RSC Advances

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

A pyrenyl linked calix[4]arene fluorescence probe for recognition of ferric and phosphate ions

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A pyrenyl linked calix[4]arene fluroionophore has been synthesized and used as ditopic chemosensing ensemble for Fe^{3+} and $H_2PO_4^-$ by emission spectra. The detection limit of synthesized receptor was found to be 0.88 pM for Fe^{3+} and 1.11 pM for $H_2PO_4^-$. Moreover, this probe has been applied for recognition Fe^{3+} in blood serum and $H_2PO_4^-$ in waste water.

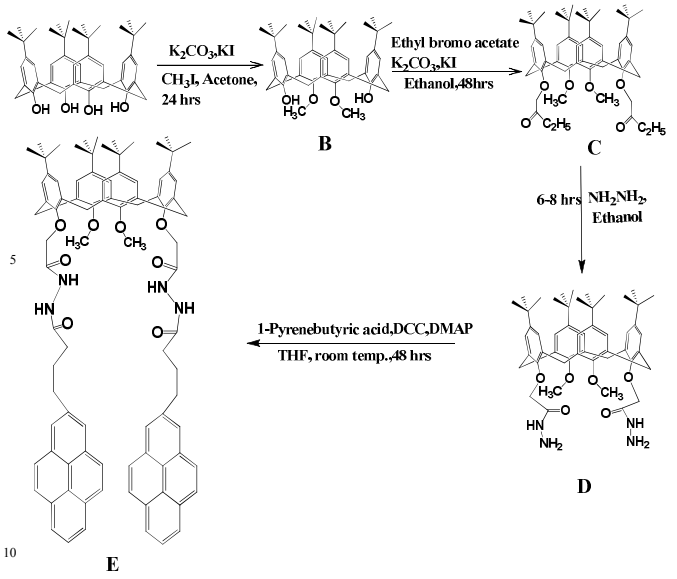
The development of synthetic receptors for cations and anions recognition has attracted considerable attention in recent years within the field of supramolecular chemistry due to the fact that a large number of biological processes involve molecular ion recognition[1]. Among all biologically important metals, iron is extensively distributed in nature and is one of the most vital elements in biological systems, which plays a fundamental role in numerous biochemical processes at the cellular level. Iron metabolism disorders have been reported to cause anaemia as well as liver and kidney damage (hemochromatosis) which might ultimately lead to liver cancer, liver cirrhosis, arthritis and diabetes or heart failure[2]. Phosphate ions and their derivatives are not only prominent for their central roles in signal transduction and energy storage in living systems but are also liable for the eutrophication of waterways[3]. Therefore, selective detection of Fe^{3+} and $H_2PO_4^-$ in biological fluids has become a significant objective and a number of methods have been developed for this purpose.

The leading issue in the design of any active chemosensor is the association of a selective molecular recognition event with a physical signal highly sensitive to its occurrence. Changes in both the absorption and emission of light can be employed as signals, provided appropriate chromophores or fluorophores are available, and two important classes of sensors are those of the optical and fluorimetric types. Fluorescence technique is commonly considered superior than other electrochemicals methods[4-11] because of its low detection limits for trace chemical detection, selectivity, fast response time and in situ monitoriochng ability. Designing of fluoroionophore is the most crucial aspect of this technique, having more number of aromatic fluorophore in the vicinity so that they will be closer enough to create van der Waals contact with π- π stacking. Under this condition, electronic excitation of one ring can cause an enhanced interaction with its neighbour, leading to what is termed as excited-state dimer or excimer for fluoroionophore .There have been so many reports on highly π delocalized planner systems such as pyrene, quinoline, coumarine, antharcene and dansyl chloride which have been used for this purpose[12].

