Electrostatic interaction enhanced chromophore reaction: cationic pyridinium functionalized pyrrolopyrrole *aza*-BODIPY fluorophore for highly efficient H_2S detection

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1 Experimental

1.1 Chemicals and instruments

^{1}H NMR, ^{11}B NMR, ^{19}F NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz. UV-*vis* absorption spectra and fluorescence spectra were recorded using a Helios Alpha UV-*vis* scanning spectrophotometer and a Hitachi F-4500 FL spectrophotometer, respectively. All reagents and solvents were used directly without further purification. MALDI-TOF mass spectra were recorded on a Bruker BIFLEX III ultra high-resolution Fourier transform ion cyclotron resonance mass spectrometer. The theoretical calculations of energy levels and ground-state structures were optimized with the B3LYP/6-31G* method. Geometry optimizations are performed at the B3LYP and TD-B3LYP levels.

1.2 Spectroscopic measurements

The anions and biological thiols were dissolved in double distilled water to afford stock solution (50 mM). The H_2S test solution is made of Na_2S aqueous solution (1 M). Probes (**PPAB-Br**, **PPAB-Py**, **PPAB-Ps**) were dissolved in dioxane to afford the stock solution (1 mM). Test solutions were prepared by placing 1 mL of probe stock solution into a 100 mL volumetric flask, adding an appropriate aliquot of each anion or biological thiols stock solution, and diluting the solution to 100 mL with dioxane. The resulting solution (10 μM) was shaken well for desired time at room temperature for absorption and fluorescence spectral analysis.

1.3 Experimental procedure for H_2S detection

Three dyes (**PPAB-Br**, **PPAB-Py**, and **PPAB-Ps**) were dissolved in THF to afford the stock solution (1 mM). The anions were dissolved in double distilled water to afford stock solution (0.1 M). The H_2S test solution is made of Na_2S aqueous solution (0.1 M). Test solutions were prepared by placing 1 mL of probes stock solution into a 100 mL volumetric flask, adding an appropriate aliquot of each anion stock, and diluting the solution to 100 mL with dry THF. The resulting solution (10 μM) was shaken well for desired time at room temperature for absorption and fluorescence spectral analysis.

1.4 Preparation of PPAB-Ps-loaded polyamide thin film and test paper

The polyamide thin film or test paper was immersed into **PPAB-Ps** dioxane solution

(50 μM) for 5 min at room temperature. After dioxane is evaporated, **PPAB-Ps**-loaded polyamide thin film or test paper was dried at oven and kept in a desiccator before use.

1.5 Preparation of PPAB-Ps coated filter paper

A volume of 20 μL of THF stock solution of the **PPAB-Ps** (10 mM) was drop-casted onto the Whatman filter paper followed by evaporation to dry. A volume of 400 μL of Na_2S solution (1 mM) was prepared and used as ink for writing.

1.6 Measurement of detection limit

The detection limit was gained from the UV-vis and FL titration data. On the basis of the results of the UV-vis and FL titrating experiment, a good linear relationship was obtained. A limit of detection (LOD) was calculated by means of Eq. (1):

$$\text{Detection limit} = (3\times\sigma) / k \quad (1)$$

Where σ is the standard deviation of blank measurements, k is the slope between maximum absorption or emission peaks versus Na_2S concentration.

1.7 Detection of the released H_2S in eggs with PPAB-Ps coated filter paper

The eggs were bought from a local supermarket. After eggshell was broken, it was kept in airtight containers at room temperature, where **PPAB-Ps** -loaded filter paper was hanged inside. After desired incubation time, the filter paper was taken out for fluorescence test.

1.8 Preparation of real samples

The lake water and tap water samples used in the experiment were taken from West Lake and laboratory in South China University of Technology, respectively. The mineral water and whole milk were brought from local supermarket. All real samples are used without any pretreatment. The H_2S test solution is made of Na_2S aqueous solution. Na_2S solid was dissolved in four real samples to afford corresponding S^{2-} aqueous solution (1 M). Test solutions were prepared by placing 1 mL of **PPAB-Ps** stock solution (1 mM in dioxane) into a 100 mL volumetric flask, adding an appropriate aliquot of each Na_2S stock solution in real samples, and diluting the solution to 100 mL with dry dioxane. The resulting solution (10 μM) was shaken well for 10 min at room temperature for absorption spectral analysis. Three parallel samples are prepared and used for the test. The sample was repeatedly detected for three times.

1.9 Detection of the released H_2S in eggs with PPAB-Ps -loaded test paper

The eggs and meat (pork, fish, shrimp) were bought from a local supermarket. The broken egg and meat were kept in airtight containers at room temperature, where **PPAB-Ps** -loaded test paper was hanged inside. After desired incubation time, the test paper was taken out for fluorescence test.

1.10 Determination of Meat Freshness with PPAB-Ps Test Strips.

Meat samples (pork and shrimp) were bought from local Supermarket (Guangzhou, China). The meat samples were placed inside glass Petri dishes (80 mL), and the **PPAB-Ps** test strips were fixed at the inner lids of the Petri dishes. The lid was put back on the Petri dishes and sealed with tape. These samples were stored at 25 °C for 0, 12 and 24 h. The fluorescence pictures of these test strips under 365 nm ultraviolet light were recorded with a mobile phone, and then RGB (red, green, and blue) values were read out with an APP software (Dragon RGB). The G/R ratios were plotted against the concentrations of cadaverine to generate a calibration curve.

1.11 Determination of Total Volatile Basic Nitrogen in Meat Samples.

The contents of total volatile basic nitrogen (TVBN) in meat samples were determined according to the Chinese Standard GB 5009.228-2016. Meat sample (1.000 g) and 50 mL of distilled water was placed in a 150 mL conical flask; then, they were shaken to make the sample dispersed. Later, it was filtered, and 0.5 g of magnesium oxide was added to 25 mL of filtrate. For the control group, 25 mL of distilled water and 0.1 g of $(NH_4)_2SO_4$ were used to replace the real sample. The content of TVBN was determined by steam distillation. Steam distillation was performed using the Kjeldahl distillation unit for 300 s. The distillate was absorbed by boric acid (30 mL, 20 g/L) and then titrated with hydrochloric acid (0.1 mol/L). The contents of TVBN were calculated according to the Chinese Standard GB 5009.228-2016.

