Supporting Information

Etching silver nanoparticles by DNA

Shengqiang Hu,[a,b] Tiantian Yi,[a] Zhicheng Huang,[b] Biwu Liu,[b] Jianxiu Wang,*[a] Xinyao Yi,*[a] Juewen Liu*[b]

[a]College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China

*E-mail: jxiuwang@csu.edu.cn; yixinyao@csu.edu.cn

[b]Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada

*E-mail: liujw@uwaterloo.ca

Materials and Methods

Chemical and materials. Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), trisodium citrate, polyvinyl pyrrolidone (PVP, Mw = 40,000), hydrogen peroxide (H_2O_2, 30 wt%) and calcein-AM were purchased from Sigma-Aldrich (St. Louis, MO). Commercial citrate-capped AgNPs (20 nm diameter) were purchased from Nanocomposix (San Diego, CA, USA). Citrate-capped AuNPs (15 nm diameter) were purchased from BBI Solutions (Cardiff, UK). Sodium fluoride (NaF), sodium chloride (NaCl), sodium bromide (NaBr), and cetyltrimethyl ammonium bromide (CTAB) were from Mandel Scientific (Guelph, ON, Canada). Fetal bovine serum (FBS) and RPMI 1640 cell culture medium were obtained from Invitrogen (Gibco, NY, USA). Phosphate buffered saline (PBS), propidium iodide (PI), Luria-Bertani (LB) broth with or without agar, and Cell Counting Kit-8 (CCK-8) were acquired from Beyotime Institute of Biotechnology (Haimen, China). Human Burkitt's lymphoma Ramos cells were obtained from Xiangya Hospital of Central South University (Changsha, China). ATCC 25922 Escherichia coli (*E. coli*) and CMCC 63501 Bacillus subtilis (*B. subtilis*) strains were purchased from Shanghai Luwei Microbial Science and Technology Co., Ltd. (Shanghai, China). All the oligonucleotides were purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA). The DNA sequences used were as follows: 5′-AAA AAA AAA AAA AAA AAA-3′ (A_{18}), 5′-TTT TTT TTT TTT TTT TTT-3′ (T_{18}), 5′-GGG GGG GGG GGG GGG GGG -3′ (G_{18}), 5′-CCC CCC CCC CCC CCC CCC-3′ (C_{18}), 5′-CCC CC-3′ (C_5), 5′-CCC CCC C-3′ (C_7), 5′-CCC CCC CCC-3′ (C_9), 5′-CCC CCC CCC CCC -3′ (C_{12}), CAC ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC AC ($(CA)_{17}C$), CCC ACC CAC CCA CCC ACC CAC CC ($(C_3A)_5C_3$), CCC CCC ACC CCC CAC CCC CC ($C_6AC_6AC_6$). Unless otherwise stated, all the stock solutions were prepared daily with a water purification system (Simplicity 185, Millipore Co., Billerica, MA).

Instrumentation. UV-vis absorption spectra were recorded on a spectrometer (Agilent 8453A). The morphology of AgNPs was examined by a transmission electron microscope (TEM, Philips CM10). Dynamic light scattering (DLS) measurements were carried out using a Zetasizer Nano 90 (Malvern) at 25 °C. The concentration of Ag^+ was determined using an Agilent ICP-MS 7700 inductively-coupled plasma mass spectrometer (ICP-MS, Agilent Technologies). The etching kinetics of AgNPs were monitored using a microplate reader (Infinite F200, Tecan). Bright-field and fluorescence images were obtained on an IX83 inverted

fluorescence microscope (Olympus). Cell viability was measured on a Thermo Scientific Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific).

Preparation of AgNPs. In addition to the commercially purchased AgNPs, citrate-capped AgNPs were also synthesized according to a previously reported method. [S1, S2] Typically, trisodium citrate dehydrate (1 mL, 0.03 M) was mixed with $AgNO_3$ (1 mL, 0.01 M) in 7 mL deionized water, followed by stirring for 30 min in the dark. Then, a freshly prepared $NaBH_4$ solution (1 mL, 0.047 M) was added quickly. After stirring for 4 h, the AgNPs with a final concentration of 108 μg/mL silver were obtained. Note that the concentrations of AgNPs was obtained by assuming all the silver ions were converted to AgNPs.

