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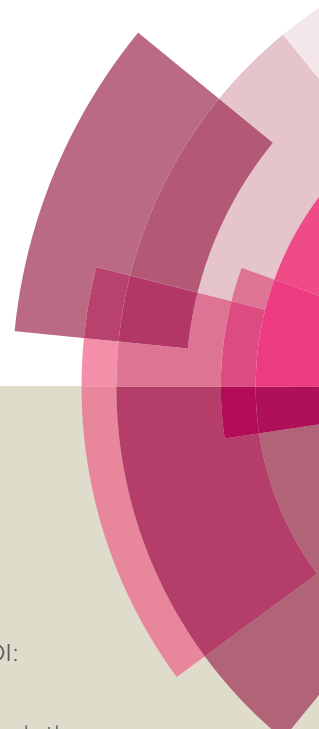
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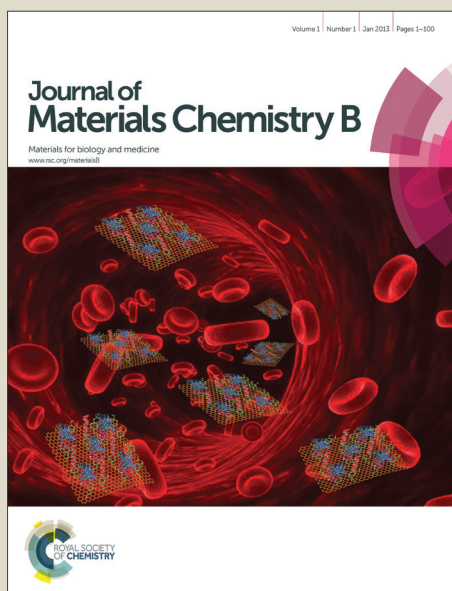
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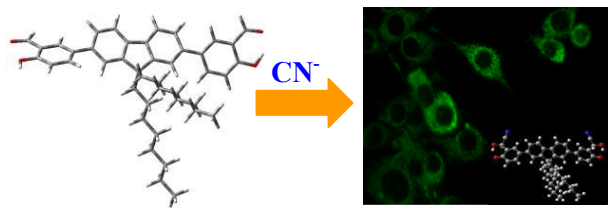
A bio-compatible fluorene based chemodosimeter (FSal) selectively detects CN^- ion by turn on fluorescence with a minimal detection limit of 0.06 ppm.

Journal Name

ARTICLE

Fluorene Based Chemodosimeter for "Turn-on" Sensing of Cyanide by Hampering ESIPT and Live Cell Imaging

Manas Kumar Bera,[a] **Chanchal Chakraborty,**[a] **Pradeep Kumar Singh,**[b] **Chandan Sahu,**[c] **Kaushik Sen,**[c] **Samir Maji,**[b] **Abhijit Kumar Das**[c] **and Sudip Malik**[a*]

A new salicylaldehyde appended fluorene based chemodosimeter (FSal) has been designed by taking consideration of the special nucleophilicity of cyanide ion. FSal shows selective affinity towards CN^- over other anions (namely F^-, Br^-, NO_3^-, ClO_4^-, N_3^-, $H_2PO_4^-$, AcO^-, I^-, Cl^-, NO_2^-) through turn on fluorescence with a minimal detection limit of 0.06 ppm. Turn on fluorescence of FSal-CN complex because of hampering ESIPT is also supported by DFT and TDDFT calculations. Biological compatibility and live cell imaging of this unique probe have also been explored.

1. Introduction

The cyanide anion (CN^-) is one of the most deadly poisons to mammals,[1-2] although it is extensively used in industrial processes, including heap leaching of gold from ore, metallurgy, electroplating. It is also used in fibers and resins industry.[3] Despite safeguards and stringent norms set by different regulatory bodies, the accidental release of cyanide contaminates drinking water and becomes a serious threat to the environment.[4] Environmental implications may also occur by some food plants like cassava and also by some Rosaceae such as apricot seeds (almond, apple, cherry, plum, pear etc.) which produce cyanogenic glycosides during natural defense mechanism. The glycosides are hydrolyzed by enzyme upon injury or stress to produce HCN.[5] Furthermore, the release of cyanide to the environment with potential dangerous effects is also an added source of concern.[6] In living cells, Cyanide binds with Fe^{3+} of heme unit, and affects the oxygen supply. The active site of cytochrome c is inactivated by CN^- and blocks the electron transport chain. As a result, cellular respiration is inhibited.[7] All the above factors demand the development of a receptor for CN^- sensing in aqueous as well as in cellular environment.

