Supporting Information

Development of a Next-Generation Fluorescent Turn-On Sensor to Simultaneously Detect and Detoxify Mercury in Living Samples

Abdul Malek[†, Φ], *Kallol Bera*[#, Φ], *Shrutidhara Biswas*[§], *Govindaraj Perumal*[¥], *Anand Kant Das*[€], *Mukesh Doble*[¥], *Tiju Thomas*[*‡] *and Edamana Prasad*[*†]

[†]Department of Chemistry, Indian Institute of Technology Madras, Chennai-600036, India.

[#]Chemical Sciences Division, Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata 700064, India.

[§]Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, India.

[¥]Department of Biotechnology, Indian Institute of Technology Madras, Chennai-600036, India.

[€]Department of Chemical Sciences, Tata Institute of Fundamental Research, Mumbai- 400005, India.

[‡]Department of Metallurgical and Materials Engineering, Institute of Technology Madras, Chennai-600036, India.

[Φ] *These authors contributed equally*

Correspondence and requests for materials should be addressed to E.P. and T.T.

*Tel: +91-44-2257-4232. Fax: +91-2257-4202. E-mail: pre@iitm.ac.in (E.P.)

*Tel: +91-44-2257-5781. Fax: +91-44-2257-4752. E-mail: tijuthomas@iitm.ac.in or tt332@cornell.edu (T.T.)

Contents:

1. Characterization of PYDMSA by FT-IR spectroscopy

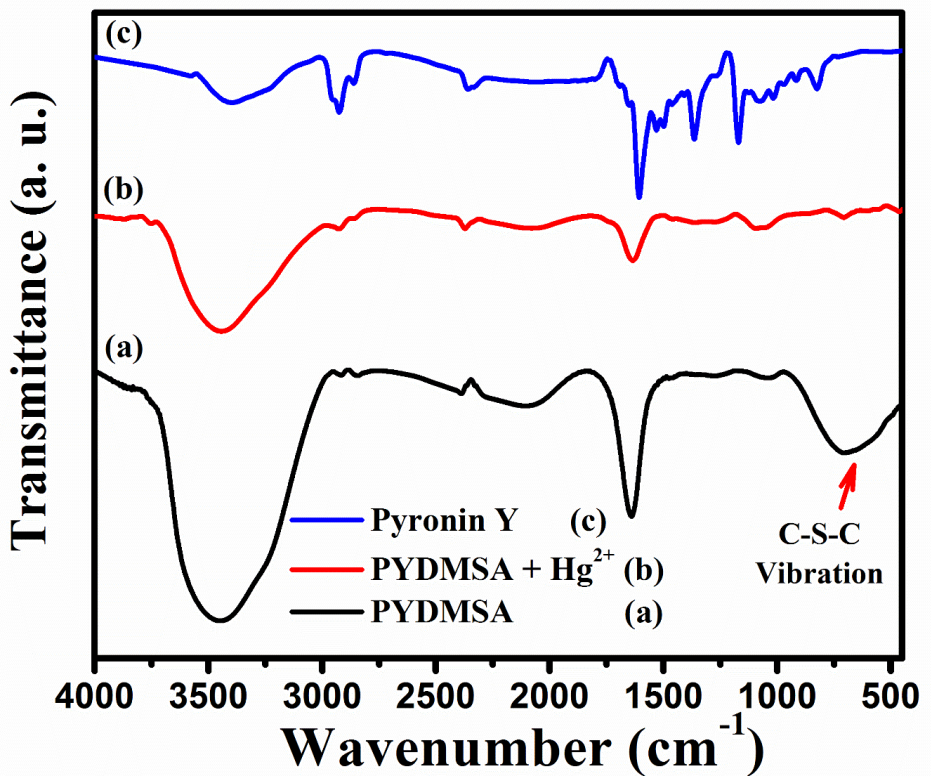
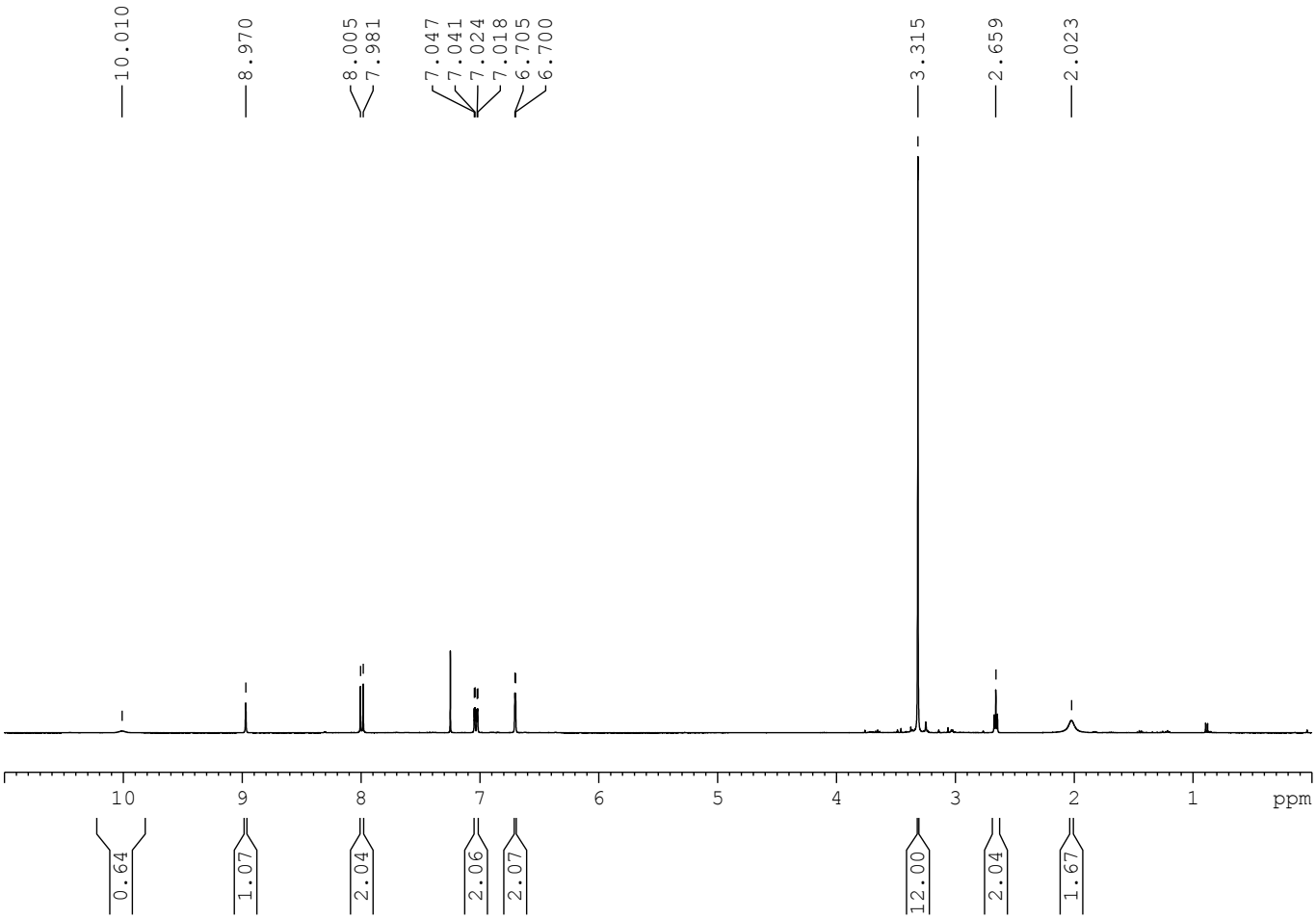


