Electronic Supplementary Material (ESI) for Chemical Communications

Colorimetric detection of apoptosis based on caspase-3 activity assay using unmodified gold nanoparticles

Yuliang Pan, Manli Guo*, Zhou Nie, Yan Huang, Ying Peng, Afang Liu, Meng Qing and Shouzhuo Yao

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P. R. China

Experimental section

Materials and instrumentation

Dithiothreitol (DTT) was obtained from Alfa Aesar. $HAuCl_4 \cdot 4H_2O$ was obtained from Shanghai Reagent Company (Shanghai, China). Other reagents of analytical grade were obtained from Changsha Chemical Company (Changsha, China) and used as received. All solutions were prepared with ultra-pure water (18.2 MΩ cm) from a Millipore system.

Human recombinant caspase-3 was purchased from R&D Systems (Minneapolis, USA), and used following the manufacturer's instruction. The sequence of peptide, Ac-Gly-Asp-Glu-Val-Asp-Cys-Cys-Arg-NH_2 (GDEVDCCR, GR-8), was designed as the substrate of caspase-3. And another sequence of peptide, Cys-Cys-Arg-NH_2 (CCR, CR-3), was used in control experiments. All peptides were synthesized and purified by GL Biochem. Ltd. (Shanghai, China). Assay buffer solution (pH 7.4) contained 25 mM HEPES, 0.1% (m / v) CHAPS, and 10 mM DTT. Lysis buffer was purchased from Sangon Inc. (Shanghai, China) and used following the manufacturer's instruction. UV-Vis absorption spectra were obtained on a Beckman Du800 spectrophotometer.

Synthesis of gold nanoparticles (AuNPs)

AuNPs were synthesized as reported previously. In brief, 50 mL of 1 mM $HAuCl_4$ solution was added into a 100 mL round flask, and was heated to boiling. Then, 5 mL of 38.8 mM sodium citrate solution was added rapidly under vigorous stirring. The solution was maintained at the boiling state for 10 min, during which

time the color changed from yellow to deep wine red. A stable and monodispersed gold nanoparticle colloidal solution was obtained and stored at 4 °C. The resulting AuNPs were characterized by transmission electron microscopy (TEM) and UV-Vis spectroscopy. The diameter of the AuNPs was 13 ± 1 nm. The final concentration of AuNPs was estimated to be 10.2 nM from the UV-Vis absorption spectrum based on an extinction coefficient of 2.7×10^8 M^{-1} cm^{-1} at λ_{520} for 13 nm AuNPs[1].

Cell culture

Jurkat cells (T-cell, human acute lymphoblastic leukemia) were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). The cells were cultured in 25 cm^2 cell culture flasks (Corning) at 37 °C with 5% CO_2 in air atmosphere. RPMI-1640 medium (Gibco) was supplemented with 10% fetal bovine serum (Gibco) and 100 U/mL penicillin-streptomycin. The cell density was determined with a hemacytometer. After the density of cells reached 5×10^6 cells/mL, they were collected by centrifugation at 1500 g for 5 min, washed with fresh medium, and redispersed in fresh medium for further use.

Caspase-3 activity assay

Fifty μL of GR-8 solution was mixed with 50 μL caspase-3 and incubated for a certain time period at 37 °C. Then 100 μL unmodified AuNPs was added to the mixture and mixed. The color change of the solution was recorded with a camera, and UV-Vis spectroscopy measurements were performed.

Detection of apoptotic Jurkat cells

Jurkat cells (2×10^6 cells/mL) were put into 24-well culture plate (1 mL per well). Cycloheximide was added at a final concentration of 100 μg/mL to induce the apoptosis of Jurkat cells. At different time intervals, the cells were collected by centrifugation and lysed with 1 mL lysis buffer. Then 200 μL lysate was treated through ultrafiltration (6000 rpm, 30 min) using 10 kD cut-off ultrafiltration tubes (Millipore). Then 150 μL assay buffer solution (pH 7.4) containing 25 mM HEPES and 0.1% (m / v) CHAPS was added to the inner tube (sample container) followed by ultrafiltration (6000 rpm, 30 min). Fifty μL of the remaining sample was mixed with 50 μL peptide substrate solution, and the mixture was incubated for 30 min at 37 °C.

Then the mixture was chilled in ice-bath for 1 min to stop the enzymolysis. One hundred μL of unmodified AuNPs was added to the solution and mixed, and images were taken and quantitative analysis was implemented.

