Supplementary Information

Multiplexed Analysis of Silver(I) and Mercury(II) Ions Using Oligonucleotide-Metal Nanoparticle Conjugates

Gioi Dong Huy, Min Zhang, Peng Zuo and Bang-Ce Ye*

1. Preparation of Citrate-Stabilized Au Nanoparticles (AuNPs)

All glassware was thoroughly cleaned overnight with freshly prepared 3:1 HCl/HNO₃ (aqua regia) and rinsed thoroughly with Mill-Q water prior to use. AuNPs were prepared according to the reported method.¹ Briefly, 100 mL of HAuCl₄ (0.01%) was added to a 250 mL round bottle and then boiled. Under rapid stirring, 3.5 mL of trisodium citrate (1%) was added and further rapidly stirred for 15 min. After stirred for 30 min, the solution was then gradually cooled to room temperature, and was filtered by 0.22 µm filter paper, which was stored in the refrigerator (4°C) before further use. The concentration of the as-prepared AuNPs is estimated to be about 2.3 nM using Beer’s law. Prepared AuNPs were concentrated from about 2.3 nM to about 11.5 nM AuNPs for further use in this work. Transmission electron microscope (TEM) measurements were performed on Jeol JEM-1230 instrument operated at an accelerating voltage of 120 kV. Samples for TEM studies were prepared by placing a drop of AuNPs solution on a copper grid. The films on the TEM grids were allowed to dry for 3 min following that the extra solution was removed using a blotting paper.

Fig. S1 (A) TEM image and (B) histograms of size distribution of AuNPs.
2. Preparation of Citrate-Stabilized Ag Nanoparticles (AgNPs)

All glassware was thoroughly cleaned overnight with freshly prepared 3:1 HCl/HNO₃ (*aqua regia*) and rinsed thoroughly with Mill-Q water prior to use. AgNPs were prepared according to the reported method with minor modifications.² Briefly, 1mL of freshly prepared NaBH₄ (0.5%) was quickly added to a 500 mL solution of AgNO₃ (0.25 mM) and trisodium citrate (0.25 mM) under vigorous stirring. The reaction was stirred for 30 min. The color of the mixture gradually changed and finally became yellow when silver colloidal is formed. The resulting solution was filtered by 0.22 µm filter paper and stored in the refrigerator (4°C) before further use. The concentration of the as-prepared AgNPs is estimated to be 4.2 nM using Beer’s law. Prepared AgNPs were concentrated from about 4.2 nM to about 21 nM AgNPs for further use in this work. Transmission electron microscope (TEM) measurements were performed on Jeol JEM-1230 instrument operated at an accelerating voltage of 120 kV. Samples for TEM studies were prepared by placing a drop of AgNPs solution on a copper grid. The films on the TEM grids were allowed to dry for 3 min following that the extra solution was removed using a blotting paper.

![Fig. S2](image.png) (A) TEM image and (B) histograms of size distribution of AgNPs.
3. **Characterization of the self-assembled of DNA onto the surface of AuNPs**

![Agarose gel electrophoresis](image)

**Fig. S3** Agarose gel electrophoresis for AuNPs (lane 1) and DNA-AuNPs (lane 2). Running buffer was 1×TBE buffer (89 mM tris base, 89 mM borate and 2 mM EDTA, pH 8.2 at 25°C).

4. **TEM characterization of aggregation of DNA-nanoparticle complex**

![TEM images](image)

**Fig S4**. TEM images of AuNPs-C-ssDNA treated with 20 μM of Ag⁺. (A) Before adding 0.05 M of NaNO₃; (B) After adding 0.05 M of NaNO₃.

![ TEM images](image)

**Fig S5**. TEM images of AgNPs-T-ssDNA treated with 20 μM of Hg²⁺. (A) Before adding 0.05 M of NaNO₃; (B) After adding 0.05 M of NaNO₃.
5. Investigating the behavior of AuNPs-C-ssDNA/AgNPs-T-ssDNA sensing system treated with different concentrations of Ag$^{+}$ and/or Hg$^{2+}$

Fig. S6. UV–vis absorption spectra of AuNPs-C-ssDNA/AgNPs-T-ssDNA sensing system treated with different concentrations of Ag$^{+}$ in NaNO$_3$-MOPS buffer.

Fig. S7. UV–vis absorption spectra of AuNPs-C-ssDNA/AgNPs-T-ssDNA sensing system treated with different concentrations of Hg$^{2+}$ in NaNO$_3$-MOPS buffer.

Fig. S8. UV–vis absorption spectra of AuNPs-C-ssDNA/AgNPs-T-ssDNA sensing system treated with different concentrations of Ag$^{+}$ and Hg$^{2+}$ mixture in NaNO$_3$-MOPS buffer.
Table 1. The absorption ratio \((A_{650}/A_{520})\) of AuNPs-C-ssDNA/AgNPs-T-ssDNA sensing system treated with different concentrations of \(\text{Ag}^+\) and \(\text{Hg}^{2+}\) mixture in NaNO\(_3\)-MOPS buffer (deduced from Fig.S8).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorption ratio ((A_{650}/A_{520}))</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Ag}^+ 0.005 , \mu\text{M})</td>
<td>0.162796 ± 0.000185</td>
<td>1.134</td>
</tr>
<tr>
<td>(\text{Ag}^+ 0.005 , \mu\text{M} + \text{Hg}^{2+} 5 , \mu\text{M})</td>
<td>0.162842 ± 0.000328</td>
<td>2.016</td>
</tr>
<tr>
<td>(\text{Ag}^+ 0.005 , \mu\text{M} + \text{Hg}^{2+} 10 , \mu\text{M})</td>
<td>0.162916 ± 0.000233</td>
<td>1.432</td>
</tr>
<tr>
<td>(\text{Ag}^+ 0.005 , \mu\text{M} + \text{Hg}^{2+} 20 , \mu\text{M})</td>
<td>0.162741 ± 0.000247</td>
<td>1.520</td>
</tr>
</tbody>
</table>

Fig. S9. UV–vis absorption spectra of AuNPs-C-ssDNA/AgNPs-T-ssDNA sensing system treated with the river water which was spiked with different concentrations of \(\text{Ag}^+\) and \(\text{Hg}^{2+}\) mixture in NaNO\(_3\)-MOPS buffer.

References: