

# Dual-mode unsymmetrical squaraine-based sensor for selective detection of Hg<sup>2+</sup> in aqueous media†

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A novel chemosensor based on unsymmetrical squaraine dye (USQ-1) for the selective detection of Hg<sup>2+</sup> in aqueous media is described. USQ-1 in combination with metal ions shows dual chromogenic and “turn-on” fluorogenic response selectivity toward Hg<sup>2+</sup> as compared to Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Al<sup>3+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>3+</sup>, Ag<sup>+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup> and Co<sup>2+</sup> due to the Hg<sup>2+</sup>-induced deaggregation of the dye molecule. A recognition mechanism based on the binding mode is proposed based on the absorption and fluorescence changes, <sup>1</sup>H NMR titration experiments, ESI-MS study, and theoretical calculations.

## Introduction

The quest to develop molecule based chemosensors for detecting metallic ions is important in biological, medical, environmental and industrial applications. In particular, the presence of Hg<sup>2+</sup> in biological tissues is a significant threat to health on a global scale, and causes central nervous system defects and erethism as well as arrhythmia, cardiomyopathy, and kidney damage.<sup>1–4</sup> Previous promising fluorescent and colorimetric sensors for Hg<sup>2+</sup> ion detection have been reported,<sup>5–11</sup> but the demand for highly sensitive and selective chemosensors is continuously increasing, and there is still a challenge to develop highly specific, rapid and inexpensive methods for monitoring this dangerous and widespread global pollutant.<sup>11</sup>

Squaraines belong to a novel class of organic dyes with resonance stabilized zwitterionic structures, which have a strong absorption ( $\epsilon > 10^5$  L mol<sup>-1</sup> cm<sup>-1</sup>) in the visible region and are photostable. They show sharp and intense absorption bands from the visible to near-IR wavelengths, arising from the donor–acceptor–donor (D–A–D) type of charge transfer and the extensively conjugated structure, and are influenced by the substituents attached to the phenyl ring and the anilino nitrogen.<sup>12</sup> Their favorable optical properties and their sensitivities to solvent polarity, temperature, and ionic inputs make them highly suitable for chemosensors. Interestingly, squaraine dyes have a high

tendency to form aggregates. The monomers can be arranged in a face-to-face fashion to form H-aggregates that give blue-shifted absorption bands (compared to the monomer absorption) or in a side-by-side fashion to form J-aggregates that give red-shifted absorption bands, together with an observable fluorescence quenching.<sup>13,14</sup> These phenomena have been successfully applied to designing sensors,<sup>15–18</sup> but there are only a few examples designed for Hg<sup>2+</sup> ion detection.

Recently, we have reported the synthesis of a symmetrical squaraine dye substituted with (phenylazanediy)l-bis-(ethane-2,1-diyl)-bis-diethylcarbamo-dithioate groups.<sup>10</sup> This is a specific Hg<sup>2+</sup> sensing agent using organic dye deaggregation as a conductive signaling mechanism for a cation binding process as measured by the dual chromogenic and fluorogenic responses. However, such squaraine dyes are notorious for their low solubility and processability. We have therefore taken advantage of the known ability of alkyl chains of appropriate lengths to enhance the solubility of such compounds, and now report a new unsymmetrical squaraine dye (USQ-1) incorporating an *N,N*-dibutylanilino group as a side arm. This exhibits high solubility and superior properties, and recognizes Hg<sup>2+</sup> selectively in an aqueous medium and signals the event through visual color change and “turn-on” fluorescence intensity due to the Hg<sup>2+</sup>-induced deaggregation of the dye molecule. Cyclic voltammetric measurements suggest this could also be designed as an electrochemical chemosensor by virtue of its dramatic electrochemical response to Hg<sup>2+</sup>. To the best of our knowledge, this is the first report which demonstrates the use of an unsymmetrical squaraine dye as a novel probe for the detection of Hg<sup>2+</sup>.

## Results and discussion

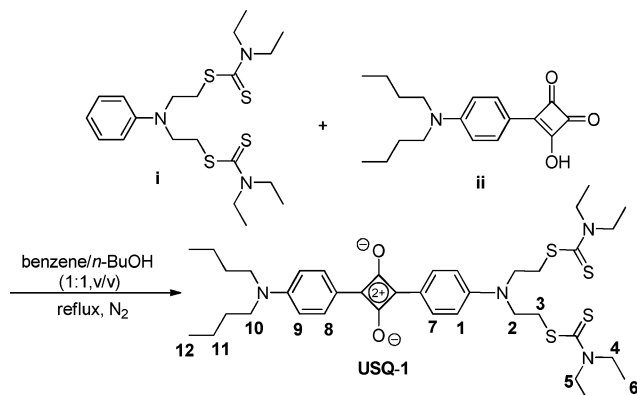
### Synthesis

Building blocks **i** and **ii** were synthesized and purified as reported in the literature.<sup>10,19,20</sup> Refluxing of **i** and **ii** in 1:1

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† Electronic supplementary information (ESI) available: Supplementary figures of absorption data, <sup>1</sup>H NMR, <sup>13</sup>C NMR and ESI-MS of USQ-1, ESI-MS of the [Hg<sup>2+</sup>·USQ-1·CH<sub>3</sub>COO]<sup>+</sup> complex, <sup>1</sup>H NMR titration spectra along with the X-ray crystal structure data. CCDC reference number 826187. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob06519j

*n*-butanol : benzene under a nitrogen atmosphere gave **USQ-1** in 58% yield after purification by silica gel column chromatography, using dichloromethane : ethyl acetate (10 : 1, v/v) as an eluent (Scheme 1). This new compound was fully characterized by NMR, elemental analysis and mass spectroscopy.

