Electronic Supplementary Information for

Sensitive and Selective Resonance Light Scattering Bioassay for Trace Homocysteine in Biological Fluids Based on Target-involved Assembly of Polyethyleneimine-capped Ag-Nanoclusters

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Chemicals and Materials. All chemicals used are of at least analytical grade. Silver nitrate was from Chengdu Chemial Reagents Co. (Chengdu, China). Formaldehyde was from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Branched polyethyleneimine (PEI) (M_w = 10000, 99%) was from Alfa Aesar (Tianjin, China). Ultrapure water was obtained from a WaterPro water purification system (Labconco Corporation, Kansas City, MO, USA). Hcy was from Aldrich (Steinheim, Germany). All the other amino acids and reduced GSH were from Newprobe Biotechnology Co. Ltd. (Beijing, China). HSA was purchased from Sigma. Aqueous solutions of K^+, Na^+, Ca^{2+} Mg^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} were prepared from KNO_3, NaCl, $Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3){\cdot}6H_2O$, $Fe(NO_3)_3{\cdot}9H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$ and $Zn(NO_3)_2{\cdot}6H_2O$ (Tianjin Guangfu Fine Chemical Research Institute, Tianjin, China), respectively. All solutions were freshly prepared before use.

Preparation of PEI-capped Ag-nanoclusters. PEI-capped Ag-nanoclusters were synthesized according to a recently reported method,[5a] but with modification. Briefly, 5 mL of 100 mM $AgNO_3$ and 1.67 mL 0.05 g mL^{-1} PEI were added into 3 mL H_2O, and homogenized by stirring for 2 min. Then, 96 μL of formaldehyde solution (35%) was added under vigorous stirring. The

colloidal Ag solution was further stirred for 10 min and was subsequently centrifugated at 12,000 rpm for 15 min four times to remove larger particles. Thus, stable solution of the as-prepared PEI capped Ag nanoclusters was obtained for further use, which contained 37 mM silver as determined by ICP-MS. 10000 Da of PEI was used for effective stabilization of the Ag-nanoclusters.

Instrumentation and characterization. The Ag-nanoclustes were characterized by HRTEM on a Philips Tecnai G2 F20 microscope (Philips, Holland) operating at a 200 kV accelerating voltage. The samples for HRTEM were obtained by drying sample droplets from PBS dispersion onto a 300-mesh Cu grid coated with a lacey carbon film. The RLS measurements were performed on an F-4500 spectrofluorometer (Hitachi, Japan) equipped with a plotter unit and a quartz cell (1 cm × 1 cm) in the synchronous mode. The slit width was 10 and 10 nm for excitation and emission, respectively. The photomultiplier tube (PMT) voltage was set at -400 V. The dynamic light scattering (DLS) measurements were carried out on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (BI-9000AT) at 636 nm at 70 ^{o}C. All samples for DLS was prepared by filtering 2 mL of aqueous solutions through 0.45 μm Millipore filters.

Detection of Homocysteine. 10 mL of 10 mM PBS in a capped tube was heated in a water bath at 70 ^{o}C for 5 min, then 100 μL of the as-prepared Ag nanoclusters and 400 μL of Hcy standard solution or ultrafiltrated serum sample were added immediately. The resultant mixture was further heated in a water bath at 70 ^{o}C for 10 min, and then the RLS signal was monitored.

Note: The above procedure led to 100-times dilution of the as-prepared Ag nanoclusters solution. Less dilution of the Ag-nanoclusters solution gave higher sensitivity, but narrower linear range (See Fig. S1).

