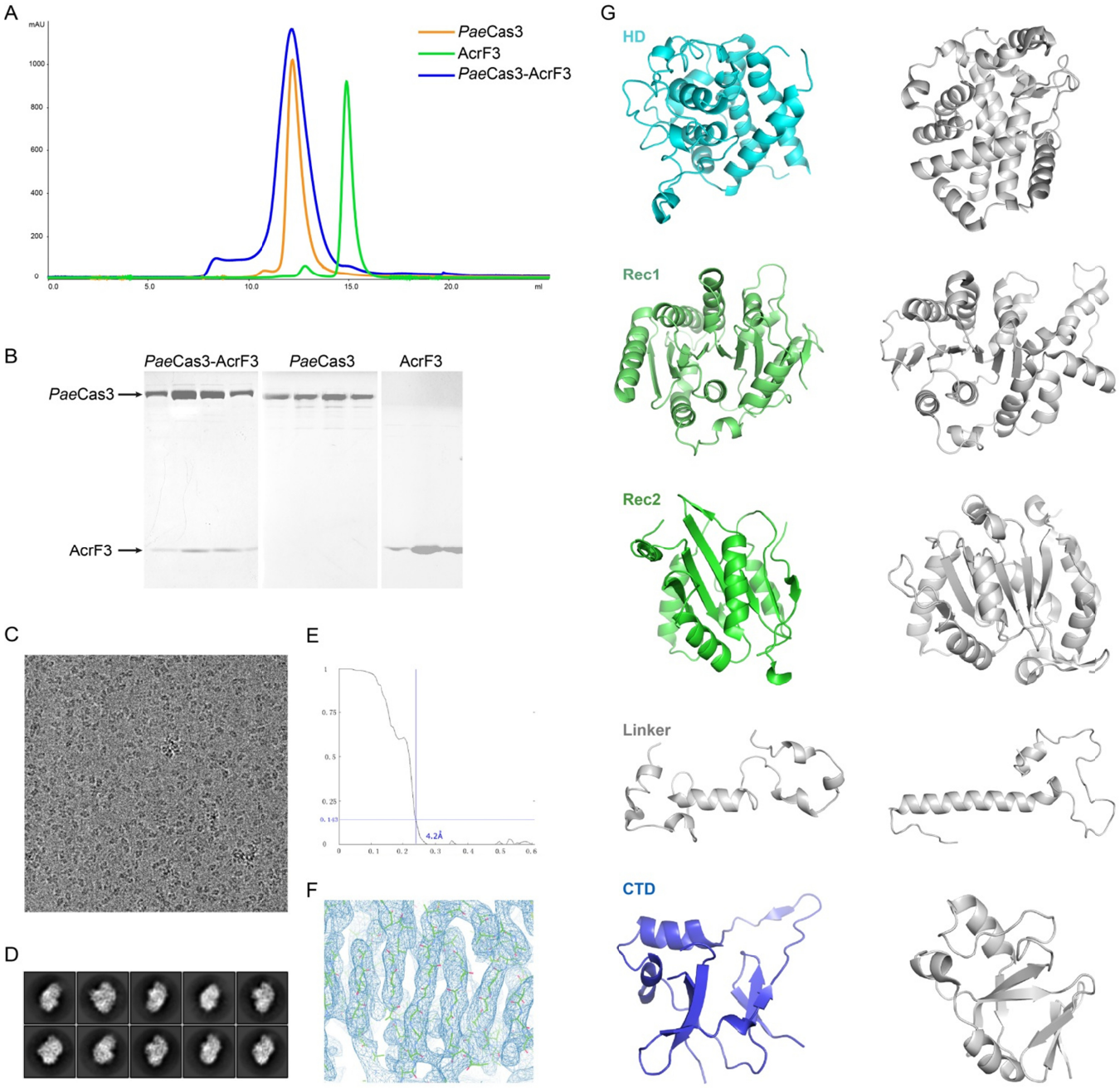


Supplementary information, Figure S1. Cryo-EM structure of *Pae*Cas3-AcrF3 complex.