Preparation of Ag nanoplates. The Ag nanoplates were synthesized using a literature reported method.[S3] Briefly, a 25 mL aqueous solution containing silver nitrate (0.05 M, 50 μL), trisodium citrate (75 mM, 0.5 mL), PVP (17.5 mM, 0.1 mL) and H_2O_2 (30 wt%, 60 μL) was vigorously stirred at room temperature in air. Then, freshly prepared $NaBH_4$ (100 mM, 250 μL) was rapidly injected into the solution. After about 30 min, Ag nanoplates were obtained.

C_{18} DNA directed AgNPs etching. In a typical experiment, a C_{18} DNA solution (50 μM, 50 μL**)** were incubated with 50 μL of stock AgNPs (20 μg/mL) at 37 °C for 1.5 h. In this case, the final DNA concentration was 25 μM. In other experiments, the length, concentration and sequence of DNA was varied. The concentration of AgNPs was also varied for some experiments.

Kinetics of etching AgNPs. A mixture of 90 μL C_{18} DNA (50 μM) and 90 μL stock AgNPs (20 μg/mL) were incubated in a 96-well plate for 5 min, followed by adding 10 μL NaCl of different concentrations (1, 10, 100 mM). After another 5 min, 10 μL NaCl of the same concentrations was added, so that the final NaCl concentrations were 0.1, 1 and 10 mM, respectively. The reason to add DNA first was to avoid salt-induced aggregation of AgNPs. Other halides were tested the same way. The absorbance intensity of AgNPs at 402 nm was monitored every 30 s under the kinetic mode using a plate reader.

Determination of Ag^+ concentration. To eliminate potential interface from other ions, the stock AgNPs (10 μg/mL) were firstly centrifuged for 10 min at 13 000 rpm. After removing the supernatant, the pellet was re-dispersed with deionized water. 50 μL of the purified AgNPs were then incubated with 7.5 or 25 μM C_{18} DNA (50 μL) at 37 °C for 1.5 h, followed by another

centrifugation at 13 000 rpm for 10 min. 50 μL of the supernatant was diluted into 15 mL with deionized water for the following ICP-MS analysis.

Cancer cell apoptosis assay. To measure cytotoxicity, the culture media containing home-made AgNPs (20.25 μg/mL) without DNA or the AgNPs pre-incubated with the C_{18} DNA of different concentrations (5, 12.5, 25 μM for 1.5 h at 37 °C) were co-cultured with Ramos cells for 24 h. Then, 5 μg/mL of calcein-AM and 5 μg/mL of PI were added and incubated for another 0.5 h. Bright-field and fluorescence images of the cells were taken on an IX83 inverted fluorescence microscope.

The colorimetric CCK-8 assay was performed to further confirm enhanced cytotoxicity of home-made AgNPs induced by the C_{18} DNA.[S4] Briefly, Ramos cells were firstly treated with the culture media containing the AgNPs (20.25 μg/mL) or AgNPs pre-treated with the C_{18} DNA as described above for 24 h. After washing with PBS, the Ramos cells were cultured in the culture media containing 10 μL CCK-8 solution for another 2 h. The absorbance of the solutions was measured on a microplate reader at 450 nm. Cells treated with PBS were used as a control. Cell viability (%) was calculated by the percentage absorbance of the AgNPs-treated groups relative to that of the control group (n = 5).

Antibacterial tests. A colony counting assay and the growth inhibition curve method were utilized for evaluating the antibacterial effect of AgNPs.[1] The colony counting assay was carried out by culturing *E.coli* and *B. subtilis* with an optical density at 600 nm (OD_{600}) of 0.01 with home-made AgNPs (20.25 μg/mL), or AgNPs pre-incubated with the C_{18} DNA as described above for 4 h, followed by spreading them onto LB agar plates and culturing for another 8 h. Then, the numbers of viable bacteria were counted. To obtain the growth curve of bacteria, the *E.coli* and *B. subtilis* cells with an OD_{600} of 0.01 were respectively introduced to the fresh LB medium. Then, AgNPs (20.25 μg/mL) or AgNPs pre-treated with C_{18} were added and co-cultured under gentle shaking. The OD_{600} value of the resulting bacteria solutions was recorded at different time points. The mixture of AgNPs and LB medium was taken as a positive control.

