

Analysis of classical and steered molecular dynamic simulations of the A_{2A} receptor bound to adenosine and two adenosine derivatives

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INTRODUCTION.

G-protein-coupled receptors (GPCRs) are proteins with seven transmembrane domains. Their activation is generally characterized by a reorganization of the cytoplasmic side that exposes an intracellular pocket. This pocket is involved in interaction with other proteins such as G proteins, GPCR kinases (GRKs) and arrestins [1].

Adenosine (ADN) is the natural ligand of adenosine receptors (ARs), with high affinity to the A_{2A} receptor type. This receptor is a GPCR characterized by stimulating the production of cyclic adenosine monophosphate (cAMP) via the adenylyl cyclase. They are mostly expressed in dopamine-rich regions of the brain, although they are also found in tissues such as endothelial and lymphoid cells, blood vessels and several types of neurons in the sympathetic and parasympathetic nervous system [2].

In this paper we describe the dynamics of the adenosine A_{2A} receptor bound to an adenosine molecule (PDB structure 2YDO [3]), and 2 adenosine derivatives that were designed to improve ligand affinity. This was done by means of classical molecular dynamics (MD) and steered molecular dynamics (SMD).

RESULT & DISCUSSION.

The first analysis involved classical molecular dynamics (MD) simulation of the A_{2A} receptor complexed with adenosine over a 1.6 ns timescale. A volumetric mapping of water molecules occupancy (water Volmap) was conducted to locate stable water molecules in the binding cavity that could be displaced by modifications of the ligand, and increase ligand contacts with the receptor (**Figure 1, A**). Subsequently, two distinct modifications to the ligand were proposed.

The initial modification consisted in the addition of a hydroxymethyl group (CH₂OH) to the C3 position of adenosine (**Figure 1, B**). This modification showed a similar capacity for water displacement as the adenosine ligand. This may be due to the minimal nature of the structural alteration.

In contrast, the second modification involved the insertion of two amino-ethyl groups (CH₂CH₂NH₂) at the adenosine N6 position, converting the primary amine to a tertiary one (**Figure 1, C**)

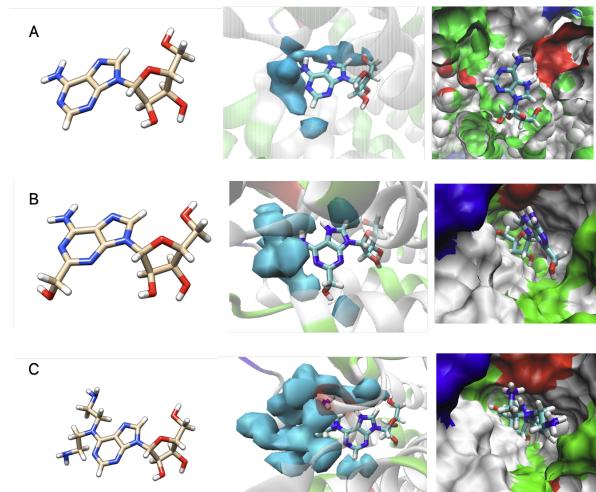


Figure 1. Ligand-Receptor interaction analysis and ligand modifications. (A) Adenosine molecule, its water Volmap (3 Å) and its position when bound to the A_{2A} receptor. (B) Modification 1, its water Volmap and its position when bound to the A_{2A} receptor. (C) Modification 2, its water Volmap and its position when bound to the A_{2A} receptor.

This modification led to a significantly higher degree of water displacement, attributable to the increased size of the modification. A greater amount of water around the ligand can be seen but it may be due its greater size. It

suggests a better interaction with the A2A receptor (**Figure 1**).

After conducting a classical MD simulation for the modified ligands over a 1.6 ns period, the dynamic stability of the adenosine ligand and its two modifications was assessed. The ADN ligand presented a lower average RMSD (0.83 Å) than the 2 modified ones (1.15 Å and 1.24 Å) (**Figure 2**). In the analysis of ligand dynamics, Modification 1 exhibited a slightly lower RMSD, which may imply a more stable interaction with the receptor. It was also observed that Modification 1 migrated within the binding site from one side to the opposite over the course of the simulation, indicative of a search for a stable binding conformation. However, conclusions regarding the overall stability of the system cannot be made due to the limited duration of the simulation.