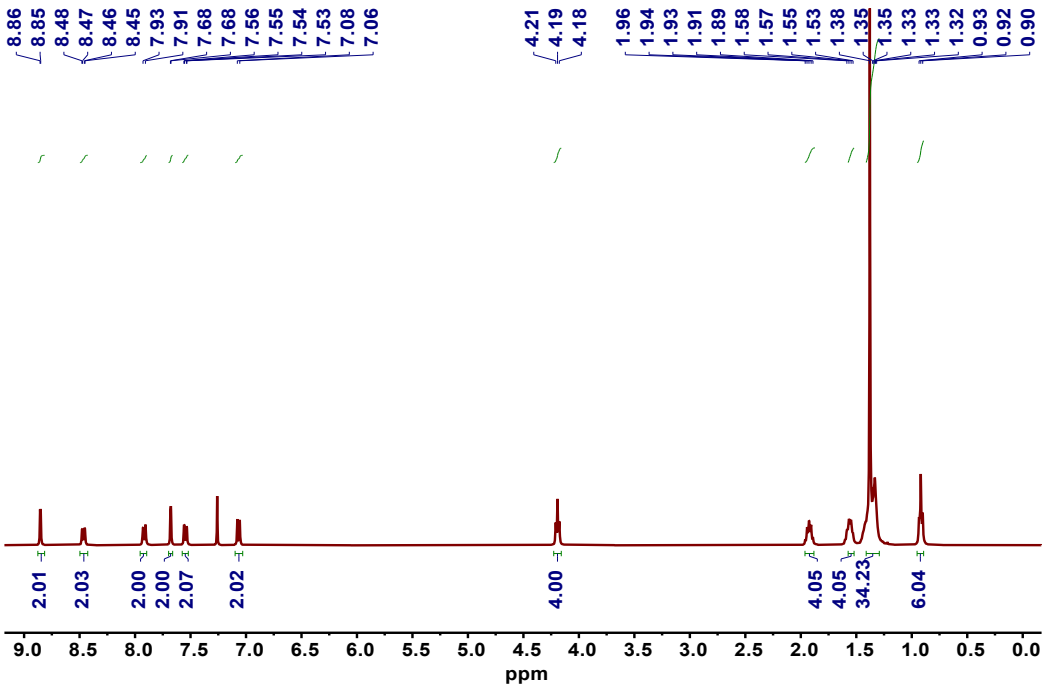


Figure S1 ^{1}H NMR spectrum of **PPAB-Br** in $CDCl_3$

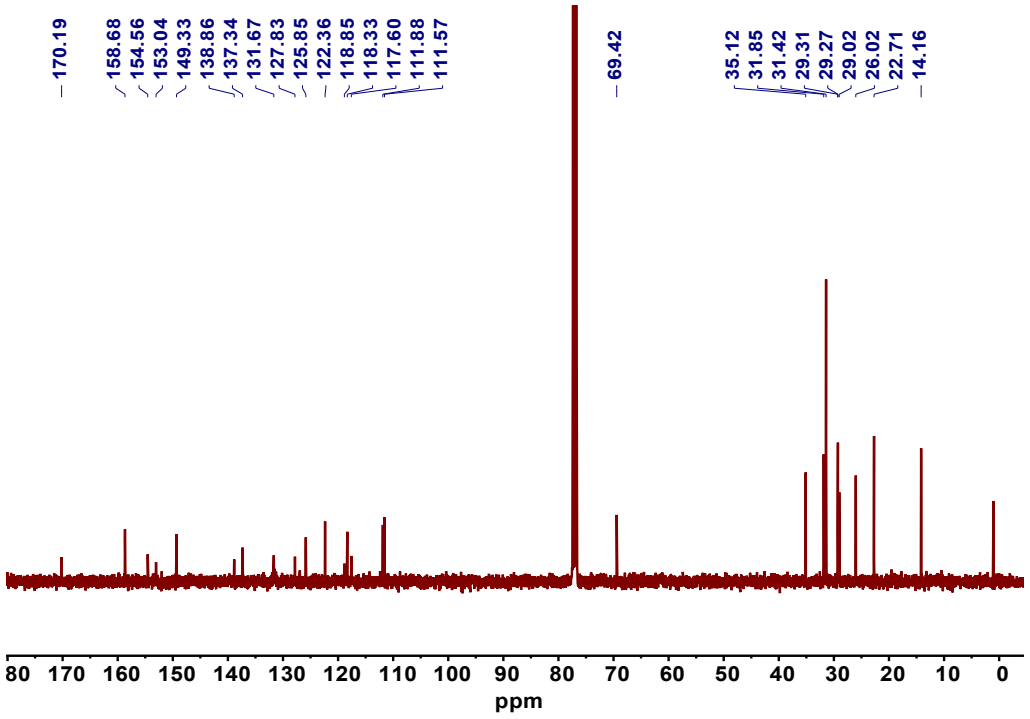


Figure S2 ^{13}C NMR spectrum of **PPAB-Br** in $CDCl_3$

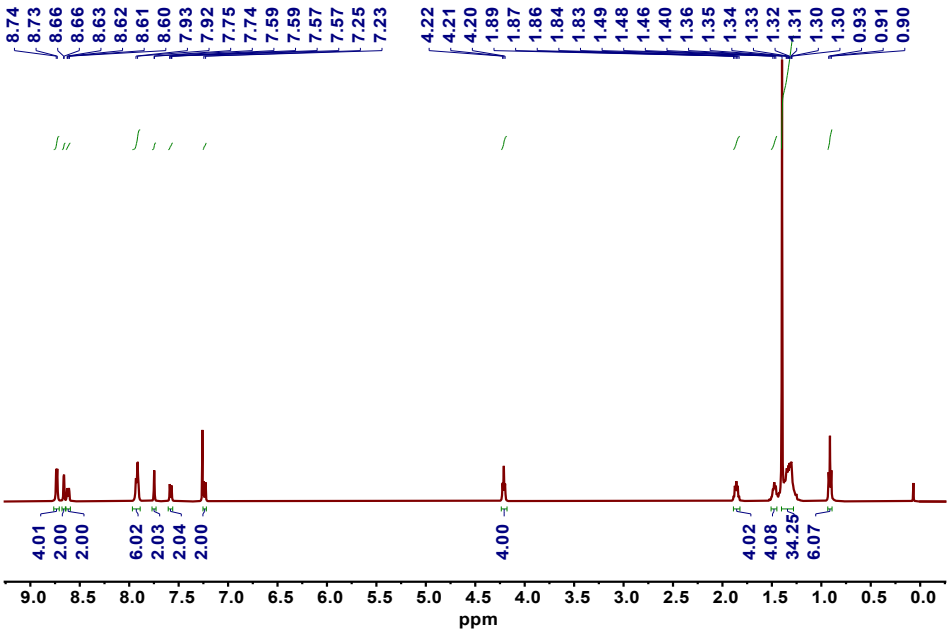


Figure S3 ^{1}H NMR spectrum of **PPAB-Py** in $CDCl_3$

