RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Effective Discrimination of GTP from ATP by a Cationic Tentacle Porphyrin through "Turn-On" Fluorescence Intensity

Suneesh C. Karunakaran,[a] Albish K. Paul[a] and Danaboyina Ramaiah[a,b]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

A cationic tentacle porphyrin selectively recognises guanosine (G) based nucleotides and which was effectively utilized as a fluorescence "turn-on" probe to discriminate GTP from ATP through fluorescence indicator displacement (FID) assay in the aqueous medium.

Selective recognition of nucleotides, especially in the aqueous media, has gained immense attention, as they form the fundamental units of all the life forms.[1] Among the various nucleotides, the selective detection and quantification of the guanosine based nucleotides, especially guanosine-5'-triphosphate (GTP) (Chart 1) is vital due to its specific functions in various biological processes.[2] For example, GTP is required for the synthesis of DNA, RNA, and proteins, and also in nutrient metabolism, and cell signalling.[3] The abnormalities in the concentrations of ATP and GTP in the body due to the defect of purine salvage enzymes, particularly, adenosine phosphoribosyl transferase (APRTase) and hypoxanthine phosphoribosyl transferase (HPRTase), result in severe combined immuno-deficiency disorder (SCID) and Lesch-Nyhan syndrome (brain gout), respectively.[4]

Most of the probes reported for the detection of nucleotides utilise complementary hydrogen bonding for the recognition. Such a recognition mechanism would be limited in the aqueous medium due to either involvement of competitive hydrogen bonding from the solvent[5] or the presence of sugar moiety of the nucleosides and nucleotides.[6] Therefore, the development of probes that are devoid of hydrogen bonding and selectively recognise nucleosides or nucleotides in the aqueous medium is quite challenging. In this context, we have synthesized a water soluble cationic porphyrin **PyP** and its zinc complex **Zn-PyP** (Chart 1), and have investigated their interactions with various nucleosides and nucleotides in phosphate buffer medium. Our results demonstrate that, the porphyrin **PyP** exhibits selective interactions with the guanosine (G) based nucleotides when compared to other nucleotides and nucleosides through changes in its fluorescence intensity. Uniquely, this system in combination with the fluorescence indicator displacement (FID) assay can be effectively utilized to develop a fluorescence "turn-on" probe for the selective detection of the G based nucleotides and also to discriminate GTP from ATP in the aqueous medium.

The pyridinium appended cationic porphyrin **PyP** was synthesized through the modified Lindsey's method,[7] while its zinc complex, **Zn-PyP** was synthesized from 5,10,15,20-tetrakis[4-(8-bromooctyloxy)phenyl]porphyrin. Reaction of the starting bromoporphyrin derivative with zinc acetate in methanol at 25 °C gave the zinc complex in *ca.* 80% yield. Subsequently, it was substituted with pyridine by refluxing at100 °C to give the pyridinium appended complex **Zn-PyP** in *ca.* 65% yield. The starting materials as well as the products **PyP** and **Zn-PyP** were purified through recrystallization and were characterized on the basis of spectral and analytical evidence (ESI†). The free base **PyP** showed the characteristic porphyrin absorption in the region 419 - 654 nm, and fluorescence emission in the region 665 - 727 nm.[8] Similarly, the zinc complex, **Zn-PyP** exhibited a bathochromically shifted (*ca.* 6 nm) Soret absorption at 425 nm and two Q-bands at 560 and 600 nm, while its emission spectrum showed two emission peaks centered at 615 and 655 nm (Figure S1, ESI†). The quantum yields of the fluorescence (Φ_F) of these derivatives were calculated using tetraphenylporphyrin (**TPP**) as the reference ($\Phi_F = 0.11$ in toluene)[9] and are found to be 0.10 and 0.03 ± 0.002, respectively for **PyP** and **Zn-PyP** in water.

