**SUPPORTING INFORMATION**

*Bordetella pertussis* FbpA binds both unchelated iron and iron-siderophore complexes

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**Iron-Alcaligin Coordination Chemistry**

Alcaligin (ALK), the natural siderophore synthesized by *Bordetella Pertussis*, is a macrocyclic tetravalent bis-hydroxamic acid chelator (see Figure S1) with a high affinity for Fe$^{3+}$. As a tetravalent ligand it readily forms an Fe$^{3+}$ complex with stoichiometry Fe$_2$(ALK)$_3$ with a single bridging ALK$^{2-}$ di-anion between two Fe(ALK)$^+$ units.\(^1\) The models of Fe$_2$(ALK)$_3$ and Fe(ALK)$^+$ in Figure S2 and Figure 5 respectively are based on the crystal structure reported by Raymond, et al.\(^2\) Relevant aqueous solution complexation equilibria for Fe$^{3+}$ and ALK are as follows:\(^1,2\)

\[
\begin{align*}
\text{Fe(OH}_2\text{)}_6^{3+} + \text{ALK}^{2-} & \rightleftharpoons \text{Fe(ALK)(OH}_2\text{)}_2^{+} + 4\text{H}_2\text{O} & K_1 &= 10^{23.5} \\
2 \text{Fe(ALK)(OH}_2\text{)}_2^{+} + \text{ALK}^{2-} & \rightleftharpoons (\text{ALK})\text{Fe(ALK)}\text{Fe(ALK)} + 4\text{H}_2\text{O} & K_2 &= 10^{17.74} \\
2 \text{Fe(OH}_2\text{)}_6^{3+} + 3 \text{ALK}^{2-} & \rightleftharpoons (\text{ALK})\text{Fe(ALK)}\text{Fe(ALK)} + 12\text{H}_2\text{O} & \beta_{230} &= 10^{64.66}
\end{align*}
\]

The large value for $K_1$ is illustrative of the high Fe$^{3+}$ affinity of the hydroxamic acid chelate unit and the preorganization of the Fe$^{3+}$ binding site in the alcaligin ring. The crystal structure of metal free alcaligin shows that there is no change in atomic positions on forming the Fe(ALK)$^+$ terminal unit in (ALK)Fe(ALK)Fe(ALK), indicative of preorganization of the binding cavity.\(^2\) The overall stability of the coordinatively saturated Fe$_2$(ALK)$_3$ complex is very high (Equation (S3)) with a log$\beta$ value of 32.3 per Fe$^{3+}$ center, in excess of that found for other hydroxamic acid siderophores such as ferrioxamine B, for example.\(^3\) The pFe value for ALK is 23.0.
Although the Fe$_2$(ALC)$_3$ complex exhibits a high thermodynamic stability, the assembly is kinetically labile and can readily dissociate to form the Fe(ALC)(OH$_2$)$_2^+$ unit (reverse of Equation (S2)). The proton driven Fe$_2$(ALC)$_3$ dissociation rate constant is $1.6 \times 10^2$ M$^{-1}$s$^{-1}$. This dimer dissociation reaction, which provides vacant coordination sites on the Fe$^{3+}$ for FbpA$_{Bp}$ protein binding to Fe(ALC)$^+$, is suggested as the cause of the spectral shift shown in Figure 4.

