**Cryo-EM for structurally enabled development of small molecule drugs and therapeutic antibodies against GPCRs**

**Authors**

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**Abstract**

GPCRs are increasingly important targets for drug discovery, both for the development of small molecule drugs and therapeutic antibodies. Moreover, structurally aided maturation of GPCR-antibody interactions can enhance target engagement and reveal receptor conformational changes associated with antibody binding. Cryo-electron microscopy (cryo-EM) is the preferred method for elucidating GPCR structures, including both agonist-bound active states and, increasingly, antagonist-bound inactive states. However, obtaining high-quality GPCR samples for small molecule drug discovery, therapeutic antibody development, and cryo-EM structure determination is challenging due to the inherent size, flexibility, and instability of GPCRs post-extraction from the membrane. Effective strategies and critical decisions in construct design, expression, and purification are essential for producing high-quality samples to be used as immunogens and for successful imaging. Here, we detail our approach to the production of high quality GPCRs for drug discovery, and present as an example a new cryo-EM structure of the 5-HT2A receptor, demonstrating scFv16 binding at its epitope and revealing a novel serotonin binding conformation.