Outer-Sphere Effects on Reduction Potentials of Copper Sites in Proteins: The Curious Case of High Potential Type 2 C112D/M121E *Pseudomonas aeruginosa* Azurin

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Supporting Information

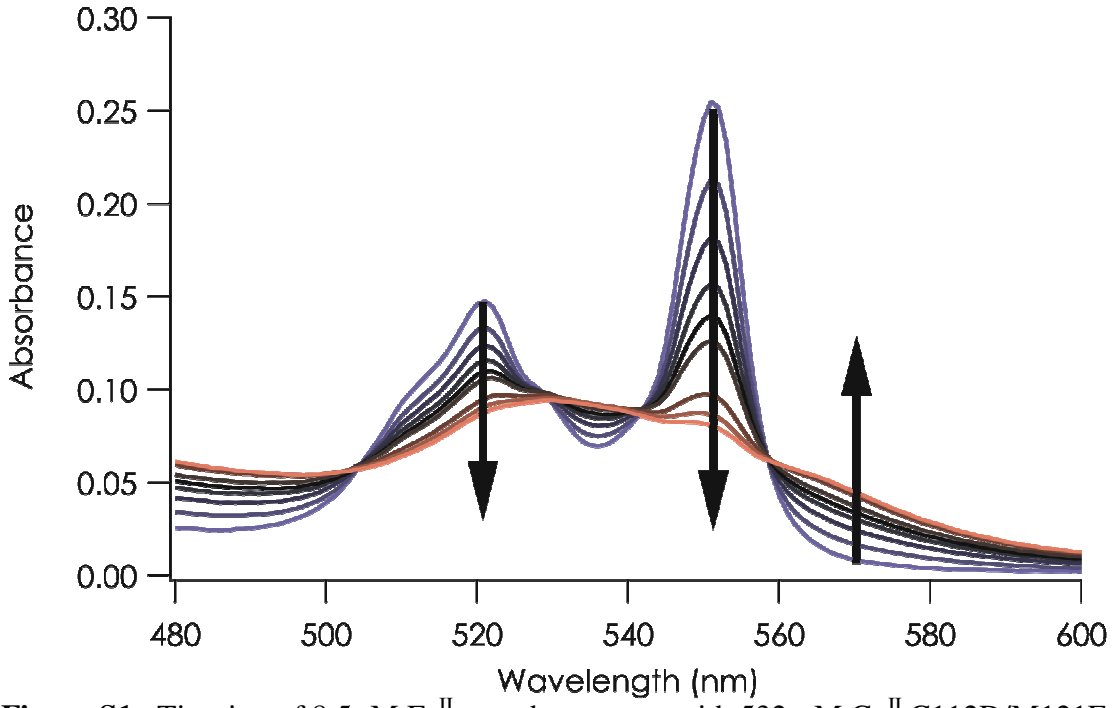

Figure S1. Titration of 8.5µM Fe^{II} cytochrome c_{551} with 532 µM Cu^{II} C112D/M121E azurin in 50 mM HEPES, pH 7.0. Generally 3-5 minutes were required after addition and mixing to achieve equilibrium across the pH range in which the titrations were conducted. Titrations were carried out in triplicate at each reported pH.

