Supporting Information

Pyridinium–based fluororeceptors as practical chemosensors for hydrogen pyrophosphate ($HP_2O_7^{3-}$) in semi aqueous and aqueous environments

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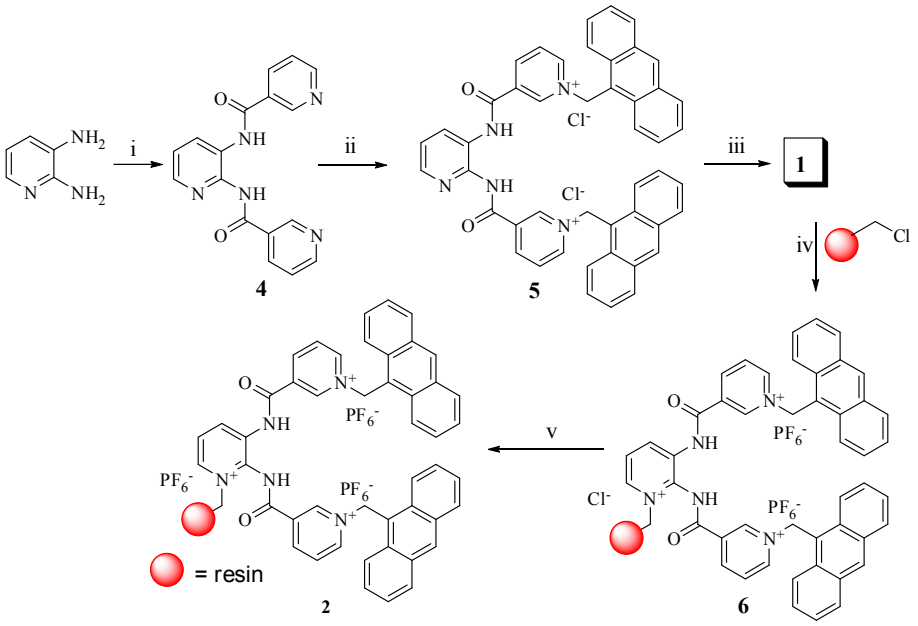
Experimental Section

1. Synthesis:



Scheme S1. (i) Nicotinoyl chloride, dry CH_2Cl_2, Et_3N. (ii) 9 - chloromethyl anthracene in dry CH_3CN and DMF mixture, reflux for 36 h (isolated yield: 22%), (iii). NH_4PF_6, aq. CH_3OH (yield 57%), (iv) DMF, polystyrene resin, refluxing condition, (v) DMF, aq solution of NH_4PF_6.

***N, N'-(*pyridine-2, 3-diyl) dinicotinamide (4)**:

The unsymmetrical hetero bis amide **4** was obtained by coupling of 2,3-diaminoaminopyridine (1 g, 8.41 mmol) with pyridine – 3- carbonyl chloride (3.57 g, 25.20 mmol) in 60 mL dry CH_2Cl_2 followed by addition of triethylamine (1 mL) under nitrogen atmosphere. The reaction mixture was stirred overnight. After completion of the reaction, solvent was removed under vacuum. The residual mass was extracted with $CHCl_3/CH_3OH$ mixture (3 x 30 mL). The organic layer was washed with $NaHCO_3$ solution (3 x 15 mL) and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The crude mass was purified by column chromatography over silica gel using ethyl acetate: hexane (7:3 v/v) to 2% CH_3OH in ethyl acetate as eluent to afford the desired diamide **3** in 64% yield (1.72 mg), mp 170 ^{0}C.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 10.95 (s, 1H), 10.13 (s, 1H), 9.13 (s, 1H), 9.00 (s, 1H), 8.75 – 8.71 (m, 2H), 8.40 – 8.30 (m, 2H), 8.24 (d, 1H, *J* = 8 Hz), 8.19 (d, 1H, J = 8 Hz), 7.56 – 7.51 (m, 2H), 7.45 – 7.42 (m, 1H). ^{13}C NMR (d_6DMSO, 75 MHz): δ 165.3, 164.5, 152.8, 149.6, 149.0, 145.4, 144.8, 144.1, 136.2, 135.8, 134.8, 130.4, 130.0, 123.9, 122.4, 118.8, 118.1. FTIR (KBr, ν cm^{-1}): 3252, 1680, 1656, 1591, 1572, 1478, and 1306. Mass (LCMS): 320.2 $(M+1)^+$.

3,3'-(pyridine-2,3-diylbis(azanediyl))bis(oxomethylene)bis(1-(anthracen-9-ylmethyl)pyridinium) hexafluorophosphate (V) (1):

To the solution of the diamide **4** (0.07 g, 0.219 mmol) in CH_3CN (15 mL) containing dry DMF (2 mL), 9-chloromethyl anthracene (0.149 g, 0.658 mmol) in CH_3CN (20 mL) was added. The resulting solution was refluxed

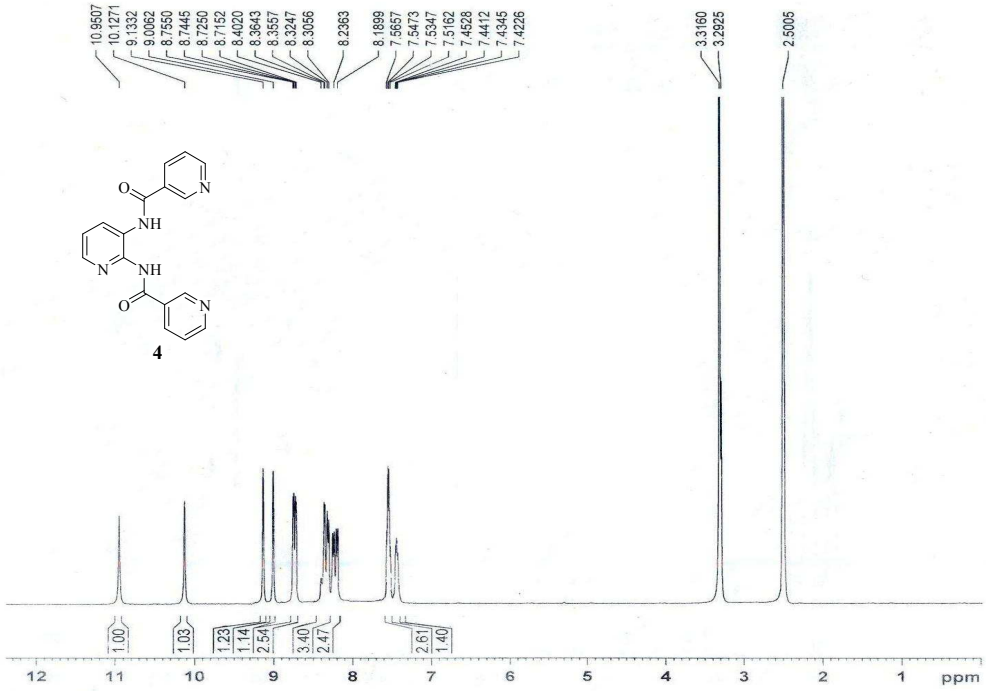
with stirring for 3 days under nitrogen atmosphere. On cooling the reaction mixture, yellow precipitate appeared. The precipitate was filtered off and washed with hot CH_3CN for several times and then with diethyl ether to have pure dichloride salt **5** (0.108 g, 63%). The pure dichloride salt **5** (0.108 g, 0.139 mmol) was dissolved in 5 mL hot CH_3OH and the volume was reduced to 2 mL. Then aqueous solution of NH_4PF_6 (0.068 g, 0.412 mmol) was added in one portion to exchange Cl^- ions. After stirring the reaction mixture for 35 min, light yellow precipitate appeared. Filtration of the precipitate followed by thorough washing with diethyl ether afforded the receptor **1** in 90% yield (0.125 g); mp 184 ºC. ^{1}H NMR (400 MHz, d_6-DMSO): δ 11.33 (s, 1H, amide NH), 10.56 (s, 1H, amide NH), 9.38 (d, 2H, J = 8 Hz), 8.97 (brs, 2H), 8.92 (s, 1H), 8.84 (d, 3H, J = 8 Hz), 8.40 – 8.35 (m, 4H), 8.29 – 8.22 (m, 5H), 8.15 (brt, 1H), 8.08 (t, 1H, J = 8 Hz), 7.63 -7.58 (m, 9H), 7.41 (brd, 1H), 7.01 (s, 2H), 6.98 (s, 2H). ^{13}C NMR (100 MHz, d_6-DMSO): δ 161.0, 145.3, 144.5, 133.7, 131.5, 131.4, 131.3, 131.1, 131.0, 129.6, 129.5, 128.4, 128.3, 128.1, 125.8, 125.7, 123.0, 121.2, 56.4. FTIR (KBr,ν cm^{-1}): 3431, 3337, 3090, 2917, 1690, 1629, 1591, 1583, 1531, and 1493. m/z (ES^+): 700.6 $(M\text{-}2PF_6^-\text{-}1)^+$. Anal. Calcd for $C_{56}H_{52}F_{12}N_4O_3P_2$: C, 56.92; H, 3.56; N, 7.06. Found: C, 56.96; H, 3.61; N, 7.11.

Synthesis of 2:

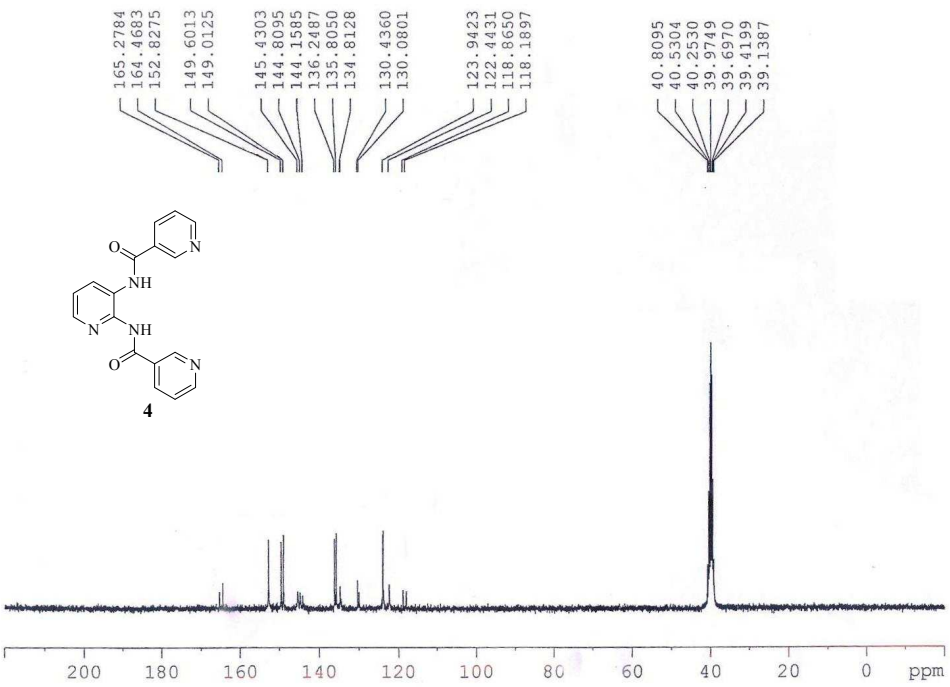
Compound **1** (100 mg) was immobilized on chloromethyl polystyrene (called as Merrifield resin) by refluxing and stirring a DMF solution of **1** with chloromethyl polystyrene. Filtration of reaction mixture followed by thorough washing with DMF to remove the excess amount of **1** was done. Then NH_4PF_6 was added to the DMF solution of **6** to carry out the anion exchange reaction. After heating with stirring of the reaction mixture for 50 min. the beads were collected through filtration. Finally, the beads were washed properly with water and diethyl ether to have the desired Merrifield resin bound host **2**. The beads were characterized by recording FTIR, fluorescence image and also SEM.

