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A fully-aqueous red-fluorescent probe for selective optical sensing of Hg^{2+} and its application in living cells

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ABSTRACT

A new red-fluorescent mercury ion sensor material is designed and synthesized, which is composed of a tweezer-shaped hydrophilic probe containing bifurcated soft-base atoms N and S coupled with 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF) unit. By virtue of the strong electron-accepting nature of TCF unit (as a push–pull chromophore), this designed sensor material can selectively detect Hg^{2+} over various tested metal ions in a 100% aqueous medium via naked-eye and photoluminescence (PL) observations. Theoretical and time-resolved photoluminescence measurements further confirmed the selectivity and reversibility of the probe towards Hg^{2+} via intramolocular charge transfer mechanism in this sensor material. Moreover, the living cell tests by confocal fluorescence images of this sensor material towards Hg^{2+} were also investigated. Finally, distinguished absorption changes and fluorescence quenching spectral appearances allowed us to present the selective optical indicator of Hg^{2+} via TCF moiety for the first time.

1. Introduction

Despite the vital roles of many metal ions in sustaining life, it is required to have precise control to maintain an ecological balance in living cells. However, the accumulations of heavy metal ions in environment within the approved environmental protection agency limits are highly challenging. Among all heavy metal ions, Hg^{2+} is extremely toxic owing to its lipophilic nature of organo-mercury (CH_3HgX) under environmental aqueous media [1–3]. Mercury ingestion could cause fatal damage to human central nervous and endocrine systems [4,5]. Due to its unique chemical properties and lethal effects on environment along with living organism, in recent years rapid, selective and sensitive detections of mercury ions are intriguing targets [6–10]. Consequently, myriads of chemosensors towards sensitive Hg^{2+} detections based on chelation-enhanced fluorescence [11–20] and fluorometric chemodosimeter [21–30] approaches have long been designed and discussed. However, conventionally most of those chemosensing probes in selective fluorometric detections of Hg^{2+} are suffered owing to a fact that fluorescence intensities of probe molecules could be altered by photo bleaching, concentration variations of probe ensembles and heterogeneities of surrounded microenvironments. Thus, developing Hg^{2+} selective optical indicators are highly demanding.

An exceptional electron-accepting capability of 3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF) make it as an excellent moiety for the applications of nonlinear optoelectronics [31–33]. However, its unique push–pull chromophoric nature stimulated scientists recently in designing novel red fluorophores for the applications of bioimaging- [34–37], pH sensing- [38,39], and chemosensing- [40–43] platforms, as well as dye sensitized solar cells (DSSCs) [44]. Liao et al. have demonstrated negative photo-chromism of a TCF chromophore [45]. Recently, Yu et al. have presented a near-infrared TCF-based probe for the colorimetric and ratiometric detection of SO_2 [46]. Cho and co-workers have reported a selective optical TCF-based indicator for Hg^{2+} via an Hg^{2+} mediated intramolecular cyclization reaction of ethynyl phenols [47]. However, to the best our knowledge the selective and sensitive optical indicator based on the

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TCF moiety towards Hg^{2+} via chelation induced large hypsochromic shifted chemosensing ensemble has never been reported.

Herein, we demonstrate the first selective optical indicator **T2** towards Hg^{2+}, which was constructed by bridging an electron-withdrawing TCF moiety with a water soluble and well-known thiophilic ligand [48,49]. The utilization of the TCF moiety in this work as a strong electron-delocalizing unit via its flexible push–pull feature leads to an unprecedented sensing ensemble **T2**. It displays a 100% water solubility with surprisingly large blue-shifted photophysical properties of the selective detection towards Hg^{2+} via a H-type self-association and alterable ICT of the TCF moiety and yields a better live cell permeability as shown in Scheme 1. Prominently, the selective detection of probe **T2** towards Hg^{2+} was completely reversible upon the addition of EDTA, which proves its practical utility.

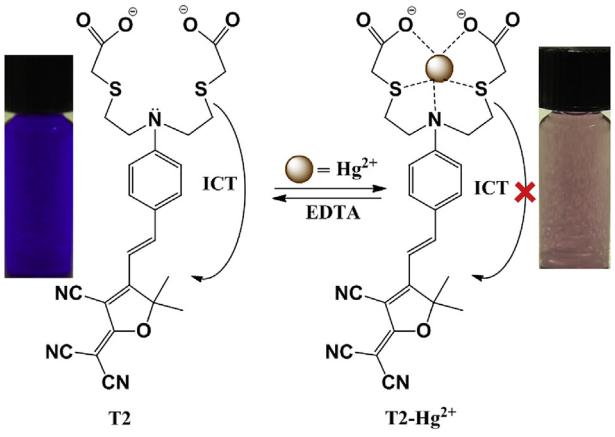
2. Experimental section

2.1. Materials

All chemicals and solvents were used are reagent grades and HPLC grades respectively and were purchased from Aldrich, ACROS, Fluka, TCI, TEDIA, and Lancaster Chemical Co. All chemicals were used without any further purification. Anhydrous solvents were obtained by passing through activated alumina column purification system, further dried by standard drying procedures. Solvents were degassed by freeze/thaw/pump cycle technique prior to use. Thin layer chromatographies (TLC) were performed on glass plate coated with silica 60 F24 (Merck). The plates were visualized using ultraviolet light (256 nm) and developed using I_2 chamber. Flash chromatographies were performed on Merck silica gel 60 (230–400 mesh) under pressure using desired solvents.

