Structural and Biochemical Characterization of the Flavin-Dependent Siderophore-Interacting Protein from *Acinetobacter baumannii*

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Supplemental Materials

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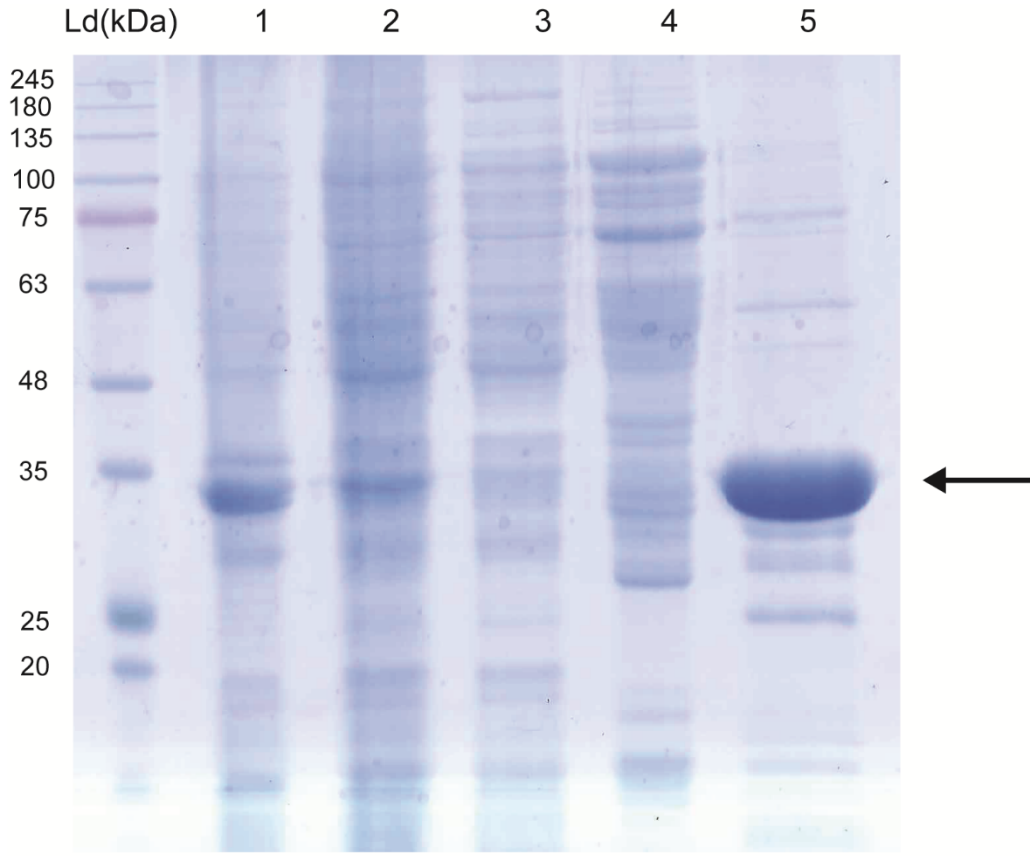

Figure S1. BauF purification with immobilized metal affinity chromatography (IMAC). Lane 1) lysate pellet, 2) lysate supernatant, 3) flow-through, 4) 30 mM imidazole wash, and 5) 150 mM imidazole elution. The molecular weight of BauF is 33 kDa (*black arrow*).

