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Mechanism of mercury detection based on interaction of single-strand DNA and hybridized DNA with gold nanoparticles

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ABSTRACT

Mechanisms of interaction of single-strand DNA and hybridized DNA on gold nanoparticles in the presence of Hg^{2+} was studied in this work. Recently the detection of Hg^{2+} using unmodified gold nanoparticles (AuNPs) combined with DNA is becoming a promising technique with the advantages of simplicity, cost-effectiveness and high sensitivity. However, few studies focused on the interaction of ssDNA and hybridized DNA on AuNPs to date. In the present work, we compared the interactions of different DNA probes on AuNPs using both absorption and fluorescence detection. It was found that there were only small partial dsDNA dissociated from the surface of AuNPs after hybridization in the presence of Hg^{2+}. Moreover, we found that the aggregated AuNPs/DNA system tended to be dispersed again with increasing Hg^{2+} concentration up to 250 μM. Based on these results, the mechanisms of mercury detection based on interaction between DNA-conjugated gold nanoparticles were investigated. Positively charged dsDNA could bind to the surface of AuNPs and dominate the electrostatic interactions and consequently aggregation of the AuNPs/DNA system.

1. Introduction

Mercury (II) (Hg^{2+}), as one of the heavy metal ions, is considered to be highly toxic and extremely hazardous for both human health and our living environment. Development of efficient methods for sensitive and selective detection of Hg^{2+} has been attracting much interest in recent years. Besides conventional methods, such as inductively coupled plasma mass spectrometry, atomic absorption/emission spectroscopy, and electrochemical sensing devices [1–3], new methods or sensors based on polymers [4], oligonucleotides [5], and proteins [6] have been developed. The method using gold nanoparticles combined with oligonucleotides is one of the most promising ideas among them [7,8].

Gold nanoparticles (AuNPs) are widely used optical sensing nanomaterials because they have high visible-region extinction coefficients and their surface Plasmon resonance (SPR) absorption is strongly distance-dependent [9,10]. When AuNPs are brought close to one another, the absorbance at the red region will increase. Visual observation of the solution changes from red to blue is feasible as a result of the changes in inter-particle distance. This makes AuNPs a good indicator for binding and hybridization events [11–13]. Studies have also shown that Hg^{2+} can bind between thymines to form stable base pairs in DNA [14–16]. It is also known that DNA is able to use their bases to be adsorbed onto AuNPs surfaces via coordination interaction between bases and AuNPs [17–19]. The adsorption of DNA onto AuNPs can significantly enhance AuNPs resistance to salt-induced aggregation. As a result, increasing numbers of colorimetric methods for detecting Hg^{2+} using combinations of DNA and gold nanoparticles (DNA/AuNPs) have been developed.

Using single thymine mismatched DNA modified AuNPs, Mirkin's group achieved Hg^{2+} assay through the changes of the melting temperature [8]. Liu's group went on to change the number of the T–T mismatch so that the detection system could work at room temperature [20]. Another novel detection method had also been developed using unmodified AuNPs based on the fact that ssDNA could adsorb on AuNPs to prevent salt-induced aggregation and only the addition of Hg^{2+} could cause the formation of hybridized DNA which leaded to aggregation [21,22]. The hybridized DNA could have either hairpin or helix complex structure, depending on how the T–T mismatch was designed in the DNA sequences. AuNPs/DNA fluorescence detection system was also introduced due to the advantage of quenching ability of AuNPs [23]. One example was the development of "turn-off" sensor by Ono's group. They modified fluorophore and quencher at opposite ends of a hairpin DNA strands so that there was quenching effect

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Table 1
Names and sequences (5′–3′ orientation) of the oligonucleotides.

Name	Sequence
Probe 1	TTCGTGTTGTGT
Probe 2	CGCTTTCTGGTTTCTCTTCTCGTT
Probe 3	TTCGTGTTGTGTTTCCTGTTTGCGT CGTGTCGTTGT
Probe 4	TTTCTTCCCCTTGTTT-FAM
Probe 5	TTCTTTCTTCTCCCCTGTTGTTTGTT-FAM
Probe 6	TTCTTTCTCTTCTTCTCCCCTGTTGTTGTGTTTGTT-FAM

Probe 1–3 are ssDNA with random sequences. Probe 4–6 are fluorescein (FAM)-labeled self-complementary ssDNA with multiple T–T mismatch.

based on fluorescence resonance energy transfer (FRET) in the presence of Hg^{2+} [5]. However, Yang's group developed a "turn on" Hg^{2+} sensor based on structural switching DNA containing T–T mismatch with FAM labeling [24]. This was preferred over "turn-off" sensor as there were some quenchers or species in the environment which could cause decrease of fluorescence and result in false positive.

