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Accelerated molecular dynamics simulations of the octopamine receptor using GPUs: discovery of an alternate agonist-binding position

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ABSTRACT

Octopamine receptors (OARs) perform key biological functions in invertebrates, making this class of G-protein coupled receptors (GPCRs) worth considering for insecticide development. However, no crystal structures and very little research exists for OARs. Furthermore, GPCRs are large proteins, are suspended in a lipid bilayer, and are activated on the millisecond timescale, all of which make conventional molecular dynamics (MD) simulations infeasible, even if run on large supercomputers. However, accelerated Molecular Dynamics (aMD) simulations can reduce this timescale to even hundreds of nanoseconds, while running the simulations on graphics processing units (GPUs) would enable even small clusters of GPUs to have processing power equivalent to hundreds of CPUs. Our results show that aMD simulations run on GPUs can successfully obtain the active and inactive state conformations of a GPCR on this reduced timescale. Furthermore, we discovered a potential alternate active-state agonist-binding position in the octopamine receptor which has yet to be observed and may be a novel GPCR agonist-binding position. These results demonstrate that a complex biological system with an activation process on the millisecond timescale can be successfully simulated on the nanosecond timescale using a simple computing system consisting of a small number of GPUs.

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Key words: GPCR; octopamine receptor; aMD simulations; molecular dynamics; GPCR active and inactive states.

INTRODUCTION

G-protein coupled receptors, or GPCRs, are seven transmembrane (TM) domain receptors that are involved in many diseases and are the target of approximately 30% of all modern medicinal drugs.[1,2] Through the study of GPCRs, multiple activation features have been discovered to hold true for the majority, many of which can be summarized as follows.[3–5] The TM3–TM6 distance increases on the cytoplasmic side of the protein; this increase causes the breaking of an ionic lock that is present in the inactive state of many GPCRs. The TM7 region containing the NPxxY motif becomes displaced. The 3–7 lock being broken, which involves two residues in the extracellular region's TM3 and TM7. A transmission switch, which has also been referred to as Trp toggle switch, occurs; this can involve several different mechanisms, but most often results in a relocation of a TM6 Trp and Phe residue toward the TM5 region. Other activation features have been found, though not as commonly, such as there being a fixed distance between a TM2 Leu and a TM7 Pro, as well as there being a slight conformational change in the protein's connector region.

Octopamine receptors (OARs) are GPCRs, which activate on the binding of octopamine, a ligand which has physiological roles in invertebrates only.[6] Our previous studies on the *An. gambiae* octopamine receptor, AgOAR45B, has led to the discovery of the importance of residues Asp100, Ser206, and Ser210 to the activation of the receptor.[7] We also found that several aromatic residues may be involved in activation as well. We also noted that while Ser206 and Ser210 are indeed important to

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the protein's activation, that it may be possible for an alternate binding position to exist.

The activation of GPCRs have been shown to be on the millisecond timescale, a timescale which is difficult to reach using long conventional molecular dynamics (MD).[8] However, accelerated molecular dynamics (aMD) simulations have been shown to capture millisecond–timescale events in only hundreds of nanoseconds.[9,10]

AMD is an enhanced sampling technique which adds a non-negative boost potential to the protein's potential energy surface.[11–13] This enables the simulation to more quickly transition between the active, inactive, and intermediate states by reducing the free energy barriers between the states. AMD simulations have already been performed successfully for a number of other systems, including the GPCR, M2 muscarinic receptor.[14–19]

In this article, it is demonstrated that the AgOAR45B octopamine receptor can be successfully simulated on GPUs using the enhanced sampling technique, aMD. It is shown that both active and inactive forms of the GPCR are reached using less than one microsecond of simulation. The simulations also lead to the discovery of a potentially novel agonist-binding position for GPCRs.

MATERIALS AND METHODS

Homology modeling and protein system preparation

The I-TASSER online server was used to generate both the initial, antagonist-based inactive, and agonist-based active conformations of AgOAR45B.[20] Many GPCR antagonist-bound and inverse agonist-bound conformations were used as a template to create the inactive structure, with a crystal structure of β_2-adrenergic receptor with partial-inverse-agonist carazolol bound [PDB: 2RH1] being the primary. The active conformation was built using the crystal structure of the β_2-adrenergic receptor–$G_{\alpha s}$ protein complex with agonist BI-167107 bound [PDB: 3SN6] as the template. Standard I-TASSER settings were used to obtain both conformations. Root-mean-square deviations (RMSDs) and distances, both used as a measure for determining protein activation or inactivation, were calculated using the VMD 1.9.2 RMSD Trajectory Tool.[21] RMSDs and distances are given in Angstroms (Å), where 1 Å = 0.1 nm. The active site residues were determined from biochemical analysis performed on the ligand as well as from previous studies.[7]

Using VMD 1.9, each conformation of the octopamine receptor was first embedded in a POPC lipid bilayer, with any lipid molecules overlapping the protein being removed. This protein–membrane system was then solvated with TIP3P water molecules and 0.4*M* KCl. It is at this point that the ligands are added to each system (octopamine in the active conformation and promethazine in the inactive conformation) using Amber 11 tleap, with AMBER gaff parameters being used for the ligands and lipids, and AMBER ff03.r1 being used for the rest of the system.[22–24] The AMBER force field was chosen as it allows the generation of parameters for the ligand (and lipid) using the antechamber module.[25] A disulphide bridge found to exist in the template PDBs was added between the residues of Cys93 and Cys194.

