Supporting Information

Blue emitting gold nanoclusters templated by poly-cytosine DNA at low pH and poly-adenine DNA at neutral pH

Thomas Kennedy[a], James L. MacLean[a], and Juewen Liu*[a]

[a] *Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada. Fax: 519 7460435; Tel: 519 8884567 Ext. 38919.*

E-mail: liujw@uwaterloo.ca

1. Materials and methods

Chemicals. All the DNA samples were purchased from Integrated DNA Technologies (Coralville, IA) and purified by standard desalting. $HAuCl_4$ and NaH_2PO_4 were from Sigma-Aldrich. Hydrochloric acid was purchased from VWR (Mississauga, ON). Trisodium citrate was purchased from Mandel Scientific (Guelph, ON). Milli-Q water was used for all experiments.

*AuNC preparation and characterization***.** In a typical synthesis with C_{30} DNA, 6 μL of 500 μM DNA (final 50 μM) was added to 24 μL water followed by the addition of 10 μL of 1 mM $HAuCl_4$ (final 100 μM). After a quick mixing, 10 μL of 500 mM citrate·HCl buffer (final 50 mM) was added and thoroughly mixed. If A_{30} DNA was used, the final DNA concentration was typically 4 μM and final $HAuCl_4$ concentration was 150 μM (added the last) with 50 mM citrate·HCl buffer (pH 6). Other synthesis conditions were tested during the optimization process. The samples were stored in dark overnight to allow AuNC to form. The citrate·HCl buffers were prepared by adding HCl to sodium citrate to achieve a stock citrate concentration of 500 mM. The AuNC samples were observed using a hand-held UV lamp at 245 nm in a dark room. Sometimes the color at ambient light was also recorded using a digital camera (Canon PowerShot SD 1200 IS). For fluorescence measurement, the samples were typically diluted 20 times to a final volume of 600 μL and measured using an Eclipse fluorometer (Varian). UV-vis spectra were collected using an Agilent 8453A spectrometer.

2. UV-vis spectrum of C_{30} templated AuNCs. AuNCs prepared with 50 μM C_{30} DNA and 100 μM $HAuCl_4$ at pH 3 were diluted by half in water and the UV-vis spectrum is shown in Figure S1. There is a strong absorption peak at 360 nm, corresponding to the wavelength for directly exciting the AuNCs. There is also a small bump between 500 and 600 nm, indicating the presence of a trace amount of larger gold nanoparticles. According to the TEM micrograph (Figure S2), the nanoparticle size should be below 2-3 nm.

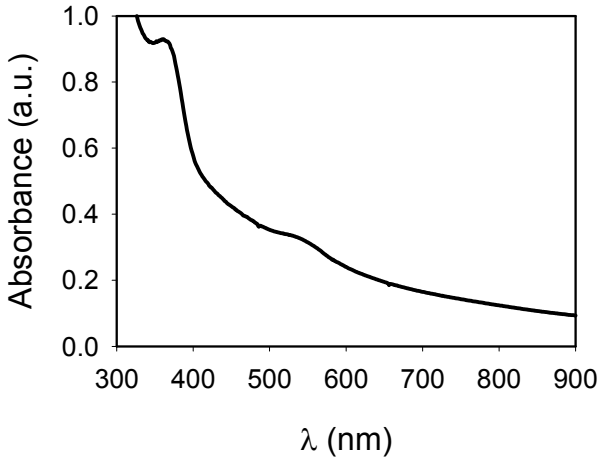

Figure S1. UV-vis spectrum of AuNCs synthesized in the presence of C_{30} DNA at pH 3.

3. TEM micrograph. The AuNCs synthesized in the presence of C_{30} DNA at pH 3 were examined using TEM after drying on a holey carbon coated TEM copper grid. The TEM experiment was carried

out on a Philips CM10 microscope. As shown in Figure S2, no large AuNPs were observed and the surface plasmon in Figure S1 is likely due to small AuNPs (circled) of just 2-3 nm.

