Supporting Information

FRET based selective and ratiometric detection of Al(III) with live-cell imaging

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1. Calculation of the detection limit:

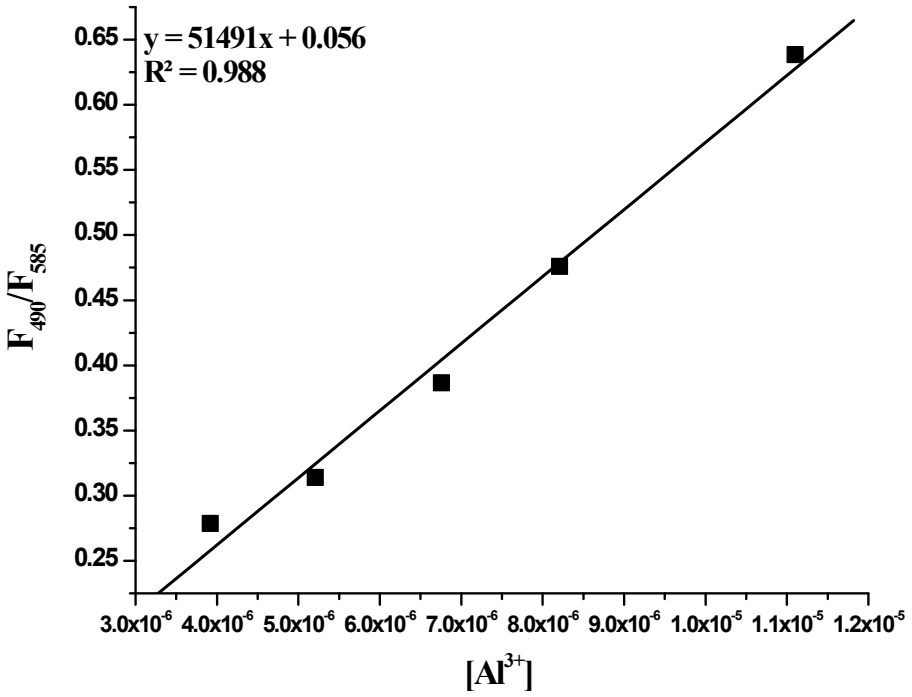


Figure S1: Fl. Intensity ratio (F_{490}/ $F_{585\ nm}$) Vs. Conc. of Al^{3+} plot.

The detection limit DL of CRH for Al^{3+} was determined from the following equation [S1].

DL = K* Sb1/S, Where K = 2 or 3 (we take 2 in this case); Sb1 is the standard deviation and S is the slope of the calibration curve. From the graph we get slope = 51491, and Sb1 value is 0.017991. Thus using the formula we get the Detection Limit = 0.69 μM i.e. CRH can detect Al^{3+} in this minimum level.

2. Job plot by fluorescence method:

Stock solution of same concentration of sensor (CRH) and Al^{3+} were prepared in the order of ≈ 2.0 x 10^{-5} mL^{-1} EtOH: HEPES buffer (3:7, v/v) at pH 7.4. The absorbance in each case with different host–guest ratio but equal in volume was recorded. Job plots were drawn by plotting $\Delta I.X_{host}$ vs X_{host} (ΔI = change of intensity of the emission spectrum during titration and Xhost is the mole fraction of the host in each case, respectively).

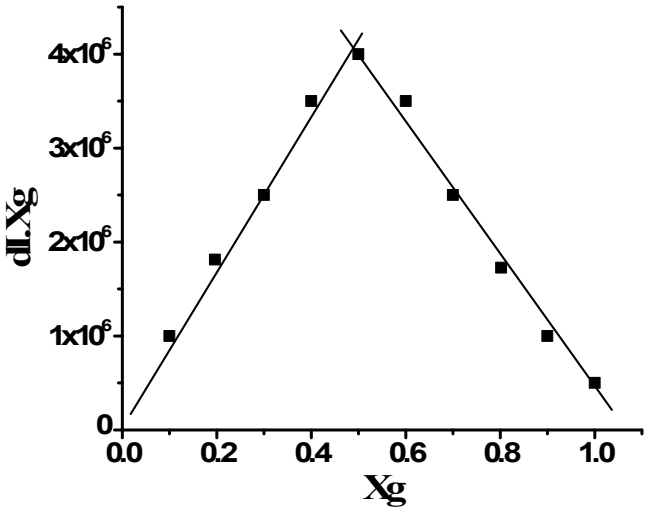


Figure S2: Job plot by fluorescence method.

3. Calculation of association constant using Emission Titration Data:

From the fluorescence titration data the association constant (Ka) for the formation of respective complex CRH-Al^{3+} was calculated by nonlinear curve fitting procedure. The non linear curve fitting was done using the following equation (1). [S2] CRH-Al^{3+} complex

