



GOLD SENSING THROUGH COUMARIN ENGINEERED FLUOROPHORE

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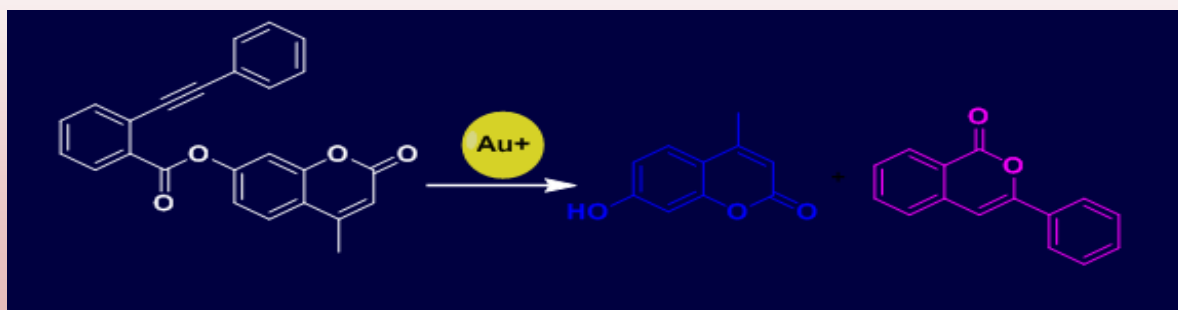
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ABSTRACT

We herewith disclose a reaction dependent strategy involving masking and unmasking of coumarin engineered fluorophore for selective sensing of ionic gold forms. Considering the alkynophilicity of gold ions, a dormant fluorophore was designed for the sensing of gold species. Activation of tripple bond, mediated only by gold ions leading to cascade and delivers active fluorophore thus makes the probe quite selective over several different metal ions.



KEYWORDS: Coumarin, Alkynophilicity, Masking-Unmasking, Fluorophore, Fluorescence.

INTRODUCTION

As we all aware with the fascinating and attractive world of glittering gold, very few of us know that Aurum in its ionic forms also being used as important reagent by organic chemist in several organic conversions as they play a vital role in organic transformations.^[1-7] Considering the pi-activation feature associated with ionic gold either in +I or +III oxidation states,^[8-11] a large number of novel functional group transformations have been reported. The high alkynophilic ability exhibited by ionic gold forms, unlike other metal ions in the elemental chart, makes them significant and distinct. Irrespective of its high stability and biofriendly nature of metallic aurum, it's ionic forms are very much reactive and substantially toxic to living beings. Amongst, several possible oxidation states +I and +III are supposed to be the most stable forms. Gold ions have anti-inflammatory action and are used as medicines in the treatment of TB, joint pains, and cancer.^[12-18] Even in Ayurveda traditional Indian medicines 'swarnabhasma' contained gold. In addition, it is well proved that gold ions are known to block activities of phagocytic cells and

granulocytes a type of white blood cell.^[19] Despite the interesting chemical and medicinal properties of gold ions, soluble gold salts such as AuCl AuCl₃ are notorious to cause damage to the liver, kidneys, and the nervous system.^[20-24] Having said this gold species may also tightly bind to biomolecules such as enzymes and DNA, and proved to be cytotoxic and may lead to cancer foration.^[25] Thus, based on the role of gold catalyzed organic transformations and the toxicity associated with gold ions it is highly desirable to develop probes for detection of gold ions in environmental and biological systems, because fluorescence-based chemosensors are simple and highly sensitive, they have been widely applied in the sensing of many biologically relevant metal ions in recent years. In particular, toxic metal ions, such as mercury⁺,^[26-28] lead,^[29-31] silver^[32] and cadmium,^[33-35] have been successfully sensed by using fluorescent probe technique. Thus, the intramolecular cyclization of the alkynes promoted by gold ions could be the strategy in the designing reaction-based fluorescent chemosensors. In continuation of our work with fluorescein based probes, we envisioned that

coumarin ester tethered with an alkyne could be used to detect gold ions (Scheme 1), where activation of the alkyne with gold ions would convert the non-fluorescent form into the fluorescent one.^[36-42]

Experimental Section

All the reagents used by us for this work were purchased from commercial suppliers and used without further purification. We have purified and dried the solvents by using standard protocols available in literature. Redistilled water was used throughout all of our reactions. The metal ion solutions were prepared from AuCl₃, AgOTf, Ba(OH)₂·8H₂O, FeCl₃·8H₂O, Bi(OTf)₃, Hg(OTf)₂, IrCl₃, KI, MnSO₄·H₂O, NiCl₂·6H₂O, CdCl₂, PdCl₂, PtCl₂, PtCl₄, CrCl₃, ZnCl₂.

