Electronic Supplementary Information

Silver-Nanoparticle-DNA Bionanoconjugates Bearing a Discrete Number of DNA Ligands

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Materials and Chemicals

DNA sequences: DNA oligonucleotides were custom-synthesized by Sangon Bioengineering Technology and Services Co., Ltd. (Shanghai, China) and purified by PAGE (unmodified DNA) or HPLC (thiolated DNA). All DNA oligos were subject to molecular weight verifications by MALDI-TOF mass spectroscopy. The purities of the DNA oligos were checked by PAGE before use. **The double stranded DNA ligands were based on various combinations of the following DNA sequences (5'-3') including L/Lc (Figure 4a, 4c, 4d) and S/Sc-1, 2, 3, 4 (Figure 4b):**

L-SH strand (24 bases), 5' thiolated:

HS-$(CH_2)_6$-TTTTTTTTTTGCGCGAACCGTATA

Lc strand (37 bases):

AGCGTAGGATAGATATACGGTTCGCGCAAAAAAAAAA

Lc-SH strand (37 bases), 3' thiolated:

AGCGTAGGATAGATATACGGTTCGCGCAAAAAAAAAA-$(CH_2)_3$-SH

S-SH strand (14 bases), 3' thiolated:

TAGCATGACCCTCA-$(CH_2)_3$-SH

Sc-1 strand (14 bases):

TGAGGGTCATGCTA

Sc-2 strand (25 bases):

TGAGGGTCATGCTACCGCGGACGCT

Sc-3 strand (35 bases):

TGAGGGTCATGCTACCGCGGACGCTCAATGACTGC

Sc-4 strand (45 bases):

TGAGGGTCATGCTACCGCGGACGCTCAATGACTGCCGTAATGAAT

Chemicals: Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), sodium acetate anhydrous (NaAc), magnesiusm acetate ($MgAc_2$), and *n*-butanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Fish sperm DNA and ethylenediamine tetraacetic acid disodium salt ($EDTA{\cdot}Na_2$) were from Sangon Bioengineering Technology and Services Co., Ltd. (Shanghai, China). Agarose, sodium chloride, boric acid, and tris(hydroxymethyl) aminomethane (Tris) were obtained from Bio Basic Inc. (BBI, Canada). Sodium citrate tribasic dihydrate was a product from Sigma.

Experimental Section

FSDNA assisted synthesis of 2 nm silver nanoparticles: Briefly, 60 mL of 0.25 mg/mL FSDNA solution was purged with nitrogen for 30 min in an ice/water bath, followed by the addition of 600 μL of 100 mM silver nitrate and 600 μL of 100 mM sodium citrate. The resulting solution was quickly mixed by magnetic stirring. An aqueous solution of $NaBH_4$ (1 mL, 300 mM) was then introduced to the above solution under vigorous stirring. For the following 3 minutes, 150 μL of 300 mM $NaBH_4$ was added in three times (50 μL each time) at 1 min interval. The solution changed from colorless to yellow. The stirring continued for 1 h in the ice-water bath. After that, the solution was kept at 4 °C for an aging of up to 23 hours before further use.

Butanol concentration of silver nanoparticles: The as-synthesized silver nanoparticles were combined with n-butanol at a suitable volume ratio and mixed well. The resulting solution was centrifuged at 1080 g for 1 min. The upper butanol layer was removed, and the concentrated silver nanoparticles as a lower phase were collected. When butanol was added in a large excess, water molecules would be completely extracted into the butanol phase so that precipitates of silver nanoparticles would form. This provided an extremely convenient way to obtain highly concentrated silver nanoparticles.

DNA conjugation of silver nanoparticles: A double stranded DNA ligand was formed between two complementary DNA strands (one or two of them were thiolated, see Materials and Chemicals section for sequence information) in a 0.5×TBE buffer (Tris, 44.5 mM; EDTA, 1 mM; boric acid, 44.5 mM; pH 8.0) supplemented with 0.1 M NaCl. The thiolated DNA ligand was then allowed to interact with silver nanoparticles in the same buffer at 20 °C for 6 hours.

Gel electrophoretic isolation of discrete silver nanoparticle/DNA conjugates: 3% agarose gel was employed to purify silver nanoparticle/DNA conjugates. The gel was run in 0.5×TBE for a given time at 16 V/cm. Corresponding gel bands representing a special valence of DNA functionalization were cut out of the gel, and the conjugated products were eluted into 0.5×TBE plus 25 mM NaAc in a dialysis tubing (MWCO 3500 Da) under an applied electric field. The recovered nanoparticle/DNA conjugates were stored at room temperature or 4 °C in 0.5×TBE supplemented with 0.1 M NaAc.

Spectroscopic and microscopic characterizations: UV-vis absorbance was measured on a Hitachi U-2910 spectrophotometer. Transmission electron microscope (TEM) imaging was conducted on a JEM-2100F field emission transmission electron microscope operated at an acceleration voltage of 200 kV. Atomic force microscope (AFM) imaging in tapping mode was performed on a Nanofirst-3000 AFM system (Shanghai Haizisi Optical-Electronics Co., Ltd., China) using a MikroMasch NSC11 tip. Samples were deposited on a carbon coated copper grid and a freshly cleaved mica substrate for TEM and AFM analysis, respectively.

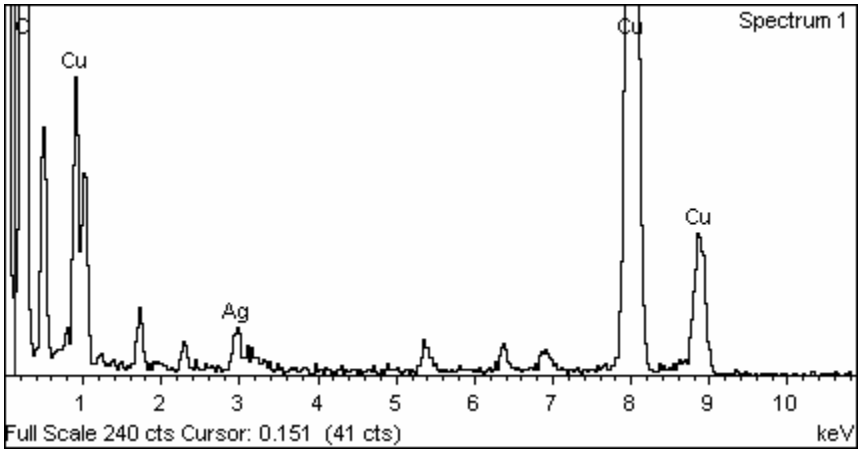

Figure S1. Energy dispersive X-ray spectroscopic (EDX) analysis of the silver nanoparticles supported on carbon coated copper grid, which evidenced the existence of silver element.