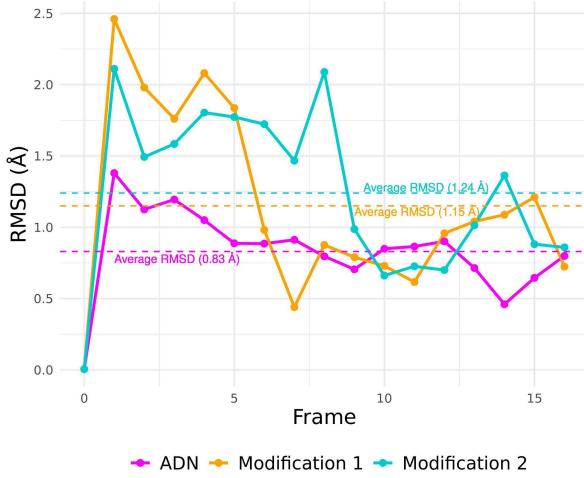


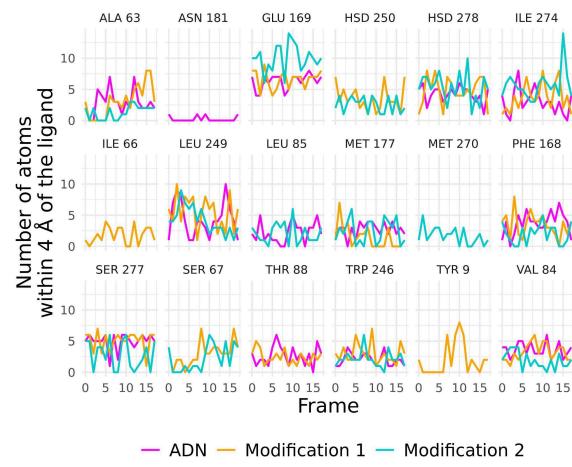
Figure 2. The Root-Mean-Square Deviation (RMSD) of the ligand and its modifications bound to the A2A receptor over 18 frames of the simulation. Magenta line: Adenosine ligand (ADN); Orange line: Modification 1; Cyan line: Modification 2.

Through the contact analysis, it was identified that both Modification 1 and Modification 2 established new contacts with the A2A receptor at certain residues such as Met 270, Tyr 9, and Ser 67 (**Figure 3, A**). Notably, despite its larger size, Modification 2 increased the number of contacted receptor residues to only 14, a minor increase over the 13 contacts observed with the original ADN ligand. Modification 1, which is a smaller molecule, made contacts with 15 residues (**Figure 3, A**). The limited increase in contacts by the bulkier Modification 2 may be attributed to the presence of additional free space in the receptor's cavity. Specifically, this space allows for the accommodation of Modification 2's larger branches without establishing new contacts.

Additionally, Modification 2 contacts are not consistently maintained across all simulation frames. In contrast, certain contacts with Modification 1 are sustained over the entire simulation (**Figure 3, B**).

Thus, Modification 1 may form more stable and persistent contacts with the receptor, potentially indicating a more stable ligand-receptor complex. However, conclusions regarding the overall stability of the system can not be made due to the limited duration of the simulation.

A



B

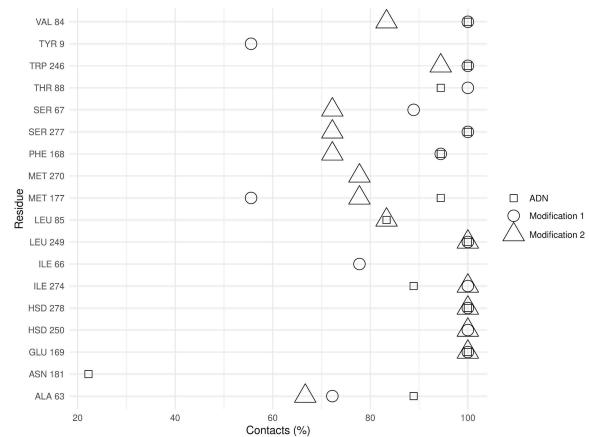


Figure 3. Contact analysis between the ligand ADN and its modifications and the A2A receptor. (A) fluctuating number of atoms within a 3 Å distance from the ligand across the classical MD simulation. (B) Percentage of simulation frames where at least one atom of the ligand is within 3 Å of each receptor residue.

