Electronic Supporting Information

A rapid and sensitive detection of ferritin at a nanomolar level and disruption of amyloid β fibrils using fluorescent conjugated polymer

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UV-Visible spectra of Metalloproteins

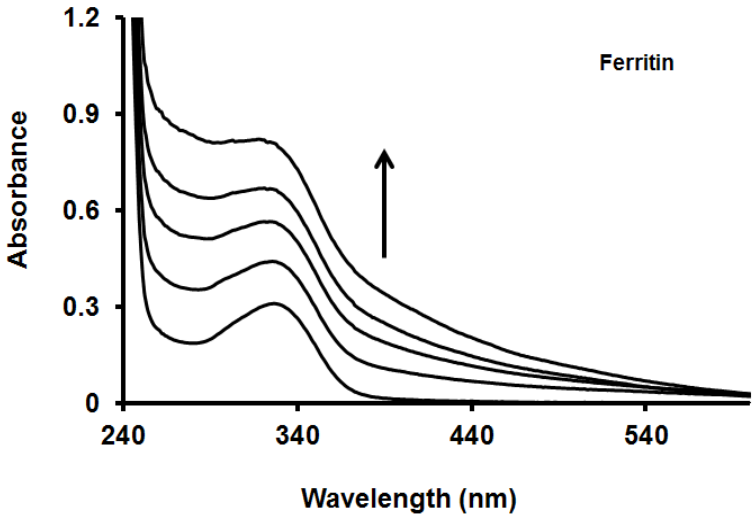

Fig. S1. UV-Vis spectra for PHQ in 4:1 THF-H_2O with increasing concentration of ferritin. Concentration of PHQ inside the cuvette was 16 μM. Final concentration of ferritin was 0.16 μM. Measurements were conducted in pH 6.5 at room temperature.

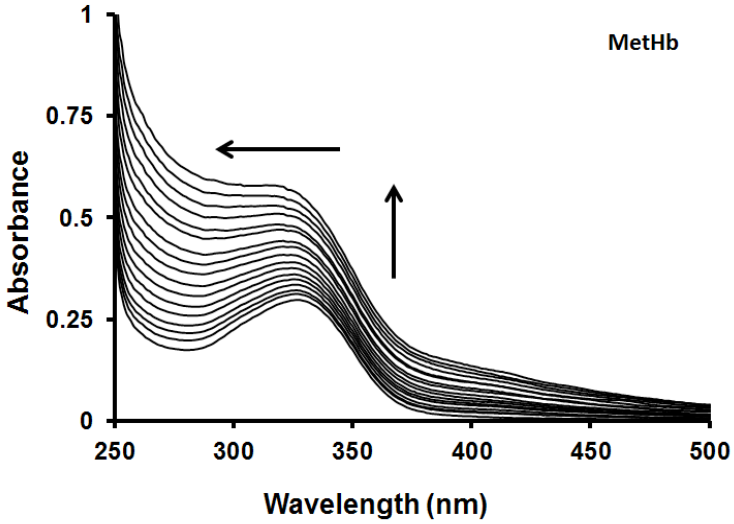

Fig. S2. UV-Vis spectra for PHQ in 4:1 THF-H_2O with increasing concentration of MetHb. Concentration of PHQ inside the cuvette was 16 μM. Final concentration of MetHb was 13.3 μM. Measurements were conducted in pH 6.5 at room temperature.

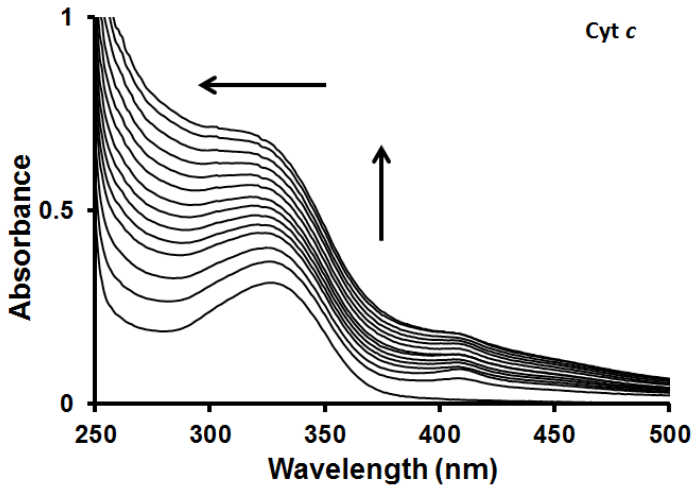

Fig. S3. UV-Vis spectra for PHQ in 4:1 THF-H_2O with increasing concentration of cyt *c*. Concentration of PHQ inside the cuvette was 16 μM. Final concentration of cyt *c* was 10 μM. Measurements were conducted in pH 6.5 at room temperature.

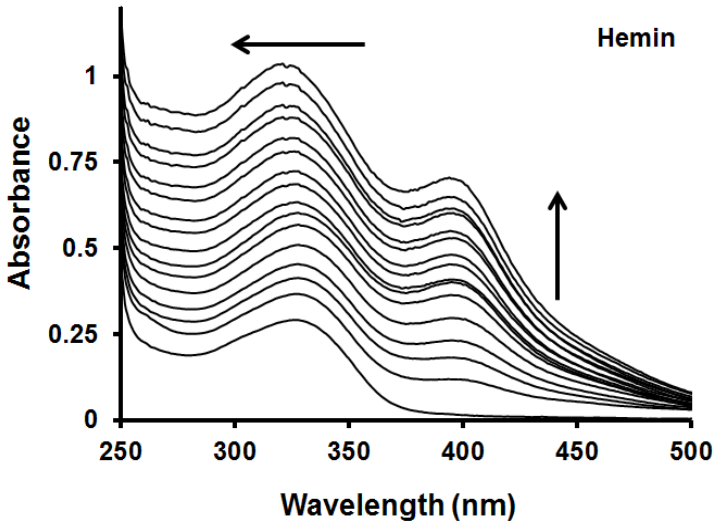

Fig. S4. UV-Vis spectra for PHQ in 4:1 THF-H_2O with increasing concentration of hemin. Concentration of PHQ inside the cuvette was 16 μM. Final concentration of hemin was 20 μM. Measurements were conducted in pH 6.5 at room temperature.

Fluorescence spectra of Non-Metalloproteins

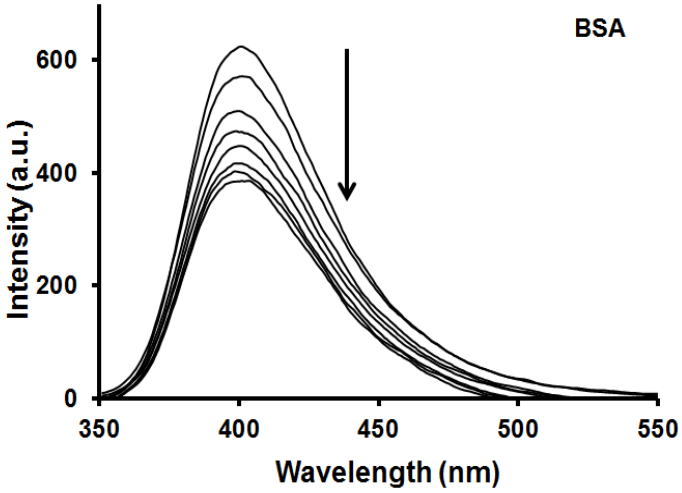

Fig. S5. Emission spectra of PHQ in 4:1 THF-H_2O with increasing concentration of BSA. Concentration of PHQ inside the cuvette was 6.6 μM. Final concentration of BSA was 50 μM. λ_{ex} and λ_{em} was 332 nm and 401 nm respectively. Measurements were conducted in pH 6.5 at room temperature.