Each complete protein system consisted of its respective AgOAR45B conformation embedded in a POPC bilayer of 168 lipid molecules, with the antagonist-bound inactive system containing 171 potassium ions, 204 chloride ions, and 22,525 water molecules for a total of 99,706 atoms (system box size: 95 × 96 × 128 Å^3), and the agonist-bound active system containing 172 potassium ions, 205 chloride ions, and 22,654 water molecules for a total of 100,077 atoms (system box size: 101 × 92 × 127 Å^3). In both protein conformations, all residues are at the normal protonation state at physiological pH.

Accelerated molecular dynamics

The equilibration and simulation steps were run using NAMD 2.9 CUDA[26] on the University of Notre Dame's Computer Aided Molecular Design (CAMD) core facility's GPU cluster of NVidia Tesla K20 GPUs.[27] For our simulations, we chose to use dual-boost aMD for the simulations over dihedral-boost aMD.[12,13] Dual-boost aMD was chosen due to its greater enhanced sampling capabilities, which would promote more sampling of the intermediate states and possibly even conversion between active and inactive states, both of which were successfully simulated on another GPCR, M2 muscarinic receptor.[19] In dual-boost aMD, two sets of two parameters are required to run the simulation: $(E_{\text{dihed}}, \alpha_{\text{dihed}}; E_{\text{total}}, \alpha_{\text{total}})$, which represent the reference energy, E, and the acceleration factor, α, of the dihedral angles and total atoms of the system, respectively. For simulations of membrane proteins including GPCRs, the input parameters for aMD are as follows[19]:

$$E_{\text{dihed}} = V_{\text{dihed\_avg}} + \lambda \times V_{\text{dihed\_avg}}, \alpha_{\text{dihed}} = \lambda \times V_{\text{dihed\_avg}}/5$$

$$E_{\text{total}} = V_{\text{total\_avg}} + 0.2 \times N_{\text{atoms}}, \alpha_{\text{total}} = 0.2 \times N_{\text{atoms}}$$

where N_{atoms} is the total number of atoms, $V_{\text{dihed\_avg}}$ and $V_{\text{total\_avg}}$ are the average dihedral and total energies calculated from short conventional MD simulations, respectively, and λ is an adjustable acceleration parameter. $V_{\text{dihed\_avg}}$ and $V_{\text{total\_avg}}$ were calculated from our previous work's simulations while λ=0.3 has been found to work best for membrane proteins.[7,19]

Before aMD simulations the systems were equilibrated using 4 GPU cores as follows: (1) MD of lipid tails for 500 picoseconds (ps) [time step = 2 femtoseconds (fs)] with protein, ligand, lipid head groups, water, and ions kept fixed; (2) equilibration for lipids, water, and ions

