Colorimetric Cu^{2+} detection with a ligation DNAzyme and nanoparticles†

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Using a Cu^{2+}-dependent DNA ligation DNAzyme, a colorimetric sensor for Cu^{2+} has been developed based on directed assembly of DNA-functionalized gold nanoparticles by the ligation product, and such ligation DNAzyme-based sensors are intrinsically more sensitive than cleavage DNAzyme systems due to the lack of background.

Cu^{2+} is an essential metal ion for biological functions. At elevated concentrations, however, it may cause adverse health effects such as gastrointestinal disturbance and liver or kidney damage.[1] Therefore on-site and real-time detection and quantification of the Cu^{2+} ion is important to take advantage of its beneficial aspects while avoiding its toxic effects. Design of fluorescent metal sensors has been shown to be a viable approach and many effective sensors for diamagnetic metal ions have been reported.[2–9] As a paramagnetic metal ion, Cu^{2+} is challenging to detect using conventional fluorescent sensor design methods due to its paramagnetic quenching effects on fluorophores.[10–14] We have recently reported a catalytic beacon strategy for metal sensing.[15–18] By using a Cu^{2+}-dependent cleavage DNAzyme to spatially separate the Cu^{2+}-binding site from the fluorophore, a highly sensitive and selective Cu^{2+} sensor was constructed.[17] Colorimetric sensors, on the other hand, are free of such quenching problems. In addition, colorimetric sensors require minimal instrumentation and thus can make on-site detection even easier.

Most reported colorimetric Cu^{2+} sensors employed organic dyes for both Cu^{2+} recognition and for signal generation.[14,19–25] For color signal generation, metallic nanoparticles are particularly attractive, as such nanoparticles possess much higher extinction coefficients compared to organic dyes, allowing sensitive colorimetric detections with minimal material consumption.[22,26] More importantly, metallic nanoparticles display distance-dependent optical properties.[27] For example, dispersed gold nanoparticles (AuNPs) are red in color, while aggregated ones are purple or blue. Based on this phenomenon, Mirkin and co-workers have developed simple colorimetric sensing methods for sequence-selective detection of DNA.[28] We have expanded the class of molecules for detection significantly by using functional DNA (DNAzymes, aptamers and aptazymes) as a template for stimuli-responsive assembly of AuNPs.[29–35] For example, a colorimetric Pb^{2+}-sensor has been developed using a Pb^{2+}-specific DNAzyme that cleaves a DNA substrate with a ribonucleotide adenosine in the scissile position, transforming blue AuNP aggregates to red dispersed AuNPs.[31,33,34]

While the above cleavage-based DNAzyme/AuNP system has been successful in designing colorimetric sensors for metal ions, one major issue remains to be addressed; the cleavage-based DNAzyme system is vulnerable to interference in samples that can cause non-specific nucleic acid cleavage, resulting in high background. In addition, because the AuNP aggregates contain many DNA cross-linked to each other, it requires many cleavage steps by the DNAzymes before the aggregates are fully dispersed, making it difficult to detect the color change quickly and thus reducing sensor sensitivity. To address these issues, in order to improve the sensor performance, we use a ligation-based DNAzyme system because many fewer interfering species can catalyze ligation reactions, and ligation generates new species against an almost zero background and thus will result in higher sensor sensitivity.

The Cu^{2+}-dependent DNA ligation DNAzyme isolated by Cuenoud and Szostak was used.[36] The secondary structure of the ligation DNAzyme is shown in Fig. 1A. The enzyme strand (in black) is named E47. Substrate S1 is activated with imidazole. Substrate S2 is hybridized to the 5′-region of E47. In the presence of Cu^{2+}, E47 catalyzes the nucleophilic attack of the phosphorus center of S1 by the hydroxyl group on S2, forming a phosphodiester bond with the imidazole acting as a leaving group. The ligation product was designed as a linker to assemble AuNPs, giving blue colored aggregates (Fig. 1B). If no Cu^{2+} was present, no aggregation occurred and the color remained red. The change of color was quantitatively monitored by UV-vis spectroscopy. Dispersed 50 nm diameter AuNPs had an extinction peak at ~532 nm (Fig. 2A, —), which rendered the nanoparticles a characteristic red color. The extinction coefficient of 50 nm AuNPs was 1.5×10^{10} M^{-1} cm^{-1}, and the concentration of dispersed AuNPs used in the experiment was 0.05 nM. In the presence of Cu^{2+}, the ligation product was formed and AuNPs were assembled, giving increased extinction at 700 nm region (Fig. 2A, ---). Therefore, the extinction ratio at 700 and 532 nm was used to quantify the color of the system, with a low ratio associated with red color of dispersed particles, and a high ratio associated with aggregated particles of blue color.

