Supplementary information, Data S1 Materials and Methods

Protein expression and purification

The full-length *Pae*Cas3 and AcrF3 genes were synthesized from Sangon Biotech and cloned into pRSFduet vector (Novagen) containing a His_6 tag at N-terminal of *Pae*Cas3. The *Pae*Cas3-AcrF3 protein complex was overexpressed in *E. coli* Rosetta (DE3) (Novagen) cells that were induced with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) at OD_{600}=0.6 at 16 ˚C for 12 hours. Cells were collected and lysed by Sonication in buffer A (20 mM Tris-HCl, pH 7.5, 150 mM NaCl). After centrifugation, supernatant was incubated with Ni Sepharose (GE healthcare). The bound protein was eluted with buffer A containing 100 mM imidazole and was further purified by Heparin column (GE healthcare) in buffer A, and eluted with buffer B (20 mM Tris-HCl, pH 7.5, 1 M NaCl). Eluted protein complex was collected and concentrated to 1 mg/ml.

The AcrF3 gene was cloned into a modified expression vector pET-28a with an N-terminal His_6-Sumo tag followed by a ubiquitin-like protein 1 (Ulp1) protease cleavage site. The AcrF3 protein was overexpressed at 18 ℃ in *E. coli* Rosetta (DE3) and purified by chromatography on Ni Sepharose (GE healthcare). The AcrF3 protein was eluted with buffer C (20 mM Tris-HCl, pH 7.5, 0.5 M NaCl) containing 200 mM imidazole and digested with Ulp1 protease to remove the His_6-Sumo tag. The protein was then passed through the Ni Sepharose column and was further purified on a HiLoad Superdex 75 16/60 column (GE healthcare) in buffer D (20 mM Tris-HCl, pH 7.5, 150 mM NaCl). AcrF3 was concentrated to 37 mg/ml and was used for crystallization. The seleno-methionine (SeMet) substituted AcrF3 was purified using an identical protocol.

Crystallization, data collection and structure determination

Crystals were obtained by mixing 1 μl protein solution and 1 μl of reservoir solution. Crystals of AcrF3 were grown from 100 mM MES/Sodium hydroxide, pH 6.0, 200 mM Calcium acetate, 20% (w/v) PEG 8000. The SeMet crystals were grown in the same condition. All crystals were cryo-protected using corresponding reservoir buffers supplemented with 10% (v/v) glycerol and flash frozen in liquid nitrogen.

All diffraction data sets were collected at beamline BL-19U1 at Shanghai Synchrotron Radiation Facility (SSRF), and processed with HKL2000[1]. The phases of AcrF3 were solved by Se single wavelength anomalous dispersion method using PHENIX Autosol[2]. The model was manually built and adjusted using the program Coot[3]. The structure refinement was performed using PHENIX. All statistics of data processing and structure refinement of AcrF3 are summarized in Table S1. The structure figures were prepared using PyMOL (http://www.pymol.org/).

Cryo-electron microscopy

Approximately 3.0 μl *Pae*Cas3-AcrF3 protein with a concentration of 1 mg/ml was placed on glow-discharged holy carbon grid (GIG). After removing the extra aliquots on the grid by 2 s blotting with filter paper, the grid was flash plunged into liquid ethane using the Leica EMGP device at 99% humidity, 16 ℃. The single particle data was collected by Gatan K2 camera using super resolution mode on a 300-kV FEI Titan Krios electron microscope. A total of 680 micrographs were imaged at a calibrated magnification of 60976 yielding a physical pixel size

of 0.82 Å on the detector with a dose rate of ~ 8 $e^-/s/Å^2$ and defocus range between 1 μm to 3 μm. The total dose of about 64 $e^-/ Å^2$ was fractionated evenly into 32 frames during the 8 s exposure.

Data processing

In each micrograph, the electron induced movement[4] and anisotropic magnification distortion[5] were corrected before calculating the contrast transfer function parameters[6]. About 4000 particles in the first 10 micrographs were automatically picked by e2boxer.py software in EMAN2[7]. The averaged images produced by the two dimensional (2D) classification of this 4000-particle data set were used as references for automatic particle picking of all micrographs by RELION program[8], yielding an intact data set with about 400,000 particles. This data set was reference-free 2D classified and good 2D classes were kept for further data processing. The atomic model of the homolog of Cas3 (PDB: 4Q2C) was used to calculate a density map. In order to eliminate the model bios, this density map was low-pass filtered to 60 Å resolution and was used as an initial model for three dimensional (3D) classification. Among the all four 3D classes, two of them converged to a similar reconstruction with extra densities that might belong to AcrF3. The particles belonging to these two classes were kept for further high resolution refinement in RELION which results in a map with an overall gold-standard resolution of 4.4 Å. Further particle based motion correction and particle polishing led a map of 4.2 Å resolution.

Model building

The predicted model of *Pae*Cas3 from the Phyre2 web server[9] was fitted into the electron density map by each single domain using UCSF Chimera[10]. All side chains were cut off after fitting and the backbones were flexibly refined in real space under tight stereochemical and secondary structural restraints in COOT. Some side-chain densities for bulky amino-acid residues were good for us to trace most of the amino-acid residues. The model of AcrF3 was generated by fitting its crystal structure into the electron density map. Model analysis and model were visualized in COOT and Chimera. Figures were made in Chimera and PyMOL.

References

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