CHARACTERIZATION OF A DIPARTITE IRON-UPTAKE SYSTEM FROM UROPATHOGENIC *Escherichia coli* STRAIN F11

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Supplemental Material

TABLES

Table S1. Primers used in this study.

Primer	Sequence
fetMP$_{prom}$ d	TTACAGCGTCTTGCCAGCGATC
fetMP$_{prom}$ u	AAGAGTTACGGAAGTTACGCACGT
pGEM *fetMP* u	GTACGGCGGGTTGAATTAAGCG
pGEM *fetM* u	GTATCCTCTCGTCTAAAACAACGGCT
pGEM Delta *fetM Nco*I d	AAACCATGGAACCCATAATCGTTGTATAGCCGT
pGEM Delta *fetM Nco*I u	AAACCATGGTTCCGCTGTTTTATCAAGACGTTG
pUC18 *lacZ Kpn*I d	TTTGGTACCCAGGAAACAGCTATGACCATGATT
pUC18 *lacZ Kpn*I u	ACGGGTACCCATGGCCTGCCCGGTTATTAT
fetP T7 new d	GAAATTAATACGACTCACTATAGGGAACCTGCAACCGATTGA TATGG
fetP u	GGCGCGTTGGTTAGTCGGTT
fetP helper probe1 (H1)	TTCAATGTGGTAGGTCACTTTA
fetP helper probe2 (H2)	CCATCGCTGGCAACCATCG
fetP capture probe (BIO)	Biotin-AGTTGCCCACGCCCATCATT
fetP detection probe (DIG)	TGATGTTCGCGCCATAGTGC
pASK3 *fetM Eco*RI d	GGCGAATTCCACGTGCGTAACTTCCGTAACTCTTT
pASK3 *fetM Pst*I u	AAACTGCAGTGGGTTATTTTTTATCGTCTCCCCGGGA
pASK3 *fetP Eco*RI d	AGCGAATTCACCATGAAGAAAACCCTGATTGCC
pASK3 *fetP Pst*I u	TCGCTGCAGGTTCAGACCGACATATTTAAACTCGTAGCTC
pET22b(+) *fetP Nco*I d	AAACCATGGGCTTTAAAGAGTACCCGGCAGGC
pET22b(+) *fetP Xho*I u	AAACTCGAGGCTGCCGCGCGGCACCAGGCCGCTGCTGTTCAG ACCGACATATTTAAACTC

Table S2. FetP is a dimeric protein[a]

Addition	s_{app}, (S)	Mr_{app}, (Da)
none	2.7	35,000
100 mM NaCl	2.8	35,400
20 mM NaCl	2.8	34,800
300 µM $FeCl_3$	2.7	34,500
300 µM $ZnCl_2$	2.7	34,600
300 µM $CuCl_2$	2.8	37,000

[a]Periplasmic FetP without any tag was incubated for 30 min with 10 mM EDTA and dialyzed against 25 mM Tris/HCl, pH 7.2 in ultrapure water. Analytical ultracentrifugation was performed with 17 µM FetP in 300 µl sample in the absence or presence of the indicated metal salts.