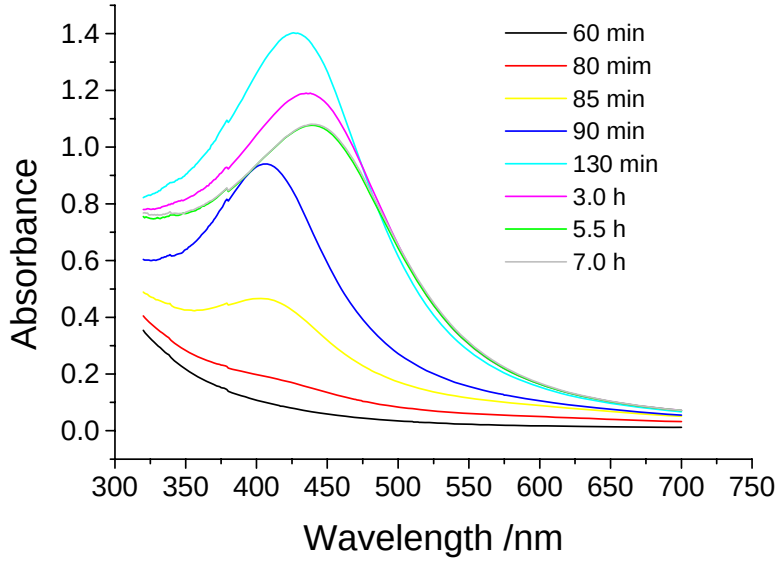

Figure S2. UV-vis monitoring of the FSDNA-templated AgNPs during an aging process at 37 °C. Note that the absorbance curve became constant after a 5.5 h reaction and aging (1 h $NaBH_4$-based reduction in ice-water bath and 4.5 h aging at 37 °C).

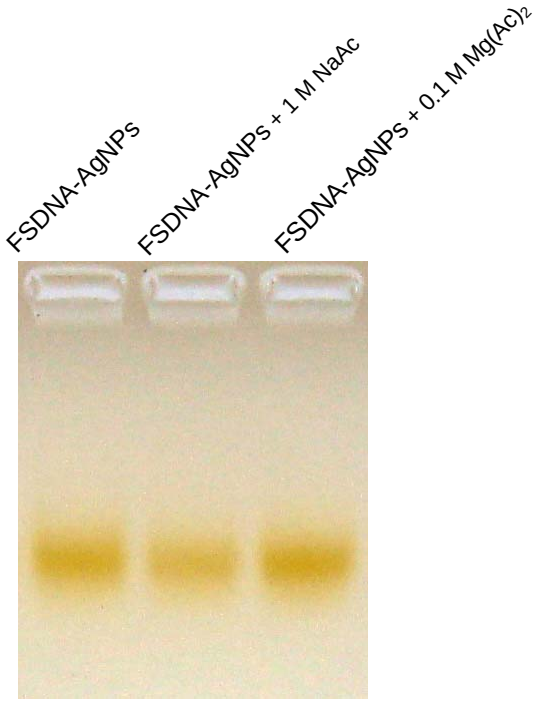

Figure S3. Gel electrophoresis test showed that the FSDNA stabilized silver nanoparticles were stable and well-dispersed even after a long time incubation with 1 M NaAc or 0.1 M $Mg(Ac)_2$ for 6 hours. The AgNPs were pre-concentrated 6 times by butanol extraction for a better visibility in the gel before interaction with the salts. The 3% agarose gel was run at 16 V/cm in 0.5xTBE for 30 min. Before electrophoresis, the salt-treated samples were dialyzed against ddH_2O for another 6 hours to remove salts.

