G-quadruplex facilitated turn-off fluorescent chemosensor for selective detection of cupric ion†

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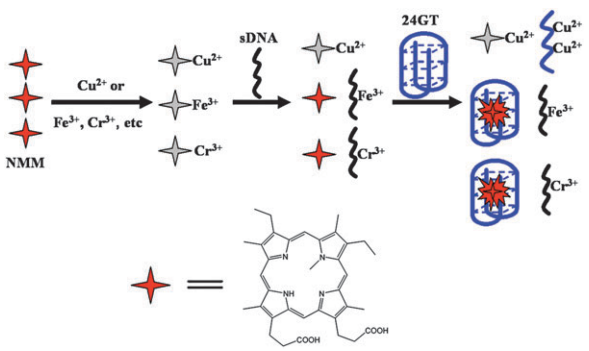
A simple turn-off fluorescent method was utilized to quantitatively detect Cu(II) which can selectively quench fluorescent dye molecules. Addition of DNA G-quadruplex into the solution significantly magnifies the discrimination, lowers the limit of detection and broadens the linear response range.

G-quadruplex applied in a biosensor can function as a versatile signaling component in combination with other small molecules, for example, catalytic DNAzyme[1] and fluorescent reagent.[2] Compared with the utility of G-quadruplex as DNAzyme, fluorescence enhancement based on G-quadruplex has its own unique characteristics, such as higher stability and reproducibility. For practical quantitative measurement, the fluorescence based detection approach is much more stable and desirable. Since the structure of G-quadruplex is highly cation-dependent, it can be employed as a fluorescent biosensor for detection of heavy transition metal ions.

Widespread usage of copper ion containing substances has posed an unanticipated threat to our ecosystem. Trace copper concentration can act as both a micronutrient and a toxicant in fresh water and the sea.[3] Copper is the third most abundant transition metal ion in the human body and plays an important role in living systems below a certain amount.[4] Discovery of a facile method to quantitatively detect copper ion is of considerable significance for environmental protection and human health, which has attracted plenty of attention recently.[5,6]

Among the variety of methods used for detection of cupric ion,[5–7] the fluorescence detection technique is popular and elicits many efforts.[5] Previous works focus on the interaction between metal ion and polynucleotides.[6b,7c,e] However, in our case, we describe a new strategy based on fluorescence quenching of G-quadruplex binding ligand to detect cupric ion in aqueous solution without entailing organic solvent or organic solvent-containing solution.[5b,d] A schematic illustration of our design strategy for fluorescence quenching of NMM (*N*-methyl mesoporphyrin IX) by metal ions is presented (Scheme 1). Upon addition of Cu^{2+}, the two proximal carboxylate groups chelate Cu^{2+}; as a result, the fluorescence of NMM turns off. Cu^{2+} ion shows the strongest quenching of the weak fluorescence emission of NMM among all the metal ions. The interference of other metal ions, such as Cr^{3+} and Fe^{3+} (or Fe^{2+}), can be obviated by subsequent addition of single-stranded DNA (sDNA), which results in the regeneration of weak fluorescence emission of NMM without any effect on the binding between Cu^{2+} and NMM. The regenerated fluorescence emission of NMM can be significantly enhanced by binding to G-quadruplex (24GT), which further magnifies the discrimination between Cu^{2+} and other metal ions. It has also been reported previously and is confirmed here (Fig. S1, ESI†) that the introduction of Cu^{2+} can reversibly unfold the G-quadruplex,[8] whereas interestingly in our case 24GT failed to snatch the copper ion from the complex composed of Cu^{2+} and NMM. Besides the increased discrimination by introduction of 24GT, another important merit of 24GT is that it facilitates the sensitivity of NMM to cupric ion and decreases the limit of detection as well.

