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A simple "clickable" biosensor for colorimetric detection of copper(II) ions based on unmodified gold nanoparticles

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ABSTRACT

A novel colorimetric copper(II) biosensor has been developed based on the high specificity of alkyne–azide click reaction to the catalysis of copper ions and unmodified gold nanoparticles (AuNPs) as the signal reporter. The clickable DNA probe consists of two parts: an azide group-modified double-stranded DNA (dsDNA) hybrid with an elongated tail and a short alkyne-modified single-stranded DNA (ssDNA). Because of low melting temperature of the short ssDNA, these two parts are separated in the absence of Cu^{2+}. Copper ion-induced azide–alkyne click ligation caused a structural change of probe from the separated form to entire dsDNA form. This structural change of probe can be monitored by the unmodified AuNPs via mediating their aggregation with a red-to-blue colorimetric read-out because of the differential ability of ssDNA and dsDNA to protect AuNPs against salt-induced aggregation. Under the optimum conditions, this biosensor can sensitively and specifically detect Cu^{2+} with a low detection limit of 250 nM and a linear range of 0.5–10 μM. The method is simple and economic without dual-labeling DNA and AuNPs modification. It is also highly selective for Cu^{2+} in the presence of high concentrations of other environmentally relevant metal ions because of the great specificity of the copper-caused alkyne–azide click reaction, which potentially meets the requirement of the detection in real samples.

1. Introduction

In recent years, great efforts have been devoted to the detection of Cu^{2+} ions in environmental and biological systems because of their possible toxic effect on public health and ecosystem. Copper is the third most abundant transition metal in the human body, and it plays an essential role in physiological processes below certain amounts, involving energy generation, dioxygen transport and activation, and signal transduction (Que et al., 2008). However, at elevated concentrations, it is highly toxic and pathogenic to living organisms, leading to serious neurodegenerative diseases, such as Menkes and Wilson diseases, hereditary aceruloplasminemia, Alzheimer's disease, and prion disease (Brown and Kozlowski, 2004; Georgopoulos et al., 2001; Vulpe et al., 1993; Waggoner et al., 1999; Zietz et al., 2003). In this regard, developing robust environment-friendly and high-throughput methods to detect trace copper with high sensitivity and selectivity has become increasingly important in biological and environmental analysis. Several methods have been developed to detect copper ions based on organic fluorophores or chromogenic sensors (Chen and Zhong, 2005; Wu and Anslyn, 2004; Wen et al., 2006; Xiang et al., 2006; Zeng et al., 2006), novel-metal nanoclusters (Chen et al., 2009; Lan et al., 2010; Shang and Dong, 2008), quantum dots or nanorods (Chan et al., 2010; Gattás-Asfura and Leblanc, 2003; Liu et al., 2010), peptides (Zheng et al., 2002) and voltammetry (Etienne et al., 2001). However, the selectivity is an important issue for copper ion biosensors because the traditional copper ions detection methods are readily susceptible to the interference by other metal ions, such as Co^{2+}, Ni^{2+}, Hg^{2+}, Pd^{2+}, and Fe^{3+} (Lan et al., 2010; Shang and Dong, 2008; Wu and Anslyn, 2004).

The copper(I)-catalyzed alkyne–azide Huisgen cycloaddition, the best example of "Click Chemistry", appears to solve this problem due to its high yielding capability under common reaction conditions with little by-product and high specificity to the catalysis of Cu^{+} (Deiters et al., 2003; Rostovtsev et al., 2002; Shen et al., 2012; Wang et al., 2003). Cu^{+} was conveniently formed through the reduction of Cu^{2+} in the presence of sodium ascorbate. Owing to these unique characteristics, the copper(I)-catalyzed "Click" cycloaddition acts as a powerful linking reaction in a wide range of applications, such as drug discovery (Kolb and Sharpless, 2003; Manetsch et al., 2004; Mocharla et al., 2005), polymer synthesis (Ladmiral et al., 2006; Malkoch et al., 2005; Parrish et al., 2005; Wu et al., 2004), nanoparticle fabrication (Joralemon et al., 2005), biomacromolecule functionalization (Beatty et al., 2005; Wang et al., 2003), and

