Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

A supramolecular assembly enables discrimination between metalloproteins and non-metalloproteins

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Thioflavin T (ThT) was obtained from Sigma-Aldrich as the chloride salt of the dye and was re-crystallized twice from methanol. The purity of the re-crystallized ThT was checked through NMR spectra. β-cyclodextrin, sulphated sodium (extent of labelling 12-14) was purchased from Sigma-Aldrich. Lysozyme (Lz) from chicken egg white, Myoglobin (Mb), Cytochrome C (Cyt C) from equine heart, Pepsin (Pep) from porcine gastric mucosa, Trypsin (Tr) from porcine pancreas, Ovalbumin (Ova) from chicken egg white, β-lactoglobulin (BLG) from bovine milk, and Ferritin (Fer) from equine spleen, type I, saline solution, bovine serum albumin, alpha amylase, and alpha chymotrypsin were also obtained from Sigma Aldrich and were used as received. DNA polymerase was obtained from thermo scientific and was used as received. Different metal salts such as sodium chloride, lithium chloride, potassium chloride, silver nitrate and ferrous sulfate were obtained for Sigma-Aldrich and were used as received. All samples were prepared using nanopure water (conductivity less than 0.1 μS cm^{-1}) obtained from a Millipore Milli-Q system and pH of all the stock solution was maintained at 7.

Ground-state absorption spectra were recorded with a Jasco UV-visible spectrophotometer (model V-650). Steady-state fluorescence spectra were obtained with a Hitachi spectrofluorimeter model F-4010. All the measurements were carried out at ambient temperature (~ 25 ^{0}C) using a quartz cell of 1 cm path length. For titrations, an incubation time of 15 mins was allowed before measurement.

The time-resolved fluorescence measurements were carried out using a diode laser based time-correlated single-photon counting (TCSPC) spectrometer from IBH, U.K. A 406 nm diode laser (1 MHz repetition rate) was used for sample excitation. A microchannel plate (MCP) detector was used for the detection of the emitted photons through a monochromator. The IRF of the TCSPC instrument was measured by collecting the scattered light from a TiO_2 suspension in water and was found be ~120 ps. The lifetime measurements were done at magic angle condition. The data analyzing software version DAS-6 from IBH was used for the reconvolution analysis of the observed decays, following suitable exponential function to obtain best fits for the decays. The quality of the fits and consequently the multi-exponential nature of the decays were judged by the reduced chi-square ($\chi 2$) values and the distribution of the weighted residuals

among the data channels. For a good fit, the $\chi 2$ value is close to unity and the weighted residuals are distributed randomly around zero line among the data channels. All measurements were carried out at pH value of 7, near physiological conditions. Principal component analysis was performed using Origin (version 8.6).

Optimization conditions for Sensing

Since our sensing platform is based on a fluorescent supramolecular aggregate assembly of a popular bio-probe, Thioflavin-T (ThT) with a versatile polyvalent sulphated β-cyclodextrin derivative (SCD). Thus, to attain the best sensing of this sensor platform, various experimental parameters such as pH, ionic strength, temperature etc. need to be optimized such that the maximum emission response of ThT-SCD system can be achieved.

pH Optimization: For a fluorescent sensing platform, its response under different pH should also be evaluated. By changing the pH values from 3 to 10, we measured the emission intensity of ThT –SCD complex. The emission properties of ThT-SCD complex were not significantly affected in the pH range from 4-10. This indicates that metalloprotein/non-metalloprotein sensing, using ThT-SCD complex as a sensing platform, meets reasonably broad pH requirements. Therefore, the pH value of 7, near physiological conditions, was chosen as the optimum experimental condition.

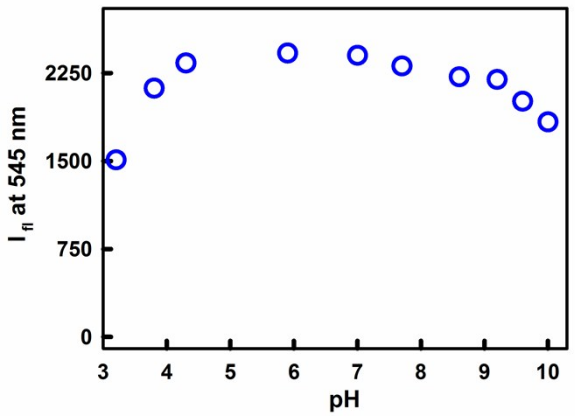


Figure S1. Variation in emission intensity (λ_{ex} = 400 nm, λ_{em} = 545 nm) of ThT-SCD complex at various pH.

