RESEARCH ARTICLE

Comparative MD Simulations Indicate a Dual Role for Arg1323.50 in Dopamine-Dependent D2R Activation

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Abstract

Residue Arg3.50 belongs to the highly conserved DRY-motif of class A GPCRs, which is located at the bottom of TM3. On the one hand, Arg3.50 has been reported to help stabilize the inactive state of GPCRs, but on the other hand has also been shown to be crucial for stabilizing active receptor conformations and mediating receptor-G protein coupling. The combined results of these studies suggest that the exact function of Arg3.50 is likely to be receptor-dependent and must be characterized independently for every GPCR. Consequently, we now present comparative molecular-dynamics simulations that use our recently described inactive-state and Go-bound active-state homology models of the dopamine D2 receptor (D2R), which are either bound to dopamine or ligand-free, performed to identify the function of Arg1323.50 in D2R. Our results are consistent with a dynamic model of D2R activation in which Arg1323.50 adopts a dual role, both by stabilizing the inactive-state receptor conformation and enhancing dopamine-dependent D2R-G protein coupling.

Introduction

Residue Arg3.50 (the superscript refers to the generic Ballesteros-Weinstein numbering[1]) belongs to the DRY-motif of G protein-coupled receptors (GPCRs), which is located at the base of transmembrane helix (TM) 3, and had been suggested to play a vital role in regulating the structure and function of these receptors.[2] The individual residues of the DRY-motif, aspartate (or glutamate), arginine and tyrosine, are highly conserved among class A GPCRs; their degree of conservation is 67.9% (D)/22.8% (E), 96.8% and 71.5%, respectively (calculated by means of the GMOS web interface, http://lmc.uab.cat/gmos/gmos.php).

Arg3.50 is part of the so-called ionic lock, an intramolecular ionic interaction between Arg3.50 of TM3 and Glu6.30 of TM6, which was originally observed in the crystal structure of dark-state rhodopsin.[3] As alanine mutation of Glu6.30 has been shown to enhance constitutive activity at the β2 adrenergic receptor (β2AR), the ionic lock was suggested to help stabilize...
the ground state of GPCRs. With the exception of rhodopsin, the dopamine D3 receptor (D3R) [6] and distinct β1 adrenergic receptor (β1AR)-ligand combinations, [7] an intact ionic lock is not observed within the majority of currently available crystal structures. However, several independently performed molecular-dynamics (MD) simulations have reported the highly dynamic nature of this interaction, with an equilibrium between intact and broken ionic-lock conformations, which is likely to reflect the basal activity of non-rhodopsin GPCRs. [5, 8–10] The existence of open and closed states of the ionic lock, connected to different conformations of TM6, had been supported by crystal structures of β1AR. [7] Nevertheless, only 34% (calculated by means of the GMOS web interface, http://lmc.uab.cat/gmos/cgmos.php) of class A GPCRs exhibit both an arginine and a glutamate residue at positions 3.50 of TM3 and 6.30 of TM6, respectively. This suggests that an intact ionic lock may not be the only determinant that stabilizes inactive-state GPCRs. Thus, it was not possible to reduce the pronounced constitutive activity of the wild-type histamine H4 receptor (H4R), which has an alanine residue in position 6.30, when trying to reconstitute the possibility of forming an ionic interaction to Arg3.50 using an Ala6.30Glu mutant receptor. [11]

Besides its (possible) contribution to the basal signaling profile of GPCRs, the crystal structure of opsin in complex with the C-terminal fragment of transducin revealed hydrogen bonds between the side chain of Arg3.50 and that of Tyr3.58 of TM5. Arg3.50 also hydrogen bonds to the backbone carbonyl atom of Cys347 of the G protein, thus attributing a key role to Arg3.50 in stabilizing active-state GPCR conformations and mediating receptor-G protein interactions. [12] Based on this structure, we recently performed computational studies on β2AR together with the C-terminal fragment of Gαs, in which direct interactions between Arg3.50 and residues of the G protein could be observed. [13] The crystal structure of β2AR coupled to the heterotrimeric Gs protein confirmed such direct interactions: the side chain of Arg3.50 was found to pack against Tyr391 of Gαs. [14] In addition, MD simulations on our previously developed homology model of the dopamine D2 receptor (D2R)-Gαi complex indicated an ionic interaction of Arg1323.50 and a C-terminal residue Asp350 of Gαi. [15] This ionic interaction was found to persist in the presence of the full agonist dopamine, but to be destabilized by aripiprazole-type partial agonists. [16] In agreement with these studies, different groups have reported reduced or abolished G-protein activation when Arg3.50 of wild-type receptors is mutated to alanine, including D2R, [17] rhodopsin [18] and H4R. [11] In addition, it was shown that mutations of Arg3.50 that cause a loss of the capacity to couple to or to activate G proteins can culminate in diseases such as autosomal dominant retinitis pigmentosa (ADRP), [18] nephrogenic diabetes insipidus [19] or hypogonadotropic hypogonadism. [20] However, no unified picture of the influence of Arg3.50 on G protein activation can be generated, as, for example, different mutations of Arg3.50 at β2AR were connected to an unchanged ability to activate Gαs (even if the capacity to recruit β-arrestin was reduced for the Arg3.50Ala mutant). [5, 21] A more detailed discussion of the effect of distinct Arg3.50 mutations at different receptors is provided in the literature. [2]

