

Supplementary Information

Spontaneous assembly of redox-active iron-sulfur clusters at low concentrations of cysteine

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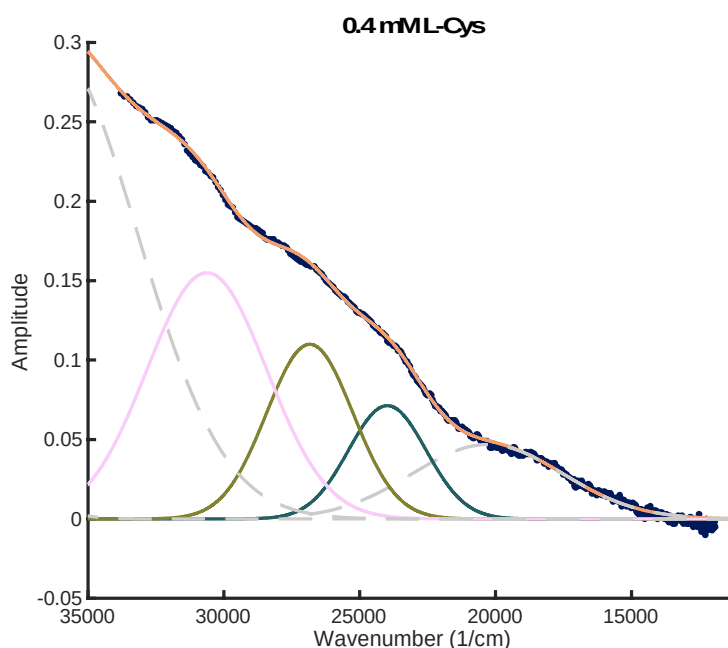
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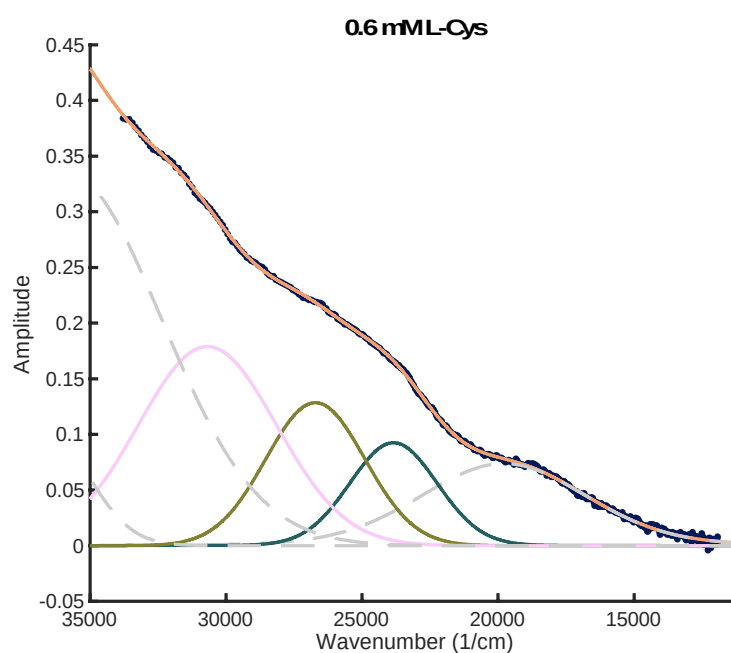
** These authors contributed equally to the work.*

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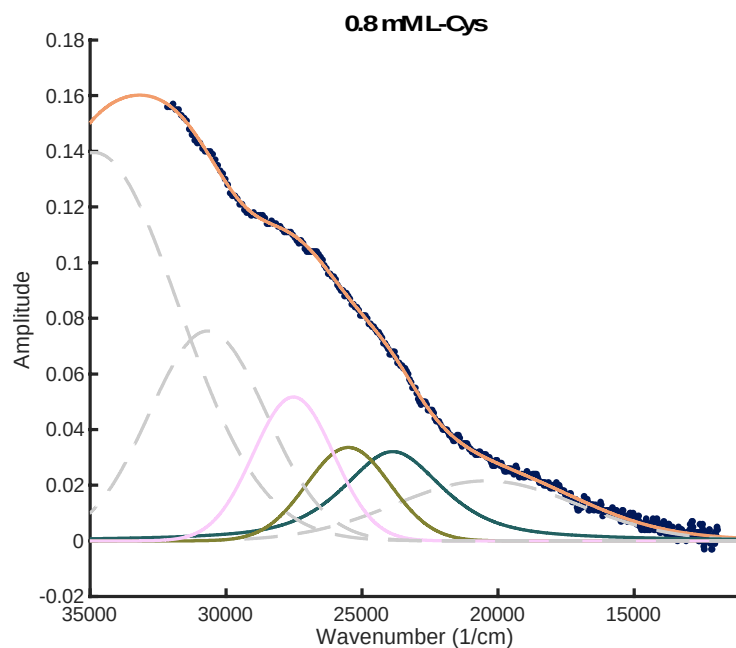
SI Results



