ACTIVATION MECHANISM OF THE VASOPRESSIN RECEPTOR 2

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1. INTRODUCTION

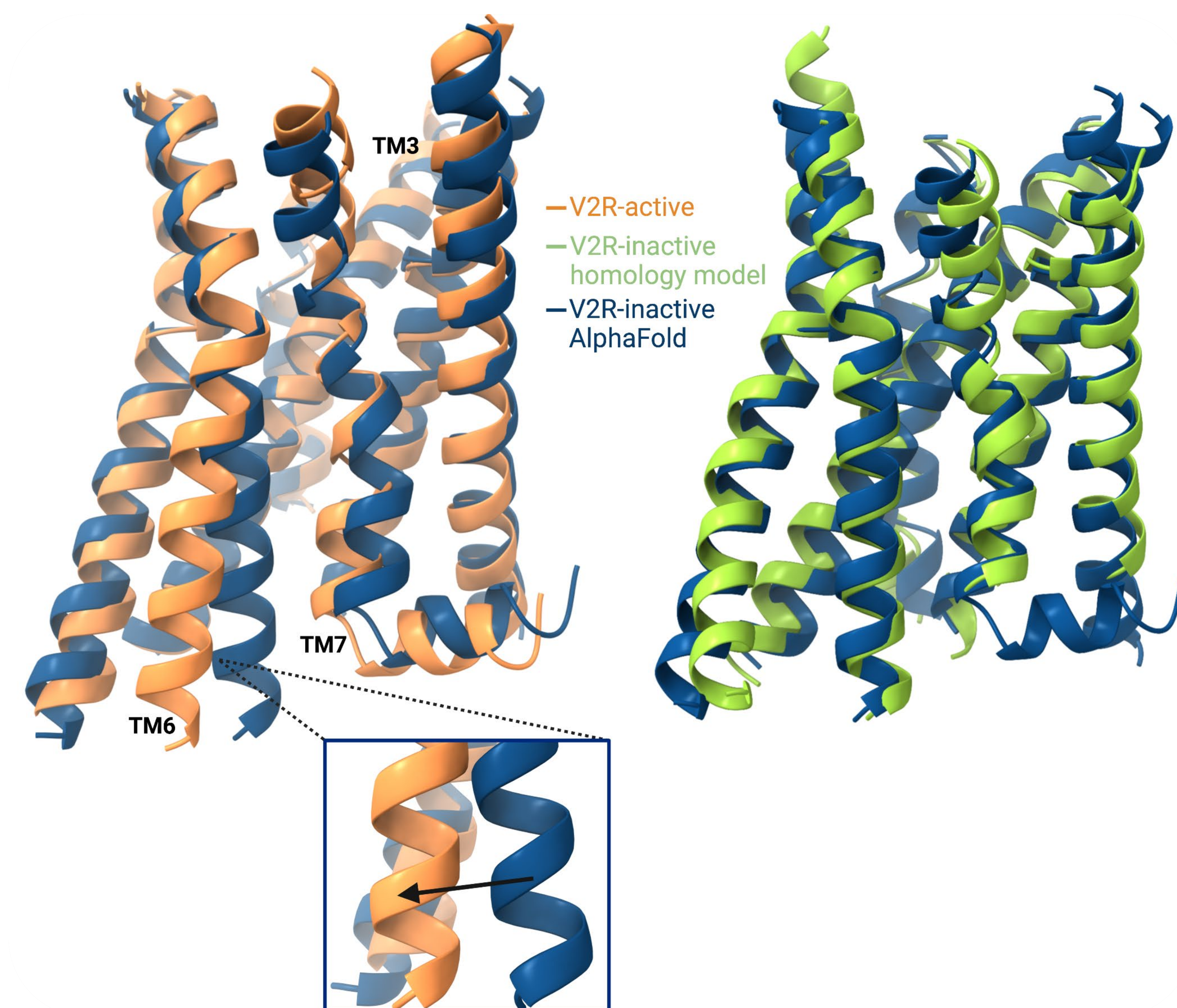
Arginine-vasopressin (AVP), a neurohypophyseal cyclic nonapeptide, plays an essential role in maintaining water homeostasis in the body. In the kidney, AVP binds to the vasopressin type 2 receptor (V2R), a G protein-coupled receptor (GPCR), at the renal collecting ducts, promoting water reabsorption back into circulation. Numerous mutations in V2R have been identified that result in severe water balance disorders linked to shifts in its activation state, making V2R attractive for targeted drug design. Detailed understanding of the structural dynamics of V2R is therefore paramount to guide discovery of novel drug candidates. However, the lack of an inactive structure for V2R has hindered analyses of its ligand binding mechanism and activation dynamics.