Fe$^{3+}$ dissociation from the preorganized ALC cavity in Fe(ALC)$^+$ is very slow, consistent with the large equilibrium constant for Eq (S1). The monomer/dimer speciation for the Fe$^{3+}$/ALC system has been investigated as a function of pH and Fe$^{3+}$/ALC ratio by ESI-MS. Thermodynamic characterization of the Fe$^{3+}$/ALC system also includes measurement of the Fe$^{3+}$/Fe$^{2+}$ redox potential. The limiting pH independent redox potential obtained above pH 9 is $E_{1/2} = -446$ mV (NHE); this potential shifts in the positive direction (Fe$^{3+}$ more easily reduced) as the pH decreases.
Figure S1. Chemical structures of alcaligin, enterobactin, desferrichrome, and desferrioxamine B.
Figure S2. A model of Fe$_2$ALC$_3$ in the binding cleft of FbpA$_{Bp}$ (open conformation). Docked structure created with Patchdock using 1Y9U as receptor and the ferric alcaligin complex (obtained from CCDC:TEQKUV) as ligand. Panel a) shows the Fe$_2$ALC$_3$ molecule in the cleft with N lobe to the left of the figure, the ERRAT score for this model is 97.377. Panel b) is an enlarged version to show the Fe$^{3+}$ binding site in the model, which shows that both Fe$^{3+}$ ions are completely sequestered by the siderophores. The coordination bonds to Fe$^{3+}$ are depicted as dashed red lines. Fluorescence emission titration indicates $\sim$0.1 µM binding affinity. Both the closed and open conformations of FbpA$_{Bp}$ were used for modeling, but the open conformation gave better results.
Figure S3. A model of Fe-ferrichrome in the binding cleft of FbpA<sub>Bp</sub>. Docked structure created with Patchdock using 2OWT as receptor and the ferrichrome structure, FCE, downloaded from Protein Data Bank as ligand. Panel a) shows the Fe-ferrichrome molecule in the cleft with N lobe to the left of the figure, the ERRAT score for this model is 95.098. Panel b) is an enlarged version to show the Fe<sup>3+</sup> binding residues in the model. This view shows that Fe<sup>3+</sup> is completely sequestered by the six donors from the hydroxamate siderophore ferrichrome. The coordination bonds to Fe<sup>3+</sup> are depicted as dashed red lines. Fluorescence emission titration indicates ~1.0 µM binding affinity. Both the closed and open conformations of FbpA<sub>Bp</sub> were used for modeling, but the closed conformation gave better results.
Figure S4. A model of Fe-DFB in the binding cleft of FbpA_{Bp}. Docked structure created with Patchdock using 2OWT as receptor and ferrioxamine B (obtained from CCDC:155586) as ligand. Panel a) shows the Fe-DFB molecule in the cleft with N lobe to the left of the figure, the ERRAT score for this model is 95.425. Panel b) is an enlarged version to show the Fe\(^{3+}\) binding residues in the model. This view shows that Fe\(^{3+}\) is completely sequestered by the six donors from the hydroxamate siderophore DFB. The coordination bonds to Fe\(^{3+}\) are depicted as dashed red lines. The protonated amine tail group of DFB is located near the conserved tyrosine residues in our model. Fluorescence emission titration indicates ~1.0 \(\mu\)M binding affinity.
FIGURE S5. A model of Fe-ENT in the binding cleft of FbpA<sub>Bp</sub>. Docked structure created with Patchdock using 1Y9U as receptor and the enterobactin structure, EB4, downloaded from Protein Data Bank as ligand. Panel a) shows the Fe-ENT molecule in the cleft with N lobe to the left of the figure, the ERRAT score for this model is 97.377. Panel b) is an enlarged version to show the Fe<sup>3+</sup> binding residues in the model, with the ion completely sequestered by the siderophore. The coordination bonds to Fe<sup>3+</sup> are depicted as dashed red lines. Fluorescence emission titration indicates ~1.0 µM binding affinity.
