Electronic Supporting Information

Phenthiazines and Phenoxazines: As Electron Transfer Mediators for Ferritin Iron Release

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Table S1: The percentage of iron(II) released within 20 min, by phenthiazines and phenoxazines at neutral and acidic pH by monitoring the formation of $[Fe(Bpy)_3]^{2+}$ complex at 488 and 522 nm.

Dyes	**pH**	**% of iron(II) released by the formation of $[Fe(Bpy)_3]^{2+}$ complex at**	
		488 nm	**522 nm**
TH	7.0	15.02	14.92
	4.0	45.00	43.00
MB	7.0	7.63	8.71
	4.0	24.00	23.00
MG	7.0	3.60	3.44
	4.0	16.50	14.00
TDB	7.0	9.10	9.66
	4.0	20.80	18.00
BCB	7.0	10.10	10.27
	4.0	21.40	18.00
CRV	7.0	3.50	5.85
	4.0	53.00	53.00
NB	7.0	5.64	5.20
	4.0	40.60	38.00

Table S2: Initial rate and percentage of iron (II) released (formation of Fe(II)-bipyridyl complex at 488 nm monitored for 2 hours; slope obtained from linear data points collected within 3 min) at various concentrations of different ET mediators employed for reductive mobilization of iron from ferritin.

[Dyes] (μM) →		**5**		**10**		**25**		**50**	
Dyes	**pH** ↓	**Initial Rate (μM min^{-1})**	**% of Iron (II) Released**	**Initial Rate (μM min^{-1})**	**% of Iron (II) Released**	**Initial Rate (μM min^{-1})**	**% of Iron (II) Released**	**Initial Rate (μM min^{-1})**	**% of Iron (II) Released**
TH	7.0	0.22	15.16	0.33	24.16	0.86	42.80	0.83	45.35
	4.0	1.14	70.00	1.49	65.00	6.95	91.80	6.52	86.00
MB	7.0	0.01	3.70	0.02	4.73	0.34	16.50	0.19	27.00
	4.0	0.35	31.60	0.87	44.70	4.15	65.00	3.45	85.00
MG	7.0	0.03	3.20	0.08	4.40	0.14	13.20	0.12	9.95
	4.0	0.31	22.80	0.32	27.40	3.31	66.40	2.70	78.50
TDB	7.0	0.05	2.63	0.05	3.35	0.46	31.20	0.10	28.28
	4.0	0.78	43.66	1.46	47.50	3.21	64.90	4.68	87.50
BCB	7.0	0.15	6.00	0.20	10.52	0.35	33.00	0.32	52.00
	4.0	0.25	12.60	0.33	18.38	1.73	58.00	3.14	58.00
CRV	7.0	0.01	2.00	0.01	3.70	0.08	15.27	0.28	35.27
	4.0	2.4	55.00	2.43	67.00	10.30	73.00	9.92	96.00
NB	7.0	0.01	1.90	0.01	2.00	0.04	15.15	0.04	20.60
	4.0	0.59	47.40	2.15	54.00	3.77	60.00	11.00	73.00

Table S3: Stern−Volmer quenching constants (K_{SV}) for Dye-Ferritin interactions at different temperatures

Dyes	298 K	302 K	306 K	310 K
TH	4.98 ± 0.43	4.02 ± 0.25	3.71 ± 0.35	3.49 ± 0.37
MB	5.32 ± 0.28	5.04 ± 0.23	4.35 ± 0.32	3.99 ± 0.26
MG	4.05 ± 0.30	3.70 ± 0.31	3.12 ± 0.27	2.88 ± 0.20
TDB	4.28 ± 0.14	3.94 ± 0.21	3.59 ± 0.18	3.35 ± 0.14
BCB	3.61 ± 0.27	3.14 ± 0.25	3.06 ± 0.25	2.81 ± 0.18
CRV	1.56 ± 0.14	1.57 ± 0.08	1.44 ± 0.11	1.59 ± 0.11
NB	1.37 ± 0.08	1.28 ± 0.06	1.19 ± 0.08	1.02 ± 0.13

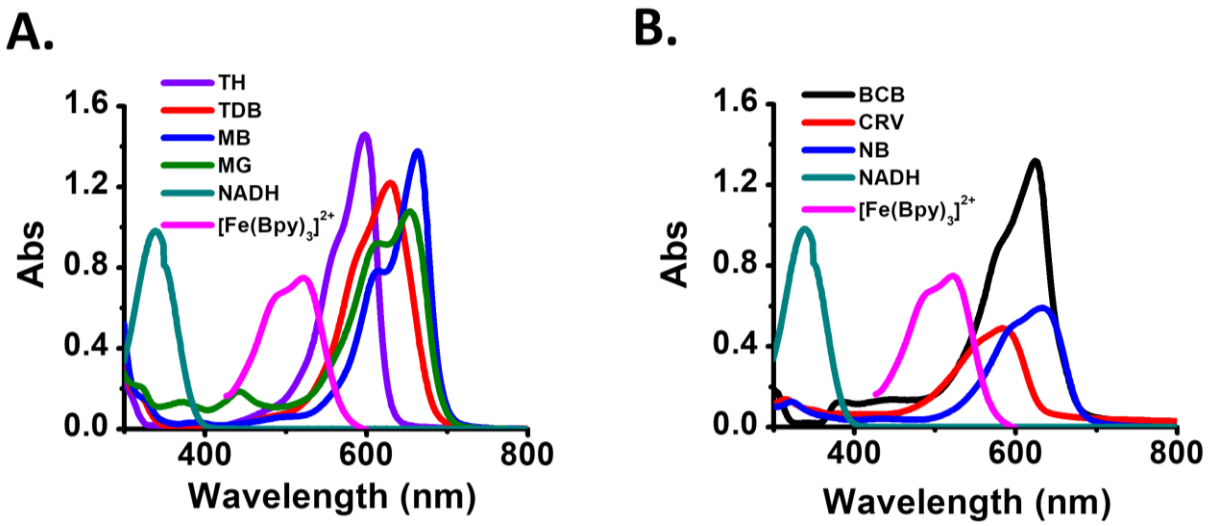
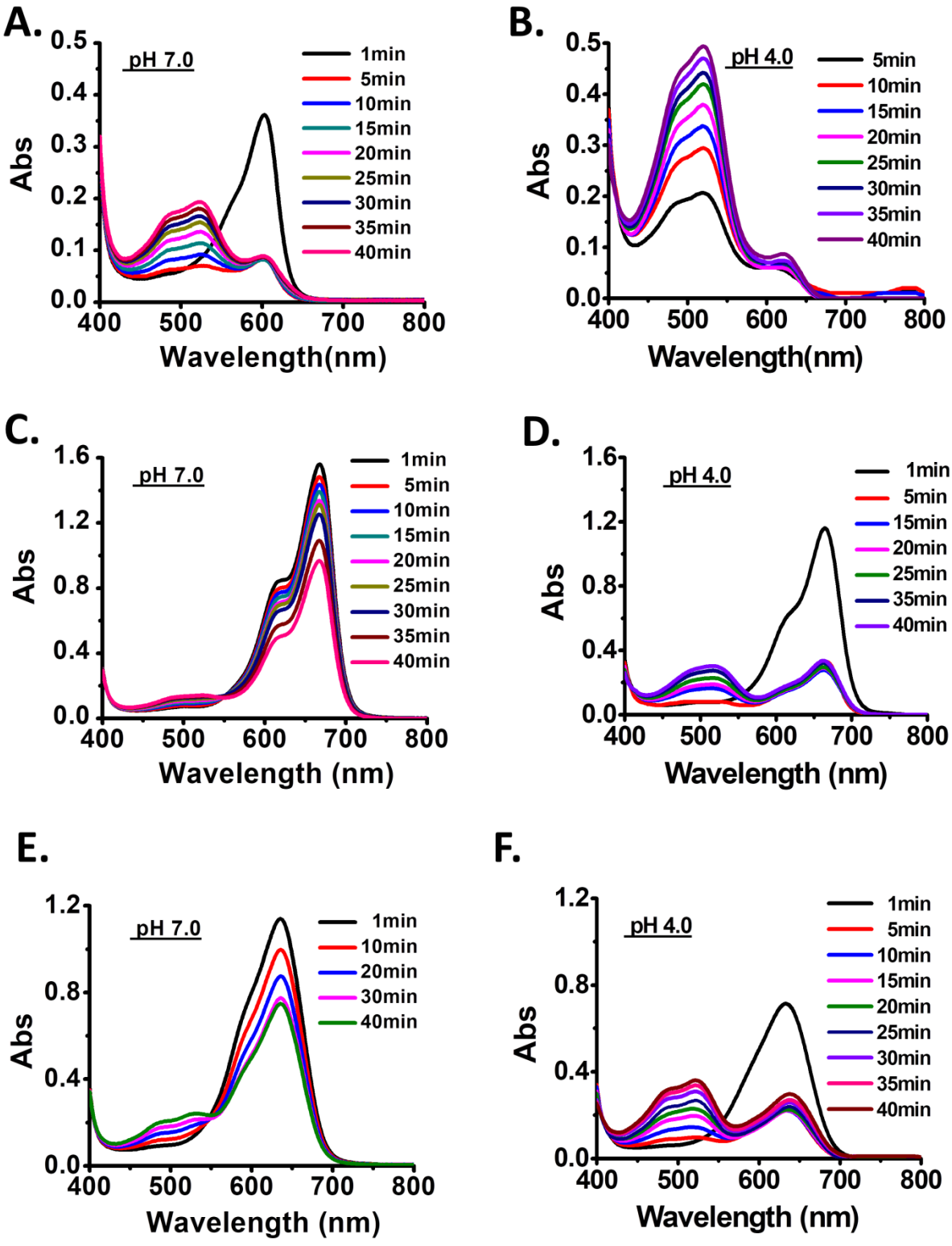
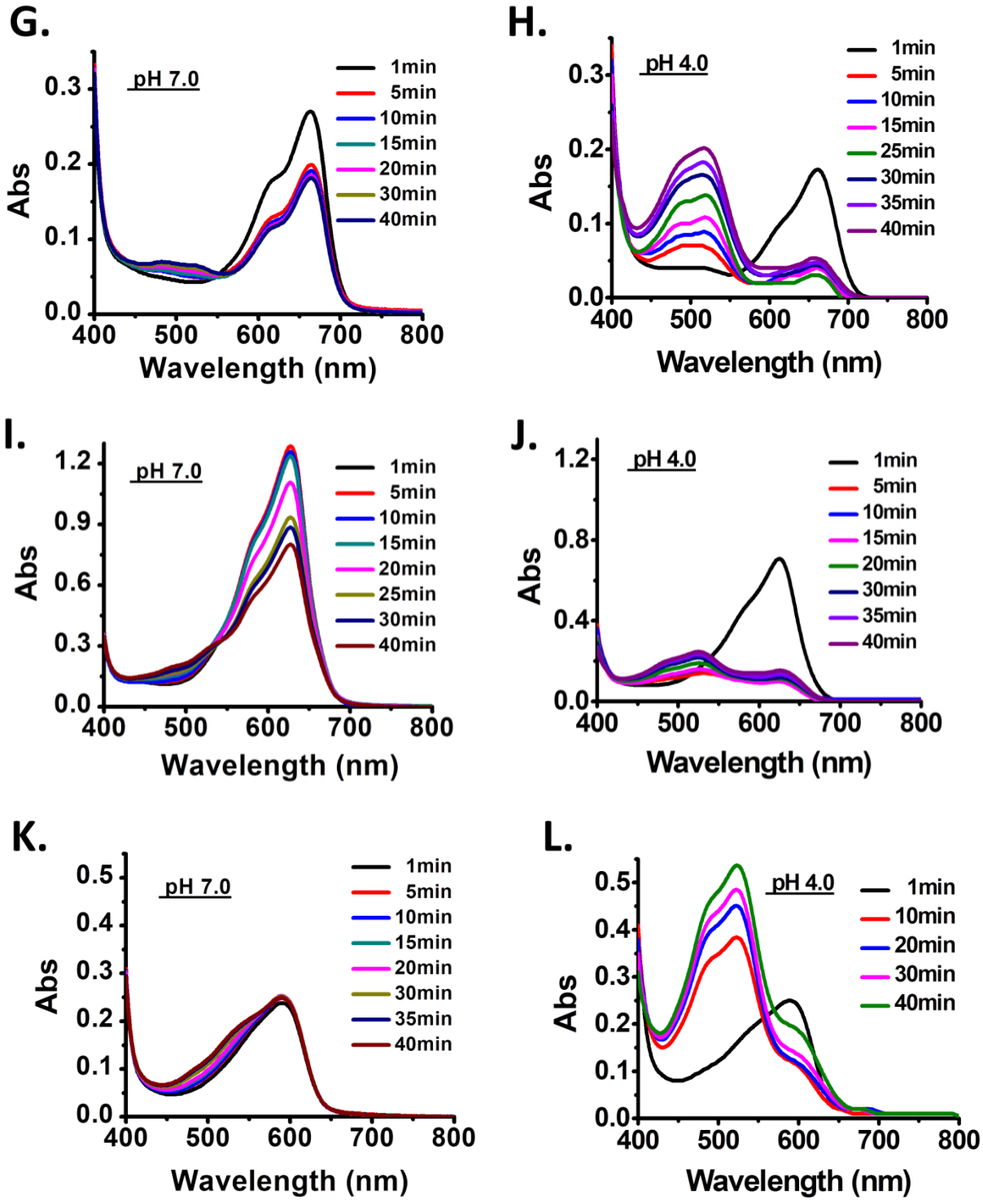


