Supporting Information

Experimental Section

Materials

300 ng/μl λ-DNA was purchased from Fermentas Life Sciences Ltd. Company (Shenzhen, China), and was extensively dialyzed in pure aqueous solution. *para*-Aminothiophenol (PATP) and all metal salts including Na_2CO_3, Na_3PO_4, $MgCl_2$, $CaCl_2$, $FeCl_3$, $CuSO_4$, and nitrate for others were A.R. grade and obtained from Shanghai Reagent Co. without further purification, and their solutions were prepared with distilled water.

Preparation of standard solutions of Hg^{2+} at nM and pM levels

To maintain a laboratory free of contamination at nM or pM levels of Hg^{2+}, the atomic fluorescence spectrometer (AFS, located in University of Science and Technology of China) as an independent measure of Hg^{2+} concentration was used to determine the concentrations of the prepared dilute solutions of Hg^{2+}. First, a stock solution of $Hg(NO_3)_2$ at 4×10^{-4} M was formulated. Then, 9 mL of distilled water and a 1 mL portion of stock solution were added into a pre-cleaned volumetric flask (10 mL), and mixed thoroughly to produce a Hg^{2+} solution of 4×10^{-5} M. Similarly, ten-fold serial dilutions were carried out till to obtain the apparent Hg^{2+} concentration of 4×10^{-10} M. The actual $[Hg^{2+}]$ in these solutions were determined by AFS (Fig. S1). The verified sub-nM concentrations of Hg^{2+} solutions were used to prepare the pM-level solutions.

General procedure for the preparation of DNA-Ag-PATP hybrid

The accurate determination of λ-DNA concentration was done by measuring the absorbance of the λ-DNA at 260 nm. λ-DNA with a final concentration of 150 ng/ul in the samples was used in the following experiments if not otherwise specified. To form a

DNA-Ag^+ complex with the desired stoichiometry, the moles of bases were first determined considering that λ-DNA has molecular weight of 31.5×10^6 daltons and contains 48502 base pairs. Based on this calculation, DNA-Ag^+ complexes with different *r* values were formed by adding the required amount of Ag^+ in a 1 μl droplet, so to minimize dilution effects.

The DNA-Ag^+ complexes were kept in dark for 1 h at room temperature, and the photoreduction was performed directly by exposure to bright sunlight. The exposure time was varied from several minutes to several hours. Then the required amount of PATP in a 1 μl droplet was added into the in-situ sunlight-reduced DNA-Ag system to form DNA-Ag-PATP hybrid.

General protocol for Hg^{2+} detection by the DNA-Ag-PATP hybrid

The as-prepared DNA-Ag-PATP hybrid with a volume of 400 μl was added into the cuvette with a 1 mm pathlength for CD measurements, and then a specific concentration of Hg^{2+} in a 1 μl droplet was added. For example, a 1 μl droplet of 4×10^{-11} M Hg^{2+} was added into the 400 μl hybrid sample, which produced a final Hg^{2+} concentration of 1×10^{-13} M, i.e. 0.1 pM, and we considered that the corresponding changes of the CD signal was induced by Hg^{2+} at this final concentration. To examine this effect cautiously, the corresponding CD signal was calibrated in terms of the changes of the total sample volume. The similar procedures were carried out in the selectivity experiments for different competing metal ions such as Na^+, Ni^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+},Co^{2+}, Cu^{2+}, Al^{3+}, and Cr^{3+} at their 10 μM levels, and for the mixtures of 0.050 pM Hg^{2+} with 1 μM for each ions.

Characterizations

CD spectra were recorded at a Jasco-810 spectrometer within the linear range of the instrument dynode voltage using the same cuvette (Hellma Ltd.) at 25 °C controlled by a circulator-bath temperature controller (Cell length: 0.1 cm; data pitch: 1 nm; band width: 1 nm; scanning speed: 100 nm/min; accumulation: 3). PATP, $AgNO_3$, and Ag NPs have

little CD signals in the FUV range of 300-190 nm, the baselines were recorded and subtracted from the spectra of samples in data processing, and the concentration of DNA was calibrated in terms of the changes of the total sample volume. All experiments were done in triplicate, the same below.