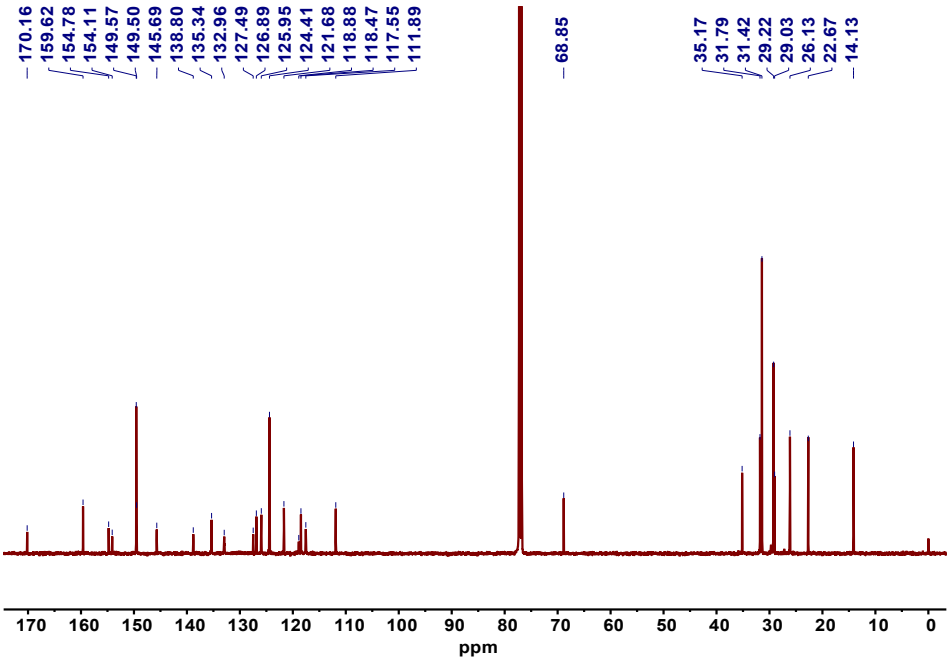


Figure S4 ^{13}C NMR spectrum of **PPAB-Py** in $CDCl_3$

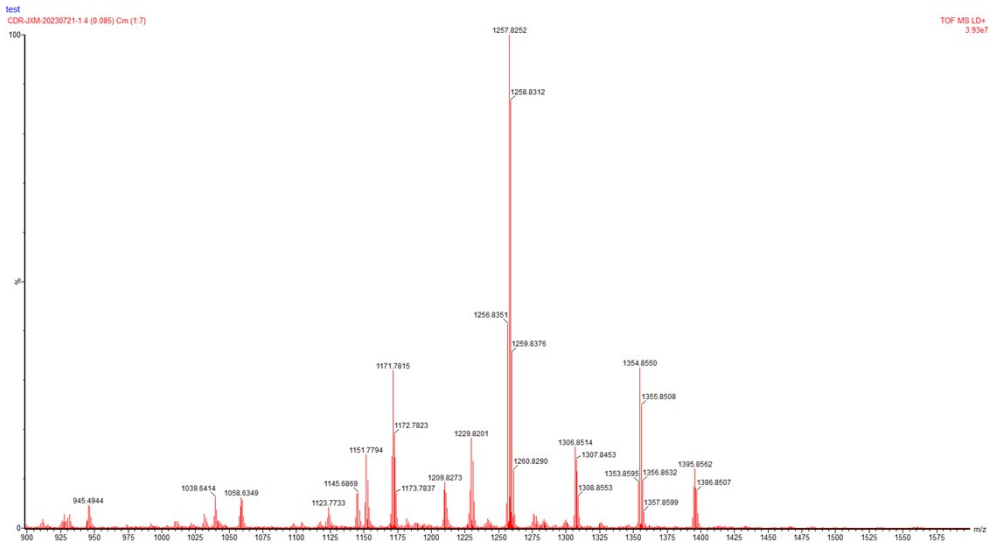


Figure S5 The MALDI-TOF spectra of **PPAB-Py**

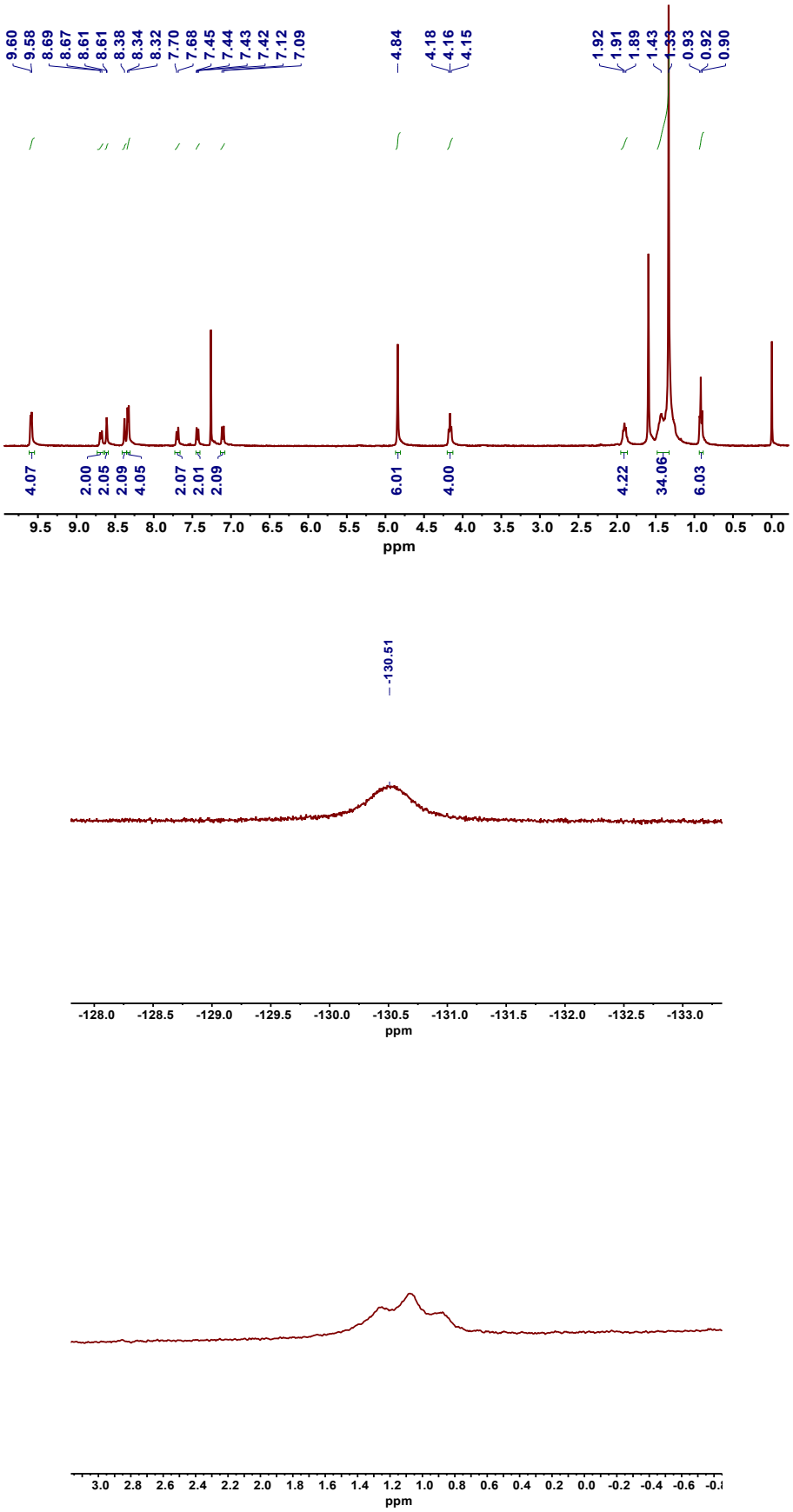


Figure S6 ^{1}H NMR, ^{19}F and ^{11}B spectra of **PPAB-Ps** in $CDCl_3$

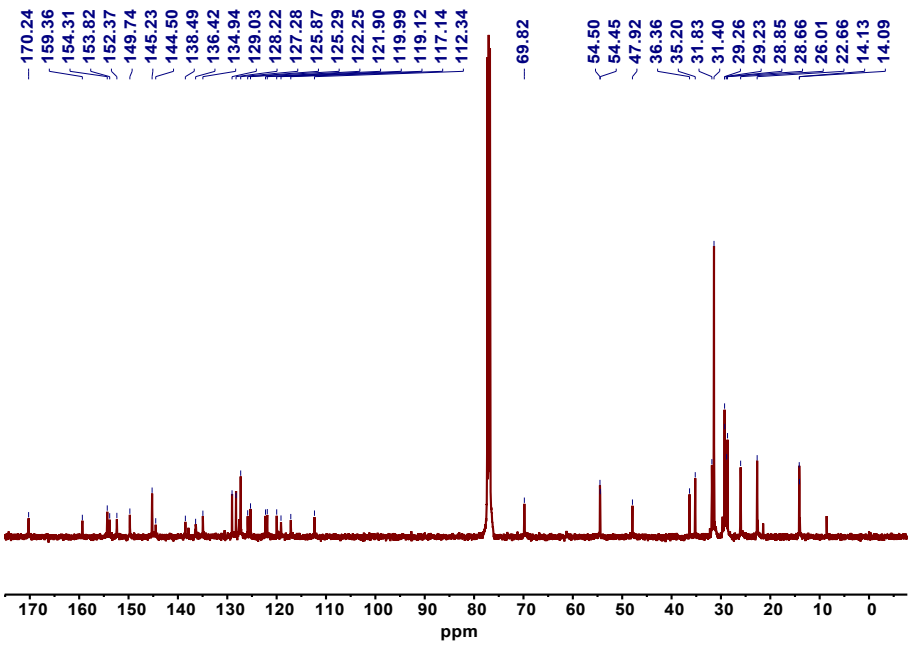


