Pyridinium-based flexible tripodal cleft: A case of fluorescence sensing of ATP and dihydrogenphosphate under different conditions and cell imaging

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1. Change in emission of receptor 1 with various anions of sodium salt in CH\textsubscript{3}CN-H\textsubscript{2}O (1:1/v/v, using 10 mM HEPES, pH-6.5).

![Graphs showing change in emission of receptor 1 with various anions of sodium salt](image)

**Figure 1S.** Change in emission of 1 \((c = 2.5 \times 10^{-5}\text{ M})\) in CH\textsubscript{3}CN-H\textsubscript{2}O (1:1/v/v, using 10 mM HEPES, pH = 6.5) upon addition of 30 equiv. amounts of (a) ADP, (b) Na\textsubscript{2}HPO\textsubscript{4}, (c) AMP, (d) NaPO\textsubscript{4}, (e) NaH\textsubscript{2}PO\textsubscript{4}, (f) Na\textsubscript{2}P\textsubscript{2}O\textsubscript{7}, (g) glucose-1-phosphate (G1P), (h) glucose-6-phosphate (G6P) [concentration of anions of sodium salts were 1 \times 10^{-3}\text{ M}].
2. Interference Study in the binding of ATP

Figure 2S. (a) Change in fluorescence ratio of 1 (c = 2.5 x 10⁻⁵ M) in absence and presence of 20 equiv. amounts of ATP in presence of sodium salts of various anions in CH₃CN–H₂O (1:1 v/v, pH = 6.5, 10 mM HEPES buffer); (b) change in fluorescence intensity of 1 in CH₃CN–H₂O (1:1 v/v, pH = 6.5, 10 mM HEPES buffer) upon addition of 20 equiv. amounts of ATP in presence of other anions; (c) Fluorescence titration spectra of 1 (c = 2.5 x 10⁻⁵ M) with tetrabutylammonium fluoride (c = 1.0 x 10⁻³ M).

3. Benesi-Hilderband plot for receptor 1 with ADP.

Figure 3S. (a) Benesi-Hilderband plot for receptor 1 (c = 2.5 x 10⁻⁵ M) with ADP ([ADP] = 1 x 10⁻³ M) at 390 nm; (b) Detection limit for ATP in CH₃CN-H₂O (1:1 v/v, using 10 mM HEPES, pH = 6.5).
4. Change in absorbance of receptor 1 with various anions of sodium salt in CH$_3$CN-H$_2$O (1:1/v/v, using 10 mM HEPES, pH = 6.5).

Figure 4S. Change in absorbance of 1 (c = 2.5 x 10$^{-5}$ M) in CH$_3$CN-H$_2$O (1:1/v/v, using 10 mM HEPES, pH-6.5) upon addition of 10 equiv. amounts of (a) ATP, (b) ADP, (c) AMP, (d) NaH$_2$PO$_4$, (e) Na$_2$HPO$_4$, (f) Na$_3$PO$_4$, (g) Na$_4$P$_2$O$_7$, (h) G1P, (i) G6P [concentration of anions of sodium salts were 1 x 10$^{-5}$ M].
5. Change in emission of receptor 1 with various anions of tetrabutylammonium salts in CH$_3$CN.

Figure 5S. Change in emission of 1 (c = 2.2 x 10^{-5} M) in CH$_3$CN upon addition of 2 equiv. amounts of (a) OAc$^-$, (b) Cl$^-$ (c) Br$^-$, (d) I$^-$, (e) F$^-$, (f) HP$_2$O$_7$$^{2-}$, (g) NO$_3$-, (h) ClO$_4$$, (i) HSO$_4$- and (j) H$_2$PO$_4$ $^-$ [anions were taken as their tetrabutylammonium salts and their concentrations were 8 x 10^{-4} M].
6. Interference study towards binding of H$_2$PO$_4^-$ ion in CH$_3$CN

Figure 6S. Change in emission of I ($c = 2.2 \times 10^{-5}$ M) upon addition of 2 equiv. amounts of tetrabutylammonium dihydrogenphosphate (TBADHP) ($c = 8 \times 10^{-4}$ M) in presence of 2 equiv. amounts of other anions as their tetrabutylammonium salts.

7. Change in absorbance of receptor 1 with various anions of tetrabutylammonium salts in CH$_3$CN.
Figure 7S. Change in absorbance of 1 \((c = 2.2 \times 10^{-5} \text{ M})\) in CH$_3$CN upon addition of 10 equiv. amounts of (a) H$_3$PO$_4^-$, (b) F$, (c) Cl$, (d) Br$, (e) I$, (f) OAc$, (g) HP$_2$O$_7^{3-}$, (h) NO$_3^-$, (i) ClO$_4^-$, (j) HSO$_4^-$ [anions were taken as their tetrabutylammonium salts and their concentrations were $8 \times 10^{-4}$ M].

8. Fluorescence Job plot of receptor 1 with H$_2$PO$_4^-$ in CH$_3$CN.

Figure 8S. Fluorescence Job’s plot for receptor 1 with H$_2$PO$_4^-$ in CH$_3$CN measured at 455 nm ([H] = [G] = $2.5 \times 10^{-5}$ M).
9. Benesi-Hilderbrand plot for receptor 1 with $\text{H}_2\text{PO}_4^-$.

![Figure 9S](image)

**Figure 9S.** (a) Binding constant curve for receptor 1 ($c = 2.2 \times 10^{-5}$ M) with $\text{H}_2\text{PO}_4^-$ ($c = 8 \times 10^{-4}$ M); (b) Detection limit for $\text{H}_2\text{PO}_4^-$ in CH$_3$CN.

10. NOESY spectrum

![Figure 10S](image)

**Figure 10S.** NOESY spectrum of $\text{1.H}_2\text{PO}_4^-$ (d$_6$-DMSO/D$_2$O, 400 MHz) ($c = 1.83 \times 10^{-3}$ M).
11. Change in $^1$H NMR of 1 in presence of H$_2$PO$_4$.$^-$.

![Diagram of molecular interactions](image)

**Figure 11S.** Partial $^1$H NMR (d$_6$-DMSO, 400 MHz) of 1 (c = 1.83 x 10$^{-3}$ M) with equiv. amount of TBADHP. The mode of interaction is shown above. Dash lines indicate the possible weak H-bond interactions or short contacts in the core in a symmetric fashion.

12. MTT assay for receptor 1.

![MTT assay graph](image)

**Figure 12S.** MTT assay of receptor 1.
$^1$H NMR of 1 (400 MHz, d$_6$-DMSO):
$^{13}$C NMR of 1 (100 MHz, d$_6$-DMSO):
HRMS OF 1: