3D Model of Crystallized Human Cannabinoid Receptors

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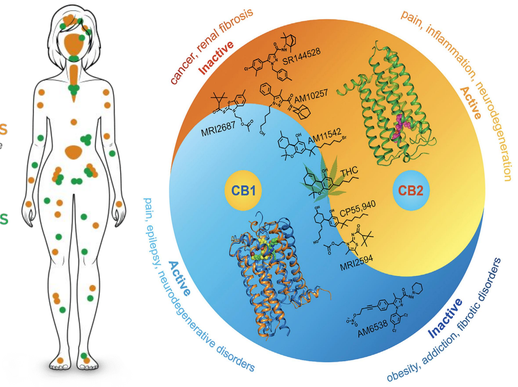
Background

- Both primary cannabinoid receptors, CB2 and CB1, share similar structures yet differ drastically in function[1].
- While CB1 is found primarily in the central nervous system, CB2 is mainly expressed in the immune system[1].
- The crystalline structure of CB2 was reported using the CB2 antagonist AM10257, and CB2 and CB1 were found to be homologous in forty-four percent of their sequencing[1].
- Due to this homology, many cannabinoids have the ability to bind to both CB1 and CB2.
- Li and colleagues tried to determine the specific interactions that are necessary to induce modulation of each receptor, particularly the lesser known CB2.

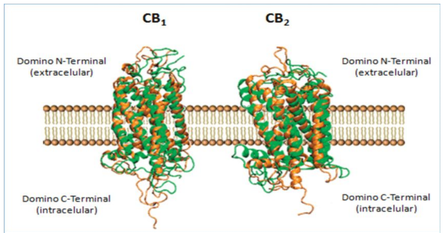
CB_1 RECEPTORS
Located in the brain and the central nervous system.

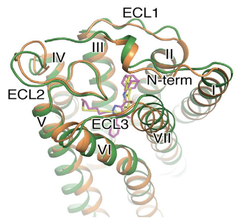
CB_2 RECEPTORS
Found on cells throughout the body's immune system.



Significance and Purpose

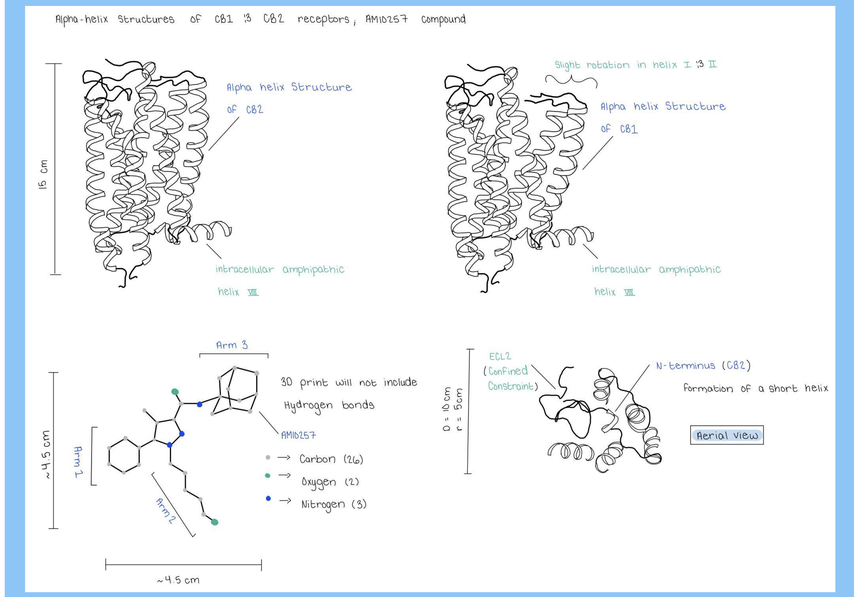
- To create a 3D model of the crystalized human cannabinoid receptors, CB1 and CB2, and demonstrate their binding with the agonist AM10257
  - We want to demonstrate the differences in binding between the CB1 and CB2 receptors
- We predicted the slight differences between the receptor would alter the way AM10257 binds thus altering downstream signaling pathways.



