

## Supplementary Material (ESI)

for

### **Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells**

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#### **Part I. Experimental Section**

**Materials.** All chemicals were purchased from Sigma-Aldrich Chemical Co. unless otherwise noted. Analytical grade organic solvents and double distilled deionized water were used throughout the experiments.

**Instrumentation.** The XRD spectrum was recorded with Philips PW1830 powder X-ray diffractometer. UV-vis absorption spectra were taken using Varian Cary 50 spectrophotometer. TEM images of the silver nanoparticles were taken with JEOL JEM-2000 transmission electron microscopy (accelerating voltage of 200 kV), Philips Tecnai 20 equipped with Oxford incax-sight EDX attachment (accelerating voltage of 200 kV), or Philips EM208s (accelerating voltage of 80 kV).

**Preparation of Silver Nanoparticles.** Aqueous AgNO<sub>3</sub> solution (50 mM, 1 mL) was slowly added to a round bottom flask with a magnetic stirring bar containing 48 mL Hepes buffer (10 mM, pH 7.4) and the mixture was refluxed for 4 h. The concentration of the silver nanoparticles was measured by Inductively-Coupled Plasma Mass Spectroscopy (ICP-MS).

#### **Biological Studies and Measurement.**

*Materials.* HIV-1(BaL) stock (300 ng/mL of p24 protein) and Hut/CCR5 cells were the gifts from Dr. D. Tao (Institute of Molecular Medicine, University of Oxford, UK). Peripheral blood mononuclear cells (PBMC) were prepared from buffy coat obtained from the Hong Kong Red Cross Blood Transfusion Service. Each unit of the buffy coat prepared from whole blood (450 mL) contained approximately  $5 \times 10^8$  cells. To isolate the PBMC, the buffy coat (15 mL) was transferred

to a centrifuge tube (50 mL) under sterile condition and was diluted by RPMI culture medium in a 1:1 (v/v) ratio. Ficoll® solution (15 mL) was gently added to the diluted blood and the mixture was centrifuged at 800 rpm for 25 min at room temperature. The PBMC at the interface were washed five times with cold RPMI culture medium. The washed PBMC were spun at first 500 rpm for 7 min, then at 250 rpm for 7 min and finally at 200 rpm to obtain a cell pellet. Hut/CCR5 cells and PBMC were maintained in the RPMI 1640 medium (Life Technologies, Inc.) supplemented with fetal bovine serum (10%, v/v), L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 µg/mL), and were incubated at 37 °C in a 5% CO<sub>2</sub>/95% air humidified atmosphere.

*Anti-HIV-1 Assay of Silver Nanoparticles.* Stock solutions (1 mM) of the silver nanoparticles were prepared in Hepes buffer and diluted to desired concentrations in growth medium. Hut/CCR5 cells in 24-well plate ( $5 \times 10^5$  cells/well) were infected with a panel of CCR5-tropic HIV-1 isolates (with the addition of 5 µL of HIV-1 stock to each well, total p24 content = 1500 pg) for 3 h. Following infection, the cells were washed to remove residual viral inoculums and cultured in the presence of silver nanoparticles (400 µL) for 3 days. Culture supernatants were harvested and viral replication was measured by determination of viral p24 antigen concentration by ELISA (HIV-1 p24 antigen Kit, Beckman Coulter). Compounds were tested in triplicate at 3 concentrations ranging from 0.5–50 µM.

*Terminal Uridyl-Nucleotide End Labeling (TUNEL) Assay.* HIV-1 infected Hut/CCR5 cells were treated with silver nanoparticles (10 nm, 5 and 50 µM) for 3 days. The TUNEL method (*In Situ* Cell Death Detection Kit, Fluorescein, Roche) combined with flow cytometry was used for the measurement of apoptosis (Gorczyca, W.; Gong, J.; Darzynkiewicz, Z. *Cancer Res.* **1993**, *53*, 1945). The quantitative analysis of 3'-OH ends was performed using the MDADS analysis program (Beckman Coulter).

*Cell Viability Assay of Silver Nanoparticles.* Assays on the cell viability of Hut/CCR5 cells and PBMC were conducted in 96-well flat-bottomed microtitre plates. The supplemented culture medium (90 µL) with cells ( $1 \times 10^6$  cells per mL) was added into a 96-well plate. Stock solution (1 mM) of the silver nanoparticles was prepared in Hepes buffer and diluted to desired concentrations in growth medium, and the diluted solutions were subsequently added into a set of wells. Control well contained only supplemented media (100 µL). Microtitre plates were incubated at 37 °C in a 5% CO<sub>2</sub>/95% air humidified atmosphere for further 7 days. All the assays were run in parallel with a negative control (i.e., vehicle control) and a positive control with cisplatin as cytotoxic agent.

