FIGURES/PRELIMINARY RESULTS

Figure 1. Preparation of transporter-Fab complexes. Top: UV trace from SEC showed NorA in PMAL-C8 amphipol (blue) and NorA in PMAL-C8 amphipol in the presence of Fab (orange). The left shift of the chromatogram shows formation of the larger NorA:Fab complex. Bottom: Analysis of the peak fraction of the NorA/Fab chromatogram by SDS-PAGE shows discrete Fab and NorA bands, further confirming complex formation.

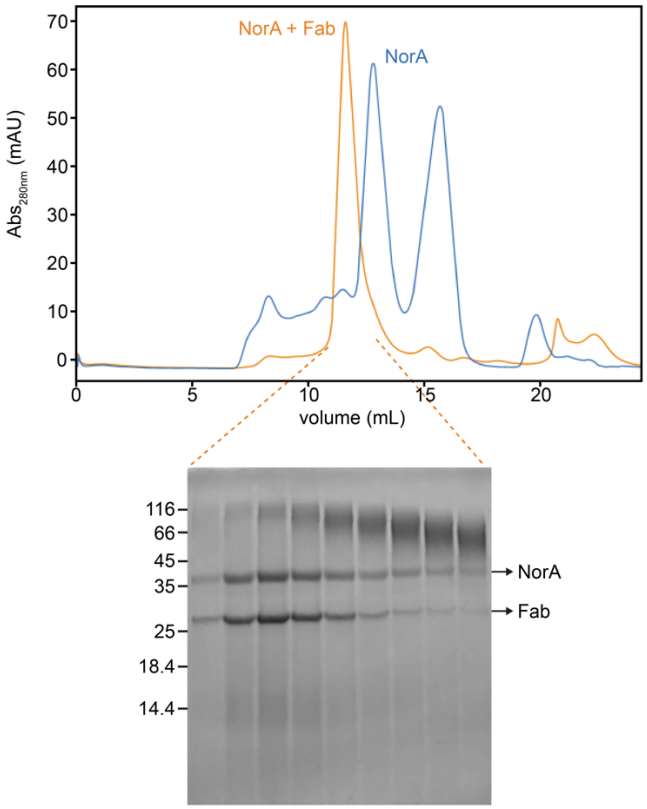

Figure 2: verification of binding affinity using MST. Representative MST binding curves of fluorescently labelled Fabs to NorA reconstituted in PMAL-C8 amphipol (i.e., that used for cryo-EM). K_d values are shown next to each dataset; "n.d." refers to not determined. This experiment confirms binding between Fabs and Nora are sequence dependent. Similar measurements are used to analyze each complex.

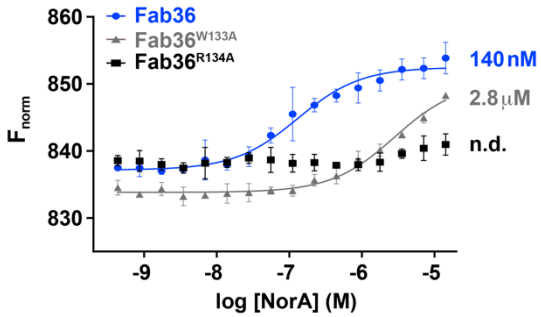

Figure 3: CryoEM data quality and processing: (a) Representative micrograph of vitrified NorA:Fab-DA1 complex recently acquired at low pH using a previously supported NCCAT cryo-EM time (unpublished data). (b) Representative 2D class averages of particles, where NorA and the Fab are clearly visible. (c) 3D reconstruction of NorA from the NorA:Fab complex from 279,282 particles; masking was used in the processing to further improve the quality of the reconstruction. (d) Estimated global resolution of the reconstruction (GSFSC = 3.26 Å).