Expanded View Figures

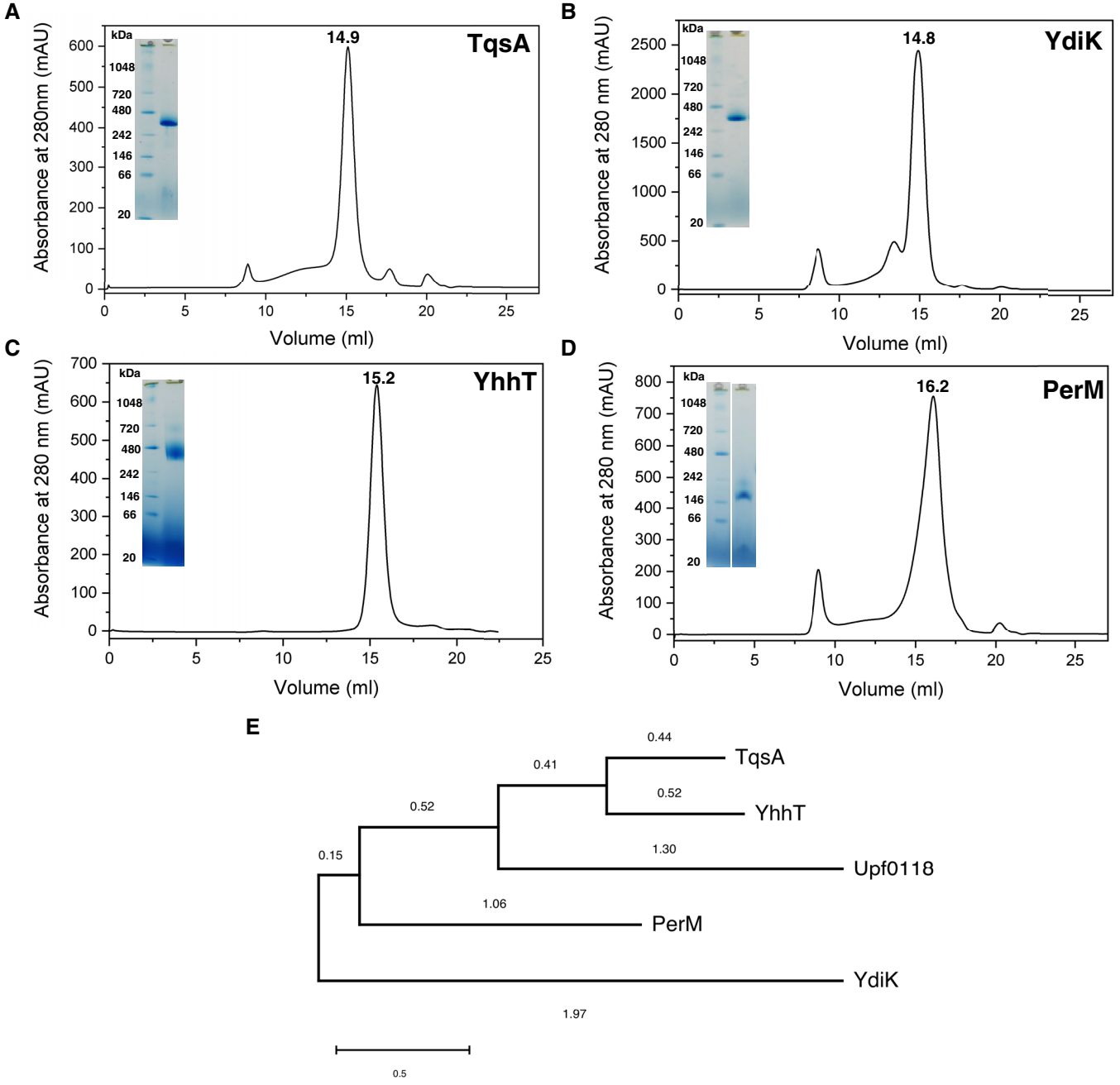

Figure EV1. Purification and phylogenetic analyses of AI-2 exporters from *Escherichia coli*.

A–D Size exclusion chromatography (SEC) and BN–PAGE profiles for the four AI-2 exporters from *E. coli*, TqsA (A), YdiK (B), YhhT (C), and PerM (D). The retention volumes for each of the proteins are included. Superose 6 increase 10/300 GE column was used for the final size exclusion step.

E Phylogenetic analysis of the four AI-2 exporters and Upf0118 family using the maximum-likelihood method. MEGAX was utilized for the analysis and the branch distances are mentioned on the respective branches of the tree.