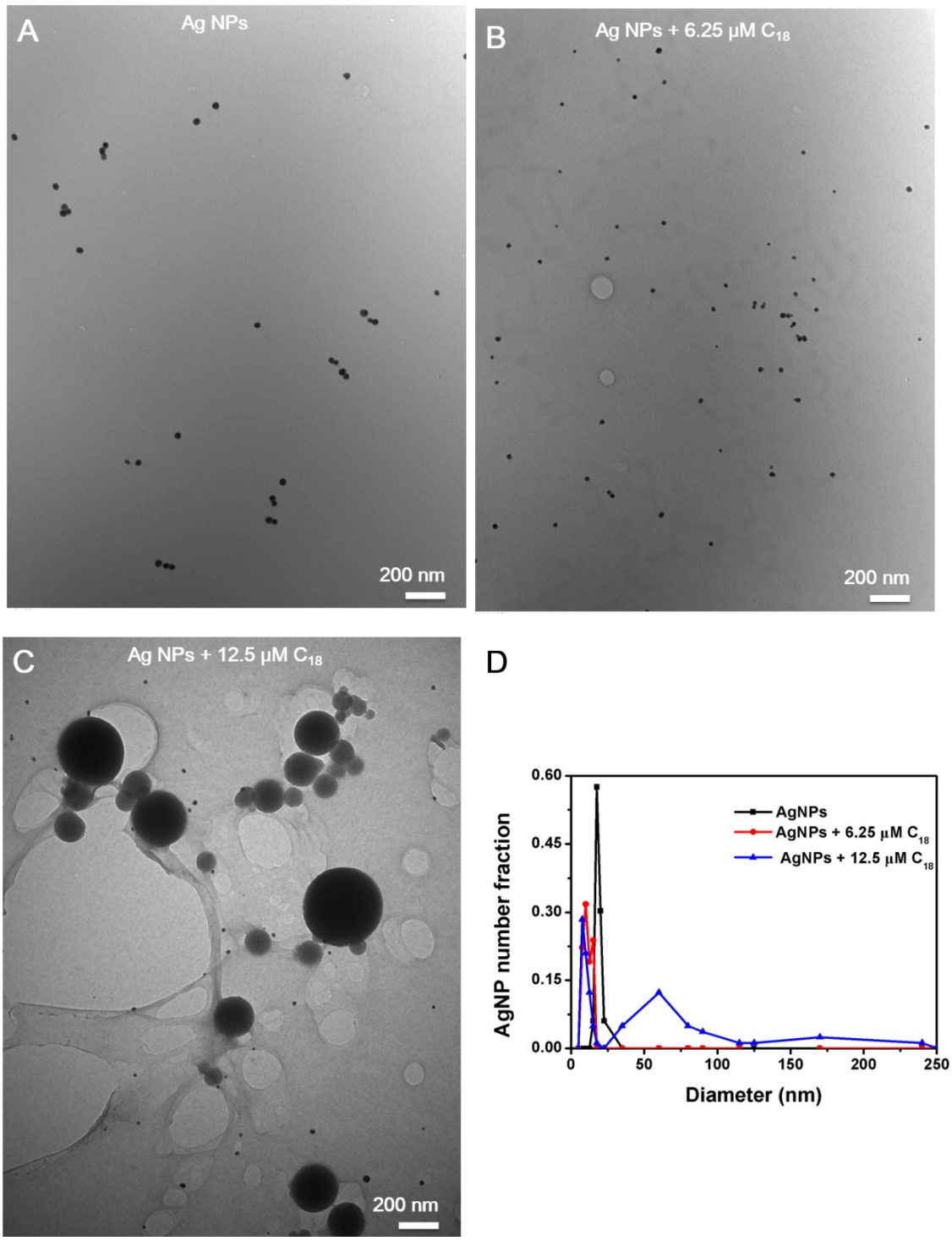

Figure S1. Wide-area TEM micrographs of 20 nm AgNPs incubated with different concentrations of C_{18} DNA: (A) no DNA; (B) 6.25 µM C_{18} DNA; and (C) 12.5 µM C_{18} DNA for 1.5 h at 37 °C. (D) Histogram of the particle size distribution from the TEM data.

