

## **SUPPORTING INFORMATION**

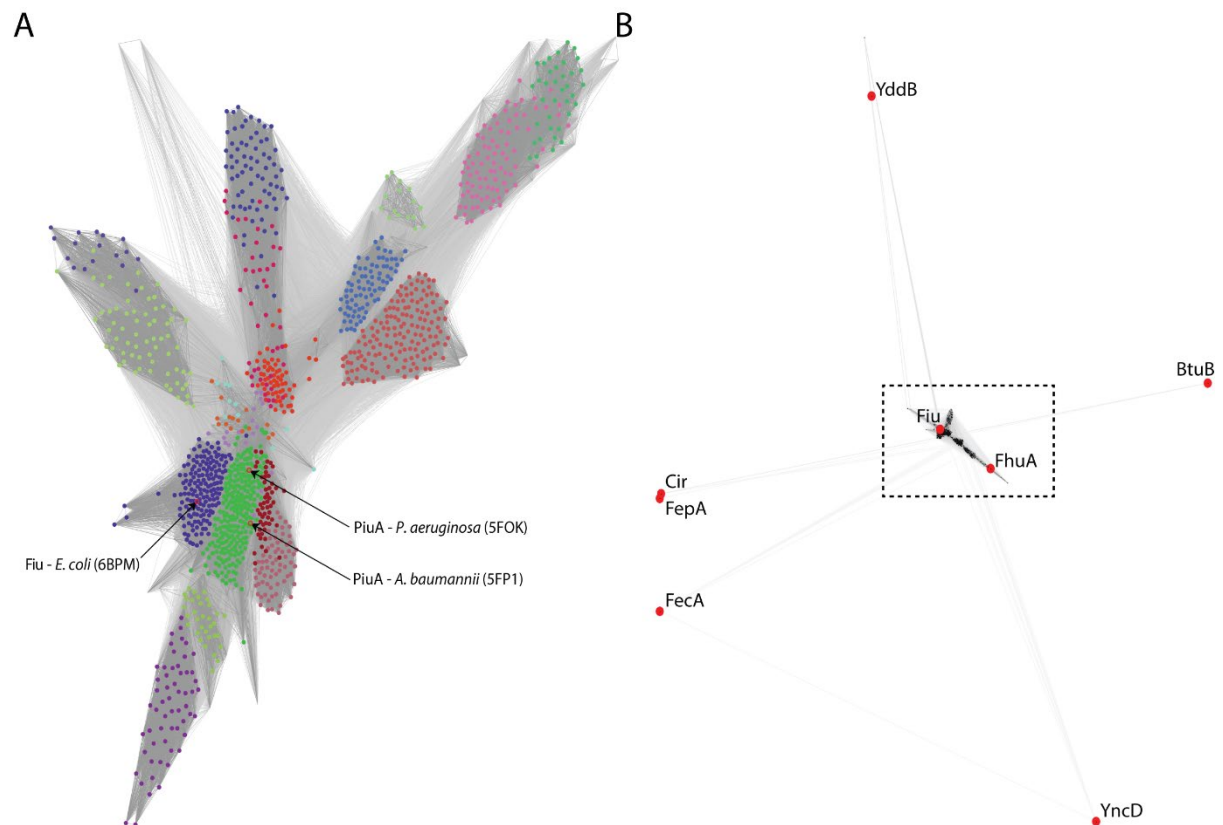
### **Digital Files:**

**Table S1 a list of sequences homologous to Fiu identified in the HMMER search (Table S1.xlsx)**

**Structural Coordinates for Fiu docked with