For carrying out all of our reactions we have made use of oven dried vials with magnetic stirring under nitrogen atmosphere. Dried solvents and liquid reagents were transferred by sterile syringes or hypodermic syringe cooled to room temperature in a desiccator. Precoated silica plates (TLC) were used for monitoring reactions, after elution plate was visualized under UV chamber. Further to confirm, the spots are exposed to KMnO₄ visualized after charring on a hot plate.

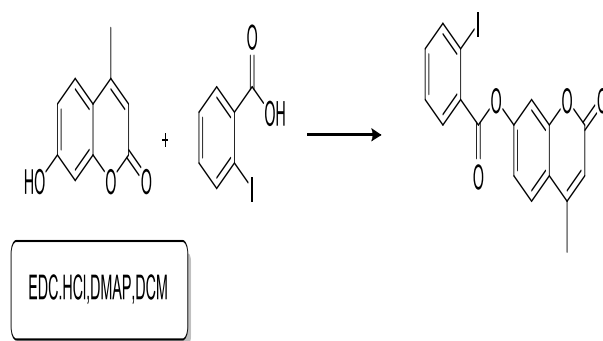
Proton NMR spectra and ¹³C NMR spectra were recorded with Bruker AV, 200/400/500 MHz instruments in appropriate solvents using Tetramethyl silane as internal standard or the solvent signals are considered as secondary standards and the chemical shifts are shown in δ scales. Multiplicities of ¹H NMR signals are assigned as s (singlet), br. s (broad singlet), d (doublet), dd (doublet of doublet), t (triplet) and m (multiplet). All measurements were carried out at room temperature. Measurement of Fluorescence spectra were performed with a Perkin-Elmer LAMBDA 950 UV/Vis Spectrophotometer and a Photon Technology International, QuantaMaster 400 Spectrofluorometer, respectively, in degassed spectral grade solvents.

RESULT AND DISCUSSIONS

Formation of The desired probe is achieved in two steps, In the first step phenolic -OH of 7-hydroxy-4-methyl coumarin undergoes esterification reaction with 2-iodobenzoic acid in presence of EDC/DMAP condition which converts the carboxylic acid group into an activated ester which later couples with -OH group at 7 position in coumarin skeleton to give desired iodoester in 70% yield (Scheme 1). The iodo ester, thus obtained, treated with phenyl acetylene under Palladium-Copper catalysis i.e. sonogashira condition to give the desired probe in 78% yield in (Scheme 2). The probe containing a triple bond functionality is activated by gold ions which, in turn, triggers a cascade and liberate the highly fluorescent fluorophore (Scheme 3).

Scheme 1: Synthesis of Probe in Two Steps.

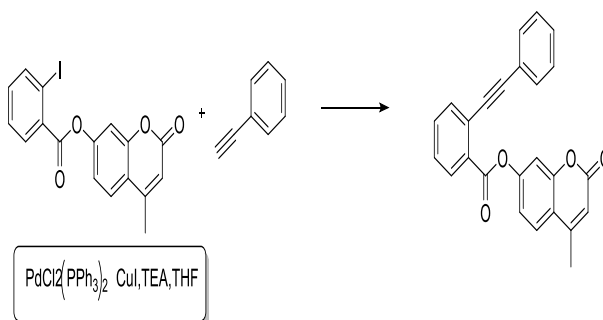
Step-1. Coupling of 7-hydroxy 4-methyl coumarin with iodo benzoic acid (Haloester): To a stirred solution of iodobenzoic acid (1.0 equiv.), EDC (1.0 equiv.) and DMAP (0.1 equiv.) in DCM, 7-hydroxy-4-methylcoumarin (1.0 equiv.) in DCM was transferred at rt. The reaction mixture was stirred for an additional 12 hrs. After completion of reaction the solvent was removed under reduced pressure and resulting residue was purified by flash column chromatography with 10/90 mixture of ethyl acetate and hexane as mobile phase to yield pure products. The yield was 70% with pale yellow solid compound. R_f value observed was 0.50 (hexane/EtOAc = 60/40).



