Perylenediimide based 'On-Off' chemosensor for detection of nucleoside triphosphate: Efficient ensemble for alkaline phosphatase activity

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SUPPORTING INFORMATION

NMR Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded on a BRUKER Biospin AVANCE-III FT-NMR HD-500 spectrophotometer using $CDCl_3$ or DMSO (d_6) as solvent. The peak values were obtained as ppm (δ) and referenced to tetramethylsilane (TMS) for ^{1}H NMR spectroscopy and the residual solvent signal for ^{13}C NMR spectroscopy. Data are reported as follows: chemical shifts in ppm, coupling constant J in Hz; multiplicity (s = singlet, bs = broad singlet, t = triplet, q = quartet, m = multiplet). ^{1}H NMR titration of **BAB-PDI** against GTP was performed in DMSO (d_6) - H_2O (9:1 v/v) on a Bruker-AVANCE-II FT-NMR AL400 spectrophotometer. Addition of higher amounts of water leads to precipitation of the compound. All the data were then processed in delta software to draw the stacking spectra of **BAB-PDI** and **BAB-PDI**+GTP complex at different concentrations.

UV-Vis and Fluorescence Spectroscopy Measurements

The absorption spectra were recorded on SHIMADZU-2450 spectrophotometer equipped with a Peltier system to control the temperature. Quartz cells of 1 cm in length were used for sample measurements. The spectral bandwidth and the scan rate were fixed at 2 nm and 140 nm min^{-1}, respectively. Fluorescence titrations were performed on a Varian Carey Eclipse fluorescence spectrophotometer (slit width: excitation = 10 nm, emission = 2.5 nm) with excitation at 500 nm, unless otherwise stated. Quartz cells of 1 cm in length were used for sample measurements. The concentration of HEPES buffer (pH 7.4) was 0.01 M. Stock solutions for various measurements of **BAB-PDI** was prepared in DMSO and dilutions of these stock solution was used for the photophysical measurements. Stock solutions (0.1 M) of NPPs were prepared in deionized

Millipore water and were diluted as required. The solution of **BAB-PDI** was added in various 10 mL volumetric flask or directly in 3 mL cuvette and subsequently different concentrations of NPPs were added. The solutions were diluted with HEPES buffer-DMSO (9:1 v/v, pH 7.4).

Detection limit

The detection limit was calculated based on the fluorescence titrations. To determine the S/N ratio, the emission intensity of **BAB-PDI** (10 μM) without GTP was measured by 3 times and the standard deviation of blank solution (without addition of GTP) measurements was determined. The detection limit was then calculated with the equation

Detection limit = $3\sigma bi/m$

Where, σbi is the standard deviation of blank solution (without addition of GTP) measurements; m is the slope between intensity versus sample concentration.

Synthesis

2.6.1 Synthesis of compound **3**: In a 100 ml two-neck RBF purged with nitrogen, CuI (0.96 gm, 10 mol%) and benzotriazole (1.21 gm, 20 mol%) was dissolved in DMSO (10 mL) and stirred at RT under N_2 atm. To this solution, 4-bromophenol (8.79 gm, 51.11mmol), benzimidazole (**4**) (5.0 gm, 42.37 mmol) and potassium *tert*-butoxide (5.69 gm, 61.01 mmol) were subsequently added at RT under N_2 atm and then resulting solution was stirred at 110^o C for 24 h. After completion of the reaction the mixture was treated with aqueous solution of EDTA and extracted with ethyl acetate. After evaporating the organic layer, the crude mixture was column chromatographed using gradient of 40% ethyl acetate:hexane to isolate pure compound **3**, as solid, 75% yield.^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 7.03 (d, 2 H, J = 9.3Hz, ArH), 7.28-7.33 (m, 4H, ArH), 7.45-7.48 (m, 1H, ArH), 7.80-7.83 (m, 1H, ArH), 8.07 (s,1H, OH), 9.50 (s, 1H, Bim-C2H); IR (ATR): ν = 3436, 2587, 1615, 1519, 1375, 1231, 924 cm^{-1}

