

Supplementary Material

Table S1. Data collection and refinement statistics

Data Collection ^a	FeCu-P19 (red)	FeCu-P19 (ox)	Cu-P19 E44Q	Cu-P19 M88I	Cu-P19 D92H	Cu-FetP E46Q	Cu-FetP M90I
Resolution Range (Å)	50.00-1.55 (1.61-1.55)	33.07-1.65 (1.74-1.65)	50.00-1.90 (1.93-1.90)	50.00-2.30 (2.34-2.30)	50.00-2.50 (2.54-2.50)	33.65-1.40 (1.47-1.40)	38.34-1.53 (1.61-1.53)
Space group	<i>P</i> 2 ₁ 2 ₁ 2	<i>P</i> 2 ₁ 2 ₁ 2	<i>P</i> 2 ₁ 2 ₁ 2	<i>P</i> 6 ₂	<i>P</i> 3 ₂	<i>C</i> 2	<i>P</i> 2 ₁
Cell dimensions (Å)	<i>a</i> = 53.91 <i>b</i> = 73.23 <i>c</i> = 74.93	<i>a</i> = 54.22 <i>b</i> = 73.72 <i>c</i> = 74.85	<i>a</i> = 55.35 <i>b</i> = 73.34 <i>c</i> = 78.96	<i>a</i> = <i>b</i> = 132.97 <i>c</i> = 104.88	<i>a</i> = <i>b</i> = 132.46 <i>c</i> = 104.59	<i>a</i> = 83.16 <i>b</i> = 36.54 <i>c</i> = 101.00 <i>β</i> = 106.5	<i>a</i> = 49.16 <i>b</i> = 51.61 <i>c</i> = 66.14 <i>β</i> = 93.3
Wavelength (Å)	1.000	0.979	1.542	1.542	1.542	1.000	1.000
Unique Reflections	43367	36846	25709	45287	69955	50962	52557
Completeness (%)	98.9 (97.1)	100.0 (99.4)	99.2 (88.8)	97.5 (77.8)	99.0 (97.3)	87.4 (84.8)	99.4 (96.8)
Average <i>I</i> /σ <i>I</i>	29.7 (4.1)	15.0 (3.1)	24.2 (1.7)	21.6 (1.4)	12.6 (1.63)	14.9 (5.0)	19.0 (2.6)
Redundancy	6.0	8.3	6.7	10.7	4.9	3.9	3.6
<i>R</i> _{merge}	0.050 (0.417)	0.082 (0.639)	0.086 (0.515)	0.104 (0.833)	0.109 (0.624)	0.053 (0.235)	0.058 (0.362)
Refinement							
<i>R</i> _{work}	0.175	0.178	0.174	0.235	0.270	0.186	0.176
<i>R</i> _{free}	0.207	0.211	0.222	0.277	0.299	0.212	0.208
No. of waters	286	260	256	385	541	274	348
Average <i>B</i> -value (Å ²)	19.9	21.9	34.0	47.0	51.0	15.4	17.1
r.m.s.d. bond lengths (Å)	0.011	0.010	0.013	0.003	0.003	0.010	0.011
Ramachandran plot							
Most-favorable (%)	91.7	92.1	97.7	95.8	96.9	93.1	91.9
Allowed (%)	8.3	7.5	2.3	4.0	2.9	6.5	8.1
Outliers (%)	0	0.4	0	0.2	0.2	0.4	0

^a Values for the highest resolution shell are shown in parenthesis

Table S2. Bacterial strains and plasmids used in this study.

Strains and plasmids	Relevant genotype	Reference
<u><i>E. coli</i> strains</u>		
XL1-Blue	<i>recA1, endA1, gyrA96, thi-1, hsdR17, relA1, supE44, lac</i> (F ϕ , <i>proAB, lacIqZ_M15, Tn10</i>)	Stratagene
BL21	F ⁻ <i>dcm, omp, hsdS, (r_B⁻m_B⁻), lon⁻, galλ, DE3, pLysS</i>	Novagen
BL21-pLysS	F ⁻ <i>dcm, omp, hsdS, (r_B⁻m_B⁻), lon⁻, galλ, DE3</i>	¹
	Genomic DNA of <i>E. coli</i> strain F11	Robert Koch-Institute, S2, Wernigerode
<u><i>C. jejuni</i> strains</u>		
81-176 wild-type reference strain		²
81-176 $\Delta p19$		³
81-176 $\Delta p19$ complemented		³
<u>Vectors & plasmids</u>		
pECD1098	pASK-IBA3:: <i>fetM</i>	¹
	pASK-IBA6	IBA GmbH
pECD1138	pASK-IBA6:: <i>fetM_PL</i>	This study
pECD1101	pET22b(+>:: <i>fetP</i>	¹
pECD1128	pET22b(+>:: <i>fetP</i> E46Q	This study
pECD1129	pET22b(+>:: <i>fetP</i> M90I	This study

