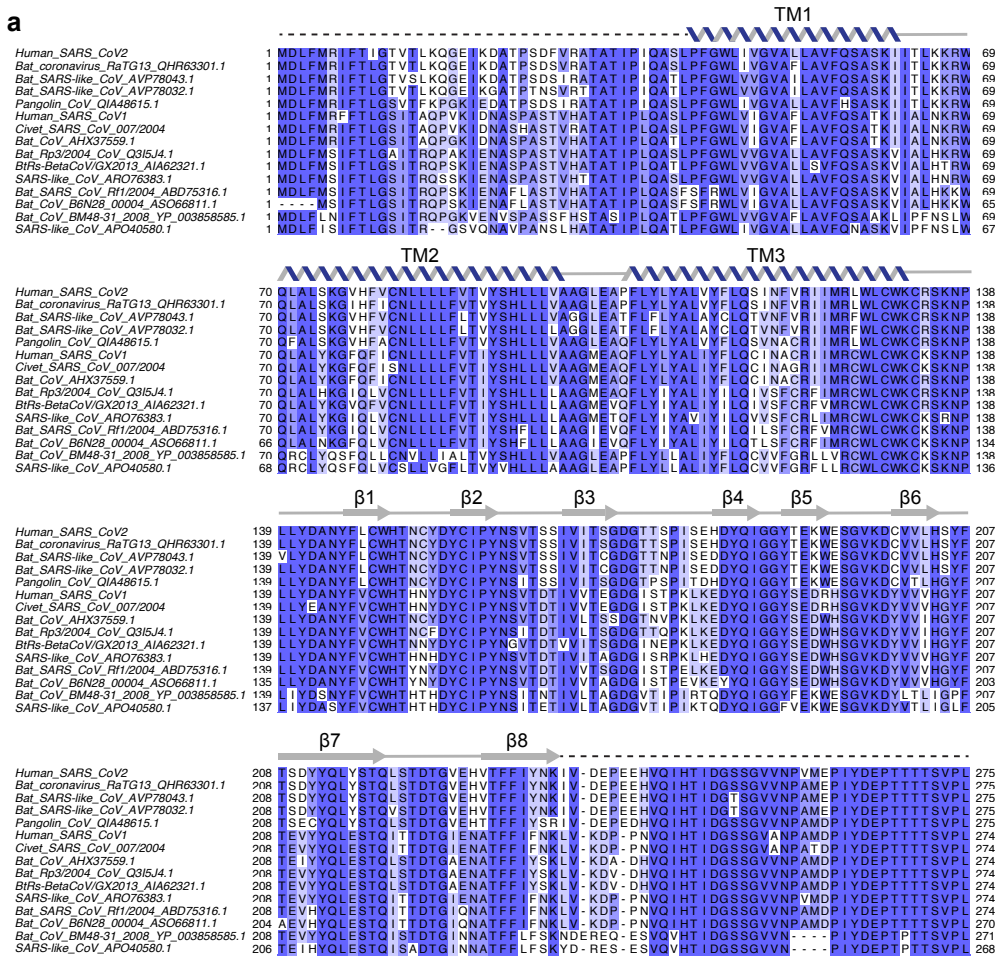
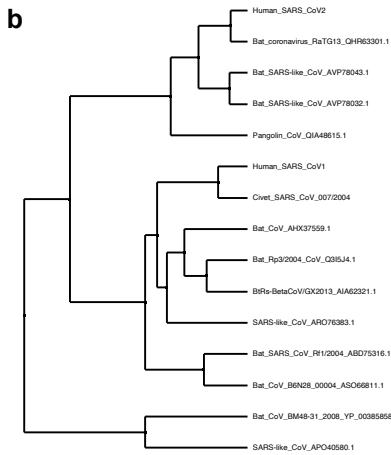


