Sensors

DOI: 10.1002/anie.200700269

Colorimetric Detection of Mercuric Ion (Hg^{2+}) in Aqueous Media using DNA-Functionalized Gold Nanoparticles**

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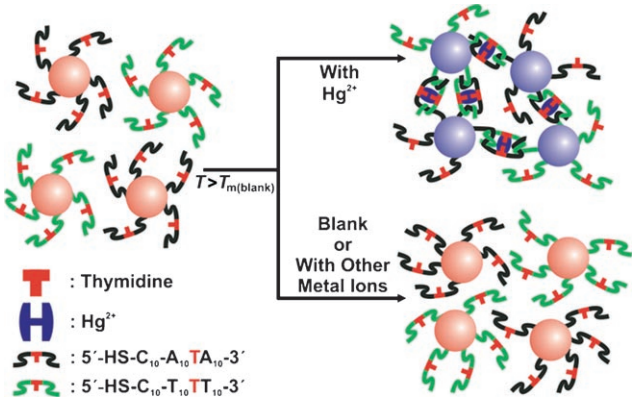
Mercury is a widespread pollutant with distinct toxicological profiles, and it exists in a variety of different forms (metallic, ionic, and as part of organic and inorganic salts and complexes). Solvated mercuric ion (Hg^{2+}), one of the most stable inorganic forms of mercury,[1] is a caustic and carcinogenic material with high cellular toxicity.[2] The most common organic source of mercury, methyl mercury, can accumulate in the human body through the food chain and cause serious and permanent damage to the brain with both acute and chronic toxicity.[3–5] Methyl mercury is generated by microbial biomethylation in aquatic sediments from water-soluble mercuric ion (Hg^{2+}).[4] Therefore, routine detection of Hg^{2+} is central to the environmental monitoring of rivers and larger bodies of water and for evaluating the safety of aquatically derived food supplies.[5,6] Several methods for the detection of Hg^{2+}, based upon organic fluorophores[7] or chromophores,[8] semiconductor nanocrystals,[9] cyclic voltammetry,[10] polymeric materials,[11] proteins,[12] and microcantilevers,[13] have been developed. Colorimetric methods, in particular, are extremely attractive because they can be easily read out with the naked eye, in some cases at the point of use. Although there are now several chromophoric colorimetric sensors for Hg^{2+},[8] all of them are either limited with respect to sensitivity (current limit of detection $\approx 1\ \mu M$) and selectivity, kinetically unstable, or incompatible with aqueous environments.

Recently, DNA-functionalized gold nanoparticles (DNA–Au NPs) have been used in a variety of forms for the detection of proteins,[14,15] oligonucleotides,[15–21] certain metal ions,[22] and other small molecules.[23,24] DNA–Au NPs have high extinction coefficients (3–5 orders of magnitude higher than those of organic dye molecules)[25] and unique distance-dependent optical properties that can be chemically programmed through the use of specific DNA interconnects, which allows one, in certain cases,[16–20] to detect targets of interest through colorimetric means. Moreover, these structures, when hybridized to complementary particles, exhibit extremely sharp melting transitions, which have been used to enhance the selectivity of detection systems based upon them.[16,18,20,26] By using such an approach, one can typically detect nucleic acid targets in the low nanomolar to high picomolar target concentration range in colorimetric format. The ability to use such particles to detect Hg^{2+} in the nanomolar concentration range in colorimetric format would be a significant advance, especially when one considers that commercial systems for detecting Hg^{2+} rely on cumbersome inductively coupled plasma approaches that are not suitable for point-of-use applications. Herein, we present a highly selective and sensitive colorimetric detection method for Hg^{2+} that relies on thymidine–Hg^{2+}–thymidine coordination chemistry[27] and complementary DNA–Au NPs with deliberately designed T–T mismatches.