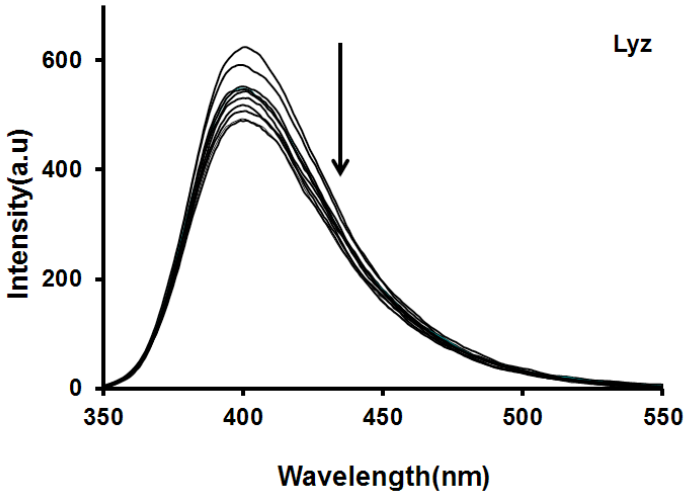

Fig. S6. Emission spectra of PHQ in 4:1 THF-H_2O with increasing concentration of Lyz. Concentration of PHQ inside the cuvette was 6.6 μM. Final concentration of Lyz was 50 μM. λ_{ex} and λ_{em} was 332 nm and 401 nm respectively. Measurements were conducted in pH 6.5 at room temperature.

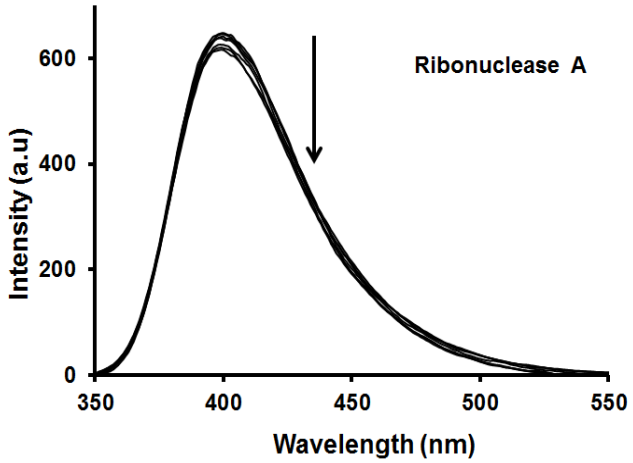

Fig. S7. Emission spectra of PHQ in 4:1 THF-H_2O with increasing concentration of Ribonuclease A. Concentration of PHQ inside the cuvette was 6.6 μM. Final concentration of Ribonuclease A was 50 μM. λ_{ex} and λ_{em} was 332 nm and 401 nm respectively. Measurements were conducted in pH 6.5 at room temperature.

UV-Visible spectra of Non-Metalloproteins

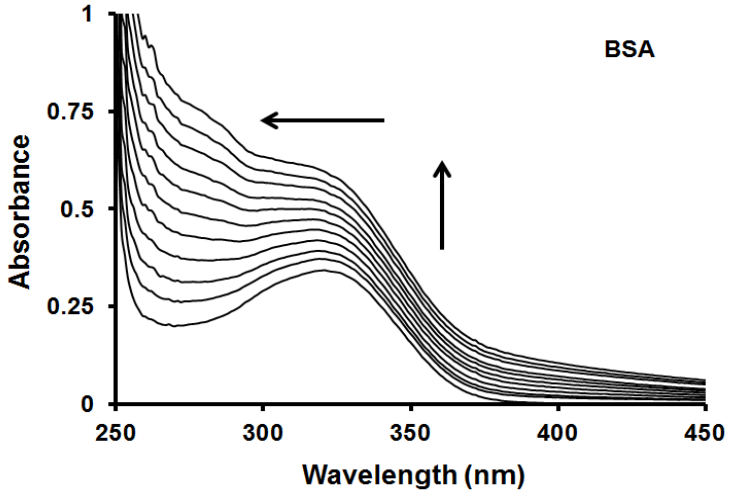

Fig. S8. UV-Vis spectra for PHQ in 4:1 THF-H_2O with increasing concentration of BSA. Concentration of PHQ inside the cuvette was 16 μM. Final concentration of BSA was 16.6 μM. Measurements were conducted in pH 6.5 at room temperature.

