

# **Rhodamine based sensor for naked-eye detection and live cell imaging of Fluoride ions**

**Gandhi sivaraman, and Duraisamy Chellappa\*<sup>a</sup>**

*<sup>a</sup> School of chemistry, Madurai Kamaraj University, Madurai-625021.  
E-mail: dcmku123@gmail.com*

**Electronic Supporting Information**

## Materials and instrumental methods:

All reagents and solvents were used without purification. Absorption measurements were carried out in JASCO V-550 UV-vis spectrophotometer. Fluorescence spectra were recorded in F-4500 Hitachi fluorescence spectrophotometer. The slit width was 5 nm for both excitation and emission. NMR spectra were recorded in Bruker (Avance) 300 MHz instrument using TMS as internal standard. ESI-MS spectral analysis was performed in positive ion as well as negative ion mode on a liquid chromatography-ion trap mass spectrometer (LCQ Fleet, Thermo Fisher Instruments Limited, US). Elemental analysis was carried out in a Perkin-Elmer 4100 elemental analyzer. Fluorescence microscopic images were taken using Zeiss LSM 510 META confocal fluorescence microscope. Tertiary butyl ammonium salts of anions ( $F^-$ ,  $Cl^-$ ,  $Br^-$ ,  $I^-$ ,  $CN^-$ ,  $AcO^-$ ,  $H_2PO_4^-$ ,  $SCN^-$ ,  $P_2O_7^{4-}$ ,  $NO_3^-$ ,  $NO_2^-$ ) were used as the source for anions.

## Synthesis of RDF-1:

Rhodamine 6G hydrazide (0.5 g, 1.09 mmol) and 2- formyl imidazole (0.5 g, 1.09 mmol) were added to 10 ml ethanol. The suspension was refluxed overnight in the presence of catalytic amount of acetic acid. The reaction mixture upon cooling to room temperature, had thrown out colorless solid. The precipitated solid was then purified by column chromatography using dichloromethane/ethyl acetate (9:1) yielded colorless solid.  $^1H$  (300 MHz,  $CDCl_3$ ) 11.50(Br, 1H), 8.00- 7.97(m, 1H), 7.76(s, 1H), 7.51- 7.42(m, 2H), 7.05- 7.02(m, 2H), 6.98(s, 1H), 6.38(s, 2H), 6.30(s, 2H), 3.50(s, 2H), 3.23- 3.15(m, 4H), 1.86(s, 6H), 1.33- 1.28(t, 6H,  $J=3.9$ Hz).  $^{13}C$  NMR (75 MHz,  $CDCl_3$ ): 166.1, 153.4, 151.2, 148.0, 144.5, 135.3, 134.1, 130.4, 128.4, 127.2, 123.7, 123.6, 118.3, 117.6, 105.1, 97.4, 65.9, 38.4, 16.8, 14.9. MS (ESI): 507.4167 ( $M+H$ )<sup>+</sup>, calculated: 506.5941. Elemental analysis for  $C_{30}H_{30}N_6O_2$ : calculated: C, 71.13; H, 5.97; N, 16.59 and found: C: 70.97; H, 5.91; N, 16.61.

## Computational details:

Density functional theory (DFT) calculations Were carried out with 6-311G\* basis set using Gaussian 09 program in order to understand the fluorescence enhancement of RDF-1 on appendage of fluoride ions. The geometries of RDF-1 and RDF-1+ $F^-$  were optimized by DFT-B3LYP using 6-311G basis sets. The TDDFT calculations on the optimized geometries of RDF-1 and RDF-1+ $F^-$  complex using above basis sets were carried out to obtain the information about absorption behaviour and corresponding transitions of RDF-1 and RDF-1 +  $F^-$ . The MO's were plotted using Gaussview 05 with the isosurface value 0.05.