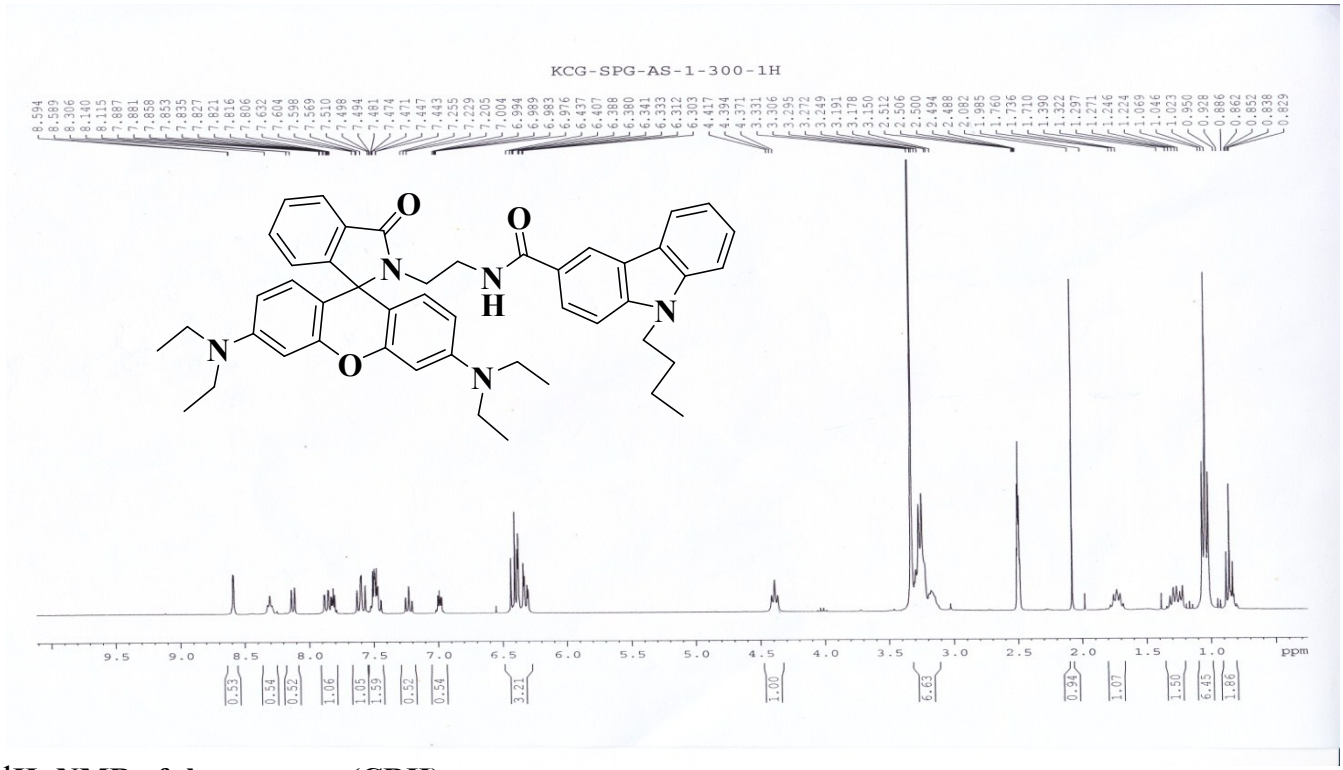
$$I = I_0 + \frac{I_{lim} - I_0}{2C_H}\left\{C_H + C_G + \frac{1}{K_a} - \left[\left(C_H + C_G + \frac{1}{K_a}\right)^2 - 4C_H C_G\right]^{1/2}\right\} \quad (1)$$

Where I_0, I, and I_{lim} are the respective emission intensity of free CRH, CRH present in the form of [CRH-Al^{3+}] in the complex, and CRH in presence of excess amounts of Al^{3+} ions where the

emission intensity reaches a limiting value. C_H and C_G are corresponding concentrations of host and cationic guest; Ka is the binding constant. The binding constant (Ka) and correlation coefficients (R) were obtained from a non-linear least-square analysis of I vs. C_H and C_G.

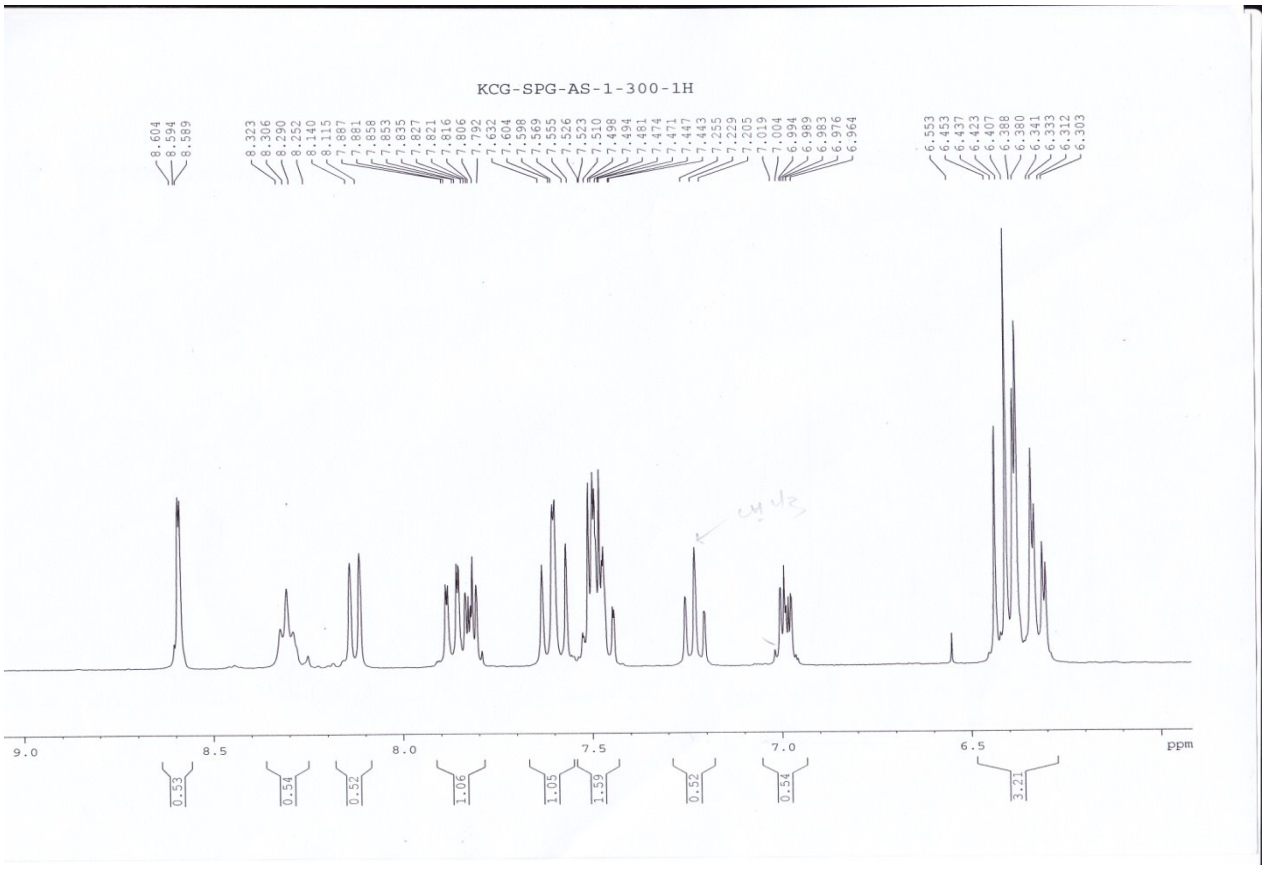
The association constant (K_a) as determined by fluorescence titration method for CRH with Al^{3+} found to be 5.03 x 10^4 M^{-1}.

