Supporting information

for

Inspiration from chemical photography: accelerated photoconversion of AgCl to functional silver nanoparticles mediated by DNA

by

Guoqing Wang,[†] Takashi Nishio,[†] Masato Sato,[†] Ayako Ishikawa,[†] Katsuyuki Nambara,[†] Keita Nagakawa,[†] Yasutaka Matsuo, [‡,§] Kenichi Niikura[‡,§] and Kuniharu Ijiro*[,‡,§]

[†]*Graduate School of Chemical Sciences and Engineering, Hokkaido University, N13W8, Kita-Ku, Sapporo 060-0810,* [‡]*Research Institute for Electronic Science (RIES), Hokkaido University, N21W10, Kita-Ku, Sapporo 001-0021 and* [§]*JST, CREST, Sanban-cho 5, Chiyoda-ku, Tokyo 102-0075, Japan*

Email: ijiro@poly.es.hokudai.ac.jp

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1.Chemicals and apparatuses

Oligonucleotides were purified using high-performance liquid chromatography (HPLC) by Hokkaido System Science Co., Ltd. (Japan). Sybr Green I was purchased from Molecular Probes (USA). Other chemicals were received from Wako Pure Chemical Industries (Japan). All reagents were used without further purification. Aqueous solutions were prepared in deionized water (18.2 MΩ cm specific resistance) purified by a Milli-Q water purification system (Millipore, USA).

XPS data was collected from a JPS-9200 Photoelectron Spectrometer (JEOL, Japan), and all spectra were calibrated with the C1s peak at 284.2 ev. A HD-2000 electron microscope (Hitachi, Japan) was applied to obtain transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images. The high-resolution TEM images and electron diffraction patterns were obtained from a JEM-2000FX transmission electron microscope (TEM) (JEOL, Japan). Atomic force microscopy (AFM) was performed with an SPA-400/SPI3800N atomic force microscope (Seiko Instruments Inc.). Zeta potential of colloidal solution was detected using a potentiometer (ELS-8000, Otsuka Electronics). A UV-1650PC UV/visible spectrophotometer (Shimadzu, Japan) was used to measure absorption spectra. The melting curves of free dsDNA and AgNP assemblies were also obtained from the spectrophotometer based on the absorbance measurement at a wavelength of 260 nm. Fluorescence spectra were collected from a RF-5300PC spectrofluorophotometer (Shimadzu, Japan).

Fluorescence images of silver nanoparticles (AgNPs) were collected from a BX51 fluorescence microscope (Olympus, Japan). The fluorescence cell images were obtained using an FV-300 confocal laser microscope (Olympus, Japan). Raman scattering signals were collected with a Renishaw invia microscope system (Renishaw, UK) coupled with a charge-coupled device (CCD) camera. During SERS spectra measurements in cells, a water-immersion 60× objective lens was installed to focus the cells. An argon ion laser operating at $\lambda = 532$ nm with a laser power of 1 mW was used for sample excitation. All Raman spectra were measured with accumulation times of 10 s.

2. Experimental

Synthesis of various AgNPs DNA-capped AgNPs were synthesized by a single-step photoreduction of AgCl at room temperature in the presence of DNA. Typically, 10 μL of 20-mer oligonucleotide and 10 μL of $AgNO_3$ (10 mM) are successively added to 980 μL Tris-HCl buffer (10 mM, pH 7.8) in a colorless glass vial (i.d. 16 mm, volume 4 mL, As One Corp., Japan), to give a 1:1 ratio ([nucleobase] = [Ag^+] = 100 μM). The thoroughly mixed dispersion was then irradiated by UV (~360 nm, 150 mW/cm^2) from a mercury lamp (Lightningcure^{TM} L9588-01, Hamamatsu) for 5 min. The distance between the lamp and the solution surface is determined to be 4.0 cm. Citrate-capped AgNPs were prepared according to Lee and Meisel's method (Lee, P. C.; Meisel, D. *J. Phys. Chem.* 1982, **86**, 3391). Briefly, 50 mL of $AgNO_3$ (1.0 mM) aqueous solution was first heated to boiling point under stirring. $Na_3Citrate{\cdot}2H_2O$ was then immediately added to give a final concentration of 6 mM. The mixture was kept boiling for additional 1 h.