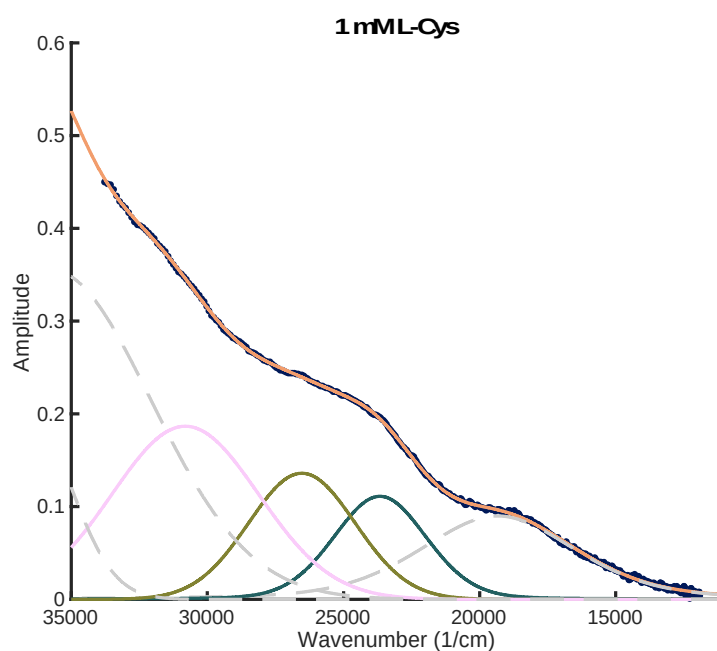
Supplementary Fig. 1. Plot of UV-Vis data for FeS clusters prepared with 0.4 mM L-cysteine in a 5:1:1 ratio with Fe and S. Fitting is based on the method of Galambas *et al.* (2019)³⁰ see also tutorials on <https://zenodo.org/record/3533467#.YIsCAxNKjAM>. Each peak corresponds to a specific excitation (From left to right: peak 1 grey dashed line – peptide charge-transfer; peak 2 grey dashed line – thiolate $RS^- \rightarrow Fe$; peak 3 pink line – thiolate $RS^- \rightarrow Fe$; peak 4 green line – $S^{2-} \rightarrow Fe$; peak 5 blue line – $S^{2-} \rightarrow Fe$; peak 6 grey dashed line – ligand field). Source data are provided as a Source Data file.



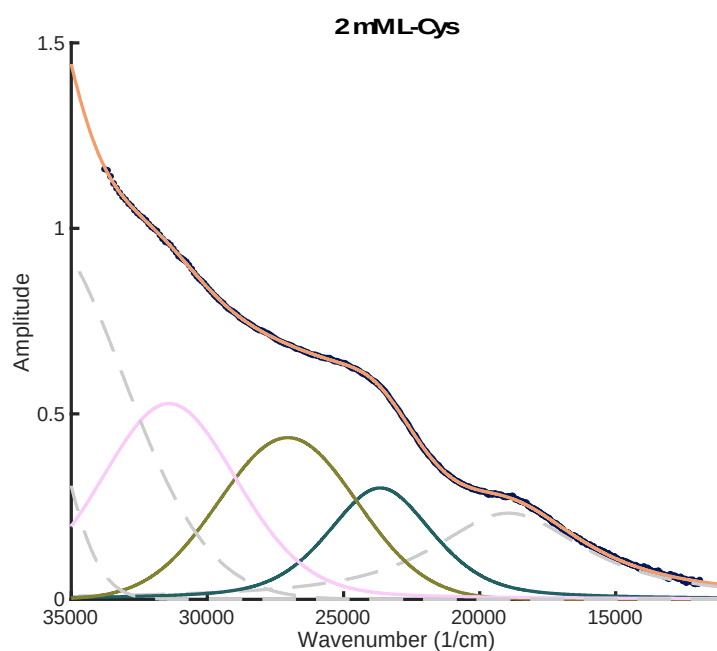
Supplementary Fig. 2. Plot of UV-Vis data for FeS clusters prepared with 0.6 mM L-cysteine in a 5:1:1 ratio with Fe and S. Fitting is based on the method of Galambas *et al.* (2019)³⁰. Each peak corresponds to a specific excitation (From left to right: peak 1 grey dashed line – peptide charge-transfer; peak 2 grey dashed line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 3 pink line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 4 green line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 5 blue line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 6 grey dashed line – ligand field). Source data are provided as a Source Data file.



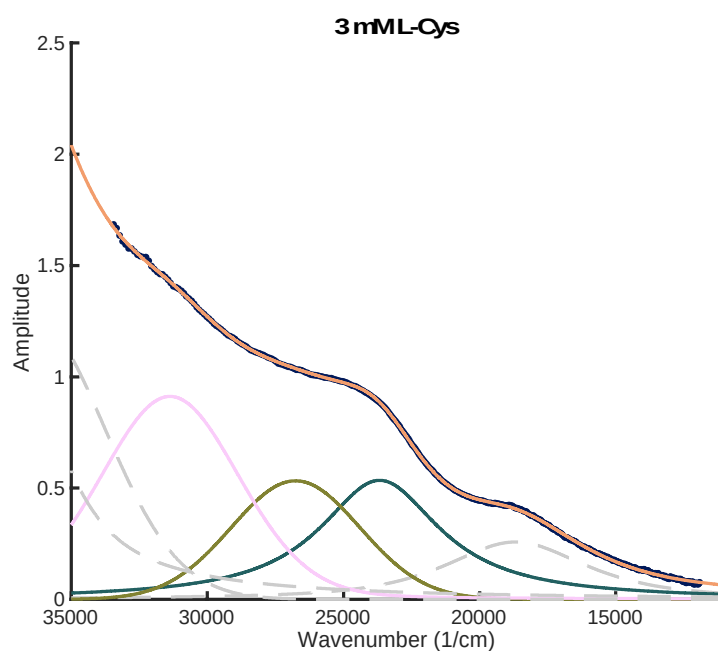
Supplementary Fig. 3. Plot of UV-Vis data for FeS clusters prepared with 0.8 mM L-cysteine in a 5:1:1 ratio with Fe and S. Fitting is based on the method of Galambas *et al.* (2019)³⁰. Each peak corresponds to a specific excitation (From left to right: peak 1 grey dashed line – peptide charge-transfer; peak 2 grey dashed line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 3 pink line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 4 green line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 5 blue line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 6 grey dashed line – ligand field). Source data are provided as a Source Data file.