## Cell culture and fluorescence imaging:

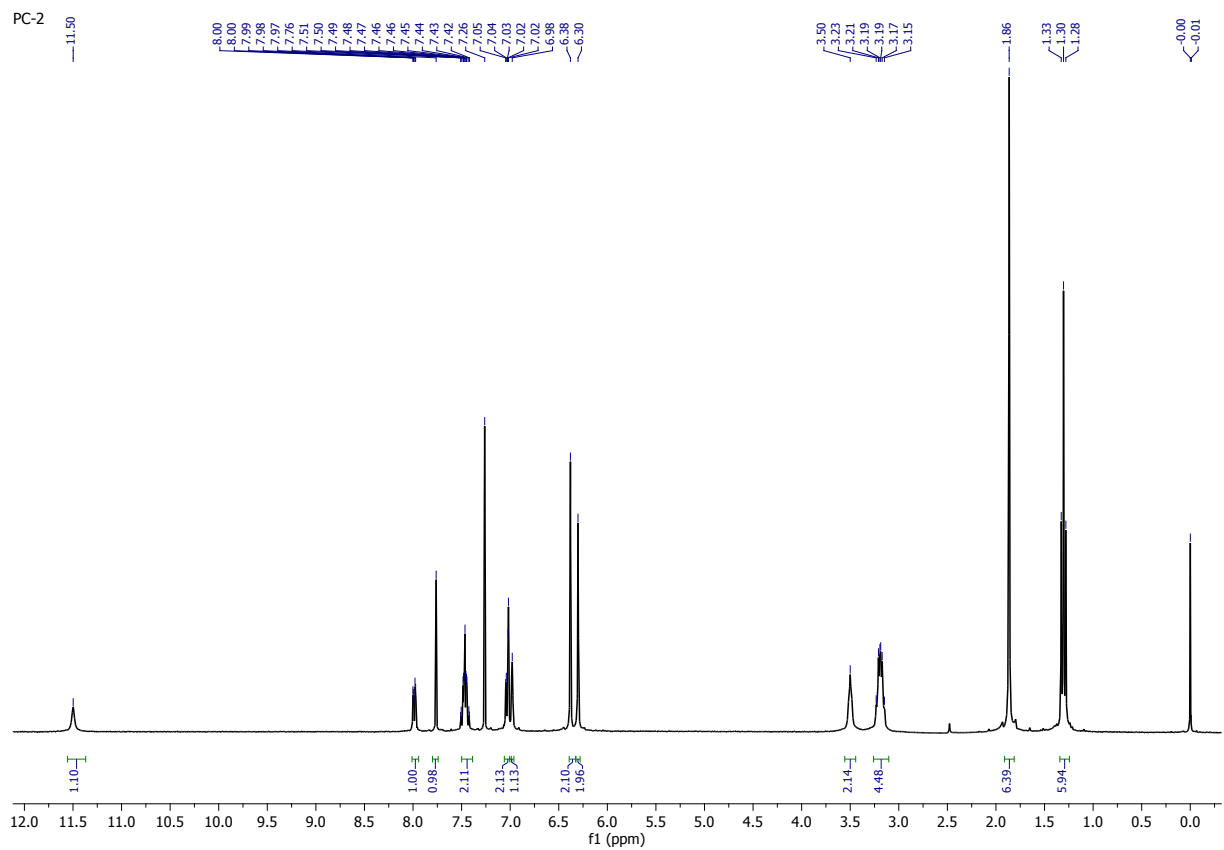
HeLa cells were grown in modified Eagle's medium supplemented with 10% FBS (fetal bovine serum) at 37°C. The cells were washed with PBS buffer. The HeLa cells were then incubated with RDF-1 (5.0  $\mu$ M in  $H_2O$ /DMSO (2:1, v/v) buffered with PBS, pH = 7.54) in the culture medium for 30 min at 37°C. After washing with PBS three times to remove the

extra cellular of the probe RDF-1 in the cells, the cells were further incubated with NaF(10.0  $\mu$ M in H<sub>2</sub>O) for 10 min at 37°C and imaged with Zeiss LSM 510 META confocal fluorescence microscope.

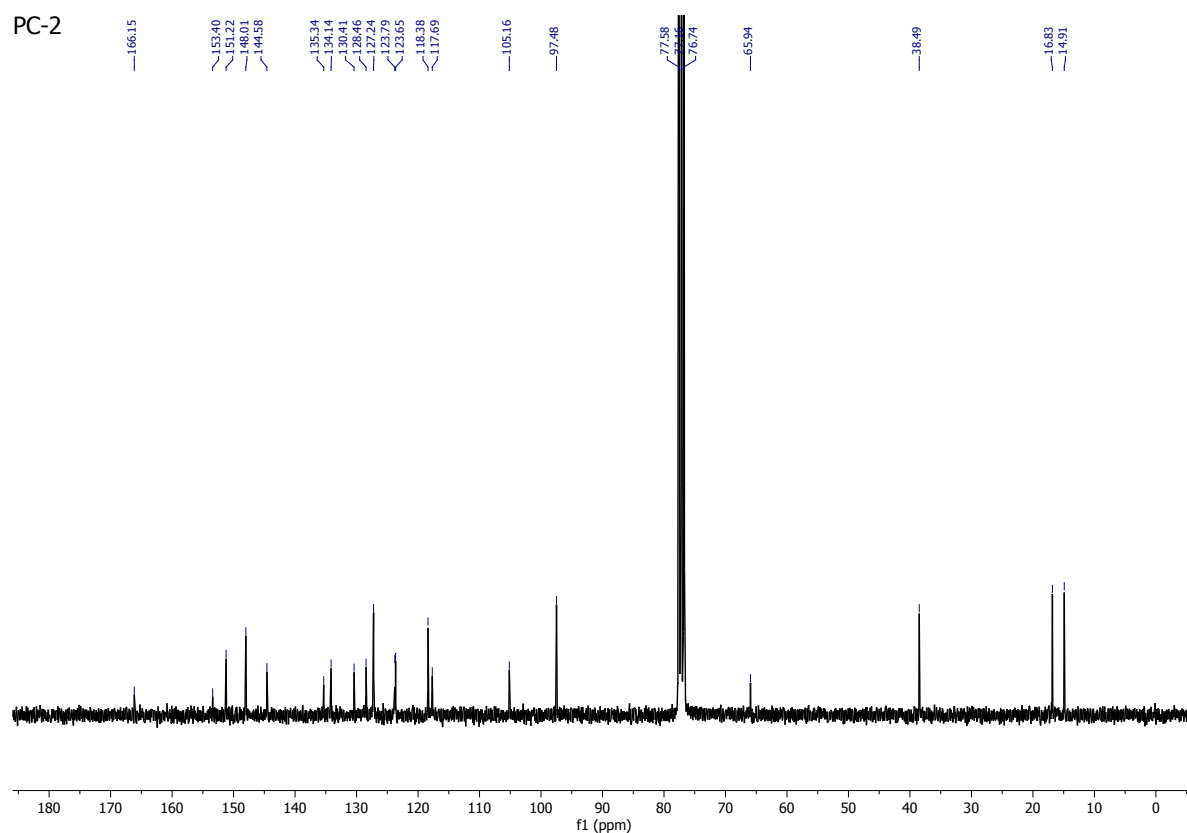
**Preparation of test Strips:**

A filter paper was immersed in the probe RDF-1 ( $5.0 \times 10^{-3}$  M) dissolved in for 10 seconds and then dried in air. The test papers was again immersed into the fluoride-containing aqueous solution for 2 min then air-dried in order to detect fluoride in real samples.

**Fig-S1: <sup>1</sup>H-nmr Spectrum of RDF-1 in CDCl<sub>3</sub>:**

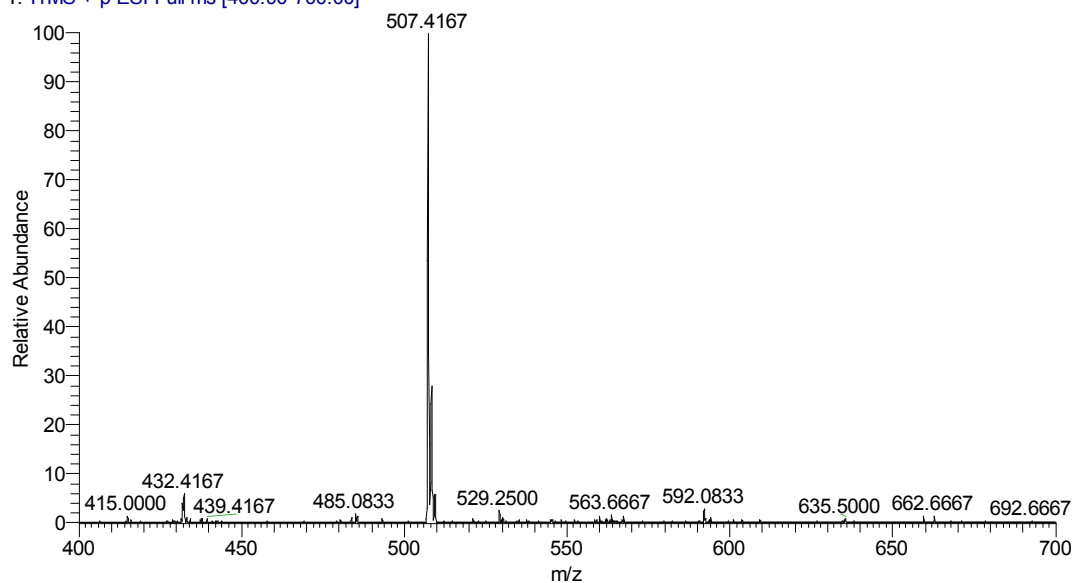


**Fig-S2:  $^{13}\text{C}$ -nmr Spectrum of RDF-1 in  $\text{CDCl}_3$ :**

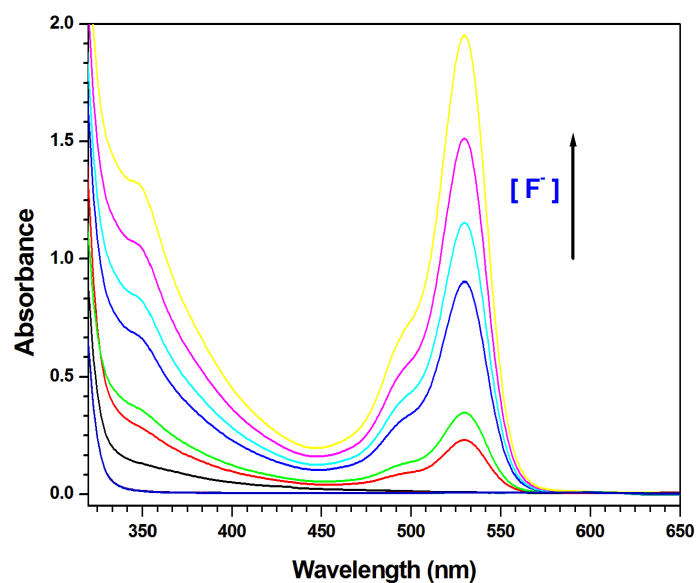


**Fig-S3: ESI-MS Spectrum of the probe RDF-1:**

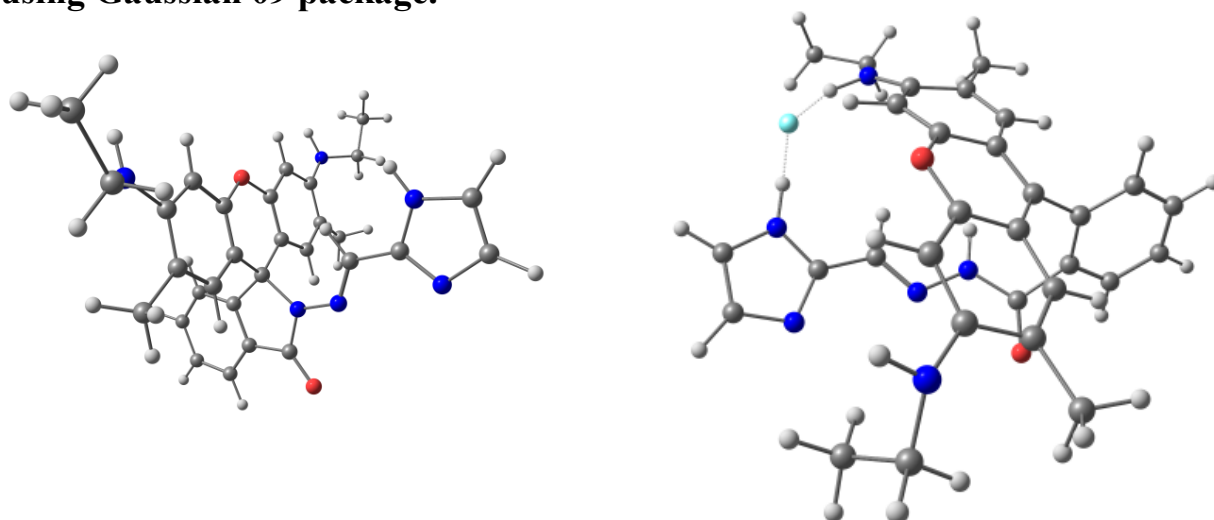
RDI\_120416230216 #196 RT: 0.40 AV: 1 NL: 1.08E4  
T: ITMS + p ESI Full ms [400.00-700.00]



