

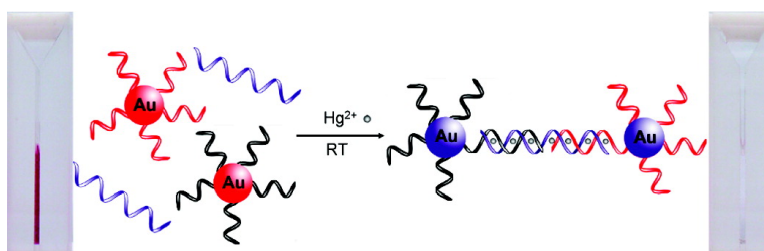
Communication

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# One-Step, Room Temperature, Colorimetric Detection of Mercury ( $\text{Hg}^{2+}$ ) Using DNA/Nanoparticle Conjugates

Xuejia Xue, Feng Wang, and Xiaogang Liu\*

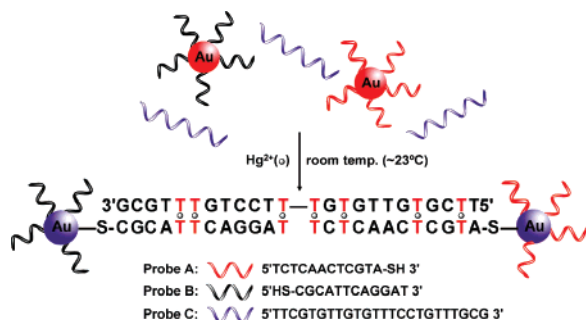
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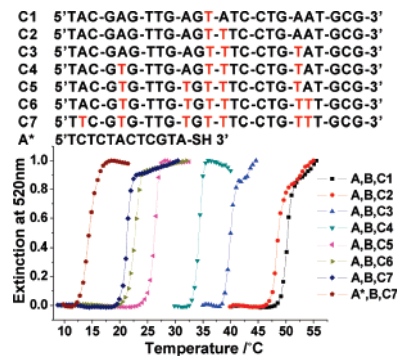
Because of the increasing threat of mercury exposure in the environment from global mercury emissions as well as various forms of contamination,<sup>1</sup> there has been a growing interest in the development of highly sensitive and selective systems for mercuric ions ( $\text{Hg}^{2+}$ ) over the past few years. Various sensor systems for detection of  $\text{Hg}^{2+}$ , based upon organic chromophores or fluorophores,<sup>2</sup> conjugated polymers,<sup>3</sup> oligonucleotides,<sup>4</sup> DNAzymes,<sup>5</sup> proteins,<sup>6</sup> thin films,<sup>7</sup> and nanoparticles (NPs),<sup>8</sup> have been reported. Most of these systems, however, have either limitations with respect to sensitivity, selectivity, and simplicity or the need of electronic read-out circuits for substantial sensitivity. Mirkin et al. recently reported colorimetric detection of  $\text{Hg}^{2+}$  in aqueous media using DNA-functionalized gold NPs.<sup>8</sup> This visual inspection method is highly selective and sensitive and also simpler than conventional methods except for the requirement of an electronic heating element coupled to the sensor system for careful monitoring of thermal denaturation temperature during the detection process.<sup>8</sup> The need of an electronic heating and read-out unit for  $\text{Hg}^{2+}$  detection at elevated temperatures clearly makes this system relatively costly and impractical for fast on-the-spot sample assays. Therefore, it should be highly desirable to develop a detection system that is not only sensitive and reliable but also simple, practical, and economical in its operation. To achieve this goal, we have taken a fundamentally different design, making use of DNA/NP conjugates and thymine- $\text{Hg}^{2+}$ -thymine (T- $\text{Hg}^{2+}$ -T) coordination chemistry<sup>9</sup> to develop a sensor system that operates within an adjustable operating temperature range and rapidly detects mercury in a single step.