One of the key features of NMM is that the structure of this molecule bears two proximal carboxylate groups which can interact with heavy transition metal ions. Interestingly, upon addition of both sDNA and 24GT, we only observe that cupric ion dramatically quenches the fluorescence of NMM in comparison with other metal ions investigated here. The high quenching efficiency by cupric ion may follow the same mechanism as cupric ion quenching of other anionic conjugated polyelectrolytes.[6a] Copper ion coordinated with the two proximal carboxylate groups of NMM forms a strong binding complex, which induces the singlet exciton of NMM to quickly diffuse to the quencher "trap site".[9] Compared to those anionic conjugated polyelectrolytes, the NMM molecule is a commercially available porphyrin analogue which eliminates the tedious synthesis in the lab. A unique characteristic of this new sensor platform is that the sensitivity toward target copper ion is highly adjustable by the introduction of G-quadruplex.



Scheme 1 Illustration of turn-off fluorescence response toward copper ion. The molecular structure of NMM containing two proximal carboxylate groups which can function as a copper ion receptor.

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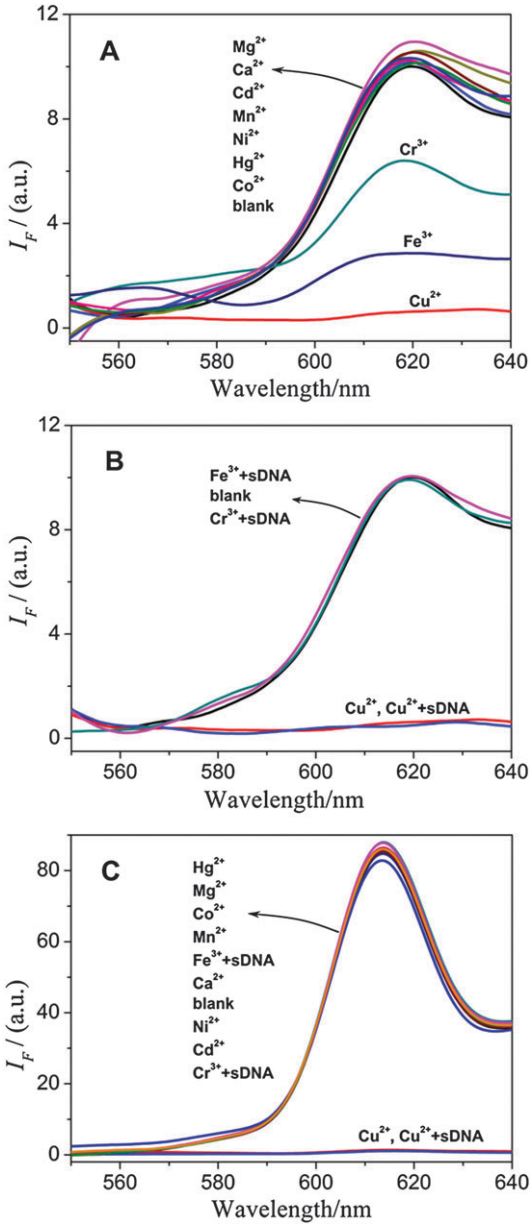

Fig. 1 (A) Fluorescence responses of NMM toward different metal ions. (B) Emission spectrum of NMM in presence of different metal ions upon addition of sDNA used as masking agent to eliminate the interference from Cr^{3+} and Fe^{3+}. (C) Fluorescence responses of NMM + 24GT toward different metal ions. Experimental conditions: 50 μM each ion, 2 μM NMM, 0.5 μM 24GT, 20 mM HEPES buffer (pH 7.0) containing 140 mM NaCl and 5 mM KCl, excitation of NMM at 399 nm.