Assessments of the cell viability were carried out using a modified method of Mosmann based 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay [Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55]. At the end of each incubation period, MTT solution (10 µL, Cell Proliferation Kit I, Roche) was added into each well and the cultures were further incubated for 4 h

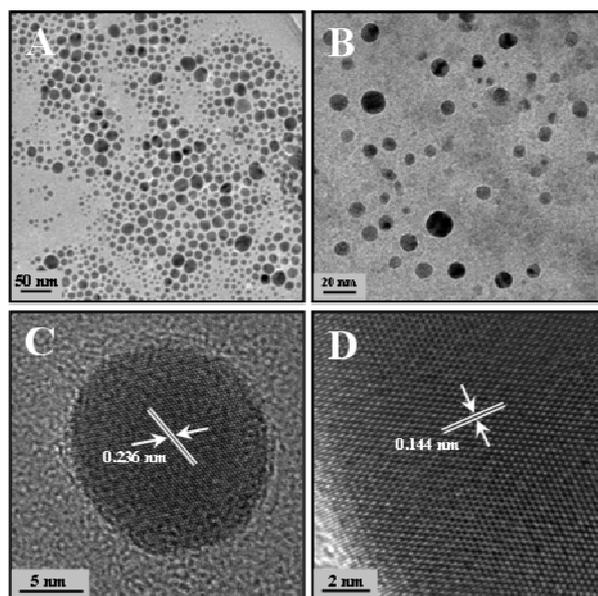
at 37 °C in a 5% CO<sub>2</sub>/95% air humidified atmosphere. A solubilizing solution (100 μL) was added into the wells to lyse the cells and to solubilize the formazan complex formed. The microtitre plates were maintained in a dark, humidified chamber overnight. The formation of formazan was measured with a microtitre plate reader at 550 nm and the percentages of cell survival were determined. The cytotoxicity was evaluated based on the percentage cell survival in a dose-dependence manner relative to the negative control.

*Inhibition Studies of HIV-1 Reverse Transcriptase (RT) by Silver Nanoparticles.* Assays on the *in vitro* HIV-1 RT inhibitory activities were conducted by using a commercial assay kit (Reverse Transcriptase Assay, Chemiluminescent, Roche). Stock solutions of silver nanoparticles and AZT-TP were prepared using Hepes buffer and mixed with a set of HIV-1 RT in the lysis buffer (2 ng, 128.7 μL) at 37 °C for 30 min. The ELISA assays were conducted by following the manufacturer's instruction [Eberle, J.; Seibl, R. *J. Virol. Methods* **1992**, *40*, 347]. The HIV-1 RT activities were evaluated based on the percentage luminescence of the solutions in a dose-dependent manner relative to the vehicle control.

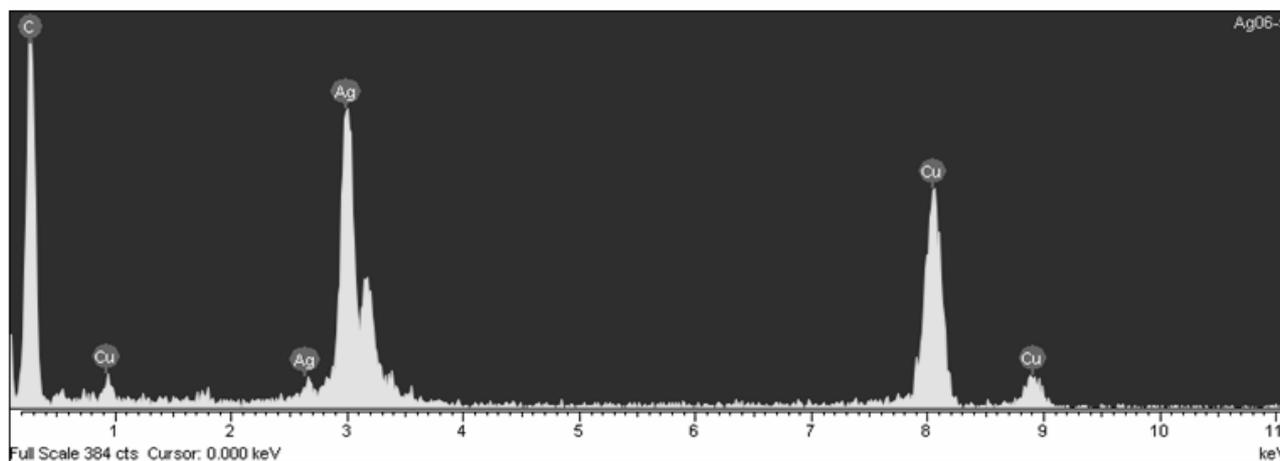
*Absorption Titration.* A solution of silver nanoparticles (5 μM) in Hepes buffer (3000 μL) was placed in a thermostatic cuvette in a UV-vis spectrophotometer. Aliquots of a millimolar stock human serum album (HSA) solution were added to the solution. Absorption spectra were recorded after equilibration for 10 min per aliquot until saturation point was reached.