Supplemental Figure 1

a



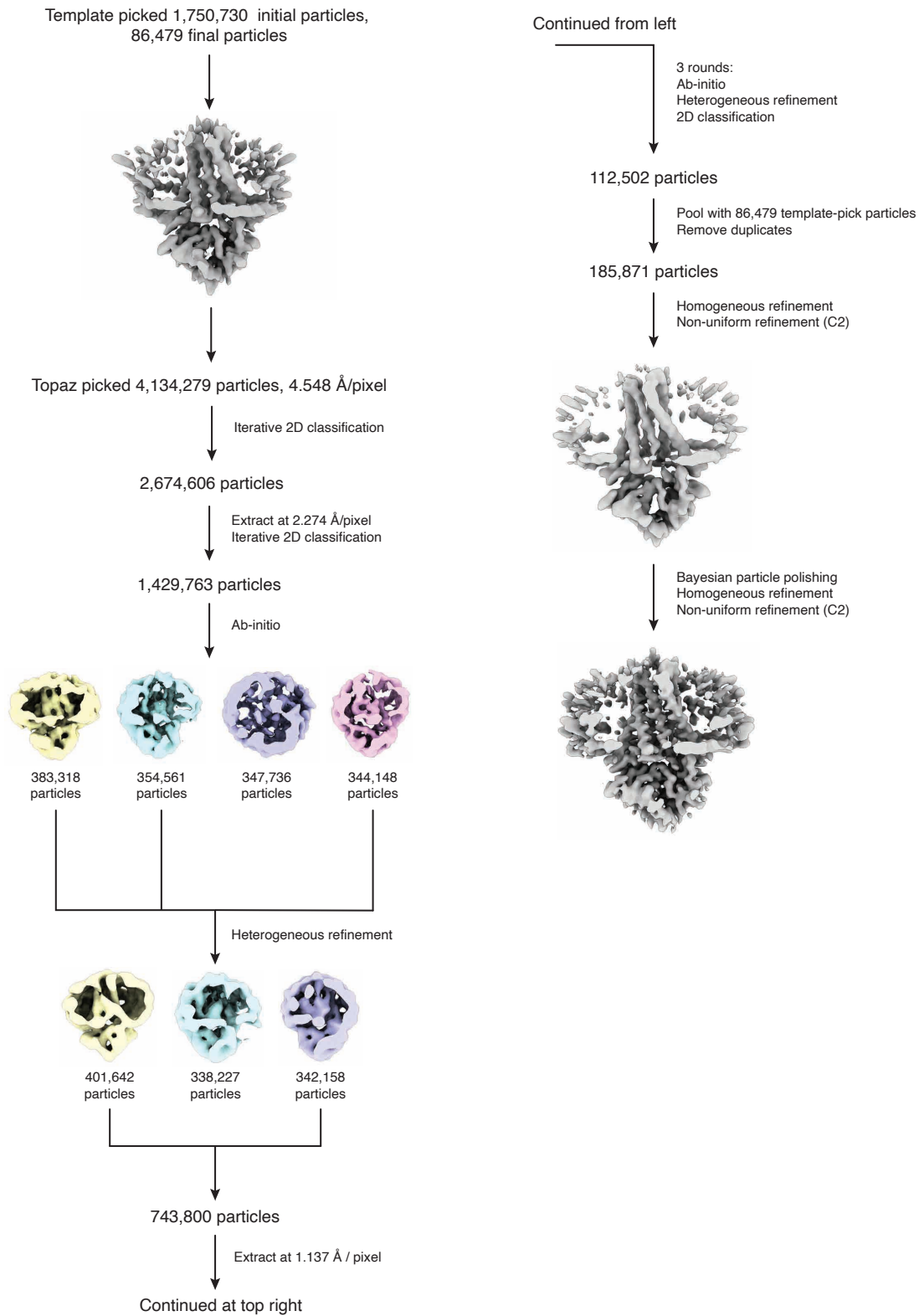
b



Supplemental Figure 1 – Sequence alignment of 3a from *Betacoronavirus Sarbecovirus*

(a) Alignment of fifteen 3a protein sequences colored by conservation in a ramp from white (not conserved) to dark blue (highly conserved). Accession numbers are indicated. Sequences were selected to maximize diversity among annotated *Sarbecovirus* 3a proteins. Secondary structure for SARS-CoV-2 is drawn above the sequence with unmodeled sequence drawn as dashed lines. (b) Neighbor-joining tree calculated from the alignment in (a).