Preparation of AgNP assemblies and measurement of melting curves The dA20-AgNPs and dT20-AgNPs were filtered through a 0.22 μm size-pore membrane. Then, 400 μL of the each surfactant is concentrated in 200 μL Tris-HCl buffer (10 mM, pH 7.8) in an eppendorf tube by 3 centrifugation/washing cycles (12, 000 rpm, 20 min). 100 μL of dT20-AgNPs were added to the resulting dA20-AgNPs. 50 mM NaCl was then introduced and the mixture was incubated overnight for DNA-directed nanoassembly. To signify the DNA hybridization behavior, Sybr Green I (1×) was incubated with above mixtures for 15 min to attain sufficient fluorescence enhancement. To measure the melting curve of the nanoassemblies and compare it with dA20/dT20 dsDNA, the temperature was increased from 20 ºC to 60 ºC at a rate of 0.5 ºC/min controlled by a F25-Refrigerated/Heating Circulator (JULABO Labortechnik GmbH, Germany).

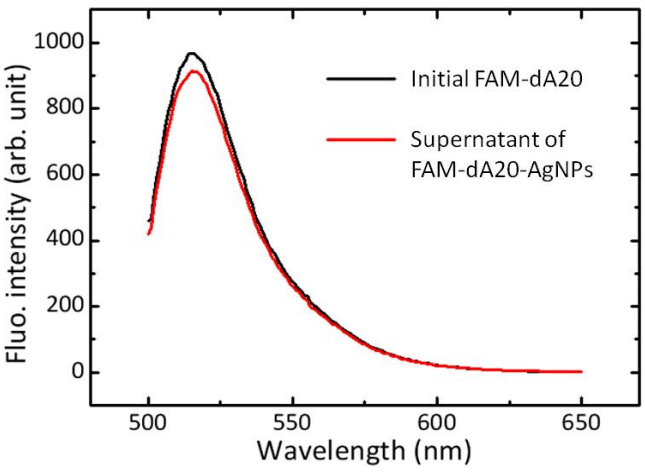
Cell culture and AgNPs internalization Hela cells (5×10^4/mL) were cultured in 10 mL of cell culture media in tissue culture dishes (~35 mm in diameter) in dark under humidified 5% CO_2/95% air at 37 ºC. DMEM supplemented with 10% FBS (heat-inactivated; GIBCO) and 100 IU/mL penicillin-Steptomycin (cell-gro) was used as cell culture medium. Before each experiment, the Hela cells were washed three times with PBS buffer, and redispersed 2 mL cell culture media in culture dishes and were left

for 1 day for cell immobilization on dish substrates. After washing, the cells were dispersed in culture media containing 0.56 nM dA20-AgNPs and incubated for about 6 h.

Optical imaging in cells The fluorescence images were obtained with a fluorescence microscope. The detection of SERS signals in cells was performed after incubation of Hela cells with dA20-AgNPs for 10 hours in culture dish. A 60× water-immersion lens was used to focus onto the cells internalized with dA20-AgNPs. To compare the resulting signals with that of glutathione, glutathione was added to dA20-AgNPs to give a final concentration of 5 mM that is comparable to its concentration in cells. The SERS signals of cell lysate mixed with dA20-AgNPs was also collected for comparison purpose.