2a. Spectral data of compounds:

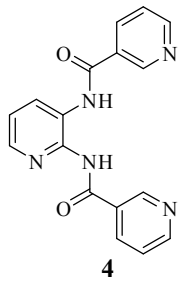
^{1}H NMR of 4 (400 MHz, d_6-DMSO):



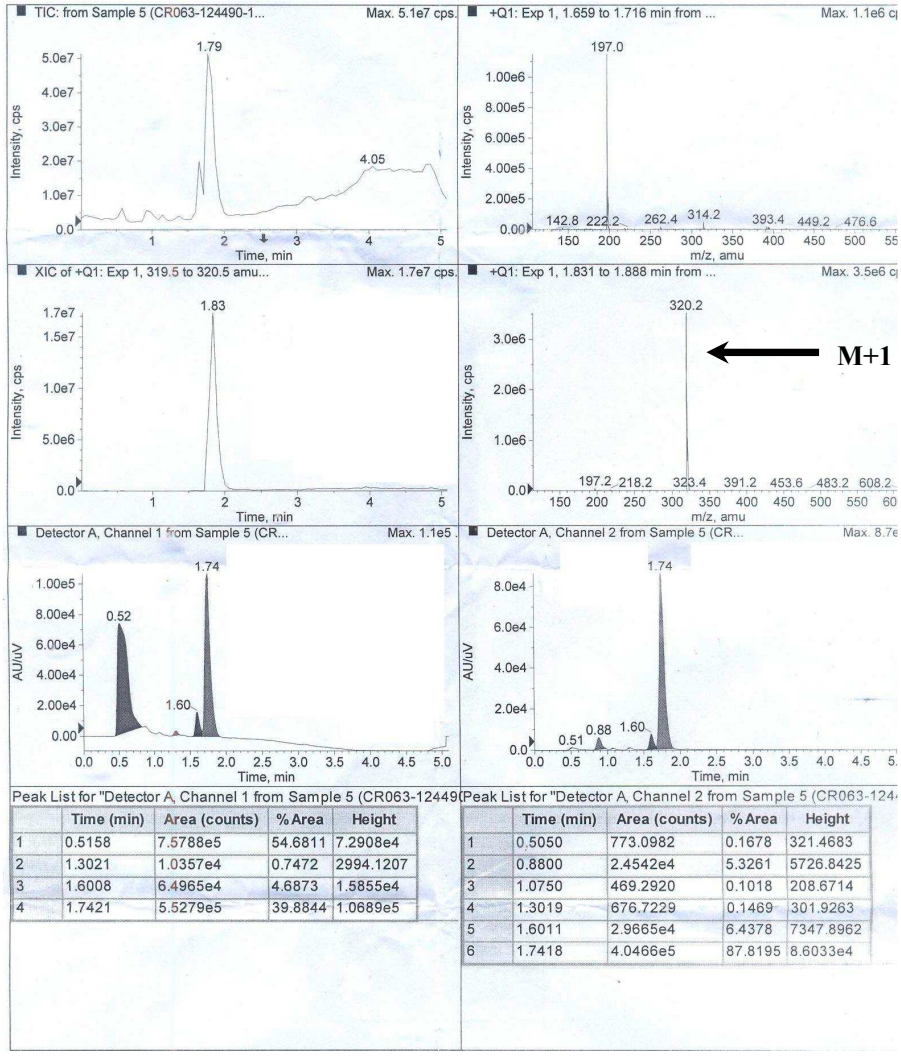
^{13}C NMR of 4 (75 MHz, d_6-DMSO):



LCMS of 4:



4



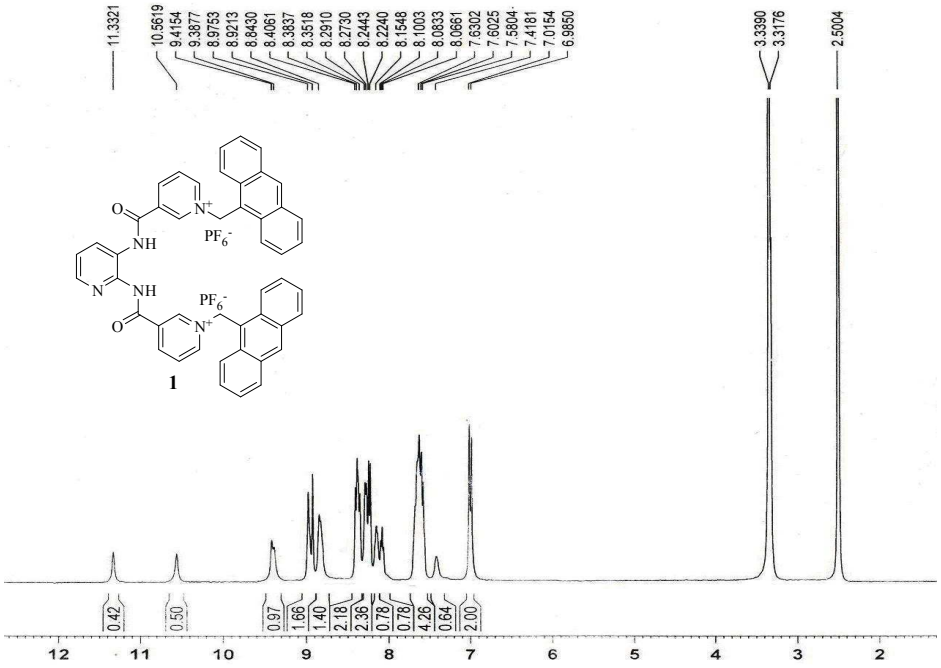
Peak List for "Detector A, Channel 1 from Sample 5 (CR063-12449

	Time (min)	Area (counts)	% Area	Height
1	0.5158	7.5788e5	54.6811	7.2908e4
2	1.3021	1.0357e4	0.7472	2994.1207
3	1.6008	6.4965e4	4.6873	1.5855e4
4	1.7421	5.5279e5	39.8844	1.0689e5

Peak List for "Detector A, Channel 2 from Sample 5 (CR063-124

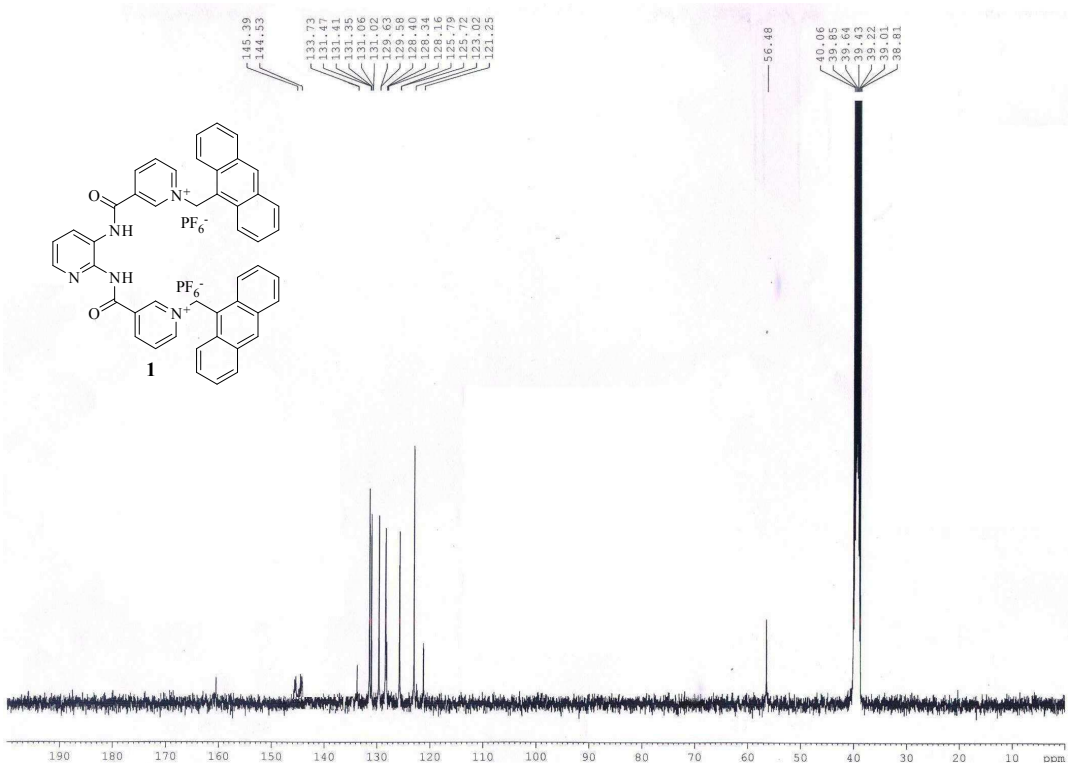
	Time (min)	Area (counts)	% Area	Height
1	0.5050	773.0982	0.1678	321.4683
2	0.8800	2.4542e4	5.3261	5726.8425
3	1.0750	469.2920	0.1018	208.6714
4	1.3019	676.7229	0.1469	301.9263
5	1.6011	2.9665e4	6.4378	7347.8962
6	1.7418	4.0466e5	87.8195	8.6033e4

^{1}H NMR of 1 (400 MHz, d_6-DMSO):



1

^{13}C NMR of 1 (100 MHz, d_6-DMSO):