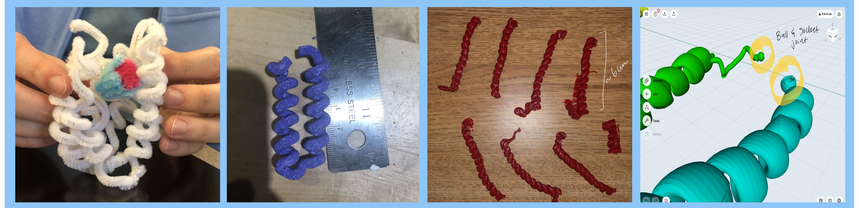


Design Process: Iteration and Troubleshooting

Original Design Sketch



Design Iterations



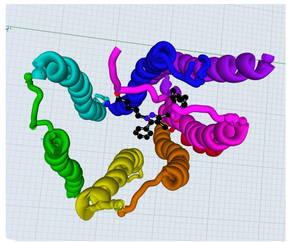
Troubleshooting

- Initially, we chose the same topic as another group, so in the interest of fairness we both switched to a new topic.
- Another problem we ran into along the way was the the scale of our original design.
  - To 3D print the size we had originally hoped for would take multiple machines over 24 hours to finish, which would not be feasible for this project.
- After printing several of our rescaled alpha-helices and ligand, we saw that the model would be too fragile to represent what we hoped it would.
  - To fix this, we decided to use magnets instead of a "ball and socket" design to represent ligand binding.
- Finally, COVID-19 cut our semester short and prevented us from printing out a final model.

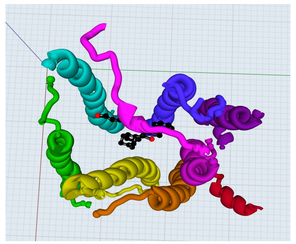
Discussion, Implications, and Visualization

- Due to the COVID-19 pandemic, our final design is a digital 3D model of the CB1-AM10257 and CB2-AM10257 complexes; although we were unable to share a physical representation with magnets to lock AM10257 into place, we were still able to share the concepts of our design and the next anticipated iteration given the BEAM Makerspace were still accessible to students. Sharing the progress of our physical iterations is valuable because it demonstrates the trial and error process that is inherent to the processes of research; future directions for our models include a final printed design complete with magnetic binding of AM10257 to illustrate residue interactions within both complexes.
- Key differences of the CB1 and CB2-AM10257 complexes are evident in the N-terminus of each receptor. The CB1 complex contains a structural V-loop which inserts into the orthosteric pocket upon ligand binding; in contrast, the CB2 complex features a helical extension that stays superior to the receptor orthosteric pocket.
- Further, helices III and IV of the CB2 receptor experience a 6.1 Å shift due to the hydrophobic interactions of AM10257 upon binding within the complex.
- Our AM10257 features a stick-and-ball model of its three arms: the first involved in affinity and stabilization, the second which alters agonism; and the third involving hydrophobic interactions
- The CB1 and CB2-AM10257 complexes illustrate the yin-yang relationship of the cannabinoid system. The binding of AM10257 serves as a partial agonist of the CB1 receptor and an anticipated inverse agonist of the CB2 receptor, given the data obtained from the antagonist-bound CB2 crystalline structure and the opposing behaviors of CB1 and CB2. This provides insight into the opposing functionality of the CB1-CB2 behaviors and future drug design for increased selectivity between receptors.

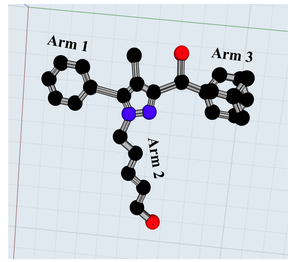
CB1-AM10257 Complex

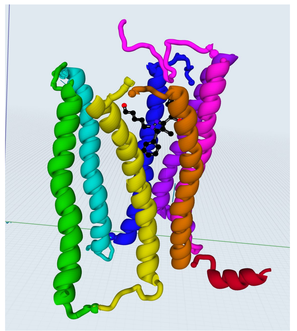


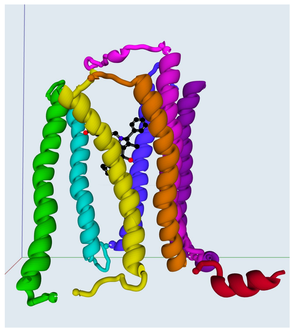
CB2-AM10257 Complex

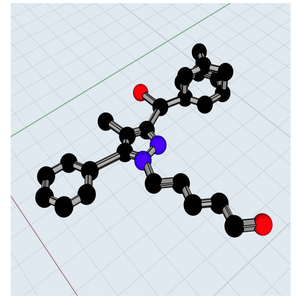


AM10257









References

1. Li, X., Hua, T., Vemuri, K., Ho, J. H., Wu, Y., Wu, L., ... & Han, G. W. (2019). Crystal structure of the human cannabinoid receptor CB2. *Cell*, *176*(3), 459-467.
2. Thingiverse