Scheme. 1.

STEP-2. Sonogashira coupling to give desired probe

To a solution of iodo-ester (1.0 equiv.) in anhydrous THF as solvent (5 mL) were added CuI (0.025 equiv) and PPh₃ (0.025 equiv) in an oven dried two neck round bottom flask with magnetic stirring bar and rubber septum. The reaction mixture was degassed by purging with nitrogen and filled with nitrogen three times and then PdCl₂(PPh₃)₂ (0.05 equiv), Et₃N (1.5 equiv), and phenylacetylene (1.5 equiv) were added to the solution. The reaction mixture was stirred for an additional 12 h at rt and then filtered through a pad of silica and Celite, and washed with DCM to remove the insoluble impurities. The solvents were removed under reduced pressure and the crude product obtained was purified by chromatography on silica gel, using a 10/90 mixture of ethyl acetate/hexane as the eluent to yield corresponding product 78% yield, The R_f value observed was 0.60 (hexane/EtOAc = 60/40).

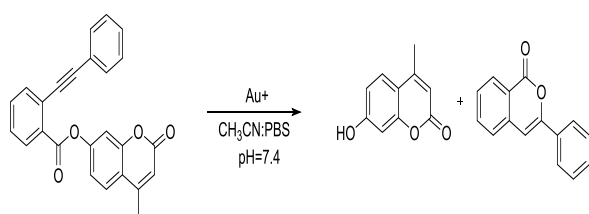
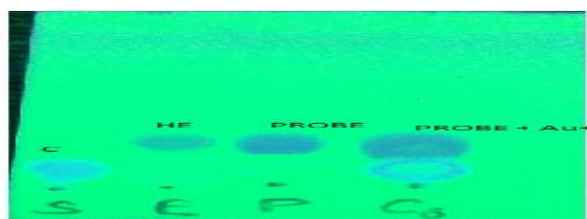


Scheme. 2.

Scheme. 3. Sensing of Gold Ions by Probe

The actual sensing experiment was performed by preparing the probe solution in CH₃CN/PBS buffer (1:1, pH = 7.4) which is colorless and displays negligible fluorescence. However, after the addition of Au¹⁺ ions to probe activates a large boost in fluorescence (λ_{max} = nm). The time reliant fluorescence response of probe (10 mM) with Au¹⁺ (100 mM) exhibited a rapid enhancement in intensity up to 30 min and then attains a steady level (Fig. 4). Further, we have studied the selectivity of probe towards various other metal ions. The study discloses that probe shows a unique selectivity towards Au¹⁺ and Au³⁺ metal ions only. The fluorescence response of other metals ions such as Ag¹⁺, Ba²⁺, Bi³⁺, Fe³⁺, Hg²⁺, Ir³⁺, K¹⁺, La³⁺, Mn²⁺, Ni²⁺, Pd²⁺, Pt²⁺, Pt⁴⁺, Ru³⁺, Sc³⁺, Yb³⁺, Zn²⁺, Cd²⁺ and Cr³⁺ is negligible. Further the selectivity of probe towards Au¹⁺ in the presence of other competing metal species has been verified. It is obvious that the intervention of other metal species is insignificant, so specifically this probe can be used as a Au¹⁺ sensor in the presence of other metal ions.

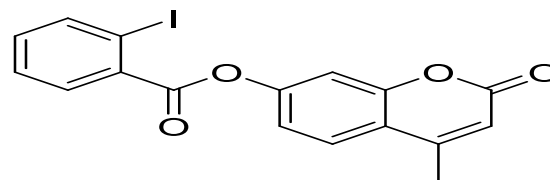
Fig. 8 shows the concentration dependent fluorescence change of Probe (10 mM) upon gradual addition of 5-100 μ M. of Au¹⁺ ion. Emission peak is seen (λ_{em} = 460 nm) immediately after addition of the Au¹⁺ ion and a large enhancement in Fluorescence intensity is observed. The increase in emission intensity is rapid and it saturates after 40 min, indicating the reaction is very fast. Gold ion triggered cascade leading to unmasking of active fluorophore is proved by exposing the probe to Au⁺. The expected mass fragments (Fig. 7) were observed in mass spectrum. As per our expectation, the desired probe showed negligible fluorescence and is colorless in H₂O-CH₃CN: PBS buffer (pH = 7.4). The probe (10 μ M) on treatment with 100 μ M solution of Au⁺ exerted strong fluorescence at 460 nm (Scheme 3).

