Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

FRET based ratiometric detection of Hg^{2+} and thiols using napthalimide-rhodamine dyad

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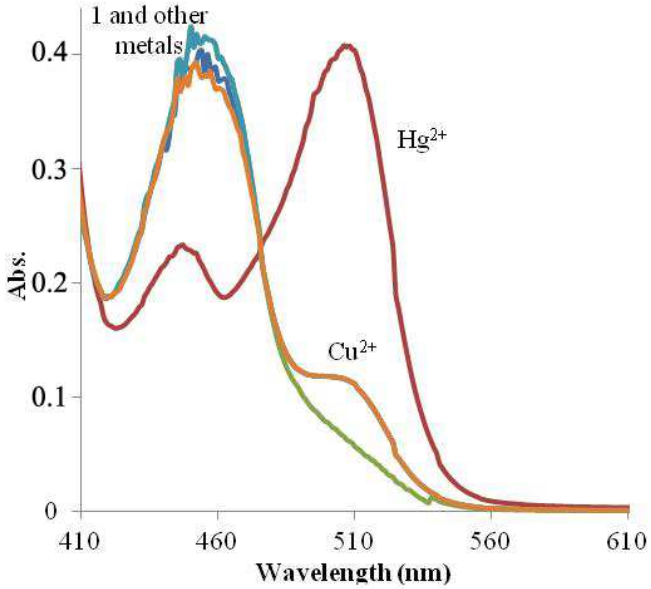
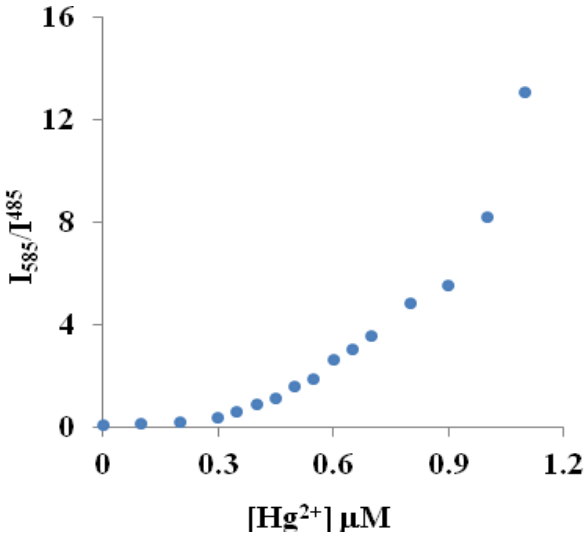
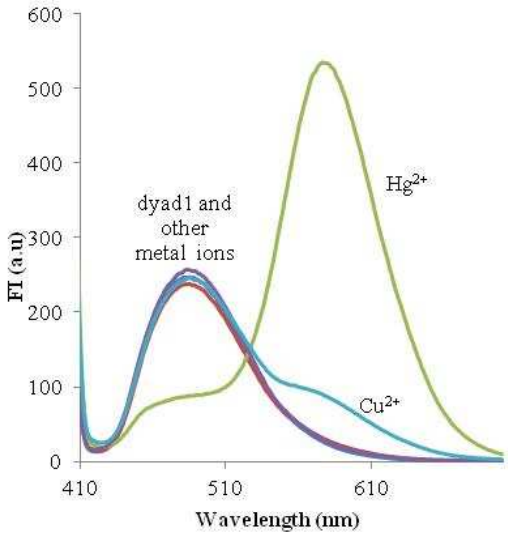


