Electronic Supporting Information

The Iron-Thiol-Oxygen Nexus for Iron Flux from Bare and Ferritin-caged Mineral and Safeguarding DNA: Impact of Thiol Structure and Protein Coat

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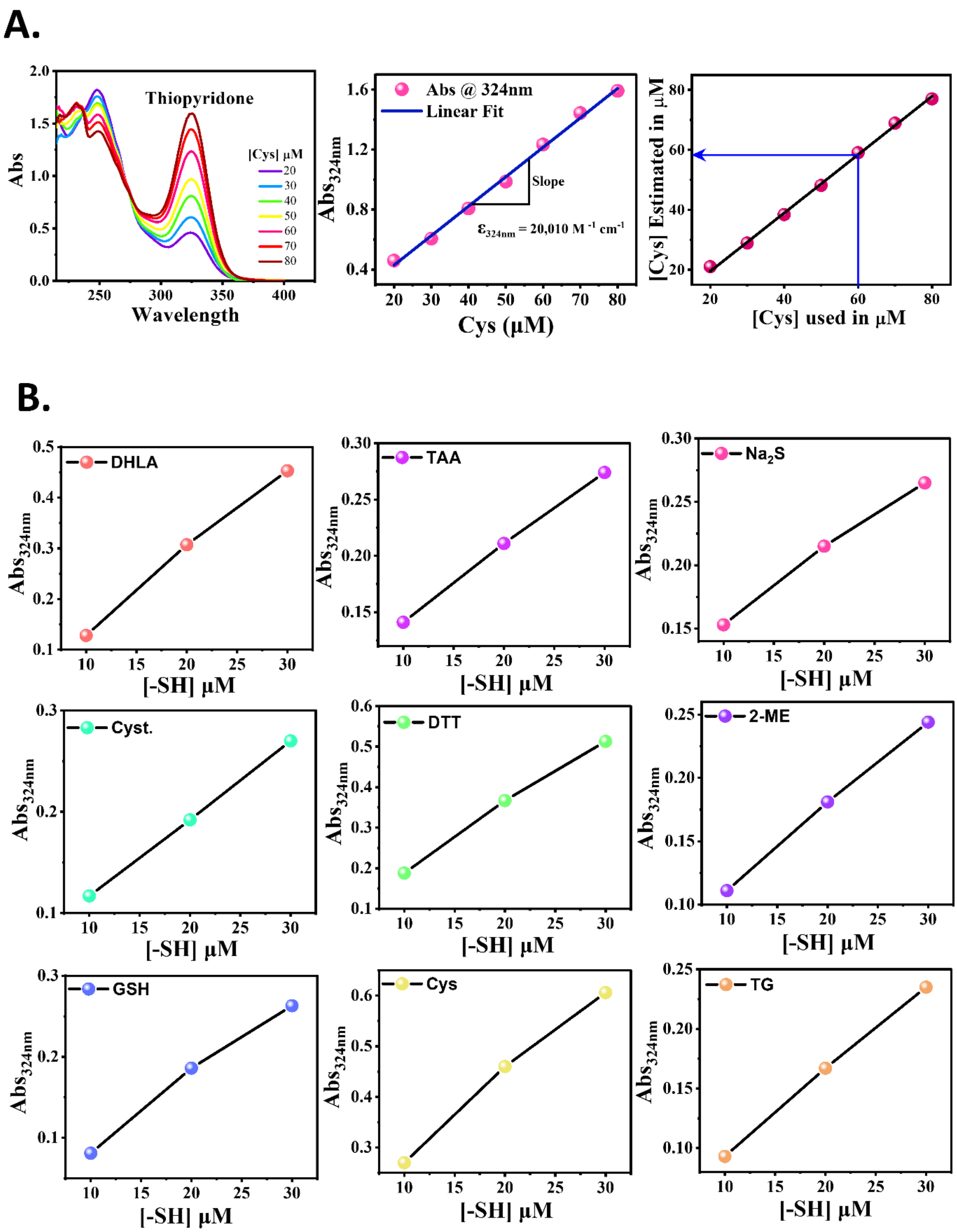

Figure S1: Thiol estimation by 4-DPS assay. (A) Standard curve for cysteine (as representative thiol) based on 4-DPS assay. The concentrations of reduced form of thiols (-SH) were quantified from the slope, i.e., molar absorptivity value (ε_{324nm} = 20,010 M^{-1} cm^{-1}). The estimated concentration of -SH was correlated with the amount of thiols used. **(B)** Quantification of reduced forms of all sulfur based reducing agents using 4-DPS assay.[1, 2]

