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**Novel anthracene and pyridine comprising schiff base probe
for selective “OFF-ON” fluorescent determination of Cu²⁺ ions
towards live cell application**

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Abstract

Novel anthracene and pyridine comprising schiff base derivative 2-(2-(anthracen-9-ylmethylene)hydrazinyl)pyridine (**AP**) has been synthesized via one-pot reaction and its fluorescent “OFF-ON” detection of Cu^{2+} ions, *via* PET based mechanism was reported firstly. The 1:1 stoichiometry of **AP**--- Cu^{2+} sensor complex was calculated from job’s plot based on PL titrations and supported by ESI (+Ve) mass analysis. In addition, the binding sites of sensor conjugate **AP**--- Cu^{2+} was buttressed by the ^1H NMR titration. The detection limit (LOD) and association constant (K_a) of **AP**--- Cu^{2+} complex was scrutinized by standard deviation and linear fittings. Furthermore, quantum yield (Φ_f), SEM analysis, pH effect, FTIR interpretation and density functional theory (DFT) studies were investigated for the **AP**--- Cu^{2+} sensing conjugate. More decisively, confocal fluorescence microscopy imaging from Raw264.7 cells indicated that **AP** could be used as an effective fluorescent probe for the analysis of Cu^{2+} ions in living cells.

KEYWORDS: Anthracene; Pyridine; Schiff base; Cu^{2+} sensors; Live cell imaging.

Introduction

Owing to the biological and environmental significance of metal ions, the chemosensors development for their detection is become a concept of interest.¹⁻³ Among the transition metal ions, copper is the third most abundant element after iron and zinc. Divalent copper ions are more attractive, because of their crucial role in the activation of dioxygen in living organisms.^{4,5} On the other hand, at higher concentration levels, it will damage biomolecules leading to oxidative stress and neurodegenerative disorders.^{6,7} With this concern, the World Health Organization (WHO) reported that the population mean consumption of copper should not exceed 10–12 mg/day for adults.⁸ Similarly, copper deficiency may also lead to some serious diseases, such as Alzheimer's, anemia, amyotrophic lateral sclerosis and prion diseases.^{9, 10}

Considering the importance of Cu^{2+} ions, several methods such as inductively coupled plasma-atomic emission spectrometry, plasma-mass spectroscopy, inductively coupled atomic absorption/emission spectroscopy, and voltammetry have been reported for its determination.¹¹⁻¹³ However, they are not suitable for on-line monitoring of Cu^{2+} ions due to their low selectivity, needing expensive instruments and delayed responses. In contrast, fluorescent turn-on Cu^{2+} detection has become more practical and favorable method in medicinal biology, and environmental chemistry.¹⁴⁻¹⁶

By means of internal charge transfer (ICT), photoinduced electron transfer (PET), excimer/excimer formation, chelation-enhanced fluorescence (CHEF) and more recently fluorescence resonance energy transfer (FRET) mechanisms, many copper selective turn-on fluorescent sensors have already been reported.¹⁷⁻¹⁹ However, due to its simplicity and applications in many opto-electronic and biological systems, PET mechanism is highly

appreciated.²⁰⁻²² Further, PET based anthracene containing “turn-on” probes are found to be very exciting because of their faster response *via* excimer/exciple formation.²³⁻²⁵ Conversely, some of those probes also have synthetic difficulties,²⁶⁻²⁸ hence the development of “turn-on” probes with lesser synthetic complications for specific analyte detection is highly anticipated. By this concern, a few anthracene based schiff base derivatives have been developed with selective sensor properties.²⁹⁻³¹ Additionally, anthracene based supramolecular dye molecules can be constructed from their sensor complexes, as reported earlier.³²⁻³⁴ Therefore, we aimed to develop such an anthracene containing schiff base derivative *via* one pot reaction for Cu²⁺ ions detection.

Herein, we have successfully developed an anthracene containing fluorescent turn-on sensor probe **AP** with demonstrated specific selectivity to Cu²⁺ ions *via* UV-Vis/PL spectral investigations. Further, to explore the optical properties and PET mechanism for the recognition of Cu²⁺ ions, B3LYP/LANL2DZ density functional calculations were adopted to investigate the electronic excitation and molecular orbitals of the probe.

Experimental studies

Materials and methods

All anhydrous reactions were carried out by standard procedures under nitrogen atmosphere to prevent the interference from ambient moisture. Solvents were dried by distillation over appropriate drying agents and reactions were monitored by thin layer chromatography (TLC). ¹H and ¹³C NMR were analyzed on a 300 MHz Bruker spectrometer. The chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz and relative to TMS (0.00) for ¹H and ¹³C NMR, (s, d, t, q, m, and dd means single,

double, ternary, quadruple, multiple, and doublet of doublet, respectively), and d_6 -DMSO (^1H and ^{13}C NMR at 2.49 and 39.51 ppm, respectively) were used as references. Mass spectrum (ESI) was obtained from the respective mass spectrometer. Absorption and fluorescence spectra were measured on HITACHI, U-3310 Spectrophotometer and HITACHI F-7000 Fluorescence Spectrophotometer, respectively. Identification and purity of the compound **AP** was characterized by NMR (^1H & ^{13}C) and ESI-Mass. Buffers with various pH values were freshly prepared as per the literature.³⁵ SEM studies were done by JEOL-JSM-6700. Fourier transform Infrared spectroscopy (FTIR) were analysed by Perkin Elmer - 100 FT-IR SPECTRUM ONE spectrometer. The powder XRD data of **AP** and **AP**--- Cu^{2+} were obtained from BRUKER AXS D2 Phaser (a26-x1-A2BOE2B). Fluorescence microscopic images were taken using Multiphoton and Confocal Microscope System, Leica, Germany, TCS-SP5-X AOBS.

Sensor titrations

Sensing probe **AP** was dissolved in CH_3CN and Ba^{2+} , Ni^{2+} , Fe^{3+} , Cr^{3+} , Cu^{2+} , Mg^{2+} , Fe^{2+} and Al^{3+} metal ions were dissolved in DI water at 1×10^{-3} M concentration from their respective chloro and perchlorate compounds. Likewise, Ag^+ , Co^{2+} , Zn^{2+} , Pb^{2+} , Mn^{2+} and Hg^{2+} metal ions were dissolved in DI water at 1×10^{-3} M concentration from their respective acetate salts.

SEM and FTIR analysis

For both SEM and FTIR studies, the samples were drop-casted on well cleaned silicon wafers, then annealed at 50°C for 15 minutes and continued for the respective analysis. We used clean Silicon wafer as background reference for FTIR measurement.