Taken together, these results suggest that the exact function of Arg3.50 is likely to be receptor-dependent and must be characterized independently for every GPCR. As (I) D2R exhibits both residues Arg1323.50 and Glu3686.30, and is thus, in principal, competent to form an ionic lock interaction, and (II) previous studies on the (dopamine-bound) D2R-Gαi complex suggested a direct ionic interaction between Arg1323.50 and the G protein, [15, 16] we chose to investigate these interactions for inactive- and active-state D2R conformations. Therefore, while taking advantage of the recent developments in the structural determination of GPCRs, a comparative analysis of MD simulations that use our inactive-state [22] and active-state homology models of D2R, [15] both bound to dopamine or ligand-free (apo), was performed to identify the function of Arg1323.50 at D2R.
Results/Discussion
Stability of the Simulation Systems

Eleven individual long-term MD simulations were performed on homology models of D2R, which were either coupled to dopamine, (and/or) Goα, or did not contain an additional binding partner (Fig 1). Data derived from previous MD simulations on a dopamine-bound D2R-Goα complex were used for comparison (system D1). The overall conformational stability of the different complexes was found to be sufficient for subsequent analyses, as indicated by RMSD analysis of the individual members of the simulation systems (S1 Fig), which did not undergo destructive conformational changes that affected the integrity of the complexes. Within the Goα-bound systems (complexes C1, C2 and D2), higher mobility was observed for Goα than for the receptor (in particular, the helical subdomain of the Goα-subunit (GoαAH), S2 Fig), which is in agreement with our previous studies on ternary complexes,[15, 16] and, as previously, Goα did not show any tendency to separate from active-state D2R (S3 Fig). Importantly, the global conformational state of the receptors (either inactive- or active-state like) did not change throughout the simulation time, as determined by measuring the distances between the intracellular tips of TM3 and TM6 (Fig 2). In the course of this study, the active-state of D2R is characterized by the outward movement of TM6 and the presence of the Goα-subunit of the G protein (systems C and D), whereas the inactive-state systems lack the latter features (systems A and B). Visual comparison of several overlaid average structures of systems A-D derived from different time windows along the simulation pathways indicated that the presence of dopamine in the systems B and D was associated with a reduced mobility of extracellular receptor domains compared to the apo-simulations. This stabilizing effect was significantly more pronounced in the active-state simulations C and D (S4 Fig). Moreover, the presence of dopamine was found to increase the conformational stability of the outward movement of TM6 in the absence of the G protein (S5 Fig).

Analysis of Dopamine Binding at Inactive- and Active-State D2R Models

Within the dopamine-bound systems B and D, dopamine was found to occupy, as expected, the same orthosteric binding pocket throughout the MD simulations in both the inactive- and active-state D2R, and to adopt a similar conformation therein (Fig 3A and 3B). The conformation of dopamine is stabilized by hydrogen bonds between its catechol moiety and Ser1935.42 and Ser1975.46 of TM5 and His3936.55 of TM6 (not shown), all of which are in agreement with previous studies reporting their importance for the binding of dopamine.[23, 24] In addition,
the canonical salt bridge between the protonated amine moiety of dopamine and Asp1143.32 of TM3 was formed persistently, an interaction that has been shown to be an irreplaceable prerequisite for specific ligand binding at dopaminergic receptors.\[25\] Although the presence of G\( \alpha_i \) did not significantly alter the nature and occurrence of intermolecular interactions between dopamine and D2R relative to inactive-state D2R, a slightly reduced dopamine mobility and an increase of 4.8 kcal/mol in its binding energy were observed (Fig 3A and 3B, S6 Fig). These observations are obviously the consequence of different shapes of the extracellular surface above the binding pocket of D2R (measured as the distance between Ile183 of extracellular loop 2 (EL2) and Tyr4087.35 of TM7, S7 Fig). A persistently closed conformation around the agonist dopamine was found in simulations of the fully active ternary signaling complex, thus facilitating the stabilization of dopamine (Fig 3C and 3D). A closed structure above the binding pocket of dopamine was originally observed in previous simulations of system D1,\[16\] and could now be confirmed by an additional MD simulation (system D2). Increased distances between EL2 and the upper part of TM7 associated with an open binding pocket to the extracellular surface were observed in both apo-D2R simulations (S4 and S7 Figs). The observation that neither the presence of dopamine (system B) nor of G\( \alpha_i \) alone (system C, representing the basally-active signaling state of D2R) were sufficient to result in a persistent and stable
contraction of extracellular domains near the binding pocket supports observations that both agonists and an intracellular binding partner are required to capture fully active-state conformations of GPCRs,[26, 27] including those of active-state binding pockets. However, the possibility that such contractions would eventually be triggered on much longer time-scales cannot be excluded.

### Dopamine-Binding at Inactive-State D2R Reduces the Stability of the Ionic Lock

The overall aim of this study was to identify the function of Arg132<sup>3.50</sup> in the pharmacologically relevant D2R, which includes in particular the investigation whether or not an intramolecular ionic interaction to Glu368<sup>6.30</sup> at the inactive-state receptor can be formed, thus stabilizing the ground-state of D2R, and whether this ionic interaction can be modulated by the presence of the endogenous agonist dopamine. Consequently, this chapter will focus on the analysis of 4 µs MD simulations at inactive-state D2R, which were performed under two different conditions: ligand-free (apo, system A) or bound to dopamine (system B).
In general, our results indicate that the inactive-state of D2R is able to adopt both formed (= closed) and broken (= open) conformations of the ionic lock, which were found to exist in dynamic equilibrium with each other (Fig 4A). These observations are in excellent agreement with previous MD simulations on closely related adrenergic receptors, which had reported alternately open and closed conformations of the ionic lock.[5, 8, 9] At β1AR, it was even possible to crystallize the different states of this motif, where such structural plasticity of intracellular receptor domains had been suggested to be a general feature of non-rhodopsin GPCRs, which exhibit varying capacities for ligand-independent signaling (also referred to as basal activity).[7] The evolution of side-chain distances between Arg132\(^{3.50}\)(C\(_{\zeta}\)) of TM3 and Glu368\(^{6.30}\)(C\(_{\delta}\)) of TM6 revealed that in MD simulations of the apo D2R-system A (representing the ligand-free ground state of D2R), the formation of an intact ionic lock between these residues is highly favored (Fig 4B). Thus, in the absence of an agonist, the ionic lock was closed most of the time, which is likely to help stabilize the inactive, ground state conformation of D2R. The latter assumption is supported by previous MD simulations studies on both carazolol-bound and apo β2AR, demonstrating that the extent of ionic-lock formation in the presence of the inverse agonist carazolol, which is known to stabilize the inactive-state of β2AR, is unchanged when compared to the ligand-free β2AR system.[8] In contrast, we observed that the presence of the endogenous agonist dopamine (system B) significantly reduced the occurrence of an intact ionic lock (Fig 4C), which is likely to result in an impaired capacity of this intramolecular interaction to stabilize the inactive-state of D2R. A comparable, agonist-dependent decreasing effect on the frequency of ionic-lock conformations was also suggested by MD simulations at the 5-HT\(_{2A}\) receptor.[10] In addition to the enhanced probability of encountering a broken ionic lock, we found that in the presence of dopamine, conformations featuring distances larger than 9.5 Å between the intracellular ends of TM3 and TM6 (measured as the C\(_{\alpha}\)-distance of Arg132\(^{3.50}\) and Glu368\(^{6.30}\), Fig 2), were increased compared to the ligand-free

![Fig 4. Distances of ionic lock residues at the inactive-state systems A and B.](https://example.com/fig4.png)

(A) Close view on representative conformations of an intact (= closed, grey) and a broken (= open, orange) ionic lock between residues Arg132\(^{3.50}\) of TM3 (light-blue) and Glu368\(^{6.30}\) of TM6 (dark-blue). In addition, Arg132\(^{3.50}\) is stabilized by Asp131\(^{3.49}\) of TM3. (B, C) Distances between the side chains of residues Arg132\(^{3.50}\)(C\(_{\zeta}\)) and Glu368\(^{6.30}\)(C\(_{\delta}\)) in the course of the simulations A and B are shown. Cumulative occurrences of certain distances for system A (B) predominantly show distances, which are consistent with an intact ionic lock (green boxes). In contrast, the latter distances are less frequently populated at the dopamine-bound system B (C), when higher occurrences were observed for larger distances, consistent with an open ionic lock.

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system A (Fig 5). Distances larger than 9.5 Å were previously shown to be associated with broken ionic lock conformations by MD simulations and crystal structures.[8, 9] The separation of TM3 and TM6 is associated with an outward movement of TM6, which represents a major hallmark of GPCR activation.[28] However, additional simulations are needed to increase the statistical significance of the result shown in Fig 5.