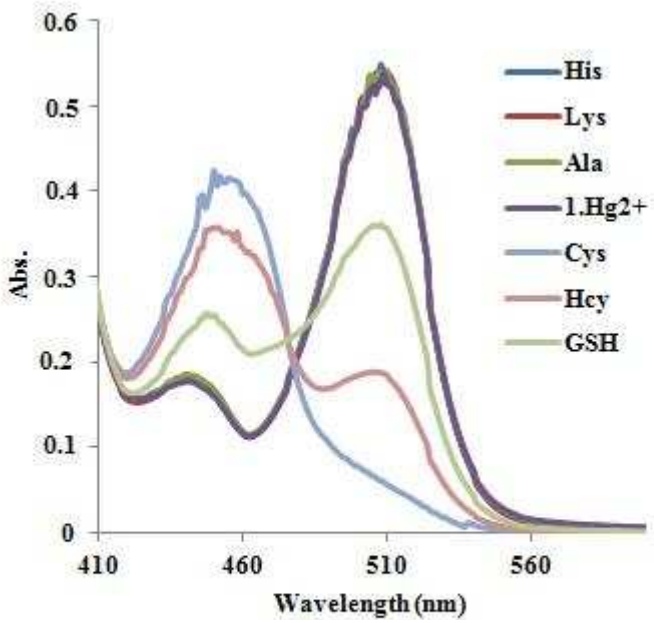
S1: UV-Vis spectra of dyad **1** in the presence of different metal ions.



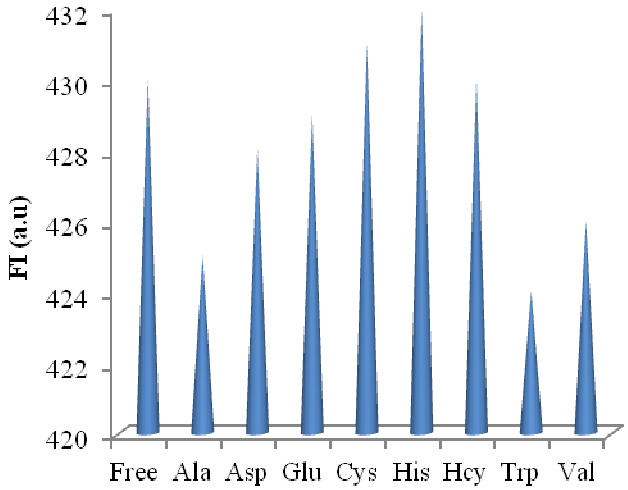
S2: Ratiometric responses of probe **1** upon incremental addition of Hg^{2+} ions



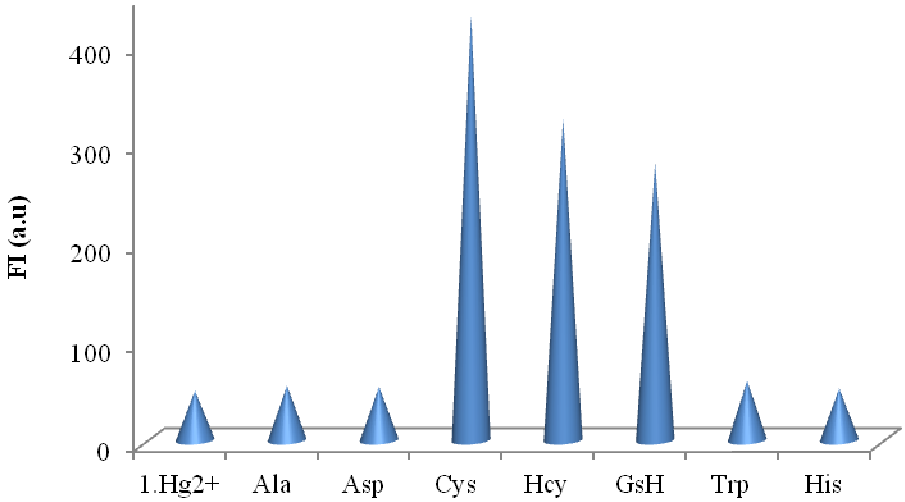
S3: Fluorescence spectra of probe **1** in the presence of different metal ions.



