Supporting Information

Instantaneous and Quantitative Functionalization of Gold Nanoparticles with Thiolated DNA Using a pH-Assisted and Surfactant-Free Route

Xu Zhang[†,‡], Mark R. Servos[‡] and Juewen Liu[†]*

[†]Department of Chemistry and Waterloo Institute for Nanotechnology, [‡]Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Email: liujw@uwaterloo.ca

1. Materials and Methods

Chemicals. All the DNA samples were purchased from Integrated DNA Technologies (Coralville, IA). DNA7-9 were purified by HPLC and the rest were purified by standard desalting. $HAuCl_4$, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), NaH_2PO_4, and KCN were from Sigma-Aldrich. AuNPs (13 nm) were synthesized based on the standard citrate reduction procedures and its concentration was estimated to be ~10 nM. AuNPs (50 and 100 nm diameter) were purchased from Ted Pella (Redding, CA). Hydrochloric acid was purchased from VWR (Mississauga, ON). Sodium citrate, sodium chloride and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonate (HEPES) were purchased from Mandel Scientific (Guelph, ON). MilliQ water was used for all experiments.

Citrate buffer preparation. Citrate has three pK_a values at 3.13, 4.76, and 6.40, respectively. Therefore, citrate is an ideal buffer from pH 3-7. Citrate·HCl buffers were prepared by dissolving trisodium citrate at a concentration close to 500 mM and concentrated HCl was used to adjust pH to designated values. The buffer volume was then adjusted to the final citrate concentration of 500 mM as the stock. For most experiments, a final of 10 mM buffer was used and the Na^+ concentration from buffer was 30 mM.

AuNP functionalization. Thiolated DNAs were treated with TCEP (50×) at pH 5.0 for 1 hr to cleave the disulfide bond. The activated DNA was desalted using a C18 Sep-Pak column and lyophilized. The dried DNA powder was dissolved in 5 mM HEPES buffer and DNA concentration (~1 mM) was quantified using UV-vis spectroscopy at 260 nm. These activated DNAs were mixed with AuNPs at designated ratios. For pH-dependent studies, a small volume of 500 mM citrate·HCl buffer was rapidly added to the DNA/AuNP mixture followed by pipette mixing. The final buffer concentration was 10 mM. For comparison, the typical salt aging method was performed by adding NaCl over 1-2 days.[S1,2]

Gel electrophoresis. The DNA-AuNP conjugates were prepared by incubating 10 nM AuNPs with 0.75 μM DNA8 with various pH citrate buffers (final concentration: 10 mM) for 10 min. The gel was prepared to contain 3% agarose and 10 mM HEPES, pH 7.6. Thirty microliters of DNA-AuNP conjugates containing 25% glycerol was added to each lane and the gel was run at 60 V. The running buffer also contained only 10 mM HEPES (pH 7.6). The AuNP bands were recorded with a digital camera (Canon Powershot SD1200 IS). The same gel was also imaged using a gel documentation system (Chemidoc-MP, Bio-Rad) under 310 nm UV excitation and the fluorescence emission from FAM was recorded using a green filter.

Kinetic experiments. First, DNA8 (37.5 pmol) was added into 50 μL AuNP solution (10 nM) with a brief vortex to form a homogeneous DNA-AuNP mixture (ratio: 75:1); then, 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 (Tecan Infinite F200Pro). 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 for fluorescence measurement. The 2-μL sampling was repeated at each of the following time points (2 min, 4 min, 6 min, and 10 min). The fluorescence intensity over time was plotted to show the binding kinetics of DNA8 to AuNPs in Figure 3. The DNA adsorption in Figure 4C was measured using the same method.

DNA loading capacity quantification.