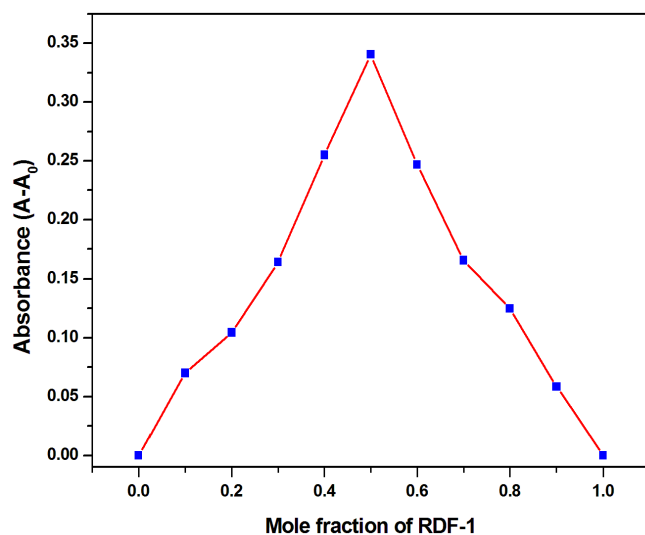
**Fig-S4: UV-visible absorption responses of RDF-1(5 mM) toward different concentrations of F<sup>-</sup> in HEPES buffer (20 mM, pH 7.4) solution (Acetonitrile/water = 3: 7).**



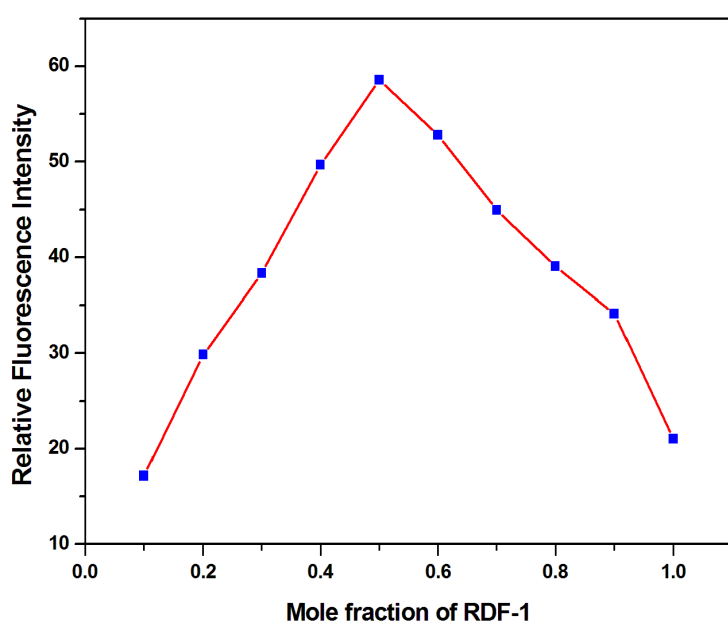
**Fig-S5: optimized geometries of RDF-1 and RDF-1+F<sup>-</sup> by DFT/B3LYP-6-311G using Gaussian 09 package.**



**Fig-S6: Continuous variation (Job's) plot mole fraction of  $\text{Zn}^{2+}$  vs change of Absorbance at  $\lambda_{\text{abs}} = 528 \text{ nm}$ .**