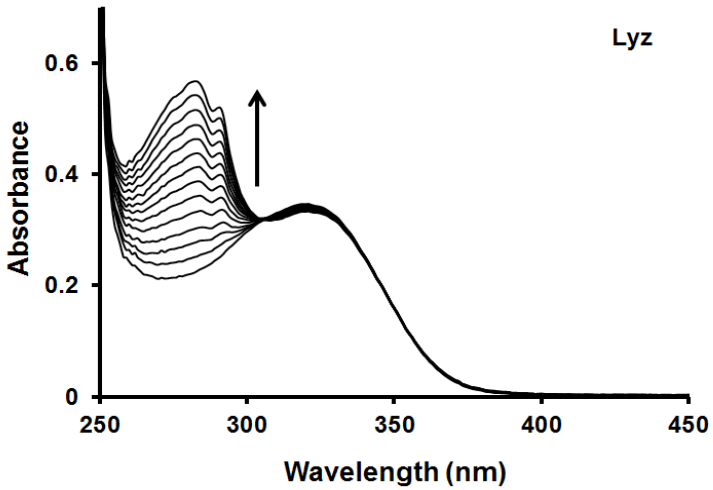

Fig. S9. UV-Vis spectra for PHQ in 4:1 THF-H_2O with increasing concentration of Lyz. Concentration of PHQ inside the cuvette was 16μM. Final concentration of Lyz was 8.6μM. Measurements were conducted in pH 6.5 at room temperature.

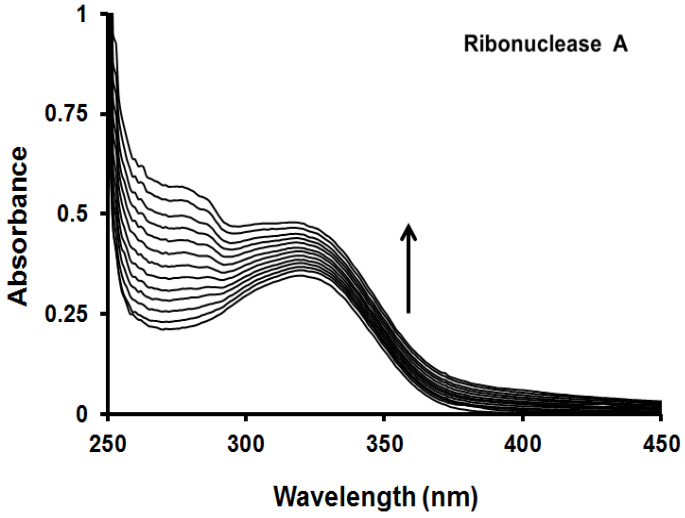

Fig. S10. UV-Vis spectra for PHQ in 4:1 THF-H_2O with increasing concentration of Ribonuclease A. Concentration of PHQ inside the cuvette was 16 μM. Final concentration of Ribonuclease A was 8.0 μM. Measurements were conducted in pH 6.5 at room temperature.

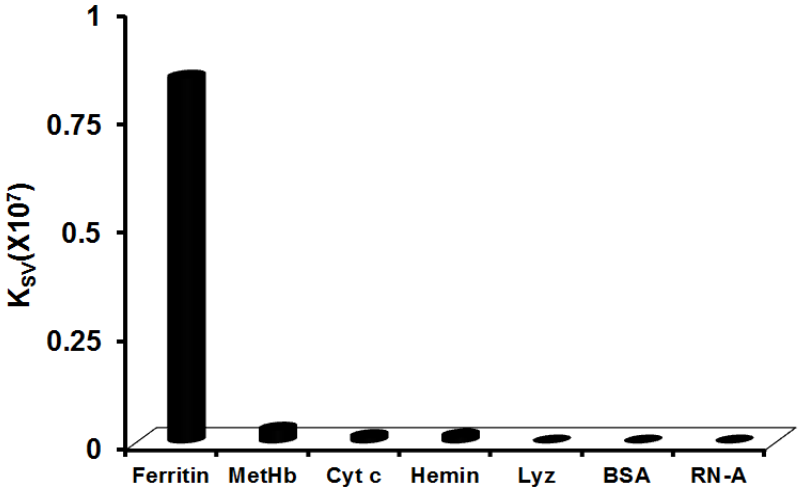

Fig. S11. Bar diagram depicting changes observed in the K_{SV} value of metalloproteins and non-metalloproteins.

