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Received: 28 October 2025

Accepted: 13 February 2026

Published online: 20 July 2026

Cite this article as: Yasar E., Dogru S., Eroglu E. *et al.* Distinct conformational dynamics of the type-2 Angiotensin II receptor bound to Angiotensin II and Angiotensin (1–7). *Sci Rep* (2026). <https://doi.org/10.1038/s41598-026-40510-w>

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Distinct Conformational Dynamics of the Type-2 Angiotensin II Receptor Bound to Angiotensin II and Angiotensin (1–7)

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ABSTRACT

The Angiotensin II type-2 receptor (AGTR2) is a G protein-coupled receptor (GPCR) that mediates vasodilatory, anti-proliferative, and cardioprotective responses as part of the renin–angiotensin system (RAS). However, the precise structural dynamics underlying its ligand-specific activation remain incompletely understood. In this study, we performed long-timescale molecular dynamics (MD) simulations to investigate how Angiotensin peptides differentially modulate the structural dynamics of AGTR2 in both inactive- and active-like conformations and to elucidate ligand-specific modulation of AGTR2 in the RAS protective arm.

Ang II stabilized a ‘Locked Active State’ through compact TM3–TM6 distances, persistent hydrogen bonding (PHE8–LYS215^{5.42}), salt bridges (ARG2–ASP279^{6.58}, ARG2–ASP297^{7.32}), hydrophobic contacts (TRP100^{2.60}, MET128^{3.36}), and rotameric locking of micro-switches. These interactions stabilized Helix 8, enhancing dynamic network connectivity and supporting ligand-dependent activation-related conformational tendencies rather than a full canonical GPCR activation transition. Conversely, Ang1–7 induced a ‘Flexible Intermediate State’ characterized by weaker interactions (PRO7–THR125^{3.33}), dynamic instability at the Helix 8 interface, and increased conformational sampling. Activation motif analyses (CWxP, PIF, E/DRY, NPxxY), DCC maps, CP, DRIN metrics, and PCA confirmed distinct signaling features for each ligand.

This study provides a dual functional profile for AGTR2, where Ang II acts as a strong conformational stabilizer promoting activation-related dynamic features, while Ang 1–7 serves as a dynamic modulator. These findings contribute to a structural and dynamic framework for understanding AGTR2 signaling and supporting its therapeutic potential in fine-tuning cardiovascular responses within the RAS.

Keywords: AGTR2, Ligand binding, GPCR activation, Molecular modeling, Conformational dynamics

1 INTRODUCTION

The Renin-Angiotensin System (RAS) is a vital hormonal system that regulates blood pressure, fluid balance, and cardiovascular function through intricate signaling pathways¹⁻³. This system maintains homeostasis by modulating vascular tone, electrolyte levels, and cardiac workload, but its dysregulation mediates to disorders including hypertension, chronic kidney disease, and cardiac hypertrophy⁴⁻⁶. These conditions often stem from excessive Angiotensin II (Ang II: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) production or signaling imbalances, making RAS a key target for pharmacological interventions like angiotensin-converting enzyme (ACE) inhibitors and receptor blockers to restore cardiovascular health⁷, though questions about its protective components persist.

Within RAS, multiple receptors mediate its diverse physiological effects, including the Angiotensin II type-1 receptor (AGTR1), Angiotensin II type-2 receptor (AGTR2), Proto-oncogene Mas receptor (MAS1), and Mas-related G-protein-coupled receptor member D (MrgD), all belonging to the class-A G-protein-coupled receptor (GPCR) family^{8,9}. AGTR1 facilitates vasoconstriction, aldosterone release, and cell proliferation, promoting hypertensive and proliferative responses, whereas AGTR2 counters these effects by supporting vasodilation, anti-inflammatory processes, and anti-proliferative actions¹⁰⁻¹². This opposition positions AGTR2 as a key regulator of cardiovascular protection, contributing to blood vessel dilation, tissue repair, and attenuation of pathological remodeling. Despite its protective potential, the precise contribution of AGTR2 to the protective arm of RAS remains elusive, as experimental evidence on its modulation by specific ligands varies widely, complicating its therapeutic exploitation^{10,13}.

Signaling within RAS involves a variety of bioactive peptides, including Ang II, Angiotensin 1-7 (Ang 1-7: Asp-Arg-Val-Tyr-Ile-His-Pro), Angiotensin III (Ang 2-8: Arg-Val-Tyr-Ile-His-Pro), Angiotensin IV (Ang 3-8: Val-Tyr-Ile-His-Pro-Phe), and Alamandine (Ala-Arg-Val-Tyr-Ile-His-Pro-Phe), generated through enzymatic processing by ACE, ACE2 and

aminopeptidases¹⁴. While these peptides diversify RAS effects, their interactions with AGTR2 are less characterized compared to Ang II and Ang 1-7, which stand out due to their pivotal roles and distinct properties. Ang II, an octapeptide formed by ACE cleavage of Angiotensin I, is widely reported to activate AGTR2 and promote vasodilatory and protective signaling, eliciting vasodilation, tissue repair, and anti-proliferative effects in cardiovascular tissues^{15,16}. In contrast, Ang 1-7, a heptapeptide produced by ACE2-mediated removal of Ang II's terminal PHE8, primarily known for mediates anti-inflammatory and vasodilatory actions via the MAS1 receptor¹⁷⁻¹⁹; however, several studies have reported its ability to interact with AGTR2 and modulate downstream signaling pathways, albeit with potentially distinct efficacy and mechanisms compared to Ang II²⁰⁻²². These functional differences motivated our comparative investigation of ligand-specific modulation of AGTR2 within the protective arm of the RAS. Ang II consistently activates AGTR2 to support protective effects, such as vasodilation in resistance arteries and anti-fibrosis^{23,24}, yet the role of Ang 1-7 at AGTR2 is less certain; some evidence suggests it acts as a partial agonist or modulator, potentially broadening its protective influence beyond MAS1^{25,26}, whereas other studies dispute its binding affinity and functional impact¹³. These conflicting data underscore a critical need to clarify how Ang II and Ang 1-7 modulate AGTR2 in the protective arm of RAS, justifying our choice of these ligands to examine their specific effects on receptor conformational dynamics and activation-related structural features.

Structural studies have provided critical insights into the binding and activation mechanisms of RAS receptors and their ligands. Crystal structures of AGTR1 (PDB codes: 4YAY, 6OS0;^{27,28}) and AGTR2 (PDB codes: 5UNG, 6JOD;^{29,30}) in ligand-bound states, alongside recent cryo-EM structures of MrgD (PDB code: 7Y15, 9DQH;^{31,32}) and AGTR1 (PDB code: 7F6G;³³) in G-protein-bound states, reveal key receptor conformations and activation features, such as TM6 outward movements and micro-switch motif transitions³⁴. Complementary NMR analyses have determined the three-dimensional structures of Ang II (PDB code: 1N9V;³⁵) and Ang 1-7 (PDB code: 2JP8;³⁶), offering detailed views of their binding configurations. Despite these advances, structural understanding of the inactive and restrained conformational states of AGTR2 remains limited, particularly regarding intracellular accessibility and activation-related dynamics. Previous computational studies using short-timescale simulations have failed to fully capture these dynamics, providing only snapshots of ligand-receptor interactions³⁷. In contrast, repeated long-timescale molecular dynamics (MD) simulations enable comprehensive analysis of conformational changes and interaction networks over extended periods, offering a robust approach to address these gaps³⁸.