Figure S7 ^{13}C NMR spectrum of **PPAB-Ps** in $CDCl_3$

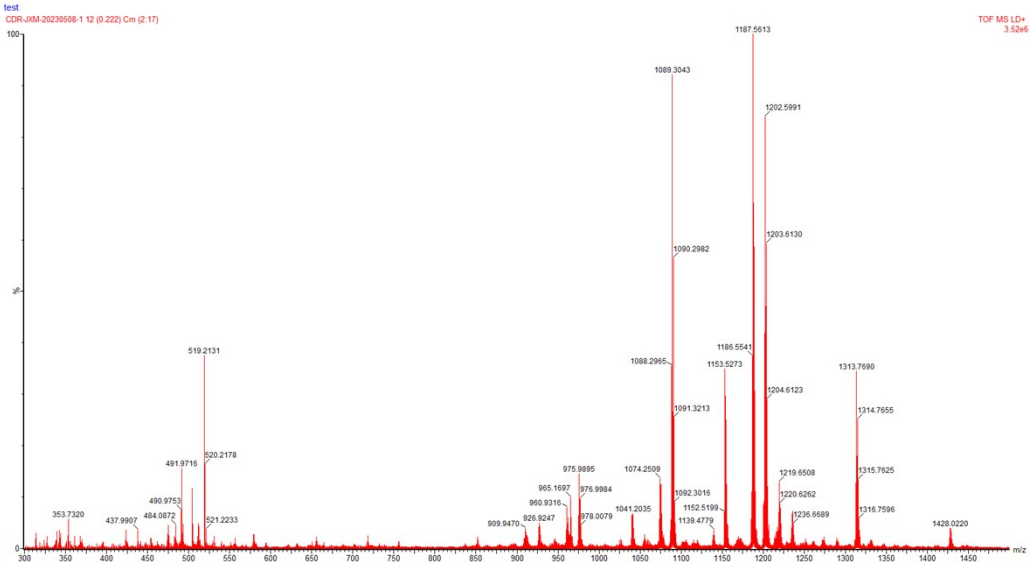


Figure S8 The MALDI-TOF spectra of **PPAB-Ps**

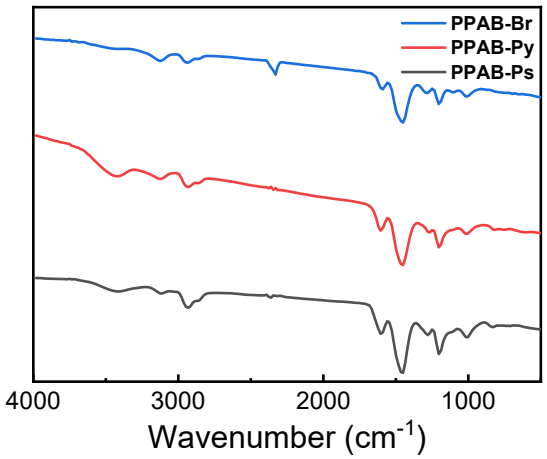


Figure S9 The FTIR spectra of **PPAB-Br**, **PPAB-Py**, and **PPAB-Ps**

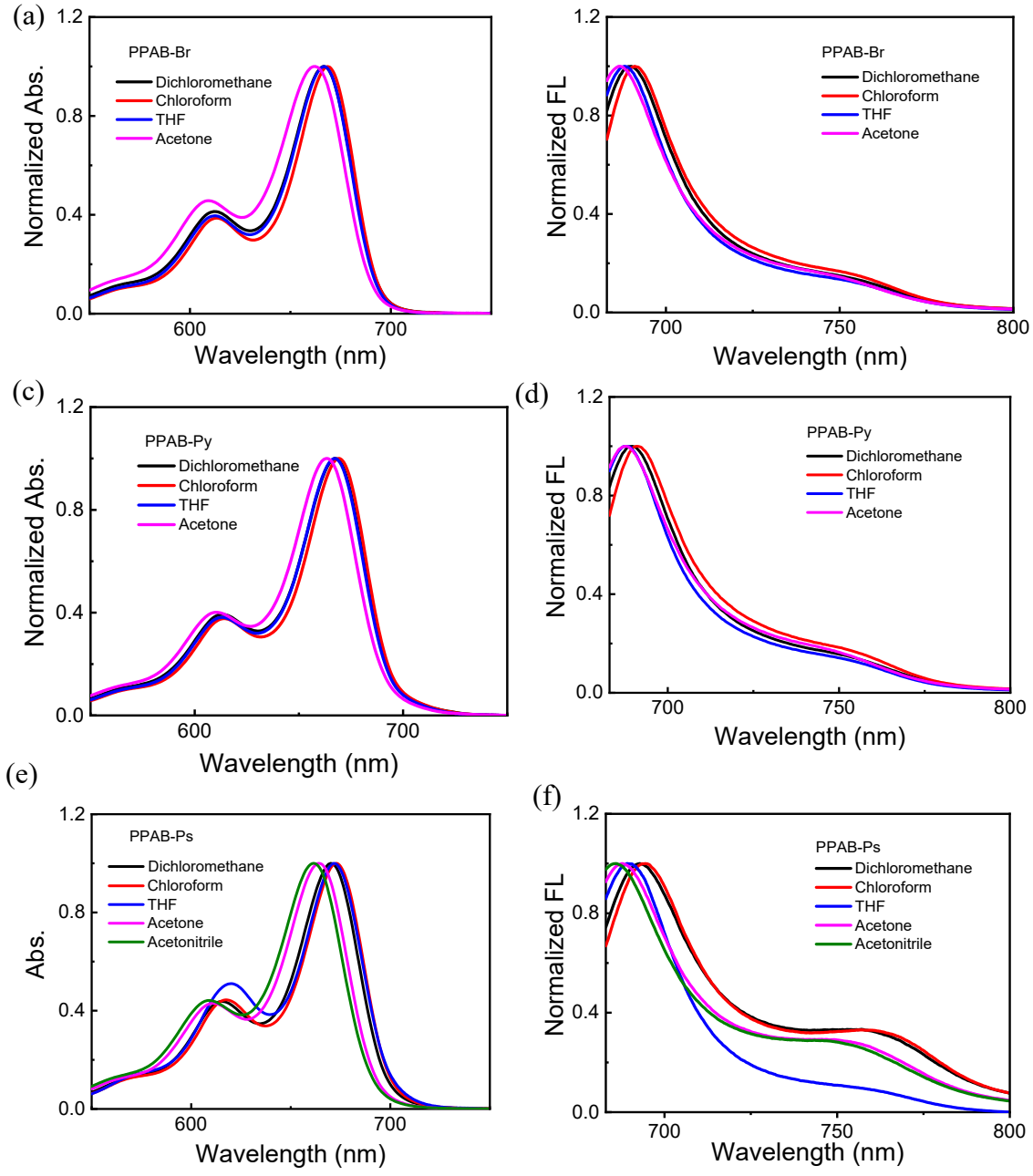