Fluorescent chemosensors have many advantages, including high sensitivity, low operational cost, ease of detection and suitability as diagnostic tools for biological concern.[8-13] There are several methods for selective detection of CN^-, which includes cyanide addition to Zn^{II}-porphyrin,[14] polymer-Cu^{II}-complex,[15] CdSe quantum dots,[16] organoboron derivatives.[17] Hydrogen-bonding interactions,[18] luminescent lifetime measurement,[19] and deprotonation technique[20] have also been adopted for this purpose. However, there is a growing interest to develop a chemodosimetric probe for CN^- to circumvent the interference of other anions and this approach is based on irreversible reaction by strong nucleophile CN^- which has been used in cyanohydrin reactions.[21] The carbonyl centers which is *ortho* with respect to a hydroxyl group act as strong electrophile due to the resonance-assisted hydrogen bonds (RAHBs) introduced by Gilli et al.[22] and the nucleophilic addition to the carbonyl centers become very flippant.[23]

Keeping these in the perspective, we have designed and synthesized a novel chemodosimetric probe FSal (Scheme 1) to detect CN^- based on fluorene as color reporting group and salicylaldehyde as an electrophile for employment of intramolecular hydrogen bond of phenolic proton. We have also synthesized two model compounds FBal and FMBal according to scheme 1 to support the sensing mechanism. To date, various chemosensors[24] which have been reported for CN^-, suffer from several limitation such as high detection limit, interference of other anions like F^- or AcO^- and high temperature. In this circumstance, it's a challenge for us to fabricate a unique chemodosimetric system which can detect CN^- from aqueous solution without affecting by other anions and below than the permissible level of cyanide (0.2 ppm) in drinking water set by Environment Protection Agency (EPA).[25] To the best of our knowledge, this is the first report where a salicylaldehyde appended fluorene-based chemodosimeter has been used for the detection of CN^- from water with high selectivity and low detection limit (0.06 ppm). Again this novel probe is biocompatible which is an added advantage for live cell application.
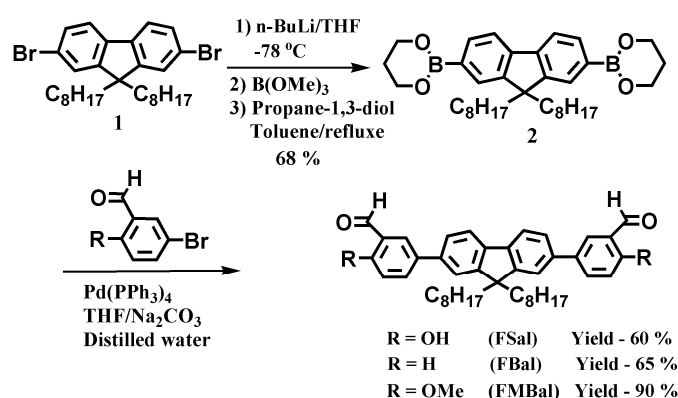
Scheme 1. Synthesis of fluorene based probe FSal and two model compounds FBal and FMBal.

2. Experimental

2.1 Materials

All these reagents (2,7-dibromo-9,9-dioctylfluorene, 3-bromobenzaldehyde, n-BuLi, tri-methylborate, 1,3-propanediol, Pd(PPh$_3$)$_4$, and tetrabutylammonium cyanide (TBACN) from Sigma-Aldrich Co. Ltd. and rest from Merck India Pvt. Ltd.) were used without further purification, and all the experiments were performed at room temperature (25 ^{0}C). All the solvents used for the synthesis were from Merck India Pvt. Ltd. and were used after distillation under N$_2$ environment. For spectral detection the HPLC grade CH$_3$CN solvent were used whereas deionized water (obtained from Millipore Milli-Q system, 18MΩ) was used to prepare salt solutions.

2.2 Characterization

All the compounds were characterized by ^{1}H-NMR, ^{13}C-NMR and Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) techniques. NMR spectra were acquired on 300 and 500 MHz Bruker DPX spectrometer using CDCl$_3$ as solvent and TMS as standard reference at room temperature, with chemical shift given in parts per million. UV–Vis spectra of all samples were studied with Hewlett–Packard UV–Vis spectrophotometer (model 8453). Photoluminescence studies of solution were recorded with a Horiba Jobin Yvon Fluoromax 3 spectrometer at an excitation wavelength 360 nm. MALDI-TOF was carried out with Bruker Daltonics FLEX-PC using diathranol as a matrix.

2.3 Preparation of test solution absorption and emission measurement

For spectroscopic measurement the solutions of FSal, FBal and FMBal (5 μM) were prepared in CH$_3$CN separately. The stock solutions of the anions (2 mM) as their potassium salt (only cyanide as tetrabutylammonium salt) were prepared in deionized water. The experiments were done by taking 2 mL probe solution followed by addition of aqueous solution of different anions. After addition of anion solution the resulting

solution was shaken well and spectra was recorded after 5 min. For emission spectra measurement the slit was 5/5.

2.4 Synthesis

9,9-Dioctylfluorene-2,7-bis(trimethylene boronates) (2).