This study employs repeated long-timescale MD simulations to examine the ligand-specific effects of Ang II and Ang 1-7 peptides on inactive and active states of AGTR2, seeking to clarify how AGTR2 modulation shapes the protective arm of RAS and address the mechanistic uncertainties surrounding role of these ligands. Through detailed analysis of ligand-receptor interactions, structural stability and activation pathways we seek to establish a direct link between ligand binding and AGTR2 conformational dynamics and downstream signaling tendencies³⁹. By elucidating the biophysical mechanisms such as the stabilizing effects of Ang II versus the modulatory influence of Ang 1-7 this work explores whether elements of class-A GPCR activation-related dynamics are recapitulated in AGTR2 while acknowledging its atypical signaling behavior and strives to provide a structural and dynamic framework for understanding protective contributions of AGTR2 within RAS. These insights may help inform the development of targeted therapeutic strategies for cardiovascular diseases, addressing a critical gap in harnessing regulatory capacity of AGTR2⁷.

2 MATERIALS AND METHODS

2.1. Structural Preparation

The active-state AGTR2 structure bound to Ang II (PDB: 6JOD; ³⁰) was sourced from the Protein Data Bank ⁴⁰. For the inactive state, although the crystal structure of AGTR2 bound to the antagonist EMA401 is available (PDB: 7JNI), this structure was not used directly as the starting coordinate for simulations focused on intracellular conformational dynamics. Structural analysis indicates that 7JNI adopts an "active-like" 7-transmembrane bundle despite antagonist binding ⁴¹, and the inward orientation of Helix 8 partially occludes the intracellular cavity. Because our aim was to investigate ligand-dependent intracellular conformational dynamics, we employed an alternative starting model in which this region remained accessible. This model was used as a complementary conformational starting point rather than a replacement for the experimental structure. Consequently, to ensure a canonical inactive starting conformation, an AlphaFold-predicted inactive model of AGTR2 was obtained from GPCRdb ⁴² and validated against the inactive AT1R structure (see Supplementary Fig. S4). Peptide structures for Ang II (PDB: 1N9V; ³⁵) and Ang 1-7 (PDB: 2JP8; ³⁶) were retrieved from NMR data in the PDB. All missing residues were modeled using MODELLER web server ⁴³, and protonation states at pH 7.0 were assigned with H++ web server ⁴⁴ to complete hydrogen atoms. Initial structures were used for molecular docking and representative starting conformations are provided in the supplemental figures (Fig S1).

2.2. Molecular Docking

Given the polypeptide nature of the ligands and the use of a homology model for the inactive state, a site-directed (focused) docking approach was employed as a comprehensive structural validation step using MDockPeP, an ab-initio protein-peptide docking program with a statistical potential-based scoring function (ITScorePeP)⁴⁵. First, to validate the blind docking protocol, the co-crystallized Ang II was computationally removed from the active-state crystal structure (PDB: 6JOD), and site-directed docking was performed as a positive control. The protocol successfully reproduced the experimental crystallographic binding pose, confirming the accuracy of the sampling and scoring algorithms for orthosteric peptide binding in this receptor system. Subsequently, the same site-directed docking strategy was applied to identify high-affinity binding poses of Ang II and Ang 1–7 to the inactive AlphaFold model. This approach allowed the flexible peptide ligands to explore the defined orthosteric binding pocket and its immediate extracellular vicinity without imposing pose bias within the grid boundaries. In all cases, the top-ranked clusters converged to the canonical orthosteric binding pocket. The poses with the lowest ITScorePeP scores within this validated binding site were selected as starting configurations for MD simulations (Fig S2).

2.3. Molecular Dynamics Simulation

To investigate the conformational dynamics of AGT2R, a total of six systems were prepared using two distinct structural templates: the inactive AlphaFold predicted model (described in Section 2.1) and the active crystal structure (PDB: 6JOD). Specifically, Apo (ligand-free) simulations were conducted in duplicate, initiated from both the inactive homology model and the active crystal structure, to benchmark intrinsic receptor dynamics and assess conformational drift. For each structural state, ligand-bound conditions (Ang II and Ang 1–7) were also simulated in duplicate. Each of the six systems was simulated in two independent replicates of 1 μ s each, yielding a total of 12 μ s simulation across all conditions. These replicates were used to assess convergence and reproducibility of ligand-specific effects. All protein structures were positioned within a lipid bilayer comprising 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), and cholesterol (CHL) in a 2:2:1 ratio, assembled using the Membrane Builder package of CHARMM-GUI^{46,47}. Membrane orientation was determined with the Positioning of Proteins in Membrane (PPM) server⁴⁸. Each ligand-protein-membrane complex was placed in a $93 \times 93 \times 117$ angstrom ($\text{\AA}$) simulation box, solvated with TIP3P water molecules⁴⁹, and

neutralized with 0.15 mM NaCl, resulting in systems of approximately 100,000 atoms, including ~253 lipids, ~63,000 water molecules, and 50-70 Na⁺/Cl⁻ ions. Simulations employed the CHARMM36m force field⁵⁰ and were conducted using GROMACS 2020.5⁵¹ on NVIDIA RTX A4000 GPUs. Systems were subjected to 5000-step conjugate gradient minimization to eliminate steric clashes, followed by 10 ns equilibration where the temperature was gradually raised from 0 K to 310 K in the NVT ensemble using a Nose-Hoover thermostat⁵² with a 1.0 ps coupling constant, and pressure was equilibrated in the NPT ensemble with a semi-isotropic Berendsen barostat⁵³. Duplicate 1-microsecond (μ s) production runs per system were performed at 310 K and 1 bar with a 2 fs timestep, using a Parrinello-Rahman barostat⁵⁴ to maintain pressure, periodic boundary conditions, Particle Mesh Ewald (PME) electrostatics⁵⁵, and SHAKE constraints⁵⁶.

2.4. Data Analysis

For each of the six systems, two independent 1- μ s replicates were performed to ensure reproducibility, assess structural and dynamic changes in AGTR2 induced by ligand binding. Analyses were conducted on one representative trajectory per system, randomly selected from the two replicates, as both runs exhibited comparable RMSD profiles and overall structural stability (Fig. S15).

Root-mean-square deviation (RMSD) was calculated using GROMACS tools⁵⁷ to evaluate overall system stability, as well as stability of individual transmembrane (TM) helices, Helix8, and ligands Ang II and Ang 1-7. Root-mean-square fluctuation (RMSF) assessed residue flexibility across the protein. Two-dimensional (2D) ligand-receptor interactions were analyzed with Maestro (Schrödinger Maestro, 2020) during a 1-month free demo⁵⁸. Hydrogen bond (2,000 frames) and salt bridge (10,000 frames) interactions were quantified using VMD 1.9.3⁵⁹, and hydrophobic interaction occupancies (%) were assessed over 2,000 frames with custom Python scripts developed by the authors, utilizing the MDAnalysis package⁶⁰ for trajectory processing, available at https://github.com/eygpcr/hydrophobic_interactions. Ligand-protein interactions were visualized through movies generated with VMD and are accessible on Zenodo (<https://doi.org/10.5281/zenodo.14697886>). Distance analyses between the terminal amino acids of Ang II (PHE8) and Ang 1-7 (PRO7) and MET128^{3,36 61}, previously identified as a key hydrophobic contact in Ang II-AGTR2 binding³⁰, were conducted to monitor simulation-long changes in ligand-receptor interactions. Advanced analyses, including Principal Component Analysis (PCA) to identify dominant conformational modes, Communication Propensity (CP) to assess residue communication efficiency, Dynamic Cross-Correlation (DCC) to detect correlated motions, Betweenness Centrality (BC) to evaluate residue signaling roles, and Dynamic Residue Interaction Network (DRIN) to map

dynamic interaction pathways, were conducted via MDM-TASK^{62,63} to characterize ligand-driven dynamics, with significant signaling residues ($\pm 3\sigma$) depicted in snake diagrams and details provided in Supplementary Methods. Center-of-mass (COM) distance measurements between intracellular TM helices (TM3, TM5, TM6, TM7), including the hydrophobic lock region (TM3-TM6), and dihedral angle shifts (ϕ , ψ , χ) in micro-switch regions (PIF, E/DRY, CWxP, and NPxxY) were analyzed using the `gmx_distance` tool and custom Python scripts developed by the authors, incorporating MDAnalysis for data handling (https://github.com/eygpcr/microswitches_dihedral_angles), to probe ligand-induced conformational shifts and transitions critical to AGTR2 activation. To characterize the conformational heterogeneity and identify dominant structural states, we performed clustering analysis using the GROMOS algorithm (via GROMACS `gmx_cluster` module) on the equilibrated trajectories. A backbone RMSD cutoff of 0.2 nm was applied to the receptor structure. The central structure of the most populated cluster (Cluster 1) was extracted as the dominant structure for each system. Additionally, the full ensemble of cluster centers was extracted to assess the range of conformational sampling and structural diversity. These representative PDB structures, along with the full clustering statistical logs and RMSD matrices, have been deposited in the Zenodo repository (<https://doi.org/10.5281/zenodo.18433392>). Trajectories are available on the GPCRmd database platform⁶⁴ (PDB: 6JOD; Uniprot: P50052; <https://www.gpcrmd.org/dynadb/search/>).

3 RESULTS and DISCUSSION

3.1. Ligand Binding Modes and Interactions

Our 1- μ s MD simulations revealed distinct binding behaviors of Ang II and Ang 1–7 with AGTR2, driven by differential interaction patterns that reflect their sequence divergence and functional specificity. Ang II, an octapeptide, differs from Ang 1–7 solely by the presence of a C-terminal last residue PHE8. Despite this minimal difference, Ang II established a significantly more extensive and stable interaction network with the receptor across both inactive and active states.

Ang II formed numerous persistent hydrogen bonds throughout the 1- μ s simulation, which were analyzed over 2000 trajectory frames. Notably, a highly stable interaction was observed between LYS215^{5,42}–PHE8 (945 frames inactive, 750 frames active), ARG182^{4,64}–HIS6 (803 and 456 frames), and THR125^{3,33}–PRO7 (811 frames inactive) (Table S1–S2, Fig. 1a). In contrast, Ang 1–7 exhibited fewer and more transient hydrogen bonds. The dominant THR125^{3,33}–PRO7 bond (1293 frames in the inactive state) declined sharply in the active state, highlighting a conformational shift associated with ligand-dependent changes in receptor conformation.

Salt bridge analysis further emphasized this contrast. Both ligands engaged the receptor via ARG2, forming electrostatic interactions with ASP279^{6,58}. However, regarding the interaction with ASP297^{7,32}, while Ang II maintained stable contact, Ang 1–7 exhibited a loss of this interaction in the active state (Fig. 1b). Consequently, these bridges were substantially more stable in Ang II-bound systems, as confirmed by lower distance fluctuations over time (Fig. 1b), suggesting a more locked-in conformation. Ang 1–7 maintained these salt bridges less consistently, indicating a looser electrostatic anchoring.

Importantly, several of our observed key interactions such as Ang II forming stable bonds involving LYS215^{5,42}, ARG182^{4,64} and ASP297^{7,32} are consistent with earlier mutagenesis studies that demonstrated the critical role of these residues in ligand binding affinity and receptor functional responses in AGTR2^{65–67}.

Hydrophobic contacts played a critical role in defining binding depth and stability. Ang II established a broad hydrophobic interaction network across both receptor states. In the inactive state, key interactions included TRP100^{2,60}–ILE5 (91%), TYR108–TYR4 (75%), LYS215^{5,42}–PHE8 (59%), and MET128^{3,36}–PHE8 (42%). In the active state, residues such as TRP283^{6,62}–ARG2 (97%) and TRP100^{2,60}–PRO7 (94%) became more dominant (Fig. 1c). Notably, three hydrophobic interactions LYS215^{5,42}–PHE8, PHE272^{6,51}–PHE8, and MET128^{3,36}–PHE8 persisted across both receptor states, highlighting their critical role in stabilizing Ang II binding. Among these, the MET128^{3,36}–PHE8 contact has previously been identified as a key anchoring interaction³⁰, and its presence here further supports the ligand's ability to stabilize specific receptor conformations. In contrast, Ang 1–7, lacking the PHE8 residue, formed fewer hydrophobic interactions, primarily involving TRP100^{2,60}–ILE5 (81%) and PHE272^{6,51}–PRO7 (74%) in the inactive state, and TYR108–ILE5 (90%) and TRP100^{2,60}–PRO7 (88%) in the active state. These interactions were more localized and exhibited lower occupancy overall, underscoring a relatively less stabilized and more flexible binding configuration. This is further corroborated by our distance analysis (Fig. S6b), which shows that the distance between MET128^{3,36} and PHE8 remains stably around ~5 Å in both receptor states, whereas the corresponding distance with PRO7 in Ang 1–7 displays greater fluctuation and increases to ~7–10 Å, reflecting a weaker and less stable hydrophobic anchoring. In addition, RMSD analysis for both ligand (Fig. S6a) demonstrates that Ang II exhibits lower overall ligand mobility compared to Ang 1–7, particularly in the active state, supporting the notion that PHE8 contributes to reduced ligand mobility and enhanced binding stability.

Two-dimensional interaction maps (Fig. S3) dynamic visualizations (Zenodo: <https://doi.org/10.5281/zenodo.14697886>) support these findings, where Ang II exhibited denser

contact patterns including hydrogen bonds, salt bridges, and π - π stacking, particularly around PHE8. Conversely, Ang 1–7 showed reduced interaction density and lacked several key contact points evident in Ang II binding.

These binding differences reflect broader functional implications. The rigid and persistent interaction network of Ang II likely facilitates a more conformationally stabilized receptor state, which may be compatible with stronger downstream signaling responses and potentially enhance AGTR2's counter-regulatory function against AGTR1^{68,69}. In contrast, Ang 1–7's sparser, more dynamic binding pattern may enable greater conformational flexibility, in line with its modulatory and protective roles via MAS1 signaling^{13,25,70}. This distinction is particularly evident in the transient nature of key hydrogen bonds and the lack of anchoring by PHE8, a feature unique to Ang II. Altogether, our long-timescale simulations provide structural and temporal resolution that surpasses previous docking-based or short-time MD studies^{37,71}, uncovering how even a single-residue difference between two closely related ligands can drastically alter binding dynamics and receptor modulation. These results position AGTR2 as a dynamic receptor capable of ligand-specific tuning, with potential relevance in targeting distinct arms of the RAS^{7,11}.

