

Table S1 Top 10 entries of Dali search results of AcrIF13

No.	PDB Chain	Z score	RMSD (Å)	Aligned residues	Molecular name
1	6n2g-A	5.1	4.5	58	Nucleosome assembly protein
2	5wlz-C	5.0	6.7	61	DNA repair protein xrcc4,myosin-7
3	2n19-A	4.6	3.6	61	Serine/threonine-protein kinase
4	5d0q-C	4.6	4.5	60	Outer membrane protein assembly
5	6w3w-A	4.2	1.8	44	Denovo ntf2
6	6mf5-B	4.2	6.9	71	Cell cycle serine/threonine- protein
7	4nkb-B	4.1	3.3	71	Probable serine/threonine- protein
8	4i0o-A	4.1	8.9	55	Protein elys
9	lp49-A	4.0	4.4	65	Steryl-sulfatase
10	6dhx-A	3.9	3.6	60	Tipc2

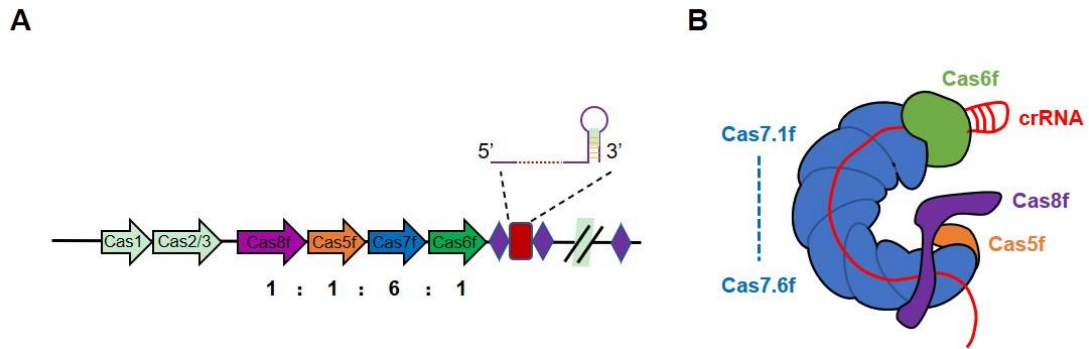


Figure S1 Schematic representation of the type I-F CRISPR-Cas system

(A) Schematic representation of the type I-F CRISPR-Cas locus. The type I-F CRISPR-Cas system contains six *cas* genes, four of which encode various Cas proteins that constitute the whole Csy complex with crRNA. The CRISPR array is comprised of invariable repeats (purple diamonds) interspaced with variable phage-derived spacer sequences (red rectangle).

(B) Schematic of 3D structure of Csy complex.

