

## **Structural and pharmacological characterisation of CXCR3 isoforms and their modulation by therapeutic antagonists**

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G protein-coupled receptors (GPCRs) are dynamic membrane proteins that convert extracellular cues into intracellular signalling. The chemokine receptor CXCR3 is a GPCR implicated in immunity, cancer, and transplant rejection, existing as two splice variants, CXCR3A and CXCR3B, with opposing effects on cell proliferation and disease outcomes. Despite its therapeutic importance, the structural basis for isoform-specific signalling and antagonist action remains poorly understood.

We systematically profiled the signalling of CXCR3A and CXCR3B using bioluminescence resonance energy transfer (BRET) biosensors to measure G protein activation (*Gai/o* and *Gaq* families) and  $\beta$ -arrestin recruitment. CXCR3B, previously assumed to lack G protein competence, exhibited robust coupling to *Gai3* and weaker coupling to *Gai1/2* and *Gao*. Chemokines displayed distinct bias: CXCL11 functioned as a full agonist, whereas CXCL9 selectively failed to recruit  $\beta$ -arrestins at CXCR3B. These findings highlight non-redundant signalling by endogenous ligands, aligning with their differential disease associations.

To advance structural studies, CXCR3 isoforms were purified in LMNG/CHS micelles and assessed by size-exclusion chromatography, dynamic light scattering, and thermal shift assays. Negative stain and cryo-electron microscopy (cryo-EM) revealed well-defined two-dimensional classes for CXCR3B-*Gai3* $\beta$ 1 $\gamma$ 2-CXCL11 complexes. To stabilise inactive states, we explored complexes with therapeutic antagonists, including monoclonal antibody Hu37 combined with binding partners such as Nb6, Fabs, and small molecules (allosteric inhibitor SCH546738 and orthosteric ligand AMG487). Antibody oversaturation and ligand binding enhanced conformational stability, with AMG487 conferring substantial thermostabilisation. These strategies provide robust approaches for preparing CXCR3 complexes amenable to high-resolution cryo-EM.

Together, our work establishes a biochemical and structural framework for CXCR3 isoforms, revealing unexpected G protein activity of CXCR3B and optimised stabilisation strategies. These findings pave the way for high-resolution cryo-EM structure determination and the rational design of precision therapies targeting chemokine receptor-driven diseases.