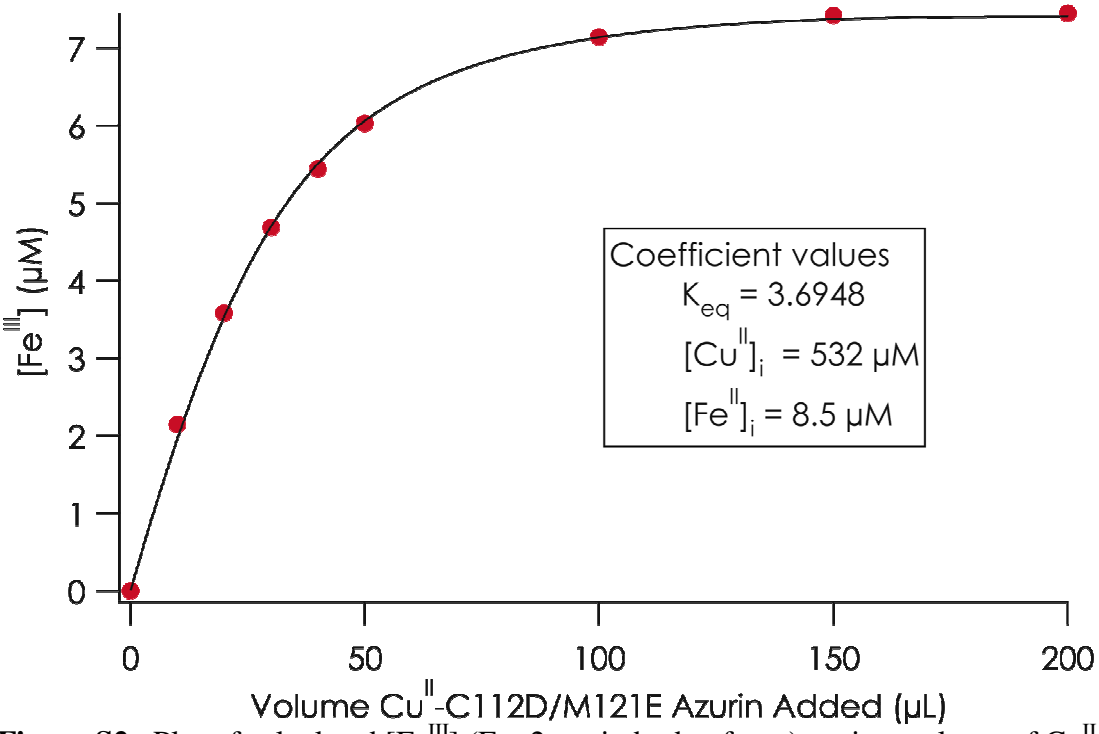

Figure S2. Plot of calculated [Fe^{III}] (Eq. 2, main body of text) against volume of Cu^{II} C112D/M121E azurin in 50 mM HEPES, pH 7.0. The data were fit to Eq. 1 (main body of text) to extract an equilibrium constant. This value was then inserted into the Nernst expression along with $E°_{1/2}$ for cytochrome c_{551} to calculate $E°_{1/2}$ for C112D/M121E azurin. Three such fits were averaged to yield the reported reduction potentials.

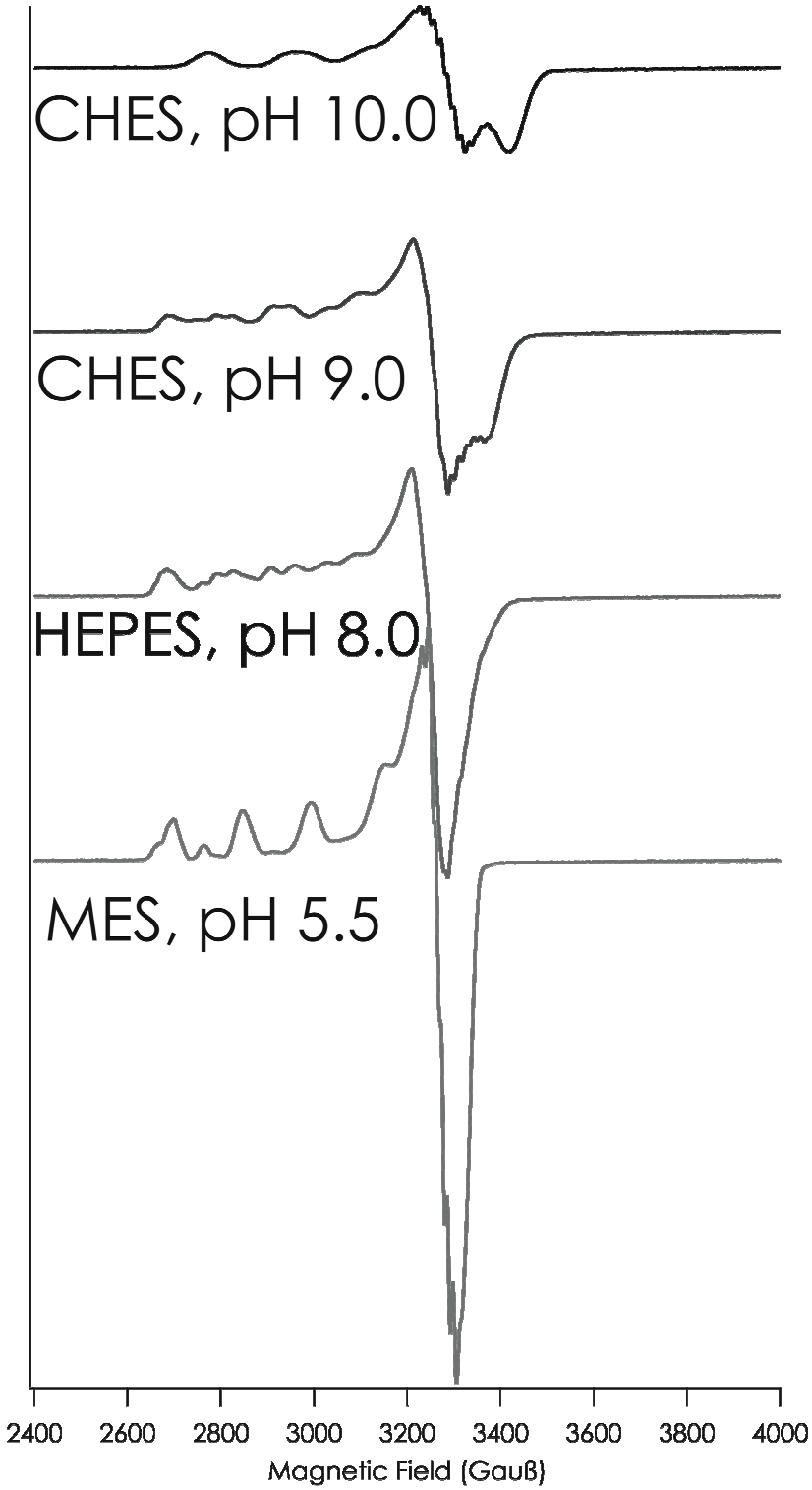

Figure S3. X-band EPRs of C112D/M121E azurin. in aqueous 77K glass containing 50 mM buffer.