Fe-DHB (DHB\_Dock\_Run1.pdb, DHB\_DockRun2.pdb, Fiu\_Dock\_Receptor.pdb)**

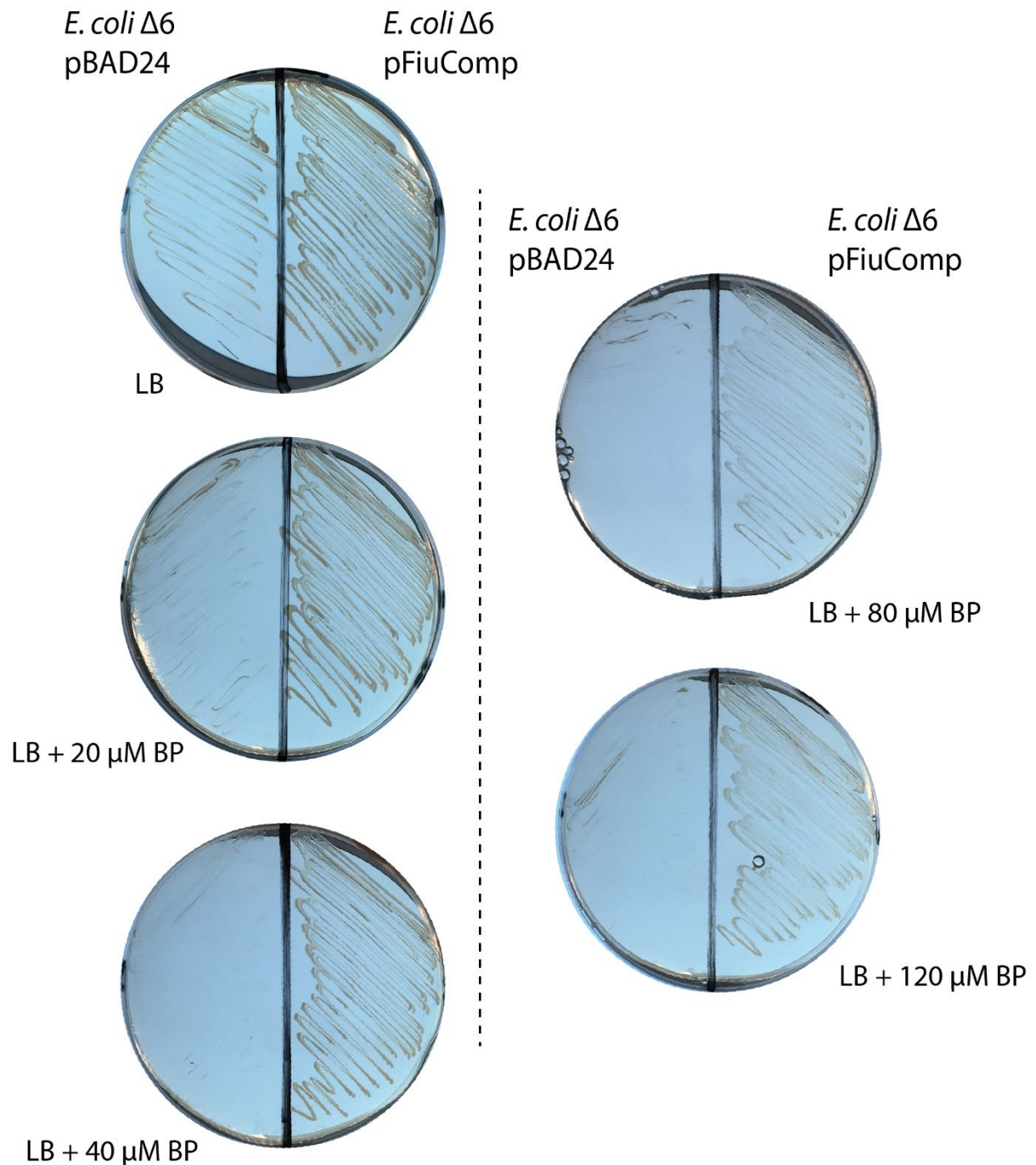
**Atomic coordinates and experimental data for Fiu Fe-DHB cocrystal (Fiu\_DHB.pdb, Fiu\_DHB.mtz, Fiu\_DHB\_refine.mtz)**