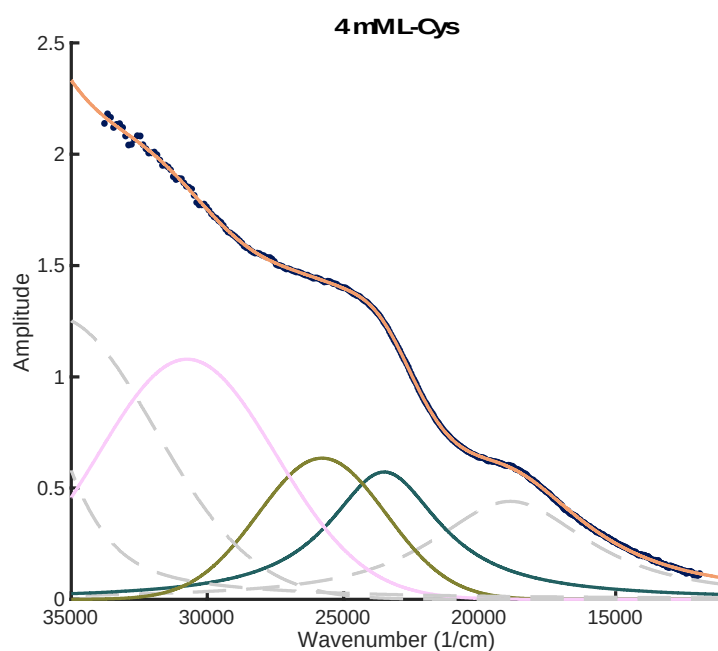
Supplementary Fig. 4. Plot of UV-Vis data for FeS clusters prepared with 1 mM L-cysteine in a 5:1:1 ratio with Fe and S. Fitting is based on the method of Galambas *et al.* (2019)³⁰. Each peak corresponds to a specific excitation (From left to right: peak 1 grey dashed line – peptide charge-transfer; peak 2 grey dashed line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 3 pink line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 4 green line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 5 blue line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 6 grey dashed line – ligand field). Source data are provided as a Source Data file.



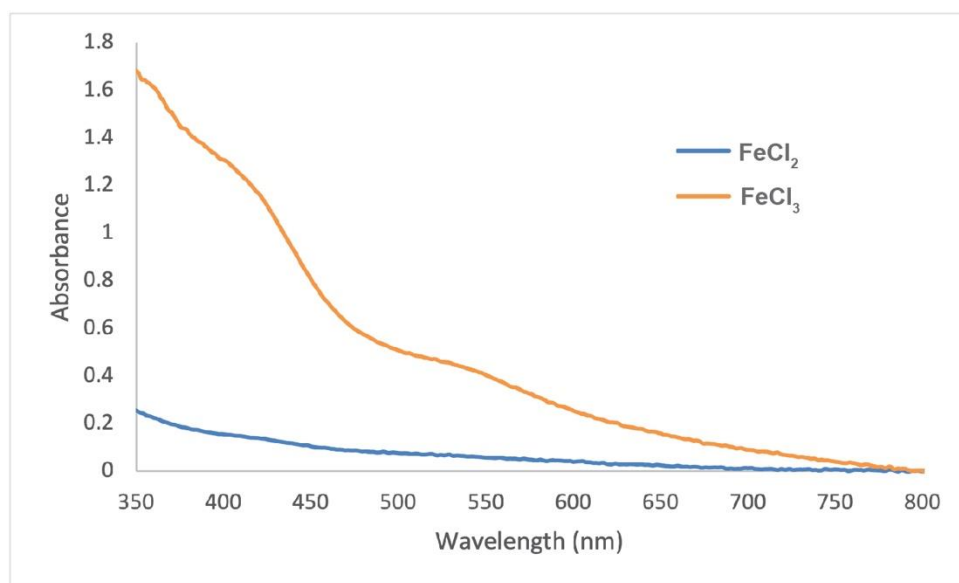
Supplementary Fig. 5. Plot of UV-Vis data for FeS clusters prepared with 2 mM L-cysteine in a 5:1:1 ratio with Fe and S. Fitting is based on the method of Galambas *et al.* (2019)³⁰. Each peak corresponds to a specific excitation (From left to right: peak 1 grey dashed line – peptide charge-transfer; peak 2 grey dashed line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 3 pink line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 4 green line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 5 blue line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 6 grey dashed line – ligand field). Source data are provided as a Source Data file.



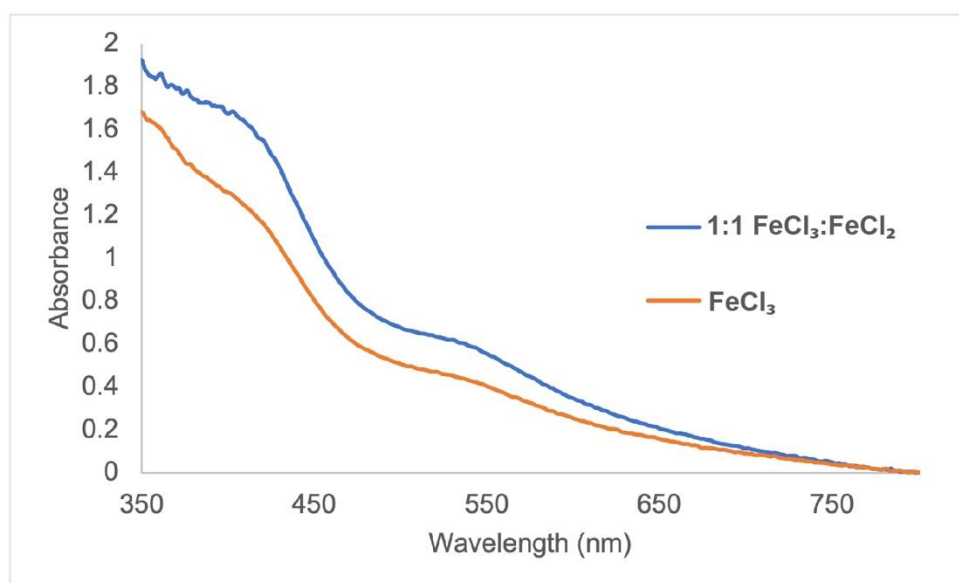
Supplementary Fig. 6. Plot of UV-Vis data for FeS clusters prepared with 3 mM L-cysteine in a 5:1:1 ratio with Fe and S. Fitting is based on the method of Galambas *et al.* (2019)³⁰. Each peak corresponds to a specific excitation (From left to right: peak 1 grey dashed line – peptide charge-transfer; peak 2 grey dashed line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 3 pink line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 4 green line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 5 blue line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 6 grey dashed line – ligand field). Source data are provided as a Source Data file.



Supplementary Fig. 7. Plot of UV-Vis data for FeS clusters prepared with 4 mM L-cysteine in a 5:1:1 ratio with Fe and S. Fitting is based on the method of Galambas *et al.* (2019)³⁰. Each peak corresponds to a specific excitation (From left to right: peak 1 grey dashed line – peptide charge-transfer; peak 2 grey dashed line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 3 pink line – thiolate $\text{RS}^- \rightarrow \text{Fe}$; peak 4 green line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 5 blue line – $\text{S}^{2-} \rightarrow \text{Fe}$; peak 6 grey dashed line – ligand field). Source data are provided as a Source Data file.

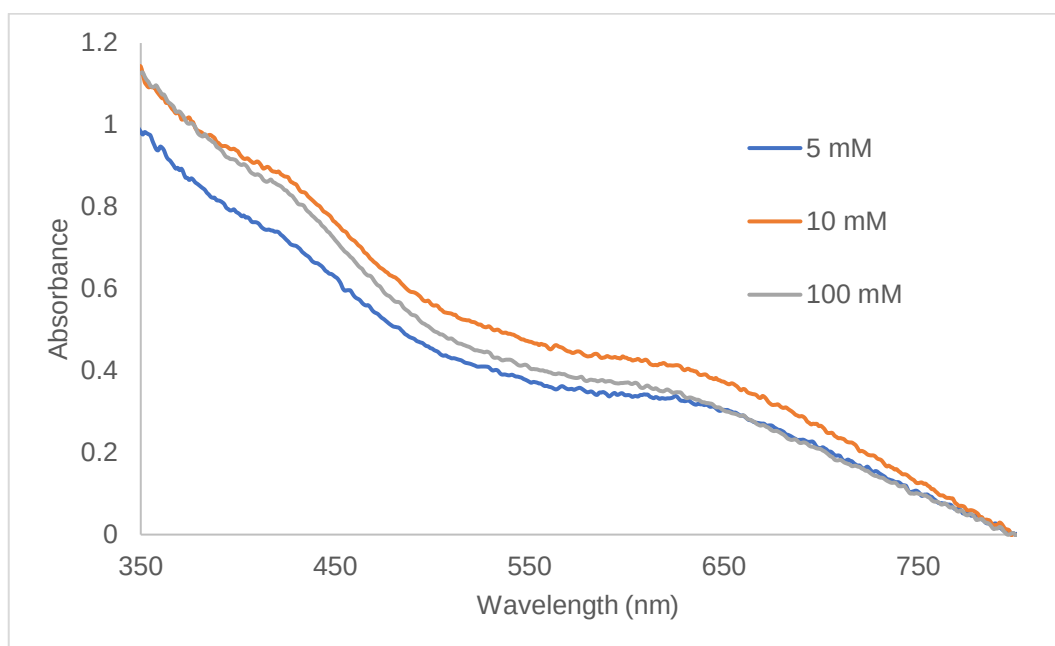


Supplementary Fig. 8. Representative UV-Vis spectrum for solution containing 5 mM L-cysteine, 1 mM FeCl₂ and 1 mM Na₂S at pH ~9 (blue line). Spectrum of solution prepared with 5 mM L-cysteine, 1 mM FeCl₃ and 1 mM Na₂S at pH 9 also included for reference (orange line). Source data are provided as a Source Data file.

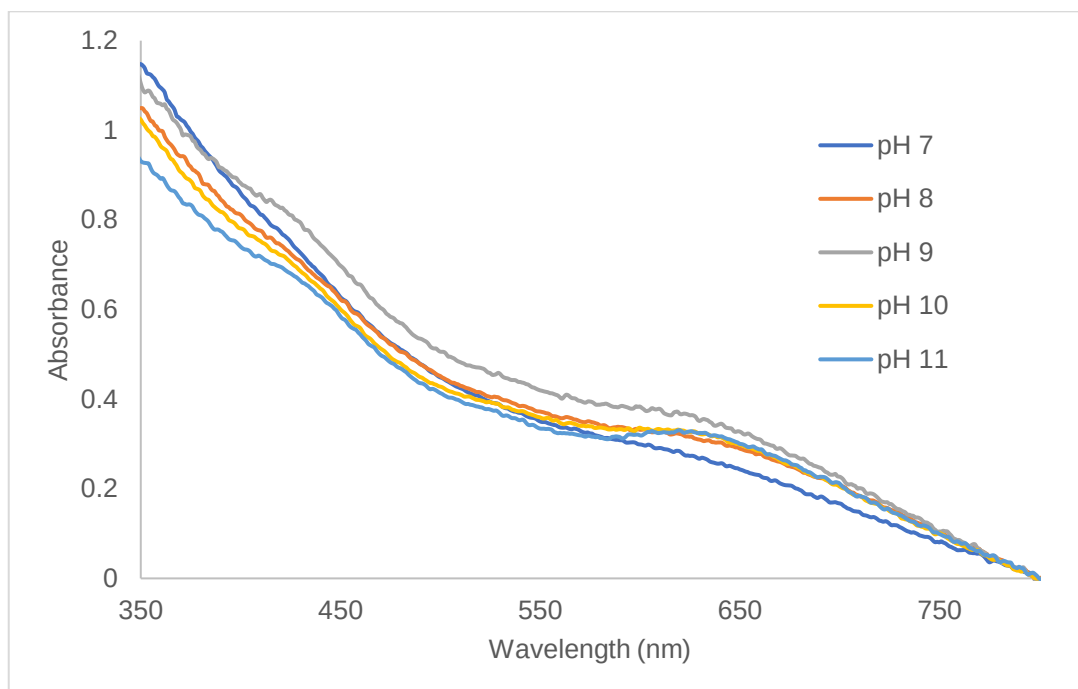


Supplementary Fig. 9. Representative UV-Vis spectrum for solution containing 5 mM Cysteine, 0.5 mM FeCl₃, 0.5 mM FeCl₂ and 1 mM Na₂S at pH ~9 (blue line). Spectrum of solution prepared with 5 mM L-cysteine, 1 mM FeCl₃ and 1 mM Na₂S at pH 9 also included for reference (orange line). Higher absorbance of mixed Fe solution is likely due to increased scattering from precipitate formation.

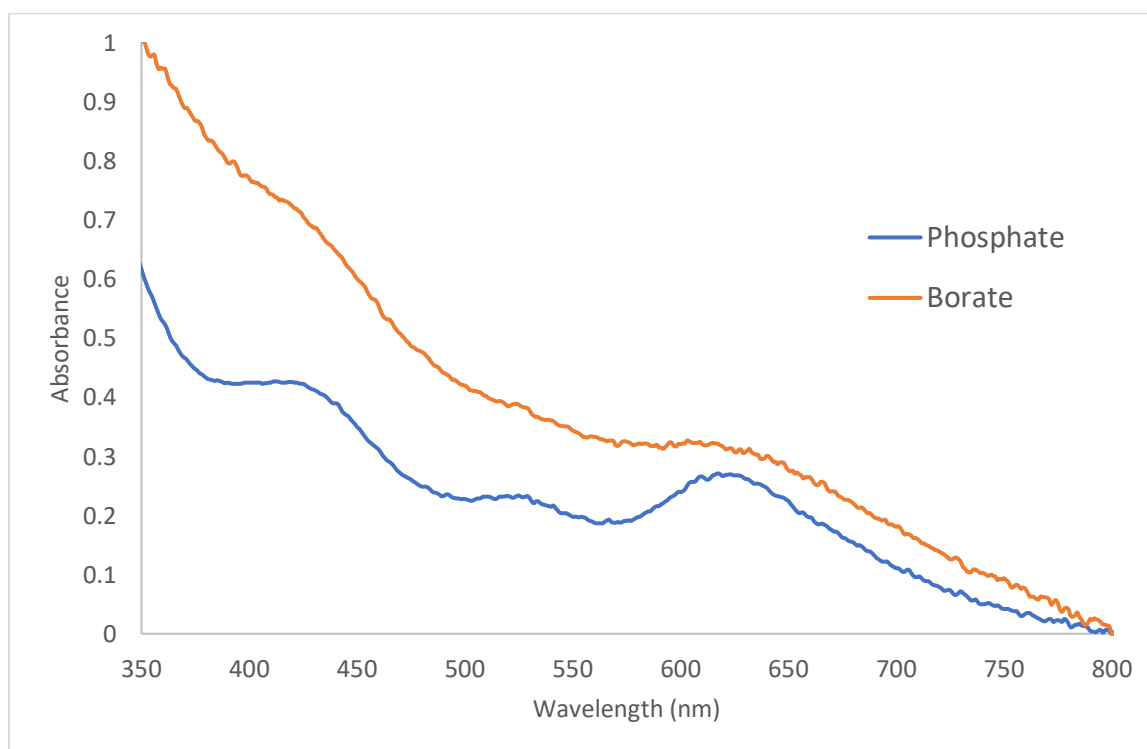
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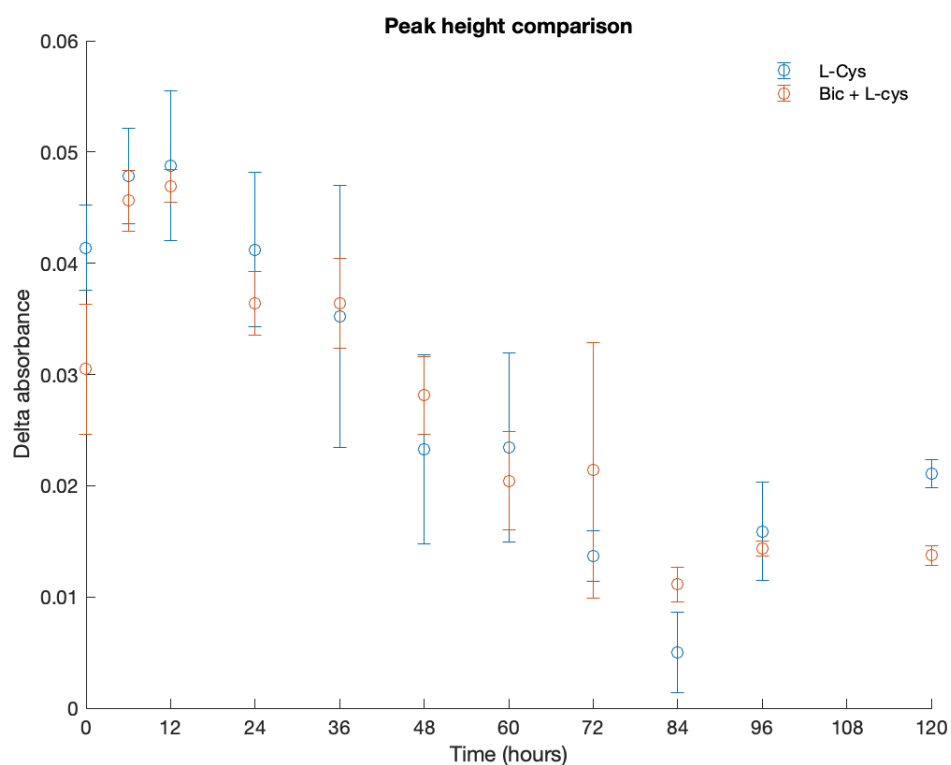
Supplementary Fig. 10. UV-Vis spectra for solutions of increasing NaHCO_3 concentration (5 to 100 mM) at pH ~ 9 . FeCl_3 and Na_2S were both maintained at concentrations of 0.5 mM. Source data are provided as a Source Data file.



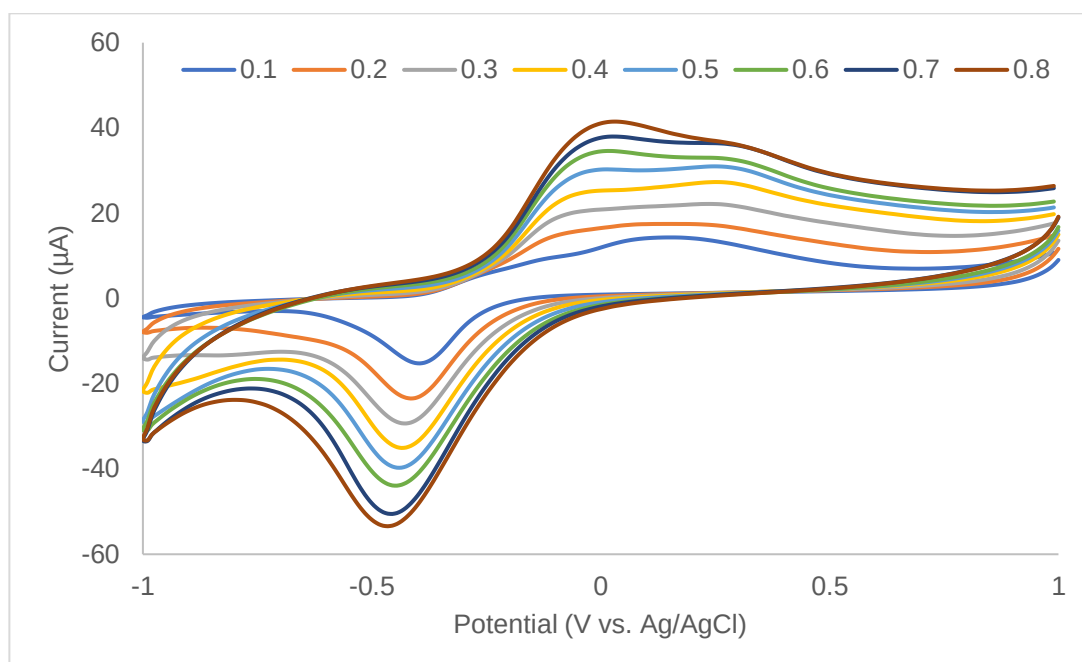
Supplementary Fig. 11. UV-Vis spectra for solutions containing 10 mM NaHCO_3 , 0.5 mM FeCl_3 , and 0.5 mM Na_2S across a pH range of ~7 to 11. Source data are provided as a Source Data file.



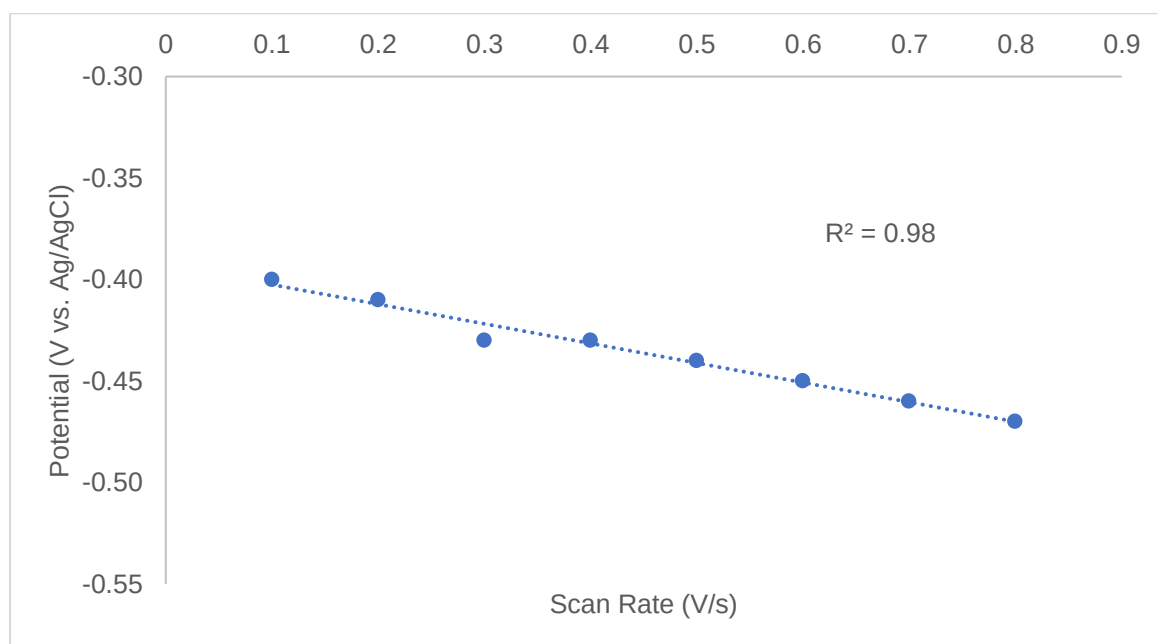
Supplementary Fig. 12. UV-Vis spectra for solutions of 0.5 mM FeCl_3 and 0.5 mM Na_2S prepared with phosphate (blue line - 80 mM Na_2PO_4) and borate (orange line - 40 mM $\text{Na}_2\text{B}_4\text{O}_7$) buffers. Source data are provided as a Source Data file.