The cyclooligomeric phenols known as calixarenes have acknowledged much interest as basic molecular scaffolds for the edifice of selective ionophores, many of which have been in corporated into fluorescent ion sensors. Calix[4] arene based chemosensors have enthralled a great deal of contemplation due to their capacity to visually sense analytes with high sensitivity as well as fast response time[13]. The mechanism of fluorescence involved in the calix[4]arene system is mainly PET[14], FRET[15], PCT[16] and ICT[17] for molecular recognition. Recenlty, we have reported an ICT based ‘‘turn on/off’’ quinoline armed calix[4]arene fluoroionophore and its sensing efficiency towards fluoride from waste water and Zn^{2+} and Cu^{2+} from blood serum[18,19] and a highly efficient PET[20] switch on-off-on fluorescence receptor based on calix[4]arene for selective recognition of Cd^{2+} and Sr^{2+}.These results prompted us to design novel ditopic fluroionophore linked with pyrene for selective detection of Fe^{3+} and $H_2PO_4^-$.

In continuation of our on-going research for the designing of calix[4]arene based fluorescent probe, herein we propose, a simple, sensitive and selective method for ferric and phosphate ions, with a low detection limit and fast response time by using synthesized 5,11,17,23 tetra-tert-butyl 26, 28 dimethoxy 25, 27 diamido pyrene 2-yl calix[4]arene (**TDPC**) receptor. This fluoroionophore has been applied for selective recognition of Fe^{3+} and $H_2PO_4^-$ in presence of other guest ions. For the first time, we have synthesized lower rim calix[4]arene diamidopyrene as fluoroionophore through modified method by using DCC reagent which was exclusively used to substitute calix[4]arene at lower rim, to provide rigidity as well as flexibility for the recognition of guest ions. There has been no report yet on TDPC which is used for ditopic recognition of Fe^{3+} and $H_2PO_4^-$

Spectroscopic properties of TDPC was examined in mixed aqueous organic medium (acetonitrile/aqueous phosphate buffer (6:4, v/v; pH=7.2). Pyrene is well established and extensively studied fluorophore for transition metal detection as well as anions[21]. The fluorescence spectra of the compound were recorded in acetonitrile in presence of 100-fold excess of various cations and anions which were prepared in acetonitrile. We evaluated the interaction of ligand with Fe^{3+} and $H_2PO_4^-$ in the presence of other cations and anions.



Scheme: 1 Synthetic route for 5, 11, 17, 23-tetra t-butyl-26, 28-dimethoxy 25, 27 diamido pyrene 2-yl (**TDPC**) calix[4]arene

To explore sensing ability of our fluoroionophore, we added 1× 10^{-6} M solutions of Fe^{3+} and $H_2PO_4^-$ into 1× 10^{-6} M solution to the fluoroionophore TDPC. We observed that Fe^{3+} and $H_2PO_4^-$ quenches the fluorescence intensity via electron transfer from free receptor to guest molecule. The sensitivity of our fluorescent probe is estimated by optimizing different concentrations of Fe^{3+} and $H_2PO_4^-$ from 0 nM (only TDPC 1× 10^{-9} M) to 100 nM with ligand and assessed quantitatively through the fluorescence emission intensity change. Before addition of Fe^{3+} and $H_2PO_4^-$, ligand has intensity of 23,000 at 470 nm wavelength. After gradual addition of Fe^{3+} and $H_2PO_4^-$ into the ligand, we observed significant decrease in the fluorescence intensity due to intramolecular charge transfer of ligand by interaction with guest ions. From these emission studies, we have calculated the limit of detection (LOD) for the synthesized probe and it was found to be 0.88 pM for Fe^{3+} and 1.11 pM for $H_2PO_4^-$ (Fig. 1A and B). Our experimental result evidently shows sensitivity and selectivity towards Fe^{3+} and $H_2PO_4^-$. To know about selectivity of our fluoroionophore in presence of other cations and anions, we have carried out emission titration in presence of other cations and anions which is displayed in **Fig.S1-S2 (ESI†).** The results indicated that other cations and anions did not produce noticeable effects on the emission spectra as compared to Fe^{3+} and $H_2PO_4^-$ **(Fig.1C and 1D).**

