Cryo-EM approaches for active-state and inactive-state GPCRs

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Introduction
Cryo-EM won the 2017 Nobel Prize in Chemistry and has since proven to be game-changing for the structure determination of GPCRs. Cryo-EM doesn’t depend on crystallization and enables structure determination of challenging proteins in their native state, which are two significant advantages over existing approaches. As a result, cryo-EM has already become the go-to method for determining active GPCR complexes and is set to overtake cryocrystallography for inactive-state GPCR structures too. Here, we highlight useful tools for robust structure determination of active-state and inactive-state GPCRs.

Figure 1. At the time of writing, there are over 400 GPCR cryo-EM In the Protein Database. Active-state structures grow exponentially after the first structure depositions in 2017. A similar trend is starting to form for fiducial-assisted inactive-state cryo-EM structures following breakthroughs in 2020. Additionally, resolutions of GPCR structures are also continuously improving as high resolution can now be routinely achieved.

Figure 2. The scFv16 antibody enables stabilization of receptor complexes with all G-protein subtypes.

Figure 3. CaptureSelect LC-kappa (Human) Affinity Ligand is a VHH that rigidifies Fab fragments.

Enablers of active-state structures

gPCR-G protein complex structures mediated by scFv16.

The scFv16 is an antibody fragment that stabilizes GPCR/G protein complexes by recognizing an interface between Gα and Gβγ subunits thereby enhancing the stability of GPCR-G protein complexes.

Figure 4. Structure of the mu-opioid receptor-G protein complex.

Enablers of inactive-state structures

Anti-BRIL Fab targeting BRIL-fusion GPCRs serve as fiducials for cryo-EM

BRIL-displayed and anti-BRIL Fabs are suited to add rigid mass to GPCRs to overcome the molecular weight barrier of cryo-EM. Anti-BRIL display structure predictions can guide the design of BRIL-fusion constructs towards rigid linker regions. The LC-kappa VH11 helps rigidity anti-BRIL Fab increases than faithful effective.

Figure 7. Cryo-EM structure and AF2 prediction of BRIL-fusion Frizzled10 receptor.

Figure 8. Inactive-state structure of histamine H2 receptor with a universal nanobody.

Nanobody 6 stabilizes inactive states of GPCRs and can be enlarged with a NabFab.

Nanobody 6 recognizes the inactive-state intracellular loop 3 of the ε-opioid receptor, which can be grafted on other receptors. Nanobody 6 can be engineered to be engaged by the nanobody-binding NabFab. Further stabilization of the NabFab using the LC-kappa VH11 is recommended (not shown).

Figure 9. Showcase studies featuring BRIL Fabs targeting BRIL.

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Conclusions

- Cryo-EM structures of GPCRs are growing at an exponential rate.
- One-day data collection on 200 kV and 300 kV microscopes is robust for routine resolution of 2.5 Å and below.
- Inactive-state structures are poised to follow the successes of active-state structures, benefiting from universal molecular tools and structure-prediction-guided construct design.

References

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