We constructed the inactive structure of V2R through homology modeling and validated the structure using equilibrium simulations of a GPCR-peptide complex and AlphaFold 2.[3] Comparison of this inactive structure with recently published cryo-EM structures of the active state provides deeper insight into the activation mechanism of V2R. Extensive molecular dynamics (MD) simulations reveal the existence of water networks, which are significant in facilitating activation, within the transmembrane segments of V2R. It is shown that the absence of G protein from the intracellular binding domain destabilizes the active state and creates multiple intermediate conformations. Also, the intracellular regions of the transmembrane segments are potential phospholipid binding sites, suggesting the role of anionic lipids as allosteric modulators.

2. INACTIVE STRUCTURE AND MECHANISM OF V2R

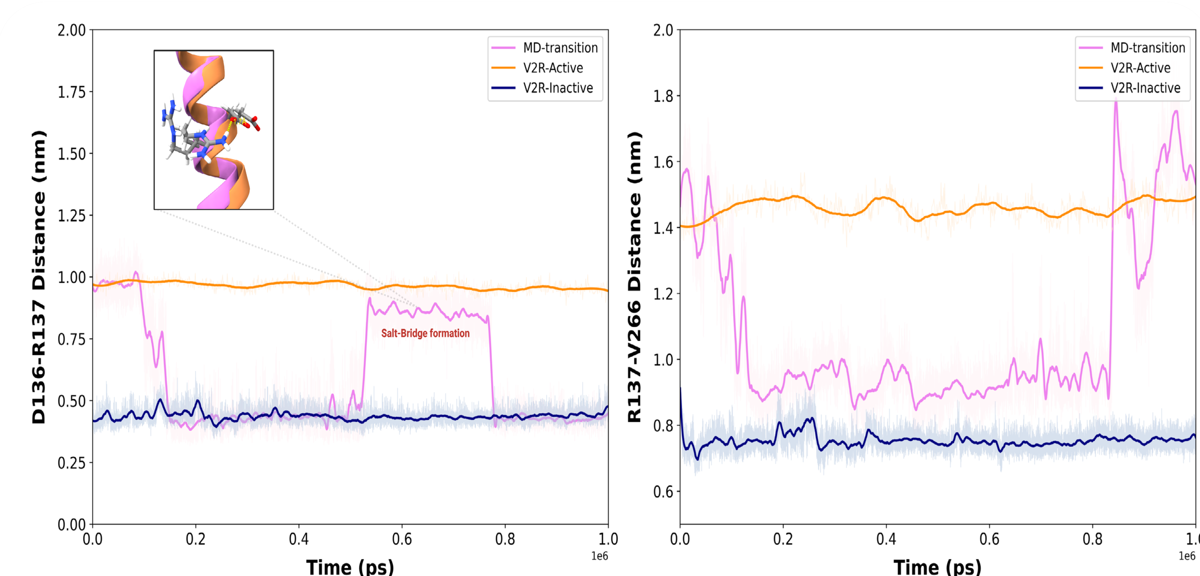
THE INACTIVE STRUCTURE OF THE VASOPRESSIN RECEPTOR 2

We modeled the inactive state of the V2R[1] (green, right) and validated our model with molecular dynamics simulations and AlphaFold 2 (blue, both figures), which predicted V2R in the inactive state. Overlay of the cryo-EM active structure of V2R[5] (orange, PDB: 7DW9) with the inactive model shows the key structural transition of the TM6 and TM7 helices, shown below.



