

Supplementary Information Appendix for

Inhibition Mechanisms of AcrF9, AcrF8, and AcrF6 Against Type I-F CRISPR-Cas Complex Revealed by Cryo-EM

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Fig. S1. Cryo-EM raw data and 2D analysis. (*A*) Csy-AcrF9 complex. Left: representative motioncorrected cryo-EM micrograph; middle: representative Fourier transform with Thon rings extending to 1.98 Å calculated by CTFFIND4; right: reference-free 2D class averages. (*B*) Csy-AcrF8 complex. Left: representative motion-corrected cryo-EM micrograph; middle: representative Fourier transform with Thon rings extending to 2.31 Å calculated by CTFFIND4; right: reference-free 2D class averages. (*C*) Csy-AcrF6 complex. Left: representative motioncorrected cryo-EM micrograph; middle: representative Fourier transform with Thon rings extending to 2.87 Å calculated by CTFFIND4; right: reference-free 2D class averages.



Fig. S2. Single-particle cryo-EM analysis of the Csy-AcrF9 complex. (*A*) Workflow of cryo-EM data processing. (*B*) Euler angle distribution of all particles used for calculating the final 3D reconstruction. (*C*) Resolution map for the final 3D reconstruction. (*D*) Gold standard FSC plot for the final 3D reconstruction.



Fig. S3. Single-particle cryo-EM analysis of the Csy-AcrF8 complex. (*A*) Workflow of cryo-EM data processing. (*B*) Euler angle distribution of all particles used for calculating the final 3D reconstruction. (*C*) Resolution map for the final 3D reconstruction. (*D*) Gold standard FSC plot for the final 3D reconstruction.



Fig. S4. Single-particle cryo-EM analysis of the Csy-AcrF6 complex. (*A*) Workflow of cryo-EM data processing. (*B*) Euler angle distribution of all particles used for calculating the final 3D reconstruction. (*C*) Resolution map for the final 3D reconstruction. (*D*) Gold standard FSC plot for the final 3D reconstruction.



Fig. S5. Resolvability of the cryo-EM structures of Csy-AcrF9 (A), Csy-AcrF8 (B), and Csy-AcrF6 (C) complexes calculated by Q-score. Q-score for each chain in models and maps of the three complexes; the black line represents the expected Q-score at respective resolution based on the correlation between Q-scores and map resolution of proteins and RNAs. The higher Q-score indicates better resolvability.



Fig. S6. Resolvability of the cryo-EM structures of AcrF9 (A, B), AcrF8 (C), and AcrF6 (D) calculated by Q-score. The model is shown as ribbon, with Q-scores colored. The higher Q-score indicates better resolvability. A sample density is shown on the right side.



Fig. S7. Interfaces of Cas7f-Cas7f and Cas7f-crRNA. (*A*, *B*) Resolvability of the cryo-EM structures of Cas7.4f (*A*) and Cas7.5f (*B*) calculated by Q-score. The model is shown as ribbon, with Q-scores colored. The higher Q-score indicates better resolvability. A sample density is shown on the right side. (*C*) Superimposition of six Cas7f subunits, with the RMSD calculated. (*D*) The interface between Cas7.4f and Cas7.5f. The residues involved in the interface are listed in Table S2. (*E*) Hydrogen bonds between Cas7.5f and crRNA are shown in black dash lines. (*F*) The direct and water-mediated interactions between Cas7f proteins and crRNA. Q-scores of the waters between the map and model are calculated and shown in magenta.

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Fig. S8. Sequence alignment of Cas7f proteins from different species. *P. aeruginosa* used in our study is shown within red box. The residues are colored by % identity. Residues of Cas7f involved in crRNA binding are shown at bottom.



Fig. S9. Interaction analysis of the crRNA 5' handle and Cas5f-Cas8f. (*A*) Sequence of crRNA. (*B*) The interface between the crRNA 5' handle and Cas5f-Cas8f, where Cas5f and Cas8f are shown in surface representation. (*C*) Hydrogen bonds between Cas5f-Cas8f and crRNA ($+2\rightarrow+7$) are shown in black dash lines.