UV-vis spectroscopy measurements were carried out on a TU-1901 model UV-vis double beam spectrophotometer (Beijing Purkinje General Instrument Co., Ltd, China) operated at a resolution of 0.5 nm.

TEM analysis were performed on a JEM -2100 electron microscope instrument (Japan Electron Co.), and the samples were prepared by drop-coating films of the solution on carbon-coated copper grids, allowing the grid to stand for 2 min, following which the extra solution was removed using blotting paper.

SERS spectra were recorded with a Renishaw 2000 model confocal microscopy Raman spectrometer (Renishaw Ltd., Gloucestershire, UK). Radiation of 514.5 nm from an air-cooled argon ion laser was used for the SERS excitation. All of the spectra reported were the results of a single 3 s accumulation. To prepare the samples, 10 ml DNA-Ag-PATP hybrid in the absence and presence of 1 μM Hg^{2+} were centrifugated (16000 rpm, 30 min), respectivley, the supernatants were removed, the pellets in bottom were dropped onto silicon sbustrates, and subsequent drying in air naturally.

The response kinetics of DNA-Ag-PATP hybrid on Hg^{2+} was measured on a planar polarization interferometer (PPI) based on TiO_2/glass composite optical waveguide (COWG) located in Institute of Electronics, Chinese Academy of Sciences.[1]

Supplementary Figures (S1-S11)

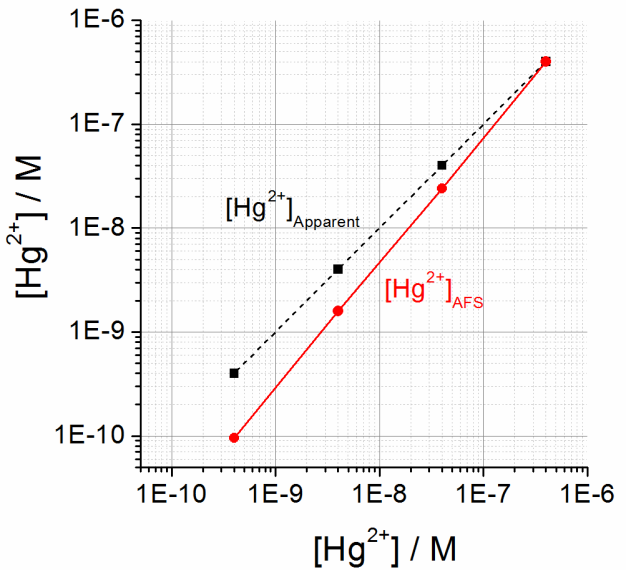

Fig. S1. The apparent [Hg^{2+}] in ten-fold serial dilution (*squares*) and the corresponding AFS-determined [Hg^{2+}] (*circles*) of the as-prepared solutions of Hg^{2+}. The log of [Hg^{2+}] was used just in order to show clearly the changes in a large scale of the Hg^{2+} concentrations.

