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Supplementary information

Cryo-EM structure of an activated GPCR–G protein complex in lipid nanodiscs

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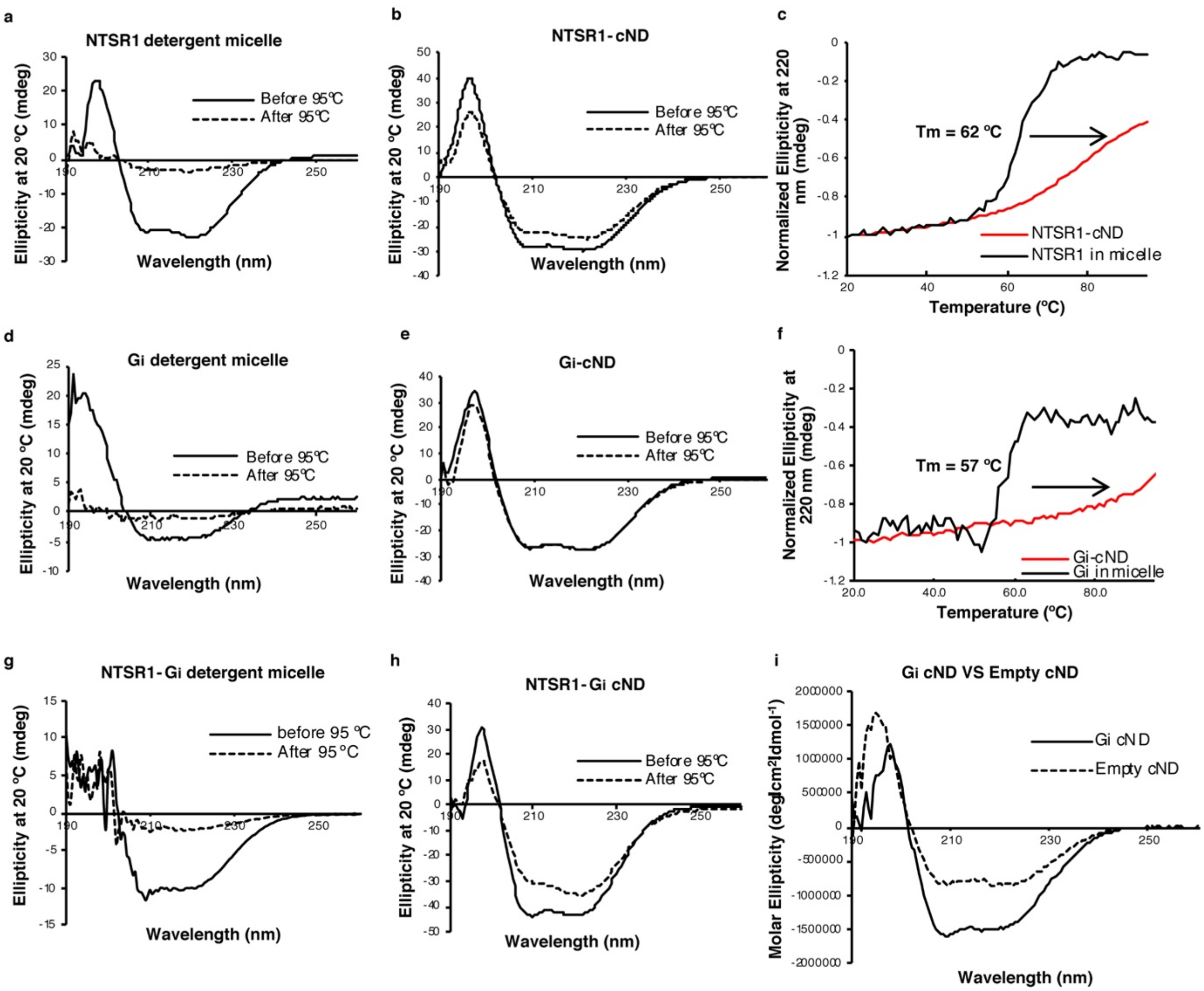
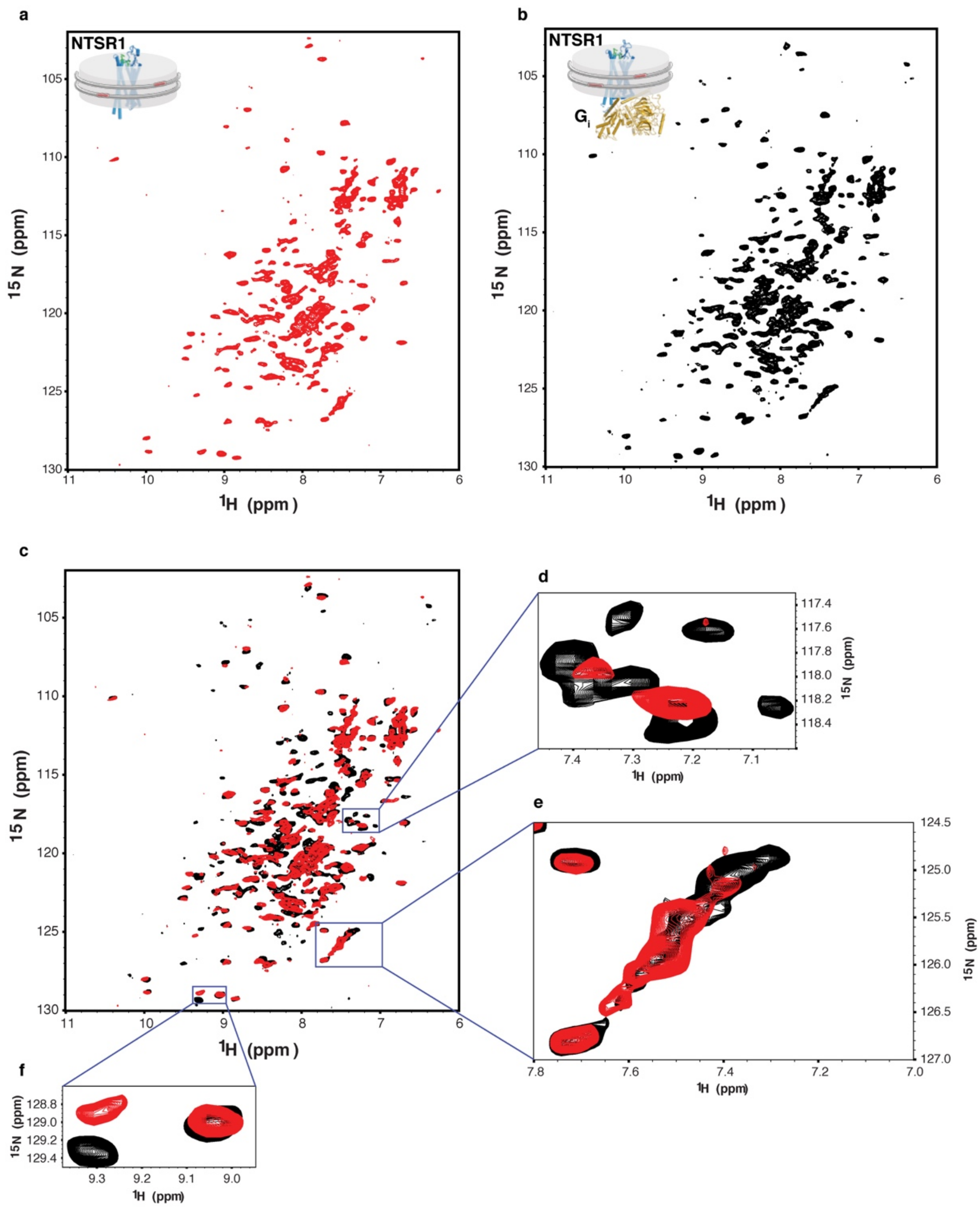


Supplementary Fig. 1 | Thermostability enhancement of NTSR1, G_i, and NTSR1-G_i complexes by incorporation into cNDs. **a-b**, Circular Dichroism (CD) spectra at 20 ºC before (solid line) and after (dashed line) treatment at 95 ºC of (**a**) NTSR1 in DH_7PC detergent micelles; (**b**) NTSR1 in cNDs. **c**, Temperature-dependent CD signals of NTSR1 in detergent micelles (black) and cNDs (red) at 220 nm. **d-e**, CD spectra at 20 ºC before (solid line) and after (dashed line) treatment at 95 ºC of (**d**) G_i in DH_7PC detergent micelles; (**e**) G_i in cNDs. G_i was reconstituted into cNDs by incubation with POPC/POPG lipid, cNW9, and cholate, followed by detergent removal and size-exclusion chromatography. **f**, Temperature-dependent CD signals of G_i in detergent micelles (black) and cNDs (red) at 220 nm. The melting temperature (Tm) of cNDs is 93 ºC (data not shown) and therefore does not affect transitions before this temperature. **g-h**, CD spectra at 20 ºC before (solid line) and after (dashed line) treatment at 95 ºC of (**g**) NTSR1-G_i in LMNG/GDN/CHS detergent micelles; (**h**) NTSR1-G_i in cNDs. **i**, CD spectra of 2 μM G_i-cND (solid line) and 2 μM empty cND (dashed line), showing nearly 50% signal contribution from G_i. NTSR1 and G_i account for at least 50% of CD signals even in the presence of cNDs. NTSR1 in detergent micelles irreversibly unfolds during temperature increase with a Tm of 62 ºC. In contrast, NTSR1-cND changes structure around 80 ºC and does not lose much secondary structure after decreasing temperature to 20 ºC. Similar observations were made for G_i, where the protein irreversibly and completely unfolds with Tm of 57 ºC in detergent micelles but displays no clear transition temperature in cNDs. For the NTSR1-G_i complex in cND, only mild unfolding was observed around 82 ºC. These observations indicate that lipid bilayers improve the stability of NTSR1, G_i and NTSR1-G_i complexes relative to detergent micelles.



Supplementary Fig. 2 | Characterization of the interaction between NTS-NTSR1 and G_i in cNDs by two-dimensional ^1H, ^{15}N-TROSY HSQC NMR spectroscopy. **a-b**, NMR spectrum of ^{15}N-labeled NTS-NTSR1 in cNDs in the (**a**) absence and (**b**) presence of G_i. **c,** Overlay of (**a**) (red) onto (**b**) (black) showing structural and dynamical changes of NTS-NTSR1 upon binding to G_i in cNDs. **d**, A region showing conformational stabilization of NTSR1. More peaks are observed in the presence of G_i, suggesting that NTSR1 is highly dynamic in the absence of G_i and resonances are averaged out among a wide range of conformers resulting in low signal-to-noise ratio and even disappeared peaks. Upon interaction with G_i, NTSR1 is stabilized into fewer conformers and becomes less dynamic, which leads to better signal-to-noise ratio and more resonances being observed. **e**, A region showing dynamically slow-exchange shift of NTSR1 upon interaction with G_i. **f**, A region showing chemical shift perturbation of NTSR1, suggesting conformational change of NTSR1 upon binding to G_i in cNDs.