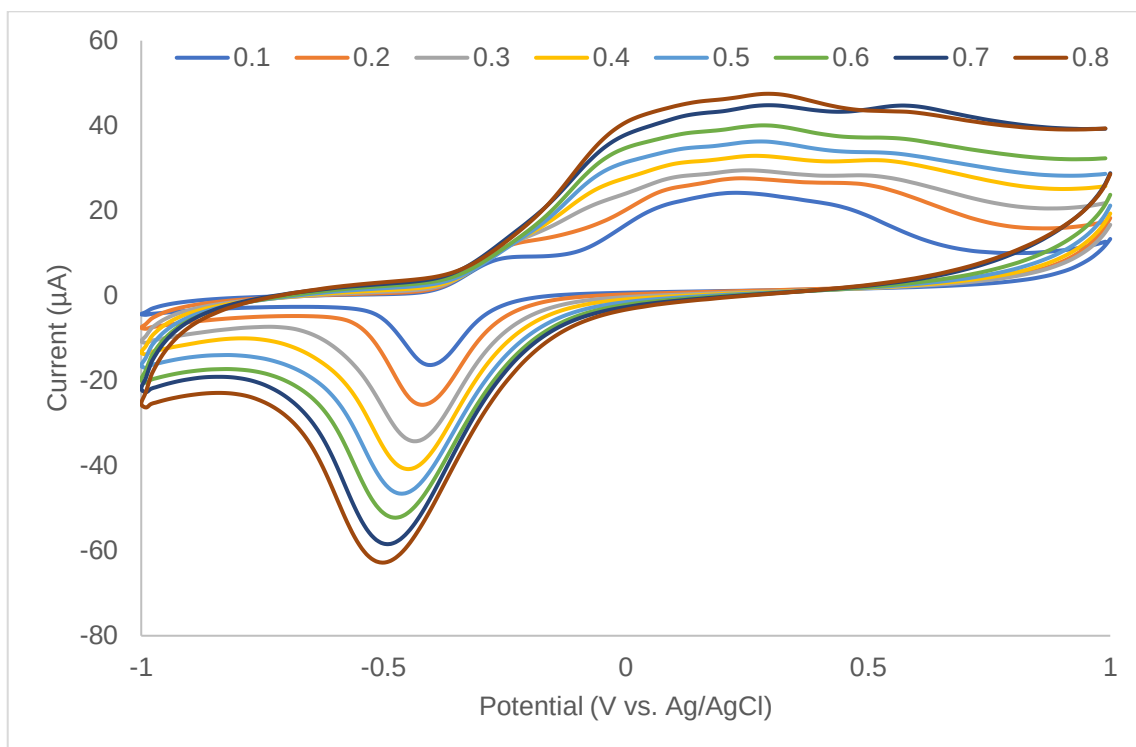
Supplementary Fig. 13. Evolution of the extracted 420 nm peak amplitude representative of clusters formed in the absence (blue) or presence (orange) of 10 mM NaHCO_3 over a 5-day period. Cys-FeS were prepared with 5 mM L-cysteine, 1 mM FeCl_3 and 1 mM Na_2S at pH 9. $N = 3$; the error bars show standard deviation around the mean (circles). Source data are provided as a Source Data file.



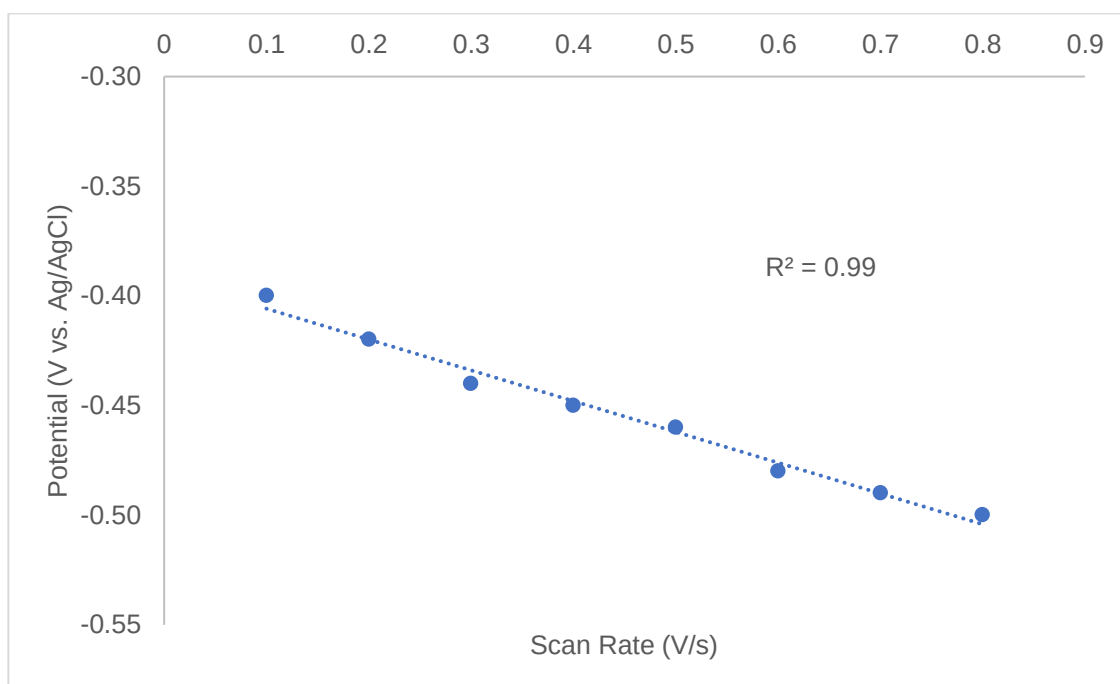
Supplementary Fig. 14. Cyclic voltammograms with increasing scan rate (0.1 to 0.8 V/s) for clusters formed with 3.5 mM L-cysteine, 1 mM FeCl₃ and 1 mM Na₂S at pH 9. Source data are provided as a Source Data file.