Figure S1: **Absorption spectra of reducing agent, iron(II) chelator and ET mediators** used for current study: (**A**) Phenthiazines such as Thionine, Toluidine blue, Methylene blue and Methylene green; (**B**) Phenoxazines such as Brilliant cresyl blue, Cresyl violet and Nile blue A; Electron source such as NADH (1 mM) and 100 μM $[Fe(Bpy)_3]^{2+}$ in 0.1 M MOPS/0.1 M NaCl (pH 7.0) was carried by SHIMADZU UV visible spectrophotometer.


A.
pH 7.0
1min
5min
10min
15min
20min
25min
30min
35min
40min
Abs
Wavelength(nm)
B.
pH 4.0
5min
10min
15min
20min
25min
30min
35min
40min
Abs
Wavelength(nm)
C.
pH 7.0
1min
5min
10min
15min
20min
25min
30min
35min
40min
Abs
Wavelength (nm)
D.
pH 4.0
1min
5min
15min
20min
25min
35min
40min
Abs
Wavelength (nm)
E.
pH 7.0
1min
10min
20min
30min
40min
Abs
Wavelength (nm)
F.
pH 4.0
1min
5min
10min
15min
20min
25min
30min
35min
40min
Abs
Wavelength (nm)


G.
pH 7.0
1min
5min
10min
15min
20min
30min
40min
Abs
Wavelength (nm)
H.
pH 4.0
1min
5min
10min
15min
25min
30min
35min
40min
Abs
Wavelength (nm)
I.
pH 7.0
1min
5min
10min
15min
20min
25min
30min
40min
Abs
Wavelength (nm)
J.
pH 4.0
1min
5min
10min
15min
20min
30min
35min
40min
Abs
Wavelength (nm)
K.
pH 7.0
1min
5min
10min
15min
20min
30min
35min
40min
Abs
Wavelength (nm)
L.
pH 4.0
1min
10min
20min
30min
40min
Abs
Wavelength (nm)