(1) R. Jin, G. Wu, Z. Li, C. A. Mirkin, G. C. Schatz, *J. Am. Chem. Soc.*, 2003, **125**, 1643.

Results and discussion

In experiments, we observed that GR-8 at concentrations higher than 50 μM would affect the stability of AuNPs, which was unsuitable for the colorimetric assay. GR-8 at concentrations ≤ 50 μM has little influence on the UV-Vis absorption of AuNPs (Fig. S3). And we considered that the more the GR-8 in the solution, the more the CR-3 released and the larger the signals. Therefore, in order to achieve large signals, 50 μM GR-8 was used in further experiments. According to the manufacturer's instruction, dithiothreitol (DTT) that contains double thiols is required in the assay buffer and caspase-3 solution, which would affect the stability of AuNPs. So the effect of DTT was also tested, and the results showed that 10 mM DTT used in this experiment would not induce the aggregation of AuNPs (Fig. S4).

The effect of incubation time between AuNPs and caspase-3 treated peptide sample on the colorimetric detection was investigated. The Abs650/Abs520 signals were detected every 1 min after mixing AuNPs with the cleaved peptide substrate. It has been found that 1 min after adding AuNPs to the caspase-3 treated GR-8 solution, significant changes in the UV-Vis spectra and Abs650/Abs520 values were observed (Fig. S5). Further increase in the incubation time could not bring about larger signals, and the signals were stable after about 2 min. So, in order to obtain steady signals, UV-Vis spectroscopy measurements were performed at 2 min after mixing AuNPs with the caspase-3 treated peptide solutions.

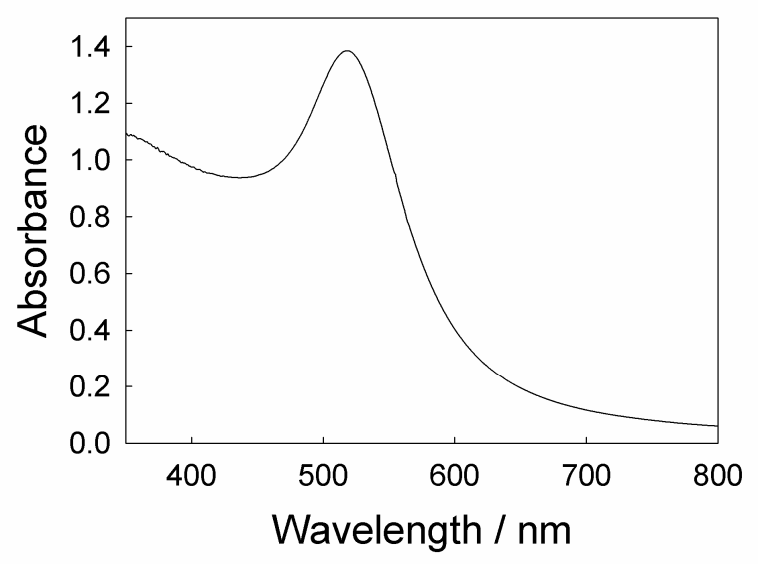


Fig. S1 UV-Vis spectrum of the synthesized AuNPs solution diluted with water (1:1). λ_{max} = 520 nm. The concentration of the synthesized AuNPs solution was estimated to be 10.2 nM.