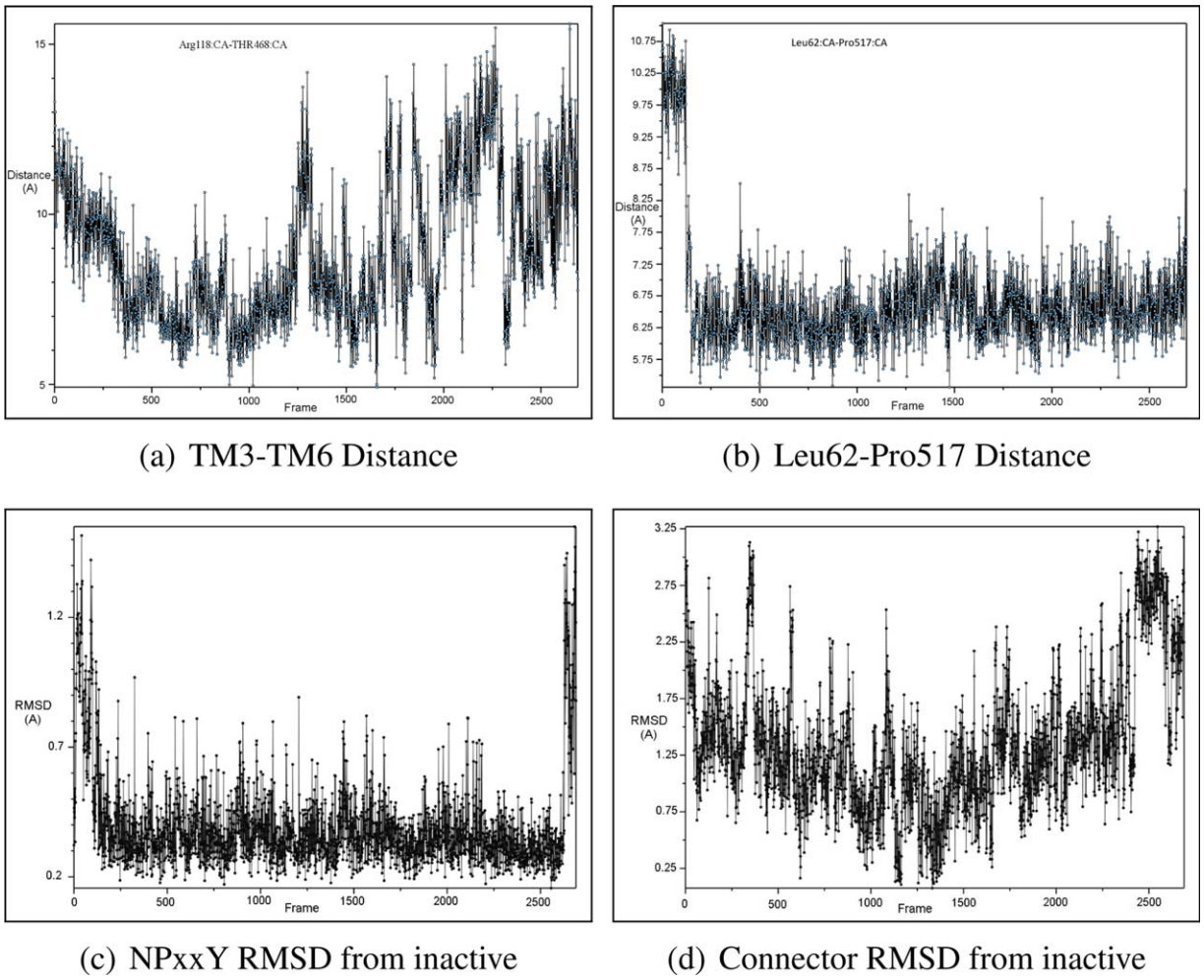

Figure 1

Data from antagonist-bound conformations after 538 ns aMD runs. Shown are the TM3–TM6 distance (**a**), Leu62–Pro517 distance (**b**), the root mean squared deviation (RMSD) of the NPxxY region (N516–A521) in TM7 from the inactive conformation (**c**), and the RMSD of the connector region (I108–F476) from the inactive conformation (**d**).

for 500 ps (time step = 2fs) with harmonic constraints on the protein and ligand; (3) equilibration of the entire system for 500 ps (time step = 2 fs) with no molecular constraints. After equilibration, 390 and 538 ns of accelerated MD (aMD) simulations were performed for the agonist and antagonist conformations, respectively, using 4 GPU cores in 2 ns followed by 24 ns increments, with time step = 2 fs and trajectory data being collected every 20 ps. With the exception of the free energy landscape (see section Free Energy Landscape) where 1 frame = 20 ps of simulation, all data shown is obtained from the trajectory data with a stride of 10, making 1 frame = 200 ps of simulation.

Free energy calculation

The potential of mean force (PMF), also known as the free energy landscape, shows how the free energy in a system changes as a function of some specific reaction coordinates; in this work, we use the TM3–TM6 distance on the cytoplasmic side of the protein and the connector region's RMSD versus the inactive state as the two reaction coordinates. The free energy landscape is calculated from the simulation trajectories using the following equation: $-k_B T \ln(P_{i,j})$ where k_B is the Boltzmann constant, T is the temperature, and $P_{i,j}$ is the percent of the protein population located at bin i, j for all trajectories; our free energy landscapes were generated using 30 bins in both x and y directions. The free energy landscape includes the entire trajectory data set, with the exception of the first 3000 frames for the agonist-bound simulation and 1230 frames for the antagonist-bound simulation to allow the protein conformations to properly orient themselves away from their initial homology models. These particular ranges

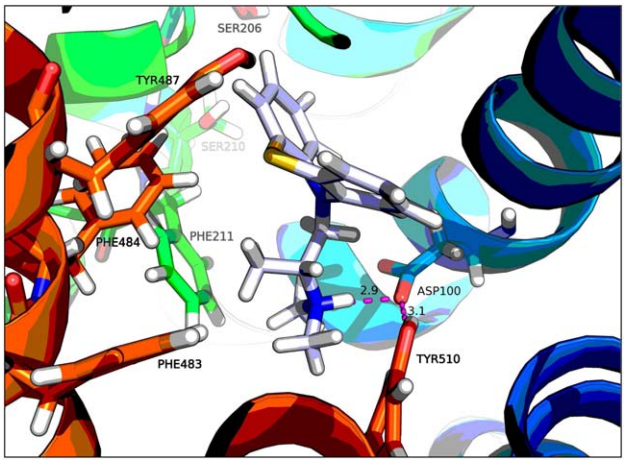

Figure 2

Active site of the inactive conformation obtained from the antagonist-bound simulation, frame 1374. H-bonds are shown in pink and D-A distances are given in Å. The agonist promethazine (shown in silver), was found to H-bond to Asp100 only. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of frames were skipped for the following reasons: for the antagonist after frame 1230 (or frame 123 in other plots), the Leu62–Pro517 distance [Fig. 1(b)] and NPxxY RMSDs [Fig. 1(c)] both stabilize at their respective values for the majority of the simulation; for the agonist before frame 3000 (or frame 300 in the other plots), the connector region RMSDs [Fig. 3(d)] stabilize, and the Leu62–Pro517 distance [Fig. 3(b)] and the seven transmembrane region RMSDs (data not shown) both have low values that are never encountered again during these frames. Note that the above equation uses the total population to calculate $P_{i,j}$, thus in Figure 6 there is a slight bias toward the antagonist-bound simulation over the agonist-bound simulation, from which 25670 and 16500 frames were used to generate the graph, respectively. This, however, did not have a significant impact as the free energy landscapes obtained from each of the antagonist- and agonist-bound simulations (see Supporting Information Fig. S2) are still clearly present on the combined free energy landscape.