2.6.2 Synthesis of PDI **1**: The mixture of compound **3** (37 mg, 0.18 mmol) and K_2CO_3 (36 mg, 0.26 mmol) was stirred in N-methyl-2-pyrrolidone (NMP) at room temperature. Then compound PDI **2** (50 mg, 0.070 mmol) was added under N_2. The reaction mixture was stirred at 80 ^{o}C for 8 h. After cooling to RT, the reaction mixture was poured into 1 N HCl and the precipitates were filtered, washed with water and then dried under vacuum to give 50 mg of crude product which

was further purified by column chromatography (SiO_2, chloroform/ethyl acetate) to isolate pure PDI **1** as red color solid, yield 33 mg (0.034 mmol, 48.5%); R_f = 0.5 (chloroform/ethyl acetate 96:4). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 1.25-1.48 (m, 6H, cyclohexyl), 1.72-1.76 (m, 6H, cyclohexyl), 1.89-1.91 (m, 4H, cyclohexyl), 2.51-2.54 (m, 4H, cyclohexyl), 4.99-5.03 (m, 2H, cyclohexyl), 7.36-7.38 (m, 8H, ArH), 7.57 (dd, J_1 = 6.0 Hz, J_2 = 3.0 Hz, 2H, ArH) 7.59-7.61 (m, 4H, ArH), 7.91 (dd, J_1 = 6.0 Hz, J_2 = 3.0 Hz, 2H, ArH), 8.14 (s, 2H, perylene-ArH), 8.41 (s, 2H, BIm), 8.66 (d, J = 8.0 Hz, 2H, perylene-ArH), 9.57 (d, J = 8.5 Hz, 2H, perylene-ArH) ppm; ^{13}C NMR (125 MHz, TFA:$CDCl_3$ (1:9), 25 °C): δ 25.3, 26.5, 29.2, 55.8, 113.0, 121.1, 122.9, 124.6, 125.6, 125.8, 126.0, 127.8, 128.5, 128.7, 129.3, 129.4, 129.8, 130.6, 131.7, 132.0, 133.6, 139.3, 154.3, 157.5, 164.5, 165.1 ppm; IR (ATR): ν = 2924, 2852, 1697, 1655, 1593, 1455, 1329, 1507, 741 cm^{-1}.

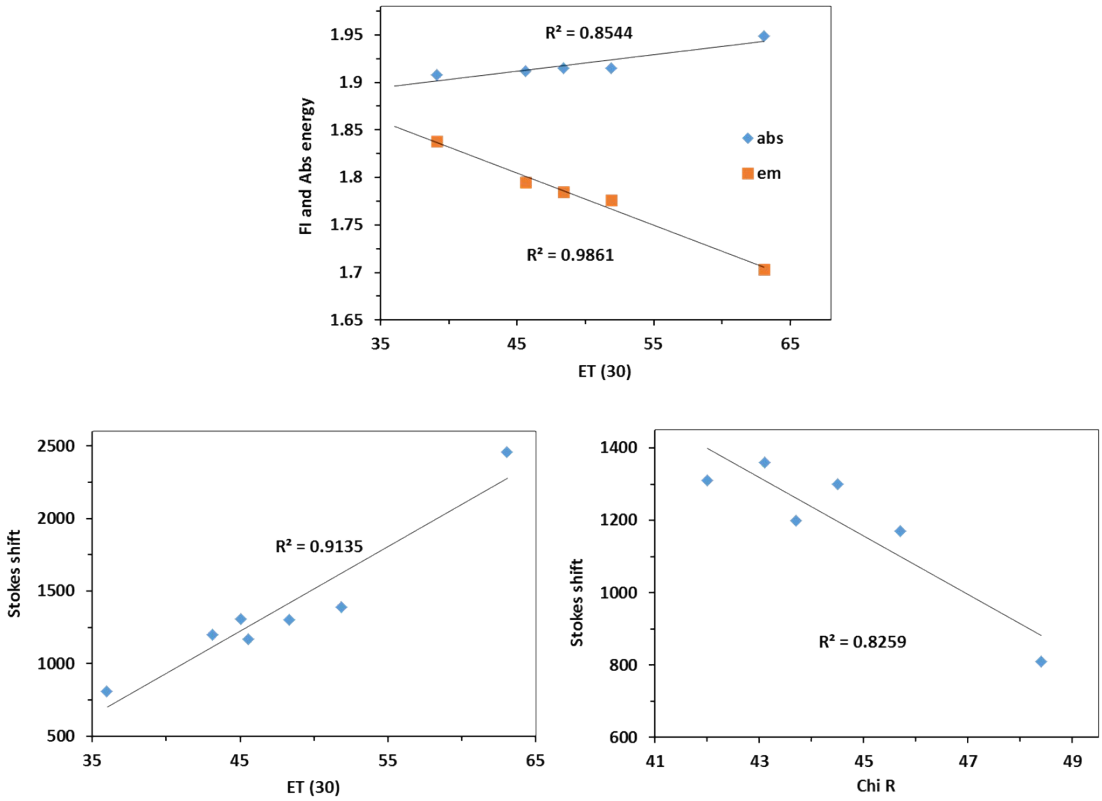

Figure S1: (Top) Plot of fluorescence and absorption energy versus ET (30) scale; (Bottom) plot of stokes shit versus ET (30) and Chi R polarity scale.

