Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

Differential detection and quantification of cyclic AMP and other adenosine phosphates in live cell

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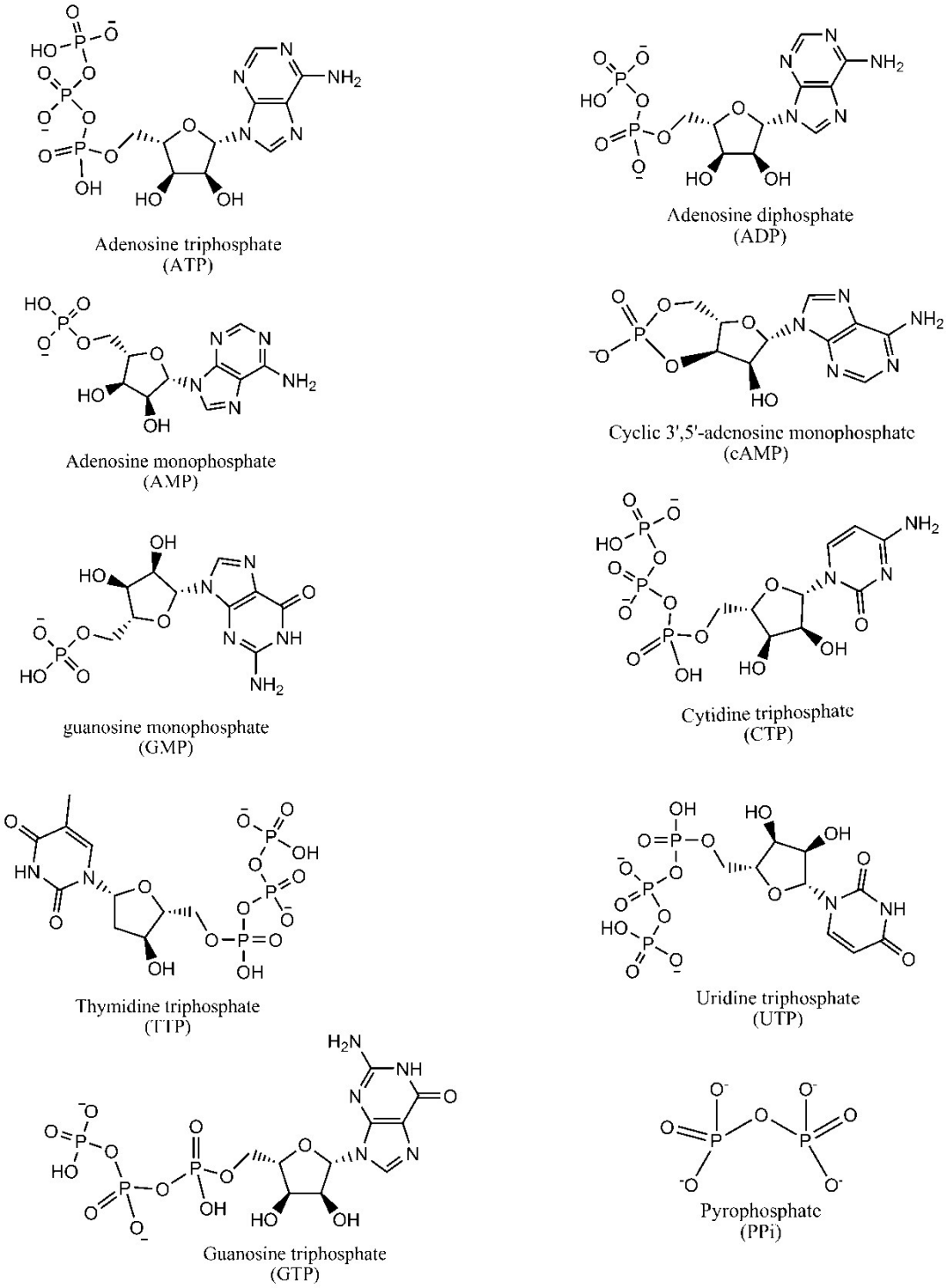


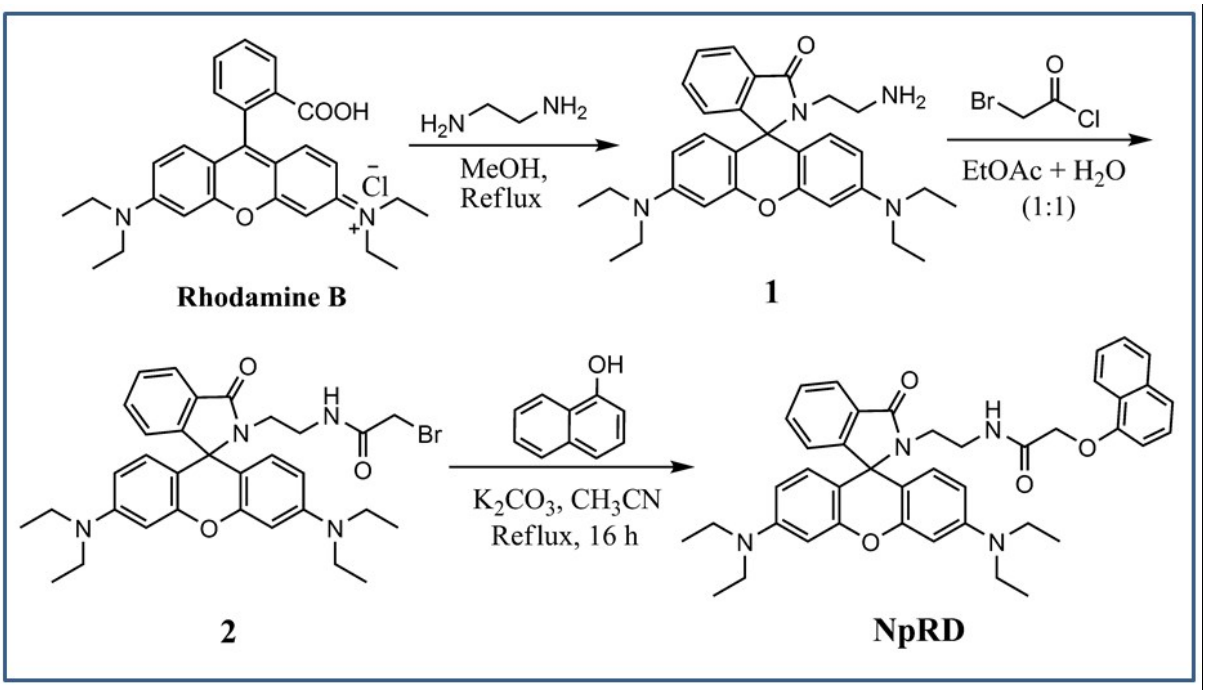
Figure S1. Structures of the biological phosphates.