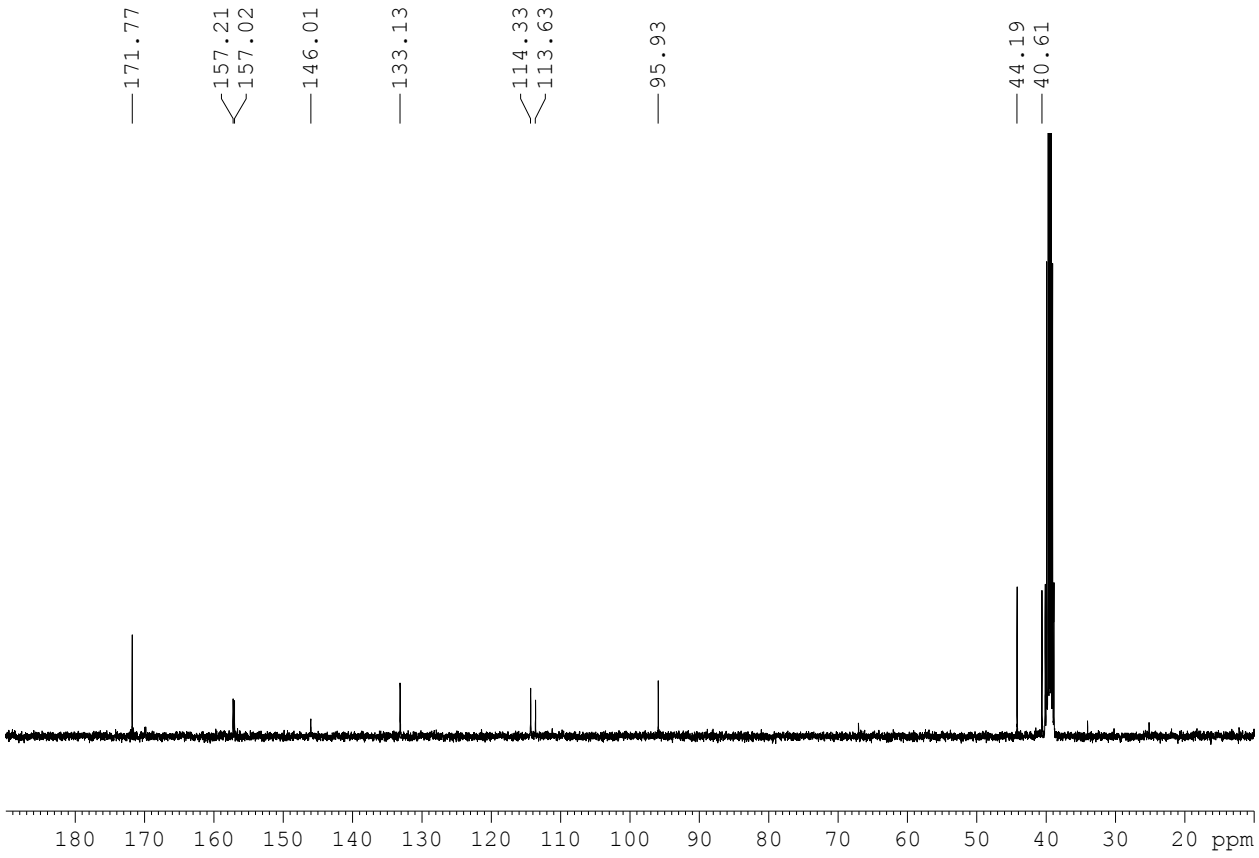
Figure S1. Fourier transform infrared spectroscopy (FT-IR) spectra of PYDMSA, PYDMSA + Hg^{2+} and Pyronin Y. Presence of CS-C[9] vibration in the FTIR spectra of the PYDMSA around 660 cm^{-1} supports the structure shown in the main manuscript.

2 (a). ^{1}H NMR of PYDMSA



^{1}H NMR ($CDCl_3$, 400 MHz): δ= 2.023 (s, 1H, SH), 2.659 (s, 2H, methylene CH), 3.315 (s, 12H, N-CH_3), 6.7025 (d, 2H, J = 6.25 Hz, ArH), 7.021 (d, 1H, J = 7.5 Hz , ArH), 7.044 (d, 1H, J = 7.5 Hz, ArH), 7.981(s, 1H, ArH), 8.005 (s, 1H, ArH), 8.97 (s, 1H, ArCH), 10.010 (s, 1H, COOH).