1. To minimize AuNP adsorption onto the container surface in the presence of high concentrations of NaCl and low pH, glass vials (2 mL capacity) were treated with 12 M NaOH and rinsed thoroughly with Milli-Q water.
2. Two DNA-AuNP solutions were prepared containing 2 μM DNA8 and 10 nM AuNPs. DNA attachment was facilitated by adding citrate buffer (10 mM, pH 3 for one sample and pH 7 for another), where the initial Na^+ concentration was 30 mM. After 10 min, 3 aliquots (25 μL each) of the mixture from each glass vial (pH 3 and pH 7) were transferred for the measurement of DNA binding capacity. Afterwards, NaCl was added to each vial to increase NaCl concentration to 100 mM and the vials were gently shaken over 20 min before collection of another 3 aliquots (25 μL each) from each vial. Finally, the NaCl concentration was adjusted to 300 mM before performing the last sampling of 3 aliquots of DNA-AuNP mixture. AuNP sticking to the glass container wall still occurred at 300 mM NaCl and therefore, the 300 mM NaCl data shown in Figure 5B are slightly lower than the true values.
3. For each of the collected aliquot (25 μL) of DNA-AuNP mixture, the free DNA was removed by three rounds of centrifugation and rinsing with 200 μL of 50 mM HEPES buffer (pH 7.6). Finally, the DNA-AuNP conjugate was re-dispersed in 100 μL HEPES buffer with 1 mM KCN to dissolve AuNP completely to release the attached DNA8. The binding capacity was obtained by the fluorescence measurement of the KCN treated solution with a standard addition calibration.
4. For the loading of thiolated DNA strands with different sequences, the procedures were essentially the same as described above. The only difference is to mix DNA9 and DNA8 with

pre-determined ratio (in this work, the molar ratios were 1:1, 1:2, and 1:3, respectively) before applying to AuNP solution. The quantification of the two DNAs was performed after centrifugation, washing the AuNPs and dissolving them with KCN. The fluorescence of both FAM and TAMRA was measured and normalized to the intensities of the 1:1 sample (for FAM, the excitation/emission wavelength is 485 nm/535 nm, while for TAMRA, it is 535 nm/595 nm).

Dynamic light scattering and UV-vis. To measure the size of AuNPs upon DNA attachment, dynamic light scattering experiments were carried out at 25 °C (Zetasizer Nano 90, Malvern). The AuNP concentration was 5 nM. The DNA-functionalized AuNPs were measured also with a AuNP concentration of 5 nM, containing either 10 mM citrate·HCl buffer, pH 3.0 after 3-min incubation or 10 mM HEPES, pH 7.6 with 100 mM NaCl after overnight salt aging. UV-vis spectroscopy was carried out for the same samples using an Agilent 8453 spectrophotometer.

2. The order of addition. The pH of as synthesized 13 nm AuNPs can be adjusted to 3 using HCl while still maintaining its colloidal stability (Figure S1). For comparison, if a final of 10 mM citrate·HCl pH 3 buffer was used, the color of the AuNPs turned blue right away. Therefore, the Na^+ in the citrate buffer caused aggregation of AuNPs by screening its surface negative charge. This same effect is responsible for its faster adsorption of DNA. With a ratio of DNA1:AuNP = 50:1, the AuNPs were stable after the pH adjustment using citrate. Therefore, in the DNA attachment experiment, it is critical to add the citrate·HCl buffer (pH 3) after mixing DNA with AuNPs. Otherwise, the AuNPs aggregate before DNA attachment. However, if only HCl (1-2 mM) was added, the order is not important, because AuNPs can survive with pH 3 HCl for at least 1 hr as shown in Figure S1.

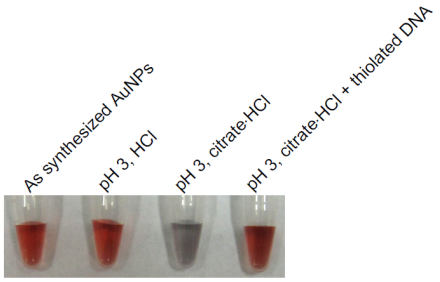

Figure S1. A photograph of citrate capped 13 nm AuNPs in various buffer conditions. Red color indicates dispersed AuNPs and blue indicates AuNP aggregation.

3. UV-vis spectra of AuNPs. In the inset of Figure 4C, we presented photographs of 13 nm AuNPs functionalized using the low pH method and at neutral pH with 100 mM NaCl. Here, their UV-vis spectra are provided to show the quality of AuNPs. Figure S2A are 13 nm AuNPs (10 nM) mixed with various concentrations of DNA1 followed by the addition of 10 mM citrate buffer, pH 3. All the samples showed extinction spectra similar to untreated AuNPs, indicating that the AuNPs remained dispersed. If 100 mM NaCl was added to the AuNPs mixed with DNA at pH 7 (~3 min incubation time), significant shifts of spectra were observed (Figure S2B). The lower the DNA concentration, the more shift observed. Even for the sample with a high DNA concentration (e.g. 150:1), a slight shift was still observed. Similar shifts were also observed upon addition of just 50 mM NaCl if the mixing was performed at pH 7. (Figure S2C). Therefore, quick addition of salt to thiolated DNA mixed with AuNPs at neutral pH cannot produce stable conjugates and slow salt aging at neutral pH must be performed.