3. Characteristics of DNA surface coverage and the binding affinity between DNA and AgNPs

To quantify the DNA surface coverage on the dA20-AgNPs, FAM-labeled dA20 (denoted as FAM-dA20) was applied to produce AgNPs by the same procedure. After centrifugation of as-formed AgNPs, the fluorescence intensity of the resulting supernatant was measured, and compared with that of the initial FAM-dA20 solution. Based on the intensity differences, we determined that the ~499 dA20 were attached on each AgNPs (Figure below).[1] To test the binding affinity between capping DNA and AgNPs, 2-mercaptoethanol (14 mM) was incubated with FAM-dA20-AgNPs (0.56 nM) overnight. After centrifugation, the fluorescence intensity of the supernatant was measured. To analyze the concentration of FAM-dA20 in the surpernatant, a standard curve was constructed for FAM-dA20 fluorescence intensity with different known concentrations in Tris-HCl buffer (pH 7.8, 10 mM) containing 14 mM of 2-mercaptoethanol.[2] According to the fluorescence intensity of the resultant supernatant, only ~39 out of 499 dA20 molecules were replaced on each AgNP, indicating highly stable conjugations.



Comparison of the fluorescence of initial FAM-labeled dA20 and the supernatant of FAM-dA20-AgNPs. The concentration of AgNPs was calculated to be 0.56 nM assuming all the silver is reduced. The surface coverage was estimated based on the amount of dA20 contributing to forming AgNPs. During fluorescence measurements, the excitation wavelength was set at 495 nm.

References: [1] Z. Wang, J. Zhang, J. M.Ekman, P. J. A. Kenis and Y. Lu, *Nano Lett.*, 2010, **10**, 1886. [2] L. M. Demers, C. A. Mirkin, R. C. Mucic, R. A. III. Reynolds, R. L. Letsinger, R. Elghanian and G. Viswanadham, *Anal. Chem.* 2000, **72**, 5535.

4. Supplementary figures

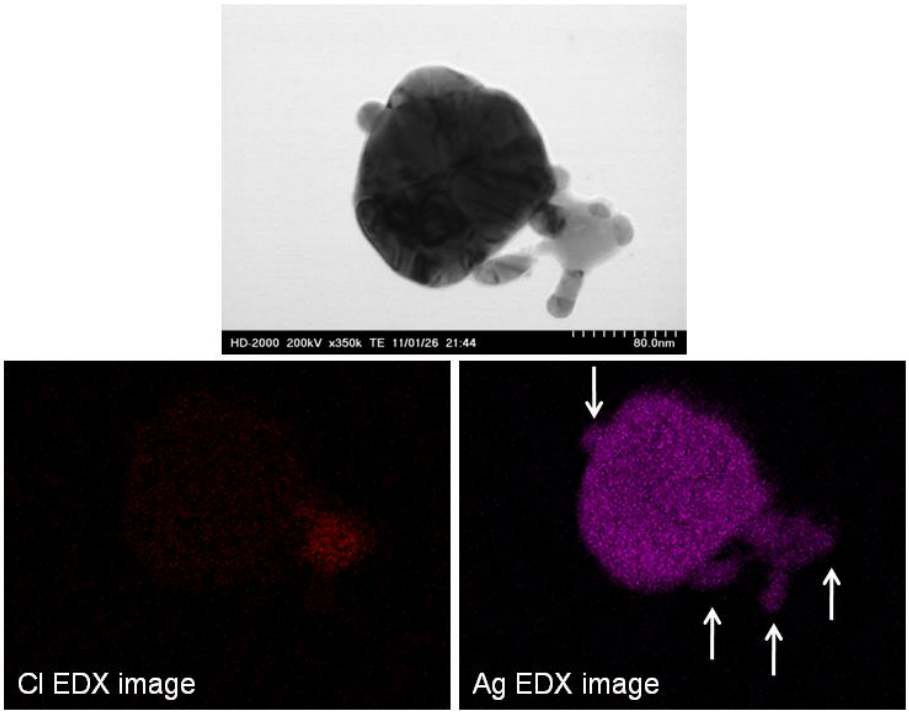


Fig. S1 Typical STEM image of the AgCl derivatives (top) obtained after UV irradiation for 1.5s in the presence of dA20, and corresponding EDX images (bottom) for the Cl and Ag elements. The arrows indicate silver nanostructures in accordance with the EDX images. The preparation of the precursor suspension included the addition of dA20 and Ag^+ ([nucleobase] = [Ag^+] = 100 μM) to a Tris-HCl buffer (10 mM, pH 7.8).