Figure EV2. Cryo-EM on AI-2 exporters.

A Raw micrographs for the three GDN-purified AI-2 exporters TqsA, YdiK, and YhhT from Titan Krios TEM at 300 kV.
B Reference-free 2D class averages for the proteins (scale bar—10 nm).
C Local resolution EM map for TqsA.
D, E Fourier shell correlation (FSC) curves for the AI-2 exporters TqsA and YdiK. The profiles for masked and unmasked maps are shown in red and black, respectively.
F Local resolution EM map for YdiK.

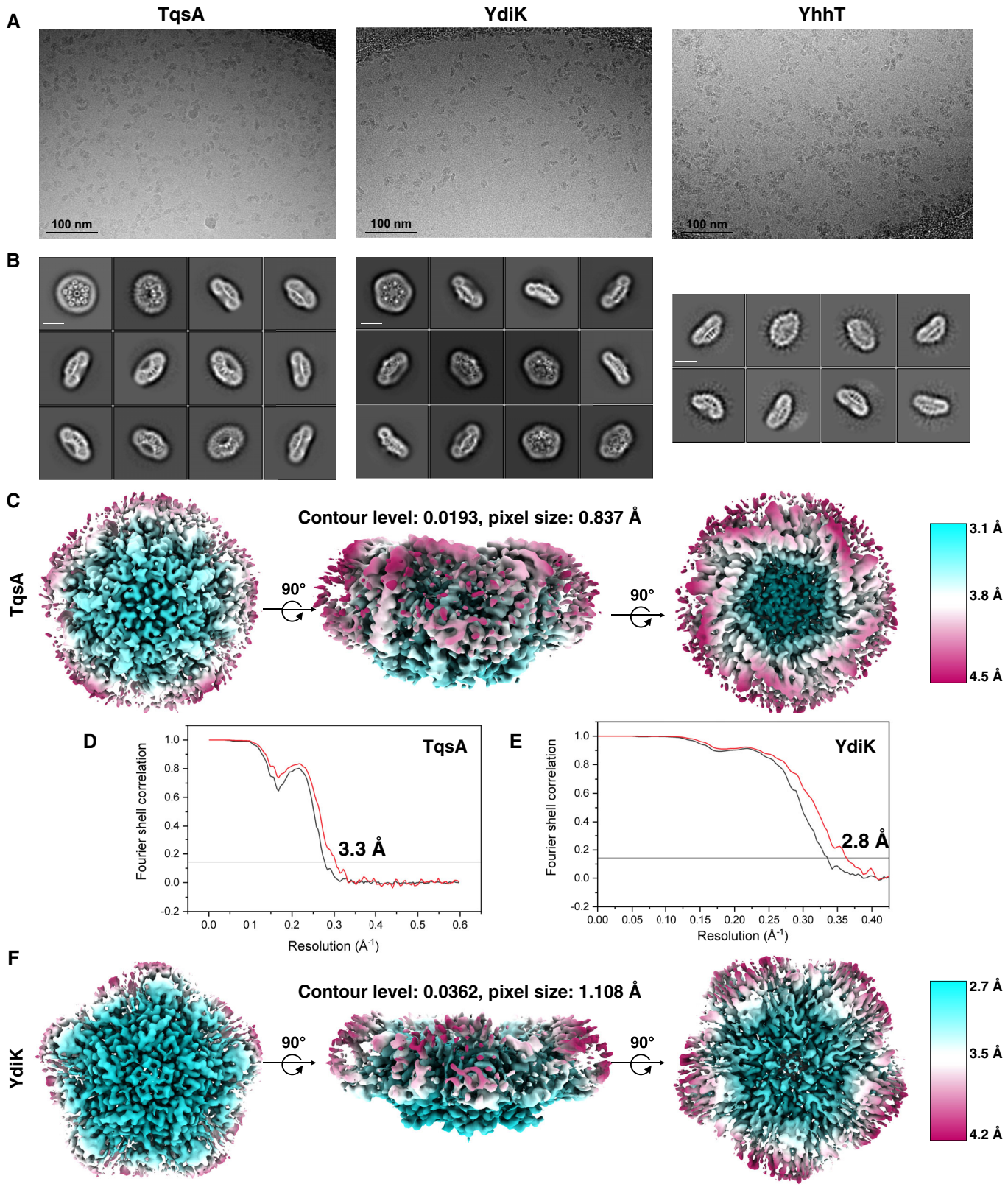

Figure EV2.