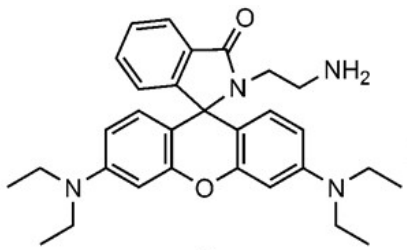
1. Experimental Section:

Materials and Methods:

RhodamineB, ethylenediamine, bromoacetyl chloride,1-naphthol, cyclic AMP, ATP and other adenosine phosphate (sodium salts)were purchased from Sigma-Aldrich Pvt.Ltd. (India). Unless otherwise mentioned, materials were obtained from commercial suppliers and were used without further purification. Solvents were dried according to standard procedures. Elix Millipore water was used in all respective experiments. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument. For NMR spectra and for NMR titrationDMSO-d_6, D_2Owere used as solvent using TMS as an internal standard. Chemical shifts are expressed in δ ppm units and ^{1}H–^{1}H and ^{1}H–C coupling constants in Hz. The mass spectrum (HRMS) was carried out using a micromass Q-TOF Micro™ instrument by using acetonitrile as a solvent. Fluorescence spectra were recorded on a Perkin Elmer Model LS 55 spectrophotometer. UV spectra were recorded on a SHIMADZU UV-3101PC spectrophotometer. FTIR spectra was recorded as KBr pellets using a SHIMADZU FTIR-8400S spectrophotometer. Elemental analysis of the compounds was carried out on Perkin-Elmer 2400 series CHNS/O Analyzer. The luminescence quantum yield in solution was measured by using Rhodamine B (Φ_F= 0.69 in ethanol) as a reference. The following abbreviations are used to describe spin multiplicities in ^{1}H NMR spectra: s = singlet; d = doublet; t = triplet; m = multiplet.

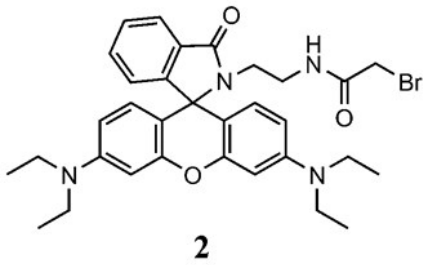
Synthetic procedures:





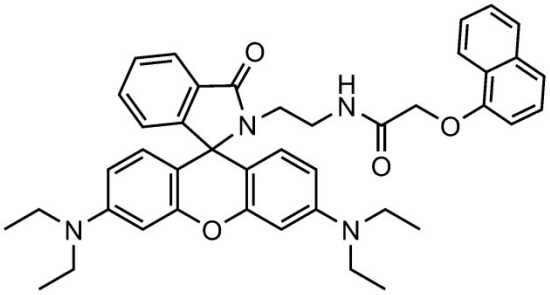
1

RhodamineB (2.40 g, 5 mmol) was dissolved in EtOH (20 mL).Ethylenediamine (5 mL,excess) was added drop-wise to the solution and refluxed overnight (15 h) until the solution loses its red color. The solvent was removed by evaporation. Water (20 mL) was added to the resultant and extracted with CH_2Cl_2(20mL × 2). The combined organic phase was washed twice with water and dried over Na_2SO_4. The solvent was removed by evaporation and dried in vacuo, affording apale-pink solid of **1**(1.9 g, yield, 79%).^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) =7.86-7.81 (m, 1H), 7.48-7.40 (m, 2H), 7.09-7.03 (m, 1H), 6.43-6.25 (m, 6H), 3.33 (q, *J*= 6.7 Hz, 8H), 3.19 (t, *J* = 6.7 Hz, 2H), 2.88 (t, *J* = 4.1 Hz, 2H), 1.16 (t, *J* = 6.7 Hz,12H). ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) = 170.07, 153.56, 153.14, 148.95, 132.96,129.89, 128.19, 123.82, 122.98, 108.53, 103.81, 97.78, 66.50, 44.42, 41.20, 40.19,12.72. HRMS: anal. calcd for $C_{30}H_{36}N_4O_2$: 484.28; found: 485.3 (M + H^+, 100%).



2

Compound **1**(1.5 g, 3 mmol) mixed with K_2CO_3(4.27 g,10mmol) is suspended into a mixture of ethyl acetate (25 mL) and water(25 mL) and stirred for 30minutes. Then, bromoacetyl chloride (1.22g, 2.5mmol) in ethyl acetate(5 mL) is added dropwise into the solution. After 4 h stirring at room temperature, the organic layer is isolated and dried by $MgSO_4$. The ethyl acetate solvent is removed by rotary evaporation to give the crude product that is purified by column chromatography (silica, 220–400 mesh, EtOAc/MeOH = 10:1 v/v). The product is isolated as a pale-pinkpowder**2** (1.26 g, 84%). 1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.90-7.94 (m, 1H), 7.45-7.48 (m, 3H), 7.05-7.09 (m, 1H), 6.37-6.47 (m, 5H), 3.71 (s,2H), 3.33 (q, *J*= 6.7 Hz, 10H), 3.02 (t, *J* = 6.7 Hz, 2H), 1.18 (t, *J* = 6.7 Hz,12H); 13C (400 MHz, $CDCl_3$) d: 172.07, 157.63, 156.02, 155.56, 151.19, 136.63, 135.04, 131.60, 130.30, 130.24, 129.51, 128.82, 128.44, 126.00, 125.85, 124.51, 120.59, 110.39, 109.06, 106.63, 99.83, 68.70, 49.21, 46.15, 40.96, 40.78, 13.69. ESI/MS: m/z calcd.for $[M+H]^+$605.57, found 606.60 ($M+H^+$, 100%). Anal.calcd.for $C_{32}H_{37}BrN_4O_3$: C, 63.47; H, 6.16; N, 9.25; O, 7.93; Br, 13.19. Found: C, 63.41; H, 6.15; N, 9.32; O, 7.92; Br, 13.20.



NpRD

To a solution of anhydrous K_2CO_3(1.1g, 8mmol) in dry acetone was added 1-naphthol (0.24 g, 1.6mmol). The mixture was stirred for 0.5 h followed by addition of compound **2**(1 g, 1.6mmol) was added to the solution and subsequently refluxed for 24 h. Then, the reaction mixture was poured into water. The solution was extracted with CH_2Cl_2(3×50 mL), and the combined organic layer was washed with 5% aqueous HCl (50 mL), 10% aqueous Na_2CO_3(50 mL) and finally with water and then was dried over anhydrous $MgSO_4$. After removing the solvents, the residue was chromatographed on silica gel (220–400 mesh) with chloroform/ethyl acetate=1:3 v/v as eluent to give 0.88 g (80%) of **NpRD** as an yellow solid.^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.37(d, J = 2 Hz,1H), 7.91(s, 1H),7.84(d,J = 8 Hz, 1H), 7.75-7.76 (d,J = 4 Hz, 1H), 7.45-7.53 (m,J = 32 Hz, 5H), 7.35-7.37(m, J = 8 Hz, 1H), 6.97(s, 1H), 6.81-6.82 (d, J = 4 Hz, 1H), 6.24-6.35 (m, 6H), 4.50 (s, 2H), 3.23-3.29 (m,J = 24 Hz, 8H),3.16-3.17 (t, J = 4 Hz,2H), 1.03-1.06(t, J = 12 Hz,12H). ^{13}C-NMR (DMSO-d_6 400 MHz):δ(ppm)167.85, 167.31, 153.81, 153.06, 152.52, 148.34, 134.02, 129.87, 128.19, 127.32, 126.52, 125.98, 125.30, 124.74, 123.55, 122.35, 122.15, 120.62, 108.06, 105.51, 104.68, 97.28, 67.09, 64.19, 43.61, 12.38.FTIR (cm^{-1}):2968.24 (N-H str.), 2928.78 (aliphatic C-H str.), 1678.92 (C=O str.), 1514.31 (N-H bending).HRMS (TOF MS):anal. calcd for $C_{42}H_{44}N_4O_4$: 668.34; found: 669.61 [M + H^+, 100%]; Anal.Calcd.For$C_{41}H_{42}N_4O_6$: C, 75.42; H, 6.63; N, 8.38; O, 9.57; Found: C, 75.42; H, 6.64; N, 8.31; O, 9.56.