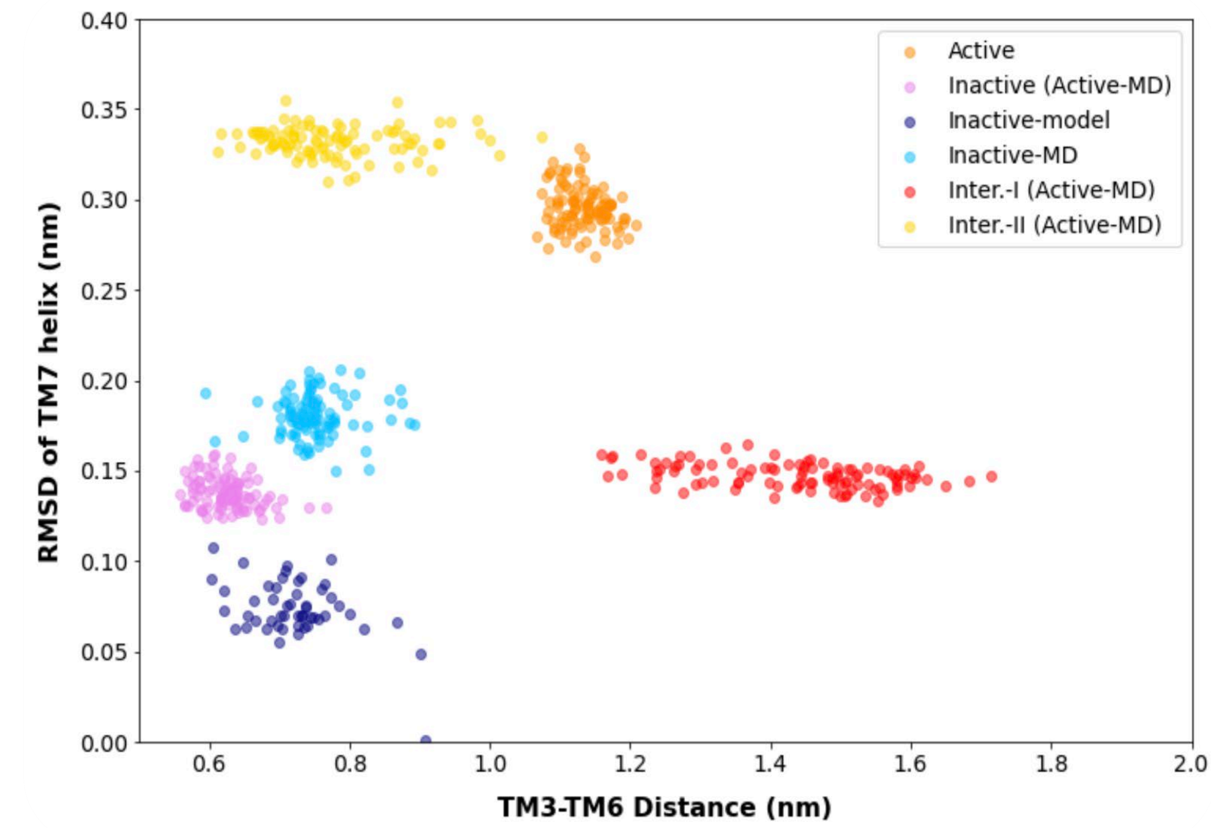
STRUCTURAL TRANSITIONS FROM ACTIVE TO INACTIVE STATE

The signature structural transition from active state to inactive state consists of: (i) formation of the salt bridge within the DRH motif in TM3 (left), (ii) increase in the distance between the intracellular region of TM3-TM6 helices (right), and (iii) secondary structure transition of extracellular region of TM7 helix (shown in the graph of the RMSD of TM7 helix vs. TM3-TM6 distance, next column).