ESI (+Ve) mass analysis

The **AP**--Cu²⁺ complex was mixed at 1:1 stoichiometry in CH₃CN and stirred at 45°C for 12 hrs, dried in oven at 100 °C for 3 hrs. The dried complex was subjected to ESI (+Ve) mass analysis.

Procedure for the synthesis of AP³⁶

To 1 equiv. of anthracene-9-carbaldehyde in 50 ml of methanol, 1 equiv. of 2-hydrazinylpyridine was gradually added with constant stirring under nitrogen and then refluxed for 12 h. The reaction was periodically monitored by TLC, after completion, the reaction mixture was cooled and solvent was evaporated to obtain the crude product, which was recrystallized from ethanol to afford the pure compound **AP** as a dark yellow solid.

2-(2-(anthracen-9-ylmethylene)hydrazinyl)pyridine(**AP**): yellow solid; 93% yield; ¹H NMR (300 MHz, d₆-DMSO) δ: 6.79-6.83 (dd; *J* = 6.1 Hz, 1H (-CH-pyridyl)), 7.22 (d; *J* = 8.4 Hz, 1H (-CH-pyridyl)) 7.55-7.72 (m, 5H (Aromatic)), 8.13 – 8.20 (m, 3H (Aromatic)), 8.65 (s, 1H(Aromatic)), 8.72 (d, *J* = 8.7 Hz, 2H (Aromatic)), 9.28 (s, 1H (-C=N-NH), 11.10 (s, 1H (-CH=N)); ¹³C NMR (75 MHz, d₆-DMSO) δ: 106.59, 115.67, 125.35, 125.92, 126.67, 127.25, 128.65, 129.42, 129.68, 131.55, 138.21, 138.66, 148.45, 157.50; ESI mass: calculated: *m/z* = 297.13 (*M*⁺, 100%); Found: *m/z* = 298.13 [(*M*+1)100%].

Fluorescence imaging and sensing

To determine the Cu²⁺ ions in living cells, Raw264.7 cells were cultured in DMEM medium (Dulbecco's Modified Eagle's Medium with high glucose) supplemented with

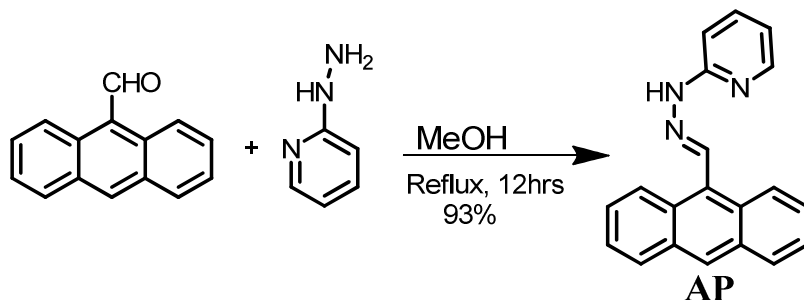
10% FBS at 37°C under 5% CO₂ environment. The cells were then cultured on glass coverslips and incubated for 24 hours to promote better adherence.

The cells cultured in DMEM were treated with 30 μM of Cu²⁺ dissolved in sterilized PBS (pH 7.4) and incubated for 30 min at 37°C and then the treated cells were washed three times with 2 ml PBS to remove the remaining metal ions. 2 mL of culture media was added to the cell culture and then treated with 20 μM of **AP** dissolved in DMSO followed by incubation (2 hours at 37°C). Thereafter, The culture medium was removed, and the treated cells were washed with PBS (2 mL) before observation. Fluorescence imaging was determined by a Multiphoton and Confocal Microscope System, Leica, Germany, TCS-SP5-X AOBS. The cells were excited with a white light laser at $\lambda_{\text{ex}} = 350 \text{ nm}$ at 6% output and emission images were collected between 410 and 440 nm (**AP**---Cu²⁺).

Results and Discussion

Synthesis and solvent selection

As illustrated in Scheme 1, **AP** was synthesized *via* one pot anthracene-9-carbaldehyde and 2-hydrazinylpyridine condensation in methanol with 93% yield and characterized with ¹H, ¹³C NMR and Mass analysis[ESI (+Ve)] (Figs. S1-S3, ESI).



Scheme 1. Synthesis of **AP** probe.

In order to choose the appropriate solvent for the sensor titrations, the selectivity test

was performed in many solvents (see Fig. S4a, ESI) and established the better “off-on” selectivity (23 fold) to Cu^{2+} ions in acetonitrile rather than other solvents. Further, to describe the $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ ratio for efficient selectivity, we have also conducted PL spectral titrations (not shown) with different ratio (0-90% with an equal span of 10%) of H_2O . In which, the **AP** probe evidenced the greater “off-on” sensory response to Cu^{2+} ions only in pure acetonitrile and observed that, the presence of water content will affect the turn-on response rapidly. Hence, we did the UV-Vis/PL titrations of **AP** ($\lambda_{\text{abs}}=350$ nm and $\lambda_{\text{em}}=430$ nm;) in acetonitrile and ^1H NMR titrations in $[\text{d}_6\text{-DMSO}]$ by adding metal ions in pure H_2O and D_2O , respectively. Further, 15 fold of PL enhancement was also noticed for **AP** in DMSO (see Fig. S4a & b, ESI), which allow us to do the cellular imaging studies by dissolving **AP** in DMSO with 2 hours incubation.

Fluorescence titrations on metal ions

Initially, **AP** probe (20 μM in CH_3CN) was investigated towards 30 μM (1.5 equiv.) of metal ions (Ba^{2+} , Ni^{2+} , Fe^{3+} , Cr^{3+} , Cu^{2+} , Mg^{2+} , Fe^{2+} , Al^{3+} , Ag^+ , Co^{2+} , Zn^{2+} , Pb^{2+} , Mn^{2+} , and Hg^{2+}) in H_2O . Upon the addition of 1.5 equiv. of metal ions, **AP** shows better selectivity towards Cu^{2+} ions, as shown in Fig 1a and 1b. Impressively, the absorption peak of **AP**--- Cu^{2+} complex was blue shifted and exhibited at 296 nm than that of **AP** probe obtained at 405 nm. The above 109 nm blue shifted absorption peak of **AP**--- Cu^{2+} complex was an added advantage for Cu^{2+} ions detection. Similarly, contrast to other metal ions, the PL spectra of **AP**--- Cu^{2+} complex was also evidenced the high fluorescence intensity peak at 430 nm. Remarkably, the PL intensity changes $[I/I_0]$ of **AP**--- Cu^{2+} was found to be 23 fold. Likewise, the quantum yield of **AP** probe $[\Phi_f = 0.01]$ was also increased for **AP**--- Cu^{2+} complex $[\Phi_f = 0.23]$. This indicated that the CHEF of

AP--- Cu^{2+} was found to be enhanced with 23 fold of quantum yield values than that of **AP** probe. The photograph of **AP**--- Cu^{2+} (visualized under UV- light irradiations at $\lambda_{\text{em}} = 365 \text{ nm}$) strongly verified its sensitivity through intense blue emission, as observed in Fig. 2.

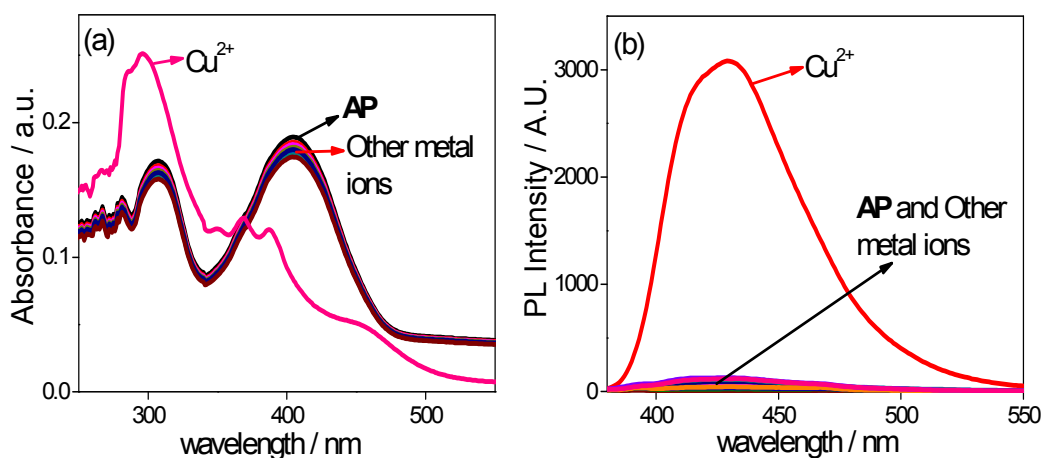


Fig. 1 (a) UV and (b) PL spectra ($\lambda_{\text{ex}} = 350 \text{ nm}$) of **AP** ($20 \mu\text{M}$) in CH_3CN with various other metal ions ($30 \mu\text{M}$) in H_2O .

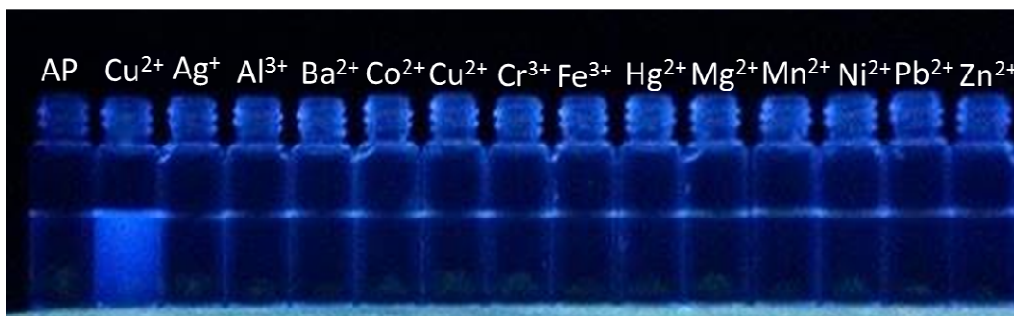


Fig. 2 Photograph of **AP** probe in presence of different metal ions.

Fluorescence titrations on Cu^{2+} sensor

By increasing the concentrations of Cu^{2+} ($0\text{--}30 \mu\text{M}$ with an equal span of $2 \mu\text{M}$ in H_2O) the sensitivity of **AP** ($20 \mu\text{M}$ in CH_3CN) towards Cu^{2+} ions were clearly witnessed as shown in Fig. 3a. The PL spectra of **AP**--- Cu^{2+} ($\lambda_{\text{em}} = 430 \text{ nm}$) demonstrated the turn-

on responses of **AP** to Cu^{2+} ions and the respective fluorescence intensity changes as a function of Cu^{2+} concentration is exposed in Fig. 3b. Similarly, the UV-Vis titration on

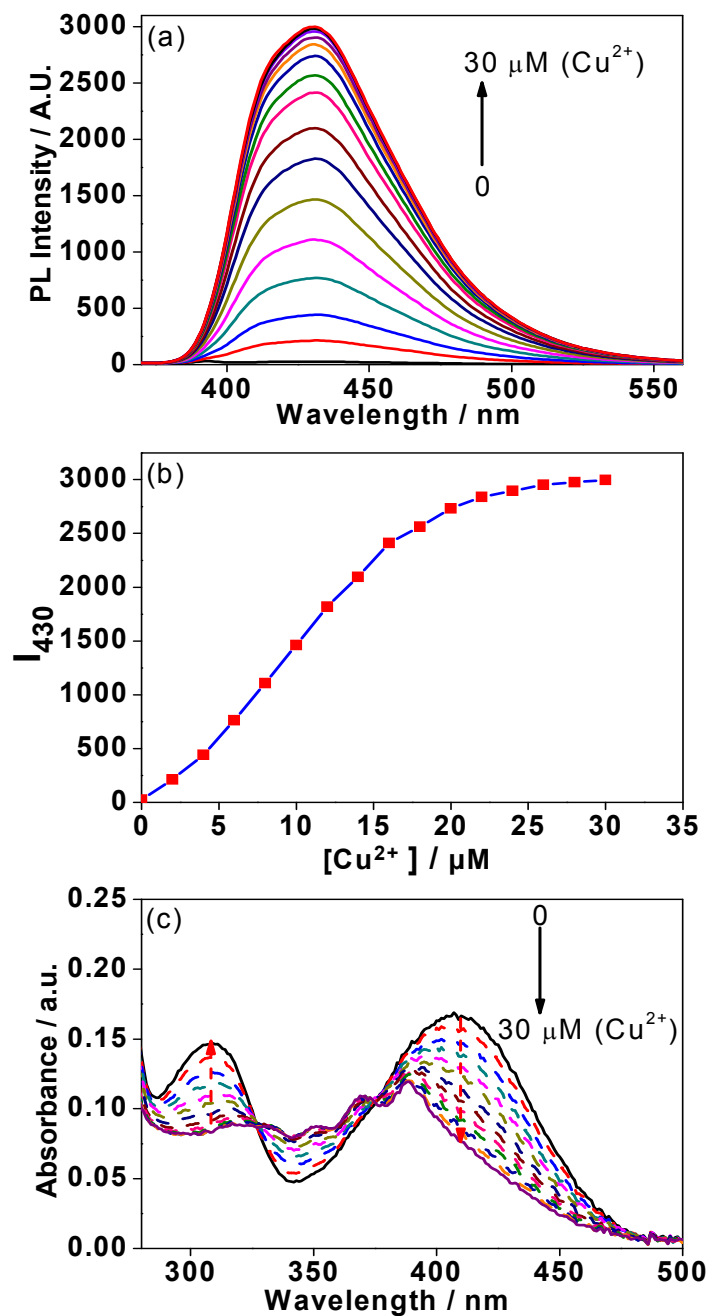


Fig. 3 (a) PL titrations ($\lambda_{\text{ex}} = 350 \text{ nm}$), (b) intensity changes as a function of Cu^{2+} concentration and (c) UV titrations of **AP** ($20 \mu\text{M}$) in CH_3CN with Cu^{2+} ions in H_2O .

Cu^{2+} ions also observed the quenched absorbance at 405 nm along with increased absorbance intensity at 296 nm, as publicized in Fig. 3c. Further, an isosbestic point at 325 nm was evidenced, which supports the possible 1:1 stoichiometry of **AP**--- Cu^{2+} complex. In addition, the selectivity of **AP** probe was further evaluated by single and dual metal studies as follows. In order to establish the specific selectivity of **AP** to Cu^{2+} , we executed the single and dual metal competitive analysis as noticed in Fig. 4. In single metal system (black bars), except Cu^{2+} ions (30 μM ; 1.5 equiv.), all the metal ions (Ba^{2+} , Ni^{2+} , Fe^{3+} , Cr^{3+} , Mg^{2+} , Fe^{2+} , Al^{3+} , Ag^+ , Co^{2+} , Zn^{2+} , Pb^{2+} , Mn^{2+} , and Hg^{2+}) concentrations were kept as 150 μM (7.5 equiv.) towards **AP**. However, for dual-metal (red bars) studies, two equal amounts of aqueous solutions of Cu^{2+} and other metal ions (30 μM + 150 μM) were combined. Moreover, 180 μM of Cu^{2+} was also considered for its effect

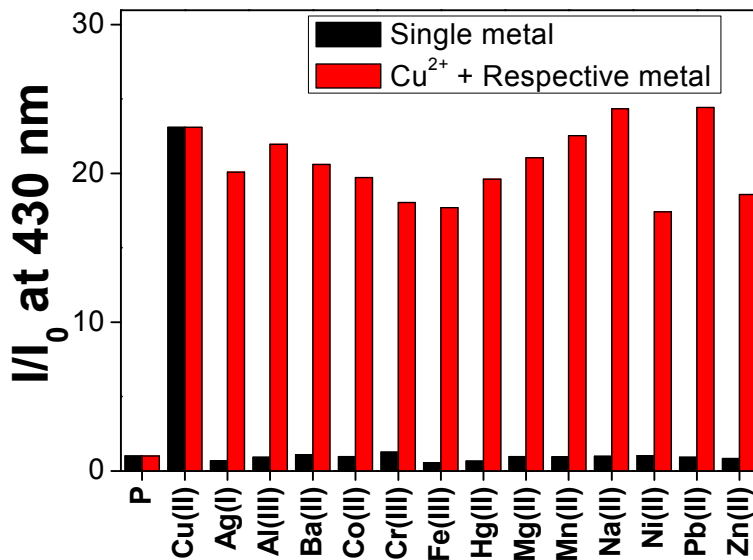


Fig. 4 Histograms on single and dual metal studies of **AP** (20 μM in CH_3CN) probe towards Cu^{2+} ions selectivity.

and the obtained results suggested specific selectivity of **AP** probe towards Cu^{2+} ions as shown in Fig. 4. Similar to the above observed results, the Cu^{2+} selectivity was also been demonstrated by single and dual metal studies in DMSO, THF and Methanol as shown in Figs S4b-c (ESI).

Stoichiometry and binding sites³⁷⁻³⁹

Stoichiometry calculations were carried out through job's plot as noticed in Fig. 5 to ensure the binding site of **AP**--- Cu^{2+} conjugate. The emission intensity at 430 nm was plotted against molar fraction of **AP** under a constant total concentration. Maximum emission intensity was notified at 0.5 molar fractions, which confirmed the 1:1 stoichiometry of **AP**--- Cu^{2+} complex. Further, the paramagnetic nature of Cu^{2+} ion also influenced on ^1H NMR proton signals (not shown) of **AP** probe that are close to Cu^{2+} binding site. Likewise, the ESI-mass peak at $m/z = 361.06$ [**AP**--- $\text{Cu}^{2+} + 1$] also supported the 1:1 sensor complex as noticed in Fig. 6. More importantly, the high

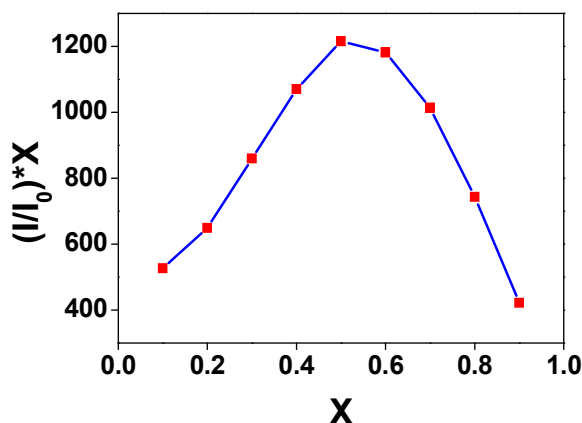


Fig. 5 Job plot of **AP**--- Cu^{2+} complex passes through $X = 0.5$. Hence 1:1 stoichiometry of **AP**--- Cu^{2+} sensor complex; $X = [\text{Cu}^{2+}] / [\text{Cu}^{2+}] + [\text{AP}]$; where X = mole fraction, $[\text{Cu}^{2+}]$ and $[\text{AP}]$ are concentrations of Cu^{2+} and **AP**. The total concentration of **AP** and Cu^{2+} was 50.0 M. The monitored wavelength was 430 nm.

resolution mass data (HRMS) was confirmed 1:1 stoichiometry of **AP**---Cu²⁺ complex. As shown in Fig. S5 (ESI), the HRMS data was well verified the complex formation *via* its isotopic pattern as well. Hence, based on job's plot, ¹H NMR titrations and mass analysis, we proposed the possible binding of **AP**---Cu²⁺ complex as shown in Scheme 2.

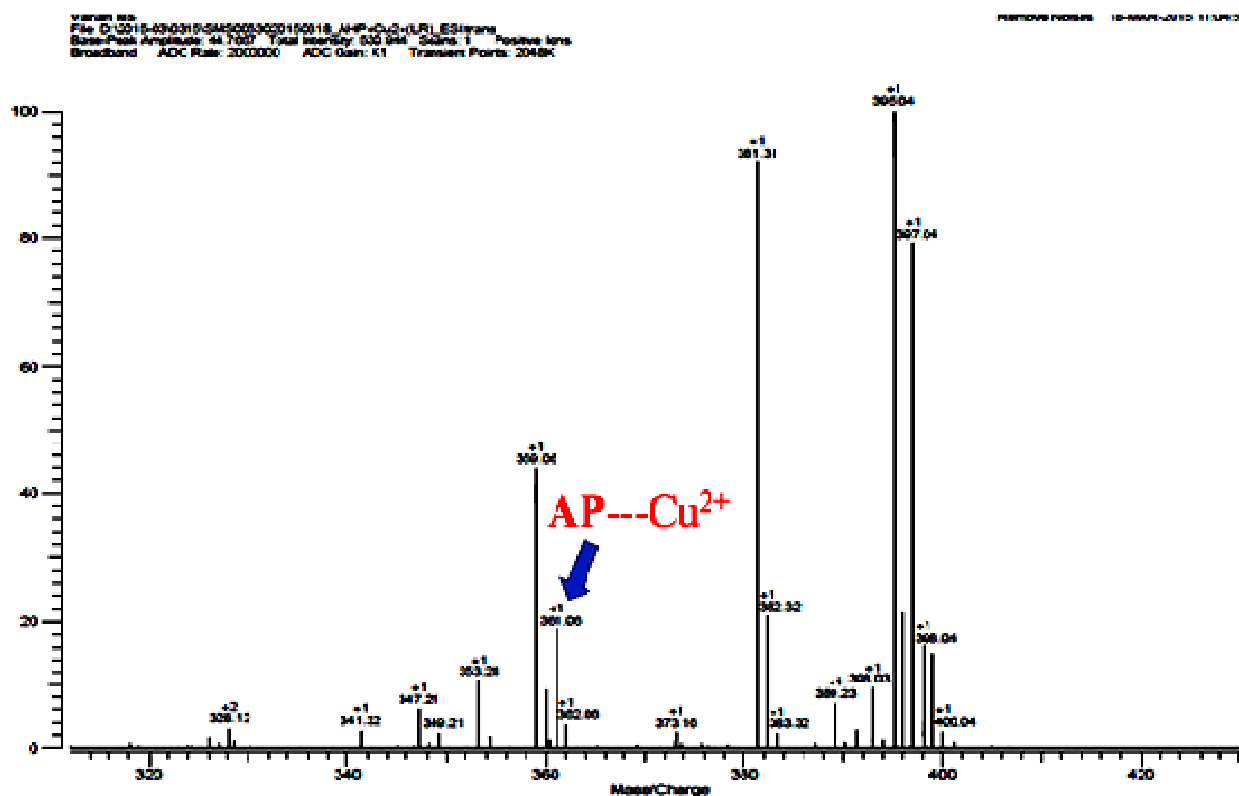
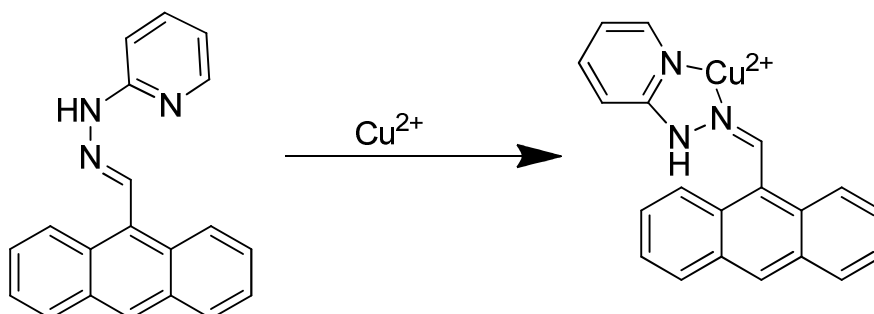


Fig. 6 Mass spectrum of **AP**---Cu²⁺ complex.



Scheme. 2 Illustration of Cu²⁺ binding with **AP** probe.

As noticed in Scheme 2, the formation stable five member ring was induced by Cu^{2+} ions, which also well buttressed by DFT studies next. Further, the above statement was well supported by many reports of two dentate Cu (II) complexes with stabilized five membered ring.^{36a, 40}

Next, the FTIR investigations of **AP** probe and **AP**--- Cu^{2+} well confirmed the 1:1 ratio complex formation of **AP** probe with Cu^{2+} metal ions as exposed in Fig. S6 (ESI). Upon formation of **AP**--- Cu^{2+} complex, the $-\text{NH}$ stretch peak became narrower. Further, the $-\text{NH}$ bend peak at 1500 cm^{-1} was also shifted to 1700 cm^{-1} and the double-bond absorption bands were newly appeared at 1629 cm^{-1} , 1552 cm^{-1} and 1494 cm^{-1} . This indicated the binding of Cu^{2+} with nitrogen of imine and pyridine groups. Hence, possible illustrated binding of **AP**--- Cu^{2+} complex (scheme. 2) was well verified. Subsequently, the morphological and crystallinity changes were established as explained next. The SEM image of **AP** probe reveals its scattered morphology. But, upon the addition of Cu^{2+} ions, the aggregated crystalline nature of **AP**--- Cu^{2+} complex was noticed as publicised in Fig. S7 (ESI).