2. NMR Studies:

^{1}H NMR ofNpRD in DMSO-d_6:

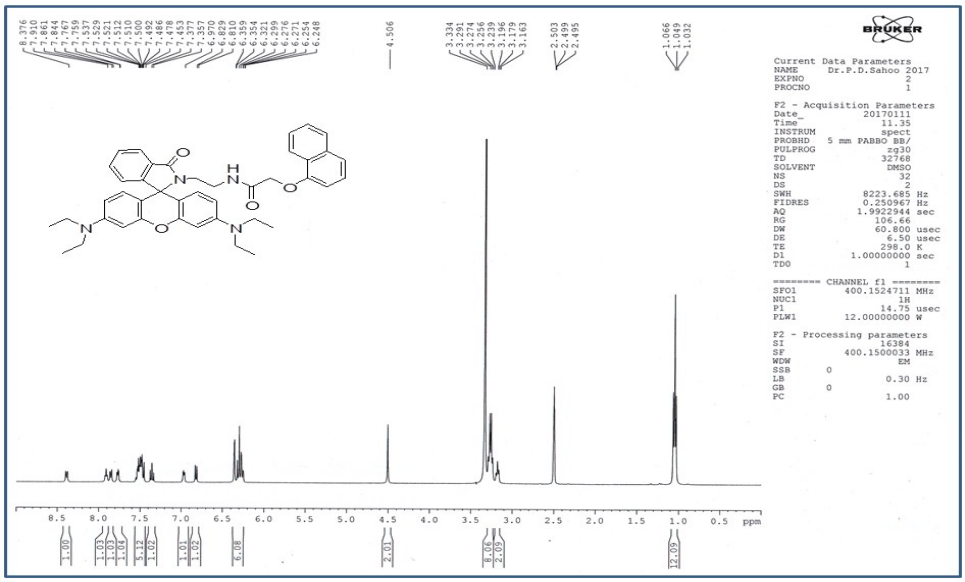


Figure S2.^{1}H NMR of **NpRD** in DMSO-d_6(400 MHz).

^{13}C NMR of NpRD in DMSO-d_6:

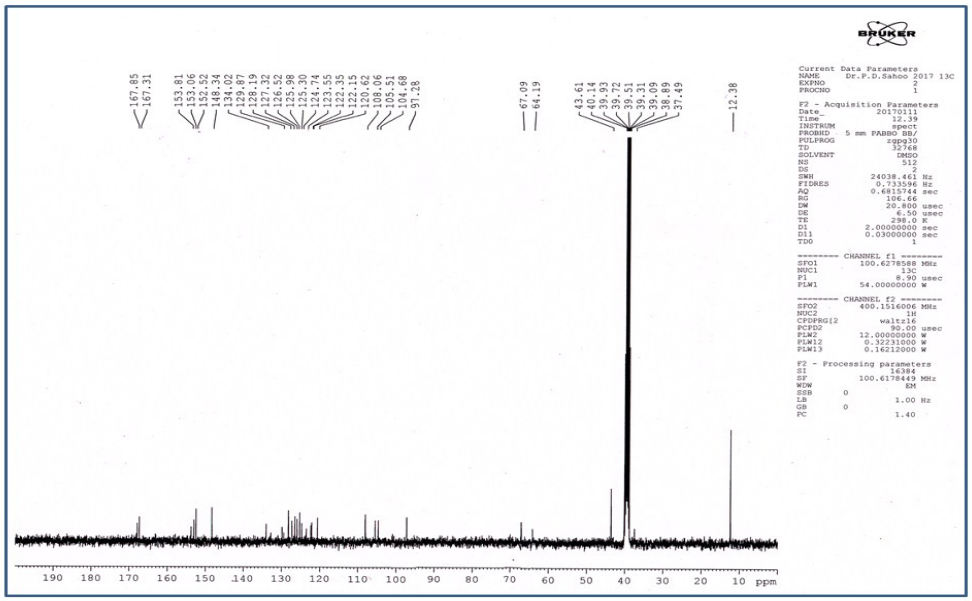


Figure S3.^{13}C NMR of **NpRD** in DMSO-d_6(400 MHz).

3. FTIR spectrum of NpRD:

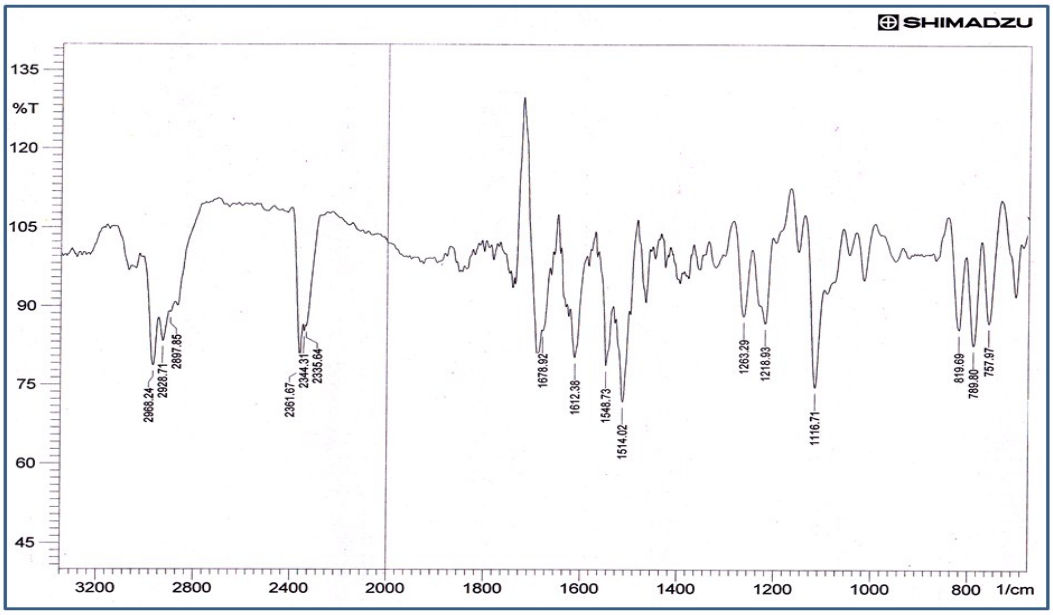


Figure S4.IR-Spectrum of **NpRD**.