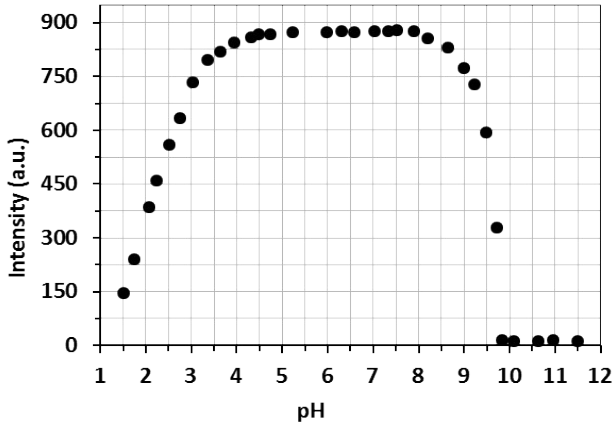

Figure S2: The plot of gradual change in fluorescence intensity of **BAB-PDI** (10 μM) in water (10% DMSO) taken at 577 nm vs. change in pH of the solution.

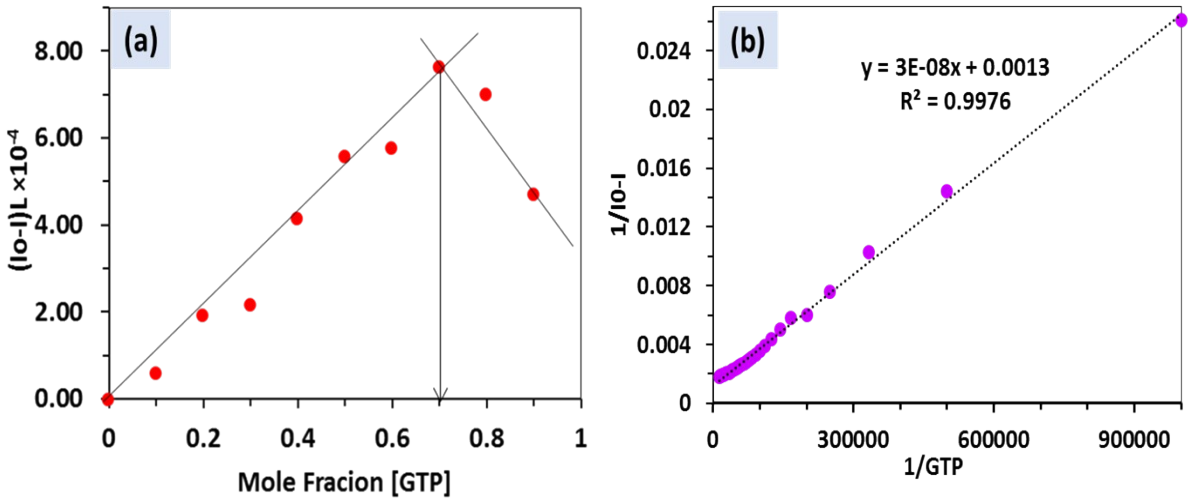

Figure S3: (a) Job's plot (fluorometrically) showing **BAB-PDI**:GTP (1:2) complex recorded in HEPES buffered solution (pH 7.4) containing 10% DMSO (v/v); (b) Benesi-Hildebrand plot of **BAB-PDI** in the presence of increasing concentrations of GTP.

