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ARTICLE

A Turn-on Fluorescent Chemodosimeter Based on Detelluration for Detecting Ferrous iron (Fe^{2+}) in Living Cells†

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A turn-on fluorescent probe for the detection of Fe^{2+} is facilely synthesized via a nucleophile substitution reaction. The fluorescent probe, N-butyl-4-phenyltellanyl-1, 8-naphthalimide (Naph-Te), shows excellent selectivity to Fe^{2+} in a mixed solution of acetonitrile and phosphate buffer under aerobic condition. The coexistence of biological abundant metal ions such as Na^+, K^+, Ca^{2+} and Mg^{2+} has little effect on the fluorescence signal. This turn-on response is achieved via a redox-involved reaction triggered by Fe^{2+} with neutral pH at room temperature, which removes the heavy-atom effect of the tellurium atom on the naphthalimide fluorophore to afford a fluorescent product (N-butyl-4- hydroxyl -1, 8-naphthalimide). The probe has excellent cell membrane permeability and is further applied successfully to monitor supplementary Fe^{2+} in live HL-7702 cells by a laser confocal fluorescence microscope.

Introduction

As the most abundant transition metal nutrient for humans, iron is of fundamental importance to a variety of physiological events.[1] The biological versatility of iron is partly attributed to its convertible redox states in vivo, where iron mainly exists in two ionic forms, i.e. ferric iron (Fe^{3+}) and ferrous iron (Fe^{2+}).[2] In particular, many perplexing diseases are attributed to the metabolically active form of cellular iron associated with the labile iron pool (LIP), which represents only a minor fraction (<5%) of the total cell iron (50 – 100μM). [3] Since the reduced form Fe^{2+} prevails in LIP under the reducing conditions inside cells,[3b] the development of analytical techniques for detecting Fe^{2+} in living samples is crucial to decipher the complicated mechanisms of iron on human health and diseases. Optical imaging with highly selective fluorescent probes is an effective and convenient methodology that allows the temporal and spatial monitoring of exchangeable metal pools in living biological specimens.[4] So far, while various fluorescent probes for detecting Fe^{3+} have been devised successfully,[5] there are still many challenges and opportunities to develop probes for Fe^{2+} for biological application. [6]

Fe^{2+} usually serves as a fluorescence quencher due to its paramagnetic nature and encounters many biological metal ions which act as competitors when binding with common ligands, therefore, it is very difficult to devise highly selective probes for Fe^{2+} via a traditional chelation strategy, especially turn-on probes which are much more efficient and reliable than turn-off ones.[5,7] Generally, for detecting metal ions with unoccupied d orbitals, a reaction-based strategy offers good selectivity against competing metal ions, as well as a way to circumvent the fluorescence quenching phenomenon caused by the paramagnetical target.[4a] Very recently, two outstanding fluorescent probes (RhoNox-1 and IP1) were reported, both utilizing reaction-based strategies that convert non-emissive chemodosimeter molecules to the emissive ones via irreversible redox-involved reactions promoted by Fe^{2+}.[8]

Herein, we report a highly selective fluorescent chemodosimeter for detecting Fe^{2+} based on a detelluration reaction in a mixed solution of acetonitrile and phosphate buffer saline under aerobic condition. The fluorescent probe, N-butyl-4-phenyltellanyl-1, 8-naphthalimide (Naph-Te), is non-emissive in aqueous solution due to the heavy-atom effect[9] of the tellurium atom on the naphthalimide fluorophore. After the detelluration reaction triggered by Fe^{2+}, a strong fluorescence emitting product (N-butyl-4- hydroxyl -1, 8-naphthalimide) is released. The reaction can be finished within half an hour at room temperature with neutral pH. The practicality of Naph-Te has been further demonstrated through the imaging of supplementary Fe^{2+} in live HL-7702 cells.