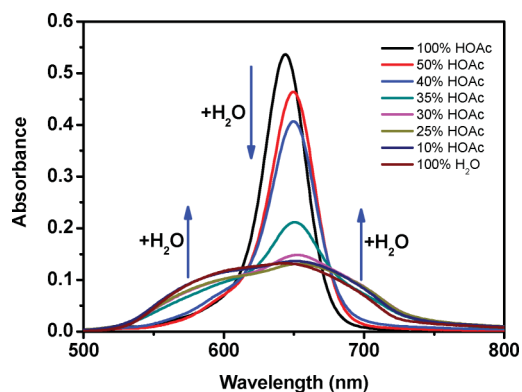


**Scheme 1** Synthesis of the unsymmetrical squaraine dye **USQ-1**.

### Physicochemical properties and absorption experiments

Squaraine dyes are insoluble in many organic solvents and water. Due to their excellent spectroscopic properties, considerable effort has been devoted to the development of water-soluble squaraines for use in biological and environmental samples.<sup>21</sup> Our approach is to introduce the dithiocarbamate (DTC) functionality into the molecule as the binding arm, and the *N,N*-dibutylanilino group to improve the solubility. **USQ-1** is highly soluble in many organic solvents, and these absorption and emission maxima are presented in Table 1. This show strong and symmetrical absorption maxima from 638 to 649 nm in various organic solvents with emission maxima from 656 to 673 nm, both of which arise from its donor–acceptor–donor type of charge transfer and the extensive conjugated structure. It is interesting to note that the absorption maximum of **USQ-1** experienced a dramatic red-shift in the polar aprotic solvent DMF.

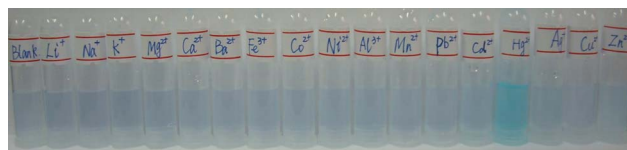
An important characteristic of squaraines is their tendency to congregate and precipitate from an aqueous medium, resulting in a dramatic color modulation and fluorescence quenching.<sup>22</sup> However, **USQ-1** shows excellent solubility after modification by incorporation of an *N,N*-dibutylanilino group, and can even dissolve in 100% aqueous solution without precipitation and remains stable. As shown in Fig. 1, the absorption near 644 nm, belonging to the monomer of **USQ-1**, decreased and shifted slightly to longer wavelengths with increasing water concentration, while the absorption in the region of 550–750 nm, belonging to the aggregates, appeared as a broad flat band, indicating that H- and J-aggregates were both present upon the addition of



**Fig. 1** Change in absorbance of **USQ-1** (2.5  $\mu\text{M}$ ) with increase of  $\text{H}_2\text{O}$  percentage in AcOH aqueous solutions.

water. These phenomena may be attributed to the unsymmetrical structure of the squaraine, which causes the dye molecules to arrange irregularly in the solvent, resulting in the generation of the mixed aggregate. It is gratifying that **USQ-1** is especially stable in the mixed solvent of acetic acid and water. It shows a good spectral reproducibility without any interference. The equilibrated formation of the aggregates and **USQ-1** monomer at 90% water concentration reached the maximum, so the subsequent investigations were carried out in AcOH-90% $\text{H}_2\text{O}$ .

Fig. 2 shows the color changes of **USQ-1** (5.0  $\mu\text{M}$ ) upon addition of 10 equiv. of physiologically and environmentally relevant metallic cations including  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ag}^+$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  ions. As depicted in Fig. 2, a  $5.0 \times 10^{-6}$  M AcOH :  $\text{H}_2\text{O}$  (10 : 90, v/v) solution of **USQ-1** displayed a lilac color, the addition of  $\text{Hg}^{2+}$  caused a vivid color change from lilac to brilliant blue while other metal cations did not result in any appreciable changes under the same conditions. This fact indicates that **USQ-1** exhibits high selectivity for  $\text{Hg}^{2+}$  compared to other metal ions, evidently due to the selective complexation of  $\text{Hg}^{2+}$  by the dithioate groups, and establishes the value of this assay for the naked-eye detection of  $\text{Hg}^{2+}$  in aqueous media quality monitoring.