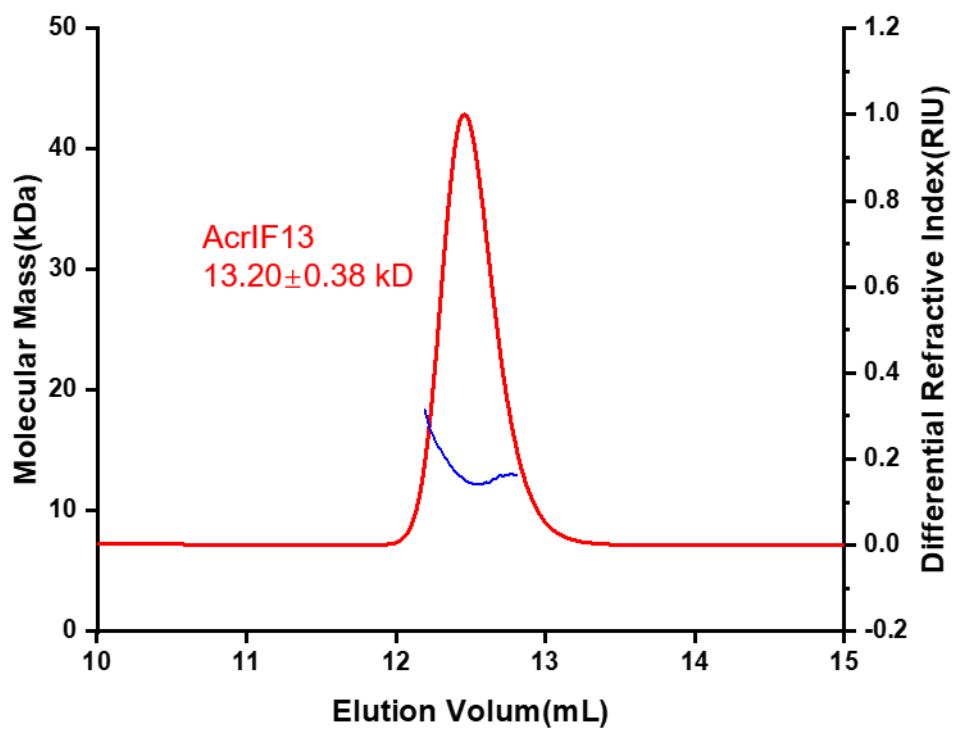


Figure S2 AcrIF13 is a monomer in solution

Static light scattering (SLS) study of AcrIF13. The calculated molecular weight of the main peak is shown.

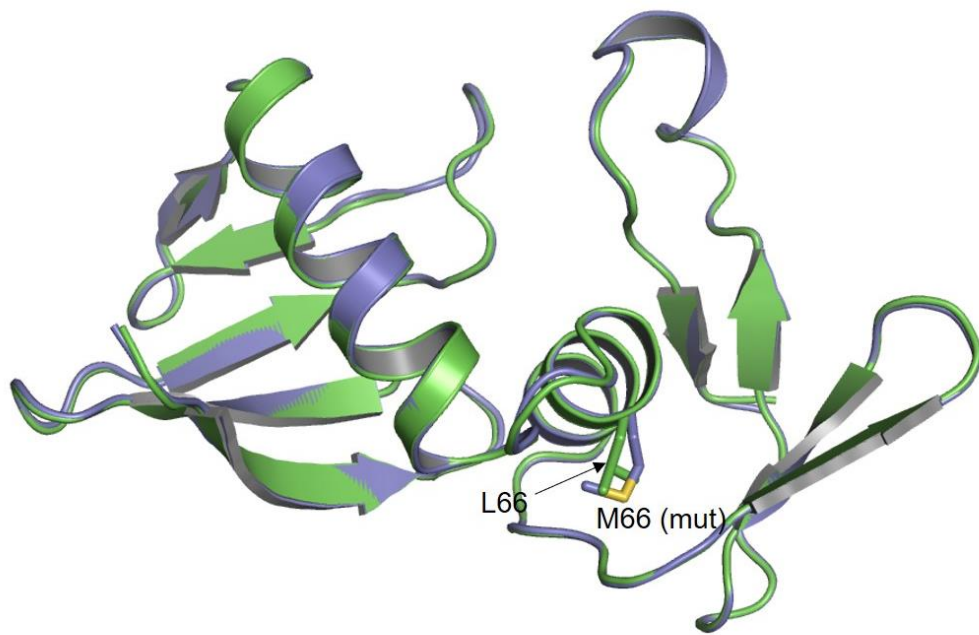


Figure S3 AcrIF13 is structurally similar as AcrIF13 L66M mutant

Structural superimposition between AcrIF13 (green) and its L66M mutant (light blue). L66 in AcrIF13 and mutated M66 in AcrIF13 mutant are shown as sticks.