Fig. S10. Interface between AcrF9.2 and Cas7.6f. The residues involved in the interface are shown.



Fig. S11. Conformational change induced by the binding of AcrF9.1 (A) and superimposition of AcrF9 with Cas2/3 (B).



Fig. S12. Ribbon representation of different Acr proteins.

	Csy-AcrF9	Csy-AcrF8	Csy-AcrF6					
Data collection and processing								
Microscope	Titan Krios	Titan Krios	Titan Krios					
Voltage (kV)	300	300	300					
Camera	Gatan K2 Summit	Gatan K2 Summit	Gatan K2 Summit					
Pixel size (Å)	0.65	1.06	1.06					
Total Dose (e-/Å ²)	56	42	42					
Exposure time (s)	6	6	6					
Number of frames per	30	30	30					
exposure								
Defocus range during data	-0.62.7	-0.63.6	-0.92.4					
collection (µm)								
Number of micrographs	9,267	6,338	1,956					
Number of initial particles	875,560	445,854	327,591					
Number of particles for 3D	595,828	288,565	301,900					
analyses								
Symmetry	C1	C1	C1					
Number of final particles	332,404	91,080	56,455					
Defocus range after data	-0.33.1	-0.53.7	-1.12.5					
processing (µm)								
Resolution (0.143 FSC, Å)	2.57	3.42	3.15					
Atomic model refinement								
Software	phenix.real space	phenix.real space	phenix.real space					
	refine	refine	refine					
Clashscore, all atoms	5.11	7.83	6.61					
Protein geometry	·		·					
Poor rotamers (%)	1.16	0.57	0.04					
Favored rotamers (%)	95.45	84.51	98.07					
Ramachandran outliers	0.07	0	0.03					
(%)								
Ramachandran favored	91.31	86.93	91.24					
(%)								
MolProbity score	1.83	2.05	1.88					
Bad bonds (%)	0.06	0.01	0					
Bad angles (%)	0.11	0.01	0.04					
Nucleic acid geometry								
Probably wrong sugar	1.67	0	0					
puckers (%)								
Bad bonds (%)	0	0	0					
Bad angles (%)	0	0	0					

Table S1. Cryo-EM data collection, processing, and model validation

Hydrogen bonds	Cas7.4f		Č I	Cas7.5f		
	Atom	Res	Res no.	Atom	Res	Res no.
	NH2	ARG	34	OE1	GLU	242
	OD2	ASP	40	NH2	ARG	63
	NH1	ARG	112	0	SER	104
	0	THR	114	NE2	GLN	241
	OG1	THR	114	0	ASP	239
	OG1	THR	114	NE2	GLN	241
	NH1	ARG	116	0	GLY	172
	Ν	CYS	127	0	SER	308
	OE1	GLU	248	OG	SER	66
	OE2	GLU	248	Ν	SER	66
	Ν	LEU	249	OG	SER	66
	OH	TYR	265	NH1	ARG	63
	OE1	GLN	276	NZ	LYS	65
	NE2	GLN	276	0	SER	66
	NZ	LYS	317	OD1	ASP	81
	OH	TYR	323	0	SER	72
Salt bridges	OE2	GLU	33	NE	ARG	168
	NH2	ARG	34	OE1	GLU	242
	OD2	ASP	40	NH2	ARG	63
	NH2	ARG	112	OD2	ASP	239
	NZ	LYS	317	OD1	ASP	81
	OD2	ASP	327	NH1	ARG	74
	OE2	GLU	356	NE	ARG	74

Table S2. The residues involved in the formation of hydrogen bonds and salt bridges between Cas7.4f and Cas7.5f subunits of the Csy complex



Movie S1. Overall structure (map and model) of the Type I-F CRISPR-Csy complex bound with two copies of AcrF9.