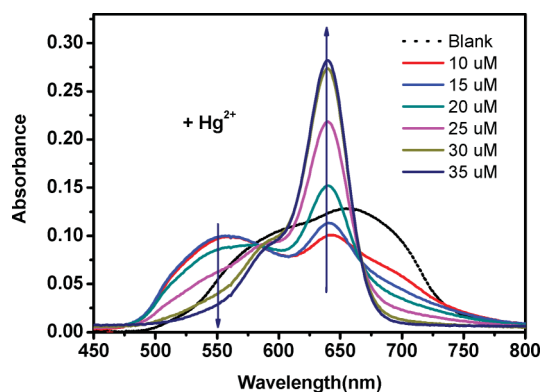


**Fig. 2** Color changes upon addition of different metal ions (10 equiv.) to **USQ-1** (5.0  $\mu\text{M}$ ) in AcOH/ $\text{H}_2\text{O}$  (10 : 90, v/v) solution.

To understand the recognition behavior between **USQ-1** and  $\text{Hg}^{2+}$ , the absorption spectral titration of **USQ-1** with  $\text{Hg}^{2+}$  in AcOH :  $\text{H}_2\text{O}$  (10 : 90, v/v) solution was carried out. As shown in Fig. 3, upon addition of  $\text{Hg}^{2+}$  (10  $\mu\text{M}$ ), the original

**Table 1** Absorption and emission parameters for **USQ-1** in different solvents

solvent	$\text{CHCl}_3$	THF	MeCN	DMF	AcOH	EtOH	MeOH
$\lambda_{\text{abs}}$ (nm)	638	638	639	649	644	643	644
$\epsilon$ ( $\text{M}^{-1} \text{cm}^{-1}$ )	$3.32 \times 10^5$	$3.44 \times 10^5$	$3.16 \times 10^5$	$3.16 \times 10^5$	$3.48 \times 10^5$	$3.44 \times 10^5$	$3.32 \times 10^5$
$\lambda_{\text{em}}$ (nm)	656	657	663	673	671	667	669



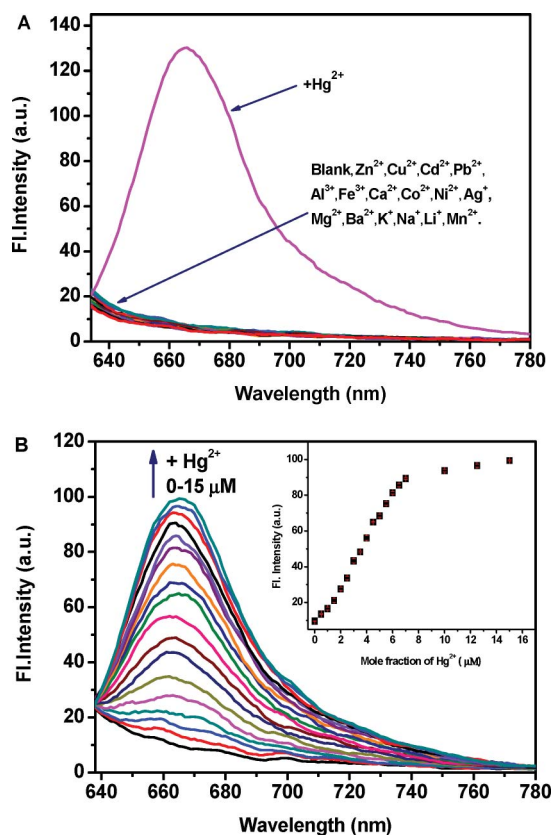
**Fig. 3** The absorption spectra of **USQ-1** (2.5  $\mu\text{M}$ ) upon addition of  $\text{Hg}^{2+}$  (0–35  $\mu\text{M}$ ) in  $\text{AcOH}:\text{H}_2\text{O}$  (10:90, v/v) solution.

absorption band is split into two individual peaks at *ca.* 640 nm and *ca.* 550 nm, ascribed to the monomer and H-aggregates, respectively.<sup>15,23–26</sup> The predominance of H-aggregate formation at low concentrations of  $\text{Hg}^{2+}$  is ascribed to the electrolyte effect,<sup>27</sup> which was further confirmed by the titration of other electrolytes (0–100  $\mu\text{M}$ ) such as  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Ag}^+$  (S3, see the ESI<sup>†</sup>), *i.e.*, they only facilitated the formation of aggregates and demonstrably showed H-aggregates of **USQ-1**. In the latter case, with increasing amounts of  $\text{Hg}^{2+}$  ions the absorption peak at 550 nm, ascribed to H-aggregates, diminished proportionally with concomitant enhancement of the 640 nm peak with isosbestic points at 590 nm as in Fig. 3. These experiments show a  $\text{Hg}^{2+}$ -induced process with unlocking of the H-aggregates, and the binding event of the  $\text{Hg}^{2+}$ -expressed in the form of a measurable absorption signal.