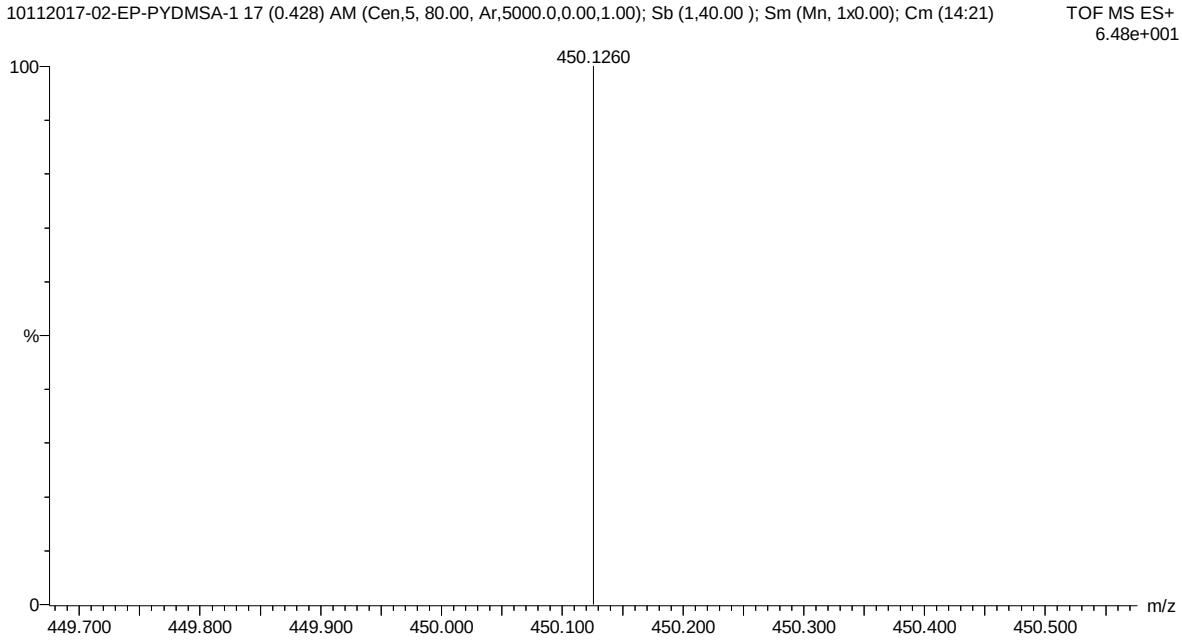
2 (b). ^{13}C NMR of PYDMSA



^{13}C NMR (DMSO-d_6, 400 MHz): δ= 40.61, 44.19, 95.93, 113.63, 114.33, 133.13, 146.01, 157.02, 157.21, and 171.77

Figure S2. (a) ^{1}H Nuclear magnetic resonance (NMR) of PYDMSA **(b)** ^{13}C NMR spectra of PYDMSA.

3. HRMS of PYDMSA



Minimum:				-1.5		
Maximum:		5.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
450.1260	450.1283	-2.3	-5.1	10.0	n/a	C21 H26 N2 O5 S2

Figure S3. High-resolution mass spectrometry (HRMS) of PYDMSA.

4. UV-Visible spectroscopic characterization of the PYDMSA

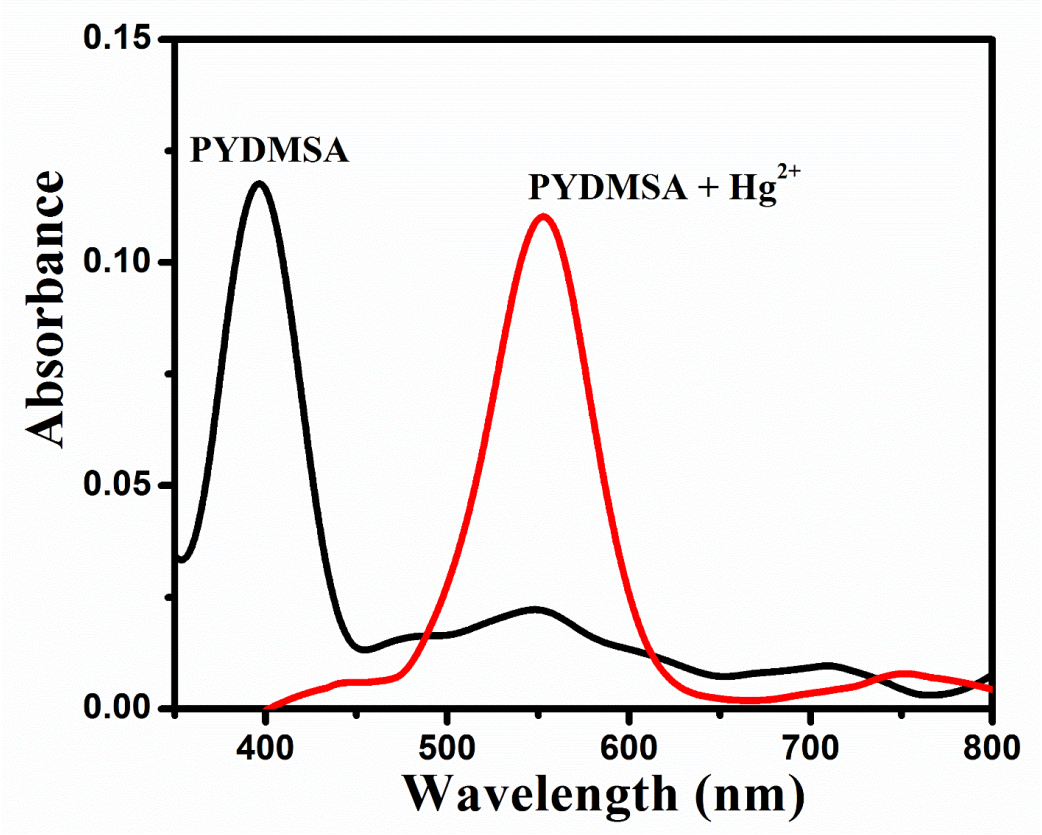


Figure S4. Black colored spectrum is the UV-Vis spectrum of PYDMSA (10 μM) in PBS buffer. PYDMSA is colorless (λ_{abs}^{max} = 390 nm) in PBS at pH 7.4. Red colored spectrum is the UV-Vis spectrum of (PYDMSA+ Hg^{2+}) the solution (10 μM of both PYDMSA and Hg^{2+}). Absorption maximum around 550 nm indicates that after interacting with Hg^{2+}, PYDMSA generates Pyronin Y; this is in accordance with the mechanism provided in the main manuscript.