Ionic strength optimization: To optimize the response of the ThT-SCD system with respect to ionic strength of the medium, we studied the response of the ThT-SCD system as a function of salt concentration. Figure S2 summarizes the results from these experiments. As evident, the addition of salt gradually decreases the emission intensity at 545 nm in a concentration dependent manner (Figure 4A) and at a higher salt concentration of >20 mM, the emission intensity reaches the situation prevailing in bulk water.. Since SCD is an anionic host, and ThT is cationic in nature, so a dominant contribution of electrostatic interaction towards the formation of these fluorescent self-assembled system is expected. These results clearly suggest that the addition of salt directly suppresses the electrostatic interaction via shielding of the charges. However, from the viewpoint of optimization and to obtain the best sensing performance with the current sensing platform, we have carried out sensing experiments in the water solution with pH maintained at 7.

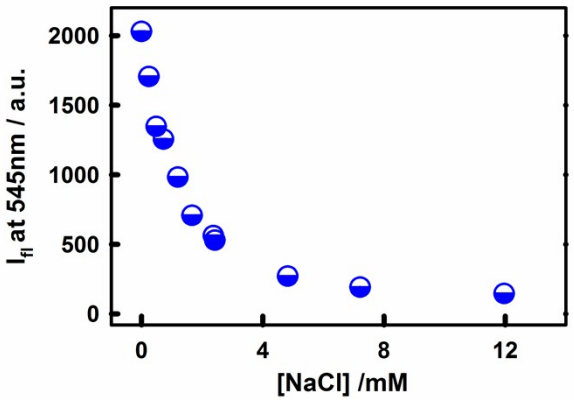


Figure S2. Variation of emission intensity of ThT-SCD system at 545 nm with increasing concentrations of NaCl.

Temperature optimization: ThT-SCD complex represents a self-assembled system which, apart from electrostatic interactions, also integrates multiple weak interactions such as van der Waals, London dispersion forces, and hydrophobic interactions. Thus, the present system is expected to be responsive to temperature. Figure S3 shows the emission response of the ThT-SCD system as a function of temperature.

It is obvious from the figure that the emission response of ThT-SCD system weakens with increase in temperature, which is a reflection of decrease in stability of the ThT-SCD complex with increase in temperature, as temperature weakens the non-covalent forces involved in the formation of the fluorescent aggregate assembly. Thus, it is obvious that at elevated temperature, the sensing performance will be affected when compared to that at room temperature leading to lower detection limit at elevated temperature. In the present work, the sensing performance was evaluated at ambient temperature of 25°C.

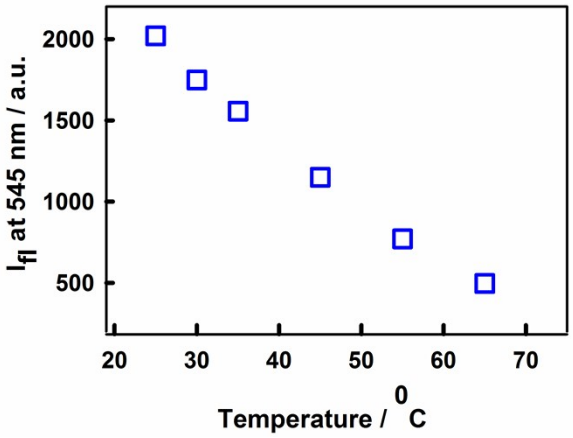


Figure S3. Variation of emission intensity of ThT-SCD system at 545 nm with increasing temperature.

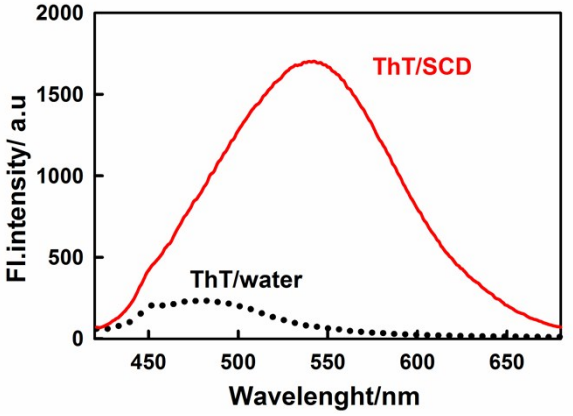


Figure S4: Steady-state fluorescence spectrum of ThT (20 μM)) in water (dotted black line) and 10 μM SCD (solid red line).