**Part II. Figures**

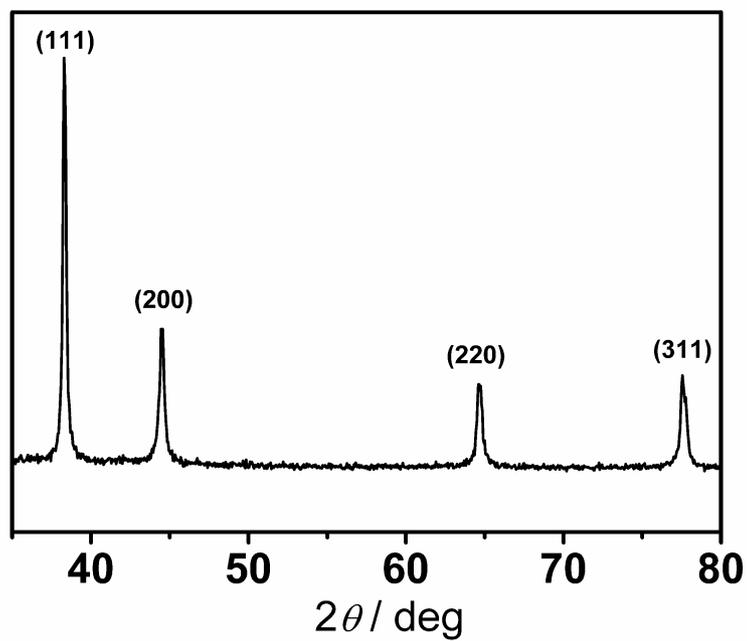
**Figure S1.** TEM (A and B) and HR-TEM (C and D) images of silver nanoparticles.



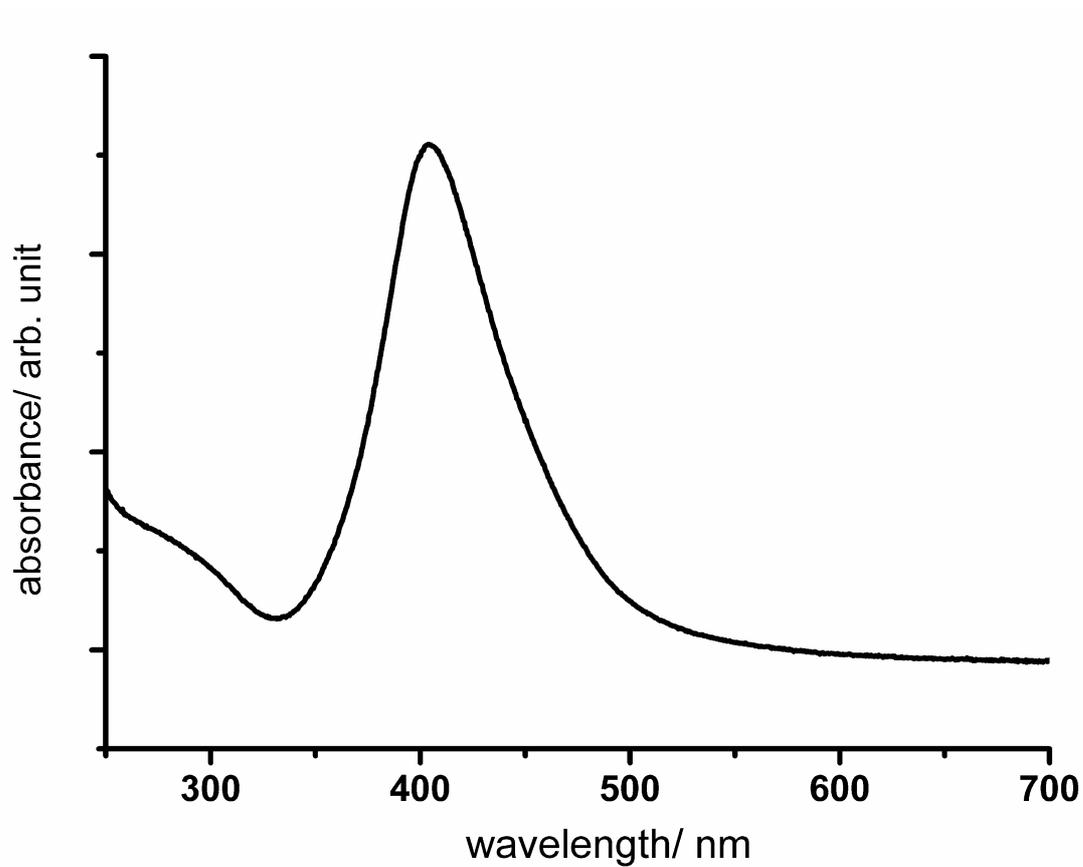
**Figure S2.** Spot-profile energy-dispersive X-ray analysis (EDX) of individual silver nanoparticles.