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biosensing (Qiu et al., 2011; Zhang et al., 2010). Meanwhile, this click reaction was intended for the development of copper ion sensors (Song et al., 2010). Hulme's group reported a luminescent sensor for detecting Cu^{+} based on the sensitized Eu(III) complex and "click" reaction (Viguier and Hulme, 2006). However, this method requires complex and expensive instruments, which inhibits its application for on-site use.

Colorimetric methods are convenient and attractive in biosensors design for their simplicity, easy observation by the naked eye and no requirement for sophisticated instruments (Rosi and Mirkin, 2005; Zhao et al., 2008). Because of their unique optical properties including high extinction coefficient and adjustable plasmon band, gold nanoparticles (AuNPs) are perfectly suitable for colorimetric biosensors (Liu and Lu, 2006; Lim et al., 2007; Li et al., 2011; Mirkin et al., 1996; Pan et al., 2012; Storhoff et al., 2000; Sato et al., 2003; Zhao et al., 2007). Jiang's group has developed a colorimetric method for the Cu^{2+} assay by azide- and terminal alkyne-functionalized gold nanoparticles using click chemistry (Zhou et al., 2008). Nevertheless, the relatively low efficiency of conventional click chemistry on AuNPs surface causes a long reaction time (24 h) and relatively high detection limit. To solve this disadvantage, Mirkin's group developed a colorimetric Cu^{2+} sensor based on DNA-functionalized gold nanoparticles and click chemistry (Xu et al., 2010). They applied DNA-templated click chemistry in their assay and achieved remarkably shorter reaction time (2 h) and excellent selectivity. However, this AuNP-based assay relies on the modification of AuNPs with dual labels of alkyne (or azide) and thiol groups at the ends of DNA. Such dual-modified DNA is rather high-cost and further effort is required to prepare DNA-functionalized AuNPs. In addition, this approach requires alternating temperature in the experimental process. Therefore, it still remains a challenge to develop simple, rapid, and specific colorimetric sensors for the detection of Cu^{2+} based on the click chemistry.

In this study, we report a simple colorimetric method for assaying Cu^{2+} ions by using copper(I)-catalyzed click chemistry and unmodified AuNPs. The rationally designed DNA probe can switch its structure from separated form to rigid dsDNA structure responsive to copper(I)-catalyzed click cycloaddition. Such copper ion-induced DNA structure variation was applied to mediate the dispersion or aggregation of unmodified AuNPs, thus the existence of copper ion can be sensitively probed by the color change of AuNPs. The DNA probe used here for click cycloaddition avoids the usage of expensive dual-labeling and the preparation of AuNPs was facile without modification. This colorimetric strategy displayed high sensitivity and excellent selectivity for the detection of Cu^{2+} because of high efficiency and great specificity of click chemistry. Moreover, the colorimetric read-out of this AuNPs-based assay can be readily observed by the naked-eye, which is promising for on-site use.

2. Experimental

2.1. Materials and measurements

Hydrogen tetrachloroaurate(III) tetrahydrate, copper(II) sulfate and sodium ascorbate were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Trisodium citrate and sodium chloride were obtained from Beijing Chemical Reagent Company (Beijing, China). All chemical reagents were of analytical grade and used without further purification. The ultra-pure water (18.25 MΩ cm) used throughout all experiments was purified with a Millipore system. All the oligonucleotides used in this work were synthesized by Takara Biotechnology Co. Ltd. (Dalian, China) and purified by high-performance liquid chromatography (HPLC). Before use, the oligonucleotide sample was dissolved in water. The concentration of the oligonucleotide was determined by measuring their UV absorbance at 260 nm. UV–Vis absorption spectra were acquired on a Beckman DU-800 spectrophotometer using quartz cuvettes with an optical path length of 1 cm at room temperature. The pH was measured with a Mettler Toledo FE20 pH-meter. The sequences of the oligonucleotides are as follows.