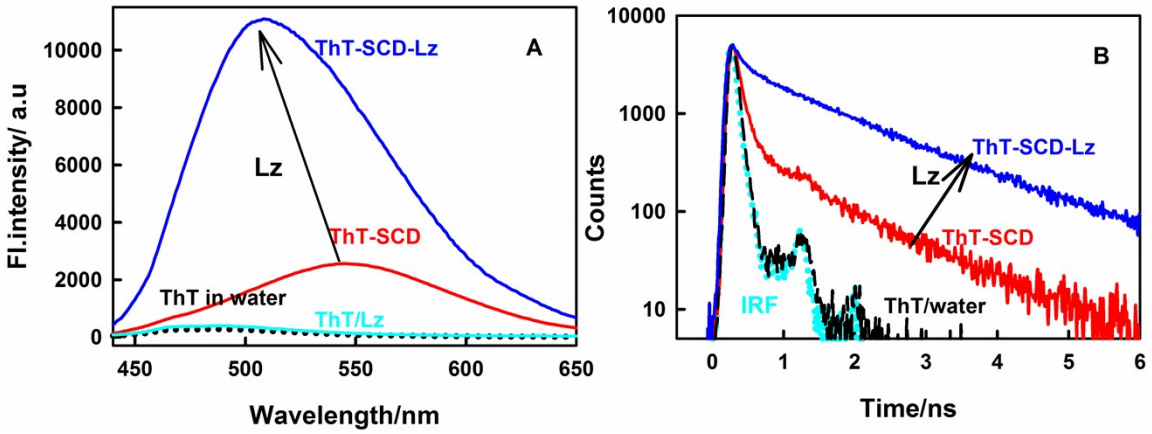


Figure S5: (A) Steady-state emission spectrum of ThT-SCD (red), ThT-SCD –Lysozyme (blue), ThT-Lysozyme (light green). The dotted line represents the emission spectrum of ThT in water. (B) Transient decay traces of ThT-SCD (red), ThT-SCD –Lysozyme (blue) and ThT-water (light green). ThT-lysozyme system shows very similar decay trace as that of ThT-water and is not presented here, to avoid crowding. IRF stands for instrument response function.

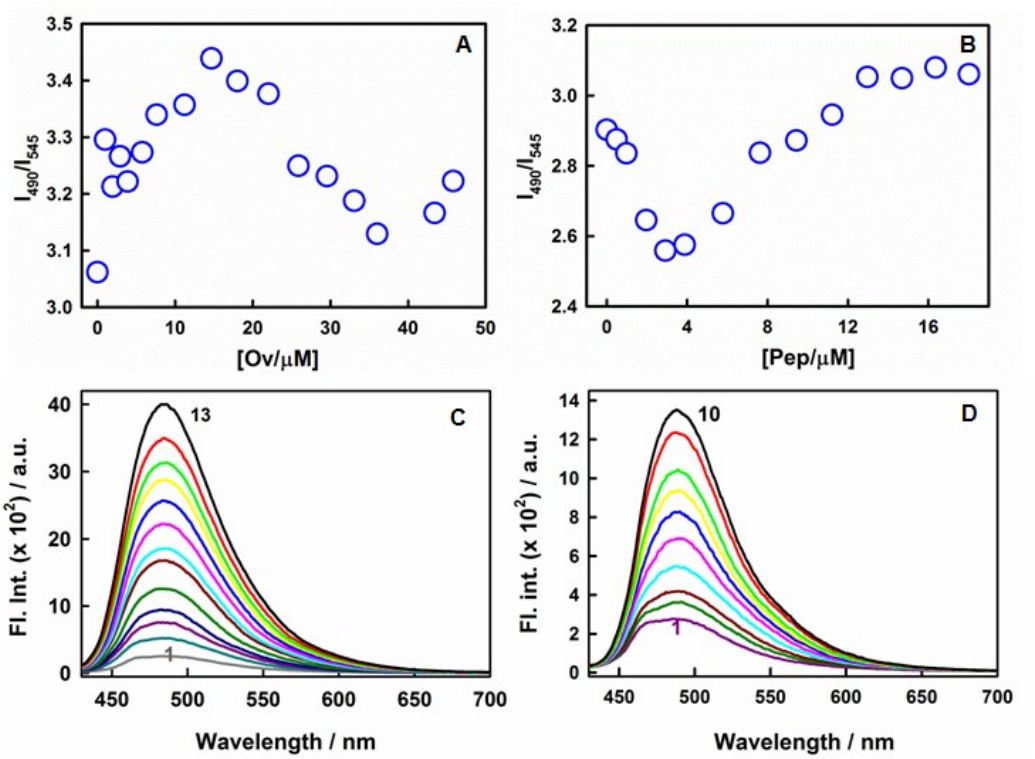


Figure S6: . Variation of emission intensity ratio (I_{490}/I_{545}) with increasing concentration of (A) Ovaalbumin (B) Pepsin. Steady-state emission spectrum of ThT (20 μM) at varying concentration of (C) Ovaalbumin (Ov) from 1) 0 μM to 13) 45 μM and (D) Pepsin (Pep) from 1) 0 μM to 13) 18 μM