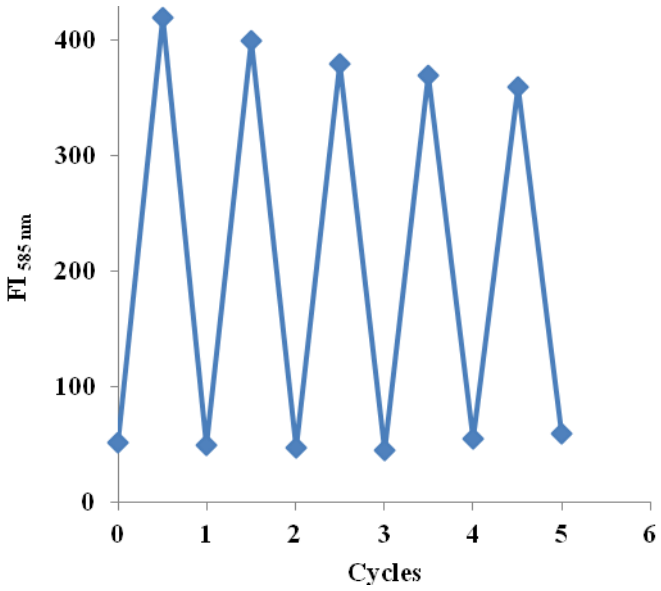
S4: Effect of addition of different amino acids on UV-Vis spectra of **1.Hg^{2+}** complex



S5: Fluorescence intensity of probe **1** (1µM) at 485 nm in the presence of various amino acids

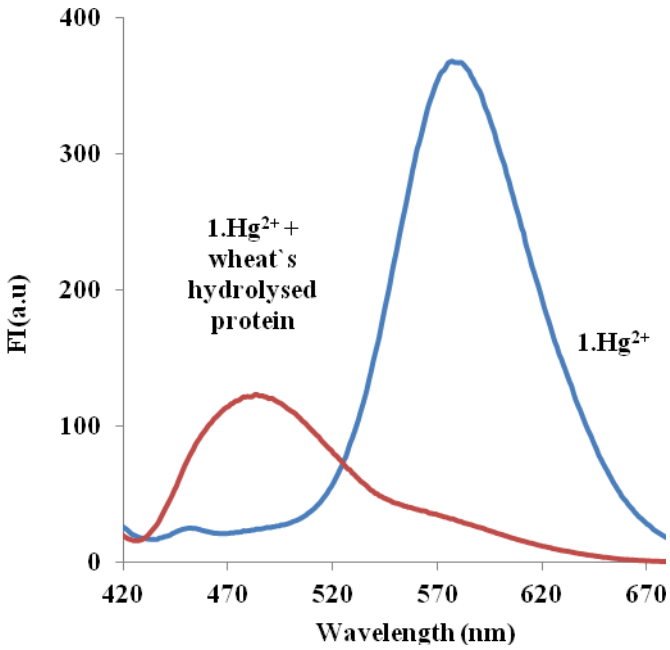


S6: Fluorescence intensity of probe **1.Hg^{2+}** complex (1µM) at 485 nm in the presence of various amino acids

