Feeling at home: Structure of the NTSR1–Gi complex in a lipid environment

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GPCR SIGNALING

Feeling at home: Structure of the NTSR1–G_i complex in a lipid environment

The interaction of G protein–coupled receptors (GPCRs) with heterotrimeric G proteins plays a critical role in signal transduction processes, and multiple GPCR–G protein complexes reconstituted in detergent micelles have been visualized using cryo-EM. A new study reports the structure of neurotensin receptor 1 (NTSR1) in complex with the heterotrimeric G_i protein, assembled in a lipid environment using circularized nanodiscs. The structure sheds light on how the lipid context may influence receptor–G protein coupling and activation.

Jagannath Maharana and Arun K. Shukla

GPCRs constitute a large family of cell surface proteins in the human genome, with more than 800 members, and they continue to be one of the most sought-after drug targets for a range of human disorders[1]. They exhibit incredible diversity in their ligand-recognition ability on the extracellular side but converge significantly on the transducers they are coupled with on the intracellular side. Agonist-induced activation of heterotrimeric G proteins associated with GPCRs is responsible for a broad spectrum of cellular signaling pathways involved in diverse physiological processes. The technological advance in cryo-EM has clearly revolutionized our understanding of the structural basis of GPCR activation and signaling, and the structural coverage of GPCR–G protein complexes continues to grow at a staggering pace[2]. This has resulted in cryo-EM structures of GPCRs from different subfamilies in complex with different G protein sub-types, leading to deep mechanistic understanding of coupling principles and the ensuing activation process[3]. However, the majority of the GPCR–G protein complex structures have been determined in detergent micelles, with the exception of the recent structure of the dopamine D2 receptor (DRD2) in complex with G_i (ref. [4]), which was assembled in a nanodisc-based lipid environment. Now, Zhang et al.[5] have determined the structure of the NTSR1–$G\alpha_{i1}$–β_1–γ_1 complex in circularized nanodiscs (Fig. 1), which provides interesting insights into the effect of the lipid context on receptor–G protein interactions and activation.

NTSR1 is a rhodopsin-like class A GPCR that is activated by a tridecapeptide neurotransmitter called neurotensin. NTSR1 signaling is involved in oncogenic growth, analgesic response and neurological

