Electronic Supplementary Material (ESI) for Analyst

Supporting Information for

A new, highly water-soluble, fluorescent turn-on chemodosimeter for direct measurement of hydrogen sulfide in biological fluids

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Sections:

I. Materials.

All reagents were purchased from Sigma-Aldrich, Fisher Scientific or TCI America unless otherwise specified and were used as received. Reactions were carried out in foil-wrapped flasks protected from light. The precursor to N_3-PTS, triammonium 8-aminopyrene-1,3,6-trisulfonic acid, was prepared in one step from 1-aminopyrene as described[1].

II. Synthesis.

Triammonium 8-azidopyrene-1,3,6-trisulfonate (N_3-PTS). A solution of triammonium 8-aminopyrene-1,3,6-trisulfonate (100 mg, 0.22 mmol) was dissolved in ice cold 3.6 N H_2SO_4 (500 μL) in a foil wrapped flask on ice. An aqueous solution of 0.66 M $NaNO_2$ (1 mL, 3 eq) was added dropwise over 10 min. A solution of ice cold 1.1 M sodium azide (1.2 mL, 6 eq) was added dropwise over 10 min. The reaction was stirred on ice for an additional 40 min and was quenched with 0.5 mL concentrated NH_4OH. The solution was concentrated via rotary evaporation and purified using a Biogel P-2 column (1.5 x 80 cm), eluted with 50 mM aq NH_4OAc at a flow rate of 0.15 mL/min. The fractions containing the product were combined and lyophilized giving a fluffy yellow powder (79 mg, 75%). IR ν_{max}/cm^{-1} (3435m, 3180s, 3199s, 2870m, 2113s (N_3 stretch), 1625w, 1416s, 1161s, 1115s, 1036s, 998s, 733m, 653s, 604s). ^{1}H NMR (400 MHz, D_2O): δ = 9.23 (s, 1H), 9.21 (d, 1H, *J* = 9.8 Hz), 9.11 (d, 1H, *J* = 9.8 Hz), 9.10 (d, 1H, *J* = 9.7 Hz), 8.73 (d, 1H, *J* = 9.7 Hz), 8.64 (s, 1H). ^{13}C NMR (150 MHz, D_2O) 139.05 (q), 135.90 (q), 130.43 (q), 129.71 (q), 127.73 (C-H), 125.68 (C-H), 125.51 (q), 125.20 (C-H), 124.87 (C-H), 124.76 (C-H), 123.44 (q), 123.40 (q), 116.60 (C-H). HRMS (ESI) $C_{16}H_7N_3O_9S_3$ calculated $[M - 2H]^{2-}$ = 240.4677; Observed = 240.4678.

III. Analytical Methods.

Fluorescence spectra. Fluorescence spectra were recorded with a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Inc.) at ambient temperature and under ambient atmosphere. Samples were contained in black, flat-bottomed 96 well plates. The PMT setting was 600 V for all readings. For samples containing H_2S, the plate was sealed immediately after addition of a NaHS solution of the appropriate concentration. Fluorescence readings were taken either with the seal removed

or with optically clear sealing tape intact. Readings taken in FBS had a final FBS concentration of 90%, with the remaining 10% aqueous solution containing the fluorophore and NaHS.

UV-Vis Absorbance. UV-Vis Absorbance spectra were recorded on a Hewlett Packard 8453 UV-visible Spectroscopy Systems instrument in disposable UV cuvettes (BrandTech) (Quantum yield measurements) or using a Nano-Drop 2000 spectrophotometer (UV-Vis absorbance profile and Beer's Law readings).

IR. IR readings were taken on a Nicolet Avatar 360 FT-IR.

NMR. ^{1}H NMR spectra were recorded on a Bruker 400 MHz NMR. ^{1}H NMR spectra were calibrated to the D_2O solvent peak which was set to 4.79 ppm. ^{13}C NMR spectra were recorded on a 600 MHz Bruker Advance III instrument using the attached proton test. ^{13}C NMR spectra in D_2O were calibrated using methanol as an internal standard set to 49.50 ppm. Even using this high field instrument there were indistinguishable ^{13}C peaks for N_3-PTS. Observed peaks were reported.