Supplemental Figure 2

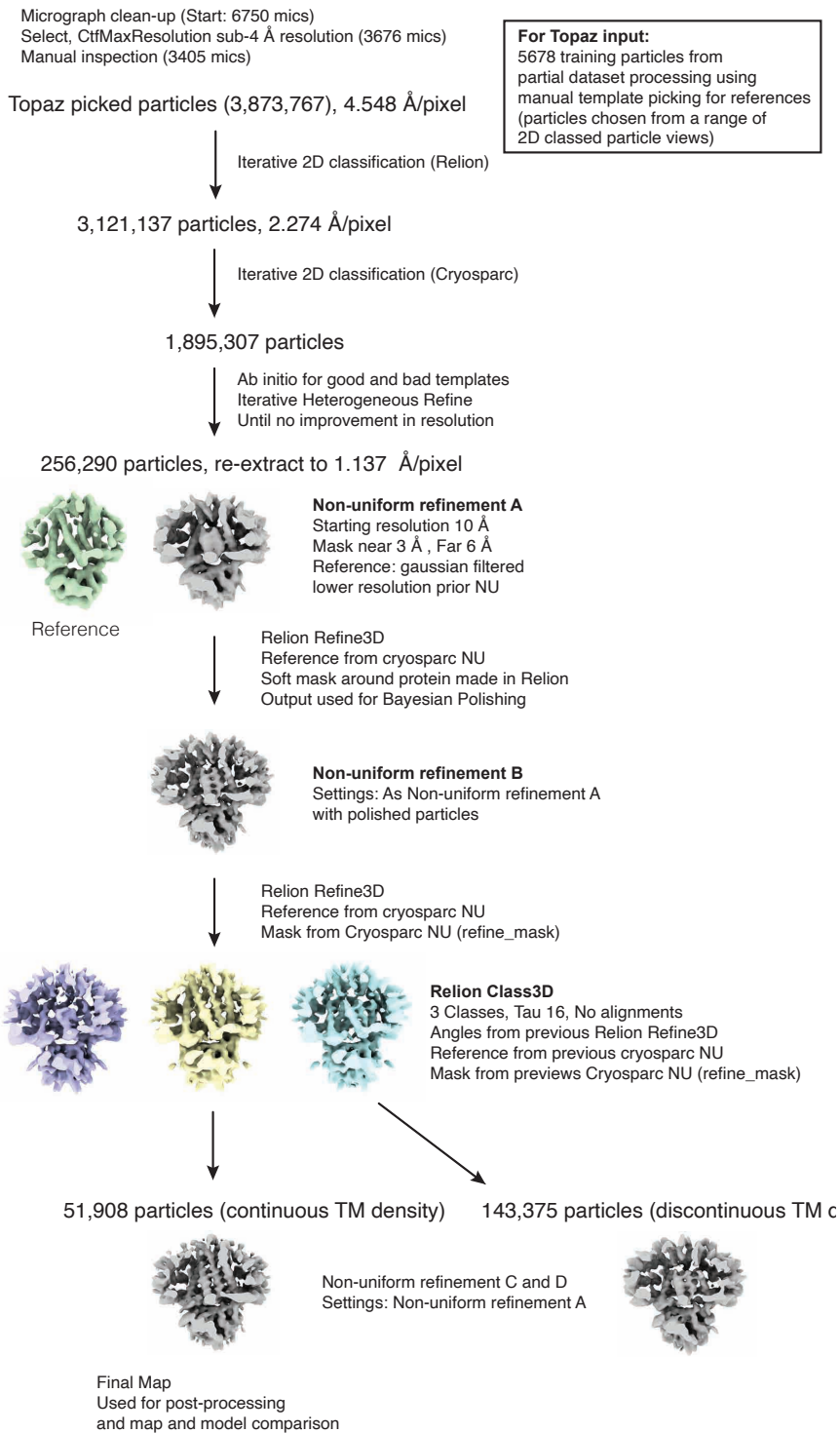


Supplemental Figure 2 - Cryo-EM processing pipeline for dimeric apo 3a in MSP1E3D1 lipid nanodiscs.

Overview of Cryo-EM data processing pipeline in cryoSPARC and Relion. See Methods for details.