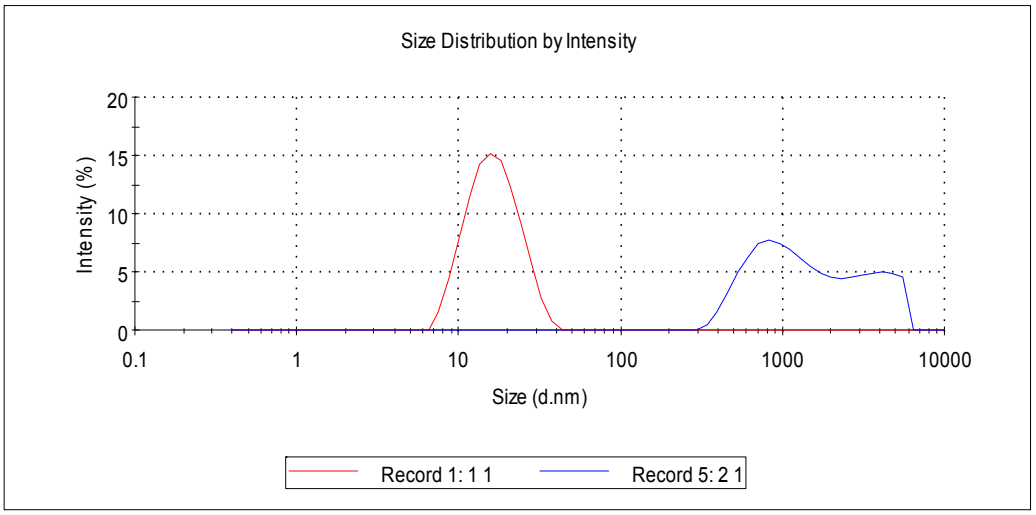


Fig. S2 Characterization of the peptide and AuNPs assembly by dynamic light scattering (DLS). Red: AuNPs mixed with GR-8 peptide; Blue: AuNPs mixed with caspase-3 treated peptide.

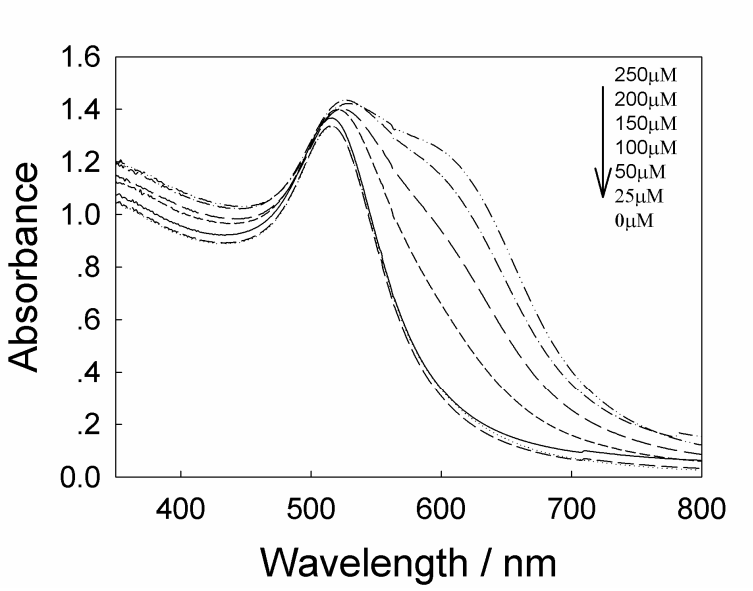
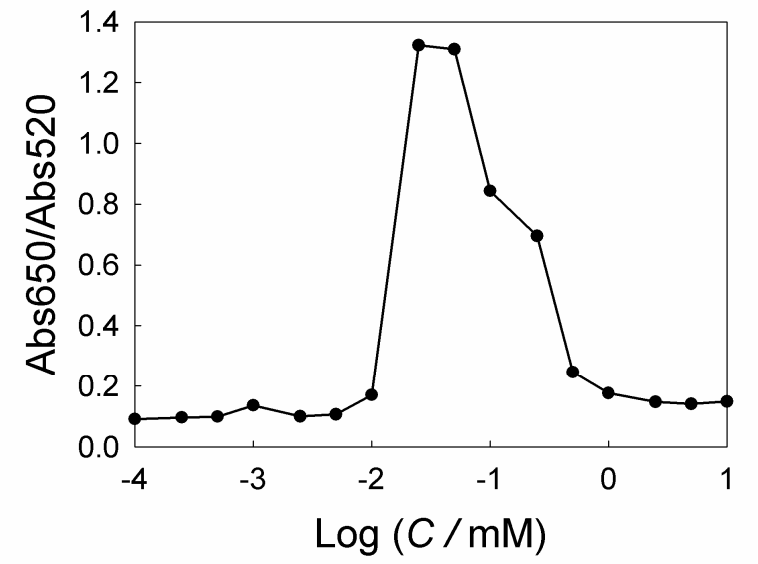
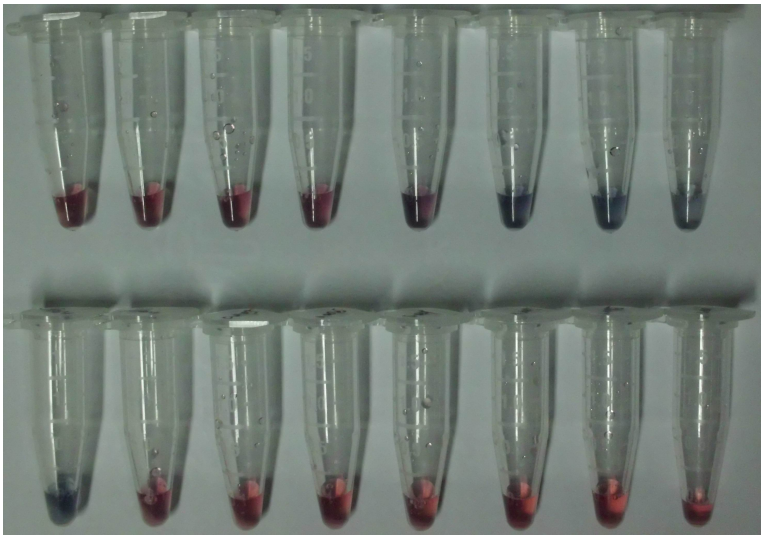