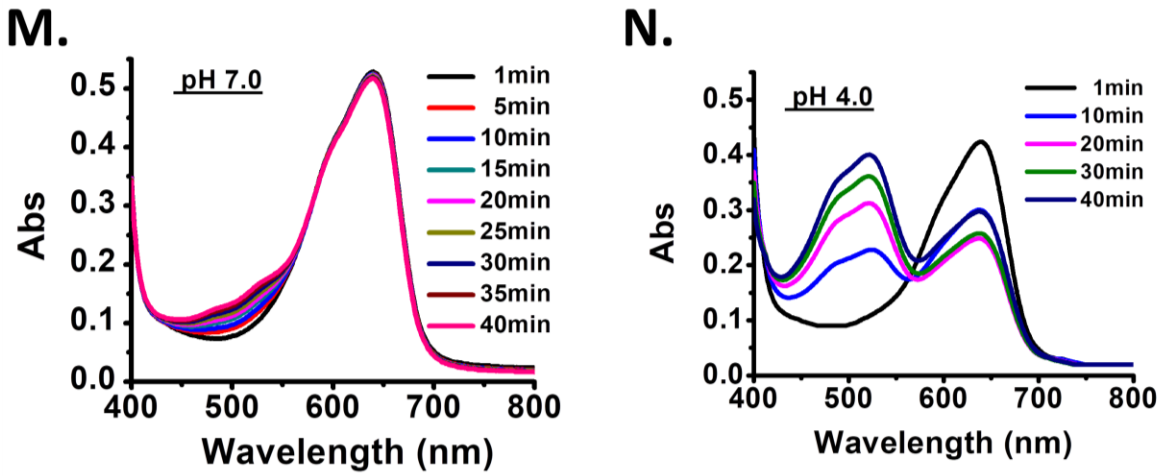
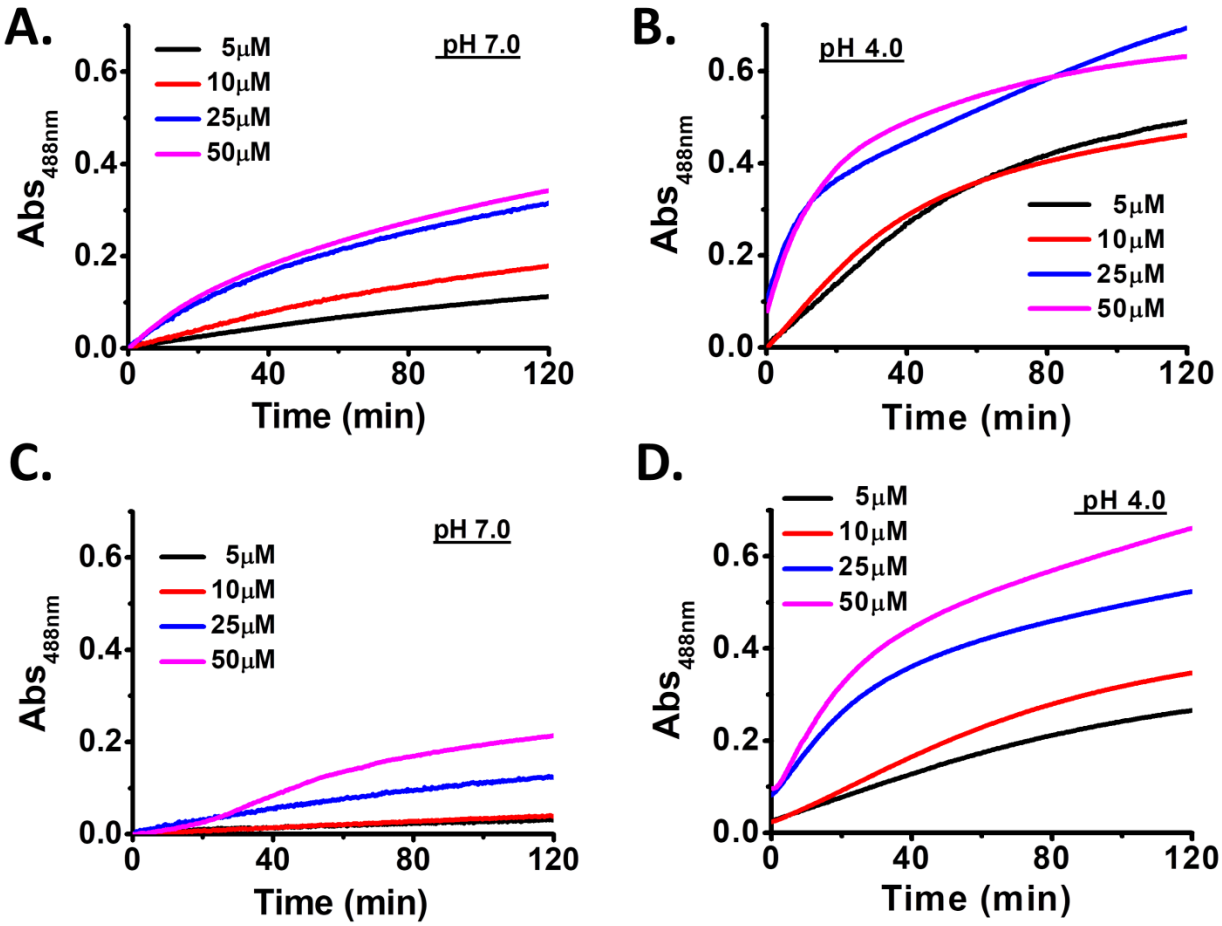
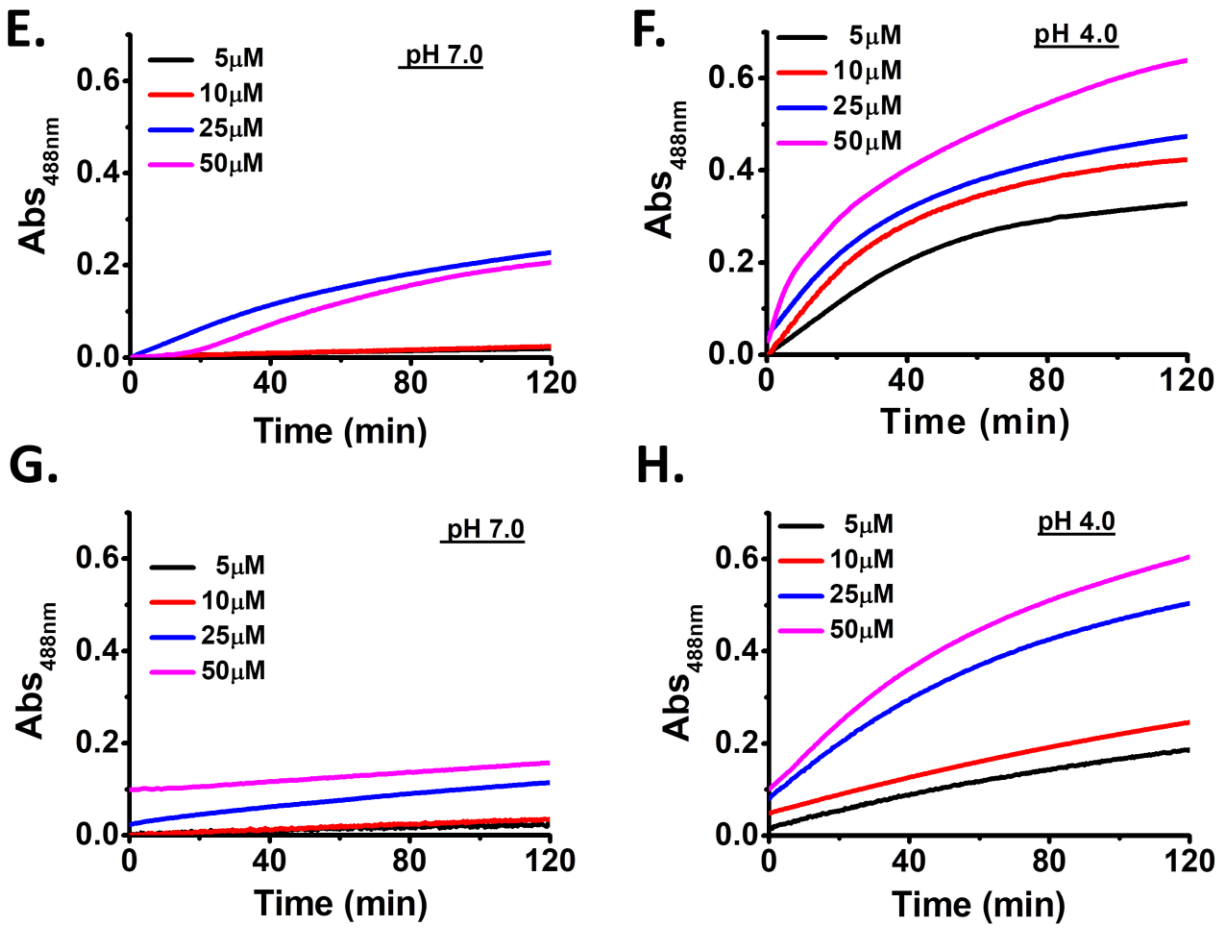


Figure S2: Dye mediated reductive iron release from recombinant frog M ferritin nanocage. Reductive mobilization of iron from ferritin measured by the formation of Fe^{2+}-bipyridine complex, $[Fe(Bpy)_3]^{2+}$ monitored by UV visible spectroscopy at 488 nm. Reductive iron release time courses on dye mediated electron transfer basis at neutral pH (**A**, **C**, **E**, **G**, **I**, **K**, **M**) and acidic pH (**B**, **D**, **F**, **H**, **J**, **L**, **N**) are shown for TH (**A-B**), MB (**C-D**), TDB (**E-F**), MG (**G-H**), BCB (**I-J**), CRV (**K-L**) and NB (**M-N**). The reaction samples were prepared by fixing the mediator concentration (25 µM) in the buffer (0.1 M MOPS/0.1 M NaCl, pH 7.0 or 0.1 M Acetate, pH 4.0) containing mineralized iron (100 µM), 2,2'-bipyridine (1 mM) and NADH (2.5 mM).