The unmodified AuNPs/DNA detection methods proposed are found to be simpler and more cost-effective, which can also provide convenience of visual observation and quantitative detection through using of analytical instruments. They have the ability to be used on-site without sophisticated instrumentation. Although the DNA/AuNPs sensors have a lot of valuable advantages as Hg^{2+} detection system and have been widely investigated, to the best of our knowledge, only a few studies referred to the structural information regarding the binding and conformation of DNA on the AuNPs surface [17–19,25,26]. Almost all of them considered that helix of dsDNA could not adsorb well on the surface of AuNPs and resulted in loss of protection for AuNPs from salt-induced aggregation, as ssDNA could uncoil sufficiently to expose its bases, whereas dsDNA had a stable double-helix geometry that always presented the negatively charged phosphate backbone [21,22,24]. So they had different abilities/"van der Waals forces" to be adsorbed on the negatively charged surface of AuNPs in solution.

However, in the present work, we found there were only small partial dsDNA dissociated from the surface of AuNPs after hybridization in the presence of Hg^{2+}, and the intercrossing link through T–Hg^{2+}–T between each AuNP might act as an important factor to cause aggregation. In the experiments, to obtain experimental evidences for attempting understandings of interaction on the surface of AuNPs, different length and types of DNA probes have been used to compare their performance in the presence of Hg^{2+} by UV/vis absorption and fluorescence detection respectively. Moreover, the performance of AuNPs-DNA in the presence of high concentration of Hg^{2+} has also been investigated and a reasonable explanation is put forward for the significantly different phenomena from that in the condition of low Hg^{2+} concentration. We believe that results of our present studies will provide better understanding of the mechanisms of interaction between AuNPs-DNA in the presence of high concentration of Hg^{2+}, and will benefit further improvement of DNA/AuNPs sensors in the future.

2. Experimental

2.1. Chemicals and instrumentations

All oligonucleotide products (shown in Table 1) were synthesized and purified by HPLC in Eurogentec AIT (Singapore) and dissolved in 10 mM HEPES buffer containing 100 mM $NaClO_4$ (pH 7.4). All the other chemicals were analytical grade and purchased from Sigma–Aldrich Chemical Co. (Singapore). All solutions were prepared using Milli-Q-quality deionised water filtered through a 0.22 μm filter (Millipore, Nepean, ON).

UV-2450 Shimadzu Vis-spectrometer and Perkin Elmer SL55 fluorescence spectrometer were used for absorption and fluorescence measurements respectively. Transmission electron microscopy (TEM) was performed on a JEM-3010F Field Emission Electron Microscope.

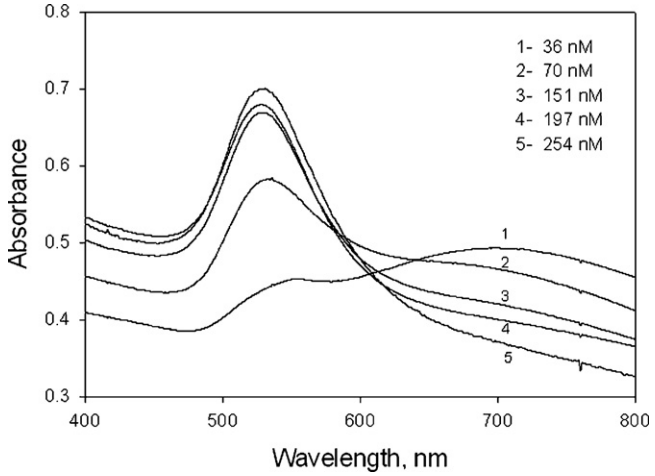

Fig. 1. Absorption spectrum of solution containing 0.8 nM AuNPs and 35 mM $NaClO_4$ in the presence of Probe 2. The absorbance increased at 520 nm while decreased at 700 nm with the increase of ssDNA concentration.

2.2. Synthesis of AuNPs

AuNPs (~13 nm) were prepared according to the literature [27]. Briefly, a solution of sodium citrate (10 mL, 38.8 mM) was added rapidly to the vigorously stirred boiling chloroauric acid (100 mL, 1 mM). The solution would turn red in approximately 5 min. Next, the solution was boiled continuously for 10 min and stirred for another 15 min with the heater switched off. The mixture was cooled to room temperature and filtered through a membrane filter, stored in refrigerator at 4 °C before use. The mean diameter of AuNPs was estimated by TEM to be ~13 nm. The concentration of the AuNPs was estimated by UV/vis spectroscopy from the characteristic absorbance peak at ~520 nm for 13 nm particles [27]. The extinction coefficient of this peak used will be $2.7 \times 10^8\ M^{-1}cm^{-1}$.

2.3. Interaction of DNA on AuNPs

For the interaction of DNA on AuNPs in the presence or absence of Hg^{2+}, optimized concentration of DNA was added into different concentrations of $Hg(ClO_4)_2$ followed with the addition of AuNPs. After 30 min, $NaClO_4$ was introduced. The final concentration of the AuNPs and $NaClO_4$ were 0.8 nM, and 35 mM respectively. The reproducibility was assessed by analyzing three replicates for each experiment.

3. Discussion

3.1. The interaction of ssDNA on AuNPs in salt solution

The AuNPs colloid is stable, red color with the surface plasma absorption peak at 520 nm in the condition of low salt concentration due to the negative electrostatic repulsion. Increasing the salt concentration of the solution, the colloid becomes unstable to aggregate with the color changing from red to blue and the surface plasma absorption decreasing and shifting to long wavelength. However, in the presence of ssDNA, the coordination interactions between DNA bases and AuNPs will enhance AuNPs' stability against the salt-induced aggregation as the typical results using Probe 2 shown in Fig. 1.

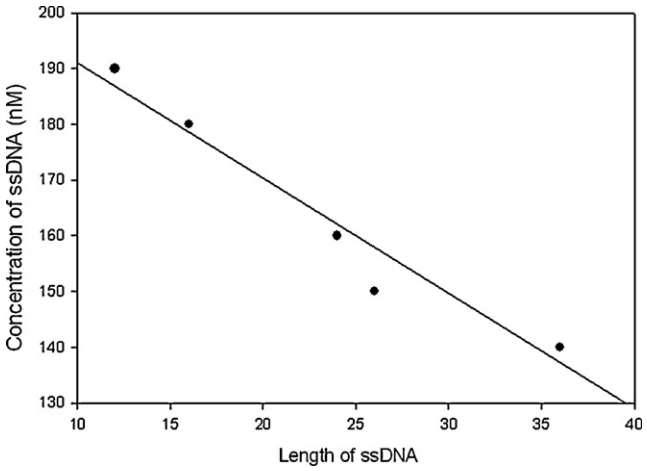

Fig. 2. The relationship of the concentration of ssDNA needed to stabilize AuNPs colloid in salt solution and the length of ssDNA.

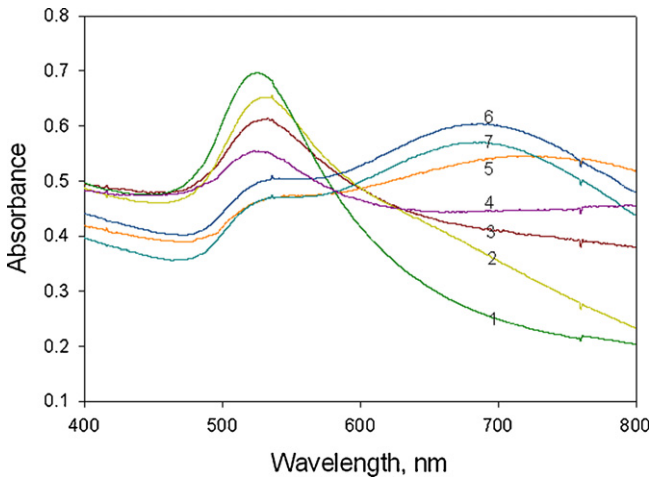

Fig. 3. Absorption spectra of DNA/AuNPs system in the presence of Hg^{2+} with different concentrations. The solution containing 150 nM DNA Probe 5, 0.8 nM AuNPs and 35 mM $NaClO_4$. The Hg^{2+} concentration is 0, 3, 4.8, 9.6, 25, 48 and 62.5 μM for curve 1–7 respectively.