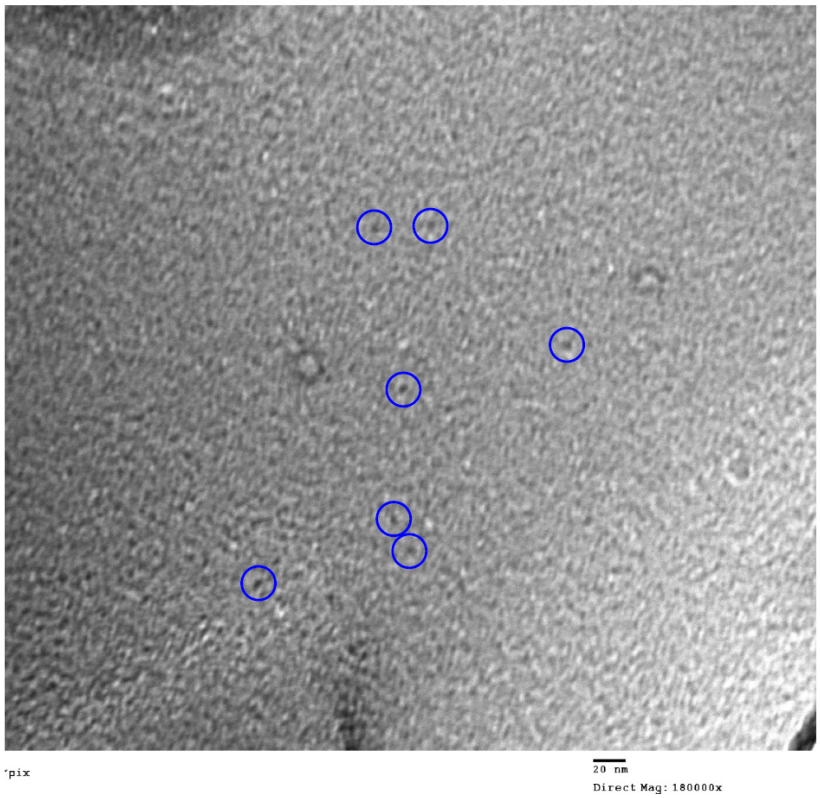

Figure S2. TEM micrograph of AuNCs synthesized in the presence of C_{30} DNA at pH 3. Scale bar = 20 nm. No large gold nanoparticles were observed and a few possible small particles are highlighted in blue circles.

4. Proposed DNA/metal interactions. At neutral pH, both the N3 nitrogen and the keto oxygen might bind to gold but at pH 3, only the oxygen can bind. It might be that at pH 7 the binding is too strong via the chelating effect, Au^{3+} cannot be effectively reduced. For adenine, the N7 position is likely to be responsible for gold binding since the AuNC synthesis was less effective at lower pH, where the N7 position is protonated. See the paper for the assignment of these interactions.

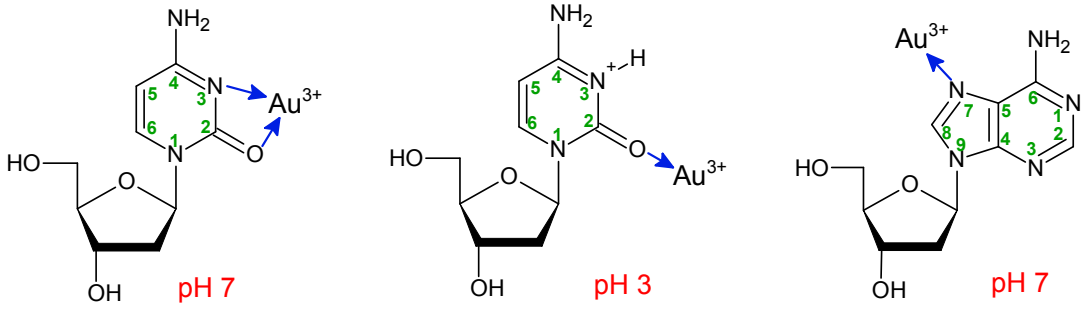

Figure 3. Schematics of the interactions between DNA bases and gold.

5. Fluorescence evolution during AuNC synthesis. Three microcentrifuge tubes were prepared (triplicate) to contain a total volume of 200 μL, each with 100 μM $HAuCl_4$, 50 μM C_{30} DNA, and 50 mM pH 3 citrate buffer. At designated intervals, 20 μL was taken from these vials, diluted 1:30 into 600 μL of water, and measured for fluorescence. The generation of fluorescence is a relatively slow process, taking ~8 h to reach a stable value (Figure S4).

