Volume 12 | Number 19 | 21 May 2014 | Pages 2981–3138

Organic & Biomolecular Chemistry

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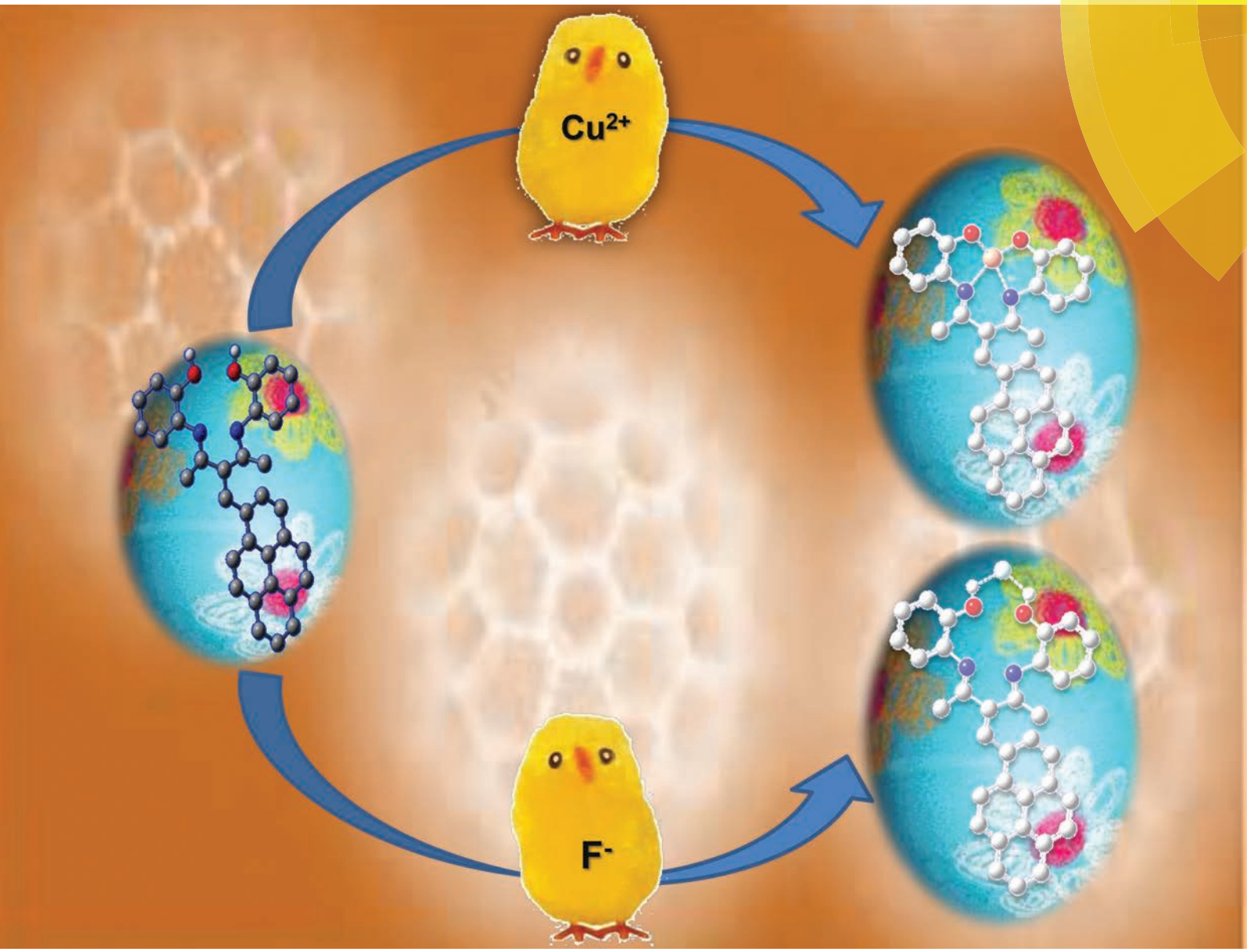

ISSN 1477-0520

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Cite this: *Org. Biomol. Chem.*, 2014, **12**, 3037

A new pyrene based highly sensitive fluorescence probe for copper(II) and fluoride with living cell application†

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Received 9th January 2014,
Accepted 20th February 2014

DOI: 10.1039/c4ob00067f

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A new pyrene based fluorescence probe has been synthesized for fluorogenic detection of Cu^{2+} in acetonitrile–aqueous media (7 : 3 CH_3CN–HEPES buffer, v/v, at pH 7.5) with bioimaging in both prokaryotic (*Candida albicans* cells) and eukaryotic (*Tecoma stans* pollen cells) living cells. The anion recognition properties of the sensor have also been studied in acetonitrile by fluorescence methods which show remarkable sensitivity toward fluoride over other anions examined.

