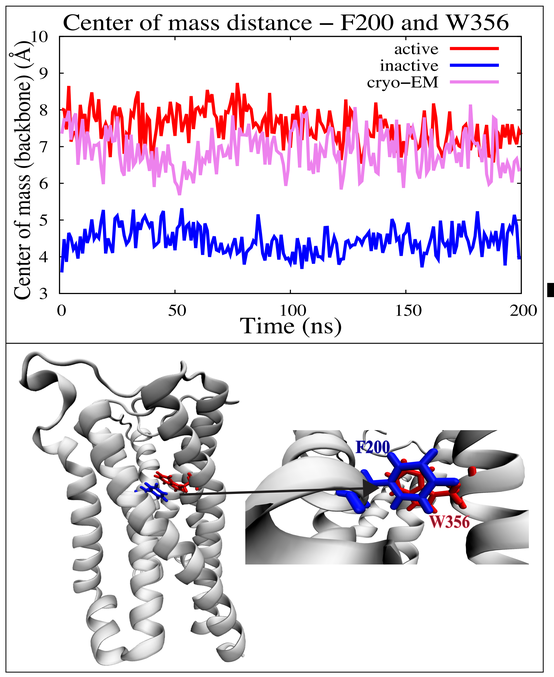
COMPARING THE DYNAMIC DIFFERENCES BETWEEN X-RAY AND CRYO-EM STRUCTURES OF CANNABINOID RECEPTOR 1 USING MOLECULAR DYNAMICS SIMULATIONS

Ugochi Isu, Adithya Polasa, Vivek Govind Kumar, Mahmoud Moradi

Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA.

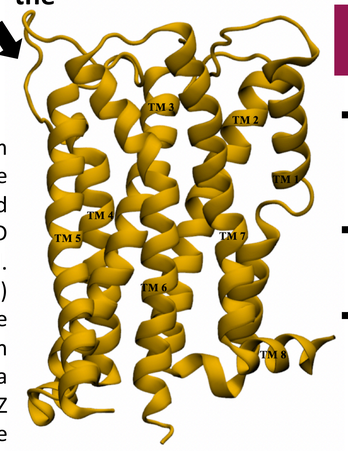
Introduction

Cannabinoid receptor (CB1), a member of class A G protein-coupled receptors (GCPRs), is a key component of the endocannabinoid system found in brain tissues. The CB1 receptor has been crystallized in active and inactive states and most recently, a Cryo-Electron Microscopy (Cryo-EM) structure has also been determined. Through the use of these structures, there has been some advancement in understanding cannabinoid receptor activation. However, a detailed knowledge of the dynamics involved in the activation mechanism remains elusive. In recent times, more protein structures have been determined by cryo-electron microscopy alongside X-ray crystallography. While X-ray crystallography produces high-quality structures, membrane proteins could take months or years to be successfully crystallized for structure determination. In contrast, Cryo-EM does not require protein crystals and could yield a more complete structure. With Cryo-EM gaining more preference in the study of membrane proteins, it is important to ascertain the structural dynamics and distinct characteristics of the Cryo-EM and X-ray structures. Using equilibrium all-atom molecular dynamics (MD) simulations and using all three determined structures of CB1 receptor, we study the structural dynamics of the 7TM region of the CB1. We have taken a step further to develop an initial restraining protocol to ensure the stability of the Cryo-EM structure.



Center of mass Distance shows an aromatic stacking between F200 and W356 in the inactive state of CB1 but disrupts in the active states. F200 (TM3) and W356 (TM6) are important molecular switches for GPCR activations. From our result, we observe first a higher distance with the active structures (red and magenta) showing they move away from the orthosteric binding site, However, we notice a reduced distance from 150ns implying that the active structures return to an inactive state .

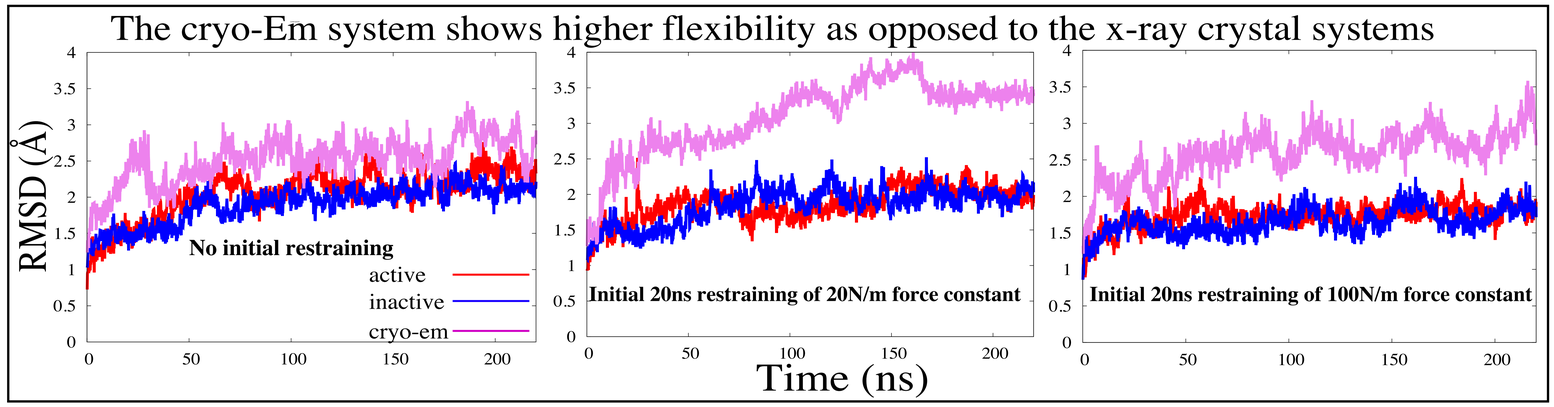
Transmembrane helices of the CB1 receptor