Into a solution of 2,7-dibromo-9,9-dioctylfluorene 1 (2 g, 3.6 mmol) in anhydrous THF (30 mL) was added n-BuLi (1.6 M in hexane, 6.7 mL, 10.8 mmol) dropwisely at -78 ^{o}C. The reaction mixture was stirred for 2 h prior to the addition of tri-methyl borate (4 mL, 36 mmol) in one portion. The mixture was warmed to room temperature, stirred overnight and was poured into crushed ice containing HCl (2 M) while stirring. The mixture was extracted with ether and the combined extracts were evaporated to give a yellowish liquid (diboronic acid). Then the mixture was refluxed with 1,3-propanediol (0.63 mL, 8.71 mmol) in 40 mL toluene for 10 h. After workup, the crude product was purified by column chromatography (silica gel, petroleum ether : ethyl acetate (4:1) as eluent) to afford a yellowish liquid (1.36 g, 68% yield). ^{1}H-NMR (300 MHz, CDCl$_3$): δ (ppm) 7.78-7.62 (m, 4H), 7.32 (m, 2H), 4.20 (t, 8H), 2.10 (m, 4H), 1.99 (m, 4H), 1.27-1.03 (m, 20H), 0.84 (t, 6H), 0.60 (m, 4H). ^{13}C-NMR (300 MHz, CDCl$_3$): δ (ppm) 150.4, 143.6, 132.6, 127.7, 127.3, 120.9, 62.7, 55.0, 40.6, 31.9, 30.1, 29.8, 29.3, 27.5, 23.8, 22.7, 14.2. Elemental analysis for C$_{35}$H$_{52}$B$_2$O$_4$ (%) calculated : C 75.28, H 9.39; found: C 75.55, H 9.30. MALDI-TOF (m/z): calculated: 558.4, found: 558.3.

9,9-dioctylfluorene-2,7-bis-(5-salicylaldehyde) (FSal).

9,9-Dioctylfluorene-2,7-bis(trimethylene boronates) (2) (0.558 g, 1.0 mmol), 5-bromosalicylaldehyde (0.4 g, 2.0 mmol) and Pd(PPh$_3$)$_4$ (0.008 g) were added to a mixture of 15 mL THF and aqueous 2M Na$_2$CO$_3$ (7 mL) under nitrogen atmosphere. The mixture was vigorously stirred at 90 ^{o}C for 24 h. After the mixture was cooled to room temperature, it was poured into 100 mL deionized water. The aqueous layer was extracted with dichloromethane three times. The combined organic layers were washed with water and dried over sodium sulfate. After vacuum evaporation of the solvent, the residue was purified by column chromatography (silica gel, DCM/Petroleum ether as eluent) to give a yellowish liquid (0.378 g, 60% yield). ^{1}H-NMR (500 MHz, CDCl$_3$): δ (ppm) 11.03 (s, 2H), 10.03 (s, 2H), 7.87–7.77 (m, 6H), 7.56–7.51 (m, 4H), 7.13–7.10 (d, 2H), 2.08–2.02 (m, 4H), 1.33-0.71 (m, 30H). ^{13}C-NMR (500 MHz, CDCl$_3$): δ (ppm) 196.8, 161.0, 152.0, 140.1, 138.4, 136.0, 133.9, 131.9, 125.7, 121.0, 120.9, 120.4, 118.2, 55.5, 40.6, 32.0, 30.0, 29.8, 29.5, 23.9, 22.8, 14.2. Elemental analysis calculated (%) for C$_{43}$H$_{50}$O$_4$: C 81.90, H 7.93; found: C 81.63, H 8.05. MALDI-TOF (m/z): calculated: 630.8, found: 630.4.

9,9-dioctylfluorene-2,7-bis-(3-bromobenzaldehyde) (FBal).

9,9-Dioctylfluorene-2,7-bis(trimethylene boronates) (2) (0.279 g, 0.5 mmol), 3-bromobenzaldehyde (0.185 g, 1.0 mmol) and Pd(PPh$_3$)$_4$ (0.006 g) were added to a mixture of 10

mL THF and aqueous 2M Na_2CO_3 (4 mL) under nitrogen atmosphere. The mixture was vigorously stirred at 90 °C for 24 h. After the mixture was cooled to room temperature, it was poured into 100 mL deionized water. The aqueous layer was extracted with dichloromethane three times. The combined organic layers were washed with water and dried over sodium sulfate. After vacuum evaporation of the solvent, the residue was purified by column chromatography (silica gel, DCM/Petroleum ether as eluent) to give a yellowish liquid (0.194 g, 65% yield). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 10.13 (s, 2H), 8.19 (s, 2H), 7.96–7.94 (d, 2H), 7.89–7.81 (dd, 4H), 7.67-7.61 (m, 6H), 2.09–2.05 (m, 4H), 1.33–0.70 (m, 30H). ^{13}C-NMR (500 MHz, $CDCl_3$): δ (ppm) 192.5, 152.1, 142.6, 140.6, 138.8, 137.0, 133.2, 129.6, 128.8, 128.1, 126.3, 121.5, 120.5, 55.6, 40.4, 32.0, 30.3, 29.8, 29.4, 23.9, 22.7, 14.1. Elemental analysis for $C_{43}H_{50}O_2$ (%) calculated : C 86.24, H 8.42; found: C 85.81, H 8.47. MALDI-TOF (m/z): calculated: 598.8, found: 598.4.