In aMD simulations, transition barriers between low-energy states are decreased, causing higher populations of the systems at these energetically less energetically-favorable states to be present in these simulations. Theoretically, the free energy landscape of aMD simulations can be reweighted using the Boltzmann factor of the boost potential applied to each trajectory frame: $e^{\Delta V(r)/k_B T}$. However, high boost potentials needed to be applied to the simulations, likely due to the large size of the system, causing overflow errors to be encountered when calculating the weights. In addition, aMD simulations performed on another GPCR, M2 muscarinic, observed that large energetic noise was encountered when attempting to reweigh, causing large fluctuations in the calculation of the free energy.[9] In this article, Miao et al. further demonstrate that the unweighted PMF profiles obtained from aMD simulations match well to PMF profiles obtained from conventional MD simulations. Thus, in this study we present our unweighted free energy landscapes.

GPCR inactivation and activation methods

GPCRs are important in the biological function of many organisms and compounds that have been developed to interfere with the natural activation and inactivation process are known to have significant effects.[1] Thus it is useful to be able to distinguish between the active and inactive conformation for GPCRs. This would help in being able to determine how compounds bind in each conformation and would aid in the development of a lead compound.

The following criteria were used to determine if the protein in the antagonist simulation was in the inactive state:

- TM3–TM6 distance below 9.0 Å;
- Any Leu62–Pro517 distance is valid. However distances equal to 8 Å or above are similar to the starting crystal-like inactive structure, while distances below 8 Å are considered to be in an alternate inactive conformation;
- Below 1.0 Å for NPxxY RMSD compared to the inactive conformation;
- Below 1.0 Å for the Ile108–Phe476 connector RMSD compared to the inactive conformation;
- Presence of the 3–7 lock (Asp100:CG-Tyr510:OH distance below 5.4 Å);
- Presence of the ionic lock (Arg118:CZ-Thr468:OG1 distance below 5.4 Å).

The following criteria were used to determine if the protein in the agonist simulation was in the active state:

- TM3–TM6 distance above 12.0 Å;
- Leu62–Pro517 distance between 7.5 and 10 Å;
- Above 1.0 Å for NPxxY RMSD compared to the inactive conformation;
- Above 1.0 Å for the Ile108–Phe476 connector RMSD compared to the inactive conformation;
- Absence of the 3–7 lock (Asp100:CG-Tyr510:OH distance above 5.4 Å);
- Absence of the ionic lock (Arg118:CZ-Thr468:OG1 distance above 5.4 Å).

As there is no crystal structure of the octopamine available at the time of this writing, a conformation was chosen from the antagonist simulation which fit all of the criteria for an inactive GPCR conformation (see section Inactive State Discovered in the Antagonist