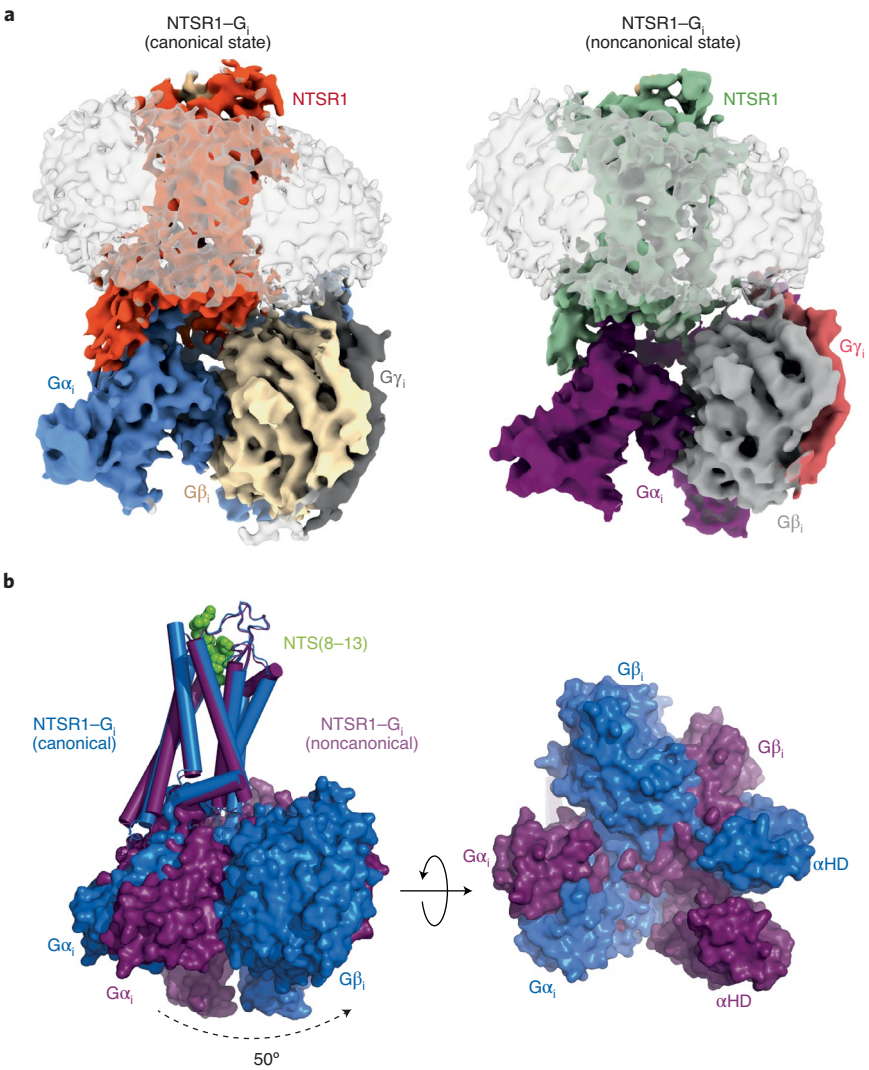

Fig. 1 | Cryo-EM analysis of the NTSR1–G_i complex reveals two conformational states. a, Surface representation of the "canonical" (left, PDB 7L0Q) and "noncanonical" (right, PDB 7L0S) states observed in circularized nanodiscs. **b**, A superimposition of the two conformational states of the NTSR1–G_i complex reveals a distinct positioning of the G_i heterotrimer (noncanonical state in purple and canonical state in marine). HD, helical domain; NTS, neurotensin.