9,9-dioctylfluorene-2,7-bis-(5-bromo-2 methoxybenzaldehyde) (FMBal).

To a solution of 9,9-dioctylfluorene-2,7-bis-(5-salicylaldehyde) (0.1 g, 0.16 mmol) in dry DMF (4 mL) under N_2 was added powdered K_2CO_3 (0.066 g, 0.48 mmol, 3 equiv) and the mixture was stirred at room temperature for 15 min. MeI (0.068 g, 0.48 mmol, 3 equiv) was added dropwise and the reaction mixture stirred at room temperature for 12 hours. It was then quenched with water (50 mL) and the solution extracted with EtOAc (4 × 25 mL). The combined organic layers were washed with water, brine, dried over sodium sulfate and concentrated. The residue was purified by column chromatography (silica gel, EtOAc/Petroleum ether as eluent) to give a white solid (0.094 g, 90% yield). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 10.55 (s, 2H), 8.16-8.15 (d, 2H), 7.90–7.86 (dd, 2H), 7.77–7.47 (d, 2H), 7.58–7.53 (m, 4H), 7.12-7.09 (d, 2H), 4.00 (s, 6H), 2.06–2.01 (m, 4H), 1.25-0.75 (m, 30H). ^{13}C-NMR (500 MHz, $CDCl_3$): δ (ppm) 190.0, 161.3, 151.9, 140.1, 138.5, 134.6, 134.4, 126.8, 125.7, 125.0, 121.0, 120.2, 112.2, 56.0, 55.5, 40.5, 31.8, 30.1, 29.8, 29.3, 23.9, 22.7, 14.1. Elemental analysis for $C_{45}H_{54}O_4$ (%) calculated : C 82.03, H 8.26; found: C 81.67, H 7.94. MALDI-TOF (m/z): calculated: 658.9, found: 658.4.

3. Results and Discussion

Starting from 2,7-dibromo-9,9-dioctylfluorene, followed by boronate ester formation and subsequent reaction with the aldehyde compounds, we have synsized fluorene based salicylaldehyde containing probe (FSal) and two model compounds (FBal and FMBal) according to Scheme 1. All the compounds are soluble in all common organic solvents like CH_3CN, DMSO, chloroform, tetrahydrofuran, methanol etc. FSal shows absorption maxima at 329 nm (π-π^*) along with a shoulder at 308 nm and emission maxima at 380 nm (λ_{ex}= 329 nm) alongwith a low intense broad peak from 520-640 nm in

CH_3CN solvent (Fig. 1b-c). The ability of FSal to complex with other anions (F[-], Br^{-}, NO_3^{-}, ClO_4^{-}, N_3[-], H_2PO_4[-], AcO[-], I[-], Cl^{-}, NO_2^{-}) is explored with the help of UV-Vis absorption and emission spectrometry. Addition of only 5 equivalent of CN^{-}, absorption peak of FSal (5 μM) shows a red shift of 14 nm (Fig. 1b) with decrease of intensity; whereas addition of other anion (excess with 10 equiv.) does not affect the absorption maxima. However, a low intense broad peak is prominent in the region 400-450 nm after addition of CN^{-} to FSal and a visible colour change (from colourless to yellow; Fig. S13 in SI) has been observed, indicating formation of FSal-CN complex.
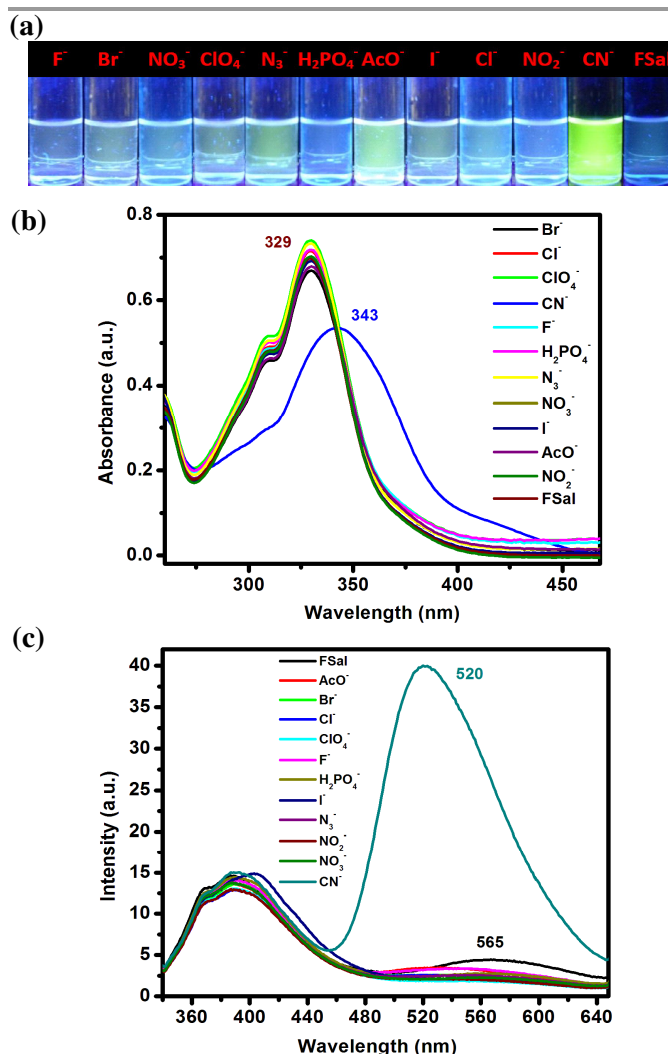