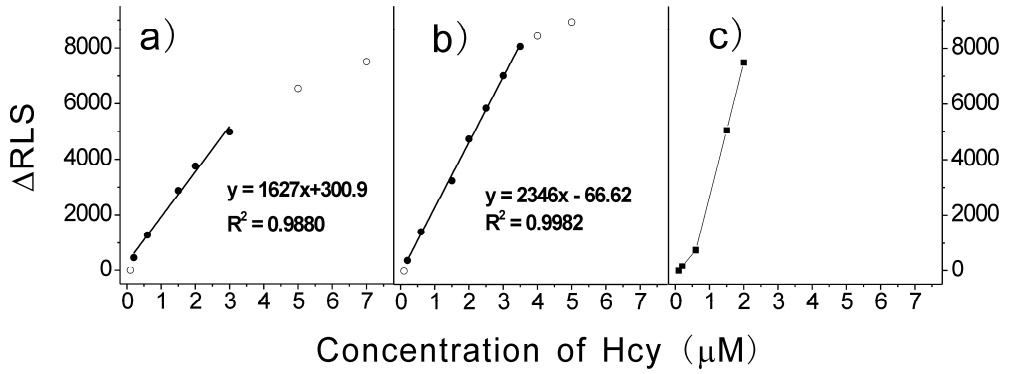

Fig. S1. Calibration curves at various times dilution of the as-prepared Ag-nanoclusters solution: (a) 200; (b) 100; (c) 50 times dilution.

Sample Collection and Pretreatment. The human serum samples were obtained from healthy volunteers in local hospital with informed consent. The serum samples are filtrated with Amicon Ultra-4 centrifugal Filter Units (30 kDa) by centrifugation at 6000 rpm for 15 min before detection.

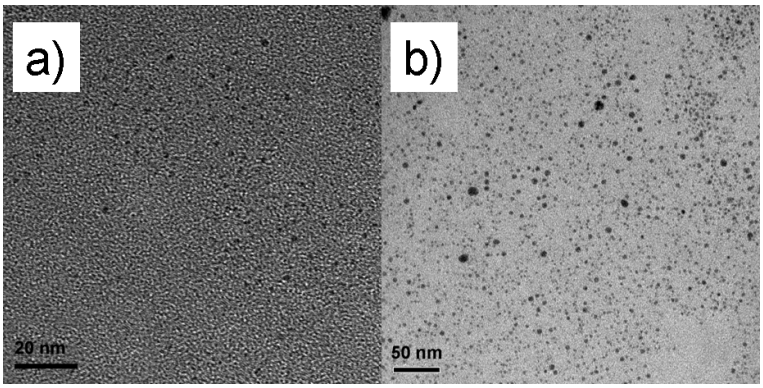

Fig. S2 HRTEM images: a) the as-prepared PEI-capped Ag-nanoclusters; b) PEI-capped Ag-nanoclusters after heating at 70 ^{o}C for 10 min. Average size of the nanoclusters/particles (nm): a) 1.8±0.2; b) 3.1±0.7 nm. The as-prepared PEI-Ag-nanoclusters are very small (1.8±0.2 nm), so no features as crystallinity of metallinity were shown by TEM.

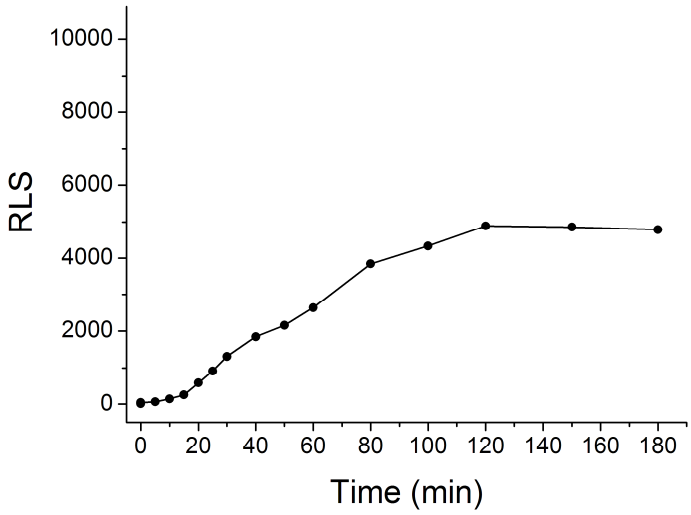

Fig. S3 The kinetics of interaction between Ag-nanoclusters and 3 μM Hcy at room temperature (20 ^{o}C).

