Ultrasensitive detection of lead (II) with DNAzyme and gold nanoparticles probes by using dynamic light scattering technique

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Experimental Section

Materials

All DNA samples were synthesized by Sangon Biotech. Co. Ltd. (Shanghai, China). $Pb(oc)_2 \cdot 2H_2O$ was purchased from Dongzheng Co.Ltd. (Guangzhou, China) and $HAuCl_4 \cdot 3H_2O$ was from Sigma-Aldrich Co. Sterilization water was homemade. All other materials used were of the highest quality available and purchased from regular sources. 25 mM of tris-acetate (pH 8.0, with 100 mM NaCl) was used in our experiments.

Preparation of gold nanoparticles

Gold nanoparticles were synthesized by reducing 20 mL of 1.0 mM $HAuCl_4$ with 2.0 mL of 38.8 mM sodium citrate, and the average sizes of them were about 13 nm, as determined from transmission electron microscopy (TEM) analysis. However, in our study, the average size of AuNPs（～16.1 nm）, which was estimated from DLS and defined based on the number average, was larger than that obtained from TEM study, the difference might be explained that DLS measured the hydrodynamic radius while TEM providing a more precise measurement of the hard AuNPs core.[1] Meanwhile, the concentration of AuNPs was about 10.6 nM, which was calculated by UV-visible absorption spectrometer based on an extinction coefficient of 2.6×10^8 $M^{-1} \cdot cm^{-1}$ at 520 nm for ~13 nm particles.

Preparation of oligonucleotides modified gold nanoparticles

Before the preparation of oligonucleotides modified gold nanoparticles, disulfide bonds at the bottom of Oligo 1 and Oligo 2 were cleaved by TCEP for about 1-2 hours.[2] Whereafter, the AuNPs-Oligo 1 and AuNPs-Oligo 2 probes were prepared

by adding 5OD of Oligo 1 and Oligo 2 to 10 mL of AuNPs aqueous solution (10.6 nM), respectively. After incubation for about 16.0 h at 25ºC, 100 μL of 5.0 M NaCl solution was added by a stepwise manner during the next 44 h incubation. Then, excess reagents were removed by centrifugation at 13800 rpm for three times (each time for 15 min) and the remained red oily precipitate containing AuNPs-Oligo 1 and AuNPs-Oligo 2 were washed with 25 mM of Tris acetate buffer each time (pH 8.0). Finally, AuNPs-Oligo 1 and AuNPs-Oligo 2 were dispersed in the same buffer for DLS analysis, and the extinction of them at 532 nm was adjusted to 1.0 (corresponding to a concentration of 3.7 nM). Meantime, the number of oligonucleotide on per AuNPs was found to be 65±3 strands for Oligo 1 and to be about 68 strands for Oligo 2, which was calculated according to literature. [3]

UV measurement, TEM images and DLS analysis

The absorption spectrum was obtained with a TU-1901 UV-visible absorption spectrometer. The TEM images of the AuNPs were recorded with a JEM-2010HR transmission electron microscope (Japan). To prepare the TEM samples, 20 μL of aqueous solutions of the sample were dropped onto a carbon-coated copper grid, excess of micellar solutions were gently removed by using absorbent paper. Samples were then kept in desiccator prior to the measurement. DLS measurements were performed by using Zetaplus/90plus Dynamic Light Scattering instrument (Brookhaven Instrument Co. USA). The DLS instrument was operated under the following conditions: temperature:25ºC, detector angle: 90^{o}, incident laser wavelength: 683 nm, and laser power:100 mW. All sizes reported here were based on the number average, and each reported particle size was the average of three times measurements.

Pb^{2+} detection

To constructed DLS analysis, 3.7 nM AuNPs-Oligo 1, 3.7 nM AuNPs-Oligo 2, 800 nM substrate and 2.0 μM enzyme were mixed firstly and the solution color changed from red to purple. Then, the sample solutions were incubated in a water bath of 60ºC for 5 min and a red color could be found because of the melting of the aggregates. At this time, different concentrations of Pb^{2+} were added and allowed them to cool slowly to 30ºC in half of an hour, and kept at 30ºC for 10 min for the cleavage of the

substrate, then EDTA was added to chelate Pb^{2+} and stop the cleavage. Finally, the sample was diluted to an appropriate concentration for DLS analysis.

