Supporting Information

Submitted to Journal of the American Chemical Society

A Colorimetric Lead Biosensor Using DNAzyme-Directly Assembly of Gold Nanoparticles

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1. Experiment details:

Lead sensor preparation. All DNA samples were purchased from Integrated DNA Technology Inc. The preparation of 13 nm diameter gold nanoparticles and the subsequent attaching of the thio-modified 12mer DNA to the nanoparticles were carried out following the literature procedures.[1] The size of the nanoparticles was verified by TEM (JEOL 2010) to be nearly mono-distributed 13 nm spheres. In a typical preparation, 500 pmol Sub_{Au} and 1000 pmol 17E were added to 3 mL DNA_{Au} (whose extinction at 522 nm was 2.2) in a 25 mM Tris-acetate buffer (pH 7.2) containing 300 mM NaCl. After the sample was heated to 70°C and allowed to cool slowly to room temperature, dark purple precipitates were formed at the bottom of the container. This color change is attributable to the aggregation of 13 nm gold nanoparticles by the DNA linkers (Sub_{Au} and 17E). The DNAzyme-gold nanoparticle precipitates were collected by centrifugation. After removal of the supernatant, the precipitates were re-suspended in 3 mL of the same buffer, and the resulting suspension can be used as a sensor for lead detection.

Lead detection. Lead detection was carried out by adding 1-2 μL of divalent metal ion stock solutions to 50 μL of the lead sensor suspension. The tube was incubated in a water bath of 50 ^{o}C for 2 min. (The melting temperature of the aggregation was determined to be 46 ^{o}C, and the 2 min. incubation resulted in a red color because of the melting of the aggregates.) and allowed to cool slowly to room temperature over 2 hours in the water bath. A red solution was obtained in the presence of Pb(II). The color change was subsequently monitored by UV-vis spectroscopy or observed directly by naked eyes when the sensor solution was spotted on TLC plates.

Sample preparation for detecting Pb(II) in leaded paint. A series of leaded paints with different concentrations of lead were made by addition of basic lead carbonate $PbCO_3 \bullet Pb(OH)_2$, (the white pigment commonly used in leaded paint) to a modern commercial white paint (Glidden, MD6000, which uses TiO_2 as the white pigment). After the paint was air dried overnight, a chip of 0.1 g was cut and soaked in 1 mL 10% HOA_C for 2 hours to release the lead from the paint. Two groups of leaded paint in different concentration ranges were examined. The soaking solution of the lower concentration group (0 to 0.5% Pb, Figure 4c) was diluted 360-fold after addition of the DNAzyme-nanoparticle sensor, while the higher concentration group (0 to 10% Pb, Figure 4d, lower) was diluted by a factor of 15,000.

2. The melting curve of the DNAzyme linked gold nanoparticle aggregates.

The extinction at 260 nm for the sensor was monitored with increase of temperature to acquire the melting curve of the aggregate (Figure S1). The sharp melting transition gave

strong evidence that the color change of the system is induced by the DNAzyme directed assembly of gold nanoparticles.[2]

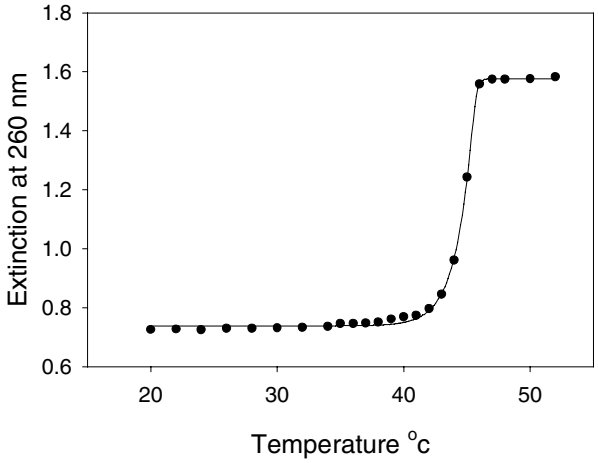

Figure S1. The melting curve of the "8-17" DNAzyme assembled gold nanoparticle aggregates. Extinction spectra were acquired in a Hewlett-Packard 8453 spectrophotometer equipped with a water bath. The DNAzyme linked nanoparticle aggregates were diluted dissolved in 25 mM Tris-acetate buffer, pH 7.2, 300 mM NaCl. The melting temperature was determined to be 46 ^{o}C in this condition.

3. DNAzyme activity assay in the presence of gold nanoparticles. To investigate whether the DNAzyme maintains the same activity in the presence of gold nanoparticles, a biochemical assay was performed using a procedure described previously.[3] As shown in Figure S2, the "8-17" DNAzyme cleaves its substrate with the same efficiency, regardless of the presence or absence of gold nanoparticles, and the reaction is essentially complete within 10 min.. The Pb(II)-dependent activities are also similar to those of the nanoparticle-free DNAzyme system reported previously (data not shown).[4]

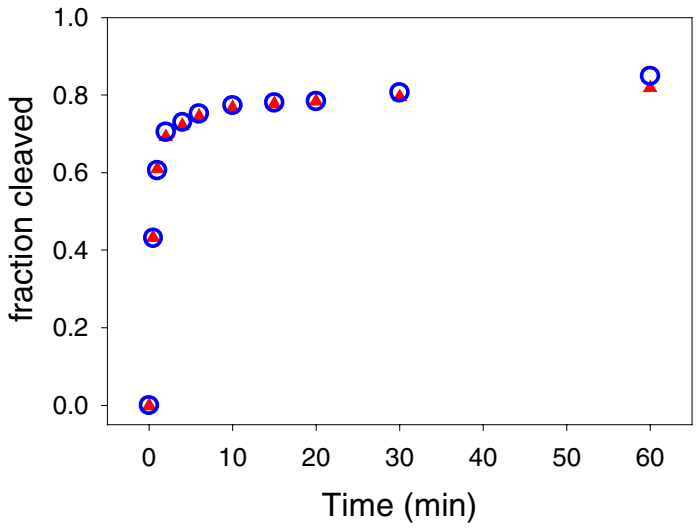

Figure S2. The "8-17" DNAzyme activity assay in the presence (red triangles) and absence (blue open circles) of 12-mer DNA-functionalized gold nanoparticles (DNA_{Au}). The reaction was carried out in 25 mM Tris-acetate buffer pH 7.2; 300 mM NaCl. 5 μM Pb(II) was added to initiate the cleavage reaction. The ^{32}P-labeled substrate (17DS) concentration was 1 nM. The 17E concentration was 5 μM. The concentration of the DNA-functionalized gold nanoparticles (DNA_{Au}) was estimated to be 8 nM. The cleaved and uncleaved substrates were separated by 20% polyacrylamide gel electrophoresis. The percentage of cleavage was quantified using a Fuji FLA-3000 PhosphorImager.

4. Sensing process analysis. The following experiments were carried out to provide further insight into the colorimetric sensing process. To investigate the time it takes for the sensing process, the DNAzyme-nanoparticle sensor assembly was placed into a water bath of 50 ^{o}C. The color transformed from blue to red within 2 minutes, indicating that the assembly dissociates into individual gold nanoparticles quickly at temperature higher than the melting temperature (T_m = 46 ^{o}C in this case). Allowing the system to cool to room temperature in the absence of Pb(II) resulted in slow conversion of red to blue color. The color change was the most apparent in the first two hours, after which the change was minimal. This is consistent with previous observations[5] that, although it takes a long time for the nanoparticle assembly to reach equilibrium and the fine-structure of the

aggregate will change to form a thermodynamically more stable state, the extinction property changes much less after initial hours of aggregation. Therefore we chose 2 hours as the detection time in a compromise between the speed and sensitivity of detection. The results were reproducible as long as the same detection time was chosen.

To investigate whether the DNAzyme cleavage occurs at room temperature when the substrate and enzyme strands are embedded in the nanoparticle aggregates, the DNAzyme-nanoparticle sensor assembly was incubated in a solution containing 5 μM Pb(II) at room temperature for 2 hours and Pb(II) was removed by addition of excess EDTA. The resulting solution was then heated to 50 ^{o}C for 2 minutes and allowed to cool to room temperature in 2 hours. The color changed from red to blue, which was almost identical to those without Pb(II) added. This result suggested that, at room temperature, the DNA substrate strands embedded in the gold nanoparticle aggregation were protected from cleavage and no cleavage reaction occurred while the substrate DNA was in the aggregated state.

We also found that, although different concentrations of Pb(II) resulted in different final colors as shown in Figures 2 and 3 in the paper, the total time required to reach the final color had little dependence on the Pb(II) concentrations. This is because once a substrate strand goes to a nanoparticle aggregate, it is protected from lead-induced cleavage. When the temperature drops to room temperature, a majority of the substrate strands are either cleaved, or in the aggregates and thus protected. Further room temperature incubation will only induce the internal structure of the aggregates to change to a thermodynamically

more stable state as mentioned above, which will not affect the extinction property of the aggregate significantly.

References:

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