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Supporting information for article:

Structural basis for oligomerization of the prokaryotic peptide transporter PepTSo2

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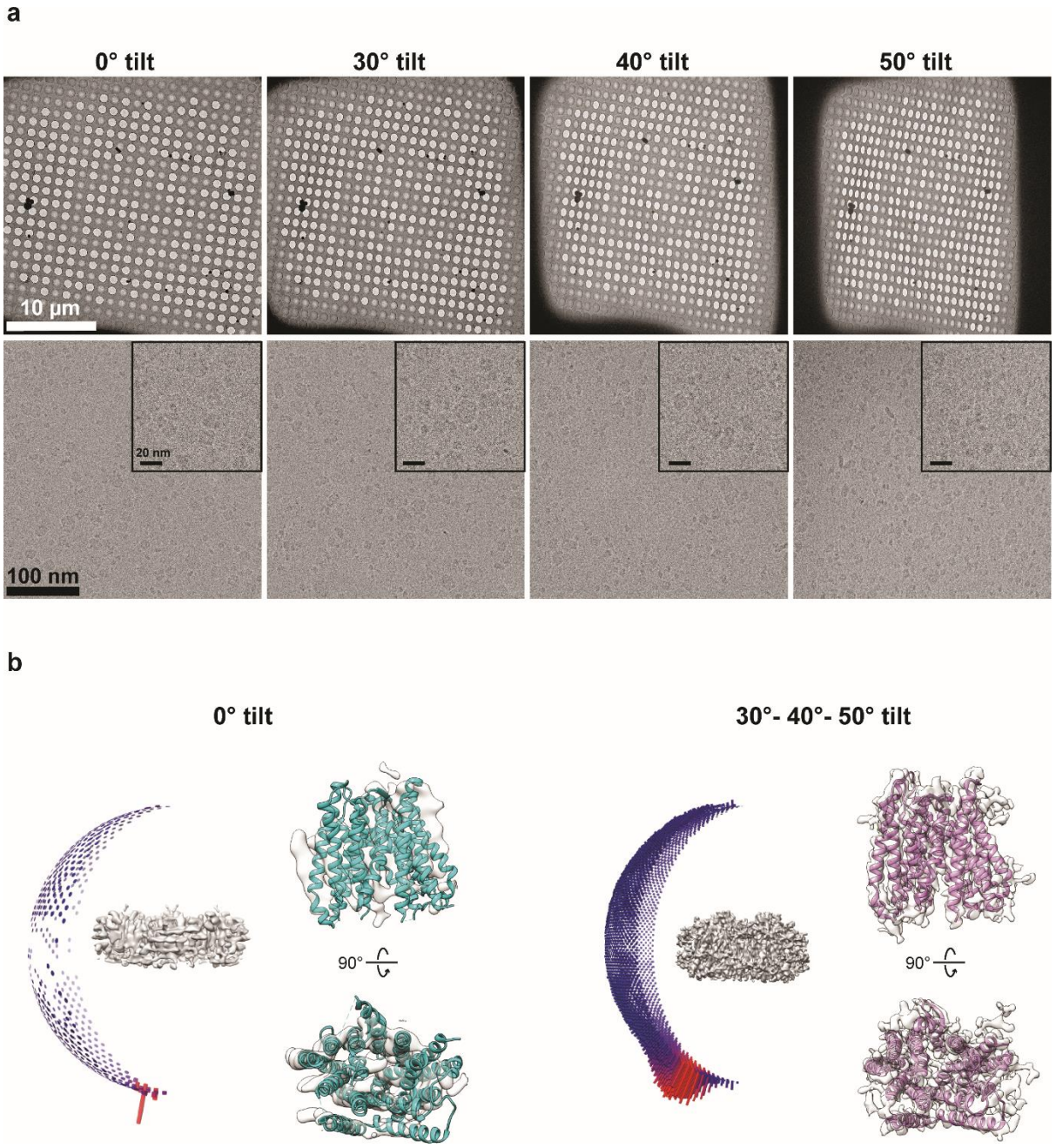

Figure S1 Per-tilt analysis of $PepT_{So2}$. (a) Cryo-EM datasets were collected untilted, or at tilts of 30°, 40°, and 50°. The grid square images of untilted, or 30° tilt, 40° tilt, and 50° tilt are shown in the upper row. Raw EM images of untilted, or 30° tilt, 40° tilt, and 50° tilt are shown in the bottom row. Magnified images of $PepT_{So2}$ are shown in the insets. (b) Euler angle distributions and 3D density maps of the monomeric $PepT_{So2}$ collected by the untilted or tilted method. The crystal structure determined in this study was fitted into each cryo-EM map.