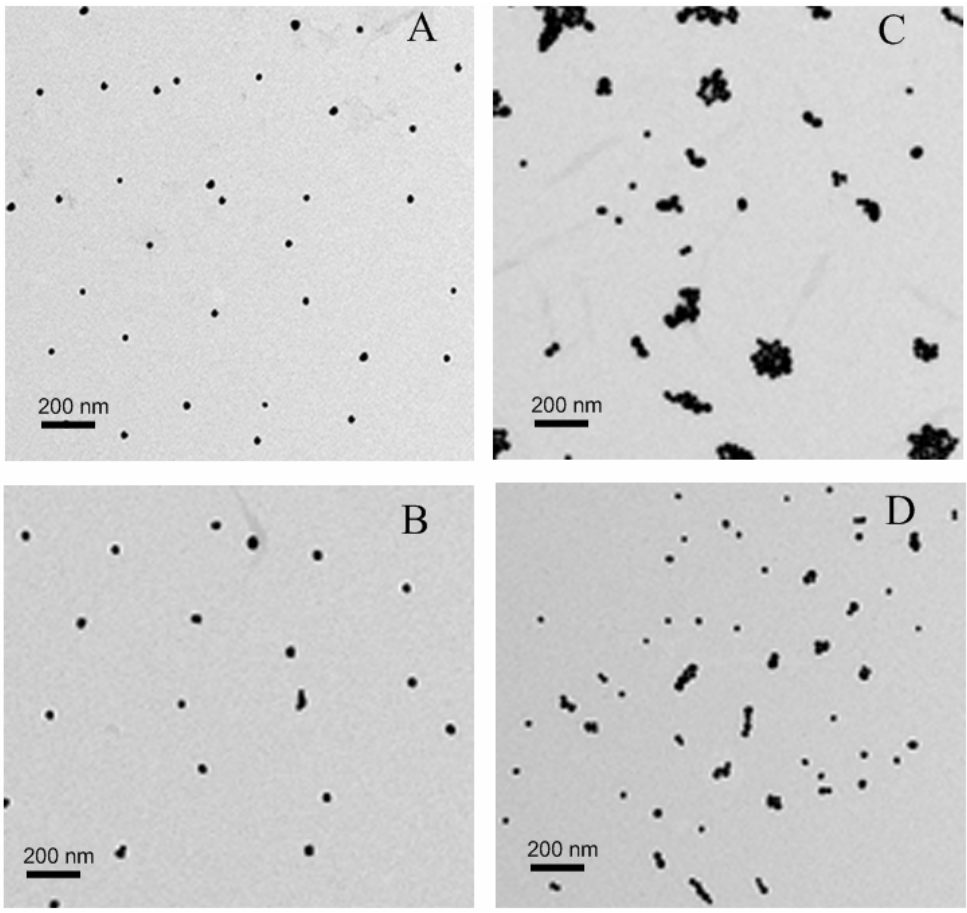

Fig. S1. The TEM images of AuNPs probes in the presence of different molecules.

A: 3.7 nM AuNPs

B: 3.7 nM AuNPs-Oligo1 + 3.7 nM AuNPs-Oligo 2

C: B+ 800 nM substrate + 2.0 μM enzyme

D: C + 1.0 nM Pb^{2+} .

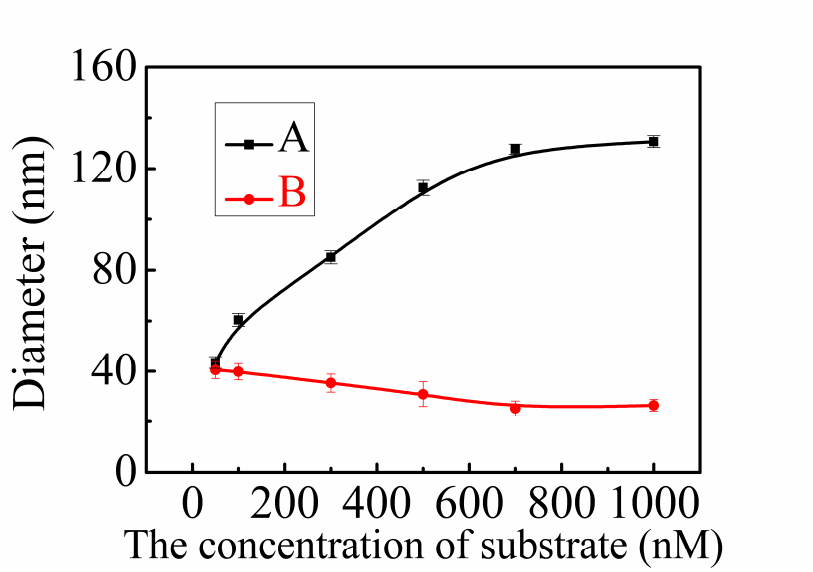

Fig. S2. Effect of the substrate concentration on average diameter of the mixture with and without Pb^{2+}. A: 3.7 nM AuNPs-Oligo 1, 3.7 nM AuNPs-Oligo 2, 2.0 μM enzyme; B: A + 1.0 nM Pb^{2+}. Experiments were conducted in 400 mM NaCl and 25 mM tris-acetate buffer(pH 8.0). The error bars represent the standard deviation for a series of three measurements.