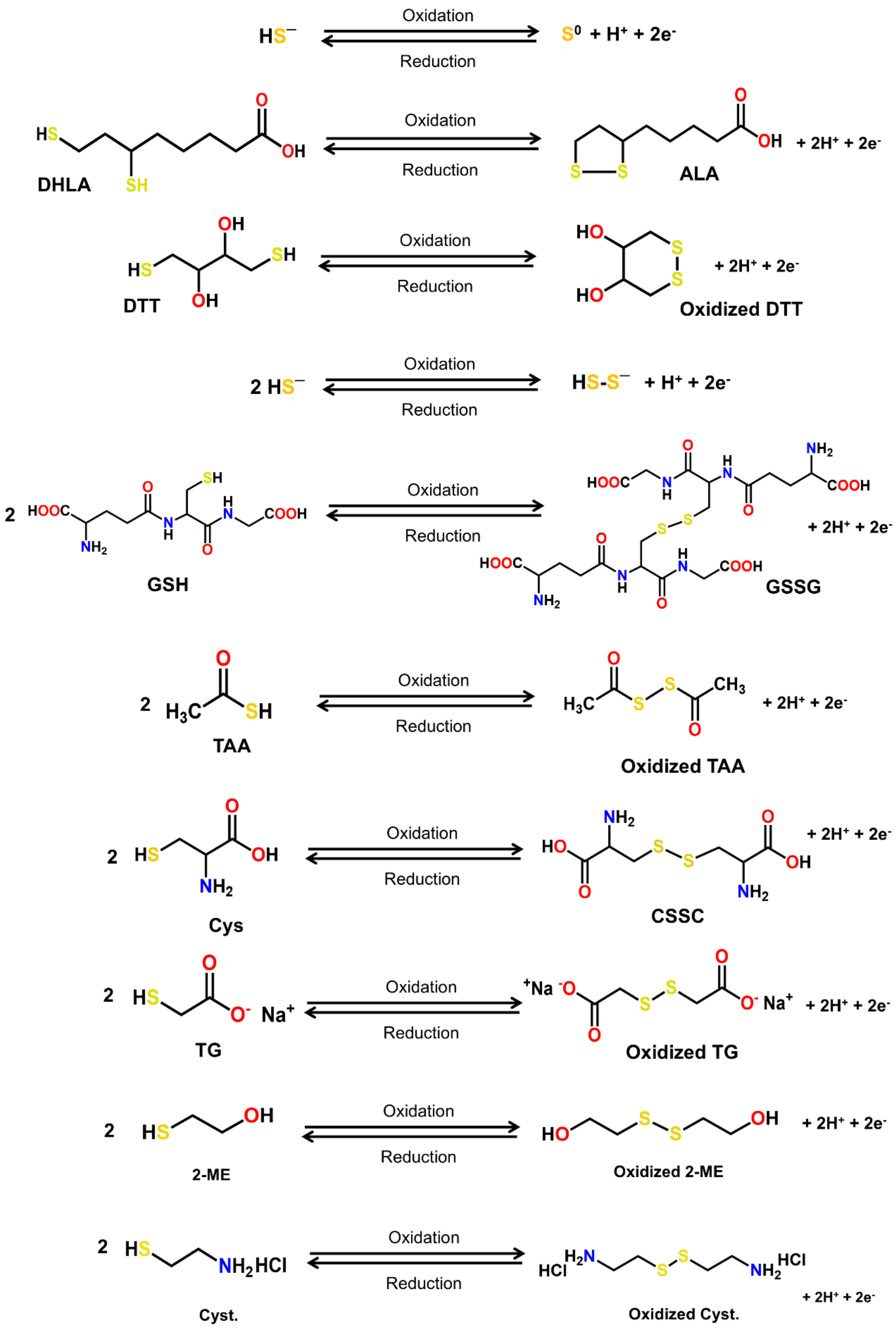

Figure S2: Balanced thiol-disulphide redox equation of all sulfur based reducing agents (used for calculation of apparent redox potential).