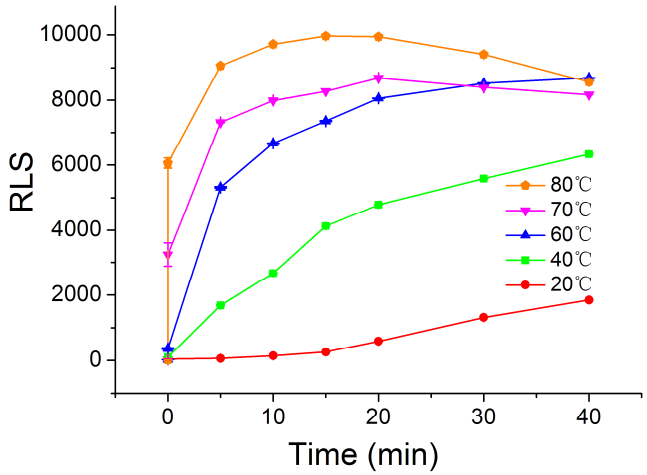

Fig. S4 Effect of temperature on the equilibrium time of the reaction of the PEI-capped Ag-nanoclusters and 3 μM Hcy. We chose 70 °C for a compromise of the sensitivity and precision although further heating resulted in a little better sensitivity, higher temperatures also accelerated water evaporation and led to worse precision.

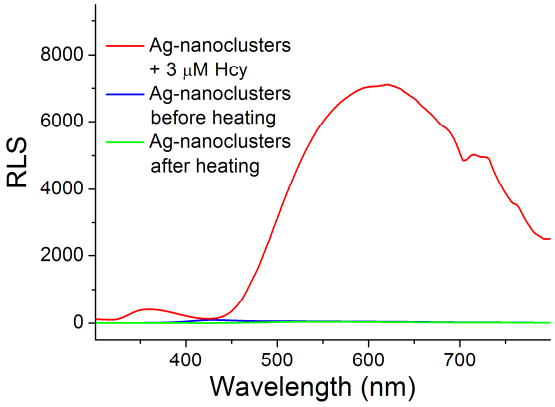

Fig. S5 RLS spectra of the as-prepared PEI capped Ag nanoclusters, the PEI capped Ag nanoclusters after heating at 70 ^{o}C for 10 min, and PEI-capped Ag-nanoclusters after incubation with 3 μM Hcy at 70 ^{o}C for 10 min.

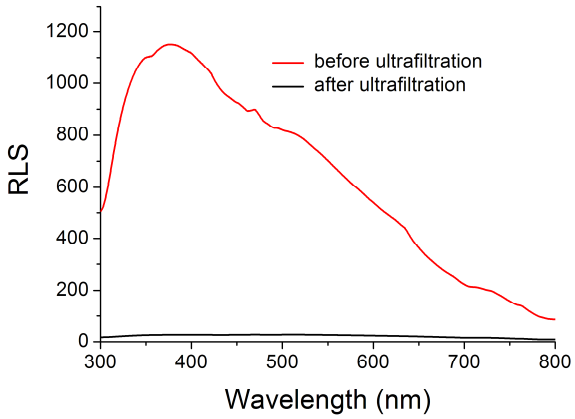

Fig. S6 RLS spectra of the human serum sample before and after ultrafiltration.

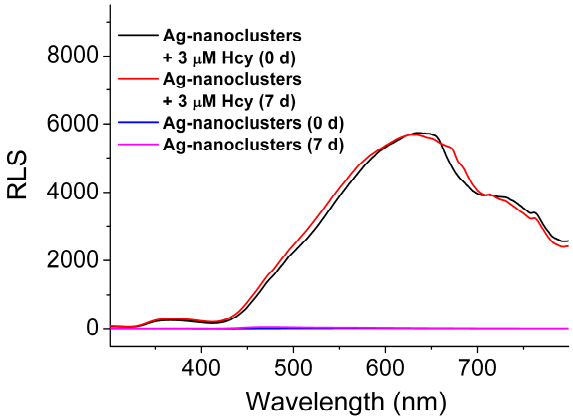

Fig. S7 Effect of time for storing the solution of the Ag-nanoclusters on the RLS of the Ag-nanoclusters in the presence and absence of 3 μM Hcy. There were no significant changes in the response of the Ag-clusters to Hcy within 7 days. However, after about half a month the Ag-nanoclusters began to aggregate and the color changed from dark red to yellow. Although the preparation of the Ag-clusters is simple and reproducible, the stability of the particle preparations needs to be improved for this assay to be useful in practice.