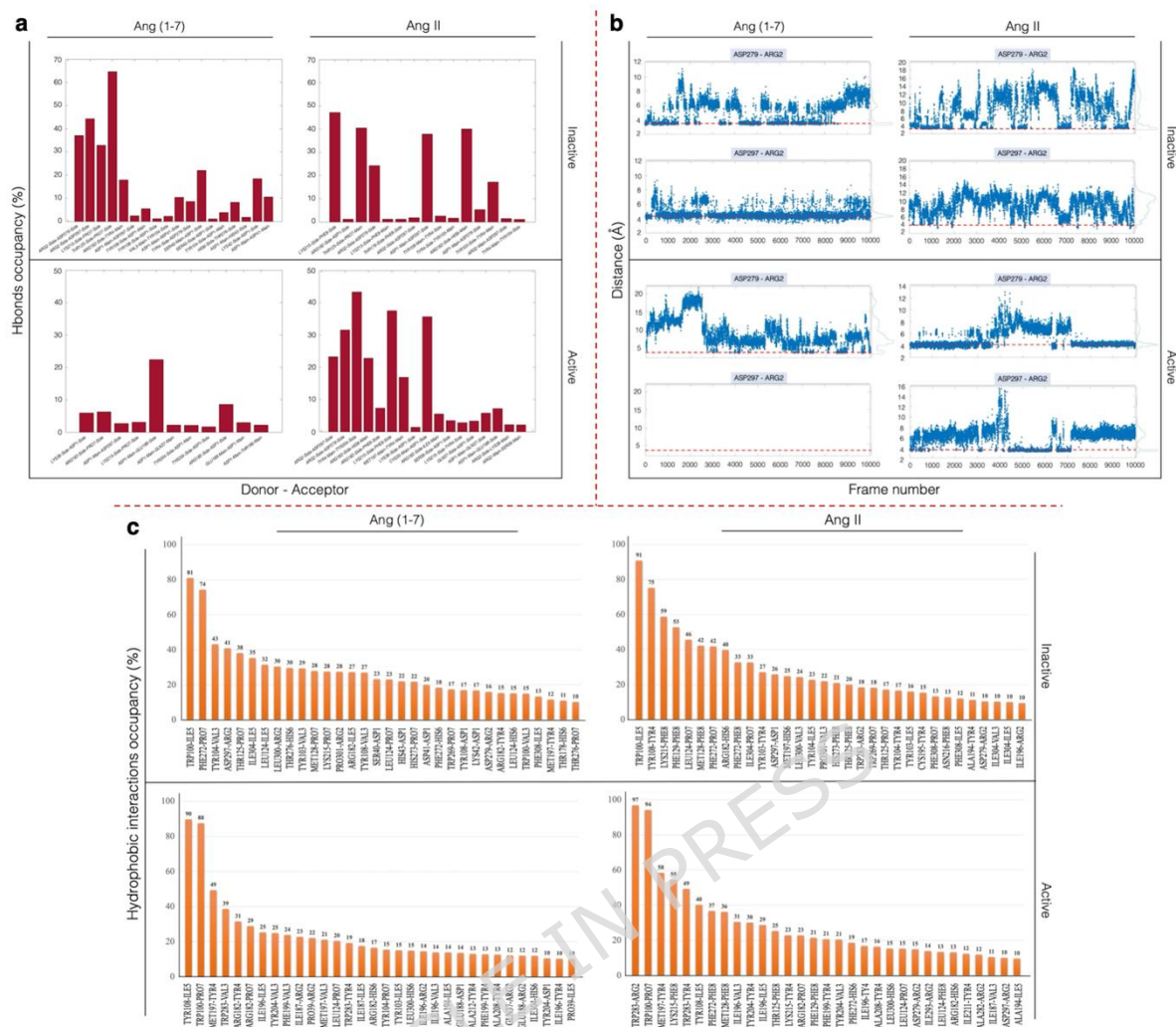


Figure 1. Interaction analyses between AGTR2 and the ligands Angiotensin 1–7 (Ang 1–7) and Angiotensin II (Ang II) over 1 μ s molecular dynamics simulation. a) Hydrogen bond occupancy percentages between ligands and AGTR2 residues. b) Distance fluctuations (Å) of key salt bridges involving ARG2-ASP279^{6,58} and ARG2-ASP297^{7,32} c) Hydrophobic interaction occupancy percentages between ligands and AGTR2 residues. Results are shown separately for inactive and active receptor conformations.

3.2. Structural Stability and Flexibility

RMSD trajectories revealed distinct conformational behaviors driven by ligand occupancy. In the active-state simulations (Fig. 2b), the Ang II-bound system maintained high structural stability with the lowest deviation (~ 3.0 Å), effectively preserving the crystallographic active conformation (PDB: 6JOD) and serving as a stable internal control. In contrast, the Apo state exhibited a progressive increase in RMSD, reflecting the expected structural relaxation

(deactivation) of the receptor from the high-energy active conformation towards an inactive-like ensemble in the absence of stabilizing agonists. Similarly, the Ang 1–7-bound state displayed a notable deviation from the starting Ang II-bound template. This indicates that Ang 1–7, as a biased ligand, does not strictly maintain the canonical active conformation but induces a distinct conformational adaptation, steering the receptor into a specific sub-state. These trends were consistent across independent replicate simulations (Fig. S15), confirming that the observed drifts represent reproducible dynamic properties rather than random equilibration artifacts.

RMSF profiles further elucidated these dynamics. Ang II significantly restricted movements in critical activation-related structural regions such as TM6 and Helix 8 (Fig. 2a–b), stabilizing the receptor in a more compact and activation-compatible conformational ensemble. Conversely, Ang 1–7 allowed elevated flexibility, particularly in TM3, TM7, and extracellular loop (ECL) regions. This distinct flexibility profile suggests a more permissive and less conformationally constrained receptor state, consistent with the hypothesis that Ang 1–7 modulates receptor function through an alternative activation-related mechanism distinct from the stabilization pattern observed with Ang II.

To further dissect these effects, we analyzed the RMSD profiles of individual transmembrane helices. Figure S4 demonstrates that Ang II consistently dampened structural deviations across nearly all TM domains compared to Ang 1–7 or apo systems, in both inactive and active conformations. In the inactive state, Ang II binding resulted in notably reduced fluctuations across the receptor core, effectively stabilizing TM1, TM3, TM4, TM6, TM7, and Helix 8 with RMSDs remaining below $\sim 3\text{--}4$ Å. In contrast, Ang 1–7 and apo systems displayed increased mobility, which was particularly pronounced in TM1, TM4, and Helix 8, exceeding the fluctuation levels observed in TM7 (Fig. S5a). In the active state, Ang II continued to stabilize TM1, TM6, and Helix 8, while Ang 1–7 and apo complexes exhibited larger RMSD excursions in these helices, often exceeding $\sim 5\text{--}6$ Å. These patterns highlight the role of Ang II in constraining the receptor's helical core, particularly in regions commonly associated with activation-related rearrangements.

In line with general principles of class-A GPCR activation³⁴, these findings suggest that Ang II stabilizes conformational features commonly associated with signaling-competent states, potentially priming AGTR2 for downstream signaling events^{29,72}. On the other hand, Ang 1–7 introduces conformational variability, suggesting that the ligand-receptor complex samples a broader conformational landscape rather than stabilizing a single active state⁷³. These ligand-

specific effects, captured over long-timescale MD simulations, reveal AGTR2's structural plasticity and underscore its capacity for fine-tuned regulation within the RAS^{10,11}.