4. Spectral data of CRH:

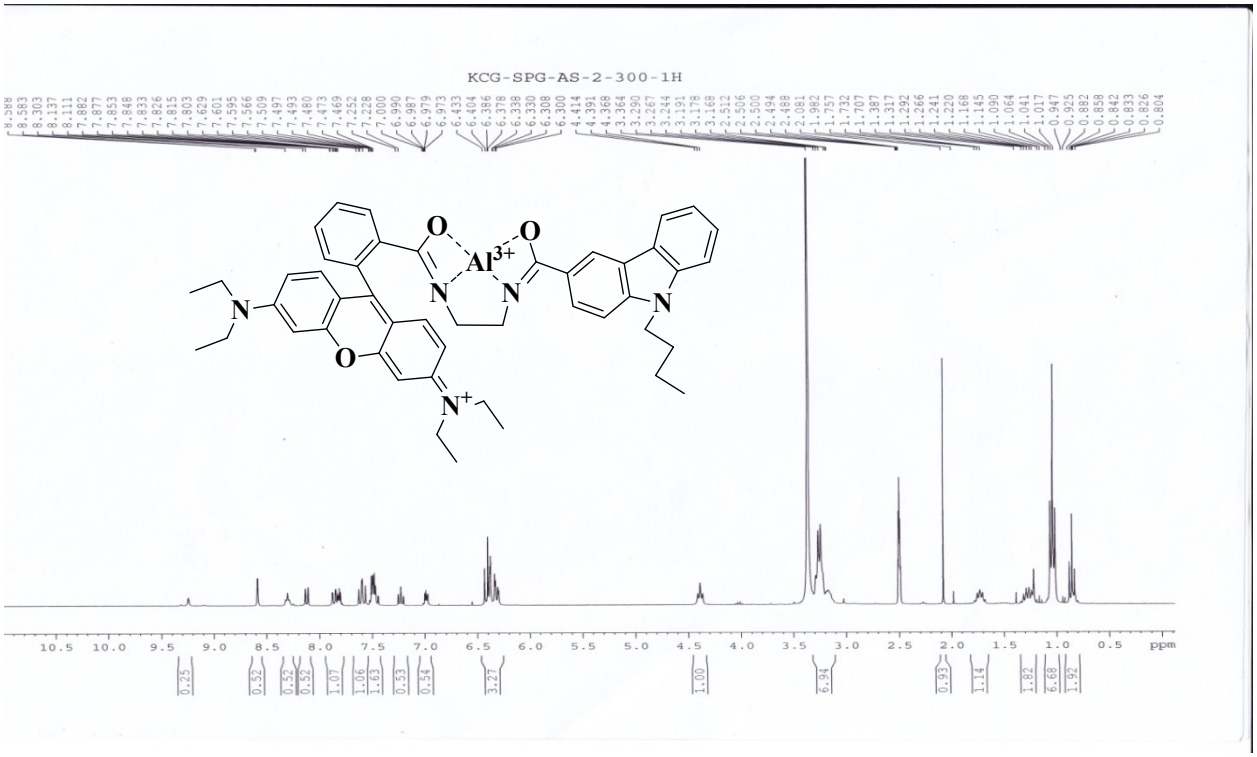


^{1}H- NMR of the receptor (CRH):

