



Cell permeable fluorescent colorimetric Schiff base chemoreceptor for detecting F⁻ in aqueous solvent



Additi Roy Chowdhury^{a,b}, Pritam Ghosh^a, Biswajit Gopal Roy^c,
Subhra Kanti Mukhopadhyay^d, Naresh Chandra Murmu^a, Priyabrata Banerjee^{a,b,*}

^a Surface Engineering and Tribology Group, CSIR-Central Mechanical Engineering Research Institute, Mahatma Gandhi Avenue, Durgapur 713209, India

^b Academy of Scientific and Innovative Research at CSIR-CMERI, Durgapur 713209, India

^c Department of Chemical Sciences, Sikkim University, Gangtok 737102, Sikkim, India

^d Department of Microbiology, The University of Burdwan, Burdwan 713104, India

ARTICLE INFO

Article history:

Received 26 March 2015

Received in revised form 13 May 2015

Accepted 16 May 2015

Available online 28 May 2015

Keywords:

Colorimetric Schiff base receptor

Fluorescence turn-on

C=N isomerization

¹H NMR

In vitro detection

Logic gate application

ABSTRACT

A novel colorimetric hydrazine based Schiff base chemoreceptor (E)-2-((2-(perfluorophenyl)hydrazono)methyl)phenol (**PMP**), for detection of fluoride (F⁻) is reported in this work. The receptor can selectively detect F⁻ by turning from colorless to yellow. The recognition event is further confirmed by solution state spectrophotometric measurements like UV-Vis, Fluorescence and ¹H NMR titrimetric studies. The fluorescence 'turn on' phenomenon of **PMP** with gradual addition of F⁻ can be well explained by inhibition of C=N isomerization process. ¹H NMR titration experiments of **PMP** with F⁻ further support the hydrogen bonding interaction between host **PMP** and guest F⁻. Interestingly, **PMP** has potential toward detection of intracellular F⁻ in cells of *Candida albicans* (diploid fungus) and pollen grains of *Techoma stans*. The recognition phenomenon is reversible and we have successfully developed two input logic network where INHIBIT gate can be considered as an AND gate with one input inverted by a NOT function. Furthermore, this logic gate represents a potential 'Write-Read-Erase-Read' memory function possessing multi writing ability.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Anion recognition has been explored in a phenomenal pace in several avenues of modern chemistry. Because of its vast application in supramolecular and biological chemistry [1–10] the interest still persists among researchers. Of several bio relevant anions, fluoride (F⁻) is one of the most important one and has received much attention due to its important contribution on several biological, medicinal and environmental related issues. Its wide range of application in industry; food and toxicity control is well known [11,12]. F⁻ is involved in dental care and its usage in treatment of osteoporosis is well acknowledged [13–15]. It is available in many anesthetic, hypnotics, and also in psychiatric drugs. The

widespread application of this particular ion has necessitated the development of simple and novel receptors which have very easy making processes and can detect F⁻ at low level too [16–18]. In this regard it is needless to say that development of potential colorimetric fluorescent receptors has innate advantages over receptors which are non colorimetric or non fluorescent in nature [19–22]. Colorimetric receptors have enormous advantages over non colorimetric ones in terms of selectivity, sensitivity, ease of detection and easy assessment [23–26]. In this perspective both fluorimetric and colorimetric dual responses have huge impact in F⁻ recognition process. Therefore efforts are made to design smart receptors such that recognition of F⁻ ions with suitable fluorophore will be achieved.

Keeping all these factors in our consideration for current research on supramolecular chemistry [4,8,27–31] we are nowadays mostly focused on selective detection of F⁻ like bio-relevant anions with Schiff base based chemoreceptor. Till date F⁻ detection has been mainly carried out by urea, amide, and imidazolium based chemoreceptor [32–36]. In recent time Schiff bases have been taken into consideration because Schiff bases are relatively easy to prepare and can be tailored structurally. In addition their spectroscopic responses in the visible region are

* Corresponding author at: Surface Engineering and Tribology Group, CSIR-Central Mechanical Engineering Research Institute, Mahatma Gandhi Avenue, Durgapur 713209, India. Tel.: +91 343 6452 220; fax: +91 343 2546 745.

E-mail addresses: pr.banerjee@cmeri.res.in, priyabrata.banerjee@yahoo.co.in (P. Banerjee).

URLs: <http://www.cmeri.res.in> (P. Banerjee), <http://www.priyabratbanerjee.in> (P. Banerjee).

very interesting and can be exploited eventually [21,25,37]. More-over Schiff bases with 'turn on' recognition are hitherto less reported in literature [31,38]. Motivated with all these factors we have synthesized a Schiff base colorimetric receptor (*E*)-2-(2-(perfluorophenyl)hydrazono)methylphenol (**PMP**) following one step synthetic protocol [39,40] which has shown visual colorimetric and interesting 'turn on' fluorescence responses with incoming F^- ion. The use of using the pentafluorophenyl subunit as a part of this receptor molecule has a few advantages [41]. First of all this pentafluoro subunit is a neutral electron deficient moiety which will exclude any electrostatic attraction between the ring and the incoming anion; Secondly it also eliminates any electronic repulsion between the aromatic unit and the concerned anion. Finally, the presence of $-OH$ and $-NH$ units with capability of forming H-bonding interaction with guest F^- is largely responsible for this recognition event [23,38]. The non covalent interaction between host **PMP** and guest F^- is duly supported by UV-Vis, Fluorescence and 1H NMR like sophisticated spectroscopic experimentation. In recent time fabrication of electronic devices and mimicking the functions of silicon chips with intelligent organic/inorganic single molecules (namely molecular logic gates), having interesting spectral responses toward an external stimulant has gained appreciable importance in sustaining the development in information sciences and related new technologies. Any smart and intelligent molecule which is capable of developing sequential logic operations is highly interesting. As a basis of current computers, electronic logic gates perform the binary arithmetic and several logical operations. Logic gates are mainly typical switches with output and input mode, where the output mode (0 or 1) depends on existing or applying input mode (0 or 1) [42–45]. Molecular logic gates actually work on input signals relying on chemical processes and shows output signals in line with spectroscopic outcomes. Spectral responses of **PMP** toward F^- inspired us to visualize different logic gate functions. In this present work; synthesis of smart organic molecule (**PMP**) and its strong binding affinity toward specific anion (F^-) can be reflected as a signaling OUTPUT, in certain solvent reaction medium which mimics the AND gate together with an exclusive INHIBIT logic gate function with different inputs. Interestingly receptor **PMP** is also found to be cell permeable and has an enormous potential toward detection of F^- in cells of *Candida albicans* (diploid fungus) and pollen grains of *Teuchoma stans*.

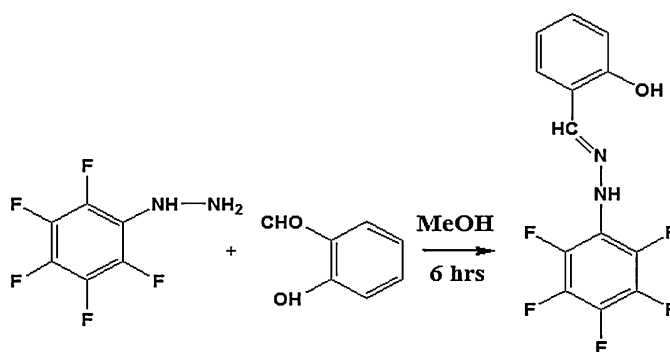
2. Experimental

2.1. Materials

All starting materials (chemical reagents, solvents) were obtained commercially and were of analytical grade. Solvents like methanol, diethyl ether, dichloromethane, hexane, etc. were received from Merck India Pvt. Ltd. and used without any further purification. All tetrabutyl ammonium salt of anions like fluoride (hydrate), chloride, bromide, iodide, nitrate, hydrogen sulfate, acetate and phosphate were purchased from Sigma–Aldrich chemical company and used as it was received. DMSO and HEPES-Buffer were of spectroscopic grade, purchased from Merck India Pvt. Ltd. and were used without doing any further purification.

2.2. Apparatus

The infrared spectra were recorded in an ATR-FT-IR (Model-Alpha, Bruker, Germany) instrument. A Perkin Elmer 2400C elemental analyser was used to collect the microanalytical (C, H, N) data. The mass spectra were done in Advion's CMS Expression (serial number: 3013-0140) compact mass spectrometer. 1H NMR was recorded in a Bruker AV-400 spectrometer. UV-Vis spectra



Scheme 1. The synthetic route of **PMP**.

were taken in ALS-SEC2000 and Agilent Cary 60 spectrophotometer. Fluorescence titration experiment was done in Perkin Elmer LS-45 spectrometer. Fluorescence imaging experiments of bio cells were performed where the imaging system comprised of a fluorescence microscope (Leica DM 1000 LED), digital compact camera (Leica DFC 420C) and an image processor (Leica Application Suite v3.3.0). The microscope was equipped with a mercury 50 watt lamp.

2.3. Synthesis of **PMP**

Pentafluorophenyl hydrazine (1 mmol, 0.198 g) was dissolved in 15 mL methanol followed by stirring for next 0.5 h. Salicylaldehyde (1 mmol, 0.122 g) was dissolved in 5 mL same solvent in another beaker and added drop wise into the stirring solution of pentafluorophenyl hydrazine. The solution was then allowed to stir for next 6 h at room temperature (Scheme 1). A white residue appeared which was washed several times with diethyl ether. Single crystals of **PMP** suitable for X-Ray study were produced after 4 days of slow diffusion from dichloromethane/hexane (2:1) layering. The ORTEP of **PMP** with atom numbering scheme is shown in Fig. 1. The receptor was furthermore characterized by FT-IR, ESI-MS, 1H NMR spectroscopy (Figs. S1, S2 and S3, Supporting information). The crystallographic details of **PMP** are depicted in Table 1. Elemental

Table 1

Crystallographic data and details of the structure determination for **PMP**.

Crystal data	
Formula	C13 H7 F5 N2 O
Formula weight	302.21
Crystal system	Monoclinic
Space group	P21/n (No. 14)
a, b, c [Angstrom]	7.095(4) 23.681(15) 7.104(4)
alpha, beta, gamma [deg]	90 102.747(8) 90
V [Ang ³]	1164.2(12)
Z	4
D(calc) [g/cm ³]	1.724
Mu(MoKa) [/mm]	0.166
F(000)	608
Crystal size [mm]	0.06 × 0.11 × 0.18
Data collection	
Temperature (K)	293
Radiation [Angstrom]	MoKa 0.71073
Theta Min–Max [Deg]	1.7, 25.0
Dataset	–8: 8; –28: 28; –8: 8
Tot., Uniq. Data, R(int)	9191, 2002, 0.075
Observed data [I > 2.0 sigma(I)]	1300
Refinement	
Nref, Npar	2002, 191
R, wR2, S	0.1265, 0.3393, 1.20
w = 1/[s ² (Fo ²) + (0.2000P) ² + 1.5792P]	where P = (Fo ² + 2Fc ²)/3
Max. and Av. Shift/Error	0.00, 0.00
Min. and Max. Resd. Dens. [e/Ang ³]	–0.91, 1.26

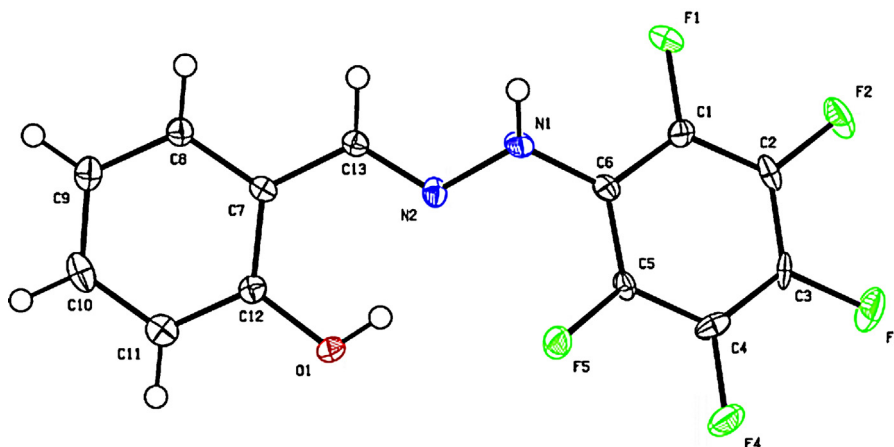


Fig. 1. ORTEP with atom numbering of PMP.

analysis for **PMP**: Calc. $C_{13}H_7N_2OF_5$: C: 51.6%; H: 2.3%; N: 9.27%; found: C: 51.23%; H: 2.26%; N: 9.72%. Yield: (0.28 g, 94%). Melting point of **PMP** is 135 °C. ESI-MS [m/z , negative mode (**PMP**-H⁺), in MeOH] Calc. 302, found: 300.89. ¹H NMR (400 MHz, DMSO-*d*₆, Me₄Si) δ = 6.86–6.90 (m, 2H), 7.23 (t, 1H), 7.46 (dd, 1H), 8.38 (s, 1H), 10.41 (s, 1H), 10.45 (s, 1H).

3. Results

The white colored microcrystalline material of **PMP** is fully characterized by solid state elemental analysis, FT-IR and single crystal X-ray and by solution state analysis like UV-Vis, ¹H NMR and ESI-MS.

3.1. X-Ray crystal structure

Single crystals of **PMP** suitable for X-ray study are obtained from dichloromethane and hexane layering (slow evaporation). The ORTEP and atom numbering scheme of **PMP** is shown in Fig. 1. Single crystal X-ray data reveals that **PMP** is crystallized in P2(1)/c space group. In a unit cell four **PMP** moieties are co-crystallized and formed a closely packed structure along crystallographic *a* axis. The molecule is non planar in nature with strong inter and intramolecular H-bonding (Fig. 2). The intramolecular H-bonding between the imine nitrogen and phenoxy hydrogen (N2-H1:1.9 Å) forms a six

membered ring structure. The C=N distance (C13–N2: 1.286 Å) is slightly greater than in ideal case (1.27 Å) [8,30,46]. The other bond distances of the ring structure *i.e.*; C–C (C13–C7: 1.445 Å), C–C (aromatic) (C7–C12: 1.408 Å), C–O (C12–O1: 1.369 Å) and O–H (0.96 Å) also deviated from ideal bond distances: C–C (aromatic) = 1.54, C–O = 1.35, C=C = 1.43 and O–H = 0.96 Å, suggesting delocalization of electrons throughout the six membered ring structure (Fig. S4, Tables S1–S3, Supporting information). In addition, the intermolecular H-bond (about 2.09 Å) between two adjacent **PMP** is propagated through the phenoxy oxygen (O1) of one unit to NH hydrogen (H1A) of another unit, forming a supramolecular 1D chain. The crystallographic details for **PMP** with selected bond distances and angles are tabulated in Tables 1 and 2.

3.2. Visual colorimetric analysis

We have examined the visual change in solutions of chemoreceptor **PMP** in DMSO-HEPES buffer before and after addition of several TBA salts. In case of F[−] a sharp color change was noticed from colorless to sharp yellow. For AcO[−] ion the change is very weak. The formation of a six membered H-bonding network between F[−], –OH and –NH is the reason for the appearance of this color. Addition of several other tetrabutyl ammonium salts (*e.g.*: Cl[−], Br[−], I[−], HSO₄[−], H₂PO₄[−] and NO₃[−]) did not result in any detectable visible change in color (Fig. 3). The recognition of colorimetric changes toward different anions was studied experimentally by visual as well as UV-Visible methods.

3.3. UV-Vis spectral responses studies

The visual and colorimetric detection study suggests that **PMP** is highly selective toward F[−] in DMSO-HEPES buffer at pH 7.4. However a slight color change of **PMP** is observed in presence of AcO[−] ion in same solvent mixture. In order to find out qualitative anion sensing behavior of this chemoreceptor the UV-Vis

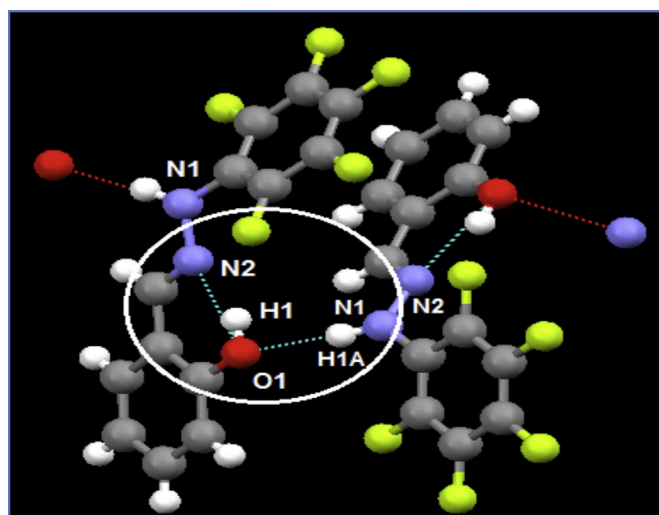


Fig. 2. Segmented view of inter and intramolecular H-bonding interaction in **PMP**.

Table 2
Selected bond distances (angstrom) and angles (degrees) for **PMP**.

Bond distances (angstrom)			
O1–C12	1.369(7)	C7–C13	1.445(7)
C7–C12	1.408(7)	N2–C13	1.286(7)
N1–N2	1.382(6)	N1–C6	1.388(7)
C5–C6	1.396(8)	F5–C5	1.341(6)
Bond angles (degrees)			
F5–C5–C6	120.6(5)	N2–N1–C6	117.7(4)
N1–N2–C13	118.0(4)	N2–C13–C7	121.3(5)
C12–C7–C13	122.8(5)	O1–C12–C7	121.2(4)

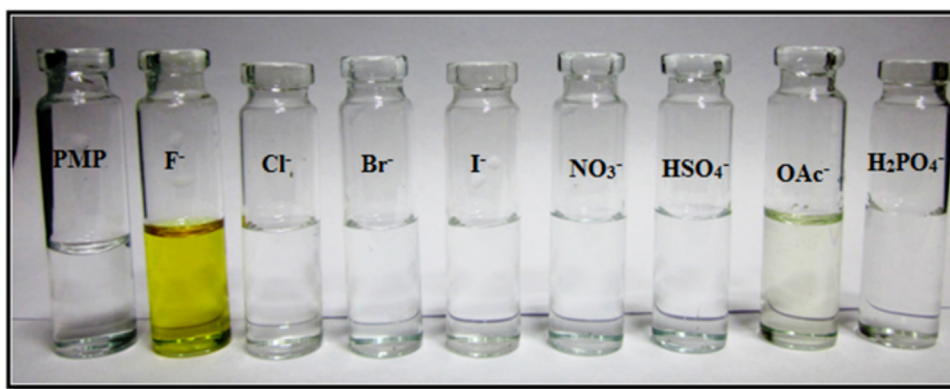


Fig. 3. Solutions of **PMP** in DMSO-HEPES buffer (9:1 v/v) at pH 7.4 in presence of various anions.

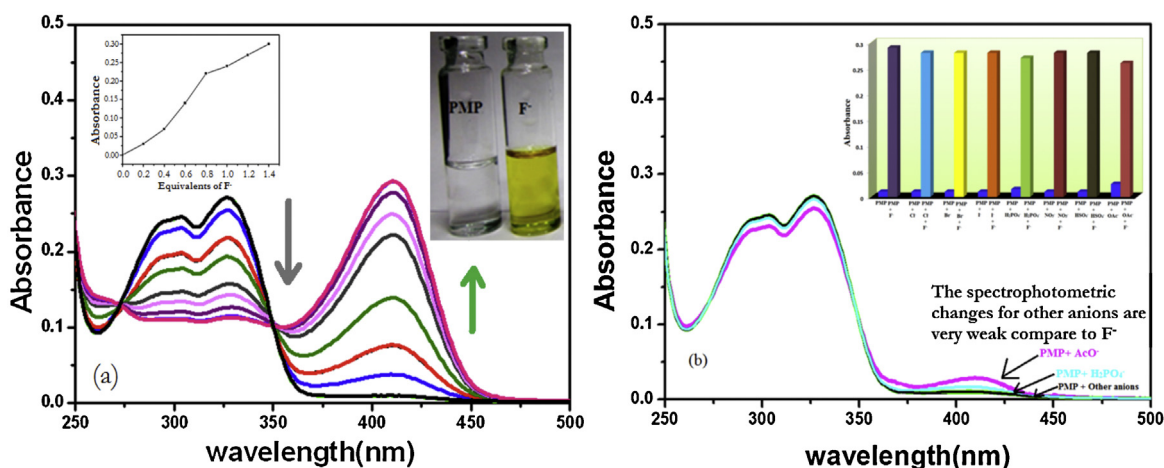


Fig. 4. (a) UV-Vis spectral changes of **PMP** (1×10^{-4} M) with TBA salts of F^- in 9:1 v/v DMSO-HEPES buffer at pH 7.4 (0–1.5 equiv.), inset: Color changes of **PMP** in presence and absence of F^- and plot of absorbance vs. equivalent of F^- addition, (b) AcO^- (0–1.5 equiv.) and other anions, inset shows the interference of F^- in presence of other anions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

titration experiments are one of the essential spectroscopic tools. In 9:1 v/v DMSO-HEPES buffer (9:1 v/v) medium (pH 7.4), **PMP** shows absorption maxima at 300 nm and 330 nm (Fig. S5, Supporting information). The band at 300 nm can be assigned to $\pi-\pi^*$ and the band at 330 nm to $n-\pi^*$ transition of the π electron localized on azomethine group ($C=N$) [47–49]. With gradual addition of F^- (Fig. 4a), a new absorption peak generates at 410 nm and attains a maxima at around 1.5 equiv. of the added anion. The formation of this new peak indicates the occurrence of hydrogen bonding interaction between **PMP** and F^- which in turn alters the overall intramolecular charge transfer inside the whole **PMP** molecule and results in this new peak (410 nm). The color turns from colorless to sharp yellow. Addition of other anions even in excess (e.g.; Cl^- , Br^- , I^- , HSO_4^- , $H_2PO_4^-$ and NO_3^-) produces no visible color change and therefore no reallocation of electronic distribution in **PMP** is noticed (Fig. 4b). UV-Vis titration of **PMP** (1×10^{-4} M) is also performed with OH^- ion to observe the effect of higher pH (8–10) on the receptor (Fig. S6, Supporting information) [50]. There is however no change at lower pH range (7–5.5) (Fig. S7, Supporting information). The anion binding equilibrium constant of **PMP** is calculated from Benesi–Hildebrand (B–H) equation. The binding constant for F^- ion has been found to be $0.1 \times 10^3 M^{-1}$ (Fig. S8, Supporting information). To establish **PMP** as a universal receptor, we have simply carried out the interference study for F^- with other tested anions. In DMSO-HEPES buffer (9:1 v/v) at pH 7.4 medium **PMP** can detect F^- in presence of other anions (Fig. 4b inset). A feeble interference from AcO^- is observed in DMSO-HEPES buffer (9:1 v/v) at pH 7.4.

3.4. Development of 2-input logic gate

The UV-Vis absorption studies in presence of anionic and cationic inputs show a detectable change in corresponding spectrum. These sensitive responses of **PMP** toward selective anion and cation initiate us to fabricate Boolean logic gates and molecular level arithmetic calculation. The UV-Vis spectral response of **PMP** with F^- has generated a new signal at 410 nm. The outcome of this spectral response is best mimicking AND logic gate function. Two chemical inputs are receptor **PMP** (In 1) and F^- (In 2) respectively. Output (Y) is a newly generated signal at 410 nm for **PMP** (In 1) after interacting with F^- (In 2). The output will be HIGH (i.e.; 1) if input is HIGH (i.e.; 1) which means if both inputs are 'ON' then only output is 'ON' otherwise 'OFF', i.e.; the output response at 410 nm will appear only if input In1 (**PMP**) and In2 (F^-) are present together [45]. The results are fitted in AND logic gate function (Table 3). Simplifying all these findings a logic symbol is shown in Fig. 5a, where In1 and In2 as input is shown along with the output Y.

Table 3
Receptor **PMP** at 410 nm.