P1: 5′-alkyne-GCGATCT-3′
P2: 5′-CCTCCACTGTTCC-azide-3′
P3: 5′-AGATCGCGGAACAGTGGAGG-3′

2.2. Synthesis of AuNPs

All glassware was cleaned with aqua regia (ratio of HCl/HNO_3=3:1 in volume), rinsed thoroughly with ultrapure water, and oven-dried prior to use. AuNPs were synthesized according to the reported method (Jin et al., 2003). A 250 ml aqueous solution of 1 mM hydrogen tetrachloroaurate(III) tetrahydrate was heated to reflux, and then 25.0 ml 38.8 mM of trisodium citrate was added quickly. The solution was boiled for another 15 min, during that time its color changed from pale yellow to deep red. This solution was cooled to room temperature under continuous stirring. The resulting deep red solution was stored in a refrigerator at 4 °C before use. The final concentration of AuNPs was estimated to be about 11 nM using UV–Vis spectrometric measurement based on an extinction coefficient of $\sim 2.7 \times 10^{8}\ M^{-1}\ cm^{-1}$ at λ 520 nm for 13 nm particles.

2.3. General procedure of colorimetric determination of Cu^{2+}

In a typical experiment, DNA-templated chemical ligation was first conducted. A volume of 1 μL of Cu^{2+} solution with different concentrations was added to 9 μL of mixture containing P1 (1 μM), P2 (1 μM), P3 (1 μM) and sodium ascorbate (100 μM) in PBS buffer (10 mM sodium phosphate, 0.2 M NaCl, pH 7.4), and the reaction mixture was incubated at room temperature for 1 h or 20 min, dependent on the Cu^{2+} concentration. After that, 7 μL of the reaction solution was transferred into a 0.2 mL microcentrifuge tube, and then 33 μL of water, and 30 μL of AuNPs were micropipetted into this solution to give a volume of 70 μL. After incubation for 2 min, 50 μL of 0.2 M NaCl was added immediately to give a final volume of 120 μL. The photographs of resulting solutions were taken and the UV–visible absorption spectra of the mixtures were recorded immediately.

3. Results and discussion

3.1. Principle for the biosensing system

The mechanism for the Cu^{2+} biosensor is shown in Fig. 1. Three ssDNA were used in the experiment. The 7-mer ssDNA (P1) was modified with an alkyne group at 5′ end and the 13-mer ssDNA (P2) was functionalized with an azide group at 3′ end. The ssDNA (P3) with 20-mer oligonucleotides was designed to complement to P1 and P2 with 7 base pairs and 13 base pairs, respectively. Since the *Tm* of the hybrid of P1/P3 and P2/P3 were estimated by Oligoanalyzer of IDT to be 24 °C and 55 °C, respectively (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/), the hybrid of P1/P3 is unstable at room temperature (25 °C), thus the P1 prefer to exist as unhybridized form. Therefore, the free 7nt ssDNA P1 can be adsorbed onto the surface of AuNPs because ssDNA is sufficiently flexible to partially uncoil its bases to interact with the surface of AuNPs through the attractive

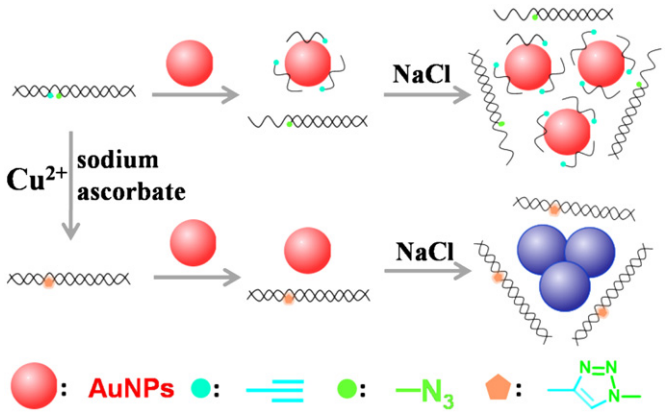

Fig. 1. Schematic representation of the strategy of colorimetric Cu^{2+} assay based on unmodified AuNPs and DNA-templated click chemistry.