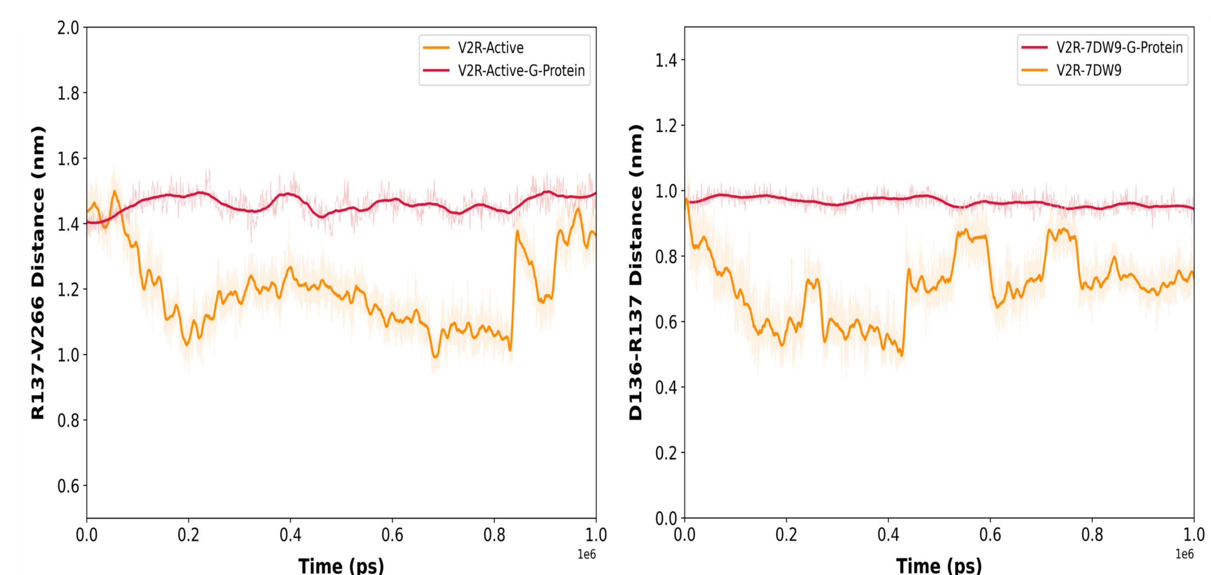
CONFORMATIONAL DYNAMICS OF V2R

Class A GPCRs exist in conformational ensembles in which the structural transitions from active to inactive states are mediated through multiple metastable intermediate states. Our MD simulations reveal that the transition of V2R from active to inactive happens through different intermediate states. In the figure below, pink indicates the transitioned inactive structure, and red and yellow indicate intermediate states.



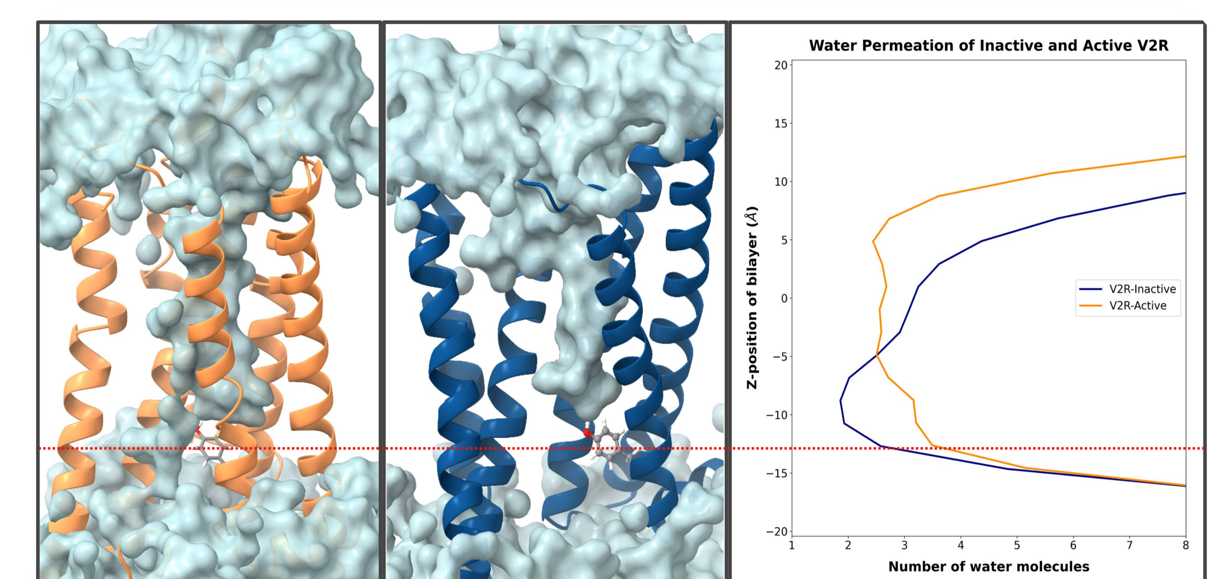
V2R STABILITY WITH G PROTEIN

The presence of G protein in the intracellular binding site stabilizes the active conformation of V2R. The distances between the TM3-TM6 helices of V2R simulated with (red) and without (orange) G protein are depicted below. In the absence of G protein, V2R loses structural stability, indicated by the large fluctuation in distance between the helices, allowing for transition into inactive or intermediate conformations.