Figure S10 Normalized UV-vis and emission spectra of (a, b) **PPAB-Br**, (c, d) **PPAB-Py**, (e, f) **PPAB-Ps** (10 μM) in different solvents (λ_{ex} = 450 nm).

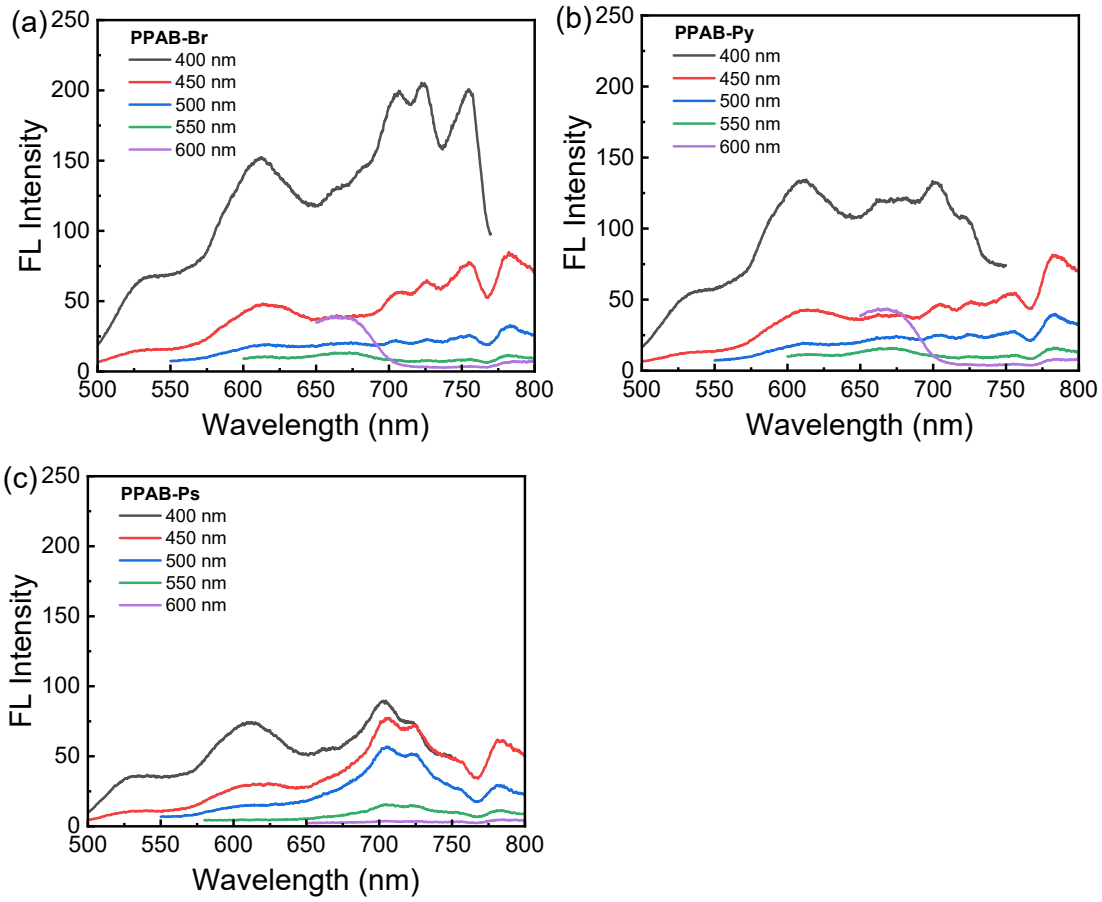


Figure S11 The excitation spectra of **PPAB-Br**, **PPAB-Py** and **PPAB-Ps** in dioxane (10^{-5} M).

