Supporting Information for

Fast pH-assisted functionalization of silver nanoparticles with monothiolated DNA

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1. Materials and methods

Chemicals. All the DNA samples were purchased from Integrated DNA Technologies (Coralville, IA). Thiolated DNAs were treated with TCEP (50×) at pH 5.0 for 1 h to cleave the disulfide bond. TCEP was then removed by passing the DNA through a Sep-Pak column. $AgNO_3$, $NaBH_4$, KCN was from Sigma-Aldrich. AgNPs and AuNPs (20 nm diameter) were purchased from Ted Pella (Redding, CA). AgNPs (10-20 nm) were also prepared in our own lab. AuNPs (13 nm) were synthesized based on the standard citrate reduction procedures and its concentration was estimated to be ~10 nM.[S1] Sodium citrate, sodium chloride and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonate (HEPES) were purchased from Mandel Scientific (Guelph, ON). Milli-Q water was used for all experiments.

Preparation of AgNPs. In addition to the AgNPs purchased from Ted Pella Inc, we also prepared our own AgNPs to minimize effects of added stabilizing agents in the commercial AgNPs. We followed the procedure by Ung *et al* with slight modifications.[S2] To 99 mL of ice-cooled water containing 0.3 mM trisodium citrate, a final of 1 mM $NaBH_4$ (freshly prepared) and then 1 mL of 10 mM $AgNO_3$ were added. The color changed to bright yellow immediately indicating formation of AgNPs. These particles were used right away. After ~30 min, the color of the colloid changed to dark yellow/black, which can be changed back to bright yellow by adding a small amount of $NaBH_4$.

Preparation of DNA-AgNPs. First, a small volume (i.e., 1-3 μL) of DNA stock solution (50 – 100 μM, treated by TCEP, in 5 mM HEPES buffer, pH 7.6) was added into 500 μL of AgNPs (size: 20 nm; concentration: 0.11 nM) and mixed by a brief vortex. After a few minutes, a small volume of 500 mM pH 3 citrate·HCl buffer (final 5 mM) was added into the AuNP solution (e.g. 1 μL citrate buffer per 100 μL AgNP solution). After a brief vortex and 5 min incubation, the same amount of the pH 3 buffer was added into the DNA-AgNP mixture again to achieve a final pH 3 buffer concentration of 10 mM. After 25-min incubation at room temperature for DNA loading, the pH of the AgNP solution was adjusted back to neutral by adding 500 mM HEPES buffer (pH 7.6). The volume of added HEPES was about 3 times of the added pH 3 buffer in last step (e.g final 30 mM HEPES buffer). Finally, the DNA-AgNP mixture was centrifuged at 15,000 rpm and the supernatant was removed. The pellet was washed 4-5 times with 5 mM HEPES buffer and centrifugation to completely remove the free DNA strands. The washed DNA-AgNP conjugate was re-dispersed in 5 mM HEPES buffer for further use.

DNA adsorption isotherm. In each of the 1.7 mL microcentrifuge tube that contained 300 μL of AgNPs (size: 20 nm; concentration: 0.11 nM), various amount of DNA3 was added to form a DNA

concentration series with DNA/AgNP ratios as 20, 100, 250, 500 and 1000 (n = 3). Then 3 μL the pH 3 citrate buffer (500 mM) was added into the mixture followed by a brief vortex. After 5-min incubation, another 3 μL pH 3 citrate buffer was added so that the final concentration of citrate buffer was 10 mM. This mixture was incubated at room temperature for an additional 25 min prior to 4 rounds of centrifugation (15,000 rpm, 12 min) and rinsed with 300 μL of 5 mM HEPES buffer (pH 7.6) to remove all free DNAs. Next, each tube of AgNP-DNA conjugates was re-dispersed in 100 μL of 5 mM HEPES buffer to have a final AgNP concentration of 0.33 nM, followed by incubation with 10 mM KCN for 30 min to dissolve AgNPs to release all adsorbed DNAs. The fluorescence quantification was performed with a microplate reader (Infinite F200 PRO, Tecan), where a calibration curve was built by plotting the fluorescence intensity of each standard over the concentration series to calculate the DNA concentrations in the KCN treated samples.