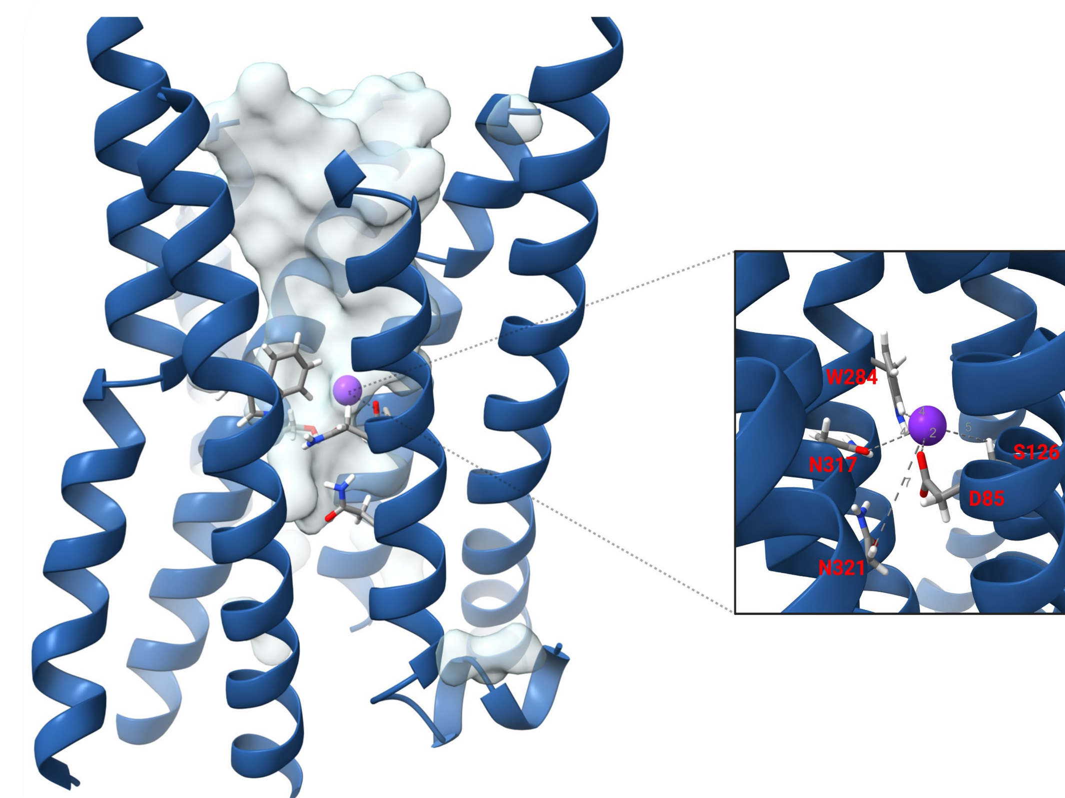
INACTIVE AND ACTIVE V2R WATER NETWORKS

Water molecules play a significant role in modulating the activation dynamics of V2R.[2] MD simulations reveal that the active and inactive states display highly distinct water density profiles. The inactive state has a narrower hydrophobic region in the intracellular part close to TYR325 (red dotted line, below), disconnecting the water network between extracellular and intracellular regions. In the active state, the water density in the binding site reduces due to the presence of its endogenous ligand, AVP. The presence of G protein in the intracellular region opens the helices and forms a water bridge network.