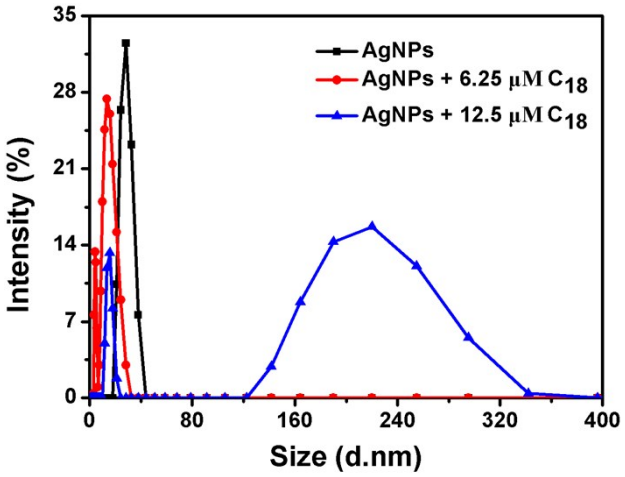

Figure S2. The average hydrodynamic diameter of of 20 nm AgNPs (10 μg/mL) mixed with different concentrations of the C_{18} DNA measured by DLS. With 6.25 μM C_{18} DNA, many small particles below 20 nm were detected, while with 12.5 μM C_{18} DNA, a strong scattering from large AgNPs was observed together with a peak for small particles. Note that the *y*-axis is the scattering intensity, and each larger particle can contribute very significantly to light scattering and the peak area is not a reflection of the number of particles. This statistics is different from the above TEM-based calculation, which is based on the particle number distribution. Nevertheless, the decreased particle size with 6.25 μM DNA and the presence of both very small and very large AgNPs with 12.5 μM DNA can be concluded from both experiments.

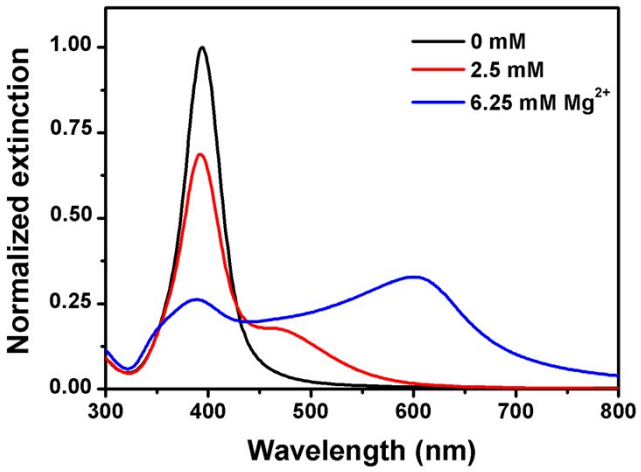

Figure S3. UV-vis spectra of 20 nm AgNPs mixed with different concentrations of $Mg(NO_3)_2$. With 2.5 mM Mg^{2+}, the characteristic surface plasmon peak of AgNPs decreased and a new peak at around 470 nm emerged. Further decrease in the peak of AgNPs was accompanied with a new broad peak at around 600 nm upon incubation with 6.25 mM Mg^{2+}. These red shifted peaks are characteristi of aggregation of AgNPs. On the other hand, etching of AgNPs produces smaller particles initially, resulting in a blue-shifted peak.

