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Graphic Abstract

The Ttitle:

A high selective and sensitive turn on fluorescent probe for detection of holmium ion and its bioimaging

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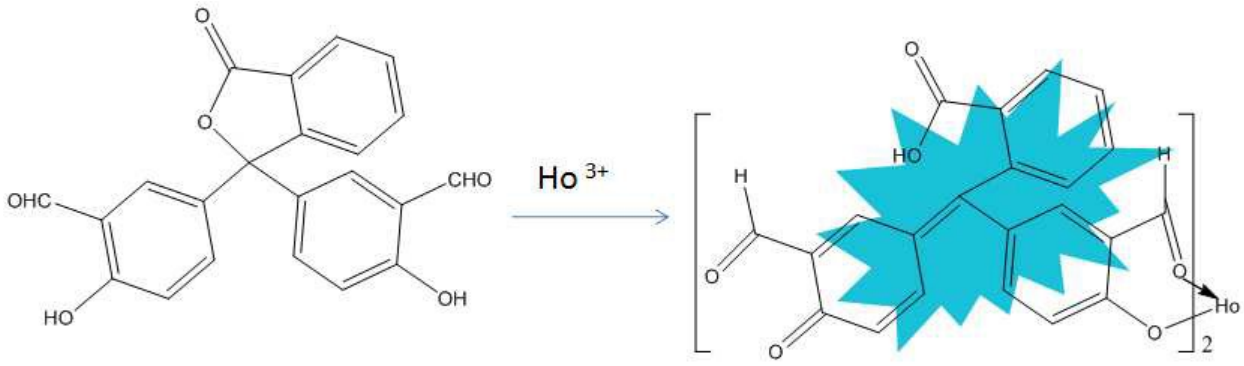
The statement:



The phenolphthalein aldehyde was synthesized and used to an turn-on fluorescent probe for the detection of holmium ion（Ho^{3+}), which is one of lanthanide ions in HEPES:DMF = 1:1 (V/V pH=7.4) solution with an excellent selectivity and sensitivity for Ho^{3+} over other metal ions.

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Communication

A high selective and sensitive turn on fluorescent probe for detection of holmium ion and its bioimaging

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The phenolphthalein aldehyde was synthesized and used to a turn on fluorescent probe for the detection of holmium ion (Ho^{3+}), which is one of lanthanide ions in HEPES:DMF = 1:1 (V/V pH=7.4) solution with an excellent selectivity and sensitivity for Ho^{3+} over other metal ions.

Rare earth (RE) is an important non-renewable resources. With the development of science, it was made various demands for materials. Application of rare earth elements, from their use of homogeneous property, extends to the use of single rare earth speciality, and has gone deep into all areas of modern technology. Because rare earth element (REE) has the special properties to promote crop production and improve crop quality, the area of application of compound fertilizer of rare earth element is more and more widely. Generally, low concentrations of REEs is present in soil, plant, water, and atmosphere, but REEs can accumulate in such environments following anthropogenic inputs because of the low mobility of these elements [1-3]. As the products containing rare earths continuously into the environment, long-term risks to plants, animals, the environment and human health has attracted widespread attention at home and abroad scientific community.

Holmium is one of lanthanide ions, however, it is 20 times more abundant than silver [4]. There is a growing trend in the uses of holmium element, due to the fact that it is studied to produce catalysts, polish glass or use for biological applications [5-7]. Lanthanides do not partake in any metabolic process. However, due to the similarity of lanthanides ionic radii to calcium ion, possessing a higher charge, they exhibit a high affinity for the Ca^{2+} sites on biological molecules and a stronger binding to water molecules [7-10]. The bioaccumulation of the holmium ions in the body can be a threat to the liver. Nowadays, it is known that holmium causes damages to the cell membranes of the water animals with negative effects on the reproduction and the nervous systems [7,8,10-12]. This makes the detections of Ho greatly important. Conventional methods of Ho ion analysis have included neutron activation analysis, ICP- MS, ICP-AES and isotope dilution mass spectrometry [13-18]. In recent years, it was reported a number of opcode and membrane sensor for selective determination of Ho^{3+} [4,10,18-25]. However, the fluorescent probe to detect Ho^{3+} has not been reported.

Herein, we report a novel fluorescent probe based on phenolphthalein aldehyde to selective detect Ho^{3+} in medium of near neutral pH value. And the recognition ability of probe for Ho^{3+} was studied. The result showed that this probe works well at physiological pH and has a high selectivity and sensitivity for Ho^{3+} over other metal ions. These desirable attributes render the sensor suitable for detection of Ho^{3+}. Furthermore, the system is used to bioimaging.

The synthesis of probe is summarized in Scheme 1. To a stirred TFA (10 mL) on ice was added phenolphthalein (636.64 mg, 2 mmol) and hexamethylenetetraamine (403.7 mg, 2.88 mmol). The solution was allowed to warm to room temperature, and then it was refluxed for 8h. The excess TFA was removed under vacaum. 30 mL water was then added to the remaining solution, and the mixture was warmed at 60□ whilst stirring for another 30 min. Upon cooling on ice, a pale-yellow solid were precipitated out from the solution which were collected by filtration. Then the crude product was purified by chromatography on a silica gel column, and give the desired product as a white solid (57 % yield). ^{1}H NMR (300 MHz, 25℃, DMSO -d_6): δ 11.04 (bs, 2H), 10.22 (bs, 2H), 7.94 (d, 1H, J = 7.2 Hz), 7.84 (m, 2H, J = 20.7 Hz), 7.68 (q, 1H, J = 14.4Hz), 7.51 (s, 2H), 7.43 (d, 2H, J = 8.7Hz), 7.02 (d, 2H, J = 8.7Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 189.9, 168.1, 160.3, 150.5, 134.6, 134.2, 130.7, 129.4, 125.7, 125.1, 123.6, 121.1, 117.5, 89.3; ESI-MS $[M-H]^-$ 373.25; Elemental analysis (calcd %) for $C_{22}H_{14}O_6$: C, 70.59; H, 3.77; Found: C, 70.38; H, 3.89. (Fig. S1, ESI†)

The recognition ability of the probe was investigated by the fluorescence spectra. The selectivity towards the metal ions is one of the most important criteria for probe design. The effect of a wide range of environmentally and physiologically active metal ions was investigated for probe using the fluorescence spectra of solutions containing probe and the metal ions (100 equiv.) in the HEPES:DMF = 1:1 (V/V pH=7.4) solution. As shown in Fig. 1, whereas metal ions such as Cu^{2+}, Cr^{2+}, Gd^{3+}, Ni^{2+}, Co^{2+}, VO^{2+}, La^{3+}, Pb^{2+}, Zr^{4+}, Mn^{2+}, Zn^{2+}, Ru^{3+}, Yb^{3+}, Cd^{2}, Sm^{3+}, Bi^{3+}, Ce^{4+}, Er^{3+}, Ca^{2+}, Mg^{2+}, Sn^{2+}, Fe^{3+}, Dy^{3+}, Pr^{3+}, Tb^{3+}, Pm^{3+}, Lu^{3+}, Tm^{3+}, Nd^{3+} and Eu^{3+} do not result in any apparent changes in fluorescence intensity, there is notable change when Ho^{3+} is involved (λ_{ex}= 365 nm) (Fig. S2, ESI†). Fig. 1 shows that fluorescence optical density at 480 nm when various metal ions are added and fluorescence color change.

The probe could specifically recognize Ho^{3+} was explained as following: peripheral electron shell configuration of Ho^{3+} is $4f^{10}$, which results in the abundant level structures, as well as some high metastable state. The nice isolation between energy levels prompts Ho^{3+} having abundant electron transition[26]. Its peculiar energy level structure inducing the bigger gap between ^{3}k8, ^{5}s2 and adjacent low level makes non-radiative relaxation be not easy to happen. What's more, absorption peak of Ho^{3+} is very rich, which resulted from two way: one is low energy photon is easy to excite Ho^{3+} ions, another is other rare earth with sensitized effect realizes inversion of high level particle number to obtain the strong blue-green glow[27, 28]. Green glow is corresponding to the energy level transition of ^{5}S2 (^{5}F4)—^{5}I8 of Ho^{3+} spectral term. The fluorescence of Dy^{3+} is easy to be quenched by high-energy

vibration of O –H bond in water molecules [29].

A titration experiment of the probe for Ho^{3+} was also carried out on fluorescence spectrophotometer. The probe displayed no emission at 480 nm. With addition and gradual increasing amount of Ho^{3+} to the HEPES/DMF = 1:1 (V/V, pH=7.4) solution containing probe, a significant increase in fluorescence intensity at 480 nm was observed with an excitation wavelength 365 nm. When 260 μM Ho^{3+} was added to the solution of the probe (20 μM), a more than 25-fold increase in fluorescence intensity at 526 nm was observed (Fig. 2) with a change of fluorescence quantum yield from φ_{probe} = 0.001 to $\varphi_{probe\text{-}Ho^{3+}}$ = 0.256. In addition, with increasing Cu^{2+} concentration, the absorption peaks at 330 gradually decreased and 380 nm increased. One isosbestic points was noted at 348 nm indicating the formation of a new species (Fig. S3, ESI†). According to linear Benesie Hildebrand expression, the measured intensity $[1/(A - A_0)]$ at 380 nm varied as a function of$1/[Ho^{3+}]$ in a linear relationship (R = 0.99797) (Fig. S4, ESI†), indicates formation of 1:1 stoichiometry between $Ho^{3+}$and probe. The association constant of probe with $Ho^{3+}$was calculated to be 1.08×10^7 M^{-1}.

The effect of pH on the fluorescence properties of the system was investigated for pH values of 2.0–13.0. The results are shown in Fig. 3. Over the pH 2.0–8.0, the free probe exhibits no fluorescence in the HEPES/DMF = 1:1 (V/V, pH=7.4) solution. It is obvious that the fluorescent signal of the probe increases significantly with increasing pH from 8.0 to 12.0. However, the intensity of the probe exhibits a non-ignorable decrease at pH 13.0. Meanwhile, the fluorescence signal for the probe solution containing Ho^{3+} increased sharply from pH 4.0 to 8.0, and then decreased from pH 8.0 to 11.0. The fluorescence intensity of the probe solution containing Ho^{3+} has a little increases at pH 12.0 compared with at pH 11.0. Holmium exsits in the form of $Ho(OH)_3$ precipitate due to the strong basicity of pH 13.0 which result in the fluorescence decrease of probe with addition of Ho^{3+}. Therefore, considering pH and fluorescence intensity, a pH of 7.4 was selected for the analytical system.

The effect of reaction time on the fluorescence emission of the system was also studied in the presence of 10 equiv. of Ho^{3+} and the results are shown in Fig. 4. The fluorescence signal for the system increases gradually with increasing reaction time, and the addition of Ho^{3+}, under the selected reaction conditions, was rapid. Therefore, a 4 min reaction time was selected for the following experiments.

To investigate the detection limit of probe for Ho^{3+}, probe (20 μM) was treated with various concentrations of Ho^{3+} (0–260 μM) and the relative emission intensities at 480 nm were plotted as a function of the Ho^{3+} concentration (Fig. S5, ESI†). The fluorescence intensity of probe is linearly proportional to the Ho^{3+} concentrations, and the detection limit, based on the definition by IUPAC (C_{DL}= 3 Sb/m) [30], was found to be 0.055 μM indicating this probe is sensitive enough to monitor Ho^{3+} compared with other method (Table 1)[18,19,23,25].

The reaction mechanism of the present system was also studied. We presumed that the fluorescence increase could be attributed to ring-open after the coordination of probe with Ho^{3+}. Namely, because of the probe with hydroxyl and aldehyde groups (their O atoms as hard bases), easily coordinating with Ln ions as hard acids and resulting in the ring open, increased the conjugate structure of intramolecular, which lead to the system became strong fluorescence. The identification of coordination product in the ESI-MS analysis made it possible to propose the signaling mechanism: a peak at m/z = 984.00, corresponding to $[probe\text{-}Ho +4H_2O+H]^+$, is clearly observed (Fig. S6, ESI†). The proposed detection mechanism and the structures of the probes, both with and without the addition of Ho^{3+}, are shown in Scheme 2.

The ability of probe reacting with Ho^{3+} within living cells was also evaluated by laser confocal fluorescence imaging using a Leica TCS SP5 laser scanning microscope. The optical window at the yellow channel (450–550 nm) was chosen as a signal output. As shown in Fig. 5a, HepG2 cells incubated with 20 μM probe for 30 min at 37 °C and washed 3 times with PBS showed no fluorescence. In a further experiment it was found that HepG2 cells displayed green fluorescence when the cells were first incubated with 20 μM of probe for 30 min at 37 ℃ and washed 3 times with PBS, then incubated with 100 μM $HoCl_3$ and washed 3 times with PBS (Fig. 5b). These cell experiments show the good cell-membrane permeability of probe, and it can thus be used to mark Ho^{3+} within living cells.

In summary, we have developed a fluorescent probe for the detection of Ho^{3+} by turn-on fluorescence over other analytes in aqueous solution. This probe is based on a phenolphthalein derivative which has a high selectivity and sensitivity for Ho^{3+} over other analytes in HEPES: DMF = 1:1 (V/V pH=7.4). Furthermore, the system is used to bioimaging.

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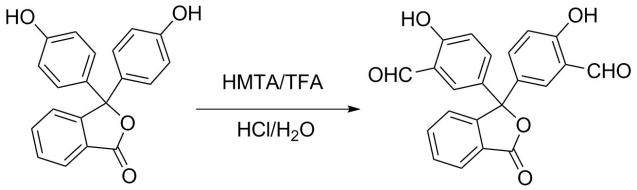
Inserting Graphics



Scheme 1 Synthesis of probe..

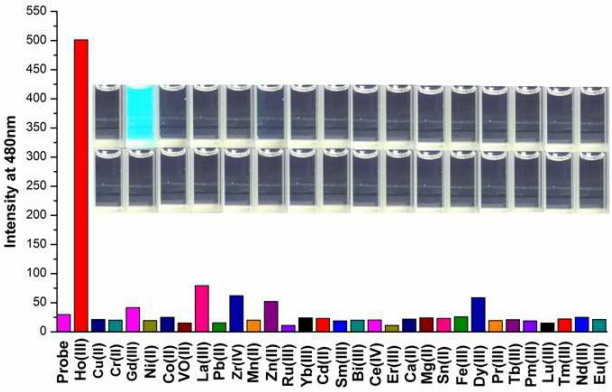


Fig. 1. Optical density two-dimensional graph of the probe at 480 nm, respectively upon the addition of several metal ions (top: from left to right, probe, Ho^{3+}, Cu^{2+}, Cr^{2+}, Gd^{3+}, Ni^{2+}, Co^{2+}, VO^{2+}, La^{3+}, Pb^{2+}, Zr^{4+}, Mn^{2+}, Zn^{2+}, Ru^{3+}, Yb^{3+}, Cd^{2+}; bottom: from right to left, Sm^{3+}, Bi^{3+}, Ce^{4+}, Er^{3+},

Ca^{2+}, Mg^{2+}, Sn^{2+}, Fe^{3+}, Dy^{3+}, Pr^{3+}, Tb^{3+}, Pm^{3+}, Lu^{3+}, Tm^{3+}, Nd^{3+} and Eu^{3+}); Inset: a color change photograph for Ho^{3+} and the other metal ions under illumination with a 365 nm UV lamp.

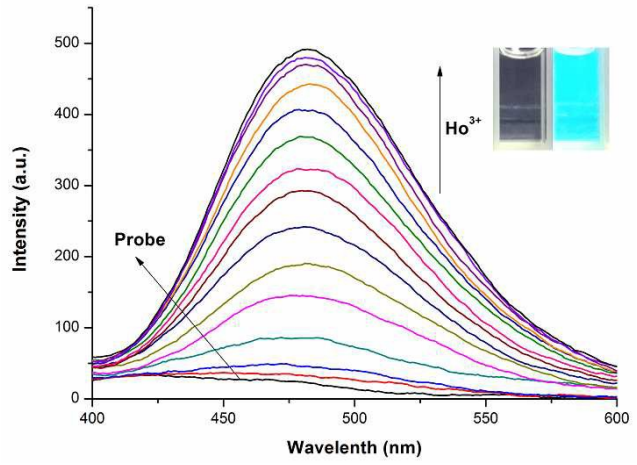


Fig. 2. Fluorescence spectral changes of probe (20 μM) in 10 mmol/L HEPES buffer /DMF (v/v=1:1, pH 7.4) (λ_{ex}= 365 nm, slit: 5 nm/5 nm) upon addition of Ho^{3+}; Ho^{3+} was added gradually with [Ho^{3+}] = 0–260 μM; Inset: a color change photograph for Ho^{3+} under illumination with a 365 nm UV lamp.

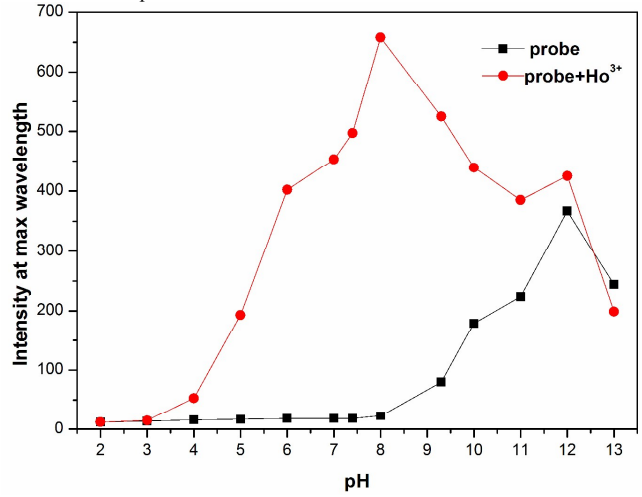


Fig. 3. Effect of pH on the fluorescence intensity of the probe and probe+Ho^{3+} (260 μM.)

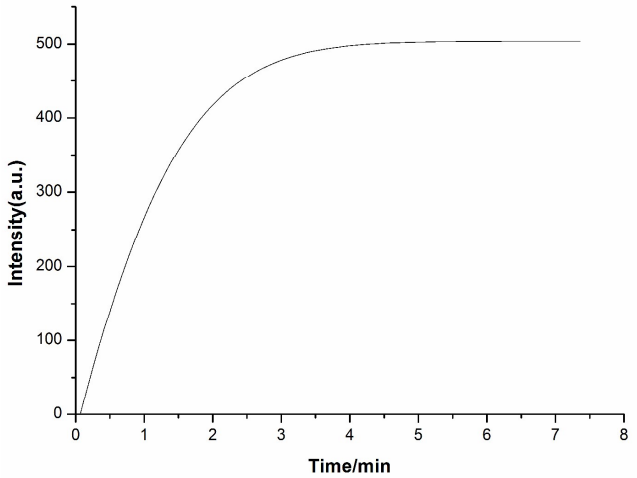
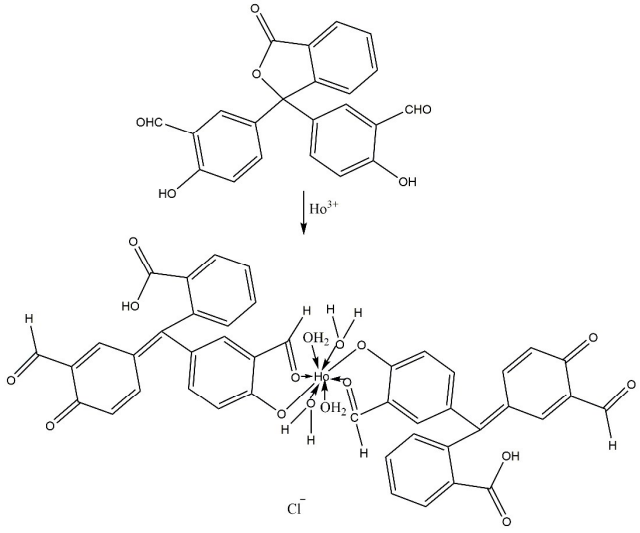


Fig.4. Reaction time profiles of probe (20 μM) and Ho^{3+} (260 μM)

Table 1. A comparison table about the detection methods for Ho^{3+}.

	method	analyte	detection limit
ref (18)	membrane sensor	Ho^{3+}	0.75μM
ref (19)	Potentiometric Membrane Sensor	Ho^{3+}	0.63μM
ref (23)	polymeric membrane sensor	Ho^{3+}	5μM
Ref (25)	membrane sensor	Ho^{3+}	0.62μM
this work	Fluorescent probe	Ho^{3+}	0.055μM



Scheme 2. The mechanism of chemosensor.

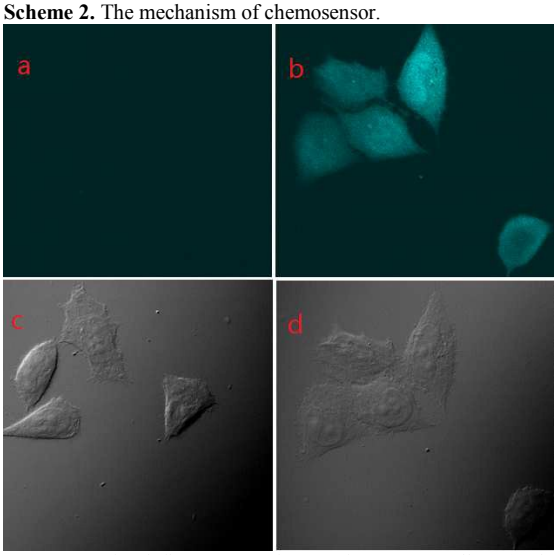


Fig. 5. Confocal fluorescence images in HepG2 cells. (a) Fluorescence image of HepG2 cells with adding probe (20 μM) and its bright field image (c); (b) Fluorescence image of HepG2 cells incubated with 20 μM probe for 30 min at 37 °C, then incubated with 100 μM $HoCl_3$ for 30 min at 37 °C and its bright field image (d)

Notes and references

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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