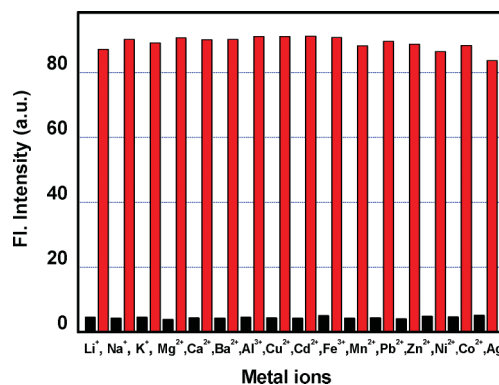
### Fluorescence experiments

Fig. 4A shows the fluorescence spectra ( $\lambda_{\text{ex}} = 600 \text{ nm}$ ) of **USQ-1** (2.5  $\mu\text{M}$ ) measured in  $\text{AcOH}:\text{H}_2\text{O}$  (10:90, v/v) solution with the respective metal cations (10 equiv.). Without cations or in the presence of the respective cations besides  $\text{Hg}^{2+}$ , **USQ-1** shows very weak fluorescence. Exhilaratingly, only after the addition of  $\text{Hg}^{2+}$ , was the intensity of the fluorescence emission significantly enhanced by approximately 10-fold at 665 nm (Fig. 4A). This demonstrates the unique selectivity of **USQ-1** for  $\text{Hg}^{2+}$ . With the titration of  $\text{Hg}^{2+}$ , **USQ-1** experienced a gradual increase in fluorescence intensity and was saturated upon addition of 3 equiv. of  $\text{Hg}^{2+}$  (Fig. 4B). Competition experiments also have been performed: metal cations (125  $\mu\text{M}$ ) such as  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ag}^+$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  caused no change in the fluorescence emission spectra, while in the presence of competing cations,  $\text{Hg}^{2+}$  ions (25  $\mu\text{M}$ ) still led to a dramatic fluorescence enhancement (Fig. 5). These findings indicate that **USQ-1** behaves as a highly sensitive and selective  $\text{Hg}^{2+}$  sensor.

Preliminary studies on the detection limit were also conducted by the titration of **USQ-1** with  $\text{Hg}^{2+}$ . A series of measured fluorescence intensities *versus*  $\text{Hg}^{2+}$  ion concentrations ranging from 1.0–3.5  $\mu\text{M}$  were obtained and linearly-fitted ( $R^2 = 0.99$ ,  $k = 1.32 \times 10^7 \text{ au M}^{-1}$ ) as shown in Fig. 6. The standard deviation ( $\sigma = 0.20$ ) was obtained from 7-fold consecutive scanning with the fluorescence spectrophotometer. The detection limit was calculated ( $3\sigma/k$ ) and

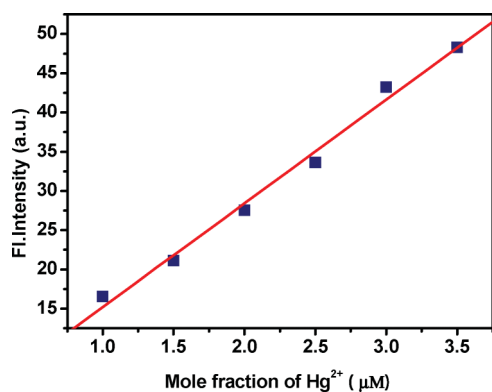


**Fig. 4** (A) The fluorescence spectra of **USQ-1** (2.5  $\mu\text{M}$ ) in the presence of different metal ions (25  $\mu\text{M}$ ) in  $\text{AcOH}:\text{H}_2\text{O}$  (10:90, v/v) solution. (B) Change in fluorescence intensity of **USQ-1** (2.5  $\mu\text{M}$ ) with increasing addition of  $\text{Hg}^{2+}$  (0–15  $\mu\text{M}$ ) in  $\text{AcOH}:\text{H}_2\text{O}$  (10:90, v/v) solution. Inset: the binding isotherm of **USQ-1** at 665 nm on the addition of  $\text{Hg}^{2+}$ . Error bars for  $n = 3$ . ( $\lambda_{\text{ex}} = 600 \text{ nm}$ , slit: 5 nm/5 nm, PMT Volts: 700).

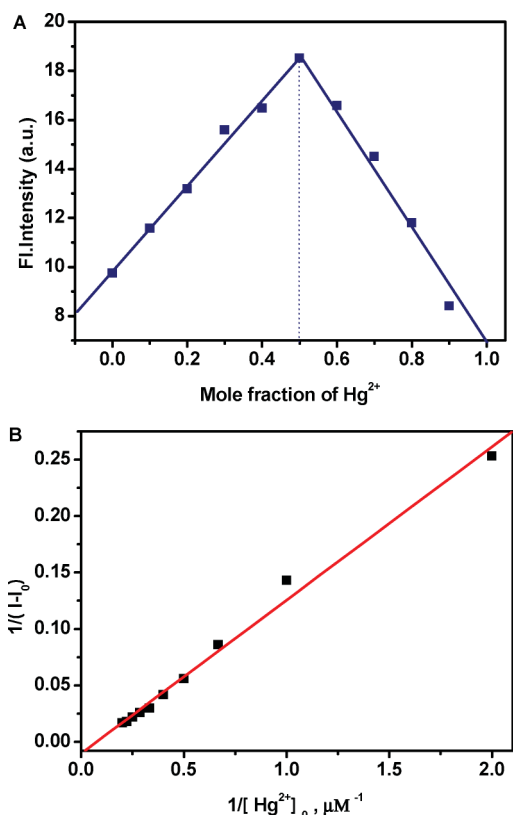


**Fig. 5** Competition experiments of  $\text{Hg}^{2+}$  with other metal ions. Black bar: fluorescence intensity of **USQ-1** (2.5  $\mu\text{M}$ ) with the addition of the respective competing cations (125  $\mu\text{M}$ ) in  $\text{AcOH}:\text{H}_2\text{O}$  (10:90, v/v) solution. Red bar: fluorescence intensity of **USQ-1** (2.5  $\mu\text{M}$ ) with the addition of the respective competing cations (125  $\mu\text{M}$ ) and the  $\text{Hg}^{2+}$  (25  $\mu\text{M}$ ) in  $\text{AcOH}:\text{H}_2\text{O}$  (10:90, v/v) solution. ( $\lambda_{\text{ex}} = 600 \text{ nm}$ , slit: 5 nm/5 nm, PMT Volts: 700).