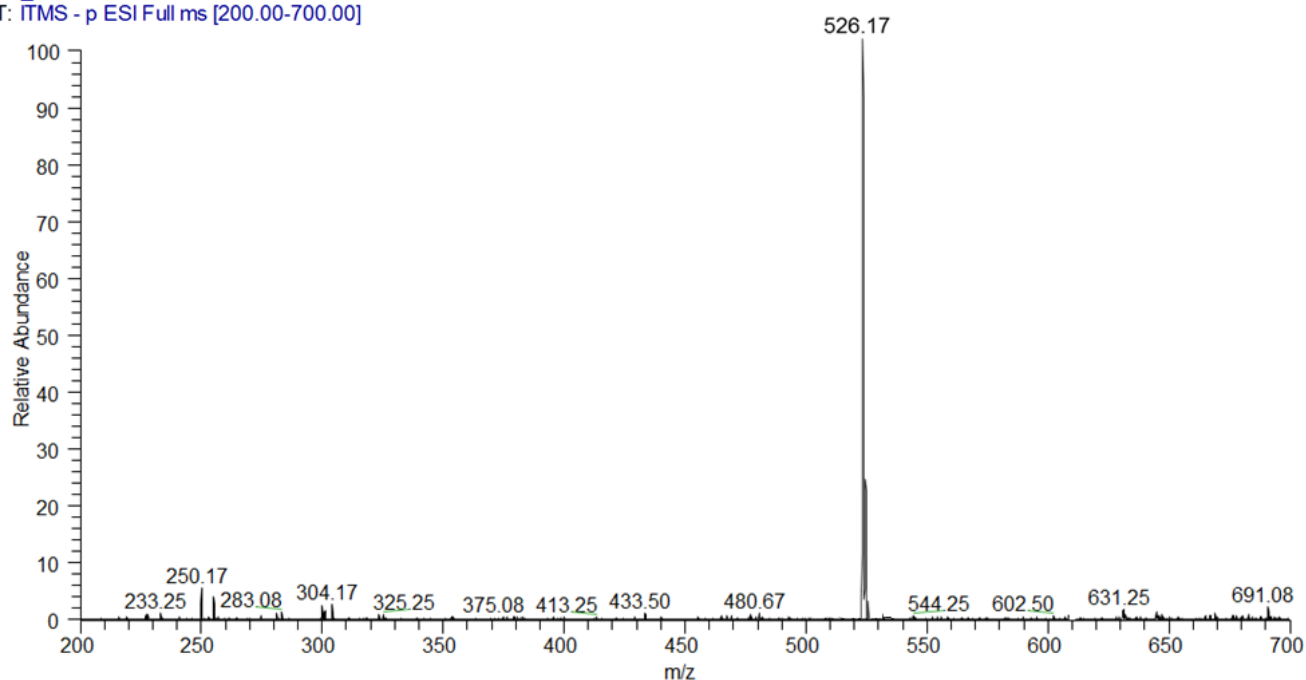


**Fig-S7: Continuous variation (Job's) plot mole fraction of RDF-1 vs change of fluorescence intensity at  $\lambda_{\text{Emm}} = 557 \text{ nm}$ .**

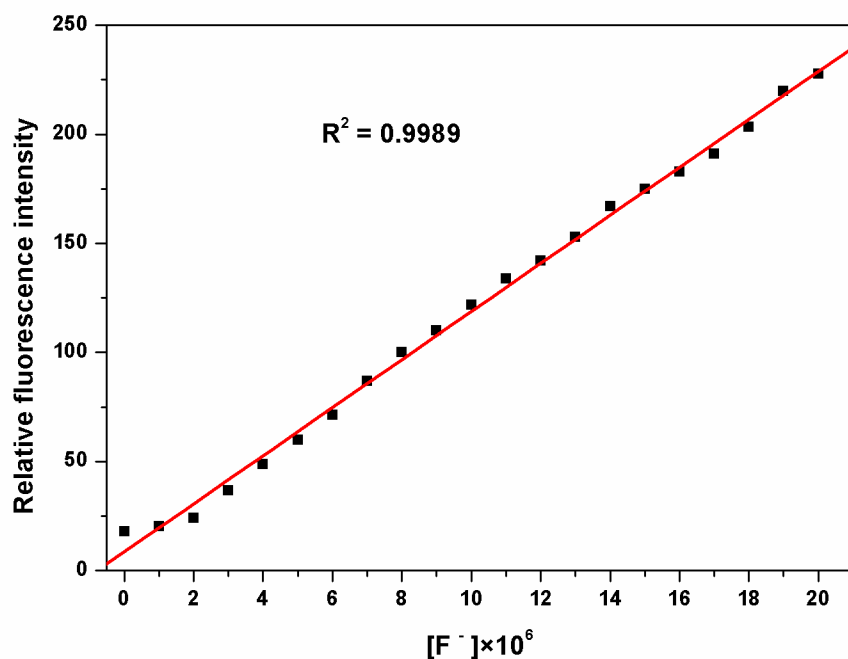


**Fig-S8: ESI-MS spectrum of RDF-1 + fluoride adduct.**

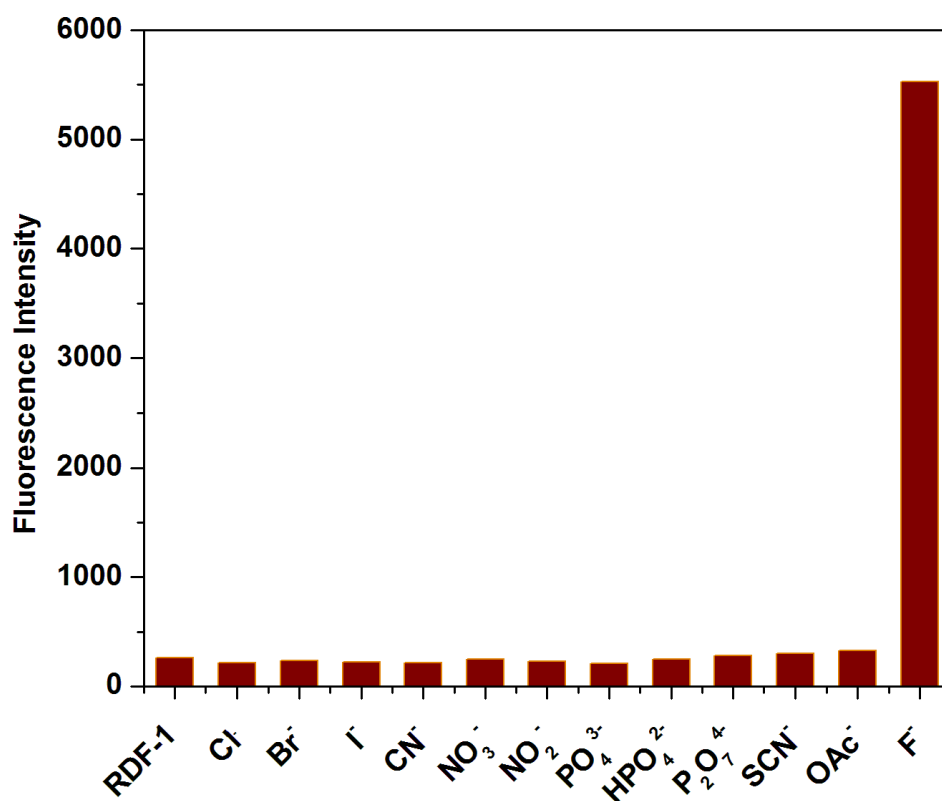
PZ\_120802223851 #283 RT: 0.73 AV: 1 NL: 4.00E1  
T: ITMS - p ESI Full ms [200.00-700.00]