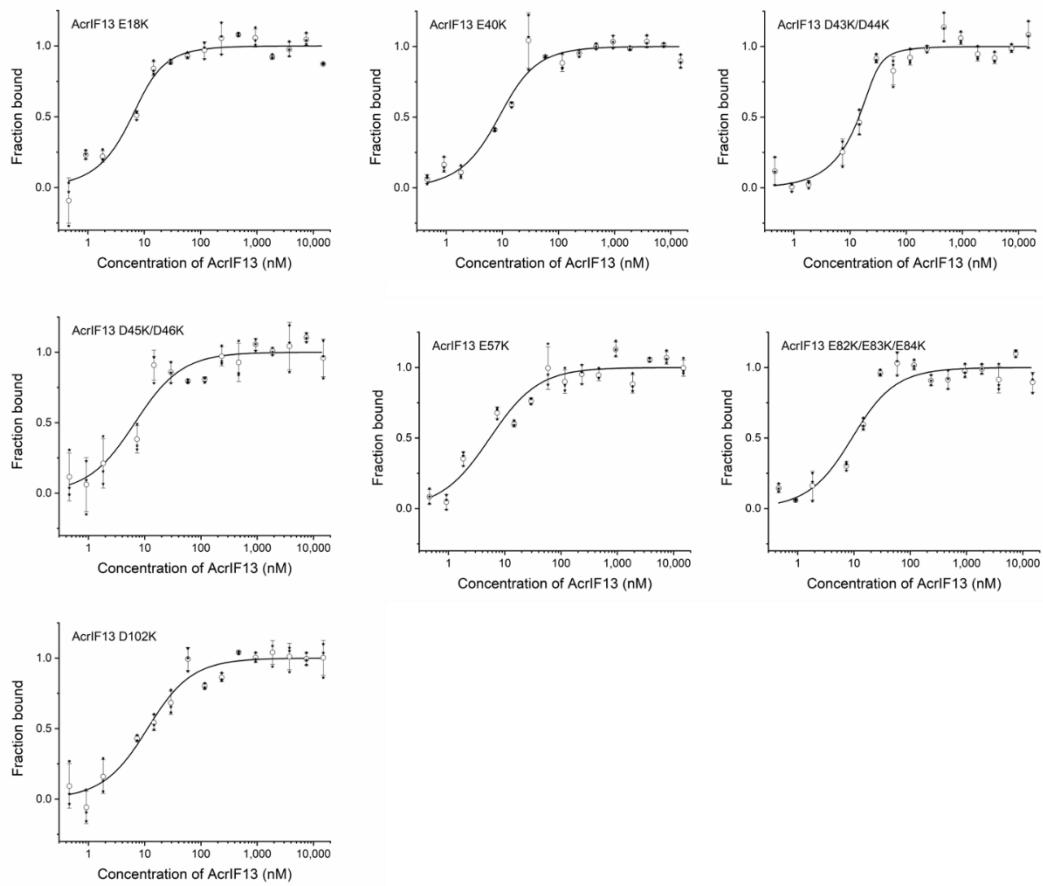


Figure S4 MST assay of the binding between Csy and AcrIF13 mutants

MST assays of the binding of Csy to AcrIF13 mutants. Error bars represent SD; n = 3. Binding curves are also shown.

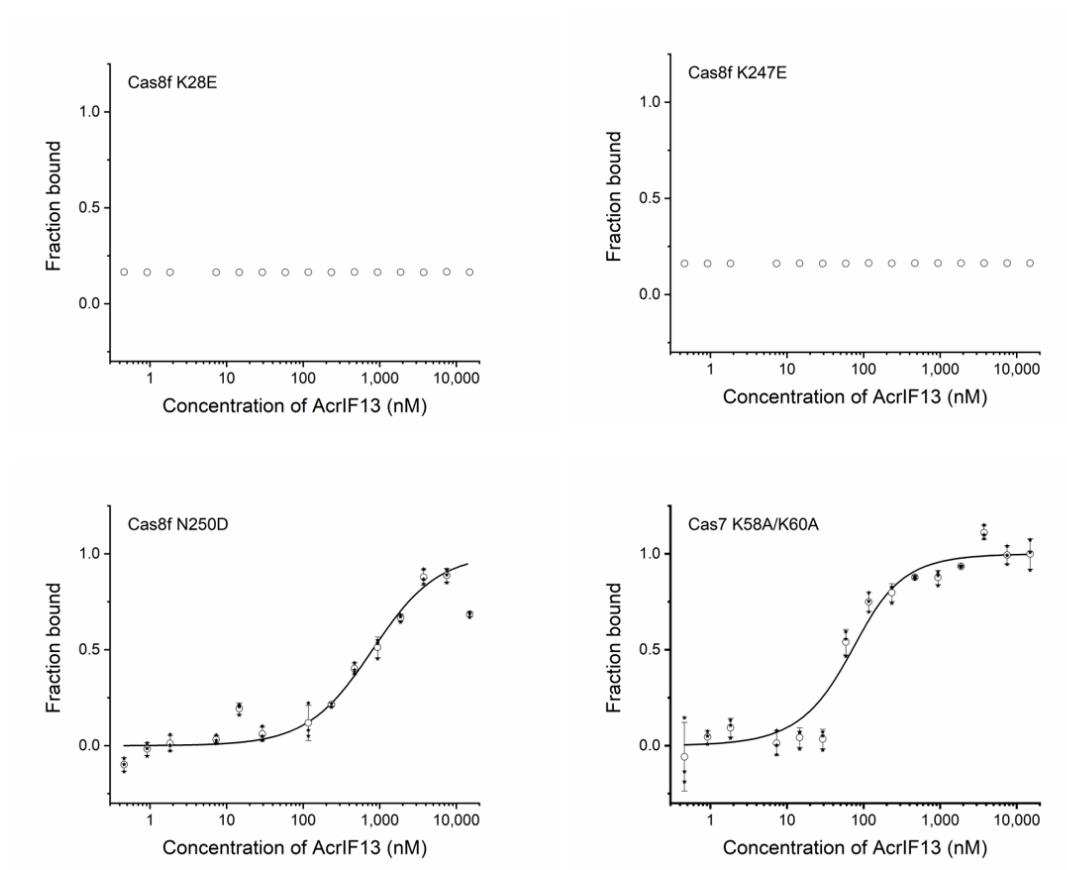


Figure S5 MST assay of the binding between AcrIF13 and Csy mutants

MST assays of the binding of AcrIF13 to the Csy mutants. Error bars represent SD; $n = 3$. Binding curves are also shown. For the K28E and K247E mutants of the Csy, K_D could not be determined.

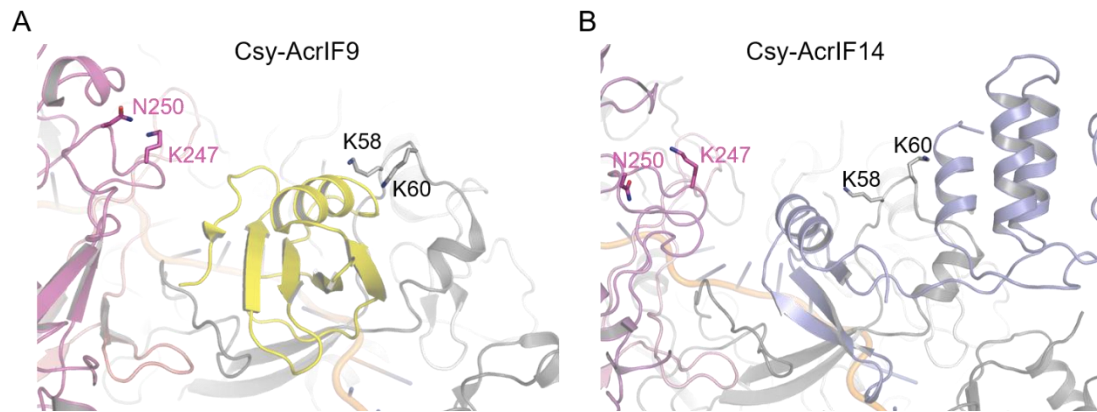


Figure S6 Structures of Csy-AcrIF9 and Csy-AcrIF14

(A and B) A closed view of the K58/K60 region of the Csy-AcrIF9 (A, PDB code: 6VQV) and Csy-AcrIF14 (B, PDB code: 7ECV) structures. Cas8f and Cas7.6f are colored in magenta and dark gray, respectively. AcrlF9 and AcrlF14 are colored in yellow and marine, respectively.