Table S1 Top 10 entries of the Dali search of AcrIF23

No.	PDB Chain	Z score	RMSD* (Å)	Aligned residues	Molecular name
1	7E58-A	8.1	6.5	62	Guanylate-binding protein 2
2	3DO9-B	8.0	3.0	60	Upf0302 protein ba_1542/gbaa1542/bas1430
3	1F5N-A	7.9	6.0	60	Interferon-induced guanylate-binding protein 1
4	6RZ8-A	7.9	4.1	66	Cysteinyl leukotriene receptor 2,soluble cytochro
5	1NG6-A	7.8	5.0	54	Hypothetical protein YQEY
6	5GOY-A	7.8	2.5	61	Methionine--trna ligase, cytoplasmic
7	1V7X-A	7.8	3.3	66	Chitobiose phosphorylase
8	1DG3-A	7.7	6.8	80	Protein (interferon-induced guanylate-binding pro
9	6W1E-A	7.7	3.2	64	Positive transcriptional regulator mutr family
10	5UHQ-C	7.7	5.0	56	Sugar transporter semisweet

*RMSD: root mean square deviation.

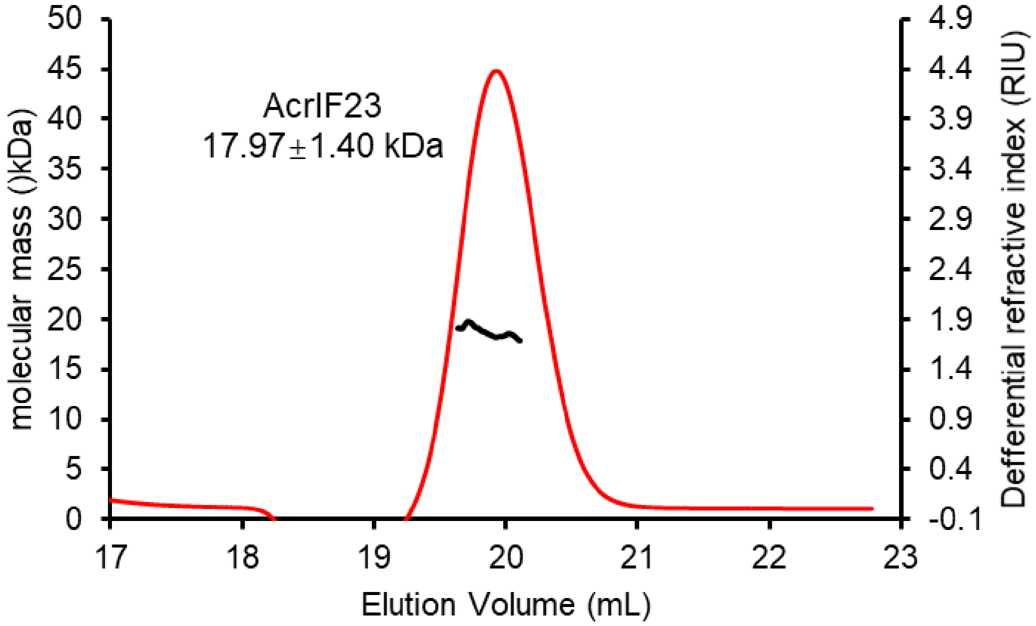


Figure S1 AcrIF23 exists as monomer in solution.

The average molecular weight was calculated to be 17.97±1.40 kDa.

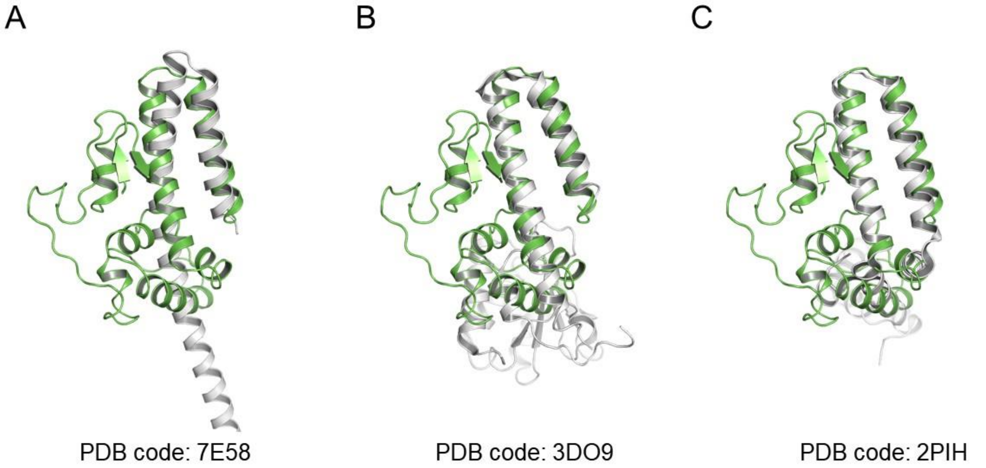


Figure S2 Structural comparisons between AcrIF23 and the returned entries from Dali search

(A-C) Structural superimposition between AcrIF23 and the guanylate-binding protein 2 (PDB: 7E58, A), Ba1542 from *Bacillus Anthracis Str.Ames* (PDB: 3DO9, B) and ymcA from Bacillus subtilis (PDB: 2PIH, C). AcrIF23 is colored in green in all the panels. All the other three structures are colored in gray.