Taken together, our results are consistent with a structural mechanism of dopamine-dependent D2R activation, by which the agonist dopamine reduces the stability of the ionic lock, thereby reducing the global conformational stability of the inactive-state of D2R, and thus increases the probability for an outward movement of TM6, which finally facilitates receptor activation. It is important to note that even in the absence of dopamine (system A), we detected open ionic lock conformations and a minor fraction of TM6 showing limited outward movement, both of which are consistent with the basal activity profile of D2R. Although we are aware that there may also be other intramolecular interactions that stabilize the inactive-state of D2R, it is tempting to assume that the breakage of the ionic lock is one crucial and necessary prerequisite in the activation process of D2R.

**Dopamine Binding at Active-State D2R-Gαi Complexes Triggers the Formation of an Ionic Interaction between D2R and Gαi via Arg132^3.50**

Crystal structures of opsin and β2AR coupled to the C-terminal fragment of transducin and the natural Gs protein, respectively, revealed direct interactions of receptors and G proteins, which were, among others, mediated by residue Arg^3.50 of the receptors.[12, 14] The structures supported various experiments employing Arg^3.50 receptor mutants, which had attributed a key role to this residue in maintaining the active-state of a GPCR.[2] In addition, by using long-term MD simulations on a dopamine-bound ternary D2R-Gα complex model based on the crystal structure of β2AR coupled to Gαs,[15] we previously detected a consistent ionic interaction between Arg132^3.50 of D2R and Asp350 of Gαi, which we suggested to help stabilize receptor-G protein coupling.[16] The latter observation is supported by experiments using an

![Fig 5. Total occurrences of distances larger than 9.5 Å between Arg132^3.50 and Glu368^6.30 at the simulation systems A and B. The fractions of simulation time within the systems A and B, in which the distances between the Cα-atoms of Arg132^3.50 and Glu368^6.30 were found to be larger than 9.5 Å. The values above the bars represent mean ± standard error of the mean of the simulation systems A and B and indicate a higher frequency of distances larger than 9.5 Å in the presence of dopamine (unpaired t-test, two-tailed P value = 0.0960).](image-url)
Arg1323.50Ala mutant of D2R, which completely lost the capacity to activate G proteins upon agonist stimulation.[17] As the final part of this study, which was designed to investigate the function of Arg1323.50 in both inactive- and active-state conformations of D2R, we now focus on the analysis of the structural properties of this latter ionic interaction, employing a total of 3.9 μs MD simulations performed on either dopamine-bound (system D) or ligand-free (system C) D2R-Gαi models.

We detected the formation of a consistent ionic interaction between Arg1323.50 of D2R and Asp350 of Gαi after approximately 200 ns in the presence of dopamine. This was further corroborated by an additional MD simulation run on the same dopamine-bound ternary D2R-Gαi complex (system D2), in which this ionic interaction was reproduced and remained, in both cases, stable for most of the simulation time (Fig 6B). In the absence of dopamine (system C), increasing distances between the corresponding residues Arg1323.50 of D2R and Asp350 of Gαi were observed in two independent simulation runs on the same apo D2R-Gαi complex, indicating that the aforementioned ionic interaction can hardly be formed in the ligand-free, basally active-state of D2R (Fig 6A). It is thus tempting to speculate that Arg1323.50 may play a crucial role in mediating a ligand-induced increase in G protein activation.

Interestingly, these results show that a valid answer whether or not an ionic interaction between Arg1323.50 and Asp350 can be formed in the particular simulation systems C1 and C2 does not become evident before a certain “induction period”, in these large systems of more than 200,000 atoms at least 200 ns (Fig 6). This is in line with ten individual MD simulations on system C (systems C3 to C12), each using the same configuration than C1 and C2, randomly attributed initial velocities and each lasting 100 ns, which do not show a clear tendency of the system to form an ionic interaction between Arg1323.50 and Asp350 (S8 Fig). These

Fig 6. Time evolution of the ionic interaction between D2R and Gαi at the active-state systems C and D. Representative snapshots of ligand-free (A, top line) and dopamine-bound (B, top line) active-state D2R conformations, showing an either broken or formed ionic interaction between residues Arg1323.50 of D2R and Asp350 of Gαi, respectively. In addition, the time evolution of distances between Arg1323.50 (Cy) of D2R and Asp350 (Cy) of Gαi in simulation systems C (A, bottom line) and D (B, bottom line) are depicted, revealing that distances, which allow the ionic interaction between D2R and Gαi (green boxes) are only formed in the presence of dopamine.