Stern-Volmer plots are worthwhile for appreciative the mechanism of emission quenching[22] and hence were utilized to probe the nature of the quenching process in the complexation of Fe^{3+} and $H_2PO_4^-$ with ligand TDPC. From the data, dynamic or static quenching processes can be determined by plotting relative emission intensities (I_0/I) against quencher concentration [Q]. Expressed by the following equation (1), the slope of the plotted line yields Ksv(the static quenching constant).

$$I_0/I = 1 + K_{sv}[Q] \text{ …….. (1)}$$

If the evolution of I_0/I plots, according to the concentration of quencher, is linear for the whole range of quencher concentrations, fluorescence quenching can be attributed either to being purely dynamic, or purely static, the latter mechanism being due to the formation of a ground-state non-fluorescent complex. In our case, typical linear plots for Fe^{3+} and $H_2PO_4^-$ with TDPC was observed which indicate that fluorescence quenching is purely static because of a non-fluorescence ground state complex between Fe^{3+} and $H_2PO_4^-$.

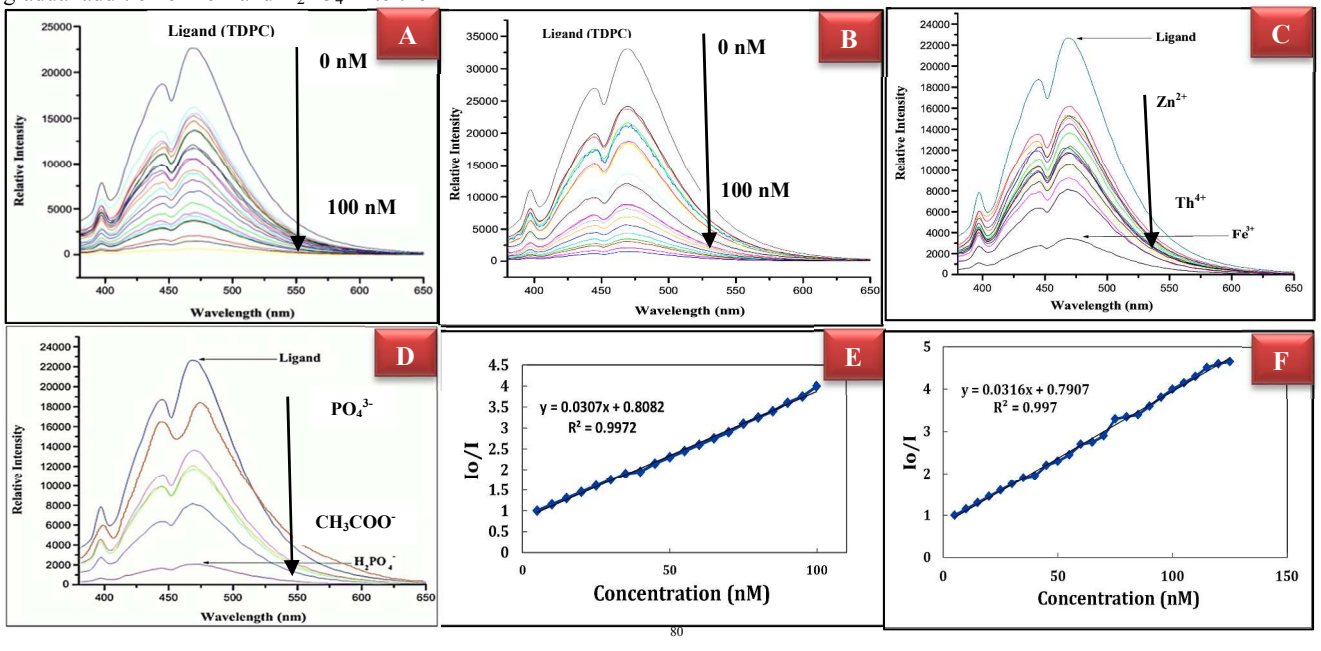


Fig.1. (A and B) Emission spectra of increasing concentration of Fe^{3+} (0, 2.5,5...100 nM) and $H_2PO_4^-$(0,2.5, 5, 10,..100 nM) with TDPC (1×10^{-8}M). **(C and D)** Selectivity plot of TDPC (1×10^{-6}M) with various cations from top to bottom (Zn^{2+}, Cd^{2+}, Fe^{2+}, Hg^{2+}, Na^+, K^+, Cu^{2+}, Ni^{2+}, Mn^{2+}, Cr^{3+}, Pb^{2+},Sr^{2+}, Ce^{3+},Co^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Cs^+, Rb^+, Ag^+, Be^{2+}, V^{5+}, Th^{4+} and Fe^{3+}) and anions from top to bottom (PO_4^{3-}, F^-, Cl^-, Br^-, I^-, CH_3COO^- and $H_2PO_4^-$) (1×10^{-6}M) in acetonitrile. **(E and F)** Stern- Volmer plot of TDPC (1×10-8M) with Fe^{3+} (0-100 nM) and for H_2PO_4- (0-100 nM) with TDPC. λex= 380 nm.

