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A squaraine and Hg^{2+}-based colorimetric and "turn on" fluorescent probe for cysteine

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ABSTRACT

A novel squaraine derivative (**SQ-1**) was synthesized and characterized for the determination of cysteine. Binding with Hg^{2+} as a fluorescent quencher to **SQ-1** leads to absorption and emission turn-off. More significantly, the **SQ-1**–Hg^{2+} complex exhibits a dual-channel chromofluorogenic responses to biologically important cysteine with a high sensitivity and selectivity over other natural amino acids, along with a low detection limit of 36.7 nM.

1. Introduction

In maintaining biological systems, cysteine (Cys) as intracellular thiol plays a pivotal role in various important biochemical functions such as biocatalysis, metal binding, detoxification of xenobiotics, and metabolic processes [1,2]. A deficiency of Cys is implicated in many health problems including retarded growth in children, hair depigmentation, lethargy, liver damage, muscle and fat loss, skin lesions and edema [3,4]. In the human blood plasma, the abnormal levels of Cys result in various diseases such as Alzheimer's disease [5], cardiovascular disease [6,7], neural tube defect, inflammatory bowel disease, and osteoporosis [8]. Therefore, it is of great importance to detect and discriminate selectively for Cys as a disease-associated biomarker in biological systems. To date, various conventional detection methods for the determination of Cys have been exploited such as high performance liquid chromatography (HPLC) [9,10], capillary electrophoresis [11] and optical detections [4]. Among these various techniques, optical detection methods have been proven to be the most convenient [12]. Fluorescent sensors or probes for Cys have especially attracted attention owing to their simplicity, sensitivity and efficiency [13]. More importantly, fluorescent probes can be used for intracellular online detection [14–16]. Recently, many fluorescent and colorimetric methods for detecting Cys have been developed, which are based on gold/silver nanoparticles [17], specific reactions between probes and amino acids [18]. Interestingly, the metal complex-based coordination fluorescent probe for selectively detecting Cys has also attracted much attention [19]. However, most of these chemosensors have one or more disadvantages, such as competing for other metal ions [20], poor aqueous solubility [21], inferior sensitivity and selectivity [22] and sensing at a rather short emission wavelength [23]. There is still a great need to develop novel and simple colorimetric and fluorescent chemosensors for Cys with high selectivity and sensitivity.

Squaraines (SQ) are versatile organic dyes used extensively as chemosensors based on their desirable optical properties associated with a resonance stabilized zwitterionic structure, especially their intense absorption and fluorescent emission properties [24]. The squaraine dye color results from a charge transfer band between two nitrogen atoms from the anilino groups and two oxygen atoms from the central acceptor four-membered ring. The coordination with protons or certain metal ions results in a rich modulation of the color of the squaraines and the electron-deficient central cyclobutene ring is susceptible to nucleophilic attack, and consequently squaraines have been developed as chemosensors in recent years [25]. Coordination with specific metal ions delocalizes π-system dramatically and leads to turn-off the squaraine fluorescence and absorption. Thiol-containing amino acids can sequester metal ions, because of the thiophilic nature of specific metal ions, thereby inducing the fluorescence and absorption of the squaraine turn-on. In addition, 8-hydroxyquinoline is one of the important chelators used to construct unique ionophoric systems for metal ions, such as Hg^{2+}, Cu^{2+}, Zn^{2+}, and Fe^{3+} [26,27], as well as organic light-emitting diodes (OLEDs) [28]. Inspired by these results, a novel squaraine-based chemosensor with quinolin-8-yloxy binding sites (**SQ-1**) was designed and synthesized.

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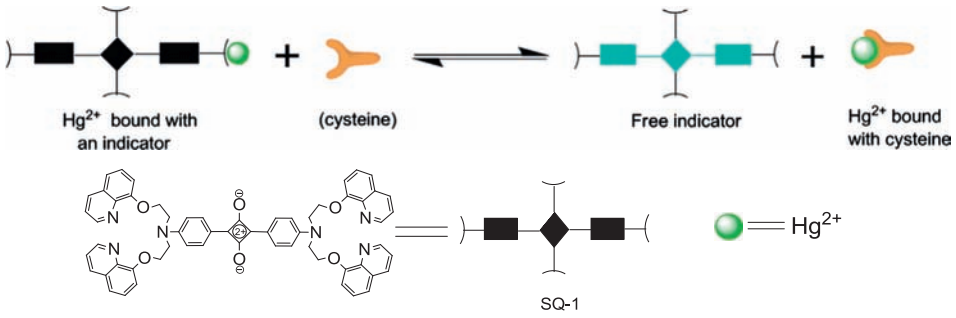


Scheme 1. Rational design of the squaraine based probe for cysteine detection.

Chelation of Hg^{2+} leads to switching off the absorbance and fluorescence intensity of **SQ-1**. Furthermore, the **SQ-1**–Hg^{2+} complex can be applied to specific detection for Cys over other natural amino acids with significant selectivity and sensitivity though a dual colorimetric and fluorescent enhancement mode in EtOH–H_2O media, as shown in Scheme 1. Additionally, an unsymmetrical squaraine with quinolin-8-yloxy unit **SQ-2** was synthesized for the control reaction.