**Scheme 3.**

TLC clearly shows that probe on reaction with gold releases fluorescent coumarin (EA: Hexane).

Spectral Data

Compound: 4-methyl-2-oxo-2H-chromen-7-yl 2-iodobenzoate (Haloester).

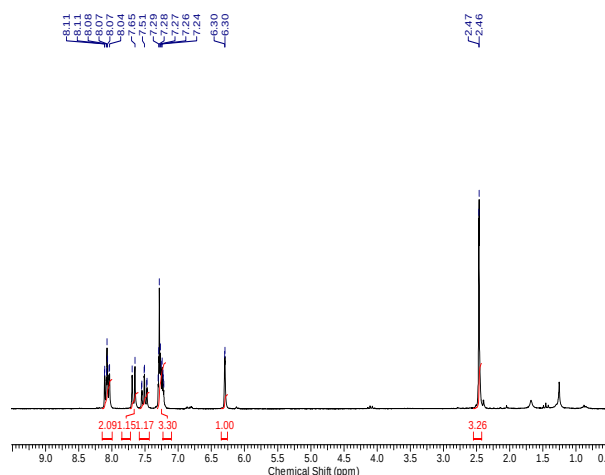
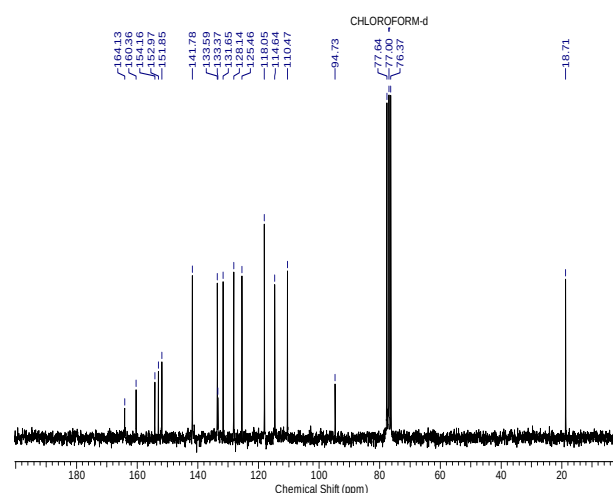


¹H NMR (200MHz, CHLOROFORM-d) δ = 8.08 (ddd, J = 1.3, 6.1, 7.7 Hz, 2 H), 7.67 (d, J = 8.2 Hz, 1 H), 7.51 (dt, J = 1.1, 7.6 Hz, 1 H), 7.32 - 7.19 (m, 3 H), 6.30 (d, J = 1.1 Hz, 1 H), 2.46 (d, J = 1.1 Hz, 3 H).

¹³C NMR (50MHz, CHLOROFORM-d) δ = 164.1, 160.4, 154.2, 153.0, 151.9, 141.8, 133.6, 133.4, 131.7, 128.1, 125.5, 118.1, 114.6, 110.5, 94.7, 18.7.

IR (film): ν_{max} 2924, 1762, 1609, 1500, 1423, 1245, 1166, 1106, 878, 759 cm⁻¹.

MS: m/z 406.9, 407.9.

**Fig. 1: NMR spectrum of haloester.****Fig. 2: ¹³C NMR of haloester.**

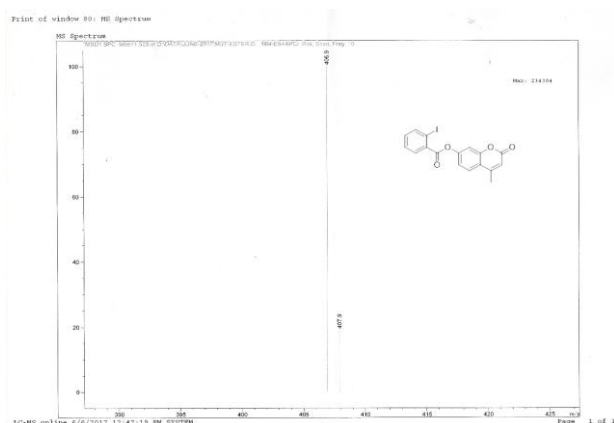
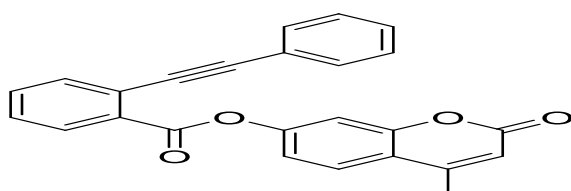


Fig. 3. Mass spectrum of haloester.

