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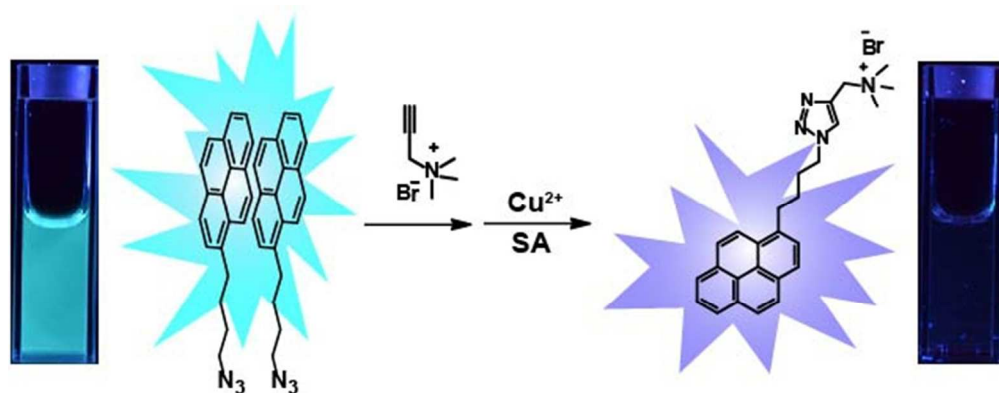
A ratiometric fluorescent probe for the visual detection of Cu^{2+} in aqueous media has been developed based on "click" reaction between a pyrene azide and a positively charged terminal alkyne.
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Visual Detection of Cu(II) Ions Based on a Simple Pyrene Derivative Using Click Chemistry

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A ratiometric fluorescent probe for the visual detection of Cu^{2+} in aqueous media has been developed based on "click" reaction between a pyrene azide and a positively charged terminal alkyne. The probe has a quick response and excellent selectivity towards Cu^{2+}, with a detection limit as low as 50 nM.

Detection of trace metal ions in drinking water, particularly for transition metal species has attracted considerable attention in recent years[1]. Copper is an essential element involved in various biological processes of organisms[2]. But on the other hand, many diseases are related to abnormal Cu^{2+} level, such as Menkes syndrome[3], familial muscular dystrophy[4] and Alzheimer's disease[5]. In the environment, it is one of the major components of the pollutants produced from industrial processes such as mining and manufacturing[6]. Therefore, developing simple and efficient approaches for the rapid and on-site detection of Cu^{2+} is still an important task in analytical chemistry.

Substantial efforts have been devoted to the development of various approaches for Cu^{2+} sensing, such as atomic absorption/emission spectroscopy[7], inductively coupled plasma mass spectroscopy[8], electrochemical assays[9], quantum dots[10], gold and silver nanoparticle probes[11], and organic fluorescent probes based on synthetic receptors[12]. Among them, fluorescent methods have been extensively investigated due to their advantages of high sensitivity, fast response, low cost, and ease of operation[13]. As one of the fluorescent sensing materials, pyrene derivatives are exceptionally useful molecular probes owing to their high fluorescence quantum yield, chemical stability, and long fluorescence lifetime[14]. Especially, the transition of fluorescence emission mode between the monomer and excimer of pyrene moieties provides a two-wavelength fluorescent responses in these probes, so called "ratiometric sensors"[15]. By inherent correction of the two emission bands, ratiometric sensors could effectively eliminate most interference from the environment. So far, many pyrene-functionalized sensing systems have been constructed by taking advantage of these properties[16]. Most pyrene-based fluorescent probes are designed in the way that analytes either link the two pyrene monomers with special receptors or detach the pyrene excimer by specific cleavage, thus inducing the formation/dissociation of the intramolecular