When two complementary DNA–Au NPs are combined, they form DNA-linked aggregates that can dissociate reversibly with a concomitant purple-to-red color change.[24,28] For our novel colorimetric Hg^{2+} assay, however, we prepared two types of Au NPs (designated as probe A and probe B, see the Supporting Information), each functionalized with different thiolated-DNA sequences (probe A: 5′HS-C_{10}-A_{10}-**T**-A_{10}3′, probe B: 5′HS-C_{10}-T_{10}-**T**-T_{10}3′), which are complementary except for a single thymidine–thymidine mismatch (shown in bold; Scheme 1). Importantly, these particles also form stable aggregates and exhibit the characteristic sharp melting transitions (full width at half maximum $< 1\,^{\circ}C$) associated with aggregates formed from perfectly complementary particles, but with a lower melting temperature T_m.[17,18] Since it is known that Hg^{2+} will coordinate selectively to the bases that make up a T–T mismatch, we hypothesized that Hg^{2+} would



Scheme 1. Colorimetric detection of mercuric ion (Hg^{2+}) using DNA–Au NPs.

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[**] C.A.M. acknowledges AFOSR, ARO, NSEC/NSF, and NIH for support of this research. C.A.M. is also grateful for a NIH Director's Pioneer Award.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

selectively bind to the T–T sites in our aggregates formed from mismatched strands and raise the T_m of the resulting structures.[27] The analogous interaction with particle-free DNA leads to significant increases in T_m ($\Delta T_m \approx 10\,^{\circ}C$).

The assay begins by adding an aliquot of an aqueous solution of Hg^{2+} at a designated concentration to a solution of the DNA–Au NP aggregates formed from probes A and B (1.5 nM each) at room temperature (see the Supporting Information). The solution is then heated at a rate of $1\,^{\circ}C\,min^{-1}$ while its extinction is monitored at 525 nm, where the Au NP probes exhibit the maximum intensity in the visible region of the spectrum. The T_m is obtained at the maximum of the first derivative of the melting transition. Without Hg^{2+}, the aggregates melt with a dramatic purple-to-red color change at about 46 °C. In the presence of Hg^{2+}, however, the aggregates melt at temperatures higher than 46 °C because of the strong coordination of Hg^{2+} to the two thymidines that make up the T–T mismatch, thereby stabilizing the duplex DNA strands containing the T–T single base mismatches.

To evaluate the sensitivity of the assay, different concentrations of Hg^{2+} from one stock solution were tested. When an Hg^{2+} sample was mixed with the Au NP probe aggregate solution, there was no noticeable change under the reaction conditions described above. Once heated, however, the aggregates melt with a significant purple-to-red color change at a specific temperature (Figure 1a), which is linearly related to the concentration of Hg^{2+} over the entire concentration range studied (Figure 1b). The present limit of detection for this system is approximately 100 nM (= 20 ppb) Hg^{2+} (Figure 1a), which, to the best of our knowledge, is the lowest ever reported for a colorimetric Hg^{2+} sensing system. Each increase in concentration of 1 μM results in an increase in T_m by about 5 °C, thus providing an easy way of determining Hg^{2+} concentration.

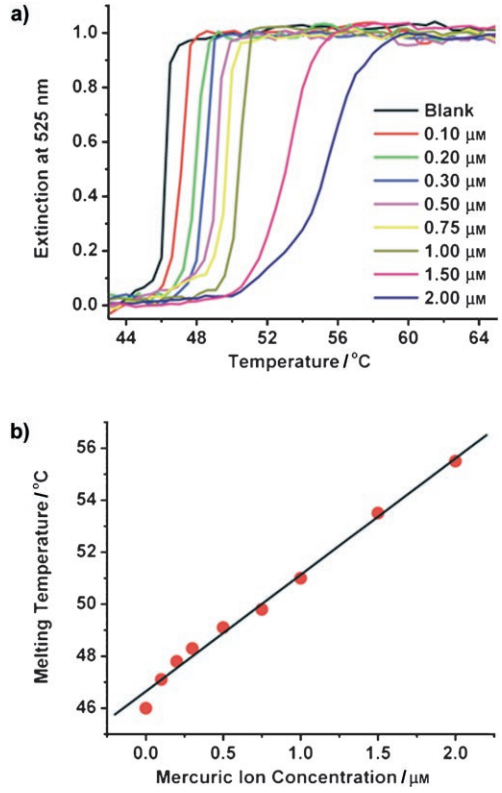

Figure 1. a) Normalized melting curves of aggregates (probes A and B) with different concentrations of Hg^{2+}. b) Graph of the T_m for the aggregates as a function of Hg^{2+} concentration.