2. Experimental

2.1. Instrumental analysis

The syntheses and manipulations of squaraine dyes were carried out under dry nitrogen. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AV-400 spectrometer (400 MHz). The chemical shifts were measured relative to TMS (0.00 ppm) for $CDCl_3$ as indicated. FTIR spectra were recorded on a Perkin Elmer Spectrum 2000 Fourier Transform Infrared Spectrophotometer. High resolution mass spectra (HRMS) were recorded on Varian 7.0 T FTICR-mass spectrometers. Absorption spectra were measured on a Perkin Elmer Lambda750 UV spectrophotometer. Fluorescence emission spectra were collected on a Cary Edipse fluorescence spectrophotometer. Melting points of compounds were determined with a SGW X-4 instrument without correction.

2.2. Regents and general methods

Twice-distilled water was used throughout all experiments. Metal salt solutions (0.01 M) of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Al^{3+}, Cu^{2+}, Cd^{2+}, Mn^{2+}, Fe^{3+}, Ag^+, Hg^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+} and Co^{2+} were prepared in twice-distilled water. The solutions of various amino acids (0.01 M) were also prepared in double deionized water. The squaraine dye **SQ-1** was dissolved in EtOH–H_2O (30:70, v/v) solution (2.5 μM). Metal ion and amino acids consecutive titrations were carried out respectively by adding small volumes (0.5–1.0 μL) into 2 mL **SQ-1** solution in a quartz cuvette each time.

2.3. Synthetic procedures

2.3.1. Synthesis of *SQ-1*

In a 100 mL three-necked flask, **3** (200 mg, 0.46 mmol) and squaric acid (26 mg, 0.23 mmol) were dissolved in 35 mL 1-heptanol. Then the stirred mixture was heated to reflux for 6 h under reduced pressure (75 mmHg, 133 °C). After cooling down, most of the solvent was first removed under reduced pressure, and then the crude product was purified by column chromatography over silica gel. Elution of the column with a mixture of chloroform and methanol (30:1, v/v) was used to afford the desired green squaraine dye **SQ-1** (57 mg) in 26% yield. m.p.: 262–264 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.33 (t, J=5.6 Hz, 8H), 4.60 (t, J=5.6 Hz, 8H), 7.00–7.02 (m, 8H), 7.33–7.40 (m, 12H), 8.11 (q, J_1=1.6 Hz, J_2=8.4 Hz, 4H), 8.41 (d, J=9.2 Hz, 4H), 8.84 (q, J_1=1.6 Hz, J_2=4.0 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.8, 65.9, 109.6, 113.0, 120.5, 120.7, 121.7, 126.6, 129.6, 133.6, 135.9, 140.3, 149.4, 153.8, 154.2, 183.0, 190.7; ESI-TOF-MS: m/z 475.2 ($[M+2H]^{2+}$), 317.1 ($[M+3H]^{3+}$); HRMS (ESI): Calcd for $C_{60}H_{50}N_6O_6^{2+}$ ($[M+2H]^{2+}$): 475.1890, Found: 475.1906.

2.3.2. Synthesis of *SQ-2*

Semisquaric acid **4** was prepared as described before [29]. In a 100 mL three-necked flask, **3** (42.5 mg, 0.097 mmol) and semisquaric acid **4** (31 mg, 0.097 mmol) were dissolved in 20 mL 1-heptanol. Then the stirred mixture was heated to reflux for 8 h under reduced pressure (75 mmHg, 133 °C). After cooling down, most of the solvent was first removed under reduced pressure, and then the crude product was purified by column chromatography over silica gel. Elution of the column with a mixture of chloroform and methanol (40:1, v/v) was used to afford the desired green squaraine dye **SQ-2** (20 mg) in 28% yield. m.p.: 90–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (t, J=7.3 Hz, 3H), 1.26 (s, 1H), 1.40 (dd, J=14.8, 7.3 Hz, 2H), 1.64 (d, J=7.0 Hz, 2H), 3.48-3.41 (m, 2H), 4.32 (s, 2H), 4.60 (s, 2H), 6.73 (d, J=9.0 Hz, 1H), 7.04-6.97 (m, 2H), 7.37 (dd, J=18.5, 7.6 Hz, 3H), 8.12 (d, J=7.9 Hz, 1H), 8.39 (d, J=7.0 Hz, 2H), 8.86 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13,84, 20.23, 29.64, 29.70, 51.33, 51.65, 65.84, 109.49, 112.57, 112.67, 119.56, 120.35, 120.83, 121.67, 126.62, 129.56, 132.89, 133.98, 135.95, 140.24, 149.39, 152.81, 154.24, 183.30, 186.31, 190.51; ESI-MS: m/z 741.3 ($[M+Na]^+$).