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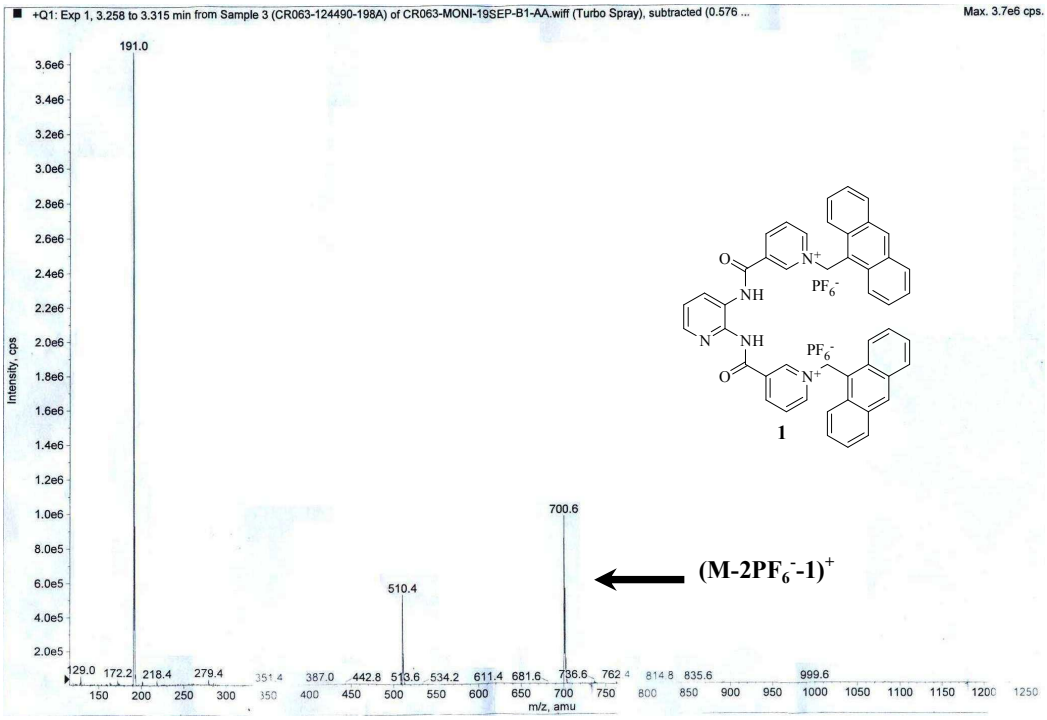
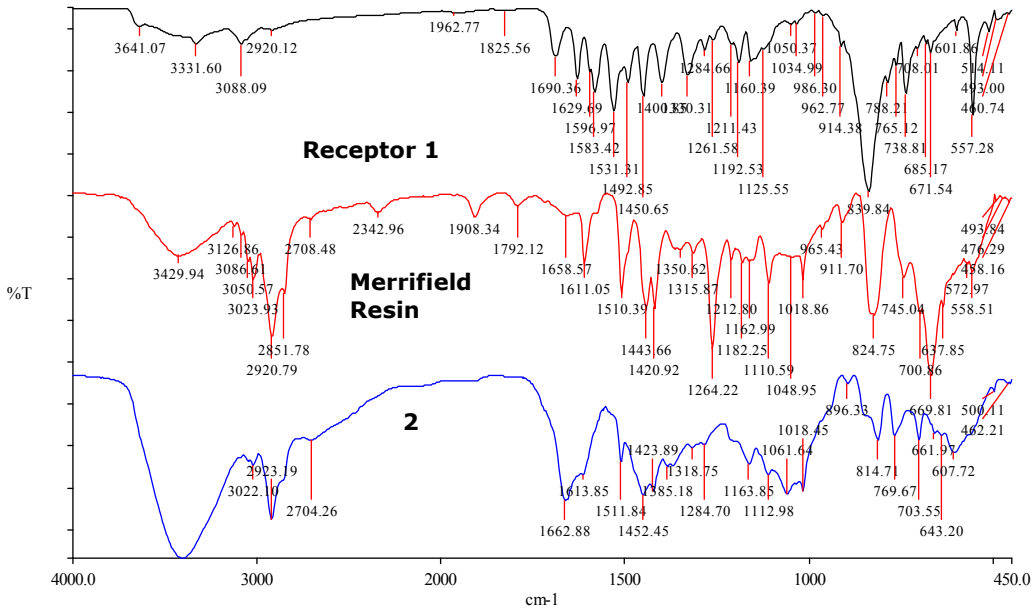
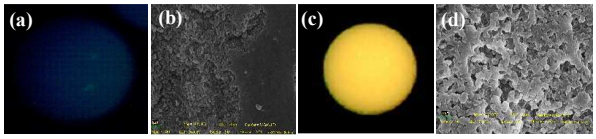
ESI mass of 1:



FTIR spectra of 1, Merrifield Resin and 2:

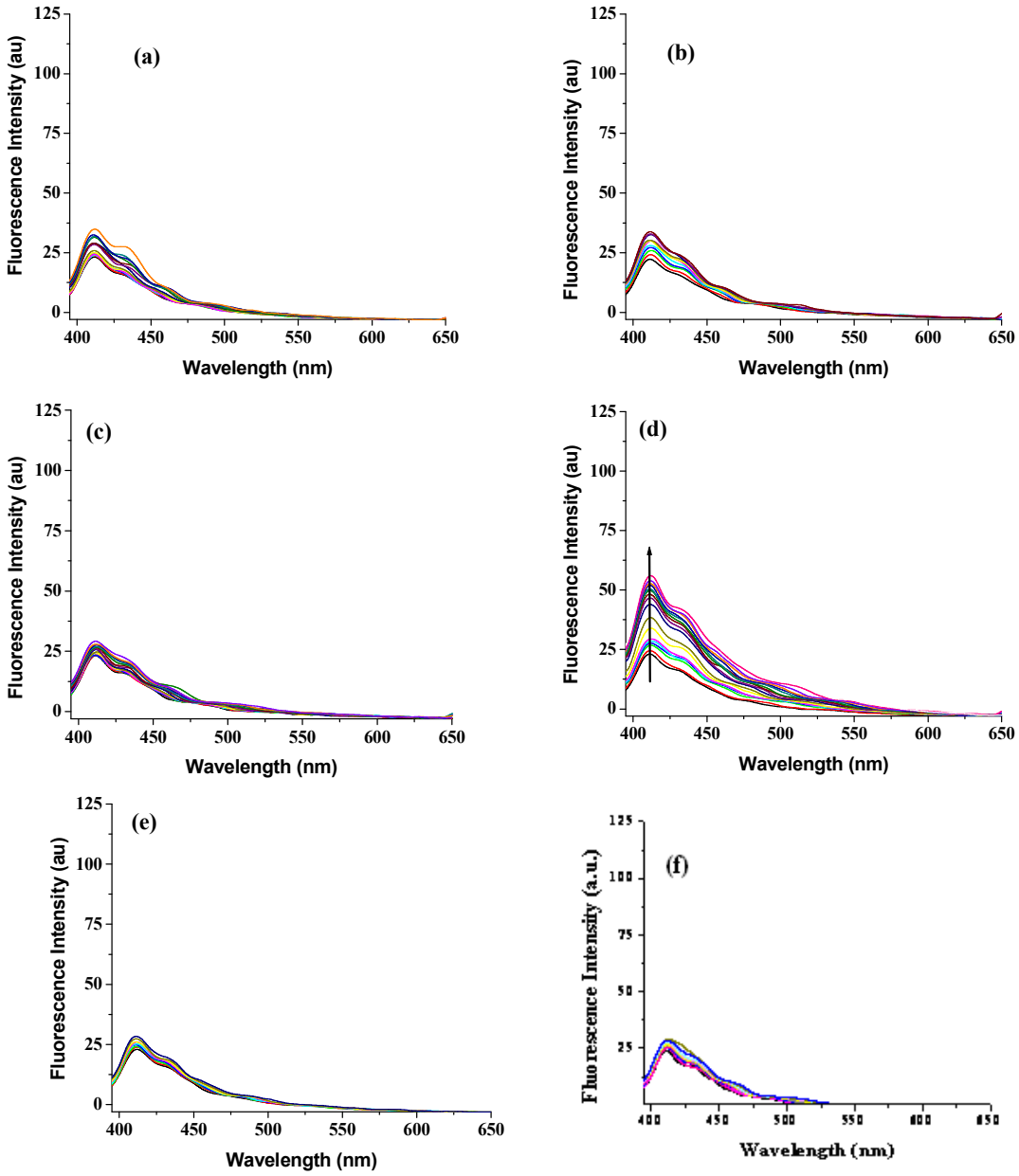


2b. Fluorescence images of (a) resin and (c) 2, irradiating the beads at 365 nm. SEM images of (b) resin and (d) 2.



Fluorescence images of (a) resin and (c) **2**, irradiating the beads at 365 nm. SEM images of (b) resin and (d) **2**.

3a. Selected fluorescence titration curves for receptor 1 in CH_3CN:H_2O (v/v) 10 mM Tris/HCl buffer pH = 6.5



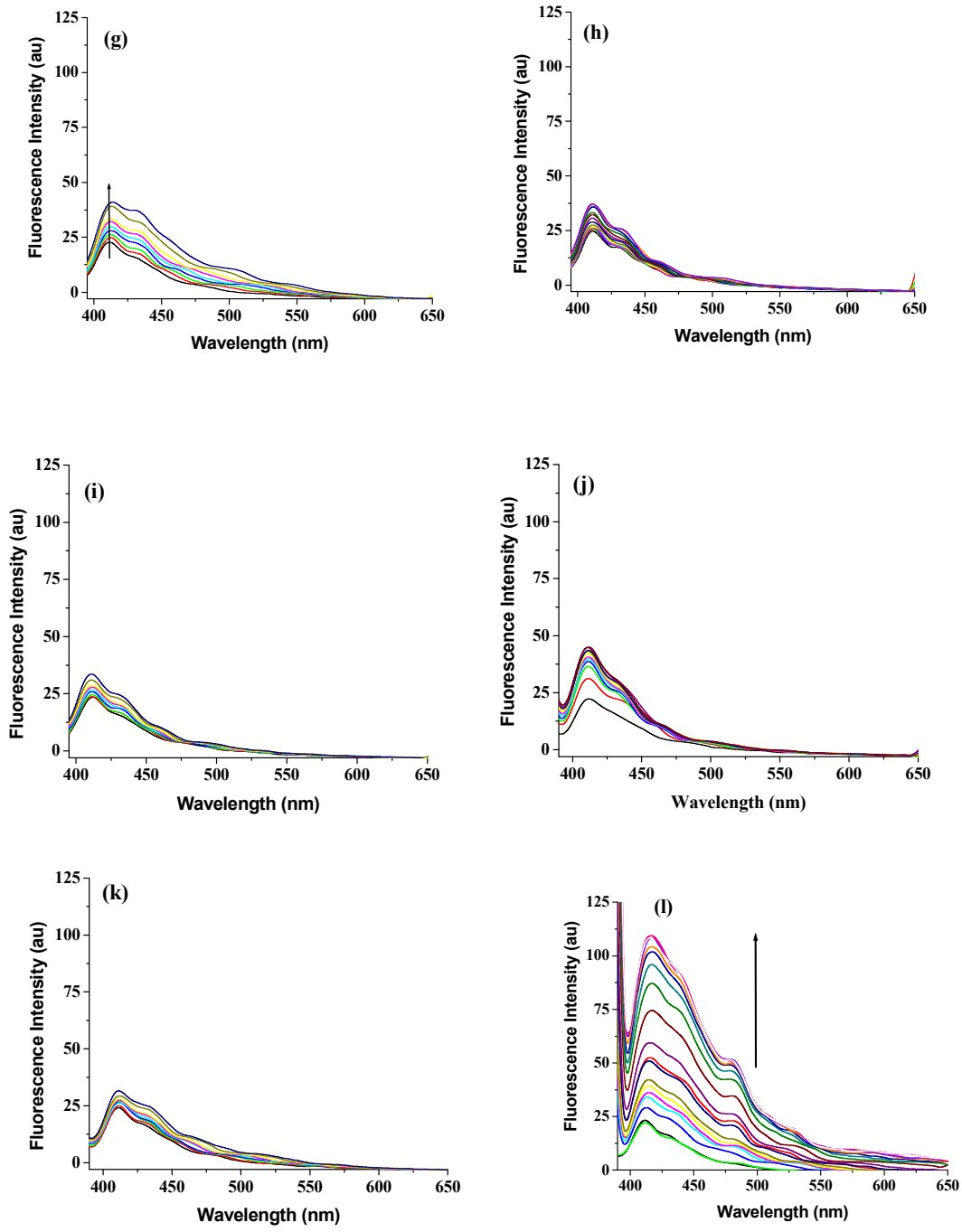
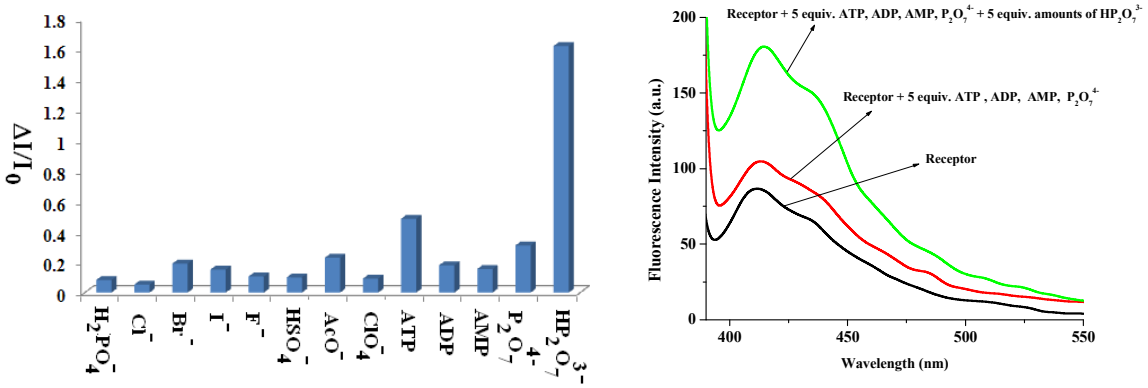