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observations support previous studies,[13, 15, 16, 29] in which our group has performed few long simulations, rather than multiple shorter ones, in order "to avoid missing conformational changes that occur with a characteristic induction period".[16]

In summary, our analyses of MD simulations on the active-state systems C and D demonstrate that the ionic interaction between D2R and Goi strongly depends on the presence of an agonist like dopamine. Only in the presence of dopamine (system D, Fig 6B) does this interaction remain stable throughout the simulation time. We previously observed a reduced stability of this particular ionic interaction once the full agonist dopamine was replaced by aripiprazole-type partial agonists.[16] As agonists are known to enhance the capacity of GPCRs to activate G proteins according to their distinct intrinsic efficacies,[30] we suggest that, at least in the case of the D2R-Goi complex, an agonist-dependent increase in the capacity of D2R to form an ionic interaction to Goi via Arg1323.50 provides one structural explanation for the question as to how this enhanced activation can be accomplished.

**Conclusion**

To identify the function of Arg1323.50 at D2R in terms of forming the intramolecular ionic lock between TM3 and TM6 and an ionic interaction to the G protein, comparative MD simulations on both inactive-state and Goi-bound active-state D2R models, which were either ligand-free or bound to the endogenous agonist dopamine (Fig 1), were used.

Within the dopamine-bound systems B and D, our MD simulations detected different conformations of the extracellular surface above the binding pocket adopting a closed conformation in the fully activated Goi-bound system D (Fig 3), although the interactions of dopamine with residues of D2R were comparable for active- and inactive-state D2R. As a result of MD simulations on the inactive-state of D2R, we found that dopamine was able to attenuate ionic-lock formation between Arg1323.50 of TM3 and Glu3686.30 of TM6 compared to the ligand-free system (Fig 4). As a consequence, higher occupancies of increased distances between the intracellular ends of TM3 and TM6 were recorded in the presence of dopamine, which are consistent with a more pronounced outward movement of TM6 (Fig 5). Both of these observations are in full agreement with the ability of dopamine to trigger D2R activation. Conducting MD simulations that used the D2R-Goi complexes, we observed a consistent ionic interaction between Arg1323.50 of D2R and Asp350 of Goi in the presence of dopamine, which was not formed within the apo simulation (Fig 6), and which we believe is one structural explanation for an enhanced G protein activation of agonist-bound D2R.

Taken together, our results lead to a model of D2R activation, in which Arg1323.50 participates in this process by adopting a dual role, both by the stabilization of the inactive-state receptor conformation and by enhancing dopamine-dependent D2R-G protein coupling (Fig 7). Although it is still not possible to capture the activation process of GPCRs in a single trajectory using classical MD simulations, this study provides a firm, dynamic model for dopamine-dependent D2R activation.

**Materials and Methods**

A schematic overview of the simulation systems and their simulation times is shown in S1 Table. In general, comparative MD simulations on either dopamine-bound or ligand-free homology models of inactive-state D2R and of the active-state D2R-Goi complex, which were based on the crystal structures of D3R[6] (PDB-ID: 3PBL) and β2AR-Goi[14] (PDB-ID: 3SN6), respectively, were performed. The homology models were generated as described previously for inactive-state D2R[22] and the active-state complex.[15] Docking of dopamine was performed manually as described.[15] The systems A, B, and C were submitted to twenty
independent MD simulation runs ranging from 100 ns to 750 ns, and complemented by one 
additional simulation run of 400 ns for complex D2 (Fig 1). The systems E and F were gener-
ated by removing Gαi from the final snapshots of the simulation systems C1 and D2, respec-
tively. Subsequently, two independent simulation runs for system E (850 ns and 700 ns) and F 
(1050 ns and 1100 ns) were performed. All systems were embedded in a lipid bilayer consisting 
of dioleoylphosphatidylcholine (DOPC) molecules as described. For comparison, the 
results of previously published long-term MD simulations on the D2R-Gαi complex in pres-
ence of the full agonist dopamine were used (simulation D1).

To carry out MD simulations, the GROMACS simulation package was used as described 
previously. Briefly, the general AMBER force field (GAFF) was used for dopamine and 
the lipids and the AMBER force field f99SB for D2R and Gαi. The SPC/E water model was 
used, and the simulations were carried out at 310 K. In the absence of Gαi, no external 
force was applied (systems A and B); in contrast, a stabilizing force (1.0 kcal mol⁻¹ Å⁻²) was 
applied to the N-terminal tail of the αN-helix of Gαi (systems C and D2). We removed water 
and DOPC molecules for data analysis. The analysis of the trajectories was performed with the 
PTRAJ module of AMBER10. Figures were prepared using PyMOL and Chimera.

Supporting Information

S1 Fig. RMS-deviations of the simulation systems. RMSD analyses in the course of the simu-
ation times for individual components of the systems A (A), B (B), C (C) and D (D) are 
shown, revealing, in general, stable simulation systems. Dopamine and D2R are fitted on the 
Cα-atoms of D2R, whereas Gαi is fitted on the Cα-atoms of Gαi. For the D2R-Gαi complexes 
(C and D), coordinates are fitted on the Cα-atoms of D2R.