## Supporting Figures:

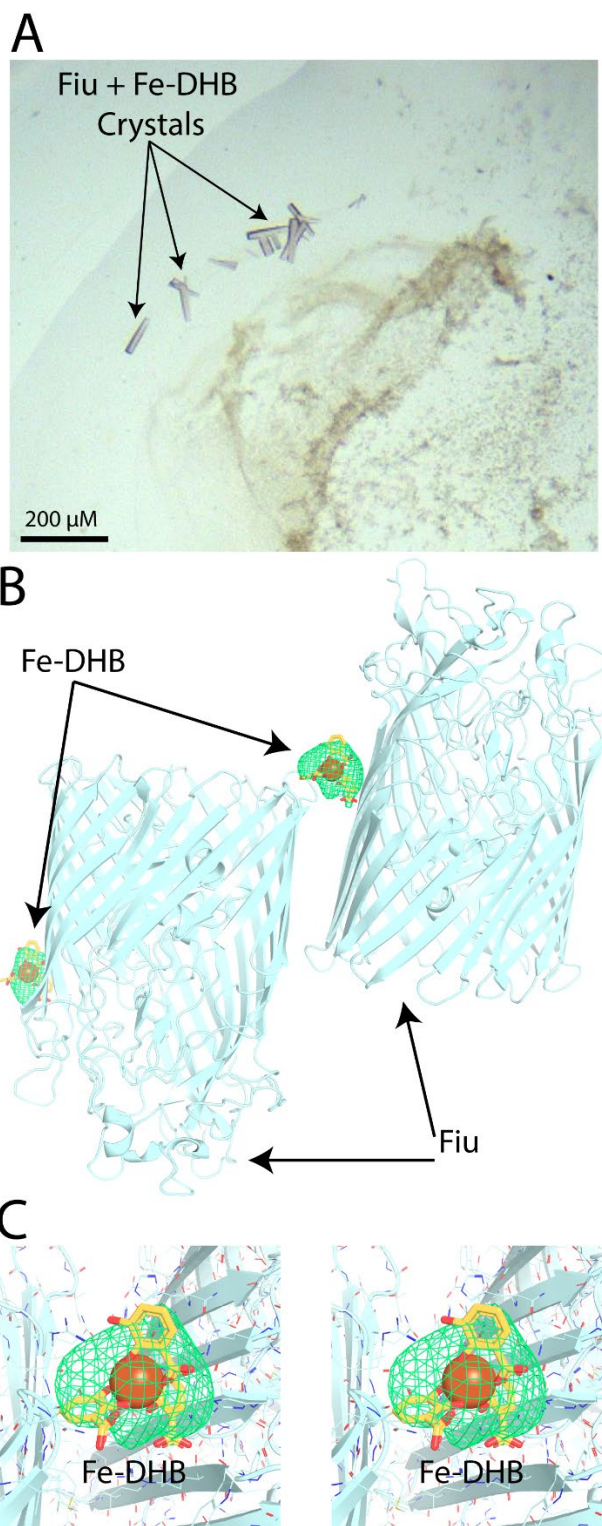


**Figure S1 CLANS clustering of Fiu homologs identified by HMMER search and other TBBDs from *E. coli*.**

(A) CLANS clustering analysis (59) of Fiu homolog sequences identified by HMMER search (Table S1), location of the points corresponding to Fiu and PiuA are indicated. Sequences in (B) are spiked with TBBD sequences from *E. coli* BW25113 to show the relationship between these receptors and the Fiu homologs identified in the search.



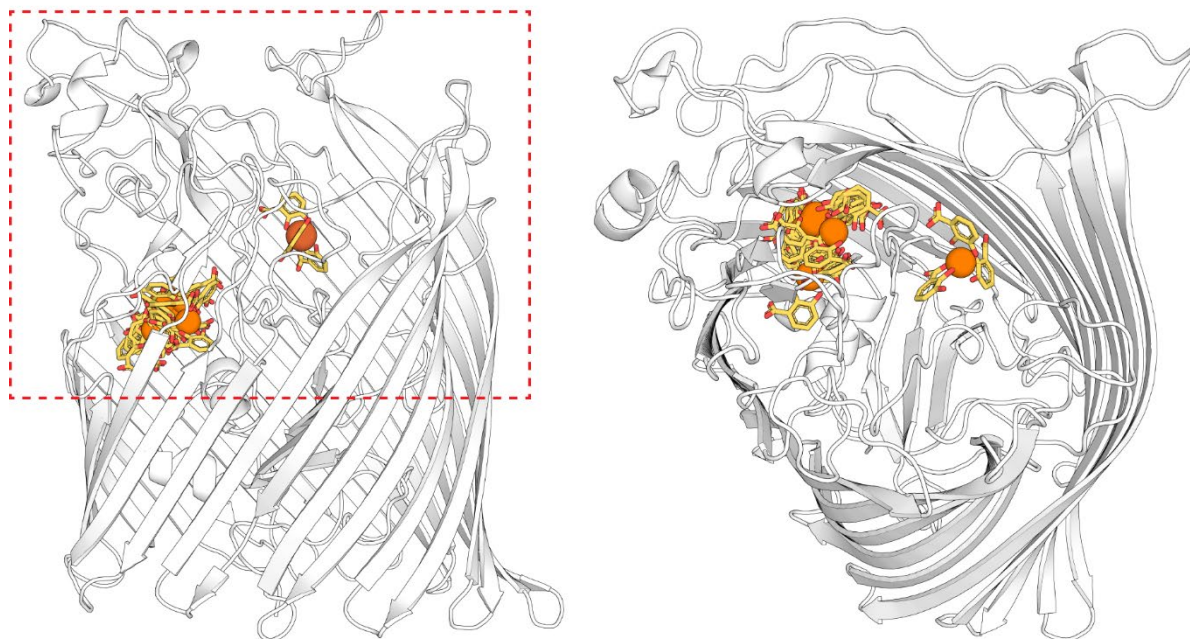
**Figure S2 Complementation of *E. coli* BW25113 TB $\Delta$ 6 with plasmid derived Fiu**  
 Growth of *E. coli* BW25113 TB $\Delta$ 6 with complementation vector (pBAD24Fiu) or vector control (pBAD24) on LB agar with 0-120  $\mu\text{M}$  2,2'-Bipyridine (BP). Vector control is unable to grow at a concentration of BP > 20  $\mu\text{M}$ , while the complemented strain can grow at a concentration up to BP = 120  $\mu\text{M}$ .