Fig. 1 (a) Fluorogenic response of FSal (5 μM in CH_3CN) with CN^{-} 26 equiv. and other anions 50 equiv. in H_2O. (b) Absorption spectra of FSal (5 μM in CH_3CN) upon addition of 5 equiv. CN^{-} and 10 equiv. of other anions in H_2O. (c) Emission spectra of FSal (5 Mm in CH_3CN) upon addition of CN^{-} (26 equiv.) and excess other anions (50 equiv.) in H_2O. Excitation at 329 nm (slit 5/5).

To explain the colorimetric response of FSal, we have taken the help of density functional theory (DFT) calculation on FSal and its product FSal-CN.[26] Geometry optimization was performed at the B3LYP/6-31G(d) level[27] and optimized geometries were considered for single point time dependent

DFT (TD-DFT) calculations at the B3LYP/TZVP level.[28] The lowest energy geometries of FSal and FSal-CN, correspond to H transfer from phenolic oxygen to formyl oxygen, are shown in the Fig. 2a-b . This proton transfer is also validated by ^{1}H NMR study (Fig. 3). ^{1}H NMR titrations were performed in CDCl$_3$, with 0.016 mmol of FSal dissolved in 0.5 mL of CDCl$_3$. TBACN was dissolved in CDCl$_3$ and added to the NMR tube via a syringe followed by shaking for 5 minutes. It reveals that after addition of CN$^-$ to FSal, the aldehyde proton is shifted to upfield (from δ = 10.03 ppm to δ = 5.6 ppm) in CDCl$_3$ at room temperature, indicating the formation of cyanohydrin due to nucleophilic attack of CN$^-$ to formyl group of FSal. As a result, a negative charge has moved on the phenolic oxygen to generate a partial quinonoidal structure for which the negative charge is delocalized over the conjugated system.
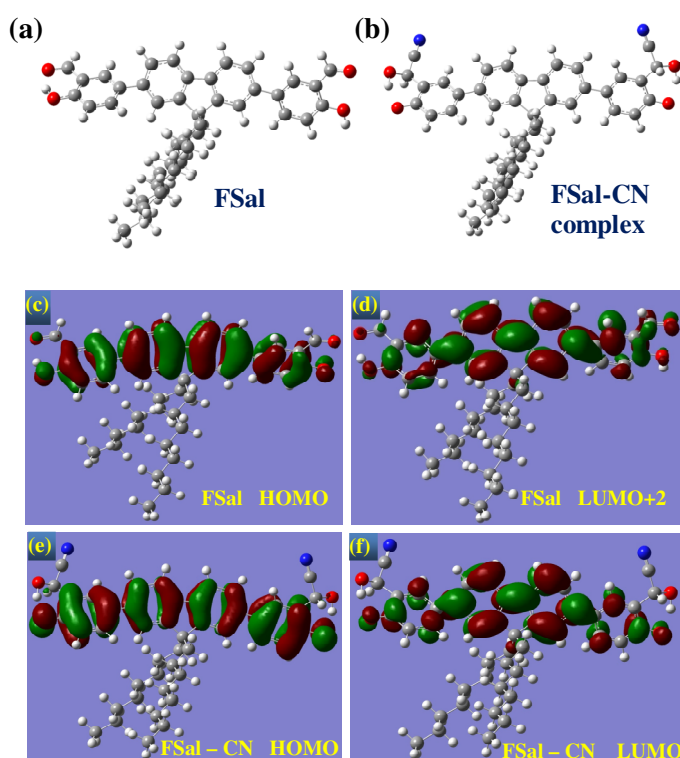

Fig. 2 (a-b)Optimized geometry of FSal and FSal-CN complex. HOMO and LUMO of FSal (c-d) and FSal-CN (e-f).