Fig. S3 UV-Vis absorption spectra of AuNPs in GR-8 solution of different concentrations.



A



B

Fig. S4 Effect of DTT concentration in the assay buffer on the stability of AuNPs. (A) Abs650/Abs520 signals and (B) color change of AuNPs in the assay buffer containing different concentrations of DTT. Upper row (from left to right): 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05 mM; lower row (from left to right): 0.025, 0.01, 0.005, 0.0025, 0.001, 0.0005, 0.00025, 0.0001 mM.

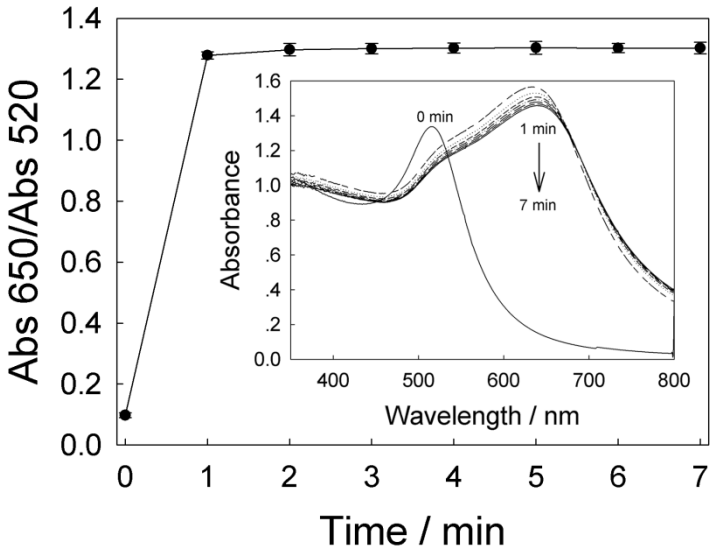


Fig. S5 Effect of the incubation time between AuNPs and caspase-3 treated GR-8 peptide on the UV-Vis detection signals.

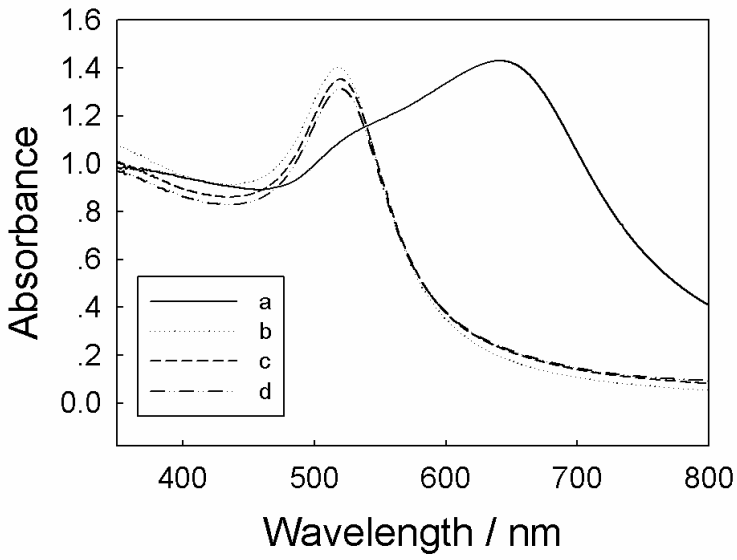


Fig. S6 UV-Vis absorption spectra of AuNPs in (a) cell lysing buffer, (b) remaining cell lysing buffer after ultrafiltration, (c) remaining cell lysate after ultrafiltration, and (d) remaining cell lysate after ultrafiltration mixed with GR-8 peptide (AuNPs was added immediately after the two solutions were mixed). The two steps of ultrafiltration are described in the experimental section.