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## A coumarin-containing Schiff base fluorescent probe with AIE effect for the copper(II) ion†

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A novel coumarin-derived Cu<sup>2+</sup>-selective Schiff base fluorescent “turn-off” chemosensor CTPE was successfully obtained, which showed an AIE effect. It could identify Cu<sup>2+</sup> by quenching its fluorescence. The lower limit of detection was 0.36 μM. CTPE can act as a highly selective and sensitive fluorescence probe for detecting Cu<sup>2+</sup>.

The specific detection of transition metal ions has received considerable attention because of their significant roles in the fields of biological, chemical, medical and environmental processes.<sup>1</sup> Among these metal ions, copper(II), acting as one of the most important micronutrients, is of particular interest due to its essential role in a variety of fundamental physiological processes. In spite of the fact that copper(II) is essential in living systems, accumulation of copper(II) can lead to serious environmental and health problems.<sup>2</sup> For instance, the metabolic balance of Cu<sup>2+</sup> in the body being destroyed can lead to neurodegenerative diseases, such as Alzheimer's, Parkinson's, Menkes, and Wilson's disease.<sup>3–8</sup> Besides, free Cu<sup>2+</sup> is regarded as a significant environmental pollutant.<sup>9,10</sup> Therefore, an increasingly convenient and fast method to detect Cu<sup>2+</sup> ion existing in environmental and biological resources is currently of considerably important. By far there existing many methods make it possible to detect and quantify Cu<sup>2+</sup> ion, such as atomic absorption spectrometry,<sup>11,12</sup> inductively coupled plasma mass spectrometry,<sup>13</sup> inductively coupled plasma-atomic emission spectrometry<sup>14</sup> and voltammetry,<sup>15,16</sup> *etc.* However, these methods require tedious sample preparation procedures and complex instrumentation, which limit their prosperous applications. Alternatively, more and more attention has been attracted to the analytical methods based on fluorescent chemosensors for the detection of the metal ions owing to their high sensitivity, selectivity, and simplicity.<sup>17</sup> As a result, various types of fluorescent probes such as organic dyes, magnetic nanoparticles (MNPs), semiconductor quantum dots (QDs), carbon dots (CDs), fluorescent metal nanoclusters (NCs), and fluorescent metal organic frameworks (MOFs) have been

designed and prepared to determine Cu<sup>2+</sup> ion.<sup>18–27</sup> Since first reported in 2000, MNPs are extensively used in the field of nanotechnology for its nonhazardous feature, strong magnetization values, superparamagnetic property, active surface that can easily assembled of biological soluble structure and targeting, imaging, and therapeutic molecules.<sup>22</sup> Unlike organic fluorescent dyes, QDs are semiconductor particles which exhibit high photochemical stability, excellent resistance to chemical degradation, outstanding photodegradation and extremely large Stokes shift.<sup>23</sup> CDs is a comprehensive term for various nanosized carbon materials including graphene quantum dots (GQDs), carbon nanodots (CNDs), and polymer dots (PDs)<sup>24</sup> which shows excellent properties including high stability, bio-compatibility, low photo bleaching and toxicity and so on.<sup>25</sup> Despite successfully applying in detecting Cu<sup>2+</sup>, traditional fluorescent probes are still puzzled by the toxicity, poor solubility, “aggregation-caused quenching” (ACQ) effect, *etc.* Thus, preparing fluorescent probes that can compensate for these deficiencies is still imminently desired in the Cu<sup>2+</sup> ion detection.

Coumarin derivatives are well known excellent fluorophores in the fluorescent probe design and synthesis with the advantages of high fluorescence intensity, excellent solubility, efficient cell permeation, high quantum yield and ease of preparation.<sup>28,29</sup> As a result, an increasing number of papers concerning coumarin derivatives fluorescent chemosensors for copper(II) ion have emerged.<sup>30–33</sup> In 2009, Lee Jin Yong and co-workers developed a novel coumarin-based fluorogenic probe, which can act as a fluorescent chemosensor with high selectivity and suitable affinity in biological systems toward Cu<sup>2+</sup> (ref. 34). Besides, in 2019, Yin Jiqui and co-workers obtained a fluorescent chemosensor concerning coumarin derivatives for copper(II) which exhibited good sensitivity, fast response time and high selectivity for Cu<sup>2+</sup> ion in the presence of other important relevant metal ions.<sup>35</sup>