gave a result of  $4.5 \times 10^{-8} \text{ M}$ , which was close to the limit of  $\text{Hg}^{2+}$  in drinking water (2 ppb) set by the U. S. Environmental Protection Agency (EPA). The complexation mode of **USQ-1** with  $\text{Hg}^{2+}$  was determined by a Job's plot (Fig. 7A),<sup>28–30</sup> which showed a



**Fig. 6** Plot of fluorescence intensity change of **USQ-1** (2.5  $\mu\text{M}$ ) against concentrations of  $\text{Hg}^{2+}$  from 1.0 to 3.5  $\mu\text{M}$  ( $\lambda_{\text{ex}} = 600 \text{ nm}$ , slit: 5 nm/5 nm, PMT Volts: 700.).  $R^2 = 0.99$ ,  $k = 1.32 \times 10^7 \text{ au M}^{-1}$ .



**Fig. 7** (A) Job's plot for the complexation of **USQ-1** with  $\text{Hg}^{2+}$  in  $\text{AcOH}:\text{H}_2\text{O}$  (10:90, v/v) solution. (B) Benesi-Hildebrand plot analysis of the emission changes for the complexation between **USQ-1** and  $\text{Hg}^{2+}$ . ( $\lambda_{\text{ex}} = 600 \text{ nm}$ , slit: 5 nm/5 nm, PMT Volts: 700.).

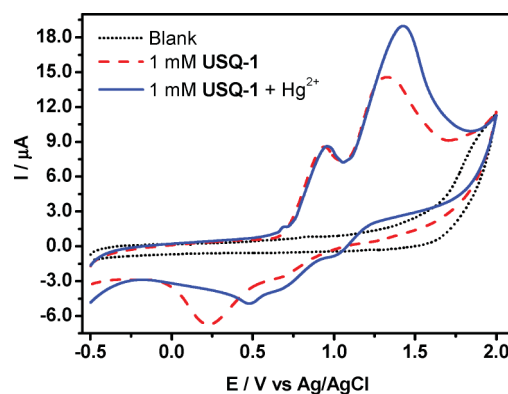
maximum value for the fluorescence intensity at 665 nm when the mole fraction of  $\text{Hg}^{2+}$  reached 0.5, which is a signature of a 1 : 1 binding between the dye and  $\text{Hg}^{2+}$ . This is also confirmed by the Benesi-Hildebrand method.<sup>31-35</sup> For a 1 : 1 association between **USQ-1** and  $\text{Hg}^{2+}$ , the Benesi-Hildebrand equation is given as follows (also see the S4 in the ESI†):

$$\frac{1}{I - I_0} = \frac{1}{K(I_{\text{max}} - I_0)[\text{Hg}^{2+}]_0} + \frac{1}{I_{\text{max}} - I_0} \quad (1)$$

In equation 1,  $I_0$  is the fluorescence intensity of **USQ-1**,  $I$  is the fluorescence intensity obtained with the titration of  $\text{Hg}^{2+}$ ,  $I_{\text{max}}$  is the fluorescence intensity obtained with excess  $\text{Hg}^{2+}$ ,  $K$  is the association constant ( $\text{M}^{-1}$ ), and  $[\text{Hg}^{2+}]_0$  is the concentration of  $\text{Hg}^{2+}$  added. As shown in Fig. 7B, the plot of  $1/(I - I_0)$  against  $1/[\text{Hg}^{2+}]_0$  shows a linear relationship, indicating that **USQ-1** does indeed associate with  $\text{Hg}^{2+}$  in a 1 : 1 stoichiometry. The 1 : 1 complex formation is also confirmed by ESI-MS analysis: a solution containing **USQ-1** and 1 equiv. of  $\text{Hg}(\text{CH}_3\text{COO})_2$  (S5, see the ESI†) shows a strong peak at  $m/z$  987.1, assigned to  $[\text{Hg}^{2+} \cdot \text{USQ-1} \cdot \text{CH}_3\text{COO}]^+$  ion, confirming that **USQ-1** actually forms the 1 : 1 complex.