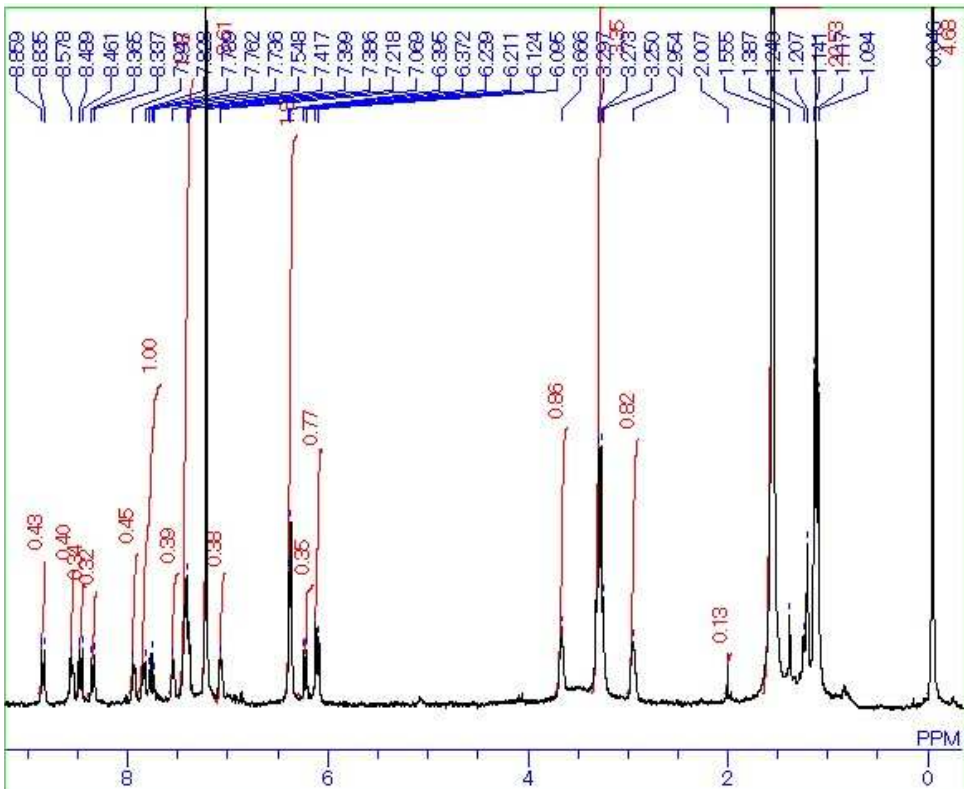


S7: Fluorescent intensity of probe **1** (1 µM) upon the alternate addition of Cys/Hg^{2+} with several concentrations (0:0, 1:0, 1:1.5, 2:1.5, 2:3, 4:3, 4:6, 6:6, 6:9, and 10:9 µM, respectively).

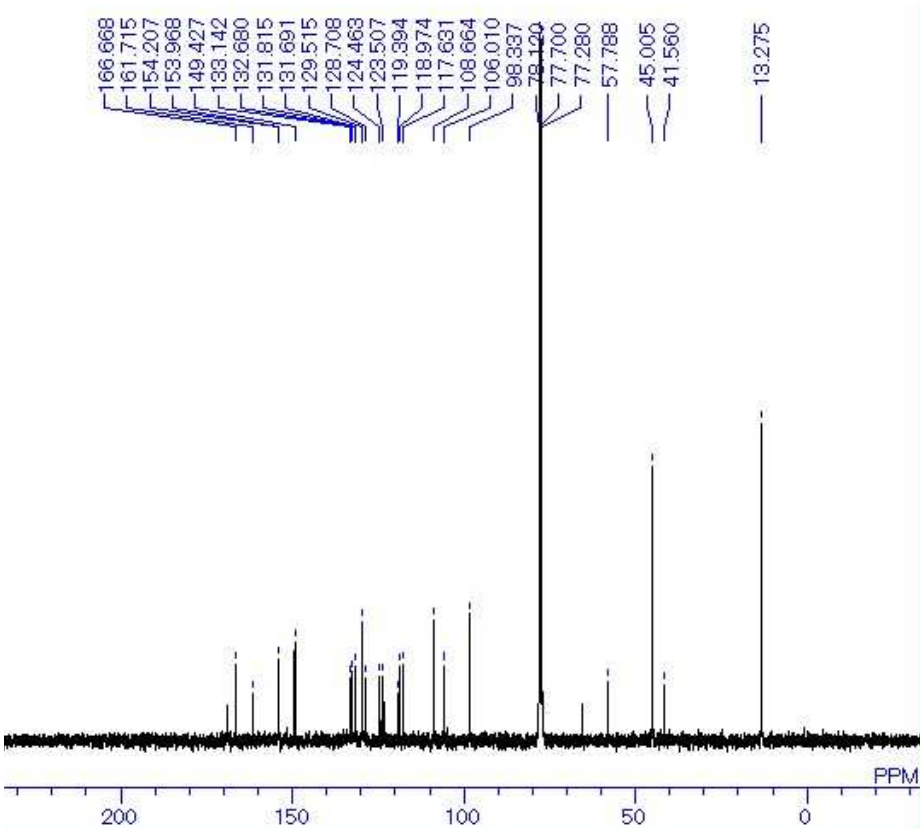
Extraction of proteins from wheat: Wheat flour sample (Triticium aestivum, cv. PBW343) was firstly defatted with hexane and defatted sample was dried under laminar hood at room temperature for 24 hrs. Different protein fractions were extracted from defatted wheat flour based on their solubility at 25 oC in distilled water (for albumin), 5% NaCl (for globulin), 0.1 M NaOH (for glutelin fraction) and 70% ethanol (for prolamin). Thus defatted wheat flour (10 g) were reconstituted in 50 ml of doubly distilled water and mixed on stirrer for 4 hrs (step 1). Albumin extract was separated by centrifugation at 5000 rpm, 4 oC for 30 min (step 2). After spinning, 5-6 drops of 1N HCl was added and kept overnight at 4 ^{o}C (step 3), followed by centrifugation at 13000 rpm at 4 ^{o}C for 20 min (step 4). The supernatant was discarded and pellet (albumin) after acetone drying was stored at 4 ^{o}C for further use (step 5). The residue left after separation of albumin was resuspended in 50 ml, 5% NaCl followed by processing through step 1 and 2. The supernatant collected after centrifugation was processed through step 3-5 to extract globulin. The residue was treated with 50 ml of 0.1 M NaOH followed by steps 1 and 2. The supernatant was used for extraction (steps 3-5) of glutelin and residue was resuspended in 70% EtOH and followed by stirring and centrifugation (steps 3-5) to separate prolamin. All soluble protein fractions from each extract were precipitated by adjusting pH to their isoelectric points. After precipitation, all the fractions were kept at 4 ^{o}C for 24 hrs to ensure complete aggregation of protein. Each extracted protein was hydrolysed and diluted million times for detection of cysteine