In 2001, Tang Benzong and co-workers proposed the concept of “aggregation-induced emission” (AIE).<sup>36</sup> Due to their

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effective in circumventing the ACQ effect, AIE-active materials provide a new path for the design and synthesis of fluorescent probes. Tetraphenylethene (TPE), one of the most commonly used aggregation-induced emission luminogens (AIEgens), has been widely favored by researchers.<sup>37,38</sup> Owing to its simple structure, simple synthesis, easy modification, and obvious AIE effect, TPE is usually used as an ideal model for construction of various fluorescent sensors.<sup>39</sup> In 2019, Zhao Feng and co-workers designed and synthesized a new tetraphenylethene-based Schiff base ligand with AIE effect, and it could be utilized as optical recording materials.<sup>40</sup> The same year, Ni Zhonghai and co-workers obtained two different polymorphs of a new tetraphenylethene-based Schiff base, which exhibited totally different photochromic and fluorescence properties.<sup>41</sup>

Many Schiff base ligands have been synthesized for the detection of  $\text{Cu}^{2+}$ , which have general fluorescence characteristics. To solve the ACQ problem, we are going to design and synthesize a Schiff base fluorescent probe with AIE effect. For this purpose, a Schiff base fluorescent probe (CTPE) incorporating the TPE group into coumarin framework has been designed and synthesized, which exhibits fast response time, simple synthetic step, lower cost and AIE effect. And it can rapidly recognize  $\text{Cu}^{2+}$  in a mixed THF/ $\text{H}_2\text{O}$  system.

Scheme 1 was the synthetic route for CTPE. Other compounds and intermediates were labelled and displayed in Scheme S1 in the ESI.† As shown in Scheme 1, CTPE was prepared in high yield *via* Maillard reaction of 8-formyl-7-hydroxy-4-methylcoumarin (M1) and 1-(4'-aminophenyl)-1,2,2-triphenylethene (TPE-NH<sub>2</sub>) in absolute ethanol. M1, TPE-NH<sub>2</sub> and CTPE were prepared according to the literatures.<sup>42,43</sup> Their chemical structures were confirmed by nuclear magnetic resonance (NMR), high resolution mass spectrometry (HRMS) and Fourier transform infrared (FTIR) (Fig. S1–S10, ESI†). In Fig. S10,† compared with TPE-NH<sub>2</sub> and M1, the spectrum of CTPE appeared an in-plane bending vibration absorption peak of C=N bond at 1620  $\text{cm}^{-1}$ , and a C=O bond stretching vibration absorption peak on the aldehyde group at 1740  $\text{cm}^{-1}$ , indicating that CTPE has been successfully synthesized.

As an AIE-based fluorescent probe, the AIE effect of CTPE was confirmed by its fluorescence spectra in THF/ $\text{H}_2\text{O}$  mixtures with various water contents. CTPE was in a good dispersion state in THF solvent, but CTPE gradually aggregated as the volume fraction of water ( $f_w$ ) increased. As shown in Fig. 1A and B, CTPE had good dispersibility and exhibited weak luminescence in dilute THF solution ( $f_w = 0\%$ ). The weak emissive nature of the luminogens in THF and aqueous mixtures with low  $f_w$  values should be ascribed to active intramolecular rotations of the genuinely dissolved compounds, which effectively

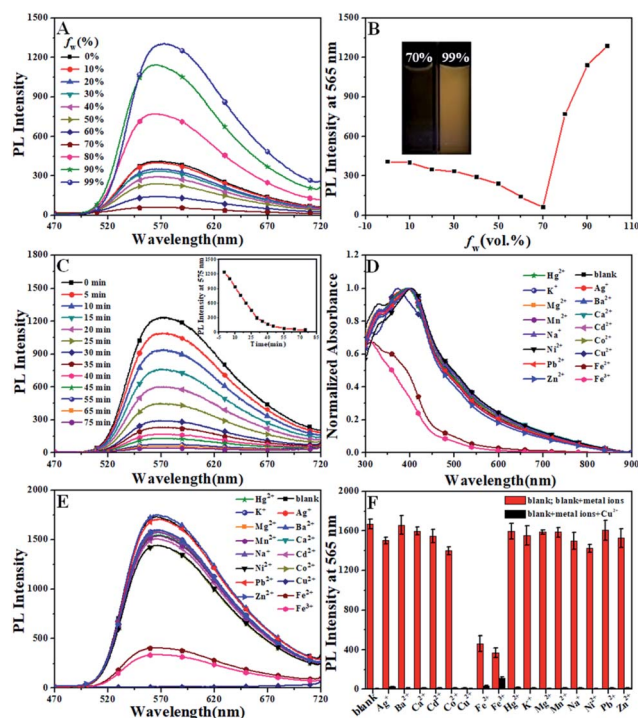
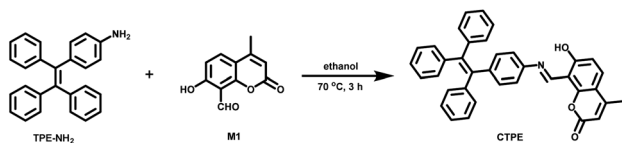


Fig. 1 (A) PL spectra of CTPE in THF/ $\text{H}_2\text{O}$  mixtures with different  $f_w$ . (B) Plots of PL intensity of CTPE versus  $f_w$  in THF/ $\text{H}_2\text{O}$  mixtures at 565 nm. Inset: photograph of CTPE in THF/ $\text{H}_2\text{O}$  mixtures under a hand-held UV lamp illumination ( $\lambda_{\text{ex}} = 365 \text{ nm}$ ). (C) PL spectra of CTPE after adding  $\text{Cu}^{2+}$  in THF/ $\text{H}_2\text{O}$  (1/99, v/v) at different reaction time. Inset: relationship between fluorescence intensities of CTPE in THF/ $\text{H}_2\text{O}$  (1/99, v/v) at 575 nm and time of addition of  $\text{Cu}^{2+}$ . (D) UV-vis absorption and (E) fluorescence spectra of CTPE with different metal ions in THF/ $\text{H}_2\text{O}$  (10/90, v/v). (F) Fluorescence responses at 565 nm of CTPE to various metal cations in THF/ $\text{H}_2\text{O}$  (10/90, v/v) solution.

consumed the energy of the excitons non-radiatively.<sup>44</sup> When  $f_w$  increased from 10% to 70%, the fluorescence intensity of CTPE gradually decreased, which was attributed to the intramolecular charge transfer (ICT) effect with the increasing polarity of the solution for CTPE.<sup>40,45</sup> When  $f_w$  was above 70%, CTPE displayed a significantly sudden increase in the fluorescence intensity owing to molecular aggregation. The fluorescence intensity at 565 nm was about 60 in THF/ $\text{H}_2\text{O}$  (30/70, v/v) mixtures, while it increased to about 1288 in THF/ $\text{H}_2\text{O}$  (1/99, v/v) mixtures with about 21-fold enhancement. Simultaneously, and the maximum emission wavelength showed a slight red shift from 565 nm to 575 nm with the increase of fluorescence intensity. The fluorescence enhancement phenomenon could be ascribed to the restriction of the intramolecular rotations. In the aggregated state, intramolecular motion (mainly including C=N isomerization and rotation of the benzene ring) was limited, and the attenuation of non-radiative energy was correspondingly blocked, so that a clear enhancement and a slight red shift of the fluorescence of CTPE were observed.<sup>40</sup> The above results clearly implied that CTPE exhibited a significant AIE effect.

The  $\text{Cu}^{2+}$ -specific binding of CTPE with various competitive metal ions was then investigated under the same experimental condition. Different from these AIE sensors that worked in the



Scheme 1 Synthesis and structure of CTPE.



aggregated state, there was not complete fluorescence quenching even after about 1 h of adding 20 equivalent  $\text{Cu}^{2+}$  in THF/ $\text{H}_2\text{O}$  (1/99, v/v) solution (shown in Fig. 1C). However, at the moment of adding  $\text{Cu}^{2+}$ , the fluorescence was immediately quenched in THF/ $\text{H}_2\text{O}$  (10/90, v/v) solution. Therefore the THF/ $\text{H}_2\text{O}$  (10/90, v/v) system was adopted to study the selectivity of CTPE to all metal ions.

Metal ion selectivity studies were performed on absorption and fluorescence spectra. Absorption spectra of CTPE recorded after the addition of each metal ion (20 eq.) was shown in Fig. 1D. In Fig. 1D, upon addition of a constant amount (20 eq.) of  $\text{Cu}^{2+}$  ion to CTPE, a significant hypsochromic shift from 395 nm to 371 nm in absorption spectrum was observed. No other metal ion induced any change under the identical conditions except  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  ions. These results demonstrated the specificity of the CTPE for selective binding interaction with  $\text{Cu}^{2+}$  ion.

To further investigate the selectivity of CTPE to  $\text{Cu}^{2+}$ , we also studied the fluorescence response of CTPE in THF/ $\text{H}_2\text{O}$  (10/90, v/v) solution. The behaviour of fluorescence “turn off” merely shown by  $\text{Cu}^{2+}$  was observed in the Fig. 1E. Surprisingly, the fluorescence was immediately quenched in THF/ $\text{H}_2\text{O}$  (10/90, v/v) solution at the moment of adding  $\text{Cu}^{2+}$ . And the fluorescence intensities decreased after adding  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$ , respectively. No obvious changes were observed upon addition of 20 eq. other competitive metal ions. The interferences from the other metal ion with CTPE in its response to  $\text{Cu}^{2+}$  were performed. As shown in Fig. 1F, the red bars represent the emission changes of CTPE in the presence of metal ions of interest (all are 20 eq.). The black bars represent the changes of the emission that occurs upon the subsequent addition of  $\text{Cu}^{2+}$  to the above solution. The fluorescent intensity of CTPE in the presence of any of the other metal ions tested after adding  $\text{Cu}^{2+}$  was decreased significantly, demonstrating little interference from the other metal ions.

Fluorescent titration experiments of CTPE in THF/ $\text{H}_2\text{O}$  (10/90, v/v) solution with  $\text{Cu}^{2+}$  ion were carried out and the changes in the fluorescence intensity at 565 nm of CTPE solution with the concentration of  $\text{Cu}^{2+}$  ion were measured and showed in Fig. 2A. It was found that the fluorescence intensity at 565 nm of CTPE solution decreased with the increase in the concentration of  $\text{Cu}^{2+}$  ion. As showed in Fig. 2B,  $F_0$  and  $F$  were the fluorescence intensities at 565 nm without adding  $\text{Cu}^{2+}$  and adding different concentrations of  $\text{Cu}^{2+}$ , respectively. In

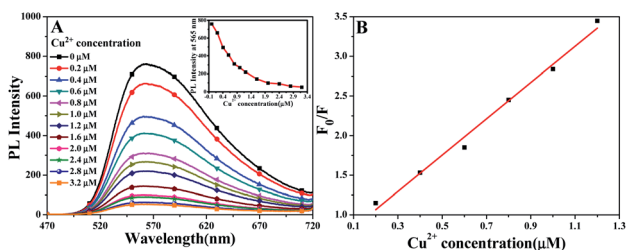


Fig. 2 (A) Fluorescence titration spectra of CTPE (10  $\mu\text{M}$ ) in THF/ $\text{H}_2\text{O}$  (10/90, v/v) solution. Inset: fluorescence intensities of CTPE (10  $\mu\text{M}$ ) at 565 nm as a function of  $\text{Cu}^{2+}$  concentration (0–3.2  $\mu\text{M}$ ). (B) Linear relationship between  $F_0/F$  and  $\text{Cu}^{2+}$  concentration.

addition, when the  $\text{Cu}^{2+}$  concentration in the solution to be tested was less than 1.2  $\mu\text{M}$ , the  $F_0/F$  of CTPE at 565 nm had a good linear relationship with the concentration of  $\text{Cu}^{2+}$ .

By taking that change in fluorescence intensity in micro molar range we have calculated the lower limit of detection (LOD) from standard deviation and the slope of calibration plot (Fig. 2B) using the equation.<sup>46</sup> It was deduced from the fluorescence titration profile that the LOD of CTPE toward  $\text{Cu}^{2+}$  ion reached 0.36  $\mu\text{M}$ .

To gain a better understanding the sensing mechanism of CTPE towards  $\text{Cu}^{2+}$ ,  $^1\text{H-NMR}$  titration experiments were performed in  $\text{DMSO-}d_6$  at room temperature (Fig. 3A). The  $^1\text{H-NMR}$  spectra of CTPE showed considerable variation with the increasing of  $\text{Cu}^{2+}$  in  $\text{DMSO-}d_6$  solvent.  $\text{Cu}^{2+}$  is a paramagnetic ion that affects the NMR resonance frequency of protons that are close to the  $\text{Cu}^{2+}$  binding site.<sup>47</sup> The downfield value of  $\text{H}_a$  (of  $-\text{OH}$ ) at  $\delta = 14.86$  ppm in CTPE was due to the intramolecular hydrogen bond between the imine-N atom of CTPE with  $\text{H}_a$  forming a six-membered transition state.<sup>48</sup> On addition of  $\text{Cu}^{2+}$  ion, the intramolecular hydrogen bonding was disturbed.<sup>49</sup> As  $\text{Cu}^{2+}$  gradually increased, the proton signal of  $\text{H}_a$  almost disappeared. The proton signal of  $\text{H}_b$  (of  $-\text{CH}=\text{N}-$ ) at  $\delta = 9.16$  ppm significantly decreased indicating the participation of the nitrogen atom in the binding with  $\text{Cu}^{2+}$  ion. The proton signals of the aromatic ring became broader and weaker with the increasing amount of  $\text{Cu}^{2+}$ . These results confirmed that  $\text{Cu}^{2+}$  binds to the CTPE chemosensor through the N atom of the imine and the O atom of phenolic hydroxyl, which were directly connected to the aromatic rings.<sup>50</sup> Thus, combined with the FTIR spectra of CTPE before and after the addition of  $\text{Cu}^{2+}$  (Fig. S11<sup>†</sup>), it was clearly illustrated that the fluorescence “turn

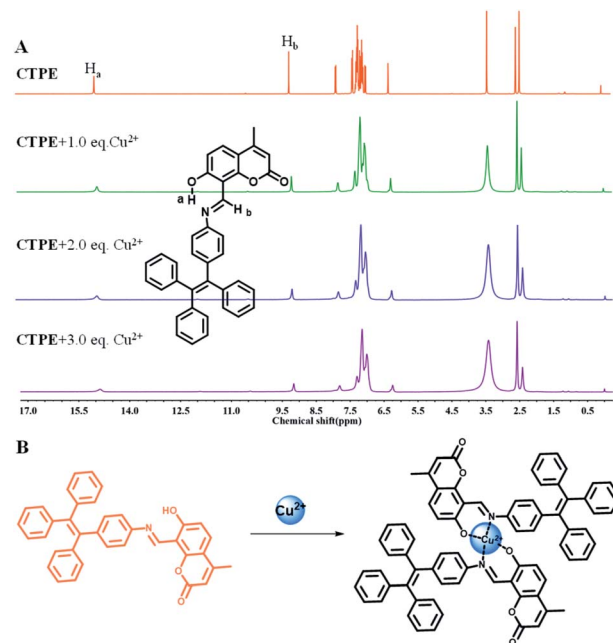


Fig. 3 (A)  $^1\text{H-NMR}$  data of CTPE in  $\text{DMSO-}d_6$  solution in the absence and presence of  $\text{Cu}^{2+}$ . (B) Proposed mechanism for CTPE upon addition of  $\text{Cu}^{2+}$ .



off" of CTPE was attributed to the chelation between imine-N atom, phenolic hydroxyl-O atom and Cu<sup>2+</sup> ion. The possible coordination modes of Cu<sup>2+</sup> and CTPE were shown in Fig. 3B.

In summary, a novel Schiff base fluorescent probe CTPE based on coumarin and TPE showing an AIE effect has been successfully synthesized and characterized. The fluorescence of CTPE was rapidly quenched by Cu<sup>2+</sup>, but no significant influence was observed for other metal ions tested. CTPE can distinguish Cu<sup>2+</sup> from other metal ions *via* the fluorescence "turn off". The LOD of CTPE for Cu<sup>2+</sup> can reach 0.36 μM. Recognition mechanism between CTPE and Cu<sup>2+</sup> was given. Therefore, CTPE can act as a potential fluorescence probe to selectively and rapidly identify Cu<sup>2+</sup>.

## Conflicts of interest

There are no conflicts to declare.

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Paper

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