excimer upon binding[17]. In this scenario, it is important to control the steric configuration of the probe structure and the distance between the two pyrene rings. Therefore, the application of this strategy is often limited. Besides, the preparation of these probes usually relies on laborious multistep organic synthesis which could be cumbersome and expensive.
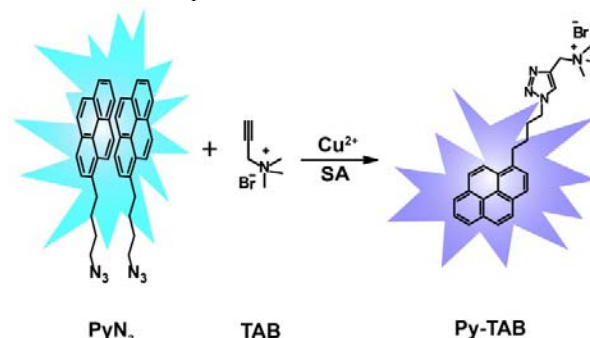
Scheme 1 Schematic illustration of the proposed sensing mechanism for the detection of Cu^{2+}. The reaction between PyN_3 and TAB caused the transformation of pyrene excimer to monomer.

It is well known that pyrene adopts a monomer conformation in good solvent whereas tends to aggregate in poor solvent along with the formation of excimer[18]. But this particular property of pyrene derivative has rarely been exploited to construct ratiometric fluorescent sensors. In this communication, we present the design of a novel pyrene-based probe with very simple structure and demonstrate its application in the detection of Cu^{2+} based on the copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction. The sensing probe is composed of two compounds, (1-(4-azidobutyl)pyrene (PyN_3) and (*N,N,N*-trimethylprop-2-yn-1-ammonium bromide (TAB) (Scheme1). In aqueous solution, the hydrophobic force drives PyN_3 molecules to form aggregates which emit excimer fluorescence. However, in the presence of Cu^{2+} and sodium ascorbate (SA) which reduces Cu^{2+} to Cu^+, the CuAAC reaction occurs within several minutes to afford a positively charged pyrene derivative, Py-TAB. These newly formed molecules possess good solubility in aqueous solution due to the positive charges. Therefore, the monomer emission of Py-TAB would be strongly enhanced along with the decrease of excimer emission caused by the

electrostatic repulsion (Scheme 1). This two wavelength output mode provides a very useful ratiometric method for the fluorescent detection of Cu^{2+} in water. Details for the sensor preparation and performance evaluation are given in the following sections.
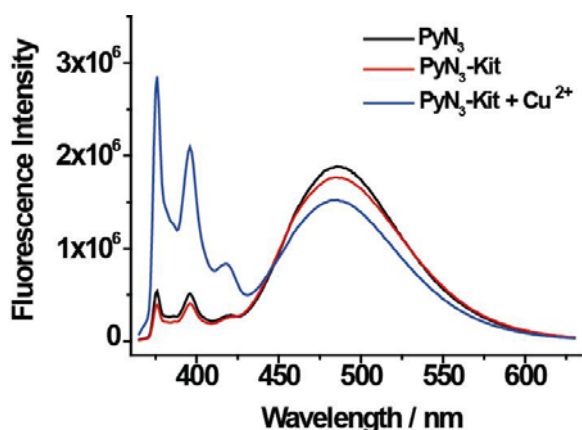

Fig. 1 Fluorescence spectra of PyN_3, PyN_3-kit and PyN_3-kit-Cu^{2+} in HEPES buffer (10 mM, pH 7.4, containing 0.05% (v/v) DMSO). $[PyN_3]$ = 5 µM, $[SA]$ = 100 µM, $[TAB]$ = 100 µM, $[Cu^{2+}]$ = 5 µM, λ_{ex}= 342 nm.