It may be because Fe^{3+} makes stronger electrostatic interaction with nitrogen atom of TDPC ligand and phosphate ion creates hydrogen bonding with amide linkage of ligand which leads to static quenching. The calibration curve shows good linearity with correlation coefficient of 0.9972 for Fe^{3+} and 0.9970 for $H_2PO_4^-$ **(Fig. 1E and F).**

Binding constants for fluoroionophore was determined by using emission titration data following the previously reported articles[23]. The titration experiment was carried out following the method described in the experimental section **(ESI †).** The plots log $[(F_0 - F)/ (F-F\infty)]$ vs. log[M] for selected compounds are shown in **Fig. S3–S4 (ESI†)**.The observed binding constant from the fluorescence spectra for Fe^{3+} is 9.73×10^8 and for $H_2PO_4^-$ 10.21×10^8.

To further support the selective sensing of Fe^{3+} and $H_2PO_4^-$ by TDPC, absorption titrations were accomplished. The absorption spectral investigation was achieved by the titration of Fe^{3+} and $H_2PO_4^-$ with TDPC in acetonitrile. With addition of 200 nM of Fe^{3+} and $H_2PO_4^-$, the absorbance at 379 nm increases dramatically and new bands at 418 nm for Fe^{3+} , 415 nm for $H_2PO_4^-$ upturn which show a bathochromic shift (λ_{max}) as shown in **Fig. S5 and S6 (ESI†)**, while other cations and anions exhibit no significant change in the absorption spectra. The changes observed in the case of Fe^{3+} and $H_2PO_4^-$ is attributable to its unique binding characteristic towards ligand with keto-enol tautomerization which is present in TDPC ligand. We also executed UV-titration by varying the concentration of Fe^{3+} (100 nM –200 nM) and $H_2PO_4^-$ (100 nM – 200 nM) to study absorption changes **(Fig. S7 and S8, ESI†)**.

A mass spectrum of TDPC was recorded in acetonitrile upon addition of an excess amount of selected metal Fe^{3+}. The formation of 1:1 complex was confirmed by ESI-MASS, where the spectrum illustrate molecular ion peak m/z of TDPC at 1361.0 and in the presence of Fe^{3+} shows peak at 1418.8 (**Fig.2.**) which will give assurance of binding of Fe^{3+} with ligand.