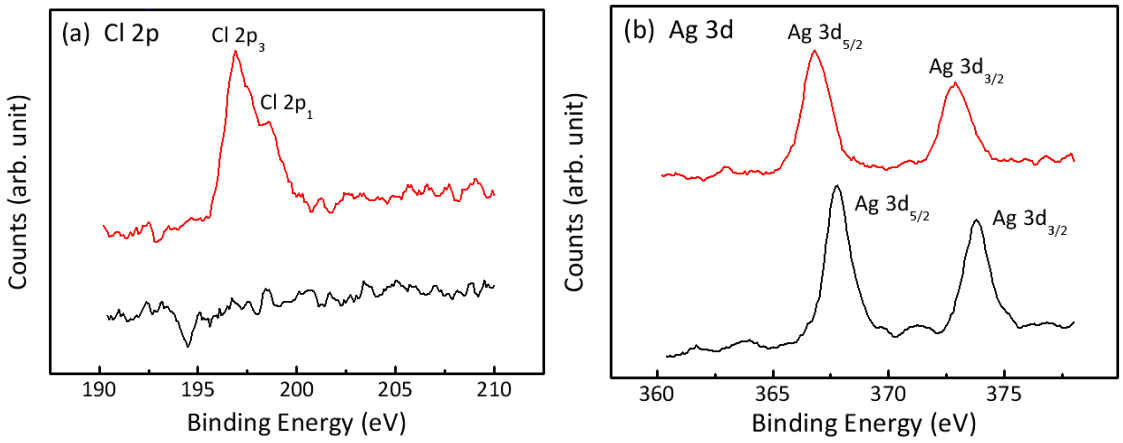


Fig. S2 XPS spectra of (a) Cl 2p and (b) Ag 3d of AgCl precursor before (red) and after (blank) the photoreduction in the presence of DNA. All spectra were calibrated with the C1s peak at 284.2 ev.