To test the sensitivity and selectivity of the system, metal-dependent color change was monitored. As shown in Fig. 2B (■), the extinction ratio increased with the increase of Cu^{2+}. The middle point for Cu^{2+} was ~5 μM. Therefore, the detection system was sensitive enough to detect Cu^{2+} in drinking water, which has a limit of 20 and 30 μM defined by the US Environmental

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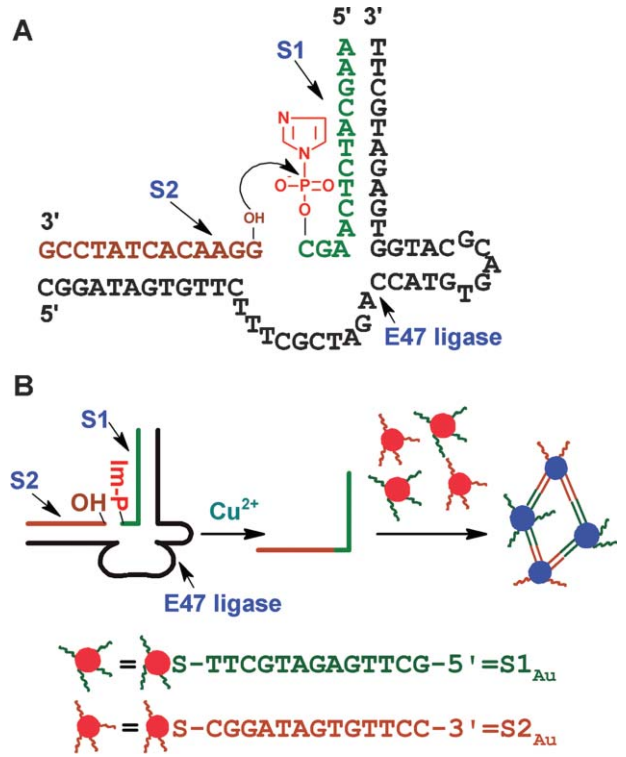

Fig. 1 (A) Secondary structure of the Cu^{2+}-dependent DNAzyme with DNA ligation activity. The 3′-end of S1 was activated with imidazole. S1 and S2 were ligated into one piece by the E47 enzyme in the presence of Cu^{2+}. (B) Schematics of the formation of the ligation product and the assembly of AuNPs with it. To facilitate release of the ligation product from E47, another piece of DNA partially complementary to E47 was added (not shown). A red-to-blue color change was observed upon assembly of AuNPs.

Protection Agency and the World Health Organization, respectively. Previous biochemical assays indicated that in the concentration range from 10 μM to 10 mM, among Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} Pb^{2+}, and Cd^{2+}, only Cu^{2+} and Zn^{2+} showed activity.[36] High metal selectivity was also observed from the nanoparticle-based sensing. In the concentration range of 0.01 to 1 mM, only Zn^{2+} showed interference at high concentrations (Fig. 2C). A Zn^{2+}-dependent response curve was also measured (Fig. 2B, □), and a middle point of 130 μM was obtained. Therefore, the sensor is 25-fold more selective for Cu^{2+} than Zn^{2+} and no other metal ions gave any signal. The response of the sensor to metal soups containing 0.1 mM or 0.5 mM of each of Mn^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Pb^{2+} and Cd^{2+} was also tested, and no color change was observed. However, if 10 μM Cu^{2+} was present in the metal soups, a color change was observed (Fig. 2D). The color of the sensor was also observed by spotting the sensor solution onto a solid substrate, such as an alumina TLC plate. With increase of Cu^{2+} concentration, color progression from red to blue was observed (Fig. 3A); while for other metal ions, except high concentration of Zn^{2+}, only red color was observed (Fig. 3B).

To confirm formation of the ligation product, the ligation reaction was followed by using a 3′-end FAM (6-carboxyl-fluorescein) fluorophore labeled S2. The ligated and unligated S2 were separated by polyacrylamide gel electrophoresis. The fraction of ligation product increased with time (Fig. 2E, inset). To confirm

