Colorimetric Cu²⁺ Detection Using DNA-Modified Gold-Nanoparticle Aggregates as Probes and Click Chemistry**

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Copper is a transition metal essential for life. At elevated concentrations, however, it is highly toxic to organisms such as algae, fungi, and many bacteria, and in humans may adversely affect the gastrointestinal, hepatic, and renal systems.[1,2] As such, the detection and measurement of copper ions in water has become increasingly important, especially in point-of-use formats. Several methods exist for the detection of Cu^{2+}, including those based on organic dyes,[3-6] semiconductor nanocrystals,[7] and spectroscopy.[8-10] The read-out mechanisms for these methods, however, often require sophisticated instrumentation. In contrast, colorimetric methods are extremely attractive for point-of-use applications since they can be easily interpreted with the naked eye or low-cost portable instruments.

DNA gold nanoparticle (Au NP) conjugates[11–13] have been used in a variety of detection formats for DNA,[14–18] proteins,[19–21] metal ions,[22–25] enzyme inhibitors,[26] small molecules,[27–29] and intracellular mRNA.[30] Carboxylate-[31] and peptide-modified[32] Au NPs have also been used for colorimetric metal ion sensing. DNA Au NPs have high extinction coefficients (about four orders of magnitude greater than typical organic dyes) and unique distance-dependent plasmonic properties. When hybridized to complementary particles, DNA Au NPs turn from red to purple and their aggregates exhibit extremely sharp melting transitions, which make them useful for the colorimetric detection of oligonucleotides.[12] These optical and melting properties have also been utilized in mercuric ion detection,[24] where deliberately designed T–T mismatches in the interparticle oligonucleotide duplexes selectively complex Hg^{2+} via coordination chemistry. The formation of the T–Hg^{2+}–T co-ordination complexes raises the melting temperature T_m of the polymeric aggregates,

so the solution color measured as a function temperature can be used to determine Hg^{2+} concentration.[23,24]

Recently, Au NP-based colorimetric detection approaches for copper ions have been developed based upon: 1) aptamers and Cu^{2+} catalyzed DNA ligation, which induces subsequent nanoparticle aggregation[33] and 2) the use of Cu^{2+}-catalyzed click chemistry off the surfaces of alkyne- and azide-terminated nanoparticles without DNA to aggregate Au NPs.[34] However, these systems suffer from limitations inhibiting their utility for on-site use. In the first example, the aptamers used a labile imidazole leaving group for oligonucleotide ligation, which requires special storage and handling. In the latter example, the assay requires overnight incubation to facilitate aggregation and was not quantitative. Herein, we report a colorimetric method for the detection of Cu^{2+} based on densely functionalized DNA Au NP conjugates and click chemistry. This new approach relies on the ligation of two 15 base pair (bp) oligonucleotide strands within polymeric aggregates of the DNA Au NPs. The oligonucleotide strands are terminated in a hexynyl or azide group, allowing them to be ligated into a 30 bp strand with click chemistry. We hypothesized that this ligation would raise the T_m of the aggregate in proportion to the amount of copper ion present after a given time (a kinetic endpoint), which could be monitored by UV/Vis spectroscopy or the naked eye. The use of click chemistry and DNA Au NPs confers three specific advantages that the previous Au NP-based systems lacked in whole or in part. First, this approach uses the oligonucleotides as a template to align the alkyne and azide groups for optimal reactivity.[35,36] Second, the sharp melting properties of the DNA Au NPs allow one to distinguish subtle differences in T_m, allowing for Cu^{2+} quantification.[37] Finally, the alkyne and azide groups are robust and their cyclization can be catalyzed specifically (almost exclusively) by copper ions.[38,39]

In a typical experiment, two sets of DNA Au NP conjugates were prepared by functionalizing two batches of 30-nm Au NPs with different alkylthiol-modified oligonucleotide strands. The first type of particle, called the template particle, was modified with 3′-propylthiol-terminated 40-mer oligonucleotides (5′ TAG GAA TAG TTA TAA GCG TAA GTC CTA ACG A_{10} SH 3′). The second particle, the alkyne Au NP, was functionalized with 3′-propylthiolated and 5′-alkylated 25-mer oligonucleotides that are complementary to half of the DNA on the template particles (5′ hexynyl TTA TAA CTA TTC CTA

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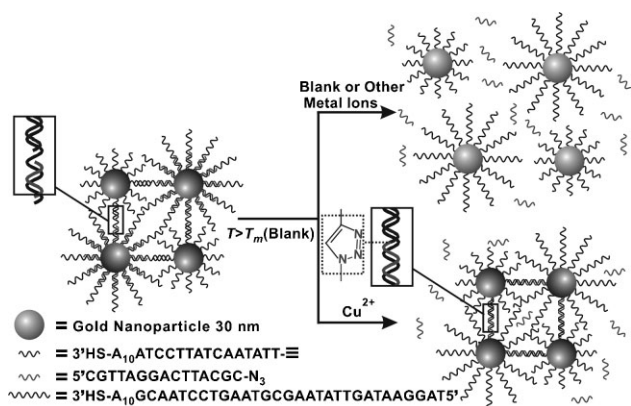
Scheme 1. Schematic illustration representing the aggregation and dissociation of the gold nanoparticle probes used in the colorimetric detection of copper ions.