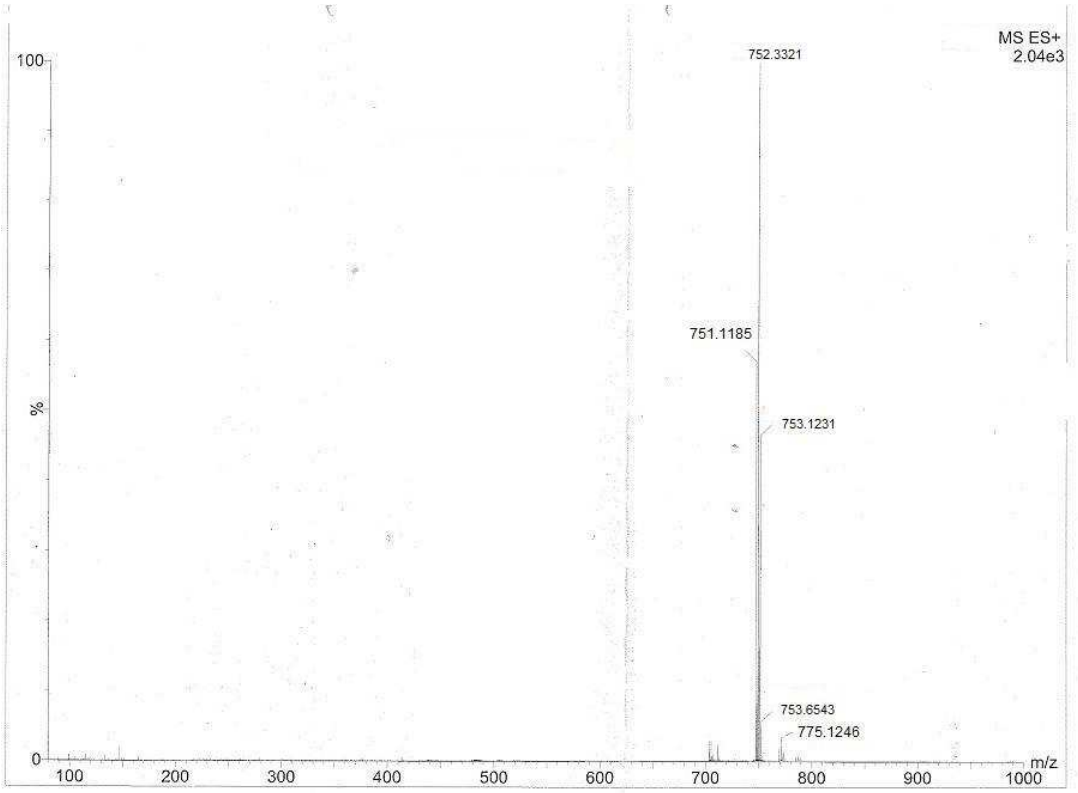
S8: Effect of addition of wheat`s hydrolysed proteins on fluorescene spectrum of **1**.Hg^{2+}