Introduction

Recognition of cations and anions is an expanding area of research which has attracted growing interest in the field of supramolecular chemistry owing to its significant role in a wide variety of environmental, clinical, chemical, and biological applications. Hence, considerable attention has been paid to the design of artificial synthetic fluorescent receptors that can selectively recognize and sense the anionic species.[1] Various structural motifs including amide,[2] pyrrole,[3] urea[4] and thiourea[5] along with the positively charged imidazolium,[6] guanidinium,[7] pyridinium,[8] *etc.* have been well studied for anion recognition. The design and synthesis of sensors capable of binding and sensing fluoride selectively have also drawn considerable attention in supramolecular chemistry,[8b] because the fluoride anion plays a very important role in clinical treatments for osteoporosis and fluoride toxicity.[8c] Recently, considerable effort has been devoted to the development of artificial fluoride sensors through visible, optical and electrochemical responses.[8d–f] Also, copper plays an important role in various biological processes. Many proteins contain copper ions as part of a catalytic center. Free copper ions in a live cell catalyze the formation of reactive oxygen species (ROS) that can damage lipids, nucleic acids, and proteins. Research has connected the cellular toxicity of copper ions with serious diseases including Alzheimer's disease,[9] Indian childhood cirrhosis (ICC),[10] prion disease,[11] and Menkes and Wilson diseases.[12,13] Due to its extensive applications, the copper ion is also a significant metal pollutant. The limit on copper in drinking water set by the US Environmental Protection Agency (EPA) is 1.3 ppm (~20 μM). Numerous methods for the detection of copper ions in a sample have been proposed, including atomic absorption spectrometry,[14] inductively coupled plasma mass spectroscopy (ICPMS),[15] inductively coupled plasma atomic emission spectrometry (ICP-AES),[16] and voltammetry.[17] Most of these methods cannot be used for assays because they entail the destruction of the sample. Consequently, more attention has been focused on the development of fluorescent chemosensors for the detection of Cu^{2+} ions.[18–24] In this regard, pyrene based frameworks represent an ideal template for the construction of new fluorescence probes for the metal ions owing to their excellent photophysical properties, such as emission wavelengths elongated to the visible region, high fluorescence quantum yield and large absorption coefficient. Taking all these properties into account, herein we report a new pyrene based probe (BMPA) which can recognize F^- and Cu^{2+} ions through chelation-enhanced fluorescence (CHEF).

The fluorescence sensor was synthesised by condensing acetyl acetone with pyrene aldehyde to afford a new dehydrated conjugated pyrenyl ene-dione (**1**). The final receptor BMPA was then synthesised from the reaction of compound **1** with 2-aminophenol (Scheme 1) which was refluxed with $CuCl_2{\cdot}2H_2O$ in methanol to yield the target complex.

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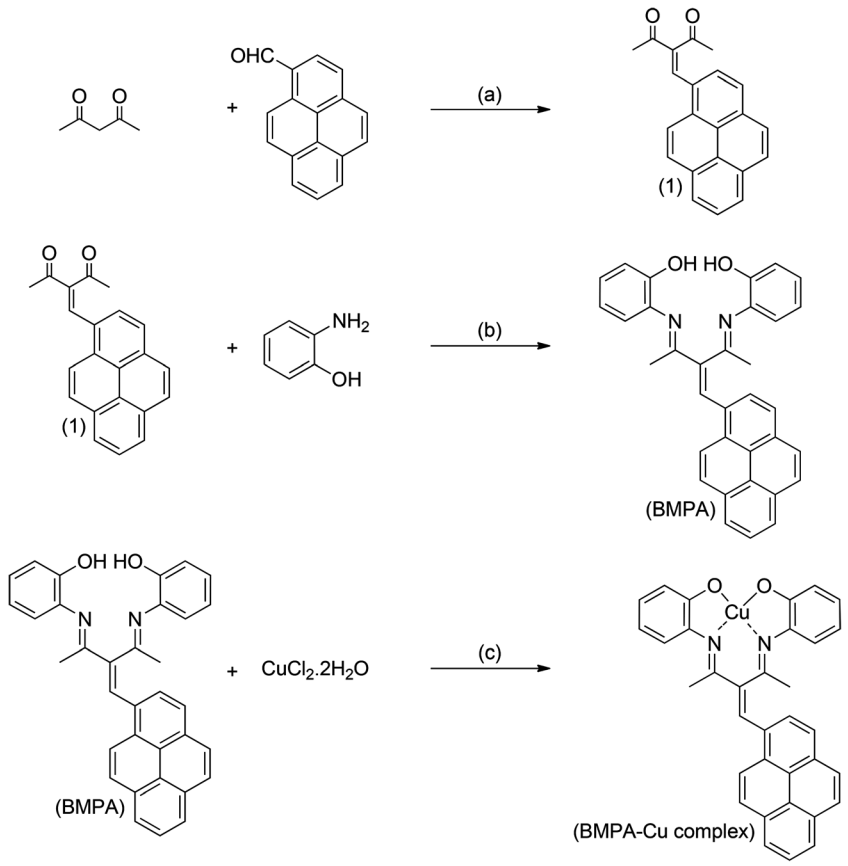
† Electronic supplementary information (ESI) available: Detailed characterization of the compound sensor BMPA along with the intermediates, additional spectroscopic details, spectra and crystallographic analysis. See DOI: 10.1039/c4ob00067f



Scheme 1 Synthetic route for preparation of the receptor (BMPA). (a) L-Proline, DMSO, room temperature, overnight. (b) Ethanol, reflux, 2 h. (c) Ethanol, reflux, 12 h.

