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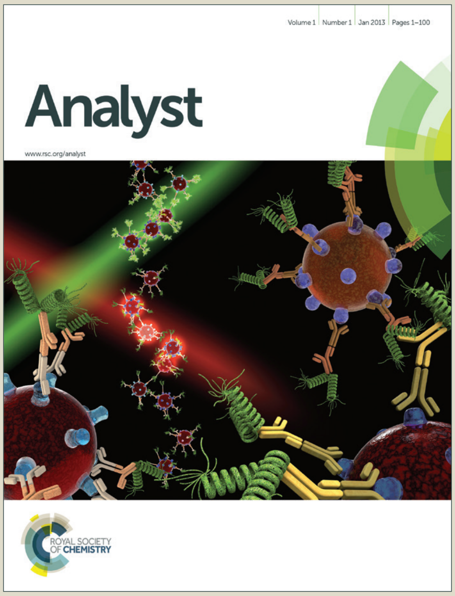

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Highly Selective Two-photon Fluorescence Probe for Determination of Mercury Ions

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In this work, we developed a new two-photon fluorescent probe, ATD (ATD = amino triphenylamine dendron), by combining two-photon fluorophore 4-(bis(4-(4-(diphenylamino) styryl)- phenyl)amino) benzaldehyde (TD) with a specific recognition molecule for Hg^{2+} - phenyl thiourea (PT), for determination of Hg^{2+}. The designed fluorescent probe emitted at ~455 nm upon one-photon and two-photon excitation at 400 nm and 800 nm, respectively. The blue fluorescence obviously dropped with the continuous addition of Hg^{2+}, and demonstrated a good linearity with the concentration of Hg^{2+} in a wide dynamic range of 5 nM - 1.0 μM. The detection limit was achieved to 0.49 nM (~0.2 ppb), which is much lower than the standard levels required by the U.S. Environmental Protection Agency (EPA) and World Health Organization (WHO). Furthermore, this probe featured high selectivity for Hg^{2+} detection over other metal ions such as Cd^{2+}, Ag^{+}, Pd^{2+}, and so on, due to the specific recognition of PT molecule. Meanwhile, the probe demonstrated long-term stability upon pH and illumination. As a result, the developed two-photon fluorescent probe with high sensitivity and selectivity, was successfully applied for on-site determination of Hg^{2+} in environmental water samples.

Introduction

Mercury, as one of the highly toxic heavy metal, is a hazardous and ubiquitous pollutants and human health.[1-3] Once mercury ions (Hg^{2+}) are introduced into the environment, bacteria convert inorganic Hg^{2+} into methyl-mercury, which has been implicated as a cause of mercury pollution related to serious irreversible neurological damage in human beings.[4-6] The U.S. Environmental Protection Agency (EPA) and World Health Organization (WHO) have set the maximum allowable levels of Hg^{2+} in drinking water at 10 nM (2.0 ppb) and 30 nM (6.0 ppb). Accordingly, exploring facile, sensitive, and rapid analytical methods for on-site monitoring of Hg^{2+} is highly desired.

Over the past years, many reliable methods have been reported for mercury analysis, including atomic absorption/ emission spectroscopy (AAS/AES), selective cold vapor atomic fluorescence spectrometry, and inductively coupled plasma mass spectrometry (ICP-MS).[7-11] These techniques are very sensitive and selective, but require expensive and sophisticated instrumentation which limits their application in on-site monitoring of Hg^{2+}. Luminescent chemosensor has become an attractive strategy for on-site Hg^{2+} detection because of its high sensitivity and rapid response time. [12-16]

Here, we developed a two-photon fluorescent probe for on-site sensing of Hg^{2+} with high selectivity and sensitivity. Recently, two-photon microscopy (TPM), a new technique that utilizes two photons of lower energy for excitation, has become more and more useful,[18-23] because this technique shows several advantages such as increased penetration depth (>500 μm), minimized autofluorescence background, and less photodamage associated with the use of near infrared excitation. To the best of our knowledge, few papers have been reported for two-photon fluorescent detection of Hg^{2+},[24-27] because the limitation in constructing the two-photon fluorescent probes for Hg^{2+} with high selectivity and sensitivity. In this work, we designed and synthesized a two-photon fluorescent probe amino triphenylamine dendron (ATD), by combining two-photon fluorophore 4-(bis(4-(4-(diphenylamino)styryl)- phenyl)amino) benzaldehyde (TD) with a specific recognition molecule for Hg^{2+} - phenyl thiourea (PT). This fluorescent probe featured emission peak at ~455 nm upon one-photon and two-photon excitation at 400 nm and 800 nm, respectively. With the continuous addition of Hg^{2+}, the fluorescence intensity gradually decreased and showed a good linearity with the concentration of Hg^{2+} in a broad range from 5 nM to 1.0 μM. The low detection limit was achieved to 0.49 nM. Moreover, the fluorescent probe exhibited high selectivity toward determination of Hg^{2+} over other metal ions such as Cd^{2+}, Ag^{+}, Pd^{2+}, and so on, due to the specific recognition of PT molecule. Finally, the developed two-photon fluorescent probe with remarkable analytical performance including high sensitivity and selectivity, was successfully applied for on-site determination of Hg^{2+} in environmental water samples. To the best of our knowledge, this is the first report for the development of a two-photon fluorescence probe suitable for detection of Hg^{2+} in environmental water.