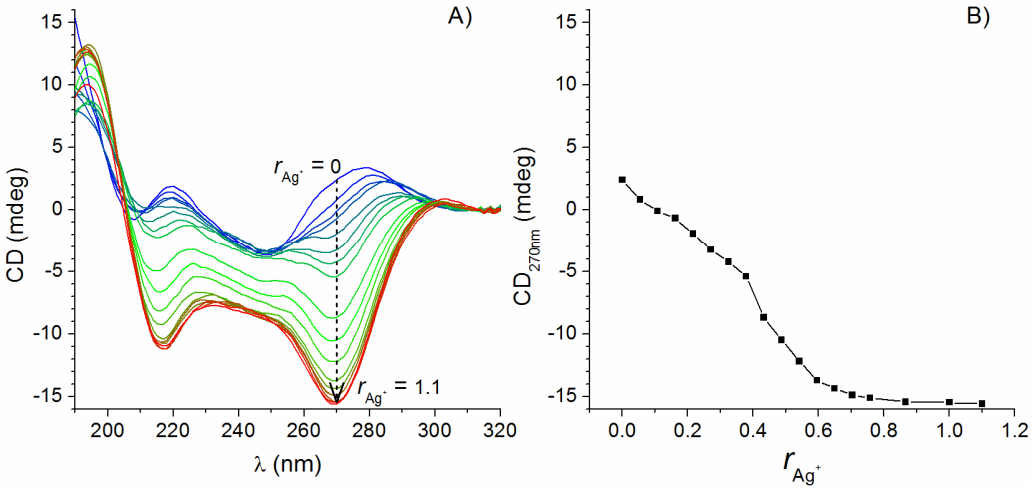

Fig. S2. A) CD spectra of λ-DNA-Ag^{+} complexes at different *r* values (*r* = the ratio of metal ion added per mole of DNA base). B) The correlation between the changes in CD magnitude of λ-DNA-Ag^{+} complexes at 270 nm and the *r* values.

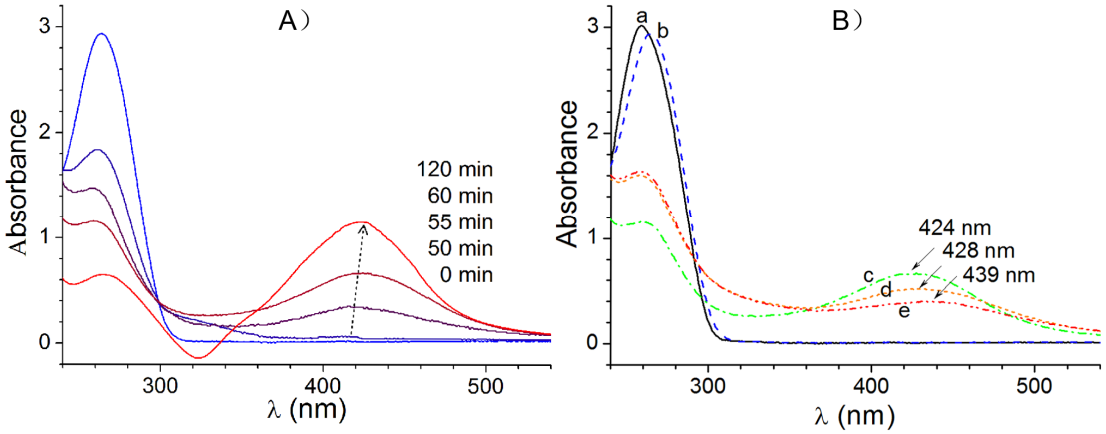

Fig. S3. A) UV-vis absorption spectra showing the photoreduction sequence for DNA-Ag^+ complex at r_{Ag+}=1.0. As the exposure time increased, a peak formed at around 420 nm demonstrating the formation of Ag NPs and its magnitude increased gradually. The absorption spectra became stable at about 3 hours, hence only partial Ag^+ was reduced after 60 min of exposure. B) UV-vis absorption spectra of DNA-Ag-PATP hybrid at different stages for detecting Hg^{2+}: a) Pure λ-DNA in aqueous solution; b) DNA-Ag^+ complexes; c) DNA-Ag synthesized by exposing b to sunlight in 60 min; d) DNA-Ag-PATP hybrid; e) Hg^{2+} with a final concentration of 0.1 μM was added into d. The conditions used in this system are as follows: [DNA bases]=460 μM, [$AgNO_3$]=460 μM (corresponding to r_{Ag+}=1.0), the exposure time=60 min, and [PATP]=10 μM.