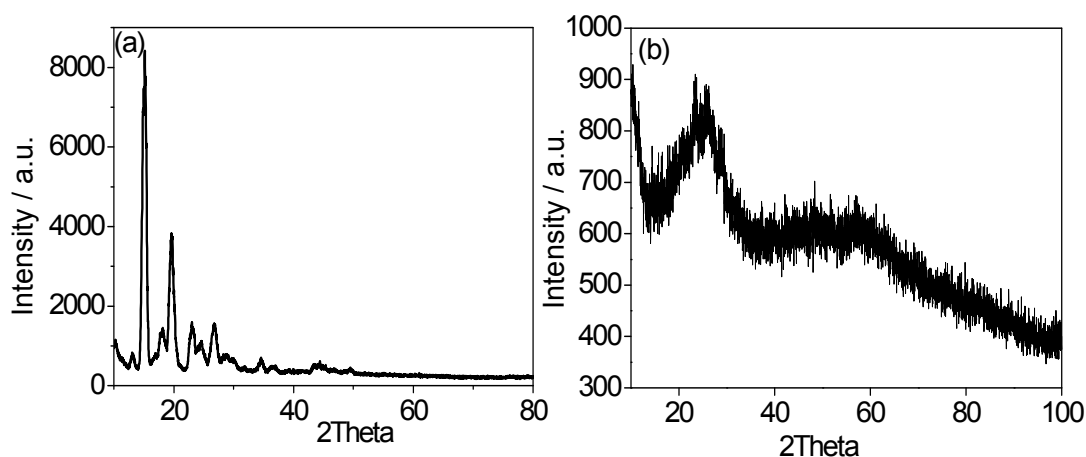


Fig. 7 XRD Analysis of (a) **AP** probe and (b) **AP**--- Cu^{2+} complex.

In addition, the XRD results evidenced that **AP**---Cu²⁺ loses crystallinity nature compared with the pure **AP** probe (Fig. 7a), which indicates the binding of **AP** with Cu²⁺ complex. The amorphous nature **AP**---Cu²⁺ of the XRD peak are shown in the Fig. 7b

In order to prove the detection capability of **AP** towards Cu²⁺ ions, the detection limit (LOD)⁴¹ calculation was performed through standard deviation and linear fittings as illustrated in Fig. 8a. By plotting the fluorescence intensity changes at 430 nm as a function of concentration of Cu²⁺ ions, the detection limit of **AP**---Cu²⁺ complex was estimated as 8.63 x 10⁻⁸ M. Assuming a 1:1 complex formation, the association constant (K_a) of **AP**---Cu²⁺ was obtained on the basis of following equation (1).⁴²

$$\alpha^2/(1-\alpha) = 1/2K_a C_F [M] \text{----- (1)}$$

Where C_F is the total concentration of probe **AP** in the system and α is defined as the ratio between the free probe **AP** and the total concentration of probe **AP**. The value “α” was obtained using Eq. (2)

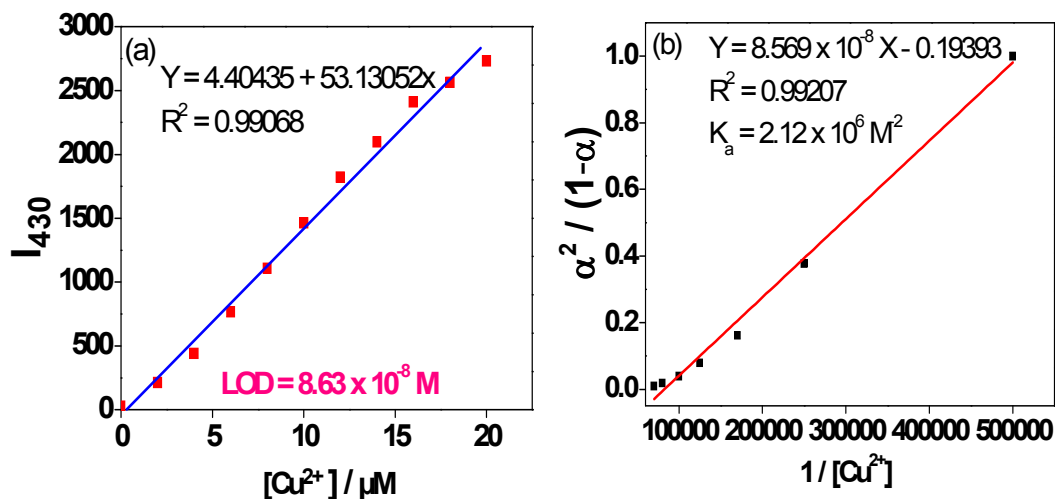


Fig. 8 (a) Detection limit calculation by plotting PL intensity changes at 430 nm vs Cu²⁺ concentration and (b) Association constant calculations by plotting $\alpha^2/(1-\alpha)$ vs $1/Cu^{2+}$ via standard deviation and linear fittings.

$$\alpha = F - F_0 / F_1 - F_0 \text{ ----- (2)}$$

F is the fluorescence intensity at 430 nm at any given Cu^{2+} concentration, F_1 is the fluorescence intensity at 430 nm in the absence of Cu^{2+} ions, F_0 is the maxima fluorescence intensity at 430 nm in the presence of Cu^{2+} . The association constant (K_a) was estimated graphically by plotting $\alpha^2/(1 - \alpha)$ against $1/[\text{Cu}^{2+}]$. The plots $\alpha^2/(1 - \alpha)$ vs. $1/[\text{Cu}^{2+}]$ are shown in Fig. 8b. Data were linearly fitted with respect to Eq. (1) and the K_a value was obtained from the slope. The K_a value of **AP**--- Cu^{2+} was estimated as $2.12 \times 10^6 \text{ M}^{-2}$.

Following, the pH titration of **AP** probe was performed to investigate a suitable pH range for Cu^{2+} sensing. As depicted in Fig. S8 (ESI), the emission intensities of metal-free **AP** probe was very low. After mixing **AP** probe with Cu^{2+} , the emission intensity at 430 nm was suddenly increased at pH 6.0 and reached maximum between pH 6 – 8. However, when pH exceeded higher than 8.5, the emission intensity started to drop towards zero. This indicates poor stability of the **AP**--- Cu^{2+} complex at higher pH ranges. For pH <6, the emission intensity is very low due to the protonation of the amine group, which prevents the formation of **AP**--- Cu^{2+} complex.