As illustrated in Fig. 1A, NMM alone which suffers from low quantum yield was not sufficient to distinguish the copper ion from other heavy metal ions, such as Cr^{3+} and Fe^{3+}, *etc.* Among all the interferents, Cr^{3+} and Fe^{3+} quench the fluorescence emission of NMM only next to Cu^{2+}, therefore, it is very important to eradicate these interferences. Upon the addition of 1 μM sDNA into the solution containing Cr^{3+} or Fe^{3+} (Fig. 1B), the interference from the two metal ions can be totally removed. In order to remove the interference of much higher concentrations of trivalent ions, masking agent (sDNA) which has to increase correspondingly should not sacrifice the copper ion detection. In our experiment, this requirement was met. As shown in Fig. S2 (ESI†), with a high concentration of sDNA (8 μM), negligible increase of the fluorescence intensity of NMM + 24GT in the presence of 50 μM Cu^{2+} was observed, indicating that the addition of masking agent (sDNA) did not degrade our sensor platform. In Fig. 1C, it is shown that further addition of 0.5 μM 24GT into the solution dramatically magnified the discrimination between copper and other metal ions. Fluorescence quenching factor (QF) defined as F_0/F (F_0 and F stand for the fluorescence intensity in the absence and presence of metal ion) was utilized to quantitatively compare the selectivity of NMM (or NMM + 24GT) towards various metal ions (Table S1, ESI†). With NMM only, metal ions such as Mg^{2+}, Ca^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+} and Co^{2+} have no discernible effect on the fluorescence emission of NMM; however, trivalent ions (Cr^{3+} and Fe^{3+}) have a distinct effect on the fluorescence quenching of NMM and a divalent ion (Fe^{2+}) has a slight effect (Fig. S3, ESI†). Addition of sDNA into solution containing Cr^{3+} and solution containing Fe^{3+} dramatically changed the fluorescence quenching factors from 1.57 to 1.01, 3.50 to 1.00, respectively, which confirmed the interaction between trivalent ions and sDNA. Interference from Fe^{2+} also can be totally removed upon addition of sDNA (Fig. S3, ESI†). The subsequent binding between the released dye molecules (NMM) and 24GT results in significant fluorescence enhancement. The quenching factor for copper ion increased to 80.06 upon addition of G-quadruplex into solution containing sDNA and NMM, whereas no discernible changes of quenching factors of other metal ions were observed.

Fig. 2 shows the quantitative response of fluorescence emission of NMM to the various concentrations of copper ion at room temperature (20 °C). There is a clear trend that the fluorescence intensity decreases with increasing concentration

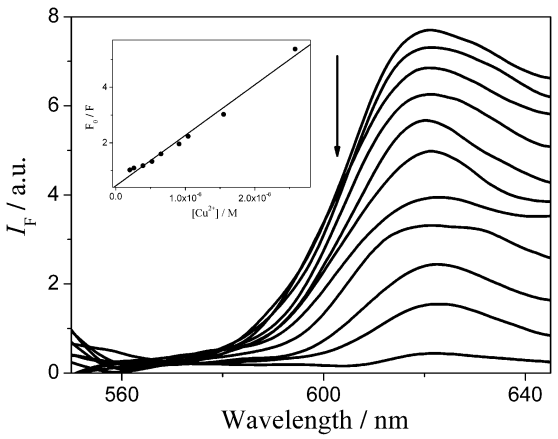

Fig. 2 Fluorescence responses of NMM (2 μM) in the presence of various concentrations of Cu^{2+}: 0, 0.2 μM, 0.26 μM, 0.39 μM, 0.52 μM, 0.65 μM, 0.91 μM, 1.04 μM, 1.55 μM, 2.58 μM and 3.1 μM. The inset shows the dependence of F_0/F (F_0 and F stand for the fluorescence intensity in the absence and presence of Cu^{2+}) at 620 nm on the Cu^{2+} concentration from 0.2 μM to 2.58 μM (R = 0.993). Experimental conditions: 2 μM NMM, 20 mM HEPES buffer (pH 7.0) containing 140 mM NaCl and 5 mM KCl, excitation of NMM at 399 nm.