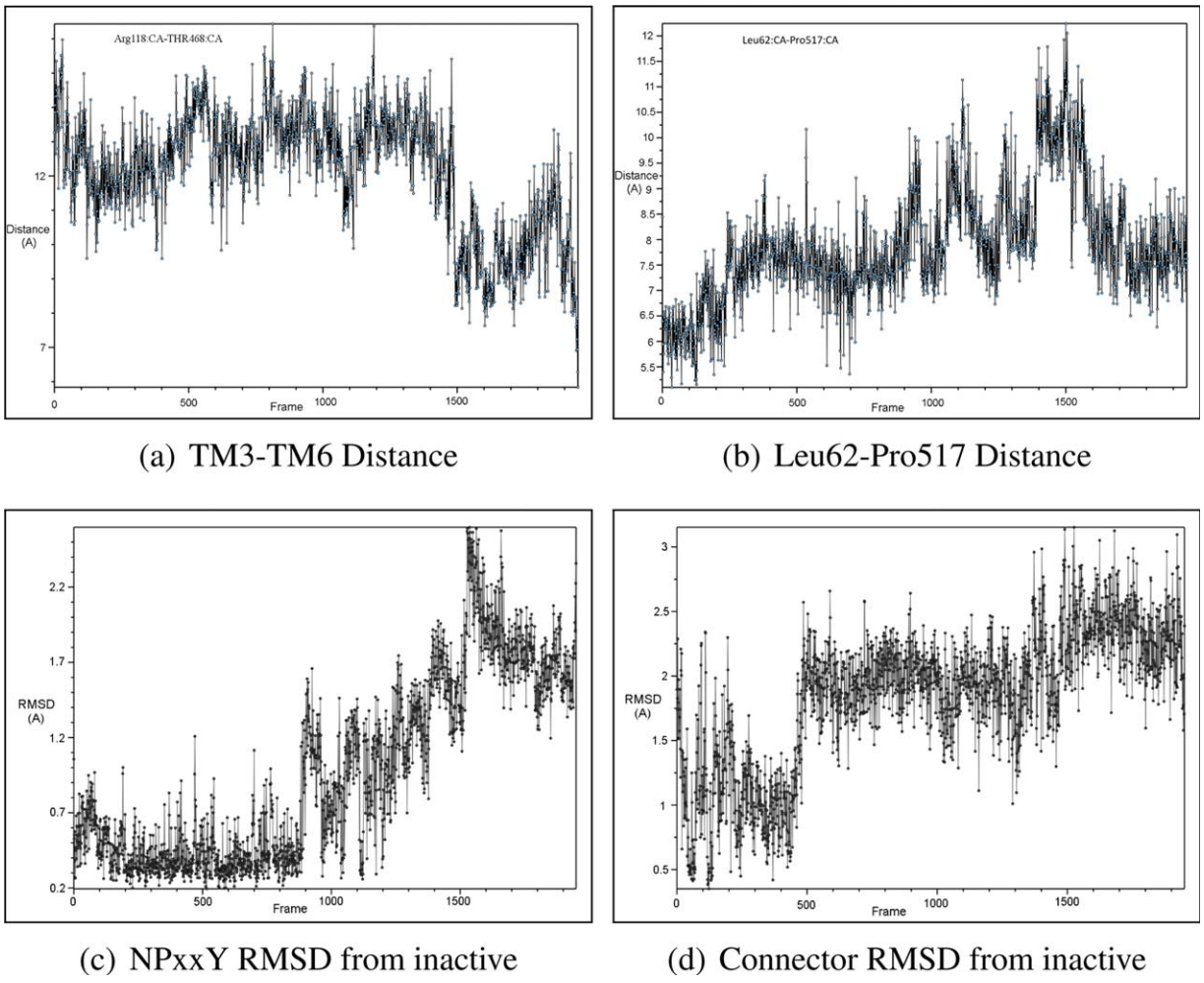

Figure 3

Data from agonist-bound trajectories after 390ns aMD runs. Shown are the TM3-TM6 distance (**a**), Leu62-Pro517 distance (**b**), the root mean squared deviation (RMSD) of the NPxxY region (N516-A521) in TM7 from the inactive conformation (**c**), and the RMSD of the connector region (I108-F476) from the inactive conformation (**d**).

Conformation Runs). Also, as there have been no known molecular dynamics simulations done on the octopamine receptor, some of the distances and RMSD values used to differentiate between active and inactive conformations were determined from Supporting Information as follows: TM3–TM6 distance and RMSDs of the NPxxY or Ile108–Phe476 connector regions versus the inactive state from Kohlhoff et al.,[4] and Leu62–Pro517 distance from Nygaard et al.[5] The 3–7 or ionic lock distances were determined via taking the maximum hydrogen bond (H-bond) D–A distance, 4.0 Å, and adding the approximate distance between a carbon atom resonantly doubly-bonded to an oxygen or nitrogen atom (1.4 Å).[28,29] Once regions were found matching each of the criteria given above, RMSDs of the protein's TM regions and regions near the active site were used to determine frames with stable ligand-binding positions as well as possible alternate positions (data not shown).

RESULTS

In our previous work, we were able to produce AgOAR45B conformations with active sites that, when combined with virtual screening and experimental methods, were able to successfully identify several potential agonists and antagonists that may be useful in drug development.[7] In this prior work, our focus was on stable active sites which could be used for virtual screening, and thus only short simulations were performed.

Our hypothesis was that longer simulations using aMD would enable the octopamine receptor to assume its full active and inactive states, and thus providing more accurate models for *in silico* lead compound discovery. To further speed up the simulations, it was decided that GPU-acceleration should also be used. This combination would allow simulations on the millisecond timescale to be run in only hundreds of nanoseconds.[30]