Figure S1: Fluorescence titration spectra of receptor **1** (c = 2.5 x 10^{-5} M) with the tetrabutylammonium (a) Acetate (b) ADP (Na-salt), (c) AMP (Na-salt), (d) ATP (Na-salt), (e) Bromide, (f) Chlorate, (g) Chloride, (h) $H_2PO_4^-$, (i) Fluoride, (j) Hydrogensulphate, (k) Iodide and (l) $HP_2O_7^{3-}$ in 4:1 CH_3CN:H_2O (v/v) 10 mM TrisHCl buffer pH = 6.5 [Concentration of all guests were 9.6 x 10^{-4} M].

3b) Change in fluorescence ratio of 1 with anions in 4:1 CH_3CN:H_2O (v/v) containing 0.1 mM Tris HCl buffer pH = 6.5



(a) (b)

Figure S2. (a) Fluorescence ratio [$\Delta I/I_0$] of **1** (c = 2.5 x 10^{-5} M) at 412 nm upon addition of 10 equiv. tetrabutylammonium and sodium salts of a particular anion in CH_3CN:H_2O (4:1 v/v, 0.1 mM TrisHCl buffer, pH 6.5); (b) Change in emission of receptor **1** (c = 2.5 x 10^{-5} M) upon addition of $(TBA)_3HP_2O_7$ to the solution of **1** in CH_3CN:H_2O (4:1 v/v, 0.1 mM TrisHCl buffer, pH 6.5) containing sodium salts of ATP, ADP, AMP and pyrophosphate.

3c) Bar plot for the change in fluorescence ratio for receptor 1 in CH_3CN

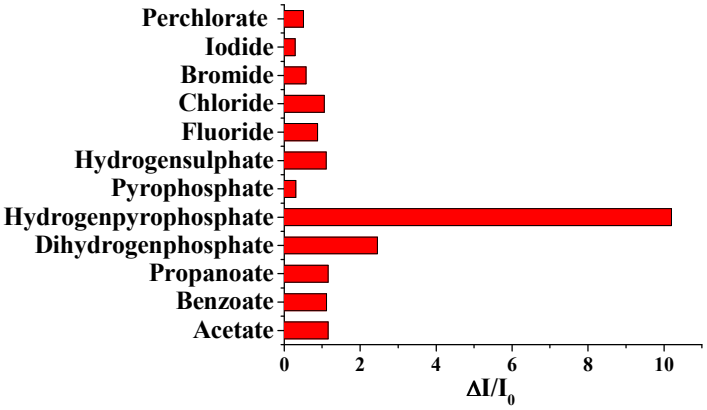


Figure S3a. Fluorescence ratio [$(I - I_0)/I_0$] of **1** (c = 2.5 x 10^{-5} M) at 412 nm upon addition of 10 equiv tetrabutylammonium salt of a particular anion in CH_3CN.

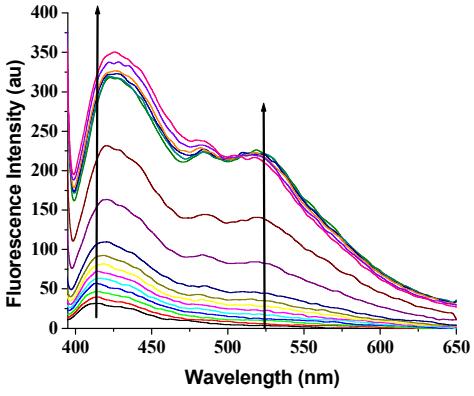


Figure S3b. Fluorescence titration spectra of receptor **1** (c = 2.5 x 10^{-5} M) with the tetrabutylammonium $HP_2O_7^{3-}$ in CH_3CN.

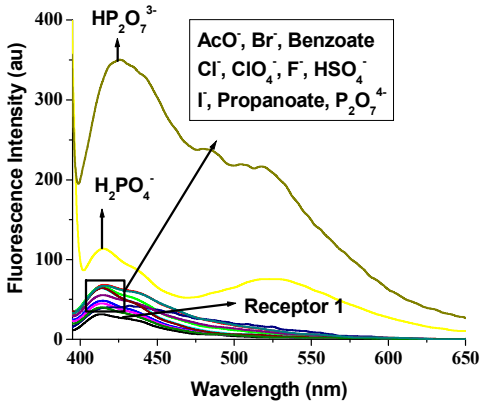
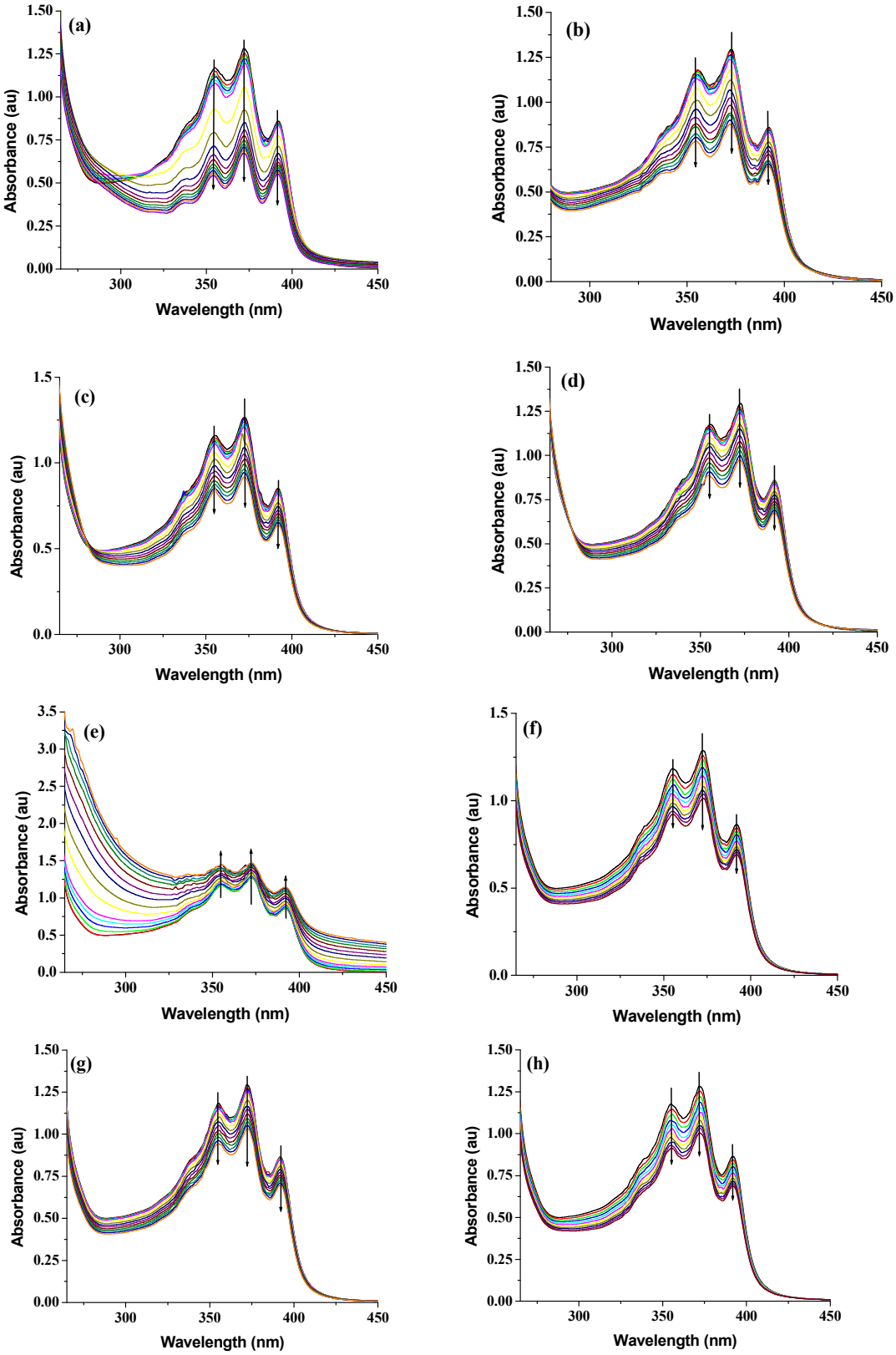


Figure S3c. Fluorescence emission change of **1** (c = 2.5 x 10^{-5} M) upon addition of various anions (10 equiv.) in CH_3CN.

3d. Selected UV-vis titration curves for receptor 1 in 4:1 CH_3CN:H_2O (v/v) 10 mM Tris/HCl buffer pH = 6.5



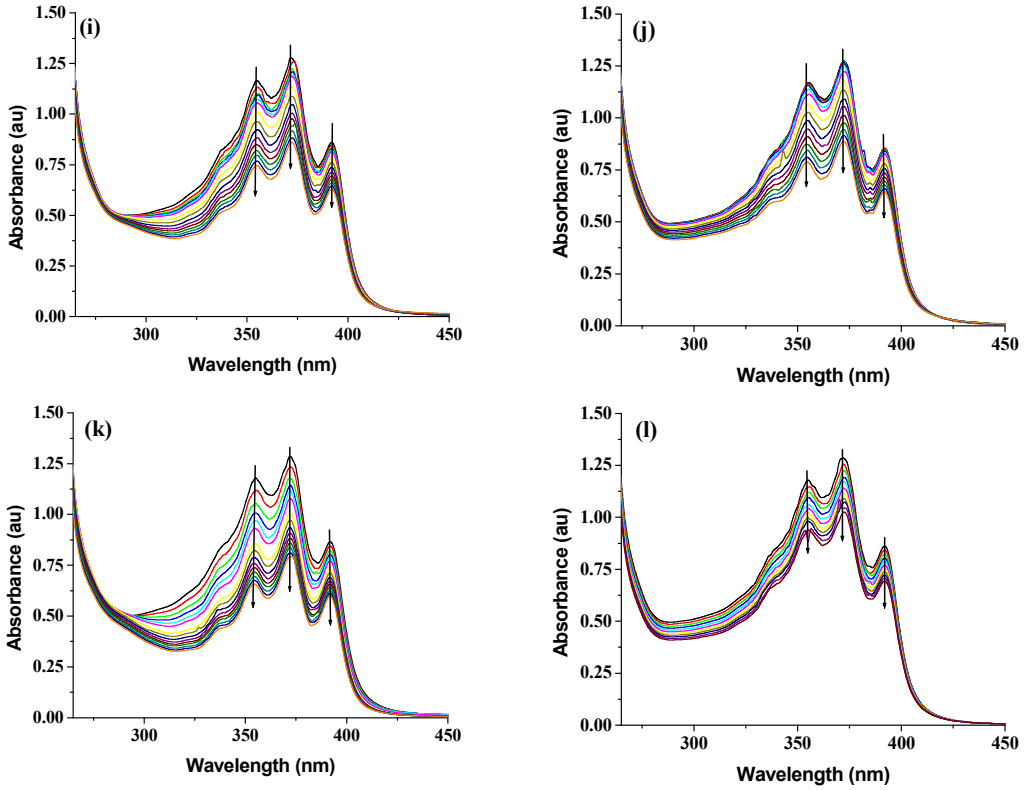


Figure S4: UV-vis titration curves of receptor **1** (c = 2.5 x 10^{-5} M) with the tetrabutylammonium (a) $HP_2O_7^{3-}$, (b) Acetate (c) ADP (Na-salt), (d) AMP (Na-salt), (e) ATP (Na-salt), (f) Bromide, (g) Chlorate, (h) Chloride, (i) $H_2PO_4^-$, (j) Fluoride, (k) Hydrogensulphate and (l) Iodide in 4:1 CH_3CN:H_2O (v/v) 10 mM TrisHCl buffer pH = 6.5 [Concentration of all guests were 9.6 x 10^{-4} M].

4. Job plots from Fluorescence and UV-vis for 1 in 4:1 CH_3CN:H_2O (v/v) 10 mM TrisHCl buffer pH = 6.5:

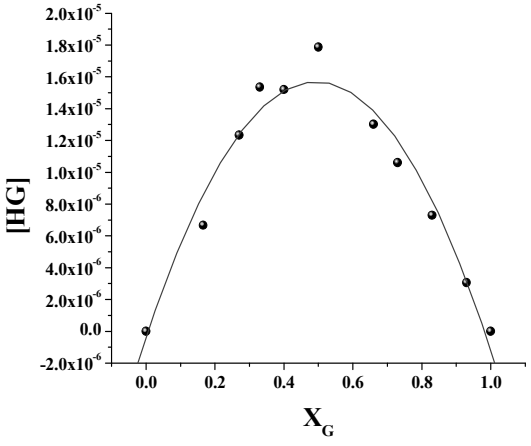


Figure S5a. Fluorescence Job plot of **1** with tetrabutylammonium salt of hydrogen pyrophosphate ([G] = [H] = 4.8 x 10^{-5} M).

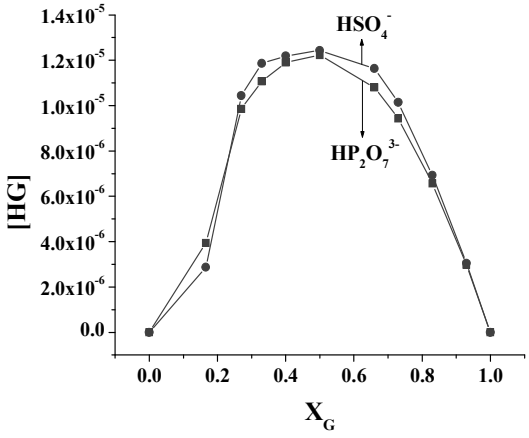


Figure S5b. UV Job plot of **1** with tetrabutylammonium salt of hydrogen pyrophosphate and hydrogensulphate ([G] = [H] = 4.8 x 10^{-5} M).

5a. Binding constant curve for receptor 1 with tetrabutylammonium hydrogen pyrophosphate in 4:1 $CH_3CN:H_2O$ (v/v) 10 mM TrisHCl buffer pH = 6.5 from fluorescence.

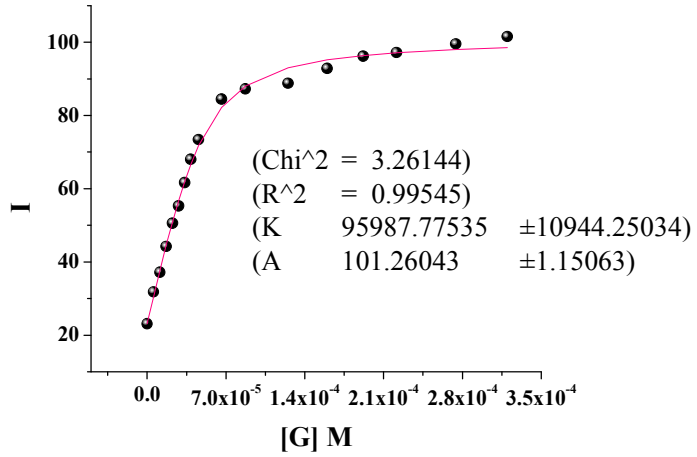


Figure S6a: Binding constant curve of **1** (c = 2.5 x 10^{-5} M) with the tetrabutylammonium hydrogen pyrophosphate (c = 9.6 x 10^{-4} M) from fluorescence titration in $CH_3CN:H_2O$ (4:1 v/v, pH = 6.5, 10 mM Tris/HCl buffer). Working formula $y=I_0+((I-I_0)/(2*x\_2))*(x\_1+x\_2+1/K-((x\_1+x\_2+1/K)^2-4*x\_1*x\_2)^.5)$, x_1= [G], x_2 = [H], y = intensity.

5b. Binding constant curve for receptor 1 with tetrabutylammonium hydrogen pyrophosphate in 4:1 $CH_3CN:H_2O$ (v/v) 10 mM TrisHCl buffer pH = 6.5 from UV-vis.

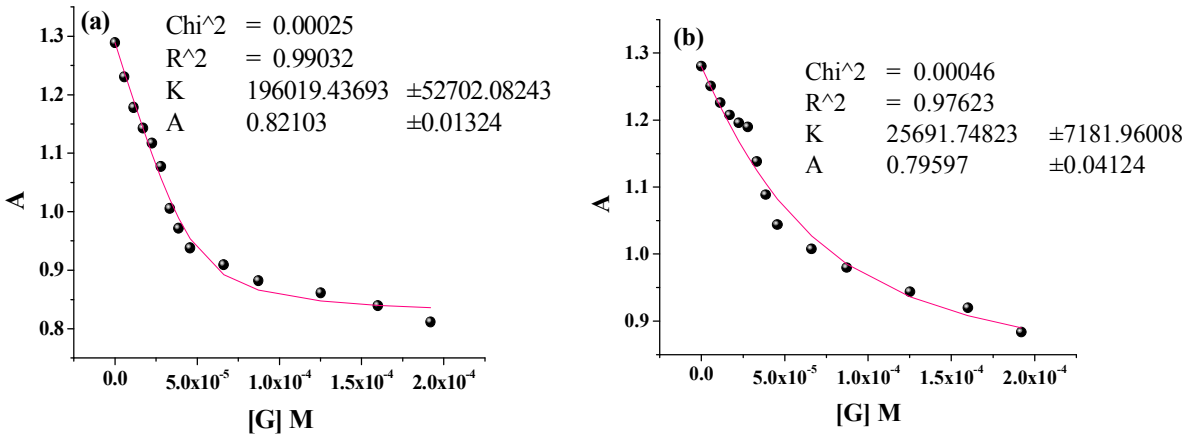


Figure S6b: Binding constant curve for receptor **1** (c = 2.5 x 10^{-5} M) with the tetrabutylammonium (a) hydrogen pyrophosphate (c = 9.6 x 10^{-4} M) and (b) dihydrogenphosphate (c = 9.6 x 10^{-4} M) from UV-vis titration in $CH_3CN:H_2O$ (4:1 v/v, pH = 6.5, 10 mM Tris/HCl buffer). Working formula: $y=A_0+((A-A_0)/(2*x\_2))*(x\_1+x\_2+1/K-((x\_1+x\_2+1/K)^2-4*x\_1*x\_2)^.5)$, x_1= [G], x_2 = [H], y = intensity, Y= absorbance.

Table S1. Binding constant values for 1 with the guests in CH_3CN: H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer).

Guests	$K_a(M^{-1})^a$	$K_a (M^{-1})^b$
$HP_2O_7^{3-}$	$(9.59 \pm 1) \times 10^4$	$(1.96 \pm 0.5) \times 10^5$
$P_2O_7^{4-}$	c	c
$H_2PO_4^-$	c	$(2.56 \pm 0.7) \times 10^4$
HSO_4^-	c	$(2.43 \pm 0.3) \times 10^4$
ClO_4^-	c	c
F^-	c	$(8.97 \pm 4) \times 10^3$
Cl^-	c	c
Br^-	c	c
I^-	c	c
Acetate	c	$(1.56 \pm 0.7) \times 10^4$

a: determined from fluorescence method; b: determined from UV
c: not determined due to irregular and minor changes.

5c. Selectivity study

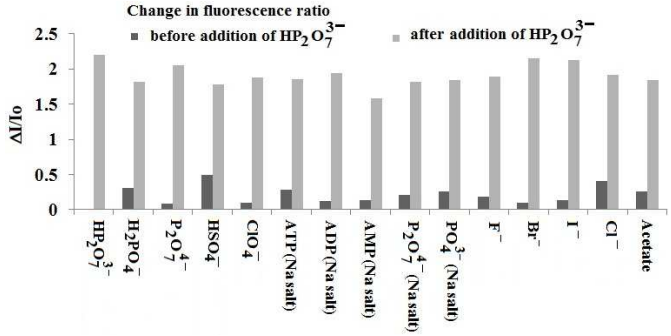


Figure S6c. Change in fluorescence ratio $[(I-I_0)/I_0]$ of **1** (6.27×10^{-5} M) at 412 nm upon addition of 5 equiv amounts of $(TBA)_3(HP_2O_7)$ in the presence of other anions (5 equiv) in CH_3CN-H_2O (4:1 v/v, 10mM Tris HCl buffer, at pH 6.5).

