Supporting Information

Phosphorylation Triggered Poly-nanoparticle Assembly for Naked-eye Distinguishable T4 Polynucleotide Kinase Detection

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Materials and Methods

Materials and reagents

T4 Polynucleotide Kinase and T4 DNA Ligase were purchased from Thermo Fisher Scientific. All DNA strands were purchased commercially from Invitrogen Life Technologies. They were either PAGE or HPLC purified by the supplier. Adenosine 5'-triphosphate disodium salt hydrate (ATP) and Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) were purchased from Sigma Aldrich.

Other chemicals and reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. All chemicals and reagents were used without further purification. The water used in all experiments was ultra-pure MilliQ water (resistance>18 MΩ cm^{-1}).

Preparation of gold nanoparticles

Approximately 13nm diameter gold particles were prepared by citrate reduction of HAuCl4.[1,2] The nanoparticles were then stabilized by complexation with BSPP in aqueous solutions. Following overnight incubation, NaCl was added to the stirring mixture until a color change from red to cloudy purple was observed, then the solution was centrifuged to collect the precipitated gold. Removed the supernatant, and resuspended the pellet in phosphine buffer (1 mg of BSPP in1 mL of MilliQ water). Nanoparticles were quantified by measuring the absorbance at λ=520 nm.

Synthesis of gold-DNA conjugates

Gold-DNA samples were prepared following a literature procedure, described briefly here.[3,4] Excess alkanethiol oligonucleotide (S3, S4) were added to gold nanoparticle solution and brought to 10 mM in phosphate (NaH_2PO_4/Na_2HPO_4). In the subsequent salt aging process, colloids were gradually salted from 0.05 M to 0.3 M in NaCl. To remove excess thiol-DNA, the solution was centrifuged for 25 min at 15 000 r.p.m. Following removal of the supernatant, the red precipitate was then washed with a solution 0.3 M NaCl in 10 mM phosphate buffer (pH 7.4) three times and redispersed. The quality of gold-DNA conjugates was also characterized using UV-Vis spectrum.

DNA phosphorylation and ligation buffer

These commercial buffer contains dithiothreitol (DTT), which was found to precipitate gold nanoparticles,[5] so a modified buffer was prepared without DTT. 10×T4 Polynucleotide Kinase buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, pH 7.6) and 10×T4 DNA Ligase buffer (400 mM Tris-HCl, 100 mM $MgCl_2$, pH 7.8) were made with MilliQ water. The solutions were stored frozen. Immediately prior to use in experiments, the buffers were thawed, and 10 mM ATP (in deionized H_2O) was added to the buffers.

Phosphorylation and ligation of DNA

S1 was phosphorylated by T4 Polynucleotide Kinase in 1×T4 Polynucleotide Kinase buffer and incubated at 37°C for 30 min, then heated at 75°C for 10 min to inactivate. The mixture was hybridized with equimolar quantities of S2 and excess hS1S2, and inserted in a heat block at 90°C, and left to cool slowly to room temperature for at least 2h or as long as overnight. To the hybridized mixture, 1×T4 ligase buffer and T4 DNA ligase were added. The mixture was vortexed briefly and incubated at 22°C for 1h or at 4°C for as long as overnight. Then heated at 65°C for 10 min or at 70°C for 5 min to inactivate T4 DNA ligase.

Hybridization to form poly-nanoparticle assembly

Following ligation, the product can be diluted 10 times with PBS (10 mM Na_2HPO_4, 10 mM NaH_2PO_4, 0.3M NaCl). The poly-nanoparticle assembly was formed with equimolar quantities of gold nanoparticle-DNA conjugates to mix with the "phosphorylation and ligation" product in room temperature.

UV-vis absorption Experiments

After briefly centrifugation, collected the supernatant as analyte. Samples were detected by monitoring UV-vis absorption (UV-Vis spectrophotometer, UV 2550, Shimadzu Co., Japan; microplate reader, SpectraMax M2, Molecular Devices Co. Ltd, USA) at the gold plasmon maximum of 524 nm.

Reference

1 G. Frens, *Nat. Phys. Sci.*, 1973, **241**, 20.

2 J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**,1959.

3 C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature* 1996, **382**, 607.

4 J. S. Li, X. Chu, Y. L. Liu, J. H. Jiang, Z. M. He, Z. W. Zhang, G. L. Shen and R. Q. Yu, *Nucleic. Acids Res.*, 2005, **33**, e168

5 S. A. Claridge, A. J. Mastroianni, Y. B. Au, H. Y. W. Liang, C. M. Micheel, J. M. J. Fre´chet and A. P. Alivisatos, *J. Am. Chem. Soc.*, 2008, **130**, 9598.