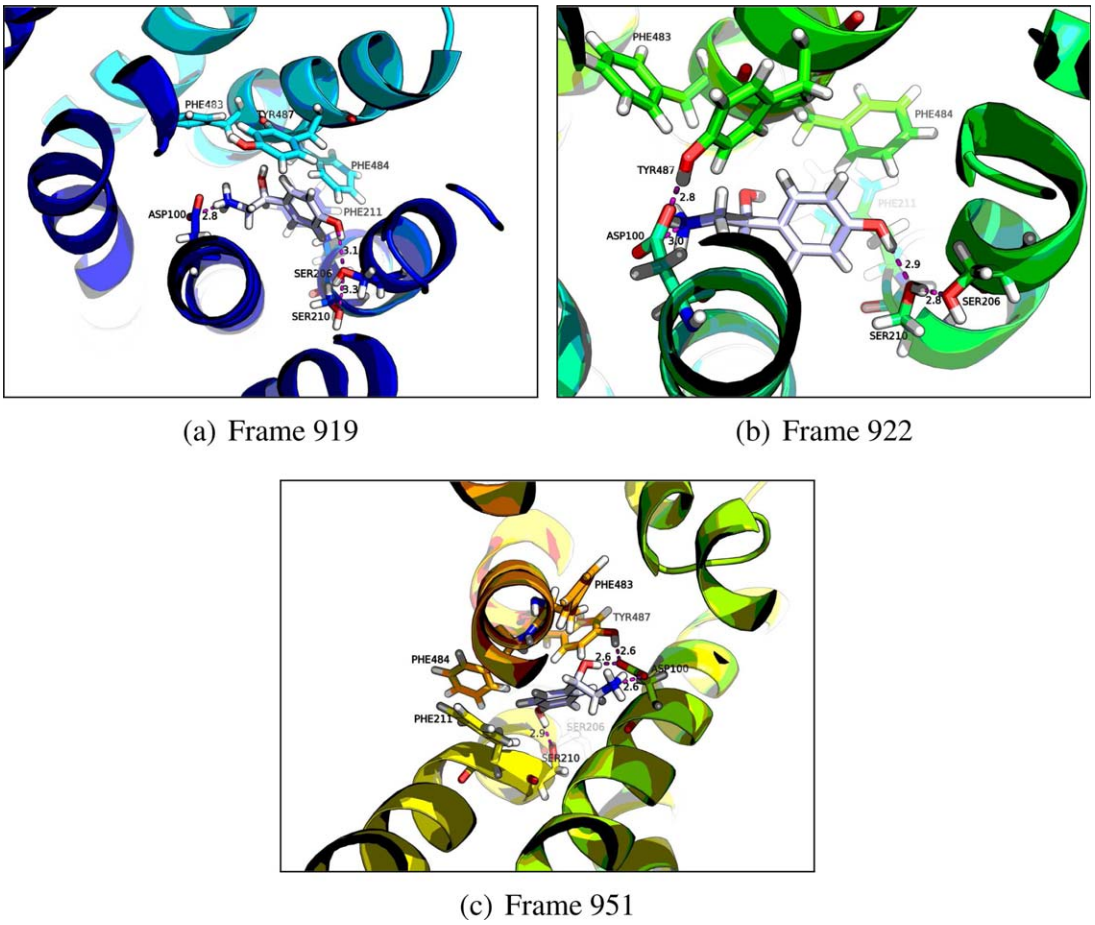

Figure 4

Active site of the active conformations obtained from the agonist-bound simulation, frames 919, 922, and 951. H-bonds are shown in pink and D-A distances are given in Å. For all of the conformations, the agonist octopamine (shown in silver), was found to H-bond to Asp100, as well as have potential π-π interactions with Phe211, Phe484, and Tyr487. In addition, octopamine was found to H-bond to either Ser206 (**a**) or Ser210 (**b**),(**c**). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

As of the writing of this article, aMD run on GPUs have not yet been performed on a GPCR.

The antagonist and agonist simulations yielded conformations that appear to be in the inactive and active states, respectively. Multiple tests were performed which prior studies have found to show GPCRs as being in the inactive or active states.

Inactive state discovered in the antagonist conformation runs

The antagonist-bound simulations contained several frames which matched the criteria of a GPCR in the inactive conformation. Measuring TM3–TM6 distance, the simulation between the frames of 300–1250, and 1300–1650 predominantly stays below 9.0 Å [Fig. 1(a)]. For Leu62–Pro517 distance, all values are valid for an inactive GPCR conformation; however, the simulation appears to only remain close to its starting inactive conformation for the first 120 frames, while the remainder of the simulation indicates that the protein has taken on an alternate conformation [Fig. 1(b)]. For the NPxxY RMSD compared to the inactive state, with the exception of the first 120 frames and the last 90 frames, the entire simulation's RMSD is below 1.0 Å [Fig. 1(c)]. For the connector region RMSD compared with the inactive state, frame ranges 950–1000 and 1300–1400 are mostly below 1.0 Å [Fig. 1(d)]. Thus, frames that fit all of the conditions for being in the inactive GPCR conformation in the antagonist simulation are frames 950–1000, and 1300-1400. These frames represent 190–200 and 260–280 ns of simulation time, respectively.

For the 3–7 lock, with the exception of an early frame the entire simulation had a distance of less than 4.75 Å, showing that this H-bond is present and in the inactive conformation throughout the entire simulation [see Supporting Information Fig. 1(a)]. For the ionic lock, the simulation drops below the distance required for an

H-bond to form often throughout the simulation, suggesting that any part of the simulation could be in the inactive state [see Supporting Information Fig. 1(b)]. As this distance oscillates between the inactive and active states, the final conformation chosen to represent the AgOAR45B inactive state, frame 1374, was ensured to have an inactive state value, which in our simulations was 3.35 Å.

The active site of these two conformation ranges were analyzed. Both ranges had the antagonist promethazine bound in the same position (Fig. 2). In this position, promethazine H-bonds with Asp100, yet does not interact with either of the TM5 serines. An S/π interaction may also exist between promethazine and Tyr487. In addtion, a potential H-bond occurs between Asp100 and Tyr510. While π–π interactions between promethazine and residues Phe211, Phe484, and Tyr487 appear possible, during these simulation frames they rarely occurred and only existed for single frames. Likewise an H-bond might appear to be possible between promethazine and Tyr487, however the O—H and S atoms do not appear to ever align properly.

Active states discovered in the agonist conformation simulation

The agonist-bound simulations contained several frames which matched the criteria of a GPCR in the active conformation. Measuring TM3–TM6 distance, the simulation between the frames of 450–1060, and 1120–1400 predominantly stays above 12.0 Å [Fig. 3(a)]. For Leu62–Pro517 distance, frame ranges 850–1400, and 1550–1700 are mostly between 7.5–10 Å [Fig. 3(b)]. For the NPxxY RMSD compared to the inactive state, frame ranges 900–960, 1060–1100, and 1260 to the end of the simulation are almost entirely above 1.0 Å [Fig. 3(c)]. For the connector region RMSD compared with the inactive state, all frames past 500 are entirely above 1.0 Å [Fig. 3(d)]. Thus, frames that fit all of the conditions for being in the active GPCR conformation in the agonist simulation are frames 900–960, and 1260–1400. These frames represent 180–192 and 252–280 ns of simulation time, respectively.

For the 3–7 lock, all of the frames after the first 130 have a distance above 8.5 Å, showing that this H-bond is absent and in the active conformation throughout the entire simulation [see Supporting Information Fig. 1(c)]. For the ionic lock, the simulation does not ever drop below 5.3 Å for the frames 900–960 and 1260–1400, suggesting that these frames are in the active state [see Supporting Information Fig. 1(d)]. As this distance does oscillate between the inactive and active states, the final conformations chosen to represent the AgOAR45B active state, frames 919, 922, 951, and 1366, was ensured to have an active state value, which in our simulations were 10.03, 10.32, 6.99, and 12.74 Å, respectively.