van der Waals forces (Li and Rothberg, 2004). Thus, the increasing electrostatic repulsion between ssDNA-adsorbed AuNPs compared to naked AuNPs could significantly enhance the resistance of AuNPs to the salt-induced aggregation. Correspondingly, the color of reaction solution remained red after addition of salt. However, in the presence of Cu^{2+}, Cu^{+} is formed through the reduction of Cu^{2+} by sodium ascorbate and catalyzes the DNA-templated "Click" cycloaddition to generate a chemical ligation of P1 and P2. This newly formed P1 – P2 ssDNA is totally complementary to P3 and yields a stable duplex at room temperature. The stiff dsDNA structure containing inner base and exposed sugar-phosphate backbone eliminates the base-AuNPs interaction and simultaneously causes a strong repulsion between dsDNA and negatively charged AuNPs, prohibiting the adsorption of dsDNA on AuNPs (Li and Rothberg, 2004). The lack of protection of adsorbed DNA makes AuNPs susceptible to salt-induced aggregation, thus inducing color change of AuNPs from red to blue in the presence of salt.

3.2. Colorimetric biosensing of Cu^{2+}

Fig. 2A depicts a typical colorimetric detection of Cu^{2+}. In the absence of Cu^{2+}, the AuNPs solution remained red after the addition of NaCl, indicating that the unhybridized ssDNA P1 was adsorbed onto the surface of AuNPs and salt-induced aggregation was prevented. On the other hand, when Cu^{2+} was added into the ligation reaction solution, the color of the AuNPs solution changed from red to blue within 1 s after NaCl was added (inset in Fig. 2A). This color change of the AuNPs suggests that the click-ligation causes the generation of a stable dsDNA of P1 – P2/P3, which hampers the adsorption of P1 onto AuNPs. Correspondingly, only one characteristic peak at 520 nm appeared in the reaction solution before Cu^{2+} treatment, whereas broad absorption (500–800 nm) was observed after Cu^{2+} treatment.

The transmission electron microscope (TEM) image demonstrates the well-dispersed AuNPs in the absence of Cu^{2+} (Fig. 2B) and the formation of aggregates in the presence of Cu^{2+} (Fig. 2C). Neocuproine, a specific copper(I)-chelator (Byrnes et al., 1992), was used to certificate the generation of Cu^{+} in click ligation solution. Incubation of click reaction solution with neocuproine induced a drastic increase of absorbance at 450 nm and a color-change from light blue to deep yellow, demonstrating the presence of Cu^{+} (Fig. S1). The control experiment showed that Cu^{2+} or sodium ascorbate had little effect on the stability of AuNPs (Fig. S2). Therefore, based on the color change of AuNPs solution, Cu^{2+} could be conveniently detected by the naked eye without the aid of any instruments.