5. Color change of the solution containing PYDMSA in the presence of Hg^{2+}

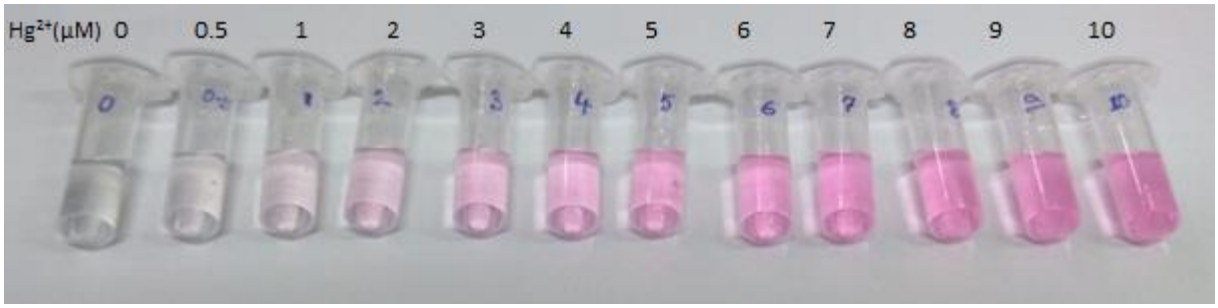


Figure S5. Color of the solution containing PYDMSA (10 μM) with varying concentrations of Hg^{2+} (0 - 10 μM). The label on the tubes indicates the Hg^{2+} concentration in micromolar (μM). The image is taken under visible light. It is observed that the naked eye can visually detect the marked color change at a concentration equal to or above 2 μM.

6. Time-dependent response of PYDMSA to Hg^{2+}

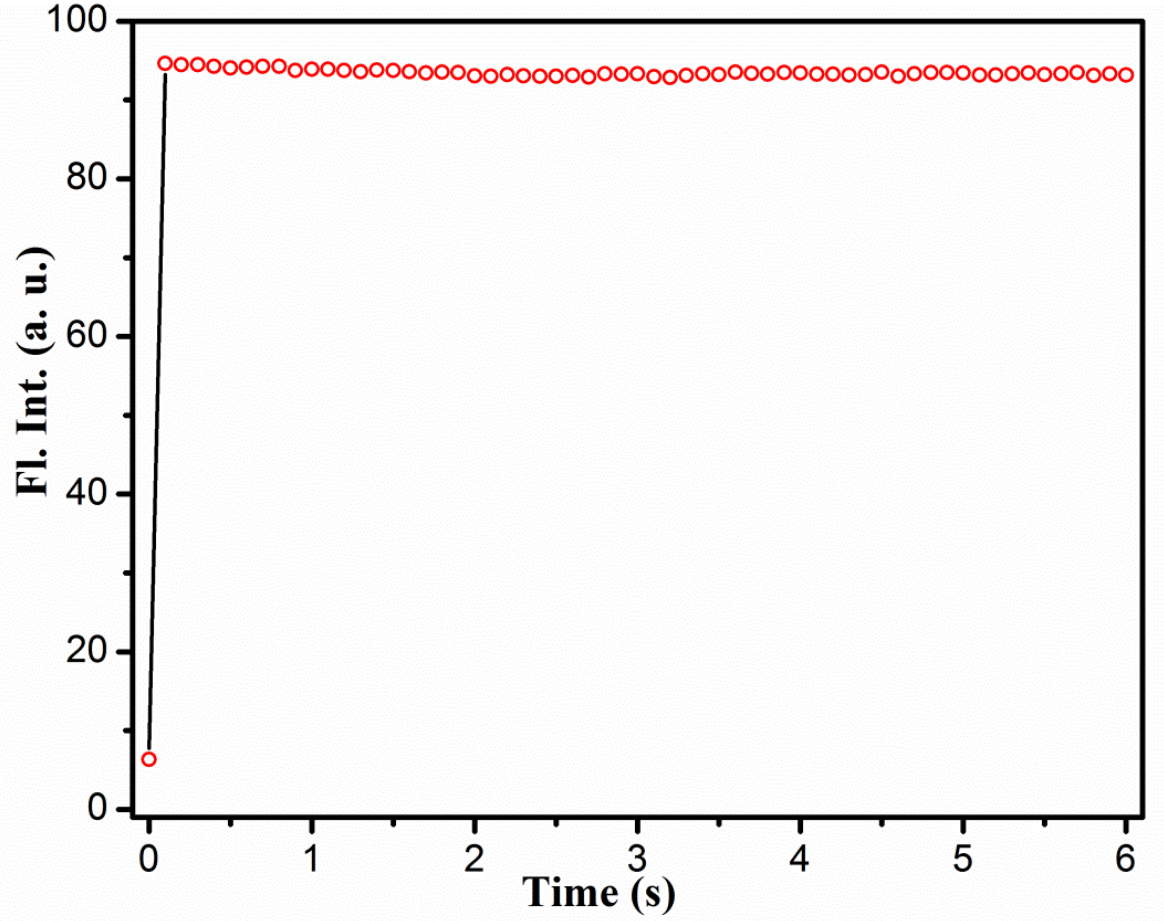
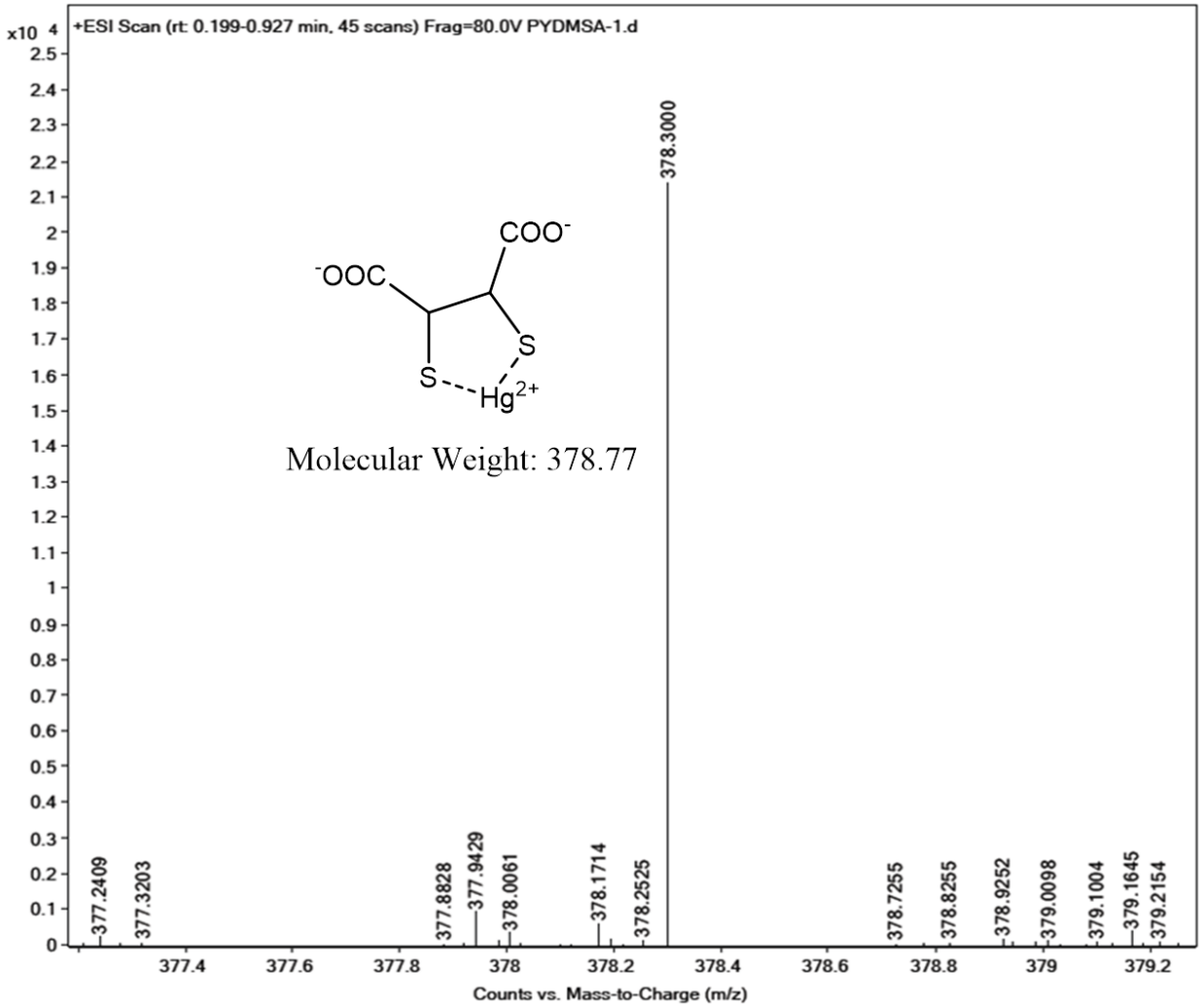


