Supporting Information

Coordination-Mediated Programmable Assembly of Unmodified Oligonucleotides on Plasmonic Silver Nanoparticles

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Supporting figures and tables

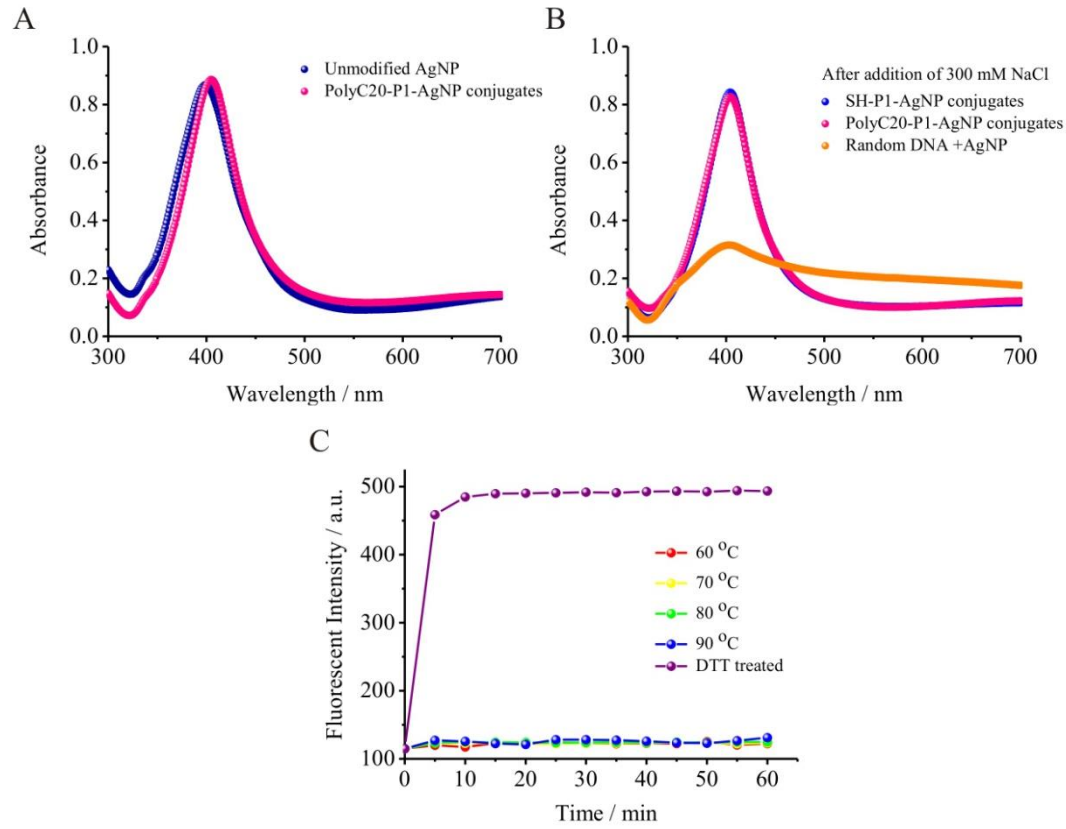

Figure S1. (A) Optical properties of bare AgNPs, polyC20-P1-AgNP nanoconjugates.(B) After the addition of 300 mM NaCl, the optical properties of polyC20-P1-AgNP nanoconjugateswas similar to SH-P1-AgNP conjugates. The optical properties of the mixture of AgNPs and random DNA indicated the aggregation of AgNPs. (C) Thermal stability of polyC20-P1-AgNPs.