Following the classical MD simulation, a SMD simulation was conducted to investigate the dissociation process of the ligand from the receptor. There is a progressive increase in the distance between the ligand and the receptor over successive frames, indicative of the pulling mechanism applied during SMD (**Figure 4**).

Analysis of the Potential of Mean Force (PMF) profiles following SMD simulations suggested a quantifiable difference in the binding affinity of the native ligand, ADN, and its two modifications to the A2A receptor. The PMF curves, which relate the work necessary to unbind the ligand from the receptor to the extension of the

ligand from its binding site, provide a measure of the ligand-receptor interaction strength.

In the presented PMF profiles, both modifications show a higher work requirement for unbinding than ADN, indicating that these modifications form more stable complexes with the A2A receptor (**Figure 5**). This stability can be correlated with an increase in binding affinity. Moreover, Modification 1 appears to present a higher potential of mean force, suggesting a more energetically favorable interaction with the receptor than Modification 2.

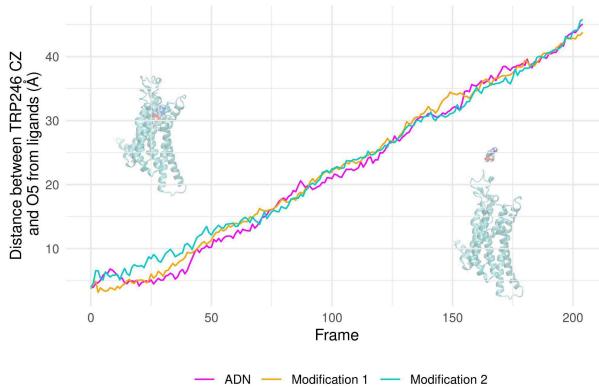


Figure 4. Trajectory plot displaying the separation distance between the CZ atom of TRP 246 on the A2A receptor and the O5 atom of the ligands across the initial 100 frames of the SMD simulation. Insets illustrate the position of the ADN ligand at the start (frame 0, left) and end (frame 100, right) of the simulation, demonstrating the extent of ligand movement.

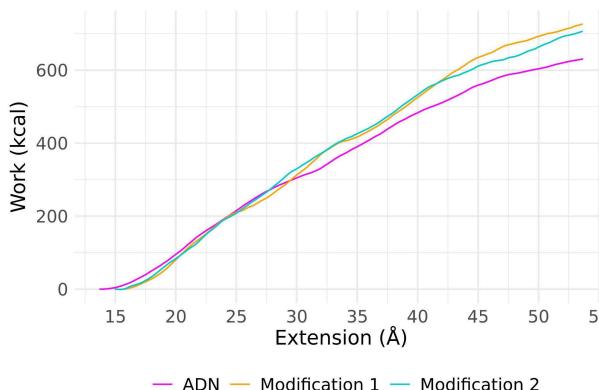


Figure 5. PMF profiles for the unbinding of ADN and its modifications from the A2A receptor.

CONCLUSION.

Classical molecular dynamics (MD) and steered molecular dynamics (SMD) were used to study the binding dynamics of adenosine and two derivatives to the A2A receptor. The introduction of a hydroxymethyl chain in Modification 1 augmented the number of receptor-ligand contacts, potentially indicating enhanced

binding stability. Modification 2, despite its increased bulk from a branching chain, was not large enough to displace a high number of water molecules due to the size of the receptor's cavity, resulting in a lower number of interactions than expected.

The SMD simulations further revealed that both modifications exhibit a greater PMF to unbind from the receptor, suggesting an increased affinity compared to the native ligand, adenosine. Particularly, Modification 1 required a slightly higher unbinding work, implying a more robust interaction with the receptor. This is complemented by the observed stability in contacts during the classical MD simulations, where Modification 1 maintained consistent interactions over time.