6. Change in fluorescence ratio for 1 at different pHs in CH_3CN:H_2O (4:1 v/v)

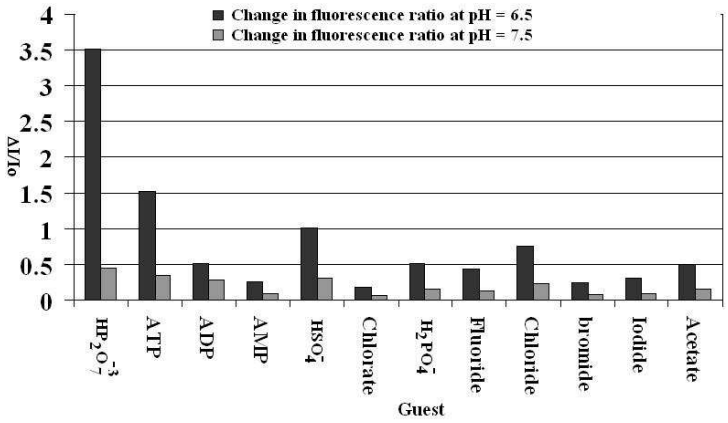


Figure S7. Fluorescence ratio $[(I-I_0)/I_0]$ of **1** (c = 6.27×10^{-5} M) at 412 nm upon addition of 10 equiv amounts of a particular anion in CH_3CN: H_2O (4:1 v/v 10 mM TrisHCl buffer) at pHs 6.5 and 7.5.

7. Change in fluorescence ratio for 1 with sodium salt of different anions and tetrabutylammonium hydrogenphosphate in at 412 nm in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer)

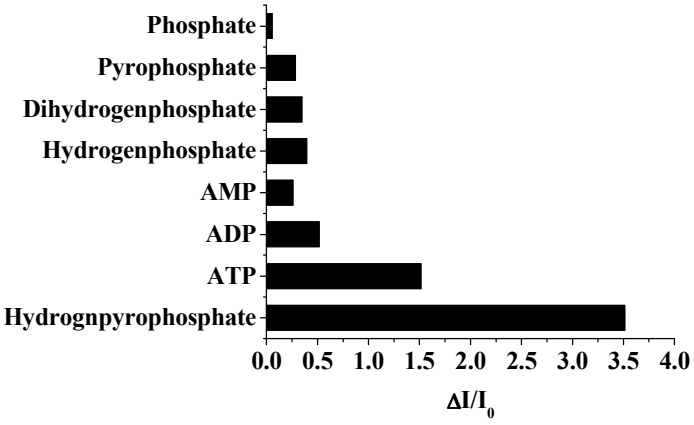


Figure S8. Fluorescence ratio $[(I - I_0) / I_0]$ of **1** (c = 6.27 x 10^{-5} M) at 412 nm upon addition of 10 equiv amounts of sodium salt of a particular anion and tetrabutylammonium $HP_2O_7^{3-}$ in CH_3CN: H_2O (4:1 v/v, pH 6.5, 10 mM Tris HCl buffer).

8. Indicator displacement experiments on 1 with Uranine dye:

From UV-vis and Fluorescence study

8a. Change in absorption upon gradual addition of 1 to the solution of uranine dye:

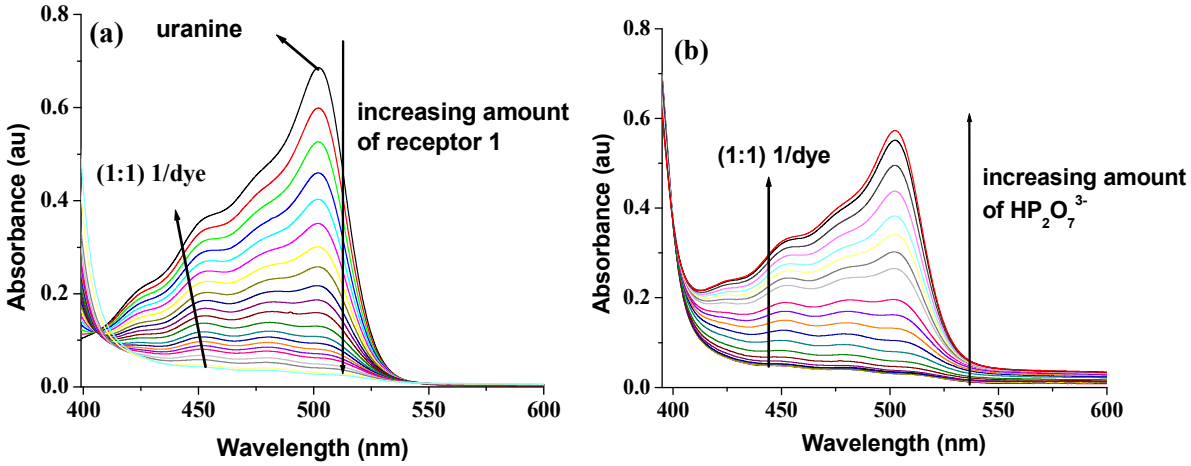


Figure S9. (a) Change in absorbance of **dye** (c = 8.5 x 10^{-5} M) upon the addition of increasing amount of **1** (c = 4.0 x 10^{-4} M), (b) gradual addition of $HP_2O_7^{3-}$ (c = 1.65 x 10^{-3} M) to the ensemble of dye **dye**/**1** (1:1). All titration are performed at 25 ^{0}C in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer).

8b. Dye displacement from the ensemble of 1/dye using various anions as their sodium and tetrabutylammonium salts and the corresponding changes in emission intensity.

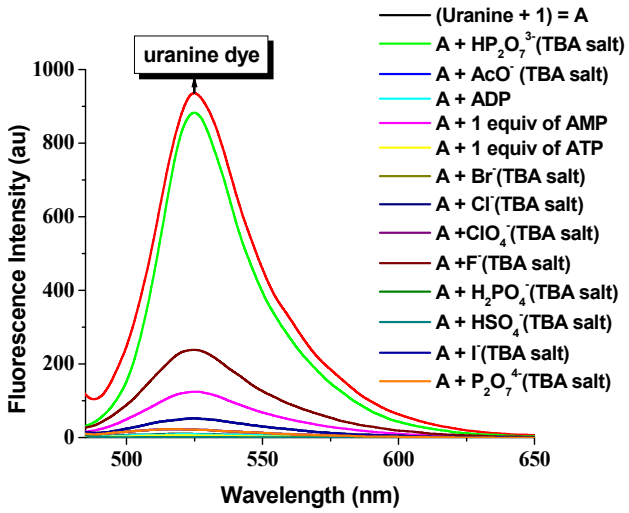


Figure S10. Change in emission intensity after addition of 1 equiv amount of different anions (G) to the ensemble of **1**/**dye** (1:1) ([G] = 4.62 x 10^{-3} M, [**dye**] = 8.5 x 10^{-5} M). All titration are performed at 25 ^{0}C in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer).

8c. Job plots for receptor 1 with uranine dye from fluorescence and UV-vis.

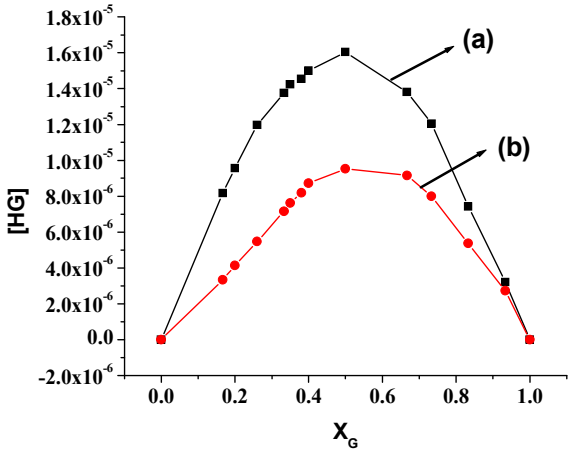


Figure S11. Job plots for **1** with **dye** from (a) Fluorescence at 525 nm and (b) UV-vis at 502 nm ([G] = [H] = 8.5 x 10^{-5} M) in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM Tris HCl buffer).

8d. Dye displacement from the ensemble of 1/dye using various anions as their sodium and tetrabutylammonium salts and the corresponding changes in absorption intensity.

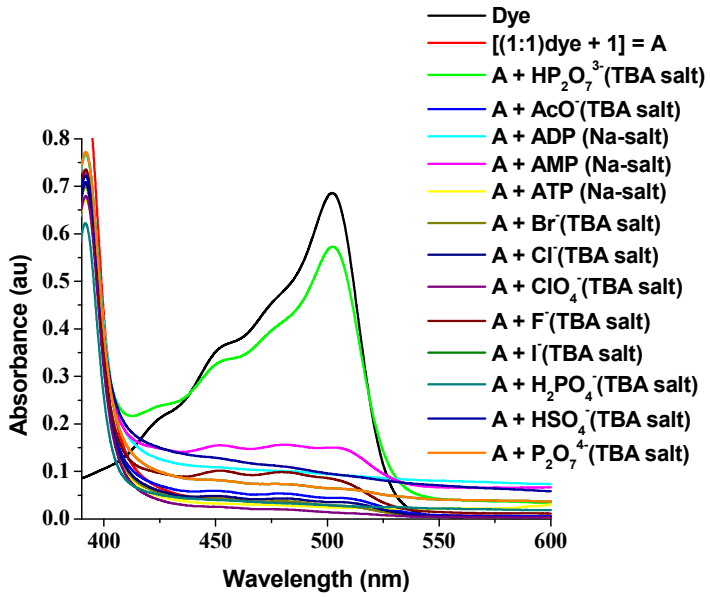


Figure S12. Change in absorption after addition of 1 equiv amount of different anions (G) to the ensemble of **1**/**dye** (1:1) ([G] = 4.62 x 10^{-3} M, [**dye**] = 8.5 x 10^{-5} M**)**. All titration are performed at 25 ^{0}C in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer).

8e. Binding constant for receptor 1 with Uranine dye in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM Tris/HCl buffer) from UV-vis.

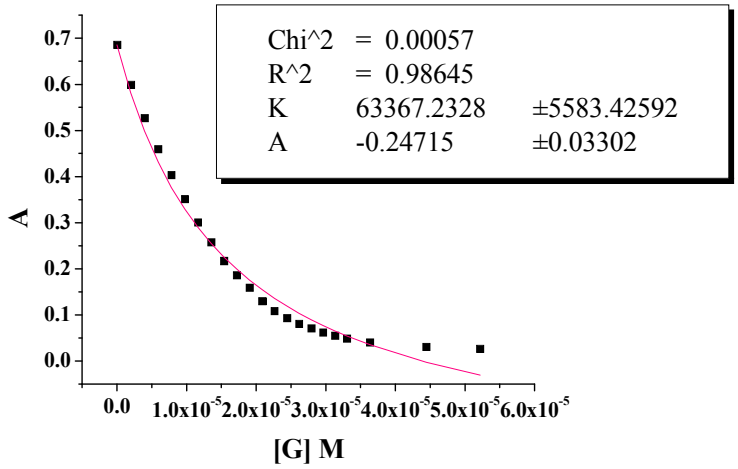


Figure S13. Binding constant at λ_{max} = 502 nm. of uranine **dye** (c = 8.5 x 10^{-5} M) with **1** (c = 4.0 x 10^{-3} M) at 25 ^{0}C [working formula: y=A_0+((A-A_0)/(2*x_2))*(x_1+x_2+1/K-((x_1+x_2+1/K)^2-4*x_1*x_2)^.5), x_1= [G], x_2 = [H], y = absorbance] in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM Tris HCl buffer).