Inputs		Output ($\lambda = 410$ nm)
In 1 (PMP)	In 2 (F^-)	Y
0	0	0
1	0	0
0	1	0
1	1	1

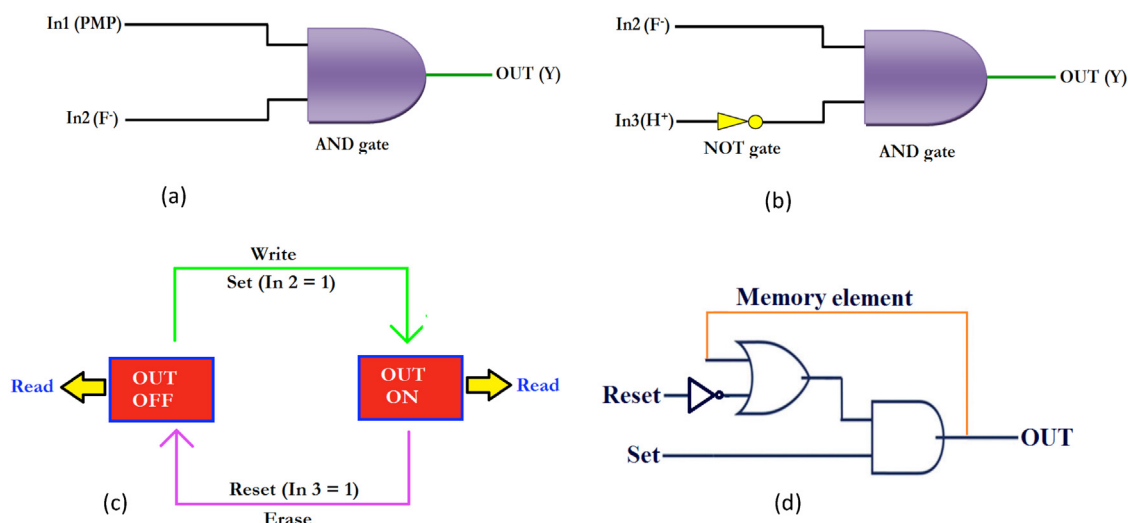


Fig. 5. The logic circuit for (a) AND (b) INHIBIT logic gate (c) proposed loop demonstrating the reversible logic operations with 'Write-Read-Erase-Read' function (d) logic circuit with memory unit.

Furthermore, another 2 input logic gate operation can be implemented with the response of **PMP** toward F⁻ in UV-Vis absorption study, where F⁻ and H⁺ (In 3) are the two inputs. It was found that **PMP** did not have any influence on its UV-Vis spectrum upon addition of H⁺. In presence of chemical input H⁺, the output signal (i.e.; 1, ON state) at 410 nm is minimized and according to Boolean arithmetic it becomes 0 (OFF state). Output is only available when In 2 (F⁻) is ON i.e.; when input 2 (F⁻) is interacting with free receptor **PMP** a significant enhancement of the 410 nm peak is noticed. Subsequent addition of input 3 (H⁺) makes the output Y to zero state (OFF), this is an implementation of NOT gate. In accordance with truth Table 4 combination of both inputs resulted in the vanishing of 410 nm signal as output 0. Thus monitoring the UV-Vis response at 410 nm, simultaneous addition of F⁻ (input 2) and H⁺ (input 3) and their combined addition leads to an INHIBIT logic gate function (Fig. 5b). Interestingly, this type of reversible colorimetric switch ON-OFF could be repeated several times by alternate addition of F⁻ and H⁺ to the **PMP** receptor solution (Fig. S9, Supporting information). In today's context this sort of reversible switch is of immense importance owing to their great impact in information technology field. In summary, this system can be properly utilized for the development of a sequential logic circuit displaying "Write-Read-Erase-Read" sequences in line with binary logic (Fig. 5c). In this present system the strong absorption at 410 nm can be defined as ON state (Out = 1) whereas the OFF state can be defined as a very weak absorption at the same wave length (Out = 0). Two chemical inputs F⁻ (In 2) and H⁺ (In 3) are defined as Set (S) and Reset (R), respectively. In high input state (S = 1) the whole system can write and memorize, on contrary the stored information will be erased by Reset input, reflecting writing and memorization of 0 binary set. The reversible and reconfigurable sequences of Set/Reset logic operations are represented by a feedback loop (Fig. 5d). These sorts of Schiff bases with dual stability state and without an appreciable

degradation in optical output stage up to a number of cycles can be treated as a potential feedstock in miniaturization and in opening new avenues under molecular logic devices.

3.5. Fluorescence spectroscopic studies

UV-Vis titration study suggests **PMP**: F⁻ hydrogen bonded adduct formation. Thus to gain further insight into the F⁻ binding ability of **PMP**, the fluorescence titration experiments have been carried out at biological pH 7.4 [DMSO: HEPES buffer 9:1(v/v)]. The Stokes shift of **PMP** from excitation-emission graph has been found to be 50 nm (Fig. 6). The quantum yield value for **PMP** has been found to be 0.05.

The appreciable enhancement in fluorescence intensity (nearly 5-fold enhancement) of **PMP** is noticed with gradual addition of F⁻ (0.1 to 1.5 equiv.) (Fig. 7). Multiple H-bonding adduct formation between incoming guest F⁻ and host receptor in such a polar solvent mixture resulted in inhibition of C–N isomerization in the receptor backbone. More specifically, two flexible N–N and C–C bond adjacent to C–N bond which can undergo a free rotation becomes blocked in presence of incoming F⁻ as a consequence of strong hydrogen bonding between –NH, –OH sites of receptor and F⁻. Thus after binding with F⁻, receptor **PMP** becomes rigid

Table 4
Receptor **PMP** at 410 nm.

Inputs		Output ($\lambda = 410$ nm)
In 2 (F ⁻)	In 3 (H ⁺)	Y
1	0	1
0	1	0
0	0	0
1	1	0

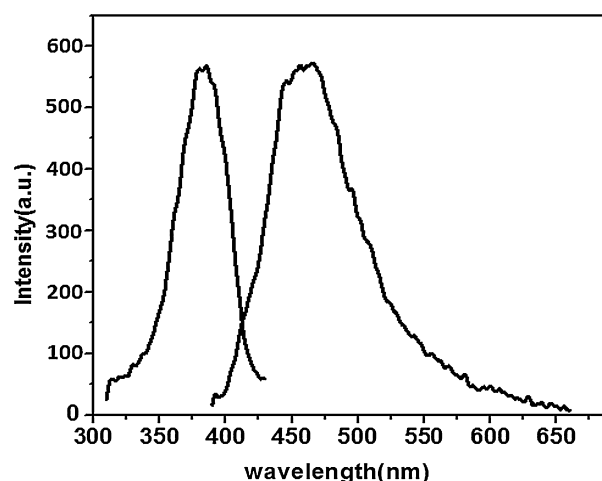
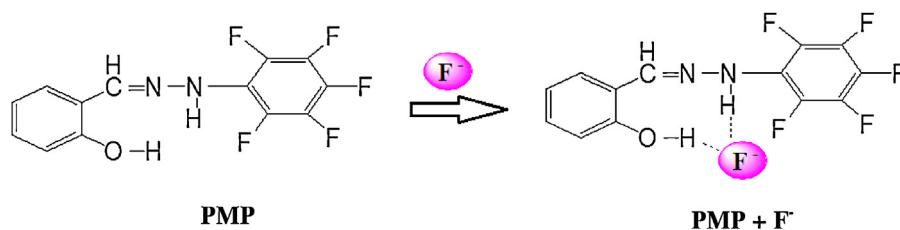


Fig. 6. Excitation-Emission graph of **PMP**.



Scheme 2. Proposed binding mode of **PMP** with F^- .

and consequently inhibits the vibrational and rotational relaxation modes of nonradiative decay. Therefore a 'turn on' fluorescence response at 440 nm is observed (Scheme 2) [38,51–55].

3.6. Job's plot using the fluorescence method

In order to determine the stoichiometric ratio [4] of **PMP** with F^- , a series of solutions containing 4×10^{-5} M TBAF and **PMP** is prepared in DMSO-HEPES buffer solvent. The total concentration of the resultant solution remains constant. The mole fractions of the added anions are varied from 0.1 to 1.0. The fluorescence at 440 nm is plotted against the mole fraction of the added anions. The curve reveals 1:1 complexation between the host receptor **PMP** and the guest anion F^- (Fig. 8).