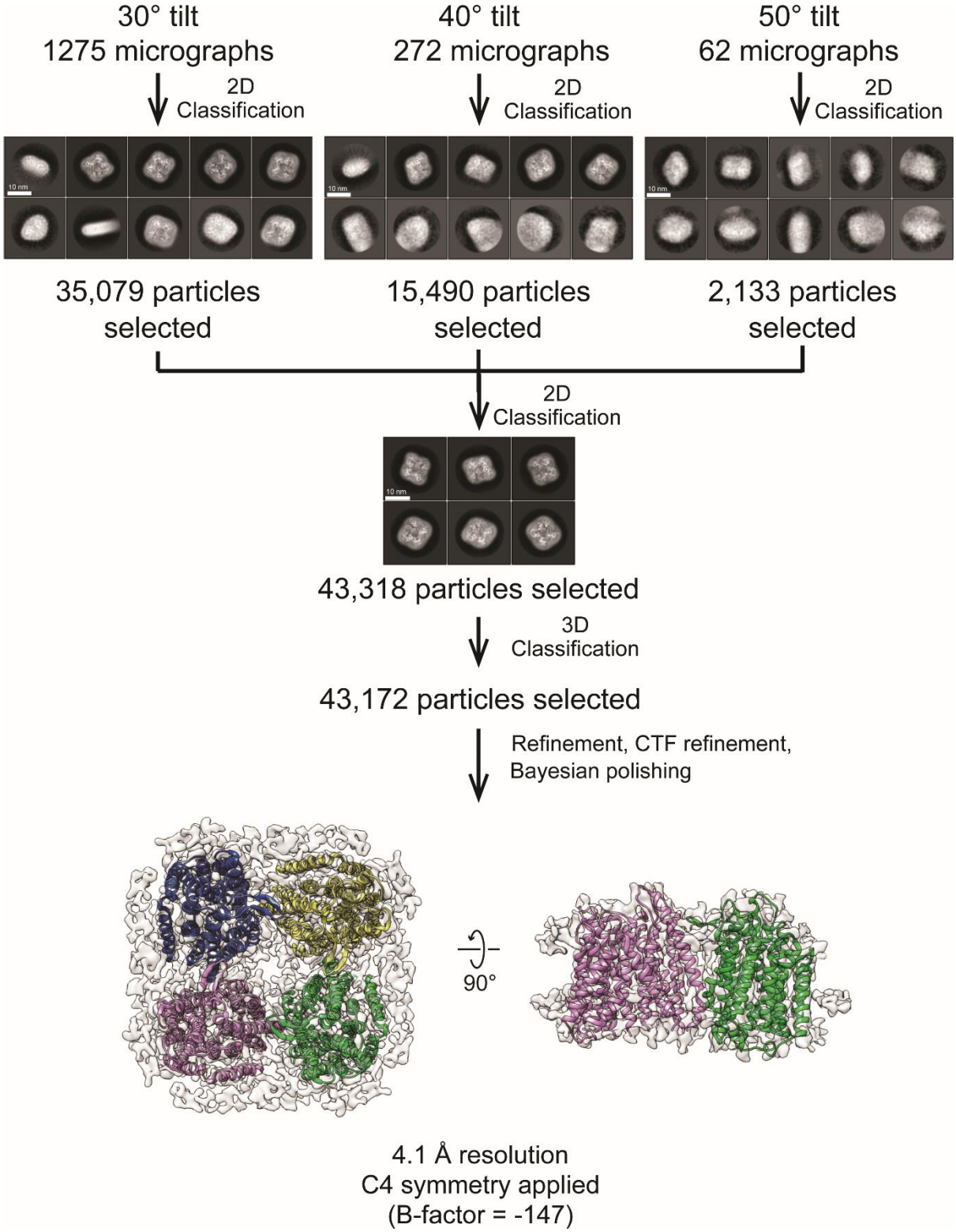
30° tilt
1275 micrographs
2D
Classification
40° tilt
272 micrographs
2D
Classification
50° tilt
62 micrographs
2D
Classification
10 nm
35,079 particles
selected
15,490 particles
selected
2,133 particles
selected
2D
Classification
43,318 particles selected
3D
Classification
43,172 particles selected
Refinement, CTF refinement,
Bayesian polishing
90°
4.1 Å resolution
C4 symmetry applied
(B-factor = -147)

Figure S2 Workflow of cryo-EM image processing. The workflow diagram illustrates the basic strategy practiced in this study. After good particles were selected from the 2D classification at each tilt angle, 43,318 good particles were selected to make the initial 3D model of the $PepT_{So2}$ tetramer and to calculate the 3D classification. Subsequently, 13,644 particles were used for the final 3D auto-refinement, CTF refinement, and Bayesian polishing, yielding a final map at 4.1 Å resolution by gold-standard FSC (Table 4).