The TD-DFT calculation of FSal also indicates that the peak at 329 nm is due to π-π* transition (Fig. 2c-d, HOMO-LUMO+2 at 338 nm with oscillator strength f = 1.45) and after addition of CN$^-$ to FSal, the low intensity broad peak is appeared in the region 400-450 nm which is for charge- transfer band from negatively charged quinone part to fluorene moiety (Fig. 2e-f). This transition calculated at 410 nm with an oscillator strength f = 1.51, corresponds to HOMO-LUMO (Table ST1 and ST2 in SI).
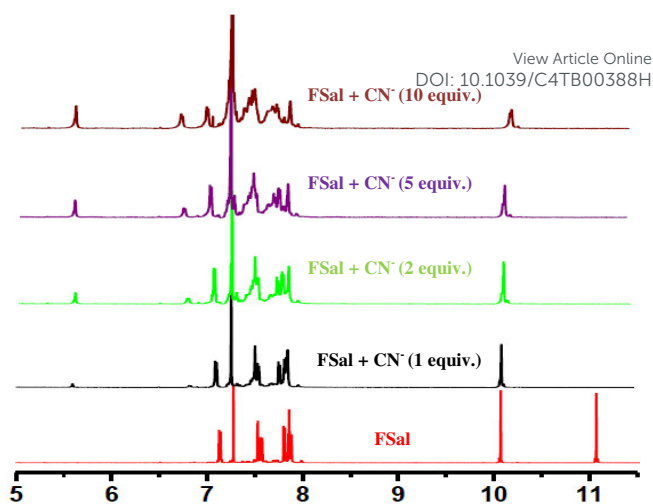
Fig. 3 Partial ^{1}H NMR spectra of FSal in presence of different concentration of CN$^-$ ion in CDCl$_3$.

The fluorescence response of FSal (5 μM in CH$_3$CN) was studied with the addition of different anions (CN$^-$ 26 equiv. and excess other anions 50 equiv. in H$_2$O). The emission maxima of FSal is shifted to 520 nm after addition of CN$^-$ with 9 fold increase in intensity whereas other anions do not lead any significant change (Fig. 1c). Due to excited state intramoleculer
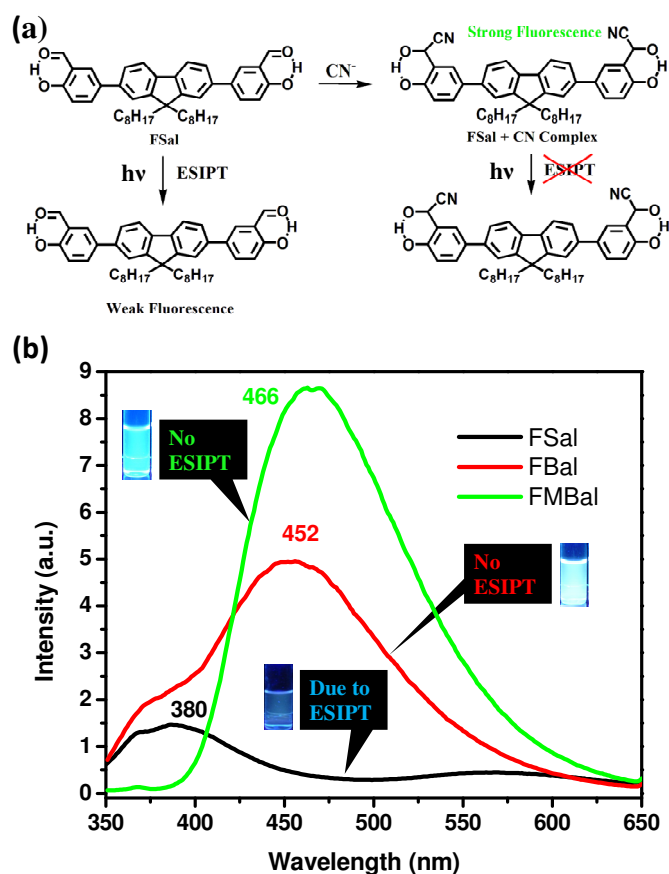

Fig. 4 (a) Scheme of sensing mechanism for FSal for CN$^-$. (b) Representative emission spectra of three probes (5 μM in CH$_3$CN).

proton transfer (ESIPT) which opens the ways for nonradiative deactivation, FSal is a weak emitter in acetonitrile.[29] After addition of CN⁻, the formyl group of FSal generates cyanoalkoxide anion (deprotonated cyanohydrin) that exchanges the phenolic proton. This process hampers ESIPT and results strong emission.[29] Excited-state intramolecular proton transfer (ESIPT) is a photoinduced procedure in which a proton jumps across the intramolecular hydrogen bond (IMHB) from a proton donor group to a proton acceptor one which first described by Waller.[30] Sensing mechanism for FSal with CN⁻ is given in (Fig. 4a). To test sensing mechanism through ESIPT, two model compounds have been synthesized (FBal and FMBal). As FBal has no phenolic-OH group and FMBal has methyl protected phenolic-OH group, the possibility of ESIPT is minimized (Fig. 4b). FBal and FMBal produce absorbance maxima 327 and 330 nm and emission maxima 452 and 466 nm (Fig. S14-S18) respectively. Even under UV irradiation (λ_{ex}= 365 nm), solution of these two moieties show strong emission.