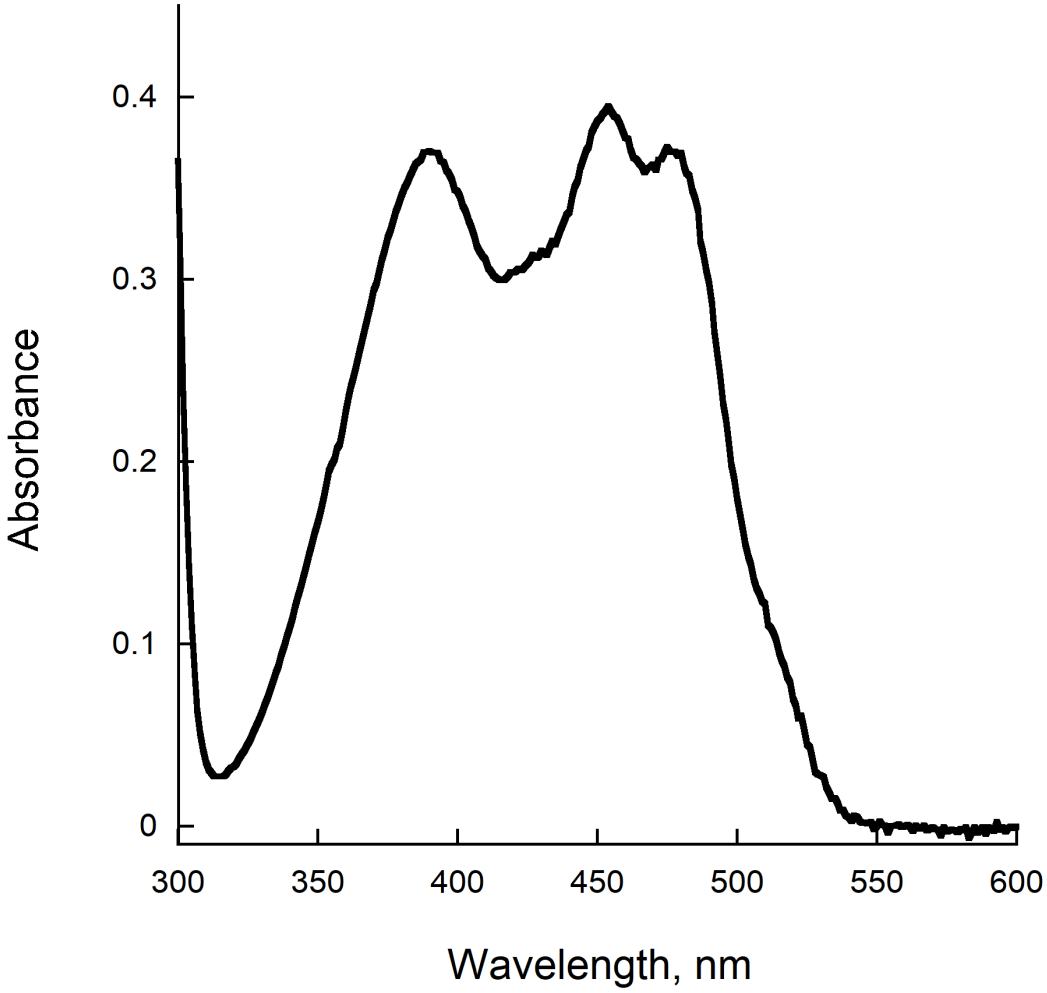

Figure S2. UV-visible spectrum of 37 µM BauF in 25 mM HEPES pH 7.5 100 mM NaCl.