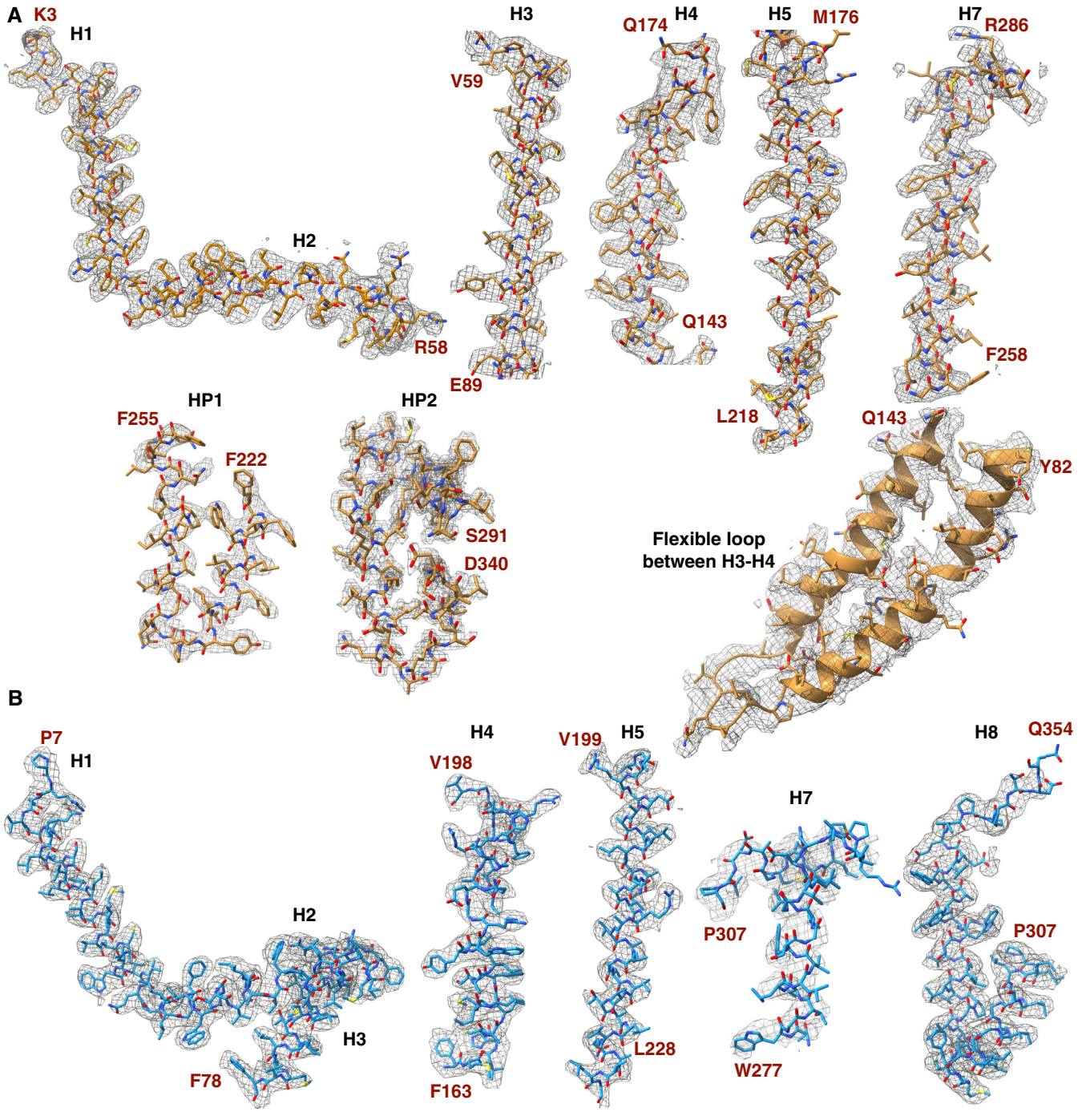

Figure EV3. Model fitting of the AI-2 exporters.

A Model fitting of TqsA in the EM density map for different regions of the monomer structure. The residues constituting a particular structural region are indicated in red.
B Model fitting of YdiK in the EM density map for different regions of the monomer structure is shown with the similar nomenclature as that used for TqsA in A.

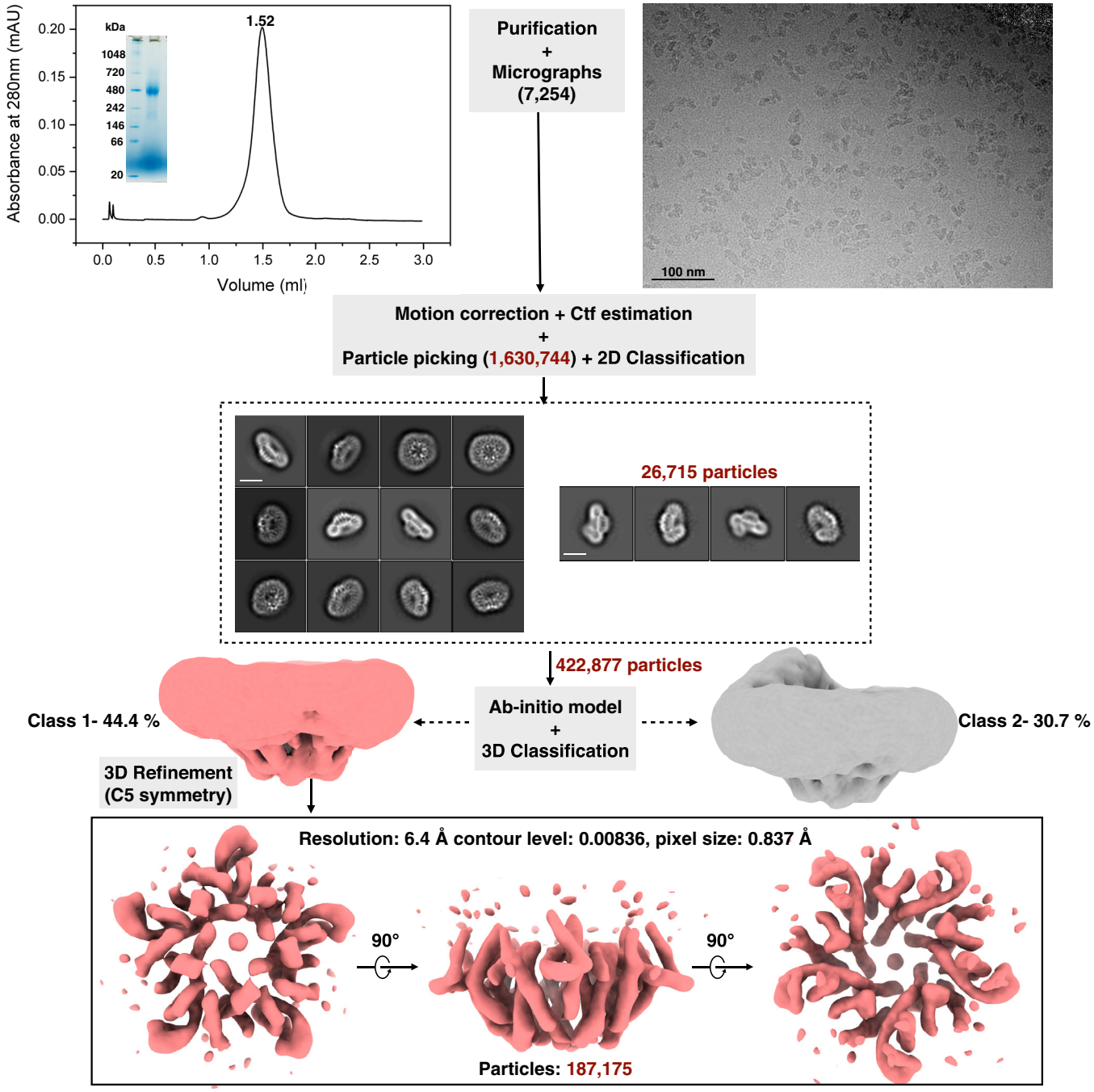

Figure EV4. Cryo-EM studies on Aq_740 from *Aquifex aeolicus*.

A flowchart for the entire workflow adopted for Aq_740 is represented in the figure. Analytical SEC and BN–PAGE profile for Aq_740 are shown in the beginning on the top of the figure. Superose 6 3.2/300 GE column analytical column was used for the final size exclusion step. An illustrative raw micrograph for GDN purified AI-2 exporter Aq_740 from Titan Krios TEM at 300 kV with 0.837 Å pixel size is shown on the right. A total of 7,254 gain-corrected movies were collected. Reference-free 2D class averages for Aq_740 (scale bar—10 nm) were obtained from RELION 3.1 with an initial particle stack of around 1,630,744 particles. Later, two different and almost equally populated 3D classes were obtained as shown. One of them depicted in light coral shade was further refined with imposed C5 symmetry. It yielded a low-resolution 3D reconstruction of 6.40 Å which is shown in different angular views at the bottom of the figure upon anti-clockwise rotation along the X-axis. The final particle stack of the map is around 187,175 particles. The processing was performed using C1 symmetry. C5 symmetry was only imposed on the later refinement stage of Class 1.