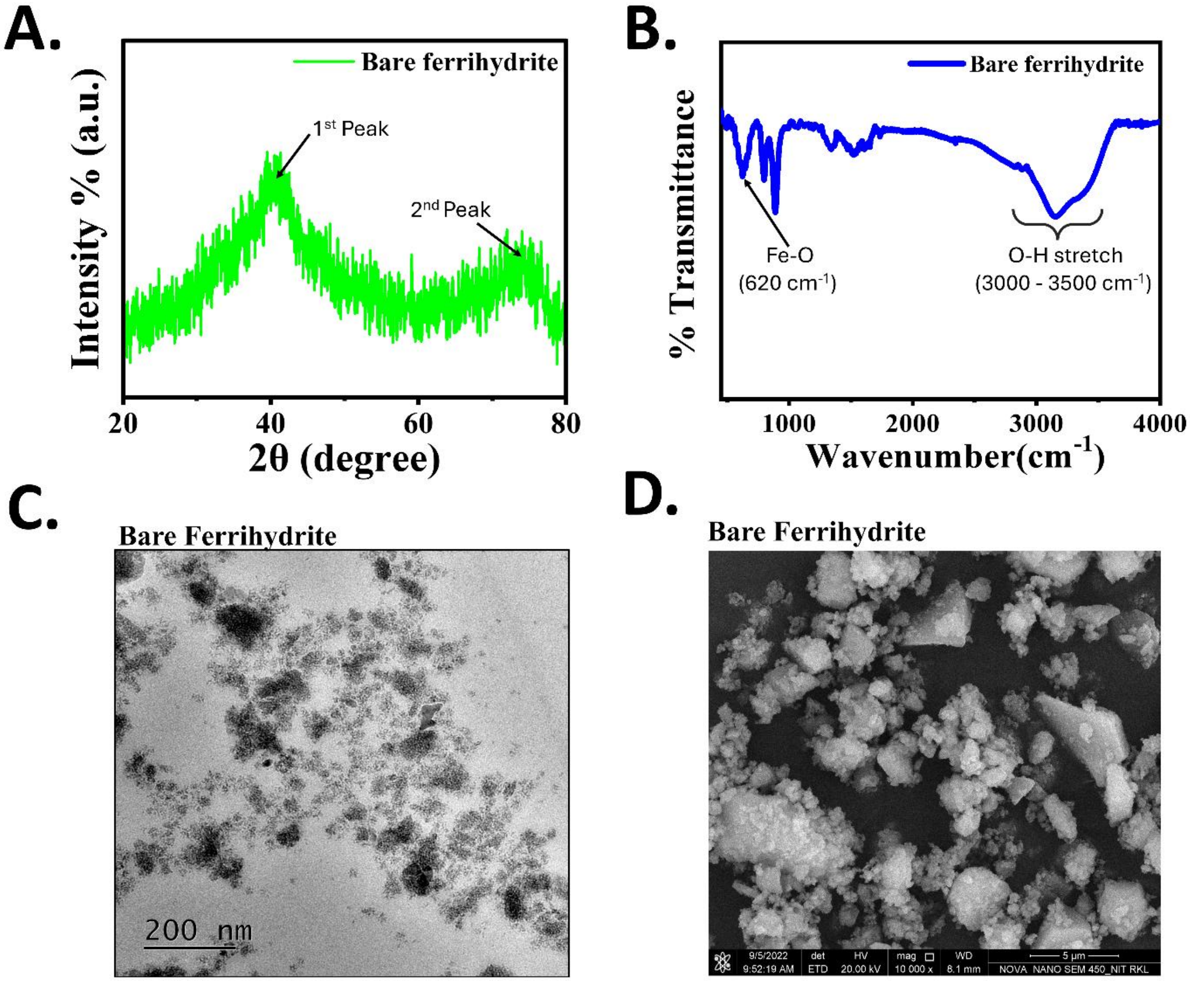

Figure S3: Characterization of synthesized bare ferrihydrite. Powder-XRD pattern **(A),** FT-IR pattern **(B)**, TEM **(C)** and FESEM **(D)** of synthesized two-line bare ferrihydrite displaying their characteristic peaks and cluster like distribution.