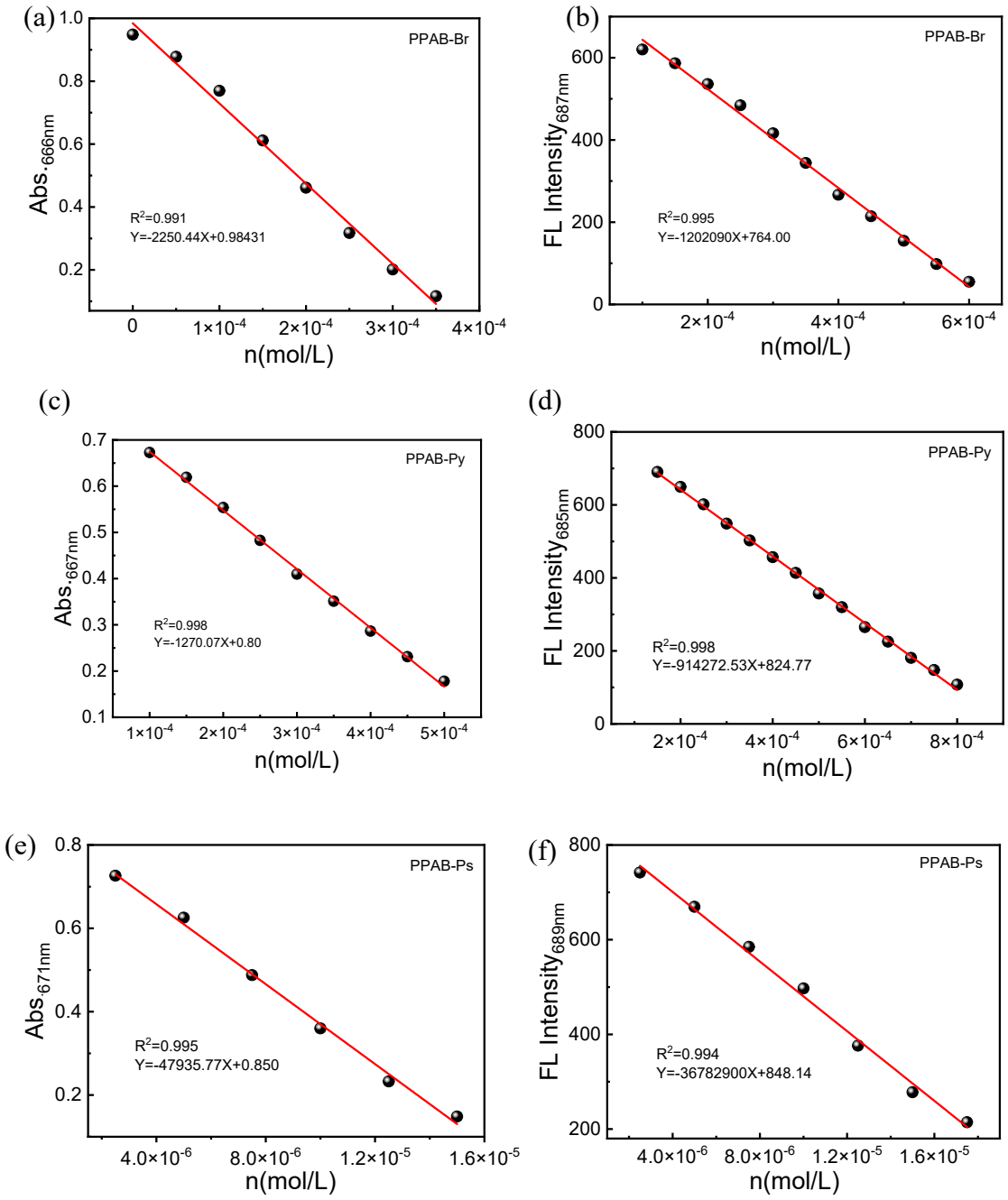


Figure S12 Concentration-dependent linear relationship of absorption and emission of **PPAB-Br** (a, b)**, PPAB-Py** (c, d) and **PPAB-Ps** (e, f) in presence of Na_2S solution at 25 °C.

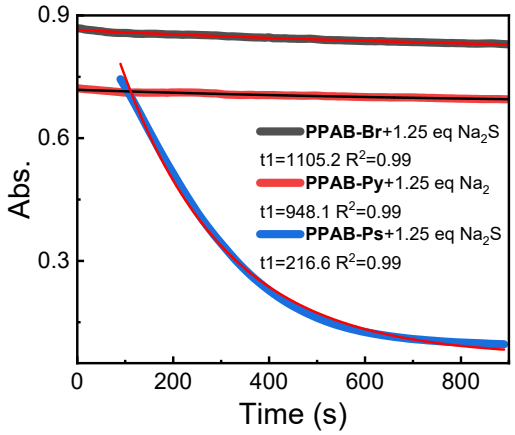


Figure S13 The kinetic curves of **PPAB-Br**, **PPAB-Py**, **PPAB-Ps** in presence of H_2S at room temperature (10 μM).

