

## Simple and rapid colorimetric enzyme sensing assays using non-crosslinking gold nanoparticle aggregation

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### Experimental

#### Materials

Calf intestine alkaline phosphatase (CIAP), phi29 DNA polymerase, and dNTPs (dATP, dCTP, dTTP, and dGTP) were purchased from MBI Fermentas. ATP and other reagents were obtained from Sigma. DNA oligonucleotides used in this study were obtained from Central Facility, McMaster University. H<sub>2</sub>O was doubly deionized and autoclaved before use. 13 nm gold nanoparticles (AuNP) were prepared according to reported procedures<sup>1</sup> and the final concentration was estimated to be about 14 nM using UV-Vis spectrometric measurements based on an extinction coefficient of  $\sim 2.7 \times 10^8 \text{ M}^{-1} \text{ cm}^{-1}$  at  $\lambda$  520 nm for 13 nm particles.<sup>1</sup>

#### Study of AuNP stability in the presence of ATP, ADP, AMP, adenosine and Inosine

100  $\mu\text{L}$  of ATP (or ADP, AMP, adenosine and inosine) solution (18 nmol, 180  $\mu\text{M}$ ) was added to 200  $\mu\text{L}$  of AuNP solution (14 nM) in a 2-mL glass vial, and the photographs, as shown in Fig. 1B vials 1-5, were taken in 1 min following the mixing. To further investigate the impact of adenosine concentration on AuNP stability, the same assay was performed with adenosine in different concentrations (90, 60, 30, 15, and 7.5  $\mu\text{M}$ ), and the results are shown in Fig. 1B vials 6-10, respectively.

We found that ATP, ADP and AMP all stabilize AuNP. To investigate the relative stability of AuNP/ATP, AuNP/ADP, and AuNP/AMP solutions, NaCl (5 M) was gradually added to the above solutions. The color change was first observed in AuNP/AMP solution, then AuNP/ADP, and finally AuNP/ATP, indicating that the AuNP stability follows the order of AuNP/ATP > AuNP/ADP > AuNP/AMP. In the present work, AuNP/ATP, AuNP/ADP, AuNP/AMP, and bare AuNP solution were found to be unstable (color change in 1 min) at NaCl concentration of  $\geq 320 \text{ mM}$ ,  $\geq 250 \text{ mM}$ ,  $\geq 170 \text{ mM}$ , and  $\geq 50 \text{ mM}$ , respectively.

The zeta potential of the nanoparticles were measured at room temperature by using a ZetaPlus (Brookhaven Instruments Corporation, Holtsville, NY). The reported values were based on 5 measurements with 15 cycles for each sample.

#### Development of calibration curve for determining the percentage conversion of ATP

ATP and adenosine with various molar ratios were mixed in a total concentration of 10 mM in 20  $\mu\text{L}$  Tris-HCl (10 mM, pH 7.5), MgCl<sub>2</sub> (10 mM) (1 $\times$  reaction buffer). 1  $\mu\text{L}$  of the mixture was diluted 100 times by ddH<sub>2</sub>O and added into 200  $\mu\text{L}$  AuNP (14 nM). UV-Vis adsorption spectra were recorded after 1 min following the mixing, and shown in Fig. S3A. The ratio of extinction at 520 and 600 nm (A<sub>520</sub>/A<sub>600</sub>) were plotted as a function of ATP/adenosine molar ratio, as shown in Fig. S3B.

#### Assay of sensing CIAP

20  $\mu\text{L}$  of enzymatic reaction solution contained ATP (200 nmol, 10 mM), 1 $\times$  reaction buffer, and various amounts of CIAP (from 10 units to 0.16 units, one unit is defined as the amount of enzyme required to catalyze the hydrolysis of 1  $\mu\text{mol}$  of 4-nitrophenyl phosphate per minute at 37 °C in 1 M diethanolamine, 10.9 mM 4-nitrophenyl phosphate, 0.5 mM MgCl<sub>2</sub> (pH 9.8)). 1  $\mu\text{L}$  of reaction mixture was taken at given reaction times, diluted 100 times by ddH<sub>2</sub>O and added into 200  $\mu\text{L}$  AuNP (14 nM). UV-Vis adsorption spectra were measured after 1 min following the mixing, as shown in Fig. S4. A<sub>520</sub>/A<sub>600</sub> was plotted as a function of reaction time and shown in Fig. 2D. The amount of ATP converted to adenosine per minute for different CIAP units added to the reaction was approximated by the following procedures: (1) Two data points were taken closest to the inflection point for each curve in Fig. 2D; (2) converted into nmol adenosine ( $200 - ((A_{520}/A_{600} - \text{lowest } A_{520}/A_{600}) \div \text{maximum } \Delta A_{520}/A_{600}) \times 200$ ); (3) then divided by the difference in the amount of adenosine produced over the two concerned time points by the time. It is important to note that the y axis in Fig. 2E, the amount of ATP converted to adenosine per minute, was not intended to represent the real reaction rate. Rather, it was only used to quantify the enzymatic activities.