Experimental

Materials and chemicals

The metal ions were aqueous solutions of NaCl, KCl, $CaCl_2$, $MgCl_2{\cdot}6H_2O$, $ZnSO_4{\cdot}7H_2O$, $Pb(NO_3)_2$, $MnCl_2$, $Hg(NO_3)_2{\cdot}H_2O$, $CuSO_4{\cdot}5H_2O$, $CoCl_2{\cdot}2H_2O$, $NiCl_2{\cdot}6H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$, $CrCl_3{\cdot}6H_2O$,

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† Electronic Supplementary Information (ESI) available: UV/Vis absorption and phosphorescence spectra of the probe Naph-Te and other additional data, ^1H NMR, ^{13}C NMR, and MS. See DOI: 10.1039/x0xx00000x

$FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. Cu^+ was from $[Cu(CH_3CN)_4]PF_4$ dissolved in acetonitrile. Fe^{2+} was from the aqueous solution of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ unless otherwise noted. $ONOO^-$ was obtained from nitrosation of H_2O_2 by isoamyl nitrite.[10] The concentrations of $ONOO^-$ and NaClO were ascertained by absorbance spectra according to literatures ($\varepsilon_{NaClO,292\ nm}$ = 350 $M^{-1}cm^{-1}$ and $\varepsilon_{ONOO^-,302\ nm}$ = 1670 $M^{-1}cm^{-1}$).[11,12] ·OH was produced by the Fenton reaction between Co^{2+} and H_2O_2.[13] Superoxide anion (O_2^-) was delivered by adding solid KO_2. NO was generated from sodium nitroprusside (SNP). $^1O_2$ was generated by the reaction between NaClO and H_2O_2. Phosphate buffer saline (PBS) was aqueous solution of 10 mM phosphate, 0.2 g L^{-1} KCl, 8 g L^{-1} NaCl. Ultrapure water (18.2 MΩ· cm) from a Millipore water purification system and HPLC acetonitrile were used in all experiments. Other solvents and chemicals were purchased from commercial sources and of analytical reagent grade.

Human liver (HL-7702) cells were purchased from the Committee on type Culture Collection of Chinese Academy of Sciences. HL-7702 cells were cultured in RPMI 1640 medium containing 12% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere containing 5% CO_2. Prior to imaging, cells were subcultured by seeding on 35 mm ×12 mm glass bottom cell culture dishes at 37 °C for 24 hours.

Instrumentation

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were taken on a Bruker 400 MHz or 500 MHz NMR spectrometer with TMS in the solvents indicated as an internal reference at ambient temperatures. Multiplicities of signals are described as follows: s --- singlet, dd --- double doublet, d --- doublet, t---triplet, m---multiplet. Mass spectra were obtained from an Agilent 6130B MSD spectrometer. All pH measurements were made with an Ohaus pH meter STARTER2100. Steady-state UV/Vis spectrum was measured on a Lambda 35 UV-visible Spectrophotometer (Perkin-Elmer) with matched 1 cm quartz cuvettes. Emission spectra were obtained from a Horiba Jobin Yvon FluoroMax-4 spectrofluorometer with 1 cm quartz cells and the excitation and emission slit widths were modified to adjust the intensity to a suitable range. Florescent images were taken by a FV1000 confocal laser-scanning microscope (Olympus) with a ×40 or 100 objective lens.

Synthesis of 4-bromo-N-butyl-1, 8-naphthalimide

This compound was synthesized by referring to a reported method.[14] A mixture of 4-bromo-1,8-naphthalic anhydride (5.0 g) and *n*-butylamine (3 mL) in ethanol (90 mL) was refluxed for 6 h. Then the mixture was filtered to obtain a wine-red solution. After evaporation, the crude product was purified by column chromatography (silica gel, ethyl acetate: petroleum ether = 1: 50) to get a pale yellow solid product (5.2 g, 86.8 %). ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 8.65 (dd, *J* = 1.0 Hz, 7.0 Hz, 1 H), 8.56 (dd, *J* = 1.0 Hz, 8.5 Hz, 1 H), 8.41 (d, *J* = 7.5 Hz, 1 H), 8.04 (d, *J* = 8.0 Hz, 2 H), 7.86-7.83 (m, 1 H), 4.17 (t, J = 7.5 Hz, 2 H), 1.75-1.69 (m, 2 H), 1.49-1.41 (m, 2 H), 0.98 (t, J = 7.3 Hz, 3 H).