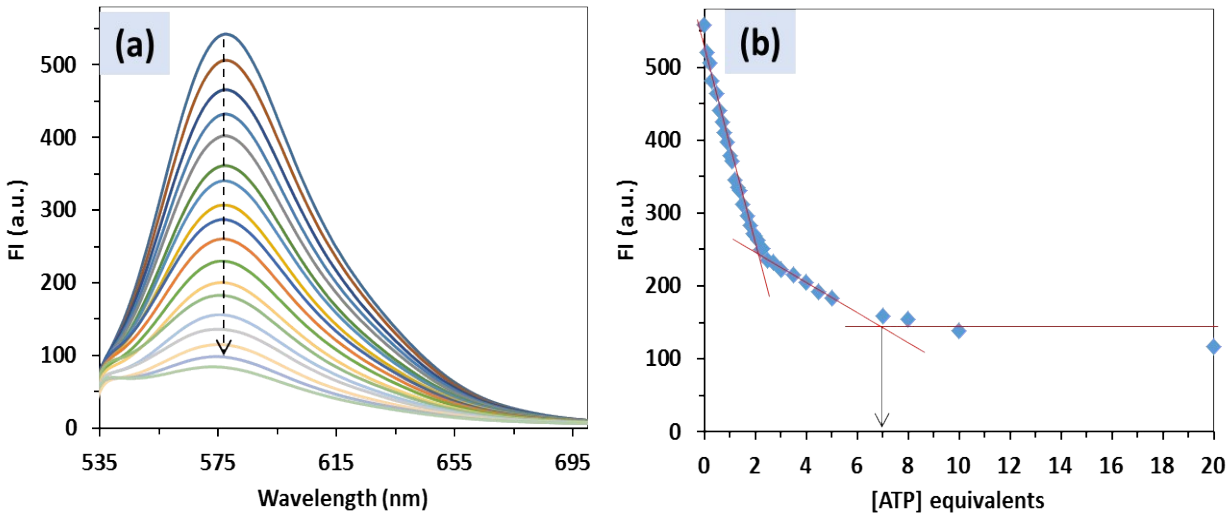

Figure S4: (a) Emission spectrum of **BAB-PDI** (10 μM) on addition of ATP recorded in HEPES buffer–DMSO (9:1 v/v, pH 7.2); (b) Plot showing the variation of emission intensity at 577 nm in response to addition of ATP.

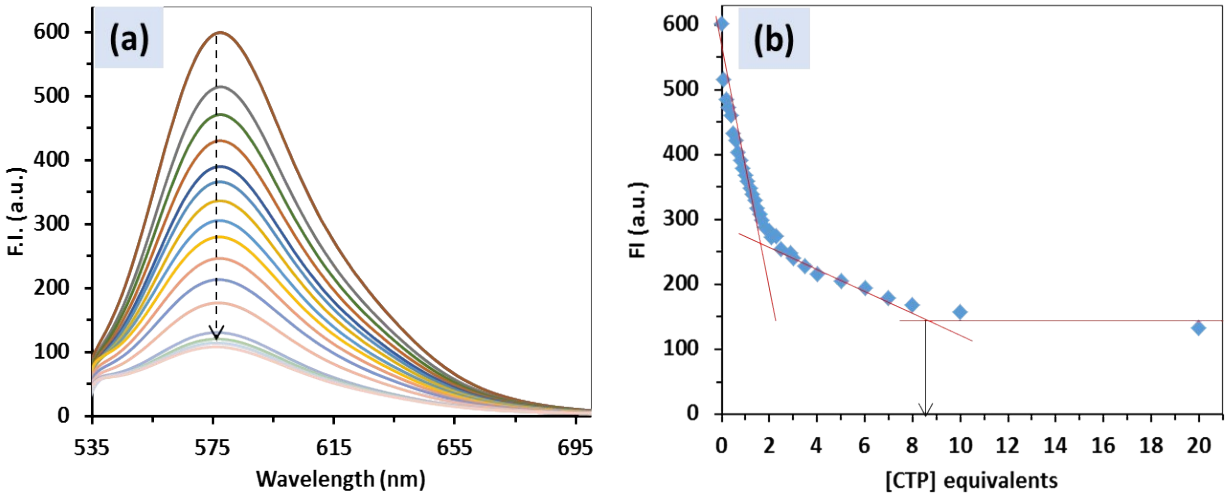

Figure S5: (a) Emission spectrum of **BAB-PDI** (10 μM) on addition of CTP recorded in HEPES buffer–DMSO (9:1 v/v, pH 7.2); (b) Plot showing the variation of emission intensity at 577 nm in response to addition of CTP.

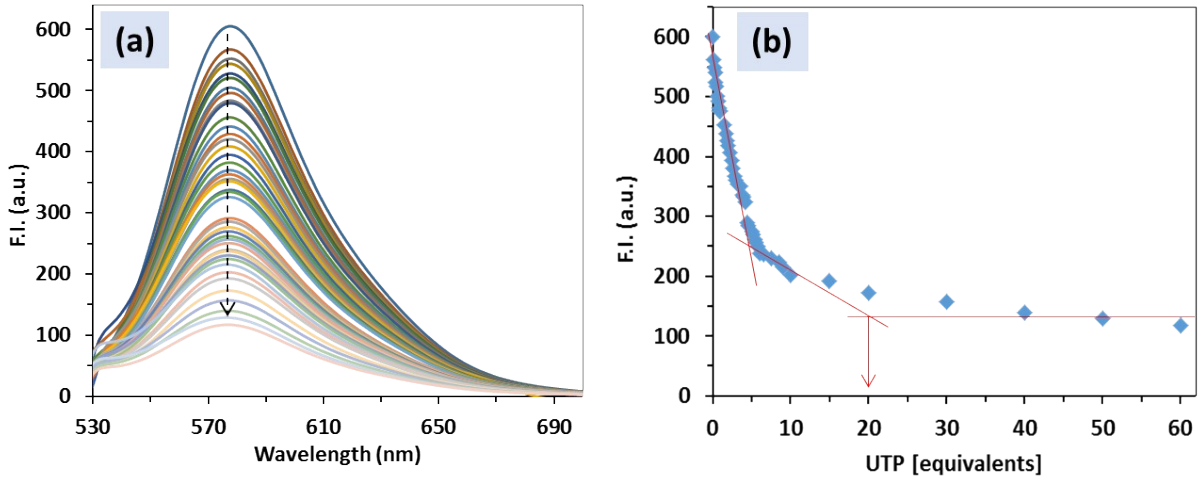

Figure S6: (a) Emission spectrum of **BAB-PDI** (10 μM) on addition of UTP recorded in HEPES buffer–DMSO (9:1 v/v, pH 7.2); (b) Plot showing the variation of emission intensity at 577 nm in response to addition of UTP.