To study the interaction of DNA on AuNPs, the screening effect of ssDNA with different lengths (Probe 1–6) on aggregation of AuNPs in salt solution was firstly studied. For each ssDNA probe, the amount needed to stabilize the AuNPs colloid was investigated by adding each ssDNA into the AuNPs (0.8 nM) followed with addition of salt (35 mM). The UV/vis absorption results (A700/520) were used to monitor the aggregation. The results shown in Fig. 2 indicate that the screening approximately depends on the length of the whole single DNA strands or the number of bases, although different bases exhibit different binding sites and strengths on the AuNPs surfaces [28,29].

3.2. Interaction of folded structure DNA on AuNPs in the presence of Hg^{2+}

The interactions of DNA probes on AuNPs in the presence of Hg^{2+} were investigated in this study using DNA Probe 4–6 which could form a self-folded helix structure after hybridization. In the experiments, the optimized amount of each DNA probe to stabilize AuNPs was applied in the presence of Hg^{2+} with different concentrations. Both absorption and fluorescence detection were used for assessing the interaction.

The AuNPs under screening of ssDNA in salt solution were quite stable without Hg^{2+}. However, with increasing Hg^{2+} concentrations, AuNPs started to aggregate with each other, resulting in the change of surface plasma absorption as shown in Fig. 3. At the same time, fluorescence signals of the systems were also investigated (Fig. 4). As expected, AuNPs could quench the fluorescence of FAM-ssDNA which was adsorbed on the surface of AuNPs. However, if the FAM-ssDNA formed self-folded structure after hybridization in the presence of Hg^{2+} and subsequently dissociated from the surface of AuNPs, the fluorescence would restore. Based on this, changes in fluorescence could be used to assess the dissociation of hybridized DNA in the system.

It was considered that the hybridized DNA presented a stable double-helix geometry exposing negatively charged phosphate backbone. Due to the electrostatic repulsion between the negative AuNPs and hybridized DNA, the DNA would dissociate from the surface of AuNPs resulting in aggregation or the change of absorption/fluorescence. As shown in the Figs. 3 and 4, the absorption value really changed significantly and the fluorescence value decreased as expected. Moreover, with the increase of the length of the probes with more T–T mismatches, the response range for Hg^{2+} became wide while the sensitivity became poor as shown in Table 2. These results indicated that more T–T mismatch sites could combine with more Hg^{2+}, while it was easy to hybridize for the short probes with less T–T mismatches in the presence of Hg^{2+}.

Table 2
Performance comparison between different DNA probes for Hg^{2+} response using absorption and fluorescence detection respectively.

Sensing system	Detection	Response region (μM)	Detection limit (μM)
Probe 4 (5 T–T mismatch)	Absorption	0–38	0.78
Probe 5 (8 T–T mismatch)		0–48	1.24
Probe 6 (11 T–T mismatch)		0–60	2.88
Probe 4 (5 T–T mismatch)	Fluorescence	0–15	0.64
Probe 5 (8 T–T mismatch)		0–25	1.04
Probe 6 (11 T–T mismatch)		0–40	1.06

Normally, fluorescence detection was considered much more sensitive compared with absorption detection. However, in this case, similar detection limits were obtained using the same DNA probes but different detection methods. There might be two possible reasons: (1) high fluorescence background; and (2) small enhancement of fluorescence after DNA hybridization. From Fig. 4, it was found that even in the absence of Hg^{2+}, the fluorescence