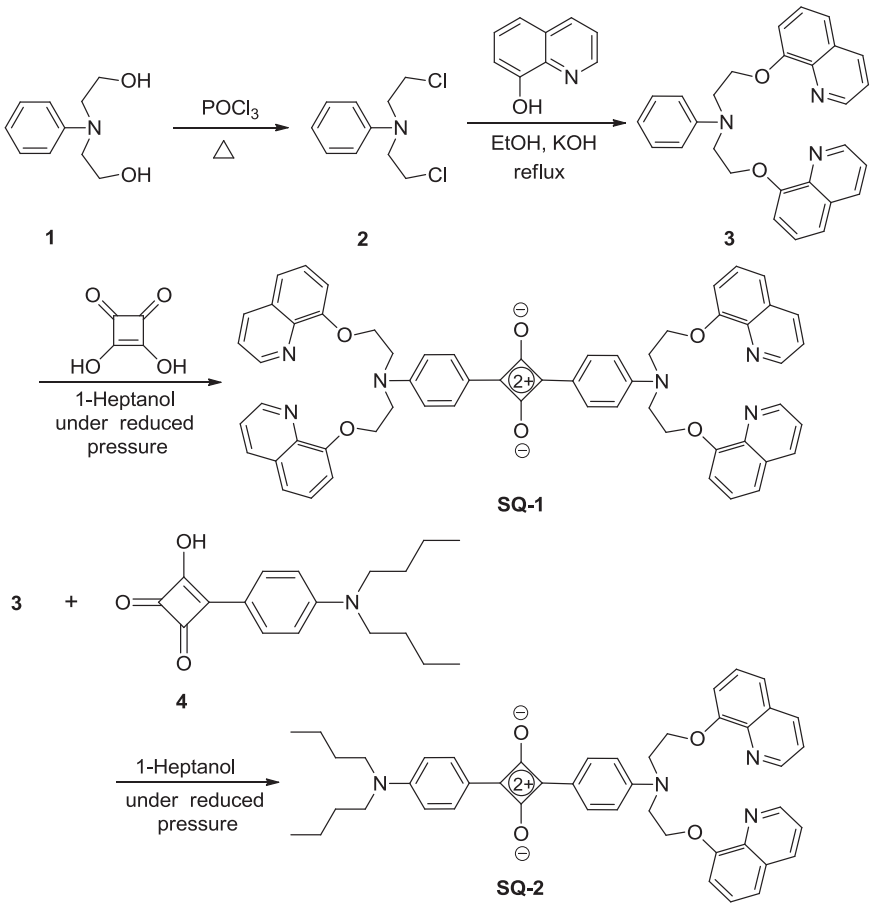
3. Results and discussion

3.1. Synthesis

SQ-1 was synthesized through a three-step process as shown in Scheme 2. By treatment of commercially available compound **1** with $POCl_3$ aniline derivative **2** was obtained. Then, a reaction between **2** and 8-hydroxyquinoline afforded the building block **3** as described before [30]. Refluxing of **3** and squaric acid in 1-heptanol under reduced pressure (75 mmHg) gave **SQ-1** in 26% yield after purification by silica gel column chromatography, using chloroform/methanol (30:1, v/v) as an eluent. In a similar way, refluxing of **3** with semisquaric acid **4** afforded unsymmetrical dye **SQ-2** in 28% yield. These new compounds were fully characterized by ^{1}H NMR, ^{13}C NMR and ESI mass spectroscopy (pp. S1–S4, Supplementary data).

3.2. Physicochemical properties and absorption experiments

Squaraine dyes exhibit excellent spectroscopic properties, which draw great attention to the development of water-soluble squaraines for use in biological and environmental samples [31]. **SQ-1** is highly soluble in many organic solvents, showing a strong



Scheme 2. Synthetic routes for the squaraine dyes **SQ-1** and **SQ-2**.

absorption band ($\varepsilon > 10^5$ L mol^{-1} cm^{-1}) with the absorption maximum from 628 to 636 nm and a fairly strong fluorescent emission with an emission maximum from 642 to 655 nm in various organic solvents (Table S1, Supplementary data).

Notably, one interesting feature of squaraines is their tendency to aggregate and precipitate in the aqueous medium, leading to a dramatic color change and fluorescence quenching [32]. Avoiding aggregation is very important to improve the optical performance of squaraine dyes. **SQ-1** shows excellent solubility and stability after modification by quinoline, which is stable even in the 100% aqueous solution without precipitation. As shown in Fig. 1, as the percent of water in ethanol increased, only tiny absorbance decreases for **SQ-1** were observed, and the spectral properties were almost preserved even in the presence of 70% H_2O. Further increasing the water ratio from 80% to 100% led to an apparent decline in the absorbance. In order to obtain excellent spectroscopic conditions with high sensitivity, the following investigation was carried out in EtOH–H_2O (30:70, v/v) due to the absorbance having negligible change at 70% water concentration.

