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

Ieva Drulyte and Mazdak Radjainia, Thermo Fisher Scientific, Materials and Structural Analysis, Achtseweg Noord 5, 5651 GG Eindhoven, Netherlands

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 crystallography 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 450 GPCR cryo-EM in the Protein Database. Active-state structures grew 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.[1]

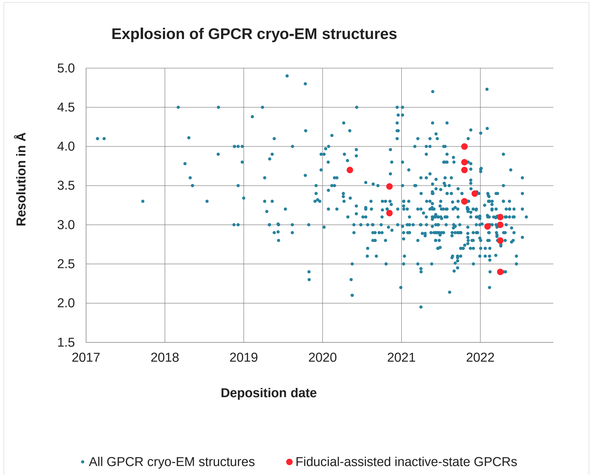


Figure 2. The scFv16 antibody enables stabilization of receptor complexes with all G-protein subtypes.

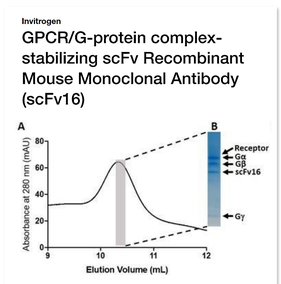


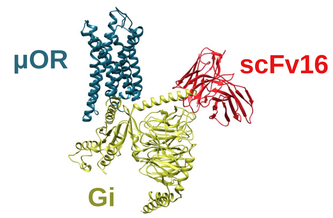
Figure 3. CaptureSelect LC-kappa (Human) Affinity Ligand is a VHH that rigidifies Fab fragments.

Enablers of active-state structures

GPCR-Gi/o 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-Gi/o complexes.[2]

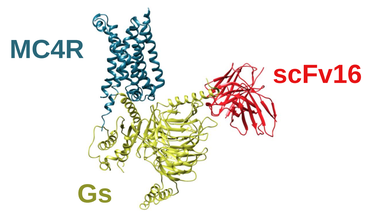
Figure 4. Structure of the mu-opioid receptor-Gi protein complex.



The scFv16 can also be used for stabilizing Gs and Gq complexes.

The stabilizing effect scFv16 can be transferred to other G-protein subtypes through minimal protein engineering and is therefore broadly applicable for structural studies of GPCR/G-protein complexes.

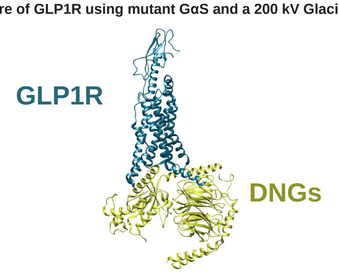
Figure 5. Structure of the melanocortin receptor 4-Gs complex.



Active-state complexes in the absence of stabilizing antibodies.

Dominant negative Gα protein is an alternative approach for the stabilization of ternary complexes.[3] The use of dominant negative mutants is not mutually exclusive with stabilizing antibodies and the combination may facilitates structures of low-efficiency compounds.

Figure 6. Structure of GLP1R using mutant GαS and a 200 kV Glacios cryo-TEM.

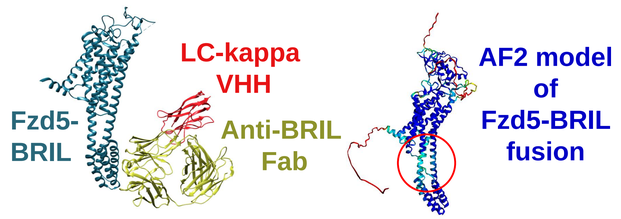


Enablers of inactive-state structures

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

BRIL-fusions and anti-BRIL Fabs are suited to add rigid mass to GPCRs to overcome the molecular weight barrier of cryo-EM.[4] AlphaFold2 structure predictions can guide the design of BRIL-fusion constructs towards rigid linker regions.[5] The LC-kappa VHH helps rigidify anti-BRIL Fabs and increases their fiducial effectivity.

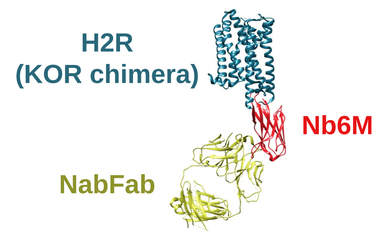
Figure 7. Cryo-EM structure and AF2 prediction of BRIL-fusion Frizzled5 receptor.



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 κ-opioid receptor, which can be grafted on other receptors.[6] Nanobody 6 can be engineered to be engaged by the nanobody-binding NabFab. Further stabilization of the NabFab using the LC-kappa VHH is recommended (not shown).

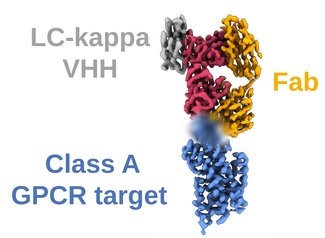
Figure 8. Inactive-state structure of histamine H2 receptor with a universal nanobody.



Showcase studies featuring inactive-state structures.

When a target-specific Fab is available or when the receptor forms homo-oligomers, inactive structures can be determined directly without needing fusion/chimera approaches.

Figure 9. Showcase studies featuring *de novo* inactive structure and epitope mapping.



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 at 2.5 Å and below.
- Inactive-state structures are poised to follow the success of active-state structures, benefitting from universal molecular tools and structure prediction-guided construct design.

References

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