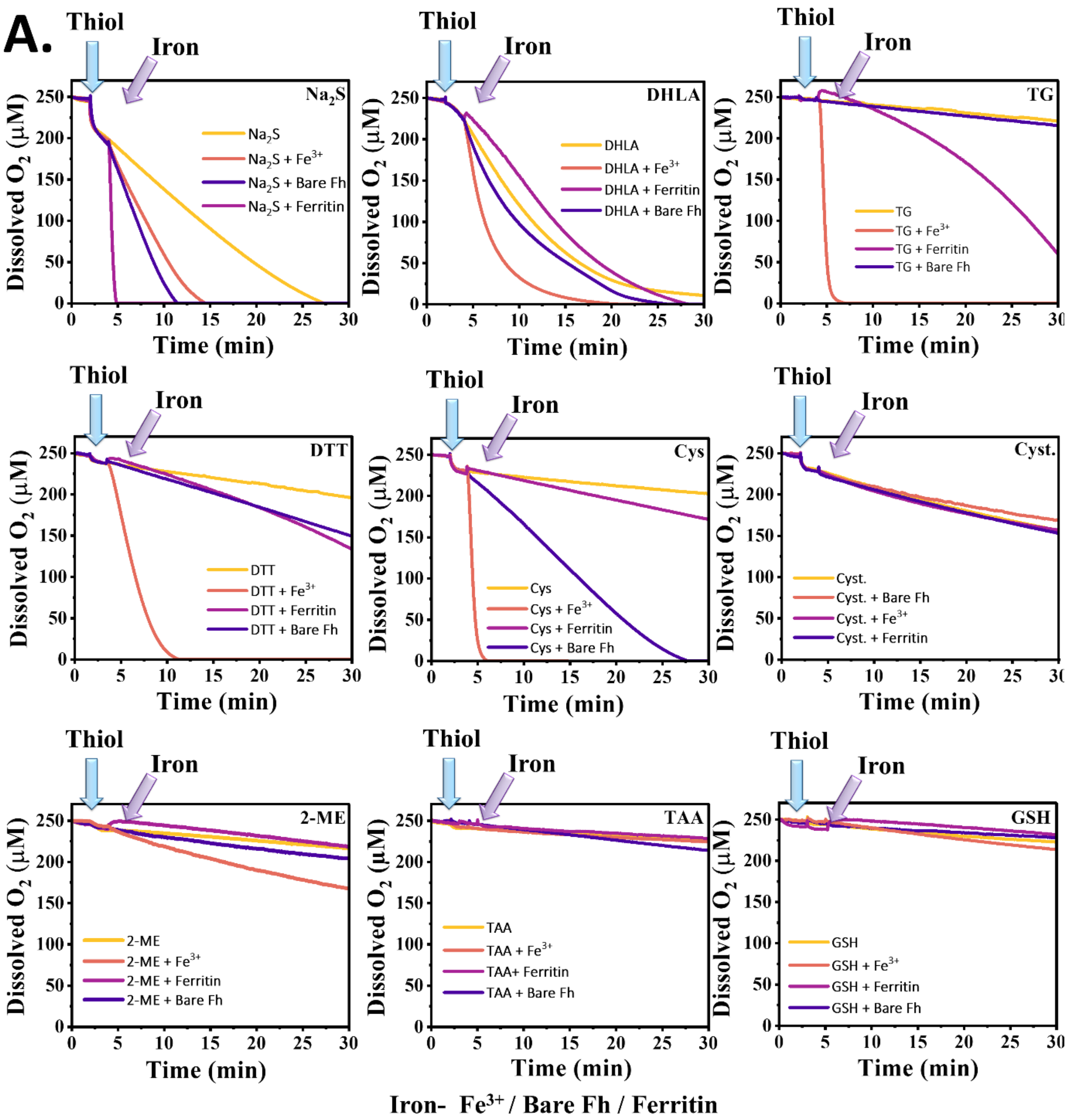

Figure S4: Impact of iron (Fe^{3+}, bare Fh, encapsulate Fh) on dissolved O_2 consumption by thiols. (A) The kinetic experiments were performed in 100mM MOPS.NaCl buffer pH = 7.0 with 2.5mM thiol and 100µM iron (Fe^{2+}/ Fe^{3+}/ bare/encapsulated iron mineral). The arrow indicates the time point of injection of the respective reactants.

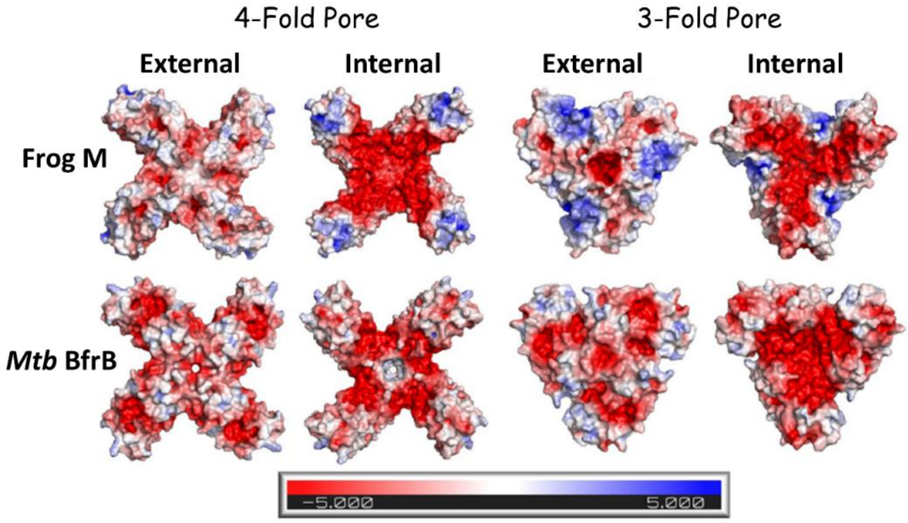

Figure S5: Structure and variable pore electrostatics of frog M and *Mtb* BfrB ferritins. Ferritin electrostatic potential surfaces along the three-fold and four-fold pores of: frog M ferritin (PDB: 1MFR), and Mtb BfrB (PDB: 3QD8). Ferritin structures are generated using PyMOL-ABPS. Figures adapted from [3]

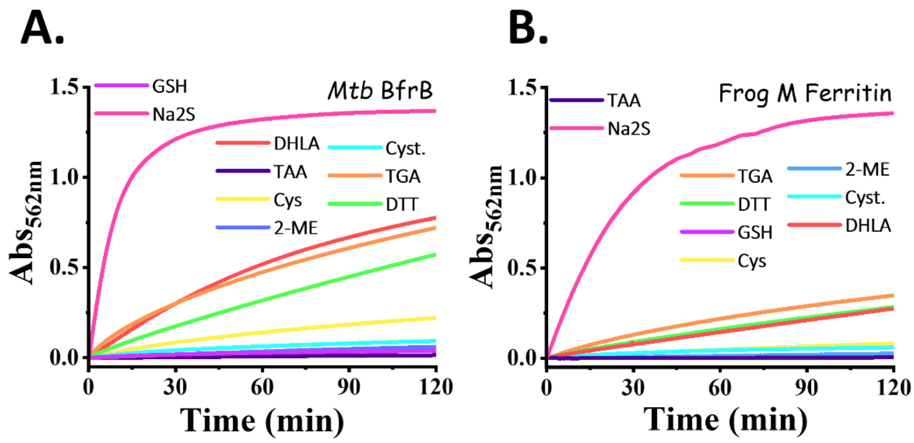

Figure S6: Impact of anaerobic condition on reductive iron release. The reductive iron mobilization was carried out in anaerobic conditions for both Mtb BfrB **(A)** and Frog M **(B)** ferritin. All the samples were deoxygenated by nitrogen purging prior to the iron dissolution kinetics. The reaction was initiated by mixing 0.2μM ferritin (100 μM of caged mineral ~500Fe/Cage) with 1mM of thiols and 1mM of ferrozine, in 100mM MOPS-NaCl buffer (pH-7.0). The trend of iron release was found to be identical with the iron release under aerobic conditions, whereas the amount of released iron was found to be more.

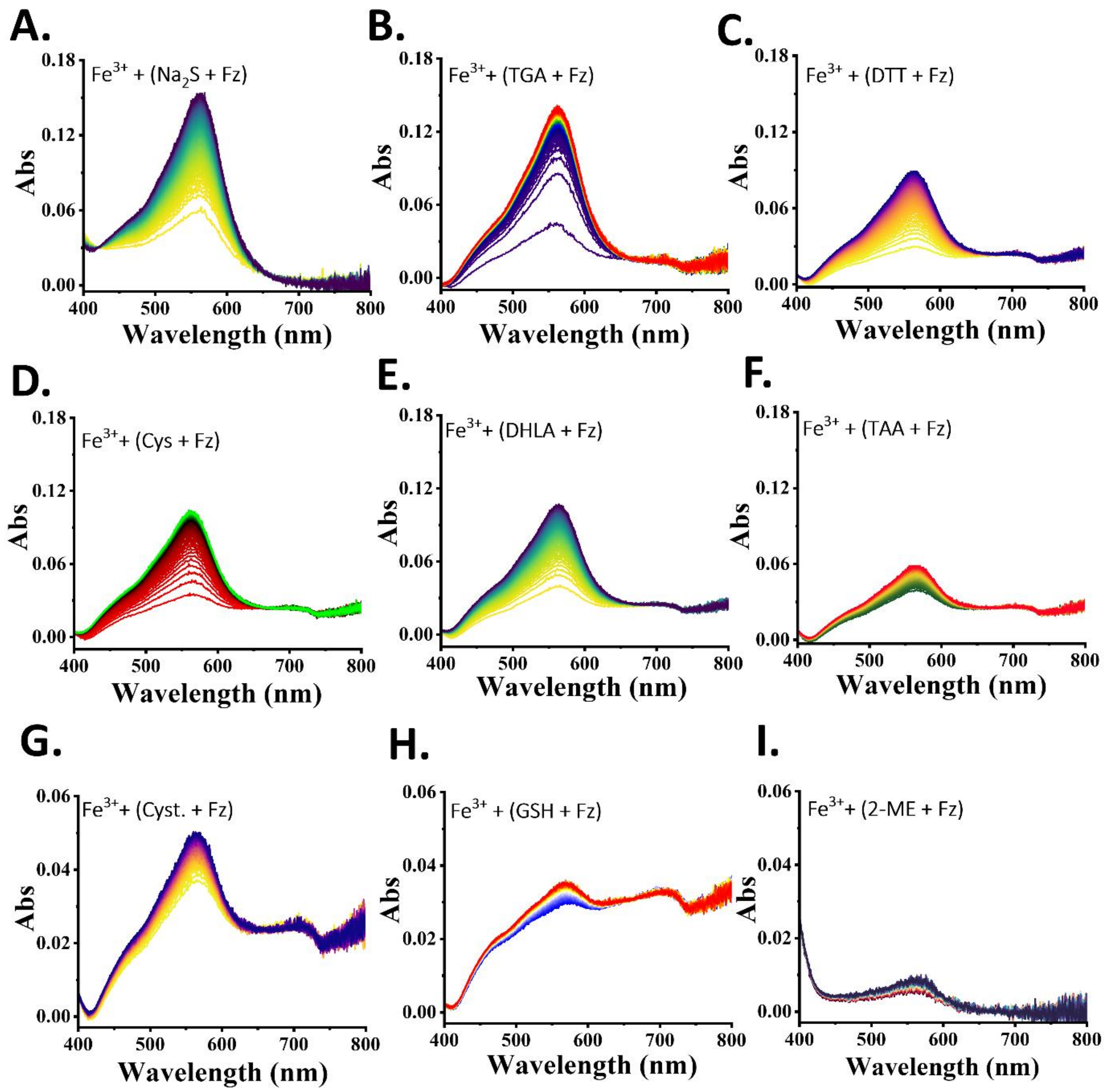

Figure S7: Stopped-flow rapid kinetics of Fe^{3+} reduction by thiols: The reduction kinetics was performed by mixing equal volumes (1:1) of freshly prepared Fe^{3+} solution (100 μM in 1mM HCl) with mixture of thiol (2.5mM) and ferrozine (Fz, 1mM) in 10mM MOPS-NaCl pH-7.0 buffer in a stopped-flow rapid mixing unit.