Figure S6. Time-dependent response of 10 μM PYDMSA to 10 μM Hg^{2+}. The fluorescence response of PYDMSA to Hg^{2+} is ~0.1 s.

7. HRMS of the Hg^{2+}-DMSA complex and pyronin Y



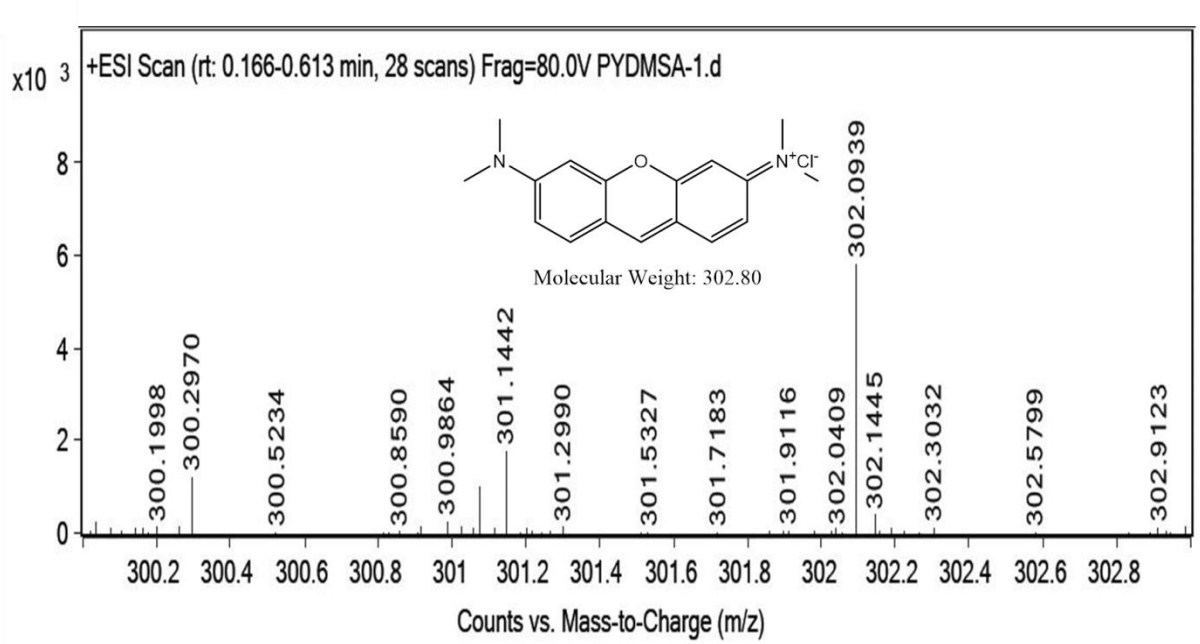


Figure S7. HRMS of PYDMSA solution after addition of Hg^{2+} (i.e. after sensing). Mass of 378.3 corresponds to DMSA-Hg^{2+} complex, and 302.093 corresponds to pyronin Y.