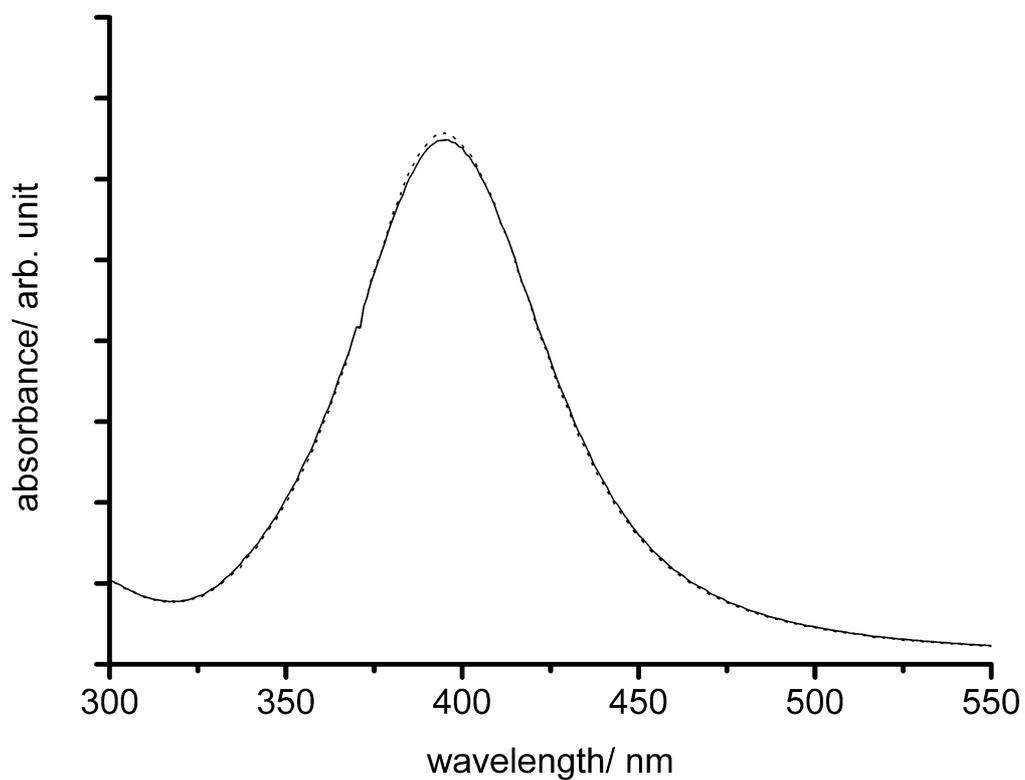
**Figure S3.** X-ray diffraction (XRD) pattern of silver nanoparticles in Hepes buffer (pH 7.4).