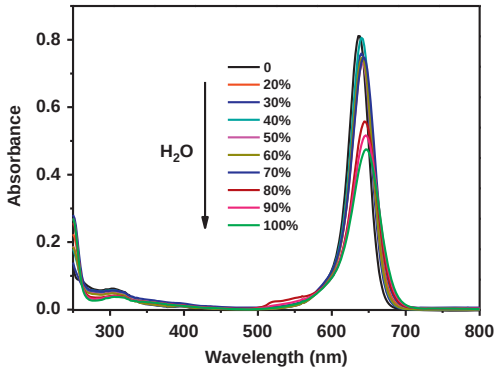


Fig. 1. Change in absorbance of **SQ-1** (2.5 μM) with increase of H_2O percentage in EtOH aqueous solution.

3.3. Fluorescence and absorption turn-off detection of Hg^{2+}

3.3.1. Absorption and fluorescence titration of Hg^{2+}

Before investigating the chemosensing behavior of **SQ-1**, the stability of **SQ-1** exposed in the air and the sunlight was recorded (Fig. S8, Supplementary data). The fluorescent intensity of **SQ-1** was almost maintained in EtOH–H_2O (30:70, v/v) solution. The free **SQ-1** has a relatively strong fluorescence emission band at 664 nm in EtOH–H_2O (30:70, v/v) solution. However, upon addition of Hg^{2+}, the fluorescence of **SQ-1** (2.5 μM) was gradually extinguished. When the concentration of Hg^{2+} reached 20 equiv., about 90% of the maximum fluorescence intensity was quenched, as shown in Fig. 2. Besides, the absorption peak of **SQ-1** at 643 nm was decreased in intensity and a weak absorption band centered at 565 nm assigned to cation coordination to the anilino nitrogen rose up gradually with increasing Hg^{2+} concentrations (Fig. S9, Supplementary data). Addition of more Hg^{2+} resulted in the decrease of 565 nm band, indicating the formation of either mixed N,O- or N,N-coordinated complexes, and the solution color changed from blue to colorless (Fig. S10, Supplementary data). Similar spectral behavior upon binding with metal ions has also been reported in other squaraine derivatives [33]. However, no detectable color change was observed when other metal ions (20 equiv.) such as Li^+, K^+, Na^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Al^{3+}, Cu^{2+}, Ag^+, Pb^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Fe^{3+}, Co^{2+} and Mn^{2+} were added. Therefore, these

results clearly validate that **SQ-1** can be exploited as a sensor for Hg^{2+} in EtOH–H_2O (30:70, v/v) solution.

Simultaneously, the Job's plot shows the **SQ-1** coordinated to Hg^{2+} in a 1:1 bonding stoichiometry under the experimental conditions though having two binding sites in **SQ-1**, which is also confirmed by the Benesi–Hildebrand method (Fig. S11, Supplementary data) [34]. Furthermore, the complex formation between **SQ-1** and Hg^{2+} was also clearly vindicated by ESI-MS spectrum analysis (Fig. S12, Supplementary data). In the ESI mass spectrum, a molecular ion peak at *m*/*z* 1209.0 corresponded to a 1:1 complex $[Hg^{2+}\bullet\textbf{SQ-1}\bullet CH_3COO^-]^+$. It is well-known that there is an extensive delocalization of the electronic charge along the squaraine molecule and the dye structure can be described by resonance structures **SQ-1a** and **SQ-1b** as shown in Scheme 3 [25b,34a,35]. We speculate that complexation with Hg^{2+} through the anilino N-Hg bond may induce the localization of the charge to the opposite N atom of the dye, reducing the affinity of the ionophore at the other side to Hg^{2+} and leading to the formation of a 1:1 complex [25b,34a]. Thus, these spectral responses to Hg^{2+} may be attributed to chelation of Hg^{2+} that destroyed the donor–acceptor–donor (D–A–D) charge transfer, and resulted in switching off the absorbance and fluorescence of **SQ-1**. For further exploration, unsymmetrical **SQ-2** with only one ionophore was synthesized for the control reaction. Under the same conditions, as shown in Fig. S13 (Supplementary data), unsymmetrical **SQ-2** shows no responses to Hg^{2+} even under high concentration (200 μM). These results are ascribed to the high electron donating ability of alkyl chain compared to quinolin-8-yloxy group, and the resonance structure **SQ-2b** is favored to **SQ-2a**, reducing the affinity of **SQ-2** to Hg^{2+}.

