Determination of antagonist-bound vasopressin receptor structures by cryo-electron microscopy

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Since 2017, cryogenic electron microscopy (cryo-EM) has revolutionized GPCR structural biology by enabling crystallization-free high-resolution structural determination of agonist-bound active state GPCRs coupled to transducer proteins. However, determining GPCRs in apo and inhibitor-bound conformations by cryo-EM remains challenging. Nevertheless, the ability to apply cryo-EM to determine apo and inhibitor-bound GPCRs would open new pharmacological avenues for drug discovery. Nb6 recognizes and binds to the κ- opioid receptor (κOR) ICL3 in the inactive state. Grafting this loop into ICL3 of other class A receptors enables Nb6 to bind to these receptors in the inactive state (1). This strategy can also facilitate the determination of inactive state GPCR structures whereby Nb6 acts as a fiducial marker to facilitate cryo-EM particle alignment of GPCRs that have limited structural features outside of the receptor TM bundle that is embedded in the micelle of detergent-purified GPCRs. Here, we utilized this Nb6 strategy to determine structures of class A vasopressin receptors bound to non-peptide antagonists. Vasopressin receptor inhibitors have the potential for the treatment of many clinical conditions including chronic hyponatremia, heart failure, polycystic kidney disease, congenital X-linked nephrogenic disease, Raynauds disease, and regulation of social behaviors, such as autism spectrum disorders.

We employed an AlphaFold-driven approach to design a range of receptor fusion constructs containing varying lengths of κOR in ICL3, which were characterized in a BRET assay to assess vasopressin (AVP)-induced dissociation of Nb6 from the receptor, and inhibition of this response in the presence of antagonists. These revealed a correlation with the AlphaFold predictions where constructs that had high prediction confidence for rigid κOR ICL3 helical fusion points with receptor TM5 and TM6 showed higher basal BRET signals and larger BRET signals for AVP-induced Nb6 dissociation, indicative of more Nb6 binding in the absence of agonist. Moreover, these responses were inhibited by antagonist binding. One construct was prioritized for structural studies that enabled the determination of antagonist-bound vasopressin receptor structures.

Reference

1. Robertson MJ, Papasergi-Scott MM, He F, Seven AB, Meyerowitz JG, Panova O, Peroto MC, Che T, Skiniotis G. Structure determination of inactive-state GPCRs with a universal nanobody. Nat Struct Mol Biol. 2022 Dec;29(12):1188-1195. doi: 10.1038/s41594-022-00859-8. Epub 2022 Nov 17. PMID: 36396979.