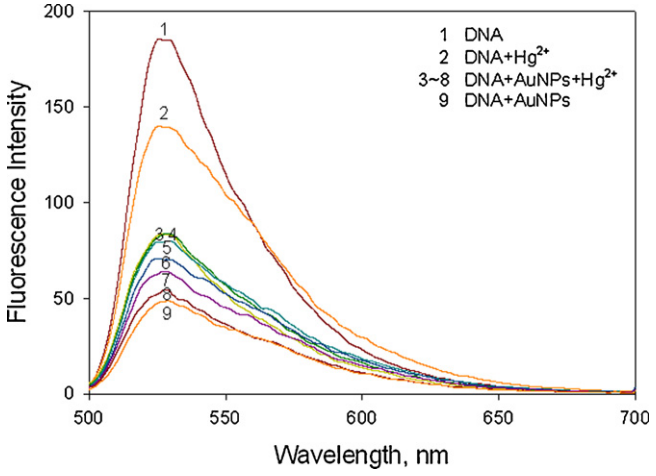

Fig. 4. Fluorescence spectrum of DNA/AuNPs system in the presence of Hg^{2+} with different concentrations. The solution containing 150 nM DNA Probe 5, 0.8 nM AuNPs and 35 mM $NaClO_4$. The Hg^{2+} concentration is 62.5, 48, 25, 9.6, 4.8 and 3 μM for curve 3–8 respectively. It contains 62.5 μM Hg^{2+} for curve 2.

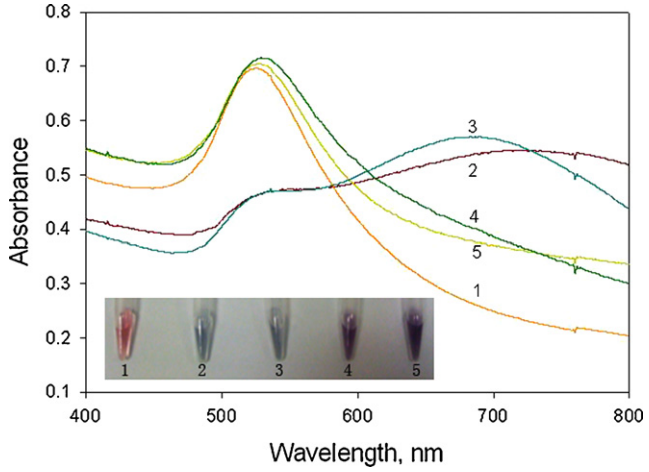

Fig. 5. Absorption spectrum and photograph of DNA/AuNPs system containing 0.8 nM AuNPs, 0.15 μM Probe 5 and 35 mM $NaClO_4$ in the presence of (1) 0 μM, (2) 25 μM, (3) 62.5 μM, (4) 250 μM, (5) 2500 μM Hg^{2+}.

background was quite high, which might be due to both the fluorescence of FAM not being quenched efficiently on AuNPs and the extra ssDNA in the solution without adsorption onto the surface of AuNPs. Moreover, the fluorescence of FAM-DNA could be quenched slightly by Hg^{2+}. And the fluorescence was quenched most with addition of AuNPs due to the adsorption, while it could be restored to a certain extent by binding with Hg^{2+}. However, according to the value of fluorescence, there were at least over-half of the FAM-DNA probes still on the surface of AuNPs, which resulted in poor fluorescence enhancement.

To determine whether hybridized helix DNA could still be adsorbed effectively on AuNPs, we proceeded to investigate the performance of DNA–AuNPs system at the condition of high concentration of Hg^{2+}. It was found that the aggregation became weak and the AuNPs tended to be stable again when the concentration of Hg^{2+} increased to 250 μM. As shown in Fig. 5, the surface plasma absorption reverted back approximately to its original value without Hg^{2+} and the color changed from blue to wine red. We believe this should be due to the binding of DNA with more Hg^{2+} on the AuNPs, and the AuNPs subsequently became totally positively charged. The electrostatic repulsion resulted in a stable AuNPs colloid. The result indicated that even though the ssDNA formed helix structures, they could also adsorb largely on the surface of AuNPs. Since there were both hybridized DNA and ssDNA coexistent on the AuNPs at low concentration of Hg^{2+}, the mechanism of aggregation should be due to not only the loss of the protection by ssDNA, but also strong electrostatic attraction. Moreover, as commonly known, there were free nitrogen atoms in the pyrimidine ring of thymine upon adsorption on AuNPs [30]. Therefore, Hg^{2+} ions could act as metal ion bridges (T–Hg^{2+}–T) the weakly bound thymine, creating an inter-connected network for the enhanced aggregation of AuNPs. Based on the above analysis, a proposed mechanism was put forwarded as shown in Fig. 6.