- 1 Koch, D. *et al.* Characterization of a dipartite iron-uptake system from uropathogenic *Escherichia coli* strain F11. *J Biol Chem* **286**, 25317-25330, doi:10.1074/jbc.M111.222745 (2011).
- 2 Korlath, J. A. *et al.* A point-source outbreak of campylobacteriosis associated with consumption of raw milk. *J Infect Dis* **152**, 592-596 (1985).
- 3 Chan, A. C. K. *et al.* Structure and function of P19, a high-affinity iron transporter of the human pathogen *Campylobacter jejuni*, *J Mol Biol* **401**, 590-604 (2010).

Table S3. Primers used in this study.

Primer	Sequence
<i>fetM</i> _PD <i>Bsa</i> I d	ATGGTAG <u>GGTCTC</u> AGCGCACCTCGACGAATTATGCGCCTTT
<i>fetM</i> _PD <i>Bsa</i> I u	ATGGTAG <u>GGTCTC</u> ATATCAACTATAGAGCAGCAGACTCCACT
<i>fetP</i> E46Q d	AAAGCCGATGTTACCTTCAGGCGGATATCCACGCTGTA
<i>fetP</i> E46Q u	TACAGCGTGGATATCCGCCTGAAGGTGAACATCGGCTTT
<i>fetP</i> M90I d	GGCACCTTCATGCCGATCGTTGCCAGCGATGGC
<i>fetP</i> M90I u	GCCATCGCTGGCAACGATCGGCATGAAGGTGCC
<i>pI9</i> E44Q phos Cj	GCAGATATTCATCTACAAGCTGACATTCACGC
<i>pI9</i> E44Q phos Rec	CTTTAGCAGATATTCACCTACAAGCTGACATTCACGCAC
<i>pI9</i> M88I phos Cj and Rec	GGAACCTTTGATGCCTATCGTGGCTGATGATGG
<i>pI9</i> D92H phos Cj and Rec	CCTATGGTGGCTGATCATGGTCCTCACTATGGTG

Underlined: restriction endonuclease recognition site

Bold letters: altered bases leading to amino acid exchange

Table S4. Taxonomic assignment of FetP-like sequences. The number (N) of predicted protein sequences in each cluster identified from a BLAST search is shown with the taxonomic assignment. The ϵ value compared to the FetP sequence of least related sequence of the cluster is indicated. Changes are truncations, non-conserved amino acid residues in metal binding site (FetP numbering) and a conserved cysteine residue in the leader of the sequences from Gram-positive bacteria, which might allow anchoring of the protein in the cytoplasmic membrane. The ratio of the sequences with these changes is also indicated. The clusters are shown in Supplementary Figure S6.