Synthesis of N-butyl-4-phenyltellanyl-1, 8-naphthalimide (Naph-Te)

To a 50 mL three-neck flask flushed with nitrogen, diphenyl ditelluride (1.02 g, 2.5 mmol) and ethanol (21 mL) were added. The suspension was cooled to 0 °C, then Sodium borohydride (0.21 g, 5.5 mmol) was slowly added. After the red color faded, the reaction mixture was heated to reflux. Then, a mixture of cuprous iodide (0.41 g, 2.2 mmol) and 4-bromo-N-butyl-1,8-naphthalimide (0.59 g, 1.8 mmol) was added. The mixture was stirred and refluxed for 20 min in a nitrogen atmosphere. After cooling to room temperature, the black mixture was filtered to remove insoluble material. Then, the black solution was vaporized on a rotary evaporator. The residue was washed and filtered again. After evaporation, the residue was purified by column chromatography (silica gel, ethyl acetate: petroleum ether = 1 : 250) to give a yellow solid product (0.72 g, 88 %).^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.63 (d, *J* = 7.2 Hz, 1 H), 8.33 (d, *J* = 8.4 Hz, 1 H), 8.25 (d, *J* = 7.6 Hz, 1 H), 7.88-7.73 (m, 4 H), 7.42 (t, *J* = 7.4 Hz, 1 H), 7.32 (t, *J* = 7.4 Hz, 2 H), 4.16 (t, *J* = 7.4 Hz, 2 H), 1.74-1.67 (m, 2 H), 1.49-1.39 (m, 2 H), 0.97 (t, J = 7.4 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 13.84, 20.39, 30.20, 40.26, 113.00, 122.27, 123.44, 126.88, 127.24, 128.09, 129.16, 129.58, 130.15, 130.66, 131.11, 131.49, 133.72, 135.83, 135.96, 139.71, 163.68, 164.03. MS (API - ES): m/z, 460.13 (calcd for $C_{22}H_{19}NO_2Te$ 460.05).

Synthesis of 4-hydroxy-N-butyl-1, 8-naphthalimide

This compound was synthesized by referring to a reported method.[14] The crude product was purified by column chromatography (silica gel, ethyl acetate: petroleum ether = 1 : 20) to give a pale green solid product (0.10 g, 62 %).^1H NMR (500 MHz, d^6-DMSO) δ (ppm) 11.84 (s, 1 H), 8.50 (dd, *J* = 1.0 Hz, 8.5 Hz, 1 H), 8.43 (dd, *J* = 1.0 Hz, 7.0 Hz, 1 H), 8.33 (d, *J* = 8.0 Hz, 1 H), 7.73 (t, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1 H), 4.01 (t, *J* = 7.5 Hz, 2 H), 1.63-1.57 (m, 2 H), 1.39-1.32 (m, 2 H), 0.94 (t, *J* = 7.5 Hz, 3 H). ^{13}C NMR (126 MHz, d^6-DMSO) δ (ppm): 13.63, 19.76, 29.69, 39.02, 109.85, 112.56, 121.71, 122.29, 125.41, 128.73, 129.07, 130.95, 133.37, 160.13, 162.89, 163.56. MS (API-ES): m/z, Calcd for $C_{16}H_{15}NO_3$: 269.11, found: $[M-H]^-$ 268.16.

Fluorescence Measurement Procedures

Typically, to a 10 mL calibrated test tube was added phosphate buffer saline (PBS, 10 mM, 5 mL, pH 7.0) and acetonitrile solution of probe Naph-Te (30 μM, 1 mL) to get a probe solution (5.0 μM); then certain amounts of Fe^{2+} (10 mM) or other testing species were added by a 100 μL pipette gun (Eppendorf) and mixed thoroughly at room temperature. After 60 min, 3.5 mL of the reaction solution was added to a quartz cell (1 cm ×1 cm ×4 cm) and sampled for fluorescence measurements with an excitation wavelength (λ_{ex}) of 445 nm and an emission wavelength (λ_{em}) of 550 nm.

Determination of Quantum Yield

The quantum yields were determined using quinine sulfate (Φ = 0.55, 0.05 M H_2SO_4) as a reference. [15] Samples were prepared with the absorption at 390 nm less than 0.05. The UV/Vis absorption spectra and the emission spectra were recorded. Then, the quantum yields were calculated according to the following Eq.

