Supplementary Material (ESI) for Chemical Communications
This journal is (c) The Royal Society of Chemistry 2011

Supplementary Information for

Quantitative detection of adenosine in urine using silver enhancement of aptamer-gold nanoparticle aggregation and progressive dilution

Zhao Fang Liu, Jing Ge, Xin Sheng Zhao[*]

Beijing National Laboratory for Molecular Sciences, State Key Laboratory for Structural Chemistry of Unstable and Stable Species, and Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

[*]Corresponding Author. Tel: +86-10-62751727, Fax: +86-10-62751708,

E-mail: zhaoxs@pku.edu.cn

Experimental

3-aminopropyltriethoxysilane (APTES) and silver staining solutions were purchased from Sigma-Aldrich. The oligonucleotides used in this study were purchased from Sangon (China) and used as received. Ultrapure water (18.2 MΩ·cm) purified by Purelab Classic (Pall) was used throughout the experiment.

The synthesis of GNPs

GNPs were prepared by the citrate-mediated reduction of $HAuCl_4$[1]. All the vessels were soaked in aqua regia ($HCl:HNO_3$=3:1 v/v) and washed by ultrapure water. A stirred aqueous solution of $HAuCl_4$ (1 mM) in 50 mL water was heated to reflux, and 5 mL trisodium citrate solution (38.8 mM) was added rapidly. The solution was heated under reflux with appropriate stirring for 10 min, and its color changed from pale yellow to claret-red. Then the heating and stirring stopped, and the solution was cooled for 15 min to room temperature. The solution was filtered (the filter unit is 0.22 μm) to remove large clusters and insoluble compounds. The mean size of the nanoparticles was 13 nm according to the TEM analysis (Fig S1), and the corresponding absorption peak was at ~520 nm.

Preparation of aggregates and spot test

The aptamer coated GNPs (Apt-GNPs) and the complementary DNA coated GNPs (Com-GNPs-) were prepared with the procedures of ref. 2. Equal amount of Apt-GNPs and Com-GNPs solutions were mixed in the present of 300 mM NaCl and 10 mM phosphate buffer (pH=7.0). The mixture was warmed to 70 ℃ for 3 min and allowed to cool slowly to room temperature in 2 h in a water bath. Nanoparticles

This journal is (c) The Royal Society of Chemistry 2011

aggregated and changed color from red to purple in this process. For the spot test, a 1 μL progressive dilution sample was added into 9 μL of the aggregates suspension described above. The 10 μL mixed solution was transferred with a pipette onto the amino-modified glass slide and allowed to dry. The procedures of amined-glass slices preparation and silver staining followed published methods[2].

Selectivity and qualitative detection

The SEAGNA-PD assay shows a significant slope for the addition of adenosine. Conversely, addition of uridine, guanidine, or cytosine did not result in significant slopes (Fig. S6). The results were attributed to the high specificity of the adenosine aptamer, which shows excellent selectivity of adenosine over other substance.

This journal is (c) The Royal Society of Chemistry 2011

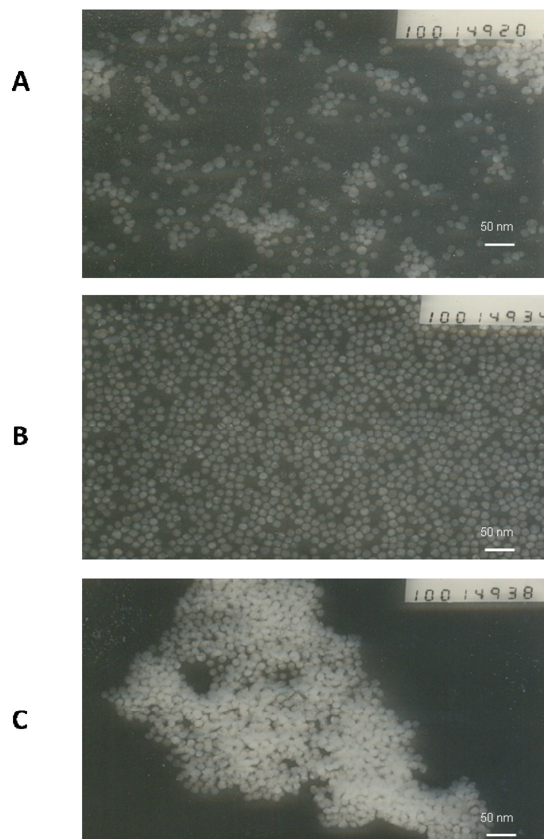

Figure S1. TEM images of (A) the citrated-capped GNPs, (B) the DNA protected GNPs, and (C) the aggregates due to the hybridization of the aptamer attached GNPs with the complementary DNA attached GNPs.