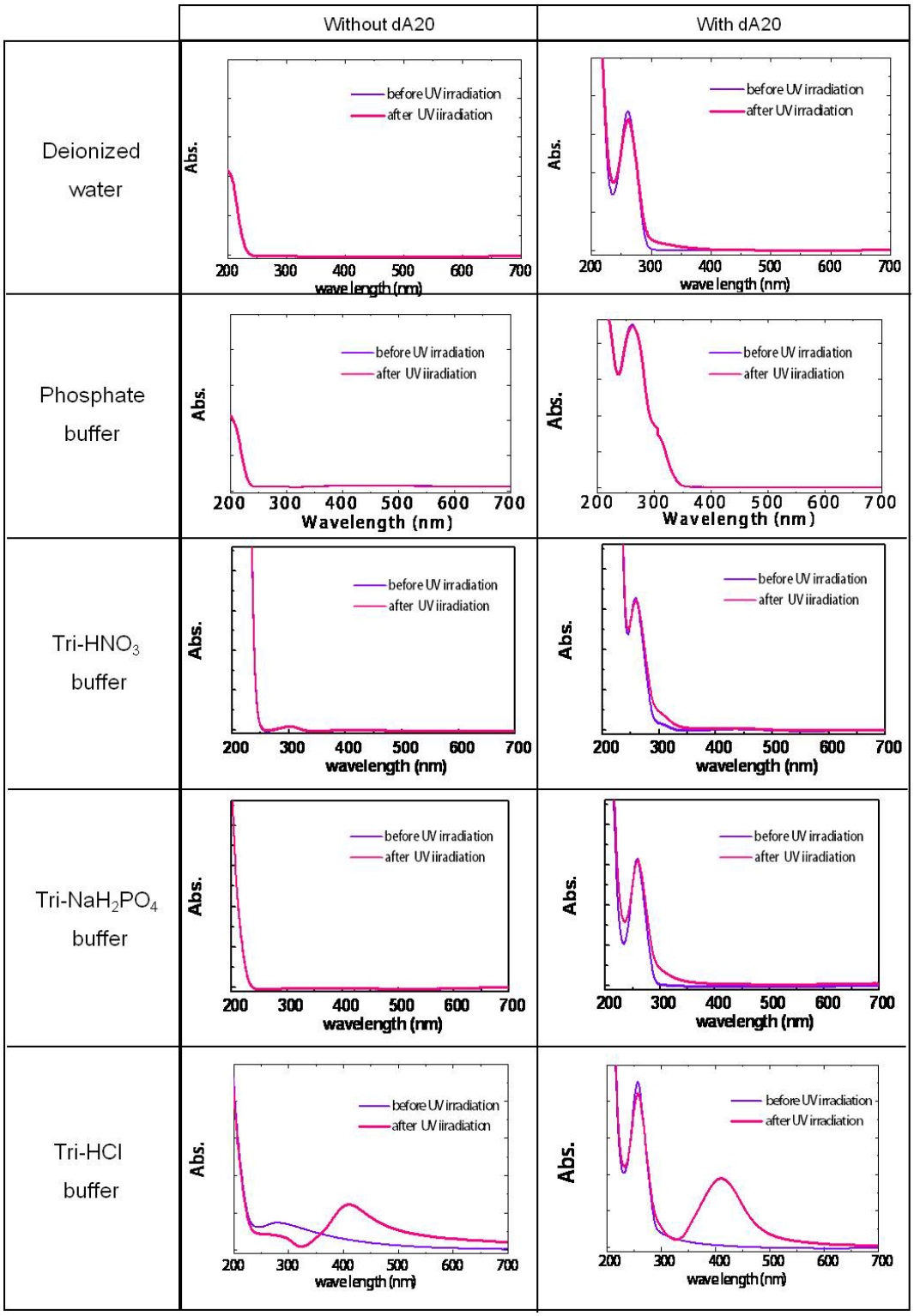


Fig. S3 Comparison of different aqueous systems used for synthesis of AgNPs in the absence (left) and presence (right) of dA20 in absorbance change after UV irradiation. The pH value of all buffers (10 mM) is adjusted to 7.8. (In all systems, [Ag] = 100 μM, where $AgNO_3$ was used as Ag source; When dA20 is used, [nucleobase] = [Ag^+] = 100 μM.)

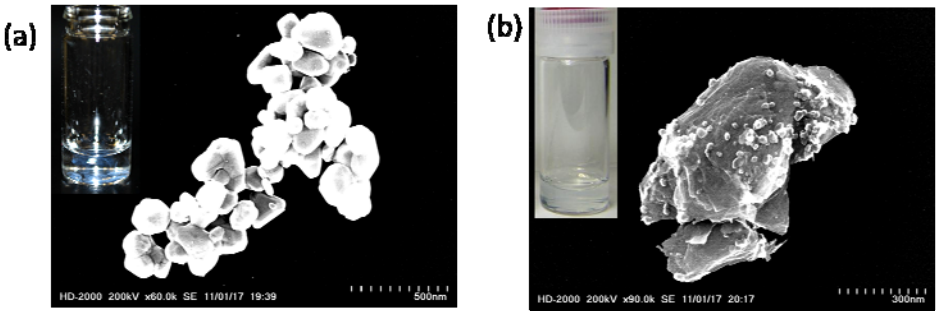


Fig. S4 Typical SEM image of AgCl dispersion in the absence of DNA before (a) and after (b) UV irradiation (365 nm, 150 mW/cm^2) for 5 min. Insert (a) indicates the photograph of Tris-HCl buffer (7.8, 10 mM) as soon as Ag^+ (100 μM) was introduced. Insert (b) shows the photograph of (a) after UV irradiation. See the absorption spectra of (a) and (b) in Fig. S3 for references.

