Electronic Supplementary Information (ESI)

Enzyme-Regulated Unmodified Gold Nanoparticle Aggregation: A Label Free Colorimetric Assay for Rapid and Sensitive Detection of Adenosine Deaminase Activity and Inhibition

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1. Experimental Section

Reagents. Adenosine, guanosine, inosine, cytidine, thymidine, uridine, adenosine deaminase (151 U/mg) and erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride were purchased from Sigma-Aldrich (Shanghai, China). Other chemicals used in this work were of analytical grade and directly used without additional purification. The solutions were prepared using ultrapure water which was obtained through a Millipore Milli-Q water purification system (Billerica, MA) and had an electric resistance >18.2 MΩ.

Preparation of Citrate-Capped AuNPs. Gold nanoparticles were prepared by citrate reduction of HAuCl₄.¹ Briefly, 3 mL of 1 % trisodium citrate solution was added quickly into a boiling solution of 100 mL 0.01 % HAuCl₄ under constant stirring. After the color of the solution changed from pale yellow to win-red within

several minutes, the solution was refluxed for another 30 min. And it was then slowly cooled to room temperature and stored at 4 °C before use. The concentration of prepared AuNPs was determined to be ~2.8 nM based on an extinction coefficient of ~ 2.7×10^8 M⁻¹ cm⁻¹ at 520 nM for 13 nm AuNPs.²

Colorimetric Assay of ADA Activity and Inhibition. The deamination of adenosine was carried out in 30 μ L of 10 mM Tris-HCl (pH 7.4) containing 37.5 μ M adenosine and various concentration of ADA at 25 °C The solutions of ADA were diluted with a storage buffer consisting of 50% glycerol and 5 mM potassium phosphate (pH 6.0). After 20 min of enzyme treatment, 60 μ L as prepared AuNPs was added and the mixture was incubated at room temperature for 1 min. After incubation, 10 μ L of 125 mM NaCl was added quickly, and the UV-Vis spectra of the resulting solution was recorded with a UV-2450 spectrophotometer (Shimadzu, Japan) 2 min later. If there is no need of ADA in optimize and control experiment, equal volume of ADA storage buffer was used instead of ADA.

For enzyme inhibition experiments, ADA (20 U/L) was first pretreated with different concentration of EHNA for 3 min. The detection procedure was the same as shown in the aforementioned experiment for ADA detection.

For ADA detection in the complex mixture, various concentrations of ADA were prepared with diluted human serum (0.25%), and then reacted with 37.5 μ M adenosine in 30 μ L of 10 mM Tris-HCl (pH 7.4) enzyme reaction buffer. The detection procedure was the same as shown in the aforementioned experiment for ADA detection in clean Tris-HCl buffer.

- (1) K. C. Grabar, R. G. Reeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, 67, 735.
- (2) R. Jin, G. Wu, Z. Li, C. A. Mirkin and G. C. Schatz, J. Am. Chem. Soc., 2003,

125, 1643.

2. Supplementary Figures



Fig. S1 Characterization of enzyme-regulated aggregation by dynamic light scattering.

Red: in the presence of ADA. Blue: in the absence of ADA.



Fig. S2 Characterization of substrate specificity of ADA. (A) Typical absorption spectra obtained with adenosine (A), guanosine (G) or cytidine (C) in the presence and absence of ADA. (B) The color changes of AuNPs. 1: A; 2: A+ADA; 3: G; 4: G+ADA; 5: C; 6: C+ADA. The concentration of each nucleotides and ADA were 37.5 μM and 50 U/L, respectively.



Fig. S3 UV-vis spectra of AuNPs obtained in the presence of ADA (50 U/L, ~10 nM) or BSA (10 nM). Inset is the comparison of A_{630}/A_{520} ratio in the presence of ADA or BSA. Error bars were estimated from four replicate measurements.



Fig. S4 The calibration curve of adenosine sensing at different NaCl concentrations.



Fig. S5 (A) UV-vis spectra of AuNPs at different incubation time of adenosine and AuNPs. An aliquot of 60 μ L AuNPs was added into 30 μ L of 10 mM Tris-HCl (pH 7.4) containing 37.5 μ M adenosine, and incubated for given times. The UV-vis spectra of AuNPs was recorded 2 min later after addition of 10 μ L of 125 mM NaCl into the mixture. (B) The ratio of A₆₃₀/A₅₂₀ as a function of the incubation time.



Fig. S6 The detection of ADA in diluted human serum. The concentrations of ADA form a to f were 0, 2.5, 5, 10, 20 and 50 U/L, respectively.



Fig. S7 The effect of EHNA on the stability of AuNPs. An aliquot of 60 μ L AuNPs was added into 30 μ L of 10 mM Tris-HCl (pH 7.4) containing various concentration of EHNA, and incubated for 1 min. The UV-vis spectra of AuNPs was recorded 2 min later after addition of 10 μ L of 125 mM NaCl into the mixture.