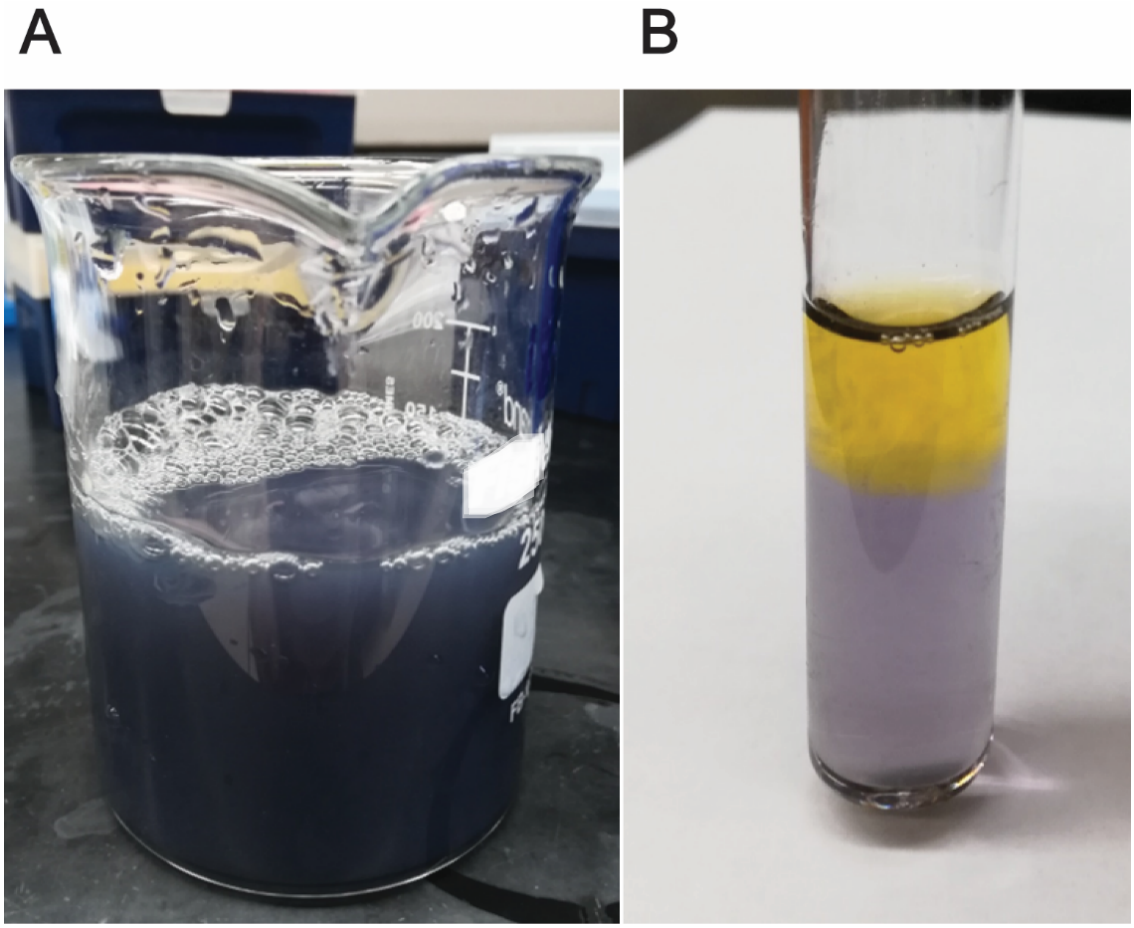

Figure S3. Color observed for BauF during purification. (*A*) BauF supernatant after cell lysis, prior to column loading. (*B)* Protein elution fraction collected during IMAC purification. The blue-gray fraction turned yellow over the period of minutes.