Supplemental Figure 3

3a in MSP1E3D1 + Emodin Dataset

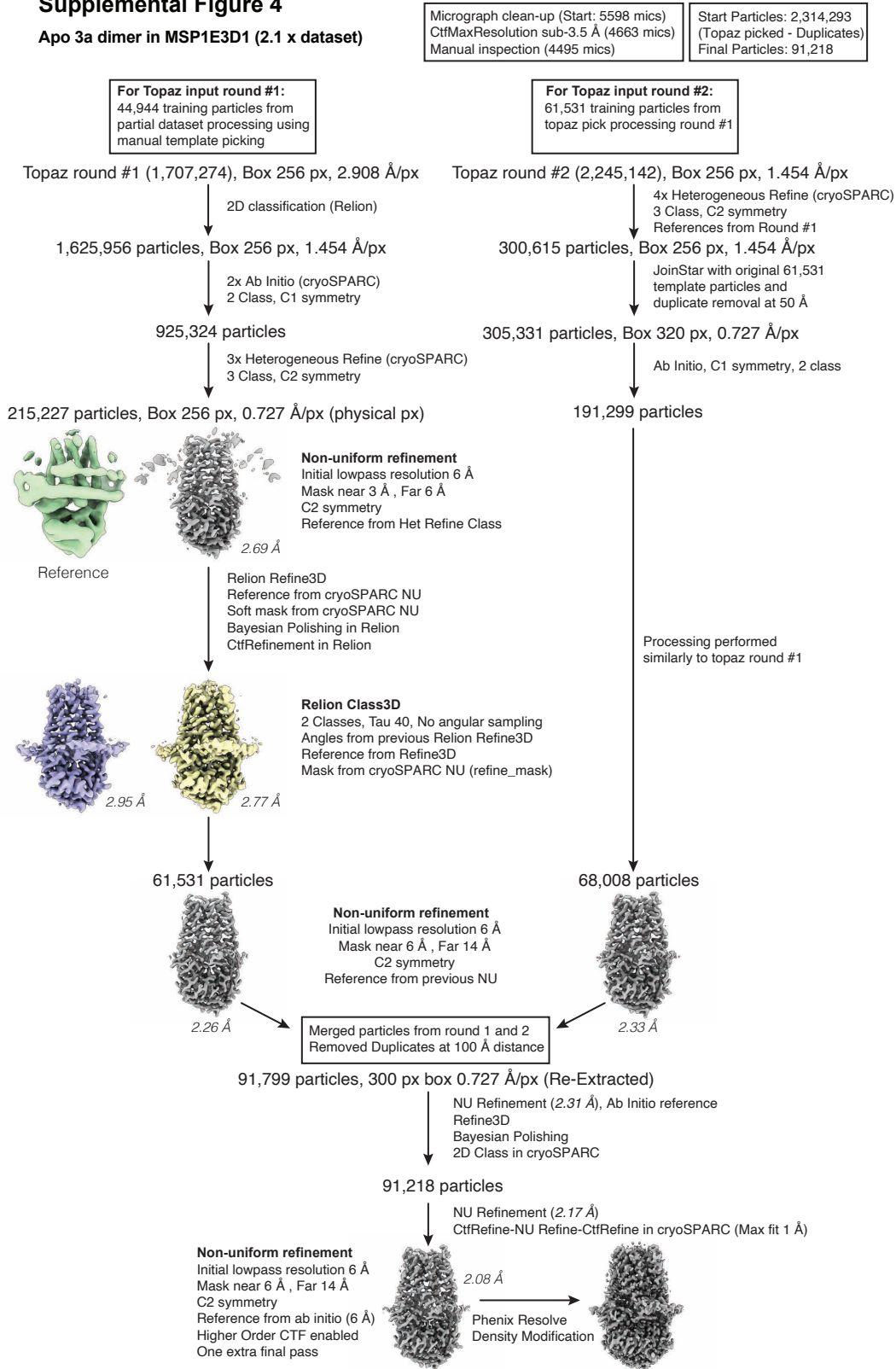


Supplemental Figure 3 - Cryo-EM processing pipeline for dimeric 3a in MSP1E3D1 lipid nanodiscs with emodin added

Overview of Cryo-EM data processing pipeline in cryoSPARC and Relion. See Methods for details.