PyN_3 was synthesized by treating 1-pyrenebutanol with PBr_3 and then substitution with NaN_3 (Fig. S1-S4). The fluorescence emission of PyN_3 exhibits a strong band at 485 nm together with two weak peaks at 376 nm and 396 nm, which is the characteristic of the pyrene excimer[16] (Fig. 1). We also measured the fluorescence of the mixture of PyN_3 and TAB together with SA (the mixture referred as PyN_3 kit *vide infra*). It was found that the fluorescence emission of the PyN_3 kit was nearly identical with that of PyN_3 in HEPES buffer. However, upon the addition of Cu^{2+}, the excimer emission decreased whereas the two peaks in the monomer emission region drastically increased. This result confirmed that the presence of Cu^{2+} initiated the coupling reaction in the PyN_3 kit and caused the disaggregation of the pyrene eximer in solution. The prominent spectral changes substantiated that the PyN_3 kit is a promising probe for the ratiometric sensing of Cu^{2+}. The response time of the PyN_3-Kit to Cu^{2+} was examined in Fig. S5. With increasing reaction time, the ratio of the intensity at 376 nm (monomer emission) and 485 nm (excimer emission) (I_{376}/I_{485}) gradually increases and reaches the maximum after 15 minutes incubation. So we chose 15 minutes as the response time for all the experiments in this study.

To obtain an optimal sensing performance of the PyN_3 kit towards Cu^{2+}, we optimised the composition ratio of the kit to be PyN_3/TAB/SA = 1:20:20. The quantitative assay was performed by examining the emission changes of the PyN_3 kit in the presence of Cu^{2+} of various concentrations in aqueous media. As shown in Fig. 2A, upon addition of increasing amounts of Cu^{2+}, the emission band at 485 nm gradually decreased along with the increasing emission at 376 nm and 396 nm. These results indicated that when the CuAAC reaction produced more positively charged Py-TAB, the main conformation of the pyrene compounds in the kit had transformed from excimer to monomer. Notably, there is a good linear relationship between I_{376}/I_{485} and the concentration of Cu^{2+} (R = 0.998 from 0.05 to 1 µM), implying that this ratiometric method could be applied to quantitative detection of Cu^{2+} within the above range (Fig. 2B). The detection limit of the PyN_3-kit for Cu^{2+} in aqueous media was determined to be 50 nM according to the 3σ rule.

To substantiate the Cu^{2+} sensing mechanism proposed in Scheme 1, we performed several control experiments to interrogate the role of each component in the detection system. The fluorescence spectra
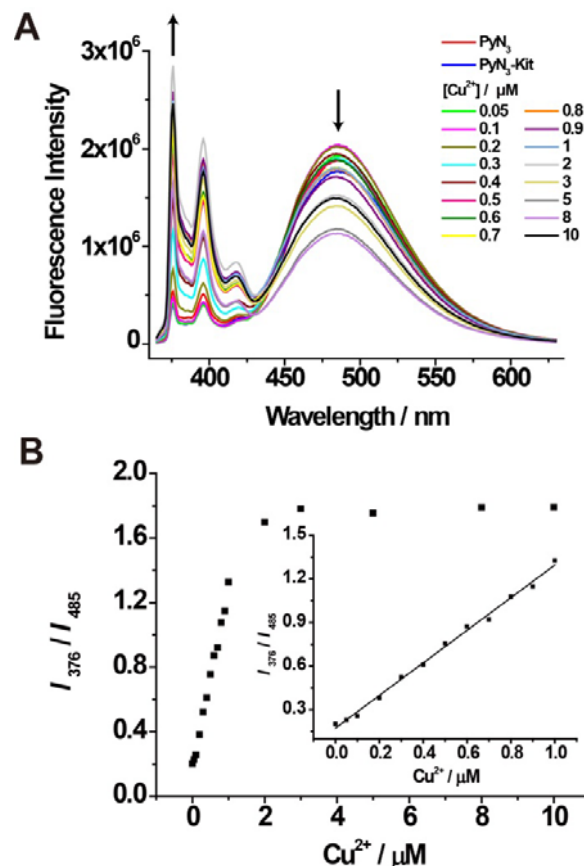