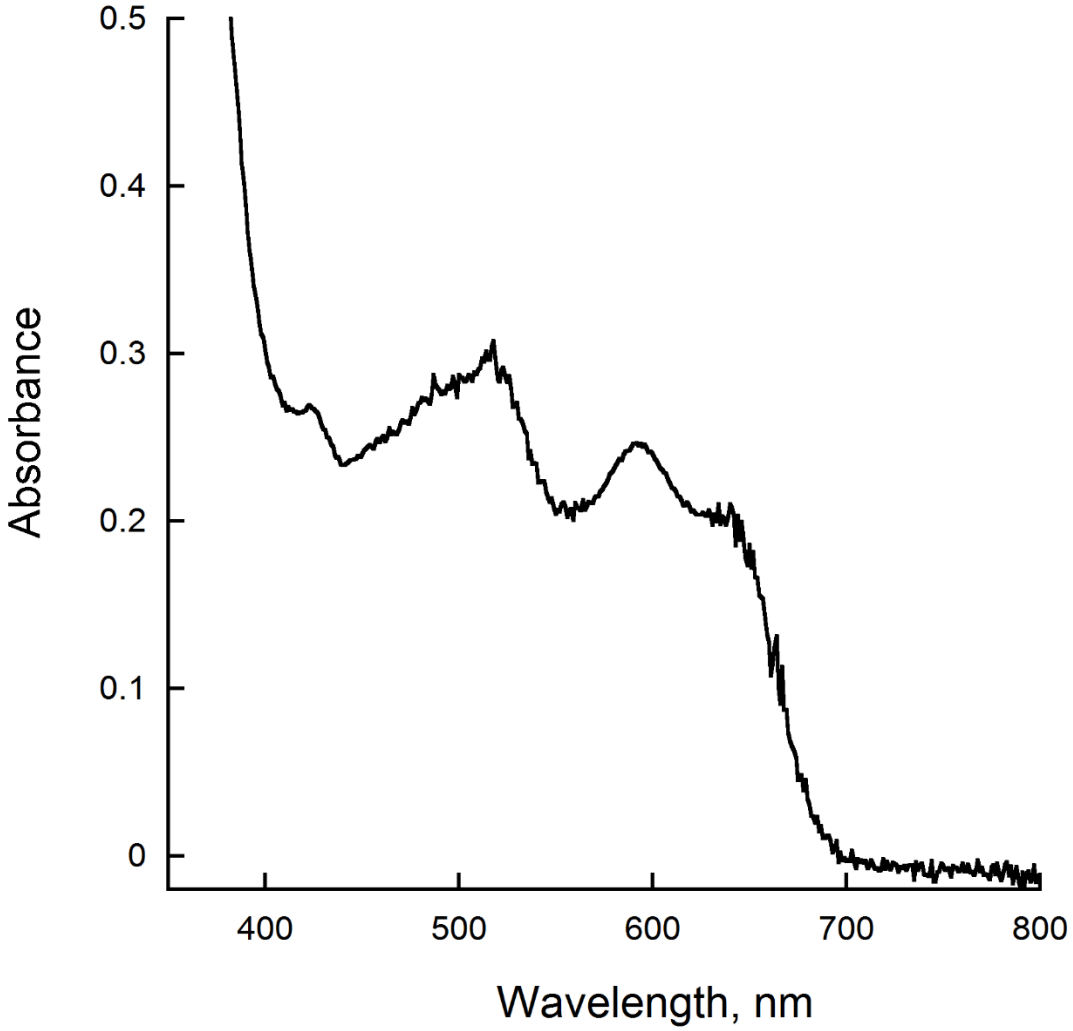

Figure S4. UV-Visible spectrum of the blue-gray protein elution fraction collected during IMAC purification.

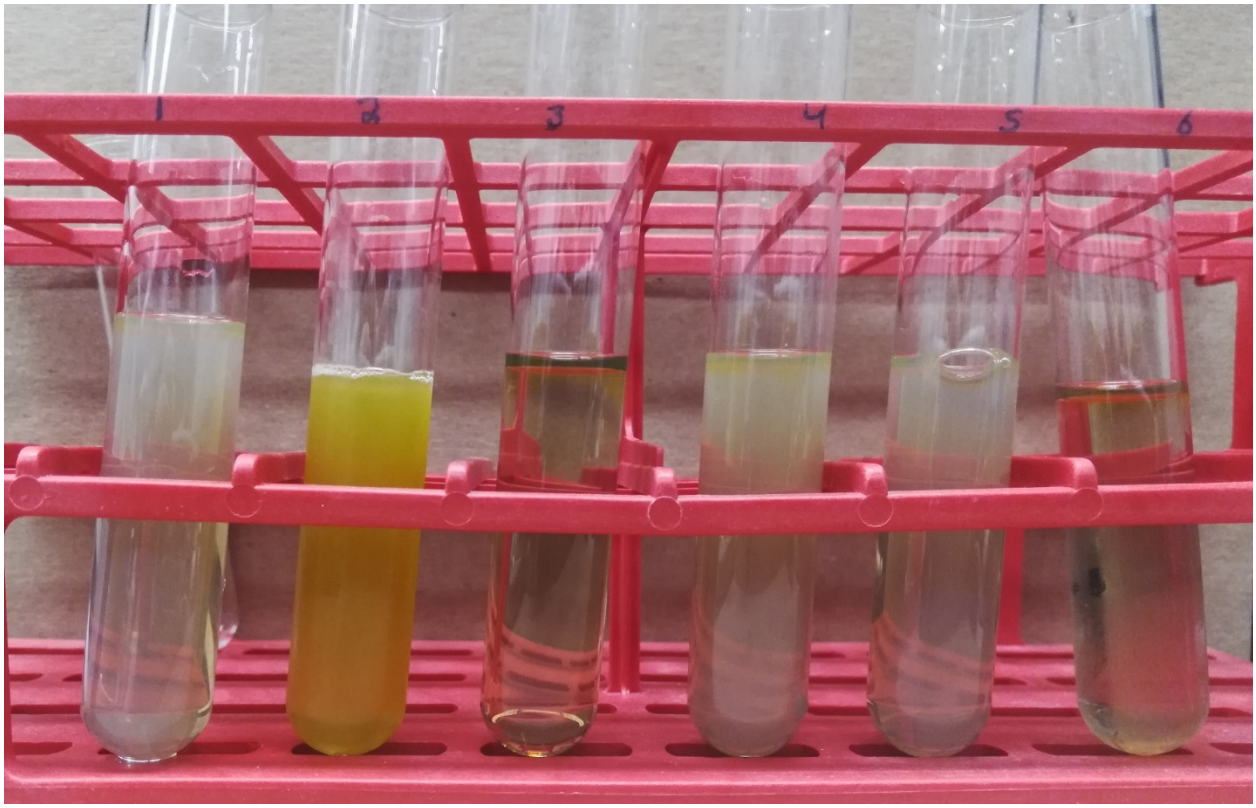

Figure S5. Supernatant of lysed ArticExpress *E. coli* cells expressing BauF. From left to right: 1) BauF supernatant in 25 mM HEPES pH 7.5, 300 mM NaCl, 10 mM imidazole; 2) supernatant after 10s vortexing; 3) supernatant supplemented with 1% Triton X-100; 4) supernatant supplemented with 1% TWEEN 20; 5) supernatant supplemented with 10 mM TCEP; 6) supernatant supplemented with 1% Triton X-100 and 10 mM TCEP.

