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A new aggregation-induced emission-based fluorescent probe for effective detection of Hg^{2+} and its multiple applications

Yongxin Pan[a,1], Yuetian Guo[a,1], Ying Li[a], Lijun Tang[a,b,*], Xiaomei Yan[c,**]

[a] College of Chemistry and Materials Engineering, Bohai University, Jinzhou 121013, China
[b] Department of Chemistry, National Demonstration Center for Experimental Chemistry Education, Yanbian University, Yanji 133002, China
[c] College of Laboratory Medicine, Dalian Medical University, Dalian 116044, China

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ABSTRACT

Detection of mercury ions (Hg^{2+}) in actual samples is of significant importance due to the toxicity of Hg^{2+} to human health. In this work, a simple tetraphenylethene (TPE) derived fluorescent probe **TPE-Hg** based on aggregation-induced emission (AIE) mechanism was synthesized. **TPE-Hg** can visually recognize Hg^{2+} in THF/HEPES (1:9, v/v, HEPES 20 mmol/L, pH 7.3) system with rapid response, strong anti-interference ability, large Stokes shift (203 nm), and low detection limit (7.548×10^{-7} mol/L). The results show that Hg^{2+} triggered elimination of **TPE-Hg** lead to releasing of an AIE-active compound **2** is responsible to the sensing mechanism. **TPE-Hg** is applicable to detect Hg^{2+} in actual water samples and image Hg^{2+} in living MCF-7 cells. In addition, **TPE-Hg** is suitable to assay the Hg^{2+} level in seafood and tea samples, and it is also applicable in test strips.

Mercury is one of the most toxic elements that widely exists in water and soil [1,2]. The human living environment is heavily contaminated with mercury ions (Hg^{2+}) as a result of industrial development [3,4]. Hg^{2+} has a persistent, non-degradable toxicity profile due to plant uptake and food chain transport [5–7]. Hg^{2+} in water is enriched in aquatic organisms (such as various seafood), and Hg^{2+} is transmitted to the human body through the food chain [8]. When excessive Hg^{2+} is enriched in the body, bodily functions are impaired, causing a range of diseases [9–12]. The Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) has proposed permissible limits for mercury in food. The limit of Hg^{2+} in different foods has been determined by the Chinese Ministry of Health [13–15]. Therefore, it is very important to detect Hg^{2+} in living cells and food samples.

During the past decades, many methods have been developed to detect mercury [16–20]. However, most of these methods require excessive financial and human resources. In contrast, fluorescence techniques are more suitable for the detection of Hg^{2+} due to their rapidity, sensitivity, selectivity and low cost [21–24]. During recent years, a tremendous amount of fluorescent probes for Hg^{2+} detection have been documented [25–29], but there are still very few fluorescent probes applied to the detection of Hg^{2+} in food, and some fluorescent probes have small Stokes shifts, and slow response speed [30–32]. On the other hand, some fluorescent probes can only recognize Hg^{2+} in a medium with high organic solvent content due to the structural characteristics of aggregation-caused quenching (ACQ), which also seriously affects the practicability of the probe [33,34]. Therefore, fluorescent probes that can rapidly detect Hg^{2+} in a solution with high water content and can overcome the ACQ effect need to be developed urgently, especially the recognition process has the characteristics of color deepening and fluorescence enhancement, and the probe can be used for the detection of Hg^{2+} in foods and living cell imaging.

Tetraphenylethylene (TPE), as a typical aggregation-induced emission (AIE) luminogen (AIEgen), can effectively avoid the adverse effects of ACQ, and is widely used in the field of fluorescent probes due to its large Stokes shift and good photostability [35,36]. In this work, we synthesized a simple fluorescent probe **TPE-Hg** based on TPE derivatives (Scheme 1). It is expected that Hg^{2+} can be specifically recognized by **TPE-Hg** at high water content. The design principle of the probe is based on the listing assumptions: (1) Compound **2**, similar to other TPE derivatives, is very likely to have AIE properties; (2) The hydroxyethyl thioether moiety in **TPE-Hg** can act as a reactive recognition site through Hg^{2+} promoted elimination reaction due to the thiophilic nature of Hg^{2+}; (3) It is speculated that Hg^{2+} can trigger the partial desulfurization in

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[*] Corresponding author at: College of Chemistry and Materials Engineering, Bohai University, Jinzhou 121013, China.

[**] Corresponding author.

E-mail addresses: ljtang@bhu.edu.cn (L. Tang), xmyan1978@sina.com (X. Yan).

[1] These authors contributed equally to this work.

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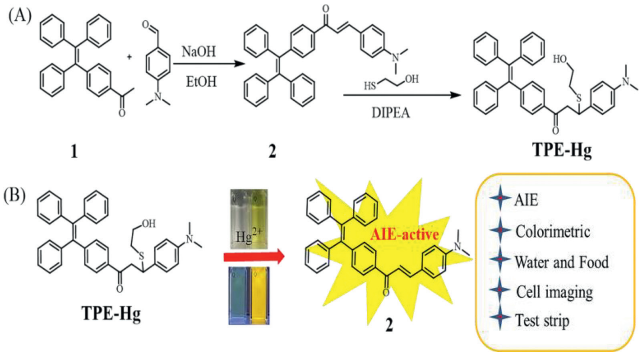

Scheme 1. (A) Synthetic route of probe **TPE-Hg**, (B) the possible sensing mechanism of **TPE-Hg** toward Hg^{2+}.

TPE-Hg and release its precursor compound **2**, thus resulting in solution color deepening and fluorescence enhancement.

Our subsequent studies reveal that Hg^{2+} can be visually recognized by **TPE-Hg** in THF/HEPES (1:9, v/v, HEPES 20 mmol/L, pH 7.3) system with advantages of strong anti-interference ability, large Stokes shift (203 nm), low detection limit (7.548×10^{-7} mol/L), and rapid response. **TPE-Hg** has been determined to be suitable for the detection of Hg^{2+} in actual water samples and living cells. In addition, **TPE-Hg** can be used as a tool to assay Hg^{2+} level in seafood and tea samples, and **TPE-Hg** also has the applicability in test strips.

Based on the design principles of the probe, the AIE characteristics of compound **2** were firstly evaluated. The changes in fluorescence behavior of **2** were examined in mixed solutions of THF/HEPES (20 mmol/L, pH 7.3) with different HEPES contents (f_W, volume fraction) (Fig. 1A, Fig. S7 in Supporting information). When f_W varied from 0 to 70%, the emission intensity of **2** gradually decreased and the emission wavelength gradually red-shifted, and the fluorescence intensity reached the lowest value when $f_W = 70\%$. With the increase of HEPES fraction, the solvent polarity was gradually increased, and compound **2** exhibits typical twisted intramolecular charge transfer (TICT) properties under this condition [37,38]. Upon further increase f_W, the fluorescence intensity at 557 nm gradually enhanced, the fluorescence intensity reached the strongest when f_W was 95%, which may due to the aggregation of compound **2**. When a solution of compound **2** in THF/HEPES (1:9, v/v) was irradiated with a laser pointer, an obvious light path was observed (Fig. S8 in Supporting information), and the Tyndall phenomenon indicates that compound **2** formed aggregates. However, in THF/HEPES (9:1, v/v) solution, no observable Tyndall phenomenon was found. Based on these results, we can conclude that compound **2** has AIE properties.

Changes in ultraviolet–visible spectrum (UV–vis) absorption and fluorescence spectra of **TPE-Hg** (10 μmol/L) induced by Hg^{2+} were recorded in a THF/HEPES (1/9, v/v, HEPES 20 mmol/L, pH 7.3) test system. Addition of Hg^{2+} caused a distinct absorption peak at 445 nm for **TPE-Hg** (Fig. 1B, Figs. S9 and S10 in Supporting information), and the fluorescence intensity at 553 nm was significantly enhanced (Fig. 1C). The fluorescence intensity and emission wavelength were similar to those of compound **2**, and it was tentatively concluded that the recognition product was probably compound **2**. When other interfering ions were present, **TPE-Hg** still showed good recognition to Hg^{2+} (Fig. 1D), indicating that **TPE-Hg** has good selectivity and interference suppression ability. According to the results of the titration experiments (Fig. 1E), the fluorescence intensity of **TPE-Hg** (553 nm) showed a good linear correlation ($R^2 = 0.996$) with the Hg^{2+} concentration (0–10 μmol/L). Based on the equation LOD = 3S/K (S represents the standard deviation of the blank solution and K represents the slope of the linear regression) [39], the limit of detection of **TPE-Hg** for Hg^{2+} was calculated to be 7.548×10^{-7} mol/L (Fig. S11 in Supporting information). These results indicate that **TPE-Hg** is highly sensitive. The pH test results demonstrate that **TPE-Hg** can recognize Hg^{2+} well in near neutral pH environments (Fig. 1F), which provides a favorable basis for ap-