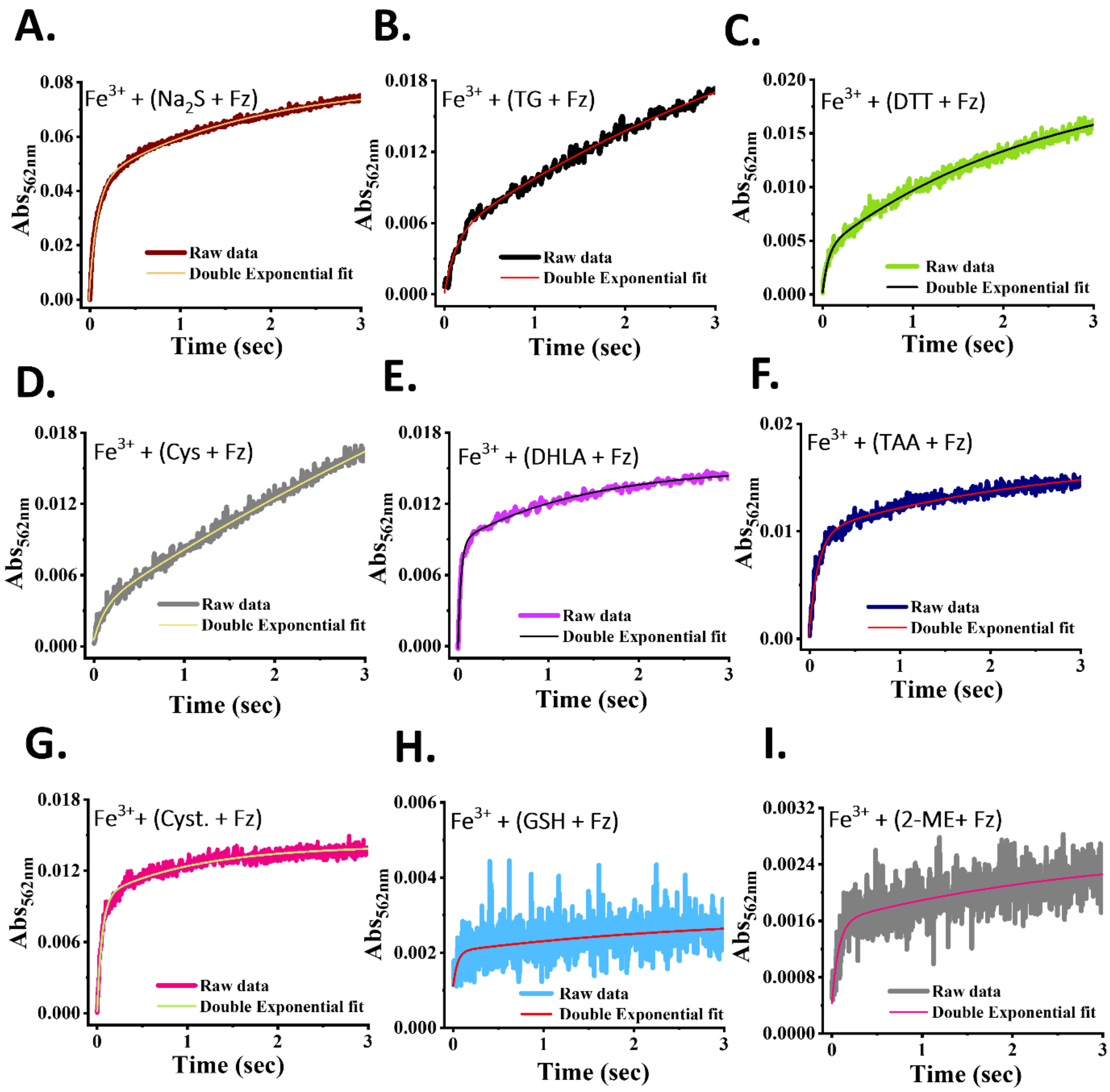

Figure S8: Stopped-flow rapid kinetics of Fe^{3+} reduction by thiols: The reduction kinetics was performed by mixing equal volumes (1:1) of freshly prepared Fe^{3+} solution (100 µM in 1mM HCl) with mixture of thiol (2.5mM) and ferrozine (Fz, 1mM) in 10mM MOPS-NaCl pH-7.0 buffer in a stopped-flow rapid mixing unit. Time courses for Fe^{3+} reduction at 562nm with nonlinear fitting with double exponential equation. The observed rate constants are listed in **Table 2**.