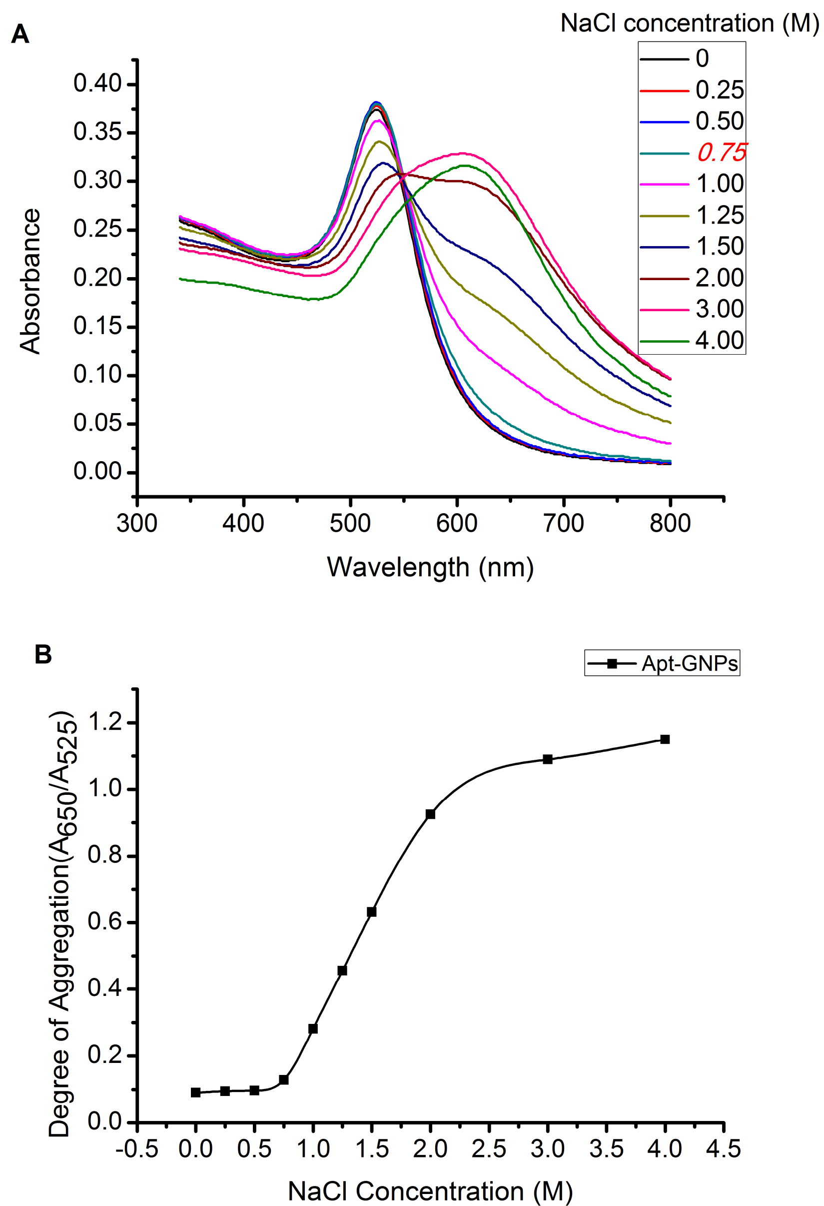

Figure S2. UV-Vis spectra of the aptamer coated GNPs (A) and the degree of aggregation (B) under different NaCl concentrations.

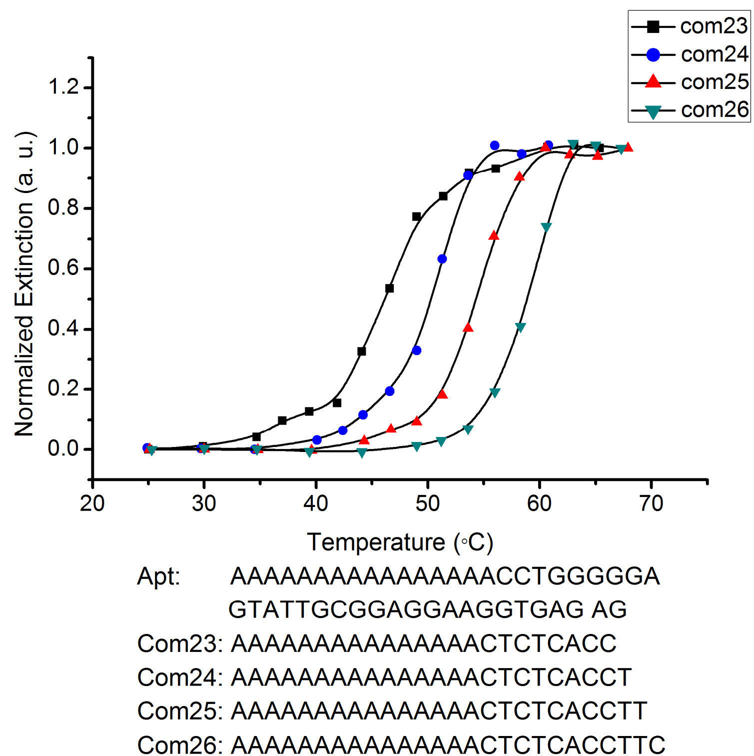

Figure S3. The T_m of different complementary DNA-GNPs hybridized with the Apt-GNPs measured by a UV-Vis spectrometer.

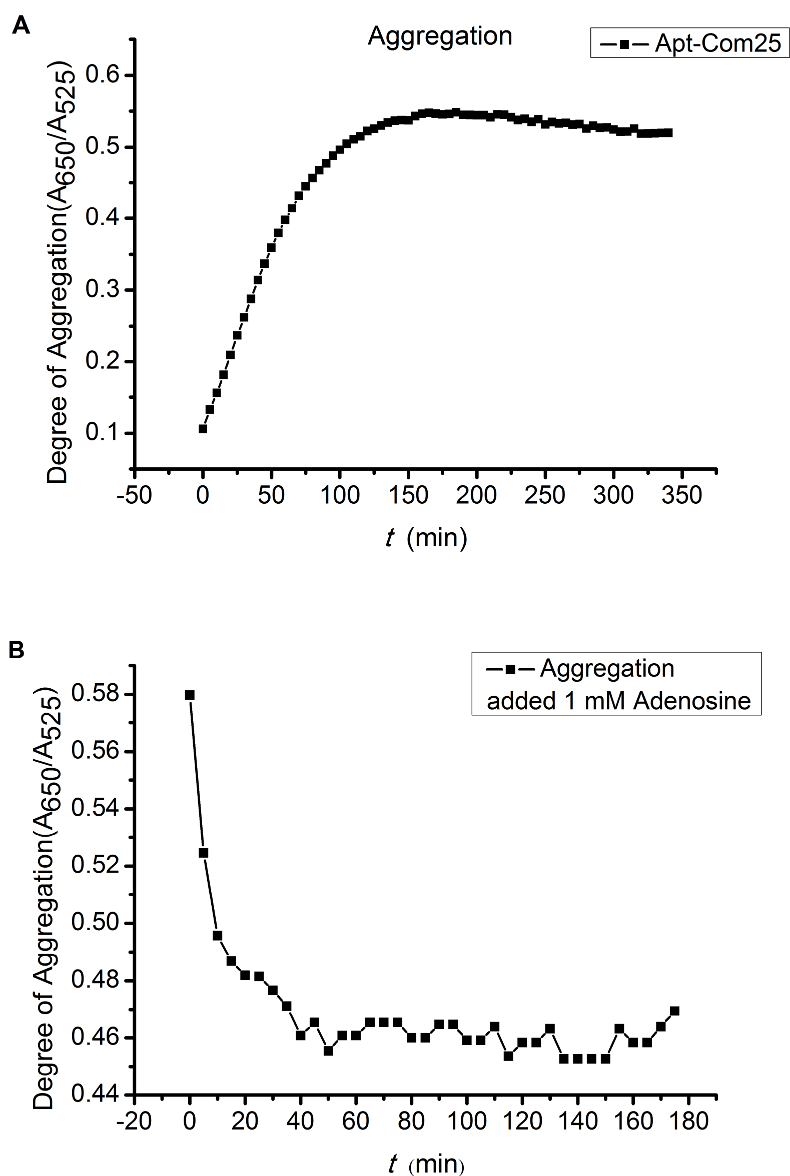

Figure S4. The time courses of UV-Vis spectra on (A) aggregation process and (B) redispersion of the aggregates due to the recognition of adenosine.