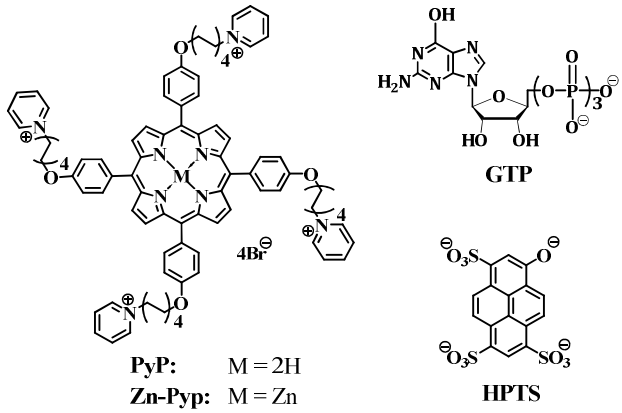


Chart 1

To understand the ability of these cationic porphyrin systems as probes for the nucleotides, we have investigated their interactions through absorption and fluorescence spectroscopy. Addition of guanosine 5'-triphosphate (GTP) to a solution of **PyP** (5 μM) resulted in gradual decrease in the Soret band absorption at 419 nm (Figure 1A). At *ca.* 450 μM of GTP, we observed *ca.* 66% hypochromicity along with 15 nm bathochromic shift in the absorption maximum of **PyP**. In the emission spectrum, we observed *ca.* 86% quenching of fluorescence intensity of **PyP** with the addition of 450 μM of GTP (Figure 1B). Similar

experiments were carried out with guanosine 5'-diphosphate (GDP) and guanosine 5'-monophosphate (GMP) under identical conditions. We observed *ca.* 37% hypochromicity with 10 nm bathochromic shift in the absorption and *ca.* 83% quenching in fluorescence intensity of **PyP** (Figure S2, ESI†) at 450 μM of GDP. Whereas with GMP under similar conditions, we observed *ca.* 16% hypochromicity with 4 nm bathochromic shift in absorption and *ca.* 16% quenching in the fluorescence intensity of **PyP** (Figure S3, ESI†). The Benesi-Hildebrand analysis of the absorption changes (Inset of Figure 1A) gave a 1:1 stoichiometry for the complex formed between **PyP** and GTP with a binding constant of K_{ass} = 8.46 ± 0.2 × 10^3 M^{-1} in water, while relatively lower values of K_{ass} = 6.75 and 4.13 ± 0.15 × 10^3 M^{-1} were observed for GDP and GMP, respectively.

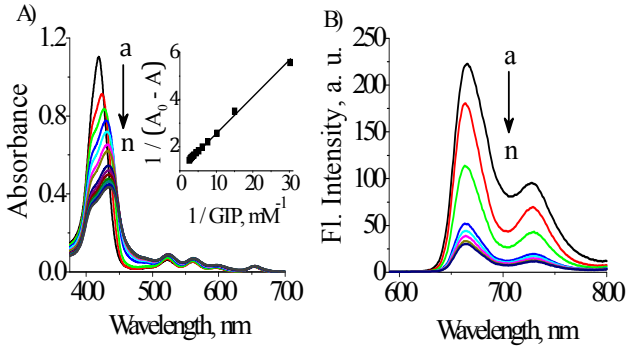


Figure 1. Changes in the (A) absorption and (B) emission spectra of **PyP** (5 μM) with the addition of GTP in phosphate buffer (pH 7.4, 10 mM KH_2PO_4, 2 mM NaCl). [GTP], (a) 0 and (n) 450 μM. Inset shows Benesi-Hildebrand plot for the binding of GTP with **PyP**. λ_{ex}, 430 nm.

To investigate the selectivity of the recognition, we have carried out the interactions of the probe **PyP** with other nucleotides such as adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP), adenosine 5'-triphosphate (ATP) (Figure S4, ESI†), cytidine 5'-triphosphate (CTP) and uridine-5'-triphosphate (UTP). Under similar experimental conditions and with the addition of these nucleotides, **PyP** showed negligible changes in its absorption and fluorescence properties. Figure 2 shows the relative changes in the absorbance of **PyP** as a function of concentration of various nucleotides. It is evident from Figure 2 that the porphyrin **PyP** shows selectivity towards the G-based nucleotides and in the order GTP > GDP > GMP. In contrast, all other nucleotides exhibited negligible interactions with **PyP**. Similarly, we have also investigated the potential of the zinc complex **Zn-PyP** as a probe for various nucleotides. We observed that **Zn-PyP** shows negligible selectivity and interactions with all the nucleotides tested under identical conditions (Figure S5, ESI†).