### Cyclic voltammetric measurements

The cyclic voltammograms (CVs) of **USQ-1** in chloroform using tetrabutylammonium hexafluorophosphate ( $\text{TBAPF}_6$ ) as the supporting electrolyte at a scan speed of  $50 \text{ mV s}^{-1}$  shows two redox waves (Fig. 8). The first oxidation wave (Epa) was at ca. 0.94 V and the second one at ca. 1.32 V, possibly due to the *N,N*-dibutylanilino groups and the DTC-modified anilino-moiety, respectively. Addition of 1.0 equiv.  $\text{Hg}(\text{CH}_3\text{COO})_2$  into the solution of **USQ-1** shifted the oxidation waves (Epa) to more positive values by 0.02 and 0.11 V, that is, from 0.94 to 0.96 V and from 1.32 to 1.43 V. This finding is in accordance with the literature,<sup>36-38</sup> which reported that the oxidation potentials of D-A molecules generally increase as the charge-transfer character of the chromophores decrease. Therefore, the observed increase in the redox potential of **USQ-1** is attributed to the coordination of the DTC arms with  $\text{Hg}^{2+}$  that decreases the charge-transfer character of the chromophore.

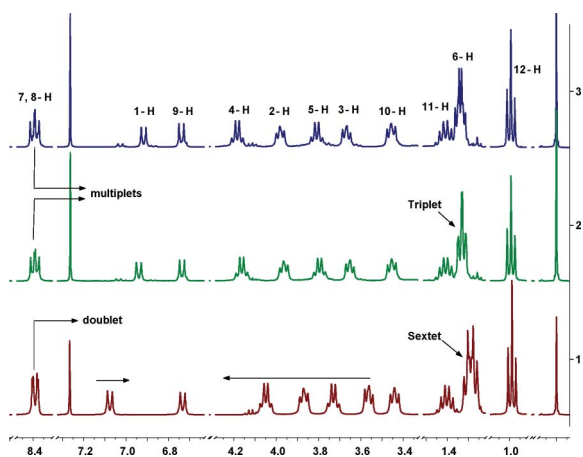


**Fig. 8** Cyclic voltammogram of **USQ-1** (1.0 mM) in chloroform using 0.1 M  $\text{TBAPF}_6$  as a supporting electrolyte; scan rate:  $50 \text{ mV s}^{-1}$ .

### <sup>1</sup>H NMR binding studies

The binding of **USQ-1** with  $\text{Hg}^{2+}$  was further confirmed by the <sup>1</sup>H NMR spectra (Fig. 9 and S6, see the ESI†). The binding effects are transmitted to the ring atoms by delocalization due to the typically zwitterionic structure of squaraines.<sup>39,40</sup> The <sup>1</sup>H NMR spectrum of **USQ-1** in  $\text{CDCl}_3$  shows four magnetically non-equivalent aromatic protons as doublets at 6.73, 7.07, 8.38 and 8.40 ppm; whereas the four different pairs of *N,S*-methylenes (located on DTC) appeared as multiplets in the region of 3.56–4.05 ppm, and the two overlapping triplets from different  $\text{CH}_3$



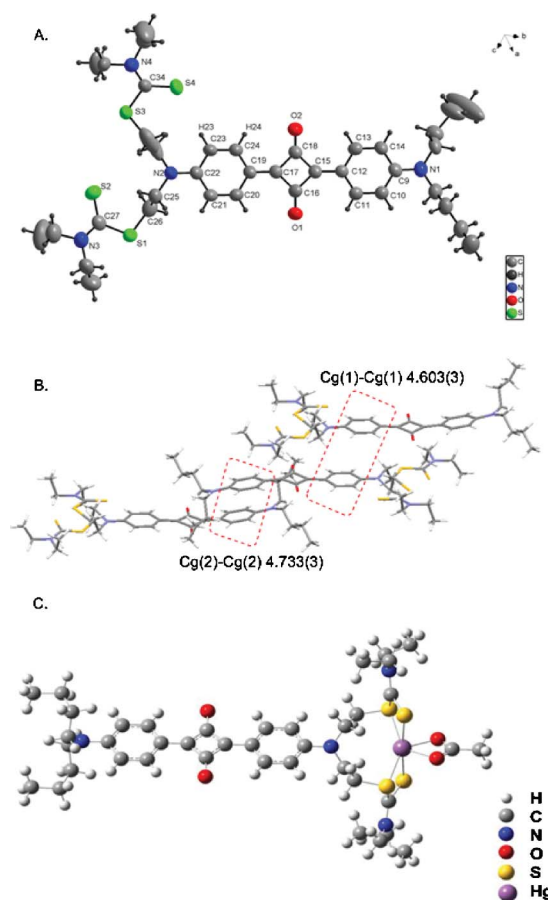


**Fig. 9**  $^1\text{H}$  NMR spectra of **USQ-1** in  $\text{CDCl}_3$ , with increasing concentration of  $\text{Hg}(\text{CH}_3\text{COO})_2$ . The mole ratio of  $[\text{Hg}(\text{CH}_3\text{COO})_2]$  to  $[\text{USQ-1}]$  is (1) 0, (2) 0.5, (3) 1.2, respectively.