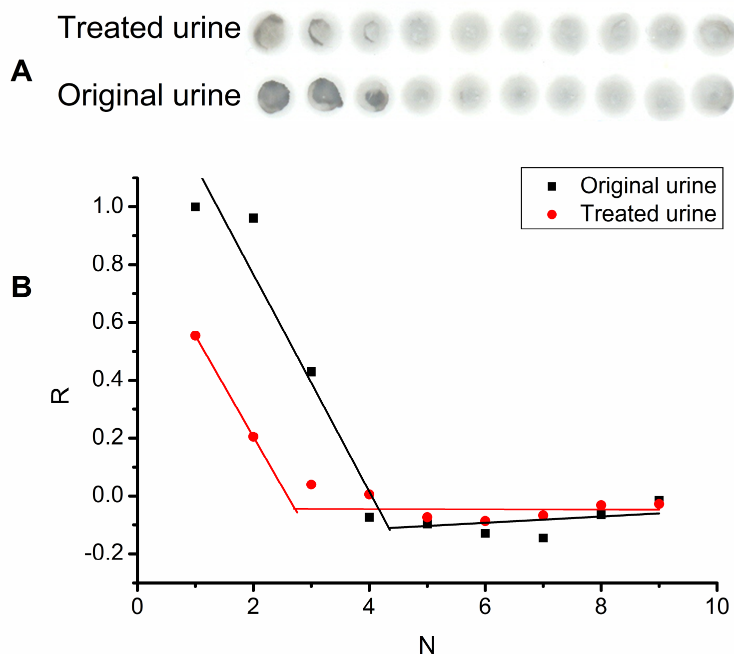

Figure S5. (A) The top row is the urine treated with the aptamer attached GNPs to reduce its adenosine concentration and the bottom raw is the original urine. (B) The grayscale obtained from (A) was plotted against the dilution times to obtain the progressive dilution curves. It shows the effective reduction of adenosine concentration after the treatment.

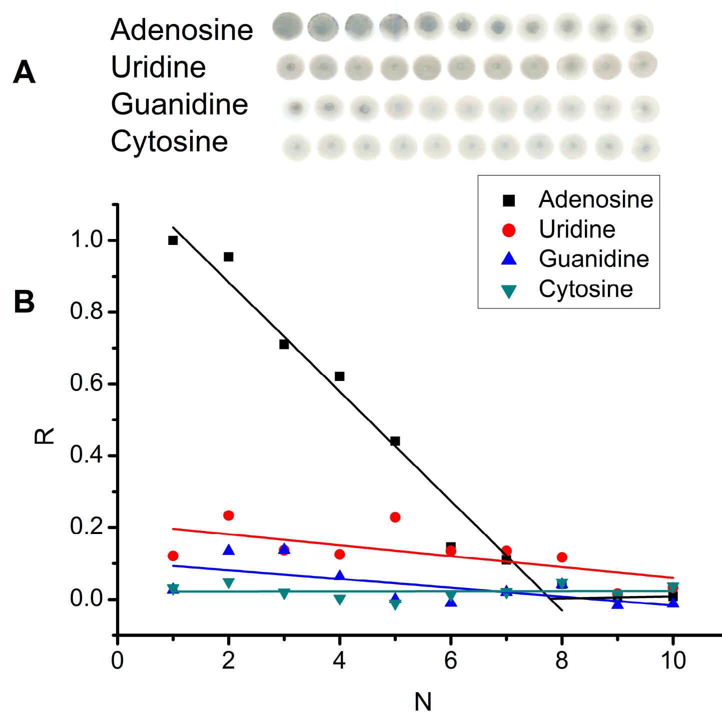

Figure S6. Selectivity of the SEAGNA-PD assay. (A) From first to the forth row are images after the silver staining of reaction solutions of adenosine, uridine, guanine, and cytosine. The samples were all progressively diluted with buffer from 10.0 mM with a dilution factor of 3. (B). The progressive dilution curves of adenosine (black solid squares), uridine (red solid circles), guanine (blue solid triangles), and cytosine (grey solid inverse triangles). From the curves it is easy to identify which sample contains adenosine.

Reference

1. G. Frens, *Nature: Phys. Sci.* 1973, **241**, 20-22.

2. Z. Zhang, C. Chen and X. S. Zhao, *Electroanalysis*, 2009, **21**, 1316-1320.