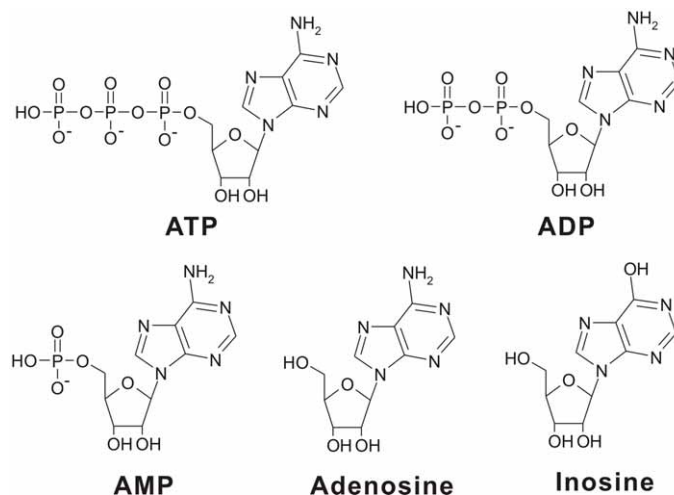
Enzymatic inhibition assay was conducted as follows: CIAP (1.25 units) was pre-incubated with desirable amount of Na<sub>3</sub>VO<sub>4</sub> in 20  $\mu\text{L}$  reaction buffer for 10 min. ATP (final concentration 10 mM) was then added to the mixture and the reaction was performed at 37 °C. At certain given reaction time, 1  $\mu\text{L}$  of reaction mixture was taken, then diluted 100 times by ddH<sub>2</sub>O and finally added into 200  $\mu\text{L}$  AuNP (14 nM). UV-Vis spectra were taken and A<sub>520</sub>/A<sub>600</sub> was calculated. The A<sub>520</sub>/A<sub>600</sub> was plotted as a function of enzymatic reaction time and the data was shown in Fig. 3A. For the inhibitor concentration assay, the enzymatic reactions with different amount of inhibitor were performed for 30 min, and 1  $\mu\text{L}$  of reaction solution was then taken for the AuNP test.

#### Assay of sensing phi29 DNA polymerase

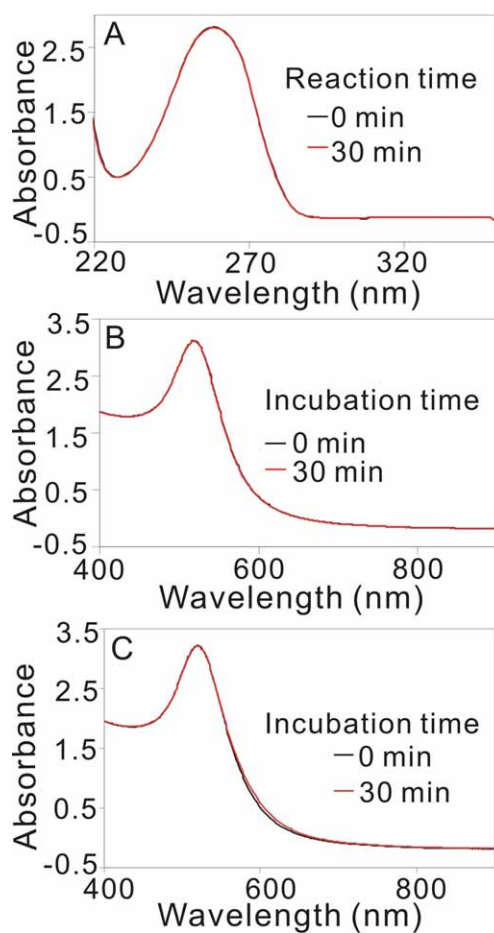
20  $\mu\text{L}$  of enzymatic reaction solution contained dNTPs (2 mM dGTP, 3 mM dATP, 0.6 mM dCTP, and 0.7 mM dTTP), phi29 DNA polymerase (40 units, one unit is defined as the amount of enzyme required to catalyze the incorporation of 0.5 pmol of dCTP into a polynucleotide fraction in 10 min at 30°C), primer (5'-GGCGAAGACAGGTGCTTAGTC, 20 pmol, 1  $\mu\text{M}$ ), circular template (5'-TGTCTTCGCCTTCTGTTTCCTTTCCTTGAACTTCTTCCTTCTTTCGACTAAGCACC, 20 pmol, 1  $\mu\text{M}$ ) (for the synthesis