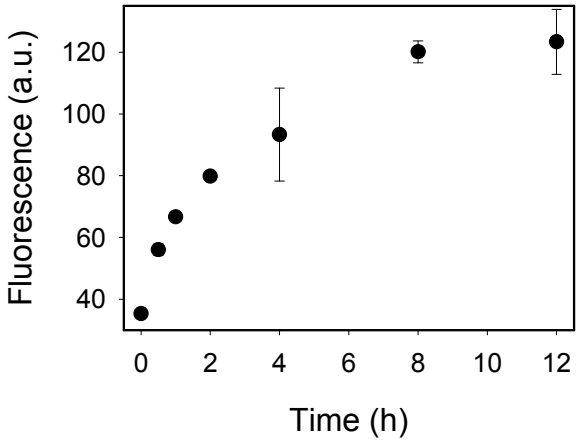

Figure S4. Kinetics of fluorescence intensity evolution during C_{30} templated AuNC synthesis.

6. Stability of AuNCs and AgNCs templated by the same DNA. Using C_{30} DNA, AgNCs were prepared by reducing $AgNO_3$ with $NaBH_4$. Red fluorescent AgNCs were obtained. Both AuNCs and AgNCs were exposed to a fluorescent lamp for 30 min. As shown in Figure S5, the AuNC fluorescence was quenched by ~30% but the AgNC fluorescence was quenched by ~70%, indicating that this AuNC is more stable than the AgNC. Since only two samples were compared, it may not be generally true that AuNCs are more stable in all the cases.

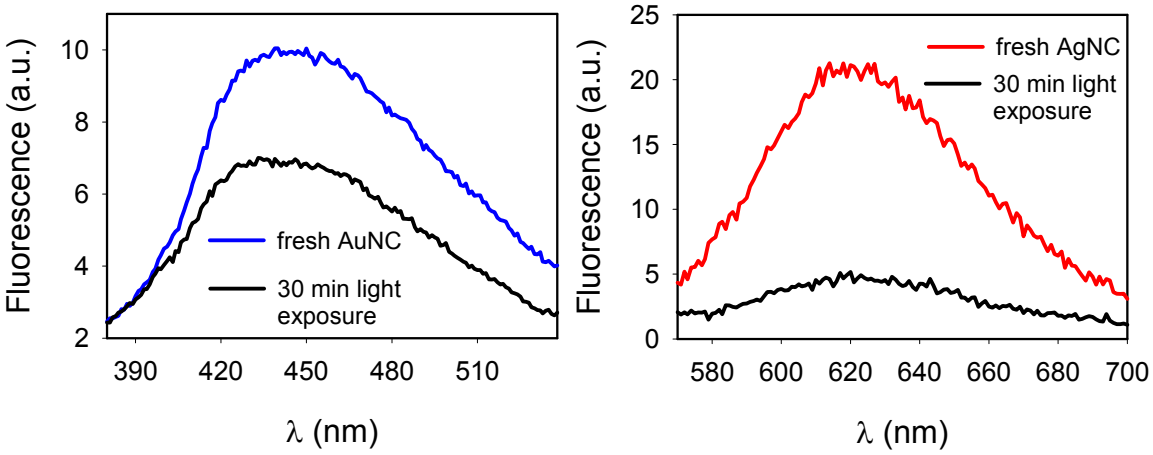

Figure S5. Stability comparison of AgNCs and AuNCs templated by C_{30}.

7. Fluorescence spectra of AuNCs templated by various DNAs. In Figure 2C of the paper, we have presented the photographs of AuNCs synthesized using various C-rich DNA sequences. Consistent with the photograph results, only DNA2 showed high fluorescence and the other sequences produced only background level of signal. All samples were prepared with a volume of 60 μL containing 50 mM pH 3 citrate buffer, 100 μM $HAuCl_4$, and 50 μM DNA. After resting overnight, the samples were diluted 1:30 with water into 600 μL and then measured for fluorescence.