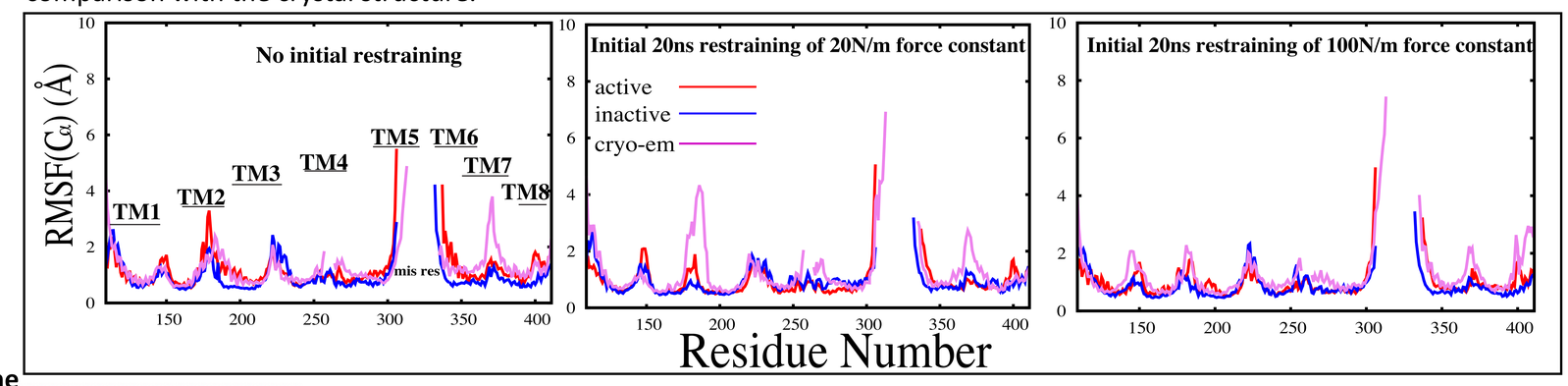
Materials and Methods

Molecular Dynamics (MD) simulations were performed based on the crystal and cryo-em structures of CB1 (PDB ID: 5XRA, 5TGZ and 6N4B). 5XRA is the active conformation, 5TGZ is the inactive conformation, while 6N4B is the cryo-em structure. All 3 structures have been simulated in apo state (without any ligand binding). The systems were built using Charmm-GUI, MD simulations were performed using NAMD 2.13 simulation package with CHARMM36 force field. The systems were solvated In a rectangular water box with 0.15M NaCl ions (192 Na^+ and 202 Cl^-) inserted into each system using the Monte- Carlo Ion placing method. Some of the systems were restrained for 20ns at a force constant of 20 and 100 N/m. Production runs were carried out in an NPT ensemble (with constant number of particles, pressure and temperature) at a temperature of 310K and a time step of 2fs for 200ns. 5XRA is made up of 104057 atoms, 5TGZ is made up of 106641 atoms and 6N4B is made up of 106913 atoms. The simulations were executed on comet super computers. VMD was used for all analysis.

Results and Discussions



Protein Stability assessment for CB1 receptors: Root mean square deviation (RMSD) plot and root mean square fluctuations (RMSF) shows a greater instability with the cryo-em (magenta) structure, in the presence and absence of restraints. We calculated the rmsd to measure the average distance between atoms. Our result shows a uniform movement with the crystal structures, that is unaffected by restraints. However, with the cryo-Em structure, we observe higher fluctuations replicated on all cases (restrained and unrestrained). The rmsf analysis suggests the fluctuations are from TM7 and TM2, which together with TM3 and TM6, make up the binding sub-pocket of the CB1 receptor. This could imply that the cryo-EM structure is unstable in comparison with the crystal structure.



Conclusion

- Our MD simulations show that the Cryo-EM CB1 structure is a highly flexible system as opposed to the X-ray crystal structures.
- The flexibility of the cryo-EM structure is less affected by the restraint protocols.
- The characteristic distancing of TM3 and TM6 in most GPCR activations is also seen in CB1 structure. This is shown in the disruption of the aromatic stacking formed between F200 and W356 in the active structures.

References

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