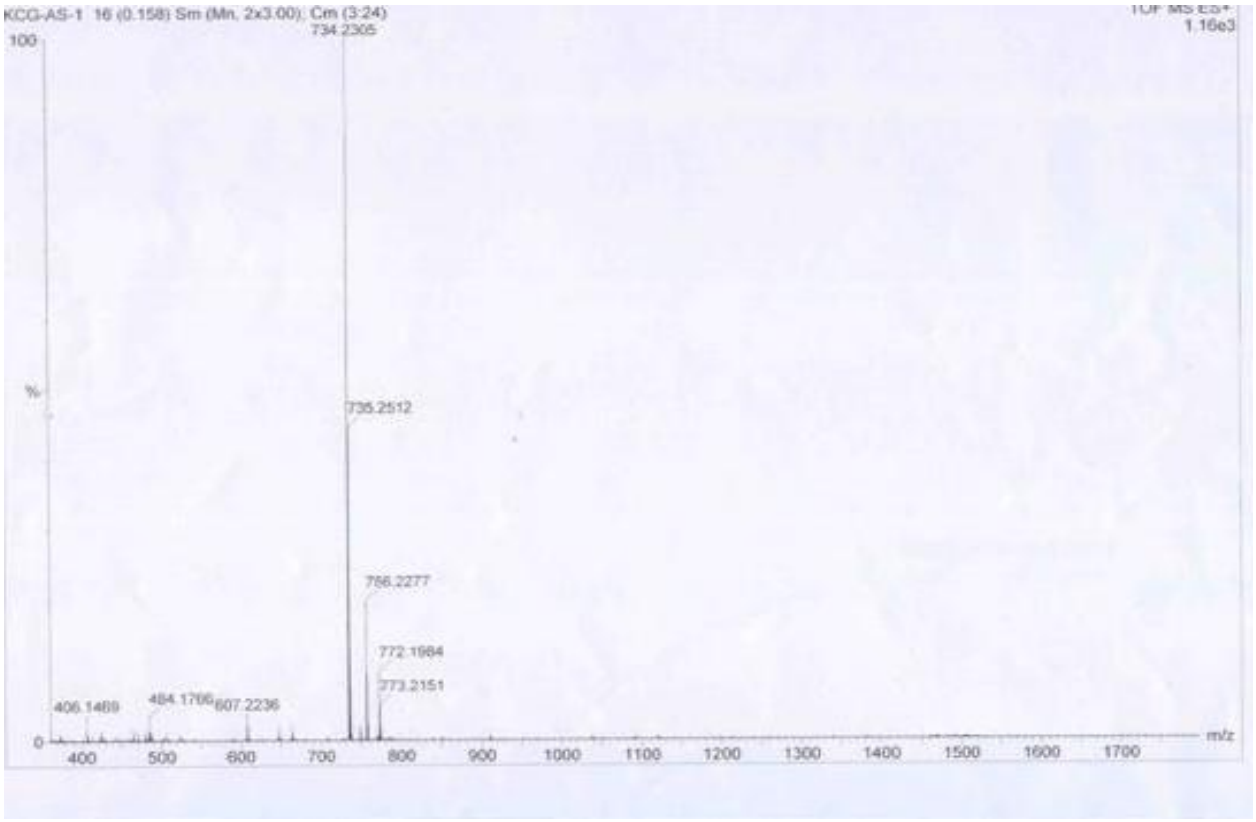
Expansion of ^{1}H-NMR of receptor:



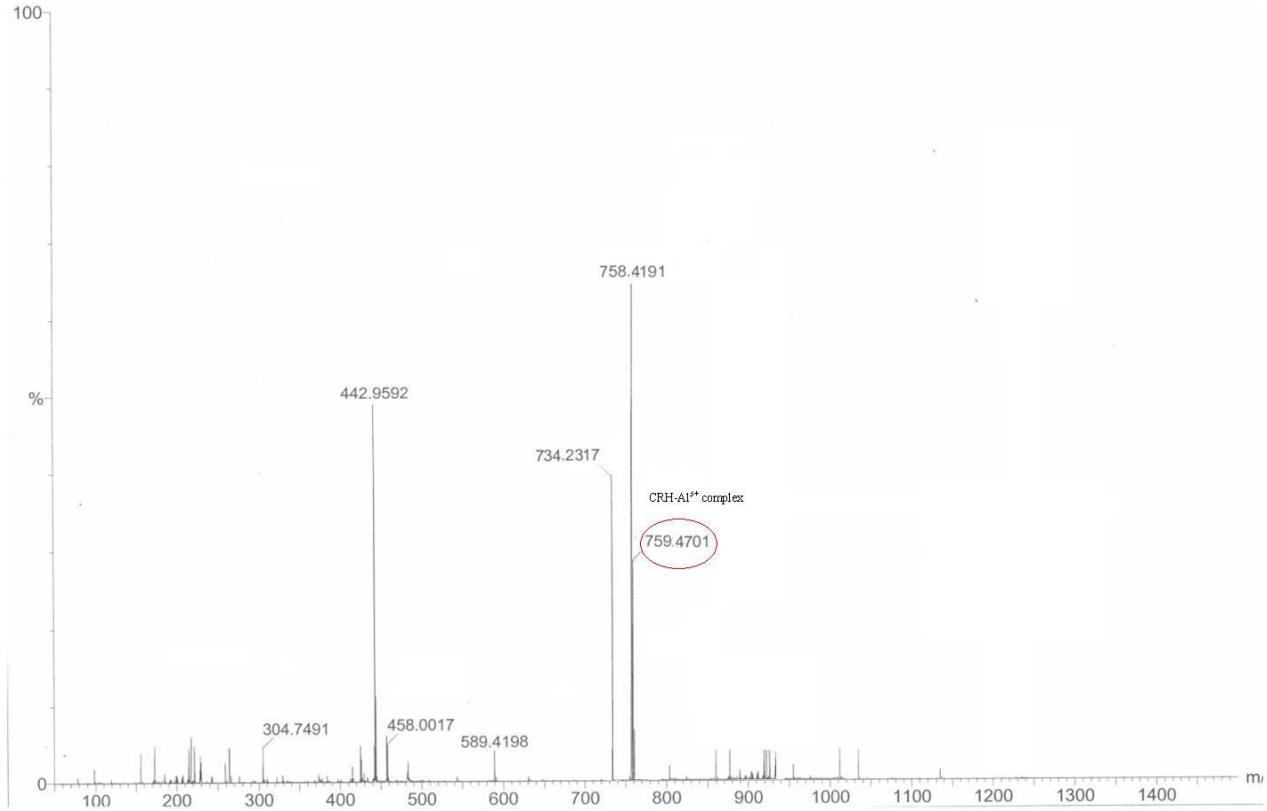
^{1}H-NMR of AL^{3+} + CRH:



ESI-MS of CRH:

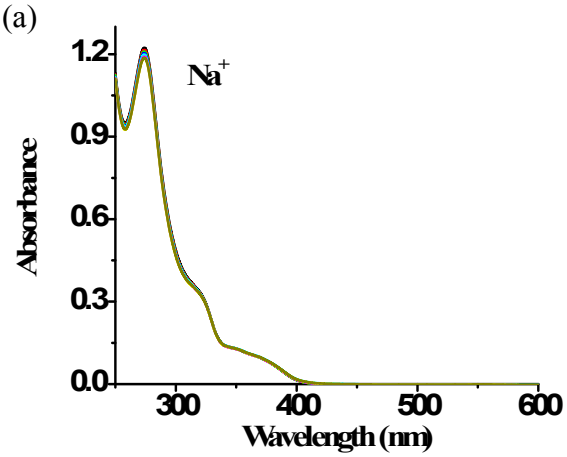


ESI-MS of AL^{3+} + CRH:

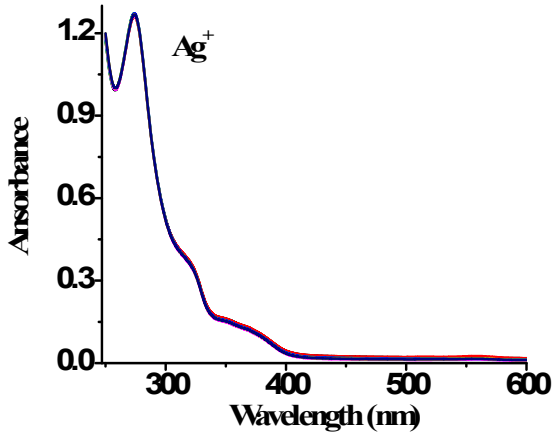


5. Absorption spectra of CRH:

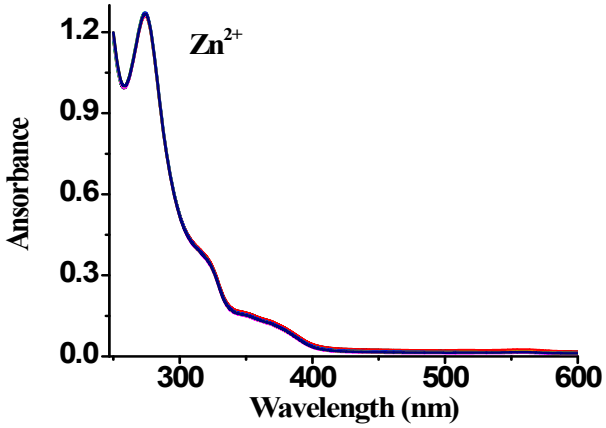
(a)



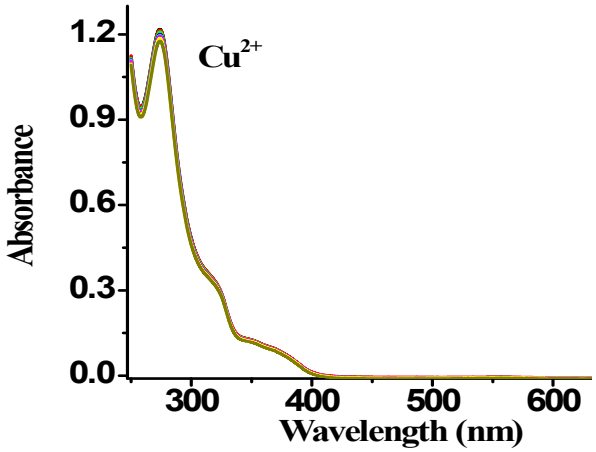
(b)



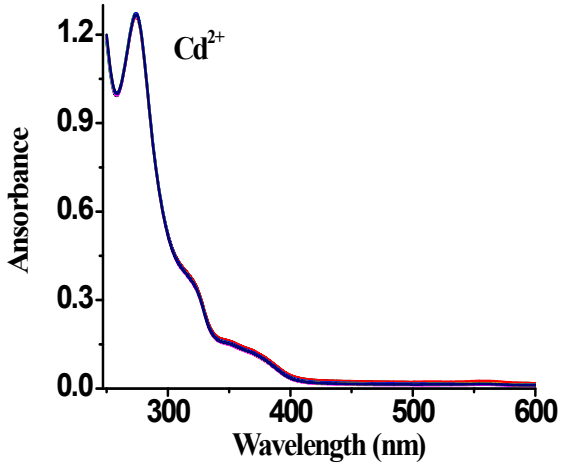
(c)