4. Mass spectrum of NpRD :

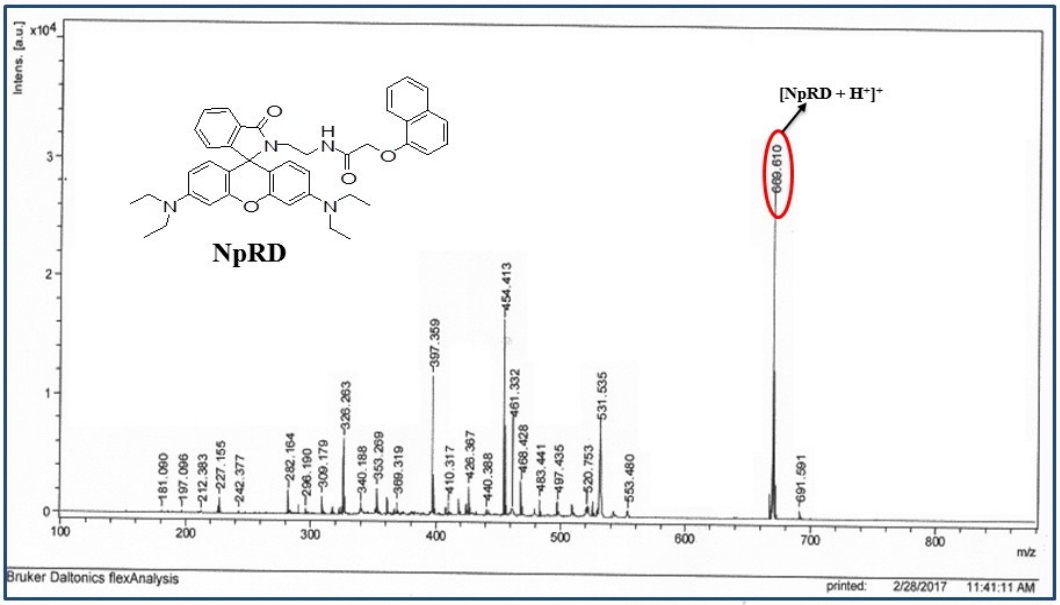


Figure S5.MALDI-TOF MS of**NpRD**

5. Crystal data for NpRD

Identification code	**NpRD**	
CCDC deposition No.	**1530419**	
Chemical formula	$C_{42}H_{44}N_4O_4$	
Formula weight	668.81 g/mol	
Temperature	296(2) K	
Wavelength	0.71073 Å	
Crystal system	orthorhombic	
Space group	P 21 21 2	
Unit cell dimensions	a = 16.3458(13) Å	α = 90°
	b = 16.7974(13) Å	β = 90°
	c = 13.2752(10) Å	γ = 90°
Volume	3644.9(5) $Å^3$	
Z	4	
Density (calculated)	1.219 g/cm^3	
Absorption coefficient	0.079 mm^{-1}	
F(000)	1424	

h,k,l_{max}+/-19,+/-20,+/-16

No. of reflections [> 2σ]6836 [5536]

Data completeness 99 %

Theta range for data collection 2.32 to 25.68°

Absorption correction Multi-scan

Refinement method Full-matrix least-squares on F^2

Refinement program SHELXL-2014/7 (Sheldrick, 2014)

R(used reflections) 0.0519 (5336)

wR2(all reflections) 0.1550 (6839)

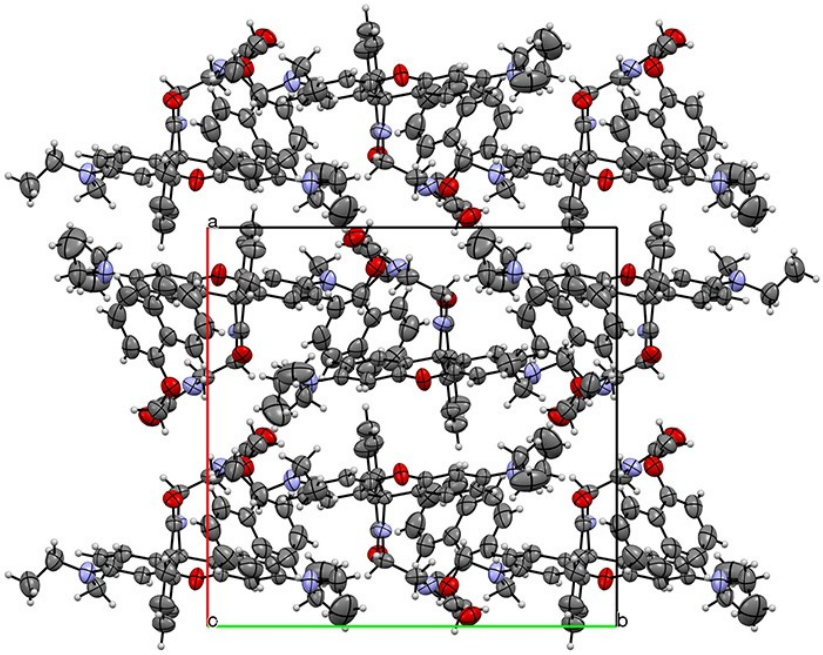


Figure S6. Crystal packing structure of **NpRD** along the *c*-axis.

7. UV-Vis and fluorescence titration studies:

UV-vis spectral studies:

A stock solution of **NpRD** (2×10^{-5} M) was prepared in acetonitrile-water (1:6, v/v). **cAMP** solution (from Cyclic 3',5'-adenosine monophosphate sodium salt monohydrate) of concentration 1×10^{-4} M was prepared in Millipore water. All experiments were carried out in aqueous medium (pH 7.0, 10 mM phosphate buffer). Duringtitration, each time a 2×10^{-5} M solution of **NpRD** was filled in a quartz optical cell of 1 cm optical path length and **cAMP** stock solution was added into the quartz optical cell gradually by using a micropipette until saturation. During the titration of **NpRD** and ATP, each time a 2×10^{-5} M solution of **NpRD** was filled in a quartz optical cell of 1 cm optical path length and ATP stock solution was added into the quartz optical cell gradually by using a micropipette. Spectral data were recorded at 2-3 min after the addition of **cAMP** or ATP.

Fluorescence spectral studies:

A stock solution of **NpRD** (2×10^{-5} M) was prepared in acetonitrile-water (1:6, v/v). **cAMP** solution (from Cyclic 3',5'-adenosine monophosphate sodium salt monohydrate) of concentration 1×10^{-4} M was prepared in Millipore water. All experiments were carried out in aqueous medium (pH 7.0, 10 mM phosphate buffer). Duringtitration, each time a 2×10^{-5} M solution of **NpRD** was filled in a quartz optical cell of 1 cm optical path length and **cAMP** stock solution was added into the quartz optical cell gradually by using a micropipette until saturation. During

the titration of **NpRD** and ATP, each time a 2 × 10^{-5} M solution of **NpRD** was filled in a quartz optical cell of 1 cm optical path length and ATP stock solution was added into the quartz optical cell gradually by using a micropipette. Spectral data were recorded at 2-3 min after the addition of **cAMP** or ATP. For all fluorescence measurements, excitationswere provided at 485 nm, and emissions were collected between 520 to 680 nm.

Absorbance and Fluorescencestudies of NpRD with cAMP and ATP:

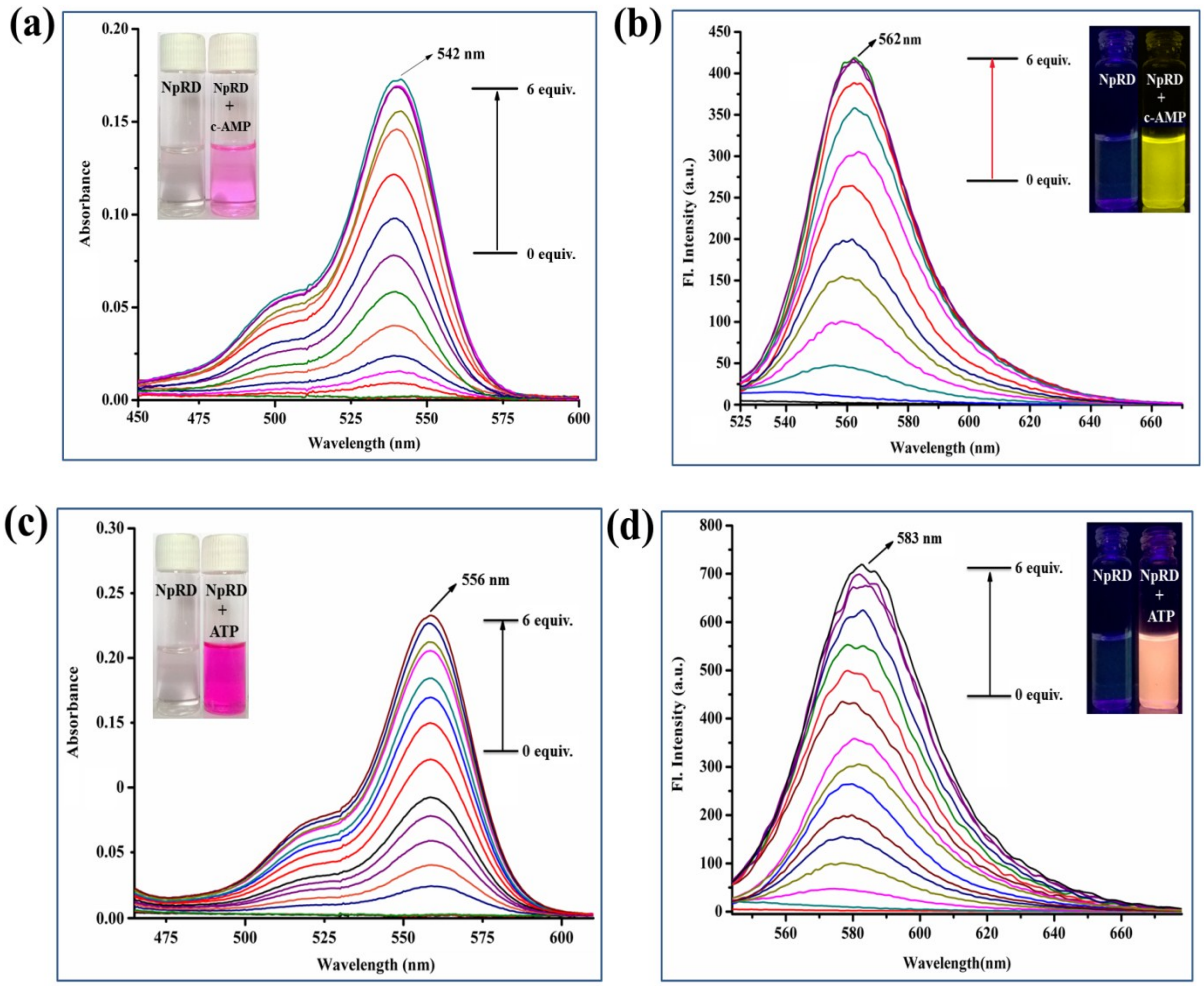


Figure S7. (a)UV-vis absorption spectra of **NpRD** (c = 2× 10^{-5}M) upon gradual addition of 6 equiv. of **cAMP**. (b) Fluorescence emission spectra (λ_{ex}= 485 nm) of **NpRD** (c = 2× 10^{-5}M) upon gradual addition of 6 equiv. of **cAMP.** (c) UV-vis absorption spectra of **NpRD** (c = 2× 10^{-5}M) upon gradual addition of 6 equiv. of ATP. (d) Fluorescence emission spectra (λ_{ex}= 485 nm) of **NpRD** (c = 2× 10^{-5} M) upon gradual addition of 6 equiv. of ATP. In each case, initial concentration of the added guest was 10^{-4}M and solvent was CH_3CN/H_2O- 1:6 (v/v) (pH 7.0, 10 mM phosphate buffer). All their respective visual and fluorescent changes are given as inset.

8.Evaluation of the Association constants for the formation of NpRD-cAMP and NpRD-ATP complex:

By UV-Vis Method:

The substrate binding interaction was calculated according to the Benesi-Hildebrand equation.

$$\frac{A_0}{A-A_0}=\left(\frac{\varepsilon_0}{\varepsilon_0-\varepsilon}\right)^2\left(\frac{1}{K_B[Substrate]^2}+1\right) \quad \ldots\ldots\ldots\ldots(1)$$

Here A_o is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest, ε_0 and ε are the corresponding molar absorption co-efficient and K_B represents the substrate binding interaction with guest.

Binding constant calculation graph (Absorption method):

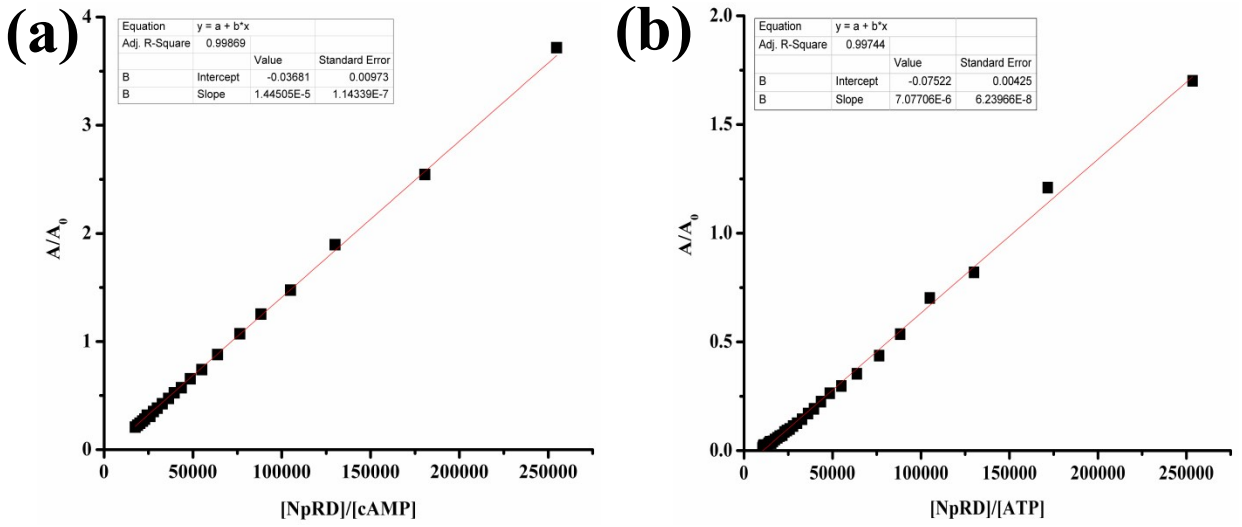


Figure S8. Linear regression analysis (A/A_0vs [H]/[G]) for the calculation of association constant values of (a) **cAMP** and (b) ATP by UV- titration method.

The association constant(K_a) of was determined from the equation: K_a = intercept/slope. From the linear fit graph we get, $K_a= 2.5 \times 10^3$ for **cAMP** and $K_a= 1.09 \times 10^4$ for ATP.

By Fluorescence Method:

Binding constant of the **NpRD-cAMP** complex also be calculated through emission method by using the following equation.

$$1/ (I – I_0) = 1/K(I_{max}– I_0) [G] + 1/(I_{max}– I_0) \quad \text{……(2)}$$

where I_0, I_{max}, and I represent the emission intensity of **NpRD**, the maximum emission intensity observed in the presence of added **cAMP** at 562 nm (for ATP, at 583 nm), λ_{ex}= 485 nm, [G] is the concentration of the **cAMP** and the emission intensity at a certain concentration of the **cAMP**, respectively.