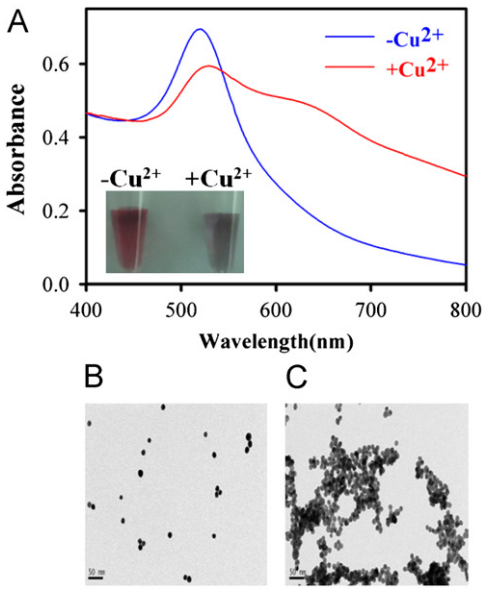

Fig. 2. Colorimetric detection of Cu^{2+}. UV–visible absorption spectra (A) and TEM image (B) and (C) of the DNA/AuNPs solution in the absence and presence of 20 μM Cu^{2+}. Inset of (A): Cu^{2+}-induced color change of AuNPs dispersion.

3.3. Sensor optimization

The short ssDNA P1 plays the role as a protector adsorbed on the surface of AuNPs against the salt-induced aggregation. Hence, its concentration is a key factor in this detection system. To optimize the concentration of P1, different amounts of P1 was added to AuNPs solution and incubated for 2 min at room temperature, then NaCl was added and their color change was monitored based on the extinction ratio between 520 and 620 nm (Fig. S3A). The extinction ratio ($A_{520\ nm}/A_{620\ nm}$) gradually increased with the increase of amounts of P1, and reached 3 when ~24 equiv of the concentration ratio of P1 to AuNP was used. As the extinction ratio change from 1 to 3 is sufficient for detection, 24 equiv of the concentration ratio of P1 to AuNP was used in the following experiments because it could efficiently stabilize the AuNPs (Fig. S3B).

The concentration of AuNPs would influence the response sensitivity of this copper ion sensor. To investigate the influence of AuNPs concentration, the net extinction ratio ($\Delta(A_{520\ nm}/A_{620\ nm}) = (A_{520\ nm}/A_{620\ nm})_{without\ Cu} - (A_{520\ nm}/A_{620\ nm})_{with\ Cu}$), the difference between the extinction ratio in the absence and presence of Cu^{2+}, was used to reflect the signal response of the sensor. As shown in Fig. S4, the net extinction ratios of the mixture of AuNPs and P1–P2–P3 DNA (1 μM for each DNA) were plotted as function of the AuNPs concentration at pH 7.4 in 0.1 M NaCl solution. The maximum signal response emerged when the concentration of AuNPs was 2.2–2.75 nM. Since higher concentration of AuNPs could contribute to more obvious colorimetric response, 2.75 nM AuNPs was chose in our experiment.

The efficiency of the azide–alkyne click chemistry is key point for the analysis performance of this copper ion sensor, thus some influential factors of the click reaction were optimized. The copper(I)-catalyzed click chemistry was influenced by the acidity of the solution. Fig. S5 shows the pH dependent extinction ratio change of the sensors responsive to Cu^{2+}. The extinction ratio of the system, $A_{520\ nm}/A_{620\ nm}$, decreased with the increasing pH from 2.0 to 4.0 and reached a plateau when the pH was ranged from 4.0 to 7.4. At pH below 4.0, Cu^{+} is unstable due to the disproportionation reaction of Cu^{+} to yield Cu^{2+} and zero-valent copper, which reduces its catalytic ability (Barceloux, 1999). However, Cu^{2+} and Cu^{+} will form hydrate in alkaline solution,

which is unfavorable for the catalytic activity of Cu^{+}, supported by the increase of the extinction ratio at pH higher than 7.4. Thus, we use phosphate buffer (pH 7.4) in our experiment.

The concentration of sodium ascorbate would influence the yield of ligation product of the copper (I)-catalyzed click chemistry. In such a click ligation, Cu^{+} is used as a catalyst and Cu^{+} is formed through the reduction of the Cu^{2+} by sodium ascorbate. Excessive sodium ascorbate could keep reducing condition in the reaction solution and thus promote the high catalytic efficiency of Cu^{+}. Hence, different concentrations of sodium ascorbate were added to the solution of click reaction and the efficiency of reaction was measured by the extinction ratio ($A_{520\ nm}/A_{620\ nm}$) of AuNPs. As shown in Fig. S6, with the increasing concentration of sodium ascorbate, the extinction ratio ($A_{520\ nm}/A_{620\ nm}$) of AuNPs sharply decreases and reaches a plateau at 100 μM sodium ascorbate. Thus, the optimized concentration of sodium ascorbate was 100 μM.