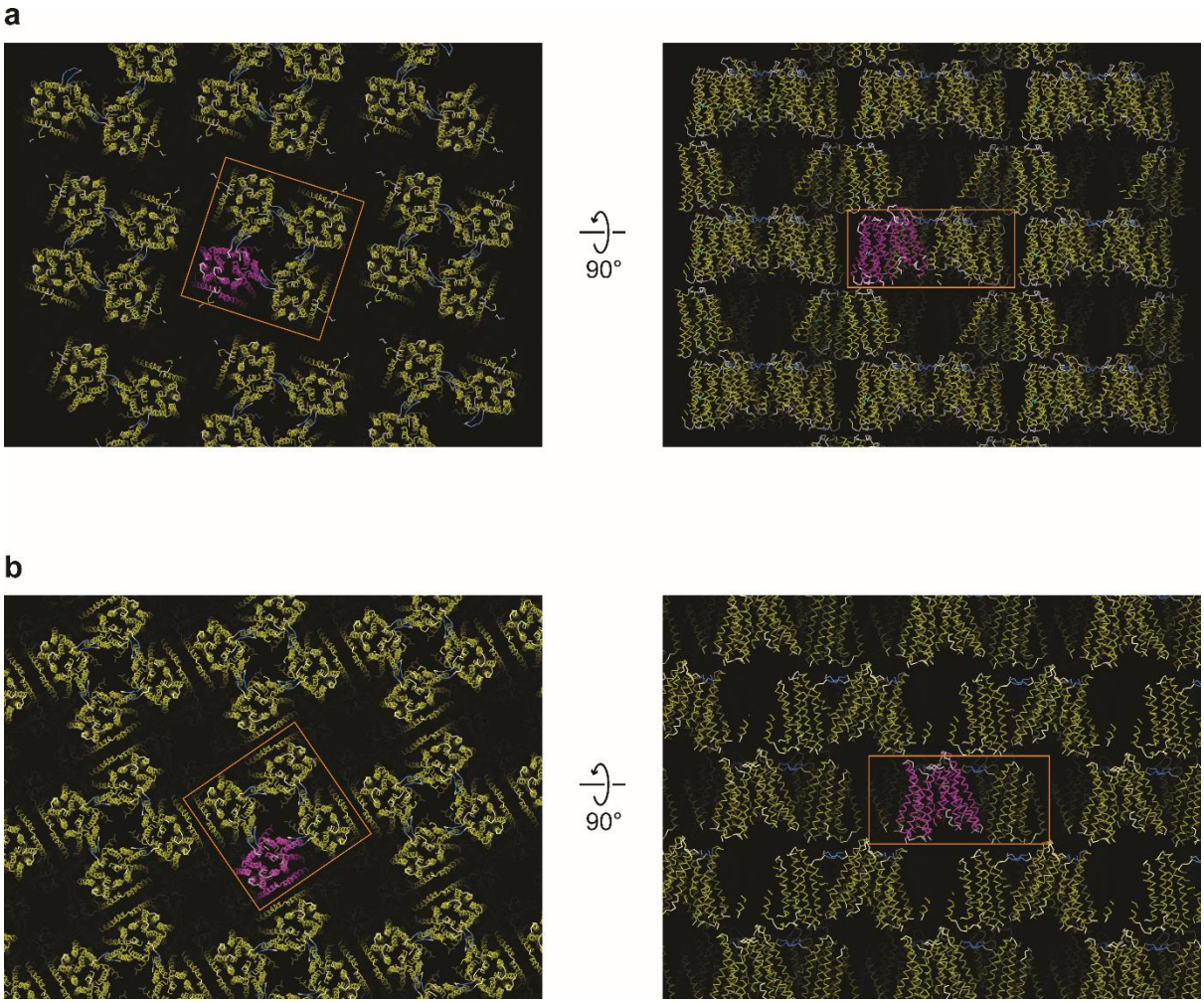

Figure S3 Crystal packings of $PepT_{So2}$ of (a) Form A and (b) Form B, respectively, as viewed from perpendicular to the bilayer and within the lipid bilayer. The $PepT_{So2}$ protomer molecule in the asymmetric unit in each crystal form are colored in pink. The approximate regions of $PepT_{So2}$ tetramer that contains this protomer are indicated in orange rectangles.