A.
pH 7.0
5μM
10μM
25μM
50μM
Abs488nm
Time (min)
B.
pH 4.0
C.
pH 7.0
D.
pH 4.0


E.
pH 7.0
5μM
10μM
25μM
50μM
Abs488nm
0.0
0.2
0.4
0.6
0
40
80
120
Time (min)
F.
pH 4.0
5μM
10μM
25μM
50μM
Abs488nm
0.0
0.2
0.4
0.6
0
40
80
120
Time (min)
G.
pH 7.0
5μM
10μM
25μM
50μM
Abs488nm
0.0
0.2
0.4
0.6
0
40
80
120
Time (min)
H.
pH 4.0
5μM
10μM
25μM
50μM
Abs488nm
0.0
0.2
0.4
0.6
0
40
80
120
Time (min)

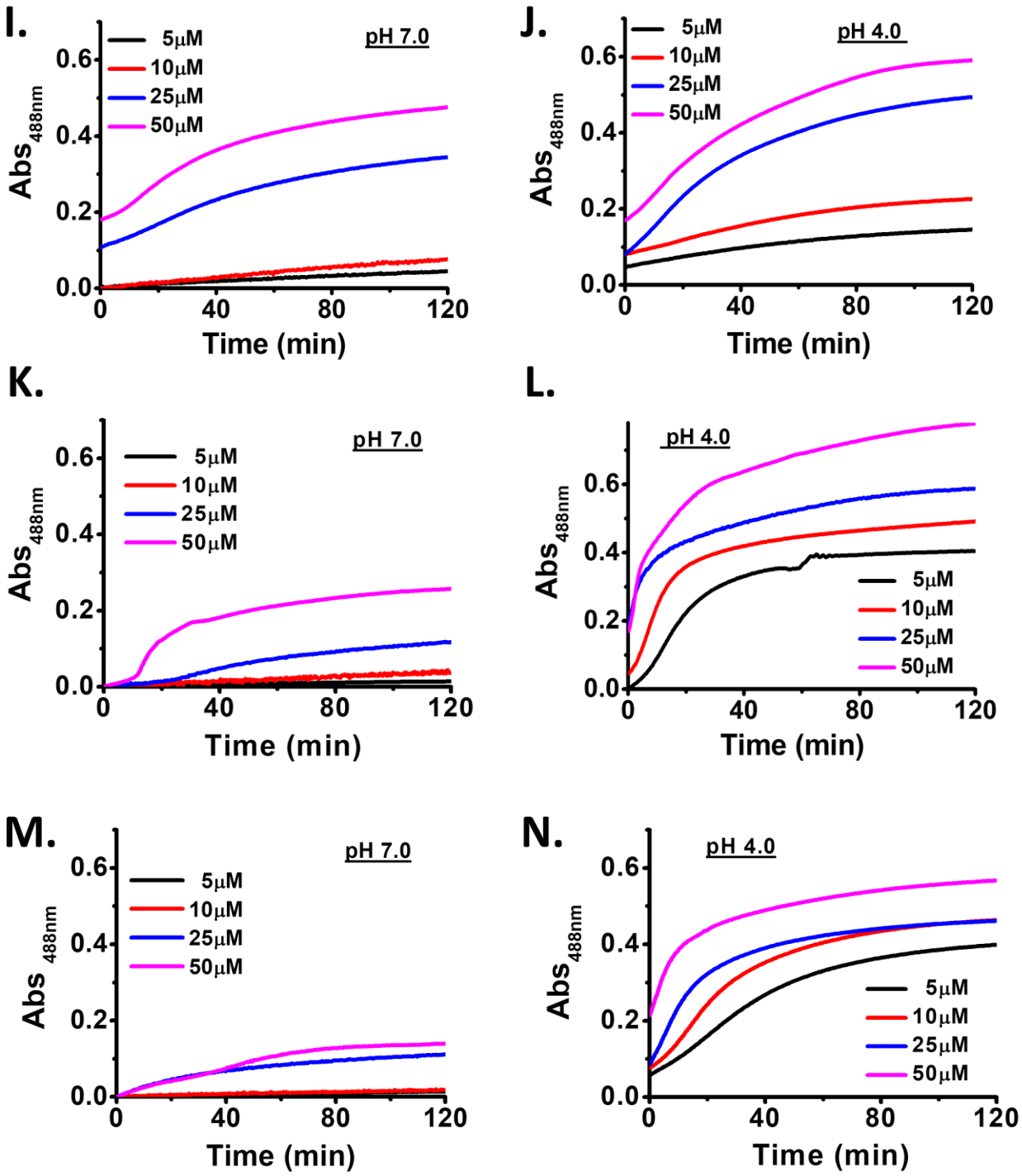


Figure S3: **Effect of dye concentration on dye mediated reductive iron release from ferritin nanocage at neutral and acidic pH.** The time course of formation of $[Fe(Bpy)_3]^{2+}$ 488 nm, resulting from the gradual iron release from ferritin, was monitored for 2 hours. Reductive iron release time courses on dye mediated electron transfer basis at neutral pH (**A**, **C**, **E**, **G**, **I**, **K**, **M**)

and acidic pH (**B**, **D**, **F**, **H**, **J**, **L**, **N**) are shown for TH (**A-B**), MB (**C-D**), TDB (**E-F**), MG (**G-H**), BCB (**I-J**), CRV (**K-L**) and NB (**M-N**). The reaction samples were prepared by varying the mediator concentration (5-50 μM) in the buffer (0.1 M MOPS/0.1 M NaCl, pH 7.0 or 0.1 M Acetate, pH 4.0) containing mineralized iron (100 μM), 2,2'-bipyridine (1 mM) and NADH (2.5 mM). Control experiments were performed in the absence of Bpy chelator for all these dyes (data not shown).

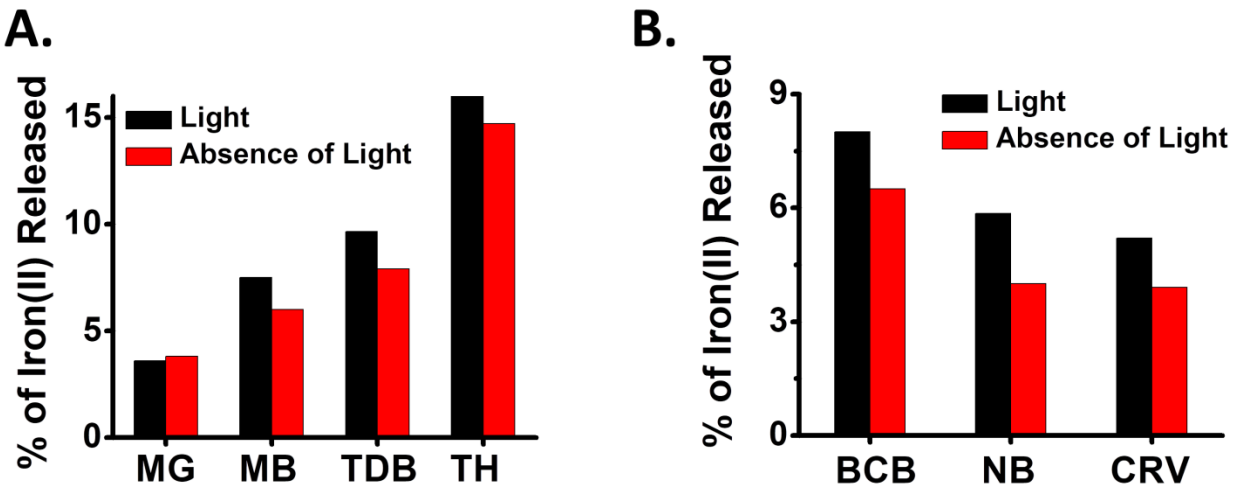


Figure S4: **Effect of light on phenthiazine and phenoxazine mediated ferritin iron release**: Iron release was carried out from mineralized frog M ferritin (480 Fe/cage) protein nanocage. 2.5 mM NADH was added to the mineralized ferritin protein solution containing 100 μM ferric iron, 25 μM phenthiazines (**A**) and phenoxazines (**B**), 1 mM 2,2'-bipyridine in 100 mM MOPS buffer (pH 7.0) containing 100 mM NaCl. Percentage (%) of iron release, after 20 min, were computed from $A_{488\ nm}$ (by monitoring the formation of $[Fe(Bpy)_3]^{2+}$ complex at 488 nm) both in the presence and absence of light.