MS. Mass spectra were acquired on a LTQ-Orbitrap Velos from Thermo Electron Corporation operating in negative ion mode.

IV. Supplementary Figures and Tables.

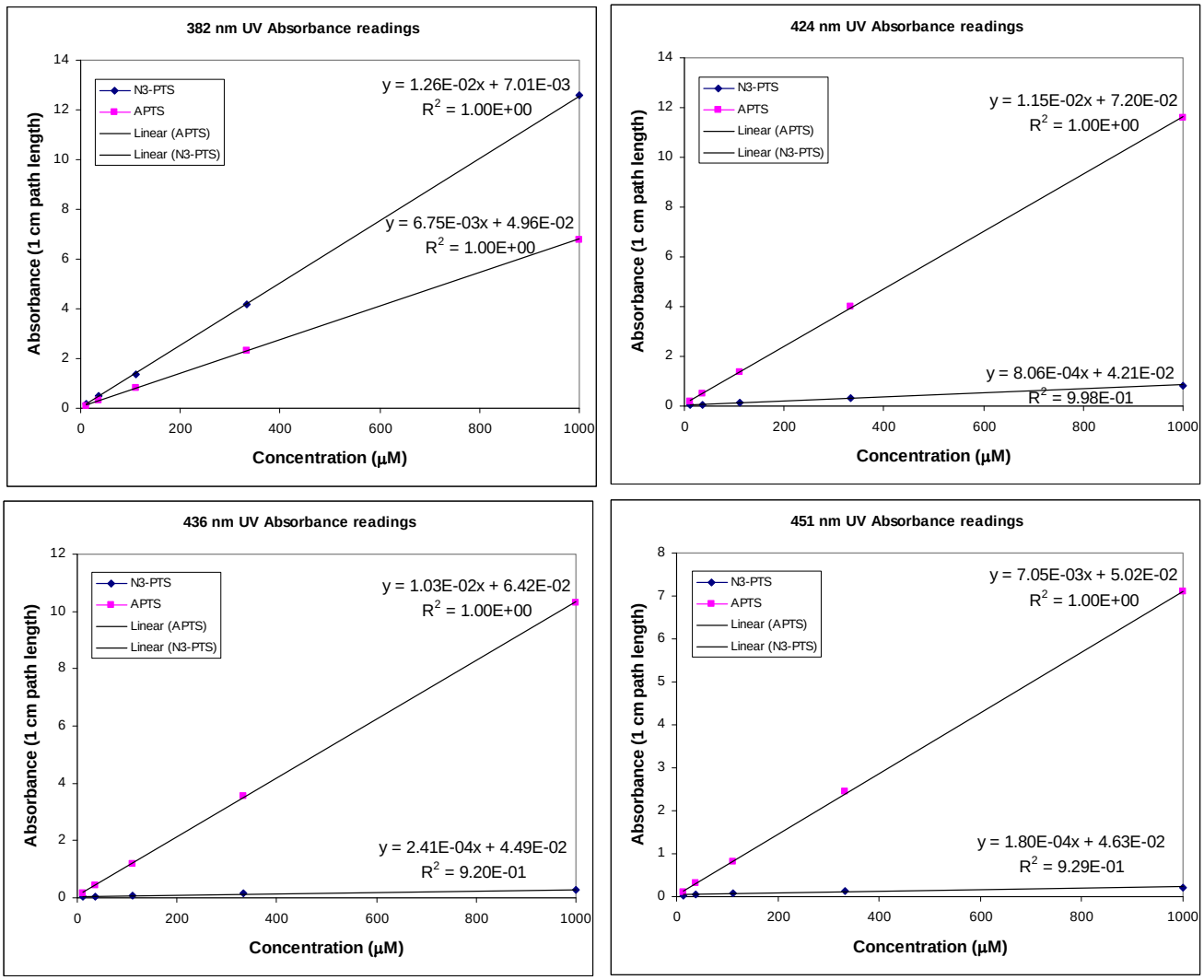


Fig. S1. Absorbance vs. concentration plots of APTS and N_3-PTS at various wavelengths in water. Absorbance parameters (Table S1) were calculated using Beer's law.

Table S1. Calculated absorbance coefficients of APTS and N_3-PTS in water

Wavelength	ε N_3PTS (M^{-1} cm^{-1})	ε APTS (M^{-1} cm^{-1})	ε APTS/N_3PTS
382 nm	12600	6750	0.54
424 nm	806	11500	14.3
436 nm	241	10300	42.7
451 nm	180	7050	39.2

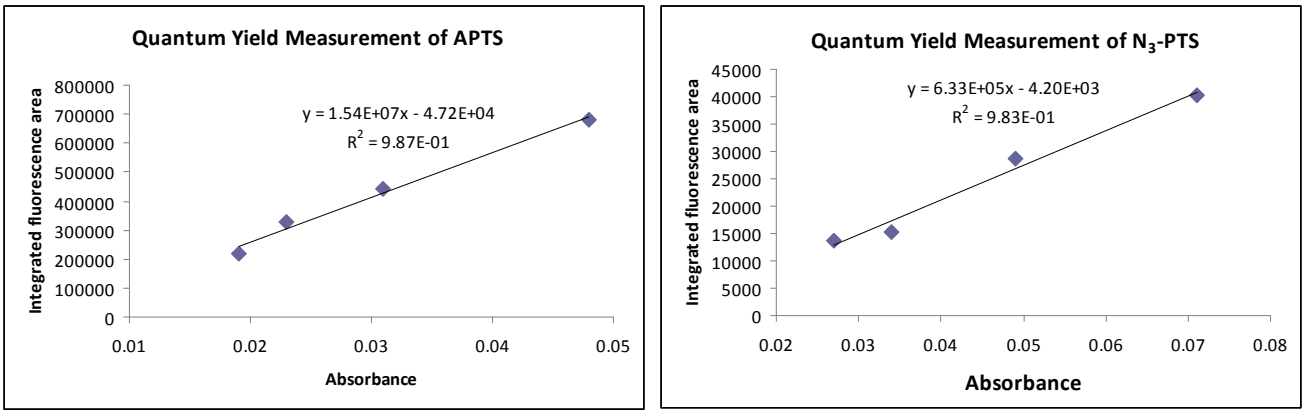


Fig. S2. Relative quantum yield measurement of APTS and N_3-PTS. Fluorescence spectra were obtained with λ_{ex} = 425 nm.

Table S2. Relative Quantum Yield Measurement

Compound	Slope	Relative Quantum Yield
APTS	1.54×10^7	**24.3**
N_3-PTS	6.33×10^5	

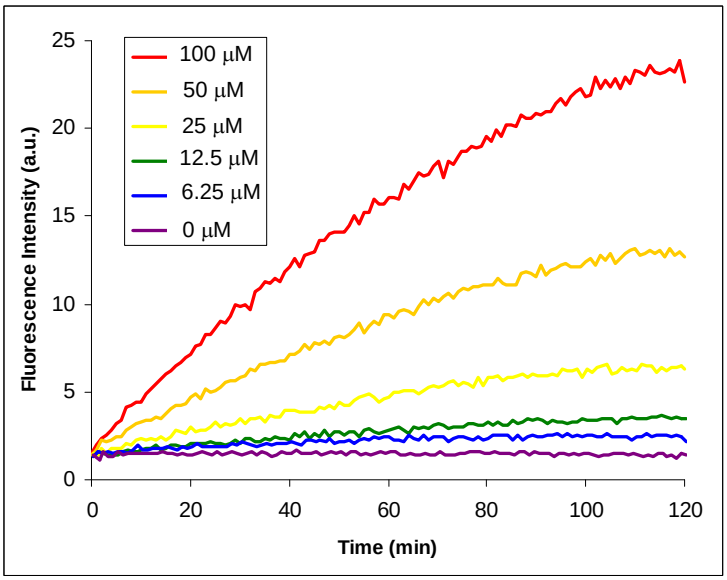


Fig. S3. Reaction of H_2S at various concentrations with N_3-PTS. The reaction was monitored at 505 nm (λ_{ex} = 435 nm), and readings were taken at 1 minute intervals.