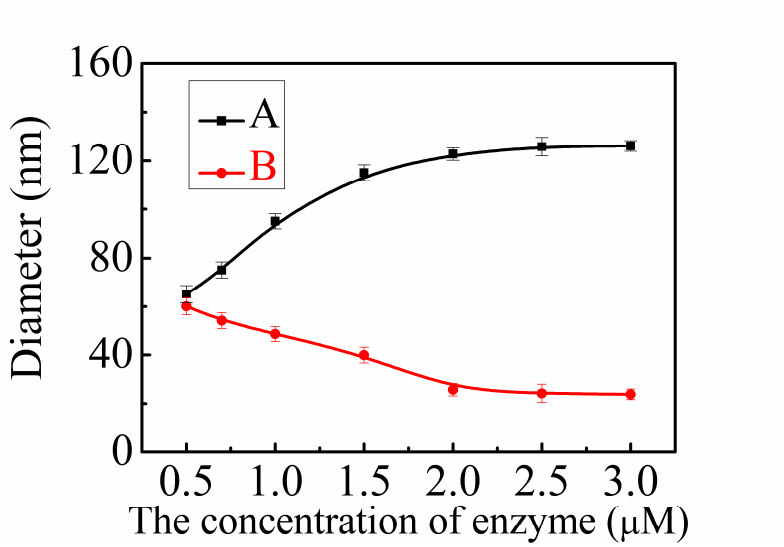

Fig. S3. Effect of the enzyme concentration on the diameter of the mixture with and without Pb^{2+}. A: 3.7 nM AuNPs-Oligo 1, 3.7 nM AuNPs-Oligo 2, 800 nM substrate; B. A + 1.0 nM Pb^{2+}. Experiments were conducted in 400 mM NaCl and 25 mM tris-acetate buffer(pH 8.0). The error bars represent the standard deviation for a series of three measurements.

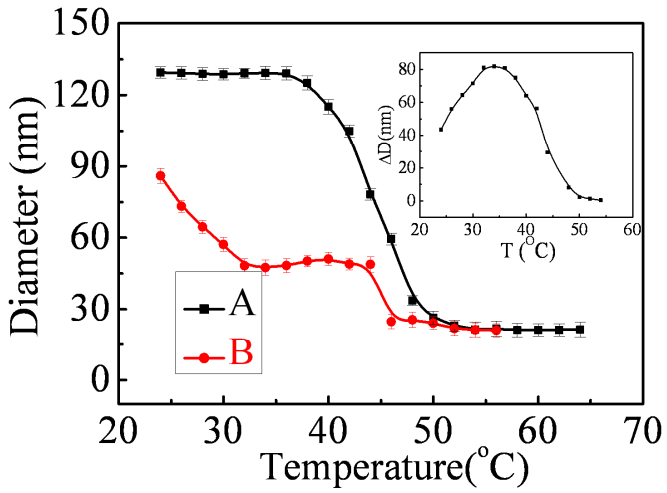

Fig. S4. Effect of the temperature on the average diameter of the mixture. A: 3.7 nM AuNPs-Oligo 1, 3.7 nM AuNPs-Oligo 2, 800 nM substrate , 2.0 μM enzyme, 25 mM tris-acetate buffer (pH 8.0, 400 mM NaCl); B: A + 1.0 nM Pb^{2+}. (inset of the figure illustrated the diameter change of nanoparticles before and after the addition of Pb^{2+}). Here $\Delta D = -(D_{Pb2+} - D_{no\ Pb2+})$, $D_{no\ Pb2+}$ denoted the average diameter of the mixture of AuNPs-Oligo probes, enzyme and substrate free Pb^{2+}, D_{Pb2+} represented the average diameter of the mixture after the addition of Pb^{2+}. The error bars represented the standard deviation for a series of three measurements.