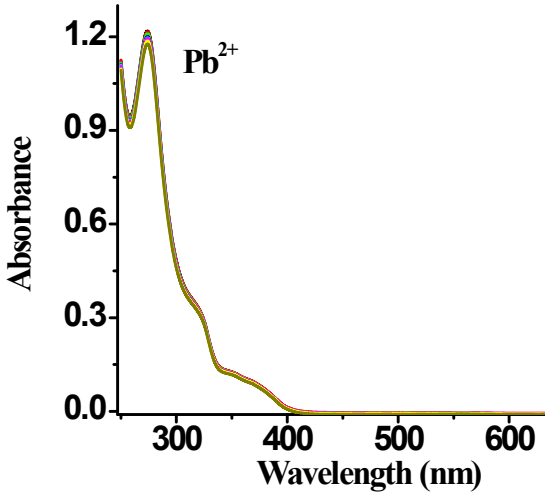
(d)



(e)



(f)



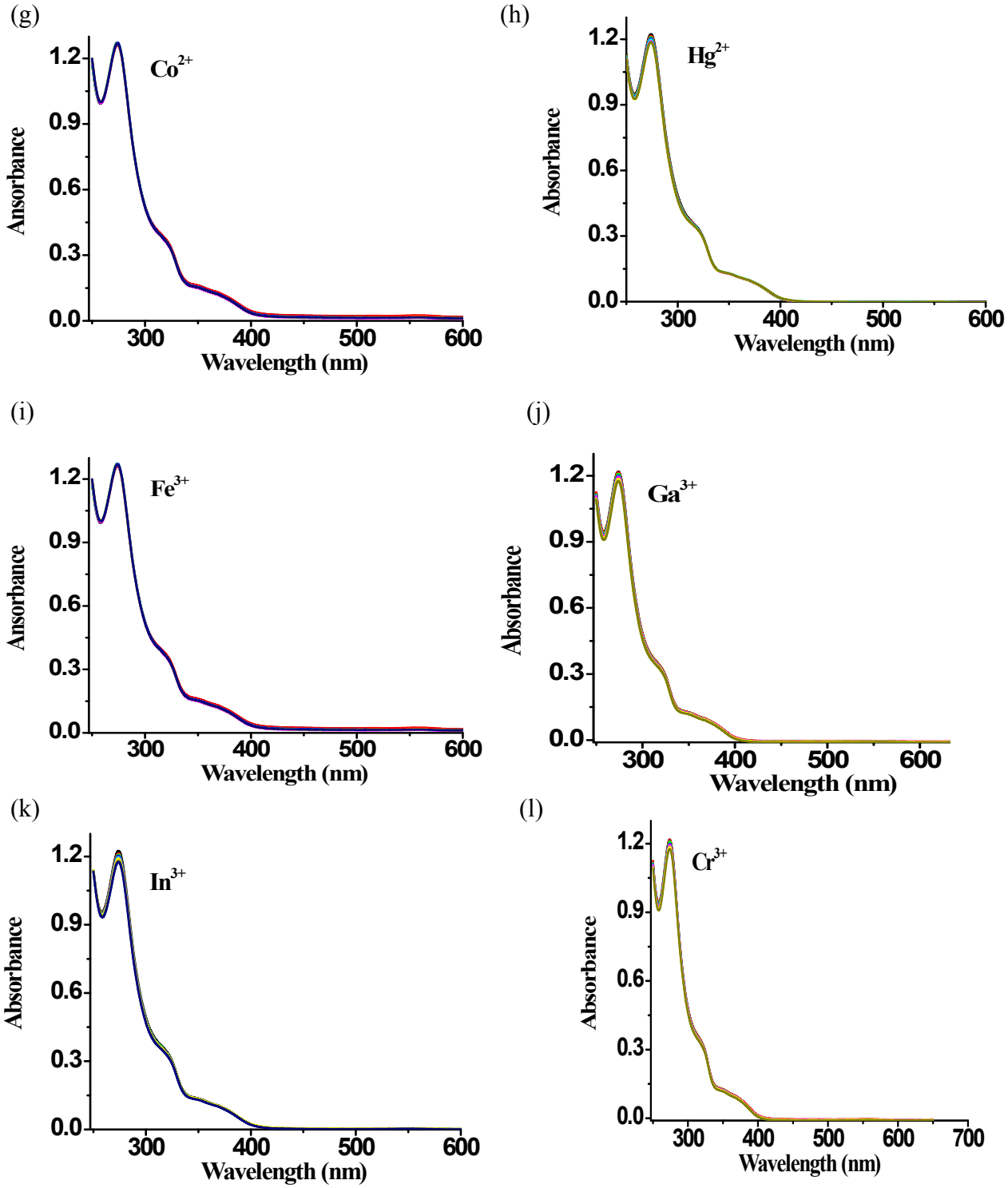
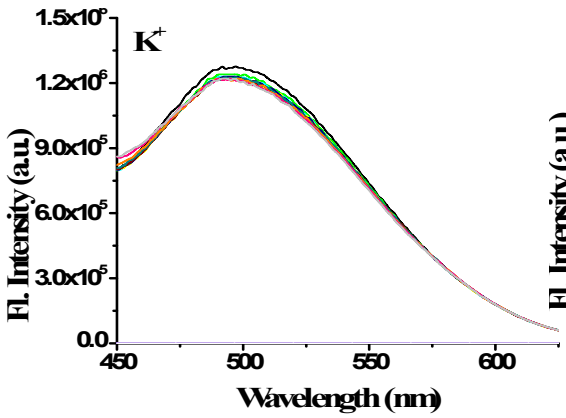


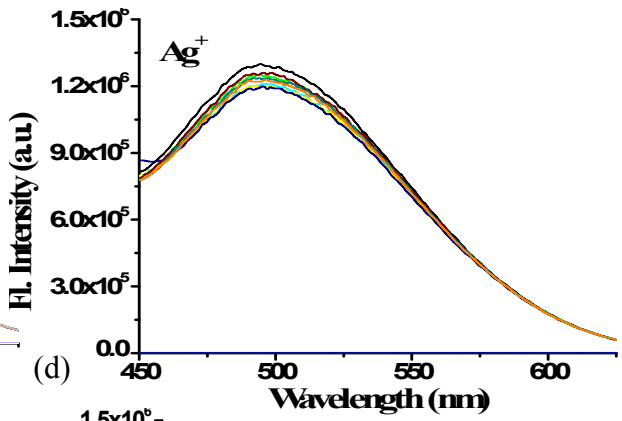
Figure S3: UV-vis response of CRH (2.0 x 10^{-5} M) towards tested cations (Al^{3+}, K^{+}, Ag^{+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Pd^{2+}, Co^{2+}, Hg^{2+}, Fe^{3+}, Ga^{3+}, In^{3+}, Cr^{3+}) at pH 7.4 in EtOH: HEPES buffer (3:7, v/v).

6. Emission spectra of CRH:

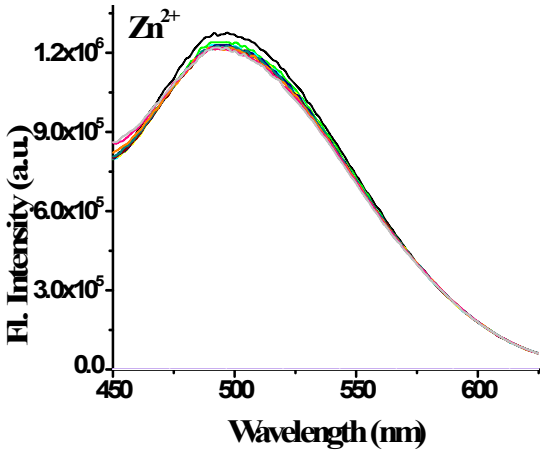
(a)



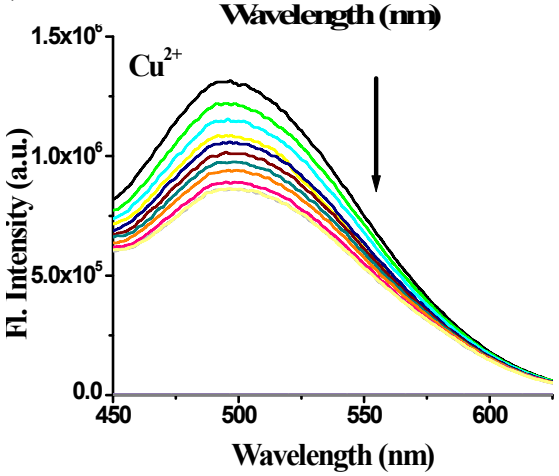
(b)



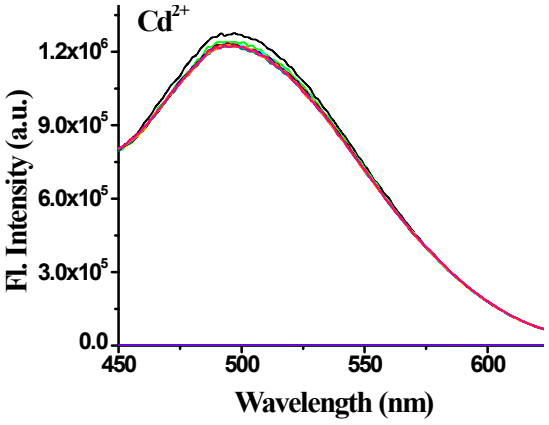
(c)



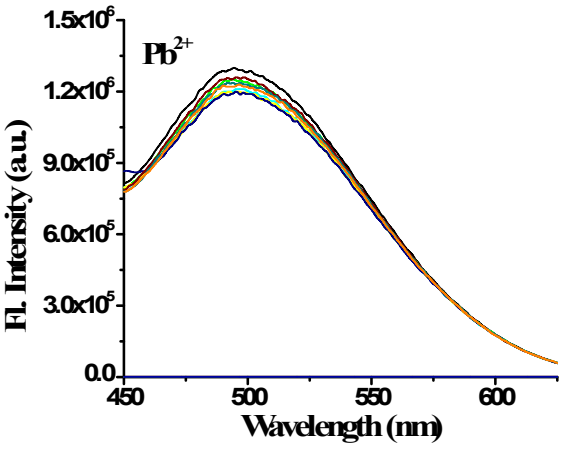
(d)



(e)



(f)