.

8f. Binding constant for receptor 1 with uranine dye in $CH_3CN:H_2O$ (4:1 v/v, pH = 6.5, 10 mM Tris/HCl buffer) from fluorescence.

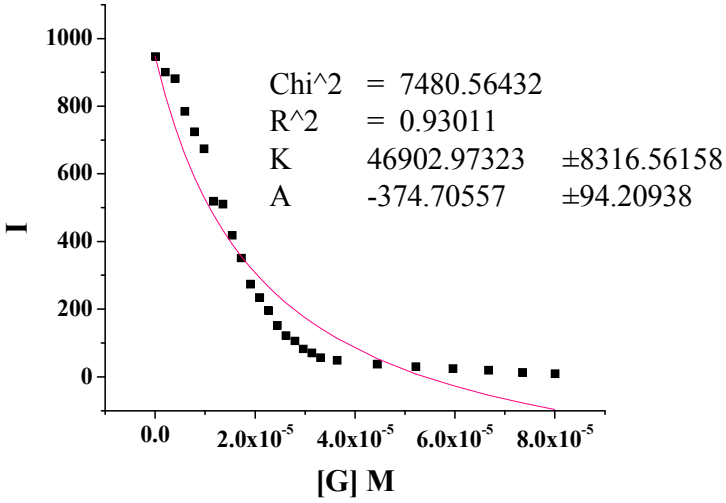


Figure S14. Binding constant at λ_{max} = 525 nm. of uranine **dye** (c = 8.5 x 10^{-5} M) with **1** (c = 4.0 x 10^{-3} M) at 25 ^{0}C [working formula: y=I_0+((I-I_0)/(2*x_2))*(x_1+x_2+1/K-((x_1+x_2+1/K)^2-4*x_1*x_2)^.5), x_1= [G], x_2 = [H], y = intensity] in $CH_3CN:H_2O$ (4:1 v/v, pH = 6.5, 10 mM Tris HCl buffer).

8g. ^{1}H study

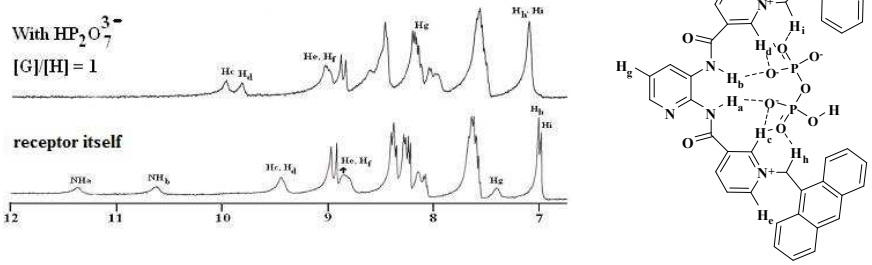


Figure S15. Partial ^{1}H NMR (400 MHz, d_6-DMSO) of (a) 1 (c = 1.86 x 10^{-3} M itself and (b) in the presence of equivalent amount of $(Bu_4N)_3HP_2O_7$.

8h. DFT optimized structure.[1]

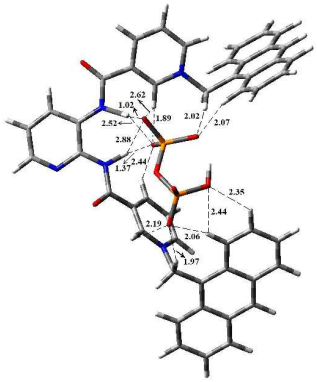


Figure S16. DFT optimized structure of the complex **1**.$HP_2O_7^{3-}$ (H-bond distances are in Å; shortest distance between two anthracenes: 5.85 Å).

9. Indicator displacement experiments on 2 with uranine dye:

From Fluorescence and UV-vis study

9a. Change in absorption and emission spectra upon gradual addition of 2 to the solution of dye 3 and then on addition of $HP_2O_7^{3-}$:

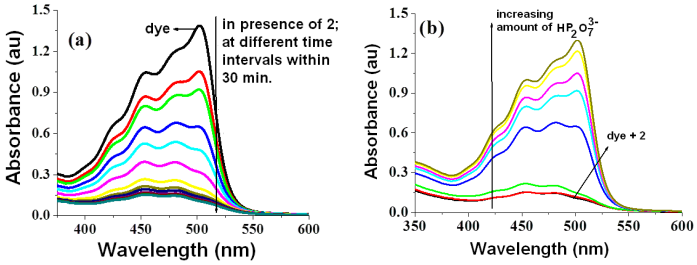


Figure S17. (a) Change in absorption intensity of dye **3** (c = 8.5 x 10^{-5} M) upon addition of **2** (5 mg) and (b) retrieval of absorption upon increasing addition of $(TBA)_3HP_2O_7$ to the ensemble **2**/dye **3** in CH_3CN / H_2O (4:1 v/v, pH = 6.5, 10 mM Tris HCl buffer).

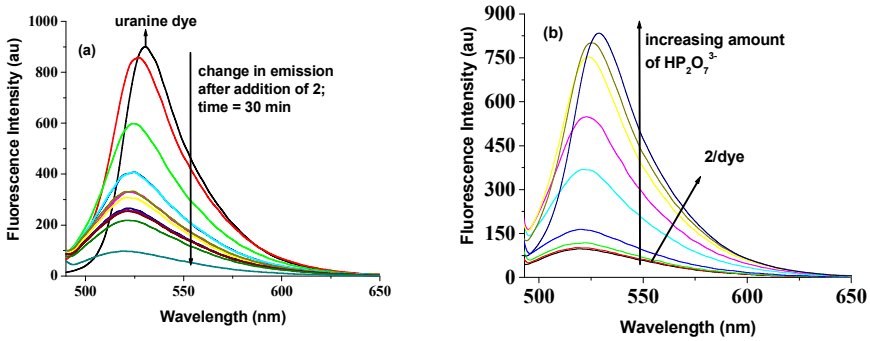


Figure S18. (a) Change in emission intensity of dye **3** (c = 8.5 x 10^{-5} M) upon addition of **2** (5mg) and (b) retrieval of emission upon increasing addition of $HP_2O_7^{3-}$ to the ensemble **2**/**dye** in CH_3CN: H_2O (4:1, v/v, pH 6.5, 10 mM Tris/HCl buffer) after 30 min.

9b. Dye displacement from the ensemble of 2/dye using various tetrabutylammonium salts and the corresponding changes in emission intensity.

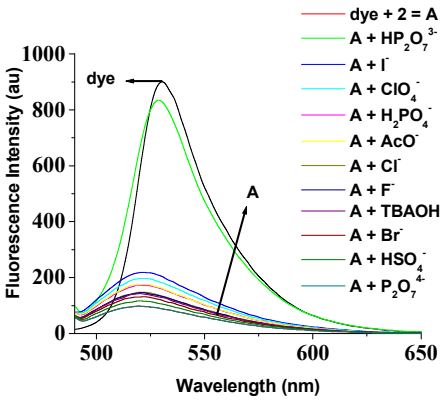


Figure S19. Change in emission intensity after addition of 1 equiv amount of different tetrabutylammonium anions (G) to the ensemble of **2**/**dye** (5 mg in 2.5 mL dye solution) ([G] = 1.65 x 10^{-3} M, [**3**] = 8.5 x 10^{-5} M). All titration are performed at 25 ^{0}C in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer).

9c. Dye displacement from the ensemble of 2/dye using various tetrabutylammonium salts and the corresponding changes in absorption intensity.

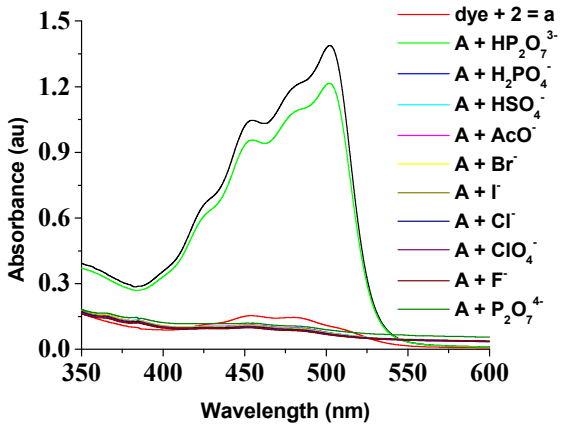


Figure S20. Change in absorption intensity after addition of 1 equiv amount of different tetrabutylammonium anions (G) to the ensemble of **2**/**dye** (5 mg in 2.5 mL dye solution) ([G] = 1.65 x 10^{-3} M, [**3**] = 8.5 x 10^{-5} M). All titration are performed at 25 ^{0}C in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer).

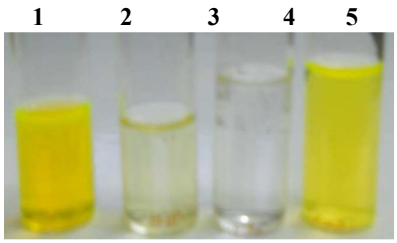


Figure S21. (1) **dye**, (2) [**dye** + **2**] = A, (3) [A + all anions (except hydrogen pyrophosphate)] = B, (4) B + hydrogen pyrophosphate in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer).

9d. Dye displacement from the ensemble of 2/dye using various sodium salts, tetrabutylammonium hydroxide, tetrabutylammonium hydrogen pyrophosphate and the corresponding changes in absorption intensity.

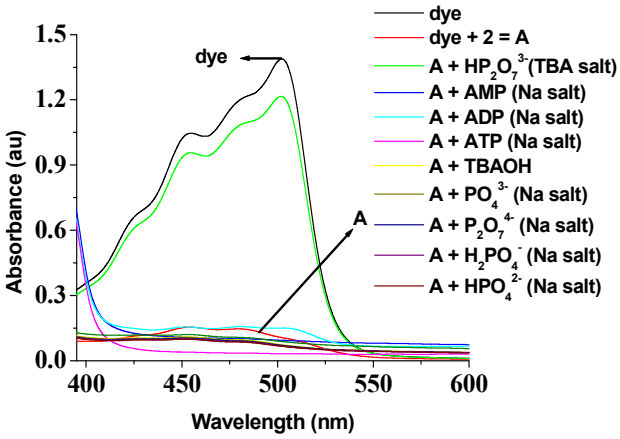


Figure S22. Change in absorption intensity after addition of 1 equiv amount of different sodium salt, tetrabutylammonium $HP_2O_7^{3-}$ and tetrabutylammonium hydroxide to the ensemble of **2**/**dye** (5 mg in 2.5 mL dye solution) ([G] = 1.65 x 10^{-3} M, [**3**] = 8.5 x 10^{-5} M). All titration are performed at 25 ^{0}C in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer).

