Supplementary Information

Visual detection of hexokinase activity and inhibition with positively-charged gold nanoparticles as colorimetric probes

Shan Ren, Baoxin Li* and Lin Zhang

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710062, P.R. China

Experimental details

Reagents and materials: Chloroauric acid ($HAuCl_4$) was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Sodium borohydride and cysteamine were purchased from Sinopharm Chemical Reagent Company (Beijing, China). Hexokinase, ATP and ADP were from Sigma. The hexokinase was dissolved in triethanolamine–HCl buffer solution (23 mM, pH 7.6) containing 0.9 mM Mg^{2+} ion and stored at 4 °C. The working solution was further diluted with water. All other solvents and reagents in this investigation were of analytical grade and used without further purification. Millipore water (18 MΩ cm) was used in all experiments.

Apparatus: UV-visible adsorption spectra were recorded on a U-3900H UV-Vis Spectrophotometer (Hitachi, Japan) at room temperature using a 500 μL black-body quartz curette with 1 cm path length. The photographs

were taken with a Cannon 500 digital camera. The pH measurements were carried out on model PB-10 digital ion analyzer (Sartorius Scientific instruments Co., Ltd., China, Beijing). Zeta potentials were recorded with a Nano ZS Laser Scattering Particles Size Analyzer (Malvern, England).

Preparation of positively-charged AuNPs: All glassware used in the following procedure was cleaned in a bath of freshly prepared 1:3 HNO_3–HCl, rinsed thoroughly in water and dried in air prior to use. The positively-charged AuNPs were prepared according to the published protocol.[1] Briefly, a cysteamine solution (400 μL, 213 mM) was added to 40 mL of 1.42 mM $HAuCl_4$ solution. After stirring for 20 min at room temperature, 10 μL of 10 mM $NaBH_4$ solution was added, and the mixture was vigorously stirred for 10 min at room temperature in the dark. Then, the mixture was further stirred 15 min, and the resulting win-red solution was stored in the refrigerator (4 °C) and ready for use. The as-prepared AuNPs were charactered with UV-Visible absorption spectra and TEM. The results of TEM showed that the average size of the AuNPs was about 34 nm. The concentration of the AuNPs solution was 10.5 nM, which was estimated by the original concentration of the gold solution.[2] The solution was stored at 4 °C until needed.

Procedure for the colorimetric assay of hexokinase: A typical colorimetric assay of hexokinase was realized by following the procedure

given in Scheme 1. First, to a 1.5 mL eppendorf tube were added 20 μL of glucose (120 mM), 20 μL of ATP (0.44 mM), 10 μL of hexokinase (appropriate concentration) and 150 μL of triethanolamine–HCl buffer (23 mM, pH 7.6, 0.9 mM Mg^{2+}),[3] and then the mixed solution was incubated for 45 min at 25 °C. Second, 25 μL of the reacted solution, 150 μL of (+)AuNPs, 200 μL of BR buffer (0.04 M H_3PO_4, 0.04 M HAc, 0.04 M H_3BO_3, pH 3.6) and 325 μL of H_2O were orderly added into a 1.5 mL eppendorf tube, and the solution was allowed to react for 10 min at room temperature (ca. 20 °C). Finally, the picture was taken and the UV/Vis spectra were recorded. The control assays contained no enzyme and were performed under the above conditions.

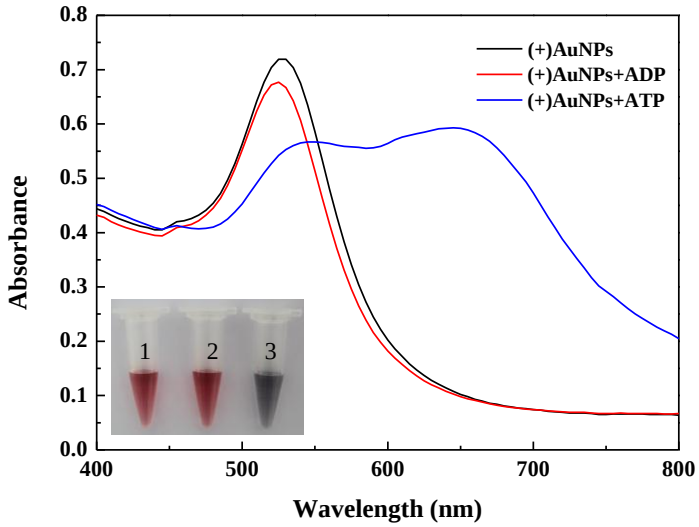

Figure S1 Absorption spectra of (+)AuNPs in the presence of ATP or ADP. Inset shows the corresponding optical photographic images: (1) (+)AuNPs, (2) (+)AuNPs+ATP and (3) (+)AuNPs+ADP. Experimental conditions: 150 μL (+)AuNPs (10.5 nM), 0.044 mM ATP, 0.044 mM ADP. All the measurement of absorption spectra has been performed in pH 3.6 Britton-Robinson (B-R) buffer solution (0.04 M H_3PO_4, 0.04 M HAc, 0.04 M H_3BO_3).