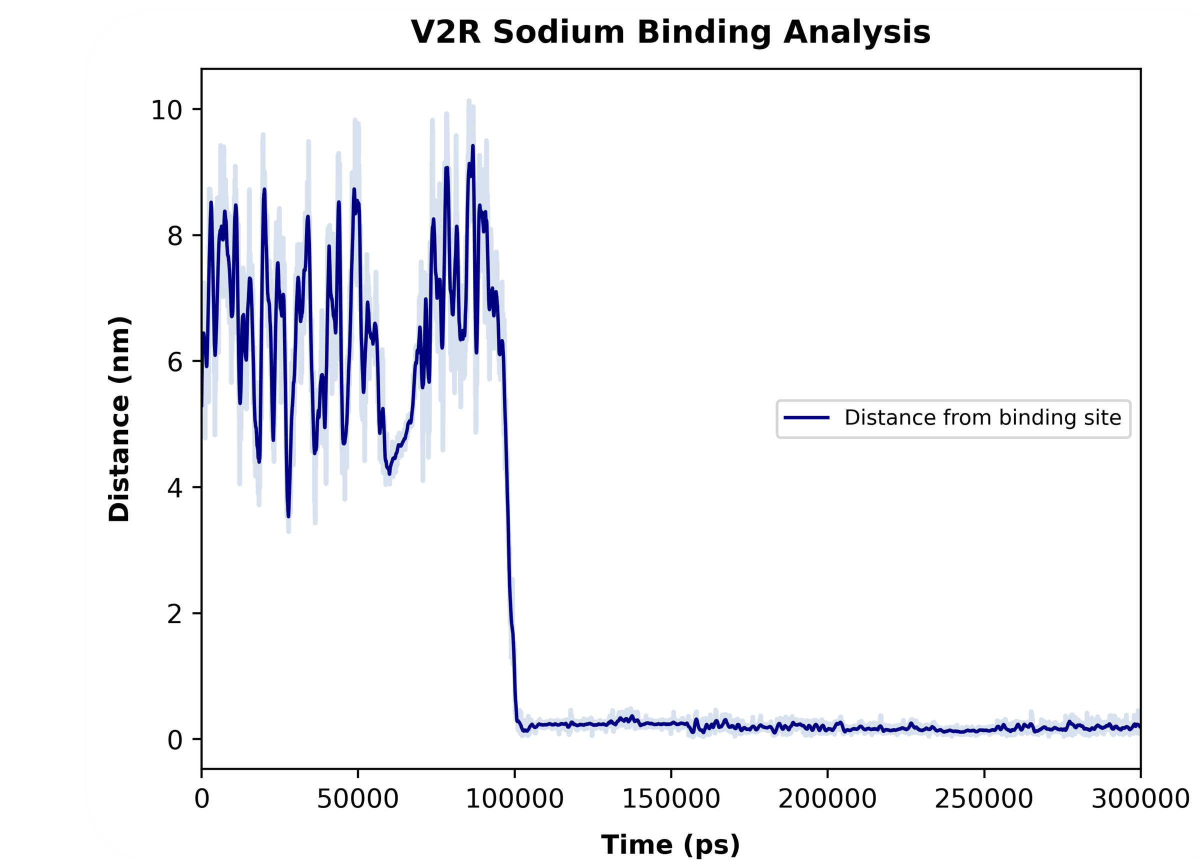
PREDICTED SODIUM BINDING POCKET

Many class A GPCRs have an allosteric sodium binding site in the intracellular region.[4] Our equilibrium MD simulations run with a normal external electric field (0.25 V/nm) show that a sodium ion is driven into the binding site. The sodium ion (violet) and the residue side chains of inactive V2R form the binding site. The translucent blue surface depicts the water density profile.