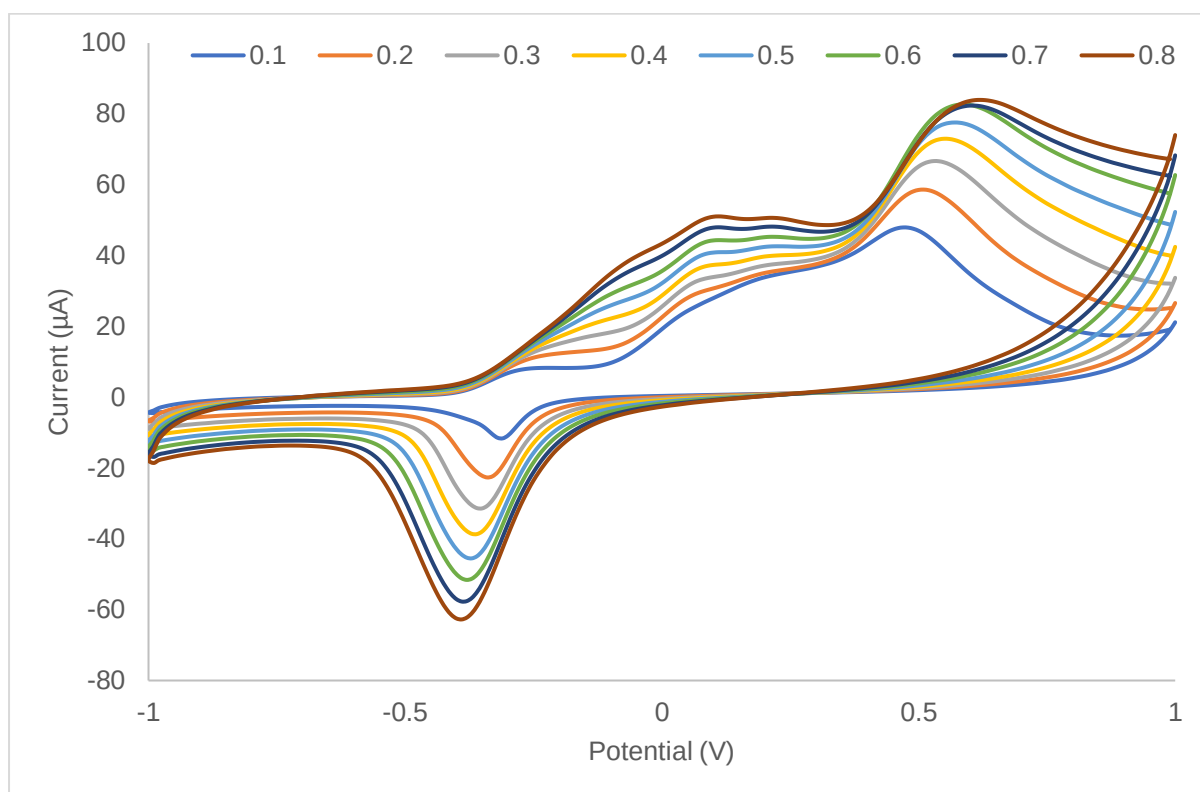
Supplementary Fig. 15. Potential vs. scan rate for reduction peak of clusters formed with 3.5 mM L-cysteine, 1 mM FeCl_3 and 1 mM Na_2S at pH 9 displaying a linear shift. Source data are provided as a Source Data file.



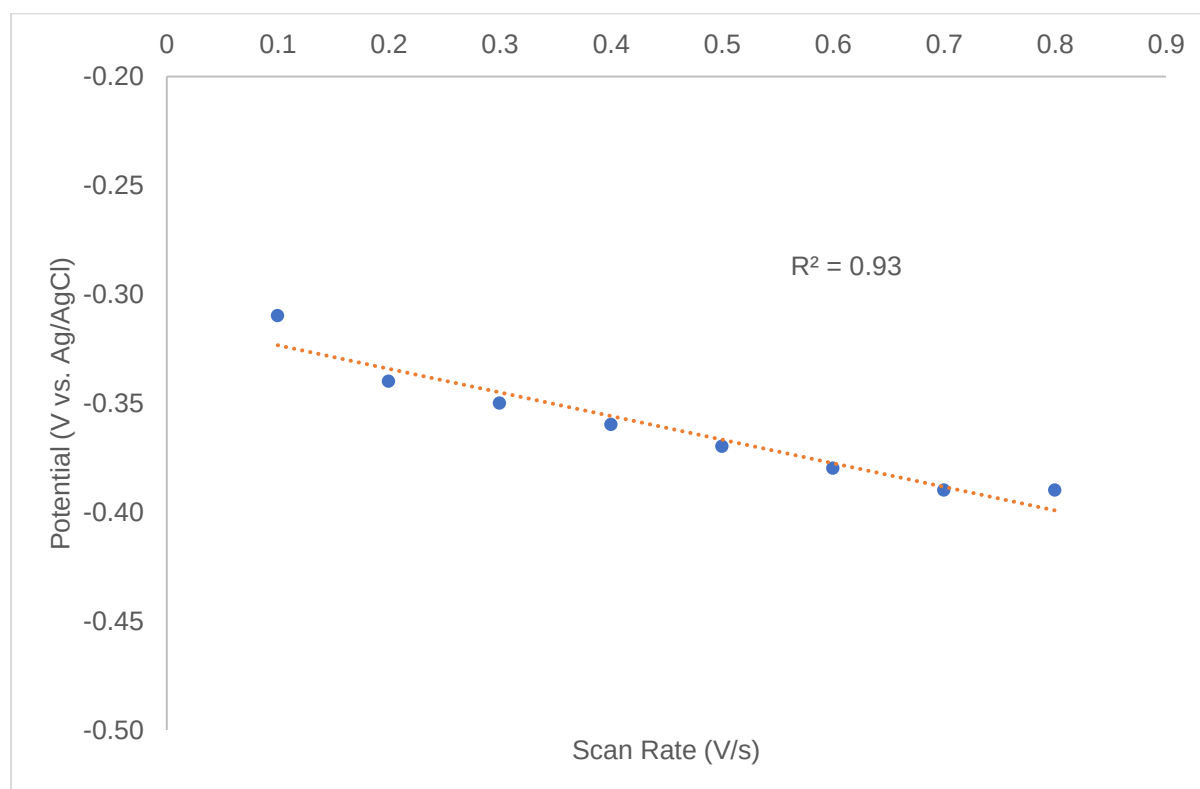
Supplementary Fig. 16. Cyclic voltammograms with increasing scan rate (0.1 to 0.8 V/s) for FeS clusters formed with 5 mM L-cysteine, 1 mM FeCl_3 and 1 mM Na_2S at pH 9. Source data are provided as a Source Data file.



Supplementary Fig. 17. Potential vs. scan rate for reduction peak of FeS clusters formed with 5 mM L-cysteine, 1 mM FeCl₃ and 1 mM Na₂S at pH 9 displaying a linear shift. Source data are provided as a Source Data file.



Supplementary Fig. 18. Cyclic voltammograms with increasing scan rate (0.1 to 0.8 V/s) for FeS clusters formed with 10 mM L-cysteine, 1 mM FeCl₃ and 1 mM Na₂S at pH 9. Source data are provided as a Source Data file.



Supplementary Fig. 19. Potential vs. scan rate for reduction peak of FeS clusters formed with 10 mM L-cysteine, 1 mM FeCl₃ and 1 mM Na₂S at pH 9 displaying a non-linear shift. Source data are provided as a Source Data file.