Cluster	N	Min ϵ value	Changes	Taxon	Genera
I A	2	2.00E-70	none	<i>Spirochaeta</i>	<i>Spirochaeta</i>
I B1	1	6.00E-72	none	δ -Proteobacteria	<i>Geobacter</i>
I B2	1	1.00E-69	none	α -Proteobacteria	<i>Rhodospirillum</i>
I B3	17	6.00E-58	none	ϵ -Proteobacteria	<i>Campylobacter</i> , <i>Wollinella</i>
I C1	1	6.00E-83	none	γ -Proteobacteria	<i>Vibrio</i>
I C2	1	1.00E-88	E5, truncated	γ -Proteobacteria	<i>Psychromonas</i>
I C3	2	2.00E-87	1/2= Δ LEADIH	<i>Chlamydia</i>	<i>Opitutaceae</i>
I C4	18	1.00E-88	none	γ -Proteobacteria	<i>Aggregatibacter</i> , <i>Haemophilus</i> , <i>Pasteurella</i> , <i>Actinobacillus</i> , <i>Cardiobacterium</i>
I C5	22	4.00E-91	none	<i>Enterobacteriaceae</i>	<i>Escherichia</i> , <i>Citrobacter</i> , <i>Shigella</i> , <i>Klebsiella</i> , <i>Yersinia</i> , <i>Brenneria</i> , <i>Pectobacterium</i> (=Seratia)
II A1	9	2.00E-49	none	<i>Enterobacteriaceae</i>	<i>Providencia</i> , <i>Proteus</i> , <i>Morganella</i>
II A2	1	2.00E-50	none	α -Proteobacteria	<i>Candidatus Liberibacter solanacearum</i>
II A3	2	3.00E-53	none	γ -Proteobacteria	<i>Vibrio</i> , <i>Photobacterium</i>
II B1	16	3.00E-42	none	γ -Proteobacteria	<i>Pseudomonas</i>
II B2	1	3.00E-52	none	α -Proteobacteria	<i>Rhodopseudomonas</i>
II B3	48	3.00E-43	1/48 E5/truncated	β -Proteobacteria	<i>Burkholderia</i> , <i>Herbaspirillum</i>
II B4	3	2.00E-46	none	α -Proteobacteria	<i>Rhodopseudomonas</i> , <i>Rhodovulum</i>
II B5	17	2.00E-27	none	α -Proteobacteria	<i>Beijerinckia</i> , <i>Brucella</i> , <i>Rhodopseudomonas</i> , <i>Rhodomicrobium</i> , <i>Methylobacterium</i>
II B6	7	1.00E-44	1/7 E5/truncated	α -Proteobacteria	<i>Hyphomicrobium</i> , <i>Mesorhizobium</i> , <i>Sinorhizobium</i> , <i>Rhizobium</i>
II C1,	11	6.00E-47	none	β -Proteobacteria	<i>Achromobacter</i> , <i>Bordetella</i>
II C2	4	2.00E-45	1/4 E5D	β -Proteobacteria	<i>Pseudogulbenkiamia</i> , <i>Chromobacterium</i> , <i>Verminephrobacter</i>
II C3	1	4.00E-50	none	β -Proteobacteria	<i>Dechloromonas</i>
II C4	2	2.00E-47	none	β -Proteobacteria	<i>Aromatoleum</i> , <i>Azoarcus</i>
II C5	1	3.00E-47	none	γ -Proteobacteria	<i>gamma proteobacterium HdN1</i>
II C6	1	5.00E-46	none	β -Proteobacteria	<i>Dechlorosoma</i>
II C7	2	4.00E-37	none	α -Proteobacteria	<i>Magnetospirillum</i>
II C8	2	2.00E-44	none	β -Proteobacteria	<i>Rubrivivax</i>
III A	1	5.00E-51	none	γ -Proteobacteria	<i>gamma proteobacterium IMCC2047</i>
III B	1	2.00E-54	none	<i>Deferribacteres</i>	<i>Flexistipes</i>
III C	16	2.00E-13	2/16: H44, E5, E46, truncated	<i>Firmicutes</i>	<i>Centipeda</i> , <i>Selenomonas</i> , <i>Dialister</i> , <i>Pelosinus</i> , <i>Megasphaera</i> ,
III D1	3	1.00E-17	1/3: H44, E5, E46; truncated	<i>Actinobacteria</i>	<i>Mobiluncus</i>
III D2	3	1.00E-27	none, Leader-Cys?	<i>Firmicutes</i>	<i>Parvimonas</i>