S2 Fig. Atomic fluctuations within the simulation systems C1, C2 and D2. Atomic fluctua-
tions for the Cα-atoms of the systems C1 (A), C2 (B) and D2 (C) are shown, which had been 
calculated as B-factors. The values are measured based on a fit to the Cα-atoms of the D2R-part of 
the complexes.
S3 Fig. Distances between D2R and the C-termini of Gαi for the systems C1, C2 and D2.
Distances between the centers of mass of D2R and the C-termini of Gαi for the simulation systems C1 and C2 (A) and D2 (B) are shown.
(TIFF)

S4 Fig. Overlay of average structures of the simulation systems focusing on the extracellular domains of D2R.
Top view on the extracellular surface of the D2R units. For clarity, TM3 is shown in blue. The average structures are calculated after the time points concretized above. Dopamine (orange, right row) was found to stabilize extracellular receptor domains compared to the apo systems (left row), which is more pronounced at active-state D2R (bottom line). Residues of D2R (Ile183EL2 and Tyr4087.35) forming a lid over the binding pocket in system D are highlighted in red.
(TIFF)

S5 Fig. Evolution of distances between TM3 and TM6 at the simulation systems E and F.
The distances between the intracellular ends of TM3 and TM6 (measured as the distances between the Cα-atoms of Arg1323.50 and Glu3686.30) are shown for system E (A) and system F (B). Mean values derived from the corresponding distances at simulation systems A-D are highlighted with dashed lines. In both systems E and F, the G protein was removed. Our results indicate a higher stability of the outward movement of TM6 in the presence of dopamine.
(TIFF)

S6 Fig. Atomic fluctuations and free energy of binding for dopamine at the simulation systems B and D.
(A) Atomic fluctuations (calculated as B-factors after a fit on the Cα-atoms of the coordinates of D2R) for the dopamine-bound systems B and D are shown. The values above the bars represent the average fluctuation of the individual simulations 1 and 2. (B) Free energy of binding calculations were performed for dopamine-binding at the simulation systems B and D using the GBSA-Method. The numbers below the bars represent average values of each simulation system and indicate an increased binding energy of 4.8 kcal/mol in the presence of Gαi (system D). The values of the individual bars are as follows (given in kcal/mol): -15.4 ± 3.9 for B1, -18.8 ± 3.9 for B2, -16.9 ± 2.8 for B3, -15.5 ± 4.1 for B4, -20.9 ± 3.0 for D1 and -22.0 ± 2.7 for D2.
(TIFF)

S7 Fig. Overview of the distances between Ile183 of EL2 and Tyr4087.35 of TM7.
The calculated distances between the side chain atoms of Ile183EL2 and Tyr4087.35 for the simulation systems A and C (A) and B and D (B) are shown. An interaction between these residues is only present in simulations of the systems D1 and D2.
(TIFF)

S8 Fig. Evolution of distances between the side chains of Arg1323.50 and Asp350 at the simulation systems C3 to C12.
The distances are highly flexible and do not offer a valid answer whether or not the ionic interaction between these residues is present at simulation system C.
(TIFF)

S1 Table. Overview of all simulation systems used within this study.
(DOCX)

Author Contributions
Conceived and designed the experiments: RCK TC PG. Performed the experiments: RCK. Analyzed the data: RCK TC PG. Wrote the paper: RCK TC PG.
References

1. Ballesteros JA, Weinstein H. Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors. Receptor Molecular Biology. 1995; 25:366–428.

2. Rovati GE, Capra V, Neubig RR. The highly conserved DRY motif of class A G protein-coupled receptors: beyond the ground state. Molecular pharmacology. 2007; 71(4):959–964. Epub 2006/12/29. doi: 10.1124/mol.106.029470 PMID: 17192495.

3. Palczewski K, Kumasaka T, Hori T, Behlke MA, Motoshima H, Fox BA, et al. Crystal structure of rhodopsin: A G protein-coupled receptor. Science. 2000; 289(5480):739–745. Epub 2000/08/05. PMID: 10926528.

4. Ballesteros JA, Jensen AD, Liapakis G, Rasmussen SG, Shi L, Gether U, et al. Activation of the beta 2-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. J Biol Chem. 2001; 276(31):29171–29177. Epub 2001/05/29. doi: 10.1074/jbc.M103747200 PMID: 11375997.

5. Valentini-Hansen L, Groenen M, Nygaard R, Frimurer TM, Schwartz TW. The arginine of the DRY motif in transmembrane segment III functions as a balancing micro-switch in the activation of the beta 2-adrenergic receptor. The Journal of biological chemistry. 2012; 287(38):31973–31982. Epub 2012/07/31. doi: 10.1074/jbc.M112.348565 PMID: 22843684; PubMed Central PMCID: PMC3442529.