DFT studies

To better understand the nature of coordination in **AP**--- Cu^{2+} , the energy-minimized structures of **AP** and its corresponding Cu^{2+} complexes (Fig. 9) were obtained with the B3LYP hybrid functional using the LANL2DZ basis set.⁴³ All the calculations have been performed using the Gaussian 09 suite of programs.⁴⁴

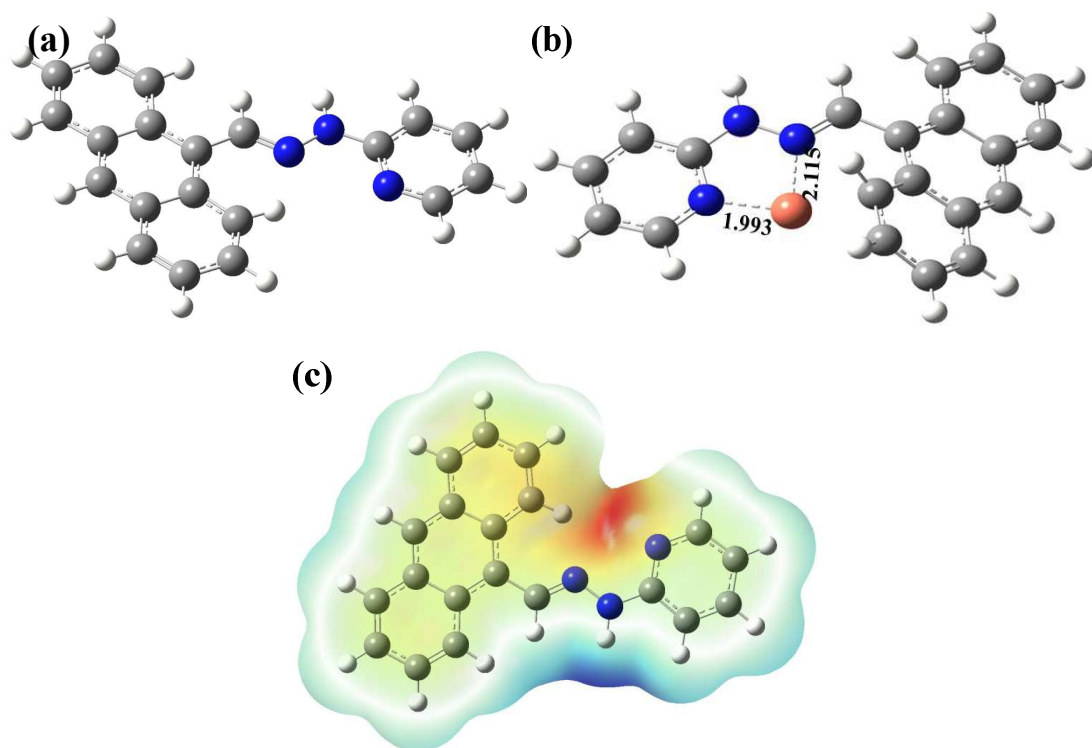


Fig. 9 (a) Optimized structure of **AP** probe (b) Optimized structure of **AP**---Cu²⁺ complex. (c) Electrostatic potential surface (ESP) of **AP**.

The optimized geometry, the ESP of the probe and the **AP**---Cu²⁺ complex are shown in Fig 9. The distances between Cu and two N atoms are observed to be 1.99 and 2.11 Å (see Fig 9). The ESP in Fig 9(b) shows that the possible position of the metal binding site in red color. The spatial distributions and orbital energies of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of **AP** and its corresponding Cu²⁺ complex were generated and the general PET mechanism for the probe and its Cu²⁺ complex has been reported in Fig.10. Further, the DFT optimized structures (HOMO, HOMO-1, HOMO-2 and HOMO-3; LUMO, LUMO+1, LUMO+2 and LUMO+3) of **AP** and **AP**---Cu²⁺ complex were supplemented in Fig. S9 (ESI).

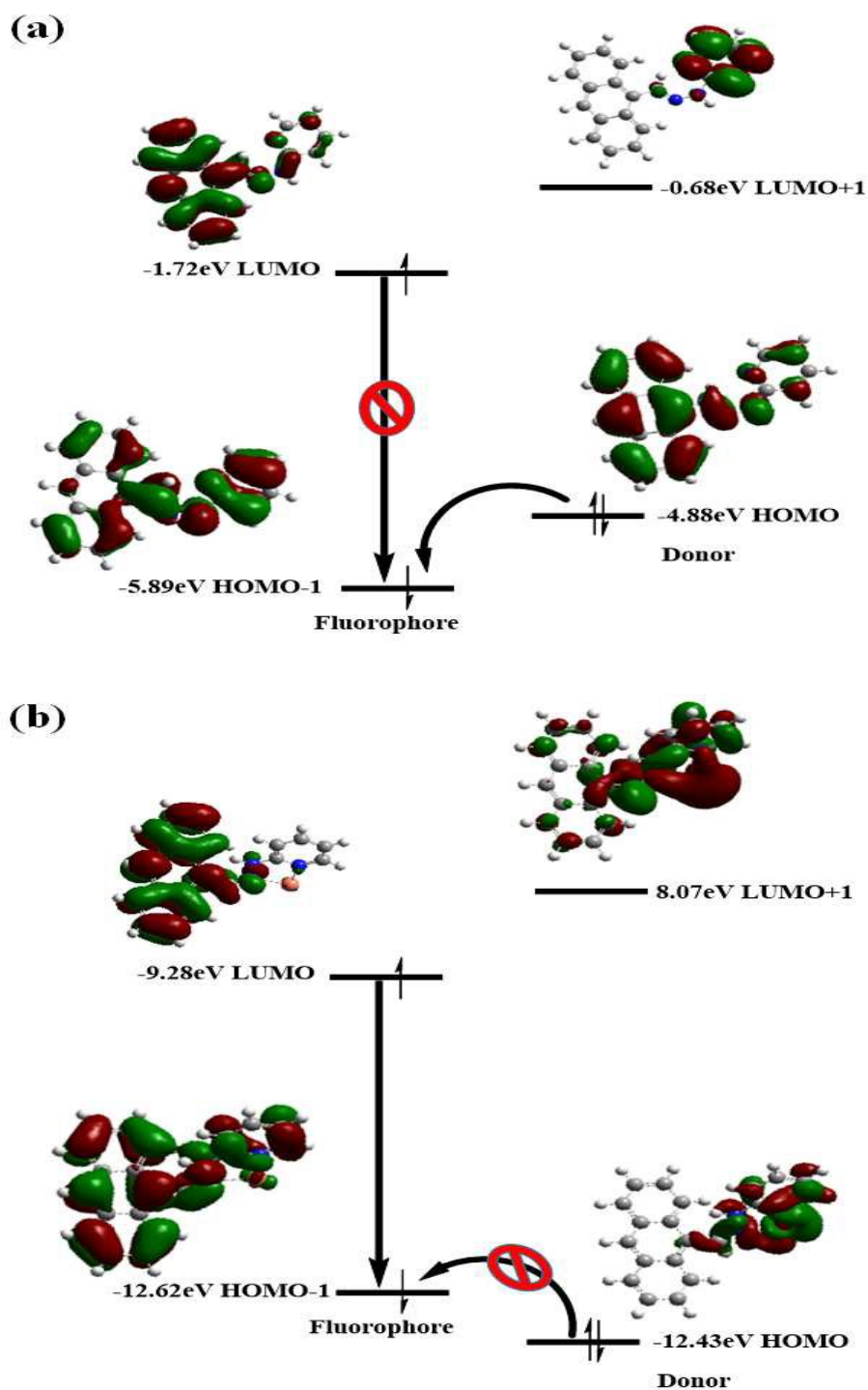


Fig. 10 General representation of PET based mechanism for (a) AP probe and (b) AP---
 Cu^{2+} sensor system.