III D3	18	1.00E-06	Cys-Leader, 2/18 M90; 1/18 M90, H97, H127, D94	<i>Fusobacteria</i>	<i>Fusobacterium</i>
III D4	1	1.00E-21	none, leader-Cys?	<i>Firmicutes</i>	<i>Clostridiales genomosp. BVAB3 str. UPII9-5</i>
III D5	3	4.00E-25	none, Cys-Leader	<i>Synergistetes</i>	<i>Synergistetes, Jonquetella, Pyramidobacter</i>
III E1	10	6.00E-28	Cys-Leader, 1/10 H127, 1/10 M90,H97	<i>Actinobacteria</i>	<i>Actinomyces</i>
III E2,	34	3.00E-34	1/34 M90, H127, E5, D94	<i>Actinobacteria</i>	<i>Actinomaces, Parascardovia, Scardovia, Bifidobacterium, Gardnerella</i>
III E3	2	1.00E-26	none	<i>Firmicutes</i>	<i>Abiotrophia, Ruminococcus</i>
III F1	1	6.00E-44	none, leader-Cys	<i>Firmicutes</i>	<i>Clostridium</i>
III F2	3	9.00E-40	none	<i>Spirochaeta</i>	<i>Treponema</i>
III F3	7	7.00E-26	1/7: M90, H97	<i>Spirochaeta</i>	<i>Treponema</i>
III F4	11	2.00E-26	none, Leacer-Cys?	<i>Firmicutes</i>	<i>Filifactor, Johnsonella, Peptinophilus, Finegoldia, Anaerococcus, Stomatobaculum</i>
IV A	1	2.00E-46	none	<i>Planctomyces</i>	<i>Singulisphaera</i>
IV B	1	7.00E-46	none	<i>α-Proteobacteria</i>	<i>Phaeospirillum</i>
IV C	1	2.00E-55	none, TwinR-leader?	<i>α-Proteobacteria</i>	<i>magnetite-containing magnetic vibrio</i>
V A	2	8.00E-35	1/2 E5/truncated	<i>α-Proteobacteria</i>	<i>Magnetospirillum</i>
V B	1	5.00E-24	none	<i>BACTERIA</i>	<i>uncultured bacterium Bio2</i>
VI A	2	5.00E-42	none	<i>γ-Proteobacteria</i>	<i>Pseudomonas</i>
VI B	11	4.00E-29	none, Leacer-Cys?	<i>α-Proteobacteria</i>	<i>Gluconobacter, Acetobacter</i>
VII A	1	3.00E-11	H44, E5, leader too long	<i>Bacteroides/Chlorobi</i>	<i>Salinibacter</i>
VII B	1	5.00E-05	H44, E5, E46, truncated	<i>BACTERIA</i>	<i>uncultured marine bacterium EB0 39H12</i>