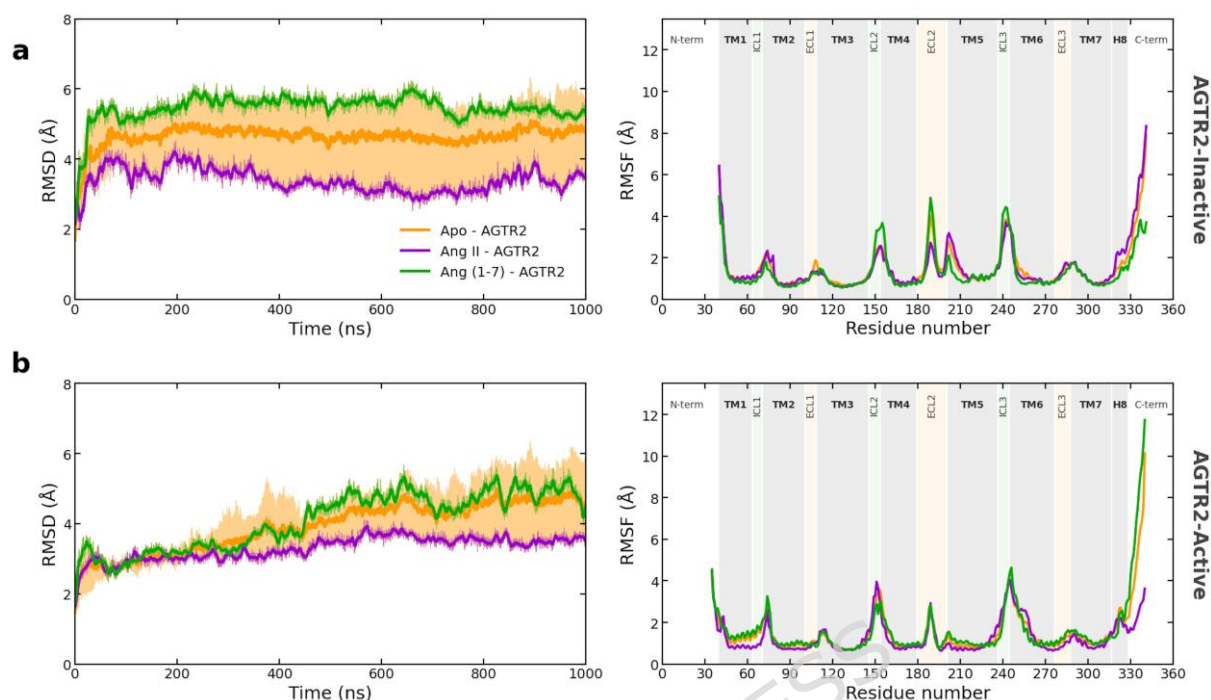


Figure 2. Root mean square deviation (RMSD) and root mean square fluctuation (RMSF) analyses of AGTR2 structures over 1 μ s molecular dynamics simulations. a) RMSD and RMSF profiles of the inactive-like AGTR2 in the Apo state (orange), Angiotensin II (Ang II)-bound state (purple), and Angiotensin 1–7 (Ang 1–7)-bound state (green). b) RMSD and RMSF profiles of the active-like AGTR2 in the Apo, Ang II-bound, and Ang 1–7-bound states. RMSF plots are shown as a function of residue number, with transmembrane (TM) helices and loop regions highlighted by transparent vertical shading. Raw trajectory data are shown in lighter shades, with smoothed trend lines overlaid to highlight average structural deviations.

3.3. Activation Pathways and Micro-Switch Dynamics

One of the most important findings of this study is how Ang II and Ang 1–7 differentially modulate the intracellular architecture of AGTR2, particularly through distances in transmembrane (TM) helix spacing and the dynamic behavior of key micro-switch motifs. A common indicator of class-A GPCR activation³⁴ is the outward movement of TM6 (increasing TM3–TM6 distance), although AT2R may not strictly follow this canonical activation pathway. In this regard, Figure 3 reveals critical state-dependent variations driven by the absence of an intracellular transducer (G-protein).

In the inactive-like state simulations (Fig. 3a), Ang II maintained a TM3–TM6 distance (~ 12 – 13 Å), effectively preventing spontaneous outward fluctuations and stabilizing the receptor in a compact intracellular conformational ensemble. By contrast, both Ang 1–7 and Apo systems, despite starting from similar TM3–TM6 distances, progressively expanded to ~ 17 – 18 Å. This indicates that without the strong conformational constraints of the full agonist Ang II, the inactive receptor undergoes spontaneous relaxation toward a more expanded intracellular helical arrangement. In the active-like state simulations (Fig. 3b), initiated from the agonist-bound active crystal structure (PDB: 6JOD), distinct ligand-dependent dynamics were observed. Ang II initially maintained a wide TM3–TM6 distance (~ 17 – 18 Å) for approximately 500 ns, reflecting a conformation commonly associated with signaling-competent states in class-A GPCRs. However, in the latter half of the simulation, this spacing gradually narrowed to ~ 12 – 13 Å. This behavior is biophysically expected; while Ang II stabilizes the active core, the fully open crystallographic conformation typically requires the steric wedge of an intracellular G-protein to maintain. Consequently, in the absence of the G-protein transducer in our simulations, the receptor relaxed from the artificially propped-open state into a compact active-like intermediate state. Crucially, the kinetics of this compaction distinguish agonist-driven stabilization from Apo deactivation. The Apo state underwent a rapid, spontaneous inward collapse from ~ 20 Å to ~ 12 Å within the first 150 ns, reflecting immediate instability in the absence of ligand. In contrast, Ang II resisted this collapse for ~ 500 ns and eventually settled into a rigid, tightly packed scaffold rather than a disordered inactive state. This implies that Ang II promotes conformational stabilization that delays closure and maintains helical packing, whereas Apo collapses due to dynamic drift. Meanwhile, Ang 1–7 consistently maintained an intermediate spacing (~ 13 – 14 Å) with greater fluctuations throughout the trajectory, consistent with a weaker or more transient stabilization pattern compared to Ang II that induces a less constrained, looser receptor conformation.

Additionally, TM7-associated distances (e.g., TM3–TM7, TM5–TM7, TM6–TM7) remained more compact in Ang II-bound systems compared to Apo, suggesting ligand-induced constraints on TM7 mobility. These results imply that Ang II stabilizes structural features often associated with the GPCR active core by restricting conformational dynamic²⁷, whereas Ang 1–7 permits greater conformational flexibility, indicating that Ang 1–7 does not enforce the rigid constraints typically observed with full agonists, consistent with a distinct activation profile⁷⁴.