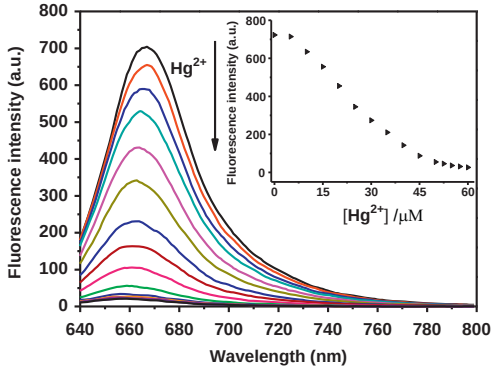
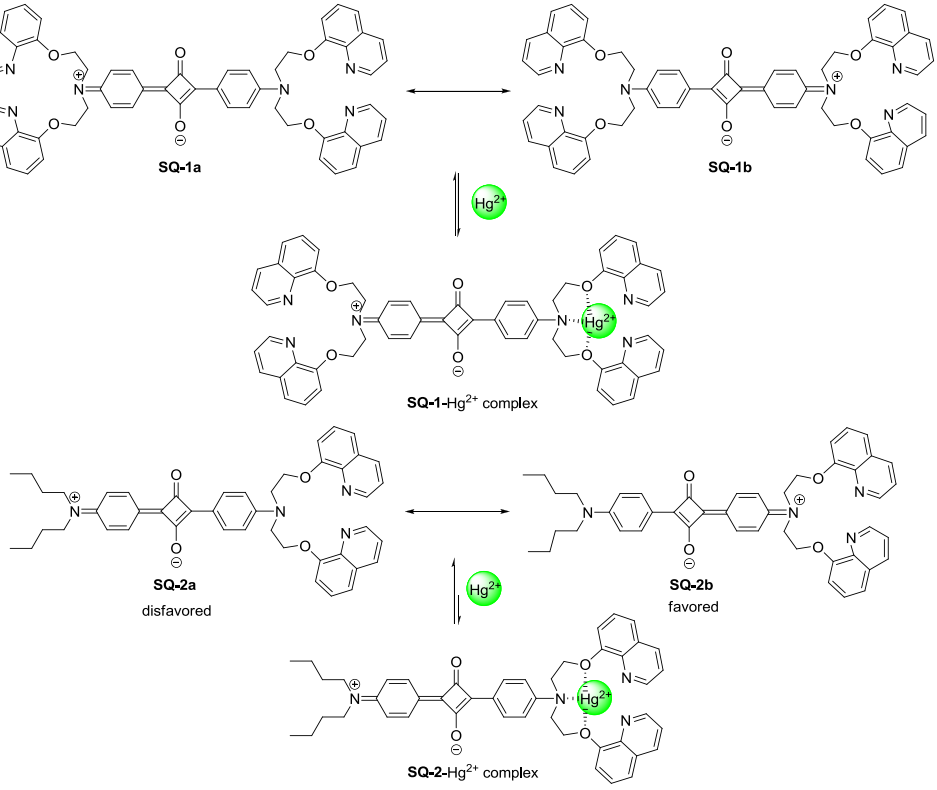


Fig. 2. The fluorescence spectra of **SQ-1** (2.5 μM) in the presence of increasing concentrations of Hg^{2+} (0–60 μM) in EtOH–H_2O (30:70, v/v) solution. Inset: the plot of fluorescence intensity at 664 nm versus the concentration of Hg^{2+}. (λ_{ex}=630 nm, slit: 5 nm/5 nm).

3.3.2. *Ion selectivity*

To investigate the selectivity of **SQ-1** towards Hg^{2+} ion, the fluorescence response toward other environmentally relevant metal ions were measured. We respectively added 100 μM metal ions including alkali (Li^+, K^+, Na^+), alkaline earth (Mg^{2+}, Ca^{2+}, Ba^{2+}, Al^{3+}), transition and heavy metal ions (Cu^{2+}, Ag^+, Pb^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Fe^{3+}, Co^{2+} and Mn^{2+}) to **SQ-1** in EtOH–H_2O (30:70, v/v) solutions. The results as shown in Fig. 3 demonstrated no apparent fluorescence quenching under identical conditions, in contrast, only the addition of Hg^{2+} ion caused the fluorescence intensity of **SQ-1** to be almost completely quenched, with the solution color changing from blue to colorless. To further explore the selectivity of **SQ-1** for Hg^{2+}, the competition experiments were also performed with the solution of **SQ-1** in the presence of Hg^{2+} (50 μM) mixed with other metal ions (500 μM) (Fig. S14, Supplementary data). The results show that no such obvious fluorescence changes occurred even under high concentrations of other metal ions. The subsequent addition of Hg^{2+} ion, however, caused the fluorescence intensity of **SQ-1** to be essentially completely quenched in the



Scheme 3. Resonance structures of **SQ-1** and **SQ-2**.

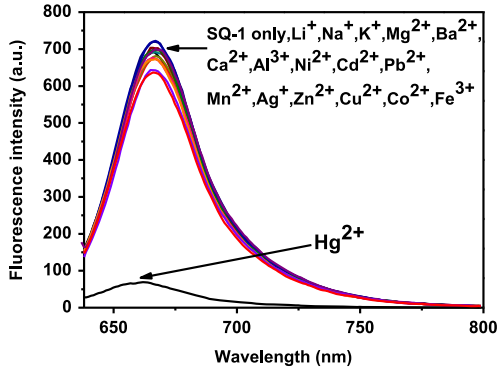


Fig. 3. The fluorescence spectrum of **SQ-1** (2.5 μM) in the presence of different metal ions (50 μM) in EtOH–H_2O (30:70, v/v) solution (λ_{ex}=630 nm, slit: 5 nm/5 nm, PMT Volts: 620.).