Supplementary Figures

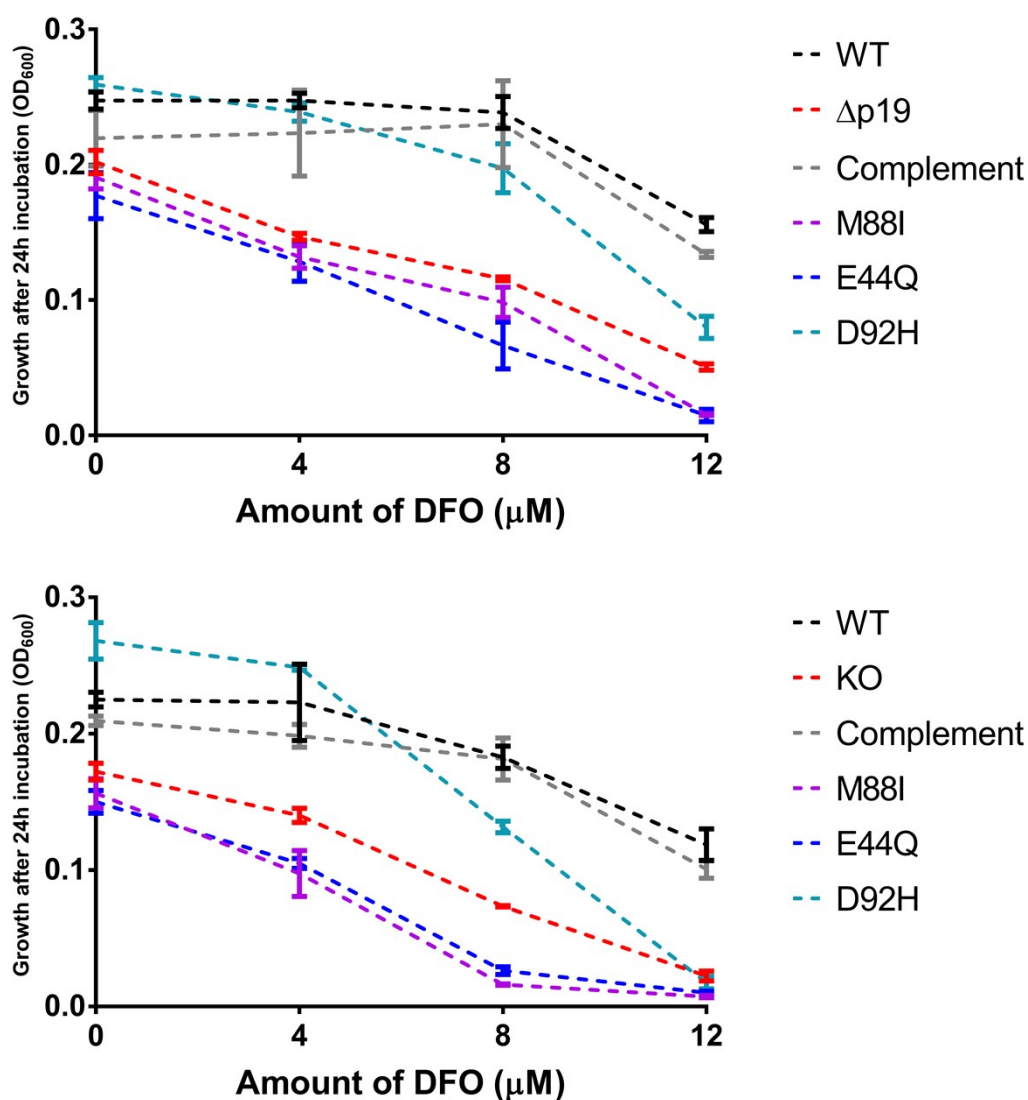


Figure S1. P19 metal site variants are unable to fully restore growth under iron limitation. *C. jejuni* wild-type 81-176, 81-176Δ*p19*, and trans-complemented strains grown under 0-12 μM DFO in a stationary 96-well plate incubated in a tri-gas incubator at 37 °C. After 24 h of growth, the plates were resuspended and OD₆₀₀ readings were taken. The two plots here and the one in Fig. 4 represent experiments performed on three separate occasions. Plot points represent the amount of bacterial growth averaged from three separate wells with standard deviation error bars drawn.

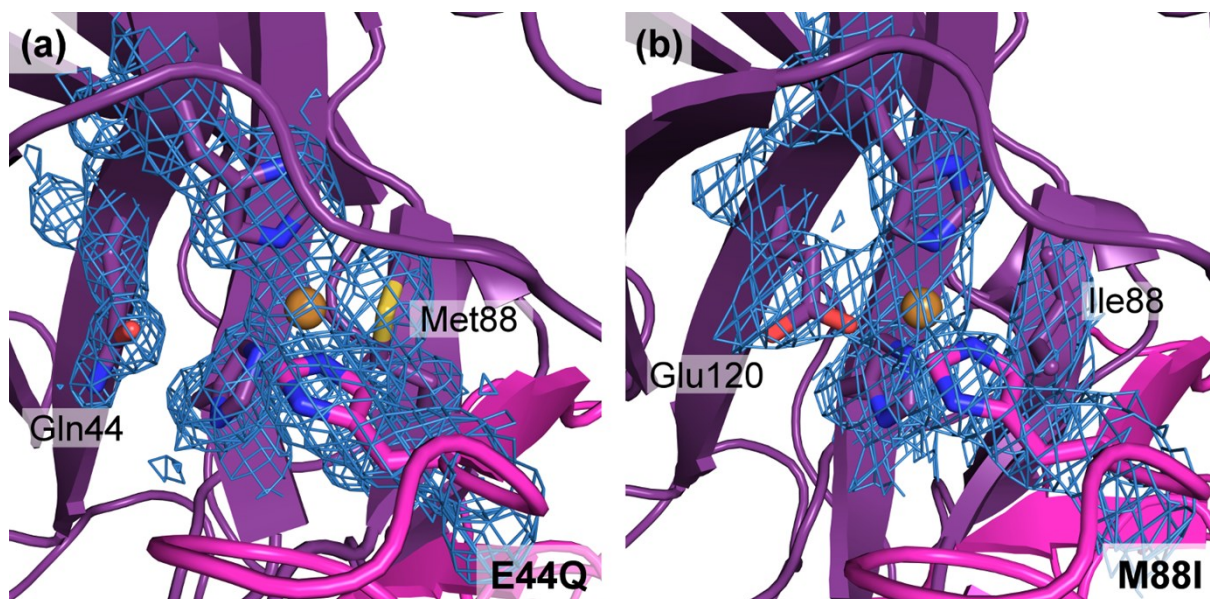


Figure S2. Cu-bound *C. jejuni* P19 (a) E44Q and (b) M88I variants do not exhibit positional plasticity. The copper cation is colored in brown. A 2Fo-Fc map contoured at 1 σ (blue) is overlaid the active site. Sulfur, oxygen and nitrogen atoms are colored yellow, red and dark blue, respectively. One protomer of the FetP dimer is shown in purple and the other in pink.

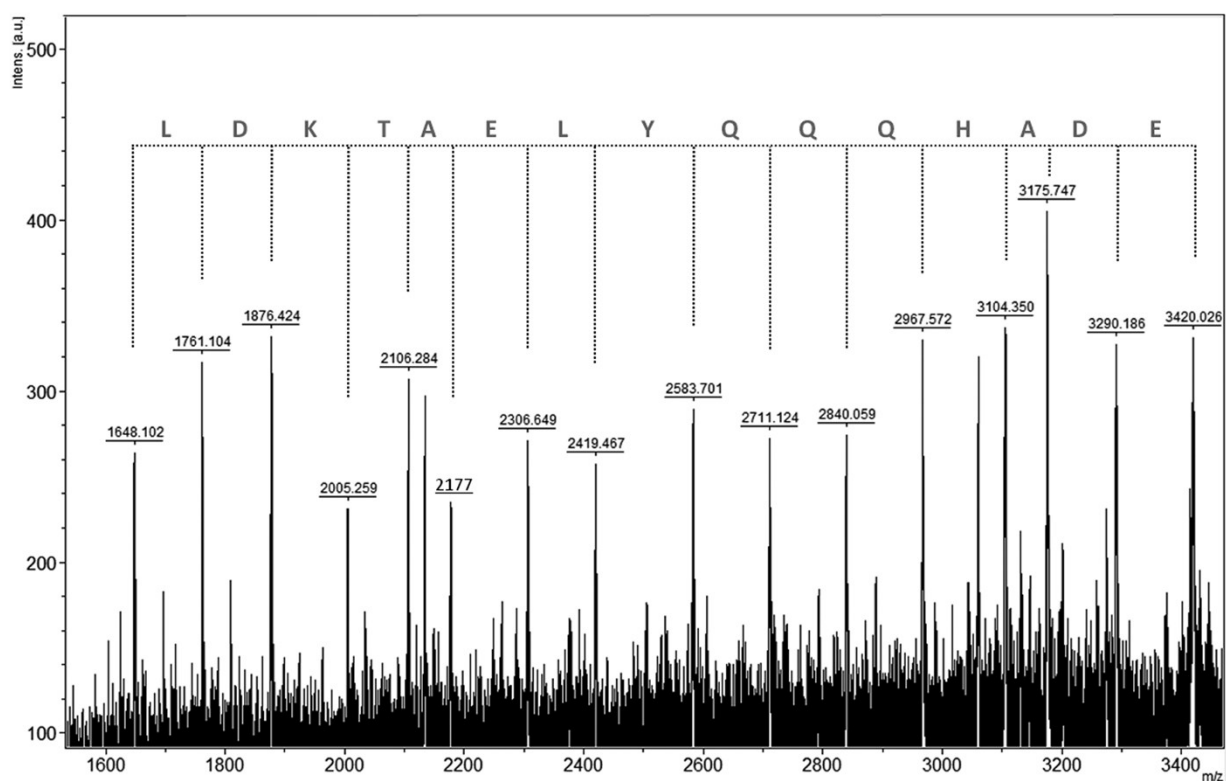


Figure S3. In source decay (ISD) MALDI-MS spectrum of ecFetM. ecFetM carrying a C-terminal strep-tag was solubilized from the membrane fraction after expression in *E. coli* and purified. In source decay led to a series of peptides and the molecular masses of these peptides (peaks) were determined with the matrix-assisted laser desorption mass spectrometer (MALDI-MS). The mass difference between the peptides identified the respective amino acid just removed from the protein with the sequence indicated above. This sequence, LDKTAEYQQQHADE, followed that of the amino acids within the smallest peptide identified with a mass of 1648.102 (left-most peak) which corresponded to the peptide STNYAPLIEDIEQR. This sequence was therefore identified as the amino-terminal sequence of the mature ecFetM-strep-tag protein as isolated from the membrane.

