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Random dsDNA-templated formation of copper nanoparticles as novel fluorescence probes for label-free lead ions detection†

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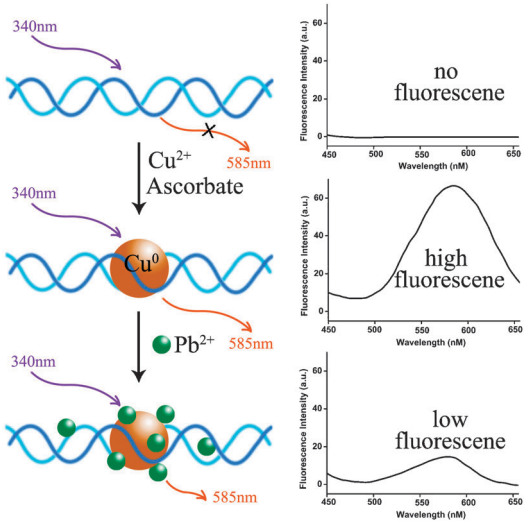
A simple label-free method for the detection of Pb^{2+} ions with high selectivity and sensitivity has been developed by using random double-strand DNA-templated formation of copper nanoparticles as novel fluorescence probes in aqueous solution.

As ideal alternatives to organic dyes and quantum dots, ultrasmall fluorescent nanoparticles including several noble metal nanoclusters, such as, Au, Ag, and Pt nanoclusters, have attracted special research interest due to their unique physical, electrical, and optical properties for use *in vivo* imaging and *in vitro* chemical/biological detection.[1] For example, Wang and co-workers have utilized DNA as scaffolds for the synthesis of fluorescent Ag nanoclusters and applied for single nucleotide mutation identification.[1c] Ying and colleagues have reported protein-directed synthesis of highly fluorescent Au nanoclusters and successful use for the inspection of Hg^{2+} ions.[1d,e] Inouye *et al.* have synthesized water-soluble, blue-emitting, and atomically monodispersed Pt nanoclusters and successful bioimaging of living Hela cells labeled with these intriguing fluorophores.[1f] Very recently, Mokhir's group reported that double-strand DNA (dsDNA) can act as an efficient template for the formation of fluorescent copper nanoparticles (CuNPs) at low concentration of Cu^{2+}, whereas single-strand DNA (ssDNA) does not support nanoparticle formation.[2] The formed dsDNA–CuNPs exhibited excellent fluorescence properties and emerged as novel fluorescent markers. Although exploration of dsDNA–CuNPs in biological analysis is still in its infancy,[3] such promising fluorescence probes are highly attractive for biochemical application because of their facile synthesis, ultrafine size, and outstanding spectral and photophysical properties. It would be of great interest to probe the application of the dsDNA–CuNPs in environmental inspection and clinical diagnosis.

Routine detection of lead ions (Pb^{2+}) is central to field monitoring and disease diagnosis because of its deleterious effects on the environment and human health. In the past few years, many exquisite strategies for Pb^{2+} analysis have been developed, most of which are based on Pb^{2+}-dependent RNA-cleaving DNAzyme[4] and Pb^{2+}-induced allosteric G-quadruplex DNAzyme.[5] However, these Pb^{2+} sensors required specific nucleic acid sequences, which limited its widespread application for routine monitoring. Hence, it is a big challenge to develop a simple, rapid, and robust Pb^{2+} sensor using random nucleic acid sequences as a sensing platform.

Herein, for the first time, we demonstrate a new and facile strategy for the highly sensitive and selective detection of Pb^{2+} using dsDNA–CuNPs as fluorescence probes. Interestingly, it was found that Pb^{2+} can quench the fluorescence of dsDNA–CuNPs. Based on such a phenomenon discovered in this work, a very simple and rapid method for Pb^{2+} monitoring has been established.