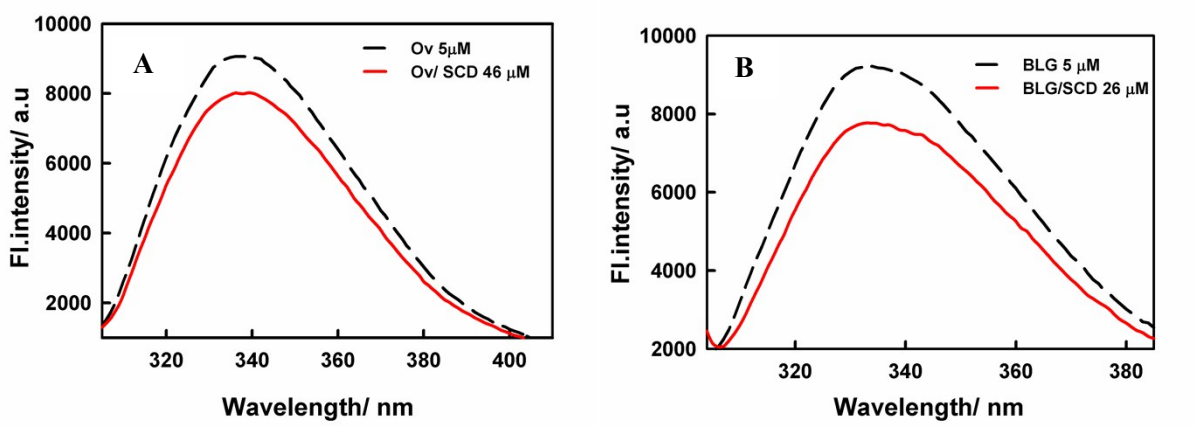


Figure S7 : Tryptophan emission intensity of (A) Ovaalbumin-5 μM (black dashed line) and in presence of 46 μM SCD (solid red line). (B) β-Lactoglobulin-5 μM (black dashed line) and in presence of 26 μM SCD (solid red line).

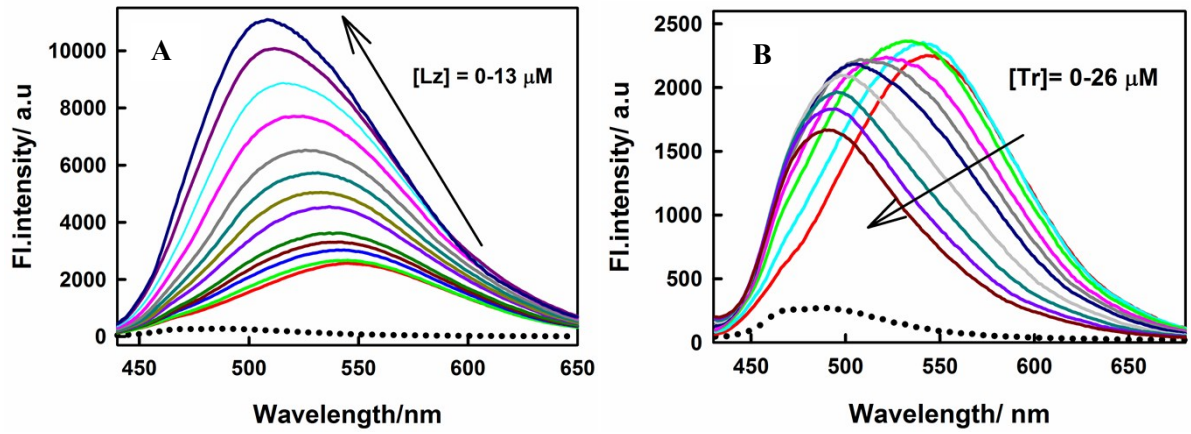


Figure S8: Steady-state fluorescence spectrum of ThT (20 μM) in SCD at varying concentration of (A) Lysozyme (Lz) and (B) Trypsin (Tr). The black dotted line represents the emission spectra of ThT in water.