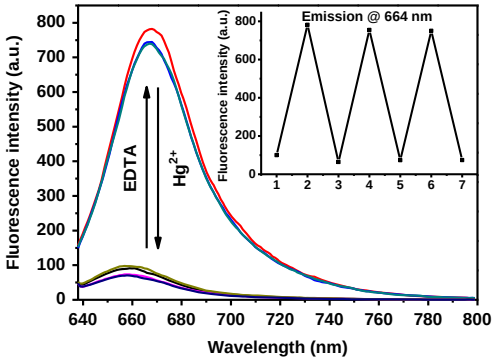


Fig. 4. Reversibility of **SQ-1** and Hg^{2+} by the introduction of EDTA to the system. The bottom lines represent the fluorescence quenching that occurred after the introduction of Hg^{2+} (100 μM) to **SQ-1** (2.5 μM) in EtOH–H_2O (30:70, v/v) solution. The top lines represent the fluorescence enhancement that occurred after the addition of EDTA (50 μM) to the system. Inset: stepwise complexation/decomplexation cycles were carried out in EtOH–H_2O (30:70, v/v) solution with **SQ-1** and Hg^{2+} (λ_{ex}=630 nm, slit: 5 nm/5 nm, PMT Volts: 620.).

presence of various competitive cations. Accordingly, the presence of other metal ions did not interfere with the fluorescent and colorimetric detection of Hg^{2+} even though some thiophilic heavy metal ions such as Ag^{+} and Cu^{2+}. **SQ-1** has a pronounced selectivity and degrees of fluorescence quenching were observed without any interference by other metal ions in EtOH–H_2O (30:70, v/v) solution.

3.3.3. *Reversible experiment*

The reversibility of the chemosensor is a particularly attractive aspect in practical application. Reversible titration by EDTA and Hg^{2+} demonstrated that the fluorescence intensity responses were also reversible, as shown in Fig. 4. Upon the addition of 50 μM EDTA into the solution containing 2.5 μM **SQ-1** and 100 μM Hg^{2+}, the fluorescence intensity was enhanced significantly, and additionally the solution color restored from colorless to blue. Addition of another 50 μM Hg^{2+} to this blue solution produced an immediate fluorescence quenching and decoloration again. Subsequent additions of Hg^{2+} or EDTA could switch the fluorescence off/on cycle at least three times without a loss of sensitivity. The process clearly revealed the high degree of reversibility of the complexation/decomplexation process.

3.4. *Fluorescence and absorption turn-on detection of cysteine*

3.4.1. *Amino acids selectivity*

Fig. 5 Based on the thiophilic nature of Hg^{2+}, Cys has been introduced to **SQ-1**–Hg^{2+} complex and to correspondingly turn on the fluorescence and absorption. To verify the selectivity, the absorption and fluorescence emission was investigated with addition of various amino acids such as Ala, Thr, Ser, Gly, Glu, Arg, Val, Trp, Lys, Pro, lle, Leu, Asp, Met, Tyr, Phe, His, and GSH in EtOH–H_2O (30:70, v/v) solution, as depicted Figs. S15 and S5 (Supplementary data). Delightedly, only after introduction of Cys (50 μM) into the solution of **SQ-1** (2.5 μM) and Hg^{2+} (100 μM), the intensity of fluorescence emission was pronouncedly enhanced whereas negligible changes were observed with other 18 amino acids. Concomitantly, the absorption also showed a significant selectivity for Cys over the other amino acids corresponding with a visual color change from colorless to blue (Fig. S16, Supplementary data). Furthermore, competition experiments also have been carried out. Amino acids (100 μM) such as Ala, Thr, Ser, Gly, Glu, Arg, Val, Trp, Lys, Pro, lle, Leu, Asp, Met, Tyr and Phe caused no change in the fluorescence emission spectra, and potential interfering agents GSH and His have only minimal interference. In contrast, in the presence of competing amino acids, subsequent introduction of Cys (50 μM) still triggered a significant fluorescence enhancement, as illustrated in Fig. 6. The further interference experiments of GSH and

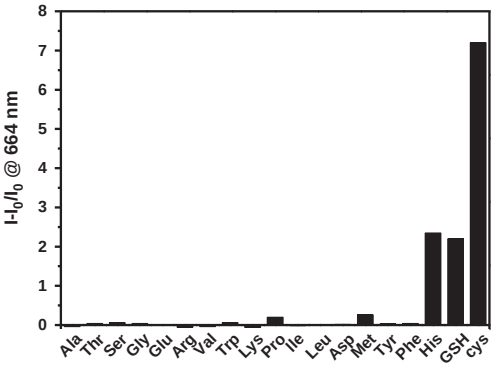


Fig. 5. Fluorescence intensity changes at 664nm, $(I–I_0)/I_0$, of the **SQ-1** (2.5 μM) and Hg^{2+} (100 μM) upon addition of various amino acids (50 μM) in EtOH–H_2O (30:70, v/v) solution.

His at high concentration level toward Cys were carried out (Fig. S17, Supplementary data). Obviously, these comparative studies clearly demonstrate that **SQ-1**–Hg^{2+} could be employed as fluorescent and colorimetric probe for specific detection of Cys.

