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Analysis of a G-Protein Coupled Receptor, CB2

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Summary

The CB2 G-protein coupled receptors (GPCR) is found in the brainstem & hippocampus and is devoid of psychotropic effects but is less studied than the CB1 receptor. CB2 is inducible in CNS microglia following inflammation or injury, indicating a role in pain response. Here we sought to analyze CB2 using ChimeraX structures and overcome GPCR protein insolubility in extraction. The pET28a-CNR2, plasmid created and transformed into *E.coli* pLysS, was confirmed by restriction digest. Purification of CB2 micelles was achieved by affinity chromatography with detergent (43 kD). We aim to utilize nanodiscs to stabilize CB2, allowing studies of the molecular underpinnings informing treatment options.

Introduction

G-protein Coupled Receptor - Cannabinoid Receptor 2

- G-protein Coupled Receptors (GPCRs) are 7 transmembrane proteins that bind to a ligand and elicit a biological response through a G-protein (3). They represent 30-60% of current drug targets.
- Endogenous cannabinoid system encompasses two GPCRs receptors, CB1 and CB2 (**Figure 1B)** (8), endogenous ligands, and enzymes to make them.
- The GPCR CB1 displays widespread expression in the CNS under normal physiological conditions, however the expression of CB2 receptors is normally expressed only in the brainstem and the hippocampal pyramidal neurons (2).
- CB2 receptor is inducible on the reactive microglia in the CNS following inflammation or injury, and does not possess the undesired psychotropic effects or addiction liability, making it a therapeutic target (2,4).
- The extracellular structure of CB2 and CB1 are significantly different when bound to antagonists as shown in **Figure 1B.**

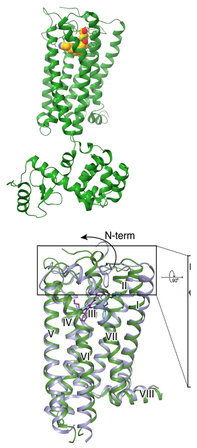


Figure 1. A. CB2 GPCR complex (PDB 6KPC). CB2 is shown in dark green, $G_i\alpha$ (periwinkle), $G_i\beta$ (salmon), $G_i\gamma$ (mint green). **B.** comparison of CB1 and CB2 (green) (8)

Recombinant E.coli pLysS Expression System

- The E. coli pLysS strain was transformed with plasmid pET28a_CB2_BamHI_NotI, encoding the gene *CNR2* for encoding CB2. The pRG/III-hs-MBP-CB2-HF, was purchased from Addgene. (6) and the CNR2 gene was subcloned into pET28a
- The 6-His tag is used for affinity chromatography to purify the CB2 protein with Ni-NTA resin **Figure 3.**

pET28a_CB2_BamHI_NotI Plasmid (6421bp)

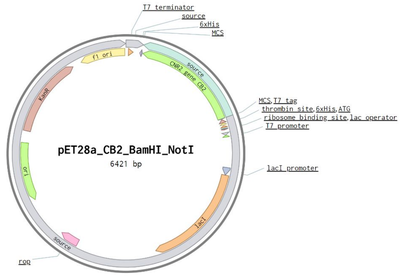


Figure 2. pET28a-hs-CB2-HF plasmid from Addgene containing lacI promoter and Kanamycin resistance. With the addition of chloramphenicol resistance in BL21 pLysS cell line. Created with Benchling.com.

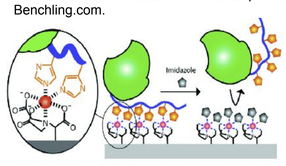


Figure 3. Ni-NTA molecules are black, red, and pink. The protein is green with an orange N-term His tag. (7)

Methods

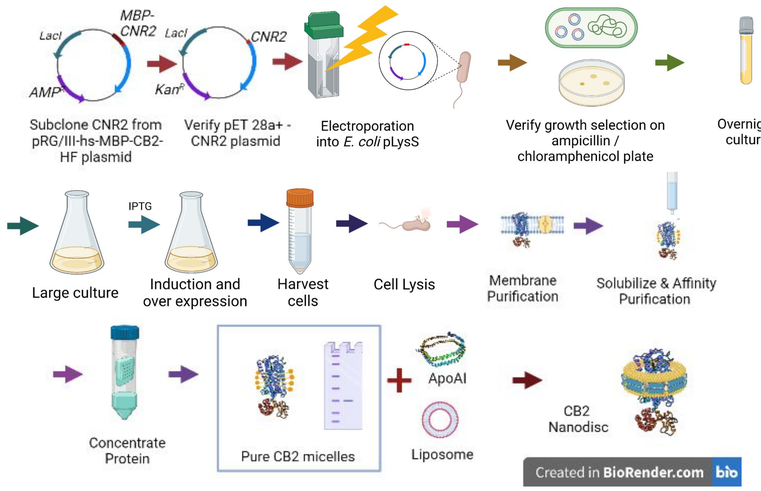
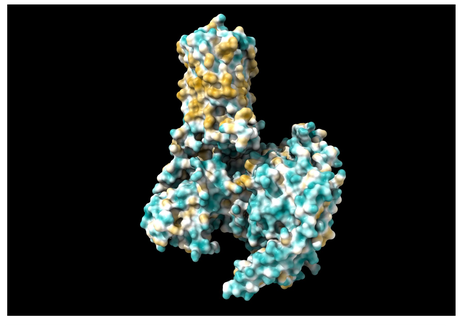
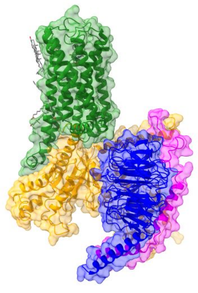