3.7. 1H NMR spectroscopic studies

Retrospective inspection and to understand the mode of interaction between receptor and F^- , 1H NMR titration is executed [4,31]. Fig. 9 displays the details of chemical shift of receptor **PMP** with gradual addition of F^- in DMSO- d_6 . Two sharp signals at 10.45 ppm and 10.40 ppm in the absence of anions can be designated as $-OH$ and $-NH$ proton signals respectively. Aromatic and imine protons have shown chemical shifts at expected region (6.5–8.5 ppm). On gradual addition of 0.25 equiv. of TBAF, $-OH$ and $-NH$ protons responded first which in turn significantly broadened upon further addition of TBAF. $-OH$ and $-NH$ protons disappeared with 2 equiv. TBAF addition while excess addition of it (up to 4 equiv.) did not show any response of HF_2^- at 16.1 ppm. Additionally, other skeletal proton responses remained unaffected. These findings suggest that multiple H-bonding interactions exist between host **PMP** and guest F^- i.e.; the interaction pathway belongs to the traditional realm of supramolecular chemistry (vide supra, Scheme 2) [38].

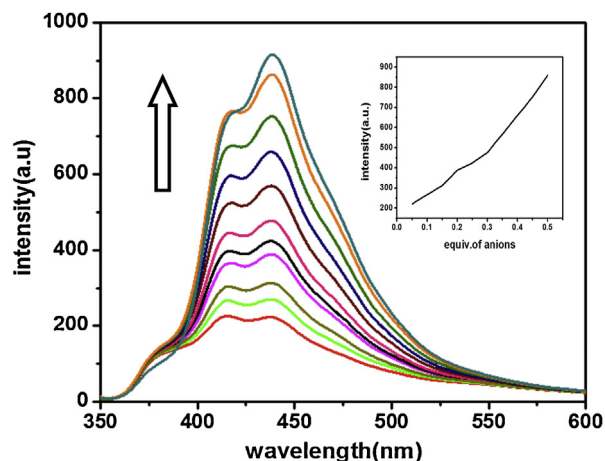


Fig. 7. Fluorescence titration spectra ($\lambda_{ex} = 330$ nm) of receptor **PMP** (2×10^{-5} M) upon gradual addition of F^- (0–2 equiv.) in DMSO-HEPES buffer (9:1 v/v) at pH 7.4; Inset: fluorescence intensity enhancement with varying concentrations of F^- anion.

3.8. Detection limit calculation

The detection limit (DL) has been calculated following fluorescence titration experimentation [56]. The fluorescence emission spectrum of **PMP** is repeated 10 times, and its standard deviation is measured. The limit of detection (LOD) is calculated from the following formula:

$$DL = \frac{3\sigma}{k}$$

σ is the standard deviation of the blank solution of **PMP**. Gradual enhancement of **PMP** emission intensity at 440 nm during fluorimetric titration with TBAF is plotted against its concentration. The slope (k) is derived from this plot (Fig. S10, Supporting information). LOD turns out to be 6.7×10^{-5} M.

3.9. Preparation of cells and detection of F^- by receptor

It is already well observed that there is a significant amount of interaction of F^- with **PMP** in aqueous organic system. In view of above we have planned to furthermore investigate the ability of **PMP** in detection of intracellular fluoride [31,57–61]. For this study two types of cells viz. *Candida albicans* (diploid fungus) and pollen grains of *Techoma stans* were used. The *Candida albicans* cells (IMTECH No. 3018) from exponentially growing culture in yeast extract glucose broth medium (\sim pH 6.0 and incubation temperature $37^\circ C$) were washed carefully in normal saline and centrifuged for 10 min. Pollen grains of *Techoma stans* were collected from buds and washed in 0.1 M HEPES buffer (\sim pH 7.4). The details of cell incubation, staining with **PMP** receptor are discussed in Supporting information. After staining, the so called controls were not visualized through the fluorescence microscope in UV filter (Fig. 10). However after incubation with a few micromolar of **PMP** for half an hour all the cells sparkled with bright blue

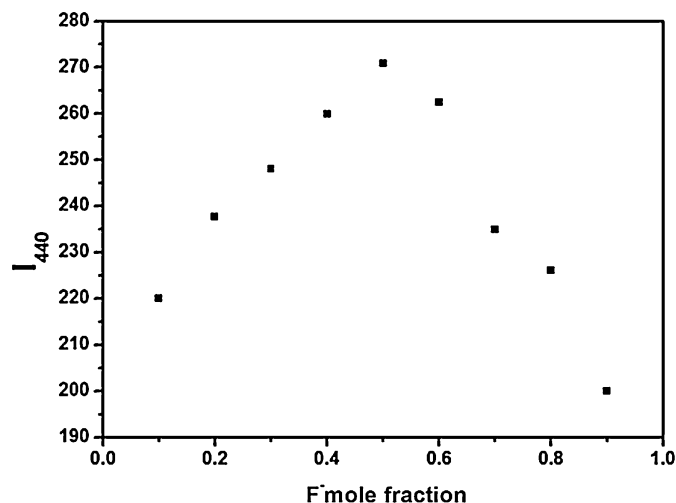


Fig. 8. Job's plot of receptor with F^- anion with a total concentration of 4×10^{-5} M.

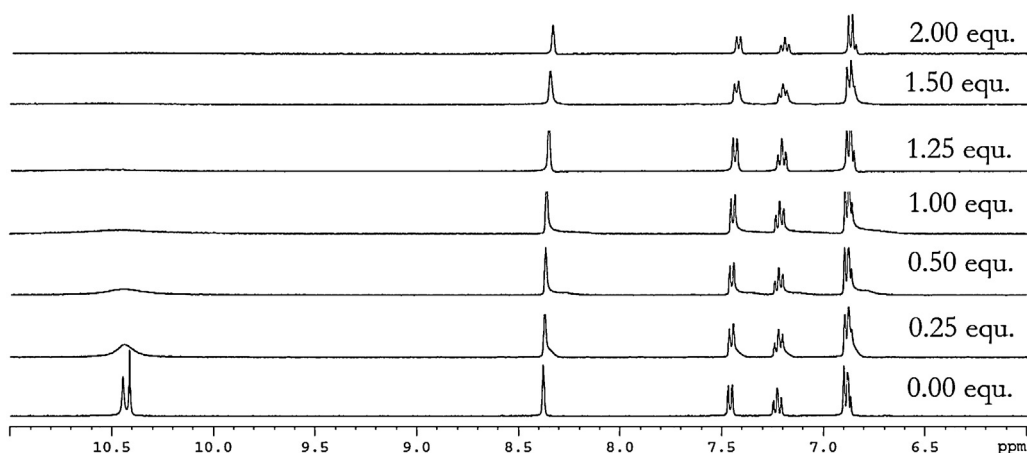


Fig. 9. ^1H NMR titration of PMP (1×10^{-3} M) in DMSO- d_6 solvent with a standard solution of F^- .

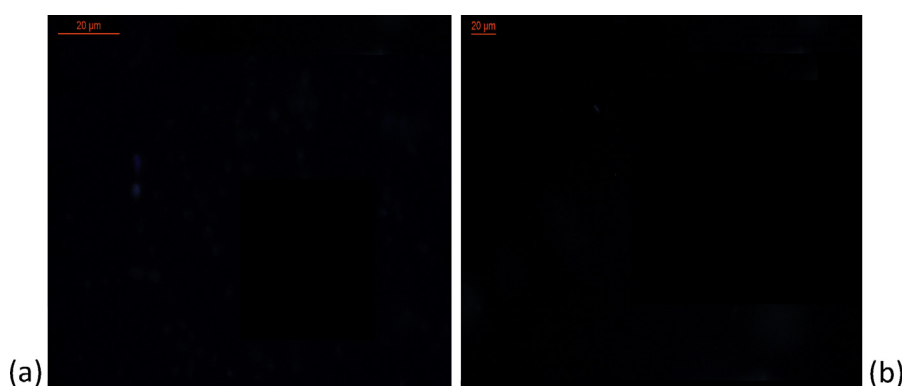


Fig. 10. Fluorescence image of the control cells (a) *Candida albicans* and (b) Pollen grains.