Fig. 2 (A) Fluorescent spectra of PyN_3-kit (5 µM) in HEPES buffer (10 mM, pH 7.4, containing 0.05% (v/v) DMSO) in the presence of increasing amount of Cu^{2+}. (B) The relationship between the ratio of I_{376}/I_{485} and the concentration of Cu^{2+}. $[PyN_3]$ = 5 µM, $[SA]$ = 100 µM, $[TAB]$ = 100 µM, λ_{ex} = 342 nm.
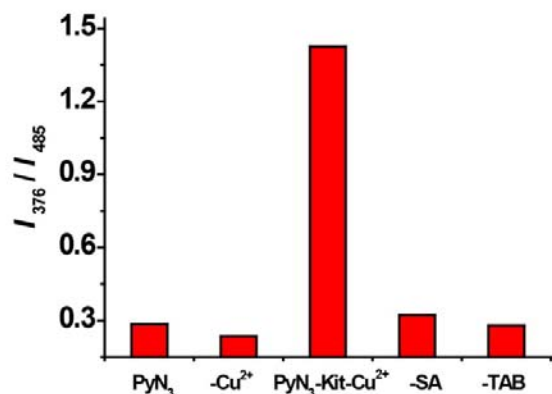

Fig. 3 The fluorescence intensity ratios of I_{376}/I_{485} of PyN_3-kit-Cu^{2+} and the mixtures with one component each missing. $[PyN_3]$ = 5 µM, $[SA]$ = 100 µM, $[TAB]$ = 100 µM, $[Cu^{2+}]$ = 5 µM.

of the PyN_3 kit in the presence of Cu^{2+} and the mixtures of PyN_3-kit-Cu^{2+} in the absence of Cu^{2+}, SA, TAB and PyN_3 each respectively were shown in Fig. S6. The ratios of (I_{376}/I_{485}) of the above measurements were displayed in Fig. 3. It is obvious that in the absence of Cu^{2+} or any of the components in the PyN_3 kit the CuAAC reaction would not occur and thus no monomer emission enhancement could be observed. Another experiment we conducted is the comparison of the emission spectra of PyN_3 in DMSO/water mixture of varying ratios (Fig. 4 and S7). It was found that with increasing volume fraction of DMSO (V_D) in the mixed solvent, the

ratio of (I_{376}/I_{485}) gradually increased and reached the maximum value at $V_D = 1$. This result clearly indicates that when water is the main component in the solvent mixture, PyN_3 adopts excimer conformation due to hydrophobicity of the pyrene rings. However, with the increasing amount of DMSO, which is a good solvent for PyN_3, the PyN_3 excimer starts to disaggregate and give rise to the formation of monomers. Besides, these spectral changes are similar to that of the PyN_3 kit caused by the addition of Cu^{2+}, which serves as an indirect support for the sensing mechanism proposed in Scheme 1. On the other hand, we also examined the fluorescence responses of PyN_3 in DMSO with the increasing ratio of water as solvent. The results showed that the disaggregated PyN_3 in DMSO started to form excimer when water was added, and was completely restored to the aggregation state when the ratio of DMSO dropped to 2% (Fig. S8). Meanwhile, we carried out one more control experiment by treating Cu^{2+} with cysteine which has a high affinity to Cu^{2+} before adding it to the PyN_3 kit. It was found that pre-incubation of Cu^{2+} with cysteine for 10 min dramatically lowered the concentration of free Cu^{2+} and addition of the Cu^{2+}-cysteine complex to the PyN_3 kit only induced very subtle changes in fluorescence spectra (Fig. S9). Finally, we run the click reaction between PyN_3 and TAB catalyzed by Cu^{2+} under the aforementioned conditions and purified the final product. The structure of the PyN_3-TAB was confirmed by ^{1}H, ^{13}C NMR and mass spectrometry characterizations (Fig. S10-S13). These results further substantiated that the CuAAC reaction is the key to the Cu^{2+} sensing strategy.
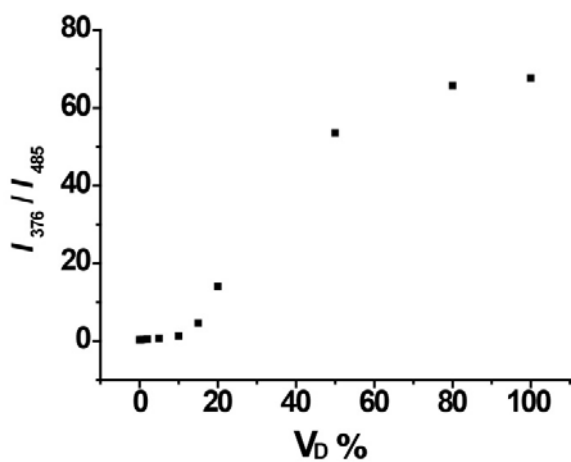