Experimental

Reagents and Chemicals

Ethylenediamine, phenyl isothiocyanate, metal salts, ethanol, dichloromethane, methanol, and silica gel (200-300 meshes) were purchased from Sinopharm Chemical Regent Co., Ltd. All chemicals were commercially available and of analytical grade. Solutions of metal ions were all prepared from their chloride salts. Ultrapure water was used from a Millipore water purification system. The solution for determination of Hg^{2+} was mixed by methanol and phosphate buffer solution (8:2). pH of the solution was adjusted by HCl and NaOH.

Apparatus and Instruments

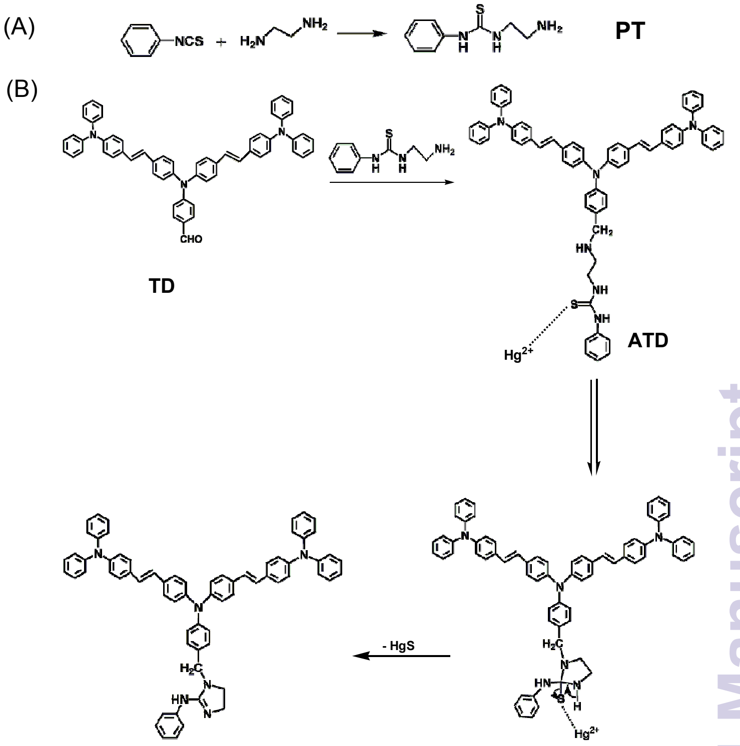
NMR spectra were collected in $CDCl_3$ or d_6-DMSO at 25°C on a Bruker AV-400 spectrometer. All chemical shifts were recorded in the standard δ notation of parts per million with chemical shifts reported in ppm. Optical absorption spectrum was noted on an Agilent 8453 UV-vis spectrometer using a quartz cuvette having a 1 cm path length. One-photon fluorescence spectra were obtained on a Hitachi F-2700 spectrofluorimeter equipped with a 450 W xenon lamp. Samples for absorption and emission measurements were conducted in 1 cm × 1 cm quartz cuvettes. The two-photon excited fluorescence spectra were measured at 800 nm excitation wavelength on a spectrometer (Horiba model iHR 550) and the pump laser beam came from a mode-locked Ti: sapphire laser (Coherent Mira 900) with a pulse duration of 80 fs and a repetition rate of 76 MHz.