Figure 4. CB2 membrane protein GPCR purification. The subcloning of the CNR2 gene encoding CB2 from plasmid pRG/III-hs-MBP-CB2-HF (addgene) into pET28a+ and purification from *Ecoli pLysS* cell lines by affinity chromatography utilizing the 6xHis tagged on the recombinant CB2 protein. Future plans (past grey box are to incorporate the receptor in a protein lipid nanodisc. Created with BioRender.com

Molecular Modeling of CB2 in ChimeraX





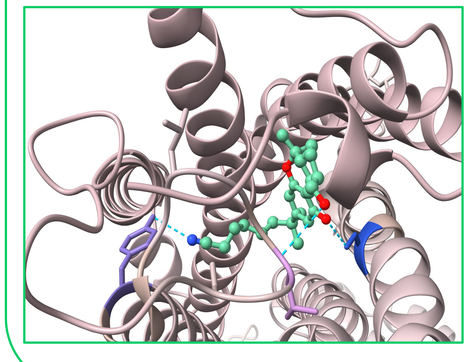


Figure 5: The molecular models of CB2 receptor. Created using the ChimeraX software, and they play a crucial role in informing our research efforts by providing a scalable, three-dimensional representation of the theoretical morphological changes that occur during protein function in situ. Specifically, we used these models to explore the conformational changes that take place in the transmembrane domain when CB2 is bound to an agonist. **A)** Surface model showing hydrophobic residues in yellow and hydrophilic residues in cyan of the CB2 complex with Gi proteins; **B**) green, CB2 seven transmembrane alpha helices; yellow, Gi alpha; blue, Gi beta; magenta, Gi Gamma binding partners. **C)** CB2 receptor binding pocket, top view,with agonist (mint) bound

Results Cloning and Expression

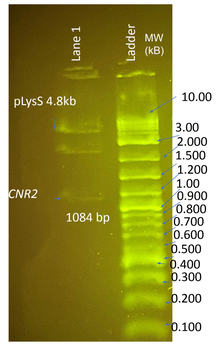


Figure 6. DNA electrophoresis of *CNR2* gene. The *CNR2* gene is ligated to the pET28a+ vector and Verification of the CNR2 gene subcloning transformed into *E. coli* FB5alpha. The plasmid pET28a+CNR2 was harvested and electroporated into BL21(DE3) pLysS an efficient protein producer. DNA electrophoresis of preparative digested plasmid

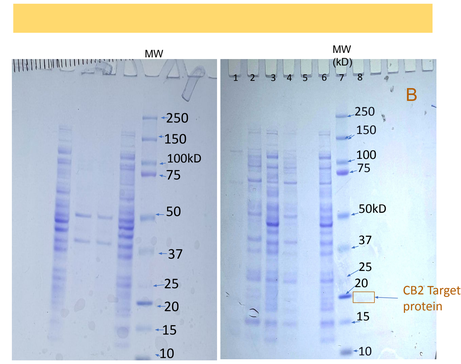
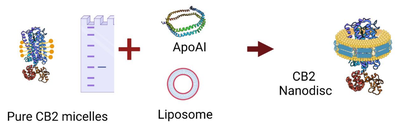


Figure 7. Mass Analysis of Denatured CB2 Protein NiNTA Purification Aliquots. A) Uninduced control **B**) Induced sample showing isolation in lane 8. SDS-PAGE Bis-Tris 4-12% gel, 35 min 200V electrophoretic separation. Lanes:
1) Wash 2.2
2) Wash 2.1
3) Ultracentrifuge Supernatant from lipid bilayer separation
4) Flow Through
5) Ultracentrifuge lipid bilayer pellet resuspended.
6) Cleared crude lysate post sonication.
7) kaleidoscope protein ladder in kilodaltons.
8) Concentrated Elution in micelles.

Conclusions

- We have analyzed molecular models of the CB2 receptor to gain a deeper understanding of the binding pocket and structure.
- We successfully produced a subclone containing the *CNR2* gene without the maltose binding protein that is viable under chloramphenicol and kanamycin resistance.
- Our team has expressed, isolated and purified small amounts of the CB2 protein in detergent micelles.
- This is a critical step in the long-term goal of assembling a nanodisc with CB2 in order to further our understanding of the protein-protein interactions CB2 performs.



Acknowledgments

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