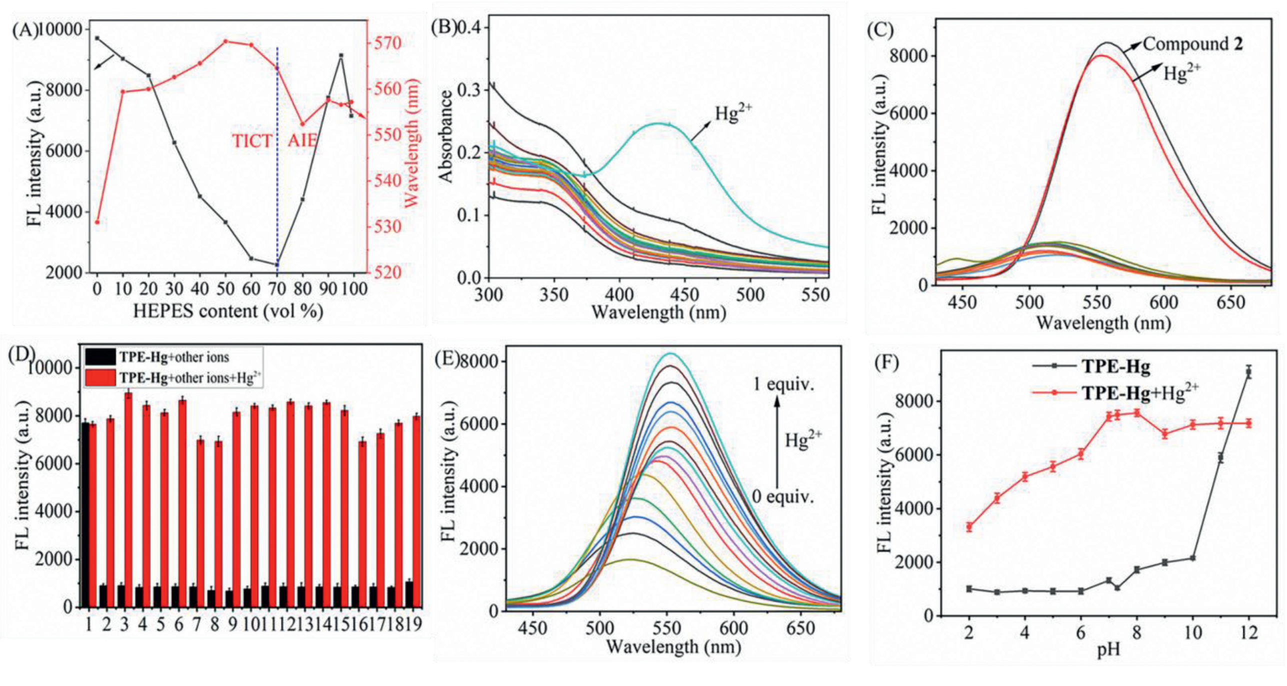

Fig. 1. (A) Changes in fluorescence intensity of compound **2** at the maximum emission wavelength in THF/HEPES with different f_W. (B) UV–vis absorption spectra and (C) fluorescence spectra of probe **TPE-Hg** with different metal ions. (D) The fluorescence intensity (at 553 nm) of **TPE-Hg** solution with various cations. 1. Hg^{2+}; 2. Al^{3+}; 3. Ba^{2+}; 4. Ca^{2+}; 5. Cd^{2+}; 6. Co^{2+}; 7. Cr^{3+}; 8. Cu^{2+}; 9. Fe^{2+}; 10. Fe^{3+}; 11. K^{+}; 12. Mg^{2+}; 13. Mn^{2+}; 14. Na^{+}; 15. Ni^{2+}; 16. Pb^{2+}; 17. Pd^{2+}; 18. Zn^{2+}; 19. Ag^{+}. (E) Changes in fluorescence spectrum of **TPE-Hg** (10 μmol/L) upon titration with Hg^{2+} (0–10 μmol/L). (F) Fluorescence intensities changes of **TPE-Hg** and **TPE-Hg**+Hg^{2+} at different pH conditions. mean ± standard deviation (SD), $n = 3$.

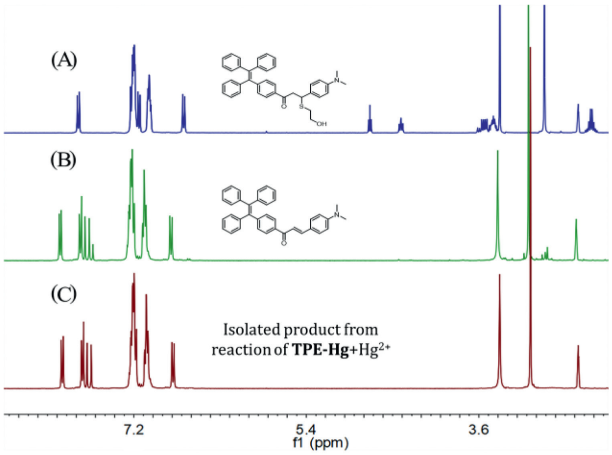

Fig. 2. Partial ^{1}H NMR spectra comparison of **TPE-Hg** (A), compound **2** (B) and isolated product from **TPE-Hg**+Hg^{2+} reaction (C) in DMSO–d_6.

plication in cellular imaging. Time-dependent experiments showed a rapid response of **TPE-Hg** to Hg^{2+} within about 4 min (Fig. S12 in Supporting information).

According to literature reports [40], the hydroxyethyl thioether moiety can be removed by Hg^{2+} and the α,β-unsaturated ketone can be obtained. To verify the recognition mechanism proposed, we examined the HRMS of **TPE-Hg**+Hg^{2+} (Fig. S13 in Supporting information). The significant peak observed at $m/z = 506.2487$ can be attributed to compound **2** ($[M+H]^+$, calcd. 