Synthesis of PT

The route for synthesis of PT is shown in Scheme 1A. Ethylenediamine (30.0 g, 0.5 mol) was first dissolved in ethanol (30 mL). Next, phenyl isothiocyanate (6.8 g, 0.05 mol) in ethanol (30 mL) was added drop-wise into ethylenediamine solution on an ice bath, and then stirred at room temperature for 5 h, resulting in white solid products. Finally, the mixture was filtrated to afford a white solid phenyl thiourea (PT) (6.2 g). Yield, 64 %. ^1H NMR (400 MHz, $CDCl_3$, ppm): δ=9.63 (s, 1H), 7.86 (s, 1H), 7.38 (d, 2H), 7.32 (t, 2H), 7.14 (t, 1H), 3.69 (s, 2H), 3.33 (s, 2H). ^{13}C NMR (400MHz, $CDCl_3$, ppm): δ=206.96, 136.62, 129.99, 126.86, 124.81, 47.57, 30.95. TOF MS EI^+: calculated for $C_9H_{14}N_3S$ $[M+H]^+$196.0911,found196.09029.

Synthesis of ATD

4-(bis(4-(4-(diphenylamino)styryl)-phenyl)amino)benzaldehyde (TD) was synthesized according to the reported procedure.[28] The route for synthesis of ATD is illustrated in Scheme 1B. The as-prepared TD (0.5 mmol) was added to a cold stirred mixture of phenyl thiourea (PT, 5 mmol), anhydrous methanol (4 mL), and anhydrous sodium sulfate (0.5 g), then further stirred for 4 h. After that, sodium borohydride (1 mmol) was added into the above products and stirred at room temperature for 10 h. Finally, the sodium sulfate was removed by filtration, and the resulting solution was concentrated and extracted with dichloromethane and aqueous ammonium chloride (wt %) (2:1 v/v). The organic phase was evaporated to dry and purify by column chromatography on silica. Yield, 60%. ^1H NMR (400MHz, d_6-DMSO, ppm): δ = 9.66 (s, 1H), 7.48 (d, 6H), 7.40 (d, 2H), 7.32 (t, 12H), 7.26 (d, 2H), 7.04 (m, 18H), 6.99 (d, 8H), 3.73 (s, 2H), 3.59 (s, 2H) 2.76 (s, 2H). ^{13}C NMR (400MHz, d_6-DMSO, ppm): δ=180.84, 167.39, 147.46, 145.83, 132.25, 132.11, 132.02, 130.01, 129.41, 129.17, 129.12, 129.08, 127.84, 126.86, 126.81, 124.79, 124.63, 124.50, 123.78, 123.63, 123.38, 123.00, 55.38, 52.45, 43.99. TOF MS EI^+: calculated for $C_{68}H_{56}N_6S$ $[M+H]^+$991.4475, found 991.4516.



Scheme 1. (A) Synthetic routes of PT and (B) ATD and reaction mechanism of ATD upon Hg^{2+} ion addition.

Results and discussion

Characterization of ATD

Synthetic routes of PT and ATD are shown in Scheme 1. The structures of the as-synthesized PT and ATD were confirmed by ^1H NMR, ^{13}C NMR, and TOF-MASS (Fig. S1-S6, Supporting Information). From UV-vis absorption spectra demonstrated in Fig. 1A, no absorption was observed for PT, while TD showed broad adsorption band from 360 nm to 420 nm. The synthesized ATD also exhibited adsorption band centred at ~400 nm. As demonstrated in Fig. 1B, PT exhibited negligible fluorescence emission, while the fluorescence emission peak for TD was observed at 450 nm. Upon both one-photon and two-photon excitation, the synthesized ATD showed a fluorescence maximum at ~455 nm (Fig. 2B). The quadratic relationship between the excitation laser power and the luminescence intensity is obvious (Fig. S7, Supporting Information), confirming that the excitation with two near-infrared photons was indeed responsible for the observed visible luminescence of ATD. Using quinine sulfate as a standard, the fluorescence quantum yield (Φ_F) of ATD was calculated to be 8.26±1.0% (Fig. S8, Supporting Information).[29] Using a femtosecond (fs) fluorescence measurement technique, the two-photon action cross section ($\Phi_F\sigma_{2P}$) of ATD was estimated to be 22±0.15 GM (Goeppert-Mayer unit), respectively.[30]

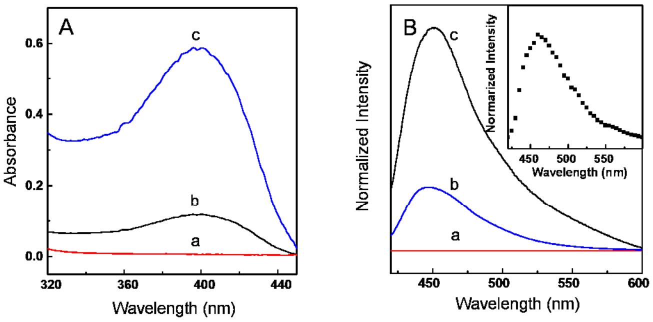


Fig. 1. (A) UV-Vis absorption spectra and (B) fluorescence spectra of (a) PT, (b) TD, and (c) ATD (400 nm excitation). Insert: Two-photon fluorescence spectrum of ATD (800 nm excitation).