Calculated HOMO, LUMO and further excitation of electrons in the probe and its complex using the *state-of-the-art* density functional theory have provided us important information on electron migration at the molecular level for sensor applications. The PET mechanism for the probe shows that the HOMO (donor) is higher than that of the HOMO-1 (acceptor) (See Fig. 10a). The energy level of HOMO is -5.08 eV and the energy level of HOMO-1 is -6.15 eV. Remarkably, the HOMO-1 (donor) energy in the **AP**---Cu²⁺ complex is lower than the HOMO (acceptor) value. It suppresses the PET process, resulting in fluorescence intensity enhanced as observed in the experiment.

Live cell application

The potential of **AP** probe for imaging Cu²⁺ in live cells were obtained using a confocal fluorescence microscope.

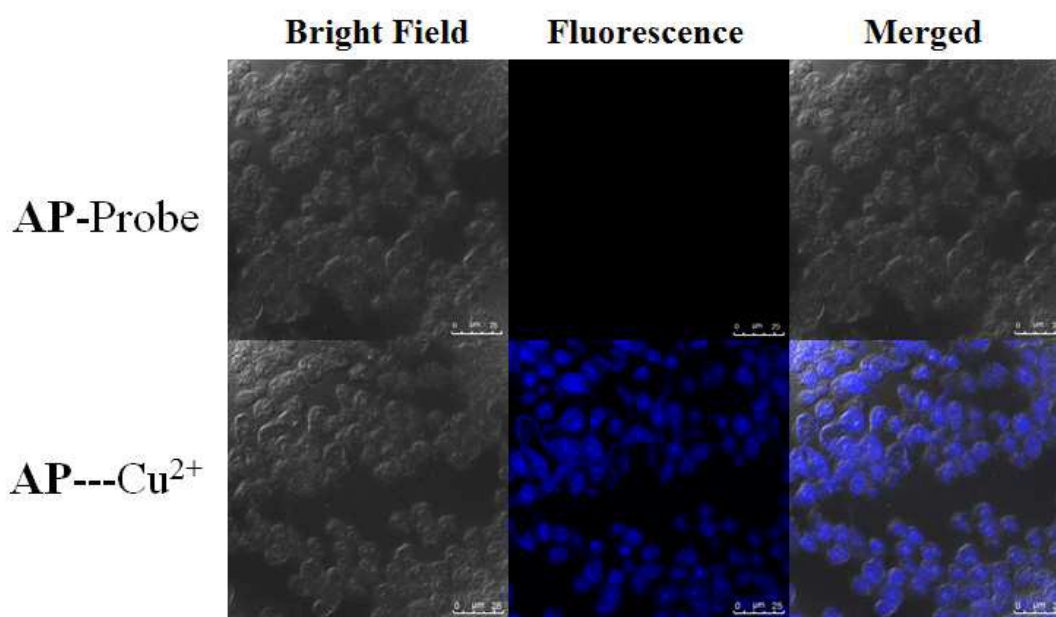


Fig. 11 Fluorescence images of Raw264.7 cells treated with **AP** and **AP**---Cu²⁺. Bright Field image (Left); Fluorescence image (middle); Merged image (right). The scale bar is 25 μ M.

When Raw264.7 cells were incubated with **AP** 20 μM), no fluorescence was observed (Fig. 11). After treatment with Cu^{2+} , bright blue fluorescence was observed in the Raw264.7 cells (Fig. 11). An overlay of fluorescence and bright-field images shows that the fluorescence signals are localized in the intracellular area, indicating a subcellular distribution of Cu^{2+} ions with good cell-membrane permeability of **AP**. Further, the cytotoxicity studies of **AP**, showed more than 70% cell viability at 40 μM concentration as exposed in Fig. 12.

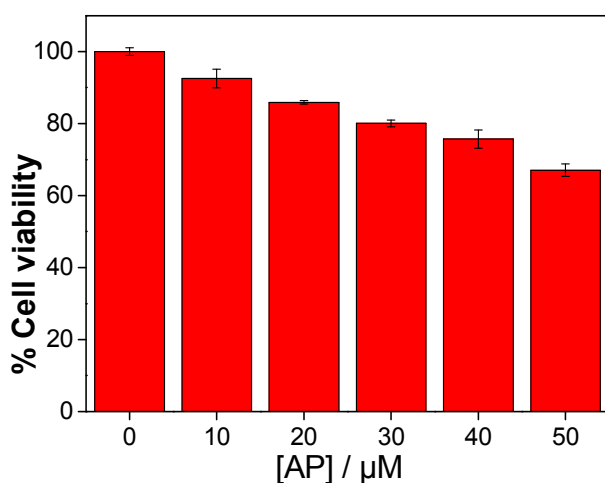


Fig. 12 Cell viability of **AP** probe.

Conclusion

In conclusion, a simple anthracene comprising schiff base derivative **AP** was successfully obtained *via* one-pot reaction. The **AP** probe showed high selectivity and sensitivity towards Cu^{2+} in a 1:1 stoichiometric manner which was confirmed by UV/PL titrations, FTIR, and ESI (+Ve) mass analysis. The LOD and K_a values of **AP**--- Cu^{2+} conjugate were estimated as 10^{-8} M and 10^6 M^{-2} , respectively. Furthermore, **AP**--- Cu^{2+}

sensor complex formation was well characterised by Φ_f , SEM, pH effect and DFT studies. Delightfully, the sensor selectivity of **AP** to Cu^{2+} ions were successfully applied in cell imaging studies with cytotoxic studies. The cell permeability of the **AP** probe with less toxicity enables its further application towards drug delivery into the cell.

Acknowledgments

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Graphical Abstract:

A simple anthracene based **AP** probe was synthesized to detect Cu^{2+} ions, via PET mechanism in live cell.