The selectivity of FSal has been further demonstrated by observing a visual greenish yellow fluorescent color only for CN⁻ (Fig. 1a) under a 365 nm UV lamp. However, FBal and FMBal have no affinity towards CN⁻ (Fig. S19 in SI). A competitive fluorescence studies were performed in order to elucidate the effectiveness of FSal under harsh condition. Fluorescence spectra of FSal (5 μM, in CH₃CN) were recorded after addition of CN⁻ anion (25 equiv. in H₂O) in presence of excess other anions (100 equiv. in H₂O, Fig. S20 in SI). A representative bar plot (Fig. 5a) showing the fluorescence response of individual anions (red bar) as well as selective sensing of cyanide by FSal in presence of excess other anions (green bar), reveals the retention of activity of FSal in different environment.
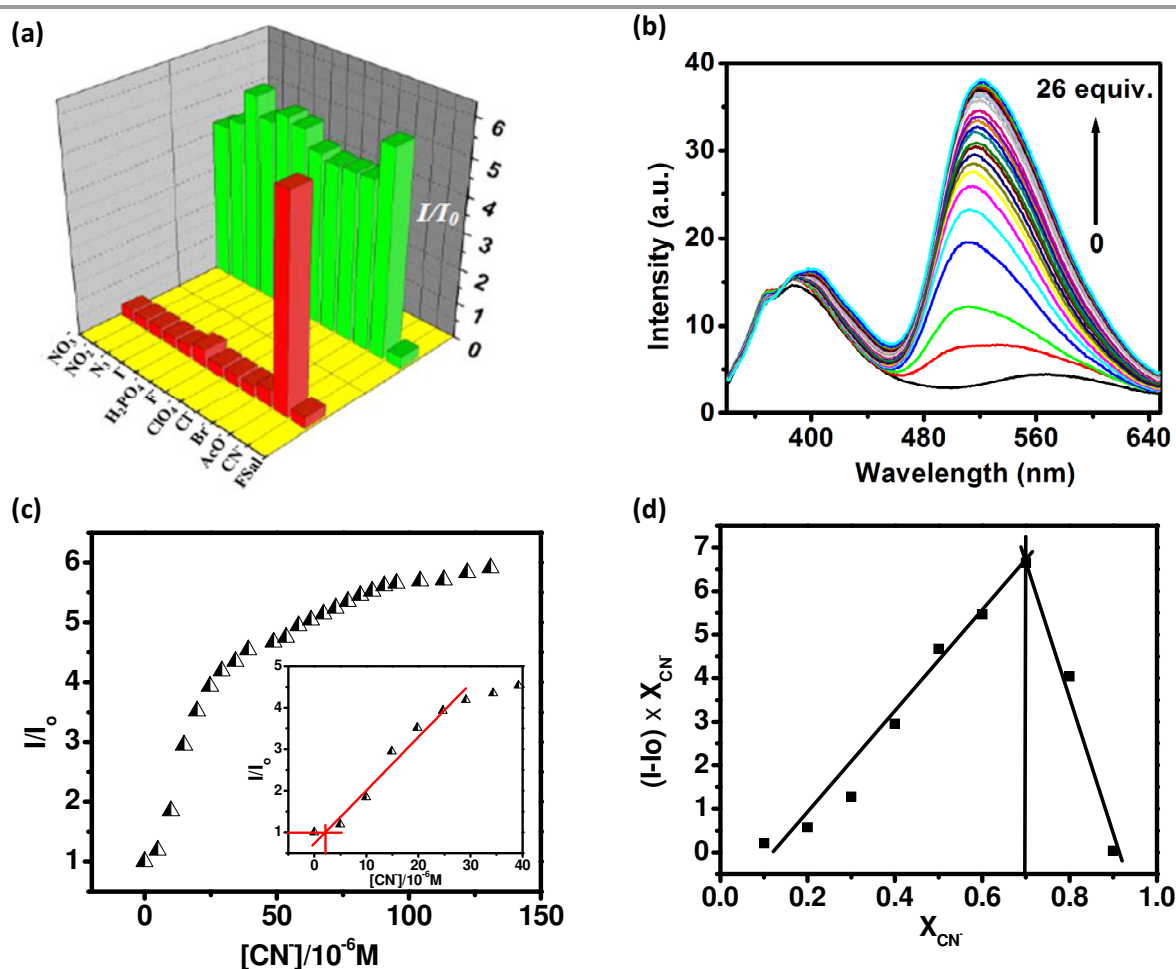
Fig. 5 (a) Bar plot of fluorescence response for individual anions (Red bar) and selectivity response towards CN⁻ in presence of excess other anions (100 equiv.) (Green bar) (b) Fluorescence titration profile of FSal (5 μM in CH₃CN) with CN⁻ (0-26 equiv.) in H₂O. (c) Plot of I/I_o vs [CN⁻] Fluorescence titration spectra. Inset: Extension of figure c. for cyanide concentration 0-8 equiv. and (d) Job's plot for stoichiometry determination of FSal to CN⁻ indicating the ratio of FSal to CN⁻ 1:2 observed from emission spectra.

