Supplementary Information for

Highly Stable, Amphiphilic DNA-Encoded Nanoparticle Conjugates for DNA Encoding/Decoding Applications

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1. Chemicals

Gold nanoparticles (20 nm) were purchased from Tedpella, Inc., (Redding, CA, USA.) PVP and PEG were purchased from Aldrich (MA, USA). Silver enhancing and initiator solutions were purchased from BBI international, USA. Thiolated oligonucleotides were purchased from IDT Inc., Coralville, IA, USA. Thiolatded oligonucleotides were reduced by using dithiolthretol (DTT, 0.1 M) in phosphate buffer according to the published procedure, and then, purified through a desalting NAP-5 column (Sephadex G-25 medium, DNA grade). All the other reagents were purchased from Sigma-Aldrich Co. (USA), and used as received without further purification.

Table 1. DNA sequences used in the experiments

Name	Sequence
DNA1	5'-ATCCTTATCAATATTTAACAATAA -$(PEG)_{18}$-A_{10}-SH-3'
DNA2	5'-CGAAGTCAGCTTTGATTAACAAAT-$(PEG)_{18}$-A_{10}-SH-3'
DNA3	5'-ACGTAGGAATGGCCAGTTAGACTC -$(PEG)_{18}$-A_{10}-SH-3'
Immobilization Capture DNA	5`-$NH_2$-$A_{10}$-$(PEG)_{18}$-GATGTAAGCCGGTAGGAATAGTTATAA-3`
Non-complementary	5'-CACGCGTTTCTCAAA-PEG_{18}-A_{10}-$(CH_2)_3$-SH-3'

2. Instruments

UV-Visible Spectroscopy

All UV-visible spectra were acquired from the UV-Visible spectrometer (Agilent 8453).

Zeta Potential Analyzer

Zeta Potential analysis was performed with the electrophoretic light scattering spectrophotometer (ELS-8000).

Microarrayer

Genetix aldehyde slides were used and the capture DNA was arrayed on the slides using the OmniGrid Accent^{TM} microarrayer (Genomic Solutions Inc, MI, USA).

3. Preparation of DNA-AuNP Conjugates.

In a typical synthesis, the freshly-reduced thiolated oligonucleotides were used for the AuNP probe preparation. The concentration of the reduced DNA was determined by the UV-Visible spectroscopy. 4 nmol of reduced DNA solution was added to 1 mL of the AuNP solution. The mixed solution was wrapped in foil and placed on an orbital shaker at room temperature overnight. The solution was then adjusted to obtain the final phosphate concentration of 10 mM and SDS concentration of ~0.1 % (wt/vol). After 30 min at an orbital shaker, the colloids were brought to a final concentration of 0.3 M NaCl by the four-time addition of 2 M NaCl solution. After the last salt addition and brief vortexing, the colloids were allowed to stand at room temperature overnight. The solution was centrifuged (12,000 rpm, 15 min), the supernatant was removed, and the precipitate was redispersed in 0.3 M PBS solution (this procedure was repeated twice). Finally, the solution was characterized by the UV-Visible spectrophotometer.

4. The UV-Visible Spectra at 60˚C and Low pH (2.5).

The storage stability of DNA1-AuNP conjugates, DNA2-AuNP conjugates, and DNA3-AuNP conjugates were tested at room temperature and 60 ˚C**.** After 5 weeks, all samples were aggregated and it showed dampening of UV-spectra. There is no DNA-sequence dependence on thermal stability. All sample solutions were centrifuged and then the supernatant was removed. The concentrated samples were diluted with pH-adjusted solution (pH 2.5). Within 5 min after solution addition, solution color was changed from wine red to blue, finally, to colorless solution along with black precipitates on the bottom, and the UV-Visible spectrum also showed dampening.

5. Dark-Field-Based Decoding of DNA-AuNP Conjugates.

100 μL of immobilization capture strands (50 μM, 5`-$NH_2$-$A_{10}$-$PEG_{18}$-GATGTAAGCCGGTAGGAATAGTTATAA-3`) were arrayed on the Genetix' aldehyde slides. The dot size was 150 × 150 μm^2, and 100 dots were spotted at once. The arrayed slides were leaved in a constant humidity environment for 4 hrs, and then the slides