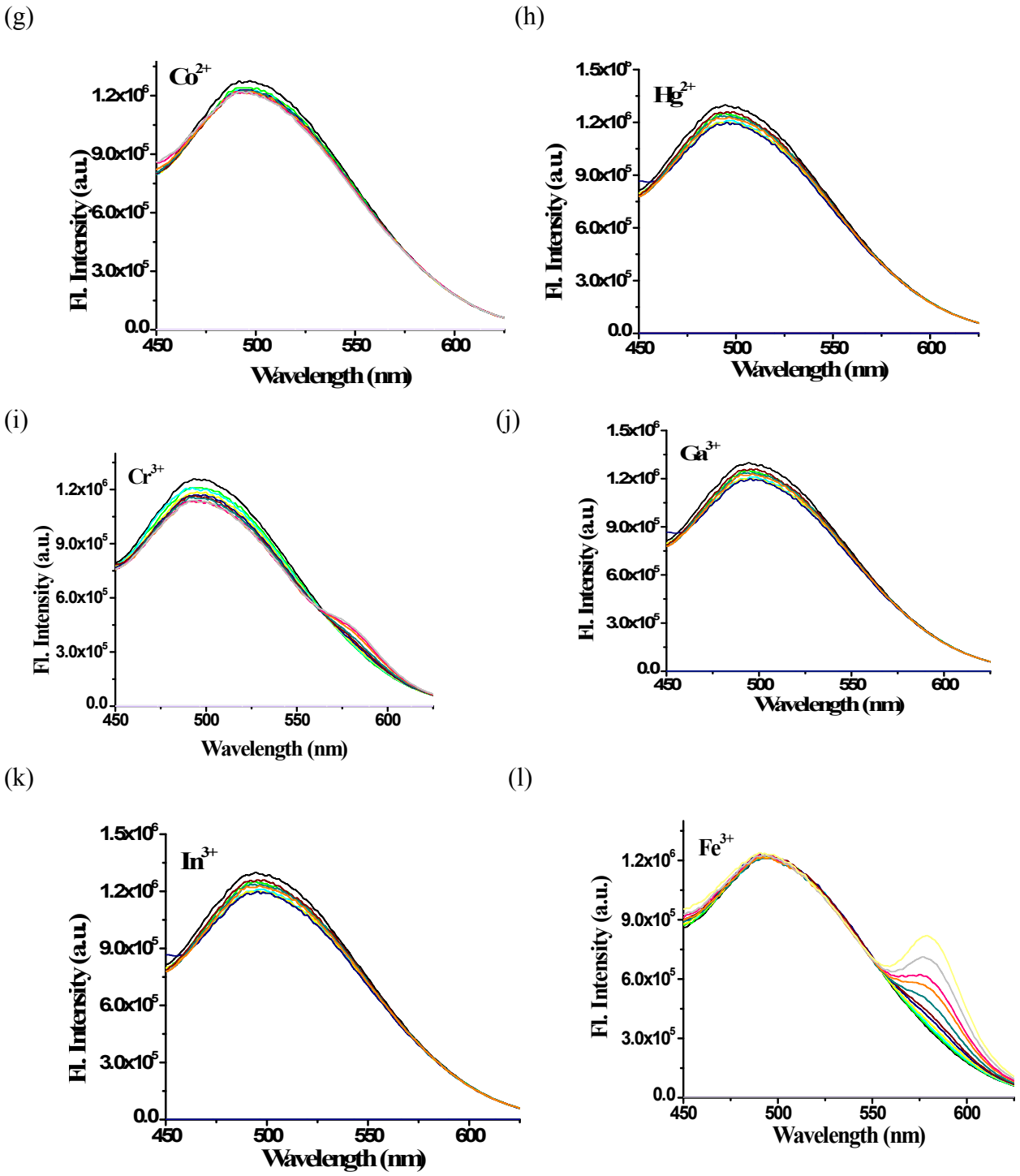


Figure S4: Ratiometric response of CRH (2.0 x 10^{-5} M) towards tested cations (K^{+}, Ag^{+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Pd^{2+}, Co^{2+}, Hg^{2+}, Fe^{3+}, Ga^{3+}, In^{3+}, Cr^{3+}) at pH 7.4 in EtOH: HEPES buffer (3:7, v/v).

6. Emission spectra of CRH:

7. Cytotoxicity experiment of CRH:

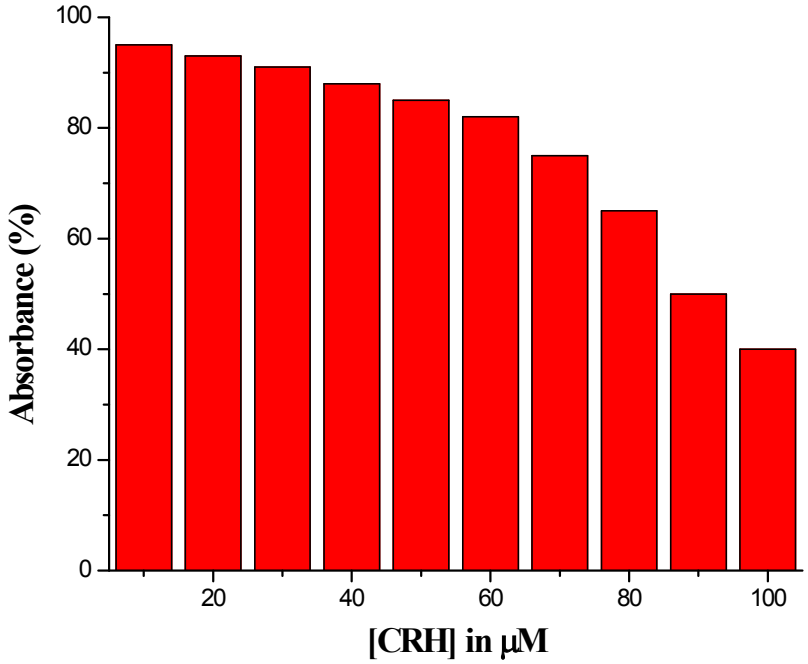


Figure S5: MTT assay with different concentration of probe (CRH) after 24h.

8. References:

[S1]. M. Zhu, M. Yuan, X. Liu, J. Xu, J. Lv, C. Huang, H. Liu, Y. Li, S. Wang, D. Zhu, *Org. Lett.* 2008**, 10***,* 1481.

[S2] (a) B. Valeur, J. Pouget, J. Bouson, *J. Phys. Chem.,* **1992**, *96*, 6545. (b) K. Ghosh, T. Sarkara, A. Samadder, *Org. Biomol. Chem.* **2012**, *10*, 3236.