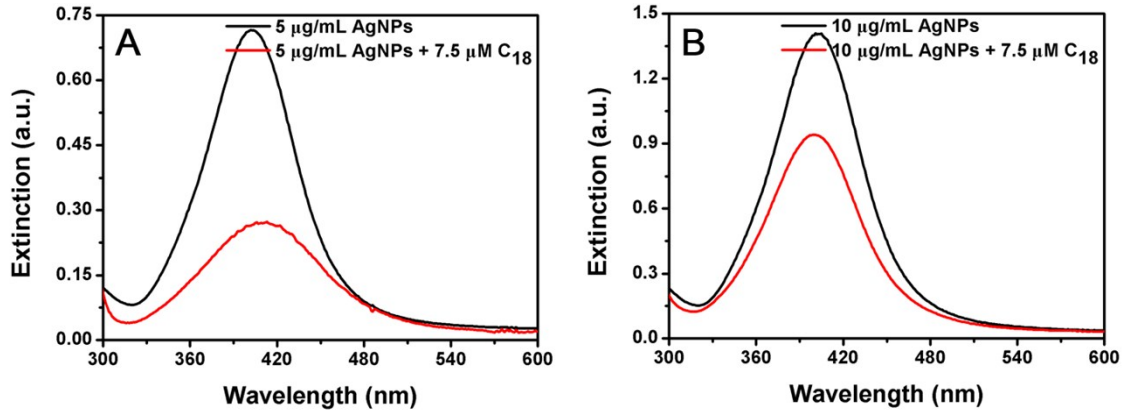

Figure S4. UV-vis spectra of 20 nm AgNPs with an initial concentraton of (A) 5 µg/mL; and (B) 10 µg/mL before and after incubated wtih 7.5 µM of C_{18} DNA at 37 °C for 1.5 h. In (A) the spectrum red shifted indicating ripening, while in (B) the spectra blue shifted indicating etching of the AgNPs. Therefore, the critical concentration for ripening to take place is a function of the initial concentration of AgNPs.

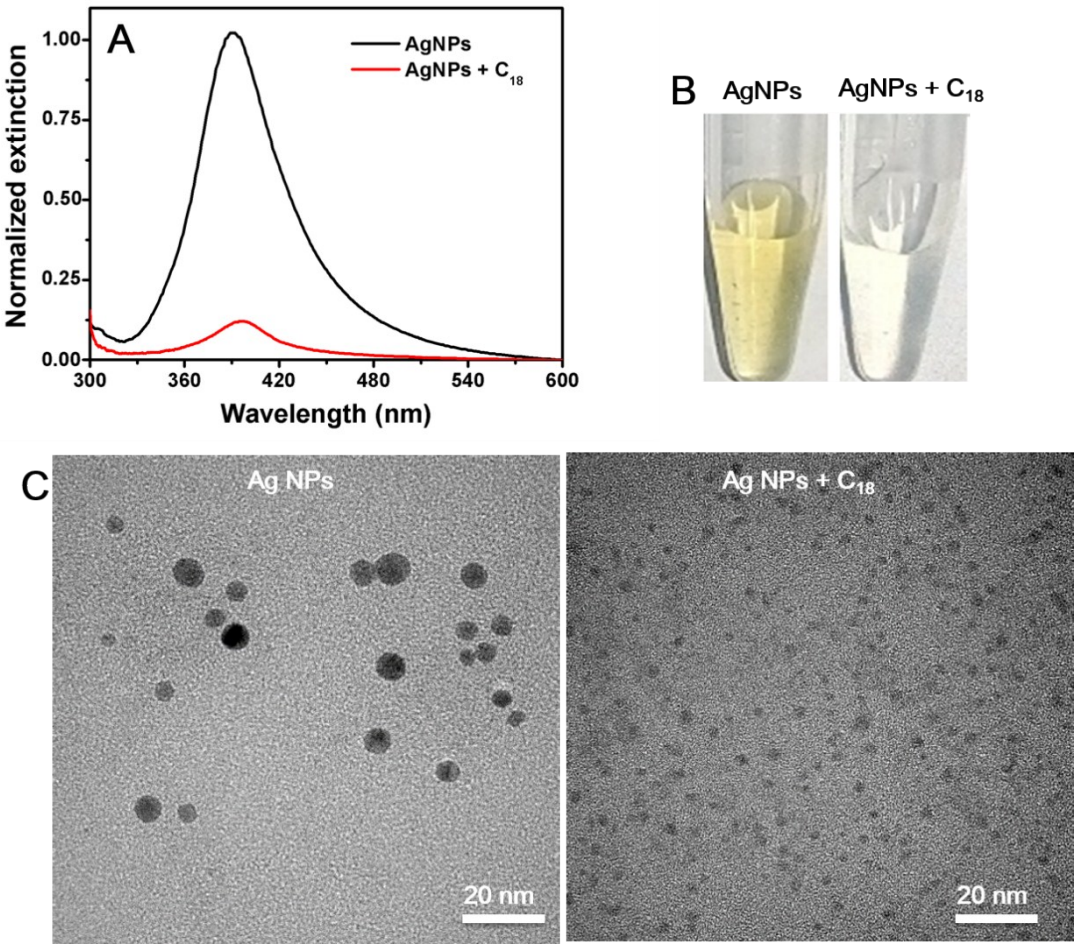

Figure S5. (A) UV-vis spectra and (B) photographs of home-made AgNPs (20.25 μg/mL) before and after incubated wtih 25 μM C_{18} DNA. The disappearance of the yellow color and the drop of the plasmonic peak intensity were indications of etching. (C) TEM micrographs of the AgNPs before and after mixing with 25 μM C_{18} DNA.