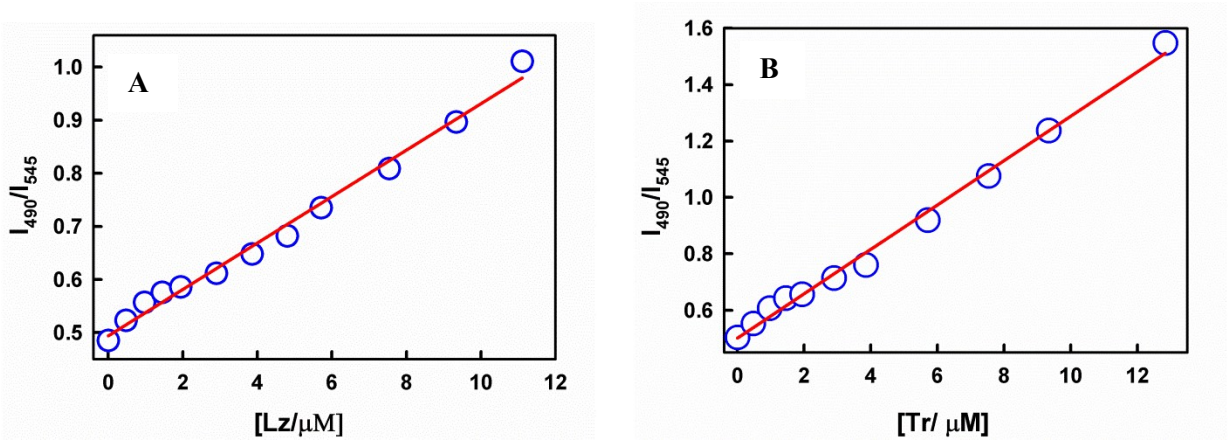


Figure S9: Variation of emission intensity ratio (I_{490}/I_{545}) with increasing concentration of (A) Lz and (B) Tr. The ratiometric analysis of the titration data reveals a linear variation of the ratio at 490 to 545 nm with increasing protein concentration and the correlation equations are represented below

$I_{490}/I_{545} = 0.45 + 0.04$ [Lz/μM]; $R^2 = 0.976$

$I_{490}/I_{545} = 0.47 + 0.08$ [Tr/μM]; $R^2 = 0.986$

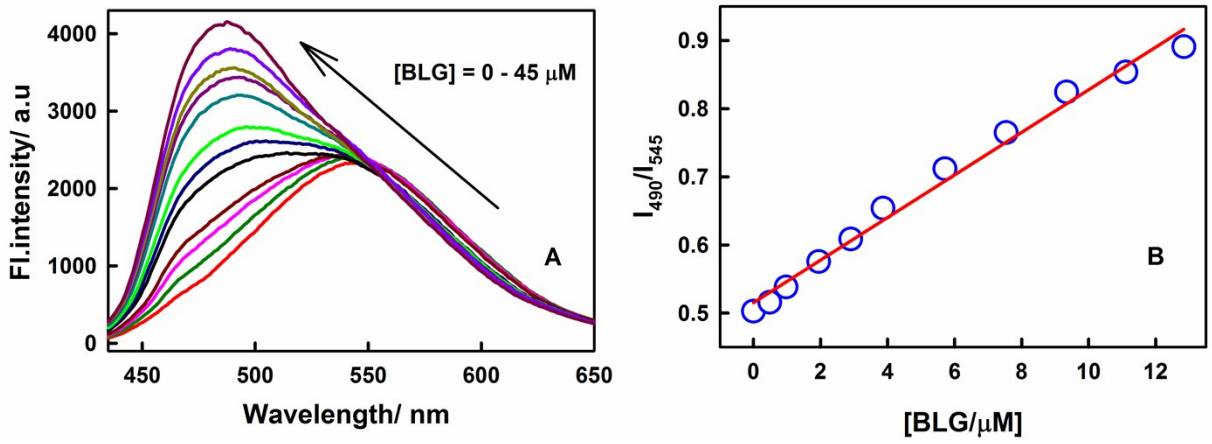


Figure S10: (A) Steady-state emission spectrum of ThT (20 μM) in SCD at varying concentration of β-Lactoglobulin (BLG). (B) Variation of emission intensity ratio (I_{490}/I_{545}) with increasing concentration of β-Lactoglobulin. $I_{490}/I_{545} = 0.47 + 0.03$ [Lz/μM]; $R^2 = 0.974$