The time of click reaction directly determines the yield of click ligation and the efficiency of this sensor. As depicted in Fig. S7, the optimized time for click reaction was found to be 1 h, much shorter than the previous click chemistry-based methods (Song et al., 2010; Zhou et al., 2008). This relatively short reaction time might be attributed by two reasons: the homogenous click reaction between azide group and alkyne group, and the fact that the DNA template mediates the proximity of those reactive groups (Gartner et al., 2004; Li and Liu, 2004; Shen et al., 2012).

After the click ligation, the resulting reaction mixture was incubated with AuNPs to implement the adsorption of free P1 on AuNPs. The incubation time of DNA with AuNPs is also one of the most important parameters for this sensor. In this work, the adsorption of DNA onto AuNPs in the presence and absence of Cu^{2+} were measured by the extinction ratio ($A_{520\ nm}/A_{620\ nm}$) of AuNPs. As shown in Fig. S8, with the increase of time, the extinction ratio ($A_{520\ nm}/A_{620\ nm}$) of AuNPs sharply increase in the absence of Cu^{2+} due to the adsorption of the 7-mer ssDNA (P1) onto AuNPs, which enhances the stability of AuNPs. However, in the presence of Cu^{2+}, the extinction ratio remained nearly unchanged for ca. 2 min, revealing the generation of the chemical ligation product of P1 + P2 (P1 − P2), which hybrid with P3 to form stable dsDNA structure. Hence, the adsorption of DNA on AuNPs was remarkably decreased, resulting in the aggregation of AuNPs after the addition of NaCl. Afterwards, the extinction ratio increased slowly with the increase of time, probably owing to the slow dehybridization of dsDNA. Additionally, in our previous experiment, the incubation of 2 min is enough for efficiently binding of the ssDNA less than 10-mer on AuNPs (Shen et al., 2009). Therefore, 2 min was chosen for the incubation time of DNA and AuNPs in the reaction solution.

Lastly, after the incubation of DNA probe and AuNPs, the salt solution was introduced to obtain the final colorimetric read-out. The effect of salt-treatment time on AuNPs in the absence or presence of Cu^{2+} was investigated by monitoring the extinction ratio change ($A_{520\ nm}/A_{620\ nm}$) of AuNPs as a function of time after the addition of NaCl. The extinction ratio ($A_{520\ nm}/A_{620\ nm}$) sharply diminished and remained stable after 4 min in the absence or presence of Cu^{2+} after the addition of NaCl (Fig. S9A). Fig. S9B clearly illustrates that the net extinction ratio ($\Delta(A_{520\ nm}/A_{620\ nm})$) remarkably decreased with the increase of time. Thus, in order to achieve a high sensitivity, the UV–visible absorption spectra were recorded immediately after the addition of NaCl.

3.4. Sensitivity and selectivity of the Cu^{2+} biosensor

Under the optimized conditions, this colorimetric biosensor was applied to detect the copper ion. The Cu^{2+} concentration-dependent color gradient from red to blue was observed (Fig. 3A). The extinction ratio value ($A_{520\ nm}/A_{620\ nm}$) versus the concentration of Cu^{2+}