were washed twice with 0.2 % SDS, Nanopure water (25 ˚C) and Nanopure water (95-100 ˚C) and cooled to room temperature. The slides were washed with $NaBH_4$ solution (1 g dissolved in 300 ml of PBS, and 100 ml of ethanol) for 5 min, washed with 0.2% SDS, Nanopure water, and then dried at room temperature. 50 μL of sample solution [control probes (non-complementary, 5'-CACGCGTTTCTCAAA-PEG_{18}-A_{10}-$(CH_2)_3$-SH-3'), PVP-coated DNA-AuNP conjugates and PVP-desorbed DNA-AuNP conjugates, respectively] was allowed to be hybridized with immobilized capture DNA sequences in a sealed hybridization chamber for 1 hr. Next, the slides were washed with copious amount of washing solution [0.5M $NaNO_3$ (1 min), 2X standard sodium citrate (SSC) solution (5 min), 0.2X SSC (5 min)], and then dried. To clearly visualize hybridized particles, additional silver-staining procedures were applied. The silver enhancing (750 μL) and initiator (750 μL) solutions were mixed, and the mixed solution was spotted onto the slide (dried for 5 min). The chips were imaged with the dark-field microscopy (Zeiss, 5x objective).

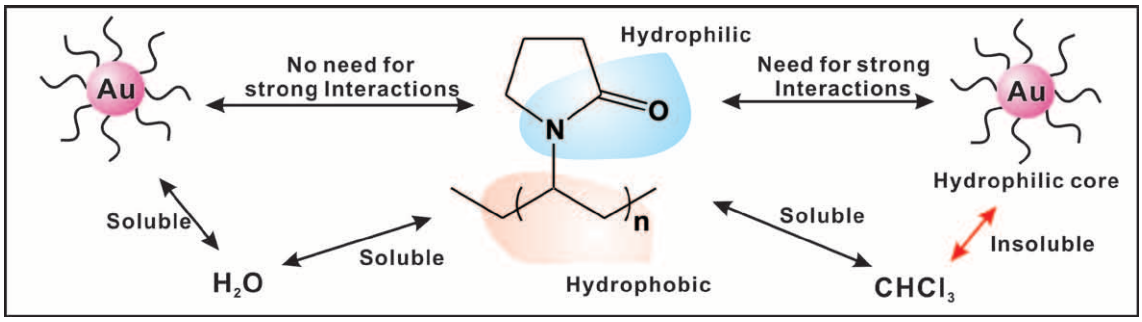

Figure S1. A dissolving mechanism of DNA-AuNPs in both water and $CHCl_3$. It is expected that the strong interactions between hydrophilic part of PVP and hydrophilic core (DNA-AuNPs) induce the formation of PVP layer around the particles in the hydrophobic organic solvent.

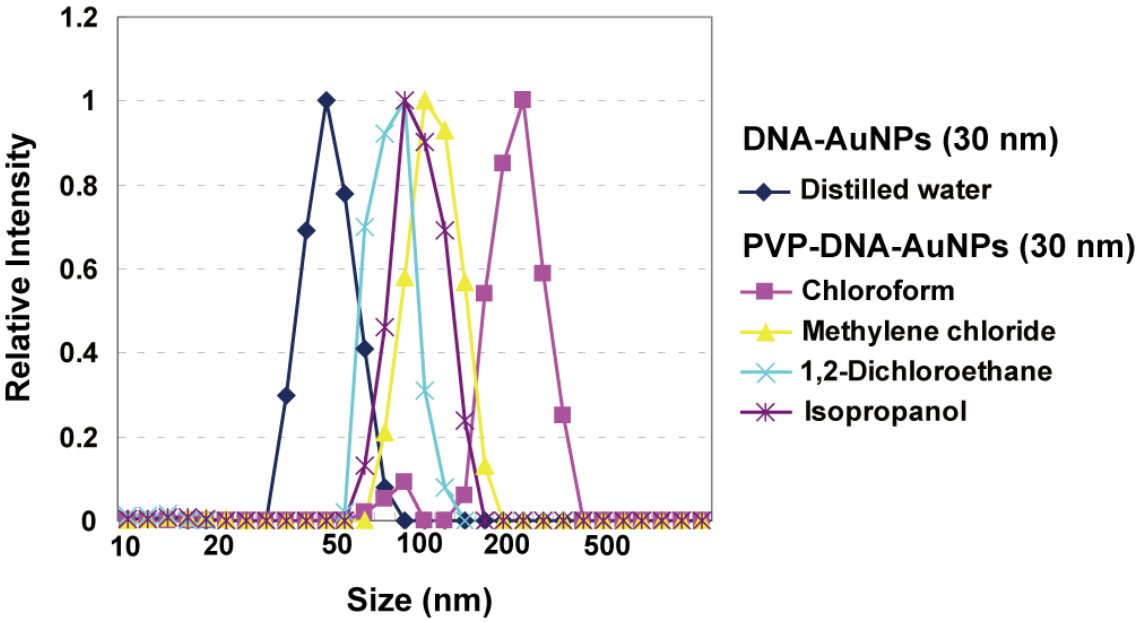

Figure S2. Dynamic light scattering-based particle size analyses for DNA-AuNPs in water and PVP-DNA-AuNPs in other solvents (chloroform, methylene chloride, 1,2-dichloroethane or isopropanol).