The basic design of our sensor system for  $\text{Hg}^{2+}$  is composed of three elements as shown in Scheme 1. We first prepared two types

## Scheme 1



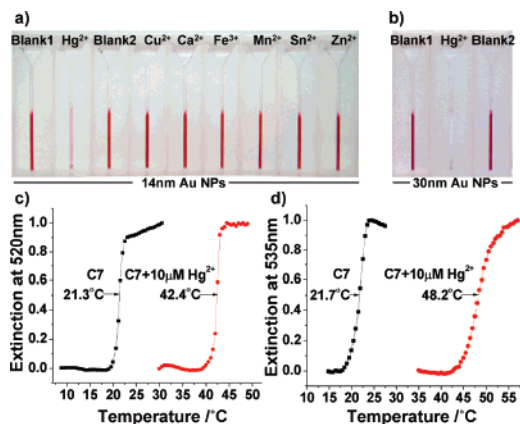
of DNA-functionalized gold NP probes<sup>10</sup> (designated as probe **A**, Au-3'S-ATGCTCAACTCT5', and probe **B**, Au-5'S-CGCATTCAGGAT3'), and an appropriate oligonucleotide linker (designated as probe **C**). To choose a specific sequence for the probe **C**, one has to ensure that it recognizes the particle probes **A** and **B** and forms stable DNA duplexes (or NP aggregated networks) only in the presence of  $\text{Hg}^{2+}$  at a given operating temperature. In the absence of  $\text{Hg}^{2+}$ , these three probes do not form DNA/NP aggregates



**Figure 1.** Normalized melting curves of solutions containing probes **A** (or **A\***), **B** and the linker probes **C**<sub>1–7</sub> with varied numbers of T–T mismatches.

because of a lower melting temperature ( $T_m$ ) than the operating temperature due to mismatches formed in the DNA duplexes. Importantly, by controlling the number of T–T mismatches in the system, one can precisely modulate the melting temperature of the three probes within a specific range. To this regard, we systematically studied temperature profiles using UV–vis spectroscopy for a series of probe solutions containing oligonucleotide linkers (probes **C**<sub>1–7</sub>) that are complementary to probes **A** and **B** except for various numbers of the T–T mismatch. As anticipated, melting temperatures of the probes **A**, **B**, and **C**<sub>1–7</sub> were decreased from 50.3, 48.6, 40.0, 34.2, 26.2, 22.8, to 21.3 °C with an increasing number of T–T mismatches (Figure 1). It should be noted that the melting temperature of the probes can be further suppressed well below the operating ambient temperature ( $\sim 23$  °C) with a solution containing eight T–T mismatches (probes **A\***, **B**, **C**<sub>7</sub>;  $T_m = 14.4$  °C) as shown in Figure 1.<sup>11</sup>

As a proof-of-concept experiment, an aqueous solution of Probes **A**, **B**, and **C**<sub>7</sub> (2 nM, 2 nM, and 1  $\mu\text{M}$ , respectively;  $T_m = 21.3$  °C) was selected for a prototype sensor system for  $\text{Hg}^{2+}$  at room temperature. Upon addition of an aqueous solution of  $\text{Hg}^{2+}$  (10  $\mu\text{M}$ ), a clear red-to-purple/pinkish colorimetric response occurred within 5 min (Figure 2a). The particle-amplified color change indicates formation of stable  $\text{Hg}^{2+}$ -mediated DNA base pairs as evidenced by an increase in the sharp melting temperature (42.4 °C) shown in Figure 2c. The UV–vis spectrum of the solution also shows a bathochromic shift of the gold nanoparticles from 520 to 565 nm, indicating the formation of particle aggregates via  $\text{Hg}^{2+}$ -induced hybridization events (Supporting Information). The NP aggregates were confirmed by transmission electron microscopy in contrast to dispersed NPs in the absence of  $\text{Hg}^{2+}$  (Supporting Information). To exclude the possibility that  $\text{Hg}^{2+}$  may directly remove the thiolated oligonucleotides from the surface of the particles because of its thiophilic nature,<sup>12</sup> we performed control experiments without the oligonucleotide linker (probe **C**<sub>7</sub>). Upon addition of various concentrations of  $\text{Hg}^{2+}$  (10, 50, and 100  $\mu\text{M}$ ), we did not observe visible color changes (or particle precipitation)