SUPPLEMENTARY FIGURES

Figure S1

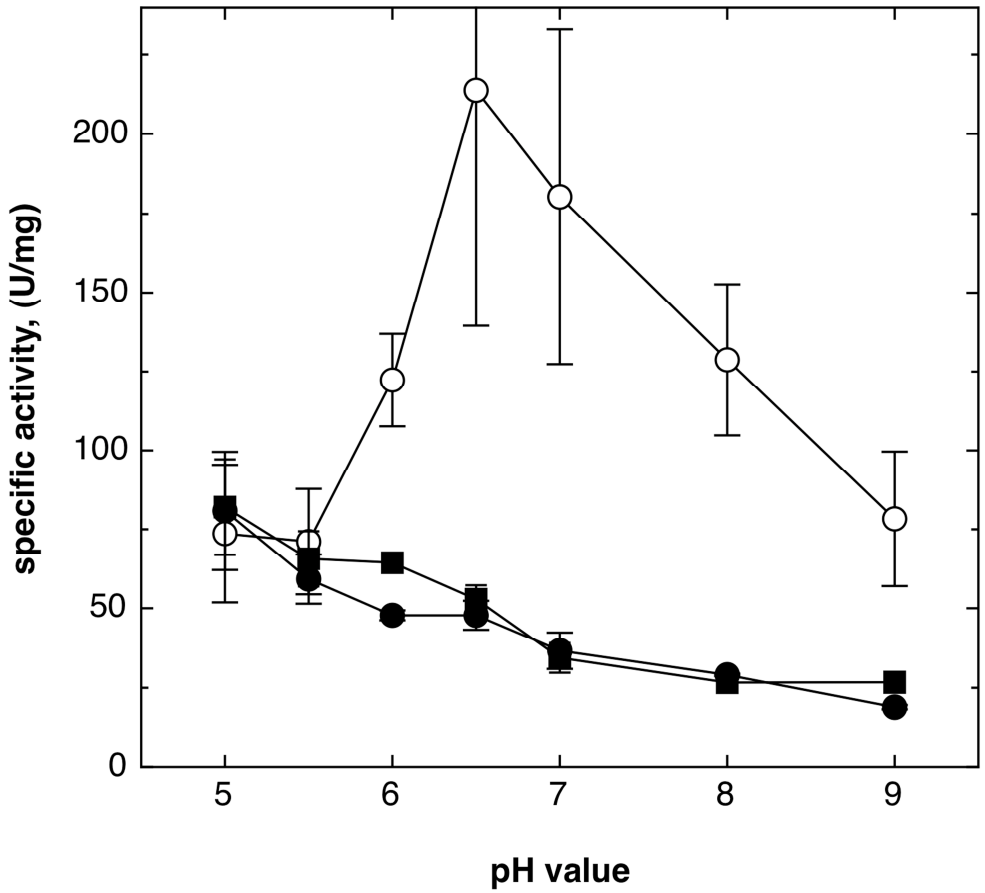

Figure S1. Expression of *fetMp-lacZ* in the natural host *E. coli* strain F11. A *fetMp-lacZ* reporter gene fusion was inserted single copy into the chromosome of the uropathogenic wild type strain F11. The cells were incubated in the presence of 50 µM DIP (open circles, ○), Fe(III)Cl_3 (closed circles, ●) or Fe(II)SO_4 (closed squares, ■) and specific β-galactosidase activity was determined. DIP, four experiments, each iron species two experiments, deviation bars shown.

Figure S2

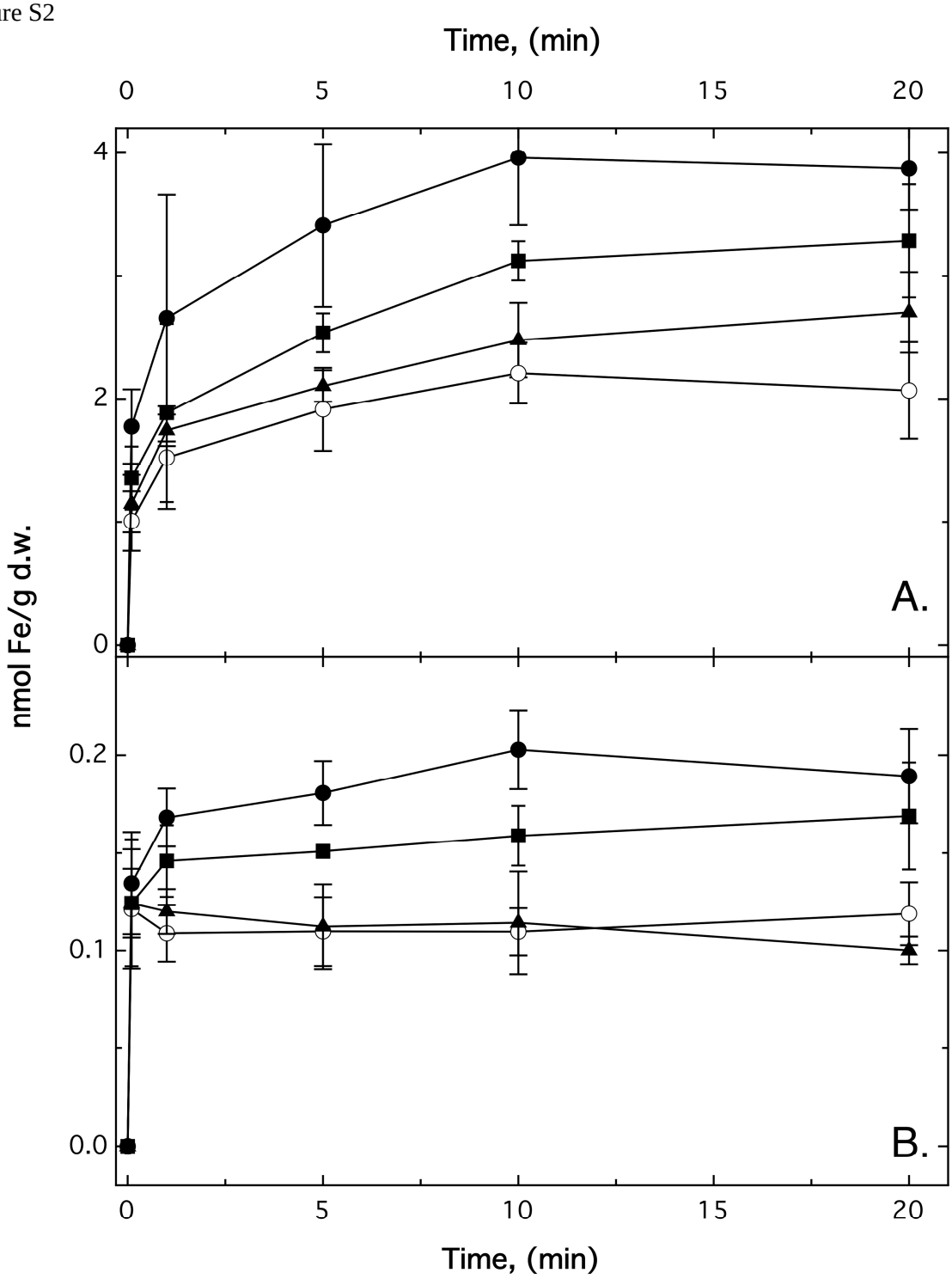

Figure S2. Iron uptake at pH 9 and 5. Uptake of ^{55}Fe by cells of strain ECA611 (*glmS-Gm*) (○), ECA612 (*glmS-fetMP*) (●), ECA613 (*glmS-fetM*) (■) and ECA614 (*glmS-fetP*) (▲) using the filtration method at pH 9 (panel A) and pH 5 (panel B). For the uptake experiment to cells were added $^{55}Fe(II)Cl_3$ at a final 1 µCi, 1 mM ascorbate, 5 µM $FeSO_4$, and samples were removed at indicated time points. Averages of three independent experiments with standard deviations (error bars) are shown.