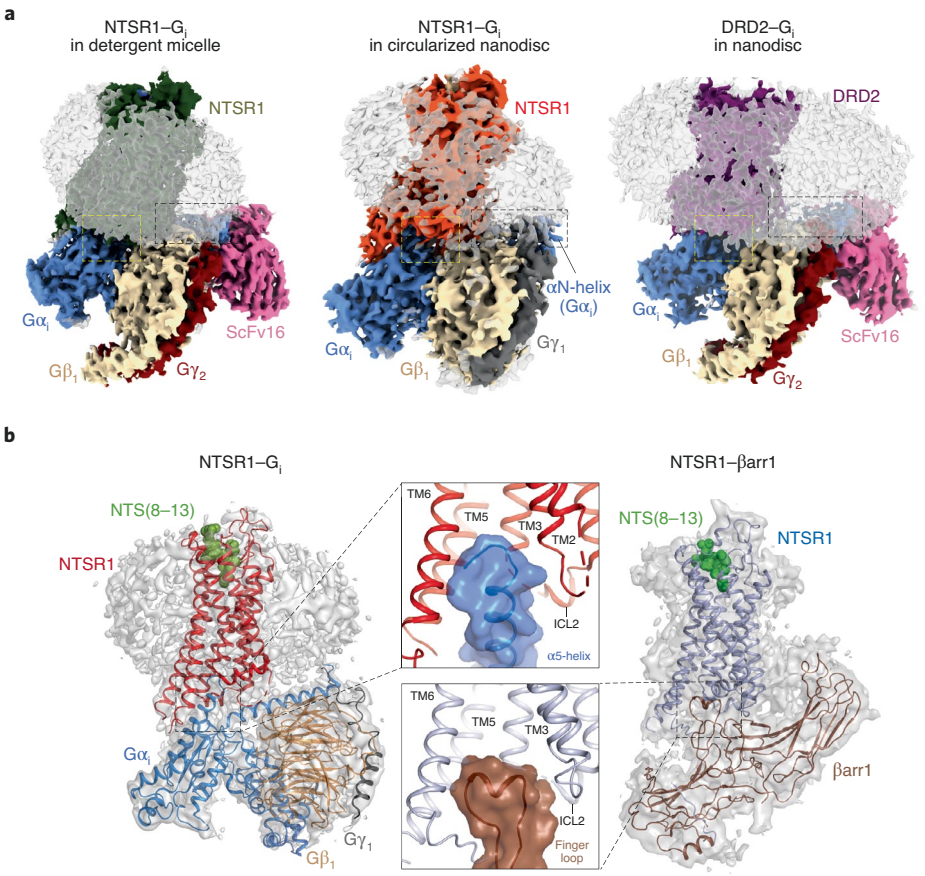

Fig. 2 | An overall comparison of the NTSR1–G_i structure with structures of the DRD2–G_i and NTSR1–βarr1 complexes. a, Surface representation of NTSR1–G_i canonical state structures in detergent micelles (left, PDB 6OS9) and circularized nanodiscs (middle, PDB 7L0P), and the structure of the DRD2–G_i complex in nanodiscs (right, PDB 6VMS). The dotted boxes are used to highlight the interaction between the G protein subunits and the membrane in a lipid context and the more extensive interactions between the receptor and $G\alpha_i$ in a lipid environment as compared to detergent micelles. **b**, The α5-helix of $G\alpha_i$ (PDB 7L0P) and the finger loop of βarr1 (PDB 6UP7) are positioned on the receptor to occupy a similar interface, which provides a structural mechanism for how βarr binding desensitizes the receptor. In addition, the αN-helix of $G\alpha_i$ and C-edge loops of βarr1 are positioned to directly interact with the lipid bilayer.

disorders, including schizophrenia and Parkinson's disease[6]. Zhang et al. reconstituted purified NTSR1 in circularized nanodiscs (cNDs) and assembled a complex with the $G\alpha_i$ heterotrimer ($G\alpha_{i1}$–β_1–γ_1) (Fig. 1). Circularized nanodiscs typically consist of lipids stabilized by a membrane-scaffolding protein, cNW9, that is covalently circularized, and they provide a membrane-mimetic environment that is similar to the conventional nanodisc but with improved sample homogeneity[7]. Interestingly, the interaction of NTSR1 with the G protein heterotrimer appears to be tighter in cNDs than in detergent micelles, and the reconstituted complex exhibits higher thermostability[5]. This enhanced stability and the stronger interaction of the receptor with the G protein heterotrimer in cNDs allows cryo-EM structure determination of the complex without additional stabilizing agents, such as nanobodies or antibody fragments, which were used for the majority of the previously determined GPCR–G protein complex structures.

Cryo-EM analysis of the NTSR1–G_i complex reveals two distinct conformational states, wherein the positioning of the $G\alpha_i$ heterotrimer relative to the receptor is significantly different, and these two arrangements of the complex are referred to as "canonical" and "non-canonical" states[5] (Fig. 1a, b). It is interesting to note that two conformational states of the NTSR1–G_i complex were also observed in a previous study that utilized a complex reconstituted in detergent micelles[8]. In the NTSR1–G_i structure reported by Zhang et al., the αN-helix of the $G\alpha_i$ subunit (Fig. 2a, middle panel) and the carboxyl-terminal prenylation site of $G\gamma_1$ are positioned to form an interface with the lipid membrane, and helix 8 in the NTSR1 also appears to be partially embedded in the lipid bilayer. Interestingly, these features were also observed in the previously reported DRD2–G_i structure determined in conventional nanodiscs[4], potentially revealing a mechanistic basis for better coupling between the receptor and G protein in a membrane context. In fact, a comparison of NTSR1–G_i structures determined in cNDs versus in detergent micelles does reveal a more extensive set of interactions between the receptor and the G_i heterotrimer. Another intriguing feature of the structure determined by Zhang et al. is that the outward movement of transmembrane helix 6 (TM6) in NTSR1 is somewhat more restricted than in previous GPCR–G protein structures. This helps position the α5-helix of the $G\alpha_i$ subunit such that it may engage in more extensive contacts with the receptor[5]. However, it remains to be experimentally determined whether the constrained movement of TM6 arises because of the lipid bilayer context or the thermostabilizing mutations present in the NTSR1.

The observation of two distinct conformational states in the NTSR1–G_i structures, both in detergent micelles and in circularized nanodiscs, is quite intriguing and raises the question of the functional relevance of the "noncanonical" conformation. Considering the less extensive network of interactions between the receptor and G_i heterotrimer in this conformation, it is plausible that it represents a less stable, transient intermediate along the pathway of forming a fully functional receptor–G protein complex. In the process of determining cryo-EM structures of GPCR–G protein complexes, only a small fraction of data is being utilized to deduce the final structure, and minor conformations may be missed during the classification procedure. It is therefore intriguing that the so-called "noncanonical" states have been observed only for NTSR1–G_i complexes. It would be interesting to probe whether this is a receptor-specific feature that is limited to NTSR1 or whether it can be also observed for other GPCRs if the complexes are assembled and visualized in lipid bilayers. As data processing algorithms for cryo-EM evolve, it is also possible that a more inclusive analysis of existing data on GPCR–G protein complexes may reveal similar conformational states for other receptors. It is also important to note that