of two nonequivalent *N*-ethyl groups are located near 1.29 ppm. Upon addition of  $\text{Hg}(\text{CH}_3\text{COO})_2$  to the solution, the 7.07 ppm aromatic signal shifts to higher field ( $\Delta\delta = 0.15$ ), and the *N,S*-methylene signals (2, 3, 4 and 5-H as in Scheme 1) move to lower field ( $\Delta\delta = 0.11$ ) incrementally. The 8.38 and 8.40 ppm aromatic signals merge and form a multiplet at 8.39 ppm. It is interesting to note that upon coordination of the DTC group the methyl signals change from a sextet to a triplet, with a very small downfield shift ( $\Delta\delta = 0.05$ ). This could be attributed to the reversible exchange between the complexed and the uncomplexed states. While these phenomena require further justification, the  $\text{Hg}^{2+}$  coordination with the DTC unit is clearly demonstrated. The signals of the 10, 11 and 12-H belonging to *N,N*-dibutylanilino moiety are not changed, either in peak values or in peak shape, indicating the absence of a significant effect upon the *N,N*-dibutylanilino group during the coordination.

### X-Ray crystallography and theoretical calculations

A crystal structure of squaraine **USQ-1** was obtained from crystals prepared by the slow diffusion of petroleum ether : ethyl acetate (1 : 1, v/v) mixture into a chloroform solution of the dye molecule for several days (Fig. 10A). The molecule has a highly planar geometry through the central cyclobutenedione and the six-membered aromatic rings. The bond lengths of C(14)–C(9), C(12)–C(13), C(13)–C(14), C(12)–C(15), C(15)–C(18), C(17)–C(18), C(17)–C(19), C(19)–C(24), C(22)–C(23), C(23)–C(24) lie in the range 1.367(7)–1.474(7) Å, which are typical for conjugated double-bond lengths, and are in good agreement with other published squaraine dyes.<sup>41</sup> However, the carbonyl bond lengths (C(18)–O(2) 1.239(6) Å, C(16)–O(1) 1.234(6) Å) are a little longer than a typical carbonyl bond length. This is attributed to the zwitterionic nature of the dye. The bond lengths of C(34)–N(4) (1.320(7) Å), C(27)–N(3) (1.324(7) Å), C(34)–S(4) (1.665(6) Å) and C(27)–S(2) (1.661(6) Å) are characterized as the double-bond, which is ascribed to the structure of dithiocarbamate attaching to the anilino side chain. It is clear from the X-ray crystal structure that **USQ-1** is planar, while the side arms (DTC) are freely arranged in space. The crystal packing shows that each dye molecule is offset, such that the two electron-rich aniline rings



**Fig. 10** (A) The crystal structure of **USQ-1**. The thermal ellipsoids are shown at 50% probability. (B) The crystal  $\pi$ – $\pi$  stacking of the aromatic rings. (C) A proposed geometry-optimized structure of the  $\text{Hg}^{2+}$  complex with **USQ-1** (geometry is optimized using a semiempirical PM3 calculation).

have  $\pi$ – $\pi$  stacking with the other dye molecule in parallel planes at the distances of 4.603(3) Å and 4.733(3) Å, respectively (see Fig. 10B and S8 in the ESI†). These experimental data indicate a weak  $\pi$ – $\pi$  stacking interaction between the dye molecules and are in agreement with the formation of aggregates in solution.

To further study the coordination geometry of **USQ-1** and  $\text{Hg}^{2+}$ , theoretical calculations were performed with the Gaussian 03 program at the semiempirical PM3 levels based on the X-ray crystal structure of **USQ-1**.<sup>42,43</sup> According to the ESI-MS analysis of the complex formation and taking the solvent effect into account, we find an octahedral structure including an acetate. As shown in Fig. 10C, the DTC arms are twisted and coordinated to  $\text{Hg}^{2+}$  associated with an acetate, *i.e.*, there are four sulfur atoms from DTC and two oxygen atoms of the acetate participating in the coordination. The structure is extended in space and the steric bulk of the complex might unfold the aggregates, resulting in the color rebound and the fluorescence enhancement. In addition, the central four-membered squarate ring and the six-membered aryl rings keep a rigid structure in the plane, which possesses a highly  $\pi$ -delocalized polymethine chromophore with partial double bond character. It confers upon the complex excellent fluorescence properties.

## Conclusion

In conclusion, an unsymmetrical squaraine dye (**USQ-1**) was synthesized as a selective colorimetric and fluorescent sensor for  $\text{Hg}^{2+}$  in aqueous media. A color change and “turn-on” fluorescence were observed only for  $\text{Hg}^{2+}$ , which can be attributed to the selective deaggregation process of the aggregates. It induced a *ca.* 10-fold enhancement in fluorescence intensity when the detection limit was as low as  $4.5 \times 10^{-8}$  M. The Job's plot and Benesi–Hildebrand plot support the 1 : 1 binding mode of **USQ-1** and  $\text{Hg}^{2+}$ . We have shown that aggregation, which is a drawback in the case of many organic dyes, becomes a useful principle in the design of a  $\text{Hg}^{2+}$  specific chemosensor. It has high potential for the design of new chromogenic  $\text{Hg}^{2+}$  chemosensors for the quantitative determination of  $\text{Hg}^{2+}$  with multiple channels of measurements.