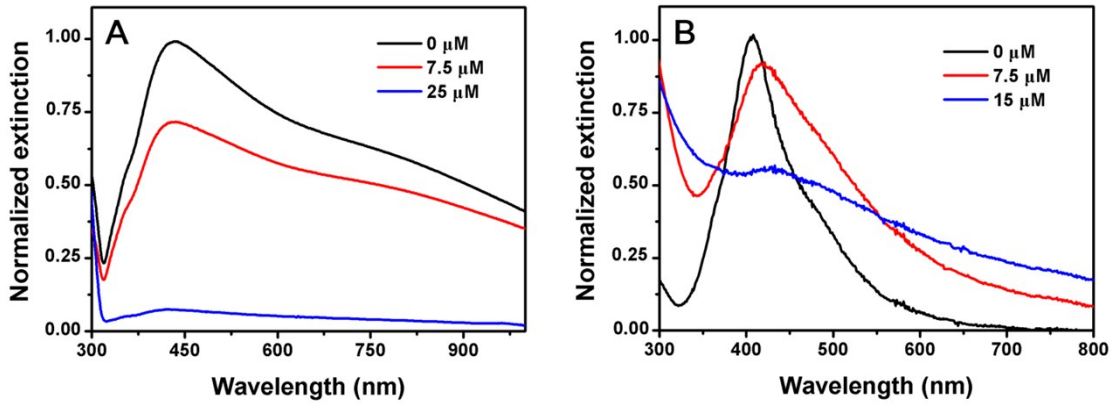

Figure S6. UV-vis spectra of (A) PVP- and (B) CTAB-capped AgNPs (20.25 μg/mL) mixed with different concentrations of C_{18} DNA. In (A) the UV-vis peak dropped and blue shifted indicating etching, while in (B) the spectra red shifted indicating aggregation of the AgNPs.

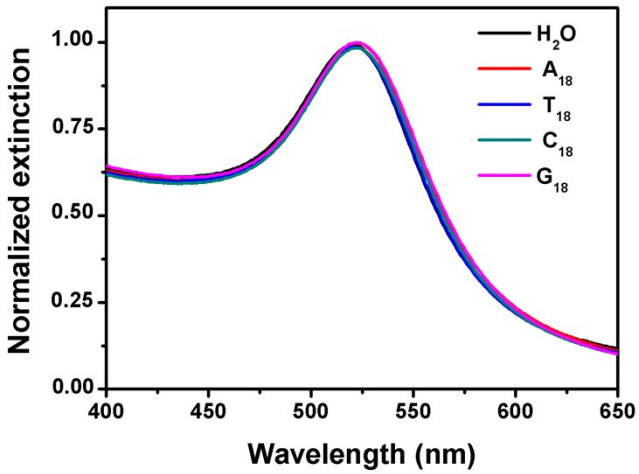

Figure S7. UV-vis spectra of citrate-capped gold nanoparticles (AuNPs) of 15 nm before and after adding 18-mer homo-DNAs of 25 μM for 1.5 h at 37 °C. Negligible changes in the spetrum were observed for all these samples indicating that the AuNPs cannot be etched by these DNAs.