Supplemental Figure 4

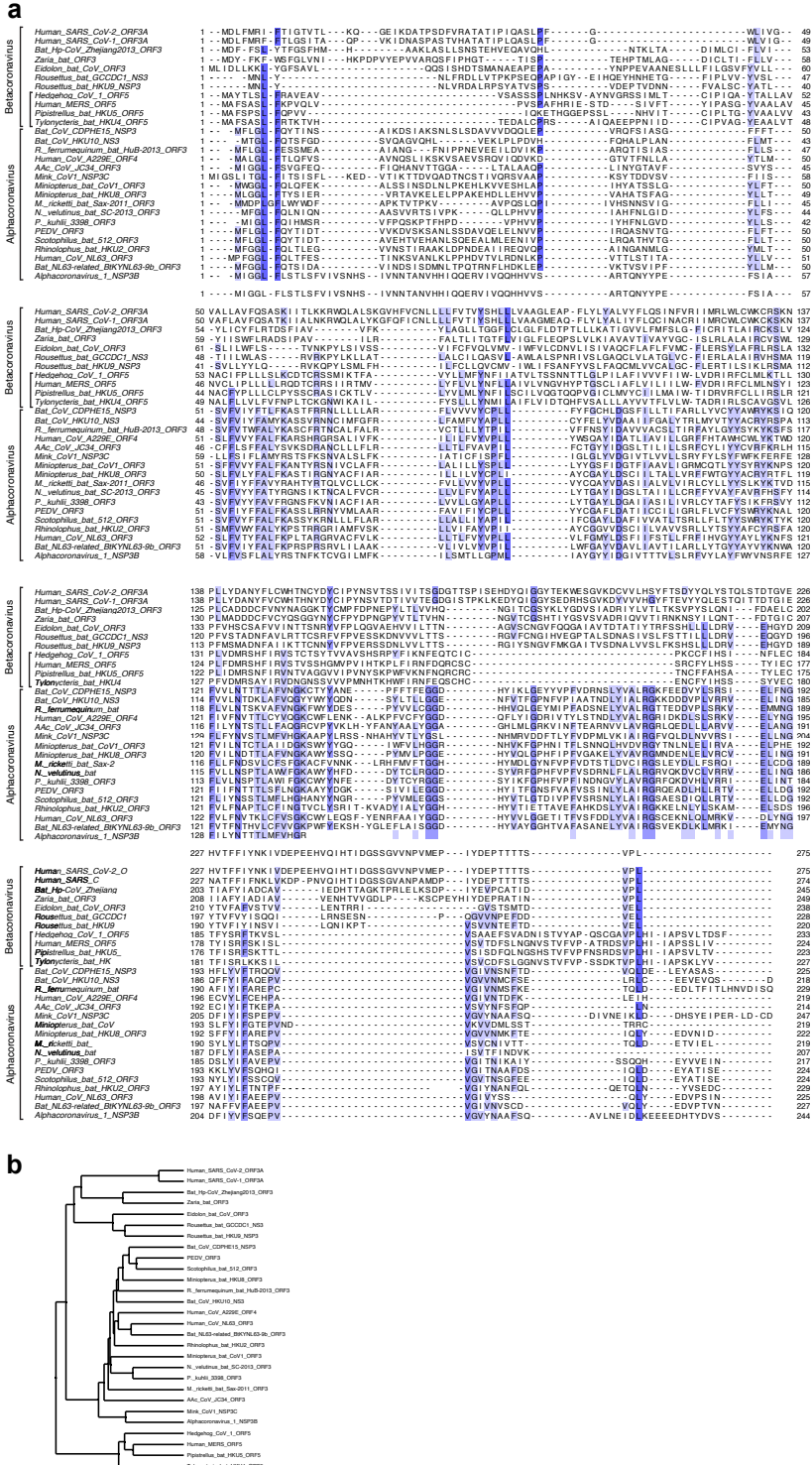
Apo 3a dimer in MSP1E3D1 (2.1 x dataset)



Supplemental Figure 4 - Cryo-EM processing pipeline for 2.1 Å dimeric apo 3a in MSP1E3D1 lipid nanodiscs.

Overview of Cryo-EM data processing pipeline in cryoSPARC and Relion. See Methods for details.

Supplemental Figure 5



Supplemental Figure 5 - Sequence alignment of 3a-like proteins across Coronaviridae

(a) Alignment of twenty-eight 3a-like protein sequences colored by conservation in a ramp from white (not conserved) to dark blue (highly conserved). Accession numbers are listed in Table 2. Sequences were selected from representative species from each Coronavirus subgenus. *Alphacoronavirus* and *Betacoronavirus* clades are indicated. Within *Betacoronavirus* the subgenus *Merbecovirus* is also indicated with a bar. (b) Neighbor-joining tree calculated from the alignment in (a).