To understand the mode of binding of the G based nucleotides with **PyP**, we have investigated the effect of ionic strength of the buffer medium on the complexation process. The decrease in absorbance of **PyP** in the presence of these nucleotides was found to be less prominent as we increased the ionic strength of the buffer from 20 to 100 mM (Figure S6, ESI†). Based on the experimental evidence and literature reports,[10] the mode of binding of **PyP** with the G based nucleotides could be attributed to the synergistic effects of both electrostatic and π-π stacking interactions. The aromatic guanine moiety of the nucleotides can undergo π-stacking interactions with the porphyrin phenyl ring, while the phosphate groups can involved in electrostatic interactions with the appended pyridinium groups.

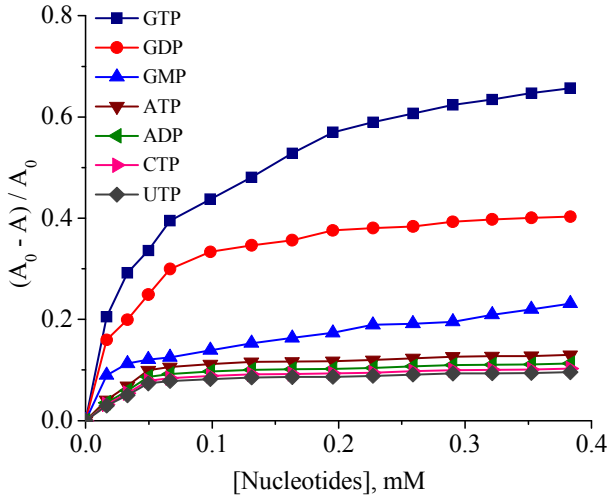


Figure 2. Relative changes in the absorbance of the porphyrin derivative **PyP** (5 μM) in the presence of various nucleotides.

To improve the sensitivity of detection of the G based nucleotides using **PyP**, we adopted a fluorescent indicator displacement (FID) assay[11] using a highly fluorescent indicator, 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS; Φ_F = 0.7). The FID assay involves the reversible binding of a fluorescence indicator with a receptor followed by a competitive binding of analyte with the receptor resulting in the displacement of the fluorescence indicator. The successive additions of the porphyrin **PyP** to a solution of HPTS (4.5 μM) in buffer resulted in a regular quenching of the fluorescence intensity of HPTS centered at 514 nm (Figure 3A). At *ca.* 5.25 μM of **PyP**, we observed complete quenching in fluorescence intensity of HPTS. The changes in the optical properties of HPTS in the presence of the cationic porphyrins are indicative of the formation a stable complex between the negatively charged HPTS and positively charged appended pyridinium moieties of the porphyrin **PyP**. We have done Benesi-Hildebrand analysis of the emission data and which gave a 1:1 stoichiometry for the complex [**PyP**•HPTS], with an association constant (K_{ass}) of 4.66 ± 0.2 × 10^4 M^{-1}. The complexation between **PyP** and HPTS was further analyzed through the picosecond time-resolved fluorescence spectroscopy. For example, HPTS alone exhibited a single exponential fluorescence decay with a lifetime of 5.3 ns, whereas, we observed a biexponential decay with lifetimes of 2.2 ns (71%) and 5.5 ns (29%) in the presence of **PyP** (Figure 3B).

To understand the nature and strength of the complex formed between **PyP** and HPTS, we have investigated the effects of ionic strength (Figure S7, ESI†) and temperature on the complexation process. For instance, the quenching of emission of HPTS by **PyP** was found to be less prominent as we increased the ionic strength of the buffer. We obtained a lower value of K_{ass} = 1.9 × 10^4 M^{-1} at higher ionic strength of the buffer (1 M). These observations confirm that the pyridinium units of the porphyrin derivative **PyP** are shielded from the sulfonate groups of HPTS by Na^+ ions, resulting in less favorable interactions between **PyP** and HPTS.