3.4.2. *Absorption and fluorescence titration of cysteine*

To investigate the fluorescence sensitivity of Cys detection by **SQ-1**–Hg^{2+} complex, fluorescence titration was carried out in EtOH–H_2O (30:70, v/v) solution. To reduce the fluorescence signal background, 100 μM Hg^{2+} was introduced into 2.5 μM **SQ-1** solution in EtOH–H_2O (30:70, v/v), in which more than 95% of the **SQ-1** fluorescence intensity has been quenched. In Fig. 7(A), with the titration of Cys, the fluorescence intensity increased proportionally and levelled off when the concentration of Cys reached to 30 μM. The fluorescence turn-on of **SQ-1** (2.5 μM) was apparent due to the competitive binding of Cys with Hg^{2+}, leading to the increase of the fluorescence recovery percentage (FRP), which is defined as the ratio ($I/I_0 \times 100\%$) of the maximum fluorescence intensity (I) after addition of Cys and the fluorescence intensity (I_0) of free **SQ-1**. The FRP of **SQ-1** was 50% at the Cys concentration of 25 μM, and when the concentration of Cys was 35 μM, the fluorescence intensity recovered to 99% of the maximum value (inset of Fig. 7 (A)). It is interesting to note that only small amount of Cys (35 μM) could nearly restore the fluorescence intensity entirely in the presence of excessive of Hg^{2+} (100 μM), indicating that no free

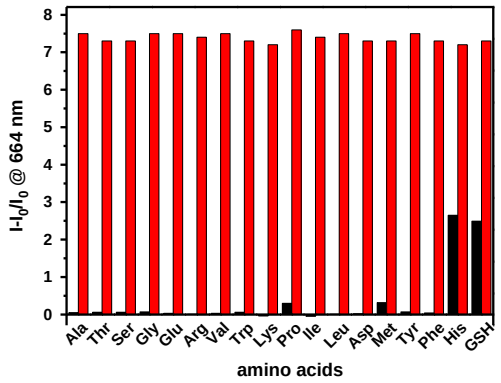


Fig. 6. The competition experiments of Cys to other amino acids in EtOH–H_2O (30:70, v/v) solution. Black bar: the fluorescence intensity of **SQ-1** (2.5 μM) and Hg^{2+} (100 μM) with the addition of the respective competing amino acids (100 μM). Red bar: the fluorescence intensity of subsequent introduction Cys (50 μM) to the system. (λ_{ex}=630 nm, slit: 5 nm/5 nm, PMT Volts: 620.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

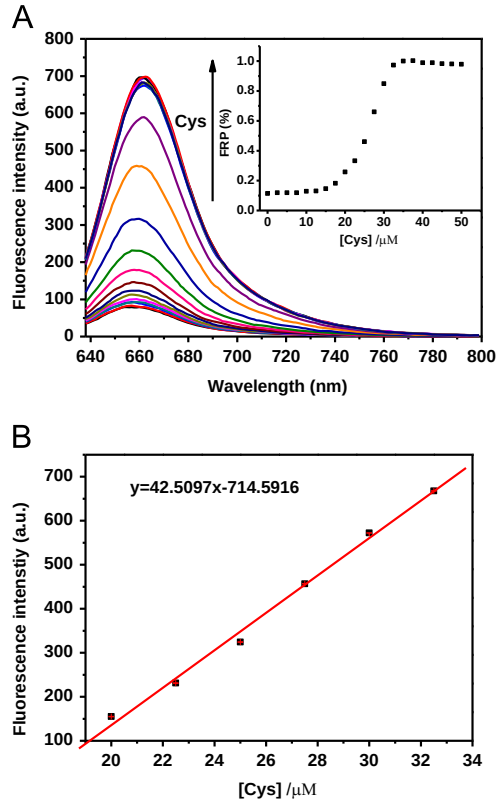


Fig. 7. (A) The fluorescence spectra of **SQ-1** (2.5 μM) and Hg^{2+} (100 μM) in the presence of increasing concentrations of Cys (0–50 μM) in EtOH–H_2O (30:70, v/v) solution. Inset: FRP versus the concentration of Cys (λ_{ex}=630 nm, slit: 5 nm/5 nm). (B) Plot of fluorescence intensity change of **SQ-1** (2.5 μM) and Hg^{2+} (100 μM) at 664 nm against varied concentrations of Cys from 20.0 to 32.5 μM at 664 nm (λ_{ex}=630 nm, slit: 5 nm/5 nm, PMT Volts: 620.). R^2=0.9940, k=4.25 × 10^7 au/M, σ=0.52, error bars for n=3.

SQ-1 was released. Instead, upon the addition of Cys, Hg^{2+} was pulled out from the ionophore by breaking the anilino N–Hg bond, resulting in the spectral restoration as shown in Scheme 4 [36].

