A diarylethene-based “on–off–on” fluorescence sensor for the sequential recognition of mercury and cysteine†

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A novel photochromic diarylethene with a quinoline unit was synthesized with multi-controllable fluorescence switching properties, which could be induced by light, mercury (Hg\(^{2+}\)) and cysteine (Cys). Because the diarylethene displayed obvious fluorescence quenching with Hg\(^{2+}\) and the fluorescence was recovered to its original state evidently with Cys, it could sequentially recognize Hg\(^{2+}\) and Cys. Additionally, a logic circuit was constructed with the fluorescence intensity at 468 nm as output, and the combined stimuli of light and chemicals as inputs.

Introduction

As a hazardous chemical in the environment from a variety of natural and anthropogenic sources,\(^1,2\) the mercury ion (Hg\(^{2+}\)) is a caustic and carcinogenic material with high cellular toxicity\(^3\) because it accumulates in the human body and a very small amount can cause serious diseases, such as cardiovascular disease, serious cognitive and motion disorders, Minamata disease and coronary heart disease.\(^4–9\) Therefore, monitoring Hg\(^{2+}\) in the environment has received increasing interest. Currently, various techniques and protocols, such as atomic absorption spectrometry, inductively coupled plasma, atomic emission spectroscopy, and capillary electrophoresis have been well developed and utilized to monitor Hg\(^{2+}\).\(^10–11\) However, most of these methods are still limited by complex instruments and complicated procedures with high cost, or low sensitivity and low selectivity. However, fluorescent sensors are very simple and sensitive in the detection of metal ions or harmful pollutants in the environment. Therefore, considerable efforts have been made to develop fluorescent molecular chemosensors for Hg\(^{2+}\).\(^12–22\)

As a small molecular weight biothiol, cysteine (Cys) plays a prominent role in various critical biological systems, such as metabolic processes, biocatalysis and detoxifications of xenobiotics.\(^23–27\) However, Hg\(^{2+}\) can bond to Cys through Hg–S to cause many health problems,\(^28–30\) such as slowed growth in children, liver damage, loss of muscle and fat, skin lesions and weakness.\(^31\) On other hand, high levels of Cys in living systems also cause many human diseases, including developmental retardation, cardiovascular, osteoporosis, Alzheimer’s disease, etc.\(^32\) Therefore, it is very important to detect Cys and Hg\(^{2+}\) in biological systems. Up to present, detection of Hg\(^{2+}\) and Cys was mainly based on photochromic materials with dual functionalities.\(^33–36\) Among these various photochromic compounds, diarylenes are one of the most promising photo-switchable molecules with excellent photochemical reactivity, thermal stability, fatigue resistance\(^37–40\) and fluorescence.\(^41–43\) For example, Tian and his co-workers have reported two photochromic diarylethene-based chemosensors with excellent optical properties to distinguish Hg\(^{2+}\) in acetonitrile.\(^44\) We also synthesized a photochromic diarylethene with a formyl group. Because of the interaction between the formyl group of its ring-closed isomer with Cys, there was a notable change in its absorption spectrum with an evident color change from blue to pale yellow if Cys was added.\(^45\) However, all of these reports were focused on recognizing Hg\(^{2+}\) and Cys individually.

In this study, we designed and synthesized a new photochromic diarylethene with a quinoline unit, which could sequentially recognize Hg\(^{2+}\) and Cys efficiently. Compared with previously reported results, this method was more feasible, economic, convenient and significant. The fluorescence of the diarylethene was quenched in the presence of Hg\(^{2+}\) in tetrahydrofuran. When a certain amount of Cys was subsequently added, the fluorescence was enhanced and restored to almost the intensity before quenching.

Experimental

General methods

NMR spectra were recorded on Bruker AV400 (400 MHz) spectrometer with CDCl\(_3\) as the solvent and tetramethylsilane (TMS) as the internal standard. Mass spectra were measured on a Bruker AnaZon SL Ion Trap Mass spectrometer. IR spectra were recorded on a Bruker Vertex-70 spectrometer. Melting
point was measured on a WRS-1B melting point apparatus. The absorption spectra were measured on an Agilent 8453 UV/Vis spectrophotometer. Photoirradiation was carried out with an SHG-200 UV lamp, CX-21 ultraviolet fluorescence analysis cabinet and a BMH-250 visible lamp. Light of appropriate wavelength was isolated through different light filters. Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer. The fluorescence quantum yield was measured on an Absolute PL Quantum Yield Spectrometer QY C11347-11. All inorganic salts (HgClO₄),·6H₂O, Cd(NO₃)₂·4H₂O, Zn(NO₃)₂·6H₂O, Mg(NO₃)₂·6H₂O, Ca(NO₃)₂·4H₂O, Ba(NO₃)₂, Cr(NO₃)₃·9H₂O, Al(NO₃)₃·9H₂O, Ni(NO₃)₂·6H₂O, MnCl₂·4H₂O, Co(NO₃)₂·6H₂O, Cu(NO₃)₂·3H₂O, Sr(NO₃)₂, Pb(NO₃)₂, Fe(NO₃)₃·9H₂O and KCl) and amino-acids (cysteine (Cys), glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), phenylalanine (Phe), proline (Pro), tryptophan (Try), serine (Ser), tyrosine (Tyr), glutamine (Glu), threonine (Thr), and histidine (His)) were purchased and used without further purification. Metal ions and amino-acids were dissolved (0.1 mol L⁻¹) and purified through distillation prior to use.

Synthesis of 1O

The synthetic route of 1-(2-methyl-3-benzothiophenyl)-2-{2-methyl-5-(4-phenyl)-8-(2-benzoylquinolines-8-benzothiazole)-3-thienyl} perfluorocyclopentene (1O) was shown in Fig. 1. The intermediate 2 was synthesized according to literature.⁴⁶

To a compound 2 (0.58 g, 1.00 mmol) solution in anhydrous acetonitrile (30 mL), 8-hydroxyquinoline-2-carboxaldehyde (0.16 g, 0.90 mmol) and K₂CO₃ (0.33 g, 2.00 mmol) were added with continuously stirring. After 6 hours of refluxing, the mixture was cooled to room temperature and concentrated under vacuum. The crude product was purified through column chromatography on silica gel with petroleum ether/ethyl acetate (10:1) as the eluent to afford compound 3 as a pale pink solid. The yield was 70%. Mp 340–342 °C. ¹H NMR (CDCl₃, 400 MHz), δ (ppm) 1.93 (s, 3H), 2.30 (s, 3H), 5.47 (s, 2H), 7.15 (t, 2H, J = 8.0 Hz), 7.30–7.36 (m, 2H), 7.44–7.49 (m, 3H), 7.52–7.57 (m, 4H), 7.73 (d, 1H, J = 8.0 Hz), 8.08 (d, 1H, J = 8.4 Hz), 8.29 (d, 1H, J = 7.8 Hz), 10.33 (s, 1H).

Synthesis of 1-(2-methyl-3-benzothiophenyl)-2-{2-methyl-5-phenyl-4-(2-formylquinoline-8-benzothiazole)-3-thienyl} perfluorocyclopentene (1O).

Compound 3 (0.34 g, 0.5 mmol) and 2-aminothiophenol (0.06 g, 0.5 mmol) were dissolved in 5 mL of ethanol. After the reaction mixture was refluxed for 6 hours, a light purple powder crude product appeared. Then, the reaction mixture was cooled to room temperature, washed with cold ethanol and dried in air. The yield was (0.31 g) 80%. Mp 438–440 °C. ¹H NMR (CDCl₃, 400 MHz), δ (ppm) 1.93 (s, 3H), 2.31 (s, 3H), 5.46 (s, 2H), 7.13–7.21 (m, 1H), 7.24 (s, 1H), 7.30–7.40 (m, 3H), 7.47–7.51 (m, 2H), 7.53–7.59 (m, 4H), 7.67 (d, 2H, J = 8.0 Hz), 7.75 (d, 1H, J = 7.7 Hz), 8.02 (d, 1H, J = 7.7 Hz), 8.14 (d, 1H, J = 8.1 Hz), 8.30 (d, 1H, J = 8.5 Hz), 8.53 (d, 1H, J = 8.6 Hz). ¹³C NMR (CDCl₃, 100 MHz), δ (ppm) 14.82, 14.89, 71.06, 112.08, 118.73, 120.35, 120.51, 122.06, 122.09, 122.13, 122.18, 122.57, 123.77, 124.51, 124.95, 125.19, 125.58, 125.73, 125.84, 126.27, 127.70, 127.92, 130.36, 132.78, 136.60, 136.97, 137.04, 138.22, 140.45, 141.58, 141.69, 142.49, 150.21, 154.46. ¹⁹F NMR (DMSO, 376 MHz), δ (ppm) −130.15 (1F), −128.96 (1F), −108.29 (1F), −106.88 (1F), −106.36 (2F).

Results and discussion

Photochromism and fluorescence of 1O

The photochromic and fluorescent behaviors of 1O were studied in THF solution (2.0 × 10⁻⁵ mol L⁻¹) at room temperature, its absorption spectral, fluorescence spectral and color changes were induced by alternating irradiation with 297 nm UV light and visible light (λ > 500 nm) (Fig. 2). As shown in Fig. 2A, the absorption maximum of 1O was at 298 nm due to its π-π* transition.⁴⁷ Upon the irradiation with 297 nm UV light, a new absorption band centered at 548 nm emerged due to the formation of the closed-ring isomer 1C of 1O with larger π-electron delocalization in the molecule, which was accompanied with a color change from colorless to purple. After 5 minutes of irradiation with 297 nm UV light, the photocyclization reaction reached a photostationary state (PSS), and the photoconversion ratio from the open-ring isomer to closed-ring isomer 1C was 84% based on the HPLC analysis. (Fig. S3†) The cyclization and cycloreversion quantum yield of the diarylethene were 0.34 and 0.027, respectively, with 1,2-bis[2-methyl-5-phenyl-3-thienyl]-perfluorocyclopentene as the reference.⁴⁸ Reversely, the absorption spectrum of 1C with purple solution could be completely bleached to the initial state of 1O upon the visible light irradiation (λ > 500 nm). Fig. 2B showed the emission spectral changes of 1O upon

![Fig. 1](image1.png) Synthetic route for 1O.

![Fig. 2](image2.png) Changes in the absorption and fluorescence of 1O upon alternating irradiation with UV and visible light in THF: (A) absorption spectra change (2.0 × 10⁻⁵ mol L⁻¹); (B) fluorescence change (2.0 × 10⁻⁵ mol L⁻¹), excited at 354 nm.

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photoirradiation when excited at 354 nm. Upon irradiation with 297 nm UV light, the emission peak of 1O was decreased significantly due to the formation of non-fluorescent closed-ring isomer 1C, which was accompanied by an obvious fluorescence intensity change from light blue to dark. In the photostationary state, the emission intensity of 1O was quenched to ca. 97%, and the fluorescence quantum yield of 1O to 1C was determined to be 0.459 to 0.008. Similarly, the fluorescence intensity of 1 could also be recovered with the irradiation of appropriate visible light (λ > 500 nm).

**Fluorescent turn-off detection of Hg\(^{2+}\)**

The fluorescence response of 1O toward 16 different metal ions (Hg\(^{2+}\), Cd\(^{2+}\), Zn\(^{2+}\), Mg\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Cr\(^{3+}\), Al\(^{3+}\), Ni\(^{2+}\), Mn\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\), Sr\(^{2+}\), Pb\(^{2+}\), Fe\(^{3+}\), K\(^{+}\)) was measured in THF (2.0 × 10\(^{-5}\) mol L\(^{-1}\)) at room temperature. As shown in Fig. 3A, diarylethene 1O displayed a strong fluorescence with an emission of 468 nm. The addition of 5.0 equiv. metal ion (Cd\(^{2+}\), Zn\(^{2+}\), Mg\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Cr\(^{3+}\), Al\(^{3+}\), Ni\(^{2+}\), Mn\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\), Sr\(^{2+}\), Pb\(^{2+}\) or K\(^{+}\)) to the solution, 1O showed no significant changes in its fluorescence, while the same amount of Fe\(^{3+}\) caused the fluorescence intensity to decrease by almost 30%. The addition of Hg\(^{2+}\) into 1O resulted in the complete quench of its fluorescence intensity. The Fig. 3B shows the fluorescence color changes of 1O when the various metal ions added. These results indicated that 1O could easily discriminate Hg\(^{2+}\) from other metal ions.

As shown in Fig. 4, when the 1O was titrated with Hg\(^{2+}\), the fluorescence intensity was significantly decreased until 3.2 equiv. of Hg\(^{2+}\) were added. With further addition of Hg\(^{2+}\), the fluorescence intensity was then decreased slowly. When 5.0 equiv. of Hg\(^{2+}\) were added, the fluorescence intensity of 1O was almost quenched completely. The quantum yields of 1O (the complex of 1O added the Hg\(^{2+}\)) from 0.459 to 0.11 were determined. Meanwhile, based on the linear Benesi–Hildebrand expression and the Stern–Volmer plot, linear relationships between 1O fluorescence at 468 nm and 1O[Hg\(^{2+}\)] were obtained. The association constant of Hg\(^{2+}\) binding to diarylethene 1O was found as 1.11 × 10\(^4\) L mol\(^{-1}\) (R = 0.99858). The limit of 1O as a fluorescent sensor to detect Hg\(^{2+}\) was 4.76 × 10\(^{-4}\) mol L\(^{-1}\), which was determined from a plot of intensity as a function of the concentration of Hg\(^{2+}\) (Fig. S4†).

Comparative, Job’s plots and the ESI-MS experiments were conducted to elucidate the binding mode of 1O with Hg\(^{2+}\). The naphthol-benzothiazole moiety (NBTZ), quinolinol-benzoxazole moiety (QBOZ) and quinolinol-benzothiazole (QBTZ) moiety were synthesized to the comparative experiments and all the \(^1\)H NMR, \(^13\)C NMR datas were conducted. (Fig. S5†) Then, the detection to Hg\(^{2+}\) in THF (2.0 × 10\(^{-5}\) mol L\(^{-1}\)) of the precursor 3, NBTZ, QBOZ and QBTZ were tested. The precursor 3 and the probe NBTZ have no effect on Hg\(^{2+}\), the probe QBOZ and QBTZ could all be quenched by Hg\(^{2+}\). (Fig. S6†) These phenomenon indicated that the Hg\(^{2+}\) could combine the atom “N” from the quinoline, the other bridging site could not be determined. While, the same structure that contains quinolinol-benzimidazole in our earlier published paper that could not recognize Hg\(^{2+}\). So, we think the other possible bridging site was the atom “S” from the benzothiazole. According to its ESI-MS spectrometry of 1O (Fig. S7†), it had a 1 : 1 complexation stoichiometry in 1O − Hg\(^{2+}\) complex with a peak at m/z 985.3 due to [1O + Hg\(^{2+}\)]\(^+\), another peak at m/z 1209.9 due to [1O + Hg\(^{2+}\) + 2ClO\(_4\)\(^-\) + Na\(^+\)]. As shown in Fig. 5 of Job’s plots, the maximum value was achieved when the molar fraction of [Hg\(^{2+}\)]/[Hg\(^{2+}\) + [1O]] was about 0.5, which further demonstrated the 1 : 1 stoichiometry between 1O with Hg\(^{2+}\).

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**Fig. 3** Competitive changes in the fluorescence of 1O in THF (2.0 × 10\(^{-5}\) mol L\(^{-1}\)) with the addition of various metal ions (5.0 equiv.): (A) emission spectral changes, (B) image of the fluorescence color changes.

**Fig. 4** Changes in the fluorescence of 1O induced by Hg\(^{2+}\) in THF (2.0 × 10\(^{-5}\) mol L\(^{-1}\)) and emission intensity changes of 1O induced by the addition of different equiv. of Hg\(^{2+}\).

**Fig. 5** Job’s plot of 1O with Hg\(^{2+}\).
Fluorescent turn-on response of 1O’ toward Cys

As shown in Fig. 7, studies on 1O’ fluorescent spectra with 17 different amino-acids, such as Cys, Gly, Ala, Val, Leu, Ile, Phe, Pro, Try, Ser, Tyr, Glu, Thr, Glu, Lys and Arg were conducted in THF. Because Hg²⁺ has high affinity towards thiol-based amino-acids, 53–54 the fluorescence intensity of 1O’ almost recovered to the original of 1O only when Cys was added into the solution, and the quantum yields of 1O’ + Cys was measured as 0.426, while other amino-acids did not change the emission spectra. The fluorescence recovery might be due to release of 1O from the 1O’ through the interaction of thiol-containing Cys with Hg²⁺. Then, others organic thiol compounds like ethylene mercaptan, 2-aminothiophenol and 2-aminoethanethiol were tested and the fluorescence could also recovery. (Fig. S8†) These results showed that 1O’ could be successfully utilized to sensor thiol-containing compounds with turn-on fluorescence.

As shown in Fig. 8, when Cys was added to the solution of 1O’, the fluorescence of the solution was enhanced rapidly. When 5.0 equiv. of Cys was added, the fluorescence recovered to the original of 1O, indicating that Cys took the Hg²⁺ away from 1O’. According to the Job’s plots (Fig. 9) and ESI-MS spectrometry (Fig. S9†), the complex 1O’ – Cys had a 1 : 2 coordination stoichiometry. In the negative-ion mass spectrum, the peak at m/z 441.4 was assignable to [2Cys + Hg²⁺ + H]⁻ and the other peak at m/z 785 was assignable to 1O. Similarly, according to Benesi–Hildebrand, the binding constant between 1O’ and Cys was 7.86 × 10³ L mol⁻¹ (R = 0.99351) and the detection limit of 1O’ was 7.06 × 10⁻⁷ mol L⁻¹ (Fig. S10†).

As shown in Fig. 10, when 1O’ was treated with 5.0 equiv. of Cys in the presence of the same concentration of other amino-acids, the emission enhancement caused by Cys was retained with Gly, Ala, Val, Leu, Ile, Phe, Pro, Try, Ser, Tyr, Glu, Thr, Glu, Lys, Arg and His, which indicated that any other amino acids could not interfere the detection of Cys by 1O’ in THF.

Application of 1O in logic circuits

Since the fluorescence intensity of 1O could be controlled by the stimulation of UV/Vis light, Hg²⁺ and Cys, a combinational logic circuit could be constructed with four inputs (In1: 297 nm UV
light, In2: λ > 500 nm visible light, In3: \( \text{Hg}^{2+} \) and In4: Cys) and one output signal (the change of fluorescence intensity at 468 nm). The emission intensity of \( 1O \) at 468 nm was regarded as the initial value.

All the four inputs could be either “on” or “off” state with different Boolean values. When the emission intensity was quenched by over 50% of the initial value, the output signal could be regarded as ‘off’ state with a Boolean value of ‘0’. Otherwise, it was regarded as ‘on’ state with a Boolean value of ‘1’. The diarylethene \( 1O \) showed an on–off–on fluorescence switching behavior under the stimuli of different inputs. For example, when the string is ‘0, 0, 1, and 0’ the corresponding input signals of In1, In2, In3, and In4 are ‘off, off, on, off’. It means that \( 1O \) was stimulated by \( \text{Hg}^{2+} \) and its emission intensity quenched dramatically. And the output signal was ‘off’ with a digit of ‘0’. All possible strings of the three inputs were listed in Table 1 and the logic circuit corresponding to the truth table was shown in Fig. 11.

### Table 1: Truth table for all possible strings of the four binary-input data and the corresponding output digit

| Input | Output \( \lambda_{\text{em}} = 468 \text{ nm} \) |
|-------|----------------------------------|
| In1 (UV) | In2 (Vis) | In3 (\( \text{Hg}^{2+} \)) | In4 (Cys) |
| 0     | 0      | 0      | 0       | 1       |
| 1     | 0      | 0      | 0       | 0       |
| 0     | 1      | 0      | 0       | 1       |
| 0     | 0      | 1      | 0       | 0       |
| 0     | 0      | 0      | 1       | 1       |
| 1     | 1      | 0      | 0       | 1       |
| 1     | 0      | 1      | 0       | 1       |
| 1     | 0      | 0      | 1       | 0       |
| 0     | 1      | 1      | 0       | 0       |
| 0     | 1      | 0      | 1       | 1       |
| 0     | 0      | 1      | 1       | 1       |
| 1     | 1      | 1      | 0       | 0       |
| 1     | 1      | 0      | 1       | 1       |
| 1     | 0      | 1      | 1       | 0       |
| 1     | 1      | 1      | 1       | 1       |

* The emission intensity of \( 1O \) at 468 nm was regarded as the initial value and defined as 1, otherwise defined as 0.

### Conclusions

In summary, a new diarylethene-based “on–off–on” fluorescence sensor for the sequential recognition of \( \text{Hg}^{2+} \) and Cys with very high selectivity and sensitivity was designed and synthesized successfully. When \( \text{Hg}^{2+} \) was added into the solution of \( 1O \), fluorescence of the \( 1O \) solution was quenched because \( 1O \) bond to \( \text{Hg}^{2+} \) into \([1O - \text{Hg}^{2+}] \) \( 1O' \) \( 1:1 \) stoichiometry. When Cys was added into \( 1O' \) solution, the fluorescence was enhanced significantly due to the reaction between \( 1O' \) and Cys in \( 1:2 \) stoichiometry. Based on the fact that the fluorescence of the diarylethene could be effectively modulated with the stimulation of light and chemical species, a logic circuit was also constructed successfully with the fluorescence intensity as the output signal, and UV/Vis, \( \text{Hg}^{2+} \) and Cys as the inputs, which should have great potentials in future fluorescent sensors for some special species.

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