8. Fluorescence intensity vs. Hg^{2+} concentration plot

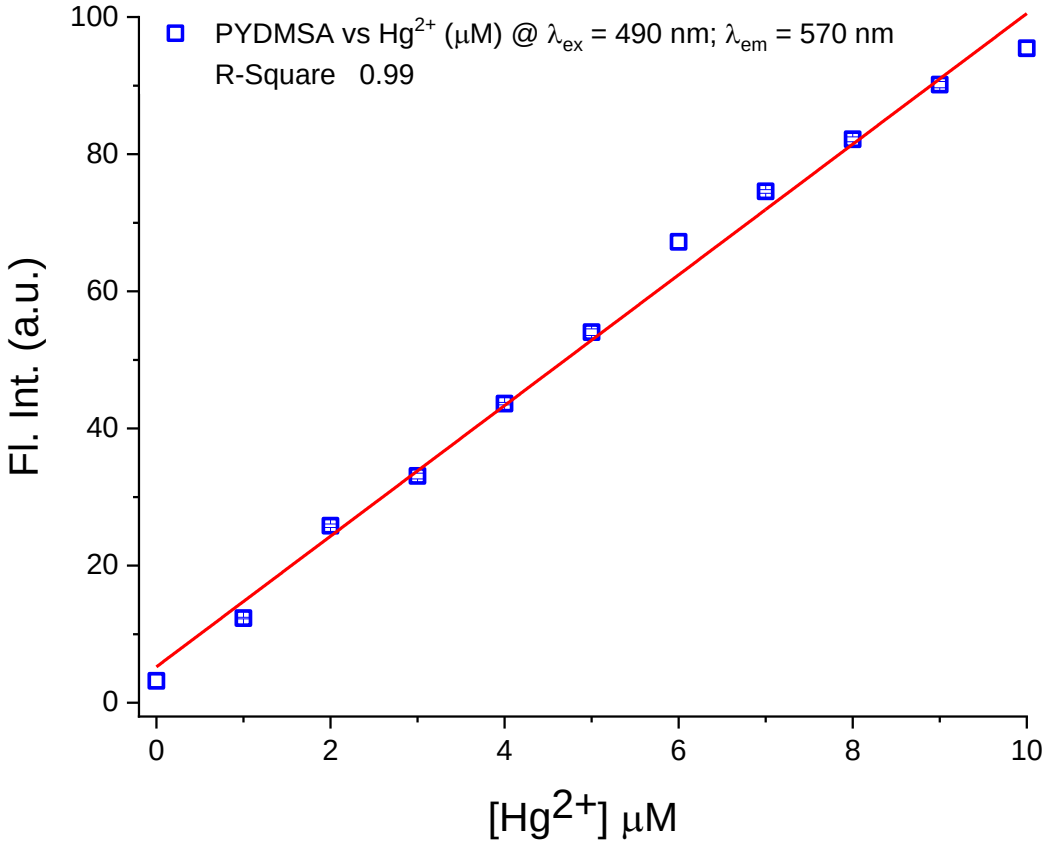


Figure S8. Fluorescence intensity vs. Hg^{2+} concentration plot. Correlation coefficient $R^2 = 0.99$.

9. Job's plot: PYDMSA vs. Hg^{2+}

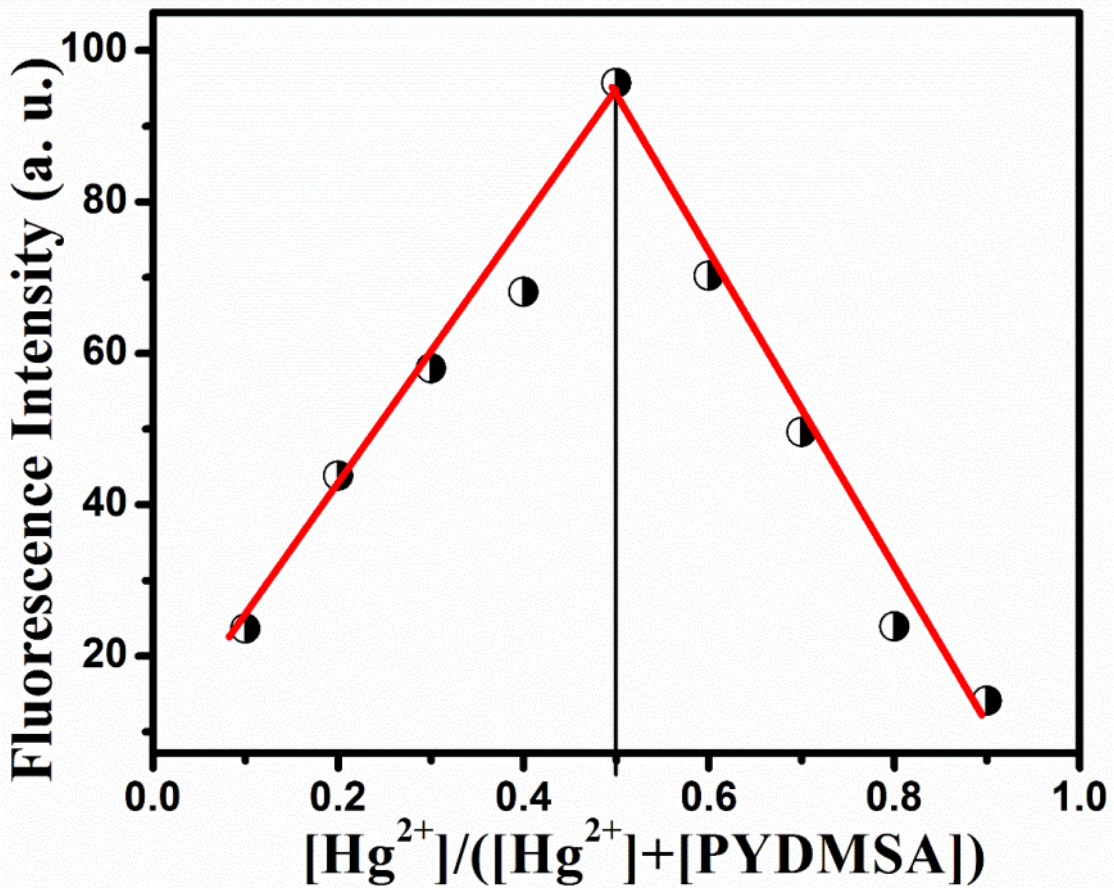


Figure S9. Job's plot for the determination of stoichiometry of PYDMSA and Hg^{2+}. It is obvious from the plot that Hg^{2+} makes 1:1 complex with PYDMSA.

10. Fluorescence titration at nanomolar (nM) concentrations of Hg^{2+}

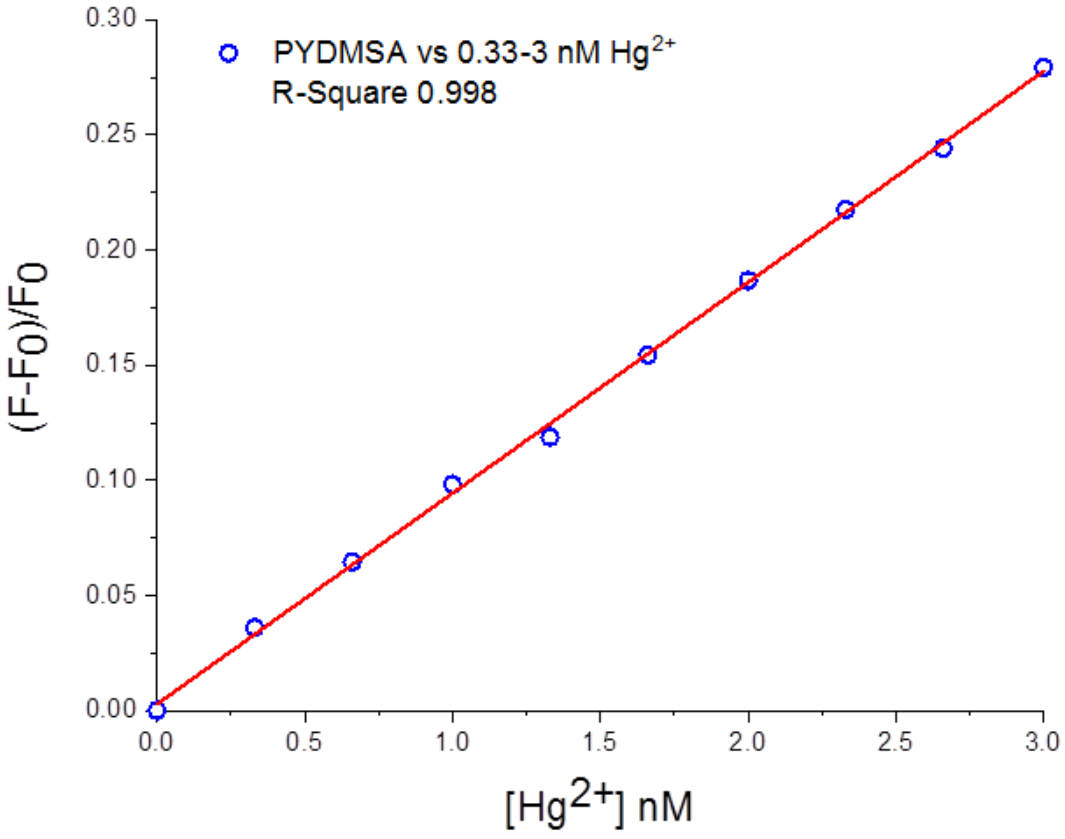


Figure S10. Plot of $(F-F_0)/F_0$ vs. $[Hg^{2+}]$. The plot is obtained upon addition of Hg^{2+} (0.33 – 3 nM) to PYDMSA (10 μM) at 25 °C in PBS, pH 7.4. [Excitation wavelength (λ_{ex}) = 490 nm and emission maxima (λ_{em}^{max}) = 570 nm].