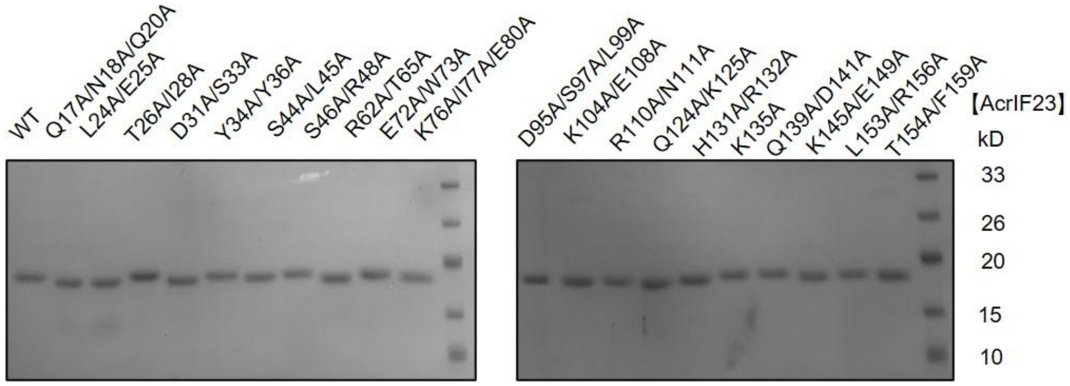
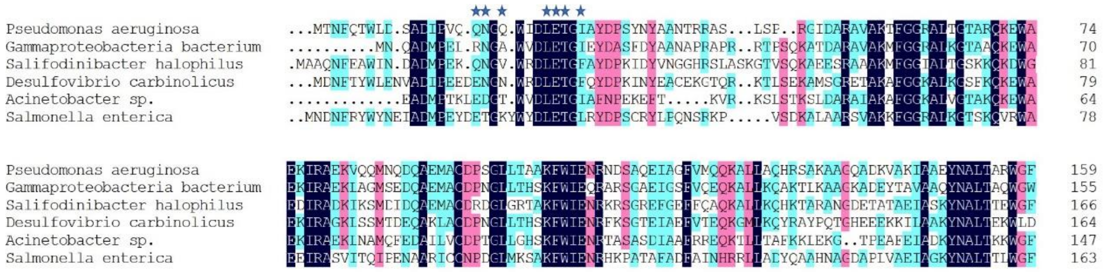


Figure S3 The purity of AcrIF23 and its mutant proteins
SDS-PAGE gel of the proteins stained by Commasie brilliant blue.



```
                                                     ★★ ★    ★★★ ★
Pseudomonas aeruginosa          ...MTNFQTWLL.SADIPVQ.QNGQ.WIDLETGIAYDPSYNYAANTRRAS...LSP..RGIDARAVAKTFGGRALTGTARQKEWA   74
Gammaproteobacteria bacterium   ..........MN.QADMPEI.RNGA.WVDLETGIEYDASFDYAANAPRAPR..RTPSQKATDARAVAKMFGGRALKGTAAQKEWA   70
Salifodinibacter halophilus     .MAAQNFEAWIN.DADMPEK.QNGV.WRDLETGFAYDPKIDYVNGGHRSLASKGTVSQKAEESRAAAKMFGGIALTGSKKQKDWG   81
Desulfovibrio carbinolicus      ...MDNFTYWLENVADIPEEDENGN.WRDLETGFQYDPKINYEACEKGTQR..KTLSEKAMSGRETAKAFGGKALKGSFKQKEWA   79
Acinetobacter sp.               .............EADMPTKLEDGT.WVDLETGIAFNPEKEFT.....KVR..KSLSTKSLDARAIAKAFGGKALVGTAKQKEWA   64
Salmonella enterica             ..MNDNFRYWYNEIADMPEYDETGKYWYDLETGLRYDPSCRYLPQNSRKP.....VSDKALAARSVAKKFGGRALKGTSKQVRWA   78

Pseudomonas aeruginosa          EKIRAEKVQQMNQDQAEMACDPSGLLTAAKFWIENRNDSAQEIAGFVMQQKALLAQHRSAKAAGQADKVAKIAAEYNALTARWGF  159
Gammaproteobacteria bacterium   EKIRAEKLAGMSEDQAEMACDPNGLLTHSKFWIEQRARSCAEIGSFVQEQKALLKQAKTLKAAGKADEYTAVAAQYNALTAQWGW  155
Salifodinibacter halophilus     EDIRADKIKSMDIDQAEMACDRDGIGRTAKFWIENRKRSGREFGEFFQAQKALLKQHKTARANGDETATAEIASKYNALTTEWGF  166
Desulfovibrio carbinolicus      EKIRAGKISSMTDEQAKLACDPNGLLTHSKFWIENRFKSGTEIAEFVTEQKGMLKQYRAYPQTGHEEEKKILAAKYNALTEKWLD  164
Acinetobacter sp.               EKIRAEKINAMQFEDAILVCDPTGLLGHSKFWIENRTASASDIAAFRREQKTLLTAFKKLEKG..TPEAFEIADKYNALTKKWGF  147
Salmonella enterica             EEIRASVITQIPENAARICQNPDGIMKSAKFWIENRHKPATAFADFAINHRRLLADYQAAHNAGDAPIVAEIAGKYNALTTIWGF  163
```

Figure S4 Sequence alignment of AcrIF23 and its homologs

Sequence information: AcrIF23 from *Pseudomonas Aeruginosa* (WP_052155777.1), and its homologs from *Gammaproteobacteria* (NCC41549.1), *Salifodinibacter Halophilus* (WP_170050866.1), *Desulfovibrio Carbinolicus* (WP_129352082.1), *Acinetobacter Sp.* (MBP9788134.1) and *Salmonella Enterica* (HAV1239868.1). Residues with 100 % identity, over 75 % identity and over 50 % identity are shaded in dark blue, pink and cyan, respectively. Residues whose mutations impairs the activity of AcrIF23 are marked with stars.