6. Chien EY, Liu W, Zhao Q, Katritch V, Han GW, Hanson MA, et al. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. Science. 2010; 330(6007):1091–1095. Epub 2010/11/26. doi: 10.1126/science.1197410 PMID: 21097933; PubMed Central PMCID: PMC3058422.

7. Moukhametzianov R, Warne T, Edwards PC, Serrano-Vega MJ, Leslie AG, Tate CG, et al. Two distinct conformations of helix 6 observed in antagonist-bound structures of a beta 1-adrenergic receptor. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(20):8228–8232. doi: 10.1073/pnas.1100185108 PMID: 21540331; PubMed Central PMCID: PMC3100933.

8. Dror RO, Arlow DH, Borhani DW, Jensen MO, Piana S, Shaw DE. Identification of two distinct inactive conformations of the beta2-adrenergic receptor reconciles structural and biochemical observations. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106(12):4689–4694. Epub 2009/03/05. doi: 10.1073/pnas.0811065106 PMID: 19258456; PubMed Central PMCID: PMC2650503.

9. Vanni S, Neri M, Tavernelli I, Roethlisberger U. Observation of "ionic lock" formation in molecular dynamics simulations of wild-type beta 1 and beta 2 adrenergic receptors. Biochemistry. 2009; 48(22):4789–4797. doi: 10.1021/bi900299f PMID: 19378975.

10. Shan J, Khelashvili G, Mondal S, Mehler EL, Weinstein H. Ligand-dependent conformations and dynamics of the serotonin 5-HT(2A) receptor determine its activation and membrane-driven oligomerization properties. PLoS computational biology. 2012; 8(4):e1002473. doi: 10.1371/journal.pcbi.1002473 PMID: 22532793; PubMed Central PMCID: PMC3330085.

11. Schneider EH, Schnell D, Strasser A, Dove S, Seifert R. Impact of the DRY motif and the missing "ionic lock" on constitutive activity and G-protein coupling of the human histamine H4 receptor. The Journal of pharmacology and experimental therapeutics. 2010; 333(2):382–392. Epub 2010/01/29. doi: 10.1124/jpet.109.163220 PMID: 20106995.

12. Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, et al. Crystal structure of opsin in its G-protein-interacting conformation. Nature. 2008; 455(7212):497–502. Epub 2008/09/27. doi: 10.1038/nature07330 PMID: 18818650.

13. Goetz A, Lanig H, Gmeiner P, Clark T. Molecular Dynamics Simulations of the Effect of the G-Protein and Diffusible Ligands on the beta2-Adrenergic Receptor. Journal of molecular biology. 2011;(0: ). doi: 10.1016/j.jmb.2011.10.015.

14. Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, et al. Crystal structure of the beta2 adrenergic receptor-Gs protein complex. Nature. 2011; 477(7366):549–555. Epub 2011/07/21. doi: 10.1038/nature10391 PMID: 21772298; PubMed Central PMCID: PMC3164188.

15. Kling RC, Lanig H, Clark T, Gmeiner P. Active-State Models of Ternary GPCR Complexes: Determinants of Selective Receptor-G-Protein Coupling. PloS one. 2013; 8(6):e67244. doi: 10.1371/journal.pone.0067244 PMID: 23826246; PubMed Central PMCID: PMC3691126.

16. Kling RC, Tschammer N, Lanig H, Clark T, Gmeiner P. Active-state model of a dopamine d2 receptor—gaip alpha complex stabilized by aripiprazole-type partial agonists. PloS one. 2014; 9(6):e100069. doi: 10.1371/journal.pone.0100069 PMID: 24933247; PubMed Central PMCID: PMC4059746.

17. Han Y, Moreira IS, Urizar E, Weinstein H, Javitch JA. Allosteric communication between protomers of dopamine class A GPCR dimers modulates activation. Nature chemical biology. 2009; 5(9):688–695. Epub 2009/08/04. doi: 10.1038/nchembio.199 PMID: 19648932; PubMed Central PMCID: PMC2817978.
18. Min KC, Zyaga TA, Cypress AM, Sakmar TP. Characterization of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa. Mutations on the cytoplasmic surface affect transducin activation. The Journal of biological chemistry. 1993; 268(13):9400–9404. PMID: 8486634.

19. Rosenthal W, Antaramian A, Gilbert S, Bimbaumer M. Nephrogenic diabetes insipidus. A V2 vasopressin receptor unable to stimulate adenyl cyclase. The Journal of biological chemistry. 1993; 268(18):13030–13033. PMID: 8514744.

20. Costa EM, Bedecarrats GY, Mendonca BB, Arnhold IJ, Kaiser UB, Latronico AC. Two novel mutations in the gonadotropin-releasing hormone receptor gene in Brazilian patients with hypogonadotropic hypogonadism and normal olfaction. The Journal of clinical endocrinology and metabolism. 2001; 86(6):2680–2686. doi: 10.1210/jcem.86.6.7551 PMID: 11397871.