**Fig-S9: plot of change of fluorescence intensity at  $\lambda_{Emm} = 557$  vs concentration of fluoride ions added to the probe RDF-1.**



**Fig- S10: Fluorescence response of 1  $\mu$ M RDF-1 to various anions**

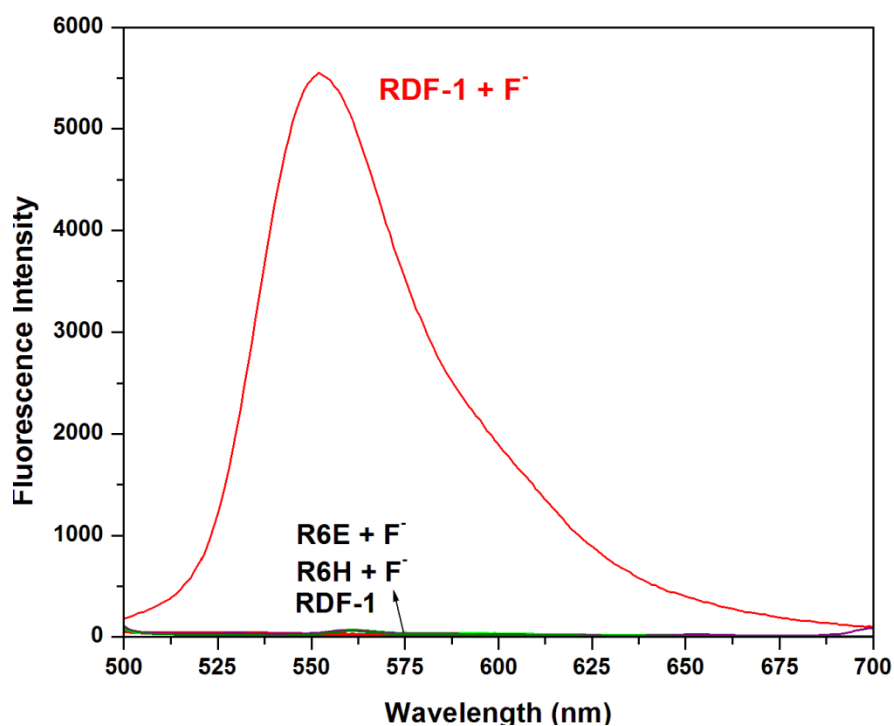


**Calculation of Binding constant:**

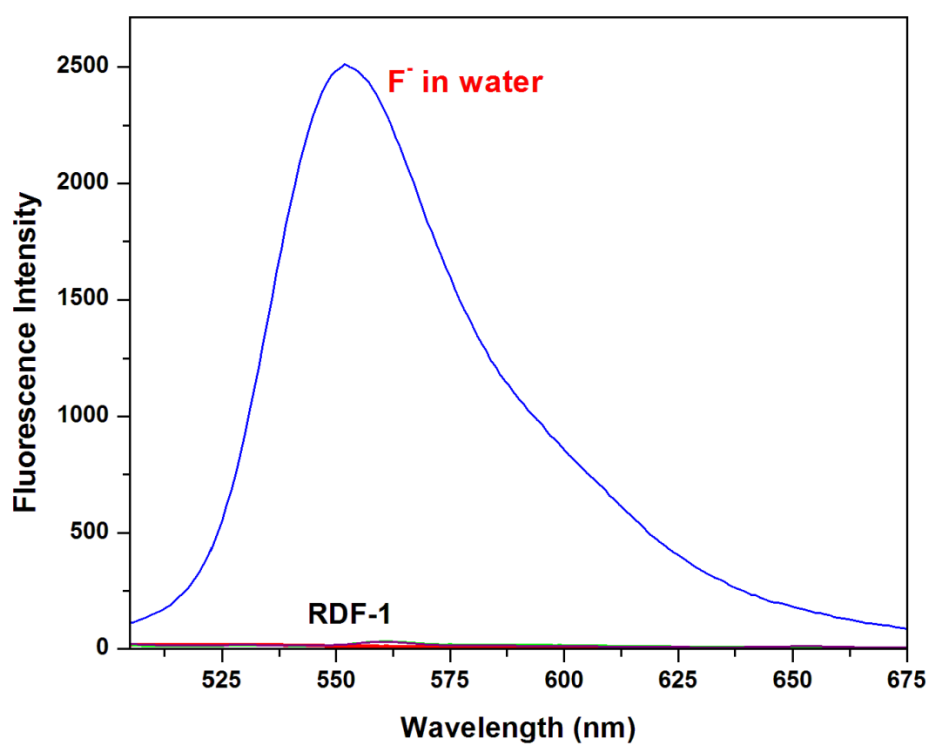
The binding constant K was determined from the plot of the linear regression of  $\log [(F - F_0) / (F_m - F)]$  vs.  $\log [M]$  in equation to obtain the intercept as  $\log K$  and the slope as n.

