

SUPPORTING INFORMATION

Tunable Loading of Oligonucleotides with Secondary Structure on Gold Nanoparticles through a pH-driven Method

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MATERIALS AND METHODS

Cell culture

HT-1080 fibrosarcoma cells were grown in a humidified incubator maintained at 37 °C with 95% air/5% CO₂. All cell lines were obtained from the American Type Culture Collection (ATCC).

Synthesis of AuNS

Gold nanostars (AuNS) were synthesized by reducing Au (III) chlorate in HEPES buffer to create biocompatible, surfactant-free gold nanoparticles. AuNS were prepared by mixing 5 μL of 40 mM HAuCl₄ (Sigma Aldrich) with 1 mL of 100 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl] ethanesulfonic acid (HEPES) buffer.^{3, 28}

Kinetic adsorption of oligonucleotides on Au surface

26 μL of dye-labeled oligonucleotides (2 μM) was first added into each well of 96-well plate containing 100 μL of AuNS solution (0.19 nM). The oligonucleotides and AuNS solution were briefly shaken to form a homogeneous mixture (2700:1). 42 μL of 100-mM citrate buffer at the desired pH conditions was added to the mixture. Immediately, the fluorescence intensity (F.I.) of the dye was measured using a Synergy 3 microplate reader indicating 0 min (t = 0 min) of the reaction. The measurement was repeated at t = 2, 5, 10, 20, 30, 60, 120, 180, 240, 300, 360, 420 and 480 min after the addition of citrate buffer. The percentage of adsorbed strands at certain time point (X) was determined by (1) and (2):

$$\% \text{ non-adsorbed DNA} = \frac{F.I \text{ at } (t=X)}{F.I \text{ at } (t=0)} \times 100\% \quad (1)$$

$$\% \text{ adsorbed DNA} = 100 - \% \text{ non-adsorbed DNA} \quad (2)$$

UV-vis spectroscopy analysis

Apt-AuNS nanoconstructs were prepared at pH = 1.7 and pH = 3.0 overnight. The solution was centrifuged twice in PBS solution to remove access free Apt. The final pellets were re-suspended in 18 Ω Millipore-filtered water. The UV-vis spectra of the Apt-AuNS were measured using the Pelkin Elmer LAMBDA 1050 UV-vis spectrometer.

Cell viability measurement

0.3 nM of Apt-AuNS nanoconstructs prepared at pH = 3 and 1.7 were incubated for 24 h with HT-1080 fibrosarcoma cells (5×10^4 cells/mL) in a 96-well plate. After 24 h treatment of cells, the cell viability was measured using MTS assay solution (Promega).

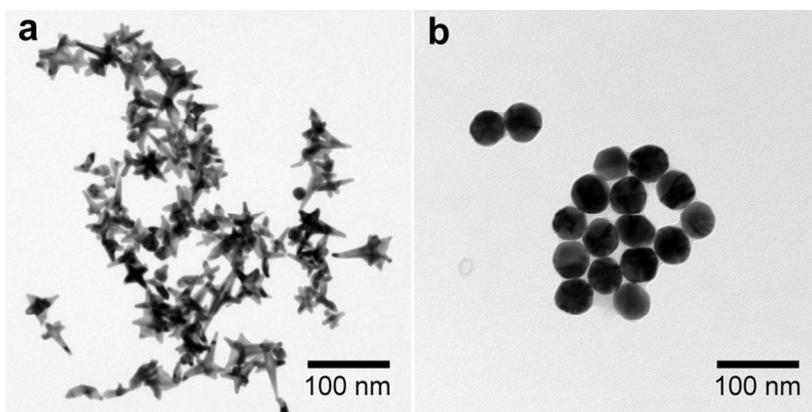


Figure S1: Transmission electron microscopy images of AuNS and AuNPs. (a) AuNS had a multi-branched structure and average diameter of 39.2 nm as measured by dynamic light scattering. (b) AuNP had a spherical shape with diameter of approximately 40 nm by dynamic light scattering.

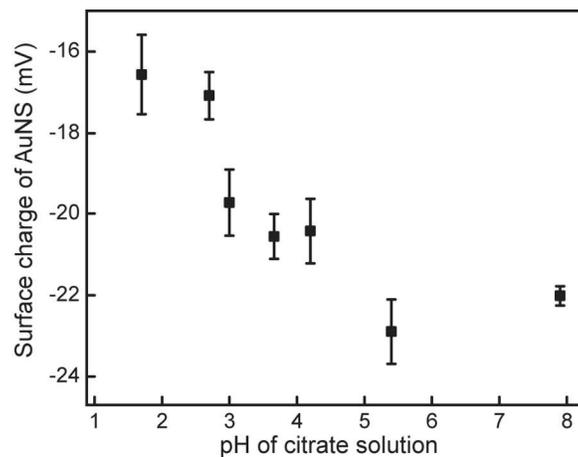


Figure S2: Surface charge of AuNS at different pH conditions. The surface of AuNS is more negatively charged at higher pH due to de-protonation at the piperazine ring (N1 and N4) of HEPES molecules. Zeta potential measurements indicated that the surface charge decreased as pH decreased.

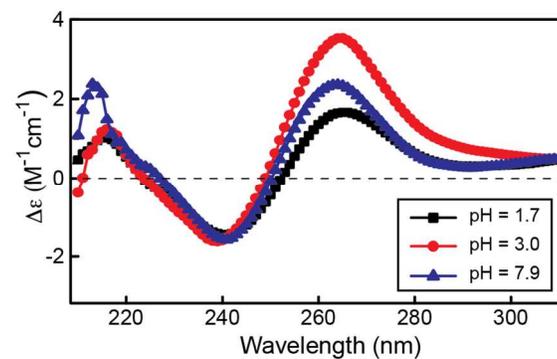


Figure S3: G-quadruplex structure of poly G at different pH conditions. Single-stranded poly G folded into a parallel G-quartet structure at pH = 1.7, 3 and 7.9.

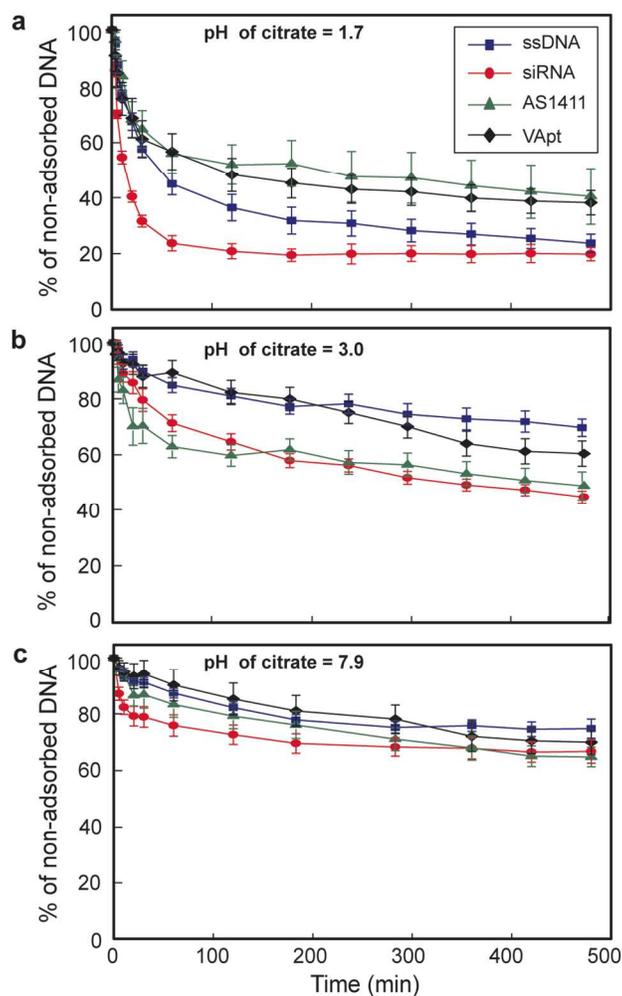


Figure S4: Percent adsorption of oligonucleotides at different pH conditions. Percentage of oligonucleotide adsorption at (a) pH = 1.7 was higher than that at (b) pH = 3, (c) pH = 7.9. The amounts of duplex and single-stranded strands adsorbed on the surface of AuNS were higher than those of G-quadruplex oligonucleotides.

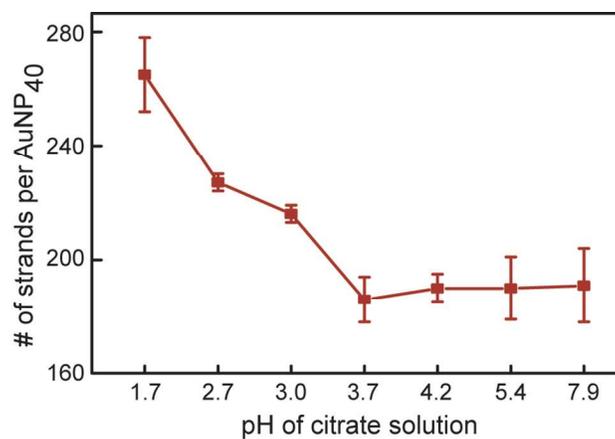


Figure S5: Loading of Apt on 40 nm citrate-capped colloidal AuNPs as a function of pH. The number of Apt loaded on AuNP₄₀ decreased as the pH of the citrate solution increased. This trend is similar to the loading of oligonucleotides on AuNS.

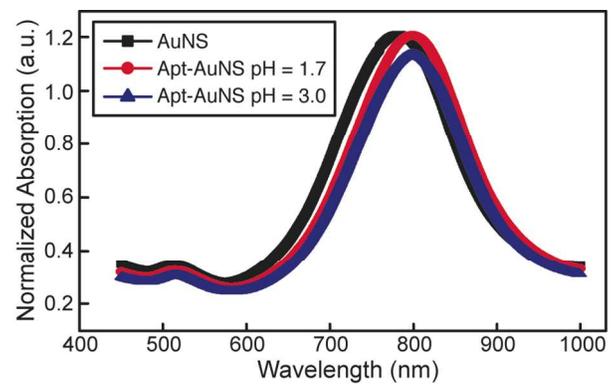


Figure S6: UV-vis measurement of Apt-AuNS prepared at pH = 1.7 and pH = 3.0. No significant difference in the spectra of Apt-AuNS at pH = 1.7 and 3.0 indicated that Apt-AuNS is stable even at low pH condition.

Table S1: Characterization of HEPES-capped AuNS and citrate-capped AuNP₄₀

| | Average hydrodynamic diameter (nm) | Surface area (nm ²) | Surface potential (mV) |
|--------------------|------------------------------------|---------------------------------|------------------------|
| AuNS | 39.2 ± 6 | 14800 ± 100 | - 25.7 |
| AuNP ₄₀ | 40.2 ± 2 | 5100 ± 10 | - 32.8 |