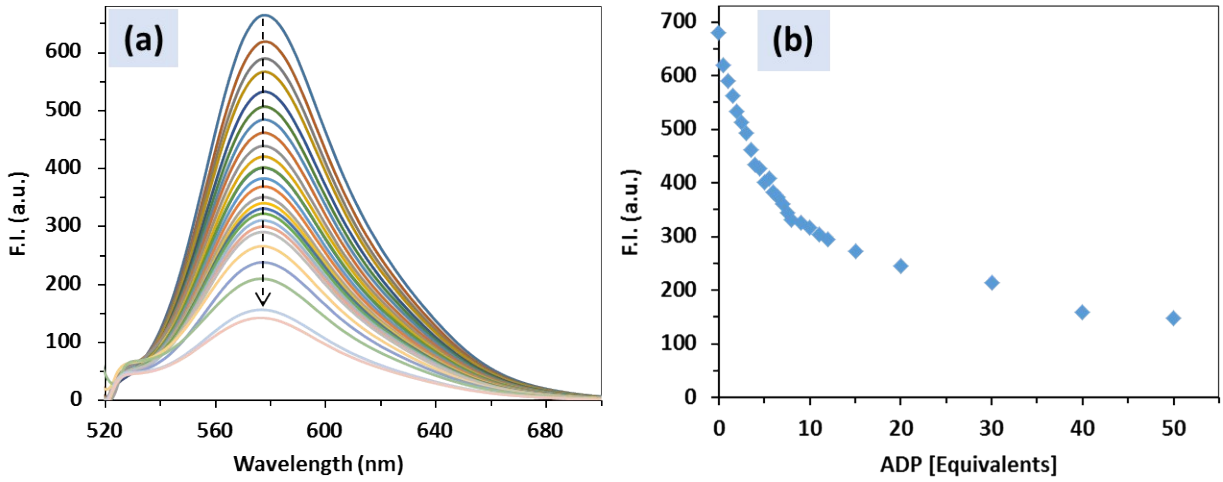

Figure S7: (a) Emission spectrum of **BAB-PDI** (10 μM) on addition of ADP recorded in HEPES buffer–DMSO (9:1 v/v, pH 7.2); (b) Plot showing the variation of emission intensity at 577 nm in response to addition of ADP.

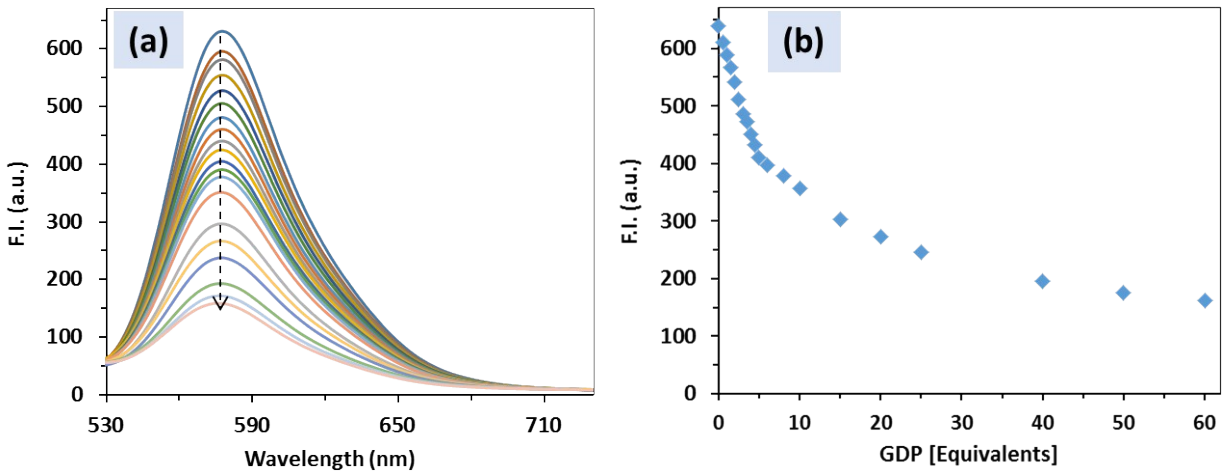

Figure S8: (a) Emission spectrum of **BAB-PDI** (10 μM) on addition of GDP recorded in HEPES buffer–DMSO (9:1 v/v, pH 7.2); (b) Plot showing the variation of emission intensity at 577 nm in response to addition of GDP.