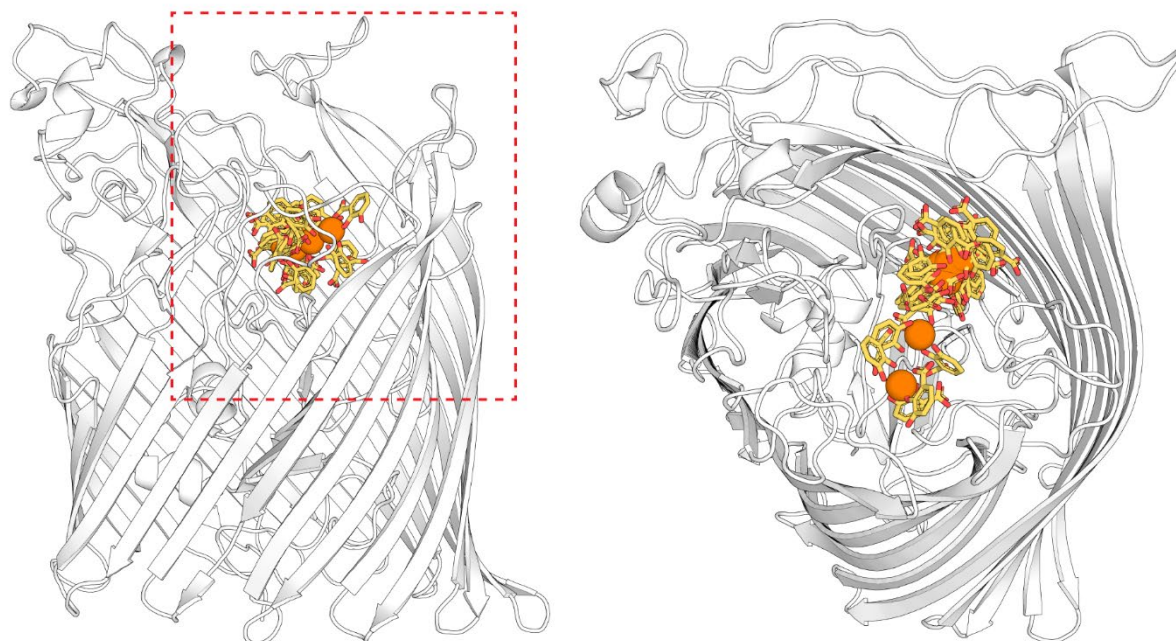
**Figure S3 Co-crystallisation between Fiu and Fe-DHB complex**

(A) Crystals of Fiu obtained in the presence of Fe-DHB, with a violet color characteristic of a Fe-catecholate complex. (B) The resultant asymmetric unit from these poorly diffracting crystals (anisotropic 3.2-5.9 Å) contained 2 molecules of Fiu in complex with 2 Fe-DHB complexes. Fiu is shown in cartoon representation, while Fe-DHB is shown as a stick/sphere model modeled into Fo-Fc density contoured to 3  $\sigma$ . (C) A zoomed stereo view of the central Fe-DHB complex from panel B, with Fiu sidechains shown as lines. Fe-DHB is coordinated by a patch of positively charged residues on one Fiu molecule.