A critical limitation of this study has been the short simulation time due to the availability of computational resources. This affected the assessment of differences in transient and enduring contacts.

Future work should aim to extend the simulation time, providing more evidence on the stability of the ligand-receptor interactions.

METHODS.

Classical Molecular Dynamics Simulation

CHARMM-GUI

System Preparation: The receptor-ligand system was prepared using CHARMM-GUI [4,5,6]. The CHARMM36m force was selected for all the simulations. The receptor's PDB and the ligand's MOL2 files were uploaded for system set up. Ligand protonation was adjusted for pH 7.0, and disulfide bonds (PROA's cysteine residues 262-259, 77-166, 71-159, and 74-146) were specified. The simulation box was set to 79 Å in the X and Y dimensions, with the system neutralized and ionized to 150 mM NaCl. A POPC (phosphatidylcholine) lipid bilayer was chosen to represent the cell membrane.

GROMACS

Classical MD simulations were performed using GROMACS [7]. The GROMACS files were obtained from CHARMM-GUI.

Energy Minimization: Prior to dynamics, the system was subjected to energy minimization to remove potential steric clashes. The maximum force was set to 1000 $\text{kJ mol}^{-1} \text{ nm}^{-1}$ and a maximum of 5000 steps. Temperature was regulated at 303.15 K. Positional restraints were applied to backbone, side-chain, and lipid atoms with force constants of 4000, 2000, and 1000 $\text{kJ mol}^{-1} \text{ nm}^{-1}$, respectively. Non-bonded interactions were handled using a Verlet cutoff of 1.2 nm for both van der Waals and electrostatic interactions, and 1.0 nm for switching. These cutoffs were maintained during the rest of the steps.

Equilibration: The system was subjected to a two-phase equilibration process under NVT (steps 1 and

2) followed by NPT conditions (steps 3 to 6). Temperature was regulated at 303.15 K.

Production: Following equilibration, a 1.6 ns production run was performed with integration via Leap-frog at a 2 fs time step.

Steered Molecular Dynamics simulations

NAMD

The NAMD files were obtained from CHARMM-GUI.

Equilibration: The system underwent two equilibration steps. The system's temperature was equilibrated at 303.15 K, ensuring a consistent thermal environment over 125,000 steps (250 ps). Periodic boundary conditions were applied with Particle Mesh Ewald (PME) for long-range electrostatics, a grid spacing of 1.0 Å. Van der Waals interactions were managed with a cutoff of 12.0 Å and force-switching.

Production: Following equilibration, the production phase was executed over 40000 steps (80 ps), with a timestep of 2 fs. Specific forces were applied to atoms, guiding the separation along the z-axis at a velocity of 0.002 Å/timestep, with a force constant of 7.2 kcal/mol/Å². The application of forces and the system's response were monitored and recorded every 50 timesteps.

Visual Molecular Dynamics (VMD)

Root-Mean-Square Deviation (RMSD): RMSD calculations were performed using the RMSD trajectory tool, aligning to the protein backbone.

Volume Mapping (Volmap): VMD's Volmap tool was employed to visualize the water occupancy at 3 Å around the ligands.

Potential of Mean Force (PMF): the PMF calculations were conducted to quantify the work associated with the ligand unbinding.

Contact Analysis: contacts between receptor residues and the ligand were identified using the inter-sel option of the timeline tool. A cutoff distance between residues and ligand of 3 Å was set.

Wrapping: Trajectories were wrapped to keep the molecules of interest within the simulation box.

ABBREVIATIONS

AR, adenosine receptor; GPCR, G-protein-coupled receptor; cAMP, cyclic adenosine monophosphate; MD, molecular dynamics; SMD, steered molecular dynamics; RMSD, root-mean-square distance; VMD, Visual Molecular Dynamics; ADN, Adenosine; PMF, Potential of Mean Force; POPC, phosphatidylcholine; PME, Particle Mesh Ewald

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