receptor, could be related to the more
14 hydrophobic interactions of arachidonic side chain.

15 A 50 ns MD simulation was then performed to investigate the interactions between the $\alpha 2C$ model
16 and the synthetic and endogenous ligands, to confirm the main binding mode, to study the effect of
17 ligands in the activated conformation of the receptor, and the time evaluation of the binding
18 process. By analyzing the results of apo-protein MD simulation, it was found that the ionic lock salt
19 bridge between the ARG in TM3 and GLU in TM6 is the characteristics of the inactive receptor.
20 The disruption of this ionic lock, which induces the TM movements and opening the binding
21 pocket would be a possible suggestion for the launch of receptor activation. The formation of new
22 lock between the ligands and ASP131 of TM3 and the establishment of some H-bonds with the
23 SER214 and ASP206, which is favored by the helices motions, led to the transition of inactive
24 receptor to active state.

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6. References

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