Results and discussion

UV-vis and fluorescence study for copper ions

The objective in designing our probe BMPA is to achieve an efficient emission signal and a change in the photophysical properties upon interaction with anions/metal ions. The sensor in the aprotic solvent, only acetonitrile, exhibits weaker fluorescence than the mixed solvent CH_3CN–HEPES buffer (7 : 3, v/v, at pH 7.5), due to the hydrogen bonding of the solvent to the N and O lone electron pairs. This weakens the intramolecular radiationless transition from the nπ^* state and makes the emission maxima (λ_{em}) undergo a gradual red shift with increasing protonation by the solvent. Therefore, the receptor appears to be a promising candidate for enhancing the fluorescence emission upon the binding of suitable metal ions if their radiationless channel could be well blocked. The detection of metal ions using fluorescent sensors based on electron donors/acceptors is usually affected by the presence of protons. The photophysical properties of BMPA are investigated by monitoring the fluorescence behaviour upon the addition of several metal ions such as Na^+, K^+, Ba^{2+}, Ga^{3+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Al^{3+}, Ag^+, Cd^{2+}, Hg^{2+}, Pb^{2+}, In^{3+}, Cr^{3+}, Fe^{3+} and Cu^{2+}, and the anions investigated are AcO^-, Cl^-, Br^-, I^-, F^-, BzO^-, SH^-, $H_2PO_4^-$, PO_4^{3-}, S^{2-}, N_3^-, $P_2O_7^{4-}$ and ADP in CH_3CN (at pH 7.5) as their salts. The

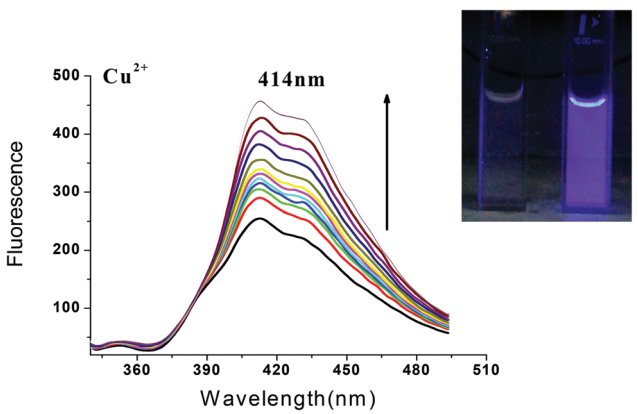


Fig. 1 The emission intensity change (λ_{ex} = 330 nm) after the stepwise and alternate 1.0 equivalent addition of Cu^{2+} (c = 2 × 10^{-4} M) to the BMPA solution of concentration c = 1 × 10^{-5} M in HEPES buffer solution in CH_3CN–HEPES-buffer (7 : 3, v/v, at pH 7.5). Visual color change of BMPA with the addition of 2 equivalents of $CuCl_2{\cdot}2H_2O$ under UV light (inset).

observed changes in the fluorescence emission spectra with Cu^{2+} are shown in Fig. 1. It seems that the fluorescence enhancement is derived from the more widespread formation of the π-conjugation system which occurs upon metal binding, and the fluorescence is sensitive to specific metal ions. The

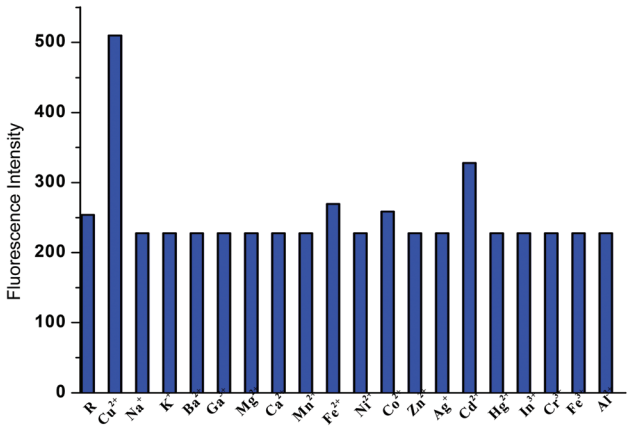


Fig. 2 The change in the emission intensity of BMPA after the addition of 2.0 equivalents of each of the investigated guest cations in CH_3CN–HEPES-buffer (7 : 3, v/v, at pH 7.5) at 414 nm and a plot of the fluorescence intensity *vs.* $[Cu^{2+}]$.

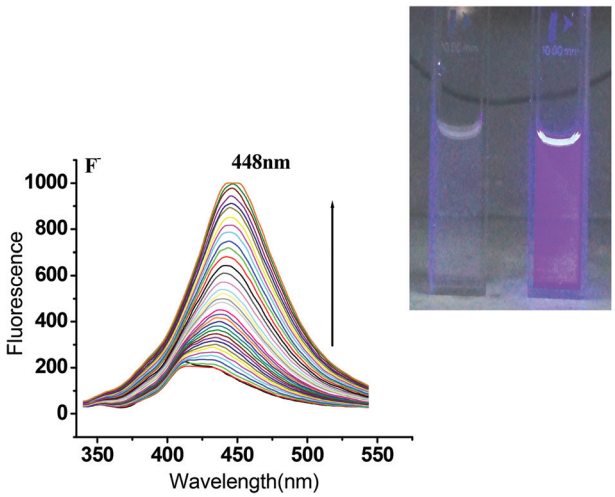


Fig. 3 The emission intensity change (λ_{ex} = 330 nm) after the stepwise and alternate 1.0 equivalent addition of F^- ($c = 2 \times 10^{-4}$ M) to the BMPA solution of concentration $c = 1 \times 10^{-5}$ M in HEPES buffer solution at pH 7.5 in CH_3CN. Visual color change of BMPA with the addition of 2 equivalents of tetrabutyl ammonium fluoride under UV light (inset).

selectivity for 2 equivalents of Cu^{2+} with 10^{-5} M of BMPA over other metal ions is plotted as a bar graph in Fig. 2. In addition, the Job's plot (Fig. S1, ESI†), which is based on the changes in the emission at 414 nm, confirmed the formation of a 1 : 1 complex of BMPA with Cu^{2+}. ESI LC-MS spectral analysis also indicates the formation of a mononuclear complex of BMPA with Cu^{2+}. The positive ion mass spectrum of BMPA upon the addition of 2 equivalents of Cu^{2+} exhibited the most intense peak at m/z = 557, which corresponds to the ion $[BMPA + Cu^{2+}]$ (ESI†).