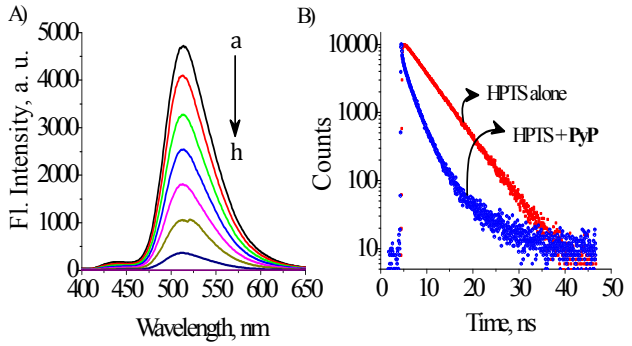


Figure 3. (A) Changes in emission spectra of HPTS (4.5 μM) with gradual addition of **PyP** in phosphate buffer (pH 7.4, 10 mM KH_2PO_4, 2 mM NaCl). [**PyP**] (a) 0 and (h) 5.25 μM. λ_{ex}, 364 nm. (B) Fluorescence decay profiles of HPTS (4.5 μM) and the complex [**PyP•**HPTS] collected at 515 nm. [**PyP**] 5.25 μM. λ_{ex}, 375 nm.

On the other hand, when the temperature of the complex [**PyP•**HPTS] was raised from 298 to 358 K, we observed negligible increase in the fluorescence intensity of HPTS, indicating thereby the stability of the complex [**PyP•**HPTS] at these temperatures.

The beneficial competitiveness of the FID assay was demonstrated by comparing the efficiency of fluorescence indicator displacement by various nucleotides. Figure 4A shows regular release of HPTS from the complex [**PyP•**HPTS] by the addition of GTP. The successive additions of GTP resulted in a regular enhancement in the fluorescence intensity corresponding to HPTS at 512 nm. In buffer, *ca*. 350-fold enhancement was observed at 450 μM of GTP. Similar experiments were carried out with the additions of other G based nucleotides, GDP and GMP to a buffer solution of [**PyP•**HPTS]. Both these nucleotides were also found to induce the revival of the fluorescence intensity, corresponding to HPTS at 512 nm upon interaction. We observed *ca*. 300 and 210-fold enhancement in fluorescence intensity of [**PyP•**HPTS] with the addition of 450 μM of GDP and GMP, respectively, in buffer. In contrast, addition of other nucleotides such as ATP, ADP, AMP, CTP and UTP showed negligible enhancement in the fluorescence intensity of HPTS at 512 nm. Relative concentration dependent enhancement of the

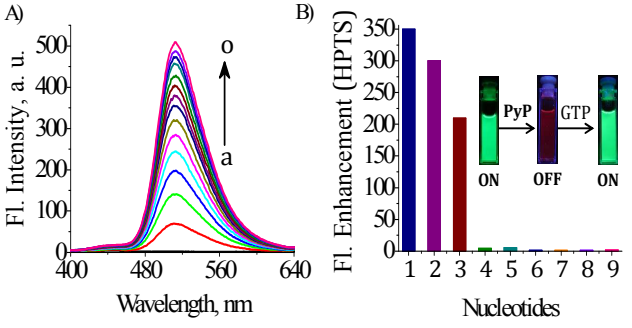


Figure 4. (A) Fluorescence indicator displacement (FID) from the complex [**PyP•**HPTS] by GTP in buffer. [GTP], (a) 0 and (o) 450 μM. λ_{ex}, 364 nm. (B) Relative concentration dependent enhancement of fluorescence intensity of [**PyP•**HPTS] complex by various nucleotides in phosphate buffer. Inset shows the visual observation of fluorescence intensity of HPTS alone, [**PyP•**HPTS] and [**PyP•**HPTS] in the presence of GTP in phosphate buffer. 1-9) GTP, GDP, GMP, ATP, ADP, AMP, CTP, TMP and UTP (450 μM).

fluorescence intensity of [**PyP•**HPTS] by the addition of various nucleotides is shown in Figure 4B. This selective interaction of **PyP** with guanosine based nucleotides was exploited for the development of a visual fluorescent system for the selective recognition of G-based nucleotides (Inset of Figure 4B) through a fluorescence "turn on" mechanism using FID assay. The sensitivity or limit of detection (LOD) of GTP by the [**PyP-**HPTS] system was calculated and is found to be 92 ppb (2.3 μM) in phosphate buffer (Figure S8, ESI†). As expected, **Zn-PyP** showed neither prominent enhancement in fluorescence intensity of [**Zn-PyP•**HPTS], nor exhibited selectivity towards G-based nucleotides Figure S9, ESI†.