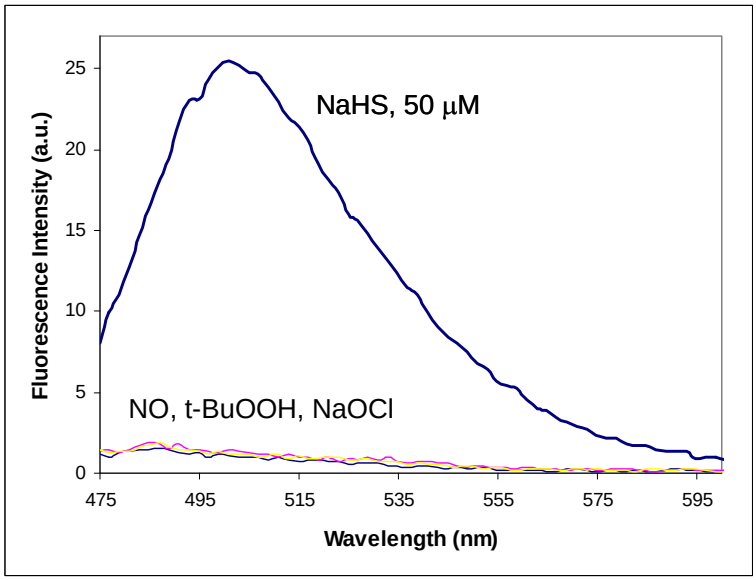


Fig. S4. Comparison of the fluorescence of N_3-PTS after incubation in 50 mM phosphate buffer (pH 7.8) along with with NaHS (50 μM) or NaOCl, Sodium Diethylamine NONOate, or tBuOOH (all 1 mM). Fluorescence emission (λ_{ex} = 435 nm) was measured after 90 min.

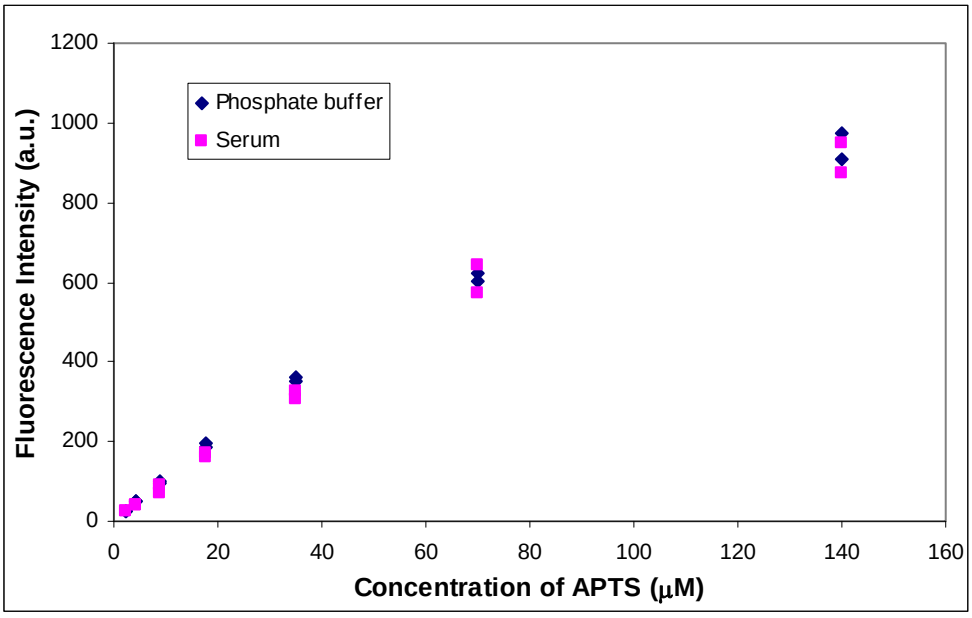
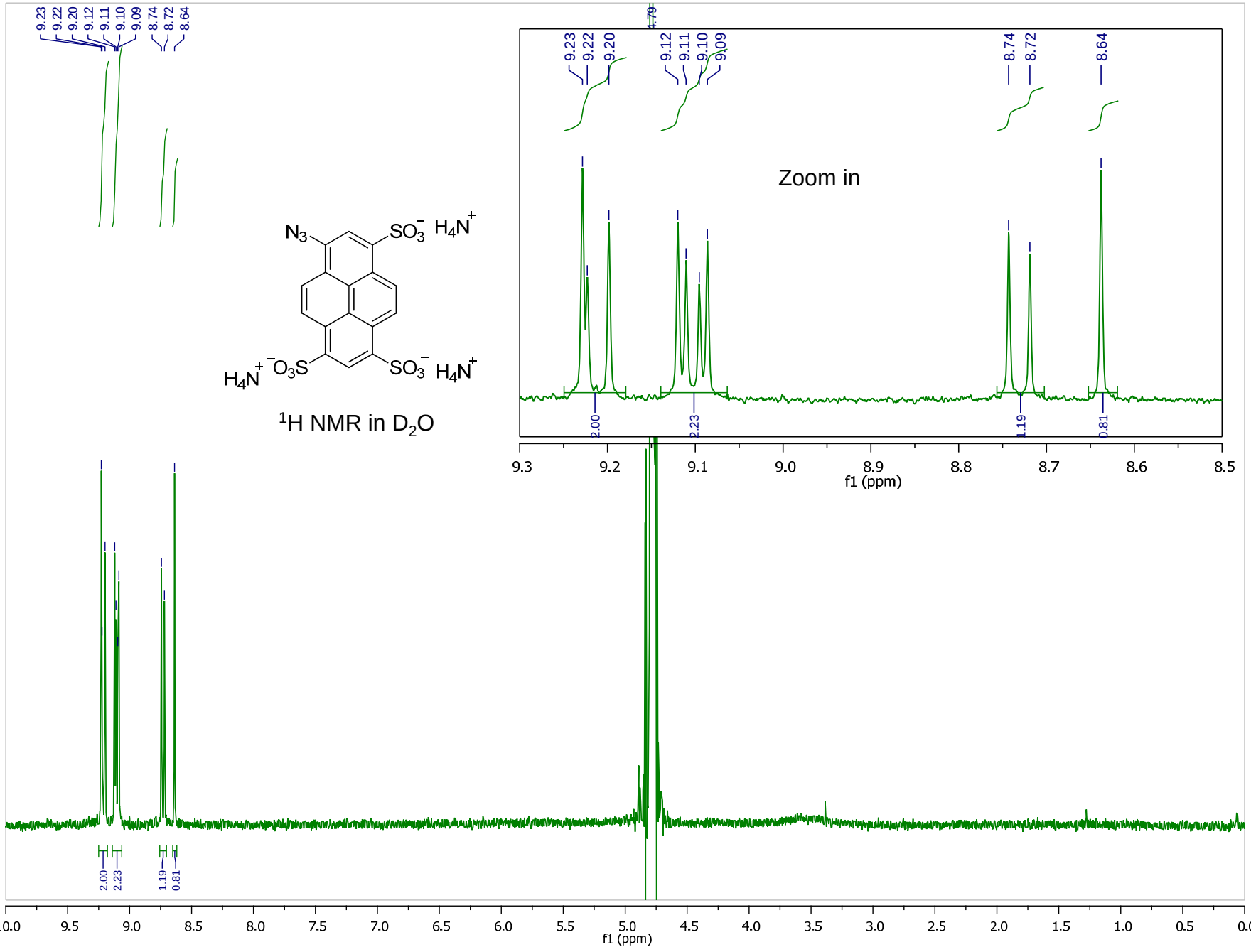


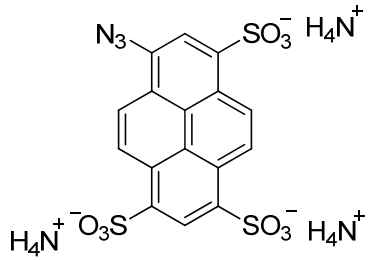
Fig. S5. Comparison of fluorescence intensity (λ_{ex} = 450 nm) of APTS at various concentrations in either phosphate buffer or Serum (FBS).

V. Reference:

1 Z. Sharrett, S. Gamsey, L. Hirayama, B. Vilozny, J. T. Suri, R. A. Wessling and B. Singaram, *Org. Biomol. Chem.,* 2009, **7**, 1461-1470.

VI. ^{1}H and ^{13}C NMR spectra for N_3-PTS





^{13}C Attached proton test in D_2O