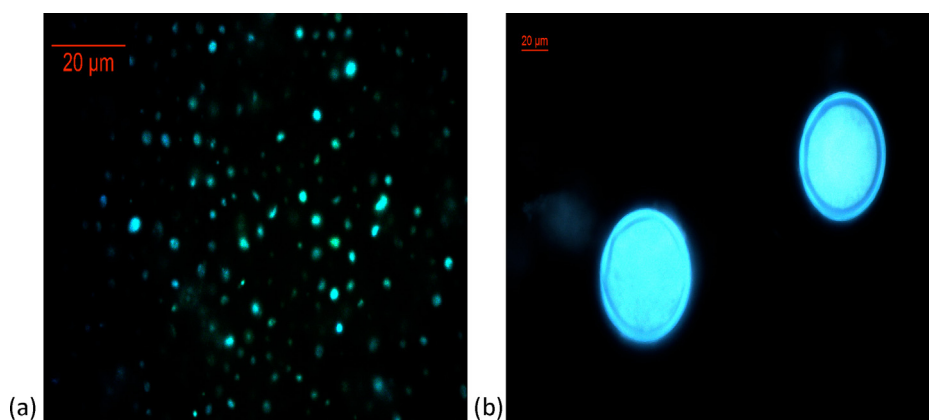


Fig. 11. Detection of F^- inside living cells under fluorescence microscope; (a) *Candida albicans* incubated in F^- solution and stained with PMP (b) Pollen grains incubated in F^- solution and stained with PMP.

emission under fluorescence microscope in UV filter. Interestingly the distribution of PMP stained cells were then observed. It is therefore concluded that PMP is cell permeable and a wonderful staining agent to monitor intracellular fluoride (Fig. 11).

4. Conclusion

In summary we have prepared a cost effective and very easy to synthesize Schiff base receptor PMP, which interestingly shows selectivity toward F^- . The receptor is colorimetric as well as luminescent and shows selective coloration and fluorescence 'turn on' response in presence of F^- . No such spectral changes are observed

with other ions. C=N isomerization is the driving force in support of fluorescence 'turn on'. Additionally, the receptor can also be used to detect intracellular F^- by fluorescence microscopic imaging. The spectrophotometric response of receptor PMP in presence of different guest entities mimics sequential integration of molecular logic gates (AND as well as INHIBIT). Our ongoing research continues for the development of more sophisticated and easy to make receptors for F^- recognition to defend practical problems. In a quest of finding several other related receptors for betterment in detection of bio-relevant anions, recently we are in constant search of the substitution effect in the receptor backbone.

Supplementary information

CCDC 1015471 contains the supplementary crystallographic data for **PMP**.

Acknowledgements

PB is thankful to CSIR-Supra Institutional research grant (ESC-0203/09) under CSIR-XIIth five year plan. PB is also thankful to Dr. Pijush Pal Roy, Director, CSIR-CMERI for his immense support. ARC is also thankful to CSIR-Supra Institutional research grant (ESC-0203/09) for her fellowship. PG is thankful to Department of Science and Technology, Govt of India for his fellowship. Thanks are due to Ms. Meenakshi Sharma, NEIST and Mr. Sourav Pal Chowdhury, NISER, for their support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2015.05.044>