**Figure 2.** (a) Color response of a 14 nm NP detection system (probes A, B, and C<sub>7</sub>) in the presence of a selection of metal ions (Hg<sup>2+</sup>, Cu<sup>2+</sup>, Ca<sup>2+</sup>, Fe<sup>3+</sup>, Mn<sup>2+</sup>, Sn<sup>2+</sup>, Zn<sup>2+</sup>; 10 μM each). Note that **Blank1** (probes A, B, and C<sub>7</sub> without Hg<sup>2+</sup>) and **Blank2** (probes A and B with Hg<sup>2+</sup>) were used as control references. (b) Color response of a 30 nm NP detection system under the same conditions. (c, d) Normalized melting curves of the solution (containing probe A, B, and C<sub>7</sub>) with or without Hg<sup>2+</sup> (10 μM) for the 14 and 30 nm NP systems, respectively.

over a period of time of 48 h (Figure 2a, **Blank2**). Taken together, these observations suggest that the rapid assembly of the nanoparticles at ambient temperature is indeed attributed to the formation of stable T–Hg<sup>2+</sup>–T base pairs in DNA duplexes.

In a further set of experiments, we have evaluated the selectivity of our system for metal ions. Solutions containing metal ions (Co<sup>2+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Ca<sup>2+</sup>, Mn<sup>2+</sup>, Sn<sup>2+</sup>, Zn<sup>2+</sup>, Ru<sup>3+</sup>, and Fe<sup>3+</sup>; each at 10 μM) were tested under the same conditions as in the case of Hg<sup>2+</sup>. Remarkably, no optical and thermal transition profile changes of the solutions were observed with these metal ions up to millimolar concentrations (Figure 2a and Supporting Information). The specific detection for Hg<sup>2+</sup> is clearly attributed to its selective binding of T–T mismatches, resulting in the formation of stable T–Hg<sup>2+</sup>–T complexes that lead to particle aggregation.

We have also investigated the sensitivity of our detection system. Various concentrations (0.5, 1.0, 2.0, 3.0, 5.0, 10, 25, and 50 μM) of Hg<sup>2+</sup> were added to a series of solutions containing 14 nm NP probes (A and B; each at 2 nM) and the oligonucleotide linker (probe C<sub>7</sub>; 1 μM). The corresponding melting temperatures were measured at 21.8, 22.3, 22.9, 24.8, 28.3, 42.4, 47.5, and 50.0 °C, respectively (Supporting Information). The limit of detection for the 14 nm NP system by the naked eye is about 3 μM of Hg<sup>2+</sup>. However, the sensitivity of the system can be further improved (~1 μM) by using larger particles or varying oligonucleotide sequences on the surface of the nanoparticles (Supporting Information). For example, upon addition of Hg<sup>2+</sup> (10 μM) to a solution containing 30 nm particles, an enhanced color change can be readily observed (Figure 2b). The signal enhancement of the 30 nm NP system was attributed to significant extinction dampening in the plasmon region of the spectrum that accompanies Hg<sup>2+</sup>-induced NP aggregation (Supporting Information). In contrast to the 14 nm NP counterpart, a larger melting transition (e.g., 27 versus 21 °C with 10 μM of Hg<sup>2+</sup>) was observed for the 30 nm NP solution between the dispersed and aggregated states (Figure 2d).

In conclusion, we have demonstrated a novel and practical system for colorimetric detection of mercury at room temperature. The system utilizes a combination of oligonucleotides and NPs at low concentrations, which readily detects Hg<sup>2+</sup> in aqueous solutions

and in the presence of an excess of other metal ions.<sup>13</sup> The method can be rationalized for use to detect other metal ions by replacing natural DNA bases with metal-dependent synthetic artificial bases.<sup>14</sup> Complementary to instrument-based ultrahigh sensitive methods with electronic read-out circuits (e.g., DNAzyme catalytic beacons<sup>5</sup>) for accurate metal ion identification, this instrument-free assay should afford a practical and convenient solution, particularly in remote areas for rapid screening of Hg<sup>2+</sup> contamination. The detection limit of sub-10 nM (U.S. EPA standard) in drinking water can be principally reached by preconcentrating the water solution through evaporation or by coupling the detection system with a signal amplification method (e.g., gold NP-promoted silver amplification<sup>10b</sup>). Our effort along this line is currently underway.

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**Supporting Information Available:** TEM images of gold probes and aggregates, UV–vis spectra of particle solutions, thermal profiles of NP probes with various concentrations of mercury and different metal ions. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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