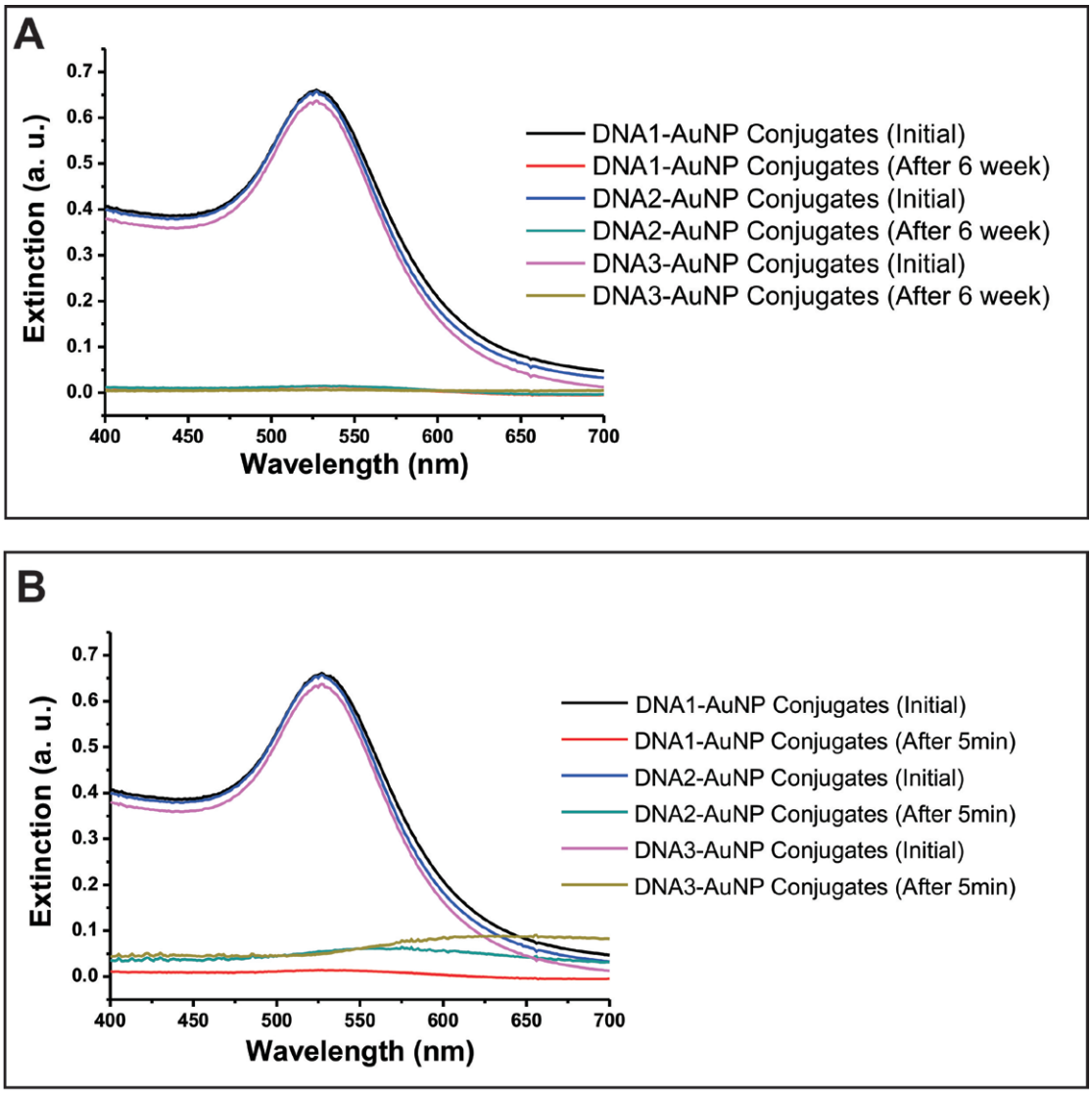

Figure S3 The UV-Vis spectrum changes after 6 weeks at 60˚C for DNA1-AuNP conjugates, DNA2-AuNP conjugates, and DNA3-AuNP conjugates, respectively, in 0.3 M PBS (A), and the UV-Vis spectrum changes in pH 2.5 solution after 5 min pH adjustment (B).