References

- [1] (a) I.-S. Ke, M. Myahkostupov, F.N. Castellano, F.P. Gabbai, Stibonium ions for the fluorescence turn-on sensing of F⁻ in drinking water at parts per million concentrations, *J. Am. Chem. Soc.* 134 (2012) 15309; (b) Y.K. François, P. Gabbai, Cationic boranes for the complexation of fluoride ions in water below the 4 ppm maximum contaminant level, *J. Am. Chem. Soc.* 131 (2009) 3363; (c) H. Miyaji, W. Sato, J.L. Sessler, Naked-eye detection of anions in dichloromethane: colorimetric anion sensors based on calix[4]pyrrole, *Angew. Chem. Int. Ed.* 39 (2000) 1777; (d) S.K. Dey, G. Das, A selective fluoride encapsulated neutral tripodal receptor capsule: solvatochromism and solvatomorphism, *Chem. Commun.* 47 (2011) 4983.
- [2] S. Chakraborty, R. Dutta, M. Arunachalam, P. Ghosh, Encapsulation of [X₂(H₂O)₄]²⁻ (X = F/Cl) clusters by pyridyl terminated tripodal amide receptor in aqueous medium: single crystal X-ray structural evidence, *Dalton Trans.* 43 (2014) 2061.
- [3] B.N. Ahmed, P. Ghosh, A chelation enhanced selective fluorescence sensing of Hg²⁺ by a simple quinoline substituted tripodal amide receptor, *Dalton Trans.* 40 (2011) 12540.
- [4] A. Roy Chowdhury, P. Ghosh, S.K. Saha, P. Mitra, P. Banerjee, Exploratory studies towards various anion recognition chemistry by two different sized cleft shaped organic ligands, *Spectrochim. Acta Part A* 124 (2014) 492.
- [5] S. Saha, B. Akhuli, I. Ravikumar, P.S. Lakshminarayanan, P. Ghosh, Recognition of fluoride in fluorophenyl attached tripodal amide sensors: structural evidence of solvent capped encapsulation of anion in a C^{3v} symmetric tripodal cleft, *Cryst. Eng. Comm.* 16 (2014) 4796.
- [6] T. Kundu, A.D. Chowdhury, D. De, S.M. Mobin, V.J. Puranik, A. Datta, G.K. Lahiri, Selective recognition of fluoride and acetate by a newly designed ruthenium framework: experimental and theoretical investigations, *Dalton Trans.* 41 (2012) 4484.
- [7] P.A. Gale, Anion receptor chemistry: highlights from 1999, *Coord. Chem. Rev.* 213 (2001) 79.
- [8] P. Ghosh, A. Roy Chowdhury, S.K. Saha, M. Ghosh, M. Pal, N.C. Murmu, P. Banerjee, Synthesis and characterization of redox non-innocent cobalt(III) complexes of a O,N,O donor ligand: radical generation, semi-conductivity, antibacterial, anticancer activities, *Inorg. Chim. Acta* 429 (2015) 99.
- [9] A. Jana, S. Mohanta, A tale of crystal engineering of metal complexes derived from a special ligand family having a cosmopolitan compartment, *Cryst. Eng. Comm.* 16 (2014) 5494.
- [10] L.S. Evans, P.A. Gale, M.E. Light, R. Quesada, Anion binding vs. deprotonation in colorimetric pyrrolylamidothiourea based anion sensors, *Chem. Commun.* 965 (2006).
- [11] P. Bose, P. Ghosh, Visible and near-infrared sensing of fluoride by indole conjugated urea/thiourea ligands, *Chem. Commun.* 46 (2010) 2962.
- [12] (a) X. Yang, Z. Huang, C.-L. Ho, G. Zhou, D.R. Whang, C. Yao, X. Xu, S.Y. Park, C.-H. Chuid, W.-Y. Wong, Dynamic dual stage phosphorescence chromatic change in a diborylated iridium phosphor for fluoride ion sensing with concentration discriminating capability, *RSC Adv.* 3 (2013) 6553; (b) P. Bose, B. Nisar Ahmed, P. Ghosh, Functionalized guanidinium chloride based colorimetric sensors for fluoride and acetate: single crystal X-ray structural evidence of -NH deprotonation and complexation, *Org. Biomol. Chem.* 9 (2011) 1972.
- [13] X. Peng, Y. Wu, J. Fan, M. Tian, K. Han, Colorimetric, Ratiometric fluorescence sensing of fluoride: tuning selectivity in proton transfer, *J. Org. Chem.* 70 (2005) 10524.
- [14] R.M.F. Batista, E. Oliveira, S.P.G. Costa, C. Lodeiro, M. Manuela, M. Raposo, Synthesis ion sensing properties of new colorimetric and fluorimetric chemosensors based on bithienyl-imidazo-anthraquinone chromophores, *Org. Lett.* 9 (2007) 3201.
- [15] M. Kleerekoper, The role of fluoride in the prevention of osteoporosis, *Endocrinol. Metab. Clin. N. Am.* 27 (1998) 441.
- [16] M.S. Kumar, S.L.A. Kumar, A. Sreekanth, Highly selective fluorogenic anion chemosensors: naked-eye detection of F⁻ and AcO⁻ ions in natural water using a test strip, *Anal. Methods* 5 (2013) 6401.
- [17] A. Mouradzadegan, F. Abadast, An improved organic/inorganic solid receptor for colorimetric cyanide-chemosensing in water: towards new mechanism aspects, simplistic use and portability, *Chem. Commun.* 50 (2014) 15983.
- [18] S.Y. Xu, X. Sun, H. Ge, R.L. Arrowsmith, J.S. Fossey, S.I. Pascu, Y.B. Jiang, T.D. James, Synthesis and evaluation of a boronate-tagged 1,8-naphthalimide probe for fluoride recognition, *Org. Biomol. Chem.* 13 (2015) 4143.
- [19] D. Sharma, A.R. Mistry, R.K. Bera, S.K. Sahoo, Spectroscopic and computational studies on the development of simple colorimetric and fluorescent sensors for bioactive anions, *Supramol. Chem.* 25 (2013) 212.
- [20] A.K. Mahapatra, S.K. Manna, P. Sahoo, Color response of tri-armed azo host colorimetric sensors and test kit for fluoride, *Talanta* 85 (2011) 2673.
- [21] M. Shellaiyah, Y.H. Wu, A. Singh, M.V.R. Raju, H.C. Lin, Novel pyrene- and anthracene-based Schiff base derivatives as Cu²⁺ and Fe³⁺ fluorescence turn-on sensors and for aggregation induced emissions, *J. Mater. Chem. A* 1 (2013) 1310.
- [22] A.K. Mahapatra, R. Maji, K. Maiti, S.S. Adhikari, C. Das Mukhopadhyay, D. Mandal, Ratiometric sensing of fluoride and acetate anions based on a BODIPY-azaindole platform and its application to living cell imaging, *Analyst* 139 (2014) 309.
- [23] H. Khanmohammadi, K. Rezaeian, Naked-eye detection of inorganic fluoride in aqueous media using a new azo-azomethine colorimetric receptor enhanced by electron withdrawing groups, *RSC Adv.* 4 (2014) 1032.
- [24] S. Goswami, S. Paul, A. Manna, A highly reactive (<1 min) ratiometric chemodosimeter for selective "naked eye" and fluorogenic detection of hydrazine, *RSC Adv.* 3 (2013) 18872.
- [25] S. Prabhu, S. Saravanamoorthy, M. Ashok, S. Velmathi, Colorimetric and fluorescent sensing of multi metal ions and anions by salicylaldehyde based sensors, *J. Lumin.* 132 (2012) 979.
- [26] I.S. Ke, M. Myahkostupov, F.N. Castellano, F.P. Gabbai, Stibonium ions for the fluorescence turn-on sensing of F⁻ in drinking water at parts per million concentrations, *J. Am. Chem. Soc.* 134 (2012) 15309.
- [27] P. Banerjee, G. Mostafa, A. Castiñeiras, S. Goswami, Two novel reactions of a ruthenium-coordinated phenazine – oxidative aromatic ring hydroxylation and dimerization via a new C–N bond formation, *Eur. J. Inorg. Chem.* (2007) 412.
- [28] P. Banerjee, A.D. Jana, G. Mostafa, S. Goswami, Organization of the Ru₂Na moiety of a phenazine ligand into a new coordination network promoted by ClO₄-aromatic-π interactions, *Eur. J. Inorg. Chem.* (2008) 44.
- [29] S. Das, P. Banerjee, S.M. Peng, G.H. Lee, J. Kim, S. Goswami, Mononuclear to polynuclear transition induced by ligand coordination: synthesis, X-ray structure, and properties of mono-, di-, and polynuclear copper(II) complexes of pyridyl-containing azo ligands, *Inorg. Chem.* 45 (2006) 562.
- [30] (a) P. Ghosh, A. Roy Chowdhury, M. Corbella, A. Bhaumik, P. Mitra, S.M. Mobin, A. Mukherjee, S. Basu, P. Banerjee, Designed synthesis of CO₂-promoted copper(II) coordination polymers: synthesis, structural and spectroscopic characterization, and studies of versatile functional properties, *Dalton Trans.* 43 (2014) 13500; (b) P. Ghosh, S.K. Saha, A. Roy Chowdhury, P. Banerjee, Recognition of an explosive and mutagenic water pollutant, 2,4,6-trinitrophenol, by cost-effective luminescent MOFs, *Eur. J. Inorg. Chem.* (2015), <http://dx.doi.org/10.1002/ejic.201500233>
- [31] P. Ghosh, B.G. Roy, N.C. Murmu, P. Banerjee, Recognition of fluoride anion at low ppm level inside living cell and from fluorosis affected tooth and saliva samples, *RSC Adv.* 5 (2015) 27387.
- [32] C. Caltagirone, P.A. Gale, J.R. Hiscock, S.J. Brooks, M.B. Hursthouse, M.E. Light, 1,3-Diindolylureas: high affinity dihydrogen phosphate sensors, *Chem. Commun.* (2008) 3007.
- [33] A. Mishra, R. Gupta, Supramolecular architectures with pyridine-amide based ligands: discrete molecular assemblies and their applications, *Dalton Trans.* 43 (2014) 7668.
- [34] N.V. Ghule, S.V. Bhosale, S.V. Bhosale, Dipyrrolyl-bis-sulfonamide chromophore based probe for anion recognition, *RSC Adv.* 4 (2014) 27112.
- [35] I. Ravikumar, P.S. Lakshminarayanan, P. Ghosh, Anion binding studies of tris(2-aminoethyl)amine based amide sensors with nitro functionalized aryl substitutions: a positional isomeric effect, *Inorg. Chim. Acta* 363 (2010) 2886.
- [36] D.K. Kumar, R. Justin Thomas, 2-Hydroxyarylimidazole-based colorimetric and ratiometric fluoride ion sensors, *RSC Adv.* 4 (2014) 56466.
- [37] M. Ghosh, T. Weyhermüller, K. Wiegardt, Electronic structure of the members of the electron transfer series [Ni]_z (z = 3+, 2+, 1+, 0) and [NiL(X)]_n (X = Cl, CO, P(OCH₃)₃) species containing a tetradentate, redox-noninnocent, Schiff base macrocyclic ligand L: an experimental and density functional theoretical study, *Dalton Trans.* 39 (2010) 1996.
- [38] (a) D. Sharma, S.K. Sahoo, S. Chaudhary, R.K. Bera, J.F. Callan, Fluorescence 'turn-on' sensors for F⁻ derived from vitamin B₆ cofactor, *Analyst* 138 (2013) 3646; (b) R. Arabahmadi, M. Orojloob, S. Amani, Azo Schiff bases as colorimetric and