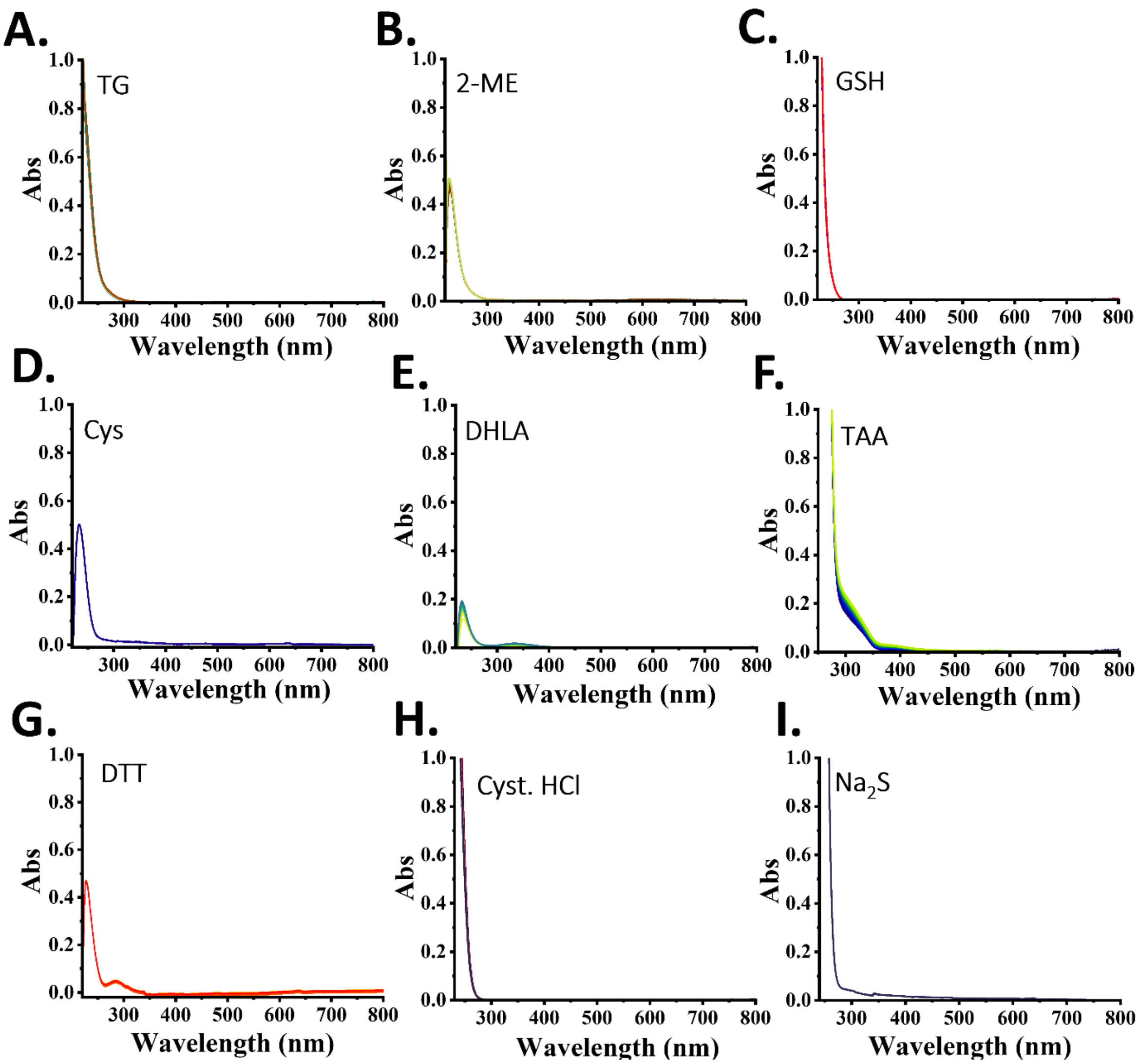

Figure S9: Thiol stability (Spectral kinetics of thiols- 2.5mM) in 100mM MOPS-NaCl, pH-7.0 buffer, control reactions.

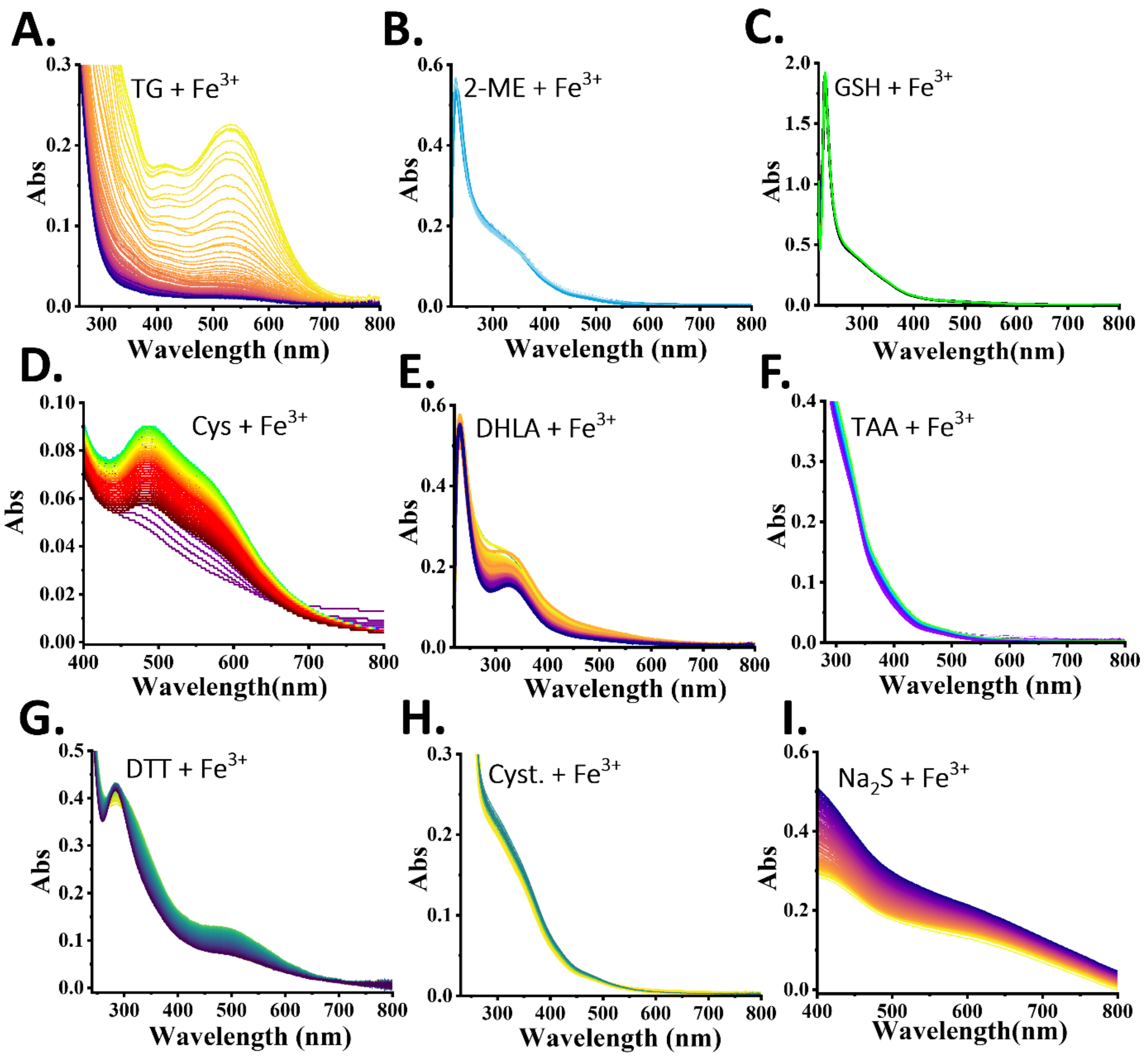

Figure S10: Fe^{3+}-thiol interaction: Kinetic analysis by manual mixing. The interaction kinetics was performed by mixing freshly prepared Fe^{3+} (100µM in 1mM HCl) with respective thiols (2.5mM) in 100 mM MOPS-NaCl, pH-7.0 buffer. **(A-I)** The spectral kinetics data for Fe^{3+} interaction with respective thiols, reveals the formation of transient species for some specific thiols.