Figure S3

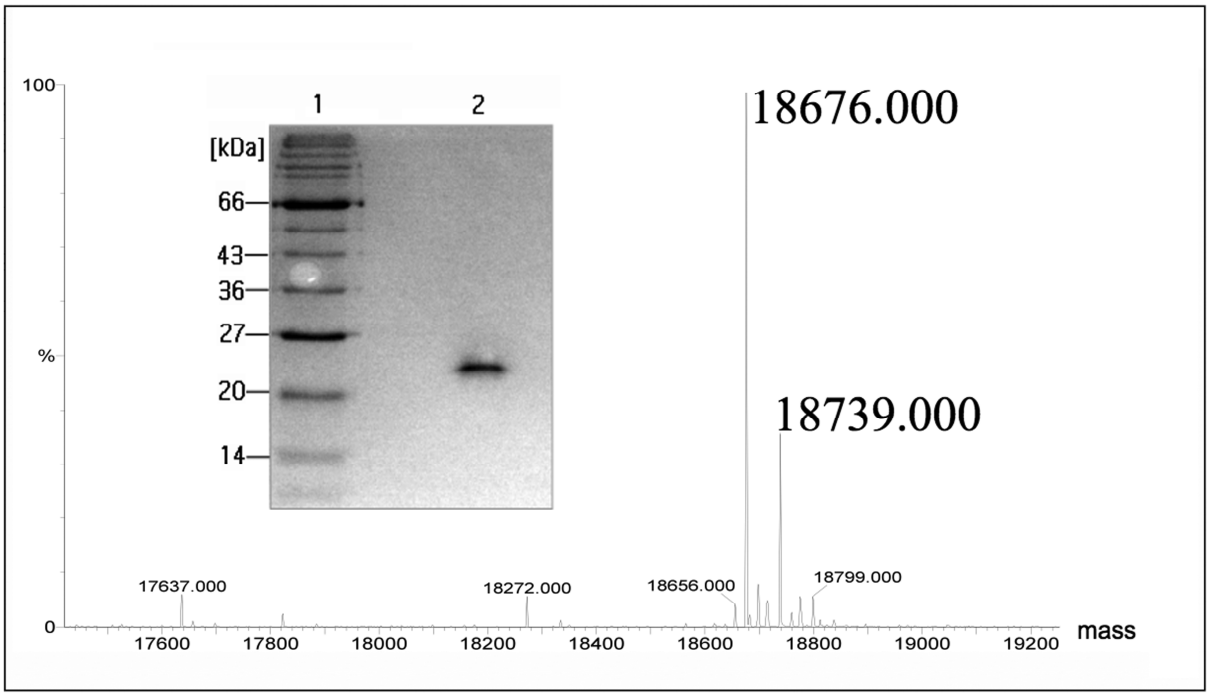

Figure S3. Purification of Strep-tagged FetP. MALDI-TOF analysis of the purified FetP protein yielding two size peaks of 18.676 and of 18.739 kDa. Inset: Coomassie-stained SDS PAGE of FetP after Strep-tactin affinity chromatography. The gel was loaded with 4 µg protein in lane 2 and a marker in lane 1 with sizes indicated on the left.

Figure S4

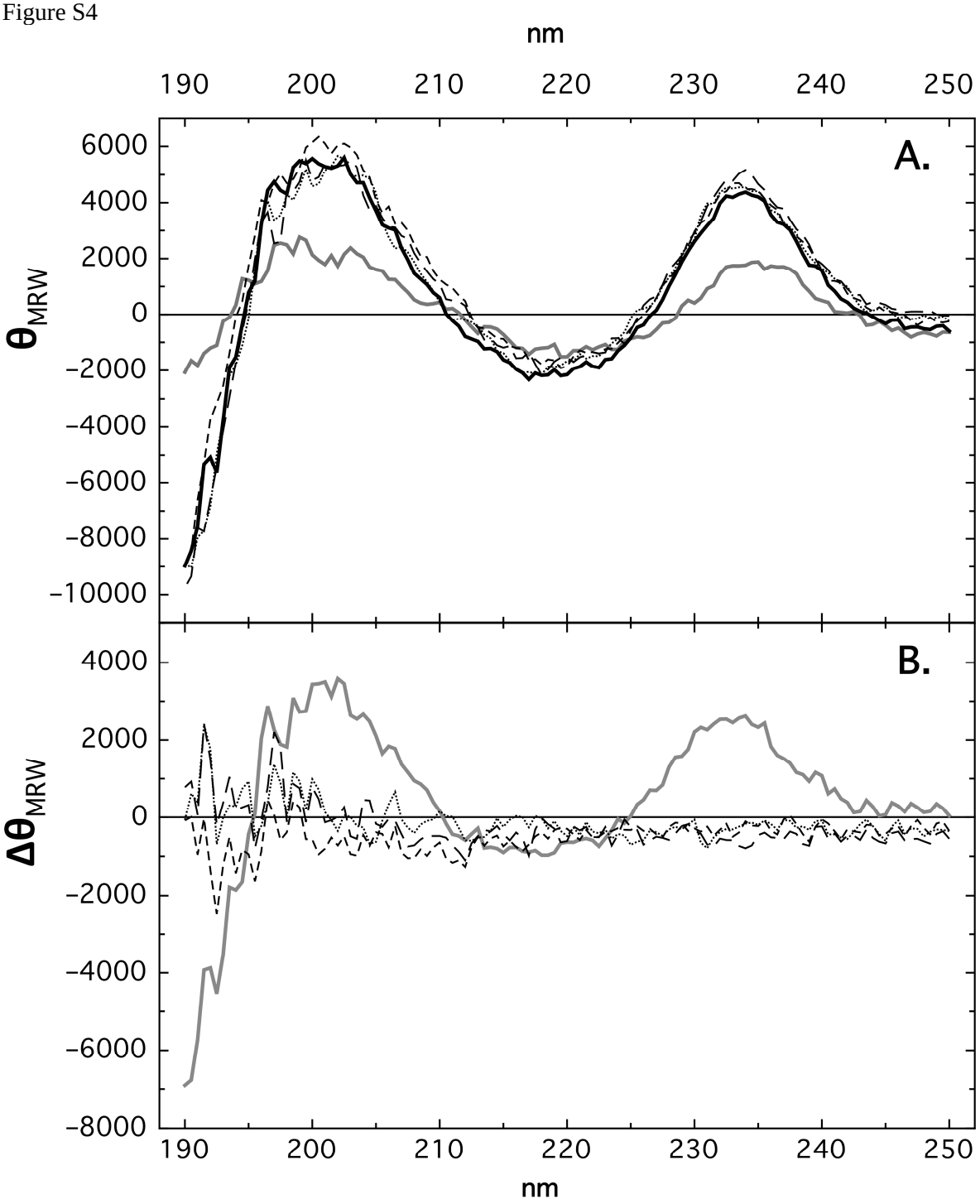

Figure S4. CD spectrum of FetP. (A) circular dichroism spectrum ($\theta_{MRW)}$ of FetP (51 µM) is shown in the absence of metals (thick solid black line), and in the presence of 150 µM $ZnCl_2$ (dotted black line), 100 µM $MnCl_2$ (short-distance dashed black line), 100 µM $FeSO_4$/1 mM ascorbate (long-distanced dashed black line) or 100 µM $CuCl_2$ (thick grey line). (B) The difference spectrum ($\Delta\theta_{MRW,}$ none minus respective metal) in 25 mM Tris/HCl buffer (pH 7.2), 25°C.

Figure S5a

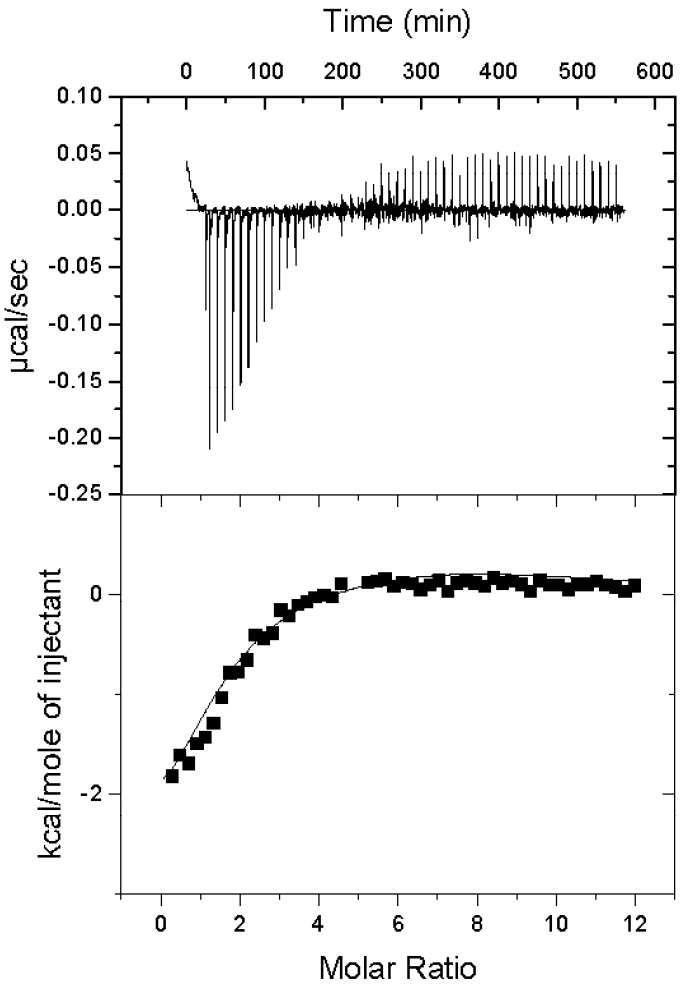

Figure S5a. Isothermal calorimetric assay of FetP titrated with copper in Bis-Tris buffer. A representative titration curve is shown. Top: Baseline-subtracted raw data. Bottom: Peak-integrated and concentration-normalized enthalpy changes vs. Cu(II)/FetP ratios. FetP protomer concentration was used for analysis.

