Supplementary Material (ESI) for Chemical Communications
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Supporting Information

Colorimetric Cu^{2+} detection with a ligation DNAzyme and nanoparticles

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EXPERIMENTAL SECTION

Oligonucleotides and reagents: All DNA samples were purchased from Integrated DNA Technologies Inc. The substrate and enzyme strands of the DNAzyme were purified by PAGE or HPLC by the company. Thiol-modified DNA were purified by standard desalting. Tri(2-carboxyethyl)phosphine hydrochloride (TCEP), $HAuCl_4$ (99.999%), 1-[3-(diethylamino)propyl]-3-ethylcarbodiimide hydrochloride 98+ % (EDC) was purchased from Aldrich.

Nanoparticle preparation: Gold nanoparticles (50 nm diameter) were prepared with the citrate reduction method. All glassware were soaked in aqua regia and rinsed with Millipore water before use.[1] To prepare 50 nm diameter AuNPs, 200 mL of 0.3 mM $HAuCl_4$ solution was heated to reflux under stir. Then 1.8 mL of 38.8 mM sodium citrate was added quickly. After color changing to red, the solution was heated for another 30 minutes and then was allowed to cool naturally to room temperature and filtered through a filter (Pyrex). Nanoparticle size may vary slightly for nanoparticles prepared in different batches. Therefore, the size of synthesized particles was confirmed by TEM to be 50 ± 5 nm.

Nanoparticle functionalization: 3′- and 5′-thiol-modified DNA were activated by incubating with TCEP. Typically, 20 μL of 1 mM DNA was incubated with 2 μL of 20 mM TCEP at room temperature for 1 hour. The mixture was then directly added into 6 mL of the above prepared AuNPs. After incubation for 16 hours at room temperature, 0.6 mL of buffer containing 1 M of NaCl and 100 mM of Tris acetate (pH 8.2) was added by drop to the nanoparticle solution under stir. After incubation for another day, the nanoparticles were centrifuged at 9000 rpm for 10 minutes. The supernatant was removed and nanoparticles were re-dispersed in buffer containing 100 mM NaCl, 25 mM Tris acetate (pH 8.2). This centrifugation process was then repeated for 3 times to remove all free DNA in solution.

Imidazole modification of DNA: S1 was 3′-end phosphorylated by the company (Fig. 1). To attach an imidazole group to the 3′-phosphate group, 20 μL of 100 μM S1 was incubated with 2.5 μL of 1 M

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imidazole (pH 6.0, pH was adjusted with concentrated HCl), and 2.5 μL of 1.5 M EDC•HCl at room temperature for 1 hour. Then the mixture was purified with a PD-10 (Amersham Biosciences) desalting column, and the fraction of 0.5 to 1.5 mL was collected. The DNA concentration of the eluted fraction was determined by monitoring the absorbance at 260 nm. Usually, the functionalized DNA was used right away or stored at -20 ºC, since the imidazole-functionalized DNA was not very stable at room temperature.

Colorimetric Cu^{2+} detection: S2, imidazole-activated S1, and E47 (200 nM of each) was dissolved in 300 mM KCl, 20 mM $MgCl_2$, 30 mM HEPES buffer, pH 7.0 at room temperature. The ligation reaction was initiated by adding 1 μL of 50× concentrated Cu^{2+} or other metal ions into 49 μL of the DNA solution. After 30 minutes, 5 μL of the solution was transferred to another tube containing 45 μL of 0.09 nM of DNA-functionalized gold nanoparticles (0.045 nM of each of $S1_{Au}$ and $S2_{Au}$, extinction at 532 nm ~1.25), 300 mM NaCl, 30 mM tris acetate buffer (pH 8.2) and 1 μM of AntiE47 DNA. AntiE47 (5´-ACCATGCGTCACATGGTCTAGCGAAAGAA-3´) forms 29 base pairs with E47 and can help releasing the ligated substrate from E47. Shortly after mixing, the tube containing nanoparticles was placed to a beaker containing 10 mL of boiling water and was allowed to cool to room temperature in 20 minutes. At the end of the 20 minutes, 40 μL of buffer (25 mM Tris acetate, pH 8.2, no NaCl) was added to quench the aggregation. The UV-vis extinction spectra of the samples were then collected or the nanoparticles were spotted onto an alumina TLC plate for visualization.

Gel-based assays: 3´-fluorescein-labeled S2, imidazole-activated S1 and corresponding enzyme strands were mixed with final concentration of 1 μM each. Ligation reactions were initiated by adding metal cofactors. At designated time points, 10 μL aliquots were transferred into 10 μL of stop buffer (200 mM EDTA and 8 M urea) to quench the ligation reaction. The ligated and unligated substrates were separated by polyacrylamide gel electrophoresis. The gel was documented by a fluorescence image scanner (model FLA-3000G, Fuji).

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1 J. Liu and Y. Lu, *Nature Protocols*, 2006, **1**, 246-252.