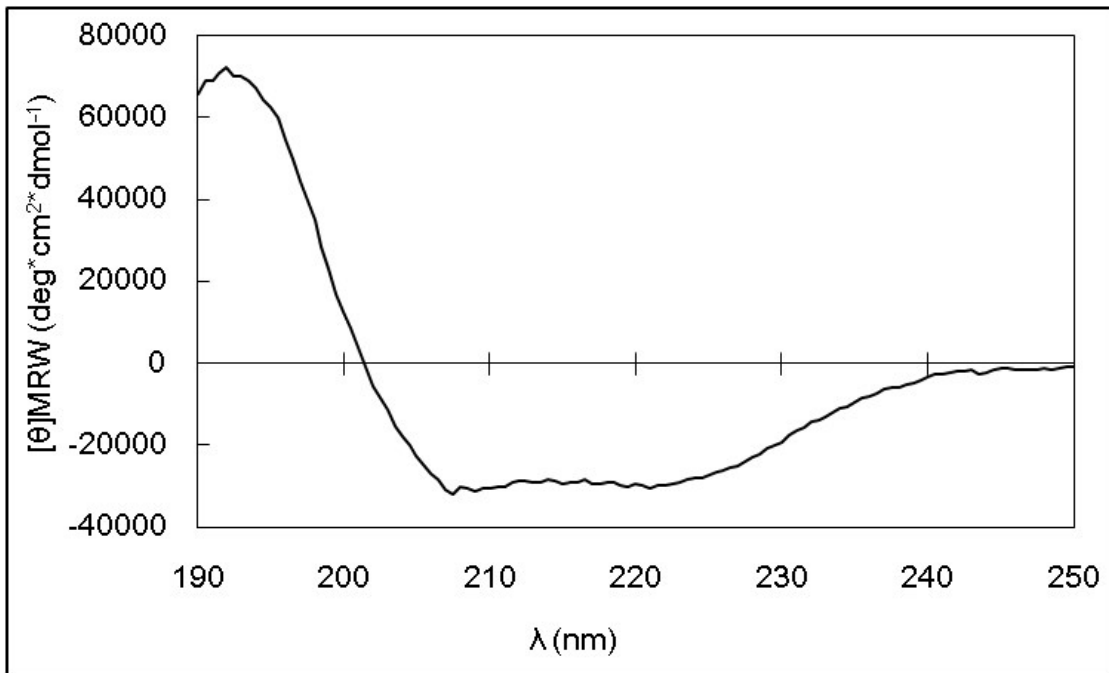


Figure S4. CD spectrum of ecFetM-PD. A circular dichroism spectrum (θ_{MRW}) of the purified periplasmic region of ecFetM, ecFetM-PD, is shown. Protein concentration was 15 μ M in 25 mM Bis-Tris/HCl, pH 7.2, 25°C. Shown is a difference spectrum plus vs. minus protein.

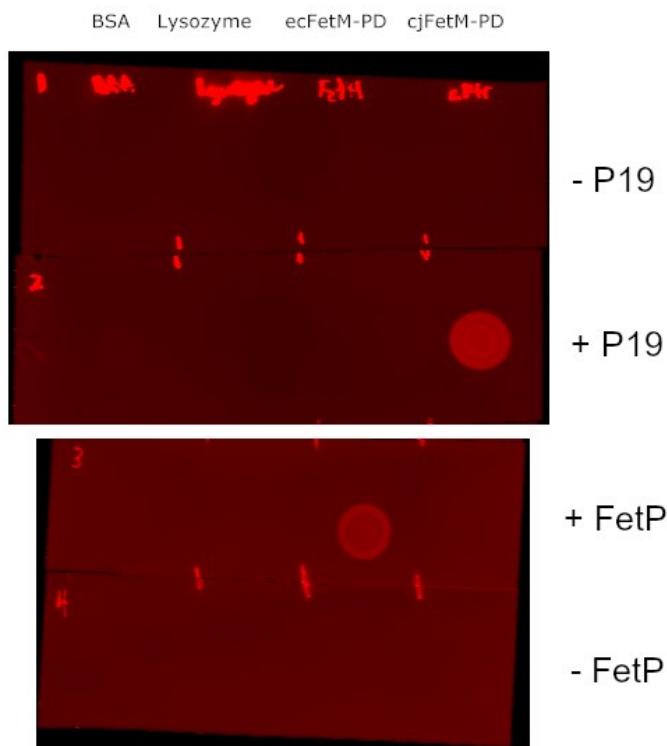


Figure S5. Original Far western blots of ecFetM-PD-FetP and cjFetM-PD-P19 interactions. Control blots (e.g. –P19) were prepared alongside non-controls (e.g. +P19) and were imaged together. The P19 and FetP experiments were performed separately.