$$\log \frac{F - F_0}{F_m - F} = \log K + n \log [M]$$

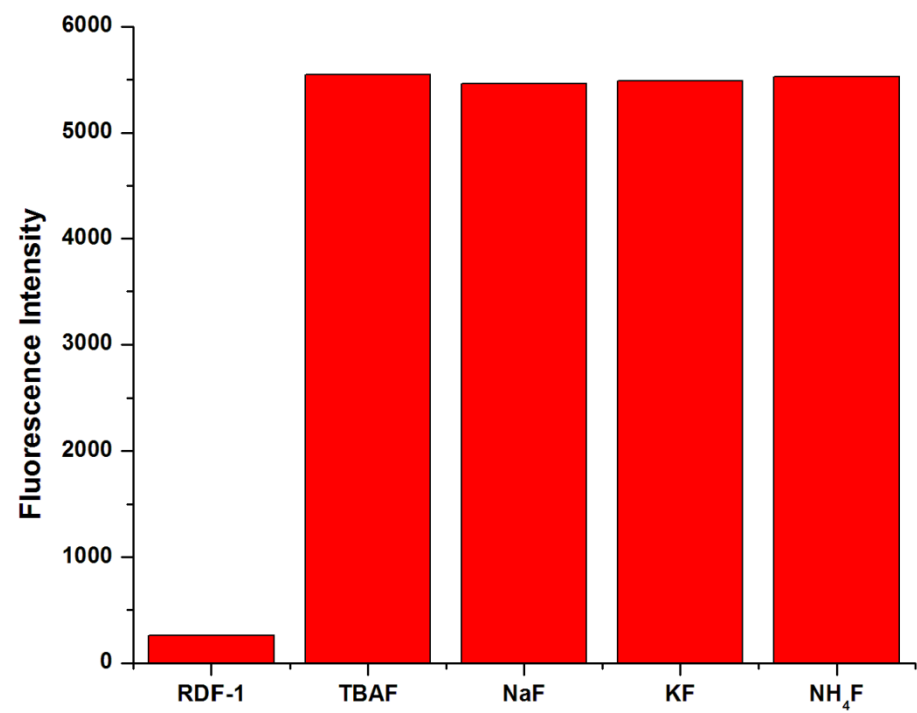
**Fig- S11: comparison of Fluorescence response of fluoride with RDF-1, R6H and R6E**



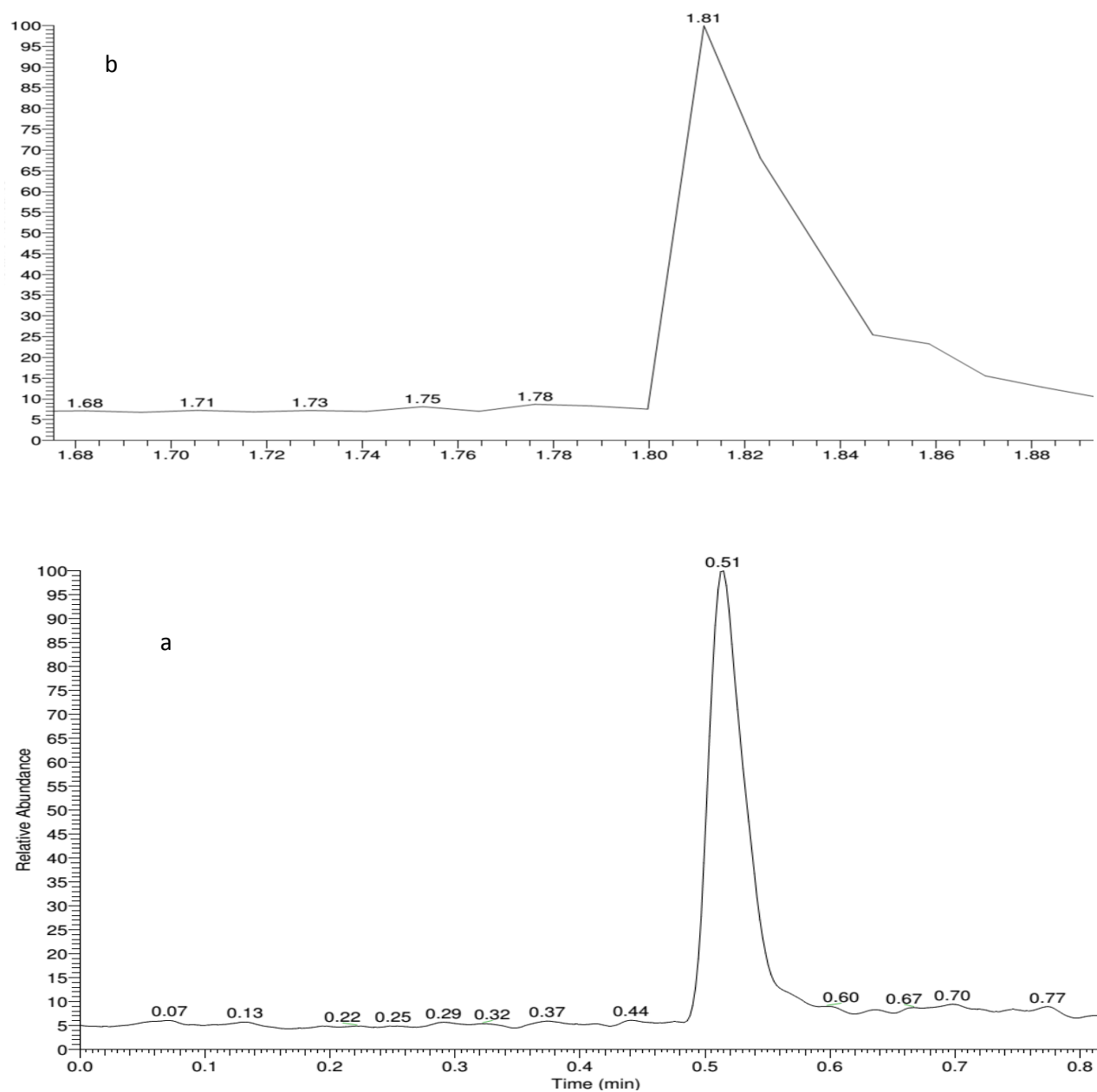
**Fig- S12: Fluorescence response of RDF-1 towards fluoride in water (fluoride added as TBAF in water)**