Figure S5b

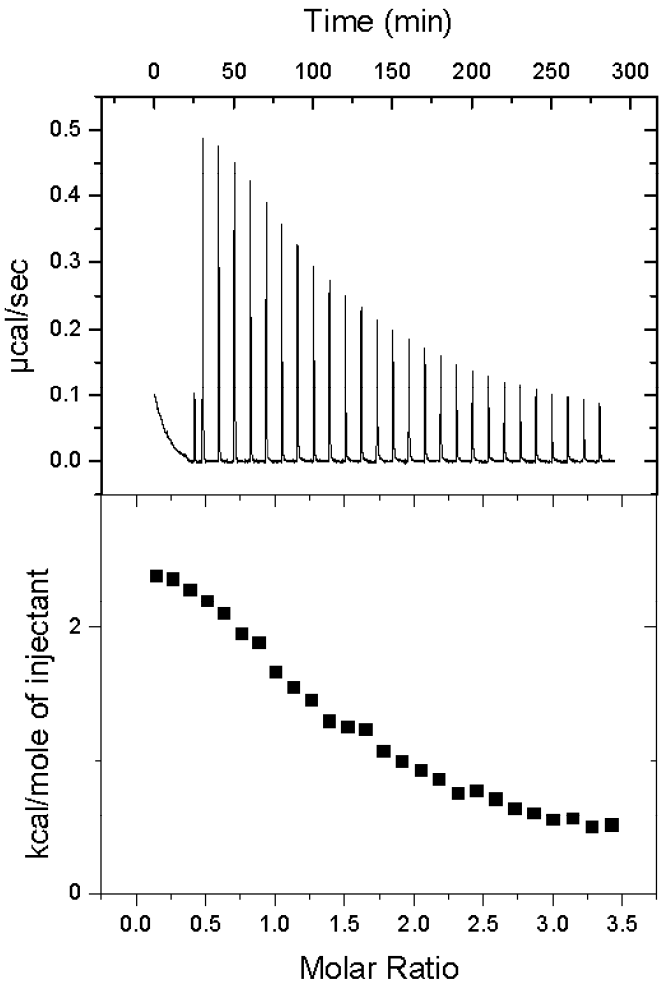

Figure S5b. Isothermal calorimetric assay of FetP titrated with zinc in ACES-buffer. A representative titration curve is shown. Top: Baseline-subtracted raw data. Bottom: Peak-integrated and concentration-normalized enthalpy changes vs. Zn(II)/FetP ratios. FetP dimer concentration was used for analysis.

Figure S5c.

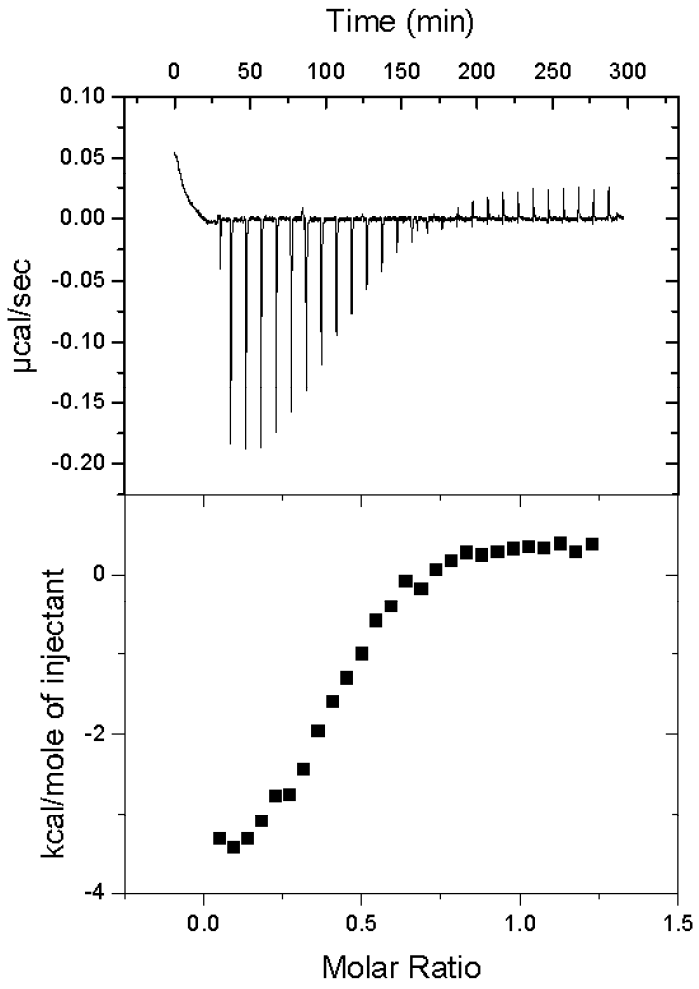

Figure S5c. Isothermal calorimetric assay of FetP titrated with manganese in Bis-Tris buffer. A representative titration curve is shown. Top: Baseline-subtracted raw data. Bottom: Peak-integrated and concentration-normalized enthalpy changes vs. Mn(II)/FetP ratios. FetP dimer concentration was used for analysis.