3.3. *Effect of other metal ions on the Hg^{2+} induced aggregation of AuNPs/DNA system*

The effect of other metal ions on the interaction of AuNPs/DNA/Hg^{2+} system was investigated. Fig. 7 shows the histogram of the absorption ratio (A700/520) in the presence of different metal ions such as Cd^{2+}, Co^{2+}, Fe^{2+}, Pb^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} and Hg^{2+}. Most of the metal ions except Hg^{2+} and Pb^{2+} had absorption ratios of less than 0.4 which was similar to the blank solution. However, the absorption change caused by Hg^{2+} was much bigger

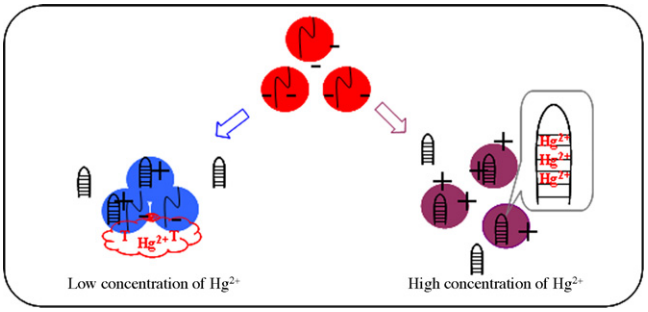

Fig. 6. The proposed mechanism for interaction of AuNPs/DNA solution in the presence of different amounts of Hg^{2+}.

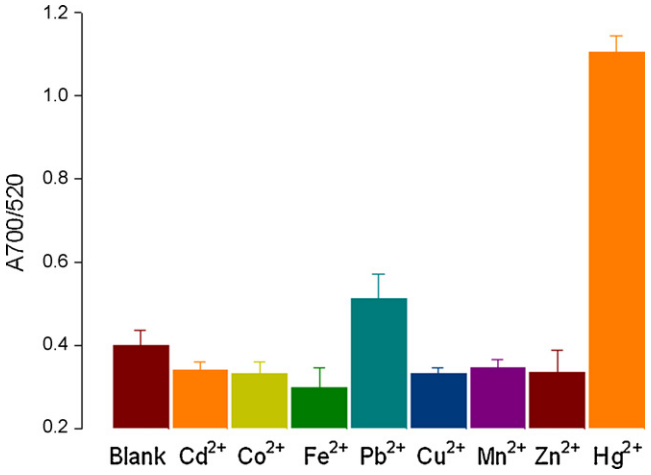

Fig. 7. Effect of other metal ions on the Hg^{2+} induced aggregation of AuNPs/DNA system. Values of A700/520 of solution containing 0.8 nM AuNPs, 0.15 μM Probe 5 and 35 mM $NaClO_4$ in the presence of 25 μM Hg^{2+} and 150 μM other different metal ions. Blank solution contained AuNPs, DNA and $NaClO_4$ only.

than that caused by Pb^{2+}, which showed that the thymine in the DNA bind selectively with Hg^{2+} even in the presence of other metal ions at high concentrations.

4. Conclusion

The interactions of different DNA probes on AuNPs were compared using optical absorption and fluorescence techniques. It was found that the AuNPs/DNA system gradually aggregated with the increase of Hg^{2+} concentration up to 62.5 μM and tended to redisperse in the high concentration over 250 μM. Interestingly, it was also found there was only small partial dsDNA dissociation from the surface of AuNPs after hybridization in the presence of Hg^{2+}. In addition to the protection effects of ssDNA, the amount of dsDNA determines the surface charge and consequently the electrostatic interactions which dominate the aggregation status of the AuNPs/DNA system. The formation of inter-connected T–Hg^{2+}–T complexes also played important role in the aggregation. Our results show that the detection of Hg^{2+} based on AuNPs and DNA is a very simple and rapid colorimetric assay. It may have great merit for rapid screening of large numbers of samples in cases of severe Hg^{2+} pollution. Moreover, it may also be applied for trace levels of Hg^{2+} detection with the aid of a sample preconcentration system. We believe that our study provides better understanding of the mechanism of interaction between AuNPs and DNA in the presence of Hg^{2+}, and will benefit further improvement of DNA/AuNPs sensors for toxic metals in the future.

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