21. Seibold A, Dagarag M, Bimbaumer M. Mutations of the DRY motif that preserve beta 2-adrenoceptor coupling. Receptors & channels. 1998; 5(6):375–385. PMID: 9826914.

22. Hiller C, Kling RC, Heinemann FW, Meyer K, Hubner H, Gmeiner P. Functionally selective dopamine d2/d3 receptor agonists comprising an enyne moiety. Journal of medicinal chemistry. 2013; 56(12):5130–5141. doi: 10.1021/jm400520c PMID: 23730937.

23. Winters Z, LANGE M, Costo EM, Bimbaumer M, Dagarag M, Dagarag M, Birnbaumer M. Dopamine D2, D3, and D4 Selective PhenyLPiperazines as Molecular Probes to Explore the Origins of Subtype Specific Receptor Binding. Molecular pharmacology. 2011; 79(3):575–585. Epub 2010/12/18. doi: 10.1124/mol.110.068106 PMID: 21163968.

24. Tscharner M, Bollinger S, Kenakin T. Efficacy at G-protein-coupled receptors. Nature reviews Drug discovery. 2002; 1(2):103–112. doi: 10.1038/nrd722 PMID: 12120091.

25. Yao XJ, Velez Ruiz G, Whorton MR, Rasmussen SG, DeVree BT, Deupi X, et al. The effect of ligand binding on the conformational dynamics of rhodopsin. Journal of computational chemistry. 2004; 25(13):1605–1612. Epub 2004/07/21. doi: 10.1002/jcc.20035 PMID: 15116359.

26. Haberl F, Lanig H, Clark T. Induction of the tetracycline repressor: characterization by molecular-dynamics simulations. Proteins. 2009; 77(4):857–866. Epub 2009/07/25. doi: 10.1002/prot.22505 PMID: 19626707.

27. Rosenbaum DM, Zhang C, Lyons JA, Holl R, Aragao D, Arlow DH, et al. Structure and function of an irreversible agonist-beta(2) adrenoceptor complex. Nature. 2011; 469(7329):236–240. Epub 2011/01/14. doi: 10.1038/nature09665 PMID: 21228876; PubMed Central PMCID: PMC3074335. Epub 2006/09/19. doi: 10.1021/jp051144h PMID: 16981200.

28. Berendsen HJC, Grigera JR, Straatsma TP. The missing term in effective pair potentials. The Journal of Physical Chemistry. 1987; 91(24):6269–6271. doi: 10.1021/j100308a038

29. Ehrlich K, Gotz A, Bollinger S, Tschammer N, Harterich S, et al. Dopamine D2, D3, and D4 Receptor Partial Agonists. Journal of medicinal chemistry. 2014; 57(11):4861–4875. Epub 2014/05/08. doi: 10.1021/jm400520c PMID: 24831693.

30. Haberl F, Lanig H, Clark T. Induction of the tetracycline repressor: characterization by molecular-dynamics simulations. Proteins. 2009; 77(4):857–866. Epub 2009/07/25. doi: 10.1002/prot.22505 PMID: 19626707.

31. Zheng Y, Chervenak MM, Wang J, Arlow DH, Aragao D, Arlow DH, et al. Structure and function of an irreversible agonist-beta(2) adrenoceptor complex. Nature. 2011; 469(7329):236–240. Epub 2011/01/14. doi: 10.1038/nature09665 PMID: 21228876; PubMed Central PMCID: PMC3074335. Epub 2006/09/19. doi: 10.1021/jp051144h PMID: 16981200.

32. Rosenbaum DM, Zhang C, Lyons JA, Holl R, Aragao D, Arlow DH, et al. Structure and function of an irreversible agonist-beta(2) adrenoceptor complex. Nature. 2011; 469(7329):236–240. Epub 2011/01/14. doi: 10.1038/nature09665 PMID: 21228876; PubMed Central PMCID: PMC3074335. Epub 2006/09/19. doi: 10.1021/jp051144h PMID: 16981200.

33. Hornak V, Abel R, Stockckine B, Rottberg A, Simmerling C. Comparison of multiple Amber force fields and development of improved protein backbone parameters. Proteins. 2006; 65(3):712–725. Epub 2006/09/19. doi: 10.1002/prot.21123 PMID: 16981200.

34. Berendsen HJC, Grigera JR, Straatsma TP. The missing term in effective pair potentials. The Journal of Physical Chemistry. 1987; 91(24):6269–6271. doi: 10.1021/j100308a038

35. Case DA, Darden TA, Cheatham TE, Simmerling CL, Wang J, Duke RE, et al. UCSF Chimera—a visualization system for exploratory research and analysis. Journal of computational chemistry. 2004; 25(13):1605–1612. Epub 2004/07/21. doi: 10.1002/jcc.20084 PMID: 15264254.