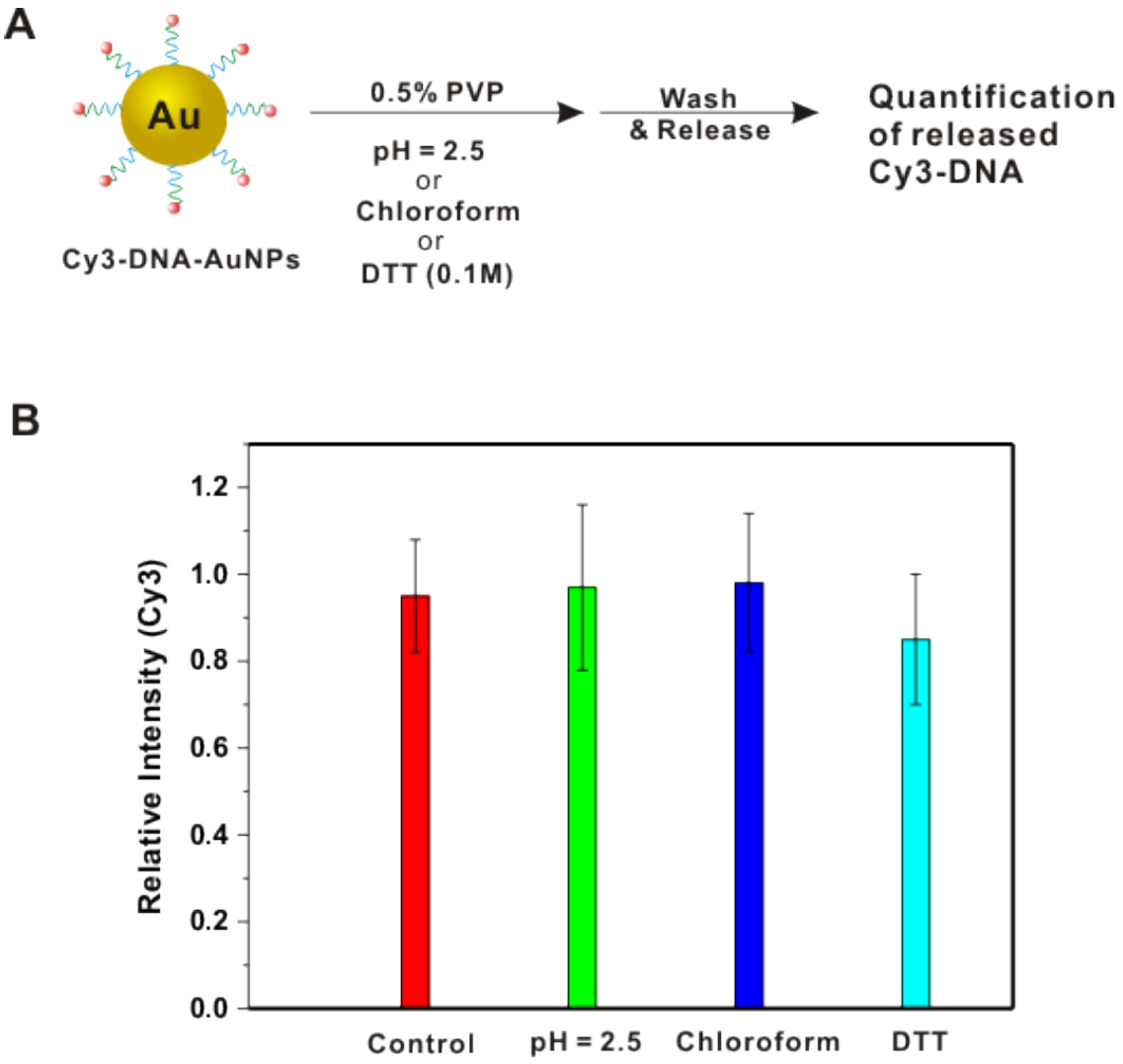

Figure S4. Quantification of the amount of released Cy3-DNA from AuNPs under various conditions (low pH, in chloroform or treated with 0.1 M DTT). All experiments were carried out in the presence of 0.5 % PVP.