## Experimental

### General methods

All solvents were purified and re-distilled by standard methods prior to use. Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers and used without further purification. The syntheses and manipulations of squaraine dyes were carried out under dry nitrogen.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker AV-400 spectrometer (400 MHz). The chemical shifts were measured relative to TMS (0.00 ppm) for  $\text{CDCl}_3$ , as indicated. Mass spectra (ESI) was recorded on DECAX-30000 LCQ Deca XP ion trap mass spectrometers. Elemental analysis was carried out by using an Elementar Vario MICRO analyzer. Absorption spectra were detected on a Perkin Elmer Lambda 750 UV spectrophotometer. Fluorescent emission spectra were collected on a Cary Edipse fluorescence spectrophotometer. Cyclic voltammograms (CVs) were recorded with a CHI 620B electrochemical analyzer (ChenHua Instrument, Shanghai, China) by a conventional three-electrode configuration. The melting points of compounds were determined with SGW X-4 without correction. Compound **i** and **ii** were obtained as reported.<sup>10,19,20</sup>

**Compound USQ-1.** In a 50 mL flask, **i** (100 mg, 0.23 mmol) and **ii** (75 mg, 0.25 mmol) were dissolved in a solution of 15 mL *n*-butanol and 15 mL benzene. Then the reaction mixture was refluxed overnight under  $\text{N}_2$  protection. After cooling, the blue powder was precipitated and filtered. After washing with ether, the crude product was purified by column chromatography over silica gel. Elution of the column with a mixture of dichloromethane and ethyl acetate (10 : 1, v/v) gave the desired unsymmetrical squaraine dye **USQ-1** as blue crystals (96 mg, 58%). m.p.: 193–195 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.98 (t,  $J = 7.4$  Hz, 6H), 1.26–1.32 (m, 12H), 1.36–1.45 (m, 4H), 1.61–1.69 (m, 4H), 3.44 (t,  $J = 7.8$  Hz, 4H), 3.56 (t,  $J = 7.4$  Hz, 4H), 3.73 (q,  $J = 7.0$  Hz, 4H), 3.87 (t,  $J = 7.4$  Hz, 4H), 4.05 (q,  $J = 7.0$  Hz, 4H), 6.73 (d,  $J = 9.2$  Hz, 2H), 7.07 (d,  $J = 9.2$  Hz, 2H), 8.38 (d,  $J = 2.2$  Hz, 2H), 8.40 (d,  $J = 2.2$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.6, 12.5, 13.8, 14.1, 20.2, 29.6, 33.1, 46.9, 49.8, 51.3, 112.5, 112.9, 119.5, 120.6, 132.9, 133.7, 153.1, 153.9, 183.4, 187.2, 189.4, 194.2; MS (ESI):  $m/z$  727.2 ( $[\text{M} + \text{H}]^+$ ); Anal. Calcd for  $\text{C}_{38}\text{H}_{54}\text{N}_4\text{O}_2\text{S}_4$ : C, 62.77; H, 7.49; N, 7.71; Found: C, 62.34; H, 7.37; N, 7.52.

## Preparation for the metal ion binding studies

Twice-distilled water was used throughout all experiments. Metal salt solutions of  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ag}^+$ ,  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  were prepared in twice-distilled water. The squaraine dye **USQ-1** was dissolved in  $\text{AcOH}:\text{H}_2\text{O}$  (10 : 90, v/v) solution (2.5  $\mu\text{M}$ ). Metal ion consecutive titrations were carried out by adding small volumes (0.5–1.0  $\mu\text{L}$ ) of the metal salt solutions into 2 mL  $\text{AcOH}:\text{H}_2\text{O}$  solution in a quartz cuvette every time. After the addition of metal salt solution to the cuvette using a microliter syringe, the solution was mixed well on a lab dancer and was left to stand for 15 s before recording the absorption and emission spectra.

## X-Ray crystallography

Crystals of  $\text{C}_{38}\text{H}_{54}\text{N}_4\text{O}_2\text{S}_4$  grew as blue-green blocks by vapor diffusion of petroleum ether:ethyl acetate (1 : 1, v/v) into a chloroform solution of the dye molecules. The data crystal was a block with approximate dimensions:  $0.25 \times 0.24 \times 0.18$  mm and was recorded at the temperature of 293(2) K. Crystal data for **USQ-1**:  $\text{C}_{38}\text{H}_{54}\text{N}_4\text{O}_2\text{S}_4$ ,  $M = 727.09$ , triclinic,  $a = 11.207(2)$  Å,  $b = 12.542(3)$  Å,  $c = 15.394(3)$  Å,  $\alpha = 70.90(3)^\circ$ ,  $\beta = 85.07(3)^\circ$ ,  $\gamma = 89.15(3)^\circ$ ,  $V = 2036.9(7)$  Å<sup>3</sup>,  $T = 293(2)$  K, space group  $P\bar{1}$ ,  $Z = 2$ , 17264 reflections measured, 8989 independent reflections ( $R_{\text{int}} = 0.0475$ ). The final  $R_1$  values were 0.1262 ( $I > 2\sigma(I)$ ). The final  $wR(F^2)$  values were 0.2922 ( $I > 2\sigma(I)$ ). The final  $R_1$  values were 0.1860 (all data). The final  $wR(F^2)$  values were 0.3362 (all data).

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