Figure S6. Representative plot of Equation (5) for FbpA_Bp fluorescence emission quenching data at 352 nm for reaction with increasing amounts of Fe-DFB. The protein and ligand were dissolved in 50 mM MES, 100 mM NaCl at pH 6.5. Excitation wavelength 297 nm; [apo-FbpA_Bp] = 3 µM; [Fe-DFB] = 0 - 23 µM. Filled circles represent actual data points corresponding to the addition of each aliquot of Fe-DFB into the FbpA_Bp solution. The solid line represents the best fit of Equation (5) to the data. The average of multiple independent determinations yields $K_d$ for Equation (3) $\sim$ 1.0 µM and a $Q_{max} \sim$ 40%.
Figure S7. Representative plot of Equation (5) for FbpA<sub>Bp</sub> fluorescence emission quenching data at 352 nm for reaction with increasing amounts of Fe-ENT. The protein and ligand were dissolved in 50 mM MES, 100 mM NaCl at pH 6.5. Excitation wavelength at 297 nm; [apo-FbpA<sub>Bp</sub>] = 0.5 µM; [Fe-ENT] = 0 - 2 µM. Filled circles represent actual data points corresponding to the addition of each aliquot of Fe-ENT into the FbpA<sub>Bp</sub> solution. The solid line represents the best fit of Equation (5) to the data. The average of multiple independent determinations yields $K_d$ for Equation (3) $\sim$ 1.0 µM and a $Q_{\text{max}}$ $\sim$ 60%. 
Figure S8. Representative plot of Equation (5) for FbpA<sub>Bp</sub> fluorescence emission quenching data at 352 nm for reaction with increasing amounts of Fe-FC. The protein and ligand were dissolved in 50 mM MES, 100 mM NaCl at pH 6.5. Excitation wavelength at 297 nm; [apo-FbpA<sub>Bp</sub>] = 3µM; [Fe-FC] = 0 - 6 µM. Filled circles represent actual data points corresponding to the addition of each aliquot of Fe-FC into the FbpA<sub>Bp</sub> solution. The solid line represents the best fit of Equation (5) to the data. The average of multiple independent determinations yields K_d for Equation (3) ~ 1.0 µM and a Q<sub>max</sub> ~ 20%.
Figure S9. Representative fluorescence emission spectra for FbpA<sub>Bp</sub> in the presence of increasing aliquots of apo-ALC. The protein and ligand were dissolved in 50 mM MES, 100 mM NaCl at pH 6.5. Excitation wavelength 297 nm; [apo-FbpA<sub>Bp</sub>] = 1µM; [ALC] = 0 – 4 µM. The ligand:protein concentration ratio at the end of the titration was 4:1. Successive addition of ligand to the protein solution gave rise to very small change in emission intensity of the 352 nm band for FbpA<sub>Bp</sub> without any change in band position. Similar quenching was observed when apo-ENT was titrated into apo-FbpA<sub>Bp</sub>.
Figure S10. FbpAbp purification. a) Coomassie blue-stained SDS-PAGE gel (12% polyacrylamide) showing DEAE CL-6B fractions. L, BRM72 (pBBR/fbpAbp) osmotic shock fluid dialysate; Ft, flow-through (unbound fraction); W, wash; 22-38, 0.0 to 0.5 M NaCl salt gradient eluate fractions. b) Coomassie blue-stained SDS-PAGE gel (12% polyacrylamide) showing purified FbpA (the dialyzed and concentrated FbpA peak fraction pool). M, molecular mass markers, masses in kDa shown at left.
Table S1. Amino acid residues in the anion binding sites from different ferric iron binding proteins

| Genera (PDB code) | Arg105 | Arg14 | Glu15 | Leu18 | Lys40 | Val62 | Asp63 | Gln140 | Pro142 | Asn193 |
|-------------------|--------|-------|-------|-------|-------|-------|-------|--------|--------|--------|
| Haemophilus (1D9V); Neisseria (1D9Y) | - | Q | H | A | - | E | Q | S | A | - |
| Pseudomonas (no model: AAG08074) | - | Q | H | T | - | E | E | S | A | - |
| Burkholderia (no model: YP_001888563) | - | Q | H | V | E | E | D | D | - | - |
| Yersinia (1XVX) | - | Q | H | - | G | E | N | G | D | Y |
| Bordetella (2OWT) | - | - | - | - | - | N | - | - | - | - |
| Mannheimia (1S10,1Q35) | - | - | Q | - | D | - | - | K | S | - |
| Vibrio (no model: WP_001885727) | - | - | Q | - | - | A | - | K | - | - |
| Campylobacter (1Y4T) | - | - | H | A | - | A | E | T | P | - |
| Synechocystis (2PT2, 2VP1) | - | - | H | T | K/S | - | - | S | E/V | - |

1. In cases where no PDB code is available, the GenBank accession number for the sequence is provided.
References

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