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Selective chemosensing of spermidine based on fluorescent organic nanoparticles in aqueous media *via* a Fe^{3+} displacement assay†

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A novel fluorescent chemosensor based on fluorescent organic nanoparticles (**F1**) has been synthesized. This tripodal framework shows significant fluorescence quenching for Fe^{3+} ions from among nineteen metal ions due to the formation of a **F1**·Fe^{3+} complex. The lowest detectable concentration of **F1** for Fe^{3+} ions was found to be 1.66 μM. Upon the addition of spermidine (a biologically active amine), the fluorescence intensity of the aqueous solution of complex increases with a detection limit of 3.68 μM indicating that spermidine can displace Fe^{3+} ions from the **F1**·Fe^{3+} complex. Moreover, the recognition of spermidine was selective with no interference from other biogenic amines studied. Thus, **F1**·Fe^{3+} acts as a potential sensor for spermidine through a cation displacement assay in aqueous media.

Introduction

The selective recognition and sensing of biologically important analytes such as metal ions and amines with artificial receptors is an important area of research.[1–3] Under the class of biogenic amines (BAs), which are products of the enzymatic decarboxylation of amino acids found in living organisms and which plays vital role in the regulation of gene expression and cell growth, histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine and spermidine are considered to be the most important BAs occurring in foods.[4,5] Despite the important physiological functions displayed by BAs, *e.g.*, aliphatic polyamines spermine and spermidine are involved in cell proliferation and histamine role in gastric secretion,[6] raised levels of these amines is undesired causing headaches, respiratory distress, heart palpitations and several allergic disorders. For example, food-borne intoxication is induced by histamine *via* ingestion of sea food;[7] increased level of spermidine and spermine in urine can be used as a tool for early diagnosis and evaluation of effectiveness of cancer therapy.[8] Therefore, keeping the concern for food safety and human health in view, the detection of these target amines is beneficially required. In contrast to the analytical tools reported for the investigation of metal ions,[9–13] elaborate procedures such as deproteination, centrifugation, and HPLC analysis are known for the determination of BAs due to which research in this area is facing challenges.[13–15] Thus, a necessary goal is the development of a rapid and concise procedure for the analysis of biologically active amines with their applicability in aqueous medium.

To achieve this goal, we focused our attention on the fabrication of fluorescent organic nanoparticles of the newly synthesized novel tripodal receptor (**1**) in 99% aqueous medium. In this context, Yao and coworkers[16] have already explained the reprecipitation method for the formation of FONs in which a monomer solution is injected rapidly into a solvent which reflects the rare solubility of the monomer. Further, it is associated with the addition of water and follows the general Ostwald type ripening process. Water and DMF are miscible, so the solubility of hydrophobic tripodal receptor **1** decreases with the increasing fraction of water, gradually reaching a critical nucleation condition at which nuclei form throughout the solution and begin to grow as nanoparticles.[17] The chemosensor comprising of association of Fe^{3+} ions with FONs is capable of detecting biological amines at a physiological pH. Upon complexation with metal ion, the fluorescence from the fluorophore is quenched. However, spermidine which was more active for Fe^{3+} reduction led to the displacement of the metal ion resulting in the restoration of the fluorescence of FONs.

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Results and discussion

Synthesis

The synthesis of receptor **1** was carried out *via* the condensation of 3-(dimethylamino)-1-propylamine and 2-pyridine carboxaldehyde followed by reduction with $NaBH_4$ in methanol and further reaction of the reduced product with 1-naphthyl isocyanate (Scheme 1).[18] The compound **1** was fully characterized using ^1H NMR, ^{13}C NMR, and mass spectroscopy (Fig. S1–S3, ESI†).

Preparation of nano-aggregates

With the growing interest in nanotechnology due to its substantial applications in all the aspects of sciences and engineering, it has been embraced most frequently in multidisciplinary fields. It also emphasizes the fabrication and characterization of substances having sizes in the nano range, as a smaller particle size leads to a high surface to volume ratio, allowing the distinct spatial domains. Moreover, nanosized particles facilitates a higher amount of binding regions on the surface making them ideal multivalent platforms for the realisation of environmentally and biologically relevant analytes.[19,20] Thus, we focused on the preparation of fluorescent organic nanoparticles (**F1**) of tripodal receptor **1** employing a simple reprecipitation method, in which a small volume fraction from stock solution of receptor in organic solvent (1 mM) was slowly injected into bidistilled water using a microsyringe. The solution was sonicated to ensure the proper mixing of two solvents, which resulted in aggregation of organic molecules due to the dramatic change of solvent properties, and an aqueous dispersion of nanoparticles was ultimately obtained. The particle size of the nanoaggregates was analysed using TEM (transmission electron microscopy) showing a spherical size of 18 nm and size distribution was confirmed from DLS (dynamic light scattering) to be in 25–60 nm range (Fig. 1).

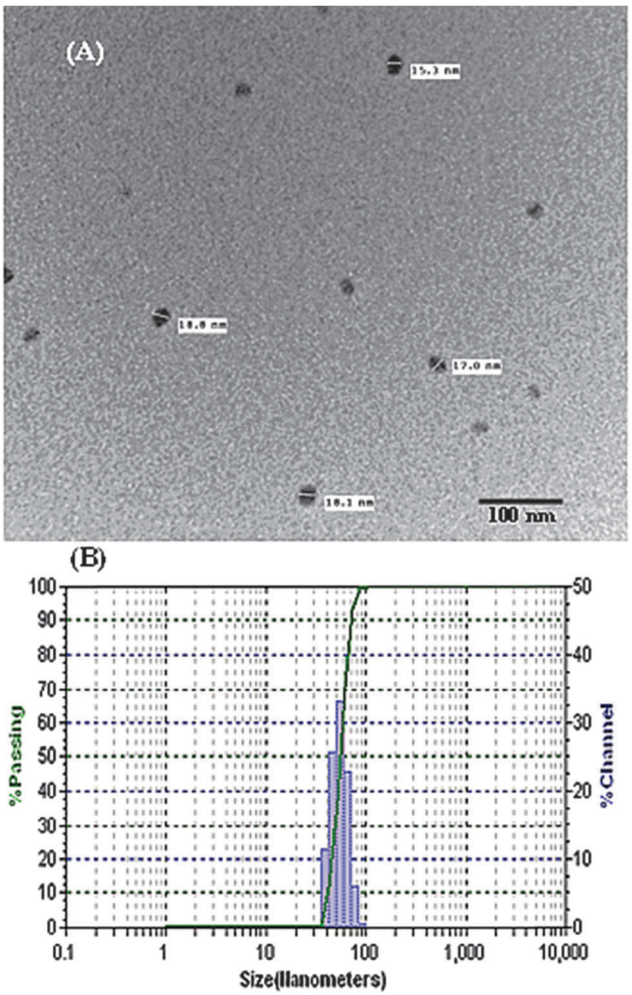
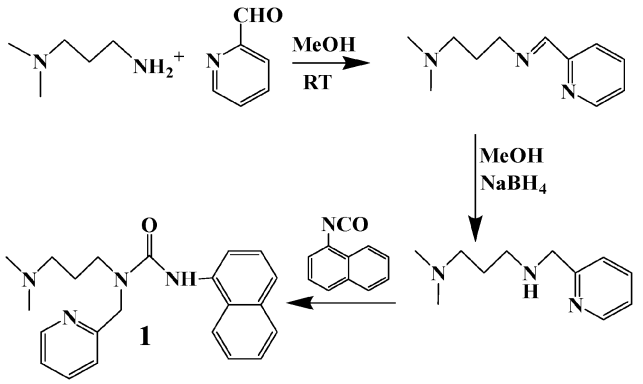


Fig. 1 (A) TEM image (B) DLS size distribution of FONs of receptor **1** prepared in aqueous solution.

Effect of water content

Using the fluorescence spectroscopic technique, the effect of water on the photophysical properties was monitored. The fluorescence spectra of receptor **1** (10 μM) in DMF exhibited an emission peak at 365 nm. As the solvent system was changed from DMF to water, the bathochromic shift of 5 nm (peak shift from 365 nm to 370 nm) was observed which could be due to the stabilization of the excited state of fluorophore undergoing π–π* transition upon increasing the polarity of the solvent system (Fig. 2A).



Scheme 1 Synthesis of receptor **1**.

Recognition studies

To study the ability of **F1** to bind metal ions, fluorescence measurements were performed in water : DMF (99 : 1, v/v) solution. The responses of **F1** (10 μM) of receptor **1** were interrogated in the presence of 10 equiv. of different metal ions such as Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Al^{3+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Pb^{2+}, Cd^{2+} and Hg^{2+}. The probe **F1** exhibits an emission band at 370 nm when excited at 301 nm. Among the metal ions, only Fe^{3+} selectively quenches the fluorescence intensity of **F1** (Fig. 2) whereas the others exhibit no fluorescence quenching response under the same spectroscopic conditions.

Moreover, upon the addition of Fe^{3+} ions from 0–140 μM, the fluorescence intensity started quenching steadily showing a good linearity having a linear coefficient of 0.9857 (Fig. 3). The detection limit calculated by 3σ method[21] was found to be 1.66 μM. In order to test whether FONs (**F1**) could be used for

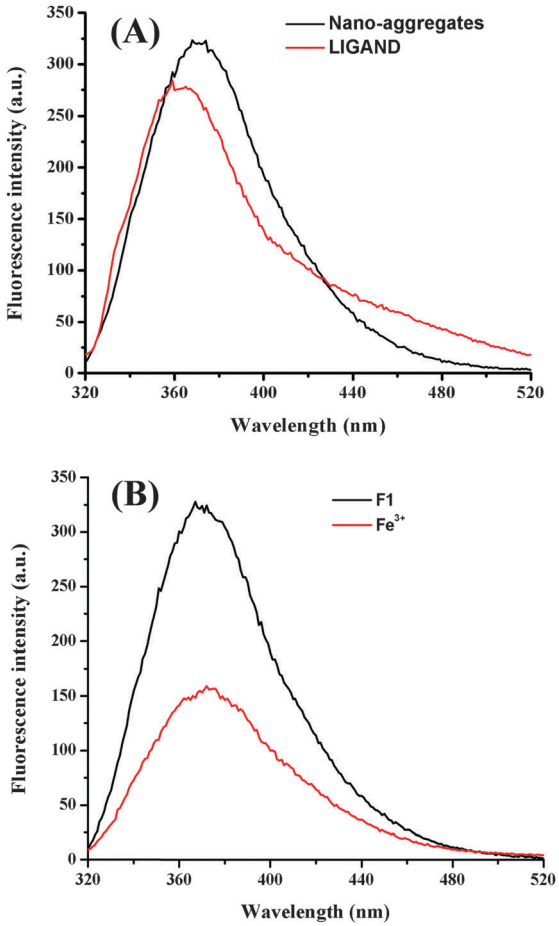


Fig. 2 (A) Emission spectrum of **F1** in water and receptor **1** in DMF; (B) fluorescence spectra of **F1** (10 μM) with Fe^{3+} ions (10 equiv.) in H_2O : DMF (99 : 1, v/v) solution.

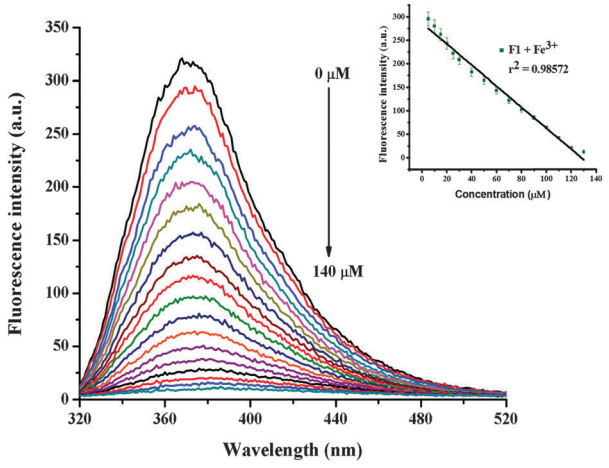


Fig. 3 Fluorescence spectra of **F1** upon addition of Fe^{3+} ions as nitrate salt in H_2O : DMF (99 : 1, v/v) solution. Inset: a plot of fluorescence intensity depending on the concentration of Fe^{3+} ranging from 0–140 μM.