Figure S5d

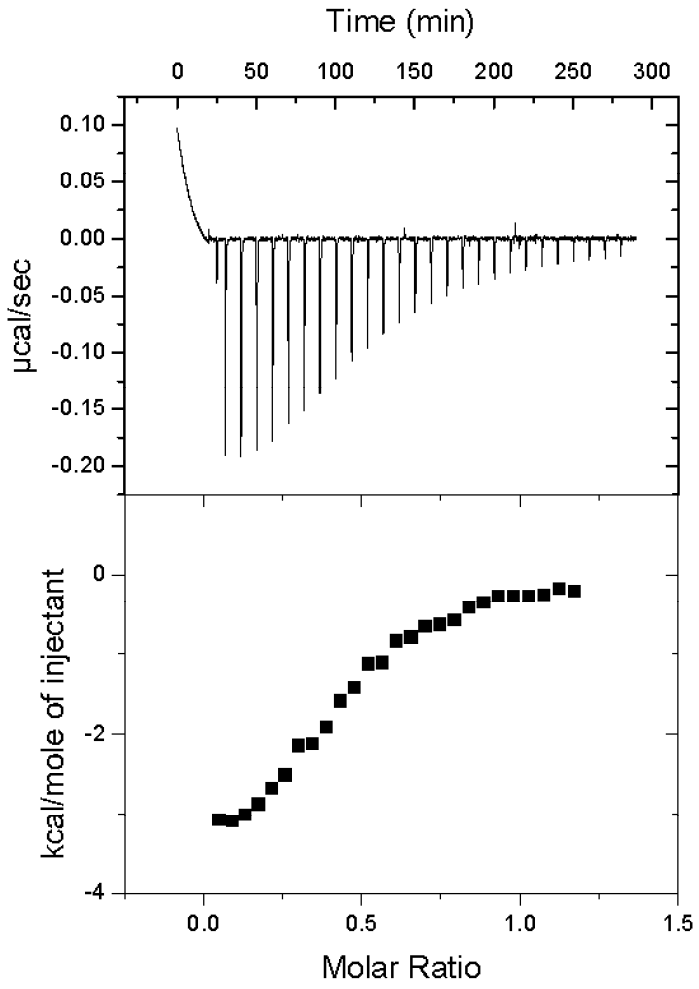

Figure S5d. Isothermal calorimetric assay of FetP titrated with manganese in ACES buffer. A representative titration curve is shown. Top: Baseline-subtracted raw data. Bottom: Peak-integrated and concentration-normalized enthalpy changes vs. Mn(II)/FetP ratios. FetP dimer concentration was used for analysis.

Figure S6

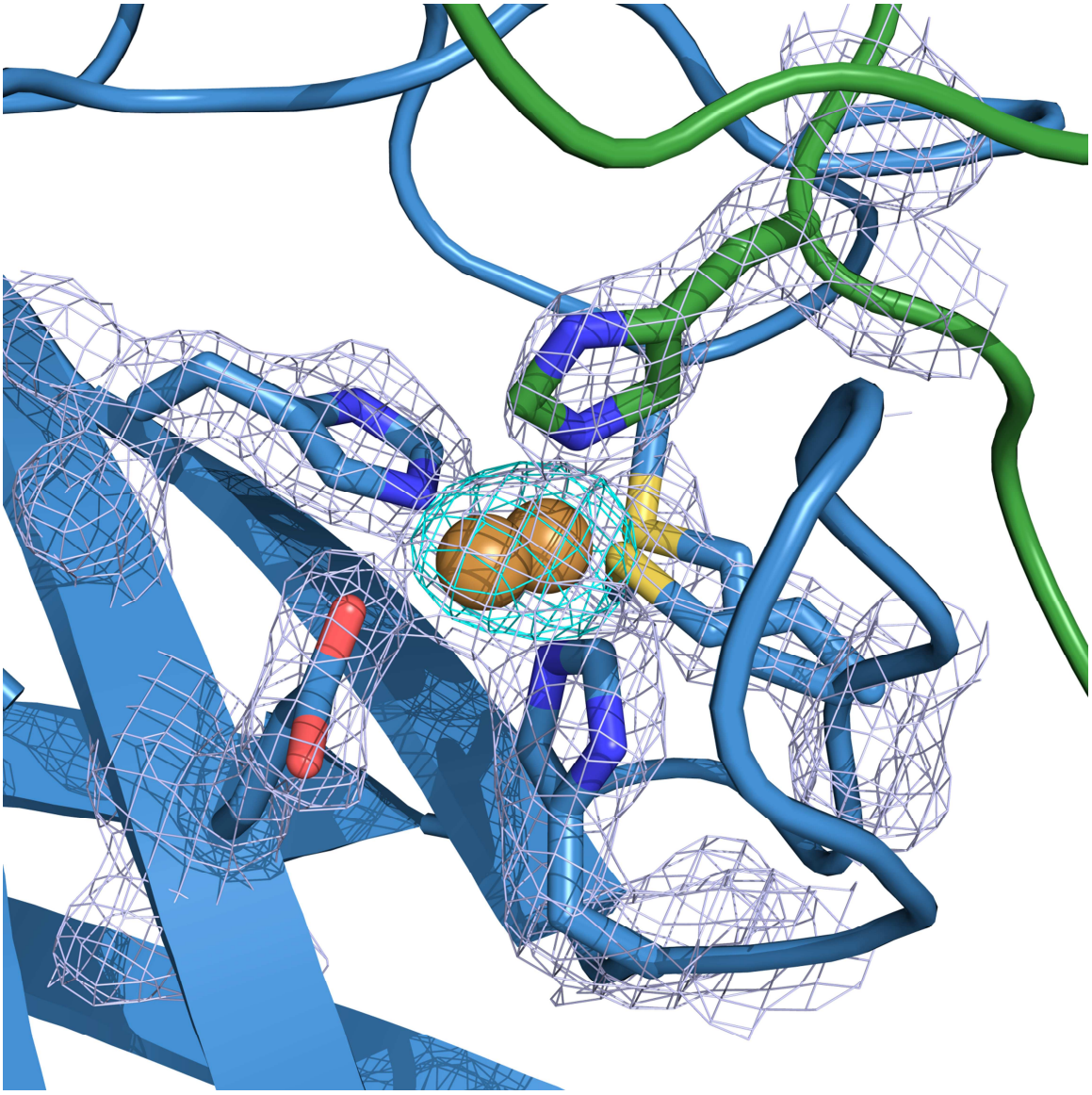

Figure S6. Electron density at the copper binding site demonstrating multiple copper positions. $2F_o$-F_c map in blue contoured at 1 σ and copper anomalous map contoured at 5 σ in teal.

