SUPPORTING INFORMATION

for

Perylene diimide–Hydroxyphenyl benzothiazole based new class of radical anion/dianions: Biochemical assay for glucose detection

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EXPERIMENTAL SECTION

Materials, instrumentation and methods

The reagent grade chemicals such as perylene-3,4,9,10-tetracarboxylic dianhydride (PTCDA), salicylaldehyde, 2-aminothiophenol, 2-ethylpropylamine, imidazole, H_2O_2, HCl were procured from Sigma Aldrich, Tokyo Chemical Industries (TCI) and Spectrochem Pvt. Ltd., India and used without further purification. The HPLC grade DMSO and water were used for analytical studies. The chloroform for organic synthesis, column chromatography and thin layer chromatography (TLC) was used after distillation. All reactions were performed under N_2 stream. The TLC for monitoring the progress of the reactions was performed on aluminum sheets coated with silica gel 60 F254 (Merck, Darmstadt, Germany). The HEPES buffer (2.38 g) was dissolved in 1 L deionized water and pH of the buffer was adjusted to *ca*. 7.4. Preparative liquid chromatography (PLC) was performed on the glass plates. All spectroscopic measurements were carried out in a 20% HEPES buffer–DMSO solution at 10 or 20 μM concentrations of BT-PDI at room temperature.

The proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on BRUKER Biospin AVANCE-III FT-NMR HD-500 spectrometer in $CDCl_3$ and referenced to tetramethyl silane (TMS) as an internal standard for ^{1}H and residual signals for ^{13}C. The spin multiplicities are reported as s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet and m = multiplet with coupling constant (*J*) values in Hz. The FT-IR (ATR) spectra were recorded on the Perkin Elmer 92035 spectrometer. The absorbance and fluorescence studies of different solutions of BT-PDI in 20% HEPES buffer–DMSO in the absence and presence of different analytes were carried out on Cary 5000 UV-VIS-NIR spectrophotometer and RF 6000 SHIMADZU Spectrofluorophotometer, respectively equipped with a Peltier to control the temperature. The UV-VIS-NIR spectra were recorded using quartz cells having 1

cm path length, 2 nm band width and 140 nm min^{-1} scan rate. The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments of BT-PDI in the absence and presence of different concentrations of H_2S were performed on Metrohm Autolab PGSTAT302N electrochemical workstation at room temperature using a standard three electrode arrangement that consists of platinum electrode as both working and auxiliary electrodes and Ag/AgCl (saturated with KCl solution) as reference electrode. All the electrochemical measurements were carried out after purging N_2 gas. Tetrabutylammonium hexafluorophosphate was used as the supporting electrolyte. The AFM micrographs were recorded on a Tosca 400 instrument developed by Anton Paar to characterize the topography of BT-PDI. Two probe Agilent B2902A precision measurement unit source meter was used to measure the resistance and data was analysed using a computer equipped with quick I–V software. The change in resistance was recorded as a function of time at different potentials. All the sensing measurements have been performed under 30–40% relative humidity.

General procedure for the formation of radical anion and dianion

The solution of BT-PDI (10 μM) was prepared in a binary mixture of 20% HEPES buffer–DMSO (pH 7.4). The freshly prepared solution of NaHS (as a source of H_2S) (0.1 M) was added at different concentrations to the BT-PDI solution. The immediate formation of radical anion (**BT-PDI**$^{\cdot-}$) and dianion (**BT-PDI**$^{2-}$) were observed with concomitant colour change from pink to green and blue, respectively. The **BT-PDI**$^{\cdot-}$ and **BT-PDI**$^{2-}$ is stable for >3h in hypoxic conditions and 1h in oxygenated conditions. The **BT-PDI**$^{\cdot-}$ and **BT-PDI**$^{2-}$ was freshly generated before recording different spectroscopic measurements.

Procedure for the detection of H_2O_2

To the solution of **BT-PDI**$^{\cdot-}$ (generated in situ upon addition of 100 equivalents of NaHS in BT-PDI) in 20% HEPES buffer–DMSO (pH 7.4), different concentrations of H_2O_2 (0–1 mM) were added and absorbance/fluorescence spectra were recorded and naked eye photographs were taken.

Biochemical assay for detection of Glucose

To the solution of **BT-PDI**$^{\cdot-}$ (generated in situ upon addition of 100 equivalents of NaHS in BT-PDI) in 20% HEPES buffer–DMSO in the absence and presence of 10% blood serum (pH 7.4), 6 μL of Glucose Oxidase (GOx) (2 mg dissolved in 1 mL of H_2O) or 6 μL of GOx (5 mg dissolved in 1 mL of H_2O) were added. To these mixtures, different concentrations of glucose (0–100 nM) were added and absorbance/fluorescence spectra were recorded and naked eye photographs were taken.

Synthesis of BT-PDI

To a reflux solution of salicylaldehyde (0.5 g, 4.1 mmol) and 2-aminothiophenol (0.56 g, 4.5 mmol) in ethanol (10 mL), 10 μL of H_2O_2 (30% v/v) and 20 μL of Conc. HCl were added and reaction mixture

was stirred for 2–3 h. The progress of the reaction was checked by the TLC and upon completion of the reaction, the mixture was cooled in an ice bath and the formed precipitates were filtered, washed with cold ethanol and dried to afford HBT as a light-yellow solid (0.72 g, 77.4%), R_f = 0.39 (Hexane–$CHCl_3$, 7:3, v/v). The compound HBT was further used without purification. To a solution of HBT (48 mg, 0.16 mmol) in CH_3CN (4 mL), K_2CO_3 (26 mg, 0.19 mmol) was added. Subsequently, PDI-Br (100 mg, 0.13 mmol) was added to the above solution and reaction mixture was stirred at 100 °C for 3–4 h. After completion of the reaction which was checked by TLC, the solvent was rotary evaporated and residue was dissolved in chloroform. The chloroform layer was washed with water (2x50 mL) and brine (2x20 mL). The chloroform layer was dried over sodium sulphate and chloroform was distilled by rotary evaporator. The obtained crude residue was purified by column chromatography (SiO_2) using 2% Ethyl acetate–Chloroform binary mixture as eluent to isolate compound **BT-PDI** as dark orange solid; Yield 54 mg (0.071 mmol, 87%), R_f = 0.65 (Ethyl acetate–Chloroform, 1:9, v/v). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 9.75 (d, *J* = 8.0 Hz, 1H, Perylene), 8.76–8.63 (m, 6H, perylene and ArH), 8.22 (s, 1H, perylene), 8.06 (d, *J* = 8.5 Hz, 1H, ArH), 7.72 (d, *J* = 8.0 Hz, 1H, ArH), 7.54–7.46 (m, 3H, ArH), 7.31 (t, *J* = 7.5 Hz, 1H, ArH), 7.13 (d, *J* = 7.5 Hz, 1H, ArH), 5.08–4.97 (m, 2H, 2xN-CH), 2.30–2.17 (m, 4H, 2xCH_2), 1.97–1.84 (m, 4H, 2xCH_2), 0.92 (t, *J* = 7.5 Hz, 6H, 2xCH_3), 0.87 (t, *J* = 7.5 Hz, 6H, 2xCH_2) ppm; ^{13}C NMR (DEPT) (125 MHz, $CDCl_3$): δ 161.53 (No peak), 155.35 (No peak), 152.64 (No peak), 152.40 (No peak), 135.64 (No peak), 134.61 (No peak), 134.52 (No peak), 133.63 (No peak), 132.70 (positive), 131.01 (positive), 129.49 (No peak), 128.94 (positive), 128.84 (No peak), 127.20 (No peak), 126.51 (positive), 126.37 (No peak), 126.21 (Positive), 125.49 (Positive), 124.17 (No peak), 123.91 (Positive), 123.43 (Positive), 122.72 (Positive), 121.43 (Positive), 120.63 (Positive), 57.99 (Positive), 57.78 (Positive), 25.14 (Negative), 25.09 (Negative), 11.47 (Positive), 11.45 (Positive) ppm; IR spectrum (ATR): ν_{max} [cm^{-1}] = 3436.6, 2998.8, 2914.8, 2363.1, 1662.4, 1431.3, 1312.0, 1140.6, 1021.3, 954.2, 700.7, 499.5; HRMS for $(C_{47}H_{38}N_3O_5S)^+$, Calculated 756.2527, Found 756.2281.

Femtosecond transient absorption (fs-TA)

We used a femtosecond pump-probe setup to carry out the fs-TA experiments.[35] Laser pulses with centre wavelength of 800 nm, pulse length of 80 fs and repetition rate of 1 kHz were generated by a regenerative amplifier (Spitfier, Spectra Physics), in which a femtosecond oscillator (Tsunami, Spectra Physics) was used as the seeding laser. We used a BBO crystal to generate the pump pulses (400 nm) by second harmonic generation. The pump pulse energies were set to 50 nJ per pulse. The spot size of the pump beam was approximately 0.4 mm^2. We focused the 800 nm laser pulses from the amplifier to generate supercontinuum as probe light. The mutual polarization between the pump and probe beams was set to the magic angle (54.7 °) by placing a Berek compensator in the pump beam. There is no photodegradation after fs-TA experiments by checking the steady-state absorption spectra. The global analysis was performed by using Glotaran software package (http://glotaran.org). A simple sequential decay model with two or three components was used to do SVD global fitting.

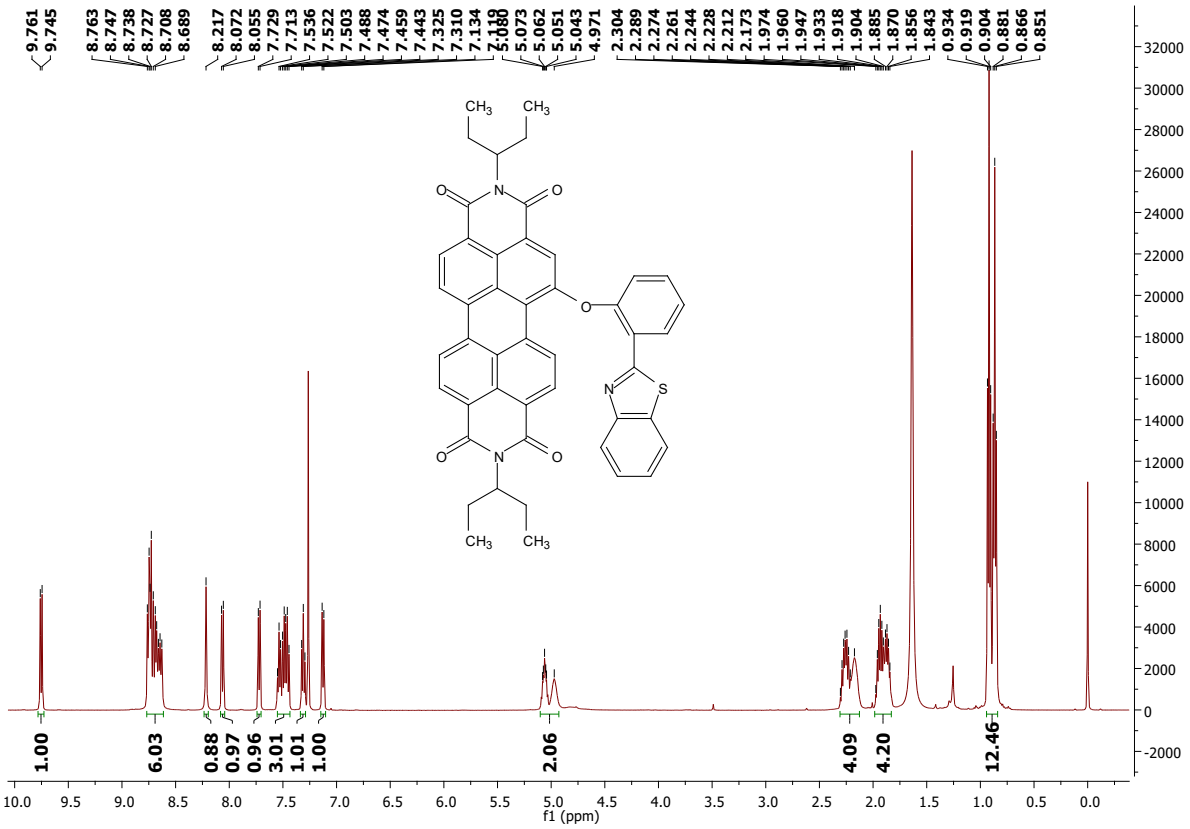


Figure S1. ^{1}H NMR data of BT-PDI.

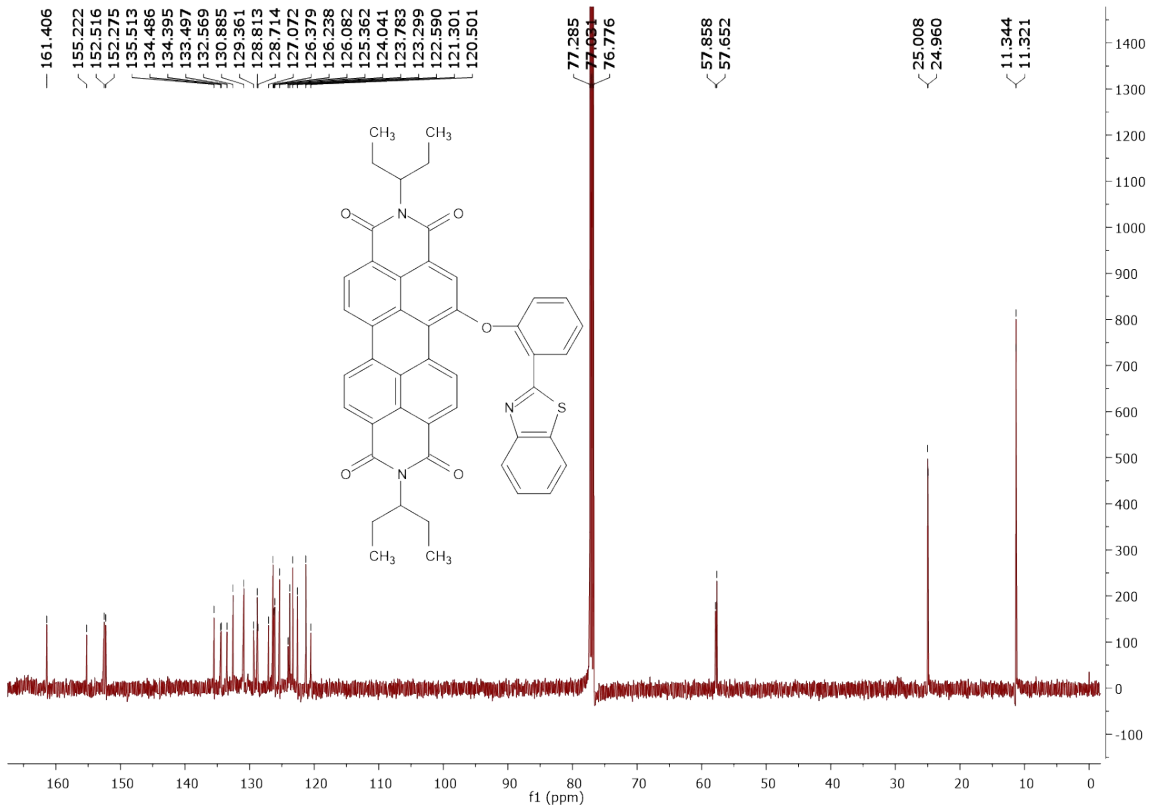


Figure S2. ^{13}C NMR data of BT-PDI.