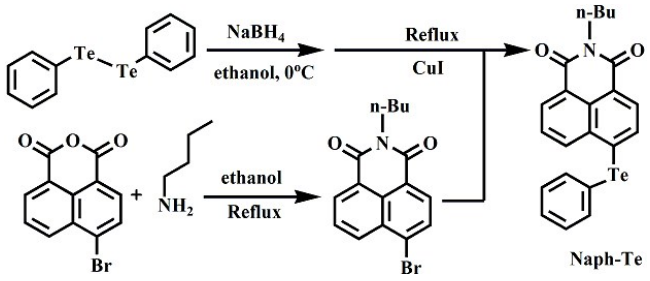
$$\Phi_s = \frac{\Phi_r \mathbf{A}_r \mathbf{F}_s}{\mathbf{A}_s \mathbf{F}_r}$$

The subscripts s and r refer to the sample and the reference, respectively. In this work, F is the integrated fluorescence intensity from 395 nm to 700 nm and A is the absorbance at 390 nm.

Results and discussion

Design of the Fluorescent Probe

In view of the limitations of traditional chelation strategy, we tried to explore a Fe^{2+}-triggered reaction under mild conditions. During the last two decades, a lot of metal–promoted desulfurisation or deselenation based chemodosimeters for heavy metal ions have been devised;[5d] we then wondered whether a dechalcogenation reaction could be used for detecting heavy metal ions such as Fe^{2+}. Considering the C-Te bond is weaker than the C-Se or C-S bond and should be fragile to break, we designed and synthesized a candidate organotellurium (Naph-Te) to test the above hypothesis. Importantly, to get an "off" state, the tellurium atom was directly connected to the 4-position of the naphthalimide platform where the heavy-atom effect could be fully utilized to quench the fluorescence. To be of considerable practical application, probes should be easily accessible by simple synthetic routes. Naph-Te was facilely synthesized via a nucleophile substitution reaction in the presence of iodide copper as the catalyst to afford a yield of 88% (Scheme 1), and was fully characterized by ^{1}H NMR, ^{13}C NMR and MS analysis.



Scheme 1 Synthesis Route of Naph-Te.

Probe Performance

Firstly, the absorption and emission properties of Naph-Te were evaluated. In acetonitrile, Naph-Te showed a broad, featureless absorption band in the visible region (ε = ca. 8000 $M^{-1}cm^{-1}$ at 412 nm) and a corresponding phosphorescence band centered at 618nm (Fig. S1, S2, ESI†). It is very interesting that the probe emitted room temperature phosphorescence (RTP); this might originated from the direct metalation of an organic chromophore.[16] RTP of Naph-Te also occurred in dichloromethane, however, in the air-equilibrated solution, the phosphorescence was quenched seriously by oxygen. In protonic solvents such as methanol, ethanol or the mixed solution of acetonitrile/PBS, no emission band appeared even though the solutions were deaired. Thus, an "off" state is achieved successfully in solutions under aerobic condition.

The fluorescence response of Naph-Te towards Fe^{2+} was investigated at room temperature in the mixed solution of acetonitrile/PBS. As shown in Fig. 1, the addition of Fe^{2+} provoked an intensive fluorescence increase at 550 nm, switching the system from an "off" state to an "on" state. The fluorescence increase was not saturated even when $Fe(OH)_2$ was precipitated. The increase of fluorescence intensity was displayed in a Fe^{2+}-concentration dependent manner, and there was a linear relationship between the fluorescence intensity of 5 μM Naph-Te and the concentration of Fe^{2+} (0 ~ 25 μM) with a high correlation effector 0.9980 (Fig. S3). The detection limit is calculated to be 0.15 μM from $3\sigma/k$, where σ is the standard deviation of the blank solutions and k is the slope of the calibration curve.[17]

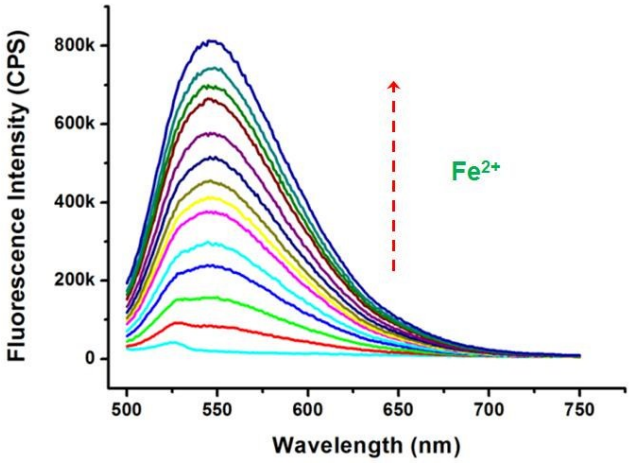


Fig. 1 Fluorescence response of Naph-Te (5 μM) to Fe^{2+}. The fluorescence spectra were measured with λ_{ex} = 445 nm, after incubating the solutions with various amounts of Fe^{2+} (0, 5 μM, 10 μM, 15 μM, 20 μM, 25 μM, 30 μM, 35 μM, 40 μM, 45 μM, 55 μM, 65 μM, 75 μM) for 60 min in acetonitrile/PBS (1/5), pH 7.0.

In contrast, other metal ions including Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+},Cr^{3+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}, Cu^{+} incurred no discernible intensity changes (Fig. 2). Importantly, Naph-Te could reliably discriminate Fe^{2+} from Fe^{3+}, enabling selective detection of Fe^{2+} in LIP of cells. These observations indicate that the probe Naph-Te can specifically recognize Fe^{2+} with high selectivity.

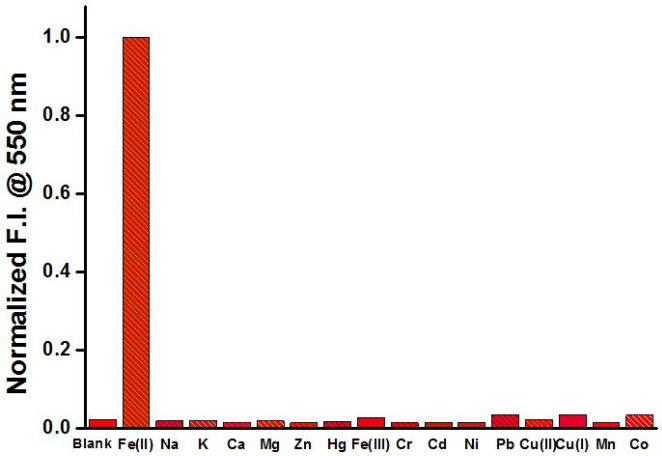


Fig. 2 Fluorescence intensity of Naph-Te (5 μM) after the addition of various metal ions. For Na^{+} , K^{+}, 1mM; for Ca^{2+}, Mg^{2+}, 0.5 mM; for Fe^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+}, Cu^{+}, Mn^{2+}, and Co^{2+}, 50 μM. Bars represent the normalized fluorescence intensity at 550 nm, λ_{ex} = 445 nm. Data were collected after incubating the solutions with various analytes for 60 min in acetonitrile/PBS (1/5), pH 7.0

An interference test was taken out to further demonstrate the utility of Naph-Te in real samples. The results show that the coexistence of biological abundant metal ions such as Na^+, K^+, Ca^{2+} and Mg^{2+} had little effect on the fluorescence response in the mixed solutions (Fig. 3a). Although Cu^{2+}, Cu^+, Mn^{2+}, Cr^{3+}or Co^{2+} could inhibit the signal to some extent, the interference of these ions in the cellular system is likely to be negligible due to their low concentrations.[18] Moreover, the fluorescence intensity of Naph-Te itself at 550 nm kept almost unchanged between pH 5.5-9.0; in the presence of Fe^{2+}, fluorescence augmentation was observed under various pH values (Fig. 3b), therefore, the interference of pH to the probe should be little in cytoplasm. Hence it is expected that the probe will work well under physiological conditions.

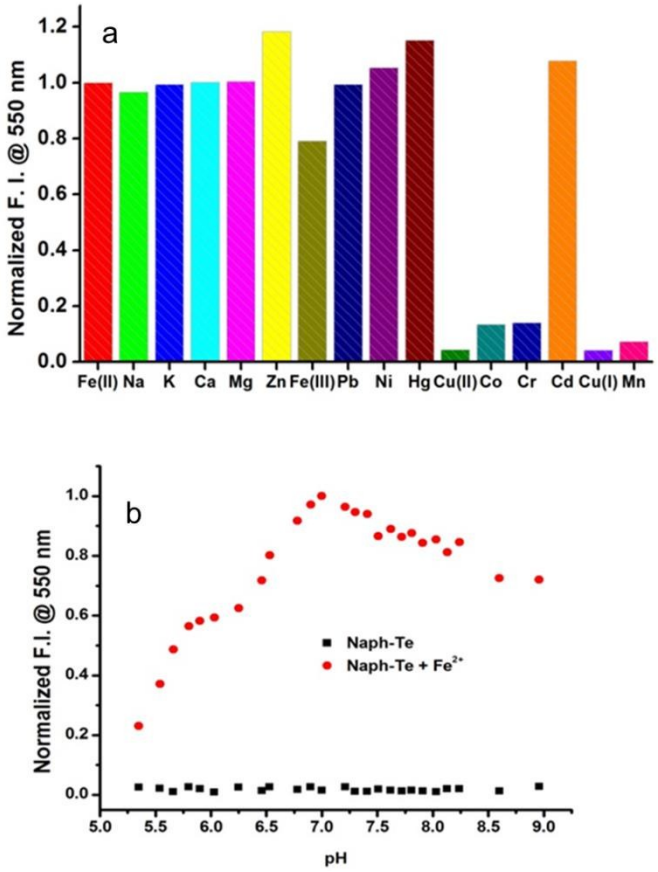


Fig. 3 (a) Fluorescence intensity of Naph-Te (5 μM) after the addition of Fe^{2+} in the presence of other metal ions. For Na^+ , K^+, 1mM; for Ca^{2+}, Mg^{2+}, 0.5 mM; for Fe^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+}, Cu^+, Mn^{2+}, and Co^{2+}, 50 μM. Data were collected after incubating the solutions with various analytes for 60 min in acetonitrile/PBS (1/5), pH 7.0 with λ_{ex} = 445 nm. (b) The effect of pH on the fluorescence intensity of Naph-Te (3 μM) and the reaction mixture (+ $FeCl_2$, 36 μM) in phosphate buffer (acetonitrile/H_2O = 1/5) at room temperature.

Kinetic experiments revealed that the fluorescence signal of the probe was stable to the medium, air, and light during the experiment, and the reaction between Fe^{2+} and the probe was finished within half an hour; however, without the presence of oxygen, Fe^{2+} was unable to turn on the emission (Fig. 4). It is noted that several chemodosimeters for metal ions use oxygen as a secondary selectivity filter,[4a, 8b, 19] so we believe that the demand of oxygen to promote this reaction may be the reason for the high selectivity of our probe.

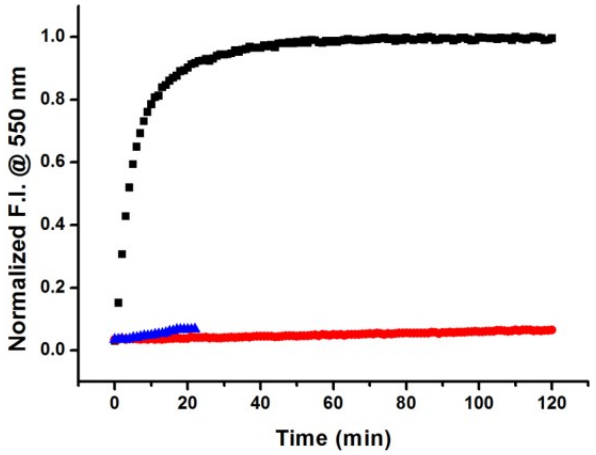


Fig. 4 Kinetic traces of fluorescence intensities at 550 nm. ▼ 5 μM Naph-Te under aerobic condition; ■ 5 μM Naph-Te + 50 μM Fe^{2+} under aerobic condition; ▲ 5 μM Naph-Te previously deoxygenated by nitrogen bubbling for 30 min + 50 μM Fe^{2+}. Data were acquired in acetonitrile/PBS (1/5) at pH 7.0 at room temperature with λ_{ex}= 445 nm.

As the above reaction relied on the existence of oxygen, reactive oxygen species (ROS) such as O_2^-, H_2O_2, $^1O_2$, ClO^-, $ONOO^-$, NO, and ·OH were also examined as potential interferers in biological systems (Fig. 5). The result indicates that the signal intensities of these ROS treated solutions were negligible compared with the Fe^{2+}-induced fluorescence increase. It is reported that organotellurium is able to detect ROS based on its redox nature.[20] In this case, the distinct response could be explained by the difference between the reaction mechanisms.

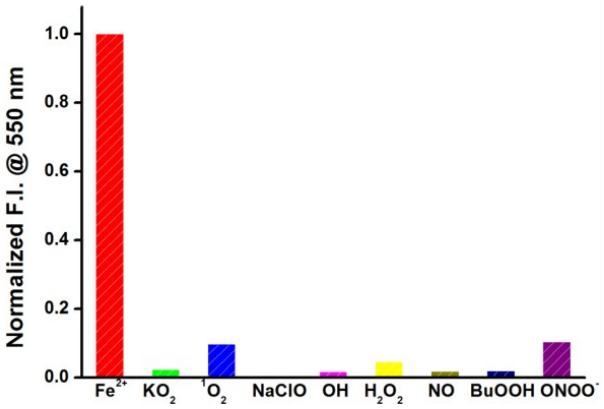
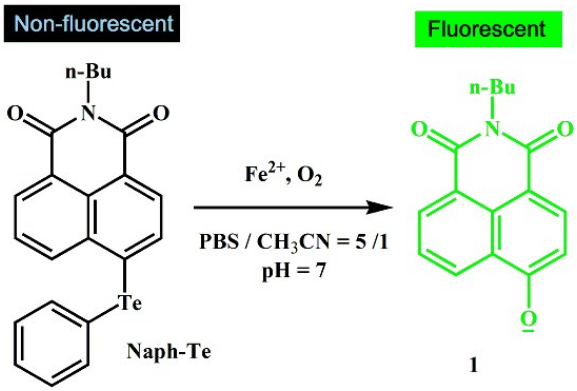


Fig. 5 Fluorescence intensity of Naph-Te (5 μM) after the addition of Fe^{2+} (50 μM) or various ROS (50 μM). Bars represent the normalized fluorescence intensity (F. I.) at 550 nm, λ_{ex} = 445 nm. Data were collected after incubating the solutions with various analytes for 60 min in acetonitrile/PBS (1/5), pH 7.0.

Fluorescence Signaling Mechanism

We confirmed that a detelluration reaction was responsible for the turn-on response. The fluorescent product of this reaction is 4-hydroxy-N-butyl-1, 8-naphthalimide, existing as the deprotonated anion (compound 1**)** at neutral pH.[21] The production of 1 was suggested by ESI-MS analysis of the reaction mixture with a corresponding peak at m/z 268.16 (Fig. S5, ESI†). We also synthesized compound 1 in an independent route to investigate its fluorescence characters under the same experimental conditions. It was noticed that both the emission and excitation fluorescence spectra of the reaction solution were essentially superimposed upon those of the authentic 1 (Fig. S6, ESI†). This result ensures the existence of 1 in its native state from the reaction, as no separation of the sample was carried out. The fluorescence quantum yield of 1 in PBS (10 mM, pH 7.0) was determined to be Φ = 0.053, which is much higher than that of the probe (Φ <0.001). It was also found that acetonitrile, which is usually used as a co-solvent to dissolve fluorescent probes when staining biological samples, [20b, 22] played an unexpected yet essential role in this reaction. Addition of Fe^{2+} to Naph-Te in PBS (pH 7.0) containing dimethyl sulphoxide, methanol, ethanol or N, N-dimethylformamide (V/V, 5/1) gave almost no emission increase. [19b] However, at this stage the detelluration mechanism still remains elusive and we are currently working on the details of this redox-involved reaction. The plausible Fe^{2+}-triggered fluorescence signaling mechanism is dipicted in Scheme 2.



Scheme 2 The fluorescence signaling mechanism.

Imaging of Supplementary Fe^{2+} in HL-7702 Cells

Having established the ability of detecting Fe^{2+} *in vitro*, the probe was applied to visualizing supplementary Fe^{2+} in normal human liver cells (HL-7702) by laser scanning confocal microscope (LSCM). The HL-77022 cell line was chosen as a model because liver is one of the major sites of iron storage (about one-third).[2b] Exposure to non-transferrin iron is a common way for elevating the level of LIP in mammalian cells and ferrous ammonium sulfate (FAS) was used as the source of Fe^{2+} to elevate the level of LIP in HL-7702 cells.[8] As expected, the image of the controlled cells (without supplemental Fe^{2+}) was almost fluorescent faint; whereas cells treated with 80 μM Fe^{2+} gave obviously brighter fluorescence than both the controlled and 20 μM Fe^{2+}-treated ones (Fig. 6, *P< 0.001). Moreover, the viability of the cells throughout the imaging studies was confirmed by bright field images and it is clearly shown that the probe was mainly localized outside the nucleus under a ×100 objective lens (Fig. 6d). In addition, MTT assay showed that 5 μM of Naph-Te had almost no toxicity to HL-7702 cells, with the survival rate of the cells incubated with our probe for 24 hours similar to that of the controls (Fig. S8). These results demonstrated that the chemodosimeter Naph-Te has excellent cell membrane permeability and is suitable for imaging supplementary Fe^{2+} in live cells.

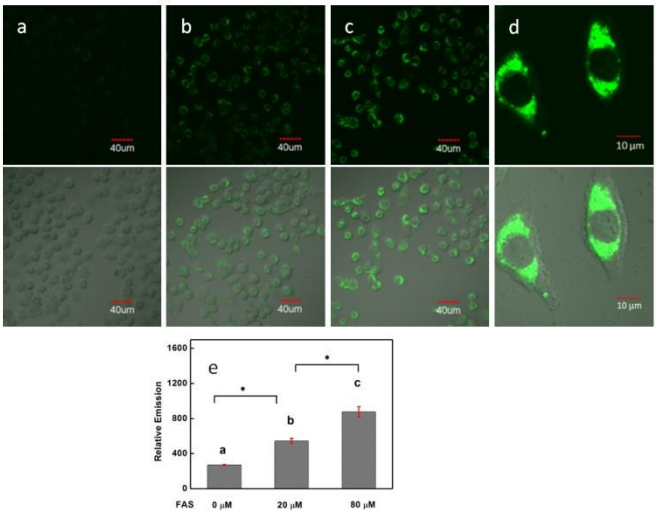


Fig. 6 Fluorescent staining of Naph-Te in HL-7702 cells in the presence and absence of supplementary Fe^{2+}. The upper panel: fluorescence images; the lower panel: overlays of the fluorescence and bright field images. (a) Vehicle controlled cells, 0 μM Fe^{2+}; (b) 20 μM Fe^{2+}-treated cells; (c) 80 μM Fe^{2+}-treated cells. After the incubation with FAS in the serum-free growth media for 60 min at 20 °C, adherent HL-7702 cells were rinsed three times with PBS (pH 7.2) to remove the extracellular Fe^{2+}; then, the cells were stained for 30 min with 5 μM Naph-Te (diluted by PBS from the stock solution of 0.55 mM Naph-Te in acetonitrile) and imaged under a ×40 objective lens. (d) 100 μM Fe^{2+}-treated cells under a ×100 objective lens to monitor the subcellular localization of the probe. (e) Statistical analysis of (a), (b) and (c) was performed with an unpaired Student's *t*-test (n =25 fields of cells). *p < 0.001, error bars are ± sem. Fluorescence was collected at 500 ~ 600 nm with λ_{ex} = 405 nm.

Conclusion

In summary, based on a redox-involved detelluration reaction under aerobic condition, we have developed a turn-on fluorescent probe (Naph-Te) for Fe^{2+} by virtue of the removal of the heavy-atom effect. Naph-Te is non-emissive in aqueous solution, but gives dramatically increased fluorescence in the presentence of Fe^{2+}. It features excellent selectivity for Fe^{2+} over biologically relevant metal ions. The probe has excellent cell membrane permeability and is successfully applied to monitoring supplemental Fe^{2+} in HL-

7702 cells. Further investigations on the details of this reaction are in progress.

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