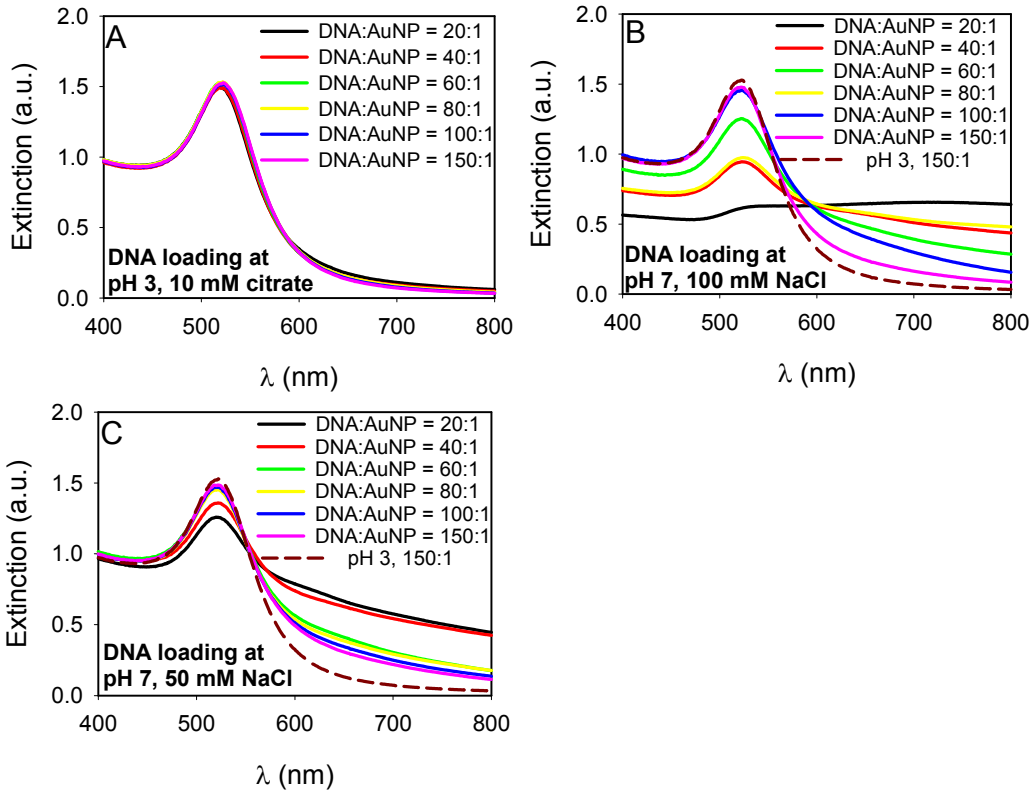

Figure S2. UV-vis spectra of 13 nm AuNPs mixed with DNA1 at pH 3 (A), or pH 7 followed by adding 100 mM NaCl (B) or pH 7 followed by adding 50 mM NaCl (C).

4. Melting curves. One of the most important features for DNA-directed assembly of nanoparticles is the sharp melting transition.[S3] To test whether the AuNPs with thiolated DNA loaded at low pH still possess such a property, we prepared AuNPs functionalized with DNA1 and DNA3 and pH 3 using 10 mM citrate·HCl buffer as previously described. After washing the AuNPs once with buffer (100 mM NaCl, 20 mM HEPES, pH 7.6), the AuNPs were mixed with 200 nM linker DNA in buffer (300 mM NaCl, 20 mM HEPES, pH 7.6) and the color of AuNPs changed to purple in a few minutes. After overnight incubation, the AuNP aggregates were harvested, washed in buffer and finally dispersed in pH 20 mM 7.6 HEPES buffer containing 25 or 50 mM NaCl. For comparison, AuNPs functionalized using the traditional salt aging methods were also prepared. The melting curves are shown in Figure S3. All the melting curves showed a similar sharpness, indicating that the DNA loading at low pH produced equally high quality and fully functional AuNPs. Lower NaCl resulted in lower melting temperature, which is also consistent with the presence of DNA linkages.