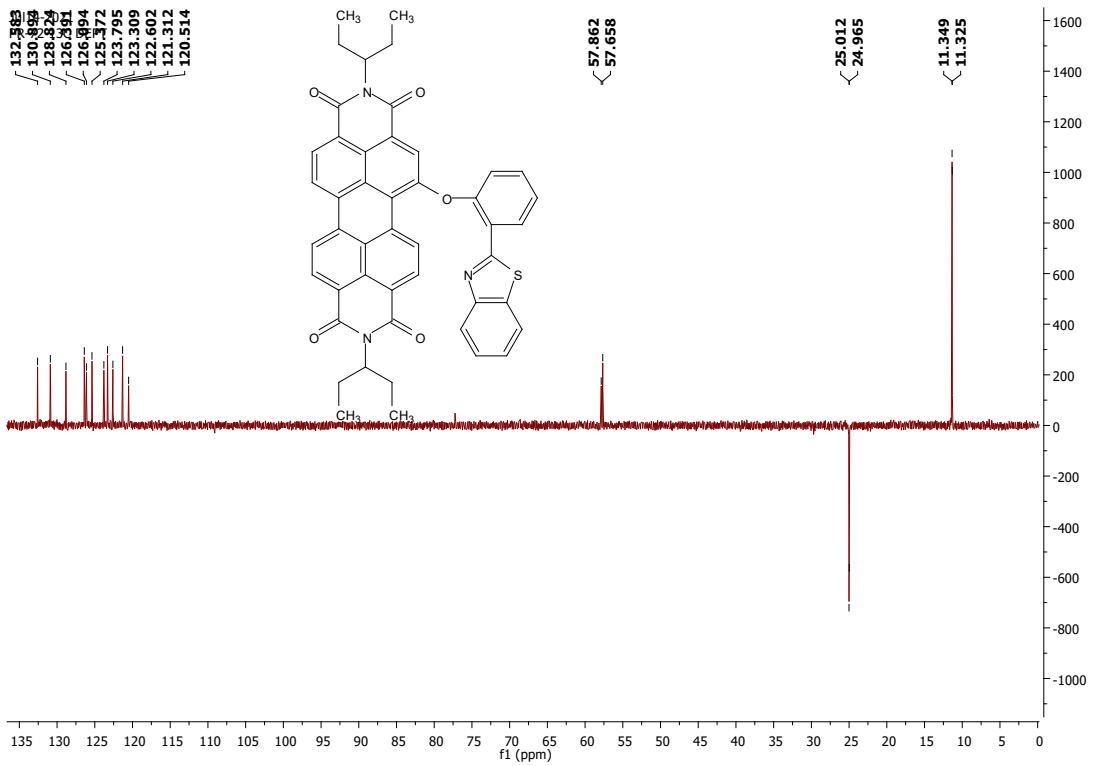


Figure S3. ^{13}C-DEPT NMR data of BT-PDI.

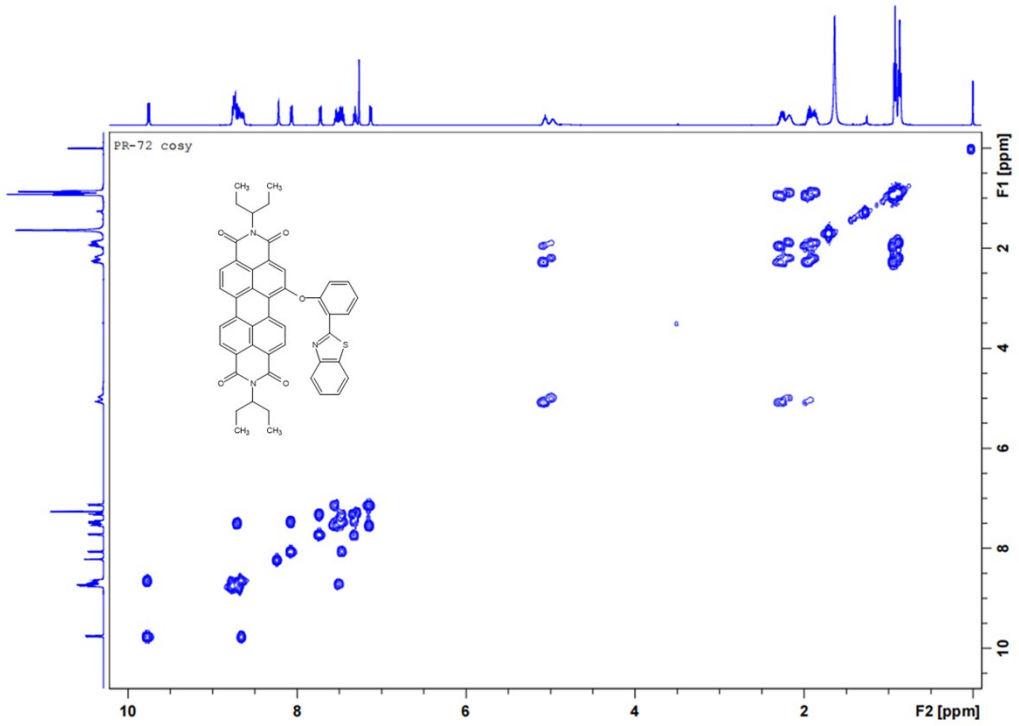


Figure S4. ^{1}H-^{1}H COSY data of BT-PDI.

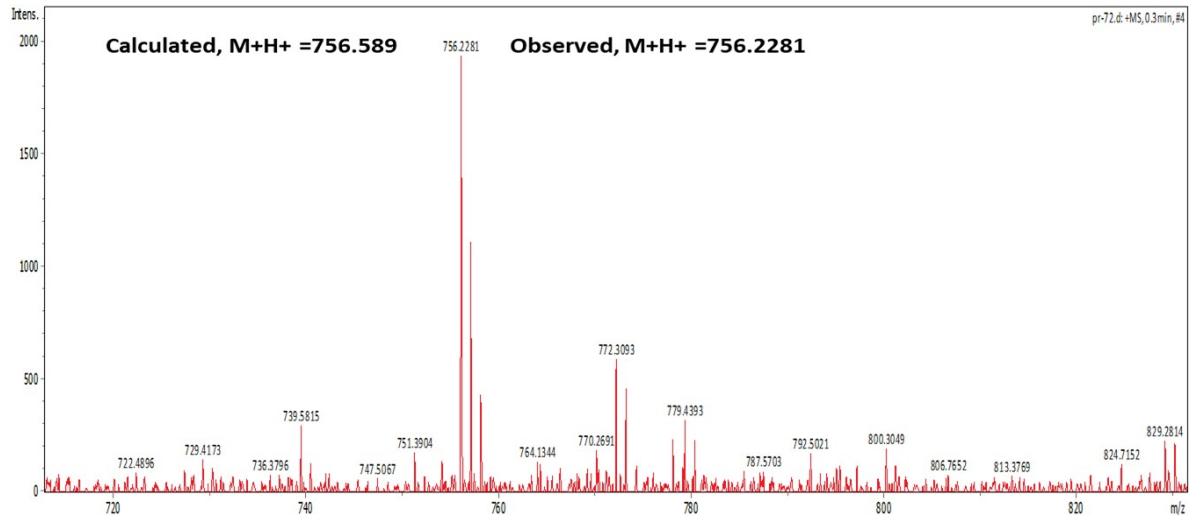


Figure S5. HRMS data of BT-PDI.

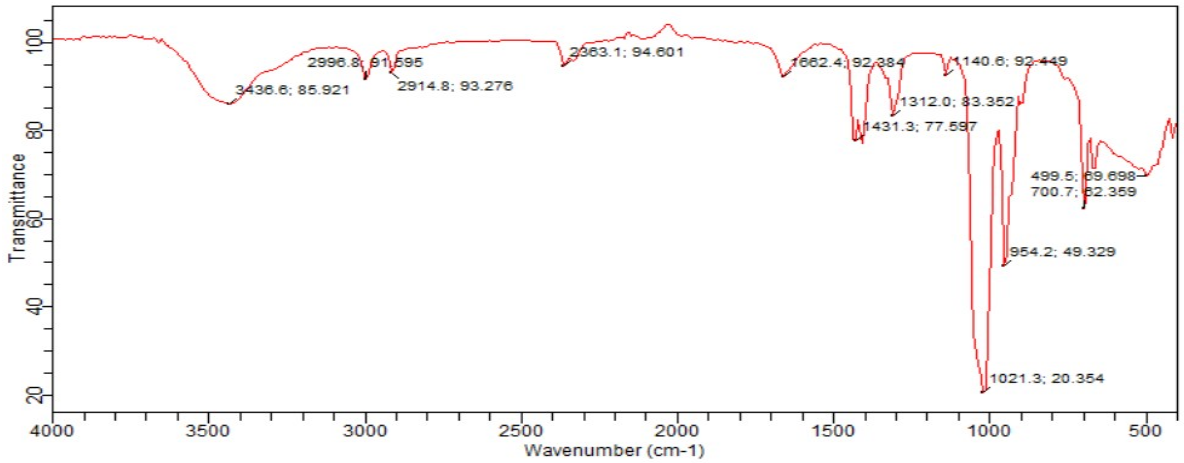


Figure S6. FTIR data of BT-PDI.

