



Figure 1: Structures of AP-3 on blot-free grids

- A) Representative 2D class averages of Apo AP-3 from previous GUP1 and GUP2 proposals
- B) Representative 2D class averages of new AP-3 “supercomplex” from GUP1 proposal, showing strong presence of 1 Arf1 GTPase
- C) Representation of AP-3 structure and current best map/structure (Depiction on right is AlphaFold generated models rigid-body docked into preliminary apo AP-3 volume determined by deepEMhancer)
- D) Representation of AP-3 supercomplex, depicting AP-3 bound to two Arf1 enzymes and associated with nanodisc. Depiction on right is AlphaFold generated models rigid-body docked into preliminary AP-3 supercomplex volume determined from previous GUP1 data, processed via deepEMhancer. Nanodisc model generated via CHARM suite.
- E) Current best resolution model of supercomplex (volume processed via deepEMhancer) with all corresponding protein models generated via AlphFold and rigid-body docked. Strong density is apparent for all AP-3 subunits as well as two copies of Arf1 and potentially some nanodisc (void volume with no model docked).
- F) Previous GUP1 data (same as E) but preference given to classes depicting one Arf1 molecule. Resolution of this intermediate-supercomplex structure is higher than in E, but lacks second Arf1 molecule and nanodisc density.