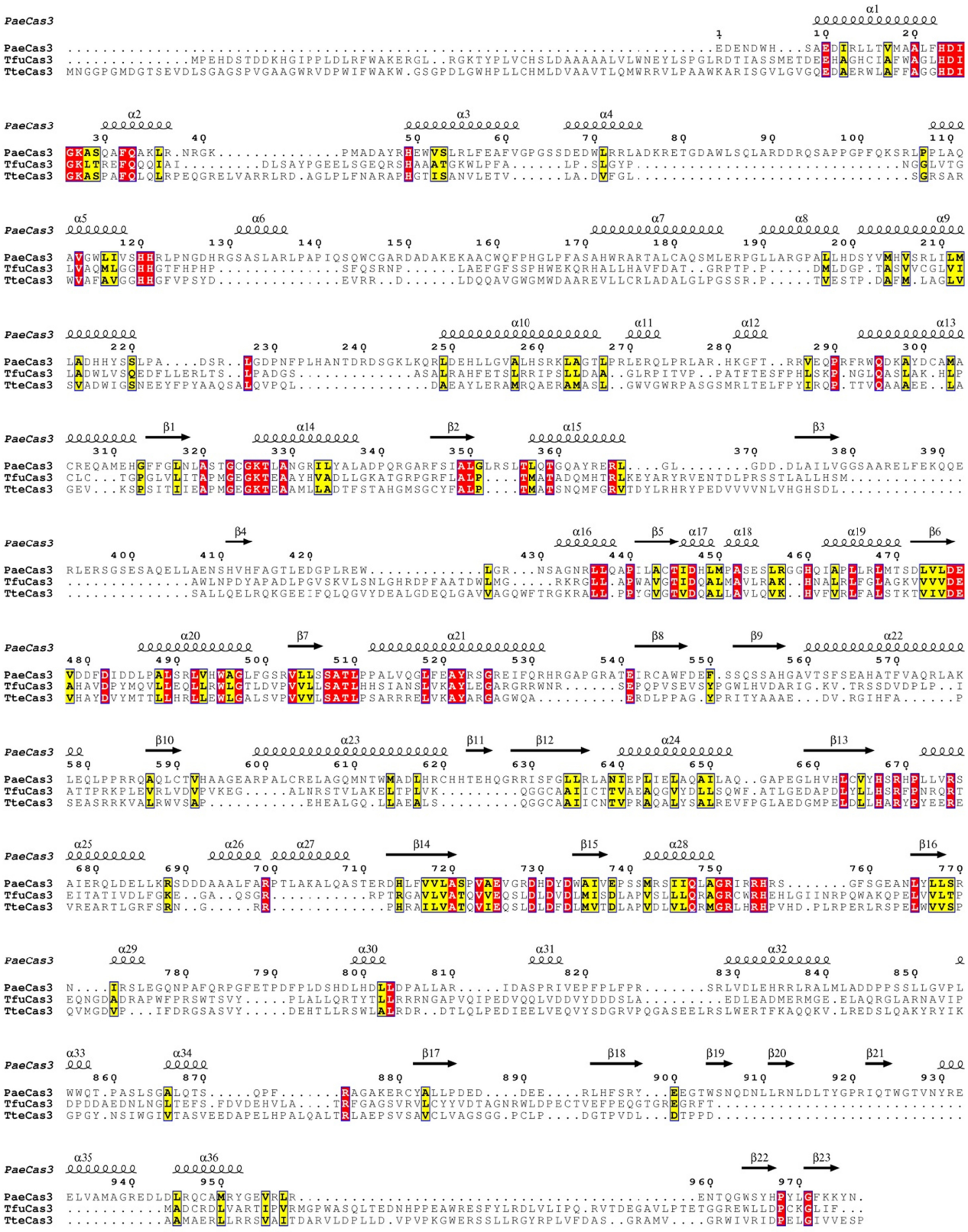
(A)-(B) *Pae*Cas3 and AcrF3 form a complex. (A) *Pae*Cas3 and AcrF3 were fractionated by Superdex 200 increase (GE healthcare) separately and together. Optical density of 280 nm UV was detected and shown in Y-axis. (B) Fractions from SEC were analyzed by SDS-PAGE. Gels were stained with coomassie brilliant blue.