NTSR1 was originally found to couple to $G_{q/11}$ G proteins, although subsequent studies demonstrated its coupling to G_s and G_i as well. Therefore, future efforts to determine the structure of the NTSR1–$G_{q/11}$ complex will be important to better understand the molecular mechanism of the G protein coupling selectivity of NTSR1, as well as the functional relevance of the two conformational states observed in the current structure.

In addition to heterotrimeric G proteins, GPCRs also couple to multifunctional proteins called β-arrestins (βarrs), which are critical regulators and transducers of downstream GPCR signaling. As the structures of NTSR1 in complex with βarr1 have also been determined previously[9,10], a comparison with the current structure offers some interesting insights. For example, the position of the α5-helix of $G\alpha_i$ and the finger loop of βarr1 are remarkably similar in the binding interfaces they form with the receptor (Fig. 2b), an observation that is also apparent when comparing the structures of β_1AR–G_s and β_1AR–βarr1 complexes[11–13]. This typical feature appears to be responsible for how the core interaction between the receptor and βarrs precludes further G protein coupling, leading to receptor desensitization[14–16]. Furthermore, the lipid interaction of G proteins and the proximal alignment of the αN-helix to the bilayer observed in the current structure are also reminiscent of the membrane interaction of C-edge loops in βarrs[11,17,18]. While an interplay between the membrane bilayer and signal transducers such as G proteins and βarrs is not surprising, the specific details observed in these structural snapshots will inform experimental strategies for probing their precise contribution to signal transduction.

In summary, the NTSR1–G_i structure determined by Zhang et al., together with the DRD2–G_i complex determined previously, underlines the importance of the lipid environment in the reconstitution and visualization of GPCR signaling complexes and demonstrates how such a structure can lead to additional mechanistic insights. As more structures of receptor–transducer complexes become available in both detergents and lipids, especially the same receptor with different transducers, comparison of these structures should guide experimental strategies for probing GPCR activation and signaling mechanisms in more detail. ❐

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References

1. Hauser, A. S., Attwood, M. M., Rask-Andersen, M., Schiöth, H. B. & Gloriam, D. E. *Nat. Rev. Drug Discov.* **16**, 829–842 (2017).
2. Congreve, M., de Graaf, C., Swain, N. A. & Tate, C. G. *Cell* **181**, 81–91 (2020).
3. Glukhova, A. et al. *ACS Pharmacol. Transl. Sci.* **1**, 73–83 (2018).
4. Yin, J. et al. *Nature* **584**, 125–129 (2020).
5. Zhang, M. et al. *Nat. Struct. Mol. Biol.* https://doi.org/10.1038/s41594-020-00554-6 (2021).
6. Mitra, S. P. *Indian J. Biochem. Biophys.* **54**, 7–23 (2017).
7. Nasr, M. L. et al. *Nat. Methods* **14**, 49–52 (2017).
8. Kato, H. E. et al. *Nature* **572**, 80–85 (2019).
9. Huang, W. et al. *Nature* **579**, 303–308 (2020).
10. Yin, W. et al. *Cell Res.* **29**, 971–983 (2019).
11. Lee, Y. et al. *Nature* **583**, 862–866 (2020).
12. Su, M. et al. *Mol. Cell* **80**, 59–71.e4 (2020).
13. Pandey, S., Saha, S. & Shukla, A. K. *Mol. Cell* **80**, 3–5 (2020).
14. Kumari, P. et al. *Nat. Commun.* **7**, 13416 (2016).
15. Cahill, T. J. III et al. *Proc. Natl Acad. Sci. USA* **114**, 2562–2567 (2017).
16. Chaturvedi, M., Maharana, J. & Shukla, A. K. *Cell* **180**, 1041–1043 (2020).
17. Lally, C. C. M., Bauer, B., Selent, J. & Sommer, M. E. *Nat. Commun.* **8**, 14258 (2017).
18. Staus, D. P. et al. *Nature* **579**, 297–302 (2020).

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Competing interests

The authors declare no competing interests.