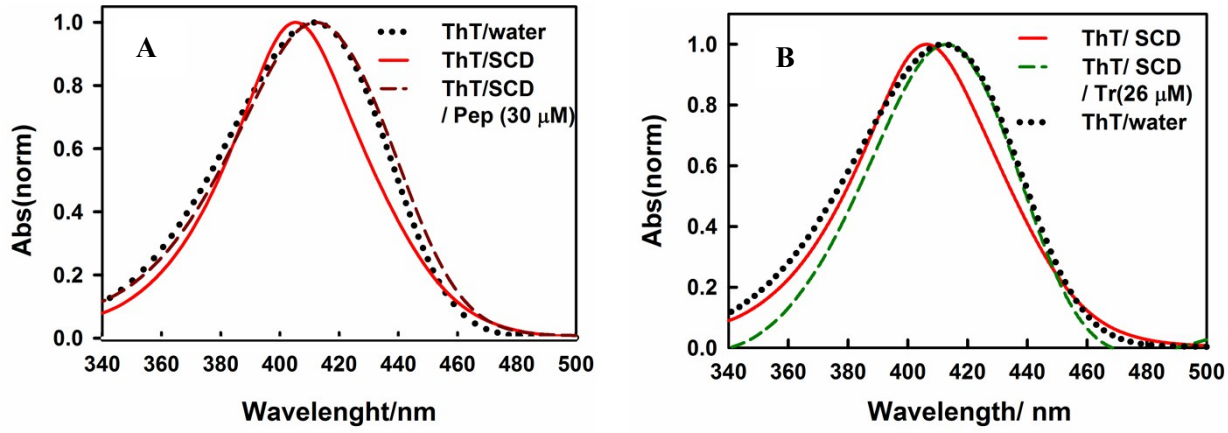


Figure S11: Normalized ground-state absorption spectrum of (A) ThT (20 μM) in SCD (solid red line, in water (dotted black line) and 30 μM of Pepsin (Pep) (dashed brown line) (B) ThT (20 μM) in SCD (solid red line, in water (dotted black line) and 26 μM of Trypsin (Tr) (dashed green line).

As depicted in Fig. S11 (ESI†), addition of pepsin and trypsin in a ThT–SCD solution resulted in a bathochromic shift of the absorption spectra. This shift in the absorption spectra is attributed to the disassembly of ThT H-aggregates from the SCD surface, induced by strong interaction between nonmetalloproteins and SCD, which results in the formation of a SCD–protein complex, followed by binding of the released ThT to the SCD–protein complex.

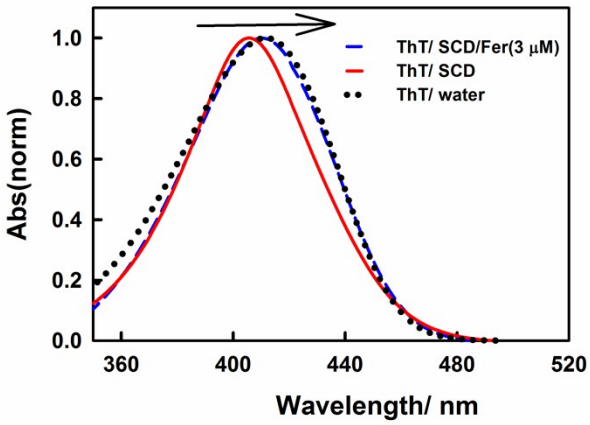


Figure S12: Normalized ground-state absorption spectrum of ThT (20 μM) in SCD (solid red line, in water (dotted black line) and 3 μM of Ferritin (Fer) (dashed blue line).

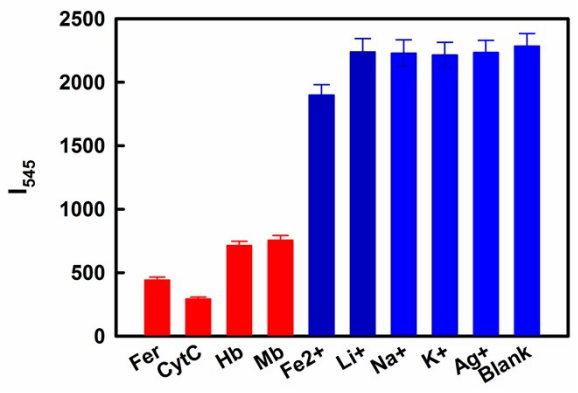


Figure S13. Selectivity test of ThT-SCD system for metalloproteins with free metal ions at analyte concentration of 20 μM.

