



Fig. S5: Cryo-EM data analysis of the active setmelanotide-MC4R-Gs-Nb35 complex.

(a) Representative cryo-electron micrograph. The scale bar represents 50 nm in the image.

(b) Distribution of projection directions as estimated during homogeneous refinement with cryoSPARC.

(c) Global resolution estimation by Fourier shell correlation calculations (FSC = 0.143 cutoff) after "gold standard" refinement. The light blue curve was calculated without masking, the black curve by applying a spherical mask and the red one after phase randomization using a soft-mask.

(d) Representative 2D class averages confirm random distribution of projection directions. The scale bar corresponds to 10 nm in the image.

(e) Representation of local resolution estimation determined with cryoSPARC. The final cryo-EM density is colored according to the local resolution ranging from dark blue (2.4 Å) to red (4.4 Å).