Binding constant calculation graph (Fluorescence method):

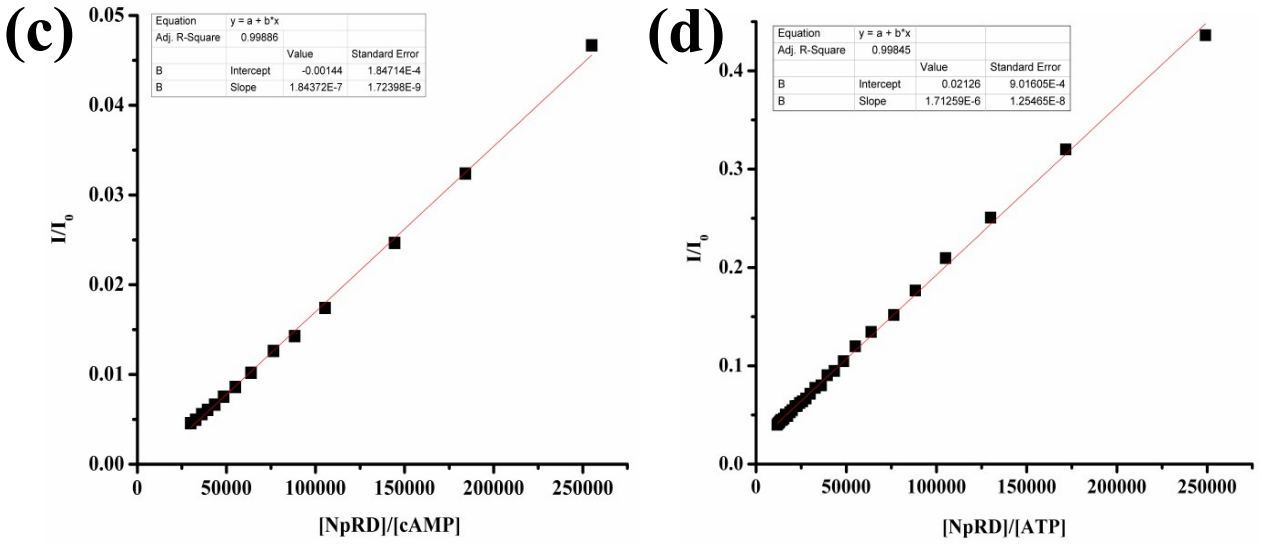


Figure S9. Linear regression analysis (1/[G] vs 1/ΔI) for the calculation of association constant values of (c) **cAMP** and (d) ATP by by Fluorescence titration method.

The association constant (K_a) of was determined from the equation: K_a = intercept/slope. From the linear fit graph we get, K_a= 7.8 × 10^3 for **cAMP** and K_a= 1.2 × 10^4 for ATP.

9. Calculation of limit of detection (LOD) of NpRD with cAMP and ATP:

The detection limit of the **NpRD** for **cAMP** was calculated on the basis of fluorescence titration. To determine the standard deviation for the fluorescence intensity, the emission intensity of four individual receptors without **cAMP** was measured by 10 times and the standard deviation of blank measurements was calculated.

The limit of detection (LOD) of **NpRD** for sensing **cAMP** was determined from the following equation:

$$LOD = K \times SD/S$$

Where K = 2 or 3 (we take 3 in this case); SD is the standard deviation of the blank receptor solution; S is the slope of the calibration curve.

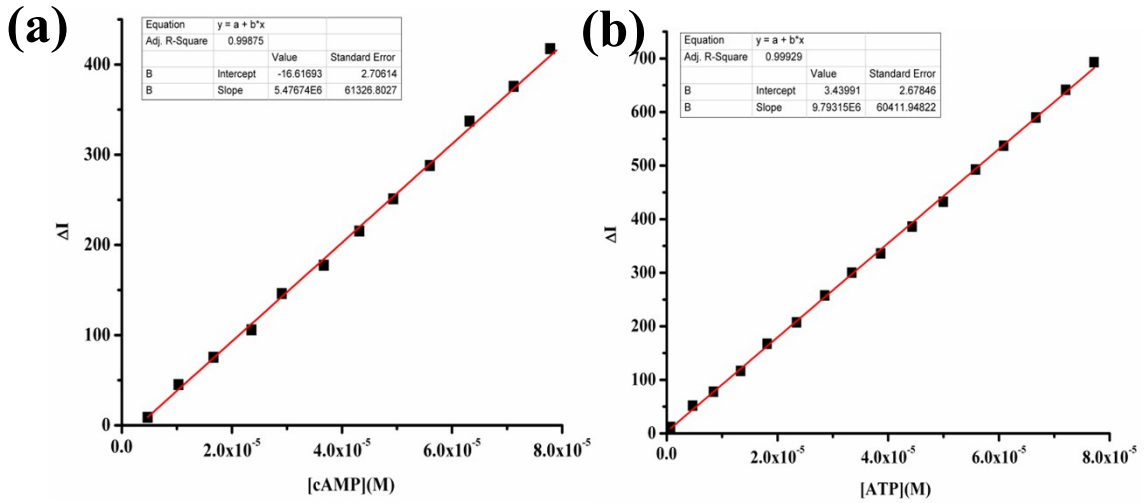


Figure S10. Linear fit curve of **NpRD**with respect to (a) **cAMP**and (b) ATP concentration.

From the linear fit graph of **cAMP** (a), we get slope = 5.47×10^6, and SD value is 4.672.
Thus using the above formula we get the Limit of Detection = 2.8×10^{-6} M or 2.8 μM. Therefore, **NpRD** can detect **cAMP** up to this very lower concentration by fluorescence techniques.

Again, from the linear fit graph of ATP(b), we get slope = 9.79×10^6, and SD value is 2.153.
Thus using the above formula we get the Limit of Detection = 0.6×10^{-6} M or 0.6 μM. Therefore **NpRD** can detect ATP up to this very lower concentration by fluorescence techniques.

10. Job's plot for determining the stoichiometry of binding by fluorescence method:

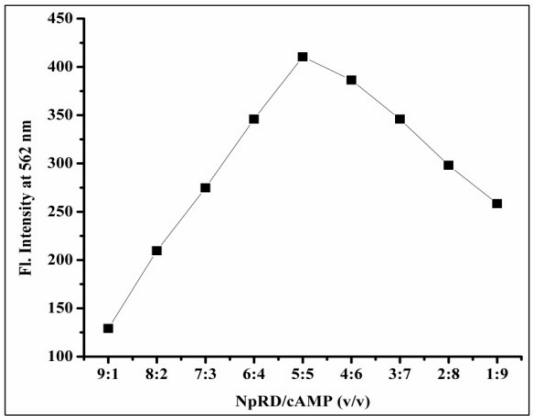


Figure S11. Job's plot of **NpRD** with **cAMP** in acetonitrile-water (1:6, v/v), neutral pH, ([**NpRD**] = [**cAMP**] = 1×10^{-4} M) by fluorescence method, which indicate 1:1 stoichiometry for **NpRD** with **cAMP**.

11. Fluorescence response ofNpRDin presence of different anions:

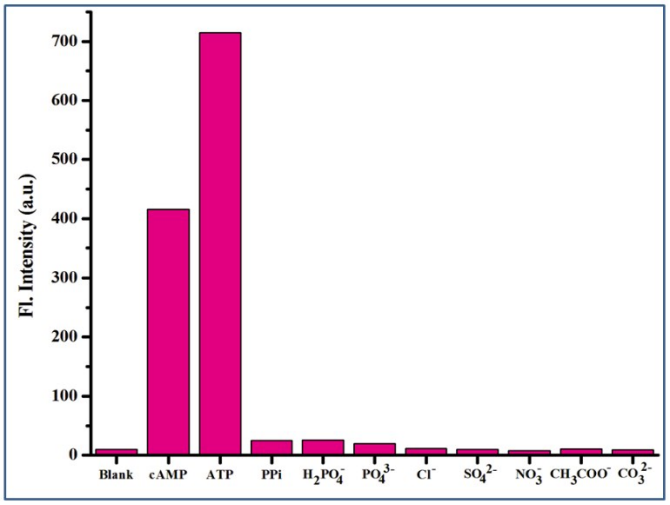


Figure S12. Fluorescence response of **NpRD** (20 μM) to various anions (100 μM)[From left to right: Only **NpRD**, **NpRD** with**- cAMP**, ATP, PPi, $H_2PO_4^-$, PO_4^{3-}, Cl^-, SO_4^{2-}, NO_3^-, CH_3COO^- and CO_3^{2-}] in acetonitrile–water (1:6 v/v, pH 7.0,10 mM phosphate buffer) solution.