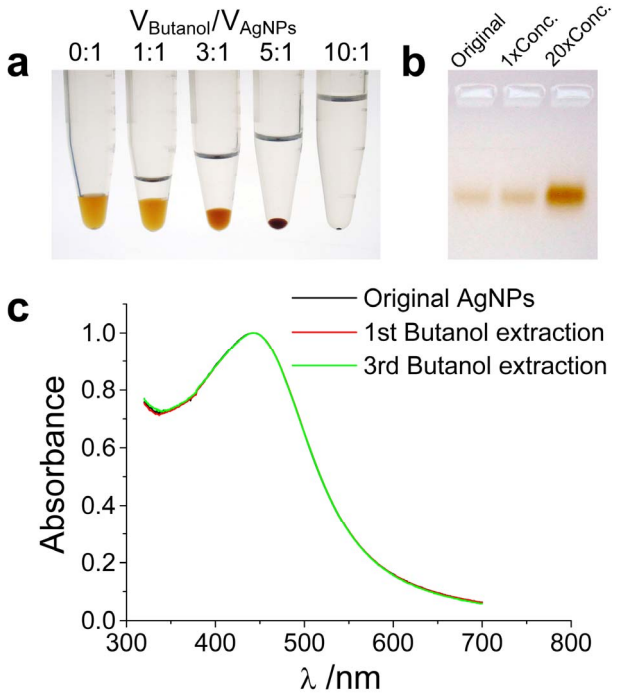

Figure S4. Butanol-extraction-based concentration of FSDNA stabilized silver nanoparticles. (a) Phase separation of the sample after addition of different amounts of n-butanol. (b) Gel electrophoresis of untreated (original) and butanol concentrated silver nanoparticles. The nanoparticles were precipitated after adding an excess amount of butanol, and the recovered silver nanoparticles were adjusted to 1x and 20x concentration relative to the original sample. (c) UV-visible spectra of the same silver nanoparticles before and after several repeats of butanol extractions.

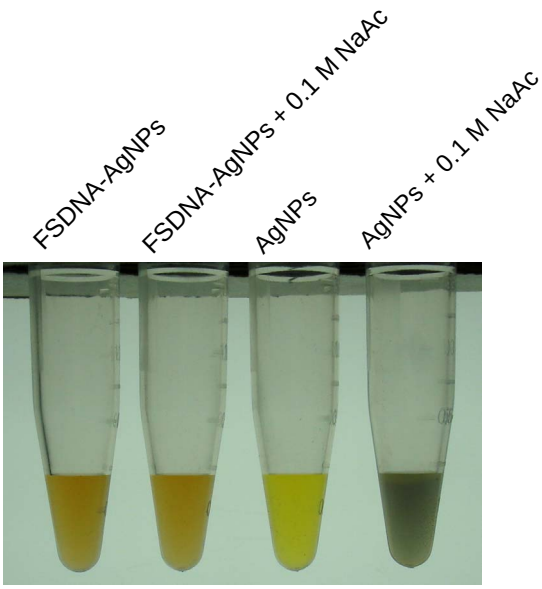

Figure S5. Salt-resistance test of the AgNPs synthesized in the presence or absence of FSDNA.

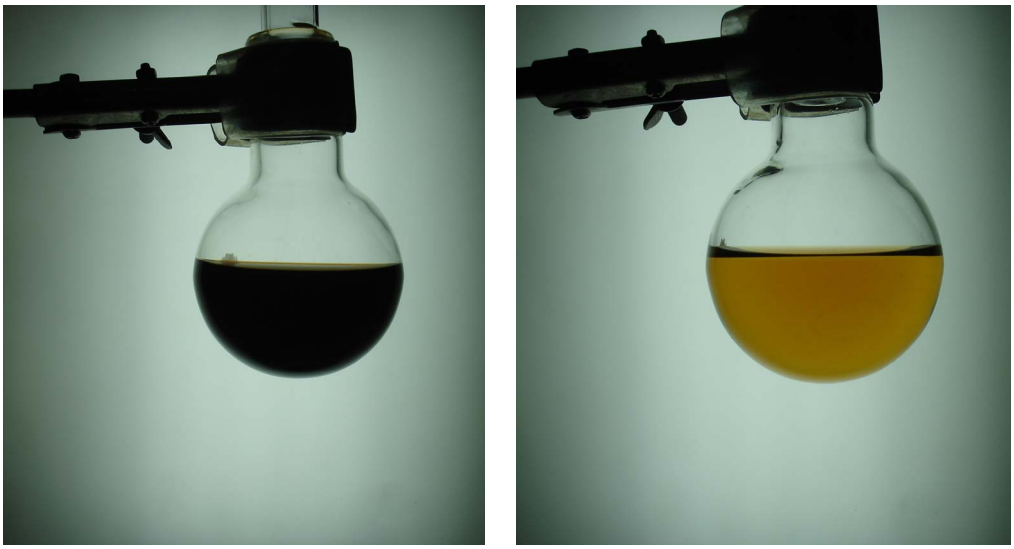

Figure S6. As-synthesized FSDNA stabilized silver nanoparticles (left picture) and its ten-time dilution (right picture).