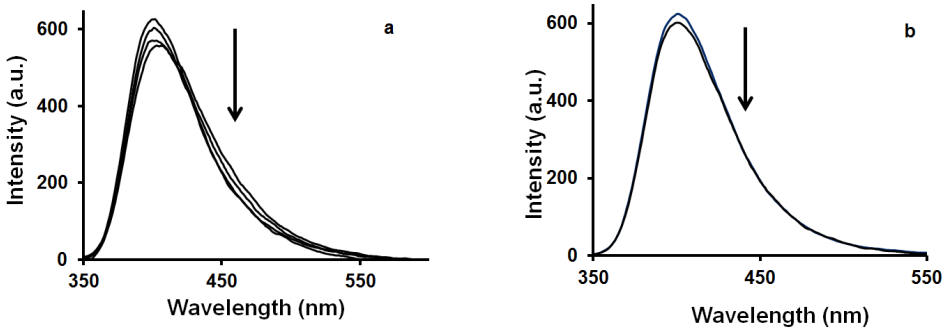

Fig. S12. (a) Gradual reduction in fluorescence intensity (12%) of PHQ (6.6 µM) occurred on addition of upto 10 µL CSF. (b) No quenching of PHQ (6.6 µM) occurred on adding CSF upto 100 µL indicating these samples lack inorganic iron or iron metalloproteins.