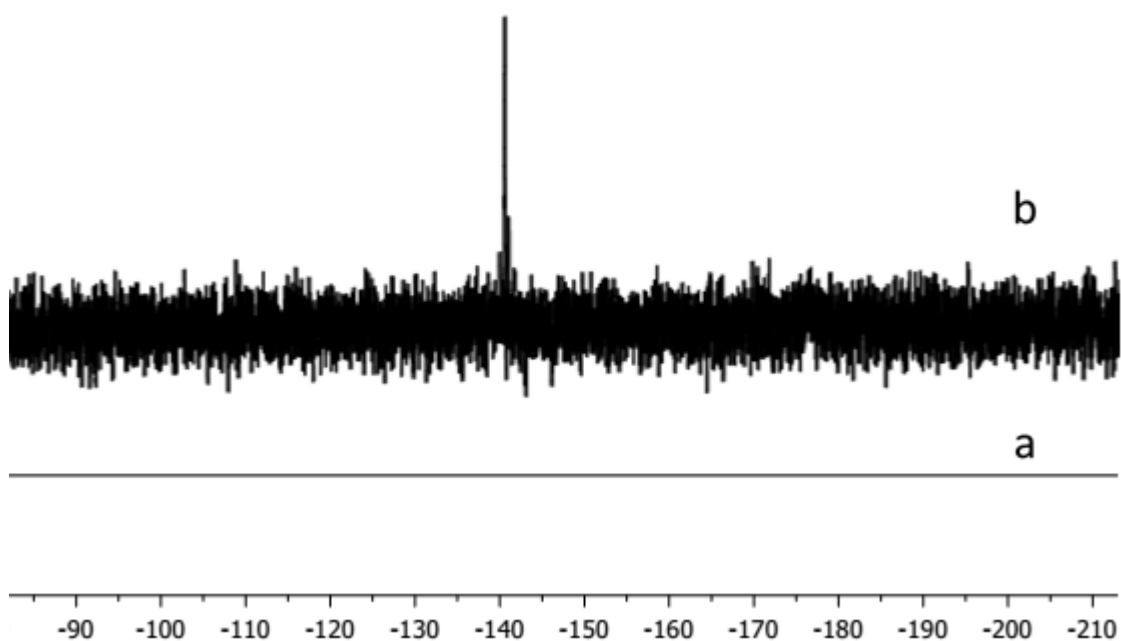
**Fig- S13: Fluorescence response of 1  $\mu$ M RDF-1 to various fluorides.**



**Fig-S14: HPLC of (a) RDF-1(50 $\mu$ M), and (b) the reaction product of RDF-1(50 $\mu$ M) with TBAF (500 $\mu$ M) after incubation of them for 15 minutes in HEPES buffered acetonitrile solution.**



**Fig-S15:**  $^{19}\text{F}$ -NMR of **1** with 1 equiv. of fluoride in DMSO at room temperature. (a) RDF-1 alone; (b) RDF-1 + 1 equiv. of  $\text{F}^-$ .



**Table-S1: The comparison of the present fluoride detection methods with the existing methods.**

| <i>Probe</i>                       | <i>Ems. Max.</i><br><i>(λ in nm)</i> | <i>Turn on/off</i><br><i>(fluorescence)</i> | <i>Detection limit</i> | <i>Ref</i> |
|------------------------------------|--------------------------------------|---------------------------------------------|------------------------|------------|
| Silylated<br>8-hydroxy quinoline   | 458                                  | off                                         | 120 nM                 | 1          |
| N-imidazolyl-<br>1,8-naphthalimide | 442                                  | off                                         | $5 \times 10^{-7}$ M   | 2          |
| Poly. oligomeric<br>silsesquioxane | 660                                  | off                                         | 10 μM                  | 3          |
| Resorfuinsulfonates                | 591                                  | on                                          | 1.9 μM                 | 4          |
| Rhodamine-Al(III)<br>Complex       | 577                                  | off                                         | NA                     | 5          |
| Stibonium Ions                     | 427                                  | on                                          | ppm level              | 6          |
| Coumarin-BODIPY<br>Platform        | 472/606                              | Ratiometric                                 | 0.12 μM                | 7          |
| Spirosilane                        | 290                                  | on                                          | 10 μM                  | 8          |
| Imidazophenazine                   | 541/498                              | Ratiometric                                 | 86 μM                  | 9          |
| Coumarintetrazole                  | 455                                  | on                                          | 207 nM                 | 10         |
| Silylated fluorescein              | 526                                  | on                                          | 1 μM                   | 11         |
| fluorene<br>triazolium ion         | 498                                  | on                                          | 1 μM                   | 12         |
| Borane–bithiophene<br>–BODIPY      | 520/606                              | ratiometric                                 | NA                     | 13         |
| Rhodamine-imidazole<br>work        | 557                                  | on                                          | 8.52 nM                | present    |

References:

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