From the fluorescence titration data it was revealed that a minimum of 1.21 μM of copper can be detected by using 10 μM of the receptor using the equation DL = $K \times Sb1/S$, where K = 3, Sb1 is the standard deviation of the blank solution and S is the slope of the calibration curve (ESI†).

UV-vis and fluorescence study for fluoride ions

With the addition of F^-, in the emission spectra, there is an enhancement in fluorescence with an emission band shift from 419 nm to 448 nm (29 nm red shift, excitation at 330 nm). With the exception of F^-, there is a negligible change in fluorescence upon the addition of other anions. The observed changes in the fluorescence emission spectra for BMPA with the addition of F^- are shown in Fig. 3 and the changes caused by the addition of other ions are shown as a bar graph in Fig. 4.

Study for complexation and binding mode for copper and fluoride ions

In addition, the Job's plot (Fig. S2†), which is based on the changes in the emission at 448 nm, confirmed the formation of a 1 : 1 complex of F^- with BMPA, and the detection limit of 2.91 μM (ESI†) suggests that the receptor is very sensitive towards F^-. However, when titrations of other anions such as $H_2PO_4^-$, PO_4^{3-}, ADP, ATP, AcO^-, Cl^-, Br^-, I^-, F^-, BzO^-, SH^-, S^{2-} and N_3^- were performed under similar experimental conditions, no significant changes were observed in the emission spectra. The selectivity for 2 equivalents of F^- with 10^{-5} M of BMPA was plotted as a bar graph in Fig. 4. Interestingly,

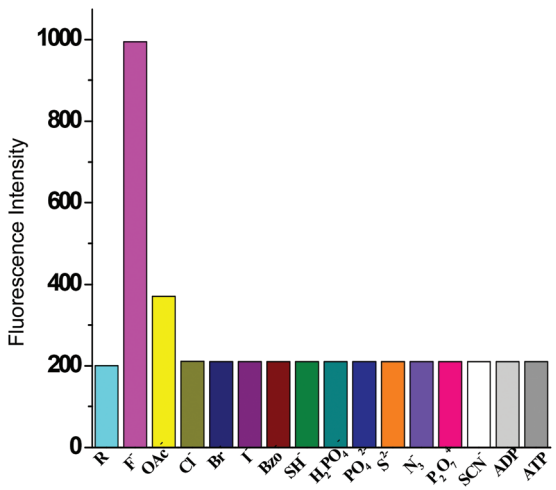


Fig. 4 The change in the emission intensity of BMPA after the addition of 2.0 equivalents of each of the investigated guest anions in CH_3CN (pH = 7.5) at 448 nm and a plot of the fluorescence intensity *vs.* F^-.

anions other than F^- do not interfere in the sensing properties (Fig. 5). Furthermore, to examine the selectivity of the sensor in a complex background of potentially competing species, the fluorescence enhancement of BMPA with Cu^{2+} was investigated in the presence of other metal ions and the fluorescence enhancement of BMPA with F^- was investigated in the presence of other common anions. The experiment is performed by adding the species under investigation, *i.e.* Cu^{2+} or F^- (2.0 equivalents), to the sensor in the presence of commonly employed interfering species, *i.e.* metal ions or anions, respectively (8.0 equivalents). With the exception of Cd^{2+} and Co^{2+} no other competing metal ions interfered in the detection of Cu^{2+} by BMPA in CH_3CN–H_2O (7 : 3, v/v, at pH 7.5) (Fig. 6). The fluorescence enhancement by Cu(II) as well as fluoride binding

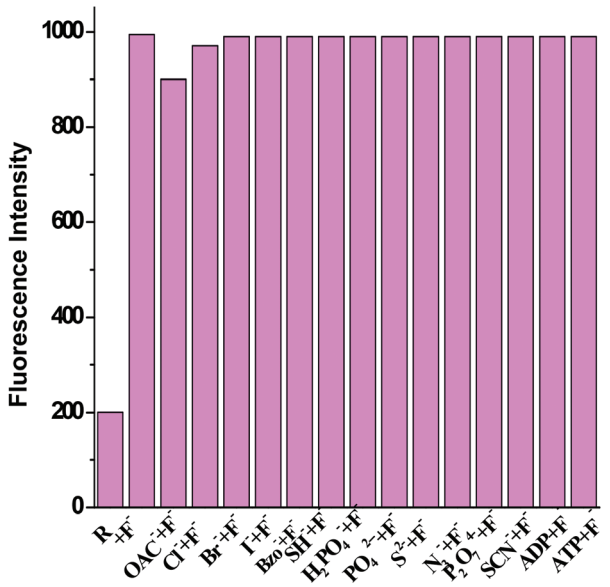


Fig. 5 The anion sensitivity profile for BMPA: the change in the emission intensity of BMPA + 8.0 equivalents of the investigated interfering anions + 2.0 equivalents of F^-.