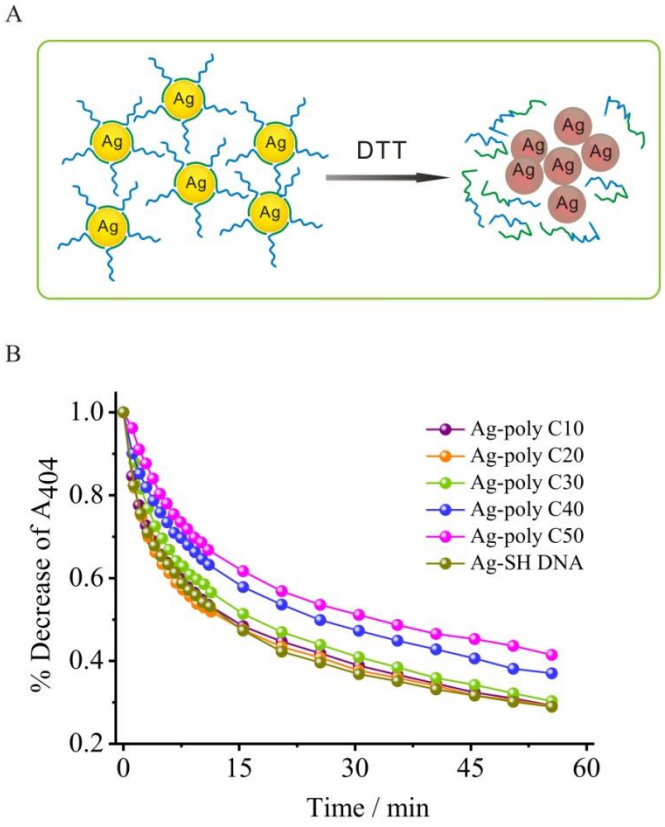

Figure S2. (A) Schematic representation of DTT-induced detachment of diblock DNA from the surface of AgNPs and aggregation of AgNPs. (B)Decrease of absorbance at 404 nm of the nanoconjugates system after treating with 1 mM of DTT.

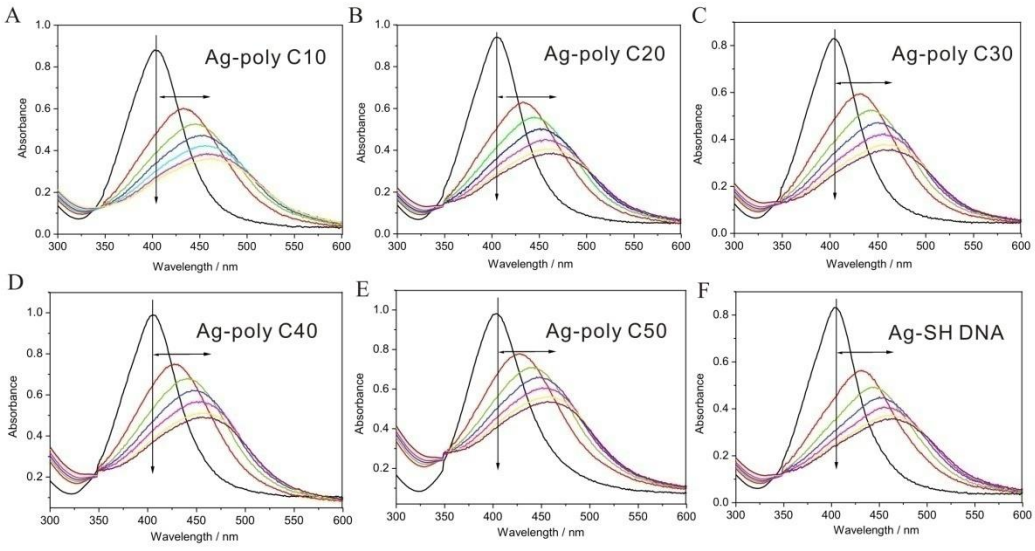

Figure S3. UV-vis spectra showing aggregation upon treatment with 1mM of DTT for (A) polyC10-AgNP, (B) polyC20-AgNP, (C) polyC30-AgNP, (D) polyC40-AgNP, (E) polyC50-AgNP and (F) SH-DNA-AgNP at 10 min intervals.