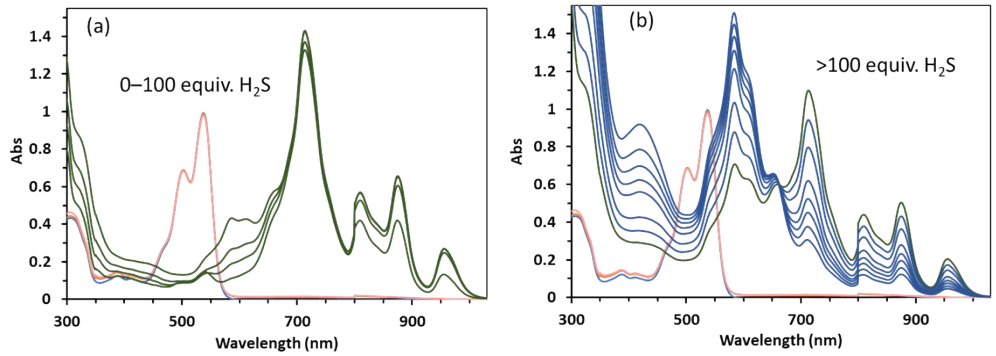


Figure S7. Absorbance spectra of BT-PDI (10 μM) upon addition of (a) H_2S (0–100 equivalents) and (b) H_2S (>100 equivalents).

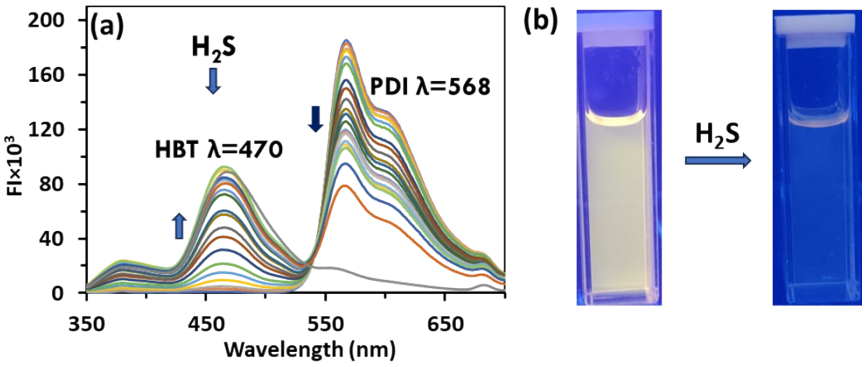


Figure S8. (a) Fluorescence spectra of BT-PDI (10 μM) upon addition of different concentrations of H_2S (0–2 mM) and (b) Photographs of BT-PDI before and after addition of H_2S under 365 nm UV illumination. All readings have been recorded in HEPES buffer–DMSO (2:8, v/v, pH 7.2) medium using λ_{ex} = 340 nm, Slit width Ex/Em = 5/5 nm).

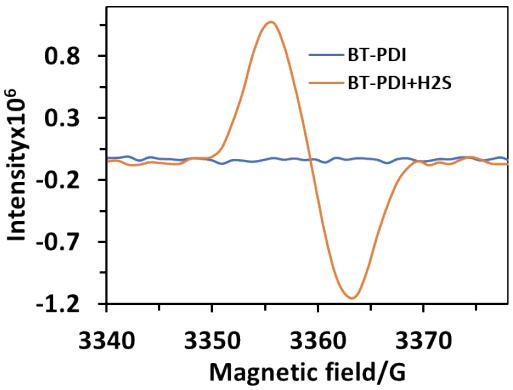


Figure S9. The EPR spectrum of BT-PDI (10 μM) upon addition of H_2S.

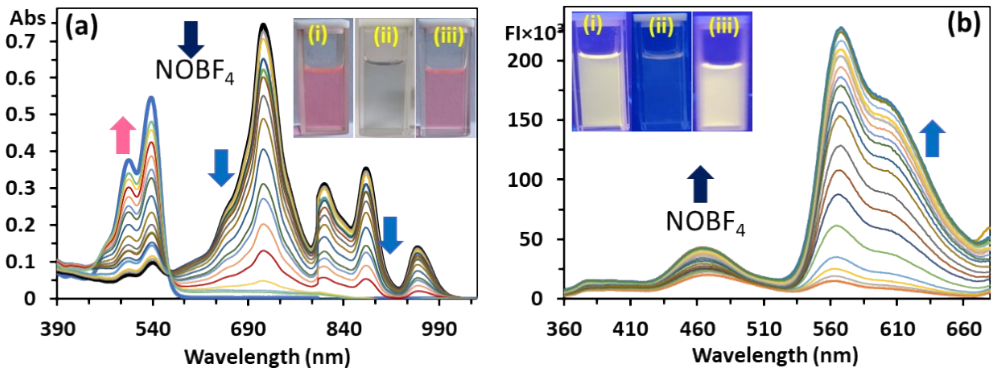


Figure S10. (a) UV–Vis–NIR and (b) emission spectra of **BT-PDI**$^{\bullet-}$ (10 μM) upon addition of different concentrations of $NOBF_4$ (0–0.5 mM) and (c); (Insets of b and d) Photographs of (i) **BT-PDI** alone (ii) **BT-PDI** + H_2S (1 mM) and (iii) **BT-PDI** + H_2S (1 mM) + $NOBF_4$ (0.5 mM) in daylight and under 365 nm UV lamp, respectively. All solutions were prepared in a 20% HEPES buffer–DMSO (pH 7.4) solution; λ_{ex} = 340 nm, slit width, Ex/Em = 5/5 nm.

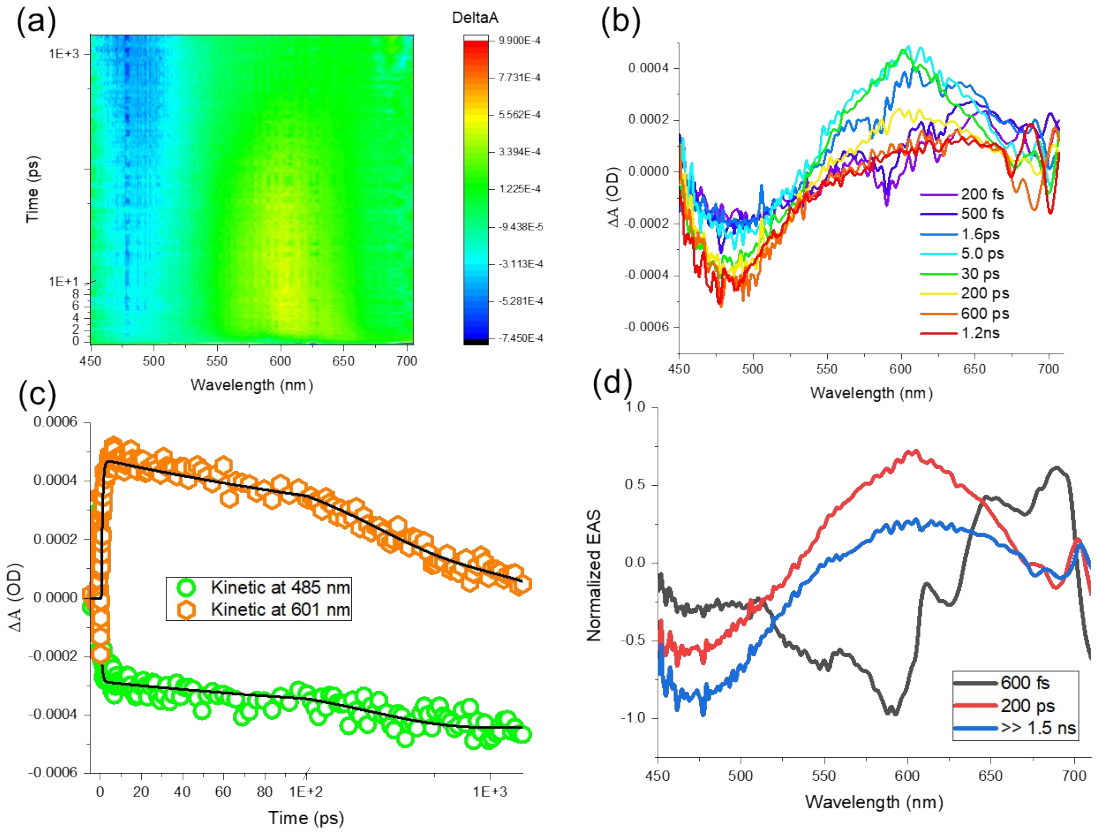


Figure S11. (a) 2D plot of fs-TA spectra of BT-PDI with H_2S; (b) fs-TA spectra of BT-PDI with H_2S at different time delay; (c) kinetics at different wavelengths, the solid line is fitted curve; (d) normalized evaluation associated (EAS) spectra.