A titration experiment was carried out by taking FSal (5 μM in CH$_3$CN) with gradual addition of aqueous solution of CN$^-$ (Fig. 5b). The intensity of the peak at 520 nm gradually increases upto 26 equiv. of CN$^-$. A plot of I/I_0 (where I is the intensity at 520 nm for FSal-CN complex and I_0 is the intensity of FSal at 565 nm) against [CN$^-$] reveals that the curve is linearly fitted from 0 to 5 equiv. of CN$^-$ (Fig. 5c), from which we estimate a detection limit of 0.06 ppm.[31] This limit of detection is much lower than the limit (0.2 ppm) set by EPA for drinking water. From the Job's plot[32] (Fig. 5d) the binding ratio of FSal and CN$^-$ is found to be 1:2.

To demonstrate the application of CN$^-$ sensing in cell, we have first tested whether this sensor (FSal) possesses any cytotoxicity. To elucidate this possibility, cytotoxicity of FSal was performed using MTT assay[33] for SH-SY5Y neuronal cells (Fig. 6a) with wide ranges of FSal concentrations. Details of the experiments are given in SI. FSal does not show any significant cytotoxicity in SHSY5Y cells. To further demonstrate the CN$^-$ sensing potential of this novel sensor in cells, intennalization of FSal was investigated by incubating them with cells. After washing the cells, cells were treated with CN$^-$. After CN$^-$ treatment (3mM) as shown in (Fig. 6c), a successful response is found to CN$^-$ in the cytoplasm of SH-SY5Y neuronal cells. The data indicate that our sensor (FSal) can detect the CN$^-$ that accumulated inside the cells.

(FSal) and first time it has been recognized as CN$^-$ sensor with high selectivity and very low detection limit (0.06 ppm which is much below the permissible level (0.2 ppm) of drinking water). TD-DFT calculations support the mechanism. Our designed probe is biocompatible and in-vitro cell imaging with SH-SY5Y neuronal cells has been explored. As our designed probe FSal is nontoxic and in-vitro cell imaging provides good response after addition of CN$^-$, it can be employed for real-life application. So our compound will cover a wide range from chemical to biological applications.

Acknowledgements

[a] Polymer Science Unit, Indian Association for the Cultivation of Science, 2A & 2B Raja S. C. Mullick Road, Jadavpur, Kolkata – 700032, India.
[b] Department of Spectroscopy, Indian Association for the Cultivation of Science, 2A & 2B Raja S. C. Mullick Road, Jadavpur, Kolkata – 700032, India.
[c] Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, Maharashtra, India 400076.
Email: psusm2@iacs.res.in

† Electronic Supplementary Information (ESI) available: Detailed NMR spectra, MALDI-TOF mass spectra for all compounds, UV-Vis, PL spectra, details of theoretical calculation and biological study. See DOI:10.1039/b000000x/

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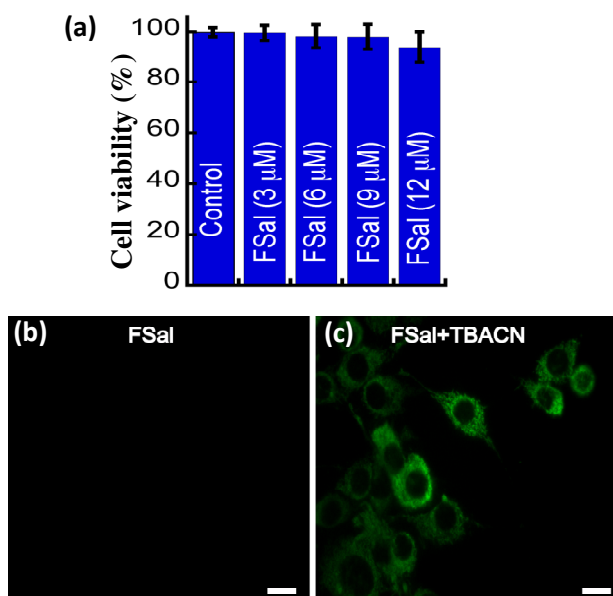
Fig. 6 (a) MTT assay for FSal. (b) Fluorescence imaging of SH-SY5Y neuronal cells which were incubated with FSal (12μM) and (c) Cells after treatment with 3mM tetrabutylammonium cyanide.

Conclusion

In summary, we have designed and synthesized a salicylaldehyde-appended fluorene-based chemodosimeter

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