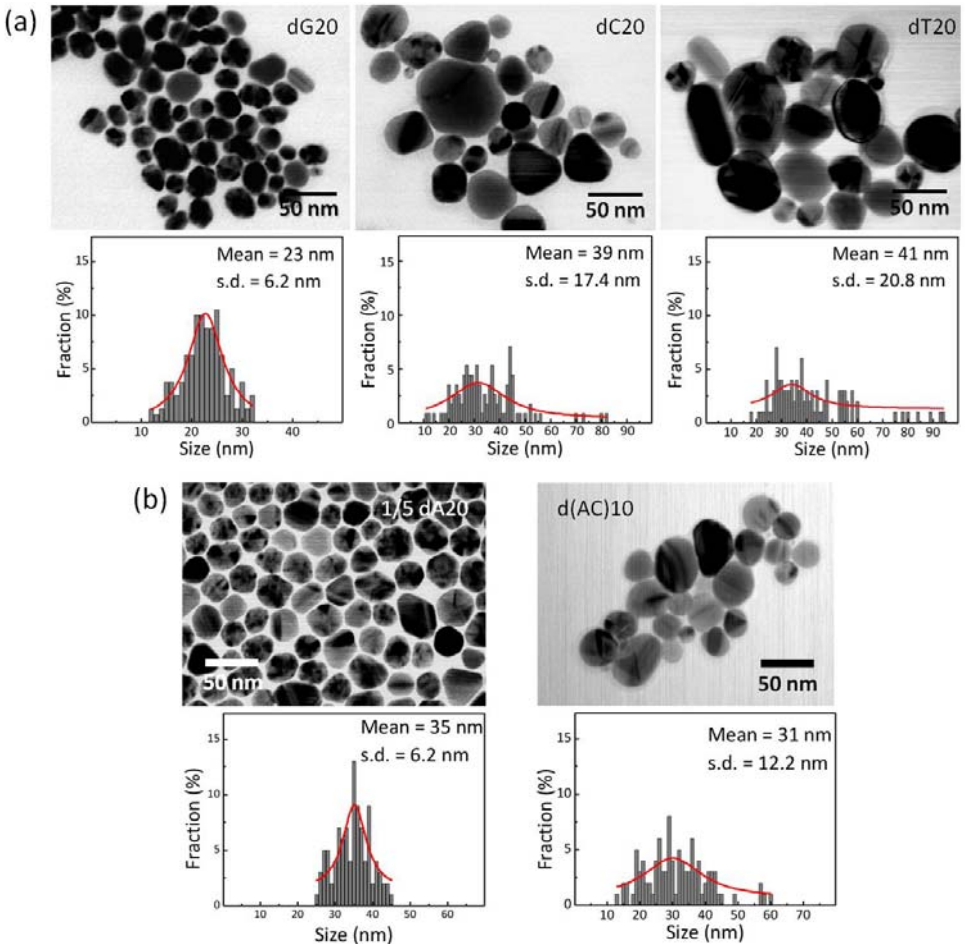


Fig. S5 (a)TEM images and size distributions (bottom) of the AgNPs synthesized with various homo-oligonucleotide. (b) TEM images and size distributions of AgNPs synthesized with dA20 at a low cocentration ([nucleobase] = 20 μM) (left), and d(AC)10-AgNPs (right) synthesized using a sequence of d(AC)10. All AgNPs were prepared by same procedure using the present method.

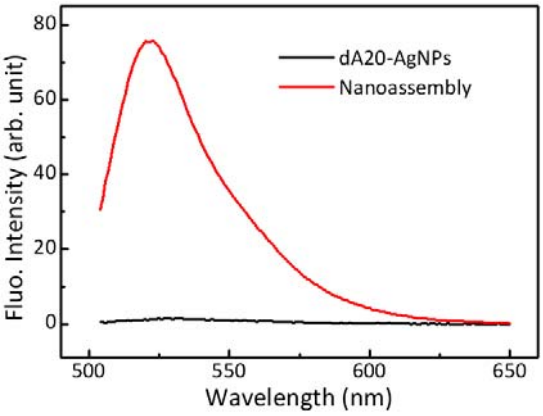


Fig. S6 Fluorescence spectra of Sybr Green I in the presence of dA20-AgNPs (black) and nanoassemblies (red), respectively. Before the fluorescence measurement (λ_{ex}=495 nm), Sybr Green I was incubated at a final concentration of 1× for 15 min.