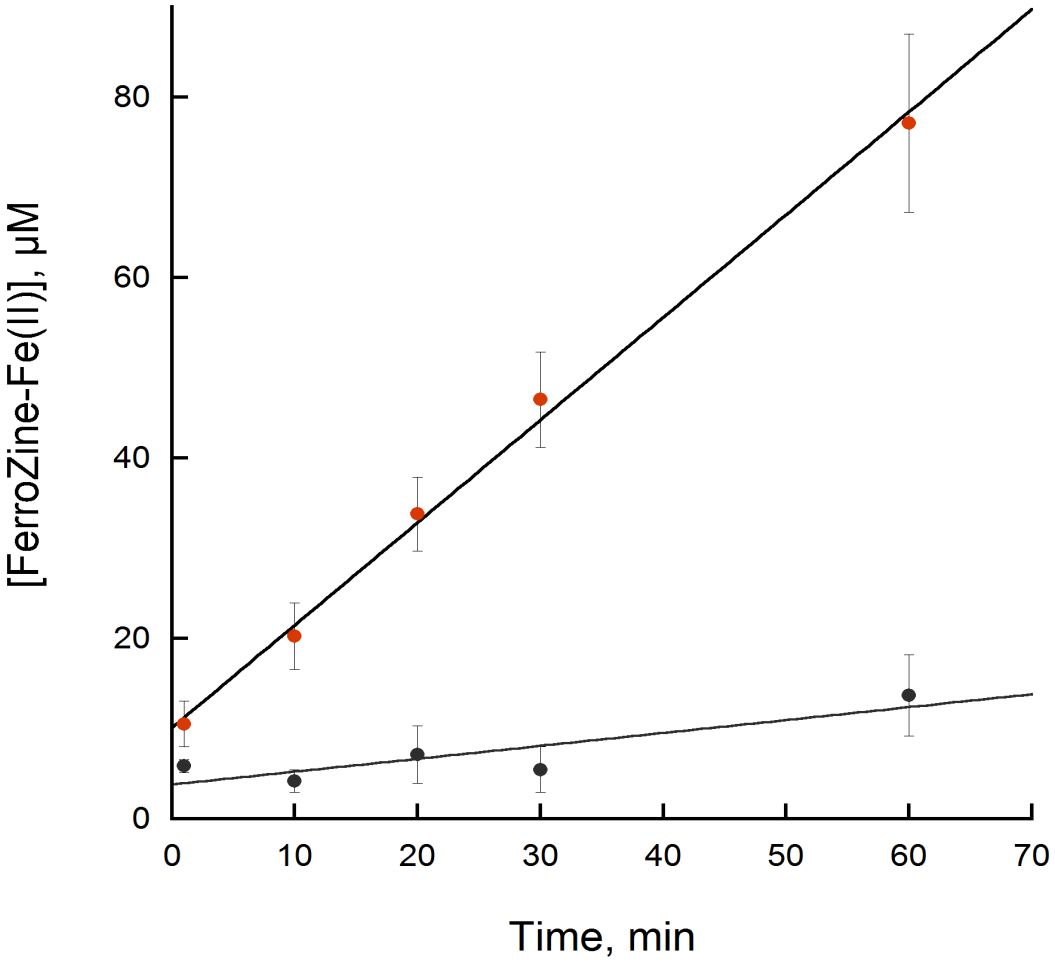

Figure S6. Detection of FerroZine-Fe^{+2} complex formation at 560 nm. The reactions contained 15 µM BauF, 0.4 mM Acb-Fe, and 1 mM NADH (*red*) or no NADH (*black*). The determined rates were $2x10^{-4} \pm 5x10^{-5}$ s^{-1}(*black*) and $1x10^{-3} \pm 3x10^{-4}$ s^{-1}(*red*). Activity with NADPH was similar to NADH (not shown).