DNA adsorption kinetics as a function of pH. First, 1 μL FAM-labeled thiolated DNA (DNA3, 1 μM) was added into 50 μL AgNP solution (0.11 nM) with a brief vortex to form homogeneous DNA-AuNP mixture (ratio: 200:1). Next, 2 μL of the mixture was transferred into 98 μL of 50 mM HEPES buffer (pH 7.6) in a 96-well plate to measure the fluorescence. Right afterwards, 1 μL citrate buffer (pH 3) was added into the DNA-AuNP solution to facilitate DNA attachment; again, 2 μL mixture solution was transferred to a fresh plate-well (n = 2) for fluorescence measurement. The 2-μL sampling was repeated at each of the following time points (2, 4, 6, 8, 12, 15, 20 and 25 min). The fluorescence intensity over time was plotted to show the binding kinetics of SH-DNA to AgNPs. The reason for this method is to avoid FAM fluorescence quenching at low pH. We assumed that 1) the fluorescence of adsorbed DNA was effectively quenched and 2) adsorbed DNA did not desorb. This method measures more tightly adsorbed DNA since the DNA/AgNP mixture was significantly diluted in pH 7.6 buffer before fluorescence measurement. Loosely associated DNA might desorb during this step.

DNA adsorption kinetics at pH 7.6 (continuous monitoring). To study the DNA adsorption kinetics onto commercial AgNP at neutral pH, 4 nM FAM-SH-DNA (FAM = 6-carboxyfluorescein, DNA 3) was dissolved in 90 μL of HEPES buffer (5 mM, pH 7.6) containing various concentrations of NaCl (0, 30, 60, 90 and 120 mM). The fluorescence intensity at 520 nm was monitored for 2 min under the kinetic mode using a plate reader (Tecan Infinite F200Pro) prior to a quick addition and mixing with 10 μL of 20-nm AgNPs (final concentration = 0.01 nM) or 20-nm AuNPs (final AuNP concentration = 0.1 nM). The change of fluorescence intensity over time was recorded with the plate reader. This method measures both tightly adsorbed and loosely associated DNA.

The salt stability of AgNP-DNA conjugates. The absorbance with and without 300 mM NaCl were recorded to illustrate the salt stability of the DNA-AgNP conjugates using a UV-vis spectrophotometer (Agilent 8453A). The sample volume was 80 μL and the AgNP concentration was ~0.4 nM. The spectra showed in the paper were normalized to have the same extinction peak at 410 nm.

DNA-directed assembly of AgNPs. AgNPs were functionalized with two 21-mer DNAs (DNA1 and DNA2), respectively, using the low-pH method with pH 3 citrate buffer as described above. Afterwards, 100 μL of each DNA-AgNP conjugate solution (AgNP = 0.5 nM) was transferred into a 0.6 mL microcentrifuge tube, and a final of 225 mM sodium citrate and 10 nM linker DNA were added. For comparison, a reference was prepared containing the same amount of DNA-AgNPs and salt, but instead

of using linker DNA, a 24-mer control DNA non-complementary to either DNA1 or DNA2 was used in the reference tube. The mixture in the sample tube changed color to gray/clear after an overnight incubation at 4 °C, which indicated forming AgNP aggregates. In contrast, the reference tube maintained the characteristic yellow color of dispersed AgNPs even after 72 h in the fridge. In addition, the linker DNA (100 nM) directed assembly of AgNPs and AuNPs was demonstrated, where AgNPs and AuNPs were functionalized with DNA2 and DNA1, respectively. The aggregates were imaged using a digital camera.

Melting curve. To test the cooperative melting property of the DNA-directed assembly, the aggregates were centrifuged and gently dispersed into the same volumes of 10 mM HEPES buffer containing 30 mM sodium citrate. Then, 150 μL of each aggregate was transferred into a quartz micro-cuvette to measure the melting curves on a UV-Vis spectroscopy (Agilent 8453A), where the temperature was controlled by a circulating water bath. The actual temperature was recorded using a thermometer. The temperature was raised at a rate of ~1 °C/min with a holding time of 2 min at each temperature. The extinction value at 410 nm was plotted as a function of temperature.

2. DNA adsorption kinetics for other DNA sequences

To test generality of our low pH method for DNA attachment to AgNPs, we employed two more FAM and thiol dual labeled DNA (DNA4: 5′-SH-T(FAM)TCCCAGGTTCTCT, where FAM was labeled on the T base right next to the thiol label; DNA5: 5′-SH-CCCAGGTTCTCT-FAM). As shown in Figure S1, both DNA showed a quick fluorescence intensity decrease after mixing with AgNPs at pH 3. It needs to be pointed out that the adsorption kinetic measurement was not performed directly to avoid FAM fluorophore quenching at low pH. See our experimental methods for descriptions.

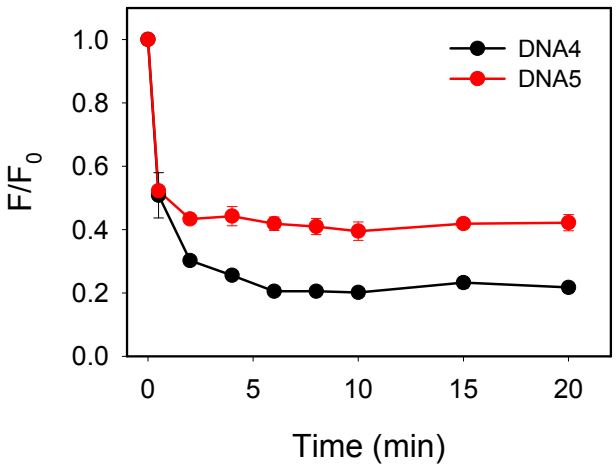

Figure S1. Kinetics of DNA4 and DNA5 adsorption onto commercial AgNPs. The DNA concentration was 20 nM and the AgNP concentration was 0.1 nM in the reaction mixture.

3. TEM of AgNPs

To characterize the size of AgNPs prepared in our lab, we dropped the AgNPs onto a TEM grid and dried the sample on a filter paper. The image was taken using a Philips CM10 TEM. As shown in Figure S2, the particles were about 10-20 nm in size.

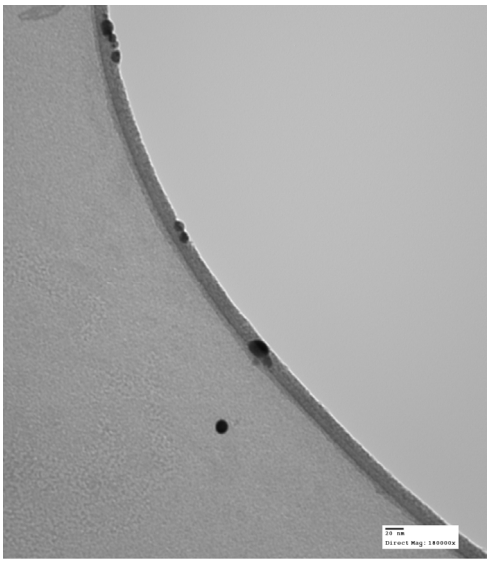

Figure S2. TEM micrograph of AgNPs prepared in our lab. The scale bar is 20 nm.

4. Dynamic light scattering of AgNPs prepared in our lab.

Right after synthesis, the particle size of AgNPs were measured using dynamic light scattering (Zetasizer Nano 90, Malvern) at 25 °C. No additional buffer or salt was added. As shown in Figure S3 (red curve), the average hydrodynamic size was ~20 nm, which agreed with the size measured using TEM (Figure S2). After ~30 min, the sample was measured again. At this time, the AgNPs were already oxidized. We only observed a slight increase of the size (green curve) and therefore we can conclude that no large aggregates were formed.

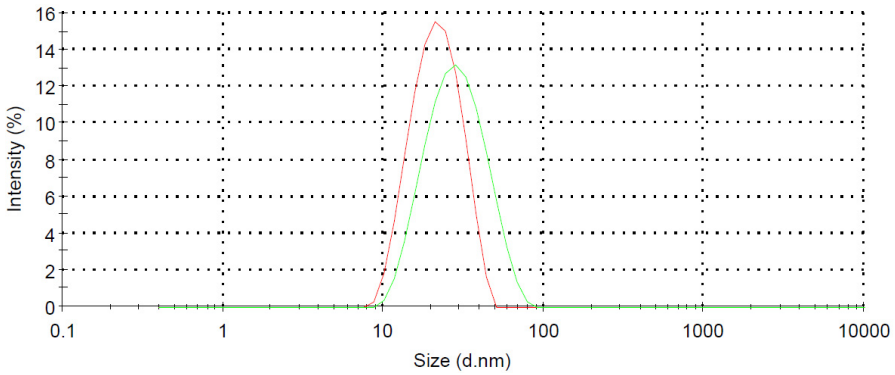

Figure S3. Dynamic light scattering data for measuring AgNPs prepared in our lab of freshly prepared sample (red) and ~30 min after preparation (green).

Additional references

S1 J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 1959.

S2 T. Ung, L. M. Liz-Marzan and P. Mulvaney, *Langmuir*, 1998, **14**, 3740.