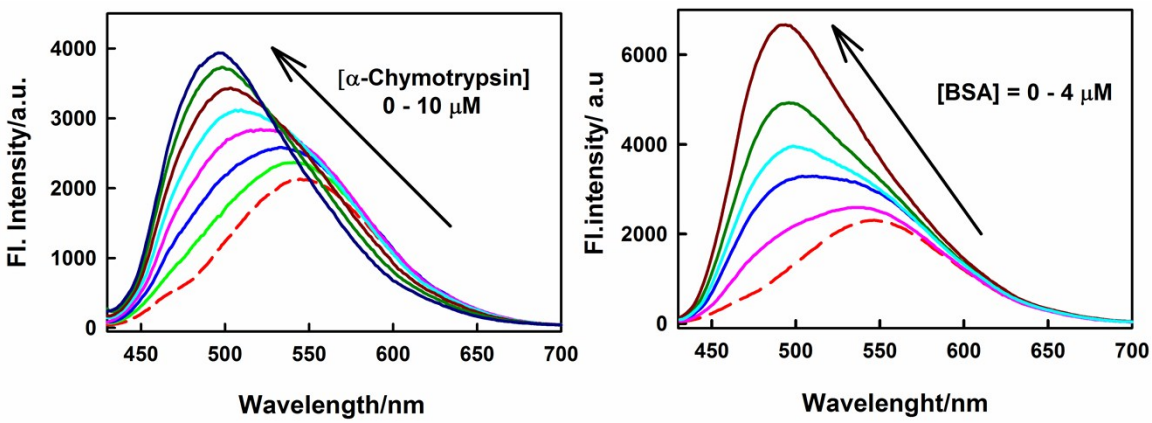


Figure S14: Steady-state fluorescence spectrum of ThT (20 μM) in SCD at varying concentration of α-chymotrypsin (left panel) and bovine serum albumin (right panel).

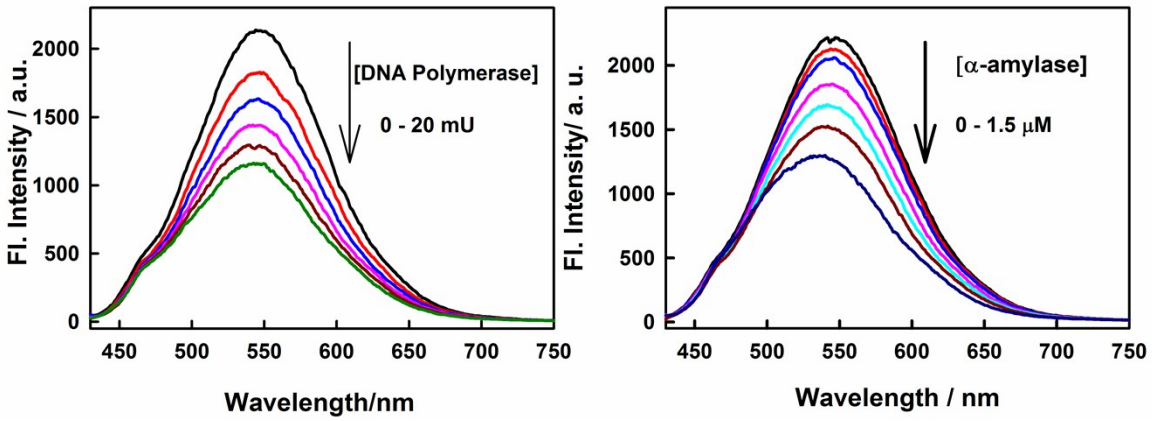


Figure S15: Steady-state fluorescence spectrum of ThT (20 μM) in SCD at varying concentration of DNA polymerase (left panel) and alpha amylase (right panel).