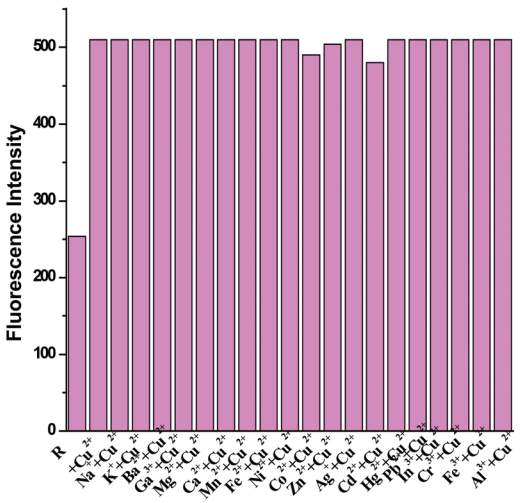


Fig. 6 The metal ion sensitivity profile for BMPA: the change in the emission intensity of BMPA + 8.0 equivalents of the investigated interfering metals + 2.0 equivalents of Cu^{2+}.

may be due to several reasons. One probable reason is that the energy gap between the ground state and the excited state of the metal bound species gets reduced by possible metal–ligand charge transfer (ICT) and chelation and another reason may be other forms of the energy decay process which is not prominent.

The binding mode of BMPA with Cu^{2+} and F^- was examined by ^1H NMR in DMSO-d_6. Spectra of BMPA before and after treatment with 0.5 and 1 equivalent of Cu^{2+} are shown in Fig. 7. On complexation of BMPA with Cu^{2+}, the −OH protons of the amino phenol moiety at δ 9.763 and δ 9.16 disappear and protons of the benzene ring of the amino phenol group are not much affected; the δ value is shifted to just ~0.017 ppm upfield with the addition of 1 equivalent of metal ions. In contrast, the amine protons are most affected in the complexation process of BMPA with metal ions. From the presence of this type of change in the NMR spectra, we can conclude that a structural change must have occurred during the complexation of BMPA with Cu^{2+}, and probably because of this, disappearance of the phenolic protons of amino phenol rings occurs due to the influence of the metal ions (Fig. 7). The protons of the aromatic ring are shifted to a lower magnetic field due to the reduction of electron density upon coordination to the metal ion and are broadened upon complexation. Thus the NMR data also demonstrate the adduct formation between Cu^{2+} and BMPA which results in these chemical shift changes. The spectra of BMPA before and after treatment with 1 equivalent of F^- are shown in Fig. 8.

The −OH protons of the amino phenol moiety at δ 9.763 and δ 9.16 disappear on addition of 1 equivalent of F^- which is the same as what happens in the case of Cu^{2+}. The ^1H NMR is much cleaner after addition of 1 equivalent of F^-, which means the complexation process has happened (ESI†). And an abrupt upfield shift occurs (0.89 ppm) to the phenolic protons (Ha) of the amino phenol ring upon the association of BMPA with F^- and an upfield shift occurs to the pyrene ring protons (0.26 ppm) with the addition of 1 equivalent of F^-. In the case of complexation with Cu^{2+} the same upfield shift is less when compared to the complexation of F^- with BMPA.

In order to investigate the practical application of the sensor (BMPA), we performed a biological study to test the ability of the receptor to image copper in living cells. As shown in Fig. 9, we used here two types of cells for the bioimaging application of BMPA. *Candida albicans* cells and *Tecoma stans* pollen grains were treated with the receptor (5 mM) for 45 minutes at ambient temperature which showed a weak green color emission from the intracellular area and then the cells were washed and treated with 5 mM BMPA solution and the green fluorescence changed to blue in both cases. These results suggest that the probe BMPA is cell membrane permeable and could be used as a sensor to detect Cu^{2+} in both prokaryotic and eukaryotic type living cells and it is also concluded that BMPA would provide detection of intracellular copper present in a biological system (Fig. 9) incubated with copper perchlorate salt (1 mg mL^{-1}) for 45 minutes.

This type of selective displacement behavior of the receptor BMPA mimics the OR logic gate by combining two different wavelengths, *i.e.* at λ_{max} = 448 nm or λ_{max} = 414 nm. The truth table and the circuit for the OR logic gate are shown in Fig. 10. Initially, when no metal ions are added, *i.e.* input 1 and input 2 are zero, the output is nothing. When we add Cu^{2+}, *i.e.* input 1 is '1' and input 2 is '0', the fluorescence is on at λ_{max} = 414, *i.e.* the output is '1'. When we add F^-, *i.e.* input 1 is '0' and input 2 is '1', the fluorescence is on at λ_{max} = 448, *i.e.* the output is '1'. When we add both Cu^{2+} and F^-, *i.e.* input 1 is '1' and input 2 is '1', the fluorescence is on at λ_{max} = 448, *i.e.* output is '1'.

