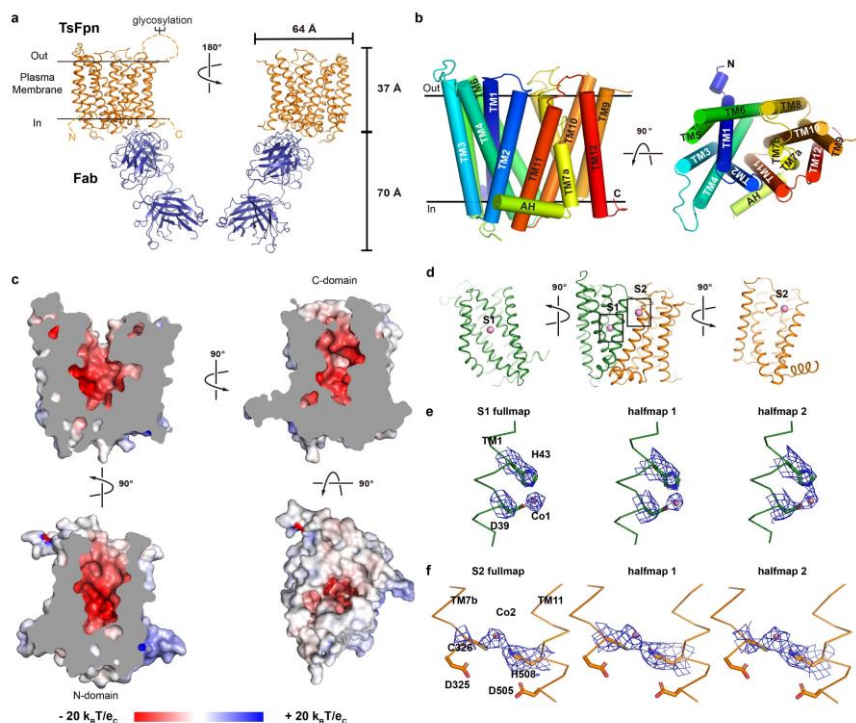
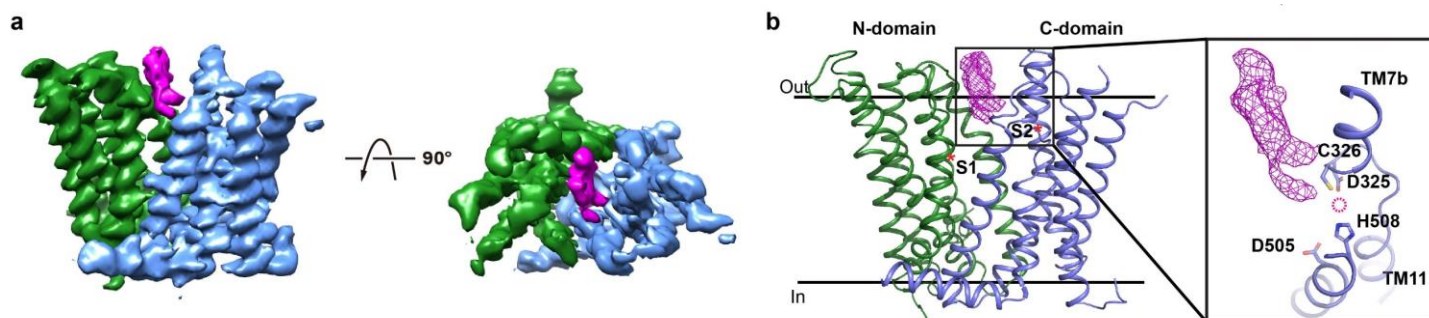


**Figure 1. Cryo-EM analysis of the TsFpn-Fab complex in nanodisc.** **a.** Representative electron micrograph and 2D class averages of cryo-EM particle images. **b.** Local resolution map for the 3D reconstruction of the TsFpn-Fab complex. **c.** The gold-standard Fourier shell correlation curve for the final map. **d.** Representative densities.



**Figure 2. Structure of TsFpn and two potential ion binding sites.** **a.** TsFpn (orange) in complex with Fab (blue) in two views. **b.** TsFpn structure shown as cylinder representation. **c.** Electrostatic potential of TsFpn mapped onto the surface representation. The cut-away views show the large cavity formed between the N- and C- domains. **d.** TsFpn in cartoon representation is shown in three orientations with S1 and S2 marked as sticks. **e.** Density maps of S1 are shown in blue mesh. Part of the TM1 is shown as trace and the side chains of Asp39 and His43 are shown in stick.  $\text{Co}^{2+}$  is shown as a magenta sphere. **f.** Density maps of S2 are shown in blue mesh.



**Figure 3. Structure of TsFpn in complex with hepcidin.** **a.** Cryo-EM map of TsFpn (green and blue) in complex with hepcidin (magenta) in two orientations. The Fab density is not shown. **b.** Structure model of TsFpn in the presence of hepcidin. Hepcidin density is shown as magenta mesh. Positions of S1 and S2 are labeled as red stars. Inset: close-up view of hepcidin density and the S2 site.  $\text{Co}^{2+}$  position is marked as a dotted circle.