Three components of the assay contribute to its high sensitivity, selectivity, and quantitative capabilities: 1) the oligonucleotides, 2) the Au NPs, and 3) the oligonucleotide–nanoparticle conjugate. From the standpoint of the oligonucleotides, the chelating ability of the thymidines that form the mismatch in the oligonucleotide duplex is extremely selective for Hg^{2+}. It is known that two thymidine residues, when geometrically preorganized in a DNA duplex, can behave as a chelate and form a tightly bound complex with Hg^{2+}.[29] From the standpoint of Au NPs, the high extinction coefficients of Au NPs (ca. $10^8\,cm^{-1}M^{-1}$ for 15-nm Au NPs) can act as an amplifier for the perturbation of the T_m upon binding Hg^{2+}, thus allowing detection limits in the ppb range. Conventional chromogenic chemosensors have relatively low extinction coefficients (typically around $10^5\,cm^{-1}M^{-1}$), which limit their sensitivity at best to the micromolar concentration range. Finally, the sharp, highly cooperative melting properties of aggregates made from oligonucleotide–Au NP conjugates enable one to distinguish subtle differences in T_m clearly, thus providing a measure of the Hg^{2+} concentration from 100 nM to the low micromolar range.[16–18]

The selectivity of this system for Hg^{2+} was evaluated by testing the response of the assay to other environmentally relevant metal ions, including Mg^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+}, Ni^{2+}, Fe^{3+}, Mn^{2+}, Ca^{2+}, Ba^{2+}, Li^{+}, K^{+}, Cr^{3+}, and Cu^{2+} (Figure 2a and 2b) at a concentration of 1 μM. Only the Hg^{2+} sample shows a significantly higher T_m ($\Delta T_m \approx 5\,^{\circ}C$) relative to that of the blank. Indeed, at 47 °C, only the aggregate solution containing Hg^{2+} is purple, whereas all others have turned bright red. Pb^{2+} is the only other metal ion that influences the T_m of the aggregate, but only by a negligible amount ($\Delta T_m \approx 0.8\,^{\circ}C$). Importantly, this selectivity can be visualized with the naked eye (Figure 2c).

Because of the thiophilic nature of Hg^{2+}, we considered the possibility that it could be removing the thiolated oligonucleotides from the surface of the gold particle, which could result in nonuniformity of the assay and a potential loss of sensitivity and accuracy. To determine if this was occurring, we utilized fluorophore-labeled oligonucleotides to evaluate the number of DNA strands per particle at various Hg^{2+} concentrations (0.5, 1, and 2 μM over 8 h; oligonucleotide sequence: 5′ HS-C_{10}-A_{10}-T-A_{10}-(6-FAM) 3′; see the Supporting Information). Significantly, Hg^{2+} shows no evidence of fluorophore quenching, whereas the gold particle is an excellent quencher of fluorescence. Therefore, if the fluorophore-labeled oligonucleotides are removed from the particles they can be easily identified and quantified by fluorescence spectroscopy. The coverage of DNA at the start of the reaction was determined to be approximately 70 strands per particle by using literature methods.[28,30] The mercuric ion, regardless of the concentrations studied, has very little effect on the surface coverage of the DNA