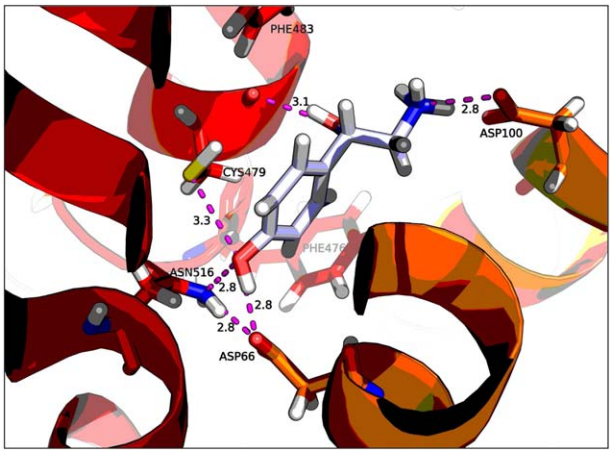

Figure 5

Active site of the active conformation obtained from the agonist-bound simulation, frame 1366. H-bonds are shown in pink and D-A distances are given in Å. The agonist octopamine (shown in silver), was found to H-bond to Asp100, as well as to residues Asp66, Cys479, and Asn516. Octopamine also has potential π-π interactions with Phe476. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The active site of these two conformation ranges were analyzed. All were found to H-bond with Asp100. Conformations in frames 900–960 were found to also H-bond to either Ser206 or Ser210 of the active site. Three slightly different conformations were found in this range, all of which still fit the criteria given above (Fig. 4). For each of these conformations, octopamine also has potential π–π interactions with Phe211, Phe484, and Tyr487. In addition, potential H-bonds exist between Tyr487 and Asp100, as well as between Ser206 and Ser210.

Potential new active conformation

Conformations in frames 1260–1400 were found to be in a completely different conformation from the ones just discussed. This conformation, while still binding to Asp100, instead of binding to either TM5 serines was found to interact with residues that are much further down in the binding pocket (Fig. 5). It was found to H-bond with Asp66, Cys479, and Asn516, and had potential π–π interactions with Phe476.

Free energy landscape

Potential of Mean Force (PMF) was calculated for both the agonist and antagonist simulations. As neither simulation appeared to contain frames that matched both active and inactive GPCR conformation criteria, PMFs from both simulations were combined so that the protein's active and inactive state energy wells could be observed as well as possible intermediate states found

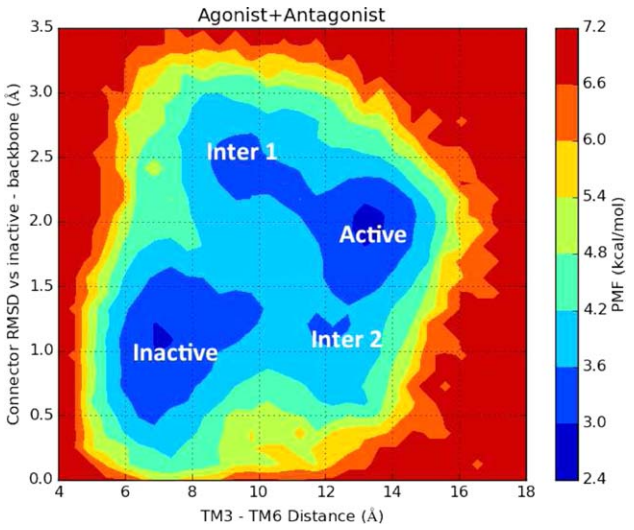

Figure 6

Combined free energy landscape of both the agonist and antagonist simulations. Four possible different energy wells are shown: Active, Inactive, and two Intermediate states (Inter 1 and Inter 2). The two parameters used were TM3-TM6 distance, and the connector region RMSD compared to the inactive state.

(Fig. 6). This combined landscape shows that there is indeed a clear distinction between the active and inactive states, while two possible intermediate state energy wells also exist.

The simulations capture transitions between the active and inactive conformations. Each simulation reaches both energy wells, indicating that there is possible overlap in the nonactive and noninactive states in the agonist and antagonist simulations, respectively (see Supporting Information Fig. S2). It should also be noted that the antagonist simulation has a larger population of conformations in the Intermediate 2 well over Intermediate 1, whereas the agonist simulation is the reverse, having a larger population in the Intermediate 1 well over Intermediate 2. This may indicate that there is a preferred pathway for when the protein activates, going through Intermediate 2, versus when the protein deactivates, instead going through Intermediate 1.

DISCUSSION

All simulation ranges in the active and inactive states have the ligand H-bonded with Asp100, however the three active state conformations from Figure 4 show the ligand spanning the active site to also H-bond with Ser206 or Ser210, an interaction which does not occur in the inactive state (Fig. 2). Previous studies have shown that these residues are not only important in the agonistic function of AgOAR45B, but in most GPCRs.[7]

Previous studies have also shown that several aromatic residues may be involved in ligand binding and potentially activation, which our simulations support. During the simulation range where these active state conformations exist (agonist simulation, frames 900–960), Phe211 keeps an orientation and distance relative to the octopamine which suggests π–π interactions. This is true even when the octopamine–serine bond appears to break during these active states (D-A distance >4 Å). However, during the inactive state frames found in the antagonist simulation, the proper orientation, and distance for Phe211 to have a π–π interaction with promethazine rarely occurred and were only shortlived when they did occur. These results suggest that, for the agonist octopamine, this Phe211 π–π interaction may be used in keeping the serines close and promoting their H-bonds with octopamine, or possibly providing an alternate binding method in place of the serines.