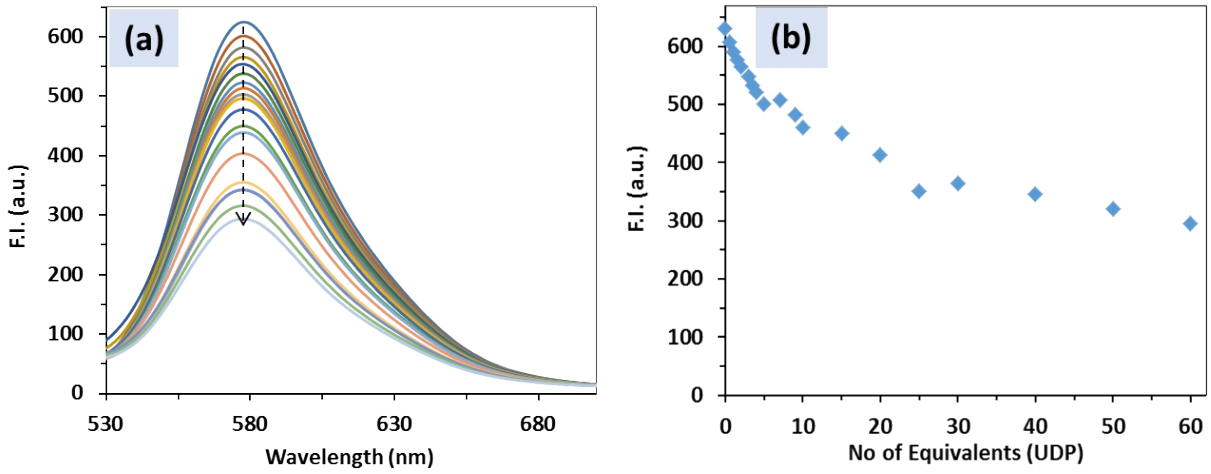

Figure S9: (a) Emission spectrum of **BAB-PDI** (10 μM) on addition of UDP recorded in HEPES buffer–DMSO (9:1 v/v, pH 7.2); (b) Plot showing the variation of emission intensity at 577 nm in response to addition of UDP.