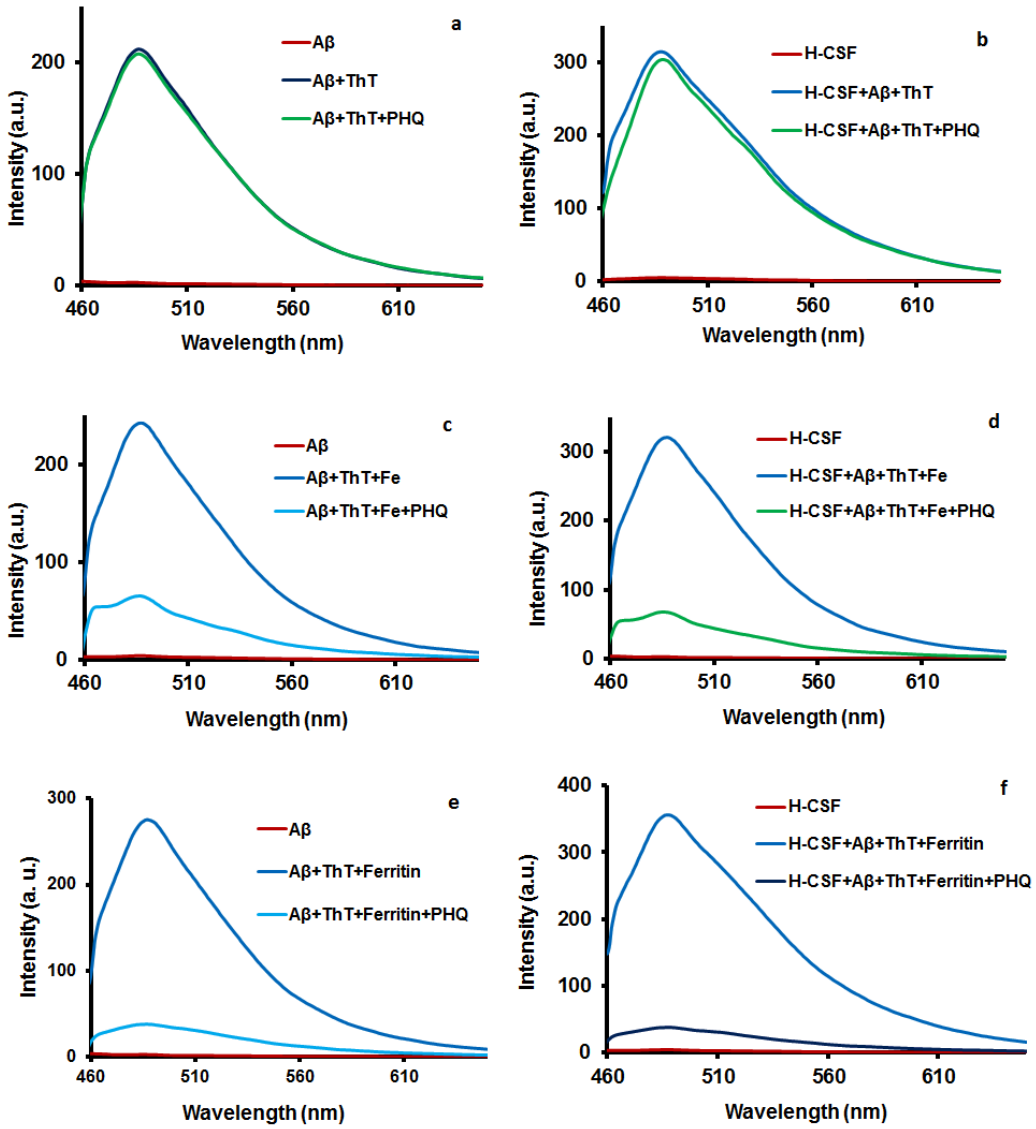

Fig. S13. ThT fluorescence changes observed in various incubated samples. No enhancement was observed in Aβ(1-40) and H-CSF without ThT (a) No significant changes in Aβ(1-40) + ThT with PHQ; (b) No significant changes in H-CSF + ThT with PHQ (c & e) Flourescence changes in Aβ(1-40) + ThT with iron and ferritin, followed by subsequent quenching with PHQ (d & f) Flourescence changes in H-CSF + Aβ(1-40) + ThT with iron and ferritin, followed by subsequent quenching with PHQ. Excitation and emission values are 440 nm and 488 nm.

Table 1. Data of analyzed CSF samples of subjects obtained from GNRC, Guwahati, India.

S. No	Sample	Age	Sex
1	Non healthy CSF (Aβ)	45	F
2	Non healthy CSF (Aβ)	40	M
3	Non healthy CSF (Aβ)	42	M
4	Healthy CSF (non-Aβ)	32	M
5	Healthy CSF (non-Aβ)	36	M
6	Healthy CSF (non-Aβ)	52	M
7	Healthy CSF (non-Aβ)	46	F
8	Healthy CSF (non-Aβ)	48	M
9	Healthy CSF (non-Aβ)	44	M
10	Healthy CSF (non-Aβ)	40	F
11	Healthy CSF (non-Aβ)	33	M
12	Healthy CSF (non-Aβ)	42	M
13	Healthy CSF (non-Aβ)	32	M
14	Healthy CSF (non-Aβ)	45	F
15	Healthy CSF (non-Aβ)	51	M
16	Healthy CSF (non-Aβ)	37	M
17	Healthy CSF (non-Aβ)	42	M
18	Healthy CSF (non-Aβ)	46	F
19	Healthy CSF (non-Aβ)	32	M
20	Healthy CSF (non-Aβ)	40	M

Experimental procedure for toxicity analysis

Hemotoxicity of PHQ: Hemotoxicity of PHQ was assessed by the method described previously[1]. In brief, 5% hematocrit was incubated with different concentration of PHQ (0-20μM) for 1hr at 37^0C in PBS-G (PBS containing 1% glucose). Post-treatment, RBCs were separated from the reaction mixture by centrifugation at 1000 rpm and supernatant was read at 540nm. RBC treated with 1% trition X-100 as 100% hemolysis.

Cyto-toxicity of PHQ: Cytotoxicity of PHQ was assessed by the method described previously[2]. In brief, 10^5 MCF-7 cells were treated with different concentration of PHQ

(0-10μM) in complete media (RPMI 1640+10% serum) for 48hrs at 37^0C. Cells were washed with PBS and viability was assessed by MTT reduction assay. Cells treated with DMSO is considered as control and used to calculate the viability of PHQ treated cells.

Toxicity analysis of PHQ: Hemotoxicity assay is used to assess the ability of the compound to cause hemolysis. Toxicity analysis of PHQ in hemotoxicity indicates no significant toxicity against RBCs to cause the release of hemoglobin in the supernatant (Fig. S14 a). RBCs are terminally differentiated cells and have no ability to proliferate. Testing the toxicity of PHQ towards fast growing breast cancer MCF-7 shows no reduction of cellular viability upto 10μM in a 48hr exposure period (Fig. S14 b). The solubility of PHQ in aqueous solvent system limits us to test higher concentrations. In conclusion, PHQ has no toxic effects against RBCs to cause hemolysis as well as it has no negative effect on the growth of proliferative MCF-7 cells.

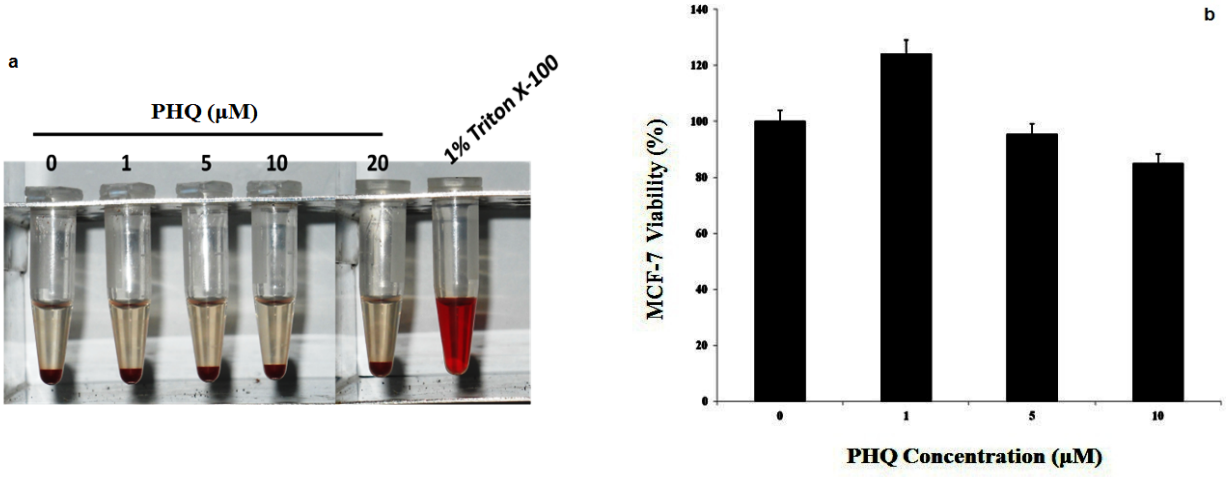

Fig. S14. Toxicity analysis of PHQ (a) Hemotoxicity test (b) Cyto-toxicity test

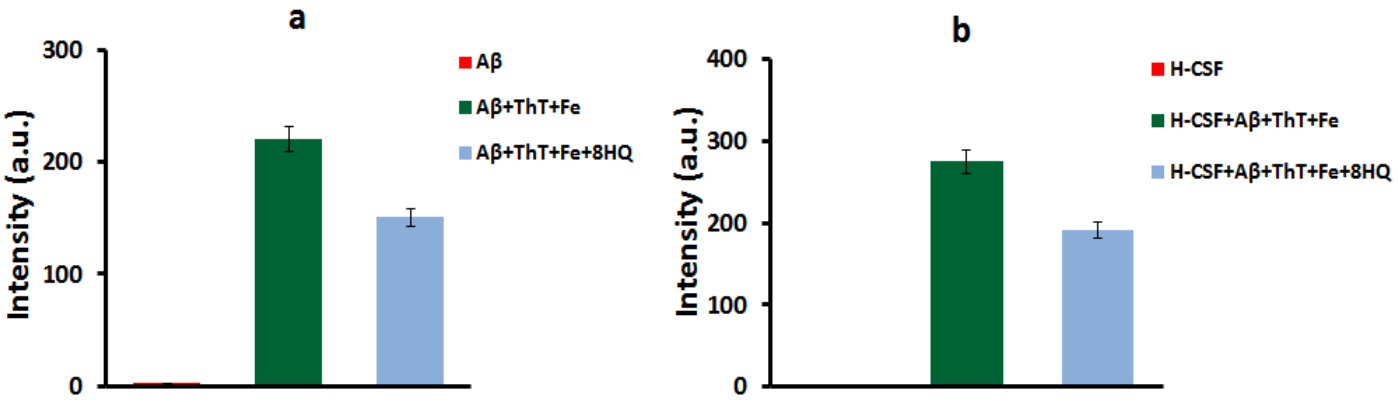

Fig. S15. ThT fluorescence changes observed in various incubated samples. No enhancement was obtained in Aβ(1-40) and H-CSF without ThT. (a) Fluorescence changes in Aβ(1-40) + ThT with Fe (10 µM), followed by subsequent quenching with 8HQ (100 µM) (d) Fluorescence changes in H-CSF + Aβ(1-40) + ThT with Fe (10 µM), followed by subsequent quenching with 8HQ (100 µM) (error bars = ± 5%).

ESI-CR-movie file attached separately.

ESI-ThT-movie file attached separately.

References:

1 S. N. Balaji and V. Trivedi, *Ind J Clin Biochem.*, 2011, **27**, 178.

2 R. Deshmukh and V. Trivedi, *Toxicology in Vitro*, 2013, **27**, 16.