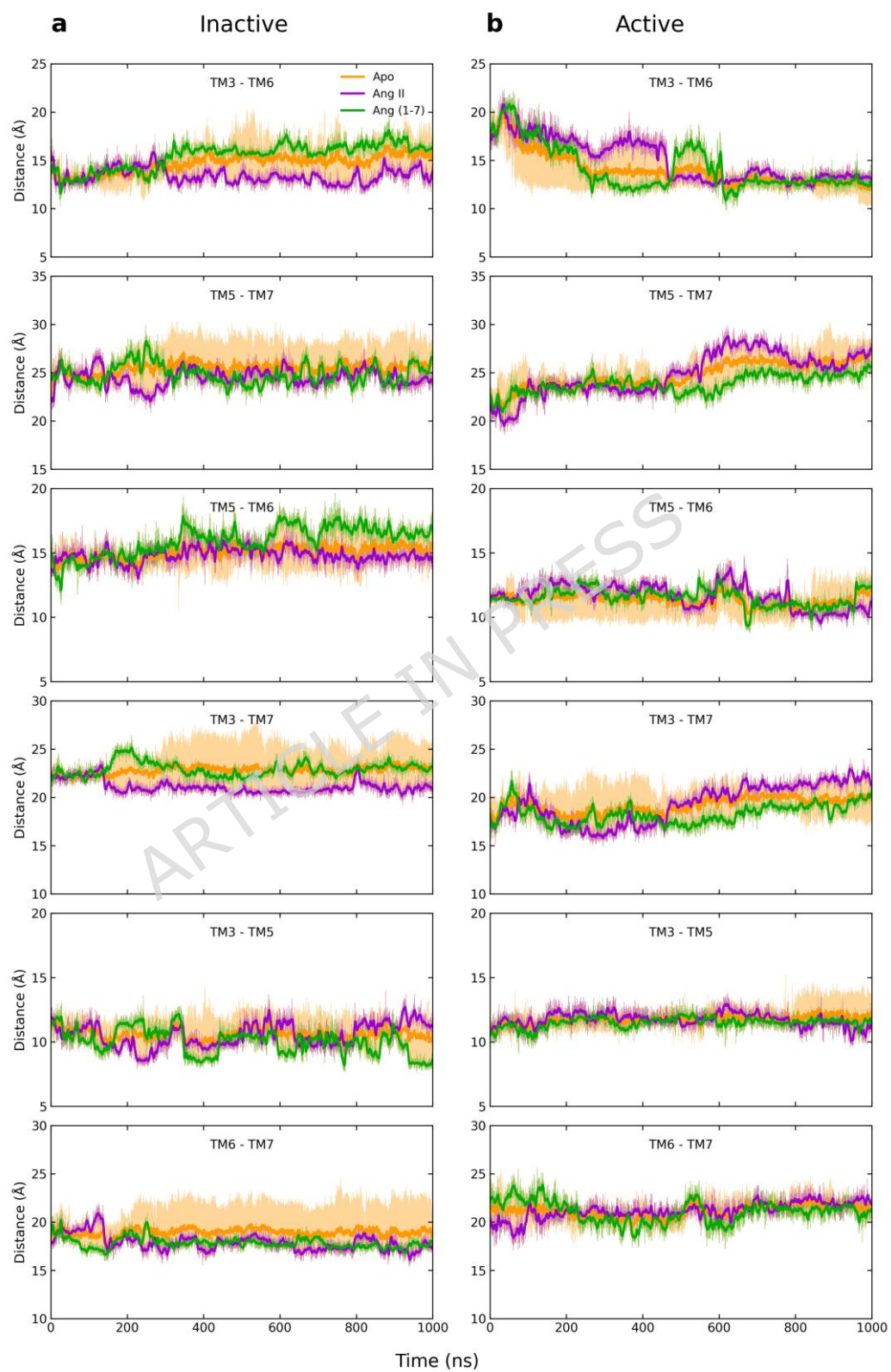


Figure 3. Hydrophobic lock distance analyses of AGTR2 during 1 μ s molecular dynamics simulations. a) Inactive-like and b) active-like states showing time-dependent variations in the intracellular distances between transmembrane helices (TM3–TM6, TM5–TM7, TM5–TM6, TM3–TM7, TM3–TM5, and TM6–TM7) in Apo-AGTR2 (orange), Angiotensin 1–7 (Ang 1–7)-bound AGTR2 (green), and Angiotensin II (Ang II)-bound AGTR2 (purple) complexes. To facilitate visualization of conformational trends, smoothed trend lines are overlaid on the raw distance fluctuation data.

To further dissect these activation differences, we analyzed the dynamics of conserved micro-switch motifs: PIF (PRO223^{5.50}–ILE132^{3.40}–PHE265^{6.44}), E/DRY (ASP141^{3.49}–ARG142^{3.50}–TYR143^{3.51}), CWxP (CYS268^{6.47}–TRP269^{6.48}–LEU270^{6.49}–PRO271^{6.50}), and NPxxY (ASN314^{7.49}–PRO315^{7.50}–PHE316^{7.51}–LEU317^{7.52}–TYR318^{7.53}) (Figs. S11–S14). Dihedral angle analyses revealed that Ang II tightly constrained ϕ , ψ , and χ_1 dihedral angles of key residues such as PHE265^{6.44}, ARG142^{3.50}, and TYR318^{7.53} within $\pm 10^\circ$ – 15° , preserving their active-state geometries. In contrast, Ang 1–7 exhibited broader angle fluctuations (up to $\pm 25^\circ$), particularly in CWxP and NPxxY motifs, and Apo systems showed the largest dihedral noise. These micro-dynamics reinforce the conclusion that Ang II promotes an active-like conformational stabilization pattern via both backbone and side-chain stabilization mechanisms, while Ang 1–7 enables a more flexible, intermediate activation profile. Crucially, this analysis demonstrates that activation-related conformational tendencies may involve precise side-chain rotameric stabilization, effectively explaining the conformational mechanics that remain masked in global backbone RMSF profiles.

These dihedral patterns align with the TM distance data and collectively reflect distinct activation modes. Ang II promotes a more stable and coordinated rearrangement of both helical spacing and micro-switches⁷⁵, hallmarks commonly associated with agonist-stabilized GPCR conformations^{39,76}. In contrast, Ang 1–7 supports partial engagement, retaining some activation features but lacking the full rigidity seen with Ang II. This duality echoes prior suggestions that Ang 1–7 has been suggested to exhibit biased or alternative signaling tendencies or allosteric modulator^{25,77,78}, allowing AGTR2 to adopt intermediate or context-dependent signaling profiles. Altogether, these dynamic, long-timescale simulations reveal that Ang II initiates a transient yet pronounced activation-related conformational stabilization pattern in AGTR2, while Ang 1–7 facilitates partial activation through selective micro-switch engagement and intermediate TM architecture. This differential modulation may underlie the complementary but distinct roles of these peptides in the RAS, where Ang II is associated with conformational features compatible with G-protein signaling, and Ang 1–7 has been associated

with protective and anti-inflammatory effects, potentially involving alternative signaling pathways.

3.4. Dynamic Network Responses

To further understand how Ang II and Ang 1–7 differentially regulate AGTR2 function, we investigated their effects on the receptor's signaling communication network using multiple trajectory-based dynamic analyses. These included dynamic residue interaction network (DRIN) metrics such as betweenness centrality (BC) and average shortest path length (ΔL), as well as communication propensity (CP), dynamic cross-correlation (DCC), and principal component analysis (PCA)^{62,63}.

DRIN results revealed that Ang II binding significantly increased BC in key micro-switch and transmembrane residues (e.g., MET128^{3,36}, PHE265^{6,44}, TYR318^{7,53}) in both inactive and active states (Fig. 4b, 4d; Table S4), surpassing the 3σ threshold and highlighting the emergence of highly centralized hubs. These residues align with previously reported activation-related hotspots in GPCRs^{28,30}. In contrast, Ang 1–7 elevated BC primarily in peripheral or flexible regions (e.g., ILE304^{7,39}, PRO315^{7,50}) (Fig. 4a, 4c; Table S3), supporting a broader but less centralized communication network. These findings were spatially mapped in snake diagrams (Fig. S10). Visual inspection reveals that Ang II engagement resulted in prominent communication hubs entrenched within the core transmembrane helices (particularly TM2, TM3, TM6, and TM7), suggesting a more centralized and continuous intramolecular communication pathway. Conversely, while Ang 1–7 also engaged transmembrane domains, it elicited a more scattered distribution of high-centrality residues that lacked the continuous helical coherence observed with Ang II, alongside notable signaling nodes appearing in extracellular loops (e.g., ECL2) and the C-terminus.

In parallel, Ang II markedly reduced ΔL values (i.e., the number of steps required for inter-residue communication) in core transmembrane segments, especially within TM6 and TM7 (e.g., PHE265^{6,44}, TYR318^{7,53}), indicating enhanced transmission efficiency. In contrast, Ang 1–7 and Apo systems exhibited longer paths and higher ΔL , particularly in active-state conformations (Table S3–S4). These differences were further corroborated by CP analyses (Fig. S6), where Ang II displayed robust CP values (15–20 Å²) across TM helices, reflecting the extensive inter-residue communication networks commonly associated with signaling-competent GPCR conformations. In contrast, Ang 1–7 produced moderate and more selective CP values (5–10 Å²), consistent with a more modulatory role compared to the stabilization

pattern observed with Ang II. The Apo condition showed minimal communication ($<5 \text{ \AA}^2$), with the most pronounced differences observed between inactive and active conformational states.

DCC maps supported this pattern (Fig. S8). In both conformational states, Ang II induced strong positive correlations ($C_{ij} > 0.7$) in organized block patterns, particularly in regions corresponding to key TM domains critical for G-protein coupling, reflecting coherent residue motions often associated with coordinated signaling-related dynamics. Ang 1–7 induced more fragmented and heterogeneous correlations ($C_{ij} \approx 0.3\text{--}0.5$) with increased anti-correlations, consistent with its modulatory role. Interestingly, Apo simulations showed relatively stable correlation patterns in the inactive state but became more disorganized upon activation, suggesting that ligand binding is crucial for maintaining coordinated dynamics in conformations associated with signaling competence.

Finally, principal component analysis (PCA) offered a global view of the conformational ensemble sampled during the 1 μs simulations, allowing us to map the ligands onto the agonist-antagonist spectrum (Fig. S9). The Ang II-bound active systems clustered into a compact and well-defined conformational space (with the first two PCs explaining over 45% of the variance) (Fig. S9b), indicating its tendency to stabilize a more compact conformational ensemble associated with signaling competence.

In contrast, Ang 1–7 generated a much broader, scattered distribution (Fig. S9b). This scattering correlates with the helix-specific RMSD analysis, where Ang 1–7 induced distinctively high fluctuations ($>10 \text{ \AA}$) in Helix 8 compared to the stable Ang II-bound state (Fig. S5b). Unlike antagonists (e.g., in PDB: 7JNI) that sterically occlude the G-protein binding site via a static inward Helix 8, our data suggests that Ang 1–7 may reduce the stability of intracellular conformations required for efficient effector coupling, potentially through increased Helix 8 mobility. Consequently, the PCA and RMSD data collectively define Ang 1–7 as stabilizing a flexible intermediate state, distinct from both the rigid active conformation of Ang II and the statically occluded inactive conformation of antagonists. Apo systems remained the most dispersed, underscoring the stabilizing role of ligand binding in signaling network integrity.

Taken together these findings, dynamic analyses converge on a consistent picture: Ang II fosters centralized, efficient, and tightly coordinated communication pathways across AGTR2, reinforcing its role as a strong conformational stabilizer associated with activation-related communication patterns⁷⁹. Ang 1–7, however, enhances flexibility and expands conformational diversity through a broader and less focused influence on the signaling

network, consistent with its reported modulatory and protective roles^{80,81}. These results highlight the ligand-dependent plasticity of AGTR2's signaling machinery and offer molecular insight into how structurally similar peptides may contribute to distinct functional outcomes within the RAS⁸².

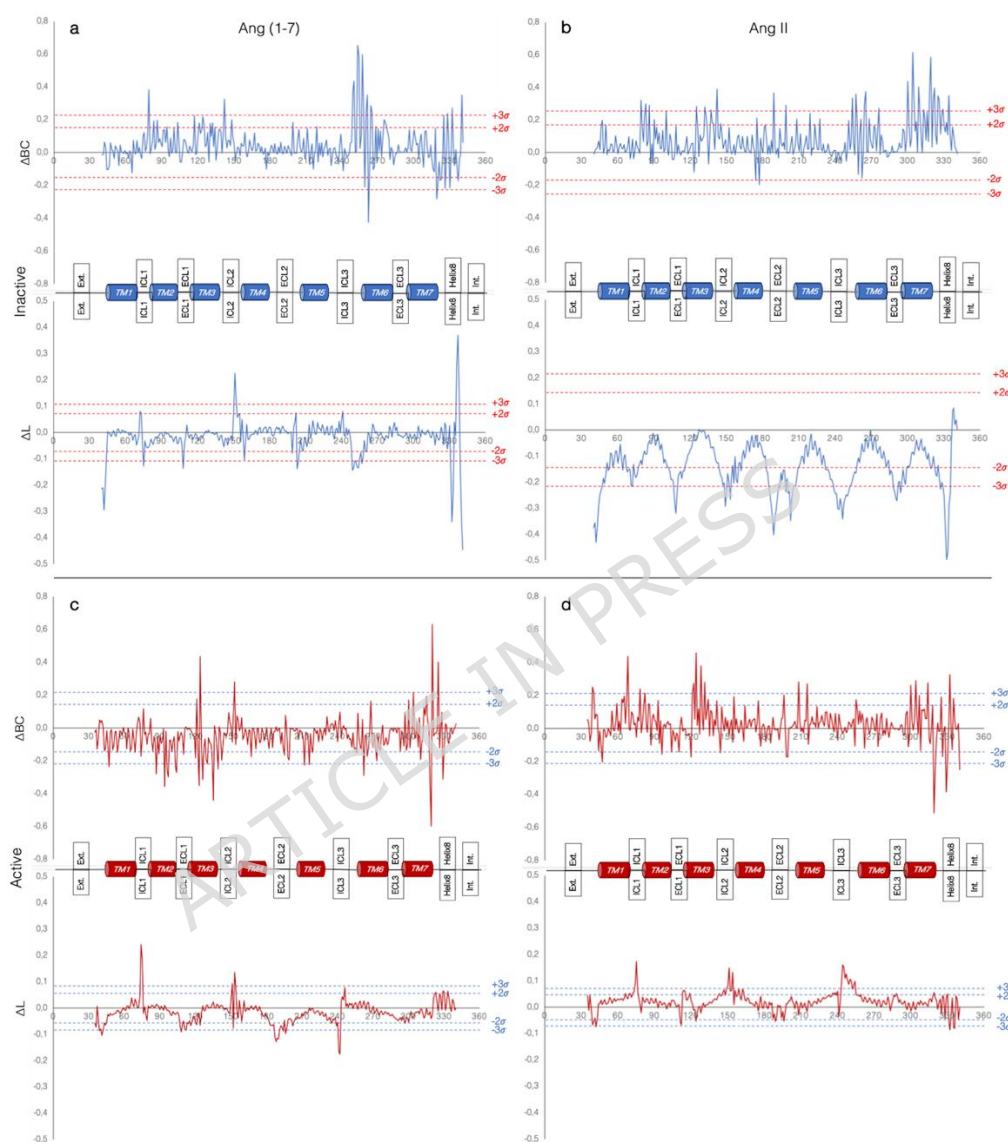


Figure 4. Dynamic residue interaction network (DRIN) analyses of AGTR2 showing changes in betweenness centrality (ΔBC) and average shortest path length (ΔL) upon ligand binding over 1 μ s molecular dynamics simulations. (a–b) ΔBC and ΔL plots for Angiotensin 1–7 (Ang 1–7) a) and Angiotensin II (Ang II) b) binding to the inactive-like state of AGTR2. (c–d) ΔBC and ΔL plots for Ang 1–7 c) and Ang II d) binding to the active-like state of AGTR2. Transmembrane (TM) helices are indicated schematically. Significant thresholds ($\pm 2\sigma$, $\pm 3\sigma$) are represented with dashed lines.