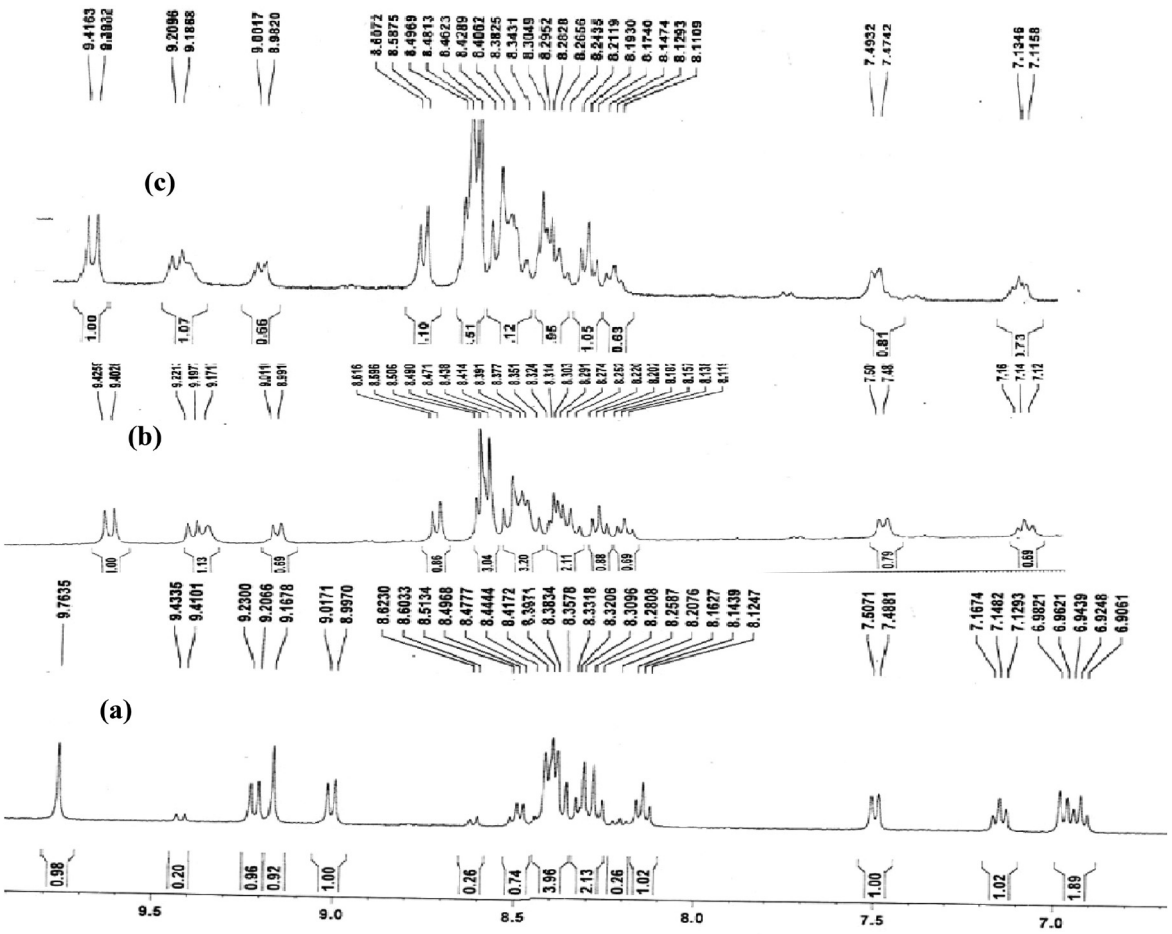


Fig. 7 Partial ^{1}H NMR spectra (400 MHz) of BMPA in DMSO-d_6 at 25 °C and the corresponding changes after the gradual addition of different equivalents of copper chloride from (a) BMPA, (b) BMPA + 0.5 equiv. Cu^{2+} and (c) BMPA + 1.0 equiv. Cu^{2+}.

Experimental section

General

The chemicals and solvents were purchased from Sigma-Aldrich Chemicals Private Limited and were used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 400 MHz instruments respectively. For NMR spectra, DMSO-d_6 was used as a solvent with TMS as an internal standard. Chemical shifts are expressed in δ units and ^{1}H–^{1}H coupling constants in Hz. UV-vis titration experiments were performed on a JASCO UV-V530 spectrophotometer and fluorescence experiments were done using a PTI fluorescence spectrophotometer with a fluorescence cell of 10 mm path length. IR spectra were recorded on a JASCO FT/IR-460 plus spectrometer using KBr discs.

Methods for the preparation of the receptor

Synthesis of the aldol dehydration product (1). To a stirred solution of acetyl acetone (260 mg, 5.21 mmol) in 3 mL of DMSO, L-proline (75 mg, 1.3 mmol) was added and stirred for one hour. Pyrene aldehyde (500 mg, 4.34 mmol) was added to the reaction mixture. The next day, TLC showed the completion of the reaction. It was worked up with water and ethyl acetate. Column chromatography was done to afford the pure yellow colored compound. Yield 67%. Color yellow. Mp >280 °C.

^{1}H NMR (DMSO-d_6, 400 MHz). δ (ppm): 8.70 (s, 1H), 8.31 (m, 5H), 8.8.21 (m, 3H), 8.12 (d, 2H, J = 8.24), 2.56 (s, 3H), 2.07 (s, 3H).

HRMS: M^{+} calculated for $C_{22}H_{16}O_2$ is 312.12; found: 313.12 $(M + H)^{+}$.

Synthesis of ligand (BMPA). To the stirred hot solution of this yellow colored compound **1** (50 mg, 0.151 mmol) in methanol, 2-amino phenol (33 mg, 0.302 mmol) was added and stirred for one hour. TLC showed the completion of the reaction and the product was filtered through a sintered funnel to afford a brick red colored compound followed by purification through column chromatography to afford a brick red colored compound ($C_{34}H_{26}N_2O_2$ from HRMS): yield 25%, mp >280 °C.

^{1}H NMR (DMSO-d_6, 400 MHz). δ (ppm): 9.76 (s, 1H), 9.43 (d, 1H, J = 9.36), 9.42 (d, 1H, J = 9.36), 8.91 (d, 3H, J = 15.5), 8.31 (d, 1H, J = 6.64), 8.26 (m, 13H), 8.18 (t, 6H, J = 10.24), 8.03 (d, 2H, J = 4.48), 7.50 (t, 1H, J = 10.53), 7.48 (t, 1H, J = 11.52),