Compound : 4-methyl-2-oxo-2H-chromen-7-yl 2-(2-phenylethynyl)benzoate(Probe).



¹H NMR (200MHz, CHLOROFORM-d) δ = 8.18 (dd, J = 1.1, 7.8 Hz, 1 H), 7.77 - 7.70 (m, 1 H), 7.68 - 7.57 (m, 2 H), 7.56 - 7.42 (m, 4 H), 7.34 - 7.28 (m, 4 H), 7.27 - 7.21 (m, 1 H), 6.27 (d, J = 1.1 Hz, 1 H), 2.43 (d, J = 1.3 Hz, 3 H).

¹³C NMR (50MHz, CHLOROFORM-d) δ = 163.8, 160.5, 154.1, 153.3, 152.0, 134.3, 132.7, 131.7, 131.1, 129.9, 128.7, 128.3, 128.0, 125.4, 124.6, 122.8, 118.2, 117.8, 114.4, 110.5, 95.4, 87.7, 77.6, 76.4, 18.6.

IR (film): $\nu_{\max}$ 2924, 2100, 1764, 1609, 1497, 1421, 1105, 1031, 756 cm^{-1} .

MS: m/z 381.0, 382.0.

Mass spectral analysis of Probe + Au (I)

MS: 117.1, 131.1, 177.0, 177.9, 222.9, 257.2

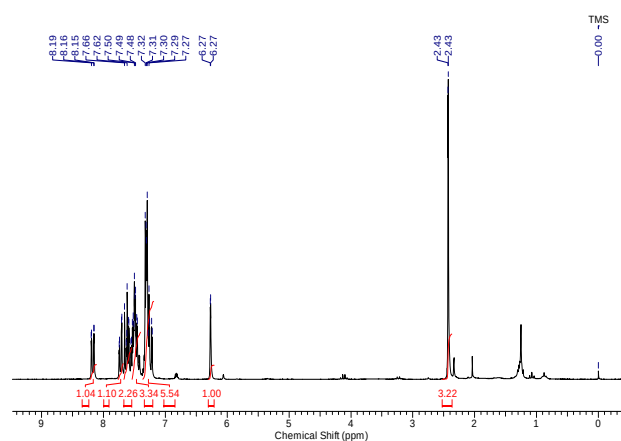


Fig. 4: NMR spectrum of Probe.