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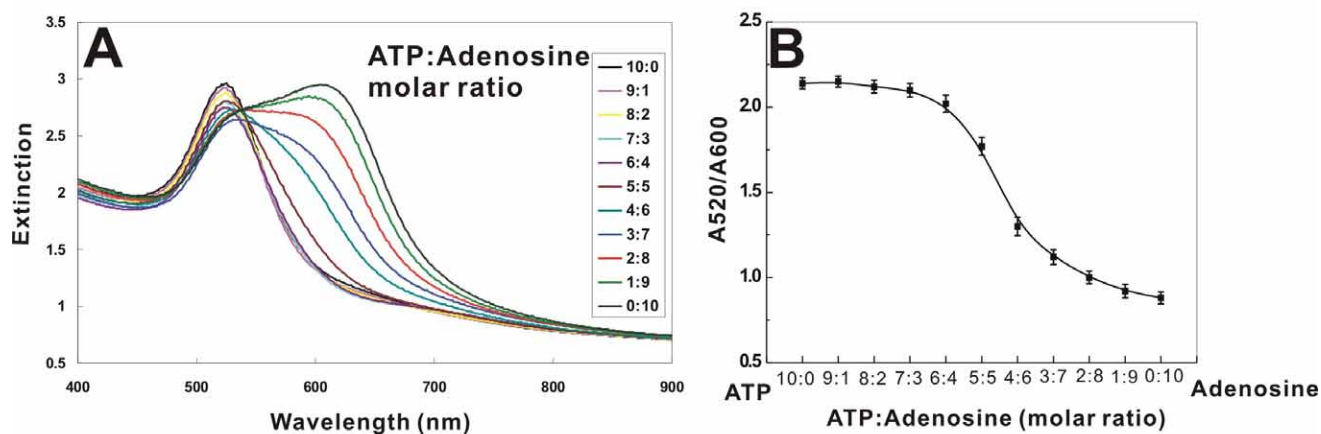
of circular template, see ref. 2), 1 × reaction buffer (33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM Mg-acetate, 66 mM K-acetate, 1% (v/v) Tween 20). The reaction was performed at 37 °C. 1 μL of the reaction mixture was taken at certain reaction time, and added to 99 μL NaCl solution (75 mM). This resulting mixture was then added to 200 μL AuNP solution (14 nM). UV-Vis adsorption spectra were measured after 1 min following mixing, as shown in Fig. S5.