selective sensing of Fe^{3+} ions even in the presence of other metal ions, a competitive experiment was conducted for determination of Fe^{3+} (10 equiv.) in the presence of various metal ion interferents like Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Al^{3+}, Cr^{3+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Pb^{2+}, Cd^{2+} and Hg^{2+} (10 equiv. each). However, no significant change in the fluorescence spectral profile of FONs.Fe^{3+} was observed. This result showed the good sensitivity and selectivity of **F1** for Fe^{3+} over other competitive metal ions (Fig. S4, ESI†).

Detection of spermidine

Since **F1** selectively recognized Fe^{3+}, the utility of the **F1**·Fe^{3+} complex was studied as a secondary chemosensor for the selective recognition of biogenic amines (BAs). In this context, the photonics of the **F1**·Fe^{3+} complex was explored *via* the addition of 15 equiv. of a series of biogenic amines (spermidine, spermine, tyramine, 2-phenylethylamine, histamine, 1,2-diaminopropane, 1,4-diaminobutane, and 1,5-diaminopentane). Under the influence of spermidine, the emission band of the solution of complex at 370 nm was greatly resurrected. In contrast, addition of other BAs did not induce any obvious change (Fig. 4A). Further addition of increasing concentration of spermidine into the **F1**·Fe^{3+} complex solution showed linear resurrection of the fluorescence profile of FONs, having good linearity with a regression coefficient of 0.9876 (inset Fig. 4B), with a detection limit of 3.68 μM calculated using the 3σ method.[21] The restored fluorescence

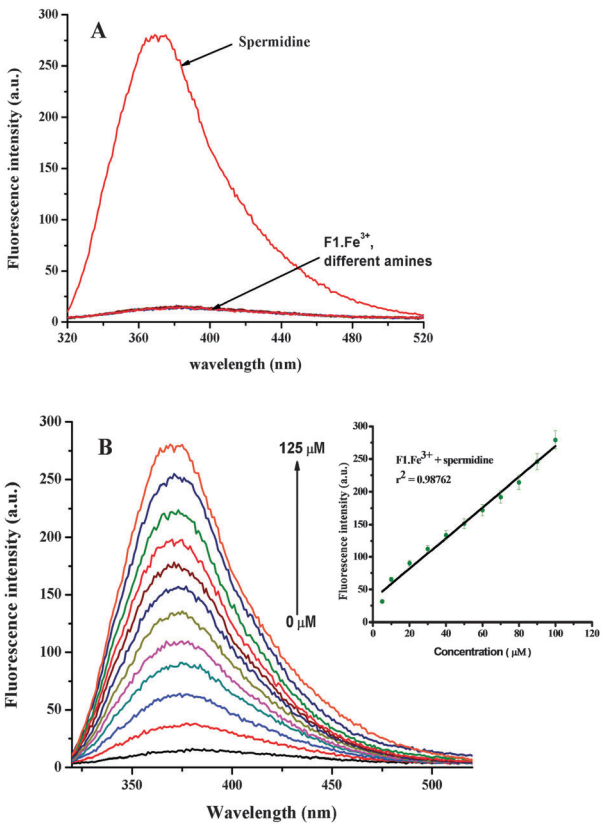


Fig. 4 (A) Fluorescence emission spectra of complex **F1**·Fe^{3+} (10 μM) with Fe^{3+} in the absence and presence of different biogenic amines in H_2O : DMF (99 : 1, v/v) solution, (B) fluorescence spectra of **F1**·Fe^{3+} complex (10 μM) in the presence of different concentrations of spermidine in H_2O : DMF (99 : 1, v/v) solution. Inset: a plot of fluorescence intensity depending on the concentration of Fe^{3+} ranging from 0–125 μM.