Table S1. Half-lives and shift of λ_{max} in 60 min for DNA-AgNP conjugates

System	$t_{1/2}$ / min	Shift of λ_{max} in 60 min / nm
C10	13.23	58
C20	13.37	58
C30	15.91	56
C40	25.08	54
C50	32.59	52
SH	13.71	58

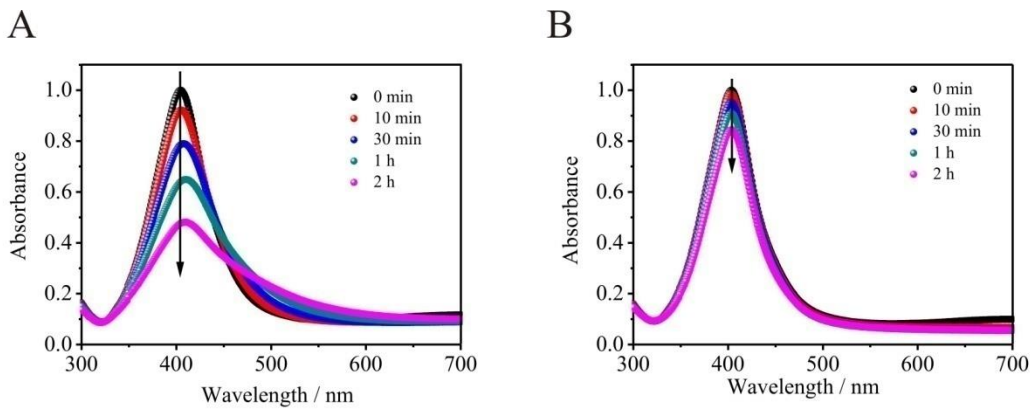

Figure S4. (A) UV-vis spectra of mixture after adding polyC20-P1-AgNPs to the complementary poly C20-T1-AgNPs at 0 min, 10 min, 30 min, 1 h and 2 h. (B) UV-vis spectra of mixture after adding SH-P1-AgNPs to the complementary SH-T1-AgNPs at 0 min, 10 min, 30 min, 1 h and 2 h.

Experimental section

Instruments and Reagents

DNA sequences were synthesized by Invitrogen Inc., (Shanghai, China) and the sequences were shown in Table S2.

Table S2. DNA sequences in this work.

DNA name	Sequence (5'-3')
Poly T10-F	TTTTTTTTTT-FAM
Poly C10-F	CCCCCCCCCC-FAM
Poly C20-F	CCCCCCCCCCCCCCCCCCCC-FAM
Poly C30-F	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-FAM
Poly C40-F	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-FAM
Poly C50-F	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-FAM
PolyA20-P1	AAAAAAAAAAAAAAAAAAAATTTTTATTATCACT
Poly A20-T1	AAAAAAAAAAAAAAAAAAAATTTTTAGTGATAAT
Poly C20-P1	CCCCCCCCCCCCCCCCCCCCTTTTTATTATCACT
Poly C20-T1	CCCCCCCCCCCCCCCCCCCCTTTTTAGTGATAAT
Poly C20-P2	CCCCCCCCCCCCCCCCCCCCTTTTTAGAAAGTCAGGCAGT
SH-T2	SH-TTTTTACTGCCTGACTTTCT
SH-P1	SH-TTTTTATTATCACT
SH-T1	SH-TTTTTAGTGATAAT
SH-P1-F	SH- TTTTTATTATCACT-FAM
Random-DNA	TCTTTCAGTCCGTCAACTGCCTGACTTTCT
Poly A20-T1	AAAAAAAAAAAAAAAAAAAATTTTTAGTGATAAT
Poly C10	CCCCCCCCCC
Poly C20	CCCCCCCCCCCCCCCCCCCC
Poly C30	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
Poly C40	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
Poly C50	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC

Silver nanoparticles (AgNPs, 20 nm and 40 nm in diameter) and gold nanoparticles (AuNPs, 20 nm and 50 nm in diameter) were purchased from Ted Pella (Redding, CA). Trisodium citrate and sodium chloride (NaCl), sodium phosphate dibasic (Na2HPO4) and sodium phosphate monobasic (NaH2PO4) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Milli-Q water was used for all the experiments.

All fluorescence measurements were performed on spectrophotometer F-4500 (Hitachi, Japan). All absorption detections were performed on Cary 100 UV-Vis spectrophotometer (Varian, USA). The DFM measurements were carried out with an inverted microscopy (Olympus IX 71, Japan) equipped with a dark-field condenser (0.8 < NA < 0.95) and a 60× or 4× objective lens (NA = 0.8), a true-color digital camera (Olympus DP70, Japan) and a monochromator (Acton SP2300i, PI, USA) equipped with a spectrograph CCD (CASCADE 512 B, Roper Scientific, PI, USA) and a grating (grating density: 300 lines/min; blazed wavelength: 500 nm).

DNA adsorption kinetics onto AgNPs

To compare the adsorption of DNA bases on AgNPs at neutral pH, poly C10-F and poly T10-F were detected in our work. The DNA sequences were modified with a FAM (carboxyfluorescein) dye (excitation, 494 nm; emission, 520 nm) at the 3' end. The procedure for fluorescence quenching measurements was as follows. First, 180 μl of AgNPs (0.11 nM) in a cell with 1 cm path-length was placed in the sample holder and 20 μl of 50 nM DNA in 0.2 M PBS (0.2 M NaCl, 0.2 M PB, pH 7.4) was then added into the solution of AgNPs. Fluorescence was detected using the "scan time" mode of fluorescence spectrophotometer immediately. The low salt concentration (final concentration of 10 mM) was set to minimize the aggregation of silver colloids. Polarization detection was performed using the "polarization scan" mode of the spectrophotometer under the same buffer condition.

Preparation of DNA-AgNPs

DNA assembly on AgNPs was achieved using the pH-assisted method developed by Liu's group[1] with modification. First, 1.5 μl of polyC-DNA (100 μM) or SH-DNA was added to 600 μl of AgNP solution (~ 0.11 nM), giving a total strand-to-particle ratio of ~ 2300:1. The solution was incubated for about 1 hour to allow the adsorption of DNA on AgNPs. After that, 6 μl of citrate buffer (500 mM, pH 3) was added into the AgNP solution to reach a final concentration of 5 mM. After incubation (30 min), the same amount of citrate buffer was added into the solution again to achieve a final concentration of 10 mM citrate. After incubation (30 min), the pH of the AgNP

solution was adjusted to neutral by adding 90 μl of PB buffer (200 mM, pH 7.4). After incubating for 5-10 minutes, excess DNA were removed by centrifuging at 15000 rpm for 20 min and the remaining was then washed 3 times with PB buffer (10 mM, pH 7.4). The prepared DNA-AgNP conjugates were dispersed in 10 mM PB for further use. Usually the DNA-AgNPs conjugates can be stable for at least one month according to our experimental experiences.

The competition between polyA/polyC adsorption onto AgNPs

To compare the preferential interaction with silver surface between polyA and polyC, we mixed 20 pmol of polyA20-M/polyC20-P1 with 20 fmol of AuNPs (10 nm) and 20 fmol AgNPs (20 nm) and allowed the adequate adsorption. After incubation for 1h, a small volume of citrate buffer (500 mM, pH 3) was added to reach a final concentration of 10 mM. The assembly and wash step was the same as previous. Besides, SH-T1 and SH-T2 modified 5 nm AuNPs were also prepared by using this low-pH approach, respectively. Hybridization was carried out between the former prepared conjugates and the prepared DNA-AuNPs (5 nm) in 0.1 M PBS (pH 7.4) overnight. After that, the mixture was dropped onto the copper grid and dried for TEM imaging.

Quantification of DNA loading on AgNPs

The surface density of ssDNA on AgNPs was measured according to the fluorescence-based method provided by Graham et al[2]. The concentration of FAM-labeled oligonucleotide conjugates were determined by measuring the absorbance of the solution by UV-visible spectrophotometer and calculated via Beer's law ($A = \varepsilon bc$). Then, this FAM labeled DNA-AgNPs were treated with DTT (final concentration of 10 mM) in PBS (0.3 M, pH 7.4) and incubated at 37 ℃ for 18 h to allow the completely aggregate of AgNPs. After centrifuging at 15000 rpm for 20 min, the supernatant was taken out to determine the fluorescence. The molar concentration of FAM labeled DNA was calculated by comparing to a standard linear calibration curve prepared with known concentrations of oligonucleotide with identical buffer pH,

ionic strength and DTT concentration. The average number of oligonuleotides per particle was obtained by dividing the measured oligonuleotides molar concentration by the AgNP concentration. All experiments were repeated at least three times.