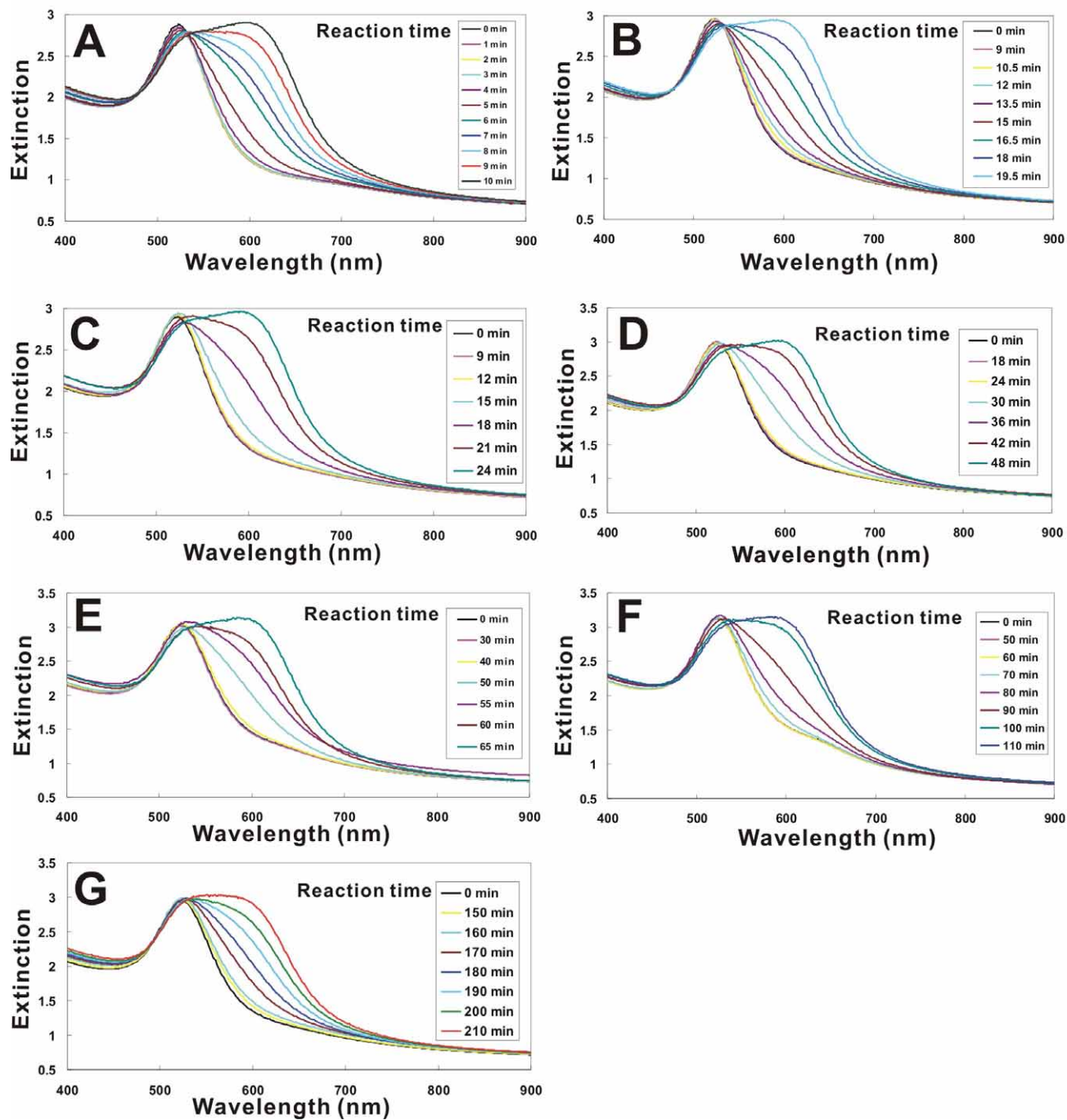
*Fig. S1.* Chemical structures of ATP, ADP, AMP, adenosine and inosine.



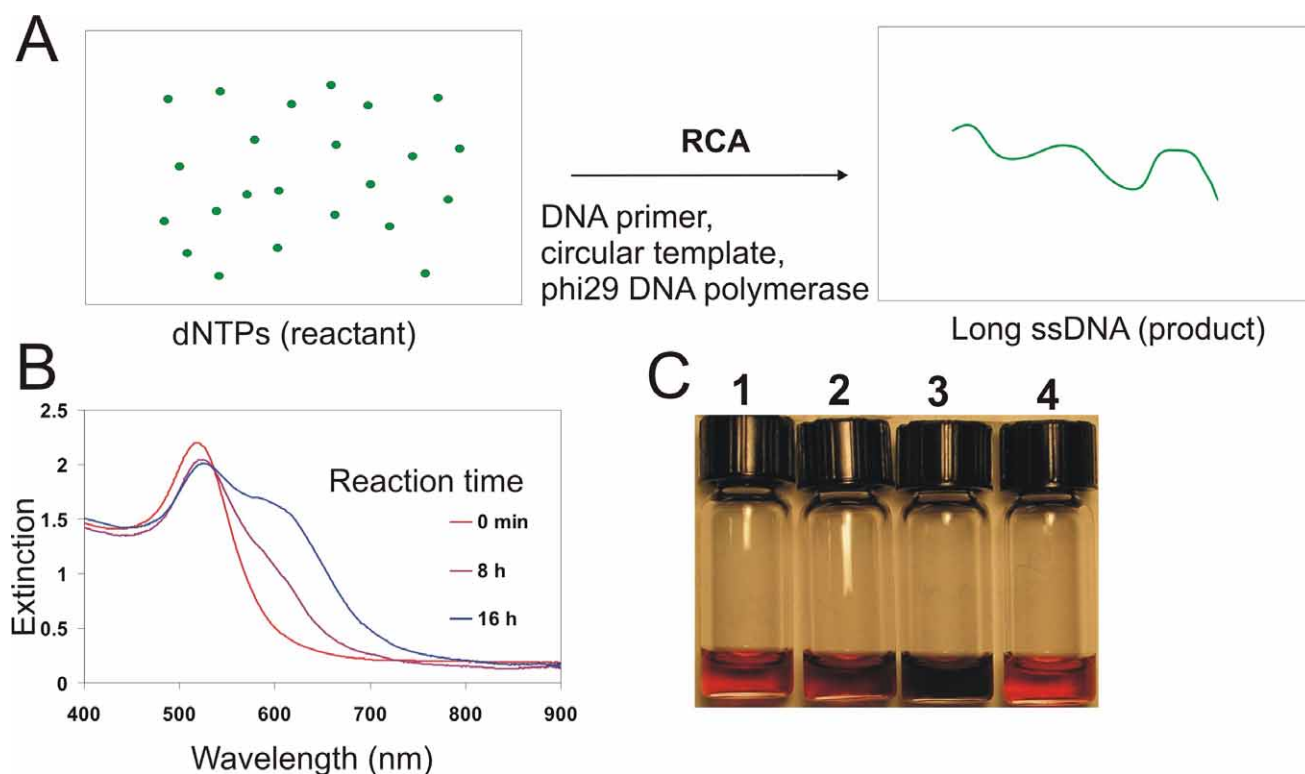
**Fig. S2.** (A) UV-Vis spectroscopy monitoring CIAP reaction without AuNPs. There was no difference in the spectra between ATP (substrate) and adenosine (product). The reaction contained ATP (180  $\mu$ M), CIAP (0.66 units/20  $\mu$ L) and 1 $\times$  reaction buffer. (B) The effect of 1 $\times$  reaction buffer on AuNP stability. 1  $\mu$ L of 1 $\times$  reaction buffer (Tris-HCl (10 mM, pH 7.5), MgCl<sub>2</sub> (10 mM)) was added to AuNP solution (200  $\mu$ L, 14 nM) without any dilution. Black and red curves are the UV-Vis spectra of the mixture after 0 and 30 min incubation time, respectively. The data indicate that 1 $\times$  reaction buffer, without any dilution, has no effect on AuNP stability. (C) The effect of CIAP on AuNP stability. 1  $\mu$ L of CIAP solution (CIAP: 0.125 units, Tris-HCl (10 mM, pH 7.5), MgCl<sub>2</sub> (10 mM)) was added to AuNP solution (200  $\mu$ L, 14 nM) without any dilution. Black and red curves are the UV-Vis spectra of the mixture after 0 and 30 min incubation time, respectively. It indicates that CIAP, without any dilution, has little effect on AuNP stability after 30-min incubation. Given that the color was read after 1 min following addition of the reaction mixture to AuNP solution in our assay, we expect that the buffer and enzyme have little effect on the AuNP stability, that is, one can directly add the reaction solution to AuNP for detection without any dilution.



