G-protein coupled receptors (GPCRs) are transmembrane proteins crucial for generating intracellular responses from extracellular signals, including hormones and neurotransmitters, as well as other signaling molecules. Around 35% of all FDA-approved drugs target GPCRs and GPCR-related proteins. This makes GPCRs one of the main targets for drug discovery and highlights the importance of understanding GPCR structure-function relationships for therapeutic applications.

Results

Figure 3. CCR6 expression in HEK-293 cells, as measured via flow cytometry. A. Single mutants, containing altered side chain charges at R42 (orange), Q192 (neon green), K200 (dark green), and R286 (tan) expressed at levels comparable with wildtype (blue). The combo mutant contains all four mutations at R42/Q192/K200/R286 (violet). B. Double mutants, with charge swap mutations at R42/Q192 (orange), K200/R286 (neon green), R42/K200 (dark green), as well as wildtype (blue), expressed poorly, as compared with untransfected cells (red). C. CCR6 expression in HEK-293 cells transfected with CCL20 or untransfected. The combo mutant fully ablated receptor activation.

Figure 4. G protein coupling as measured by NanoBiT assay. A. When a ligand binds to the receptor, two peroxidases linked to the receptor and G protein forms the functional heterotrimeric enzyme, which catalyzes substrate into the cell. This assay measures the amount of luminescence coming from the cell, with higher luminescence indicating higher receptor activation. B. Mutations at K200/Q192 (green), R286/Q192 (purple), and R42/Q192 (orange) also fully ablated GPCR activities. *RLU: Relative Luminescence Units.

Conclusion

- Identified residues critical for CCR6 binding/activation
- Single mutants had insignificant effects on protein expression
- The combo mutant strongly disturbed G-protein coupling but also disrupted protein folding, leading to lower receptor expression.
- Double mutants strongly disrupted G-protein coupling
- Developed a framework for studying structure-function relationships in a broad class of GPCRs
- Design mutations -> measure protein expression -> G-protein coupling assay -> functional assay

Design Rationale

- Selected CCR6 residues that form strong hydrogen bonds and salt bridges with their ligand complements
- Used charge-swap mutagenesis to swap out positively charged amino acid residues for negatively charged amino acids, and vice versa
- Designed multiple single, double, and combo mutational variants

Methodology

- Designed mutant receptors and amplified using PCR
- Measured protein expression, G-protein coupling, and transwell cell migration

Common inflammatory diseases in the human body:
1. Gastroenteritis
2. Endometriosis
3. Type 1 diabetes mellitus
4. Type 1 diabetes mellitus
5. Inflammatory bowel disease (IBD)
6. Asthma
7. Rheumatoid arthritis
8. E. coli
9. Alicecia’s and Parkinson’s disease
10. Cancer

Figure 9. Common inflammatory diseases in the human body.