The active state in the 900–960 frame range of the agonist simulation also has Tyr487 with potential π–π interactions with octopamine [Fig. 4(a)], or H-bonding with Asp100 [Fig. 4(b,c)], both of which bring the extracellular side of TM6 inward. In the inactive state, a potential S/π interaction exists between Tyr487 and promethazine, but due to the orientation of the ligand, this keeps the extracellular side of TM6 pushed outward. Both of these support our earlier hypothesis that the TM6 region moving inward toward the TM3 region promotes activation of AgOAR45B, likely via causing the cytoplasmic side of TM6 to move outward to bind to the G-protein.[7]

In addition, the active state in the 900–960 frame range of the agonist simulation has either Phe484 or Phe483 interacting with octopamine during most of the frames (π–π for Phe484 and N/π for Phe483), in an alternating manner as they are not both able to bind simultaneously (Phe483 interaction with octopamine not shown). The inactive state's Phe483 never interacts with the promethazine and the proper orientation and distance for Phe484 to have a π–π interaction with promethazine rarely occurred and were only shortlived when they did occur, same as Phe211. It is likely that these interactions promote movement of the extracellular TM6 inward toward TM3 along with Tyr487 during activation.

A potential alternate active state exists during the agonist simulation, frames 1260–1400 (Fig. 5). The active site of this active state form of the AgOAR45B protein once again has the agonist octopamine H-bonding to Asp100, yet not interacting with either of the TM5 serines. Instead it reaches further into the binding pocket and appears to interact with residues Asp66, Asn516, Cys479, and Phe476, which can also be named Asp-2.50, Asn-7.49, Cys-6.47, and Phe-6.44, respectively, using the Ballesteros-Weinstein numbering scheme. All of these residues have been found in other works to have been involved in the activation of GPCRs, or be in the same region as these residues.

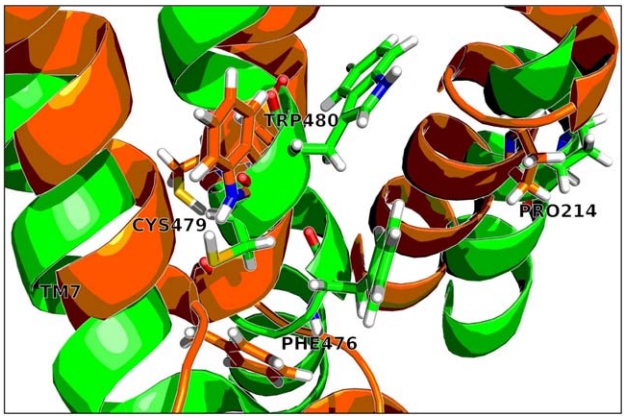

Figure 7

Transmission Switch of AgOAR45B. The inactive state (orange) Trp480 and Phe476 residues are oriented toward the TM7 helix, while the active state at frame 1366 (green) residues are oriented toward TM5s Pro214. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Asp66 and Asn516 appear to H-bond not only with octopamine, but also with each other. It has been suggested that this H-bond is important for the activation of the thyrotropin receptor.[31] Urizar et al. hypothesized that the presence of the negatively charged Asp-2.50 increases the polarity of Asn-7.49's O_δ atom and allows this atom to H-bond with another positively charged residue. This does indeed occur in our AgOAR45B agonist simulation during all active states, with Asn516's O_δ atom appearing to H-bond with Arg591 (not shown). In this alternate active state, the octopamine's presence potentially serves the dual function of both bringing these two residues together to form this H-bond as well as further increasing the polarity of Asn516's O_δ atom.

Cys479 and Phe476, along with Trp480 (Trp-6.48), are part of the GPCR region where the transmission switch, or rotamer toggle switch, is located. During activation, a relocation of the Trp-6.48 and Phe-6.44 residues toward Pro-5.50 occurs.[32] In our simulations, the inactive state Phe476 and Trp480 residues are turned away from Pro214 (Pro-5.50) and are instead facing toward TM7, while Cys479 faces away from the protein interior (Fig. 7). However, when activated the TM6 helix rotates so that both Phe476 and Trp480 are turned toward Pro214. In this alternative active state, Cys479's H-bond with octopamine likely facilitates this rotation, activating the protein.

CONCLUSION

Here, two separate conformations of the same AgOAR45B receptor, one containing the native agonist octopamine and the other containing known antagonist promethazine, were simulated on GPUs using the aMD enhanced sampling technique. At the time of this writing, no known aMD simulations have been performed on GPUs for not only the octopamine receptor, but for any GPCRs. Our results show that, running aMD simulations on GPUs, we were able to find conformations that agree with the active and inactive state conformations obtained in other GPCR studies.[4,5] The ligand-binding positions in the conformation active sites also support our previous findings, and also include a possible alternate agonist binding position previously undiscovered for the octopamine receptor, and may potentially be an undiscovered, GPCR-agonist activation method (Fig. 5).[7] Our simulations appear to not have run long enough to fully reach both the active and inactive conformation in a single simulation. Yet our PMF data suggests that intermediate states were likely reached in both simulations (Fig. 6). Our hypothesis is that running these simulations for longer would result in active and inactive conformations existing in both simulations. Obtaining these conformations would not only further validate our methods, but would also give us insight into possible effects of the active conformation on antagonists as well as of the inactive conformation on agonists.

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