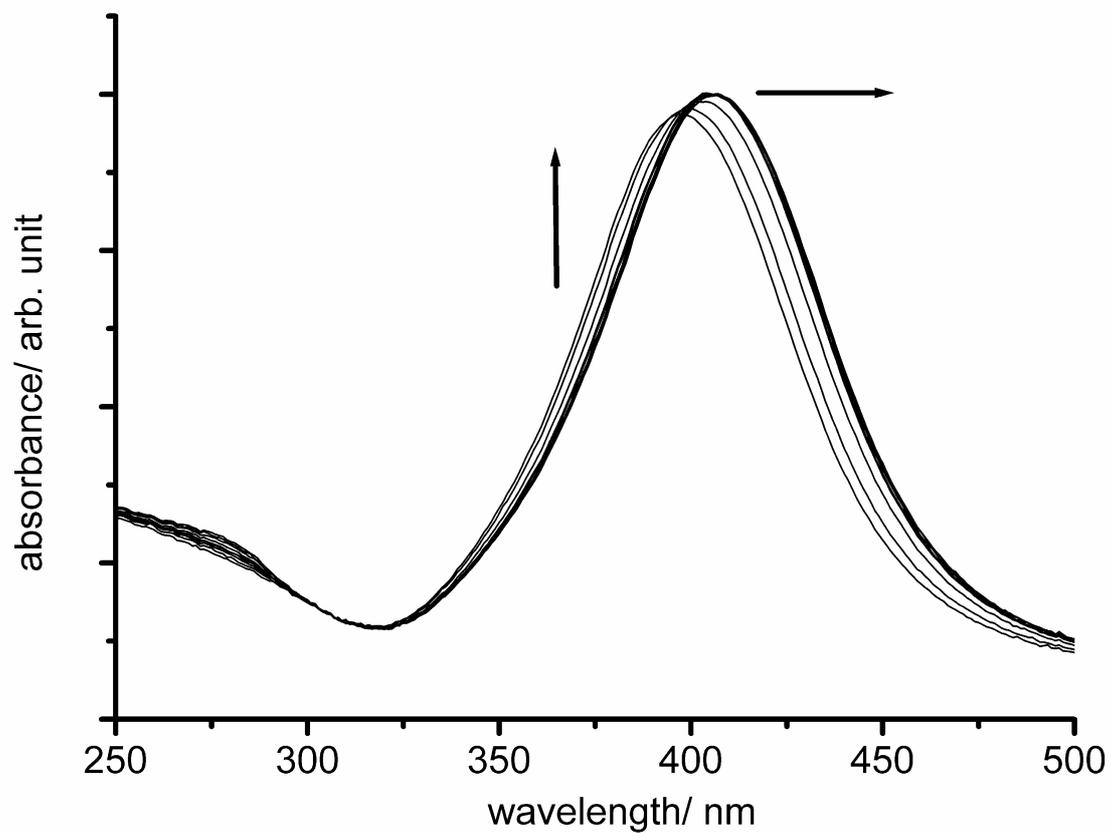
**Figure S4.** UV-vis spectrum of silver nanoparticles ( $50 \mu\text{M}$ ) in Hepes buffer (pH 7.4).



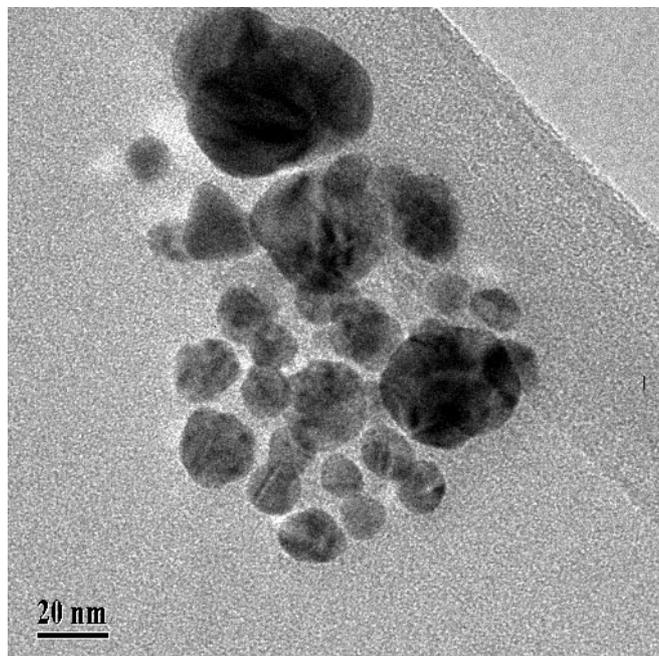
**Figure S5.** UV-vis spectra of silver nanoparticles (1 mM stock solution) in Hepes buffer (pH 7.4) containing HSA (2 mM) at time = 0 day (solid line) and time = 7 days (dotted line).



**Figure S6.** UV-vis spectral changes of silver nanoparticles ( $50 \mu\text{M}$ ) in Hepes buffer (pH 7.4) with increasing concentration of human serum albumin (HSA).



**Figure S7.** TEM image of silver nanoparticles after treatment with HSA (2 mM) for 3 days at 37 °C.



**Figure S8.** Percentage survival of Hut/CCR5 cells in the presence of silver and gold nanoparticles.