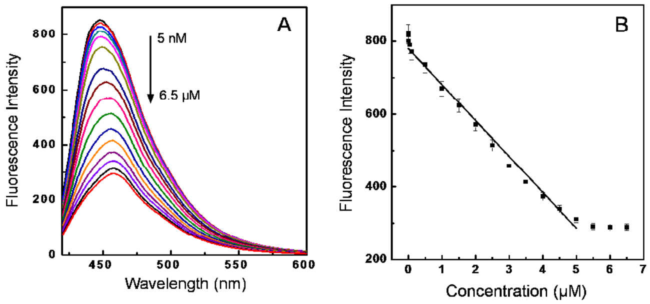


Fig. 2. (A) One-photon fluorescence spectra of ATD probe (5 μM, methanol:phosphate buffer solution=8:2) with the addition of different concentrations (5 nM - 6.5 μM) of Hg^{2+} under excitation of 400 nm. All spectral data were recorded at 20 min after Hg^{2+} addition. (B) The relationship between the integrated fluorescence intensity and Hg^{2+} concentration taken in Fig. 2A.

One-photon and Two-photon Fluorescent Responses of ATD for Hg^{2+} Determination

The response of ATD fluorescent probe for determination of Hg^{2+} upon one-photon excitation was first carried out to prove the working principle. As demonstrated in Fig. 2A, with the continuous addition of Hg^{2+}, the blue emission centred at 455 nm ascribed to ATD gradually quenched. Furthermore, the fluorescence intensity decreased with the increasing concentration of Hg^{2+} and the signal showed a good linearity with Hg^{2+} concentration in the range of 5 nM - 5.0 μM, as plotted in Fig. 2B. The detection limit was achieved to 0.49 nM (0.2 ppb), which is much lower than the standard levels of WHO and EPA for the maximum allowable levels of Hg^{2+} in drinking water. The detection of Hg^{2+} using ATD by two-photon excitation was also examined. As shown in Fig. 3, the fluorescent emission peak at 455 nm of ATD dropped with the rising concentration of Hg^{2+}, and showed good linearity in the concentration range of 5 nM - 1.0 μM. In addition, the optimized response time was checked by using ATD with concentration of 100 nM Hg^{2+} (Fig. S9, Supporting Information). The fluorescence signal of ATD decreased with the reaction time after added with Hg^{2+}, and reached a minimum within ~18 min.

The complexity of environmental system presents a great challenge to the analytical methods for detection of metal ions not only in detection limit, but more importantly in selectivity. The selectivity and competition experiments were carried out by monitoring the fluorescent intensity of the developed probe for Hg^{2+} against other metal ions such as Ag^{+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, and so on, with the same concentration. As shown in Fig. 4, F_0 represents the original fluoresce intensity of the ATD probe, while F represents the fluorescence intensity after addition metal ions. Remarkably, no obvious changes of the fluorescent signals were observed for the other metal ions, compared with that obtained for Hg^{2+}. Furthermore, these potential metal ion interferences showed not obvious effects on the detection signal for Hg^{2+} sensing, as demonstrated in Fig 6B. The results indicate that the present ATD probe showed a good selectivity for Hg^{2+} sensing against other metal ions, because of the specific recognition element of PT.

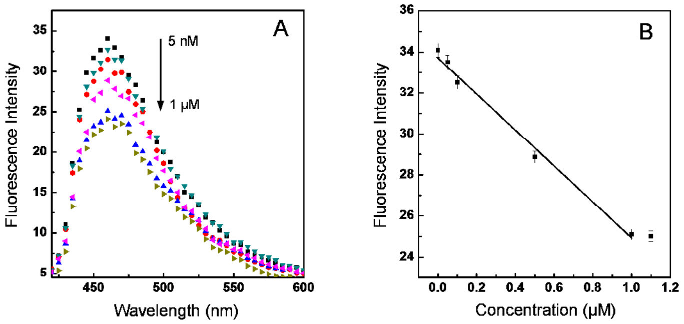


Fig. 3. (A) Two-photon fluorescence spectra of ATD probe (5 μM) with the addition of different concentrations (5 nM - 1.0 μM) of Hg^{2+} under excitation of 800 nm. (B) The relationship between the integrated fluorescence intensity and Hg^{2+} concentration taken in Fig. 3A.