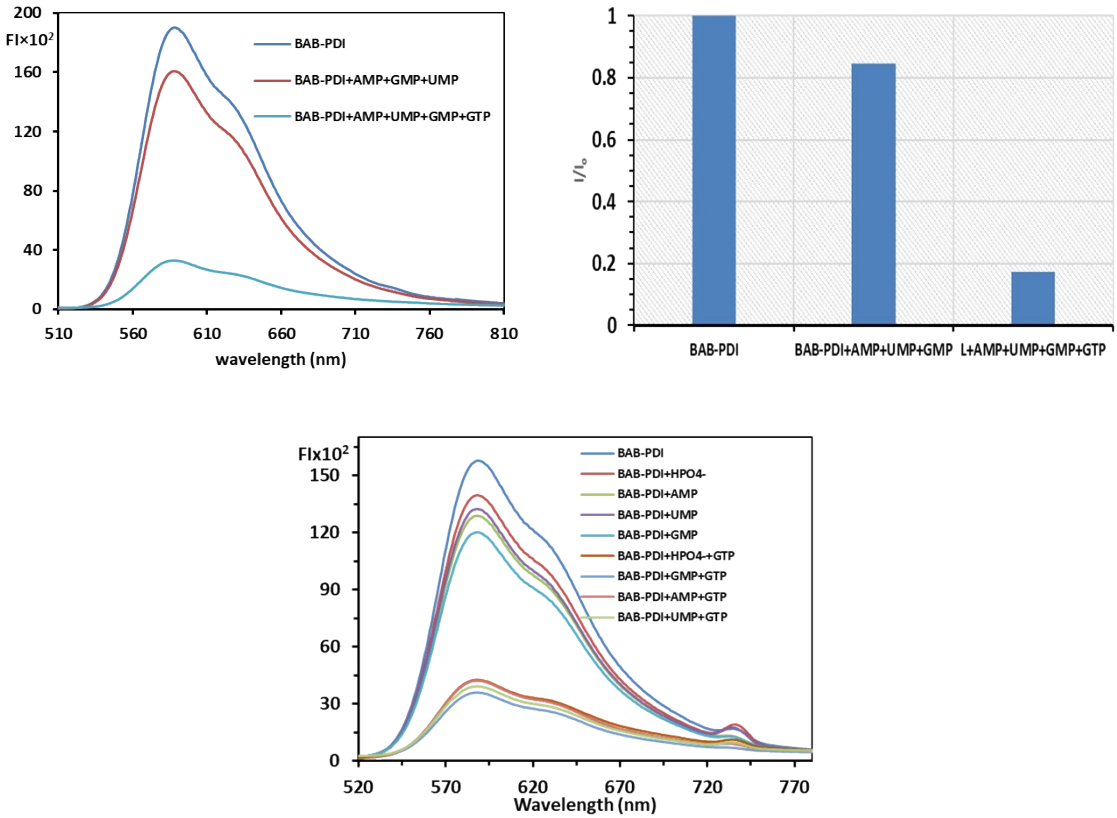

Figure S10: (upper panel) Emission spectrum of **BAB-PDI** (10 μM) on addition of GTP in the pool of monophosphates (50 μM each); (lower panel) emission spectrum of **BAB-PDI** (10 μM) on addition of GTP in the individual monophosphates (100 μM) recorded in HEPES buffer–DMSO (9:1 v/v, pH 7.2).

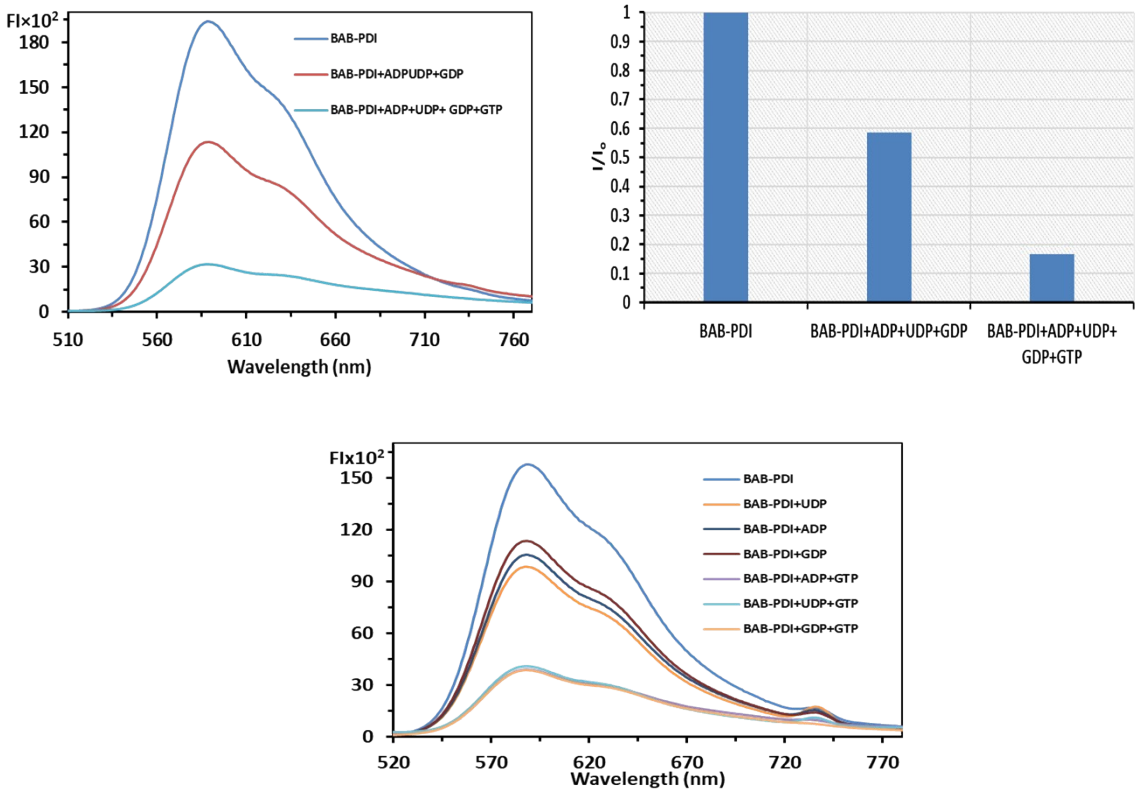

Figure S11: (upper panel) Emission spectrum of **BAB-PDI** (10 μM) on addition of GTP in the pool of diphosphates (25 μM each); (lower panel) emission spectrum of **BAB-PDI** (10 μM) on addition of GTP in the individual diphosphates (50 μM) recorded in HEPES buffer–DMSO (9:1 v/v, pH 7.2).