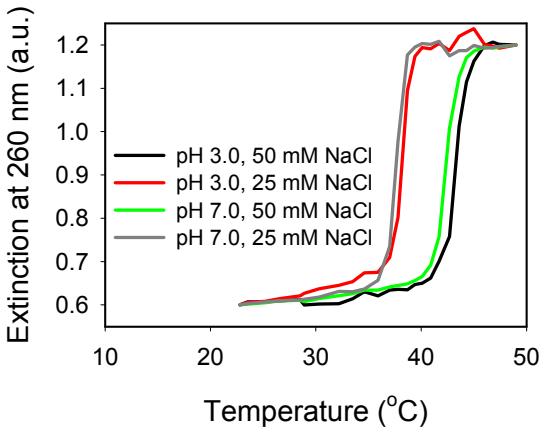

Figure S3. Melting curves of DNA-functionalized AuNPs.

5. UV-vis spectra of large AuNPs. In Figure 5D of the paper, we presented photographs of 50 nm and 100 nm AuNPs functionalized with DNA at pH 3. Here their extinction spectra are also presented. For both 50 and 100 nm AuNPs, the extinction peak dropped slightly due to dilution by the addition of salt. There was also a slight shift in the spectra, indicating a slight aggregation of AuNPs (Figure S4). However, if no DNA was added, significant aggregation occurred in both cases. It needs to be pointed out that it is often difficult to determine the stability of large AuNPs in high salt concentration based on

its color. With a densely functionalized DNA layer, these AuNPs could undergo DNA-mediated aggregation even in the absence of linker DNA.[S4] For example, if the DNA sequence can partially hybridize to itself (even with just two or three base pairs), large AuNP aggregates can form. These aggregates, however, can be disrupted upon removing salt.

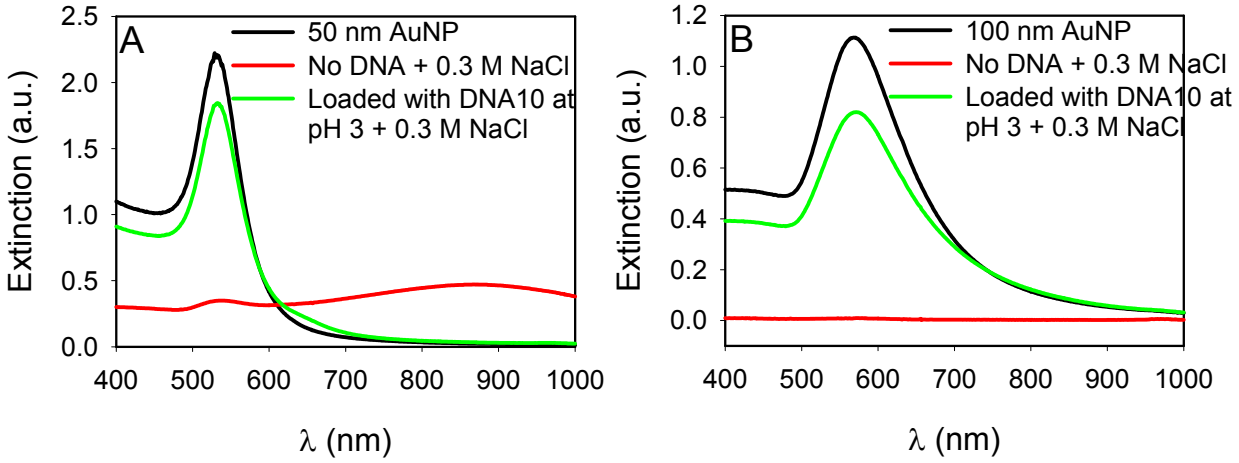

Figure S4. UV-vis spectra of 50 nm (A) and 100 nm (B) AuNPs (black lines), AuNPs loaded with DNA10 at pH 3 followed by treating with 0.3 M NaCl (green lines), or unmodified AuNPs with 0.3 M NaCl (red lines).

Additional references:

(1) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959-1964.
(2) Liu, J.; Lu, Y. *Nat. Protoc.* **2006**, *1*, 246-252.
(3) Jin, R.; Wu, G.; Li, Z.; Mirkin, C. A.; Schatz, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 1643-1654.
(4) Hurst, S. J.; Hill, H. D.; Mirkin, C. A. *J. Am. Chem. Soc.* **2008**, *130*, 12192-12200.