12. ^{1}H NMR titration spectrum of NpRD with cAMP:

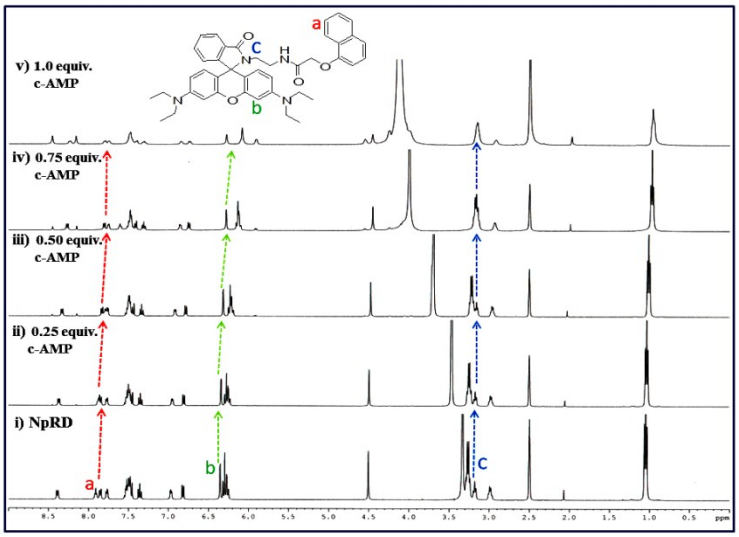


Figure S13.^{1}H NMR titration [400MHz] of **NpRD** in DMSO-d_6 at 25^{0}C and the corresponding changes after the gradual addition of one equiv. of **cAMP** in D_2O from (i) only **NpRD**, (ii) **NpRD** + 0.25equiv. of**cAMP**, (iii) **NpRD** + 0.5 equiv. of **cAMP**, (iv) **NpRD** + 0.75 equiv. of **cAMP, (v) NpRD** + 1 equiv. of **cAMP.**

13.^{13}C NMR titration spectrum of NpRDwithcAMP:

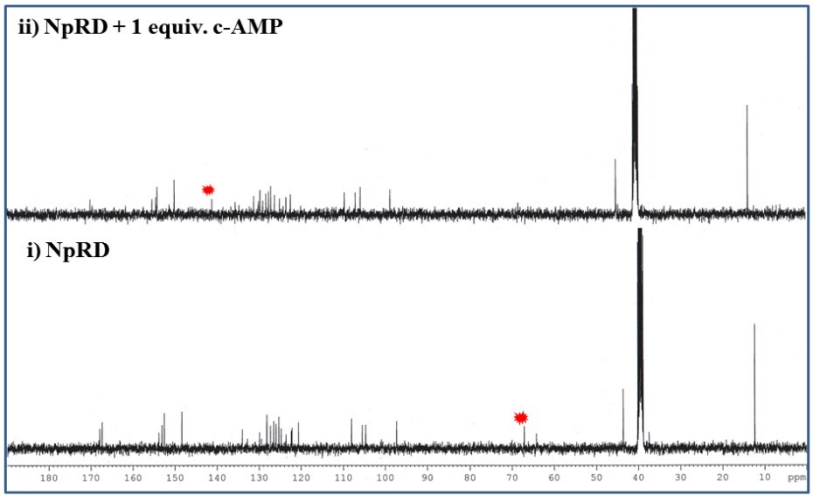


Figure S14.^{13}CNMR titration [400MHz] of **NpRD** in DMSO-d_6 at 25^{0}C and the corresponding changes after addition of one equiv. of**cAMP** in D_2O.

14. FT-IR titration spectrum of NpRD with cAMP:

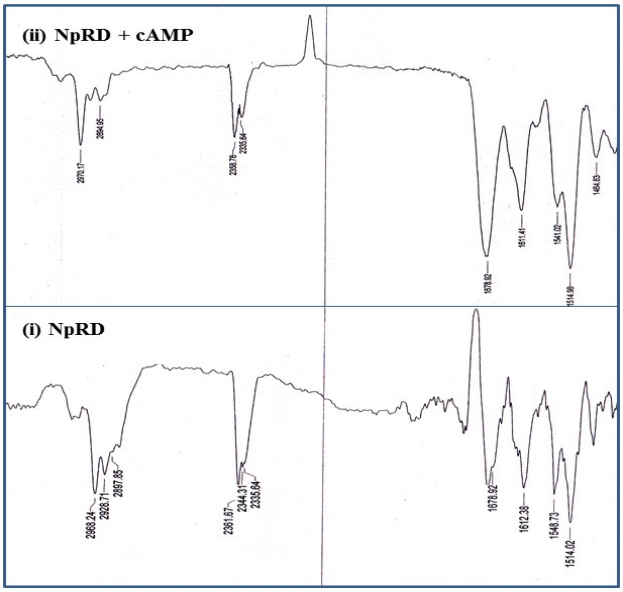


Figure S15.IRtitrationof **NpRD** in CH_3CN at 25^0C and the corresponding changes after the addition of one equiv. of **cAMP**.

15. Cytotoxicity Assay

In vitro studies established the ability of probe **NpRD** to detect **cAMP** in biological system with excellent selectivity. Hep-2 cells (Human cell ATCC: CCL-23) were used as models (Experimental section). However, to materialize this objective, it is a prerequisite to assess the cytotoxic effect of **NpRD**, **cAMP** and the complex on live cells. The well-established MTT assay, which is based on mitochondrial dehydrogenase activity of viable cells were adopted to study cytotoxicity of above mentioned compounds at varying concentrations detailed in method section. Cytotoxicity measurements for each experiment shows that **NpRD** does not have any toxicity on the tested cells, **cAMP** and the complex does not exert any significant adverse effect on cell viability either.

16. Live Cell Imaging:

Cell line and cell culture

Cell Culture: Hep-2 cells (Human cell ATCC: CCL-23)were prepared from continuous culture in Dulbecco's Modified Eagle's Medium (DMEM, Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Invitrogen), penicillin (100 µg/mL), and streptomycin (100 µg/mL). The Hep-2 cell were obtained from the American Type Culture

Collection (Rockville, MD) and maintained in DMEM containing 10% (v/v) fetal bovine serum and antibiotics in a CO_2 incubator. Cells were initially propagated in 75 cm^2 polystyrene, filter-capped tissue culture flask in an atmosphere of 5% CO_2 and 95% air at 37°C in CO_2 incubator. When the cells reached the logarithmic phase, the cell density was adjusted to 1.0 x 10^5 per/well in culture media. The cells were then used to inoculate in a glass bottom dish, with 1.0 mL (1.0 x 10^4 cells) of cell suspension in each dish. After cell adhesion, culture medium was removed. The cell layer was rinsed twice with phosphate buffered saline (PBS), and then treated according to the experimental need.