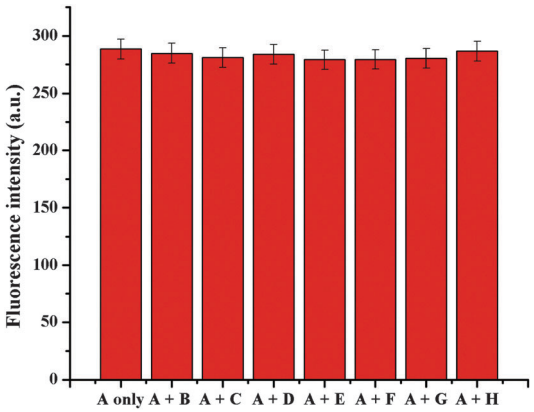


Fig. 5 Competitive binding assay of **F1·Fe^{3+}** complex towards spermidine (15 equiv.) in the presence of other biogenic amines, (A) spermidine only, (B) spermine, (C) tyramine, (D) 2-phenylethylamine, (E) histamine, (F) 1,2-diaminopropane, (G) 1,4-diaminobutane, (H) 1,5-diaminopentane.

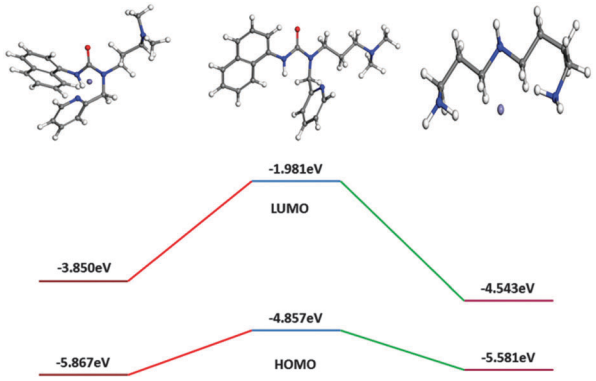


Fig. 6 Density functional theory calculations and their HOMO & LUMO energy comparison.

can be attributed to the fact that spermidine caused the displacement of Fe^{3+} from the **F1·Fe^{3+}**, indicating that **F1** can be used as a reversible chemosensor (Fig. 4B) for the determination of both Fe^{3+} ions and spermidine.

The competitive experiments were also performed for sermidine determination using the **F1·Fe^{3+}** complex, *via* addition in the presence of 15 equiv. of potentially interferent biogenic amines (spermine, tyramine, 2-phenylethylamine, histamine, 1,2-diaminopropane, 1,4-diaminobutane, and 1,5-diaminopentane). As shown in Fig. 5, the emission spectra of the complex with spermidine remain unaffected by different biogenic amines proving **F1·Fe^{3+}** as a selective sensor for the detection of spermidine in aqueous medium.

pH and salt effect

The effect of pH on the fluorescence emission spectrum of developed FONs (**F1**) solution was studied (Fig. S5, ESI†). The emission intensity remains unchanged within a wide pH range covering the physiological pH. However, a slight change in the intensity at very high or very low pH values may be related to the protonation or deprotonation of the molecule. The experiment was also conducted to see the effect of high ionic strength, therefore, tetra butyl perchlorate salt (0–100 equiv.) was added but no change in the fluorescence intensity of emission spectra of **F1** (10 μM) was observed (Fig. S7, ESI†). A similar behaviour was observed for the **F1·Fe^{3+}** complex (Fig. S6 and S8, ESI†). Thus, both **F1** and **F1·Fe^{3+}** complexes exhibited solubility as well as good stability under a wide pH range with no hindrance from the salts present in the environment and this feature proves their practical application for the determination of spermidine in aqueous medium.

Authentication of the complexation and decomplexation of F1

For authentication of the complexation of **F1** with Fe^{3+} to form **F1·Fe^{3+}** and its decomplexation upon addition of spermidine were studied using mass spectra. It was quite clearly observed that the addition of Fe^{3+} ions in F1 generated an altogether new peak at m/z = 542.3 [ligand + Fe^{3+} + 2NO_3^-] (Fig. S9, ESI†), which signifies the formation of 1 : 1 complex of **F1·Fe^{3+}**. The quenching in the fluorescence can be attributed to the open shell effect of Fe^{3+} ions.[22] Similarly, when spermidine is added to the solution of complex **F1·Fe^{3+}**, a clear shift in the peak and formation of a peak at m/z = 325.4 is observed (Fig. S10, ESI†), which is due to the formation of a complex between Fe^{3+} and spermidine along with two nitrate ions. Moreover, in the same mass spectrum, the pak at m/z = 363 also signifies the recovery of ligand peak. The stoichiometry and the binding constant of the complex was calculated using the Lehrer–Chipman equation,[23,24] (Fig. S11 and S12, ESI†). The plotted curves clearly show the stoichiometry of 1 : 1 for both the complexes, with a binding constant of 3.08 × 10^4 M^{-1} for **F1·Fe^{3+}** and 4.29 × 10^4 M^{-1} for the complex of spermidine with Fe^{3+}.