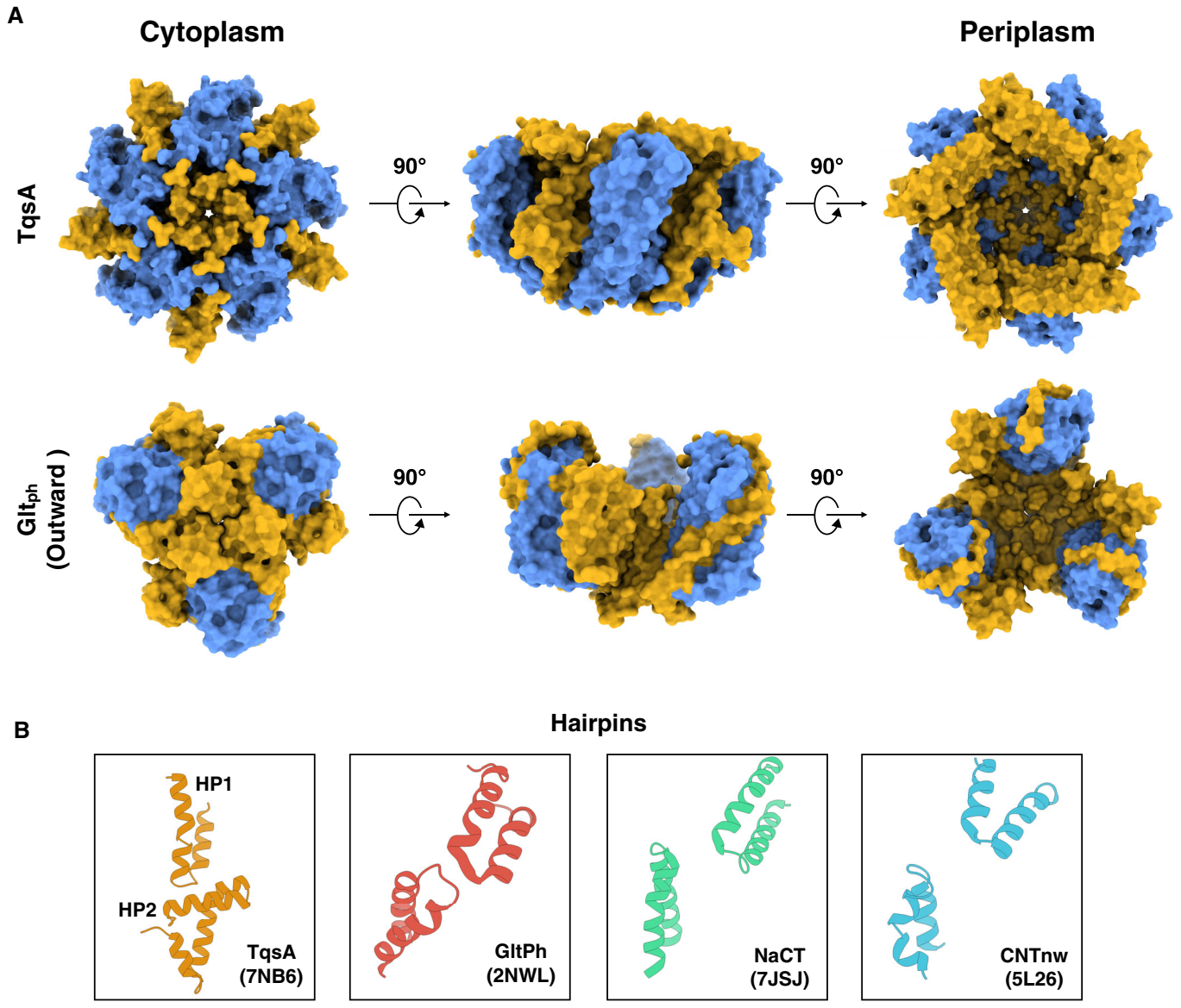

Figure EV5. Comparison between AI-2 exporter and other transporter families adopting elevator mechanism.

A Contrast between AI-2 exporter TqsA (PDB: 7NB6) and glutamate transporter Glt_{ph} (PDB: 2NWL) is shown. The scaffold domain in both the surface representative models is shown in yellow and the transport domain is illustrated in blue.

B The figure focuses on the differences in the helical hairpins in four different transporter families (AI-2 exporter (TqsA); 7NB6, glutamate transporter; 2NWL, sodium citrate transporter; 7JSJ, and nucleoside transporter; 5L26).