Cell Imaging Study: For confocal imaging studies, 1 x 10^4Hep-2 cells in 1000 µL of medium, were seeded on sterile 35 mm glass bottom culture dish (ibidi GmbH, Germany), and incubated at 37°C in a CO_2 incubator for 10 hours. Then cells were washed with 500 µL DMEM followed by incubation with 2.0 x 10^{-5} M **NpRD** dissolved in 1000 µL DMEM at 37°C for 1 h in a CO_2 incubator followed by washing with phosphate buffered saline PBS (pH 7.4) to remove any free **NpRD** and observed under an Olympus IX81 microscope equipped with a FV1000 confocal system using 1003 oil immersion Plan Apo (N.A. 1.45) objectives. Images obtained through section scanning were analyzed by Olympus Fluoview (version 3.1a; Tokyo, Japan) with excitation at 534 nm monochromatic laser beam, and emission spectra were integrated over the range 572 nm (single channel). The cells were again washed thrice with phosphate buffered saline PBS (pH 7.4) to remove any free **NpRD** and incubated in PBS containing **cAMP** to a final concentrations of 1.0 x 10^{-4} M, incubated for 20 min followed by washing with PBS three times to remove excess **cAMP** outside the cells and images were captured. For all images, the confocal microscope settings, such as transmission density, and scan speed, were held constant to compare the relative intensity of intracellular fluorescence.

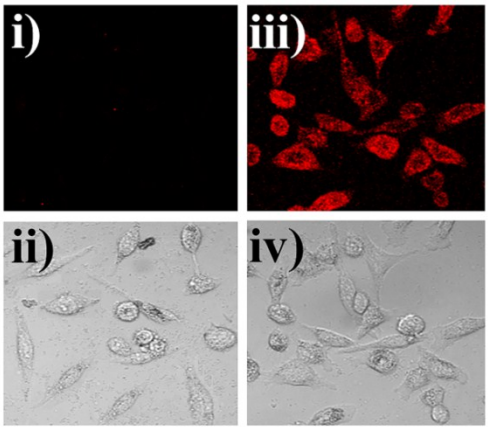


Figure S16. Confocal microscopic images of Hep-2 cells treated with **NpRD** and ATP. (i) Cells treated with **NpRD** at 1.0 × 10^{-5} M concentration. (ii) Bright field image of (i). (iii) Cells treated with **NpRD-**ATP at concentration 1.0 ×10^{-5} M. (iv) Bright field image of (iii).All images were acquired with a 40X objective lens with the applied wavelengths: $E_{excitation}$=570 nm, $E_{emission}$=590 nm.

17. Quantification of cAMP in human blood by Fluorescence method:

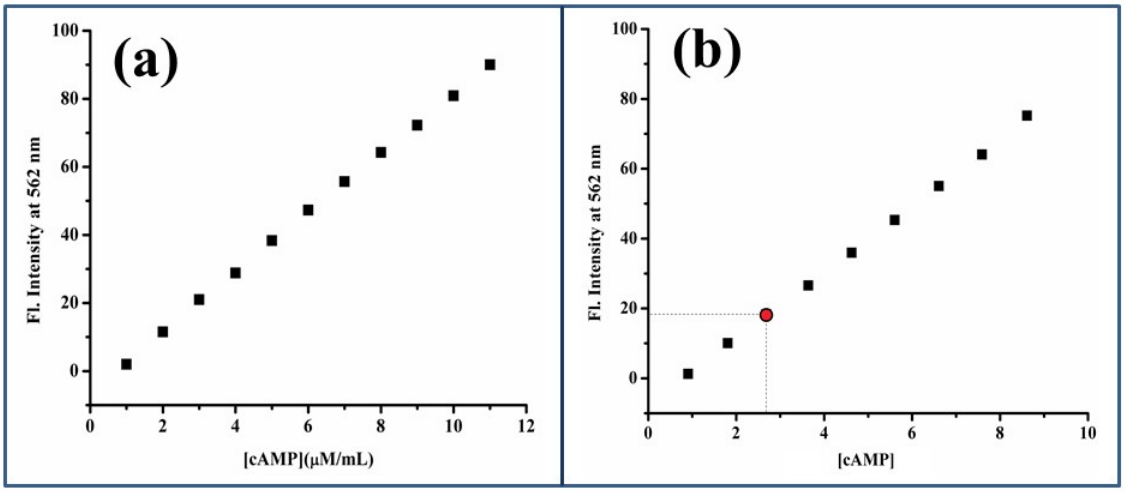


Figure S17. (a) Standard curve obtained for the estimation of **cAMP** (b) Estimation of unknown concentration of **cAMP** (red point) in human blood from the standard curve.

Table S1. Performance comparison of existing bio-sensors and chemosensor (NpRD) for detection of cAMP:

	Biosensors							**Chemo-sensor**
	PKA-based		Epac-based		CNGC-based			**Rhodamine-based fluorophore (NpRD)**
Basic methodology	FRET	BRET	FRET	BRET	FRET	Patch clamp	Ca^{2+}	**Direct binding with cAMP**
Mode of delivery	Microinjection, expression plasmid, adenovirus	Expression plasmid	Expression plasmid, adenovirus	Expression plasmid, retrovirus	Expression plasmid, transgenic mice	Adenovirus	Adenovirus, expression plasmid	**simple diffusion**
Dynamic range	0.02–5 μM	0.1–10 μM	5–100 μM	0.5–100 μM	0.5–100 μM	0.1–5 μM (E583)	0.1–5 μM (E583M)	**2.8 μM**
Localization	Cytosolic, plasma membrane, AKAP-tethered	Cytosolic	Cytosolic, plasma membrane, mitochondrial, nuclear	Cytosolic	Cytosolic	Plasma membrane	Plasma membrane	**Cytosolic, both *in-vivo* and *in-vitro***
Temporal resolution	Limited (several seconds)	-	Good (<2 s)	Limited	Good	Excellent (<15 ms)	Good	**Excellent (turned on immediately)**
Signal to noise	20% FRET ratio change	80% BRET ratio change	30% FRET ratio change	70% BRET ratio change	20% FRET ratio change	1-nA current increase	100% fura-2 ratio change	**40 fold**
Suitable for HTS	-	yes	-	yes	-	-	yes	**Highly suitable**
Environment to work	Only cellular	Only cellular	Only cellular	Only cellular	Only cellular	Only cellular	Only cellular	**Cellular and in solution**

Table S2. Determination of cAMP in Human blood sample

Human Blood Sample			
Sample	**Added(10^{-6}M)**	**Found (10^{-6}M)[a]**	**Recovery (%)**
1	3	2.96 ± 0.03	98.6
2	5	4.87 ± 0.05	97.4
3	7	6.73 ± 0.07	96

[a]An average of three replicate measurements with standard deviation.

Table S3: Optimization and validation of the screening procedure for cAMP level in various test blood sample using NpRD chemosensor.

Samples	Fluorescence Intensity			Mean	Standard Deviation	Signal*/Noise**	Z' score
	set 1	set 2	set 3				
Control	9.60	10.20	9.90	9.90	0.30	----	----
S 1	156	164	160	160.00	4.00	16.16	0.91
S 2	186	190	182	186.00	4.00	18.79	0.93
S 3	179	176	178	177.67	1.53	17.95	0.98
S 4	198	190	191	193.00	4.36	19.49	0.92
S 5	199	201	212	204.00	7.00	20.61	0.89
S 6	171	178	174	174.33	3.51	17.61	0.93
S 7	198	188	197	194.33	5.51	19.63	0.91
S 8	165	160	170	165.00	5.00	16.67	0.90
S 9	206	212	201	206.33	5.51	20.84	0.91
S 10	188	181	187	185.33	3.79	18.72	0.93

*fluorescence intensity for **NpRD-cAMP** interaction.
fluorescence intensity for **NpRD.

All experiments were performed in compliance with the relevant laws and institutional guidelines. Institutional committee (Visva-Bharati University) has approved the experiments. The informed consent was obtained for all experiments involving human subjects.