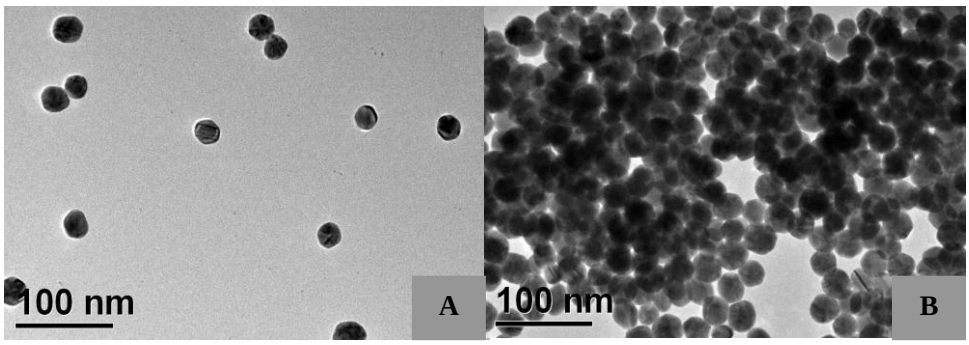

Figure S2 TEM images of (+)AuNPs in the absence (A) and presence (B) of 0.044 mM ATP.

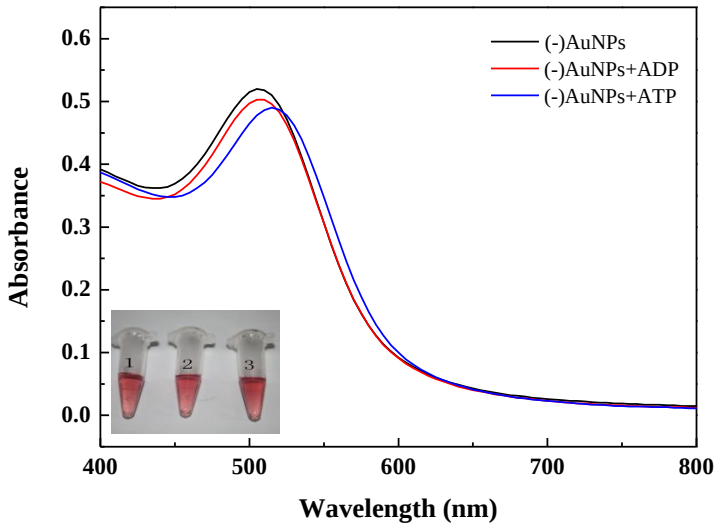

Figure S3 Absorption spectra of the negative-charged AuNPs ((-)AuNPs) in the presence of ATP or ADP. Inset shows the corresponding optical photographic images: (1) (-)AuNPs, (2) (-)AuNPs+ADP and (3) (-)AuNPs+ATP. Experimental conditions: 150 μL (-)AuNPs (17 nM), 0.044 mM ATP, 0.044 mM ADP. All the measurement of absorption spectra has been performed in pH 6.5 HAc-NaAc buffer solution (0.01 M).

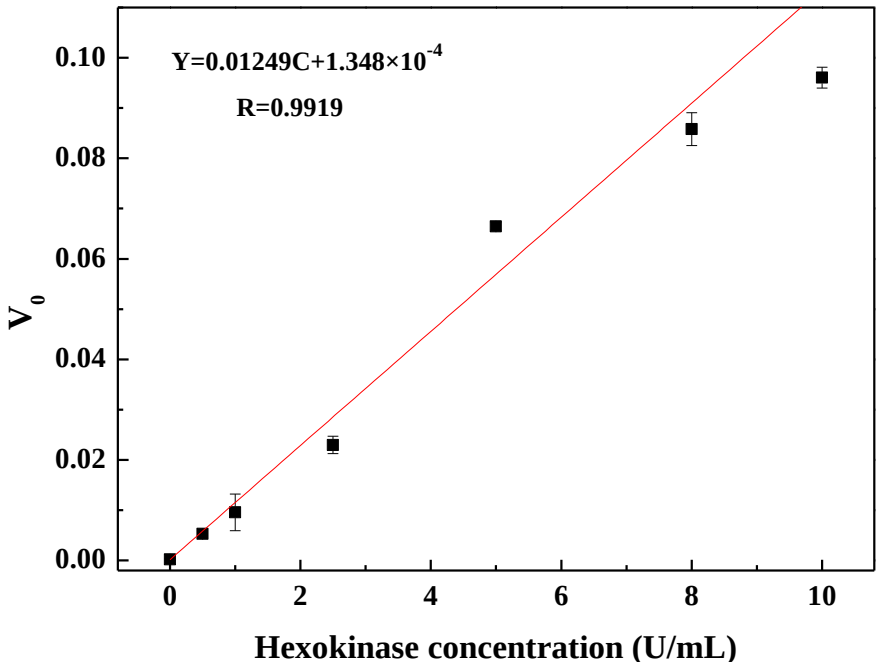

Figure S4. The linear relation between the initial rate of glucose phosphorylation reaction (V_0) and hexokinase concentration.

References

1 T. Niidome, K. Nakashima, H. Takahashi, and Y. Niidome, *Chem. Commun.*, **2004**, 1978–1979.

2 B. Neiman, E. Grushka and O. Lev, *Anal. Chem.*, **2001**, *73*, 5220–5227.

3 Yanli Tang, Fude Feng, Minghui Yu, Lingling An, Fang He, Shu Wang, Yuliang Li, Daoben Zhu, *Adv. Mater.*, **2008**, *20*, 703–705.