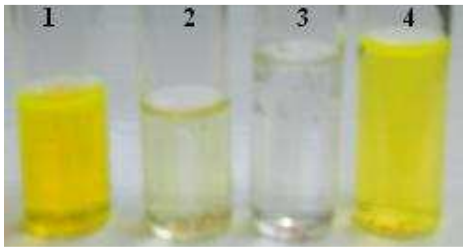


Figure S23. (1) **dye**, (2) [**dye** + **2**] = A, (3) [A + all anions Na salt of ATP, ADP, AMP, $H_2PO_4^-$, HPO_4^-, $P_2O_7^{4-}$, PO_4^{3-} and tetrabutylammonium hydroxide (except TBA hydrogen pyrophosphate)] = B, (4) B + hydrogen pyrophosphate in CH_3CN:H_2O (4:1 v/v, pH = 6.5, 10 mM TrisHCl buffer)..

9e. Absoption spectra, emission spectra and SEM images of 2 in the solid state before and after exploitation in IDA experiment.

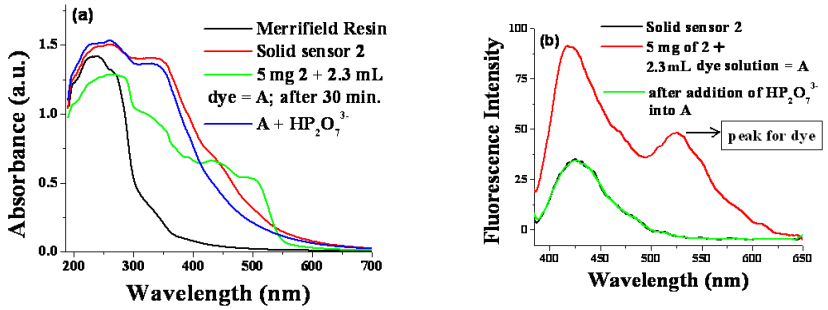


Figure S24. Comparative view of indicator displacement experiment of **2** from (a) UV-vis and (b) fluorescence (λ_{ex} = 370 nm) in solid state.

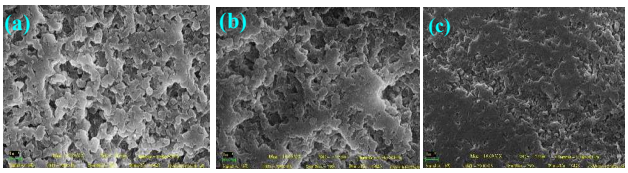


Figure S25. SEM images of (a) **2**, (b) 5 mg of **2** incubated in 2.3 mL **dye** solution (c = 8.5 x 10^{-5} M) and (c) **2** when $HP_2O_7^{4-}$ was added into the solution of **2** / **dye** ensemble.

10. Change in absorption intensity upon gradual addition of 2 to the solution of dye 3 in H_2O (pH = 6.5, 10mM Tris HCl buffer):

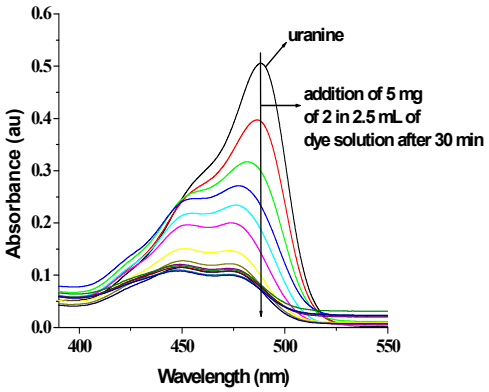


Figure S26. Change in absorbance of dye **3** (c = 2.0 x 10^{-5} M) on addition of **2** (5 mg).

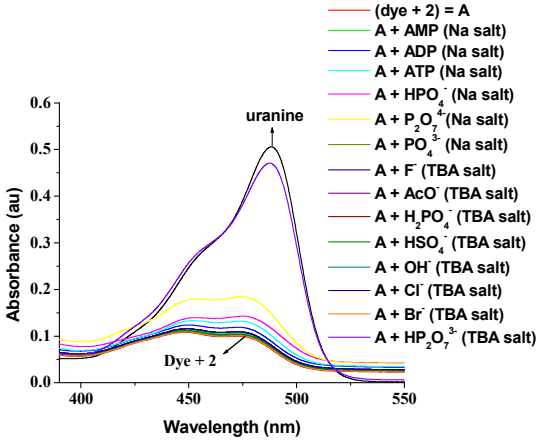


Figure S27. Change in absorption
1 equiv amount of different tetrabu
salts (G) to the ensemble of **2**/**dy**
solution) ([G] = 1.65 x 10^{-3} M,
titration are performed at 25 ^{0}C i
TrisHCl buffer).

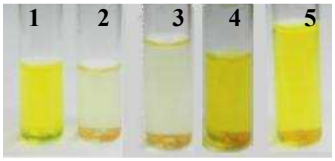


Figure S28. (1) **dye**, (2) [**dye** + **2**] = A, (3) [A + Na salt of ATP, ADP, AMP, HPO_4^-, $P_2O_4^{4-}$, PO_4^{3-} and tetrabutylammonium hydroxide , $H_2PO_4^-$, Acetate, F^-, Br^-, Cl^-, HSO_4^- (except TBA hydrogen pyrophosphate)] = B, (4) A + hydrogen pyrophosphate and (5) B + hydrogen pyrophosphate in H_2O (pH = 6.5, 10 mM TrisHCl buffer).

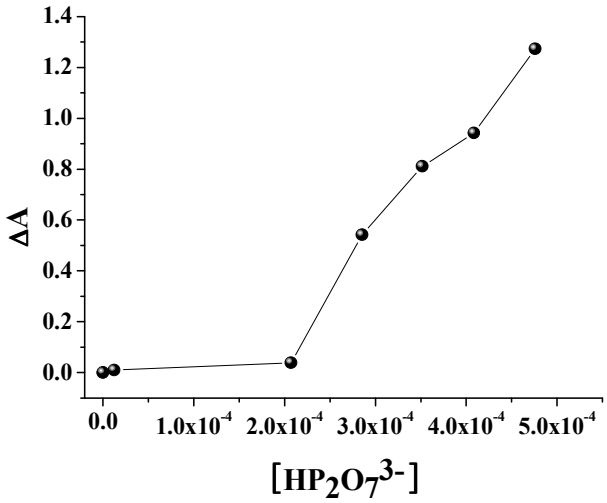


Figure S29. Change in absorption intensity (λ_{max} = 502 nm) for ensemble **2**/**dye** (5 mg in 2.5 mL dye solution; [dye] = 8.5 x 10^{-5} M) with increasing amount of tetrabutylammonium hydrogenpyrophosphate in 4:1 CH_3CN : H_2O (v/v) at pH 6.5 using 10 mM Tris HCl buffer.
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11. Experiments with serum

Materials and method

Animals

We randomly selected healthy inbred strain of Swiss albino mice (*Mus musculus*) (6/8 weeks: 25 g), housed for at least 14 days in an environmentally controlled room (temperature, 24–26 ± 2ºC; humidity, 55 ± 5%, 12-h light/dark cycle) with access to food and water *ad libitum* as the materials for the present study. We followed the guidelines approved by the Animal Ethics Committee, University of Kalyani, and conducted the experiments under the supervision of the Animal Welfare Committee, University of Kalyani (Vide: Certificate for Proposal No. KU/IAEC/Z-11/07, dated 18.5.2007).

Collection of blood serum

Three mice were sacrificed and blood was collected after cardiac puncture. The blood samples were allowed to stand for 10 min after which it was centrifuged at 2000g for 5min and serum was collected for the further experimental purpose.

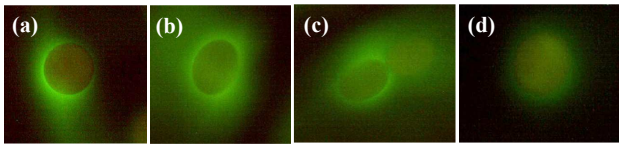

Figure S30. dye/**2** ensemble in serum contaminated with different concentrations of tetrabutylammonium hydrogen pyrophosphate: (a) 10^{-3} M, (b) 10^{-4} M, (c) 10^{-5} M and (d) 10^{-6} M.

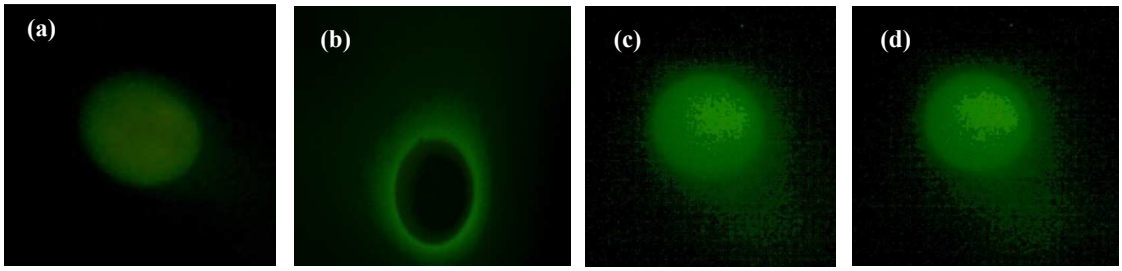

Figure S31. dye/**2** ensemble in serum contaminated with sodium salt of (a) ADP, (b) ATP, (c) HPO_4^{-2} and (d) PO_4^{-3} in neutral medium (concentrations of all guests were 1.65 x 10^{-3} M).

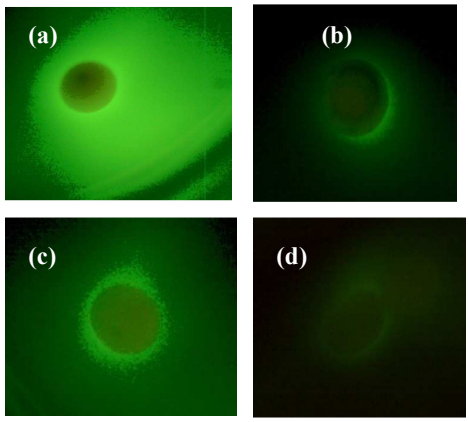

Figure S32. dye/**2** ensemble in serum contaminated with sodium salt of pyrophosphate. (a) concentration of pyrophosphate 10^{-4} M in neutral medium (b) concentration of pyrophosphate 10^{-4} M in acidic medium pH = 6.5, (c) concentration of pyrophosphate 10^{-5} M in neutral medium and (d) concentration of pyrophosphate 10^{-5} M in acidic medium pH = 6.5.

Reference

1. Gaussian 03, Revision C.01, Frisch M. J. *et al*., Gaussian, Inc., Wallingford CT, 2004.