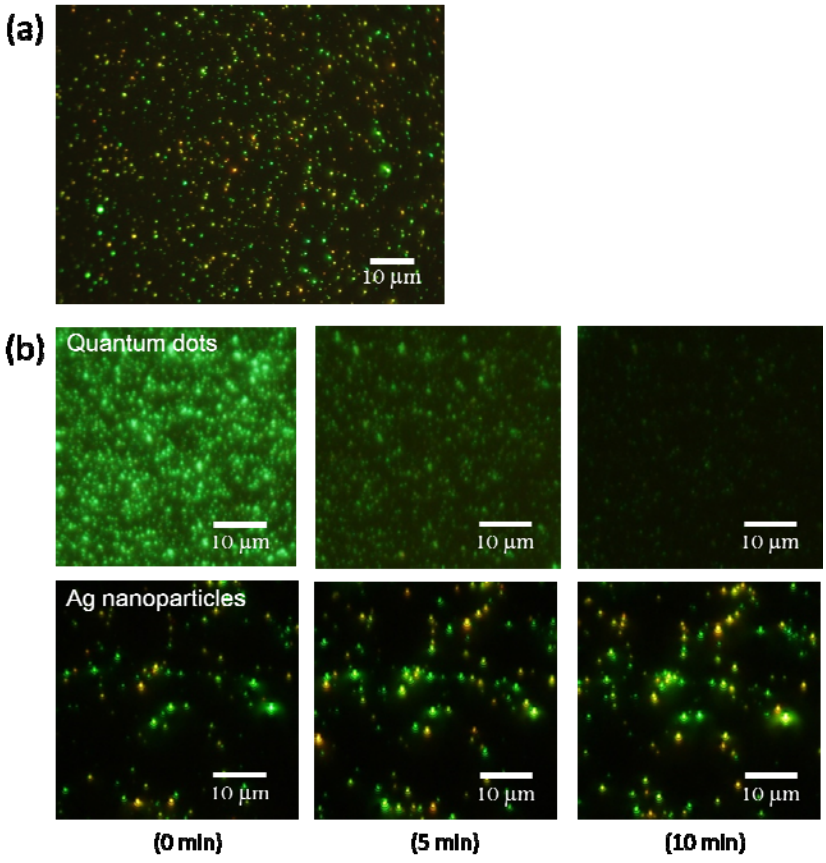


Fig. S7 (a) Luminescent image of dA20-AgNPs on a glass substrate under the excitation of blue light (460−490 nm) measured using a fluorescence microscope. (b) Photostability comparison between CdSe/ZnS core-Shell QDs and dA20-AgNPs under blue light excitation. The luminescent images were obtained under the blue light illumination for different times (0 min, 5 min and 10 min).