Fig. 4 Solvent composition dependence of the ratios of I_{376}/I_{485} in DMSO/water mixed solvents. $[PyN_3] = 5$ μM, $[SA] = 100$ μM, $[TAB] = 100$ μM, $[Cu^{2+}] = 5$ μM.
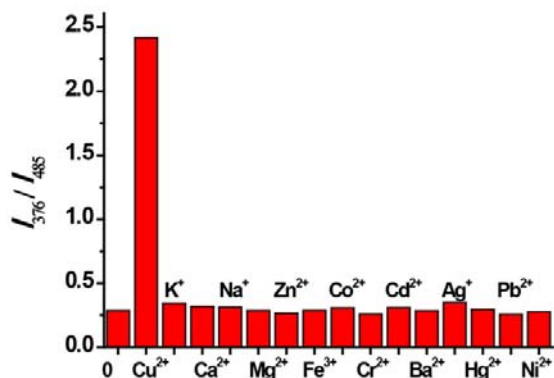

Fig. 5 Relative fluorescence intensity of PyN_3 kit in the presence of Cu^{2+} and other metal ions in HEPES buffer (10 mM, pH 7.4, containing 0.05% (v/v) DMSO). $[PyN_3] = 5$ μM, $[SA] = 100$ μM, $[TAB] = 100$ μM, $[Cu^{2+}] = 5$ μM, [other ions] = 20 μM.
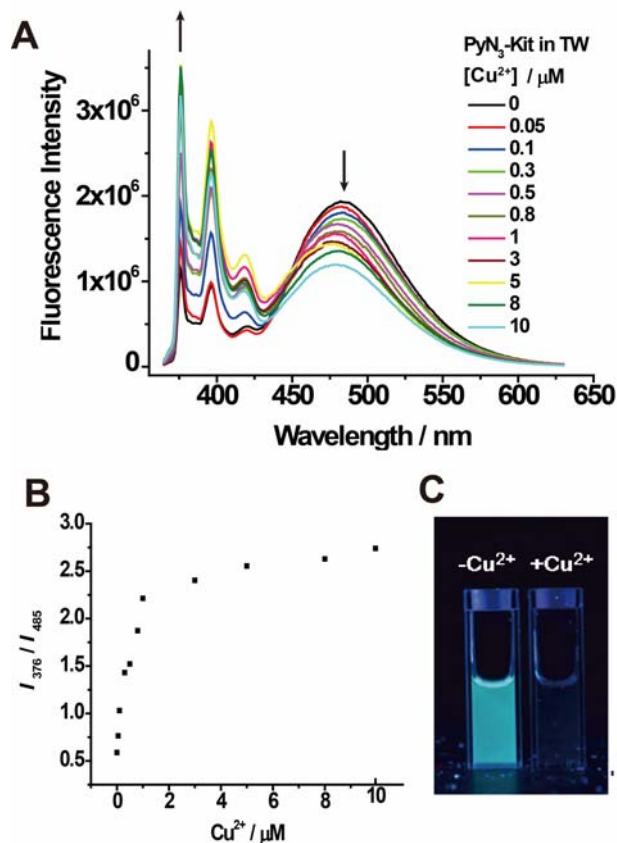