As shown in Scheme 1, dsDNA can be used as efficient templates for the formation of CuNPs through the reduction of Cu^{2+} by ascorbate, and the formed dsDNA–CuNPs have excellent fluorescence. After addition of Pb^{2+} into the dsDNA–CuNPs sensing system, the fluorescence decreased dramatically. Such selective quenching effect of Pb^{2+} on the fluorescence of the Cu nanoparticles has been validated for



Scheme 1 Schematic representation showing the protocol for the label-free detection of Pb^{2+} using dsDNA–CuNPs as the novel fluorescence probes.

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analyses of human urine and river water samples. The sensing mechanism to be hypothesized is as follows. The surface of the dsDNA-stabilized CuNPs was surrounded by many Cu^+ in the presence of ascorbate as a reducing agent and stabilizing ligand. The Pb^{2+} could react with Cu^+ at the surface of the copper nanoparticles *via* the $5d^{10}(Pb^{2+})$–$3d^{10}(Cu^+)$ metallophilic interactions to induce fluorescence quenching.[1e,2,6] However, due to the high complexity of the dsDNA–CuNPs system and the lack of adequate understanding of the chemical properties and fluorescence characteristics of dsDNA–CuNPs, exploration of dsDNA–CuNPs applied in biological- or chemical-sensing is still at an early stage.[2,3] It is still a challenge for us to fully understand the quenching mechanism of Pb^{2+} on dsDNA–CuNPs and this is a subject for further investigation. Based on the fluorescence characteristics of dsDNA–CuNPs and the quchening effects of Pb^{2+}, a novel sensor for Pb^{2+} detection has been designed in the presented study.

The dsDNA-templated CuNPs were synthesized according to previous reports[2,3] (ESI†). The obtained fluorescent CuNPs showed excitation and emission peaks at 340 and 585 nm, respectively (Fig. S1, ESI†). The maximum fluorescence emission peak was utilized to evaluate the effects of Pb^{2+} on the fluorescence emission of the dsDNA–CuNPs. The reaction pH would play a significant role in the sensitivity of the dsDNA–CuNPs sensing system toward Pb^{2+}. As shown in Fig. S2 (ESI†), no obvious fluorescence properties were observed under acidic conditions, while striking fluorescent CuNPs have been successfully synthesized in alkaline pH. Over the pH range from 5.5 to 8.5, it was found that pH 7.5 provided the optimal fluorescence signal of dsDNA–CuNPs and obtained the maximal quenching effect enhancement for Pb^{2+} detection.

Reduction of Cu^{2+} by ascorbate in the presence of dsDNA templates was completed in a few minutes after the start of the reaction (Fig. 1A). ssDNA could not be serviced as an effective template for the formation of CuNPs and no fluorescent signal could be detected. We observed that the fluorescence intensity of dsDNA–CuNPs increased with the increasing of the number of base pairs in dsDNA templates (Three pairs of random dsDNA templates with different lengths and base composition are listed in Table S1, ESI†). It was reported that a larger size of CuNPs was formed in the longer dsDNA template, which induced higher fluorescence quantum yield.[2] In order to obtain a high sensitivity and broad dynamic range for Pb^{2+} detection, 35 bp dsDNA was used in all further experiments.

The quenching kinetics of the dsDNA–CuNPs to different concentrations of Pb^{2+} was further investigated. As shown in Fig. 1B, the quenching effects occurred immediately once the quencher was added to the dsDNA–CuNPs solution, and the enhancement of quenching effect reached a constant value in 5 min. Such fast response is of particular importance in field application where individual samples must be analyzed in a short time. Without Pb^{2+}, dsDNA–CuNPs displayed no significant change in fluorescence intensity during the analysis process, which confirmed that quenching effect was actually due to the Pb^{2+} introduction. The as-prepared dsDNA-stabilized copper nanoparticles are highly stable under the synthesis conditions. And there was no obvious decrease in the fluorescence intensity of the dsDNA–CuNPs at room temperature within 3 h, making them suitable for analytical applications. Considering the fast and intensive quenching effect of Pb^{2+} to dsDNA–CuNPs, it is possible to develop a new Pb^{2+} sensor that can be adapted to hand-held instruments.