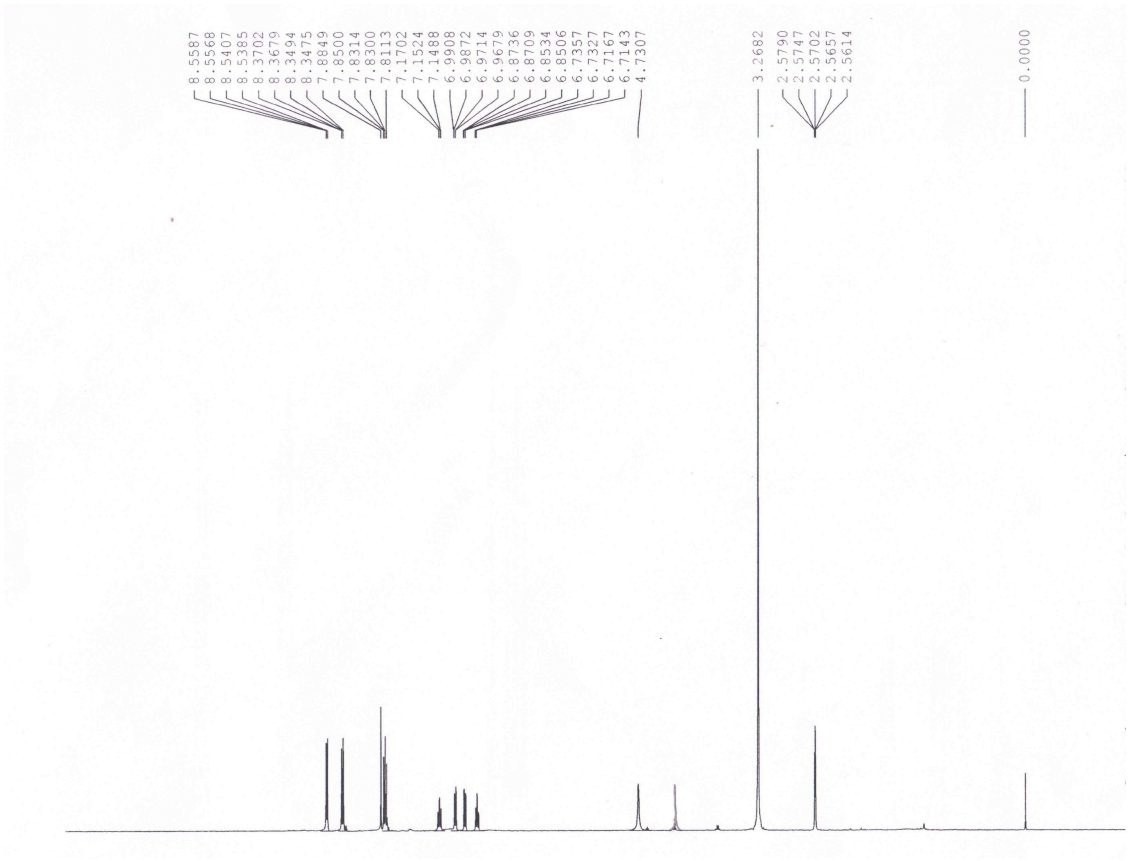
S9: ^{1}H NMR spectrum of probe **1**



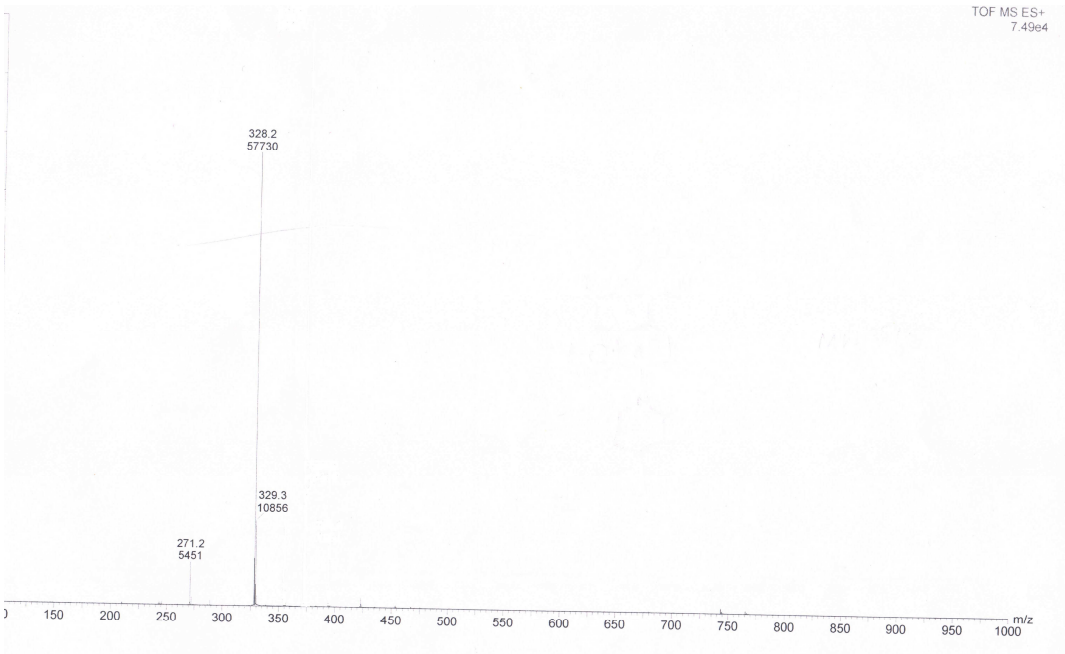
S10: ^{13}C NMR Spectrum of probe **1**



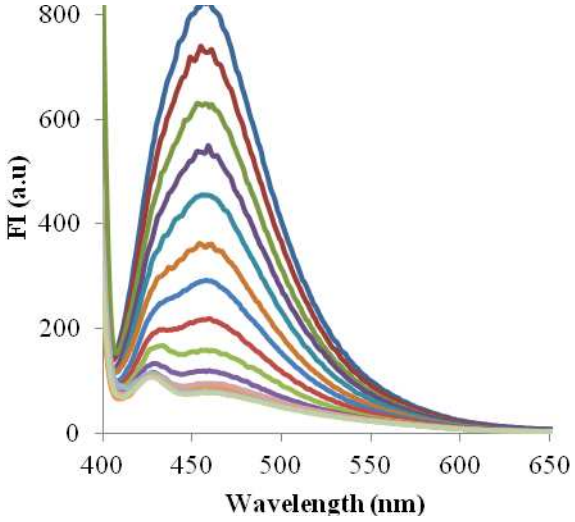
S11: HRMS spectra of Probe **1**



S12: 1H NMR of compound **5**



S13: Mass spectra of compound **5**



S14: Effect of incremental addition of Hg^{2+} to solution of **5**

Evaluation of Different Parameters for FRET Process

Förster distance R_0 was calculated using the expression shown in eq 1,

$$R_0=0.211[(J)Q(n^{-4})(\kappa^2)]^{1/6} \quad (1)$$

where, n is the refractive index of the medium in between donor and acceptor and was taken approximately to be equal to 1.4. κ^2 is the dipole orientation factor. Depending upon the relative orientation of donor and acceptor, the value ranges from 0−4, and it is often assumed to be 2/3. Q is the fluorescence quantum yield of the donor in the absence of acceptor. J is the spectral overlap integral between the emission spectrum of the donor and the absorption spectrum of the acceptor and is shown in the following eq 2,

$$J=\int fD(\lambda)\varepsilon(\lambda)\lambda 4d\lambda \quad (2)$$

where f D(λ) is the normalized emission of the donor and ε(λ) is the molar absorption coefficient (M^{-1} cm^{-1}) of the donor.

Energy transfer efficiency (ηEET) was evaluated using the expression shown in eq 3,

$$\eta EET = 1-\Phi_{F(donor\ in\ dyad\ 1)}/\Phi_{F(free\ donor)} \quad (3)$$

Here, $\Phi_{F(donor\ in\ dyad)}$ is the fluorescence quantum yield of the donor part in dyad **1** and $\Phi_{F(free\ donor)}$ is the fluorescence quantum yield of the donor when not connected to the acceptor.