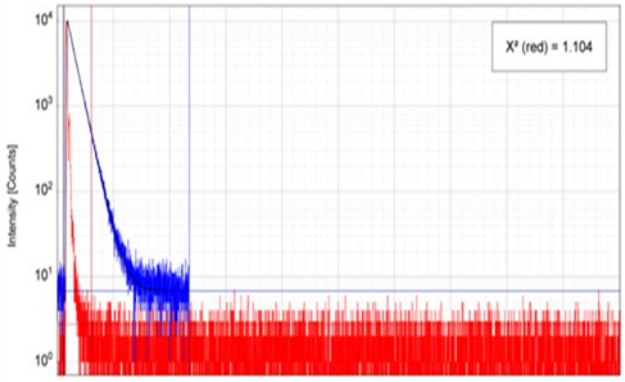


Figure S12. The fluorescence lifetime spectrum of BT-PDI in the absence of H_2S.

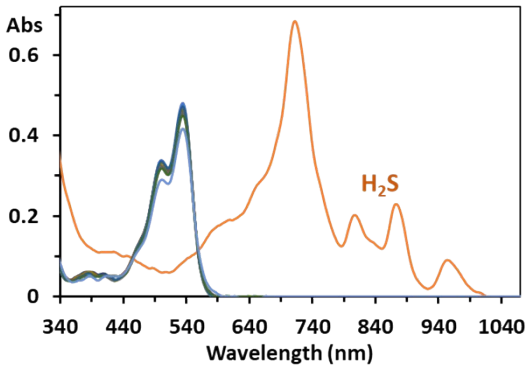


Figure S13. Absorbance spectra of BT-PDI (10 μM) upon addition of different analytes (100 equivalents). All readings have been recorded in HEPES buffer–DMSO (2:8, v/v, pH 7.2) medium using λ_{ex} = 340 nm, Slit width Ex/Em = 5/5 nm).

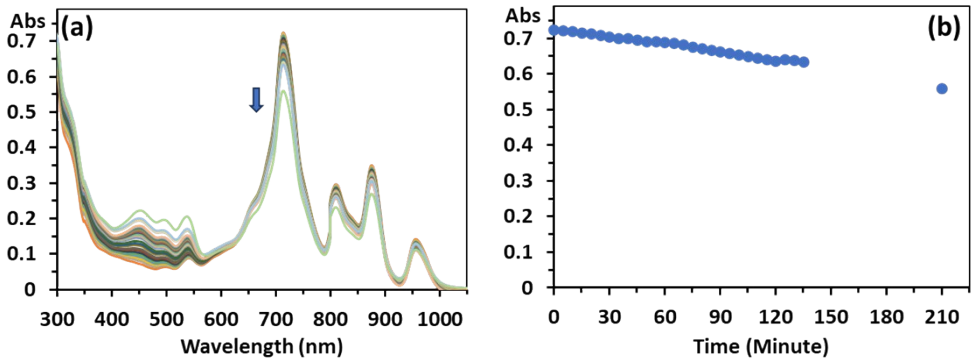


Figure S14. (a) Absorbance spectra and (b) absorbance intensity plot at 714 nm of **BT-PDI**$^{\bullet-}$ (10 μM, generated by addition of 100 equivalents of H_2S) recorded after regular interval of time under hypoxic conditions. All readings have been recorded in HEPES buffer–DMSO (2:8, v/v, pH 7.2) medium.

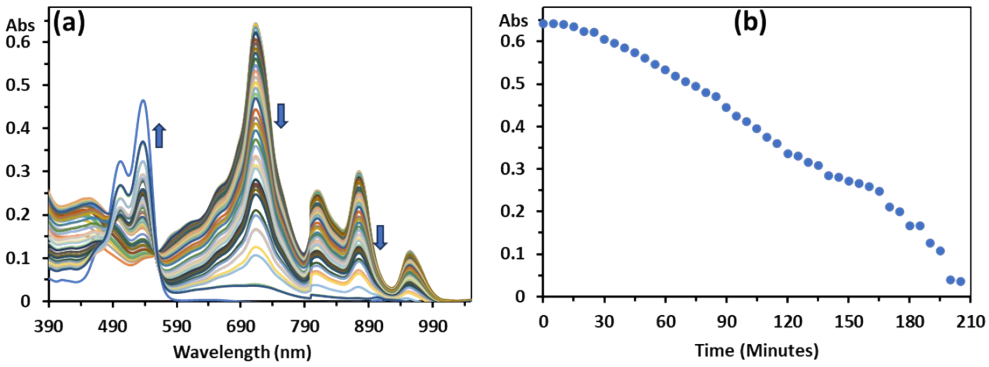


Figure S15. (a) Absorbance spectra and (b) absorbance intensity plot at 714 nm of **BT-PDI**$^{\bullet-}$ (10 μM, generated by addition of 100 equivalents of H_2S) recorded after regular interval of time under ambient air (oxygenated) conditions. All readings have been recorded in HEPES buffer–DMSO (2:8, v/v, pH 7.2) medium.

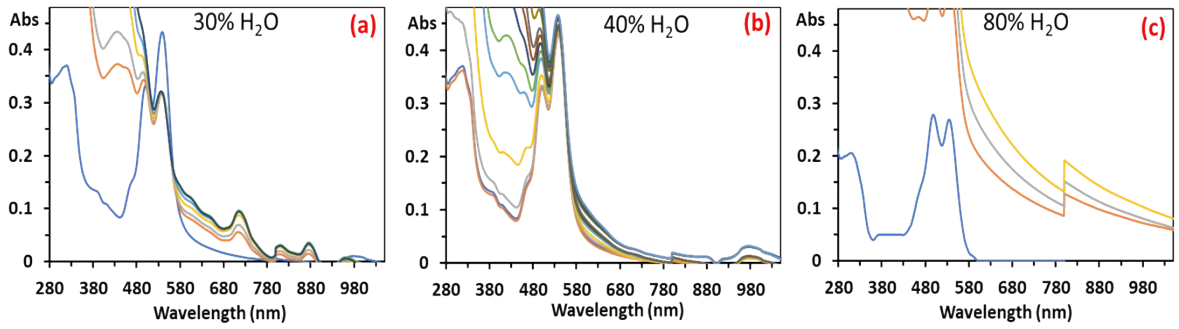


Figure S16. UV–Vis–NIR spectra of BT-PDI (10 μM) upon addition of different concentrations of H_2S (0–2 mM) in (a) 30%; (b) 40% and (c) 80% HEPES buffer–DMSO (2:8, v/v, pH 7.2) medium.

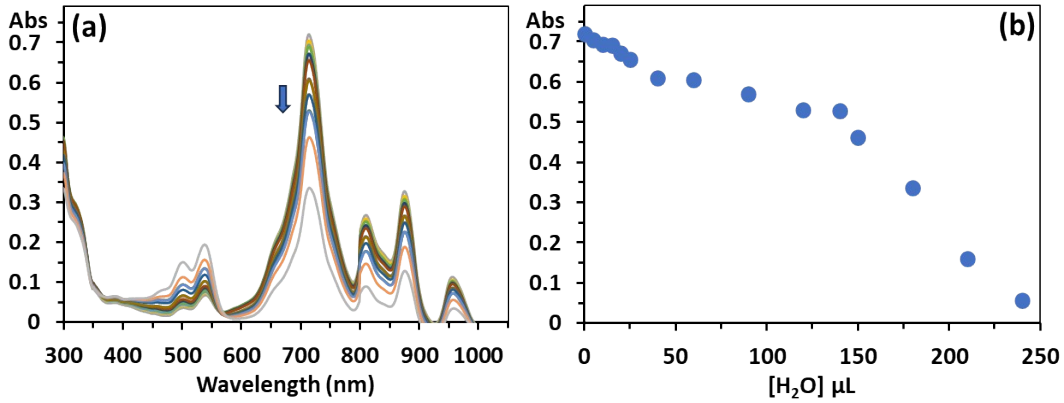
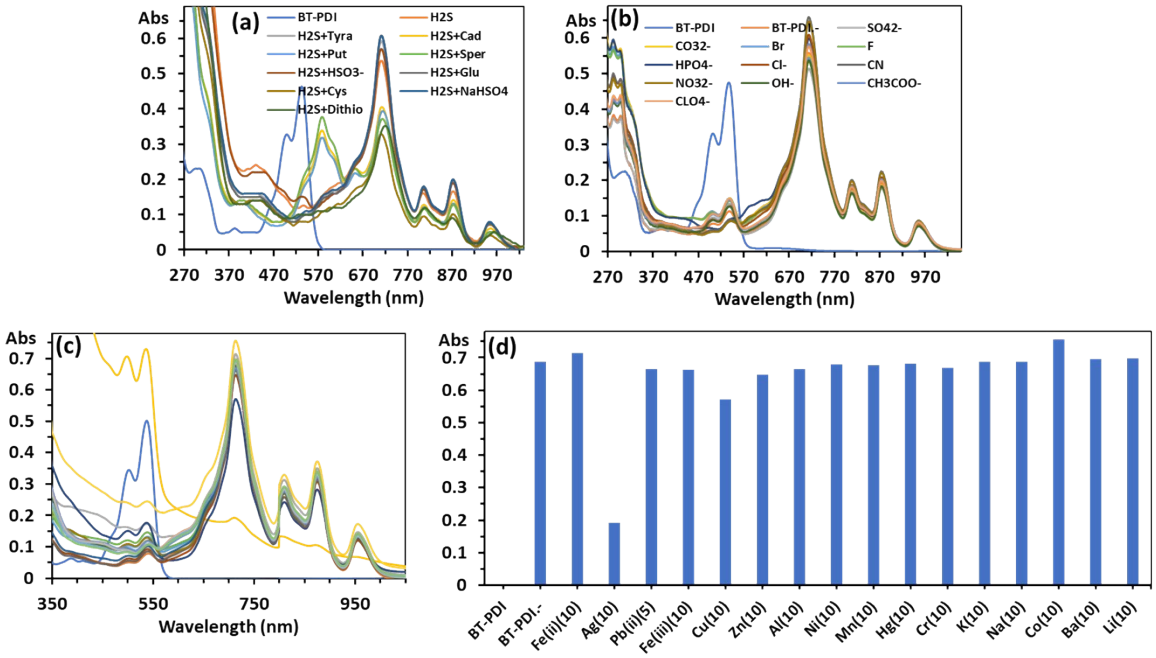