A series of different dilutions of the as-prepared dsDNA–CuNPs displayed a similar response pattern to Pb^{2+} (Fig. S3, ESI†). With the gradual dilution of the solution, although the sensitivity seemed to be increased, the linear range became worse, which resulted from less amount of the fluorescent CuNPs formed in the presence of dsDNA. And thus, "1X" dsDNA–CuNPs, which provided the best compromise between high sensitivity and broad dynamic range, was used for all subsequent experiments.

The sensitivity and linearity of the dsDNA–CuNPs–Pb^{2+} reaction system was investigated by varying the Pb^{2+} concentration. As shown in Fig. 2A, the fluorescence intensity was sensitive to and proportionately decreased with the increasing concentration of Pb^{2+} and no noticeable change in the maximum or shape of emission spectra accompanied the fluorescence decreasing. However, when the concentration of Pb^{2+} is greater than 200 nM, the fluorescence intensity decreased no further and a plateau was reached. Fig. 2B outlines the relationship between the fluorescence intensity at 585 nm and the concentration of Pb^{2+}. The linear relation (range was from 5 nM to 100 nM) with an R^2 of 0.985 could be described by the Stern–Volmer equation,[7] $F_0/F = 1 + K_{sv}[Q]$,

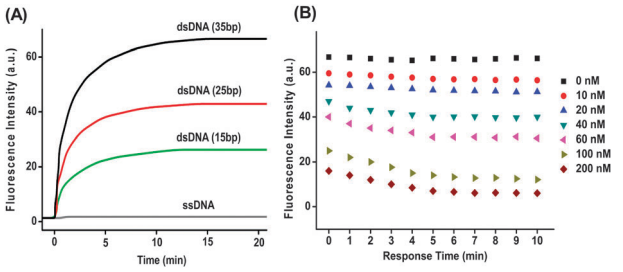

Fig. 1 (A) Time dependence of the fluorescence intensity at λ_{em} = 585 nm (λ_{ex} = 340 nm) after addition of Cu^{2+} (100 μM) to buffered solutions (10 mM MOPS, pH 7.5; NaAc 150 mM; sodium ascorbate 1 mM) of dsDNA (500 nM) of different lengths. (B) The response time of dsDNA–CuNPs to different concentrations of Pb^{2+}. The buffered solutions as the same in (A).

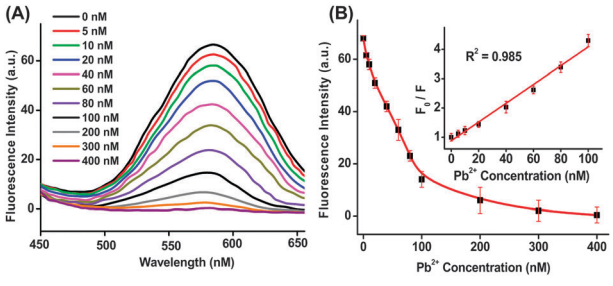

Fig. 2 (A) Fluorescence emission spectra from analyzing different concentrations of Pb^{2+}. (B) The fluorescence intensity at 585 nm against Pb^{2+} concentration. The inset is the Stern–Volmer plot of fluorescence quenching of the dsDNA–CuNPs by Pb^{2+}. The error bars represent the standard deviation of three independent measurements.