(C)-(F) Cryo-EM single particle analysis of *Pae*Cas3-AcrF3 complex. (C) A representative

image of *Pae*Cas3-AcrF3 complex, recorded using the K2 Summit camera. The image shown was motion-corrected. (D) Typical 2D reference-free class averages showing characteristic particle projection views. (E) Gold standard Fourier shell correlation (FSC) curves of the final map. The resolution is 4.20 Å at FSC=0.143. (F) Typical electron density map of some of the β-sheets are shown overlaid with the corresponding model.

(G) Pairwise structural comparison between each of the five domains of *Pae*Cas3 and *Tte*Cas3.

H

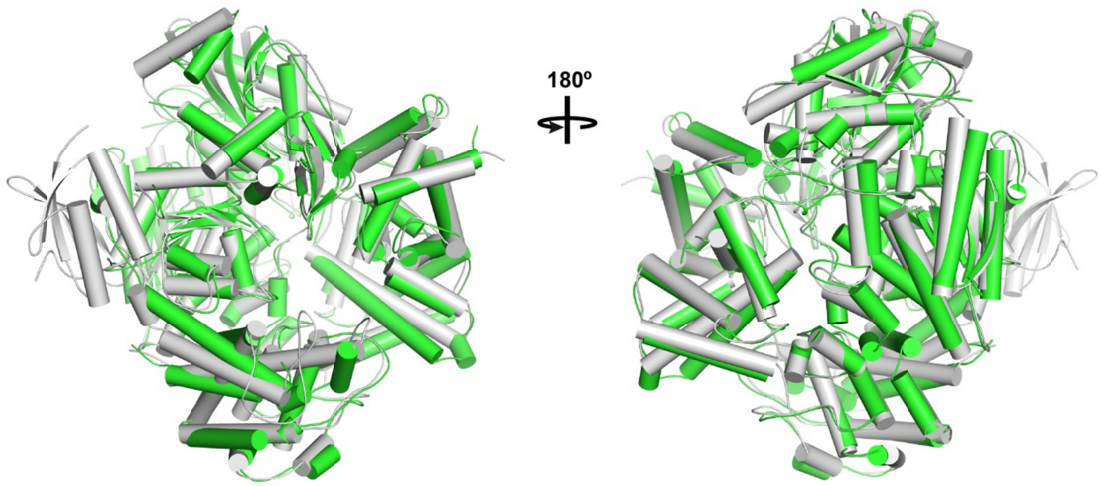


Supplementary information, Figure S1. Cryo-EM structure of *Pae*Cas3-AcrF3 complex.

(H) Sequence Alignment of Cas3 Proteins. The *Pae*Cas3 without 1-100 amino acid residues was aligned with its homologs. The aligned sequences (Genebank ID, designated GI and PDB

ID) are in the order of *Pae*Cas3 (*Pseudomonas aeruginosa* Cas3, GI: 122259486), *Tfu*Cas3 (*Thermobifida fusca* Cas3, GI: 499611287 PDB ID: 4QQW) and *Tte*Cas3 (*Thermobaculum terrenum* Cas3, GI: 502639365, PDB ID: 4Q2C). Secondary structure diagram for *Pae*Cas3 is shown on the top. Conserved residues are shaded in red, whereas essentially invariant residues are shown in yellow. The regions of *Pae*Cas3 interacting with AcrF3 are highlighted by blue boxes.

I



Supplementary information, Figure S1. Cryo-EM structure of *Pae*Cas3-AcrF3 complex.

(I) Comparison of cryo-EM model with the crystal structure of PDB: 5B7I. Superposition of the model based on cryo-EM density map (green) with the crystal structure of PDB: 5B7I (gray). The r.m.s.d. value is 2.4 Å.