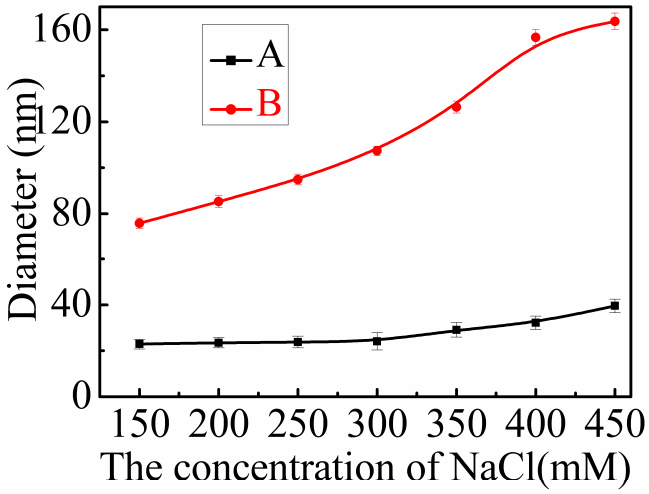

Fig. S5. A: Effect of the NaCl concentration on the diameter of AuNPs with and without DNAzyme. A: 3.7 nM AuNPs-Oligo 1 + 3.7 nM AuNPs-Oligo 2; B: A + 800 nM substrate + 2.0

μM enzyme. Experiments were conducted in 25 mM of tris-acetate buffer(pH 8.0). The error bars represent the standard deviation for a series of three measurements.

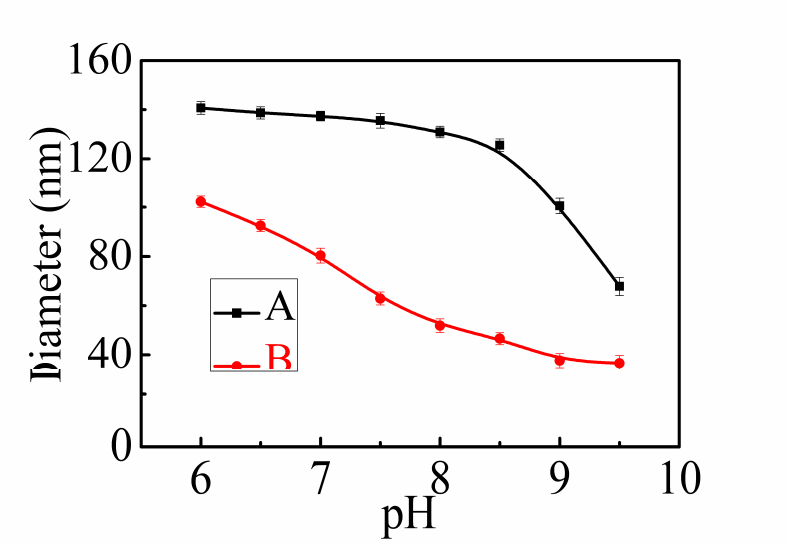

Fig. S6. Effect of the pH value on the average diameter of the mixture. A: 3.7 nM AuNPs-Oligo 1 + 3.7 nM AuNPs-Oligo 2 + 800 nM substrate + 2.0 μM enzyme + 400 mM NaCl; B: A+1.0 nM Pb^{2+}. Experiments were conducted in 25 mM tris-acetate buffer with different pH value. The error bars represent the standard deviation for a series of three measurements.

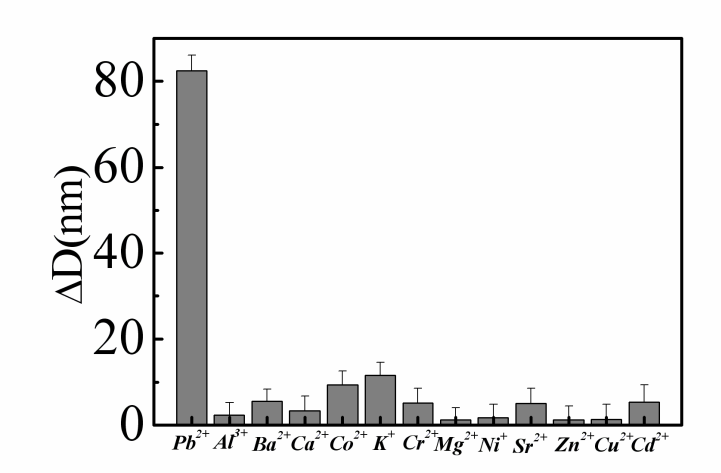

Fig. S7. The diameter change of nanoparticles before and after the addition of 1.0 nM Pb^{2+} or other divalent metal ions (Al^{3+}, Ba^{2+}, Ca^{2+}, Co^{2+}, K^{+}, Cr^{2+}, Mg^{2+}, Ni^{+}, Sr^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}). The diameter at the blank value was detected after the hybridization of 1:1 ratio of mixture of 3.7 nM

AuNPs-Oligo 1, 3.7 nM AuNPs-Oligo 2, 2.0 μM enzyme, 800 nM substrate and 400 mM NaCl in 25 mM of tris-acetate (pH 8.0). The error bars represented the standard deviation for a series of three measurements.

References

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