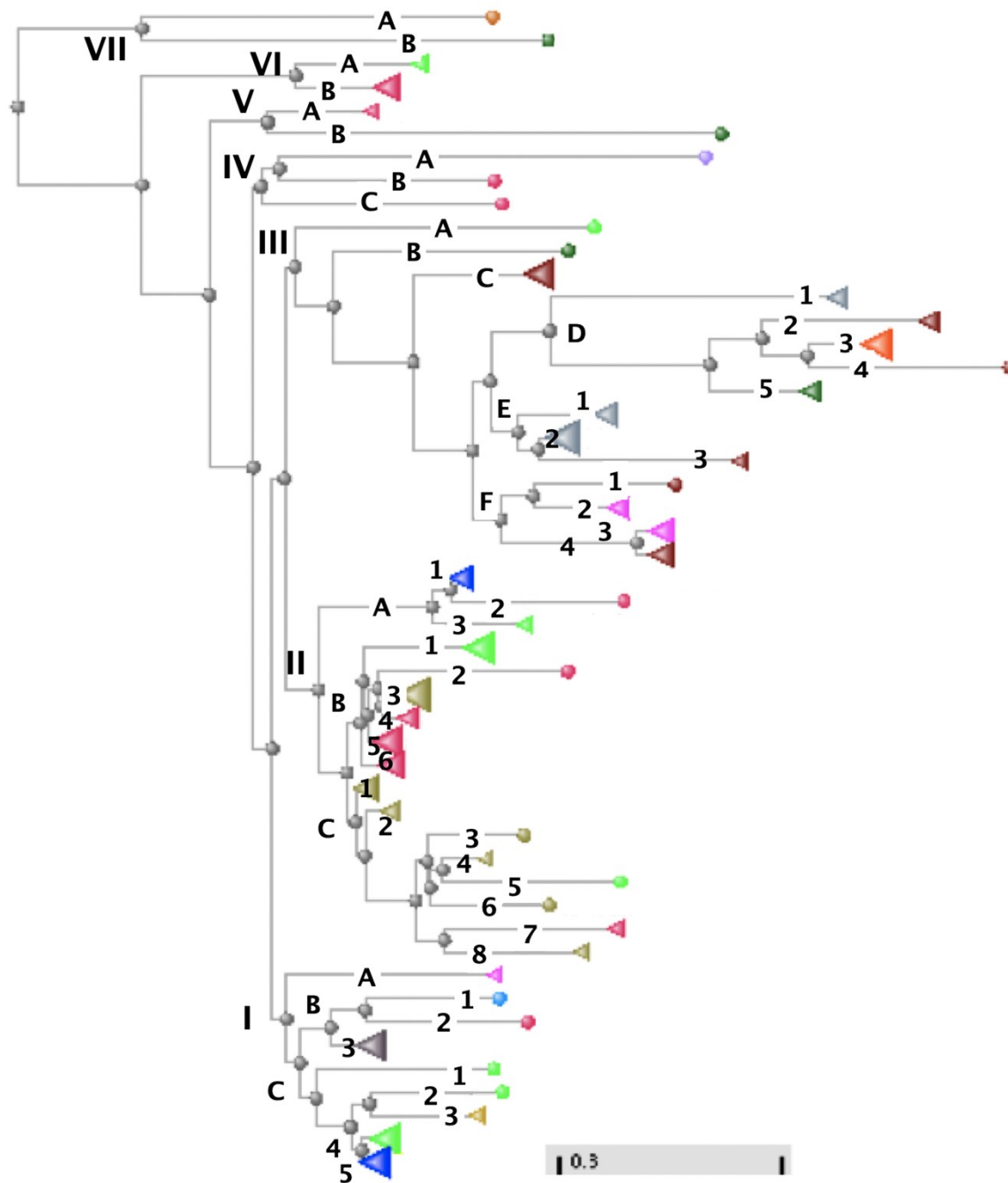


Figure S6. Clusters of FetP-like sequences. The data resulting from a BLAST search (1) was further analyzed by COBALT (2) and Geneious (www.geneious.com) to sort the sequences into clusters (I to VII), sub-clusters (A, B, C,...) and sub-sub-clusters (1, 2, 3, ...). The predicted FetP-like proteins within each sub-sub-cluster are listed in Table S4. Filled circles and squares are single sequences, arrowheads are two or more sequences with the size of the arrowhead indicating the number of sequences. Enterobacteria in dark blue, γ -proteobacteria in green, α -proteobacteria in red, β -proteobacteria in yellow, spirochetes in magenta, actinobacteria in grey and firmicutes in brown.

1. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acid Res* 25:3389-3402.
2. Papadopoulos JS, Agarwala R. 2007. COBALT: constraint-based alignment tool for multiple protein sequences. *Bioinformatics* 23:1073-1079.

Figure S7. Genomic environment of genes encoding P19-like proteins (orange, ‘COG-Tpd’) and genes encoding FetM-like transporters (light blue, ‘COG-FTR1’). The scale is in kbp and the organism is indicated above the gene symbols, which are in the direction of transcription.