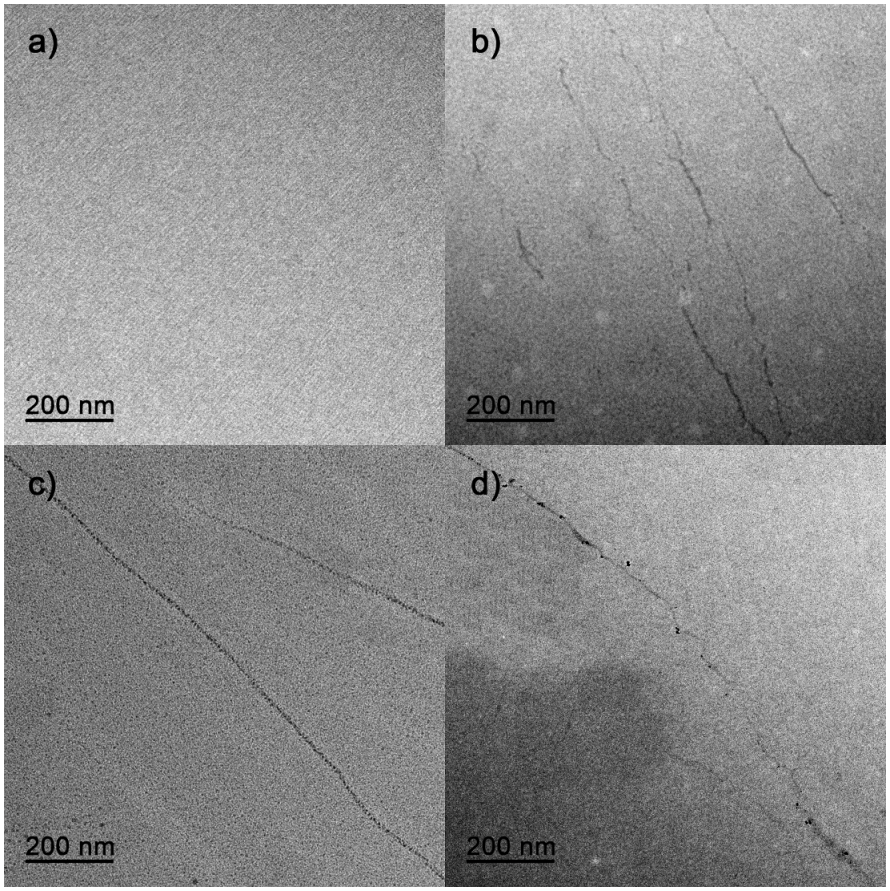

Fig. S4. TEM images of a) 0 min, b) 50 min, c) 60 min, and d) 120 min of exposure. DNA-Ag^{+} complexes could not be observed in our TEM experiments, after 50 min of exposure, the shape of DNA could be vaguely visible although the SPR peak at 420 is not yet to emerge apparently. At about 60 min, the Ag NPs can be seen clearly, which are arranged in a long linear pattern and reflects the shape of DNA molecules. After 2 hours of exposure, much larger Ag NPs is formed.

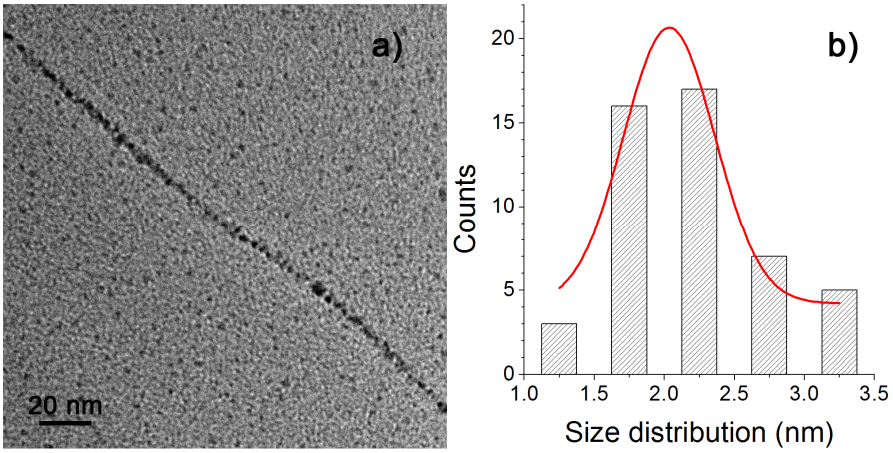

Fig. S5. A) TEM image and B) the Size distribution histogram of Ag NPs reduced by 60 min of exposure. 48 particles were counted in A), and it was obvious that the diameters of most visible particles were less than 3 nm with a mean value of about 2 nm, which is similar large to the diameter of DNA molecule.