The salt stability of DNA-AgNP conjugates

The salt stability of DNA-AgNP conjugates were characterized by UV-Vis spectrophotometer. The concentration of AgNPs was about 0.2 nM.

The thermal stability of DNA-AgNP conjugates

The thermal stability of FAM labeled DNA-AgNP conjugates were characterized by fluorescence spectrophotometer with a temperature controller. The concentration of AgNP was about 0.08 nM. For the DTT treated assay, the concentration of DTT was 1 mM.

DNA-directed aggregation of metal nanoparticles

First, two batches of AgNPs were functionalized with complementary sequences (poly C20-P1 and poly C20-T1) using the low-pH assisted method as described above, respectively. 30 μl of each DNA-AgNP conjugates solution ([AgNP] = 0.3 nM) was transferred into a microcentrifuge tube (1.5 ml) and 0.1 M of PBS was added to adjust the final volume to 100 μl. A reference was prepared containing the double amount of poly C20-P1 AgNPs and the same amount of salt. After incubation at room temperature overnight, the mixture in the uncomplimentary DNA remained the yellow color while the complementary system changed color to clear. After heating at 95 °C for 5 min, the solution of complementary system returns to bright yellow, indicating the dehybridization of probes on AgNPs in the system. For DNA-directed aggregation of AgNPs and AuNPs, AgNPs was functionalized with C20-P1 and AuNPs was functionalized with A20-T1, respectively.

Melting curve

The aggregation of AgNPs were centrifuged and dispersed into PBS (0.1 M). Then the

change of absorbance at 405 nm was monitored by UV-Vis spectrophotometer equipped with a temperature controller. The temperature was raised at a rate of 3 °C / min with a holding time of 1 min at each temperature.

Kinetics of DNA hybridization on AgNPs

For kinetics of polyC and thiolated oligonucleotide hybridization on AgNPs, two batches of AgNPs were functionalized with complementary sequences (poly C20-P1 and poly C20-T1 or SH-P1 and SH-T1) using the low-pH assisted method as described above, respectively. Equal molar at 0.05 nM of each DNA-AgNP conjugates were mixed in 0.1 M PBS (pH 7.4) and UV-Vis spectroscopy was recorded every 10 min for 2 h.

Dark-field microscopy (DFM) imaging and scattering spectroscopy measurements

For Dark-field microscopy imaging, AgNPs (40 nm) were respectively functionalized with complementary polyC20-P1 and polyC20-T1 following the above low-pH assisted method. AuNPs (50 nm) were modified with complementary polyA20-P1 and polyA20-T1 sequences using the similar method, respectively. AgNPs aggregation and AuNPs aggregation were prepared by mixing the same amount of complementary DNA-metal conjugates in 0.1 M PBS, respectively. AuNP-AgNP hybrid nanostructures was prepared by mixing polyC20-P1-AgNPs and polyA20-T1-AuNP in 0.1 M PBS.

10 μL of aqueous solution of DNA-metal nanoparticles (~ 0.025 nM) was drop-casted on the silanized glass slides and incubated for 5 min. After that, the nanoparticles-modified slides were rinsed with water and dried with N_2. Then 50 μL of water was dropped on the resulting nanoparticles-modified slides for Dark-field imaging. The true-color scattering images of AuNPs and AgNPs were taken by a 60× objective lens (NA=0.8) with a white light illumination from a 100 W halogen lamp. The scattering spectra of nanoparticle were corrected by subtracting the background spectra taken from the regions without AuNP or AgNPs and dividing with the calibrated response curve of the entire optical system. The spectra were integrated in 10 seconds.

References

(1) Zhang, X.; Servos, M. R.; Liu, J. W. Instantaneous and Quantitative Functionalization of Gold Nanoparticles with Thiolated DNA Using a pH-Assisted and Surfactant-Free Route. *J. Am. Chem. Soc.* **2012**, *134*, 7266-7269.

(2) Dougan, J. A.; Karlsson, C.; Smith, W. E.; Graham, D. Enhanced Oligonucleotide-nanoparticle Conjugate Stability Using Thioctic Acid Modified Oligonucleotides. *Nucleic Acids Res.* **2007**, *35*, 3668-3675.