## Docking Run 1

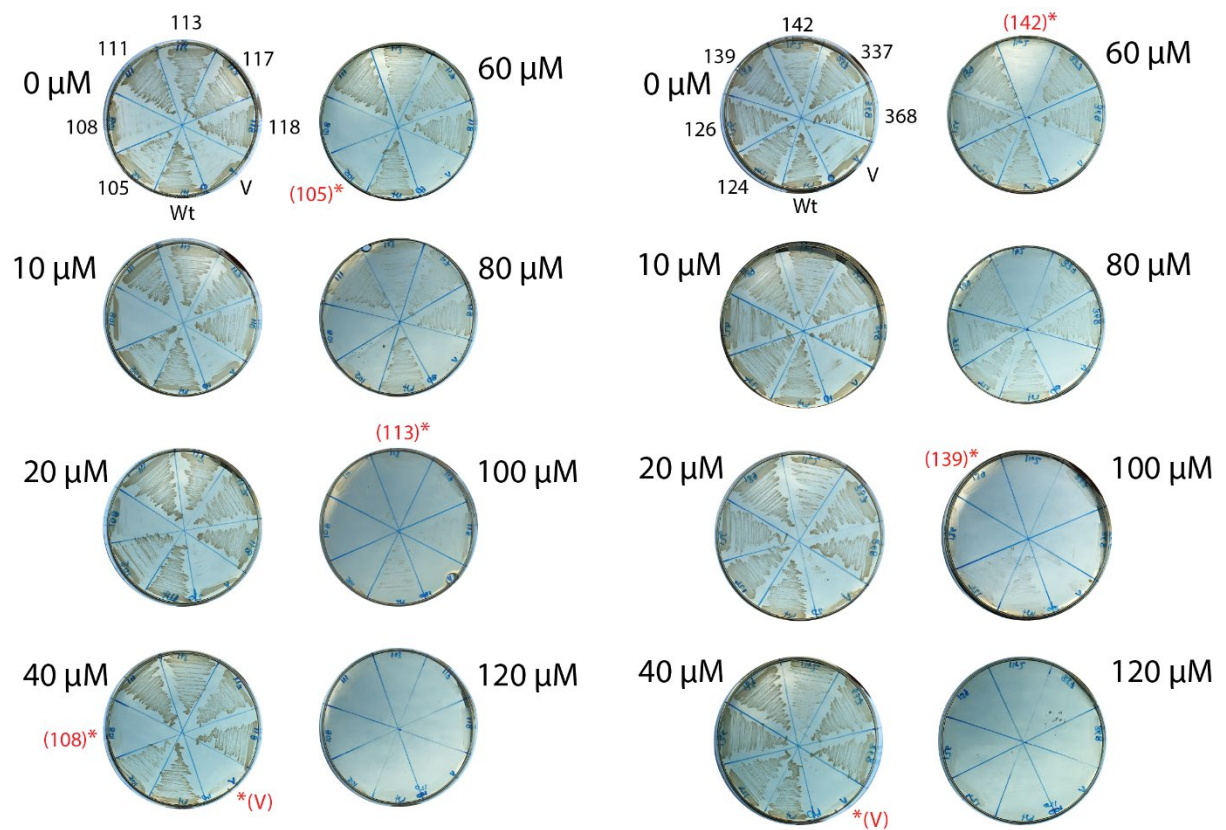


## Docking Run 2



### **Figure S4 All docking modes obtain between Fiu and Fe-DHB**

All 9 docking modes obtained for docking runs 1 and 2, shown with Fe-DHB as a stick/sphere model with Fiu with a cartoon representation. The dashed red box shows the docking search area along the Z-axis relative to the membrane. In both docking runs the majority of binding modes, including the top-ranked mode, cluster at a single location.



**Figure S5 The effect of substrate binding site mutations on *in vivo* function of Fiu**

The growth of *E. coli* BW25113 containing the complementation vector (Wt), vector control (V) or complementation vector mutants (numbers) on LB agar with 0-120 μM 2,2'-bipyridine (BP). Mutant numbers represent the position of the amino acids mutated (F105A, E108A, N111A, T113W, A117W, Y118A, D124A, S126W, S139W, R142A, Y337, L368A). Starred sections show the maximum BP concentration at which the corresponding mutated construct or control is able to grow.

## Supporting Tables:

**Table S2 Sequence identity matrix of a selection of Fiu homologs from bacteria belonging to Enterobacteriaceae.**

	<i>P. dispersa</i>	<i>P. wallisii</i>	<i>P. sp. (strain At-9b)</i>	<i>M. sp. MFB070</i>	<i>K. pneumoniae DSM 30104</i>	<i>P. gergoviae</i>	<i>E. coli (strain K12)</i>	<i>E. coli ISC11</i>	<i>L. adecarboxylata</i>	<i>R. ornithinolytica</i>	<i>K. ascorbata ATCC 33433</i>	<i>S. blattae DSM 4481</i>	<i>S. sp. M24T3</i>	<i>S.sp. Leaf50</i>	<i>E. gerundensis</i>
<i>Pantoea dispersa</i>	100	94	91	86	85	80	79	79	80	80	76	74	78	79	81
<i>Pantoea wallisii</i>	94	100	88	84	84	79	78	78	78	78	74	73	77	78	80
<i>Pantoea sp. (strain At-9b)</i>	91	88	100	86	84	79	79	80	80	81	78	74	78	80	78
<i>Mangrovibacter sp. MFB070</i>	86	84	86	100	83	80	77	79	80	79	76	72	78	79	78
<i>Klebsiella pneumoniae DSM 30104</i>	85	84	84	83	100	79	77	76	78	77	75	72	75	77	77
<i>Pluralibacter gergoviae</i>	80	79	79	80	79	100	74	73	74	73	71	69	72	73	74
<i>Escherichia coli (strain K12)</i>	79	78	79	77	77	74	100	89	86	83	82	77	77	77	78
<i>Escherichia coli ISC11</i>	79	78	80	79	76	73	89	100	87	86	84	76	80	78	79
<i>Leclercia adecarboxylata</i>	80	78	80	80	78	74	86	87	100	85	82	75	77	78	79
<i>Raoultella ornithinolytica</i>	80	78	81	79	77	73	83	86	85	100	83	77	78	79	80
<i>Kluyvera ascorbata ATCC 33433</i>	76	74	78	76	75	71	82	84	82	83	100	74	78	78	75
<i>Shimwellia blattae DSM 4481</i>	74	73	74	72	72	69	77	76	75	77	74	100	73	72	73
<i>Serratia sp. M24T3</i>	78	77	78	78	75	72	77	80	77	78	78	73	100	86	77
<i>Serratia sp. Leaf50</i>	79	78	80	79	77	73	77	78	78	79	78	72	86	100	78
<i>Erwinia gerundensis</i>	81	80	78	78	77	74	78	79	79	80	75	73	77	78	100

**Table S3 Data collection and refinement statistics for fully refined Fiu crystal structures**

	<b>Crystal State 1</b>	<b>Crystal State 2</b>	
<b>Data Collection<sup>a</sup></b>			
Space Group	<i>C222(1)</i>	<i>C2(1)</i>	<i>P1</i>
Cell Dimensions			
<i>a, b, c</i> (Å)	107.93, 148.94, 105.06	191.43, 75.67, 136.87	81.67, 133.16, 135.10
<i>α, β, γ</i> (°)	90, 90, 90	90, 91.89, 90	101.5, 107.42, 92.88
Wavelength	0.976	0.976	0.976
Resolution (Å)	48.00-2.10 (2.16-2.10)	49.34-2.50 (2.56-2.50)	50.31-2.9 (2.95-2.90)
R <sub>merge</sub>	0.152 (0.886)	0.156 (1.026)	0.222 (1.120)
R <sub>pim</sub>	0.058 (0.349)	0.111 (0.740)	0.151 (0.802)
<i>I</i> / <i>s(I)</i>	10.5 (2.4)	5.4 (1.1)	3.6 (1.1)
CC <sub>1/2</sub>	0.997 (0.811)	0.988 (0.536)	0.963 (0.491)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	99.0 (98.5)
Redundancy	7.7 (7.4)	3.6 (3.5)	3.7 (3.7)
Reflections	49686 (4026)	68066 (4595)	116009 (5714)
<b>Refinement statistics</b>			
R <sub>work</sub> /R <sub>free</sub>	17.4/22.0	19.9/25.9	21.8/26.9
No. atoms			
<i>Protein</i>	5025	10823	21644
<i>Ligand / ions</i>	146	235	40
R.m.s deviations			
Bond lengths (Å)	0.007	0.007	0.008
Bond angles (°)	0.817	0.999	1.09
PDB ID	6BPN	6BPM	6BPO

<sup>a</sup> Values in parentheses are for highest-resolution shell.

Data from one crystal was collected for each structure



**Table S4 Data collection and refinement statistics for partially refined low resolution Fiu/Fe-DHB cocrystal****Fe-DHB Co-crystal****Data Collection<sup>a</sup>**

Space Group	<i>P2(1)2(1)2(1)</i>
Cell Dimensions	
<i>a, b, c</i> (Å)	72.60, 129.32, 200.77
<i>α, β, γ</i> (°)	90, 90, 90

Wavelength	0.976
Resolution (Å)	49.21-3.23 (3.43-3.23)

**Anisotropy correction<sup>b</sup>**

Resolution truncation (reflections with $F/\sigma < 3.0$ discarded)	
<i>a*</i> , <i>b*</i> , <i>c*</i> (Å)	5.58, 5.88, 3.23
Reflections discarded	
<i>Original, discarded, final</i>	62488, 51875, 10613
Completeness after truncation (%)	
<i>49.21-5.71 Å, 5.71-4.54 Å, 4.54-3.23 Å</i>	99.8, 50.0, 10.0

**Refinement statistics**

$R_{\text{work}}/R_{\text{free}}$	31.1/34.9
No. atoms	
<i>Protein</i>	10854
<i>Ligand / ions</i>	0
R.m.s deviations	
Bond lengths (Å)	0.012
Bond angles (°)	1.377

<sup>a</sup> Values in parentheses are for the highest-resolution shell.

<sup>b</sup> Correction applied using 'Diffraction Anisotropy Server'[1]

**Table S5 Fiu Fe-DHB Autodock docking statistics**

<b>Mode</b>	<b>Affinity (kcal/mol)</b>	<b>Distance from RMSD l.b.</b>	<b>Best Mode RMSD u.b.</b>
<i>Docking run 1</i>			
1	-10.6	0.00	0.00
2	-10.0	5.08	8.56
<b>3</b>	<b>-9.8</b>	<b>15.87</b>	<b>18.46</b>
4	-9.7	2.51	7.08
5	-9.7	2.49	2.90
6	-9.7	1.75	5.92
7	-9.6	2.78	6.48
8	-9.6	2.45	7.08
9	-9.6	1.35	6.91
<i>Docking run 2</i>			
<b>1</b>	<b>-9.8</b>	<b>0.00</b>	<b>0.00</b>
2	-9.0	5.96	11.28
3	-9.0	1.39	6.35
4	-9.0	11.14	15.41
5	-8.9	1.99	2.54
6	-8.9	2.66	6.46
7	-8.9	2.33	6.83
8	-8.8	2.00	2.41
9	-8.8	1.95	6.03

**Table S6 The crystal structures of TBDT-substrate complexes utilized for binding site analysis.**

<b>PDB Code</b>	<b>Organism</b>	<b>Receptor</b>	<b>Substrate</b>
1BY5	<i>E. coli</i>	FhuA	Ferrichrome
1KMP	<i>E. coli</i>	FecA	Ferric Citrate
1NQH	<i>E. coli</i>	BtuB	Cyanocobalamin (Vitamin B12)
3QLB	<i>Pseudomonas fluorescens</i>	FptA	Ferri-enantiopyochelin
2IAH	<i>Pseudomonas aeruginosa</i>	FpvA	Ferri-pyoverdine
4RDT	<i>Neisseria meningitidis</i>	ZnuD	Zinc ion
6I96	<i>P. aeruginosa</i>	FoxA	Ferroxiamine
6E4V	<i>E. coli</i>	FhuE	Coprogen
6I2J	<i>P. aeruginosa</i>	PfeA	Enterobactin
6FOK	<i>P. aeruginosa</i>	OprC	Copper Ion
6H7F	<i>Acinetobacter baumannii</i>	BauA	Acintobactin
4AIQ	<i>N.meningitidis</i>	FrpB	Ferric Ion

**Table S7 Oligonucleotide primers used in this study**

<b>Primer Name</b>	<b>Sequence (5'-3')</b>	<b>Purpose</b>
Fiu Expr F	CCATCCCATGGGTGCCGAAGGGCAAACCTAACGC	Fiu amplification for pET20b protein expression
Fiu Expr R	CCATCCTCGAGTCAGAAATGCATATTGGCTGTGAG	Fiu amplification for pET20b protein expression
$\Delta$ FepA F	AGCGTGTTGCAGTTGTGCGTA	FepA KO cassette amplification
$\Delta$ FepA R	CCGGGCCATGTTTCGACTG	FepA KO cassette amplification
$\Delta$ FhuA F	CAACAGCAACCTGCTCAGCAA	FhuA KO cassette amplification
$\Delta$ FhuA R	CAAACGCTTTGCTGCTCCAGC	FhuA KO cassette amplification
$\Delta$ FhuE F	CAGCGAAATGGCACCGGCT	FhuE KO cassette amplification
$\Delta$ FhuE R	CGCCACACTGCGAGTATAGA	FhuE KO cassette amplification
$\Delta$ CirA F	AGGACAAAAATGCATGGCTGGAAT	CirA KO cassette amplification
$\Delta$ CirA R	GAAATAAGTTTCCTCCCTTCCTTG	CirA KO cassette amplification
$\Delta$ FecA F	TTCCGACGGACTGCCACGG	FecA KO cassette amplification
$\Delta$ FecA R	ACCGCTGGGTGAGGTGATAG	FecA KO cassette amplification
$\Delta$ fiu F	ACAGCGGCGTGGAAGGGTA	Fiu KO cassette amplification
$\Delta$ fiu R	GTTCGGTACTGCAACCATCTAC	Fiu KO cassette amplification
Fiu Comp F	GTACGGAATTCATGGAAAACAATCGCAATTTCCCTG	Fiu amplification for pBAD24 complementation
Fiu Comp R	GTACGAAGCTTTCAGAAATGCATATTGGCTGTGAGC	Fiu amplification for pBAD24 complementation
Fiu F105A F	GGCGTGGGTGCGTTTGCTGCGGGTGAGAACGGT	Fiu Mutagenesis F105A
Fiu F105A R	ACCGTTCTCACCCGCAGCAAACGCACCCACGCC	Fiu Mutagenesis F105A
Fiu E108A F	GCGTTTTTTGCGGGTGCGAACGGTAACTCCACCA	Fiu Mutagenesis E108A
Fiu E108A R	TGGTGGAGTTACCGTTCGCACCCGCAAAAAACGC	Fiu Mutagenesis E108A
Fiu N111A F	CGGGTGAGAACGGTGCCTCCACCACTGGCGAC	Fiu Mutagenesis N111A
Fiu N111A R	GTCGCCAGTGGTGGAGGCACCGTTCTCACCCG	Fiu Mutagenesis N111A