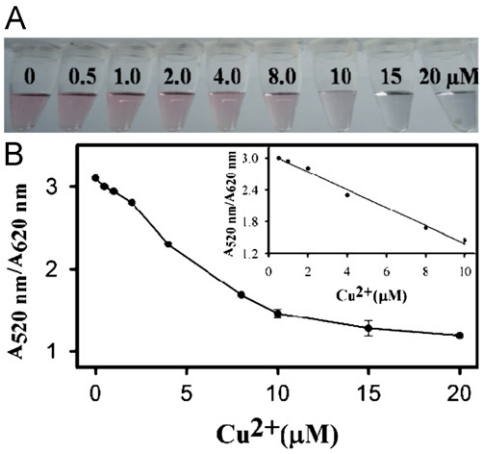

Fig. 3. (A) Color change with the increase of Cu^{2+} concentrations. (B) Plot of the absorption ratio ($A_{520\ nm}/A_{620\ nm}$) against Cu^{2+} concentration. Inset: derived calibration curve.

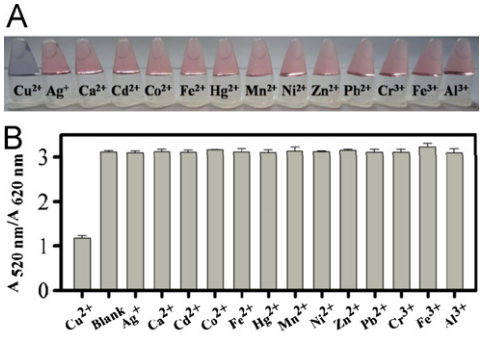

Fig. 4. The selectivity of the Cu^{2+} sensing system. (A) Color change of the solution in the presence of 20 μM Cu^{2+} or 50 μM other metal ions. (B) The absorption ratio ($A_{520\ nm}/A_{620\ nm}$) change for Cu^{2+} or other metal ions.

(0–20 μM) was recorded and illustrated in Fig. 3B. The $A_{520\ nm}/A_{620\ nm}$ value gradually decreases with the increasing concentration of Cu^{2+} and reaches a plateau at 15 μM Cu^{2+}. The linear range is 0.5–10 μM with calibration equation $A_{520\ nm}/A_{620\ nm} = -0.1691\ C_{Cu} + 3.0824$ (regression coefficient $R = 0.990$) (Fig. 3inset), and the detection limit is determined to be 250 nM on the basis of the 3σ/slope, which compared well with the copper ion assays based on florescent dyes (Sang et al., 2007; Song et al., 2007). In addition, this detection limit is much lower than that of previously reported Cu^{2+} assays using click chemistry (Song et al., 2010; Zhou et al., 2008). Moreover, the linear range of this sensor can be readily tunable by simply changing the click reaction time. When the click reaction time is shortened from 1 h to 20 min, $A_{520\ nm}/A_{620\ nm}$ is linearly related to the Cu^{2+} concentration with the linear range 10–50 μM (Fig. S10). The calibration equation is $A_{520\ nm}/A_{620\ nm} = -0.0417C_{Cu} + 3.2750$ (regression coefficient $R = 0.994$) with the detection limit of 6.5 μM (3σ/slope). The maximum contaminant level is ~20 μM in the United States, 15 μM in Canada and 30 μM in the European Union; they all are located within this linear range. Hence, our proposed colorimetric sensor can readily achieve one-step detection of copper ion contamination in drinking water.

The selectivity of this biosensor for Cu^{2+} was examined by visual assay to other metal ions, including Ag^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+}, Fe^{3+} and Al^{3+} at concentration of 50 μM. As shown in Fig. 4A, only Cu^{2+} cause a red-to-blue color change while other metal ions remain red. The corresponding absorbance data show that other metal ions cause a negligible absorbance response (Fig. 4B). Moreover, the

coexistence of other metal ions (Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+}, Fe^{3+} and Al^{3+}; 50 μM each) shows no noticeable interference for the detection of Cu^{2+} (Fig. S11). The selectivity of our system is much better than those of other sensors for Cu^{2+} ions (Lan et al., 2010; Shang and Dong, 2008; Wu and Anslyn, 2004). The excellent selectivity of this biosensor is attributed to the great specificity of the alkyne–azide click reaction to the catalysis of copper ions (Rostovtsev et al., 2002; Shen et al., 2012; Wang et al., 2003). These experimental results suggest that the proposed sensing system could be applicable for Cu^{2+} detection in complicated real samples.