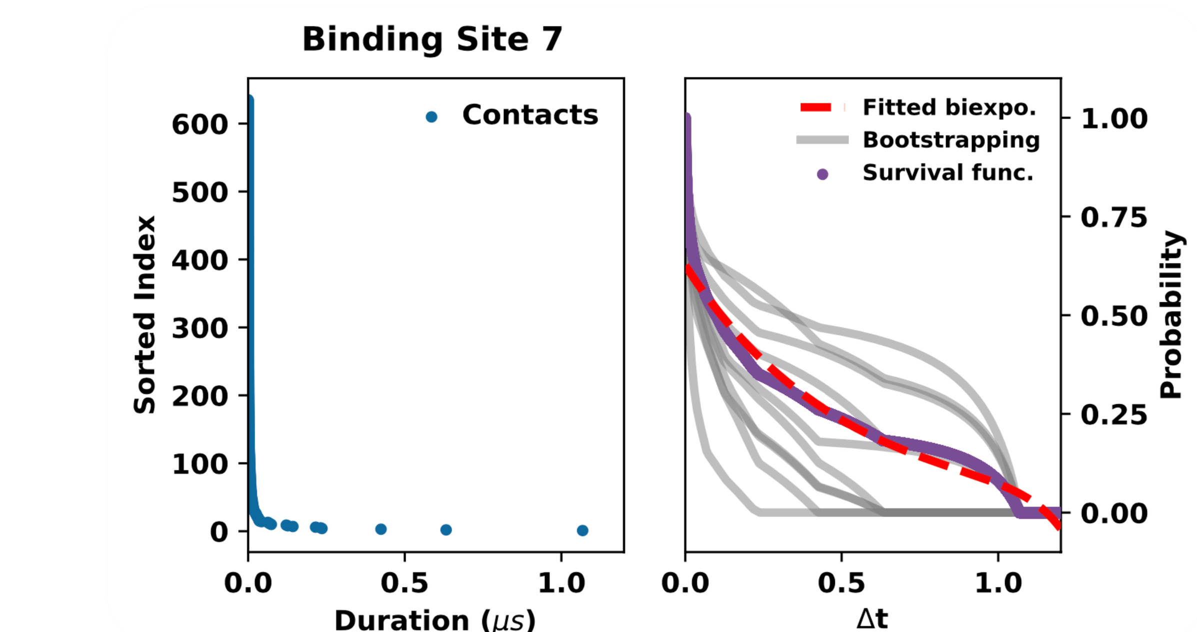
SODIUM DISTANCE FROM BINDING SITE

The sodium ion's position along the bilayer normal from the binding site clearly shows when it enters the binding site, where it remains.



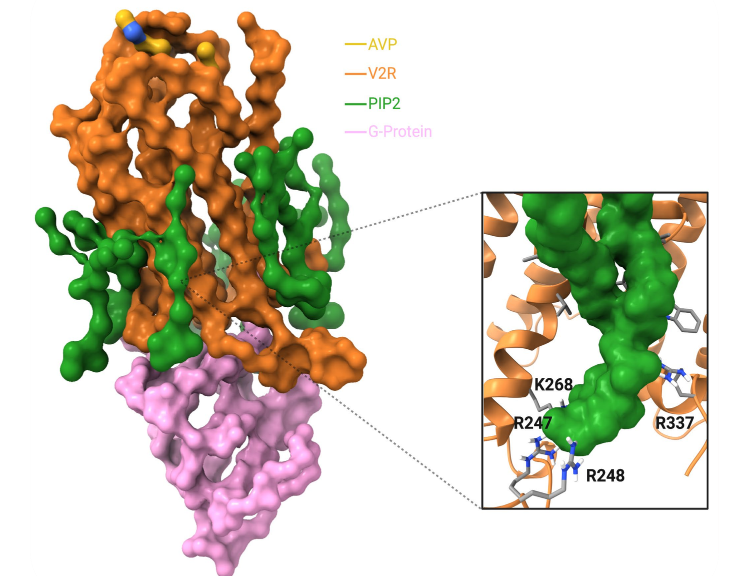
PIP2 CONTACT AT BINDING SITE

Anionic phospholipids can participate in allosteric modulation of GPCRs. The negatively charged phosphate head groups are shown to have electrostatic interactions with basic residues in the intracellular domains of the receptor and sterically hinder conformational changes. The duration of PIP2 contact at the binding site and the residence time plot corroborate the strong electrostatic interaction.