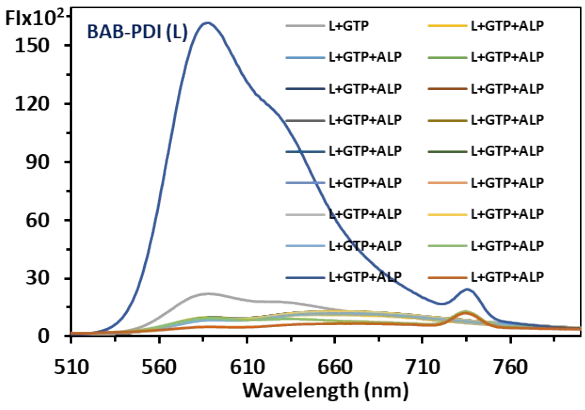

Figure S12: Fluorescence spectra of **BAB-PDI**+GTP complex (ensemble) (10 μM) with ALP (inactive) in HEPES buffer–DMSO (9:1 v/v, pH 7.4) recorded at regular interval of time.

Table S1: Comparison of BAB-PDI Sensor and ALP activity assay in Literature:

S.No.	Paper details	Solvent medium	λ_{abs}	λ_{em}	NPP sensing and LOD	Mechanism	ALP activity assay sensitivity	Bio imaging
	Present Manuscript	90% HEPES Buffer	510 nm	577nm	19.3 nM (GTP)	Fluorescence method	YES	No
1.	Talanta, 2019, 195, 566–572	Alkaline Phosphatase Buffer (500 mM Tris-HCl, pH 9.0, 10 mM $MgCl_2$)	494 nm	530 nm	ssDNA with poly-T tail could form stable DNA duplex with the addition of Hg^{2+}	Fluorescence method	0.025 mU mL^{-1}	No
2.	Talanta, 2019, 197, 451–456	Tris-HCl buffer(pH=7.3)	380 nm	428 nm and 515 nm	2.0×10^{-8} mol L^{-1} (ATP)		No	No
3.	Analytica Chimica Acta, 2019, 1057, 51-59	aqueous buffer solution (MES or MOPS in appropriate pH intervals)	289 nm	340 nm	0.12 µM for ATP	Spectrophotometric, fluorometric and electrochemical method	No	No
4.	Sensors & Actuators B. Chemical, 2019, 289, 85–92	10 mM Tris−HCl buffer (850µL, pH = 8.0)	540 nm	-	Ca^{2+} and PPi	Colorimetric assay	5.4 U/L ALP Detection	No
5.	Microchimica Acta, 2019, 186 240	PBS (10 mM, pH 7.4)	-	-	13 fM	Electro-chemical apta sensing	No	No
6.	Sensors & Actuators B. Chemical 2018, 275, 43–49	10 mM pH 8.0 Tris−HCl buffer (containing 5 mM $MgCl_2$ and 0.1 mM ZnCl2)	652 nm	-	GTP Hydrolysis	Colorimetric method	0.009 U/L	No
7.	Analytica Chimica Acta,	HEPES buffer (10 mM, pH	420		pyrophosphate	Colorimetric	0.19 U/L	No

	2018, 1004, 74-81	7.4)	nm		(PPi)	method		
8.	ACS Appl. Mater. Interfaces, 2017, 9, 722−729	HEPES buffer (10 mM NaCl, 1 mM MgCl2 , pH 7.4)	325 nm	545 nm	ATP Sensing but LOD not mentioned	Time-Resolved Luminescence	No	No
9.	Talanta, 2017, 169, 64–69	Tris-HCl, pH 7.0	325 nm	524 nm	3′-phosphorylated (DNA-P) Binding	Fluorescence method	0.1 mU/mL ALP Detection	No
10.	Biosensors and Bioelectronics, 2016, 77, 242–248	Tris–HCl (pH 7.33) containing 0.5 mM MgCl2	490 nm	510 nm	-	fluorescence probe	ALP activity assay	No
11.	Anal. Chem. 2015, 87, 2966−2973	Tris-HCl, pH = 7.4	446 nm	518 nm	3.0 mL CQDs and adenosine triphosphate (ATP 50.0μL)	Fluorescence method	1.4 U/L	No
12.	New J. Chem., 2014, 38, 4574-4579	Tris-HCl buffer, pH = 7.0	400 nm	528 nm	0.03μmol L^{-1} for ATP	Fluorescence assay	0.01 U mL^{-1}	No
13.	Chem. Commun., 2014, 50, 4438-4441	HEPES buffer (10 mM, pH = 7.2)	613 nm	675 nm	5.4 ppb (1.04 $\times 10^{-8}$ M) for GTP	colorimetric and fluorescent sensor	(0–200 mU mL^{-1})	Yes
14.	Bioorganic & Medicinal Chemistry Letters 2011, 21, 5088–5091	10 mM sodium acetate (pH 7.0)	488 nm	520 nm	ATP	Fluorescence method	0.02 units/L for ALP	No