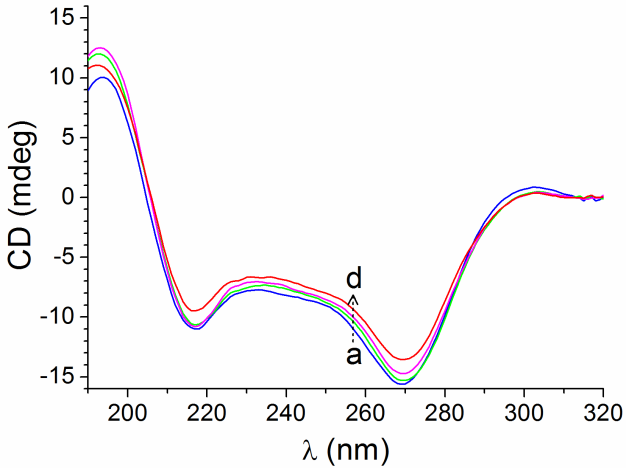

Fig. S6. Effects of bright sunlight on the CD spectra of DNA-Ag^{+} complex at r_{Ag+}=1.0 for different exposure time: a) 0 min, b) 60 min, c) 90 min, d) 120 min. 60 min of exposure nearly has no effects on the CD spectra but an increase of the 195 nm peak. As the exposure time was further extended, the magnitudes of 215 and 270 nm peaks were decreased and the magnitude of 195 nm peak was increased. These results imply that small Ag NPs formed on DNA molecules did not much change the CD spectra of DNA-Ag, whereas the larger Ag NPs induce a compensatory stress on the embedded template and consequentially alter the DNA structure (data not shown).[2]

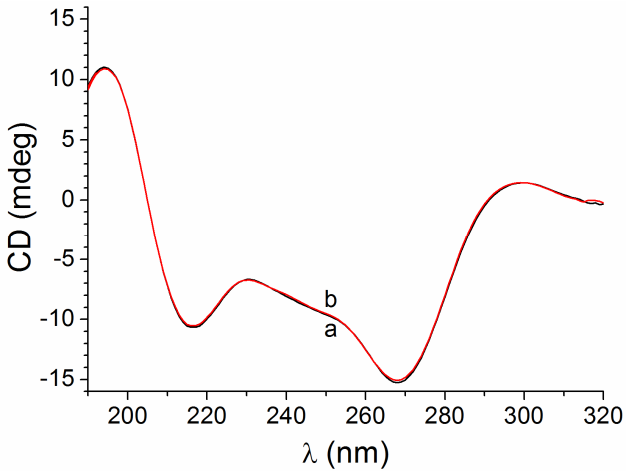

Fig. S7. CD spectra of DNA-Ag in the absence a) and presence b) of Hg^{2+} at a final concentration of 1 μM.

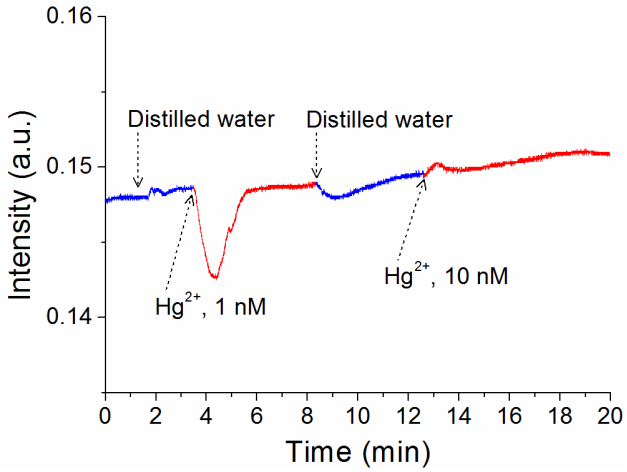

Fig. S8. The PPI based on TiO_2/glass composite optical waveguide (COWG) was used for real-time and in situ measurement of Hg^{2+}. Prior to measurement the COWG chip surface was modified by DNA-Ag-PATP hybrid solution. Then the distilled water as the background was pumped into the fluidic chamber. When the sensor signal become stable, the distilled water was exchanged into the Hg^{2+} aqueous solution at a concentration of 1 nM. The interference pattern of the sensor induced by interaction between Hg^{2+} and DNA-Ag-PATP hybrid was clearly observed. When the sensor signal was no longer changed, the distilled water was once again pumped into the chamber to remove the Hg^{2+} that did not adsorbed on the surface, resulting in a very small signal change. It can be

concluded that the binding between Hg^{2+} and DNA-Ag-PATP hybrid is strong and irreversible.[1] This conclusion is further proofed by the observation that the injecting again of Hg^{2+} solution with a concentration of 10 nM nearly did not change the signal. Similar experiment was carried out when the COWG chip surface was modified by DNA-Ag in the absence of PATP, and Hg^{2+} at a concentration of 1 nM did not change the sensor signal, indicating no binding was occurred between the DNA-Ag and Hg^{2+}.