The displacement of HPTS from the complex [**PyP•**HPTS] by G-based nucleotides was confirmed by the picosecond time-resolved fluorescence studies. When GTP was added to the complex [**PyP•**HPTS], we observed a biexponential decay with lifetimes 5.4 ns (98%) and 9.6 ns (2%) (Figure S10, ESI†). The former life time has been attributed to the free HPTS in solution. Similar observations were made with the addition of GDP and GMP. In contrast, negligible changes in the lifetime of the complex [**PyP•**HPTS] was observed with the addition of other nucleotides, which showed negligible affinity for the porphyrin **PyP**. The selectivity of the cationic porphyrin **PyP** for G-based nucleotides can be explained on the basis of their better electron cloud and low ionization potential when compared to other nucleotides, which facilitates the complex formation through synergistic electronic, π-stacking, and electrostatic interactions.

In conclusion, we investigated the biomolecular recognition properties of a novel cationic porphyrin having pyridinium substitution, **PyP** and its zinc complex **Zn-PyP**, in the aqueous medium. Interestingly, the free base porphyrin **PyP** showed selective interactions with the G-based nucleotides, even in the presence of other nucleotides, through the synergistic effects of both electrostatic and π-π stacking interactions. The unique property of **PyP** was effectively utilized to develop a fluorescence "turn on" probe for the selective recognition of G-based nucleotides as well as to discriminate GTP from ATP through the fluorescence indicator displacement assay using HPTS as the fluorescent indicator. To the best of our knowledge, this is the first report, which describes a cationic porphyrin which can be used as a probe for the on-site visual detection of G-based nucleotides in the aqueous medium.

This work was supported by CSIR, India (CSC-0134) and CSIR-National Institute for Interdisciplinary Science and Technology, Trivandrum. This is Contribution No. PPG-357 from CSIR-NIIST, Trivandrum.

Notes and references

[a]*Photosciences and Photonics, Chemical Sciences and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Trivandrum–695019, Kerala, India*
[b]*CSIR-North East Institute of Science and Technology (CSIR-NEIST) Jorhat - 785 006, Assam, India*
E-mail: rama@niist.res.in, d.ramaiah@gmail.com

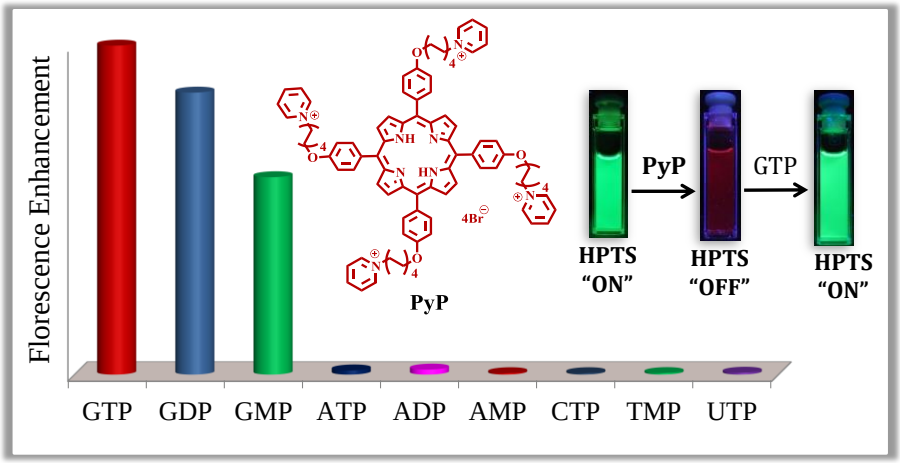
†Electronic Supplementary Information (ESI) available: Details of synthesis, calculations and Figures S1-S9 showing changes in the photophysical properties of the cationic pophyrins in the presence of various nucleotides are available in the supporting information See DOI: 10.1039/b000000x/

1. Y. Zhou, Z. Xu and J. Yoon, *Chem. Soc. Rev.*, 2011, **40**, 2222–2235; A. P. de Silva, H. Q. Gunaratne, T. Gunnlaugsson, A. J. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.* 1997, **97**, 1515-1566; M. Strianese, S. Milione, A. Maranzana, A. Grassi and C. Pellecchia, *Chem. Commun.*, 2012, **48**, 11419-11421; L. E. Santos-Figueroa, M. E. Moragues, E. Climent, A. Agostini, R. Martinez-Manez and F. Sancenon, *Chem. Soc. Rev.* 2013, **42**, 3489-3613; E. Kuruvila, J. Joseph and D. Ramaiah, *J. Phys. Chem. B* 2005**, 109**, 21997-22002.
2. N. Wu, J. Lan, L. Yan and J. You, *Chem. Commun.*, 2014, 50, 4438-4441; P. P. Neelakandan, M. Hariharan and D. Ramaiah, *Org. Lett.*, 2005, **7**, 5765-5768; P. P. Neelakandan, P. C. Nandajan, B. Subymol and D. Ramaiah, *Org. Biomol. Chem.*, 2011, **9**, 1021-1029; E. Kuruvila and D. Ramiah, *J. Phys. Chem. B* 2007**, 111**, 6549-6556; E. Kuruvila, P. C. Nandajan, G. B. Schuster and D. Ramaiah, *Org. Lett.* 2008, **10**, 4295-4298.
3. M. A. Moosavi, R. Yazdanparast and A. Lotfi, *J. Biochem. Mol. Biol*., 2006, **39**, 492-501; M. Ren, G. Drivas, P. D'Eustachio and M. G. Rush, *J. Cell Biol.*, 1993, **120**, 313-323.
4. E. Messina, V. Micheli and A. Giacomello, *Neurosci. Lett.*, 2005, **375**, 97-100; T. C. Pogson, *Am. J. Clin. Nutr.*, 1974, **27**, 380-402; B. Ullman, M. A. Wormsted, M. B. Cohen and D. W. Martin Jr, *Proc. Natl. Acad. Sci.* U. S. A., 1982, **79**, 5127-5131; R. W. E. Watts, Adv. *Enzyme Regul.*, 1985, **23**, 25-58.
5. A. K. Nair, P. P. Neelakandan and D. Ramaiah, *Chem. Commun.*, 2009, 6352-6354; J. Rebek Jr, *Angew. Chem. Int. Ed.*, 1990, **29**, 245-255; J. L. Sessler, V. Krá, T. V. Shishkanova and P. A. Gale, *Proc. Natl. Acad. Sci.* U. S. A., 2002, **99**, 4848-4853.
6. M. Inouye, K. Kim and T. Kitao, *J. Am. Chem. Soc.*, 1992, **114**, 778-780.
7. J. S. Lindsey, H. C. Hsu and I. C. Schreiman, *Tetrahedron Lett.*, 1986, **27**, 4969-4970; J. S. Lindsey, I. C. Schreiman, H. C. Hsu, P. C. Kearney and A. M. Marguerettaz, *J. Org. Chem.*, 1987, **52**, 827-836.
8. C. S. Prasanth, S. C. Karunakaran, A. K. Paul, V. Kussovski, V. Mantareva, D. Ramaiah, L. Selvaraj, I. Angelov, L. Avramov, K. Nandakumar and N. Subhash, *Photochem. Photobiol.* 2014, **90**, 628–640.
9. D. J. Quimby and F. R. Longo, *J. Am. Chem. Soc.*, 1975, **97**, 5111-5117.
10. Z. Kejik, K. Zaruba, D. Michalik, J. Sebek, J. Dian, S. Pataridis, K. Volka and V. Kral, *Chem. Commun.,* 2006, 1533-1535.
11. P. P. Neelakandan, M. Hariharan and D. Ramaiah, *J. Am. Chem. Soc.*, 2006, **128**, 11334-11335; D. Ramaiah, P. P. Neelakandan, A. K. Nair and R. R. Avirah, *Chem. Soc. Rev.*, 2010, **39**, 4158-4168.

Table of Contents

Effective Discrimination of GTP from ATP by a Cationic Tentacle Porphyrin through "Turn-On" Fluorescence Intensity

Suneesh C. Karunakaran,[a] Albish K. Paul[a] and Danaboyina Ramaiah[a,b]*



Cationic porphyrin selectively recognises guanosine based nucleotides was utilized as fluorescence "turn-on" probe to discriminate GTP from ATP through FID assay