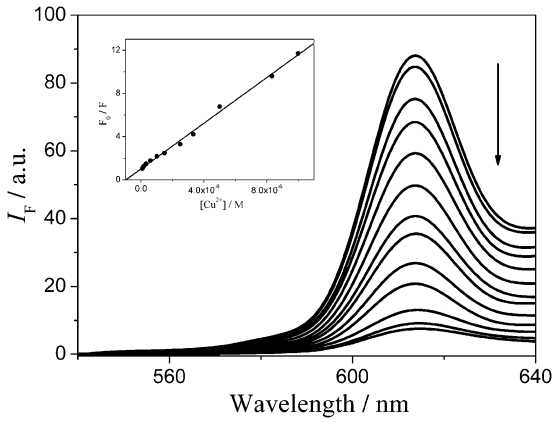

Fig. 3 Fluorescence responses of NMM + 24GT in the presence of various concentrations of Cu^{2+}: 0, 0.083 μM, 0.13 μM, 0.2 μM, 0.33 μM, 0.6 μM, 1 μM, 1.5 μM, 2.5 μM, 3.3 μM, 5 μM, 8.3 μM and 10 μM. The inset shows the dependence of F_0/F at 614 nm on the Cu^{2+} concentration from 0.083 μM to 10 μM (R = 0.997). Experimental conditions: 2 μM NMM, 0.5 μM 24GT, 20 mM HEPES buffer (pH 7.0) containing 140 mM NaCl and 5 mM KCl, excitation of NMM at 399 nm.

of copper ion. A distinct decrease in the emission spectrum is observed upon addition of as little as 200 nM of Cu^{2+}. This sensitivity is higher than that of most detection methods reported previously.[6] The inset in Fig. 2 depicts the emission intensity ratio (F_0/F) plotted against the concentration of copper ion, showing a good linear response toward Cu^{2+} in the range from 0.2 to 2.58 μM. A Job plot shows a 1 : 1 stoichiometry of Cu^{2+} to NMM in the complex (Fig. S4, ESI†). Therefore, it can be calculated that the association constant (K_a) of NMM with Cu^{2+} is 1.82×10^6 M^{-1},[7f] corresponding to a stronger binding capability of NMM toward Cu^{2+} in comparison with a rhodamine spirolactam derivative-based probe (with a K_a value of 2.08×10^4 M^{-1})[5a] or a naphthalimide-based probe (with a K_a value of 2.94×10^5 M^{-1}).[5d]

In order to improve the sensitivity and lower the limit of detection of the above-mentioned chemosensor, we utilized the fluorescence enhancement of NMM by 24GT. The propriety of this strategy is also verified by the decrease of fluorescence intensity with increasing concentration of copper ion. Fluorescence titration experiments (Fig. 3) showed that a better detection limit of 83 nM could be readily gained, with a broader linear range from 0.083 to 10 μM (Inset in Fig. 3). We tested the applicability of our chemosensor for copper ion detection in real samples (Table S2, ESI†), which shows excellent agreement with inductively coupled plasma mass spectrometry (ICP-MS). The recovery was also tested and found to be in the range of 94.5–102%. These results showed that our chemosensor can be used in real samples.

In comparison to the results reported previously *via* other approaches (Table S3, ESI†), the distinct advantage of our strategy based on fluorescence enhancement of NMM by 24GT lies in that the sensitivity, linear range and limit of detection are amenable to facile adjustment. Furthermore, this design removes the need for tedious synthesis,[5a,7f] complicated modification[7d,e] and use of organic solution.[5b,d]

In summary, a straightforward route to selectively detect copper ion was demonstrated, in which fluorescence enhancement of NMM by 24GT is interrupted by the interaction between Cu^{2+} ion and the two carboxylate groups of NMM. Excellent selectivity, comparable detection limit and adjustable detection range of target are conferred by the strategy we employed in this study. It is envisaged that in the near future G-quadruplex binding small molecules with different chelate groups can be developed to selectively detect other heavy transition metal ions.

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