Figure 1. Normalized melting curves of the aggregates in the presence of Cu^{2+} concentrations ranging from 0 μM to 500 μM. The change in the extinction was monitored at 525 nm.

Figure 2. Normalized melting curves of the aggregates in the presence of 200 μM of various metal ions. The change in extinction was monitored at 525 nm.

A_{10} SH 3′). These functionalized Au NPs formed polymeric networks upon mixing due to their complementary DNA. A third oligonucleotide, the azide strand, was added. These strands are 3′ azidobutyrate labeled 15-mer oligonucleotides (5′ CGT TAG GAC TTA CGC azidobutyrate 3′) complementary to the other half of the template strand. Together, these oligonucleotides formed three stranded aggregates with a concomitant red-to-purple color change (Scheme 1). These aggregates serve as the Cu^{2+} probes in our novel assay. In a proof-of-concept detection experiment, the Cu^{2+} concentration was increased to 200 μM and excess sodium ascorbate and a water soluble tris-triazolylamine Cu^+ binding ligand[35,40] were added to the solution of aggregates and incubated for two hours to allow for the click-chemistry ligation to occur. The melting temperature of these aggregates, which was monitored at the Au NP surface plasmon resonance maximum of 525 nm, was 62.6 °C. In a control experiment performed in the absence of copper ion, the aggregates melted at 50.4 °C, approximately 12 °C below the T_m after ligation (Figure 1). This increase in T_m was due to the conversion of the three-strand structure to a ligated two-strand 30 bp duplex. The presence of the ligated strand was confirmed with mass spectrometry (Figure S1 of the Supporting Information).

The sharp melting properties of DNA Au NPs allowed the measurement of small differences in T_m, which was proportional to the concentration of the Cu^{2+} added in the range of 20 to 100 μM (Figure 1). Greater concentrations of the ion increase the number of ligated 30 bp interstrand duplexes, increasing the T_m of the aggregates. The limit of detection of the assay is 20 μM and its dynamic range is 20 to 100 μM (Figure S2). The Environmental Protection Agency-defined maximum contaminant level for copper in drinking water is 20 μM, making it relevant for testing drinking water.

To evaluate the selectivity of this assay, it was challenged with other environmentally relevant metal ions, including Li^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Ba^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Cr^{3+}, and Fe^{3+}. In a typical experiment, one of these metal ions was added to a solution of the DNA Au NP aggregates at a final concentration of 200 μM and incubated for two hours in the presence of the tris-triazolylamine Cu^+-binding ligand and sodium ascorbate. Only the Cu^{2+} sample
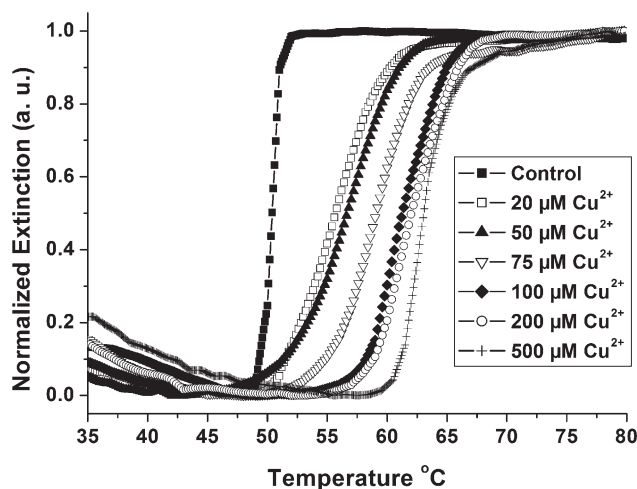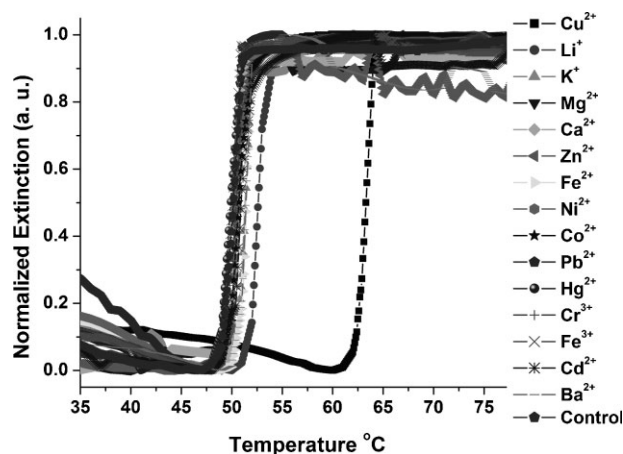
showed an increased melting temperature relative to the blank (Figure 2). No obvious melting temperature increase was observed for other metal ions. Importantly, the large extinction coefficient of the DNA Au NPs allowed for specific copper ion detection through visual inspection, which is particularly attractive for on-site use (Figure 3).