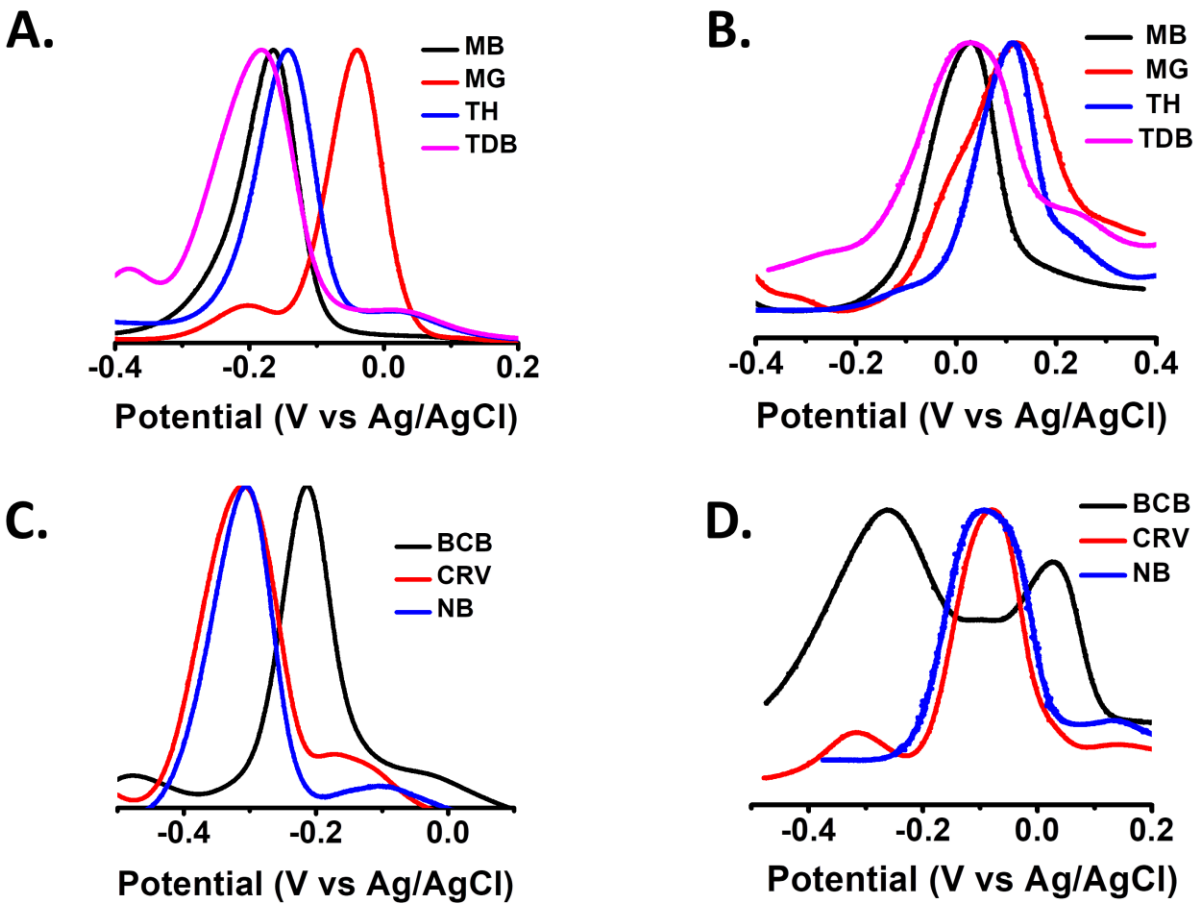


Figure S5: **Square wave voltammetry (SWV)** of phenthiazine (**A-B**) and phenoxazine (**C-D**) dyes. SWV of the ET mediators such as Phenthiazines/Phenoxazines at pH 7.0 (**A/C**) and at pH 4.0 (**B/D**). The reaction samples were prepared using final mediator concentration as 50 µM in the buffer (0.1 M MOPS/0.1 M NaCl, pH 7.0 or 0.1 M Acetate, pH 4.0).

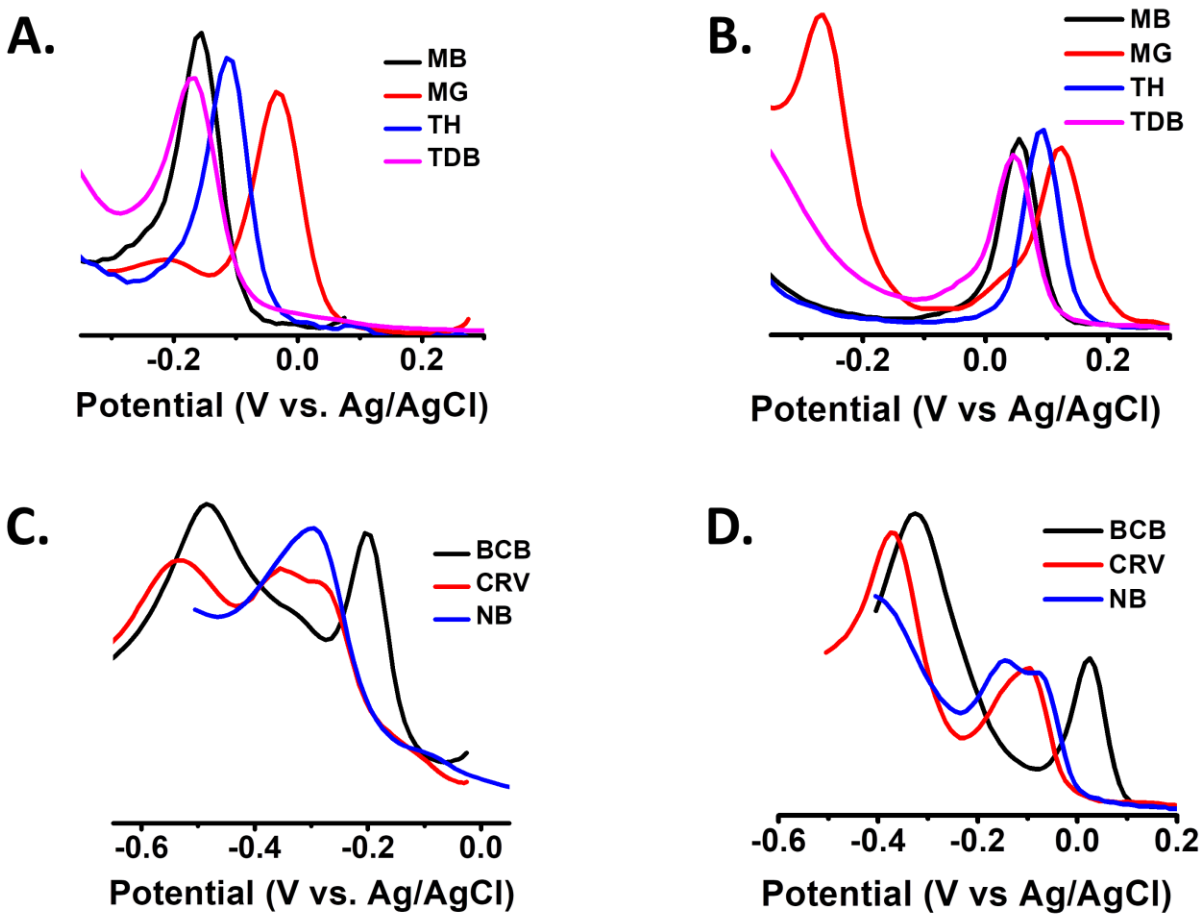


Figure S6: **Differential pulse voltammetry (DPV)** of phenthiazine (**A-B**) and phenoxazine (**C-D**) dyes. DPV of the ET mediators such as Phenthiazines/Phenoxazines at pH 7.0 (**A/C**) and at pH 4.0 (**B/D**). The reaction samples were prepared using final mediator concentration as 50 µM in the buffer (0.1 M MOPS/0.1 M NaCl, pH 7.0 or 0.1 M Acetate, pH 4.0).

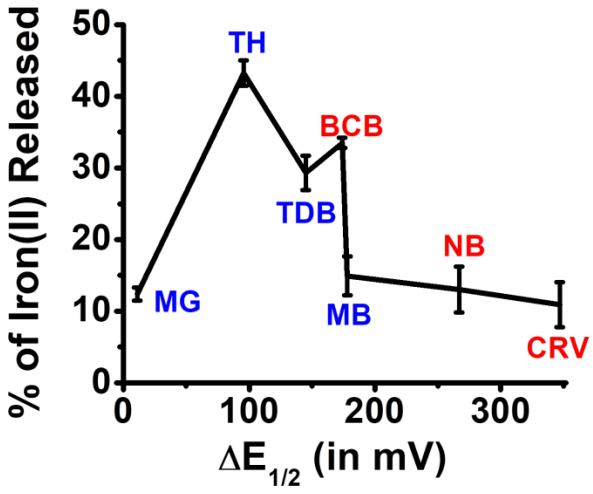


Figure S7: Iron (II) released as a function of redox potential difference between dyes and ferritin iron. Percentage of iron (II) released at neutral pH was plotted with respect to their corresponding $\Delta E_{1/2}$ value (between the dye and ferritin mineral core obtained from CV analysis). Phenthiazine and phenoxazine dyes have been labelled in blue and red color respectively.