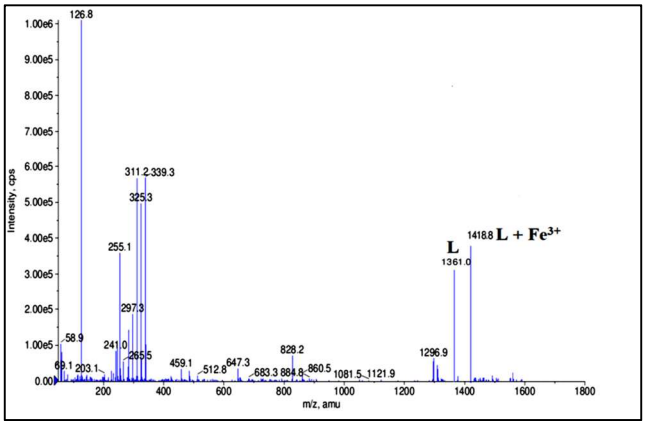


Fig.2. ESI mass spectrum showing the isotopic peak pattern of molecular ion peak for 1:1 complex formed between TDPC and Fe^{3+}

As some of the metal ions are paramagnetic in nature and also perchlorate salts contain water, the ^1H NMR investigation was performed with $H_2PO_4^-$ only. ^1H NMR investigation was performed to get insight into the interaction of $H_2PO_4^-$ with TDPC. For phosphate ion, due to hydrogen bonding with –NH group of amide linkage for TDPC ligand, the –NH peak at δ =8.18 ppm and 8.17 disappears by addition of 10 fold excess $H_2PO_4^-$ into TDPC ligand (**Fig.3**).This indicates interaction of $H_2PO_4^-$ with –NH protons via hydrogen bonding. We have also perceived that fluorescence sensor gives maximum quenching at pH 4-14, shown in **Fig.S9-S10 (ESI†)**. The stoichiometry of the complex formed (1: 1) has been also derived based on the Job's plot (**Fig.S11-S12, ESI†).** The proposed mechanism shows that TDPC recognizes Fe^{3+} and $H_2PO_4^-$ ions resulting in the changes of fluorescence intensity **(Fig.4)**.

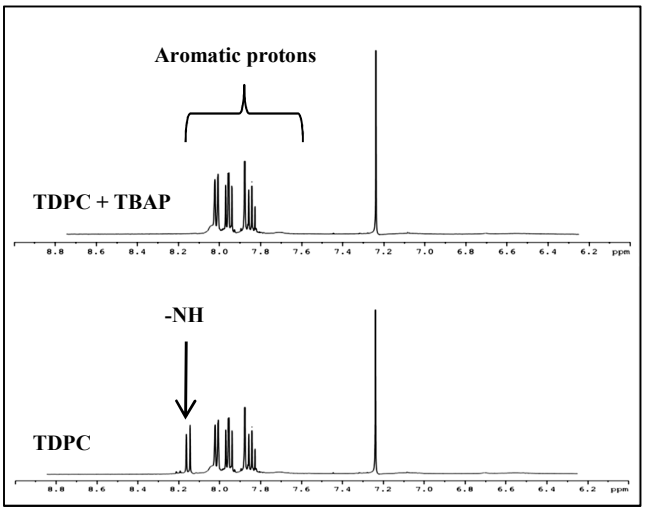


Fig.3. Selected portion of the ^1H NMR spectra for TDPC ligand and recorded in $CDCl_3$ upon addition of 10 equivalent amount of $H_2PO_4^-$ (TBAP).