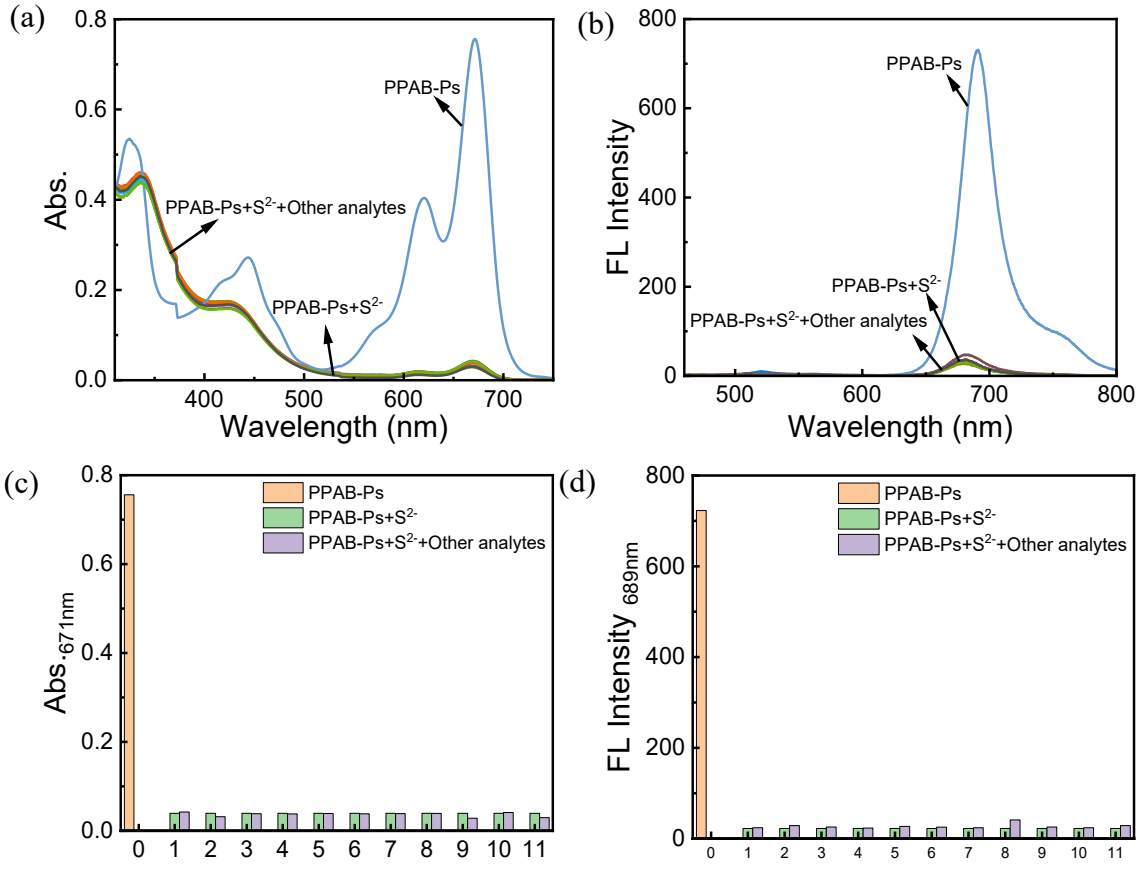


Fig. S14 (a) UV-vis absorption, (b) emission spectra of **PPAB-Ps** (10 μM) in presence of S^{2-} (30 μM) and other analytes (30 μM) at room temperature. The bar chart based on Abs at 671 nm (c) and fluorescence intensity at 689 nm (d) (0. **PPAB-Ps**; 1. NO_3^-; 2. NO_2^-; 3. SO_4^{2-}; 4. SO_3^{2-}; 5. HSO_3^- 6. F^-; 7. Cl^-; 8. Br^-; 9. AcO^-, 10. GSH, 11. Cys).

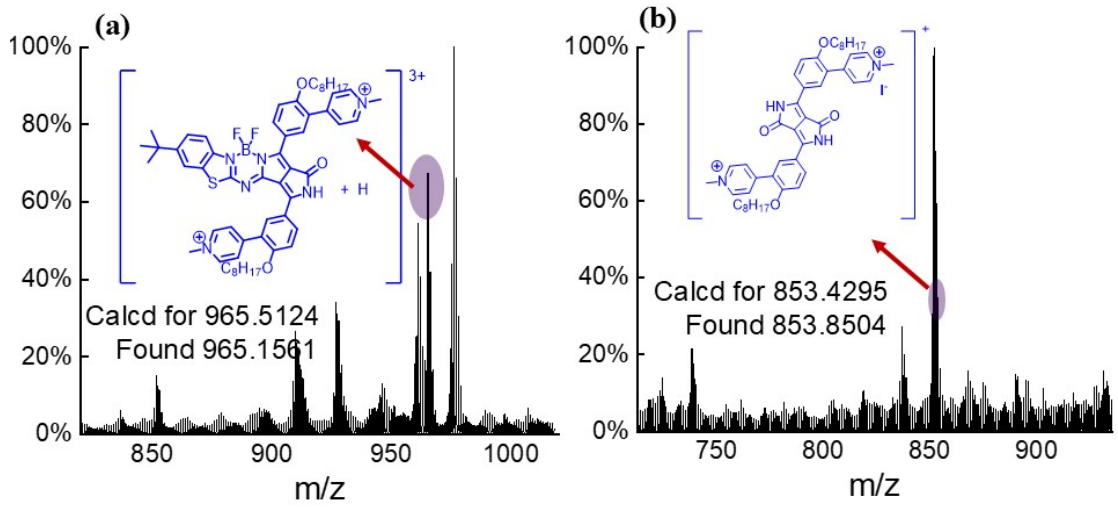


Figure S15 The MALDI-TOF spectra of **PPAB-Ps** with (a) less and (b) excess Na_2S.

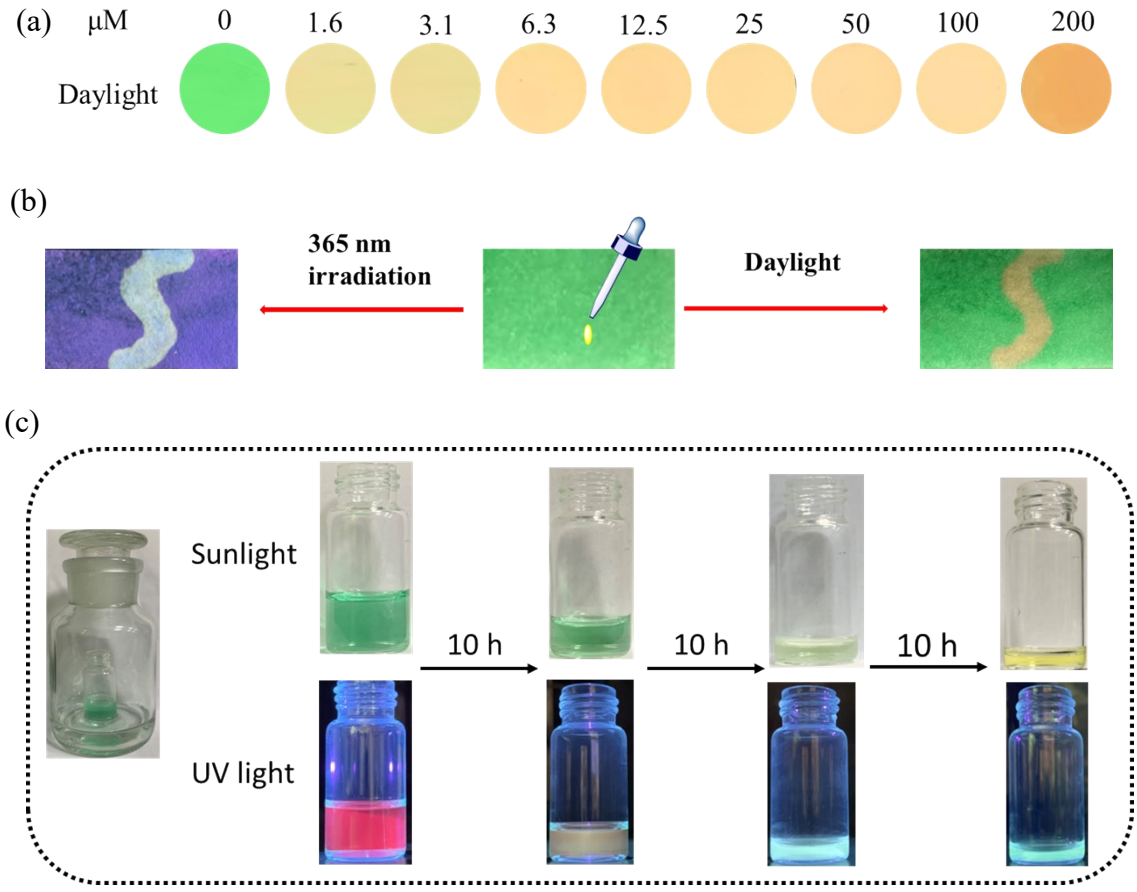


Figure S16 **PPAB-Ps**-loaded polyamide thin film (a) and test paper (b) in presence of Na_2S aqueous solution. (c) Colorimetric and fluorescent colorimetric responses of **PPAB-Pys** (10^{-5} M) solution exposure to H_2S generated from Na_2S and HCl.

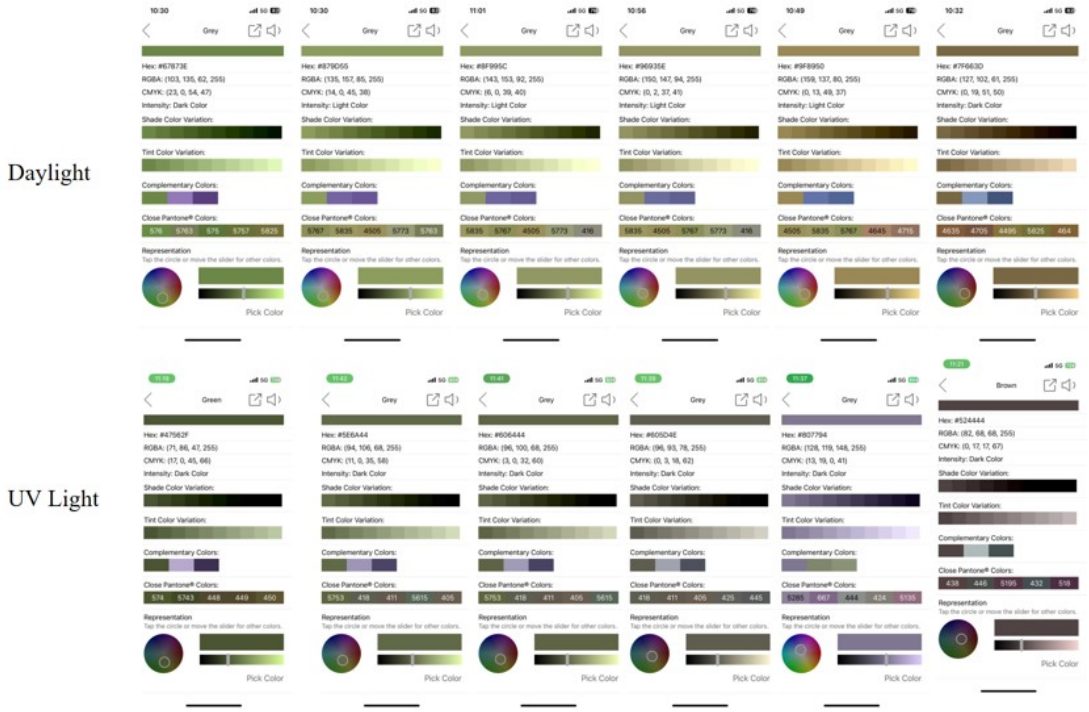


Figure S17 The picture of R, G, B value by use of Color Finder APP from the photos of **PPAB-Ps** - loaded test paper in presence of Na_2S solution in Figure 5a under day light and UV light (365 nm), respectively.

Table S1 Spectroscopic data of **PPAB-Br**, **PPAB-Py** and **PPAB-Ps** (10 μM) in different organic solvents.

Compound	Solvent	$\lambda_{max, abs}$ (nm)	$\lambda_{max, em}$ (nm)	Stokes shift (cm^{-1})	ε $M^{-1}cm^{-1}$
PPAB-Ps	Chloroform	673	694	450	1.1×10^5
	Dichloromethane	670	692	475	9.1×10^4
	Acetone	664	687	504	8.6×10^4
	Acetonitrile	661	685	530	8.4×10^4
	THF	672	689	367	5.9×10^4
PPAB-Br	Chloroform	668	691	498	1.2×10^5
	Dichloromethane	667	690	522	9.3×10^4
	THF	666	688	480	1.0×10^5
	Acetone	662	686	528	7.7×10^4
PPAB-Py	Chloroform	669	691	476	1.1×10^5
	Dichloromethane	667	689	479	8.3×10^4
	THF	667	688	458	9.2×10^4
	Acetone	663	687	527	1.2×10^5

Table S2 The comparison of probes for H_2S detection

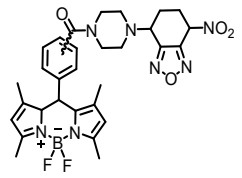
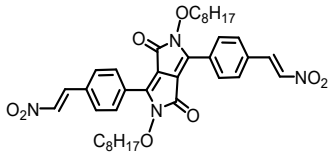
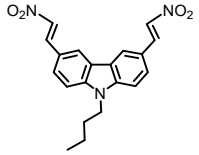
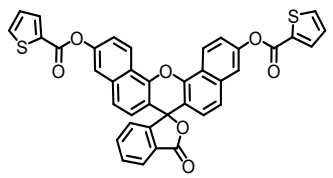
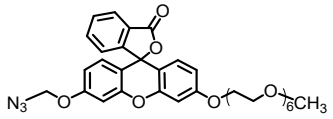
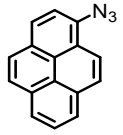
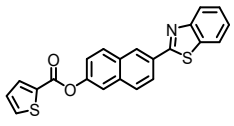
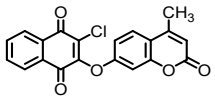
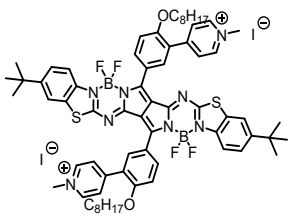
Chemical structure	Sensing mechanism	Remarks	Ref.
	Thiolysis	Turn-on fluorescence enhancement, LOD = 105 nM	Spectrochim., Acta A 2022, 272, 121007
	Reduction reaction	Fluorescence intensity at 550 nm, LOD = 5.2 nM	New J. Chem., 2017, 41, 3367
	Reduction reaction	Fluorescence of the probe turned on LOD =21 nM	Tetrahedron 2020, 76, 131317
	Thio-phenecarboxylic ester cleavage	LOD = 0.50 μM (FL)/ 0.10 μM (UV)	Journal of Molecular Structure, 2020, 1207, 127822
	Reduction reaction	Fluorescence “Turn on” at 515 nm, LOD =3.99 μM	Molecules, 2023, 28, 6195
	Reduction reaction	off-on fluorescent sensor, LOD= 56 nM	Chin. J. Chem., 2017, 35, 477
	Thenoic acid ether group cleavage	Fluorescence “Turn on” at 504 nm, LOD = 0.10 μM	Journal of Analytical Methods in Chemistry, 2019, 47,1915
	Thiolysis	Fluorescence “Turn on” response, LOD = 130 nM	Dyes and Pigments, 2021, 196, 109765
	Chromophore reaction	Dual channel detection, LOD = 88.4 nM	This work

Table S3 The R, G, B value by use of Color Finder APP from the photos of **PPAB-Ps** - loaded test paper written in presence of Na_2S solution in Figure 5a under day light and UV light (365 nm), respectively.

H_2S concentration (μM)		5	10	15	20	25	30
Daylight	R	103	135	143	150	159	127
	G	135	157	153	147	137	102
	B	62	85	92	94	73	61
	G/(R+G)	0.56723	0.53767	0.51689	0.49495	0.46284	0.44541
365 nm irradiation	R	71	94	96	96	128	82
	G	86	106	100	93	119	68
	B	47	68	68	78	148	68
	G/R+G	0.54777	0.53	0.51020	0.49206	0.48178	0.45333