**Fig. S3.** (A) Representative UV-Vis spectra of AuNP solutions with various amounts of ATP and adenosine. All spectra were recorded after 1 min following the addition of ATP and adenosine mixture to AuNP solution. (B) The A520/A600 ratios are plotted as a function of ATP and adenosine molar ratios.



**Fig. S4.** Representative UV-Vis spectra of AuNP solution after addition of CIAP-ATP reaction solution with 10 (A), 5 (B), 2.5 (C) 1.25 (D), 0.625 (E), 0.31 (F), and 0.16 (G) units of CIAP (in 20  $\mu$ L reaction volume) at different reaction times. All spectra were recorded after 1 min following addition of the relevant reaction mixture to AuNP solution. Note that the spectra which overlap with the spectrum at 0 min reaction time are not shown for clarity.



**Fig. S5.** (A) DNA polymerization via rolling circle amplification (RCA): dNTPs (reactant) are converted into long ssDNAs (product). See ref. 3 and 4 for more detailed information of RCA. (B) Representative UV-Vis spectra of AuNP solution after addition of phi29 enzymatic reaction mixtures taken at different reaction time. (C) Photographs of AuNP solutions after the addition of phi29 enzymatic reaction solutions with reaction times at 0 (vial 1), 8 h (vial 2) and 16 h (vial 3), respectively. Vial 4 was the AuNP solution after addition of a control enzymatic reaction solution quenched at 90 °C for 10 min right after the addition of phi29 DNA polymerase. The control reaction was conducted for 16 h.

#### References

- (1) R. Jin, G. Wu, Z. Li, C. A. Mirkin, G. C. Schatz, *J. Am. Chem. Soc.* 2003, **125**, 1643.
- (2) W. Zhao, Y. Gao, S. A. Kandadai, M. A. Brook, Y. Li, *Angew. Chem. Int. Ed.* 2006, **45**, 2409.
- (3) A. Fire, S. Xu, *Proc. Natl. Acad. Sci. USA* 1995, **92**, 4641.
- (4) D. Liu, S. L. Daubendiek, M. A. Zillman, K. Ryan, E. T. Kool, *J. Am. Chem. Soc.* 1996, **118**, 1587.