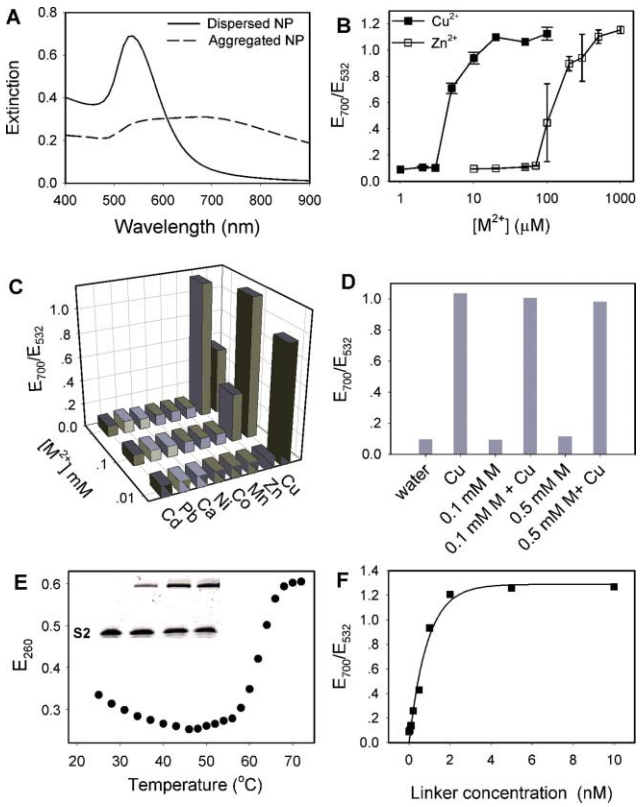

Fig. 2 Performance of the ligation DNAzyme-based Cu^{2+} sensor. (A) Extinction spectra of dispersed and aggregated AuNPs. (B) Cu^{2+}- and Zn^{2+}-dependent color change of the sensor. (C) Metal selectivity of the sensor. (D) Selectivity of the sensor for Cu^{2+} in metal soups. (E) Confirming formation of DNA-assembled AuNPs by measuring the melting curve and confirming formation of the ligation product by gel electrophoresis assays (inset). Under experimental conditions (300 mM NaCl, 25 mM Tris acetate, pH 8.2, aggregates composed of 50 nm AuNPs), the melting temperature was determined to be 62 °C. (F) Color of 50 nm AuNPs in the presence of varying concentrations of the ligation product. The change of the extinction ratio with the linker (ligation product) concentration was fit to an exponential decay curve.

that the color change was due to the assembly of AuNPs by DNA, the melting property of the aggregates was investigated by monitoring the extinction of the resulting aggregates at different temperatures. A sharp melting transition was observed (Fig. 2E), which was consistent with the property of DNA-assembled AuNPs.[28,37]

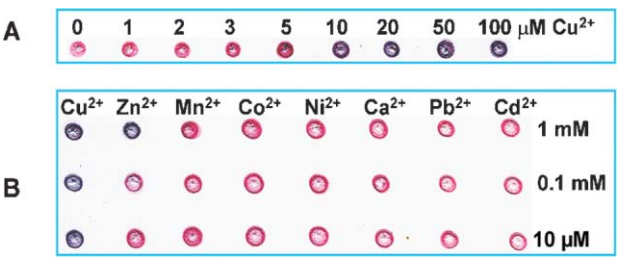

Fig. 3 Visual observation of the color of the sensor in the presence of different metal ions developed on an alumina TLC plate. (A) Cu^{2+}-dependent color change. (B) Metal selectivity of the sensor.

To experimentally prove that for such nanoparticle-based colorimetric detections, ligation reactions have higher sensitivity compared to those based on cleavage reactions, AuNPs ($S1_{Au}$ and $S2_{Au}$) were assembled by varying concentrations of the ligation product and the extinction ratios of the nanoparticles were plotted in Fig. 2F. The change of extinction ratio can be fit to an exponential growth curve with the equation: $y = 1.289(1 - e^{-1.122x})$. Therefore, $dy/dx = 1.446\ e^{-1.122x}$. For ligation reactions, the initial ligation product (linker) concentration x is close to zero, and dy/dx has the highest value close to 1.446. For cleavage reactions, the linker starting concentration is high. In the current plot, $\sim$5 nM linker was needed to form fully assembled AuNPs (Fig. 2F). At this condition, dy/dx equals only 0.0053. Although it is difficult to compare different enzymes, from this experiment and calculation, under the conditions described in this system, sensors based on detecting the generation of ligation products should have $\sim$270 times higher sensitivity than those based on detecting the decrease of linker DNA due to cleavage.

In summary, we demonstrated the possibility of designing nanoparticle-based colorimetric sensors using DNAzyme-catalyzed ligation reactions. Compared to DNA or RNA cleaving enzymes, ligation enzymes are intrinsically more sensitive due to the lack of background. This detection system is sensitive enough to detect Cu^{2+} in drinking water and no analytical instruments are needed. Sensors with better performance could be obtained by *in vitro* selection of DNAzymes under more stringent conditions, such as with lower metal concentrations and with negative selections to remove competing metal activities.[38,39]

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