In conclusion, we have developed a colorimetric copper ion detection system with high selectivity and high sensitivity for a colorimetric assay. The concentration of Cu^{2+} can be determined by the change in solution color at a given temperature or through measurement of the melting temperature of the DNA Au NP aggregates. In contrast to other Au NP-based detection systems, this method does not require labile reactive groups or long incubation times due to the robustness of the alkyne and azide functionalities and the fact that those groups are templated together for optimal reactivity via oligonucleotide hybridization, respectively. Taken together, these advantages make this assay simple and robust and
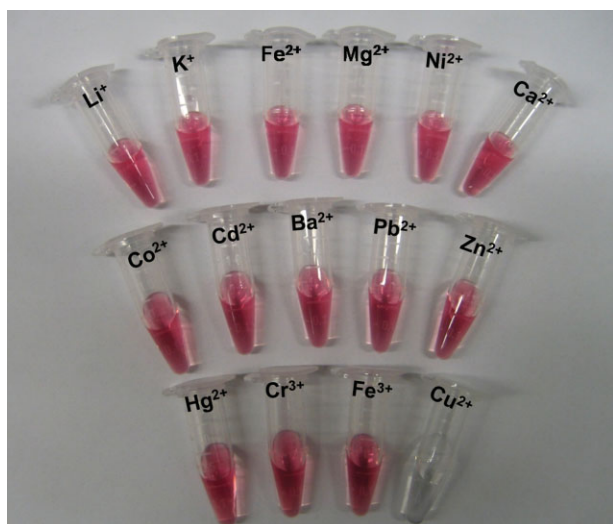
Figure 3. A photograph of the DNA Au NPs at 55 °C in the presence of different metal ions after ligation.

therefore promising for on-site water testing. As such, the strategy presented in this work represents a promising new addition to the growing library of NP-based probes for protein, nucleic acid, metal ion, and small-molecule detection.[13,41–48]

Experimental Section

Materials: All reagents and solvents were purchased from Sigma–Aldrich unless otherwise noted. 30-nm-diameter Au NPs were purchased from Ted Pella. Oligonucleotides were synthesized on an Expedite 8909 Nucleotide Synthesis System by standard solid-phase phosphoramidite synthesis techniques. All bases and reagents were purchased from Glen Research. The oligonucleotides were purified using reverse-phase high-performance liquid chromatography (RP-HPLC). After purification, the oligonucleotides were lyophilized and stored at −78 °C until used. Before nanoparticle conjugation, the 3′ disulfide functionality was reduced with dithiothreitol (DTT) following published procedures.[37]

Synthesis of alkyne-modified oligonucleotides: Alkyne-modified oligonucleotide (5′ hexynyl-TTA TAA CTA TTC CTA A_{10} SH- 3′) were synthesized using 3'-thiol-modifier C3 S-S CPG and 5′-hexynyl phosphoramidite. The oligonucleotides were characterized by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS; Supporting Information).

Synthesis of azido-modified oligonucleotides: Azido-modified oligonucleotides were obtained by conjugating an amino-modified oligonucleotide with an azide *N*-hydroxysuccinimide (NHS) ester (azidobutyrate NHS ester, Glen Research). Lyophilized amino-modified oligonucleotides (1 μmol) were dissolved in 0.5 mL of 0.1 M Na_2CO_3/$NaHCO_3$ buffer (pH 8.5). To this solution, 5 mg of azide NHS ester in 100 μL of DMSO was added. The resulting mixture was incubated overnight at room temperature, purified by RP-HPLC and characterized by MALDI-MS (Supporting Information).

Copper ion detection assay: Au NP aggregates were formed by mixing the template particles (modified with 5′ TAG GAA TAG TTA TAA GCG TAA GTC CTA ACG A_{10} SH 3′; 0.5 mL, 1 nM), the alkyne Au

NPs (modified with 5′ hexynyl TTA TAA CTA TTC CTA A_{10} SH 3′; 0.5 mL, 1 nM), and 3′ azidobutyrate labeled oligonucleotides (5′ CGT TAG GAC TTA CGC azidobutyrate 3′). These oligonucleotides formed three stranded aggregates with a concomitant red-to-purple color change. The Au NP aggregates were washed (3×) with PBS buffer (10 mM sodium phosphate, 0.1 M NaCl, pH 7.0). They were then resuspended in 950 μL of PBS buffer. Tris-hydroxypropyl triazolyl ligand (2.0 μmol), sodium ascorbic acid (2.0 μmol), and copper (II) sulphate pentahydrate (at predetermined concentrations) were then added to a solution of the aggregates, respectively, and incubated for 2 h. The melting transitions were monitored by UV/Vis spectroscopy (Cary 5000, Varian) and the solution was continuously stirred with a magnetic stir bar to keep the aggregates suspended.

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