Fiu T113W F	CGGGTGAGAACGGTAACTCCTGGACTGGCGACGCCATTTATATG	Fiu Mutagenesis T113W
Fiu T113W R	CATATAAATGGCGTCGCCAGTCCAGGAGTTACCGTTCTCACCCG	Fiu Mutagenesis T113W
Fiu A117W F	GGTAACTCCACCACTGGCGACTGGATTTATATGCGTGGTGCC	Fiu Mutagenesis A117W
Fiu A117W R	GGCACCACGCATATAAATCCAGTCCAGTGGTGGAGTTACC	Fiu Mutagenesis A117W
Fiu I118A F	CACCACTGGCGACGCCGCTTATATGCGTGGTGCC	Fiu Mutagenesis Y118A
Fiu I118A R	GGCACCACGCATATAAGCGGCGTCGCCAGTGGTG	Fiu Mutagenesis Y118A
Fiu D124A F	GCCATTTATATGCGTGGTGCCGCTACCTCTAACAGTATTTATA,	Fiu Mutagenesis D124A
Fiu D124A R	TATAAATACTGTTAGAGGTAGCGGCACCACGCATATAAATGGC	Fiu Mutagenesis D124A
Fiu S126W F	ATGCGTGGTGCCGATACCTGGAACAGTATTTATATTGATGGCA	Fiu Mutagenesis S126W
Fiu S126W R	TGCCATCAATATAAATACTGTTCCAGGTATCGGCACCACGCAT	Fiu Mutagenesis S126W
Fiu S139W F	CATTCGCGATATCGGCTGGGTCTCGCGCGACACCT	Fiu Mutagenesis S139W
Fiu S139W R	AGGTGTCGCGCGAGACCCAGCCGATATCGCGAATG	Fiu Mutagenesis S139W
Fiu R142A F	TATCGGCAGCGTCTCGGCCGACACCTTCAATACCGA	Fiu Mutagenesis R142A
Fiu R142A R	TCGGTATTGAAGGTGTCGGCCGAGACGCTGCCGATA	Fiu Mutagenesis R142A
Fiu Y337A F	CGCGCGTAAAGCAGGATGCCCTGATGACGGCGATTATG	Fiu Mutagenesis Y337A
Fiu Y337A R	CATAATCGCCGTCATCAGGGCATCCTGCTTTACGCGCG	Fiu Mutagenesis Y337A
Fiu L368A F	TGGTCACGCACGGCGAATACCGCAGATGTGAGTAATAAAATTC	Fiu Mutagenesis L368A
Fiu L368A R	GAATTTTATTACTCACATCTGCGGTATTCGCCGTGCGTGACCA	Fiu Mutagenesis L368A

**Table S8 Strains and plasmids used or generated in this study**

<b>Strain or plasmid</b>	<b>Relevant genotype and description</b>	<b>Reference or source</b>
<i>Escherichia coli</i> BW25113 Wt	F-, DE(araD-araB)567, lacZ4787(del)::rrnB-3, LAM-, rph-1, DE(rhaD-rhaB)568, hsdR514	[2]
<i>Escherichia coli</i> BW25113 TBDT $\Delta$ 1	As Wt + FhuA(del)	This study
<i>Escherichia coli</i> BW25113 TBDT $\Delta$ 2	As Wt + FhuA(del),FecA(del)	This study
<i>Escherichia coli</i> BW25113 TBDT $\Delta$ 3	As Wt + FhuA(del),FecA(del),CirA(del)	This study
<i>Escherichia coli</i> BW25113 TBDT $\Delta$ 4	As Wt + FhuA(del),FecA(del),CirA(del),FepA(del)	This study
<i>Escherichia coli</i> BW25113 TBDT $\Delta$ 5	As Wt + FhuA(del),FecA(del),CirA(del),FepA(del),FhuE(del)	This study
<i>Escherichia coli</i> BW25113 TBDT $\Delta$ 6	As Wt + FhuA(del),FecA(del),CirA(del),FepA(del),FhuE(del),Fiu(del)	This study
p20bFiu	pET20b + Fiu -minus signal peptide ligated at NcoI/XhoI sites	This study
pBAD24Fiu	pBAD24 + Fiu ligated at EcoRI/HindIII sites	This study
pBAD24FiuF105A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuE108A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuN111A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuT113A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuA117A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuI118A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuFD124A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuS126A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuS139A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuR142A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuY337A	As pBAD24Fiu with mutation as labelled	This study
pBAD24FiuK368A	As pBAD24Fiu with mutation as labelled	This study

## References

1. Strong M, Sawaya MR, Wang S, Phillips M, Cascio D, Eisenberg D. Toward the structural genomics of complexes: crystal structure of a PE/PPE protein complex from *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences*. 2006;103(21):8060-5.
2. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, et al. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular systems biology*. 2006;2(1).