2.2. General characterization methods

All reactions and operations were carried out under an atmosphere of inert argon or nitrogen using Schlenk techniques unless otherwise stated. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-300 Avance series or on a Varian Inova 400, Inova 500, and Inova 600 Series (^{1}H: 300, 400, 500, and 600 MHz; ^{13}C: 75, 100, 125, and 150 MHz) at a constant temperature of 298 K. Chemical shifts were reported in parts per million from low to high field and referenced to residual solvent ($CDCl_3$, DMSO-d_6: ^{1}H δ = 7.26, 2.49 ppm and ^{13}C δ = 77.23, 39.52 ppm, respectively). Coupling constant (J) were reported in Hertz (Hz). UV–Vis spectra were recorded on the Jasco UV-600 spectrophotometer using 1 cm quartz cuvette. Fluorescence measurements were conducted with HITACHI 7000 Series Spectrophotometer. All emission and excitation spectra were corrected for the detector response and the lamp output. Melting points were determined using a Fargo MP-2D apparatus and are uncorrected. Elemental analyses were conducted on HERAEUS CHN-OS RAPID elemental analyzer. Time resolved photoluminescence (TRPL) spectra were measured using a home built single photon counting system with excitation from a 400 nm diode laser (Picoquant PDL-200, 50 ps fwhm, 2 MHz). The signals collected at the excitonic emissions of all sample solutions were connected to a time-correlated single photon counting card (TCSPC, Picoquant Timeharp 200). The emission decay data were analyzed for **T2** and complex **T2**-Hg with biexponential kinetics, from which two decay components were derived; the lifetime values of (τ1, τ2) and pre-exponential factors (A1, A2) were determined. Confocal imaging was carried out using Leica TCS SP8 confocal fluorescence microscope, confocal fluorescence imaging with using 60 × times oil objective. DFT M06-2X method calculations were calculated using Gaussian-09 suite.



Scheme 1. Proposed sensing mechanism of **T2** towards Hg^{2+}.

2.3. Cell culture and imaging

The human cervical cancer cell line (HeLa cells) were seeded onto cover slips at a concentration of (2 × 105 cells/mL) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) and 10% fetal bovine serum in an incubator (37 °C, 5% CO_2, and 25% O_2). After 30 h, the cover slips were rinsed slightly 3 times with PBS to remove the media and then cultured in PBS for later use. In view of imaging procedure, initially cells were incubated with 5 μM of probe **T2** alone for 20 min at 37 °C and observed under microscope and then again the samples were treated with $HgCl_2$ (10 μM) incubated for 20 min and then again observed under microscope and then the samples were treated with EDTA (10 μM) incubated for 20 min and moved to the confocal stage. All the samples were slightly rinsed for 3 times with PBS buffer before observing them under the microscope. All the cell images were obtained with Leica TCS SP8 confocal fluorescence microscope using 60 × times oil objective.

2.4. Stock solutions

Standard solution of probe **T2** (100 mM) were prepared in double distilled water and $HgCl_2$, other metal ions stock solutions with a concentration of 10 mM were prepared, respectively in water. Before the titrations probe and analytes were diluted to their desired volumes.

2.5. Synthesis of probe *T2*

2.5.1. Synthesis of diethyl2,2′-((((4-formylphenyl)azanediyl) bis(ethane-2,1-diyl))bis(sulfanediyl))diacetate-(4)

In a 25 mL dried round bottomed flask, sodium (0.48 g, 20 mmol) was added to anhydrous ethanol (12 mL). After sodium was dissolved completely, ethyl-2-mercaptoacetate (2.40 g, 23 mmol) was added dropwise. The mixture was stirred for 2 h at 40 °C. Then a solution of 4-(bis (2-chloroethyl) amino) benzaldehyde (**3**) (2.24 g, 9 mmol) in DMF (5 mL) was added. The stirring was continued for another 3 h. Water (20 mL) was added to the residual and extracted with dichloromethane (3 × 10 mL). The combined organic phase was washed twice with water and dried over anhydrous magnesium sulfate. The solvent was removed by evaporation and dried in vacuum, yielding a yellow oil of compound **4** (3.58 g, 95%). The structure of **4** was confirmed by ^{1}H NMR and ^{13}C NMR, which are shown as follows: ^{1}H NMR (500 MHz, CDCl3): δ 9.58 (s, 1H), 7.61 (d, J = 8.5 Hz, 2H), 6.63 (d, J = 9.0 Hz, 2H), 4.07 (q, J = 14.2 Hz, 4H), 3.56 (t, J = 7.5 Hz, 4H), 3.17 (s, 4H), 2.76 (t, J = 7.5 Hz, 4H), 1.16 (t, J = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, CDCl3):