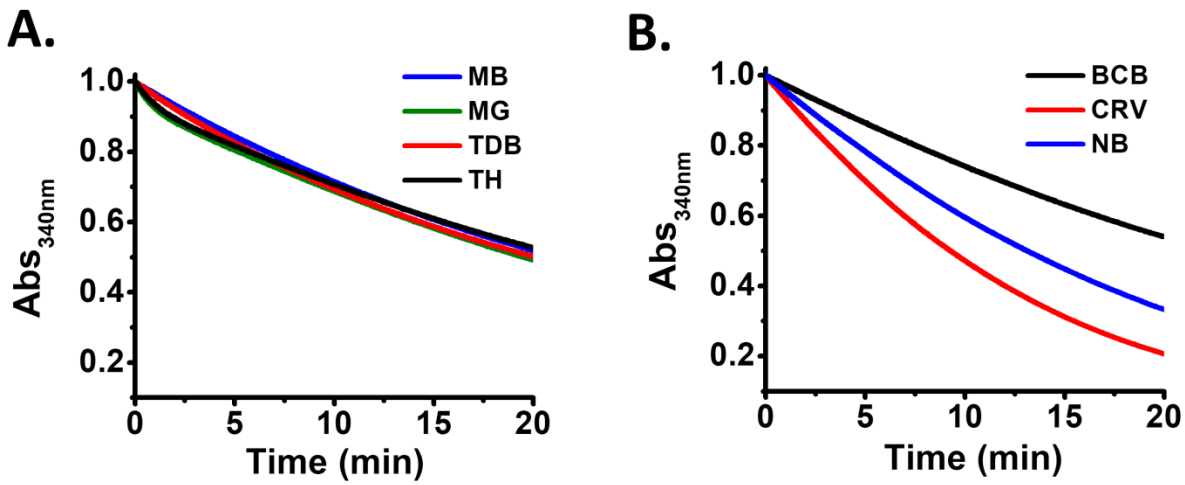


Figure S8: **NADH oxidation kinetics in the presence of different dyes at pH 4.0**. NADH oxidation by different mediators (TH/MB/MG/TDB/BCB/CRV/NB) measured by monitoring $A_{340\ nm}$ peak as a function of time. NADH oxidation time courses obtained at 340 nm by different mediators are shown in (**A**) Phenthiazines (TH/MB/TDB/TH) and (**B**) Phenoxazines (BCB/CRV/NB). The reaction samples were prepared by mixing 25 µM mediator and NADH

(160 µM final concentration) in the 0.1 M Acetate buffer pH 4.0 at room temperature. NADH oxidation kinetic traces ($A_{340\ nm}$ vs. time) for different dyes were obtained by manual mixing using SHIMADZU UV-Visible spectrophotometer under aerobic condition using quartz cuvette having pathlength 1 cm.

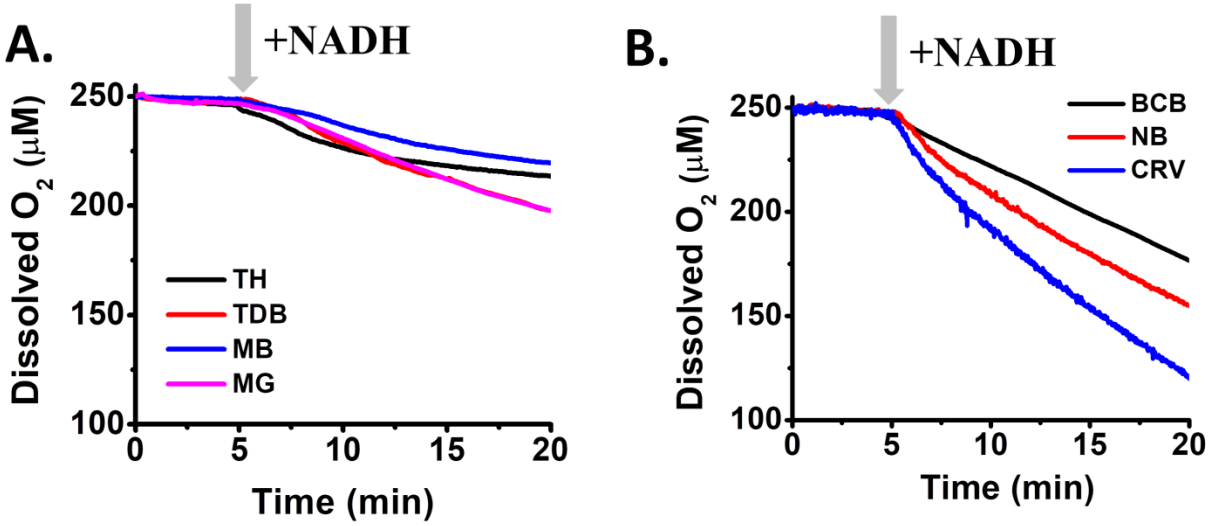


Figure S9: **Oxygen consumption time courses under acidic condition** monitored by Clark type polarographic sensor during the oxidation of NADH by (**A**) phenthiazine and (**B**) phenoxazines at 25 °C. The reaction samples were prepared by mixing of 25 µM mediator with NADH (2.5 mM) in the 0.1 M Acetate buffer, pH 4.0. Required amount of concentrated NADH was added after 5 minutes of start of the data acquisition.

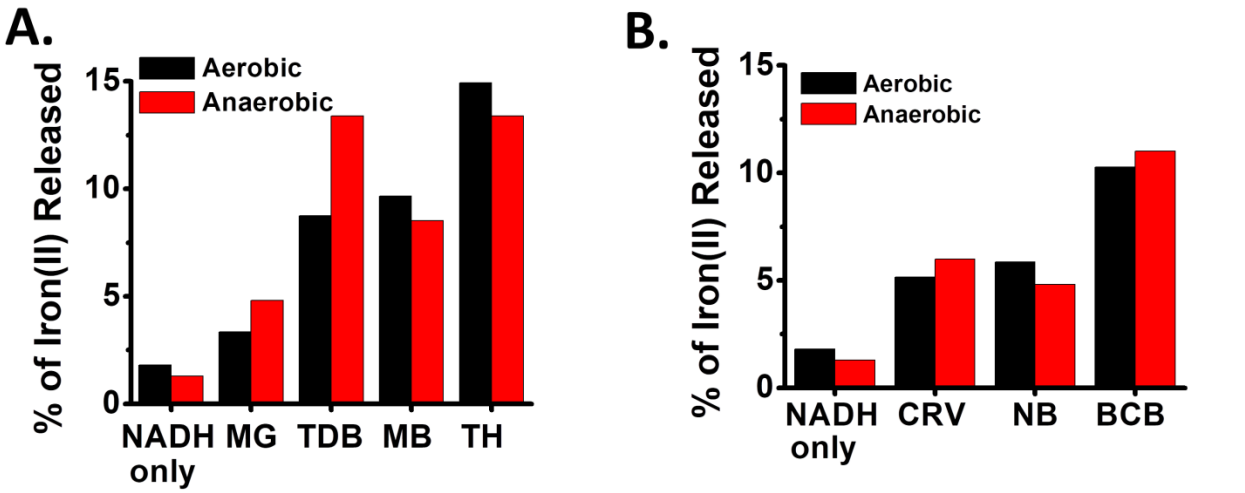
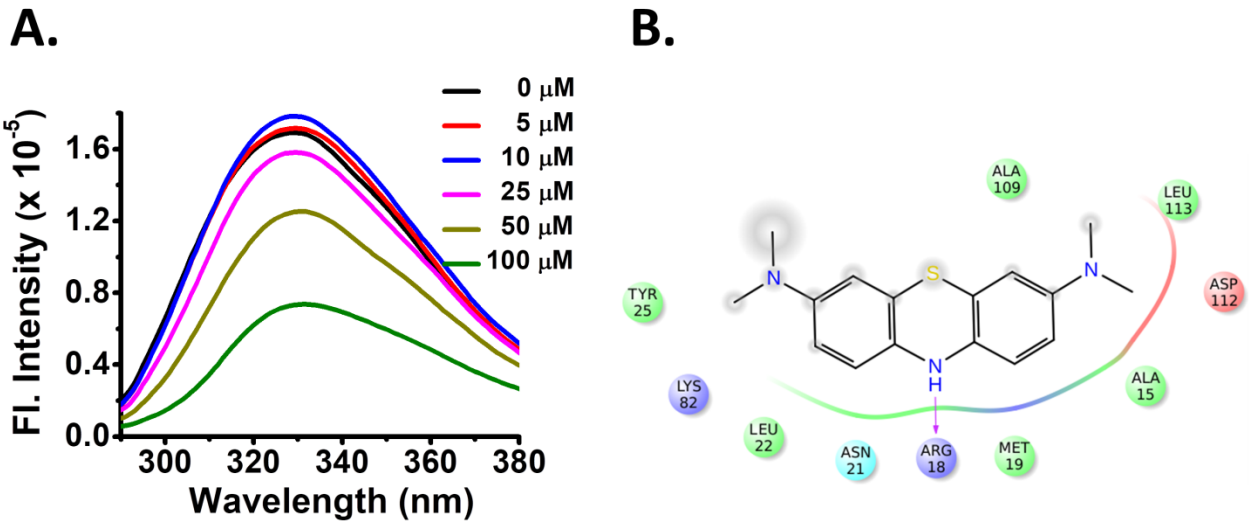
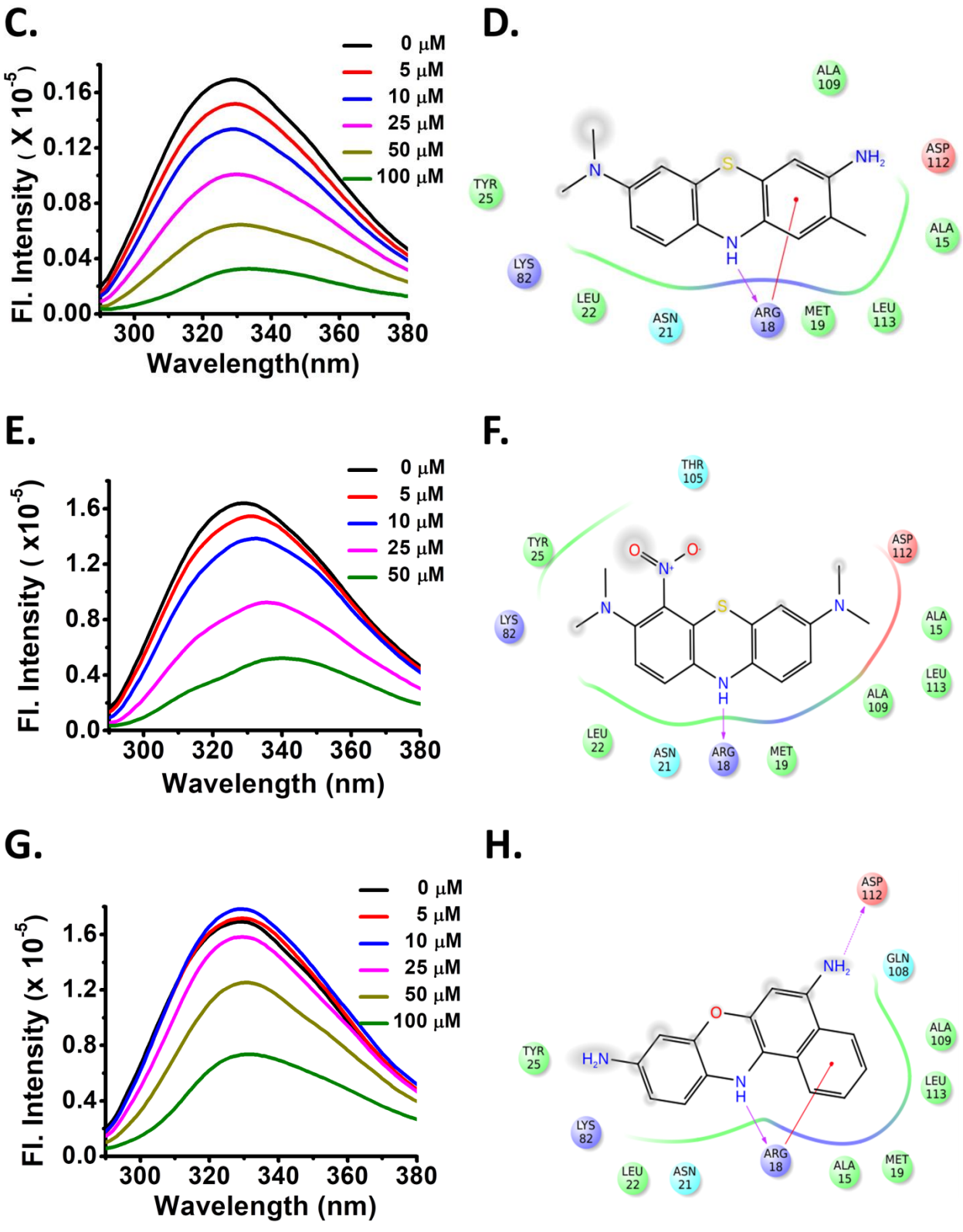