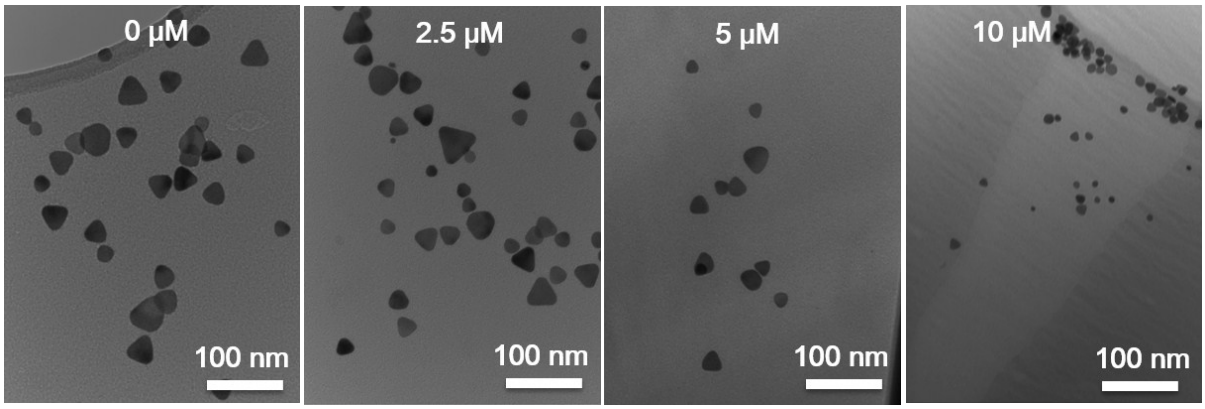

Figure S8. Wide-area TEM micrographs of Ag nanoplates before and after mixing with different concentrations of C_{18} DNA. The increase of the C_{18} DNA concentration caused an initial decrease in size of the triangular nanoplates and subsequent conversion to spherical morphology.

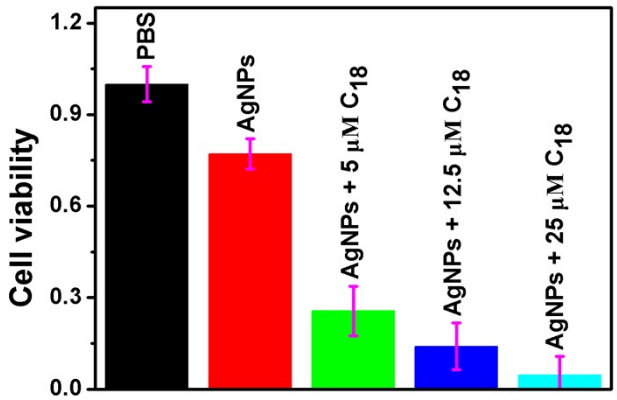

Figure S9. Viability of Ramos cells after treatment with different chemicals measured using the colorimetric CCK-8 method.

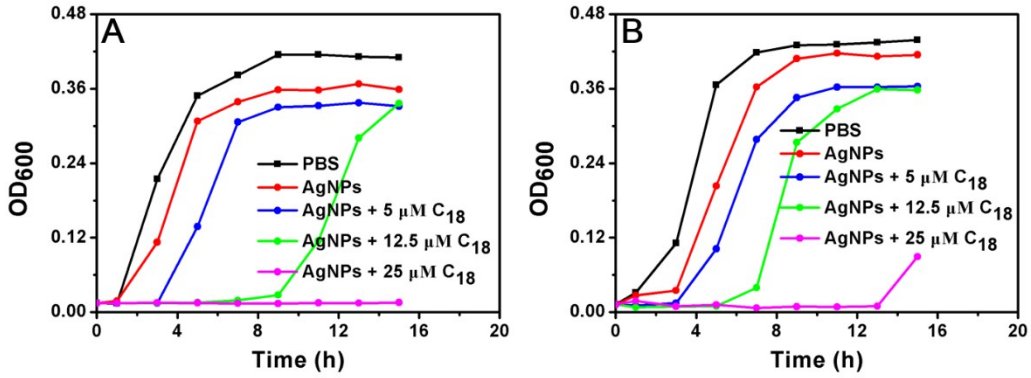

Figure S10. Cell growth curves of (A) *E. coli* and (B) *B. subtilis* after different treatments monitored at 600 nm using a UV-vis spectrometer. A faster growth is accompanied with more turbidity and thus a higher light extinction at 600 nm. The effect of AgNPs plus C_{18} DNA was more potent on the *E. coli* cells.

Additional References

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S2. S. Hu, B. Ye, H. Tang, F. Wu, X. Yi, T. Yi, D. Wu, L. Wu and J. Wang, *J. Mater. Chem. B*, 2018, **6**, 1187-1194.

S3. Q. Zhang, N. Li, J. Goebl, Z. Lu and Y. Yin, *J. Am. Chem. Soc.*, 2011, **133**, 18931-18939.

S4. L. Zhang, W. Zheng, R. Tang, N. Wang, W. Zhang and X. Jiang, *Biomaterials*, 2016, **104**, 269-278.

S5. J. Thiel, L. Pakstis, S. Buzby, M. Raffi, C. Ni, D. J. Pochan and S. I. Shah, *Small*, 2007, **3**, 799-803.