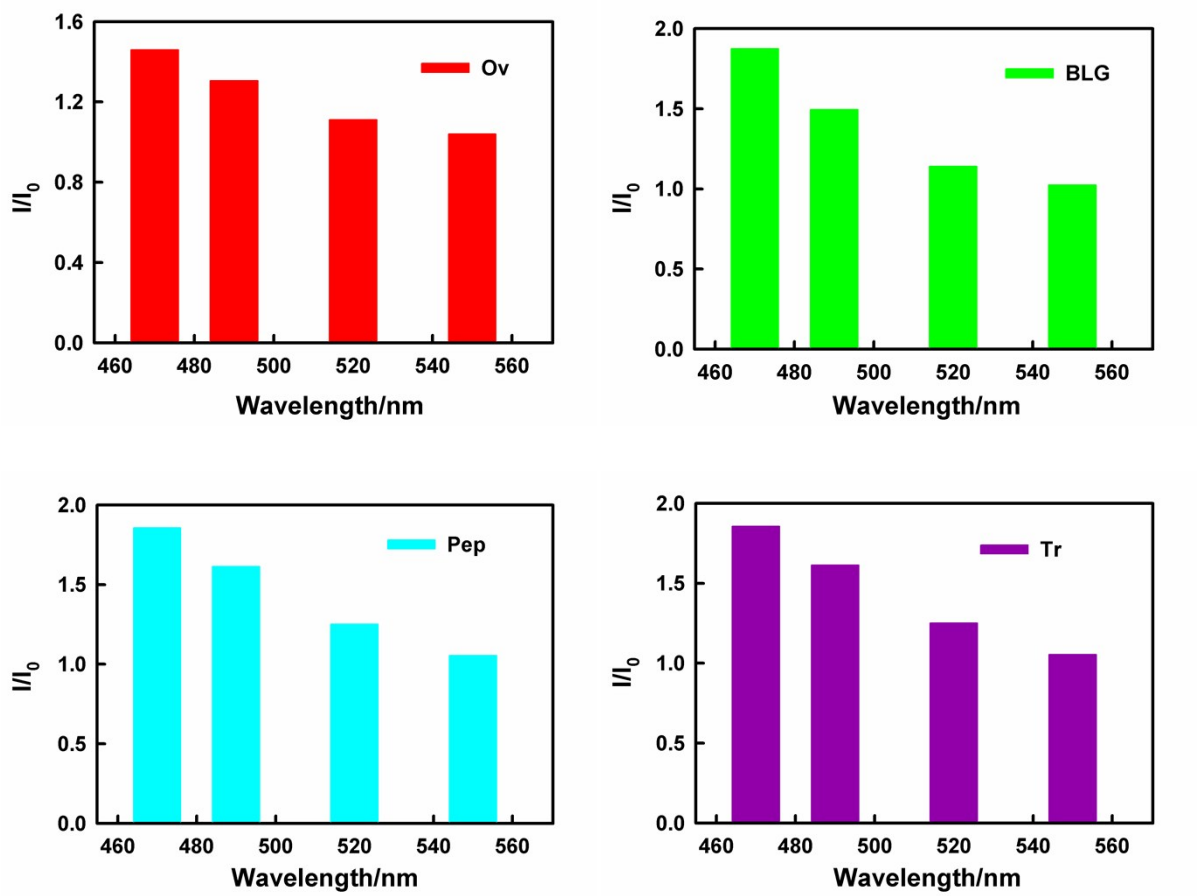


Figure S16: Response patterns for non-metalloproteins-BSA, Lz, Ov, BLG, Pep and Tr (4 μM) in terms of emission intensity ratios (I/I_0) at four different wavelengths (470, 490, 520 and 550 nm).

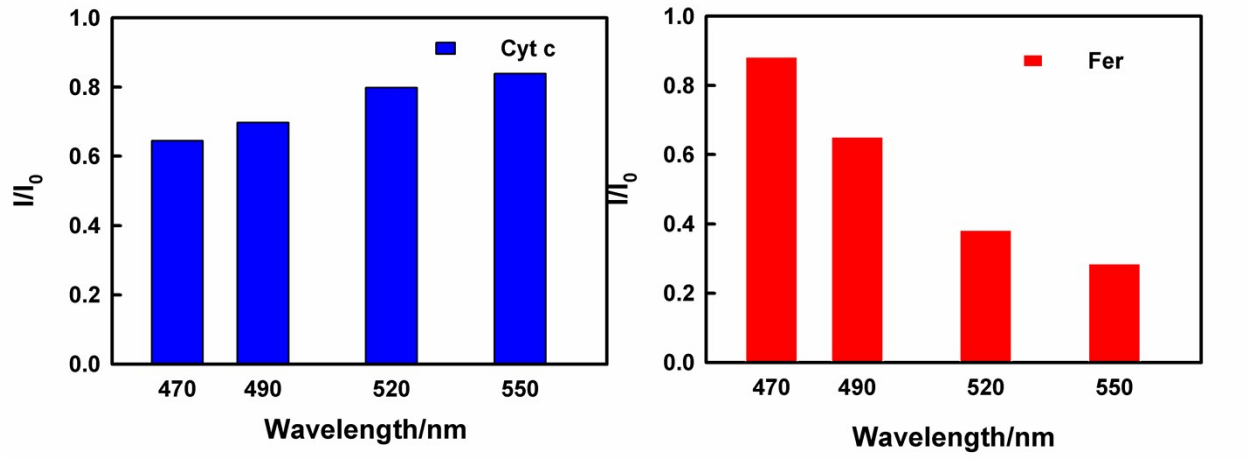
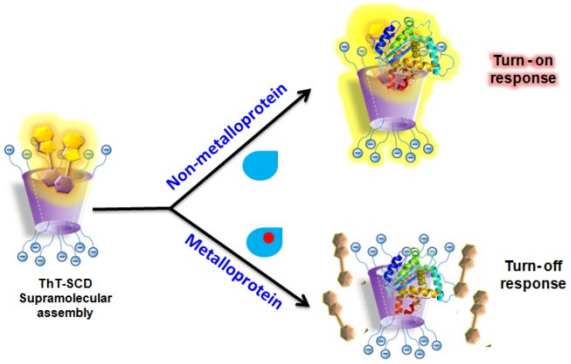


Figure S17: Response patterns for metalloproteins: Cyt C and Fer (4 μM) in terms of emission intensity ratios (I/I_0) at four different wavelengths (470, 490, 520 and 550 nm).



Scheme 1: Schematic presentation of fluorescence response mechanism of ThT-SCD supramolecular assembly towards metallo and non-metalloproteins