Figure S7

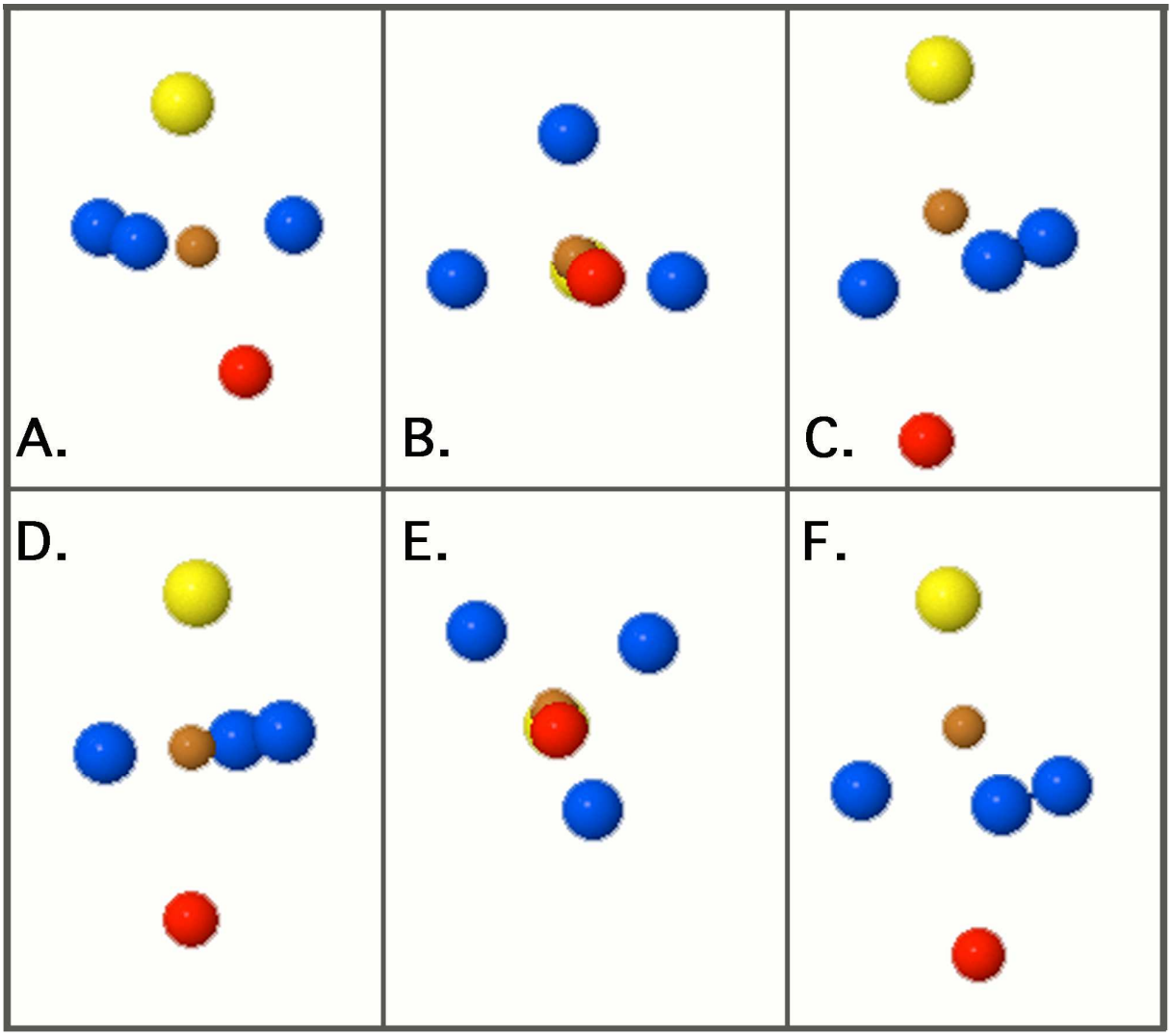

Figure S7. Arrangement of of the copper atoms in the copper centers. Panel A shows CuA1 from the side and panel B from the bottom. The O-Cu distance is 2.6 Å and the N-Cu distance is about 1.9 Å. The angle between the N-Cu bonds are close to a right angle. Cu in CuA1 is slightly below the plane defined by the three N atoms. CuA2 is shown in panel C. Panel D shows CuB1 from the side and panel E from the bottom. The O-Cu distance is 3.0 Å and the Cu is located in the plane defined by the N atoms. Again, the N-Cu bonds come close to forming right angles. Panel F shows CuB2. Atoms are shown with different colors: brown (Cu), red (O from Glu), blue (N from His) and yellow (S from Met). The picture was prepared with Geneious 4.8.5 (www.geneious.com).