Under identical conditions, the absorption peak at 664 nm increased proportionally and led to a visual color change from colorless to blue with the increase of the Cys concentration from 0 to 50 μM that was easily perceived by the naked eye (Fig. S18, Supplementary data). Furthermore, by carrying out a titration of Cys, the plot shown in Fig. 7(B) implied a linear response (R^2=0.9940, k=4.25 × 10^7), and the detection limit was measured as low as 36.7 nM (based on 3σ/k, where σ is the standard deviation of the blank measurements, and k is the slope of the intensity ratio versus sample concentration plot). All the above results indicate that the colorimetric and "turn-on" fluorescent sensor expressed outstanding desirable sensitivity towards Cys in EtOH–H_2O (30:70, v/v) solution. In addition, the pH effect to this sensing system has also been investigated. The results show that the fluorescence response was observed with the biologically relevant pH range (6.0–9.0) in EtOH/HEPES buffer (30:70, v/v) solutions (Fig. S19, Supplementary data).

3.4.3. *Dynamic measuring range of cysteine*

It is particularly interesting to investigate whether the concentration of Hg^{2+} can serve as the control of the measuring range for detecting Cys. As shown in Fig. 8, the fluorescent responses were explored by maintaining a content concentration of **SQ-1** (2.5 μM) and introducing 20, 25, 30, 35 equiv. of Hg^{2+}, respectively. As the concentration of Hg^{2+} was 100 μM, the fluorescence turn-on started at 20 μM of Cys and was complete at 35 μM of Cys, with a desirable linear range from 20 μM to 35 μM. When the concentration of Hg^{2+} was 125 μM, the fluorescence turn-on was observed in the concentration range of Cys from 30 μM to 45 μM. Similarly, with the increasing concentration of Hg^{2+}, the responding Cys concentration can shift to a higher level. For example, at 150 μM and 175 μM Hg^{2+}, the measuring range of Cys can be achieved to 50–65 μM and 70–85 μM, respectively. In this way, the absorption dynamic response was also modulated by the concentration of Hg^{2+} (Fig. S20, Supplementary data). Such

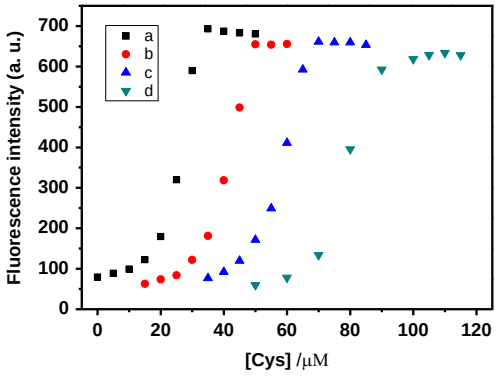
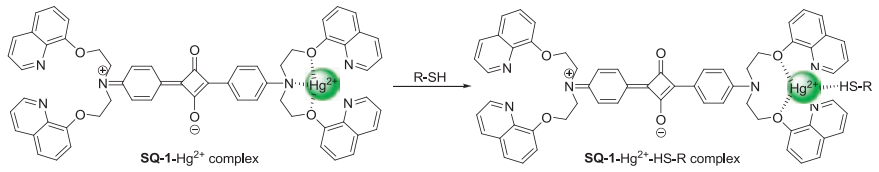


Fig. 8. Fluorescence intensity of **SQ-1** (2.5 μM) and Hg^{2+} at 664 nm in the presence of varied concentration of Cys in the EtOH–H_2O (30:70, v/v) solutions. The concentration of Hg^{2+} are (a) 100 μM, (b) 125 μM, (c) 150 μM, (d) 175 μM, successively.



Scheme 4. Proposed binding mode of **SQ-1**, Hg^{2+} and thiol.

findings may have a potential application for developing new Cys chemosensors with tunable measuring ranges.

Finally, sensor **SQ-1**–Hg^{2+} were applied for the detection of Cys in a commercially available amino acid beverage (Table S2, Supplementary data). Further evaluation of this chemodosimetric fluorescent probe with biological samples are still under progress in our laboratory.

4. Conclusion

In summary, a novel symmetrical squaraine dye **SQ-1** was synthesized as a selective fluorescent and colorimetric sensor for Hg^{2+} and Cys in EtOH–H_2O (30:70, v/v) medium. Upon addition of Hg^{2+}, the fluorescence intensity underwent proportional quenching, thereby allowing for a sensitive and selective Hg^{2+} sensing based on the fluorescence "turn-off". More importantly, subsequent addition of Cys into the **SQ-1**–Hg^{2+} complex solution induced its fluorescence turn-on and allowed for straightforward "naked-eye" Cys detection. These results show that a symmetrical squaraine dye **SQ-1** coordinated with Hg^{2+} was successfully utilized to probe Cys with high selectivity over other natural amino acids, in which **SQ-1** shows a tunable measuring range by changing the concentration of Hg^{2+}. Therefore, a symmetrical squaraine dye **SQ-1** can serve as a dual-channel chromofluorogenic chemosensor for both Hg^{2+} and Cys. These findings lead to a broad potential application for developing Hg^{2+} and Cys chemosensors.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.04.006.

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