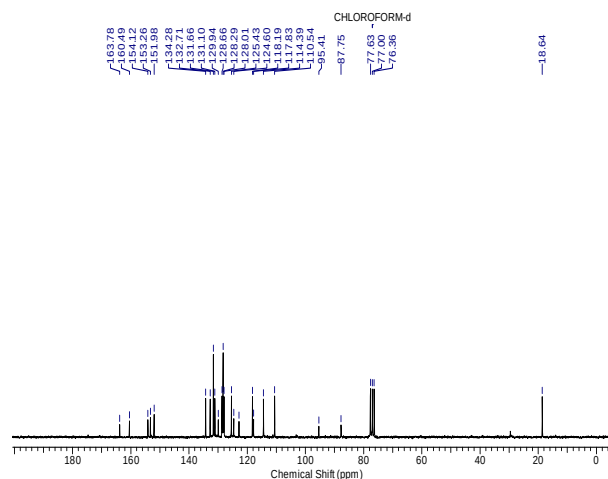
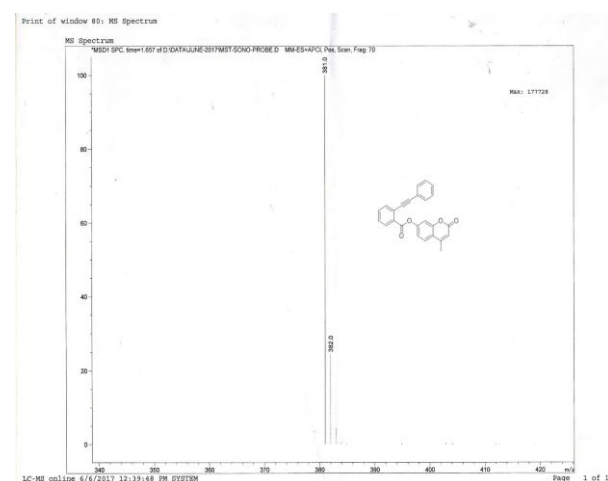
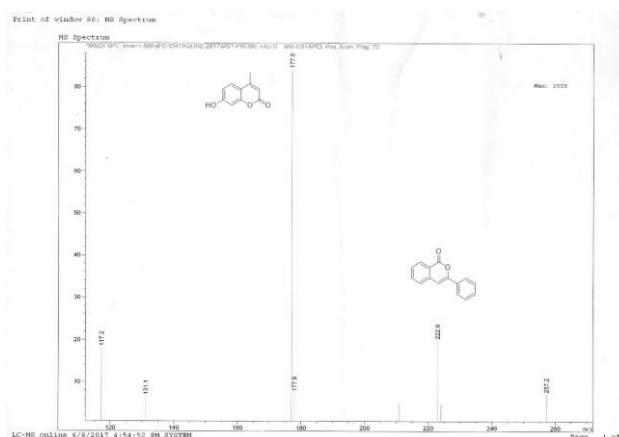
Fig. 5 :¹³C NMR of Probe.

Fig. 6: Mass spectrum of Probe.

Fig. 7. : Mass Spectra of Probe + Au⁺ : m/z : 222.9 and 177.0.

Emission Spectral Study

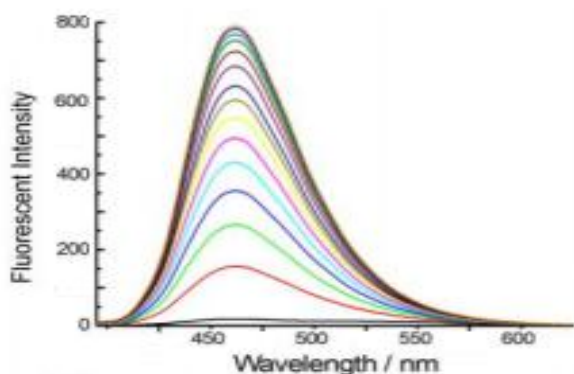


Fig. 8: Concentration dependent fluorescence spectra obtained for a mixture of probe (10 μM) upon addition of Au^{1+} (5 μM -100 μM) in CH_3CN :PBS buffer (1:1, 0.1M, pH = 7.4). λ_{excit} = 360 nm. $\lambda_{\text{emission}}$ 460nm.

CONCLUSION

In conclusion, we have successfully developed a new, facile, efficient and useful protocol for sensing gold ion, based on masking-unmasking strategy of a fluorophore. The probe is highly sensitive towards gold ion over several other metal ions. We strongly believe that this new protocol will catch the attention of the scientific community and therefore many probes based on the present approach will appear in the future.

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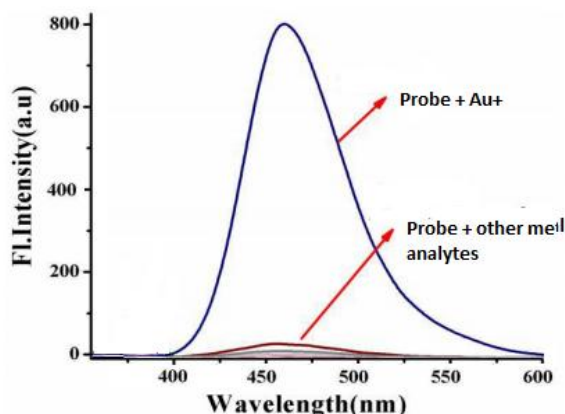


Fig. 9: Fluorescence response of Probe (10 μM) in the presence of various metal ions (100 μM).) in CH_3CN :PBS buffer (1:1, 0.1M, pH = 7.4). λ_{excit} = 360 nm. $\lambda_{\text{emission}}$ 460.

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