To evaluate whether TDPC can be of any use in the recognition of guest ions by naked eye detection, TDPC was titrated with various metal ions (Zn^{2+}, Cd^{2+}, Fe^{2+}, Hg^{2+}, Na^+, K^+, Cu^{2+}, Ni^{2+}, Mn^{2+}, Cr^{3+}, Pb^{2+},Sr^{2+}, Ce^{3+},Co^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Cs^+, Rb^+, Ag^+, Be^{2+}, V^{5+}, Th^{4+} and Fe^{3+}) and anions (F^-, Cl^-, Br^-, I^-, CH_3COO^- and $H_2PO_4^-$) in acetonitrile by conserving a 1:1 mole concentration and spotting the corresponding colour changes. The colourless TDPC turned pale yellow on addition of Fe^{3+} and no significant colour change was observed by addition of other cations and anions. The sensing property of Fe^{3+} and $H_2PO_4^-$ by TDPC have been supported by observing the fluorescent change visually in the presence of different cations and anions under incident light of 365 nm. It was found that blue fluorescent of ligand was almost disappearing dramatically in the case of Fe^{3+} and $H_2PO_4^-$, as presented in **FigS13-S14(ESI†)**. Further, this study has been carried out in the presence of other ions added to initial solution possessing a 1:2 TDPC to Fe^{3+} ratio and found no changes in fluorescence **Fig.4.** This analysis is signifying that the ability of fluoroionophore for sensing Fe^{3+} in the presence of other competitive ions and this is supported by competitive emission titration of TDPC and Fe^{3+} in the presence of other cations which is shown in **Fig.S15 (ESI†)**. Proposed binding mechanism through hydrogen bonding and electro static interaction with TDPC ligand by Fe^{3+} and $H_2PO_4^-$ are recorded in **Fig.S16-S17(ESI†).**

This probe is applied for its biological applicability to sense Fe^{3+} in blood serum using fluorescence titration and also confirmed the stability of metal-complex in presence of blood

serum. The standard addition method was applied to evaluate the validity of the proposed sensor. The preparation of serum samples and analytical results for the blood samples are shown in **Table S1 (ESI†).** The result obtained with excellent recovery of spiked Fe^{3+} ranged from 102 to 118%, illustrating the validity of the developed technique. Furthermore, this fluoroionophore has been applied for phosphate ion detection from waste water and their results are shown in **Table S2 (ESI†).** The concentration of each sample was measured by this probe, using the calibration method. The proposed technique is compared with reported methods for determination of Fe^{3+} and $H_2PO_4^-$ (**Table S3, ESI†**).

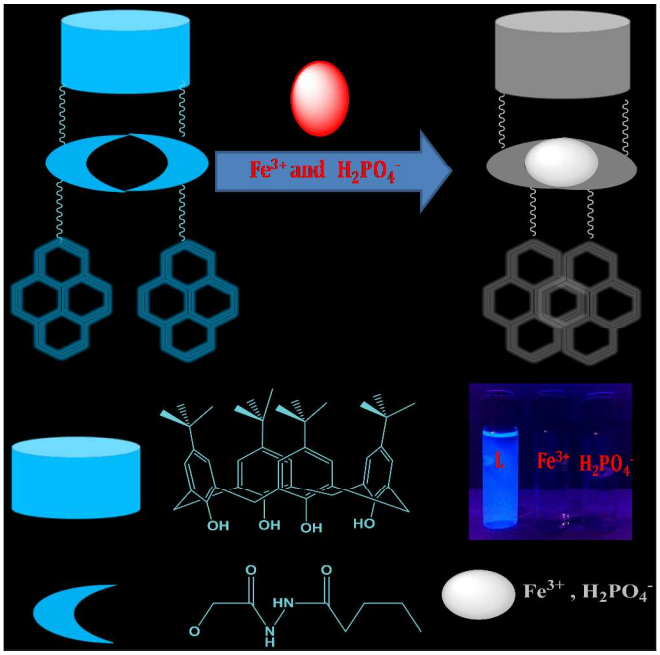


Fig.4. Schematic design of TDPC probe for Fe^{3+} and H_2PO_4- , via static quenching mechanism.

In conclusion, we have reported for the first time highly selective and sensitive fluorescence probe for Fe^{3+} and $H_2PO_4^-$. Proposed fluorescence probe has lower sensing limit as well as high selectivity towards Fe^{3+} (0.8 pM) and $H_2PO_4^-$ (1.11 pM). Furthermore, the present system has been applied for blood serum sample for selective detection of Fe^{3+} and $H_2PO_4^-$ with 102 to 118 % recovery. This highly sensitive, selective, easy and cost-effective fluorometric method will provide great interest for routine analysis of Fe^{3+} and $H_2PO_4^-$.