Figure S17. (a) Absorbance spectra and (b) absorbance intensity plot at 714 nm of **BT-PDI**$^{\bullet-}$ (10 μM, generated by addition of 100 equivalents of H_2S) recorded after regular addition of water in HEPES buffer–DMSO (2:8, v/v, pH 7.2) solution.



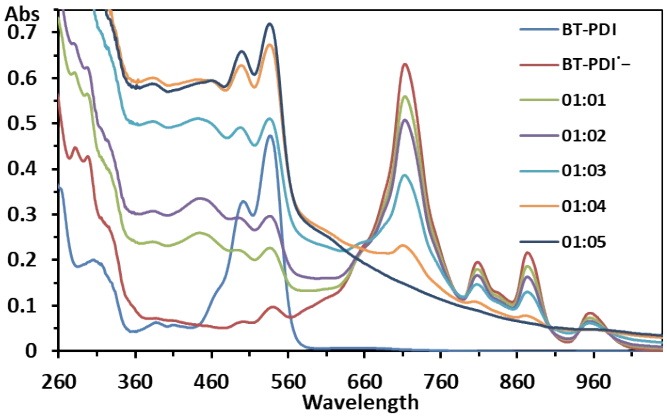


Figure S18. Absorbance spectrum of **BT-PDI**$^{\bullet-}$ upon addition of (top, a-d) amines, amino acids, anions and metal ions (bottom) hydroxyl radical anions recorded in 20% HEPES buffer–DMSO solution. Hydroxyl radical has been generated in situ based on following reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot-}$). The labels in the inset shows stochiometric ratio of H_2O_2 (0.1 µM) and Fe^{2+} (0.1 µM) as 01:01 (1:1); 01:02 (1:2); 01:03 (1:3); 01:04 (1:4); and 01:05 (1:5).

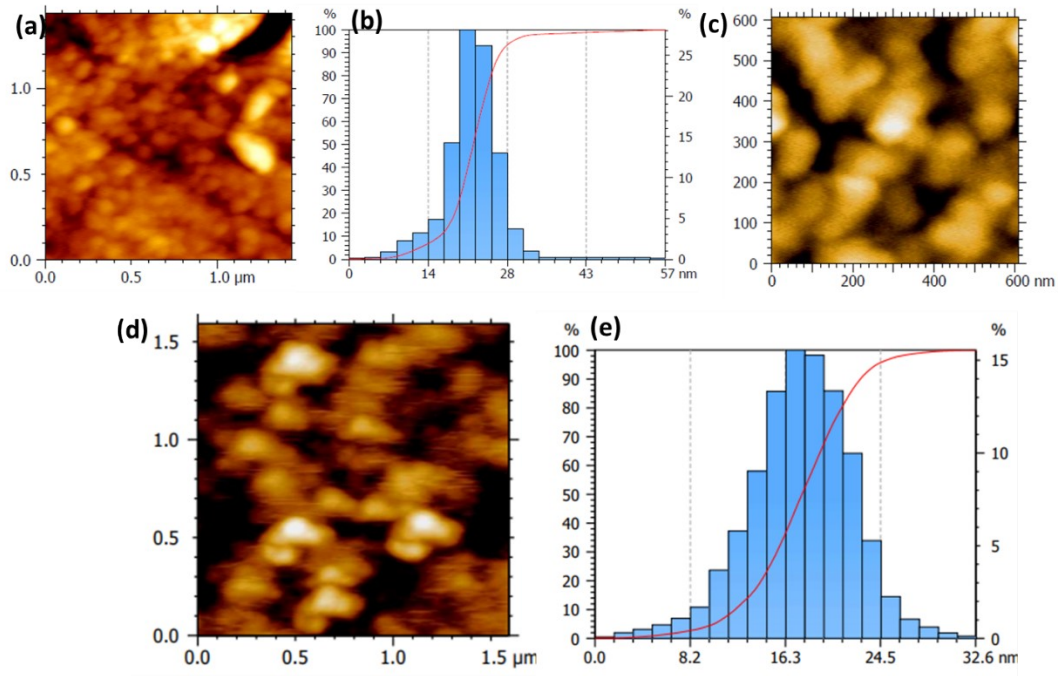


Figure S19. AFM morphology of (a-c), **BT-PDI**, (d,e) **BT-PDI**$^{\bullet-}$ in a HEPES buffer–DMSO (2:8, v/v, pH 7.4) solution.

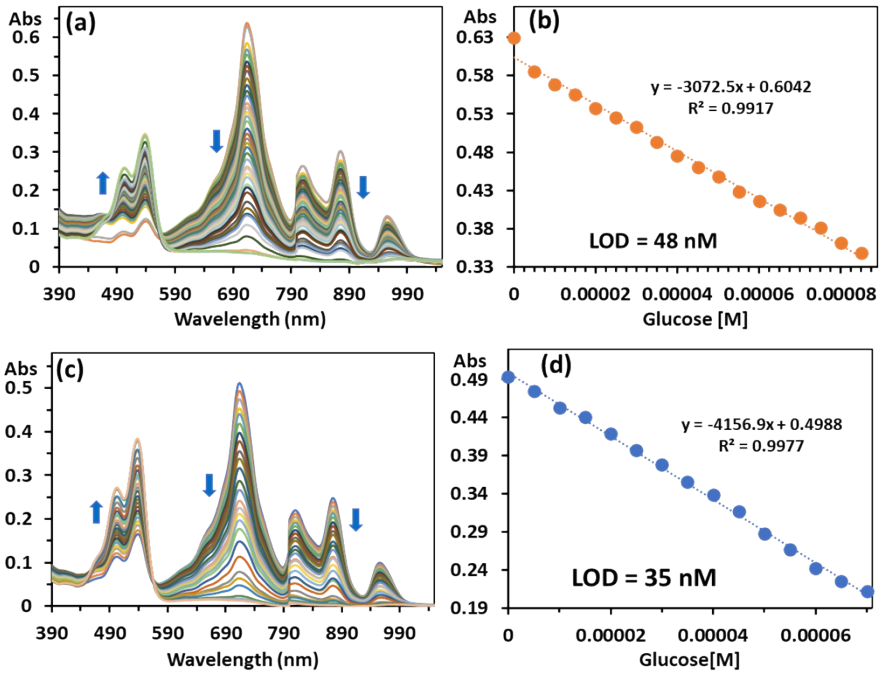


Figure S20. UV-Vis-NIR spectra of **BT-PDI**$^{\bullet-}$ upon gradual addition of glucose in the biochemical assay consisting of **BT-PDI**$^{\bullet-}$ and 3 µL of (a,b) 1 mg/mL GOx (c,d) 2 mg/mL GOx. All spectra were recorded in a 20% HEPES buffer–DMSO (pH 7.4) solution, λ_{ex} = 340 nm, slit width, Ex/Em = 5/5 nm.