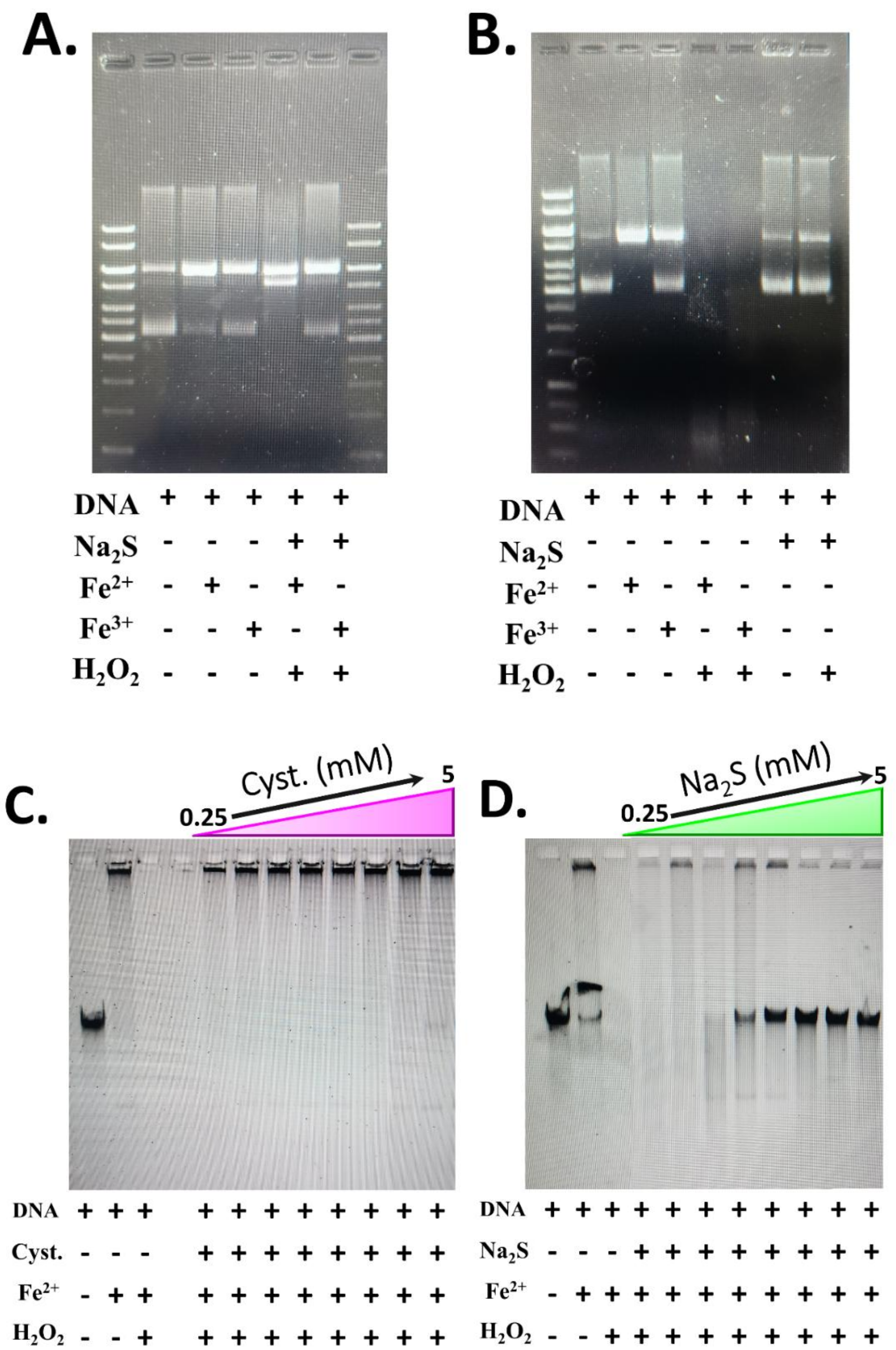

Figure S11: Agarose gel of control experiments and PC_{50} determination. The "+/-" signs indicate the "with/without" addition of respective constituents. Control reactions of DNA cleavage activity **(A-B).** Agarose gel depicting concentration variation of Cyst. and Na_2S respectively for PC_{50} determination **(C-D).**

References

1. R. E. Hansen, H. Østergaard, P. Nørgaard and J. R. Winther, *Analytical Biochemistry*, 2007, **363**, 77-82.
2. J. R. Winther and C. Thorpe, *Biochimica et biophysica acta*, 2014, **1840**, 838-846.
3. P. K. Koochana, A. Mohanty, A. Parida, N. Behera, P. M. Behera, A. Dixit and R. K. Behera, *JBIC Journal of Biological Inorganic Chemistry*, 2021, **26**, 265-281.