- fluorescent sensors for recognition of F^- , Cd^{2+} and Hg^{2+} ions, *Anal. Methods* 6 (2014) 7384.
- [39] S.K. Saha, P. Ghosh, A. Roy Chowdhury, P. Samanta, N.C. Murmu, A.K. Lohar, P. Banerjee, Corrosion control of chrome steel ball in nitric acid medium using Schiff base ligand and corresponding metal complexes: a combined experimental and theoretical study, *Can. Chem. Trans.* 2 (2014) 381.
- [40] S.K. Saha, A. Dutta, P. Ghosh, D. Sukul, P. Banerjee, Adsorption and corrosion inhibition effect of Schiff base molecules on the mild steel surface in 1 M HCl medium: a combined experimental and theoretical approach, *Phys. Chem. Chem. Phys.* 17 (2015) 5679.
- [41] (a) M. Giese, M. Albrecht, K. Wiemer, A. Valkonen, K. Rissanen, Controlling the position of anions relative to a pentafluorophenyl group, *New J. Chem.* 36 (2012) 1368;
(b) I. Ravikumar, P.S. Lakshminarayanan, M. Arunachalam, E. Suresh, P. Ghosh, Anion complexation of a pentafluorophenyl-substituted tripodal urea receptor in solution and the solid state: selectivity toward phosphate, *Dalton Trans.* (2009) 4160.
- [42] M. Irie, Diarylethenes for memories and switches, *Chem. Rev.* 100 (5) (2000) 1685.
- [43] J. Millman, A. Gabel, *Microelectronics*, McGraw-Hill, New York, 1988 (Chapter 6).
- [44] M.M. Mano, C.R. Kime, *Logic and Computer Design Fundamentals*, 4th ed., Prentice-Hall, Upper Saddle 5 River, 2000.
- [45] (a) B.L. Feringa, *Molecular Switches*, Wiley-VCH Express, New York, 2001, pp. 37;
(b) S. Salivahanan, S. Arivazhagan, *Digital Circuits and Design*, 2nd ed., Vikas Publishing House Pvt. Ltd., 2004 (Chapter 3);
(c) K. Rezaeian, H. Khanmohammadi, Two and three input molecular logic operations mediated by a novel azo-azomethine based chromogenic probe through intramolecular charge transfer processes, *New J. Chem.* 39 (2015) 2081.
- [46] S.R. Presow, M. Ghosh, E. Bill, T. Weyhermüller, K. Wieghardt, Molecular and electronic structures of new iron complexes containing N,S-coordinated o-iminothionebenzosemiquinonate(1-) π radical ligands: an experimental and density functional theoretical study, *Inorg. Chim. Acta* 374 (2011) 226.
- [47] R. Sivakumar, V. Reena, N. Ananthi, M. Babu, S. Anandan, S. Velmathi, Colorimetric and fluorescence sensing of fluoride anions with potential salicylaldehyde based Schiff base sensors, *Spectrochim. Acta Part A* 75 (2010) 1146.
- [48] D. Saravanakumar, S. Devaraj, S. Iyyampillai, K. Mohandoss, M. Kandaswamy, Schiff's base phenol-hydrazone derivatives as colorimetric chemosensors for fluoride ions, *Tetrahedron Lett.* 49 (2008) 127.
- [49] H.H. Hammud, A. Ghannoum, M.S. Masoud, Spectral regression and correlation coefficients of some benzaldimines and salicylaldehydes in different solvents, *Spectrochim. Acta Part A* 63 (2006) 255.
- [50] L.Z. Zhao, G.K. Wang, J.H. Chen, L.M. Zhang, B. Liu, J.F. Zhang, Q.H. Zhao, Y. Zhou, 1,8-Naphthalimide-based visible colorimetric sensor for the selective sensing of fluoride, acetate and hydroxyl anions, *J. Fluorine Chem.* 158 (2014) 53.
- [51] J.S. Wu, W.M. Liu, X.Q. Zhuang, F. Wang, P.F. Wang, S.L. Tao, X.H. Zhang, S.K. Wu, S.T. Lee, Fluorescence turn on of coumarin derivatives by metal cations: a new signaling mechanism based on C=N isomerization, *Org. Lett.* 9 (2007) 33.
- [52] D. Maity, T. Govindaraju, Naphthaldehyde-urea/thiourea conjugates as turn-on fluorescent probes for Al^{3+} based on restricted C=N isomerization, *Eur. J. Inorg. Chem.* (2011) 5479.
- [53] J. Wu, W. Liu, J. Ge, H. Zhang, P. Wang, New sensing mechanisms for design of fluorescent chemosensors emerging in recent years, *Chem. Soc. Rev.* 40 (2011) 3483.
- [54] S. Goswami, S. Paul, A. Manna, A differentially selective chemosensors for a ratiometric response to Zn^{2+} and Al^{3+} in aqueous media with applications for molecular switches, *RSC Adv.* 3 (2013) 25079.
- [55] S. Goswami, A. Manna, S. Paul, K. Aich, A.K. Das, S. Chakraborty, Dual channel selective fluorescence detection of $Al(III)$ and Pp_i in aqueous media with an 'off-on-off' switch which mimics molecular logic gates (INHIBIT and EXOR gates), *Dalton Trans.* 42 (2013) 8078.
- [56] A. Gogoi, S. Samanta, G. Das, A benzothiazole containing CHEF based fluorescence turn-ON sensors for Zn^{2+} and Cd^{2+} and subsequent sensing of $H_2PO_4^-$ and $P_4O_7^{4-}$ in physiological pH, *Sens. Actuators B* 202 (2014) 788.
- [57] S. Sen, M. Mukherjee, K. Chakraborty, I. Hauli, S.K. Mukhopadhyay, P. Chattopadhyay, Cell permeable fluorescent receptor for detection of $H_2PO_4^-$ in aqueous solvent, *Org. Biomol. Chem.* 11 (2013) 1537.
- [58] A. Sahana, A. Banerjee, S. Guha, S. Lohar, A. Chattopadhyay, S.K. Mukhopadhyay, D. Das, Highly selective organic fluorescent probe for azide ion: formation of a "molecular ring", *Analyst* 137 (2012) 1544.
- [59] S. Guha, S. Lohar, A. Sahana, A. Banerjee, D.A. Safin, M.G. Babashkina, M.P. Mitoraj, M. Bolte, Y. Garcia, S.K. Mukhopadhyay, D. Das, A coumarin-based "turn-on" fluorescent sensors for the determination of Al^{3+} : single crystal X-ray structure and cell staining properties, *Dalton Trans.* 42 (2013) 10198.
- [60] A. Sahana, A. Banerjee, S. Lohar, A. Banik, S.K. Mukhopadhyay, D.A. Safin, M.G. Babashkina, M. Bolte, Y. Garcia, D. Das, FRET based tri-color emissive rhodamine-pyrene conjugate as an Al^{3+} selective colorimetric and fluorescence sensors for living cell imaging, *Dalton Trans.* 42 (2013) 13311.
- [61] A. Banerjee, A. Sahana, S. Das, S. Lohar, B. Sarkar, S.K. Mukhopadhyay, J.S. Matlobos, D. Das, An INHIBIT logic gate from a thiophene derivative using iron and zinc ions as the input: tuning the efficiency on moving from naphthalene to anthracene to pyrene for the green luminescent detection of the intracellular iron, *Dalton Trans.* 42 (2013) 16387.

Biographies

Additi Roy Chowdhury has received her M. Sc. degree in 2010 from West Bengal State University, West Bengal, India. Her research is mostly focused on synthesis and characterization of selective anion sensor under the supervision of Dr. Priyabrata Banerjee.

Pritam Ghosh has received his M. Sc. degree in 2010 from Visva Bharati, a Central University, West Bengal, India. His research is mostly focused on synthesis and characterization of selective anion sensor as well as radical stabilized molecular complex development under the supervision of Dr. Priyabrata Banerjee.

Biswajit Gopal Roy has received his Ph.D. degree from Indian Institute of Chemical Biology (IICB), Kolkata in 2007. He did his postdoctoral research in South Korea; University of California, Riverside, USA and Queen's University, Canada. Presently he is an assistant professor in chemistry at Sikkim University, a Central University. His current research focus: Stereoselective synthesis of heterocycles, Synthetic methodology development, Natural product synthesis.

Subhra Kanti Mukhopadhyay has received his Ph. D. degree in 1997 from Burdwan University, West Bengal, India. He is an associate professor of microbiology at Department of Microbiology, Burdwan University. His current research focus – Bio-prospecting of extremophiles; Fluorescent ligand based detection of heavy metals and bio-molecules; Biological control of tea pests.

Dr. Naresh Chandra Murmu is working as Head and Principal Scientist at Surface Engineering and Tribology Division, CSIR-CMERI, Durgapur, India. He obtained his B.E. from BESUS (then Calcutta University) (1992), M.E. from IISC (1994) and PhD from IIT-BHU (2010) in Mechanical Engineering, served as Scientist at NAL-Bangalore, India, (1994–2001), visiting Scientist at Friedrich-Alexander-University, Germany (2001–2003) and short term visiting scholar at Northwestern University, USA in 2011. His current research interest includes design and development of micro and nano-scale devices.

Priyabrata Banerjee has received his Ph.D. degree in 2007 from Indian Association for Cultivation of Science, Jadavpur, Kolkata. He did his post doctoral research at Max Planck Institute for Bio-Inorganic Chemistry, Muelheim, Germany during 2007–2010. Presently, he is a Scientist in CSIR-Central Mechanical Engineering Research Institute, Durgapur, West Bengal. Dr. Banerjee is assistant professor of Chemical Science under AcSIR, New Delhi also. His current research focus is selective bio-relevant anion detection; metal mediated C-heteroatom bond fusion and metal-organic complexes and their hitherto unexplored radical chemistry development.