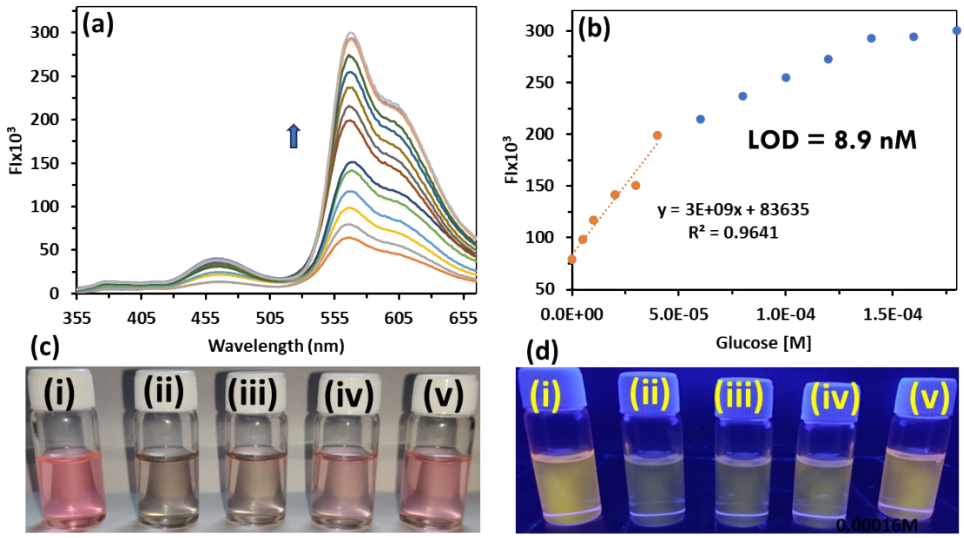


Figure S21. (a) Emission spectra and (b) point graph of **BT-PDI**$^{\bullet-}$ upon gradual addition of glucose in the biochemical assay consisting of **BT-PDI**$^{\bullet-}$ and 6 µL of GOx (5 mg of GOx dissolved in 1 mL of H_2O; Photographs of (i) **BT-PDI** (ii) **BT-PDI**$^{\bullet-}$ (iii) **BT-PDI**$^{\bullet-}$ + GOx (iv) **BT-PDI**$^{\bullet-}$ + GOx + glucose (40 µM) and (v) **BT-PDI**$^{\bullet-}$ + GOx + glucose (1600 µM) in (c) daylight and (d) under 365 nm UV lamp. All spectra were recorded in a 20% HEPES buffer–DMSO (pH 7.4) solution, λ_{ex} = 340 nm, slit width, Ex/Em = 5/5 nm.

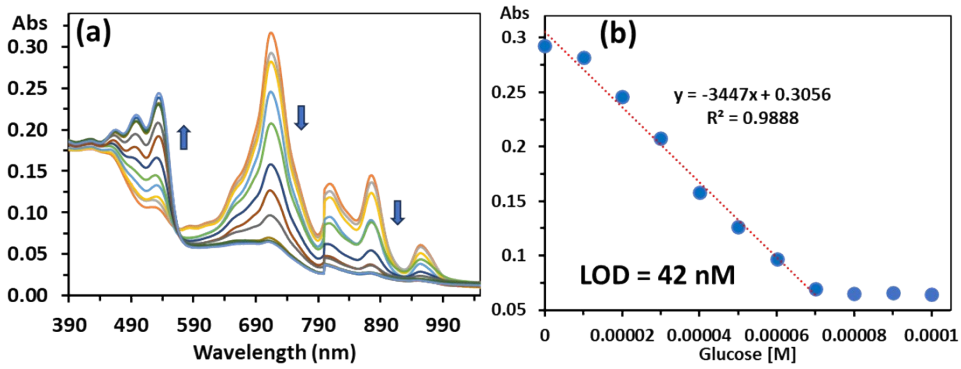


Figure S22. (a) UV-Vis-NIR spectra and (b) point graph of **BT-PDI**$^{\bullet-}$ upon gradual addition of glucose in the biochemical assay consisting of **BT-PDI**$^{\bullet-}$ and 6 µL of 2 mg of GOx dissolved in 1 mL of H_2O. All spectra were recorded in a 20% HEPES buffer–DMSO (pH 7.4) solution containing 10% blood serum, λ_{ex} = 340 nm, slit width, Ex/Em = 5/5 nm.

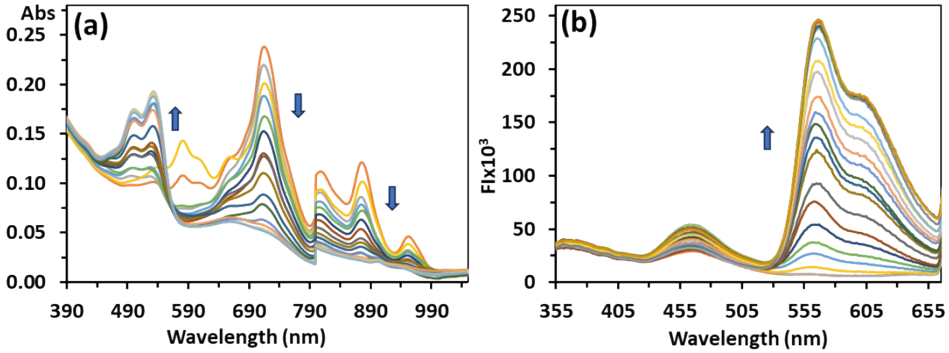


Figure S23. (a) UV-Vis-NIR and (b) emission spectra of **BT-PDI**$^{\bullet-}$ upon gradual addition of glucose in the biochemical assay consisting of **BT-PDI**$^{\bullet-}$ and 6 µL of 5 mg of GOx dissolved in 1 mL of H_2O. All spectra were recorded in a 20% HEPES buffer–DMSO (pH 7.4) solution containing 10% blood serum, λ_{ex} = 340 nm, slit width, Ex/Em = 5/5 nm.

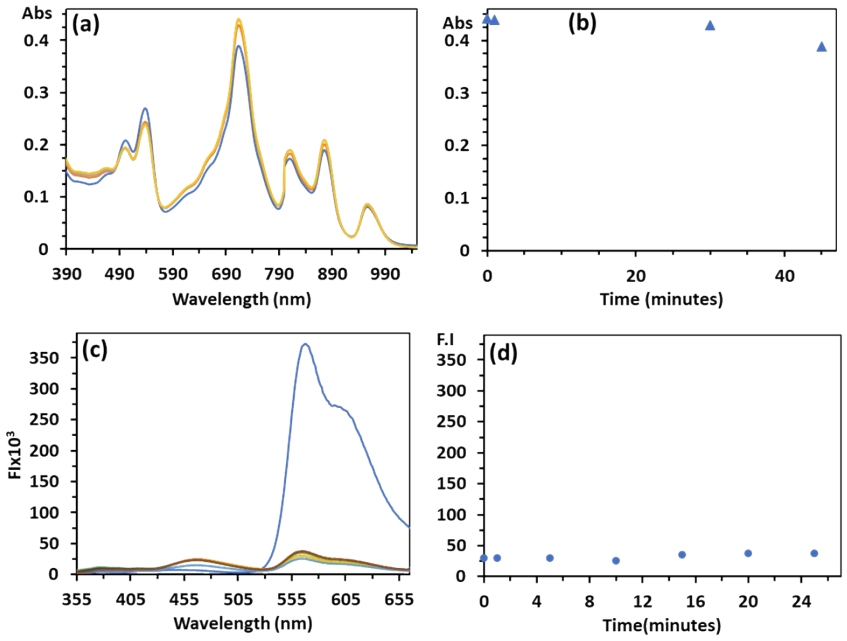


Figure S24. (a) UV-Vis-NIR; (b) fluorescence spectra and (b,d) their corresponding point graphs of **BT-PDI**$^{\bullet-}$ upon addition of glucose (30 equivalents) recorded at regular interval of time. All spectra were recorded in a 20% HEPES buffer–DMSO (pH 7.4) solution, λ_{ex} = 340 nm, slit width, Ex/Em = 5/5 nm.