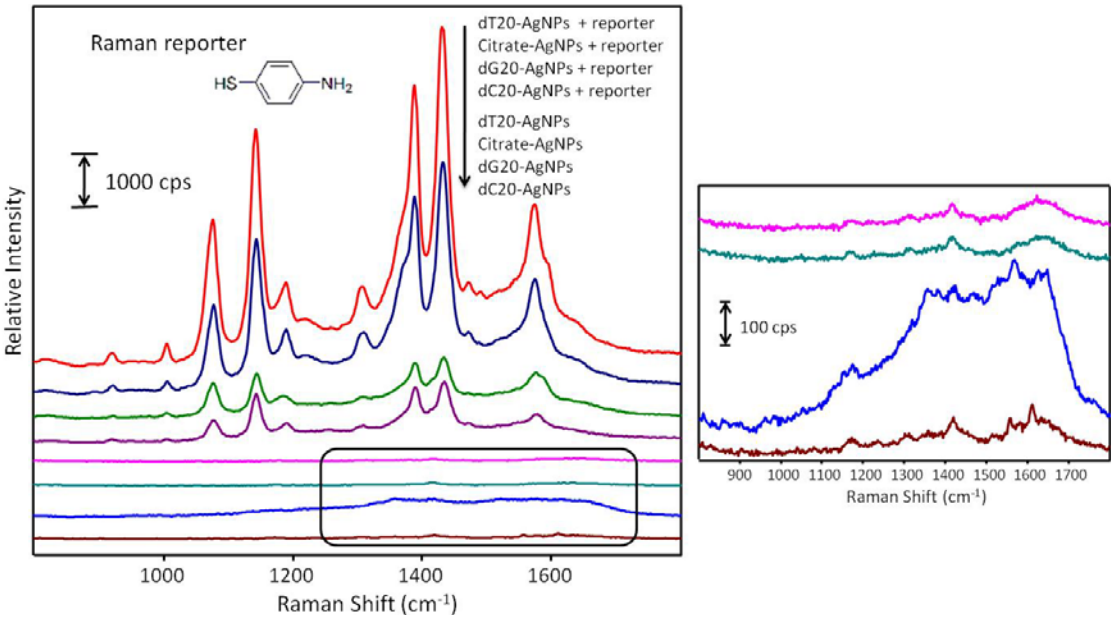


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Fig. S8 Left: SERS background signals (bottom four) and SERS spectra of p-aminothiophenol adsorbed on various AgNPs (top four), as indicated by the arrow and sample names. For measurements of p-aminothiophenol SERS spectra, the concentration was set at 1.0×10^{-5} M. Insert shows the structure of p-aminothiophenol. Right: The enlarged background signals of various nanoparticles prepared using homo-oligonucleotides.

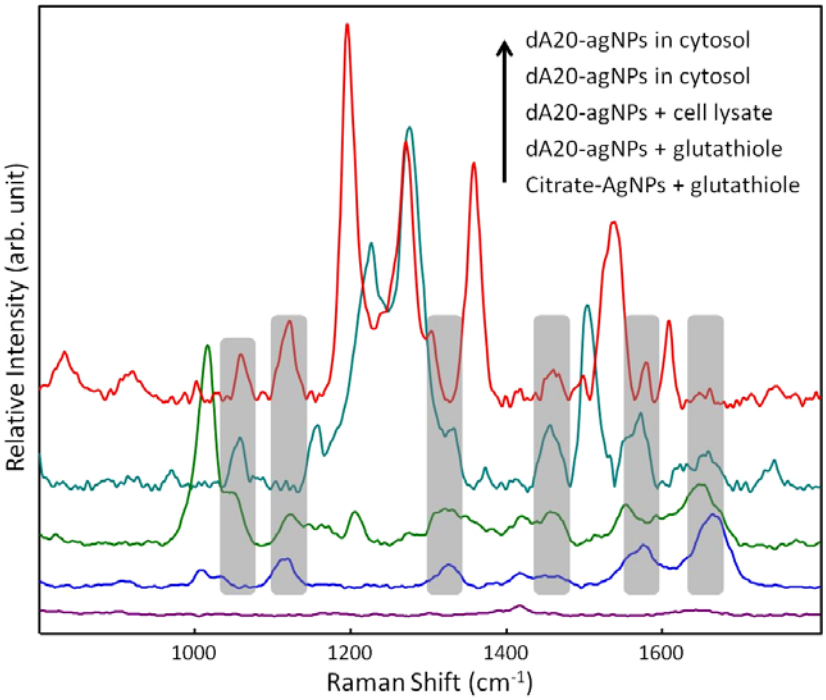

Fig. S9 Comparison of SERS spectra dA20-AgNPs internalized in the cell cytosol with that of dA20-AgNPs incubated with glutathione and cell lysate, respectively. The final concentration of glutathione was 5 mM, which is comparable to that in the cell. The mixing ratio of cell lysate and dA20-AgNPs was 20:1. The consistency of the Raman bands among the dA20-AgNPs internalized in cell, dA20-AgNPs incubated with cell lysate, and dA20-AgNPs incubated with glutathione demonstrates that the high concentration of glutathione in the cytosol affects the SERS signals of dA20-AgNPs.