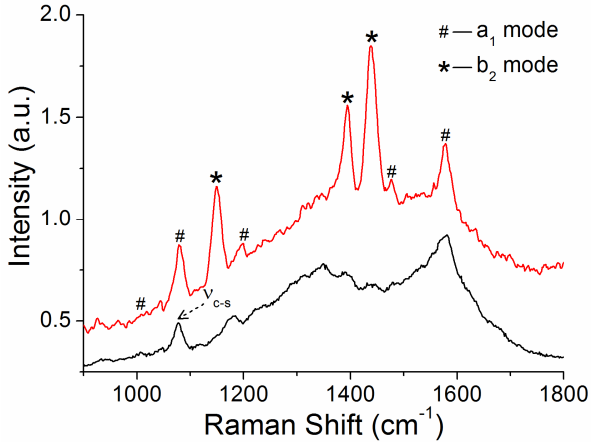

Fig. S9. SERS spectra of PATP absorbed on DNA-Ag in the absence (down) and presence (top) of Hg^{2+} at a concentration of 1 μM.

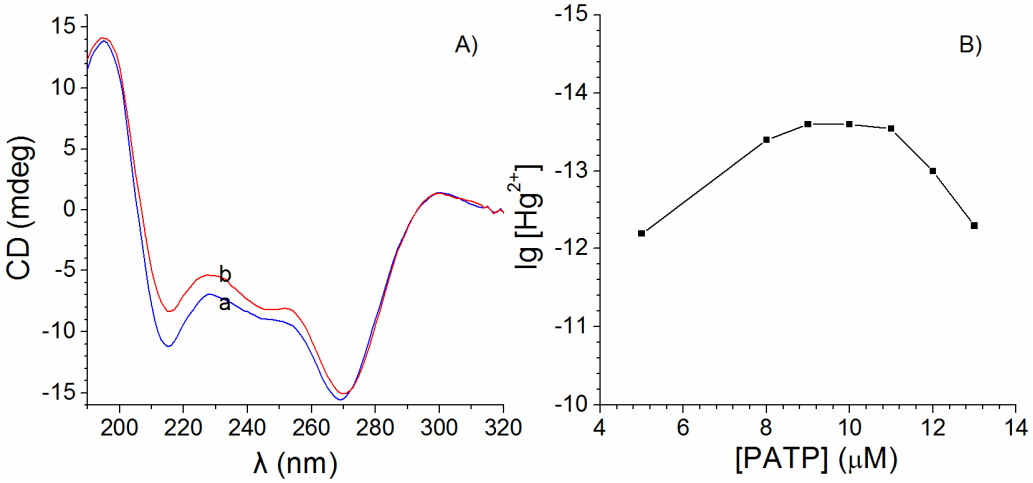

Fig. S10. A) CD spectra of a) DNA-Ag and b) DNA-Ag-PATP hybrid with a PATP concentration of 10 μM. B) Effects of PATP concentrations on the detection limit of Hg^{2+} at [bases]=460 μM, r_{Ag^+}=1.0, and exposure time=60 min.

References

[1] Y.M. Shirshov, B.A. Snopok, A.V. Samoylov, A.P. Kiyanovskij, E.F. Venger, A.V. Nabok, A.K. Ray, Analysis of the response of planar polarization interferometer to molecular layer formation: fibrinogen adsorption on silicon nitride surface, Biosensors & Bioelectronics 16 (2001) 381-390.

[2] S. Roy, S. Basak, A.K. Dasgupta, Nanoparticle Induced Conformational Change in DNA and Chirality of Silver Nanoclusters, Journal of Nanoscience and Nanotechnology 10 (2010) 819-825.