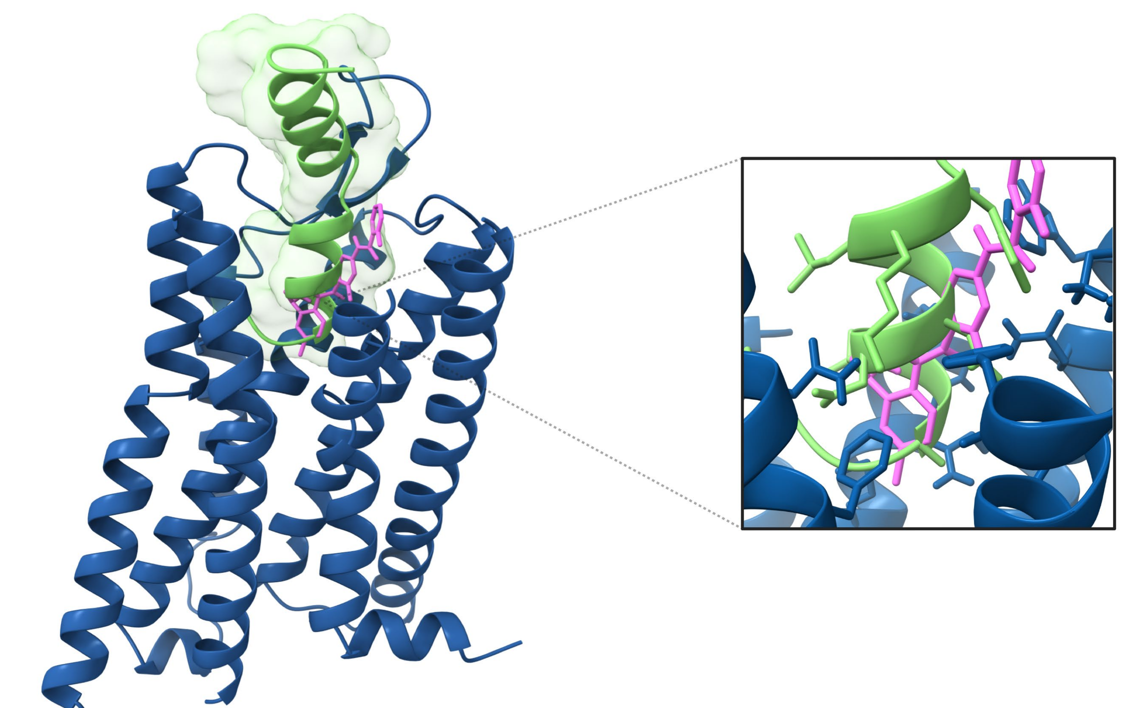
PIP2 INTERACTIONS WITH V2R

Coarse-grained MD simulation shows the coupling between V2R (orange) and G protein (pink), mediated through PIP2 lipids (green). The zoomed-in portion depicts the electrostatic interactions of basic side chain residues in the intracellular region of TM1, TM3, TM7, and H8 of V2R with the inositol headgroup of PIP2. This suggests that PIP2 lipids stabilize the active state of V2R.



3. APPLICATION TO AI-ENABLED PEPTIDE DRUG DESIGN

The activation mechanism and inactive structure of V2R were applied to study the binding mode of Peptilogics' novel AI-generated peptides. The figure below compares the predicted binding pose of an AI-generated peptide (green) to the docked structure of a commercial small-molecule V2R antagonist (pink).



4. CONCLUSIONS

A structure for the inactive state of V2R and the mechanism of activation were determined using molecular dynamics and homology modeling. Water networks within the transmembrane segments of V2R modulate its activation dynamics. A sodium ion stabilizes the inactive state of V2R through allosteric binding at the binding pocket, and binding of G protein to the intracellular binding site and interactions with PIP2 lipids stabilize the active state of V2R.

This study provides novel insights into structural differences between targets' activation states in the absence of adequate structural information. The methods can be used to guide in silico screening through prediction of a peptide's preferential affinity toward a specific conformational state.

REFERENCES

1. Bender, B. J., Marlow, B. & Meiler, J. Improving homology modeling from low-sequence identity templates in Rosetta: A case study in GPCRs. Plos Comput Biol 16, e1007597 (2020).
2. Bertalan, É., Lešnik, S., Bren, U. & Bondar, A.-N. Protein-water hydrogen-bond networks of G protein-coupled receptors: Graph-based analyses of static structures and molecular dynamics. J Struct Biol 212, 107634 (2020).
3. Jumper, J. et al. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583–589 (2021).
4. Vickery, O. N. et al. Intracellular Transfer of Na+ in an Active-State G-Protein-Coupled Receptor. Structure 26, 171-180.e2 (2018).
5. Wang, L. et al. Cryo-EM structure of the AVP–vasopressin receptor 2–Gs signaling complex. Cell Res 31, 932–934 (2021).

ACKNOWLEDGMENTS

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