Figure S8

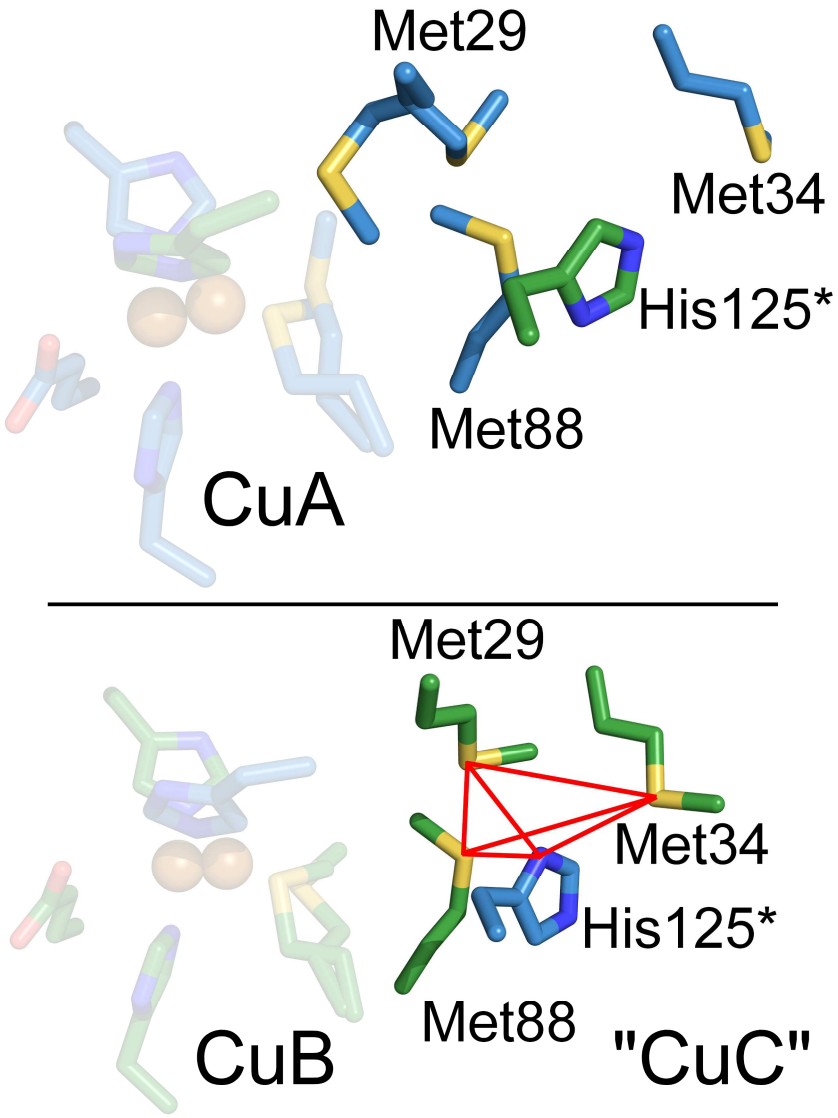

Figure S8. A putative third metal binding site "CuC" exists adjacent to CuB. A Met-rich third metal binding site ("CuC") may exist when the Cu site is in the CuB conformation. "CuC" is indicated by the red tetrahedron and is formed by Met_{29}, Met_{34}, and Met_{88} from the same protomer and His_{125}* from the other protomer. Adjacent to CuA, Met_{29}, Met_{88} and His_{125}* are also close together but M_{34} is far away.

Figure S9

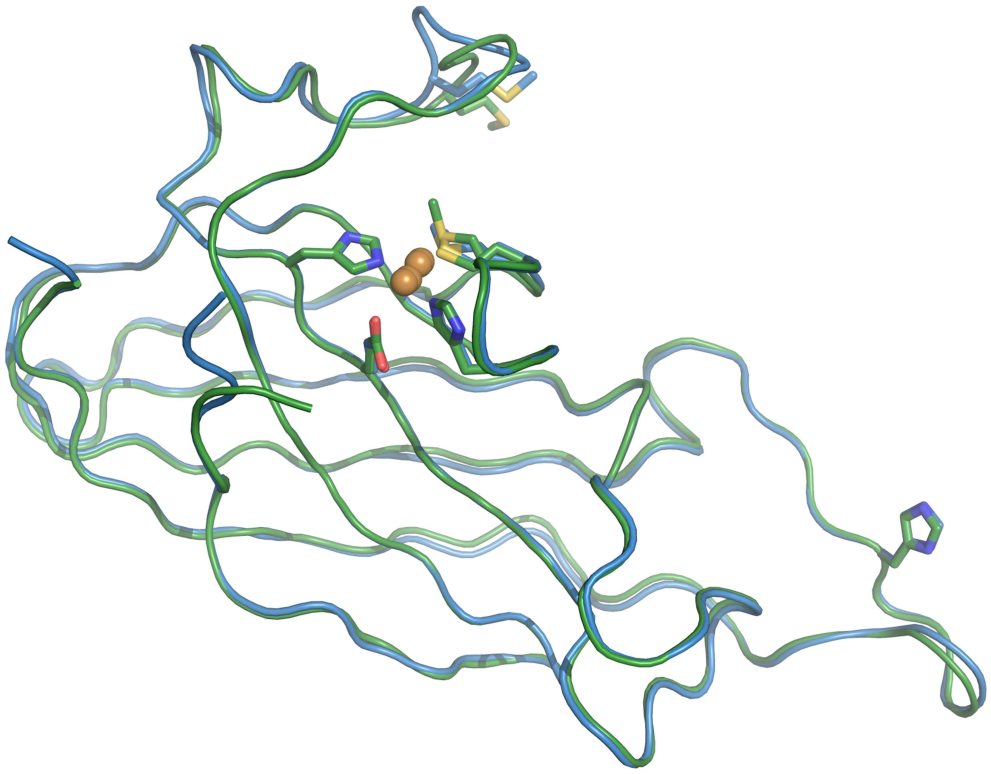

Figure S9. Superposition of the two Cu-FetP protomers.