506.2478). Furthermore, the reaction product was separated and its proton nuclear magnetic resonance (^{1}H NMR) spectrum was compared with that of **TPE-Hg** and compound **2**. The results show that the isolated product and compound **2** have almost identical ^{1}H NMR spectrum (Fig. 2), indicating that Hg^{2+} triggered elimination of the hydroxyethyl thioether moiety in **TPE-Hg** to afford compound **2** (Scheme 1B).

To verify the practical application of **TPE-Hg** in environmental systems, we selected different actual water samples for fluorescence testing. For each sample, there is a good linearity between the emission intensity and the added Hg^{2+} concentration ($R^2 > 0.99$, Fig. S14 in Supporting information). As shown in Table S1 (Supporting information), for the detection of Hg^{2+} in actual water samples, the recovery of this method is in the range of 82.10%–105.33%, the relative standard deviation (RSD) is between 0.93%–3.90%, manifesting that **TPE-Hg** has good accuracy for assaying Hg^{2+} in real water samples and can be practically applied in water environmental systems.

To further explore the potential applicability of **TPE-Hg**, its use in detecting Hg^{2+} in some food samples was examined. For each sample, there is a good linear function between the emission intensity and the added Hg^{2+} concentration ($R^2 > 0.99$, Figs. S15 and S16 in Supporting information). As shown in Table S2 (Supporting information), for the detection of Hg^{2+} in seafood and tea samples, the recovery of this method is in the range of 89.88%–105.06%, the RSD is between 2.26% and 9.71%, indicating that **TPE-Hg** has good accuracy for assaying Hg^{2+} in actual food samples.