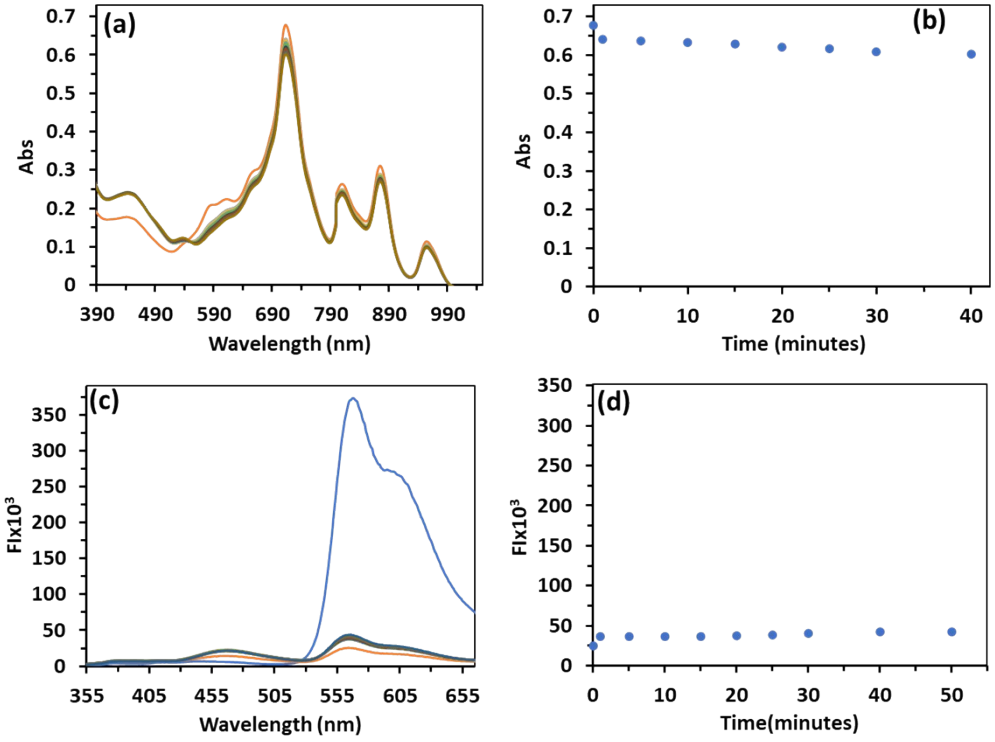


Figure S25. (a) UV-Vis-NIR; (b) fluorescence spectra and (b,d) their corresponding point graphs of **BT-PDI**$^{\bullet-}$ upon addition of 6 µL of 5 mg of GOx dissolved in 1 mL of H_2O recorded at regular interval of time. All spectra were recorded in a 20% HEPES buffer–DMSO (pH 7.4) solution, λ_{ex} = 340 nm, slit width, Ex/Em = 5/5 nm.

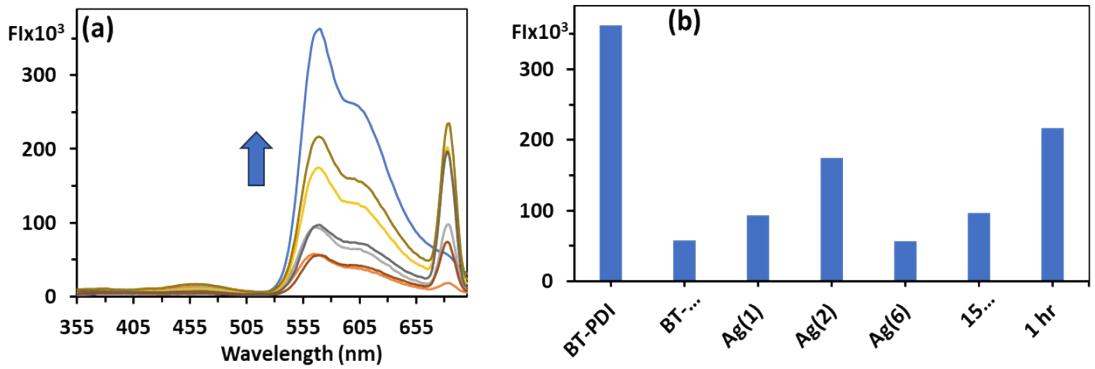


Figure S26. (a) Emission spectra and (b) corresponding bar graph of **BT-PDI**$^{\bullet-}$ upon gradual addition of Ag^{+} ions. All spectra were recorded in a 20% HEPES buffer–DMSO (pH 7.4) solution.

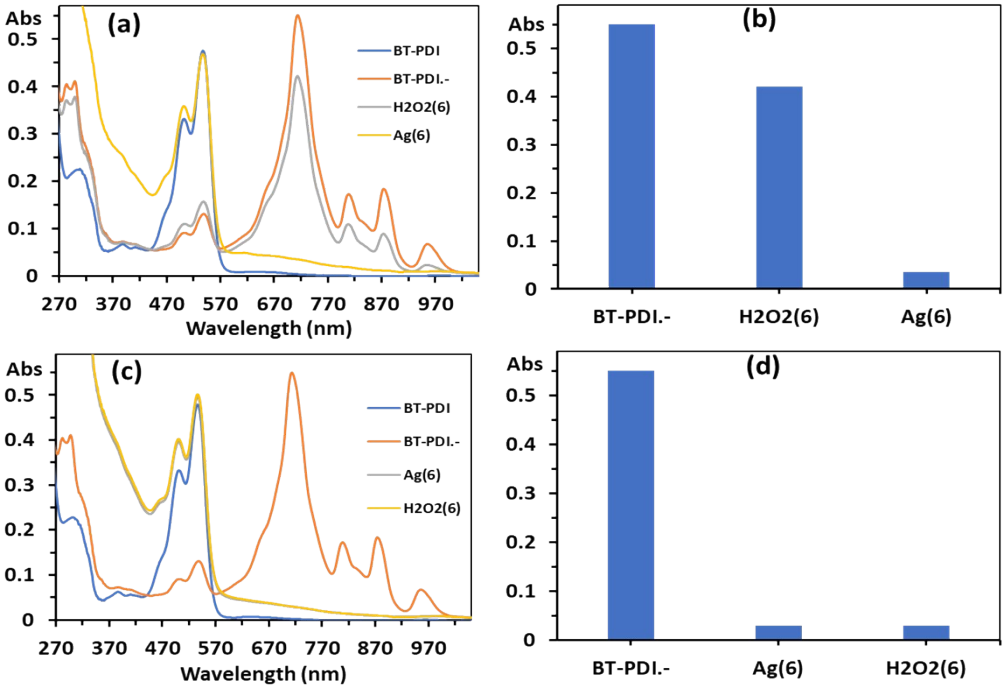


Figure S27. (a,c) UV-Vis-NIR; and (b,d) corresponding bar graph of **BT-PDI**$^{\bullet-}$ upon addition of Ag^{+} and H_2O_2. All spectra were recorded in a 20% HEPES buffer–DMSO (pH 7.4) solution.

Table S1: The comparison of PDI-based material with other materials.

Citation	Radical anion	Solvent mixture	Analytes	LOD(M)	Application
Present manuscript	**YES**	20% HEPES Buffer	H_2O_2/ Glucose/ Ag^{+}	10 nM/ 3.8 nM/ 5.7 nM	Biochemical assay

Dyes and Pigments 165 (2019) 319–326	Yes	NMP	Ag^{+}	0.05 mol/L	NA
Chem. Commun., 2023, 59, 12775	No	60% PBS buffer	H_2O_2	1.37 μM	Live cell
ACS Appl. Mater. Interfaces 2023, 15, 39−47	No	1% PBS	H_2O_2	87 nM	In-vivo, In-vitro imaging
Sensors and Actuators: B. Chemical 406 (2024) 135452	No	1% PBS	H_2O_2 (in presence of Aβ42 fibrils)	38.8 nM	In-vivo, In-vitro imaging
Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 304 (2024) 123394	No	70% PBS buffer	H_2O_2	3.01 μM	In cells
ACS Omega 2021, 6, 14819−14823	No	PBS Buffer	H_2O_2	0−200 μM	NA
Talanta 269 (2024) 125459	No	PBS buffer	H_2O_2	25.2 nM	In-vivo, in-vitro imaging
Talanta 271 (2024) 125669	No	EtOH/PB solution (1:1, v/v)	H_2O_2	62 nM	In-vivo, in-vitro imaging