3.5. Assay of Cu^{2+} in river water samples

To investigate whether the proposed method was feasible to real samples, we tested real and spiked river water samples (Xiangjiang River, Changsha, China) with various concentrations of Cu^{2+}. The river water samples collected were filtered through a 0.45 μm membrane. Then, we employed the proposed method to evaluate the concentration level of Cu^{2+} in river water. However, we could not detect the Cu^{2+} in the river water using this proposed method partially as the concentration of Cu^{2+} in the river water is less than the detection limit given by the proposed method. Thus, the as-prepared water samples were spiked with Cu^{2+} at different concentration levels and then analyzed with the proposed method. As shown in Table 1, the samples (1–4) containing low concentration of Cu^{2+} were analyzed using 1 h click reaction time as well as the calibration equation $A_{520\ nm}/A_{620\ nm} = -0.1691C_{Cu} + 3.0824$, while the samples with high concentration of Cu^{2+} (5–8) were analyzed using 20 min click reaction time and the calibration equation $A_{520\ nm}/A_{620\ nm} = -0.0417C_{Cu} + 3.2750$. Satisfactory recoveries of standard addition were obtained using the proposed method. The results revealed the potential applicability of this Cu^{2+} colorimetric biosensor in real samples.

4. Conclusions

In summary, we have developed a novel biosensing strategy based on the high specificity of copper(I)-catalyzed click chemistry and unmodified gold nanoparticles for colorimetric detection of Cu^{2+}. The structure-switchable DNA probe was designed to change its structure from separated form to entire dsDNA in response to copper(I)-catalyzed click ligation. Unmodified gold nanoparticles were used as a colorimetric indicator to probe Cu^{2+}-induced DNA structural variation. This sensor shows high sensitivity and excellent selectivity due to the high yielding capability and great specificity of click chemistry. Unlike previously reported AuNPs-based Cu^{2+} assays using click chemistry which relies on interparticle crosslinking aggregation, our method is a novel design using noncrosslinking aggregation mediated by electrostatic stabilization. More importantly, this design without dual-modifying DNA and surface modification of gold nanoparticles significantly reduces the cost and simplifies the biosensor fabrication and detection process. Furthermore, the assay of Cu^{2+} can be simply visualized by the naked eyes without requirement of complicated instruments. All these features make our biosensor facile, low-cost and particularly useful for resource-limited conditions. On the other hand, this design strategy can be readily extended to sensing various targets by simply changing the DNA-modified reactant groups capable for covalent bond formation. Therefore, taking this method as an example, we demonstrate that the rational application of click reaction and DNA-nanomaterial interaction is a promising platform for developing biosensors.

Table 1
Determination of Cu^{2+} in water samples using the proposed procedure.

Samples[a]	Added (μM)	Mean found[b] (μM)	Mean recovery[c](%)	RSD[d] (%)
1	0.50	0.54	108	3.4
2	2.00	1.96	98	2.5
3	5.00	4.83	97	2.1
4	8.00	8.18	102	1.8
5	15.00	15.45	103	3.4
6	20.00	19.67	98	2.6
7	30.00	31.62	105	3.0
8	40.00	40.56	101	1.7

[a] Samples 1–4 were analyzed using calibration equation $A_{520\ nm}/A_{620\ nm} = -0.6191C_{Cu} + 3.0824$ with 1 h click reaction time; Samples 5–8 were analyzed using calibration equation $A_{520\ nm}/A_{620\ nm} = -0.0417C_{Cu} + 3.2750$ with 20 min click reaction time.
[b] Mean concentration of three replicates.
[c] Mean recovery (%) = 100 × ($c_{mean\ found}/c_{added}$).
[d] Relative standard deviation of three determinations.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.09.032.

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