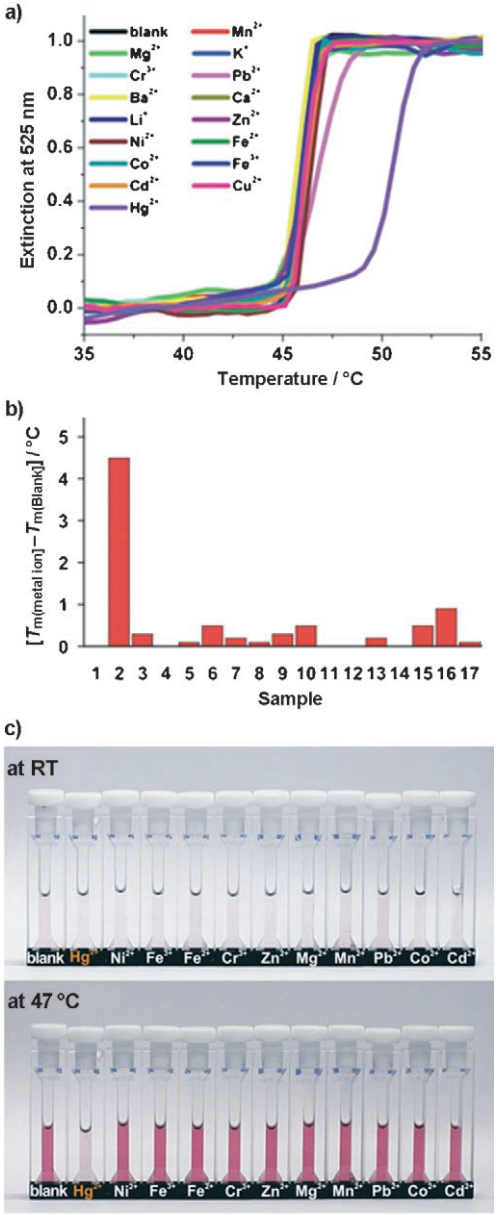

Figure 2. a) Normalized melting curves of the aggregates (probes A and B) in the presence of metal ions (each at 1 μM). b) Graph showing the difference between the T_m of the aggregates of the blank and that of the aggregates with different metal ions: 1: blank; 2: Hg^{2+}; 3: Li^{+}; 4: Cd^{2+}; 5: Ca^{2+}; 6: Ba^{2+}; 7: Mn^{2+}; 8: Mg^{2+}; 9: Zn^{2+}; 10: Ni^{2+}; 11: Fe^{2+}; 12: Co^{2+}; 13: Fe^{3+}; 14: K^{+}; 15: Cr^{3+}; 16: Pb^{2+}; 17: Cu^{2+}. c) Color change of the aggregates (probes A and B, each at 1.5 nM) in the presence of various representative metal ions (each at 1 μM) upon heating from room temperature (RT) to 47 °C. The colorimetric results for Cu^{2+} are not shown, as the data were taken after initial submission of the manuscript; see the Supporting Information.

(Table 1). Even at elevated temperature (50 °C), there is less than 10% loss of DNA from the surface of the particle even after prolonged heating (8 h) (Table 1),[30] which suggests that the particle probes will be stable over any reasonable assay conditions.

In conclusion, we have developed a colorimetric method to detect Hg^{2+} using DNA–Au NPs in aqueous media with very high selectivity and sensitivity. This method is enzyme-free and does not require specialized equipment other than a temperature control unit. The concentration of Hg^{2+} can be determined by the change of the solution color at a given temperature or the melting temperature (T_m) of the DNA–Au NP aggregates. Unlike most of the chemosensors for Hg^{2+} which have been evaluated in organic media or organic–aqueous mixtures owing to their low water-solubility, the high water solubility of the oligonucleotide-modified gold nanoparticle probes allow this assay to be performed in aqueous media without the need for organic cosolvents. Significantly, this method can in principle be used to detect other metal ions by substituting the thymidine in our study with synthetic artificial bases that selectively bind other metal ions.[31]

Table 1: The number of fluorophore-labeled DNA strands per particle before and after exposure to Hg^{2+} at room temperature or 50 °C for 8 h.

Temp.	Portion	Mercuric Ion Concentration		
		0.5 μM	1 μM	2 μM
RT	in the supernatant	2.1 ± 1.0	1.6 ± 1.3	1.8 ± 1.2
	on the particles	68.0 ± 0.9	68.9 ± 1.8	68.1 ± 2.0
50 °C	in the supernatant	5.9 ± 0.2	6.0 ± 0.8	6.1 ± 1.3
	on the particles	64.7 ± 1.1	64.1 ± 0.8	65.0 ± 2.2

Received: January 19, 2007
Published online: April 27, 2007

Keywords: colorimetric detection · DNA · mercury · nanoparticles · sensors

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