Acknowledgements

Financial assistance from UGC, New Delhi is gratefully acknowledged and CSMCRI for ^{1}H NMR as well as fluorescence study.

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† **Electronic Supplementary Information (ESI)** contains materails and metods, Synthesis procedure, UV-spectra, real sample analysis result table, selectivity plot by emission spectra,competative titration, Job's plot, FT-IR spectra, visual colour images under fluorescecnt light, binding mechanism of TDPC ligand with guest ions and pH study graph.

A pyrenyl linked calix[4]arene fluorescence probe for recognition of ferric and phosphate ions

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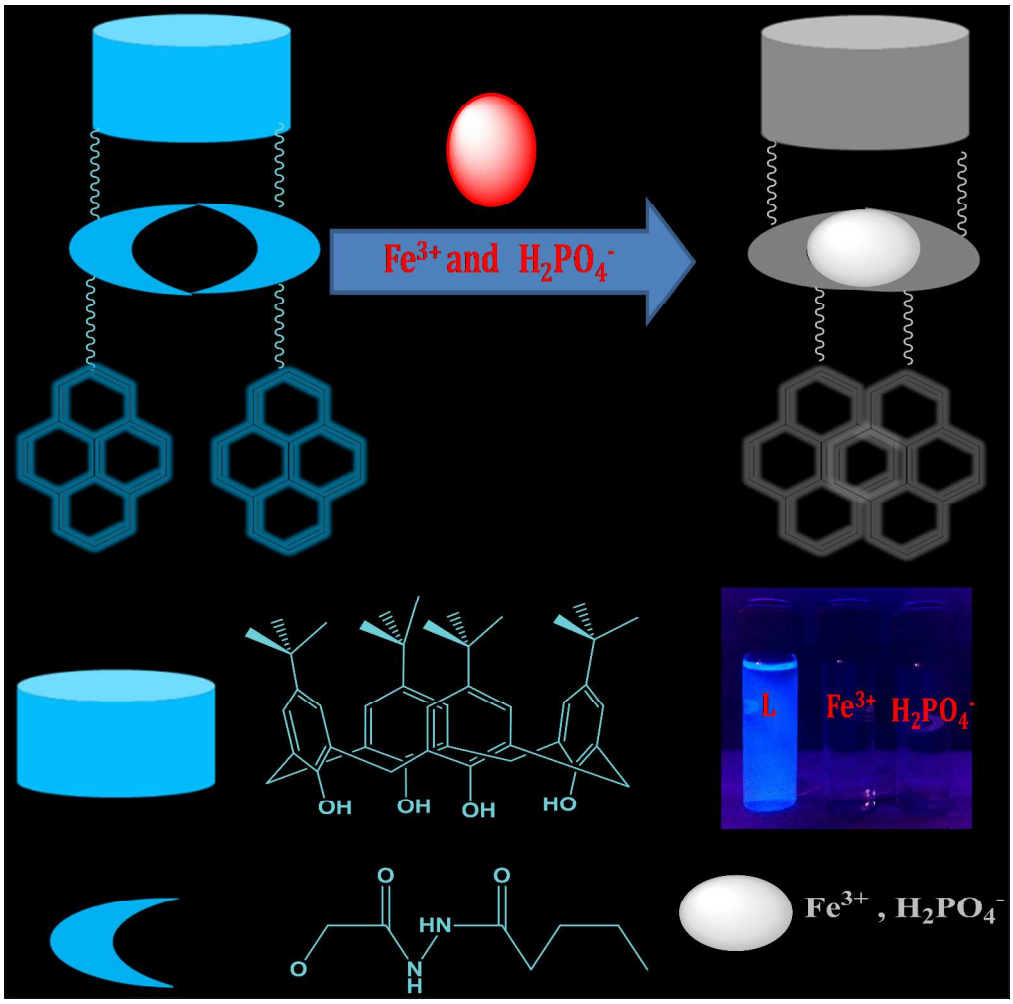
Graphical Abstract



Abstract:

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