11. Response of PYDMSA towards various metal ions

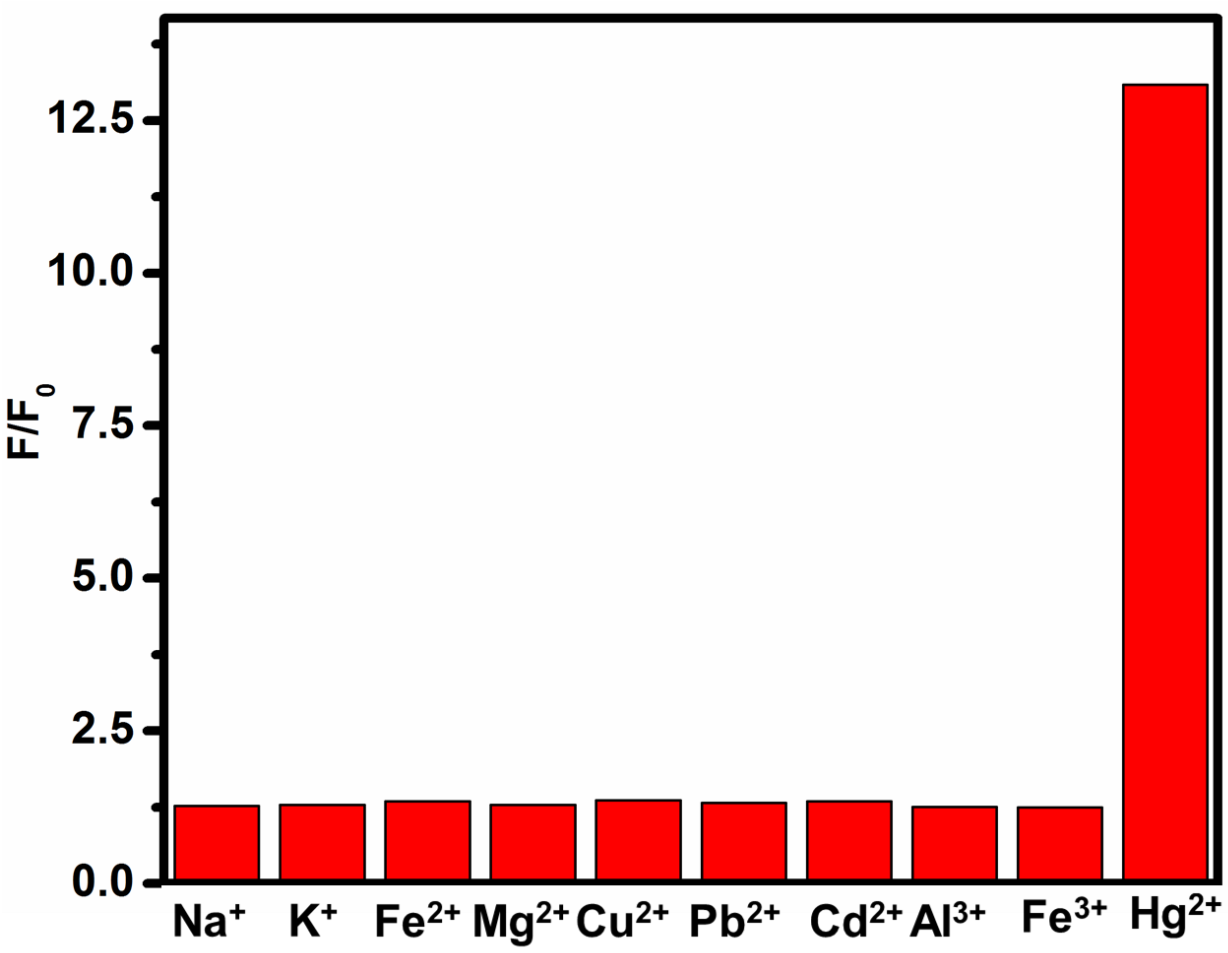


Figure S11. Relative fluorescence intensity changes after addition of 50 μM of various interfering metal ions to 5 μM PYDMSA.

12. MTT assay for the cell viability test

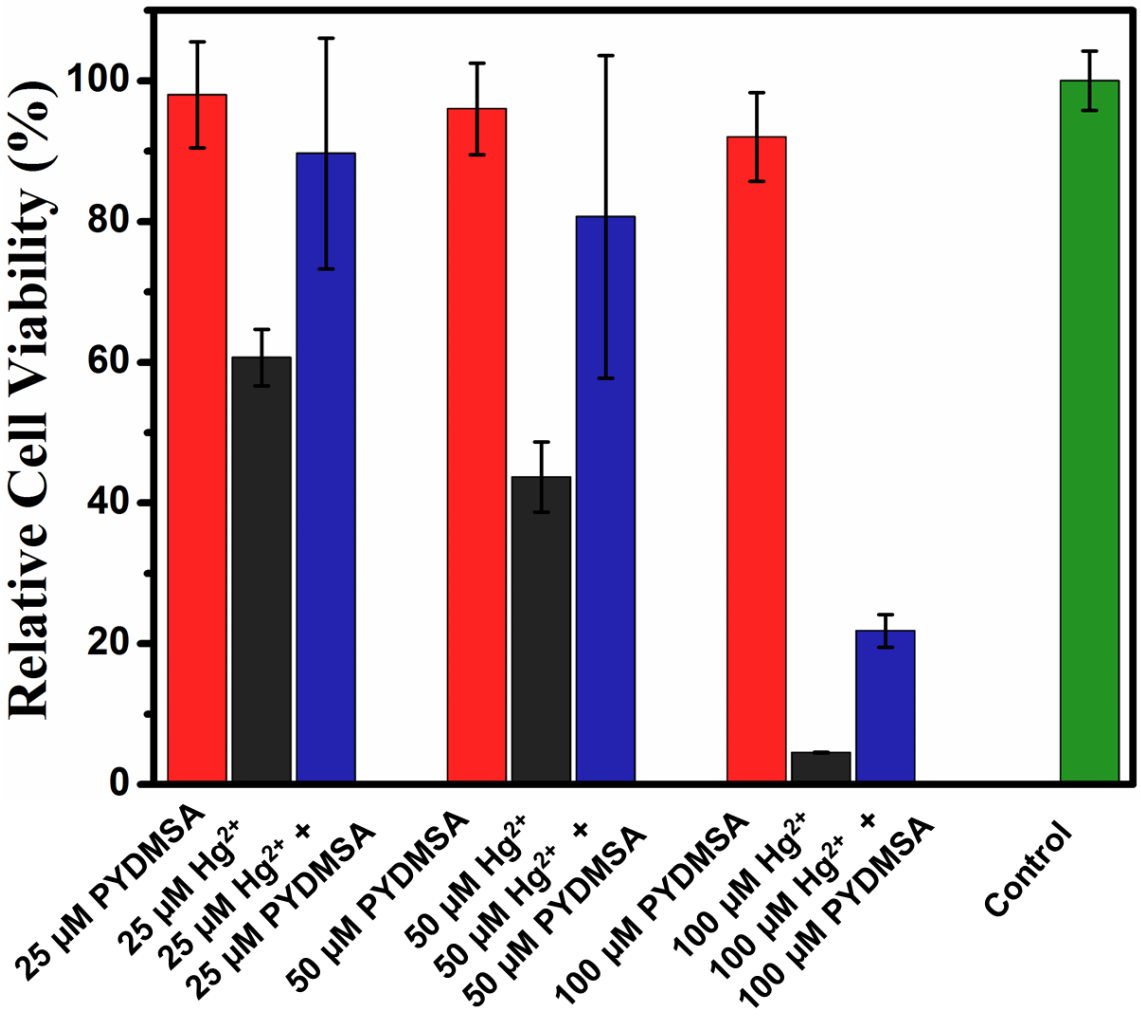


Figure S12. MTT assay is carried out to verify the detoxifying capability of the probe. It is found that the probe is capable of detoxifying Hg^{2+} as hypothesised. The trend is similar to what is found in CCK-8 assay.