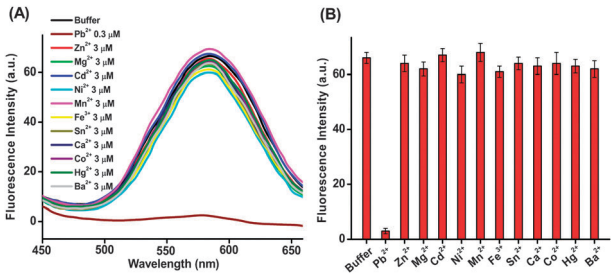

Fig. 3 (A) Fluorescence emission spectra of dsDNA–CuNPs in the absence and presence of different metal ions. (B) Selectivity of the dsDNA–CuNPs to different metal ions. The concentration of Pb^{2+} was 0.3 μM; the concentration of each of the other metal ions was 3 μM. The error bars represent the standard deviation of three independent measurements.

where F_0 and F are, respectively, the fluorescence intensity at 585 nm in the absence and presence of Pb^{2+}, K_{sv} is the Stern–Volmer quenching constant (inset in Fig. 2B). It is found that there is an obvious change in the fluorescence spectra upon addition of 5 nM Pb^{2+}, namely, the limit of detection (LOD) for Pb^{2+} analysis with the dsDNA–CuNPs probe is 5 nM, which is much lower than the maximum level (72 nM) of Pb^{2+} in drinking water permitted by the United States Environmental Protection Agency (EPA).[8] Our present work provided an LOD value that is comparable to other reported fluorescence detection methods.[9]

For an excellent analytical system, high specificity is a matter of necessity. To investigate the selectivity of the as-prepared dsDNA–CuNPs for Pb^{2+} analysis, several other divalent metal ions are tested as a control. Fig. 3 shows a high selectivity of the novel fluorescence probe for Pb^{2+} against other metal ions. The presence of Zn^{2+}, Mg^{2+}, Cd^{2+}, Ni^{2+}, Mn^{2+}, Fe^{3+}, Sn^{2+}, Ca^{2+}, Co^{2+}, Hg^{2+}, Ba^{2+} (each 3 μM) could not quench the fluorescence of the copper nanoparticles, while only 0.3 μM Pb^{2+} led to almost 100% quenching of CuNPs fluorescence. In addition, the CuNPs fluorescence probes are highly robust towards various anions (*e.g.* PO_4^{3-}, CO_3^{2-}, Ac^-, Cl^-, SO_4^{2-}, and $B_4O_7^{2-}$) and buffers (*e.g.* PBS and Tris). These results demonstrate that the dsDNA–CuNPs can serve as novel fluorescence probes for highly selective and reliable Pb^{2+} monitoring.

The potential application of the proposed sensor is demonstrated by applying it to detecting Pb^{2+} in human urine samples and in real freshwater samples (river water). The results of the Pb^{2+} determination in human urine samples are summarized in Table S2 (ESI†), revealing that no significant differences existed between the values measured using the proposed novel dsDNA–CuNPs fluorescence probes and the standard inductively coupled plasma/mass spectroscopy (ICP/MS) method. In addition, we also applied the sensor to analyze river water samples obtained from Zhujiang River (Guangzhou, China). The samples collected were simply filtered and found that the Pb^{2+} content in the river water was too low to be detected by the sensor. The recovery experiments with spiked Pb^{2+} were carried out and the analytical results are shown in Table S3 (ESI†). Satisfactory values between 86 and 108% for Pb^{2+} were obtained for the recovery, which confirmed that the proposed sensor was applicable for practical Pb^{2+} detection in real samples with other potentially competing species coexisting. The above results demonstrate that our introduced dsDNA–CuNPs fluorescence probes can be applied to the analysis of Pb^{2+} in clinical diagnosis and environmental monitoring.

In conclusion, we have developed a novel, simple, and label-free method for the detection of Pb^{2+} using random dsDNA-templated formation of CuNPs as fluorescence probes in aqueous media. The dsDNA–CuNPs provided excellent selectivity for Pb^{2+} over other metal ions, and also high sensitivity, with a detection limit of 5 nM. This approach avoids the need to adopt specific Pb^{2+}-dependent DNAzyme or G-quadruplex for Pb^{2+} detection and eliminates the use of sophisticated experimental techniques and equipment. As biocompatible, environmentally-friendly, and facile fluorophores, copper nanoparticles are promising to become versatile tools for fluorescent sensing. Future work will focus on exploring new applications of the dsDNA–CuNPs for the detection of other analytes.

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