3.5. Integrative Mechanisms and Therapeutic Implications

Our integrative analysis results construct a coherent structural and dynamic framework detailing how Ang II and Ang 1–7 distinctly modulate AGTR2 structure, activation-related conformational dynamics, and signaling tendencies. Across all analyses; ligand binding, conformational dynamics, activation switches, and signaling communication Ang II emerges as a strong conformational stabilizer, inducing a tightly coordinated active-like conformational ensemble. This is evidenced by a compact TM3–TM6 distance ($\sim 12\text{--}13\text{ \AA}$, Fig. 3a), stable RMSD values ($\sim 3.0 \pm 0.3\text{ \AA}$ across inactive and active conformations, Fig. 2), and restricted dihedral fluctuations in critical micro-switches such as PIF and E/DRY (Fig. S11–S12). Persistent hydrogen bonds (e.g., PHE8–LYS215^{5,42}, 945/750 frames, Tables S1–S2), hydrophobic anchoring via MET128^{3,36} ($\sim 6\text{ \AA}$, Fig. S5), and high betweenness centrality in TM6 and TM7 (Table S4, Fig. 4d, Fig. S10) reflect a more conformationally reinforced signaling-related core. In contrast, Ang 1–7 adopts a more flexible, modulator-like conformational profile, characterized by wider TM3–TM6 spacing ($\sim 17\text{--}18\text{ \AA}$), higher RMSD ($\sim 3\text{--}6\text{ \AA}$ inactive, $\sim 3\text{--}7\text{ \AA}$ active, Fig. 2), and broader dihedral variability (Fig. S13–S14). Its interactions are less persistent (e.g., PRO7–THR125^{3,33} drops from 1293 to 0 frames, Table S1–S2) and less centered (ILE304 $>3\sigma$ BC increase, Table S3). Dynamic network analyses confirmed elevated communication fluctuations ($\sim 15\text{--}20\text{ \AA}^2$ for Ang II, Fig. S7) and highly organized motion coordination ($C_{ij} \sim 0.7$, Fig. S8), aligning with PCA results showing broader conformational sampling (PC1–PC2 $\sim 45\%$ variance, Fig. S9). These contrasting profiles reinforce AGTR2's dual functionality within the RAS. Ang II activation-related conformational stabilization, potentially enhancing AGTR2-mediated vasodilatory and anti-fibrotic signaling in contexts such as hypertension or organ protection^{6,10}. Conversely, Ang 1–7 enables regulatory plasticity, possibly supporting MAS1 co-signaling or modulating AGTR1-driven stress responses^{83,84}. Unlike earlier static or short-timescale studies³⁷, our long-timescale simulations capture both the robustness of Ang II-induced activation-related conformational stabilization and the subtle flexibility of Ang 1–7 modulation, offering a comprehensive biophysical perspective. These insights may help inform future ligand-specific therapeutic strategies targeting AGTR2 stabilization in cardiovascular disease, or leveraging Ang 1–7's flexibility for fine-tuned receptor modulation and anti-inflammatory effects.

4 CONCLUSION

Our study provides a structural and dynamical analysis of AGTR2 conformational regulation, defining the structural and dynamical principles that distinguish the stronger conformational stabilizer Ang II from the more flexible ligand Ang 1–7. Through long-timescale MD simulations, we demonstrate that Ang II behaves as a strong conformational stabilizer by promoting active-like conformational features via persistent hydrogen bonds, compact TM domain organization, and reinforced communication networks. This stabilization extends beyond global TM compaction and involves the rotameric locking of key micro-switches (e.g., PHE265^{6,44}, TYR318^{7,53}) together with the rigid stabilization of Helix 8 in a conformation commonly associated with signaling competence, giving rise to a highly stabilized active-like conformational ensemble.

In contrast, Ang 1–7 stabilizes a distinct flexible intermediate state, characterized by broader conformational sampling and reduced network centrality, consistent with its reported modulatory or biased signaling tendencies. Our analyses reveal that Ang 1–7 modulates receptor function through a mechanism of dynamic instability at the Helix 8 interface. Unlike antagonists that block signaling through static steric occlusion, Ang 1–7 markedly destabilizes Helix 8, generating a highly mobile yet less structurally stable intracellular interface that may reduce the efficiency of productive G-protein coupling. This behavior reflects a shift toward a more flexible and less coherently organized signaling architecture.

To ensure reproducibility, two independent 1- μ s replicates were performed for each of the six systems, and representative trajectories were selected based on RMSD similarity (Fig. S15). Although full transitions between inactive and active states were not observed, reflecting the intrinsic timescale limitations of classical MD the distinct dynamical fingerprints identified here clearly differentiate the two ligands: network-stabilizing side-chain locking by the more stabilizing ligand Ang II versus interface destabilization and dynamic modulation by the more flexible ligand Ang 1–7.

Taken together, our findings demonstrate that Ang II and Ang 1–7 remodel micro-switch engagement, inter-helical stability, and communication networks in fundamentally different ways. This structural and dynamical divergence provides a structural rationale for the functional flexibility of AGTR2 and contributes to a structural and dynamic framework that may support the rational design of pathway-selective and bias-oriented agonists or modulators. We believe that this mechanistic insight may hold translational relevance for fine-tuning cardiovascular outcomes via targeted modulation of AGTR2 signaling.

DATA AND CODE AVAILABILITY

The codes used for analyzing micro-switch dihedral angles and hydrophobic interactions during molecular dynamics simulations are publicly available on GitHub at the following links:

https://github.com/eygpcr/microswitches_dihedral_angles

https://github.com/eygpcr/hydrophobic_interactions

The representative PDB structures of dominant conformational clusters and full ensemble datasets (Cluster PDBs) generated in this study have been deposited in the Zenodo repository (Version 2) and are available at:

<https://doi.org/10.5281/zenodo.18433392>

Trajectory files of the simulations and related data for AGTR2 can be accessed through the GPCRmd database using the following identifiers: PDB code: 6JOD, Uniprot ID: P50052

<https://www.gpcrmd.org/dynadb/search/>

SUPPORTING MATERIAL

Supporting Materials and Methods, which illustrate detailed analyses, including ligand-receptor interactions, structural dynamics and activation mechanisms, include fifteen figures, four tables. Additionally, the full trajectory movies visualizing the conformational dynamics are available in the Zenodo repository (Version 1): <https://doi.org/10.5281/zenodo.14697886>

AUTHOR CONTRIBUTIONS

E.Y. designed and performed the research and wrote the manuscript. S.D., E.E., and N.Y. designed the research and wrote the manuscript.

ACKNOWLEDGEMENTS

This research was conducted as part of Dr. Ekrem Yasar's PhD dissertation at Akdeniz University Medical School, under the supervision of Prof Nazmi Yaras.

FUNDING

This work was supported by Akdeniz University Scientific Research Projects Fund [Grant No. TDK-5449, 2021].

DECLARATION OF INTEREST

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used *ChatGPT* [OpenAI, L.L.C., San Francisco, CA] as well as *DeepL Write* [DeepL SE, Cologne, Germany] in order to writing process for spelling and grammar corrections. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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