Figure S10: **Dye mediated reductive iron release from recombinant frog M ferritin nanocage under oxygen deprived condition**. Iron release measured by the formation of Fe(II)-bipyridyl complex, $[Fe(Bpy)_3]^{2+}$ at 488 nm. All the samples were purged with nitrogen gas for 10 min prior to the start of the experiment to maintain the anaerobic condition. Comparison of the percentage of Fe(II) released from ferritin by (**A**) phenthiazines (TH/MB/MG/TDB) and (**B**) phenoxazines (BCB/CRV/NB) under aerobic and anaerobic condition at 20 min. The reaction samples were prepared by fixing mediator concentration (25 µM) in the buffer (0.1 M MOPS/0.1 M NaCl, pH 7.0) containing mineralized iron (100 µM), 2,2'-bipyridine (1 mM) and NADH (2.5 mM). Control experiment were performed using only NADH without any ET mediator.




C.
Fl. Intensity (X 10-5)
0.16
0.12
0.08
0.04
0.00
300
320
340
360
380
Wavelength(nm)
0 µM
5 µM
10 µM
25 µM
50 µM
100 µM
D.
ALA 109
ASP 112
TYR 25
ALA 15
LYS 82
LEU 22
ASN 21
ARG 18
MET 19
LEU 113
E.
Fl. Intensity (x10-5)
1.6
1.2
0.8
0.4
0.0
Wavelength (nm)
0 µM
5 µM
10 µM
25 µM
50 µM
F.
THR 105
TYR 25
ASP 112
LYS 82
ALA 15
LEU 113
ALA 109
LEU 22
ASN 21
ARG 18
MET 19
G.
Fl. Intensity (x 10-5)
Wavelength (nm)
0 µM
5 µM
10 µM
25 µM
50 µM
100 µM
H.
ASP 112
GLN 108
TYR 25
ALA 109
LEU 113
LYS 82
ARG 18
LEU 22
ASN 21
ALA 15
MET 19

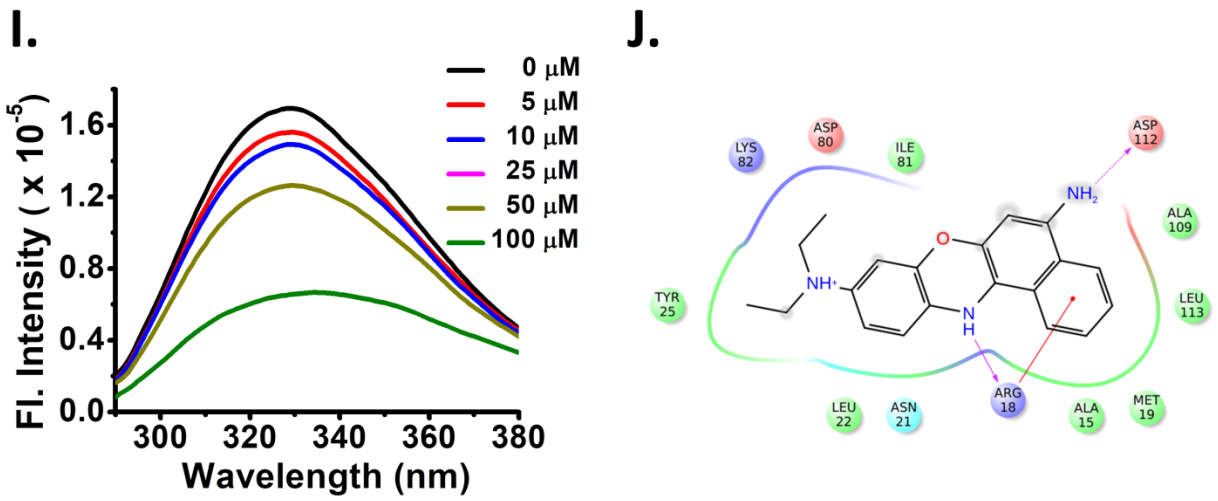
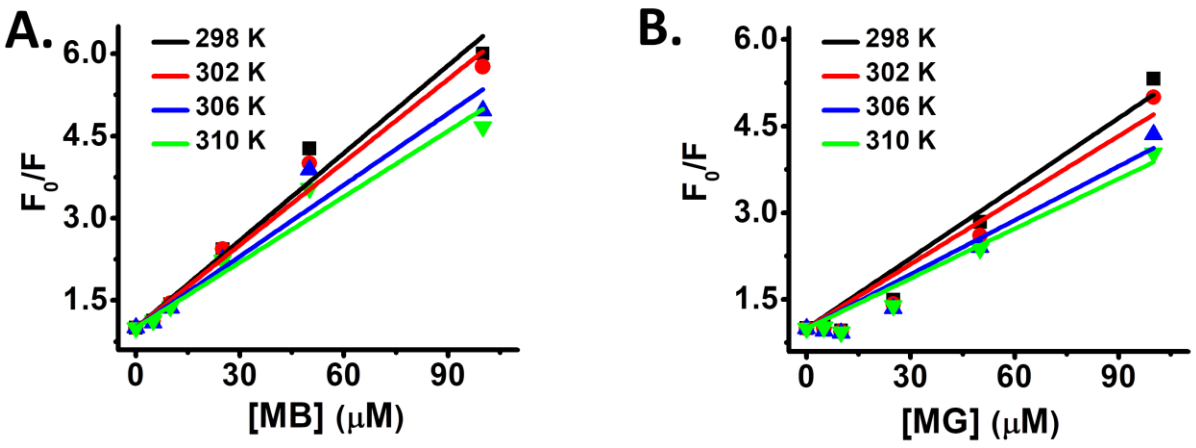


Figure S11: **Ferritin-dye interaction by fluorescence spectroscopy and molecular docking.** The study of reduced form of ET mediator's interaction on the external surface of ferritin protein nanocage by fluorescence spectroscopy and molecular docking studies. Fluorescence spectra of the ferritin protein cage in the absence and presence of dyes such as MB (**A**), TDB (**C**), MG (**E**), CRV (**G**) and NB (**I**). The mixing of ferritin protein (0.2 μM cage) with various concentrations of mediators (5, 10, 25, 50 and 100 μM) in the 0.1 M MOPS and 0.1 M NaCl (pH 7.0) were used for this study. The two dimensional representation of the various types of interaction between MB (**B**), TDB (**D**), MG (**F**), CRV (**H**) and NB (**J**) with different residues present on the ferritin surface.



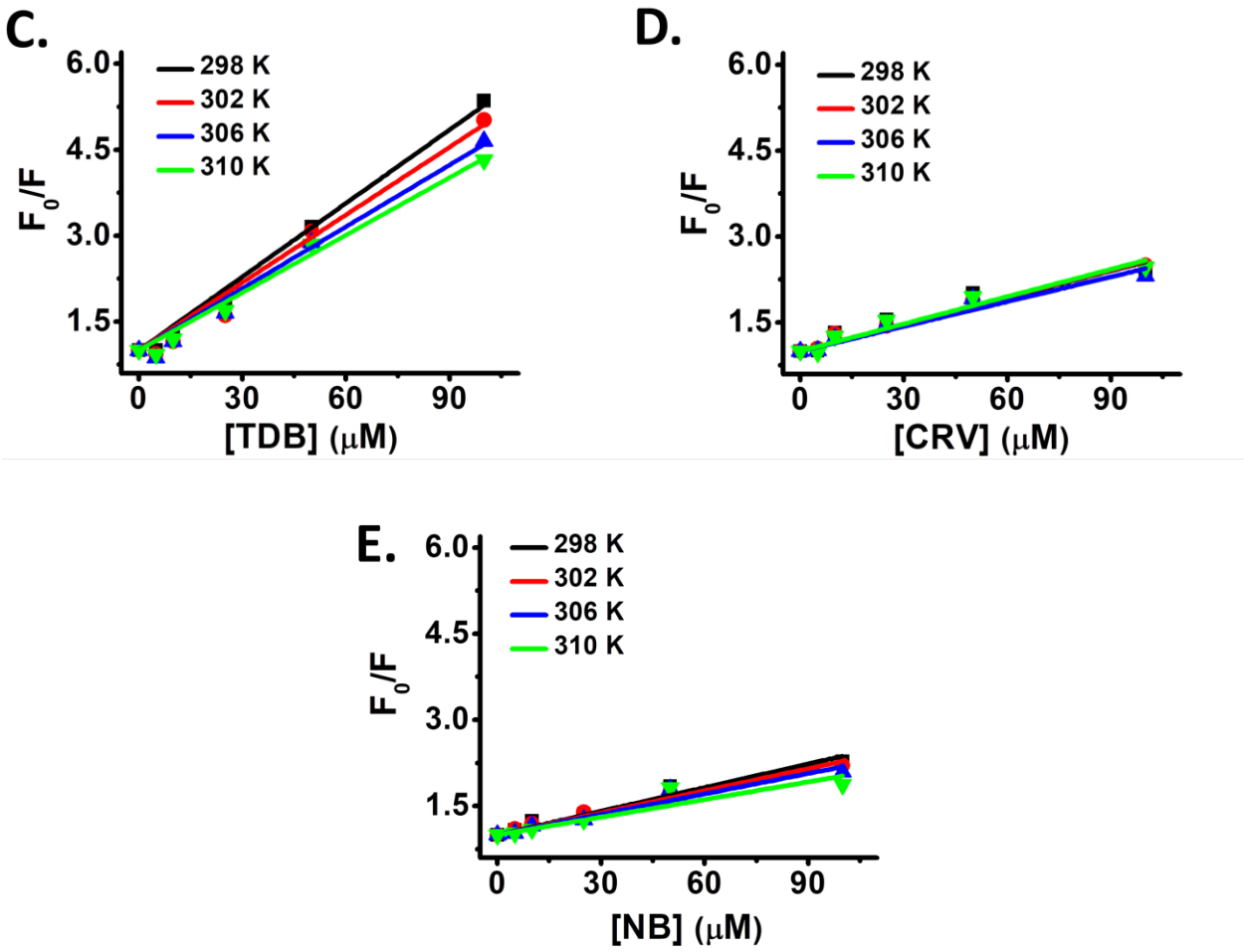


Figure S12: Stern−Volmer quenching constants (K_{SV}) for Dye-Ferritin interactions at different temperatures. Depiction of Stern-Volmer plot for the interaction of different concentration (0-100 μM) of dyes such as MB (**A**), MG (**B**), TDB (**C**), CRV (**D**) and NB (**E**) with frog M ferritin (0.2 μM cage) at different temperature (298-310 K) in 100 mM MOPS/100 mM NaCl buffer (pH 7.0).

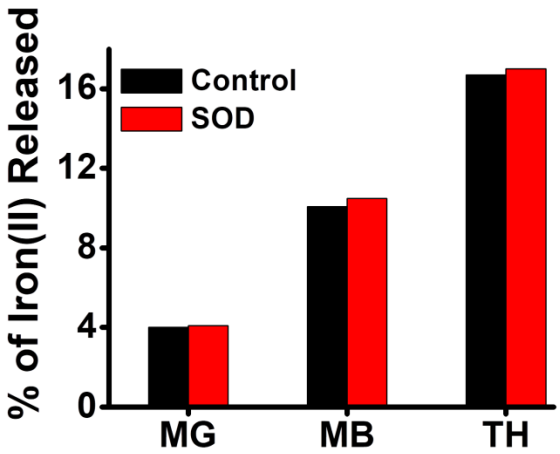


Figure S13: **Effect of superoxide scavenger (superoxide dismutase, SOD) on ferritin iron release**: Iron release was carried out from mineralized frog M ferritin (480 Fe/cage) protein nanocage. 2.5 mM NADH was added to the mineralized ferritin protein solution containing 100 μM ferric iron, 25 μM dyes, 1 mM 2,2'-bipyridine in 100 mM MOPS buffer (pH 7.0) containing 100 mM NaCl. Percentage (%) of iron release, after 20 min, were computed from $A_{488\ nm}$ (by monitoring the formation of $[Fe(Bpy)_3]^{2+}$ complex at 488 nm) both in the presence and absence (control experiment) of superoxide scavenger.

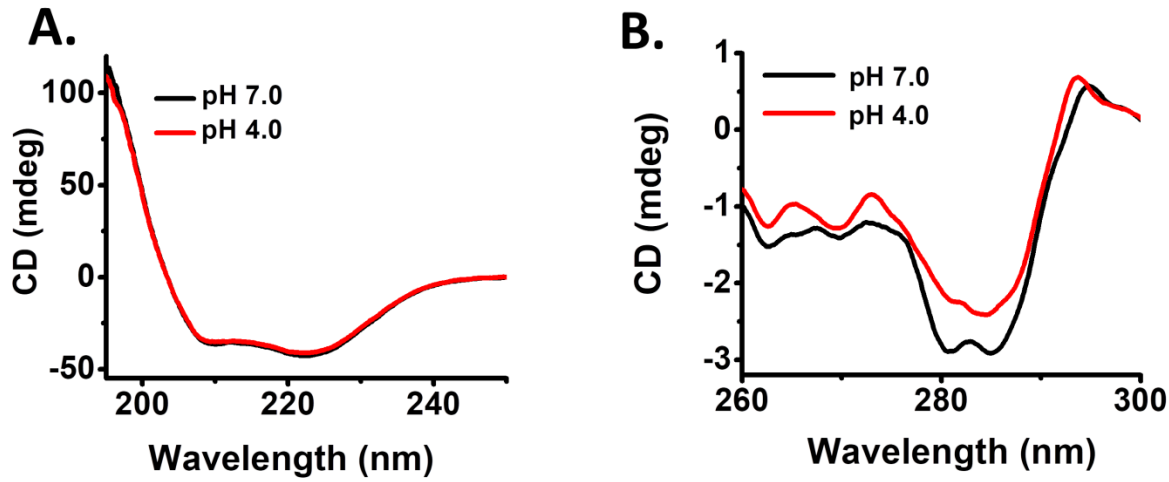


Figure S14: CD spectra of frog M ferritin at neutral and acidic pH. (**A**) Far-UV and (**B**) near-UV CD spectra of frog M ferritin at neutral (pH 7.0, 100 mM MOPS/100 mM NaCl buffer) and acidic (pH 4.0, 100 mM Acetate buffer) conditions. CD spectra were collected on a Jasco J-1500 CD Spectrometer in the far UV (190–250 nm) and near-UV (250–350 nm) range using 2 mm quartz cuvette at 20 °C. Each of the spectra obtained were the average of five scans.