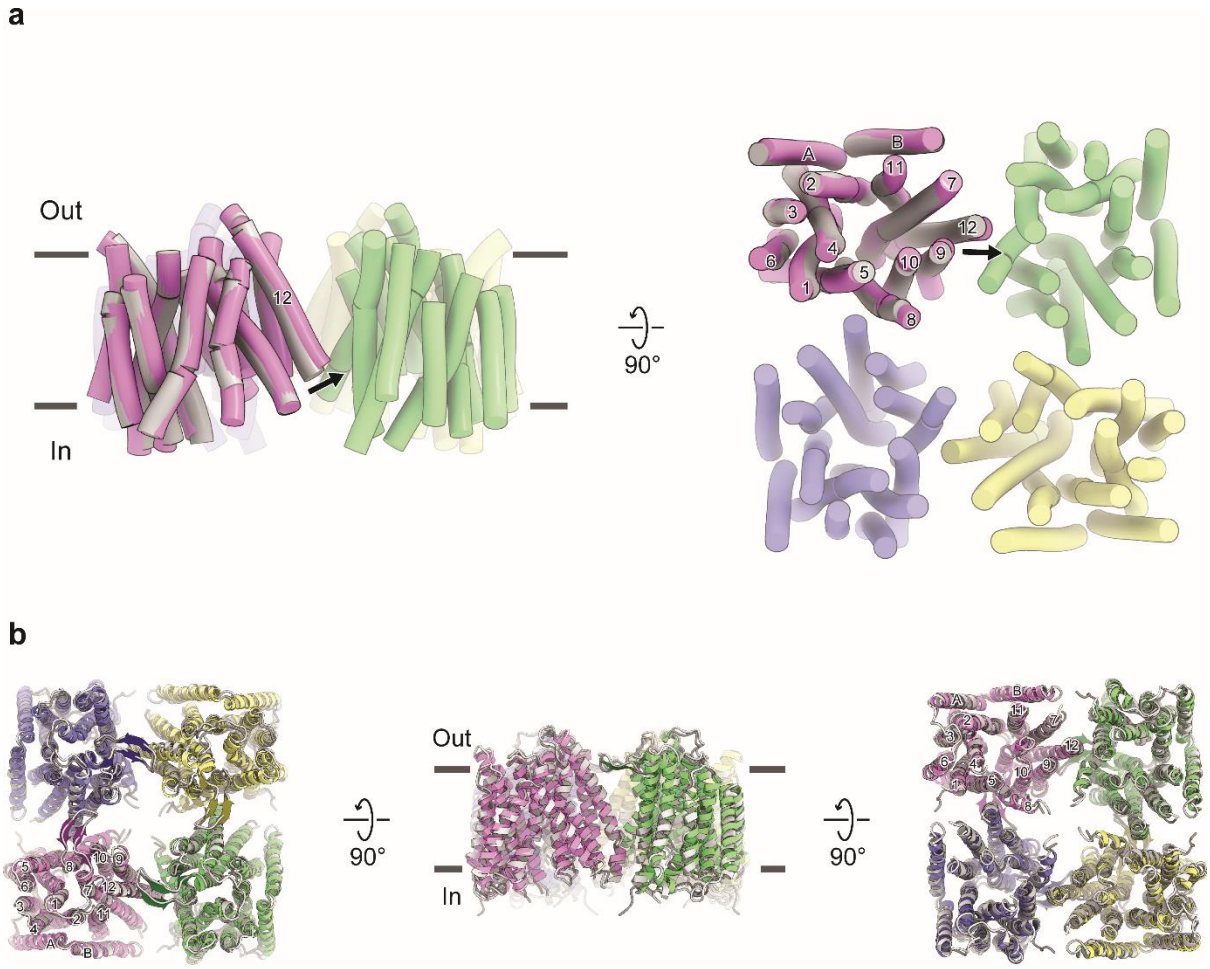

Figure S4 Molecular superposition. (a) Structural comparison of the present tetrameric $PepT_{So2}$ structure (Form B) with the same color scheme as in Fig. 4 and the previously reported dimeric structure colored in grey (PDB accession code: 4LEP). A protomer molecule of the dimeric structure is shown. Sliding motion of H12 is indicated by the black arrow. Two additional helices HA and HB are omitted for clarity in the left panel. The TM helices are shown in cylinder representation and the loop regions between the TM helices are omitted for clarity. (b) Structural comparison of the crystal structure of tetrameric $PepT_{So2}$ (Form B) with the same colour scheme as in Fig. 4 and the cryo-EM atomic model colored in grey.