Fig. 6 (A) Fluorescence spectra of the PyN_3 kit (5 μM) in tap water (5% DMSO) in the presence of increasing amount of Cu^{2+}. (B) The relationship between the ratio of I_{376}/I_{485} and the concentration of Cu^{2+}. $[PyN_3] = 5$ μM, $[SA] = 100$ μM, $[TAB] = 100$ μM, ($\lambda_{ex} = 342$ nm). (C) The photographs of the PyN_3 kit in the absence and presence of 5 μM Cu^{2+}, excited by a hand-held blacklight ($\lambda_{ex} = 365$ nm).

Next, we examined the selectivity of the PyN_3 kit for the detection of Cu^{2+}. A variety of metal ions including K^+, Ca^{2+}, Na^+, Mg^{2+}, Zn^{2+}, Fe^{3+}, Co^{2+}, Cr^{3+}, Cd^{2+}, Ba^{2+}, Ag^+, Hg^{2+}, Pb^{2+} and Ni^{2+} were tested with the kit under otherwise identical conditions (Fig. S14). Comparison of the emission spectra clearly showed that the introduction of other metal ions, even though four times more concentrated than Cu^{2+}, into the PyN_3 kit solution had negligible effects on the PyN_3 emission profile. Figure 5 shows the fluorescence intensity ratios of I_{376}/I_{485} in the presence of various metal ions. The intensity ratio bar of Cu^{2+} is significantly higher than that of all other metal ions, indicating that there is virtually no interference from other metal ions for the detection of Cu^{2+} with the PyN_3 kit. Futhermore, we also tested the specificity by mixing Cu^{2+} with each interfering metal ion. The Cu^{2+} sensing performance of the PyN_3 kit was little affected by the presence of interfering metal ions (Fig. S15). Comparisons of the response time, detection limit and advantages/disadvantages of the PyN_3 kit with other currently available methods are listed in Table S1. It is clear that the overall performance of our new probe is among the best reported for Cu^{2+} sensing.

While our new Cu^{2+} sensing kit has quick response time, excellent sensitivity and selectivity, we sought to explore its practical applicability in the rapid detection of the samples of environmental relevance. Thus, we carried out the detection of Cu^{2+} in tap water with 5% (v/v) DMSO instead of HEPES buffer solution. It is interesting to see that the Cu^{2+}-mediated spectral changes of the PyN_3 kit was barely affected by the unpurified solvent (Fig. 6A). The detection limit also retains at about 50 nM which is much lower than

the safety limit of Cu^{2+} by WHO standards (Fig. 6B). To further substantiate if the sensing kit could be employed for real-time and on-site detection without using the fluorescence spectrometer, we conducted the visual detection of Cu^{2+} by excitation with a hand-held black light (365 nm). To our delight, a distinct colour change of the PyN$_3$ kit solution from cyan to dark blue was observed in the presence of 5 µM Cu^{2+} (Fig. 6C). This result confirmed that our new sensing probe can be developed into a practically useful kit for rapid, visual and low-cost detection of Cu^{2+} in aqueous media.

Conclusions

In conclusion, a simple and rapid sensing platform for the ratiometric fluorescent detection of Cu^{2+} in aqueous media was successfully developed based on the CuAAC click reaction and subsequent excimer-monomer transformation of the pyrene moieties. This sensor features rapid response, low cost, high sensitivity and selectivity, and excellent anti-interference ability in real samples. We expect that the assay reported herein could be developed into a practically useful kit for the real-time, on-site detection of Cu^{2+}.

Acknowledgments

Notes and references

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