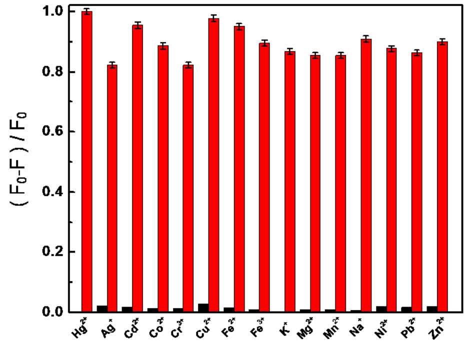


Fig. 4. Fluorescence responses of 1 μM ATD toward various metal ions. Black bars represent the addition of an excess of metal ions,1 mM for Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}; 1 μM for other cations. Red bars represent the subsequent addition of 1 μM Hg^{2+} to the solution.

In addition, the fluorescent signal of ATD was found to be independent of solution pH in broad pH range of 2.0 - 10.0 (Fig. 5A). Photostability of the fluorescent responses was also investigated at 455 nm, by excitation at 400 nm using Xenon lamp or at 800 nm by laser illumination. Furthermore, Negligible changes (<5%) were observed upon irradiation for 2 h (Fig. 5B), suggesting long-term photostability of the ATD probe. These observations indicate that the present fluorescent probe exhibited excellent stability against light illumination and pH variation, which fulfills the requirements for on-site determination of Hg^{2+}.

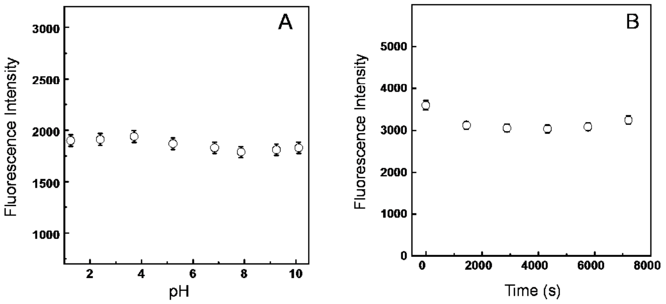


Fig. 5. (A) Effect of pH value on fluorescence response of ATD. (B) Photostability of ATD continuously illuminated using Xenon lamp for 2 h.

Determination of Hg^{2+} in Environmental Water Samples

As demonstrated above, the developed fluorescent probe having high sensitivity, selectivity, and low detection offered an on-site strategy for monitoring of Hg^{2+} in water sample. The ATD probe was then employed to determination of Hg^{2+} in drinking, tap, and lake water. The measured results were summarized in Table 1. The obtained data were compared with those measured by traditional method AAS. The present method showed more sensitive than AAS in the measurements of drinking water and tap water. According to the statistic calculation by a t test (a=0.05), the concentration of Hg^{2+} in lake water samples determined by the present probe agreed well with those detected by AAS method.

Table 1. Determination of Hg^{2+} in Environment Water Samples Using the Present Method and AAS

Samples	The present method (ppb)	AAS (ppb)
Tap water	2±0.06	Undetectable
Tap water	4±0.12	Undetectable
Lake water	23±0.69	22±1.01
Lake water	40±1.20	39±1.98

Conclusions

In summary, we have developed a new two-photon probe (ATD), which demonstrates a clear blue emission colour change in response to Hg^{2+}. The present fluorescent probe for Hg^{2+} shows high selectivity against other metal ions, as well as high sensitivity with low detection limit, and broad dynamic linear range. Meanwhile, the fluorescence probe is also inert to pH over a wide pH range from 2.0 to 10.0 and exhibits long-term photostability. Accordingly, the probe has been successfully applied in determination of Hg^{2+} in environmental water. This work has established a novel approach to detection of Hg^{2+} concentration, which is lower than the standard values required by WHO and EPA. This investigation had also provided a methodology to designing and constructing two-photon fluorescent biosensors with high selectivity for monitoring of metal ions and other environmental species.

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Notes and references

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† The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Electronic Supplementary Information (ESI) available: ^{1}H NMR, ^{13}C NMR, and TOF MS spectra of PT, ATD; Quantum yield of ATD; and Optimized reaction time. See DOI: 10.1039/b000000x/

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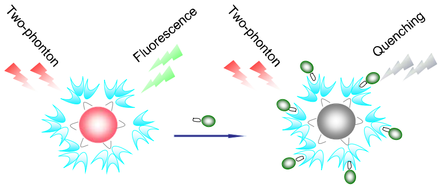

A two-photon fluorescent sensor ATD was developed for Hg^{2+} determination with high selectivity and low detection limit, in which two-photon fluorophore TD was designed and synthesized with a specific recognition molecule for Hg^{2+} - PT.