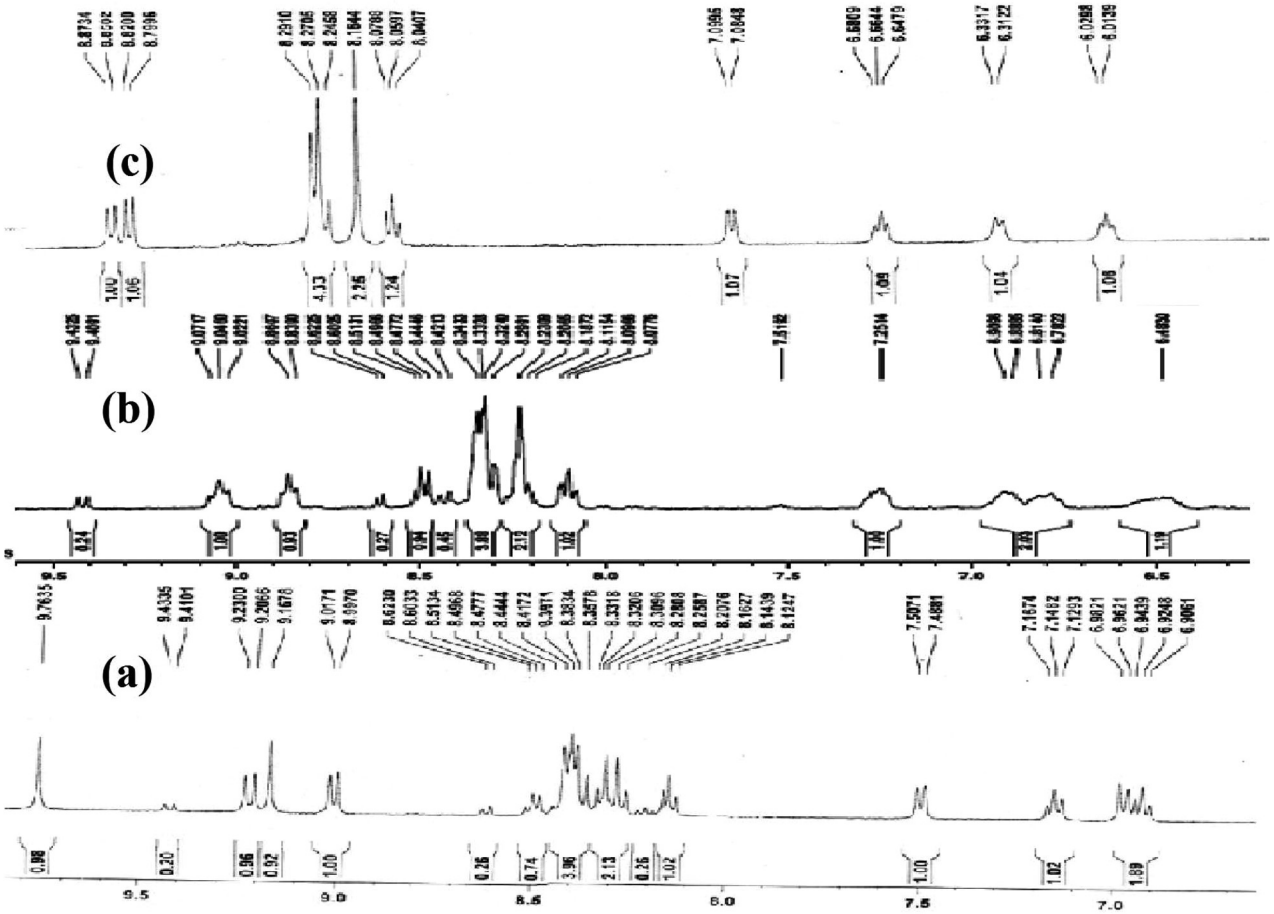


Fig. 8 Partial ^{1}H NMR spectra (400 MHz) of BMPA in DMSO-d_6 at 25 °C and the corresponding changes after the gradual addition of different equivalents of tetrabutyl ammonium fluoride from (a) BMPA, (b) BMPA + 0.5 equiv. F^- and (c) BMPA + 1.0 equiv. F^-.