The selectivity for decomplexation of **F1·Fe^{3+}** is explained on the basis of DFT calculations carried using the $DMol_3$ package[25–28] with DFT (density functional theory) calculations run through GGA with the basis set DNP (double numeric plus polarization). It is quite evident from the HOMO and LUMO energies of both complexes *i.e.* **F1·Fe^{3+}** and the complex of spermidine with Fe^{3+} ions that the spermidine.Fe^{3+} complex has stable HOMO and LUMO as compared with that of that receptor or its complex with Fe^{3+} ions (Fig. 6).

It is also concluded from the studies that spermidine engulfs the Fe^{3+} ion by forming a cage-like cavity around it. The lone pair of nitrogen present in spermidine donates their lone pair to electron deficient Fe^{3+} ions. However, other compatriot biogenic amines do not have such multiple heteroatoms forming a cavity for complexation with Fe^{3+} ions. Although, spermine has similar nitrogen atom but the atoms are in opposite orientation to that of the centre of the molecule. Therefore it does not converge to a stable configuration. Iron gets decomplexed from **F1·Fe^{3+}** and the open shell effect gets cancelled leading to enhancement in the fluorescence intensity.

Real sample analysis

The artificially prepared samples of spermidine were prepared by adding known concentrations of spermidine into water solution. The prepared samples were analysed using proposed sensors and results are given in Table 1. It is quite evident from

Table 1 Real sample analysis of artificially prepared samples of spermidine and their percentage recovery using proposed sensor

Entry no.	Sample	Concentration added	Concentration of spermidine[a]	Recovery (%)
1	Sample 1	20 μM	18.2 ± 0.37 μM	91
2	Sample 2	25 μM	23.2 ± 0.51 μM	92.8
3	Sample 3	10 μM	9.36 ± 0.24 μM	93.6

[a] Mean of three determinations.

the Table 1 that proposed sensor can determine spermidine in given solution with utmost accuracy having a recovery percentage of more than 90% in all the samples.

The prepared sensor was compared with the reported sensor in the literature. It is quite evident from the comparison table (Table S1, ESI†) that proposed sensor for selective determination of spermidine clearly holds an edge over the reported sensors, as it determines spermidine in lower micromolar range selectively in total aqueous systems.

Conclusions

In conclusion, we have developed a fluorescence sensor **F1** which is selective and sensitive for detection of Fe^{3+} ions in aqueous medium. The influence of Fe^{3+} ions results in the quenching of the fluorescence intensity of **F1** with no interference from other metal ions. Further **F1**·Fe^{3+} system has been used as a chemosensing ensemble for the sensing of biogenic amine spermidine at a physiologically relevant pH *via* the displacement approach *i.e.* by removal of the metal ion from the complex, thus, restoring the fluorescence profile of **F1**.

Experimental

General information

Analytical grade chemicals purchased from Sigma-Aldrich Co. were used for the experimentation. ^{1}H and ^{13}C NMR spectra were recorded using an Avance-II (Bruker) instrument (400 MHz and 100 MHz, respectively). Mass spectra were obtained from a quadrupole, time-of-flight mass spectrometer (LC-MS Spectrometer Model Q-ToF Micro Waters) and CHN analysis was performed using a Perkin Elmer 2400 CHN Elemental Analyser. The fluorescence emission spectra were taken on a Shimadzu RF-5301Pc Fluorescence spectrophotometer. TEM images were recorded on a Hitachi (H-7500) instrument worked at 120 kV. This instrument has a resolution of 0.36 nm (point to point) with a 40–120 kV operating voltage. A 400 mesh formvar carbon-coated copper grid was used for sample preparation. The particle size of nano-aggregates was determined using Dynamic Light Scattering (DLS) using the external probe feature of the Metrohm Microtrac Ultra Nanotrac Particle Size Analyzer.