The low toxicity of **TPE-Hg** was confirmed by cell counting kit-8 (CCK-8) assay before cell imaging experiments (Fig. S17 in Supporting information). On this basis, it was investigated whether **TPE-Hg** could visually recognize exogenous Hg^{2+} in MCF-7 cells. **TPE-Hg** (20 μmol/L) and live cells were incubated in the medium at room temperature for 30 min, no fluorescence was observed in the dark field, and weak green luminescence can be seen in the dark field. When 10 μmol/L Hg^{2+} was added, with the increase of Hg^{2+} concentration (30, 50, 100 μmol/L), the yellow-green fluorescence in the dark field gradually increased (Fig. 3A). These results prove that the probe **TPE-Hg** is capable to image Hg^{2+} in living MCF-7 cells.

As shown in Fig. 3B, the paper strips gradually turns yellow under natural light when the Hg^{2+} concentration reached 100 μmol/L. On irradiation at 365 nm, the color of the filter paper without Hg^{2+} was similar to that of the ordinary filter paper. As the Hg^{2+} concentration increasing, the fluorescence color of the test strips gradually changed from light green to bright yellow. It shows that **TPE-Hg** can be developed into a portable Hg^{2+} detection test paper for practical detection.

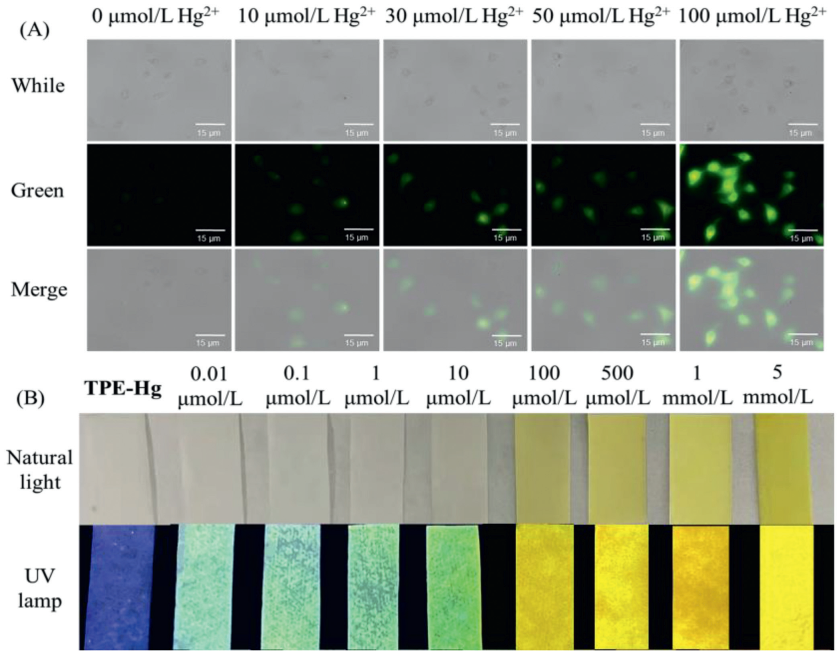

Fig. 3. (A) Fluorescence images of MCF-7 cells incubated with probe **TPE-Hg** (20 μmol/L) for 30 min and then incubated with Hg^{2+} (0, 10, 30, 50, 100 μmol/L). Scale bar: 15 μm. (B) Photographs of test strips added with different concentrations of Hg^{2+} under natural light and UV light (365 nm).

In conclusion, we have developed a new TPE derived fluorescent probe **TPE-Hg** based on AIE mechanism. The probe can specifically recognize Hg^{2+} in THF/HEPES (1:9, v/v, HEPES 20 mmol/L, pH 7.3) solution with distinct color and fluorescence emission changes. The recognition process has some advantages including good anti-interference ability, rapid response (4 min), applicable in a wide pH range, low detection limit (7.548×10^{-7} mol/L), and a large Stokes shift (203 nm). **TPE-Hg** is applicable to detect Hg^{2+} in real water samples, seafood and tea, and also can be applied to test strips for assay Hg^{2+} content. The results show that the probe **TPE-Hg** has good potential applicability.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.108237.

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