

**Figure 2 – supplement 1. Workflow of masked classification of C3-symmetry imposed map of AcrABZ-TolC pump in the presence of puromycin with residual signal subtraction.** (A)A soft mask used in the target region of the complex for focused classification. (B) Generation of three maps with the part of the complex to be ignored in focused classification. (C) Generation of modified particle images for focused classification by subtraction of the 2D projections of the maps in b from raw images. The modified images are CTF corrected. (D) Three rounds of focused 3D classification yielded classes (1,2 and 4) with good density for the AcrB subunit. (E) Particles were combined according to the features of the density map of the AcrB subunit. The map for the full pump assembly was reconstructed without any imposed symmetry using the original particle images from this combined particle subset.