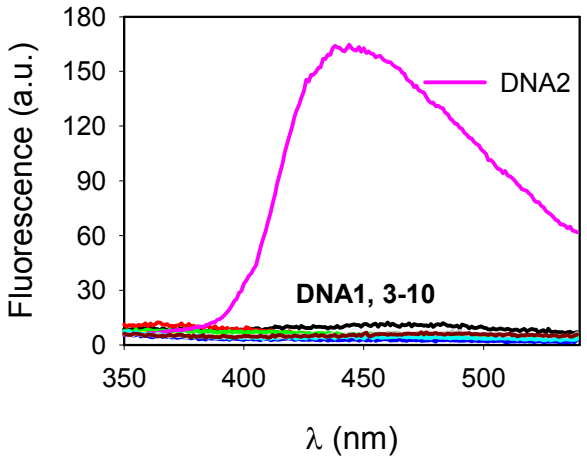

Figure S6. Fluorescence emission spectra of AuNCs templated by DNA1-10. See Figure 2C for DNA sequences.

8. Effect of citrate concentration on A_{30} templated AuNCs. As shown in Figure S7, higher concentrations of pH 7 citrate buffer produced stronger fluorescence. However, the fluorescence change beyond 50 mM citrate was quite small and thus 50 mM citrate was chosen for most of the work.

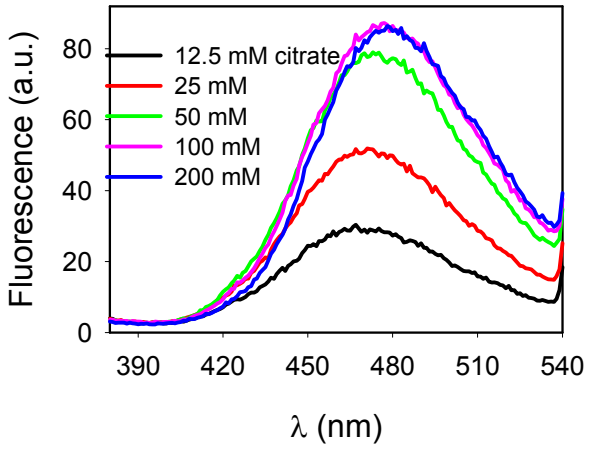

Figure S7. Fluorescence of A_{30} templated AuNCs as a function of citrate concentration.

9. Absorption spectrum of A_{30} templated AuNCs. Figure S8A shows the UV-vis spectra of free A_{30} and AuNCs synthesized using A_{30}, where the peak shifted and broadened to the longer wavelength, explaining the shape of its fluorescence excitation spectrum. The increase of absorption below 250 nm was attributed to citrate (Figure S8B).

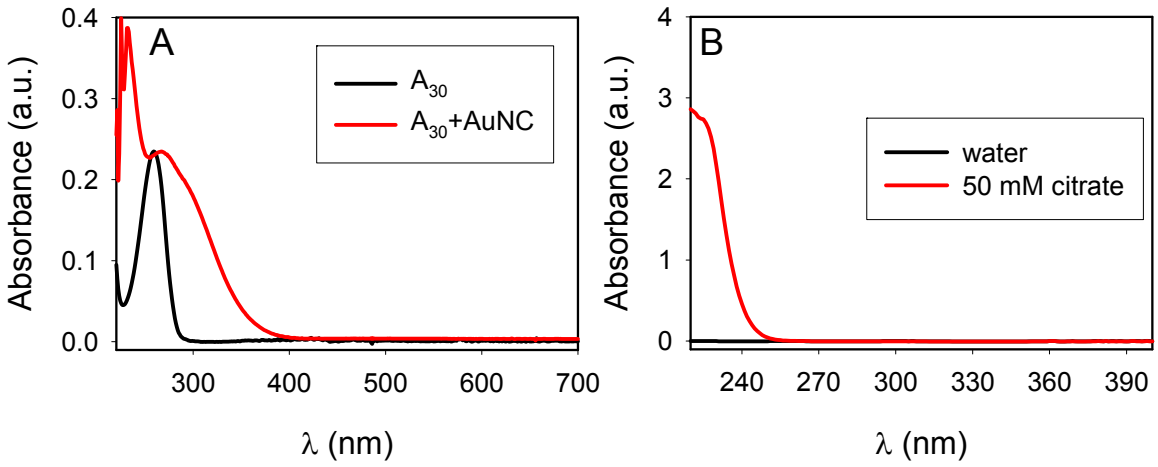

Figure S8. (A) UV-vis spectra of free A_{30} and AuNCs synthesized using A_{30} at pH 7. (B) Citrate ions contribute to light absorption below 250 nm.