Synthesis of receptor 1

To a solution of 2-pyridinecarboxaldehyde (0.95 ml, 10 mmol) in MeOH (10 mL) was added (3-dimethylamino)-1-propylamine (1.25 ml, 10 mmol) at room temperature. After the reaction mixture was stirred overnight, the liquid product (Schiff's base) obtained was reduced with $NaBH_4$ (1.5 g, 40 mmol), diluted with MeOH (10 mL) and kept stirring overnight at room temperature. The solvent was concentrated under reduced pressure, and the residue was extracted using $CHCl_3$ and water mixture. The organic layer was extracted and dried over Na_2SO_4 and concentrated to acquire the reduced amine. Further, the reaction of reduced amine with 1-naphthyl isocyanate (425 μL, 2.96 mmol) taken in $CHCl_3$ (12 mL) at 75 °C was refluxed overnight and solvent was removed under reduced pressure to obtain a residue which was recrystallized using EtOH to yield compound **1**. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.83 (q, 2H, CH_2), 2.08 (s, 6H, CH_3), 2.40 (t, 2H, CH_2), 3.63 (t, 2H, CH_2), 4.70 (s, 2H, CH2), 7.17 (t, 1H, ArH), 7.44 (m, 5H, ArH), 7.63 (t, 1H, ArH), 7.82 (t, 1H, ArH), 8.06 (t, 1H, ArH), 8.56 (d, 1H, ArH), 10.30 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 400 MHz): 14.71, 25.11, 31.06, 44.37, 61.41, 119.50, 122.09, 122.53, 122.83, 123.74, 125.35, 125.59, 126.05, 128.55, 134.30, 135.70, 137.17, 148.95, 158.18, 158.99. CHN analysis: calculated = C 72.88, H 7.93, N 15.46. Obtained = C 72.90, H 7.98, N 15.27, ESI-MS m/z = 363.3 $[M + H]^+$.

Synthesis of nano-aggregates

The formation of fluorescent organic nanoparticles (**F1**) of receptor **1** in aqueous solution was carried out successfully using reprecipitation method. During this method, 0.1 ml of stock solution of **1** was injected slowly into 100 ml of double distilled water followed by sonication for 10 min. retaining the constant concentration of 634 nm in 1 ml DMF which was found to be appropriate amongst various concentrations of compound **1** dissolved in DMF.

Recognition studies

To study the binding ability of the **F1**/complex of **F1** toward different analytes, fluorescence measurements were recorded at 25 ± 1 °C. The standard solutions of 5.0×10^{-3} M concentration of analytes were all prepared in double distilled water. For analysis, the 5 ml solutions of **F1** (10 μM) with added analytes (100 μM) were kept for half an hour to obtain uniformity and shaken well before using it for studying the cation binding behaviour of **F1** in aqueous medium. Titrations were performed using different concentrations of $Fe(NO_3)_3$ in volumetric flasks. Following this, competitive experiment was also conducted for Fe^{3+} estimation by preparing solutions of **F1** (10 μM) with and without other metal ions (100 μM each). Further, determination of spermidine was carried out using a similar pattern for the **F1**·Fe^{3+} complex (10 μM) and the evaluation of the interference behaviour in spermidine was performed using 150 μM solution of each biogenic amine. The effect of pH and ionic strength was studied for both **F1** and **F1**·Fe^{3+} complexes. Different concentrations of a TBA salt of perchlorate (0–100 equiv.) were employed to observe the effect of ionic strength.

Theoretical studies

The geometry of the complex was optimized using the DMol3 package[25,26] with GGA-DFT, using double numerical plus polarization (DNP) as the basis set. All electrons of the system were

treated using the BLYP[27,28] local functions for the exchange–correlation potential.

Real sample analysis

Three different samples with known concentrations of spermidine were prepared and were analysed using the proposed sensor.

Acknowledgements

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