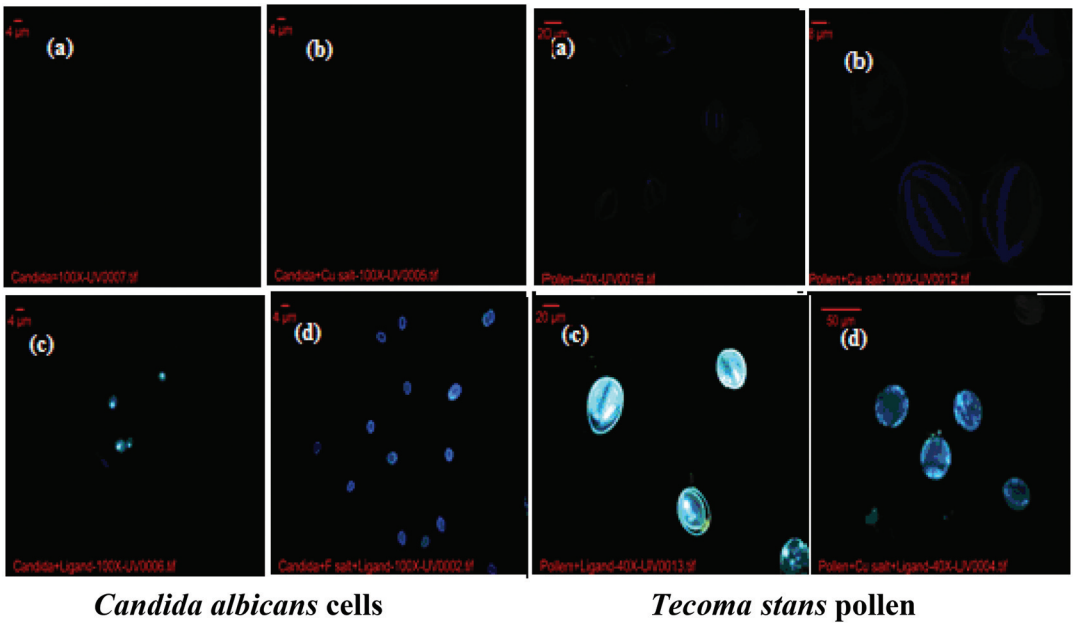


Fig. 9 Fluorescence microscopic photographs of (left) *Candida albicans* cells and (right) *Tecoma stans* pollen grains, (a) cells without any treatment, (b) cells treated with copper perchlorate, (c) cells treated with 5 mM BMPA, (d) cells treated with 5 mM copper perchlorate, washed and then treated with 5 mM BMPA. The emission intensity change for copper at 414 nm (λ_{ex} = 330 nm).

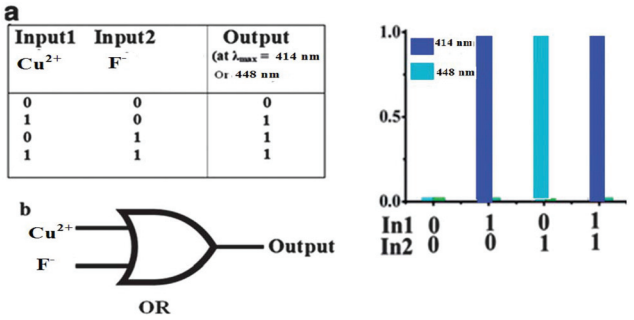


Fig. 10 (a) The truth table of the OR gate and (b) the logic scheme of the following gate, with the change in the emission spectra and the output intensities (bar diagram) of BMPA upon reversing the chemical inputs of Cu^{2+} and F^-.

6.98 (d, 1H, J = 7.60), 6.97(t, 2H, J = 7.68), 1.28 (s, 3H), 1.25 (s, 3H). HRMS: M^+ calculated for $C_{34}H_{26}N_2O_2$ is 494.2; found: 518.21 $(M + 23 + H)^+$.

^{13}C NMR (DMSO-d_6, 500 MHz). δ (ppm): 193.32, 155.75, 152.85, 135.93, 134.09, 131.69, 130.85, 129.61, 129.30, 128.50, 127.83, 127.57, 127.19, 126.93, 126.56, 125.11, 124.92, 123.42, 122.74, 122.26, 120.61, 116.35, 115.48. Anal calculated: C 82.57; H 5.30; N 5.66.

Syntheses of the complexes (BMPA-Cu complex). To a hot 1.0 mL methanolic solution containing 20 mg (0.40 mmol) of the ligand, 1.0 mL of a methanolic solution containing 70 mg (0.40 mmol) of $CuCl_2{\cdot}2H_2O$ was added. A deep blue turbidity appears immediately. After stirring for 1.0 h at 50 °C the blue complexes were filtered, collected and then washed several times with cold methanol. The complex was dried in a desiccator over anhydrous $CaCl_2$ under vacuum. The dried ligand and complexes were subjected to spectroscopic analyses. The complexes are air-stable, non-hygroscopic, and soluble in H_2O ethanol, methanol, DMSO, and DMF. CuBMPA ($C_{34}H_{26}N_2O_2Cu$): yield 80%. Colour blue. Mp >280 °C.

MS (FD). M^+ calculated for $C_{34}H_{26}N_2O_2Cu$ is 557.74; found: 558.10 (BMPA + Cu^{2+} + H^+).

Calculation of the detection limit

The detection limit (DL) of BMPA for Cu(II) and F^- was determined using the following equation. DL = $K \times$ Sb1/S where K = 2 or 3 (we take 2 in this case), Sb1 is the standard deviation of the blank solution and S is the slope of the calibration curve.

General method of UV-vis and fluorescence titrations

By UV-vis and fluorescence method. For UV-vis and fluorescence titrations, a stock solution of the sensor was prepared ($c = 2 \times 10^{-5}$ mL^{-1}) in CH_3CN–HEPES-buffer (7 : 3, v/v, at pH 7.5) for cation titration. The solution of the guest cation was prepared (2×10^{-4} mL^{-1}) in CH_3CN–H_2O (7 : 3, v/v, at pH 7.5) at pH 7.5 by using 20 mM HEPES buffer. For anion titration, a stock solution of the sensor was prepared ($c = 2 \times 10^{-5}$ mL^{-1}) in CH_3CN (pH = 7.5). The solution of the guest anion was prepared (2×10^{-4} mL^{-1}) in CH_3CN. The original volume of the receptor solution is 2 mL. Solutions of the sensor of various concentrations and increasing concentrations of cations and anions were prepared separately. The spectra of these solutions were recorded by means of UV-vis and fluorescence methods.

Conclusions

Thus, in conclusion, we have designed and synthesized a pyrene based fluorophore that easily recognizes Cu^{2+} and F^- over other interfering cations and anions in CH_3CN–HEPES-buffer (7 : 3, v/v, pH = 7.5) and in acetonitrile solution respectively by the fluorescence change which makes it an efficient sensor for possible analytical and biological use in the detection and determination of Cu^{2+} and F^-.

Acknowledgements

Authors thank the DST (SR/S1/0C-58/2010) (Govt. of India) and UGC (for a fellowship to S. P.) for financial support and SC thanks TCG Life Sciences Ltd (Chembiotek), Kolkata for spectral help and Mr M. Adak for assistance.

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