Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B
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Supporting Information

Gold nanoparticle-enhanced fluorescence polarization biosensor for amplified detection of T4 polynucleotide kinase activity and inhibition

Yong Huang,* Jia Chen, Ming Shi, Shulin Zhao,* Zheng-Feng Chen and Hong Liang*

Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources (Ministry of Education), College of Chemistry and Chemical Engineering, Guangxi Normal University, Guilin 541004

E-mail:zhaoshulin001@163.com; hliang@gxnu.edu.cn; huangyong_2009@163.com

Tel: (+86) 773 5856104

Fax: (+86) 773-583-2294

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Materials and Methods

Materials: All oligonucleotides were purchased from the Sangon Biotech Co. (Shanghai, China) and purified by HPLC. Sequences of oligonucleotide probes used in this work are listed as follows: hairpin DNA, 3'-SH-TTT TTT TTT TCC GTA CAC AGA CTC AGC TTT AAA TTT GCT GAG TCT GTG TAC GG-5'; single stranded DNA (ssDNA), 5'-FAM-GGC ATG TGT CTG AGT CG-3'. T4 polynucleotide kinase (T4 PNK; 10 units/μL) and λ exonuclease (λ exo; 5 units/μL) were purchased from New England Biolabs. Tris(hydroxymethyl)aminomethane (Tris), adenosine triphosphate (ATP) and adenosine diphosphate (ADP) were obtained from Sigma-Aldrich. AuNPs 44 nm in size were synthesized by citrate reduction of $HAuCl_4$ as previously reported.[1] All other chemicals were of analytical grade. The water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout the work.

Preparation of DNA-functionalized AuNPs: The DNA-functionalized AuNPs were prepared by the previously reported procedure with minimal modification.[2] Briefly, the hairpin DNA was first added to a AuNPs solution (10 nM) to reach a final concentration of 1 μM DNA. After being incubated overnight, the mixture was diluted with 20 mM phosphate buffer solution (PBS; 50 mM NaCl, 20 mM phosphate, pH 7.4) and allowed to stand for 24 h at room temperature. Unconjugated oligonucleotides were removed by centrifugation at 14000 rpm for 30 min at 4 ℃ and by washing three times with 20 mM PBS. The DNA-functionalized AuNPs obtained was redispersed in a tris(hydroxymethyl)aminomethane (Tris) buffer solution (pH 8.0) containing 50 mM Tris-HCl, 10 mM $MgCl_2$ and 1% BSA.

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Procedures for fluorescence polarization measurements: Fluorescence polarization (FP) is a technique that provides a quantitative measure for the rotational motion of a fluorescent molecule-labeled molecule. The FP value ***P*** can be calculated by Perrin equation.[3] In a particular surrounding, the rotational motion of the fluorescent molecule-labeled molecule is closely related to the size of the molecule. For example, if a fluorescent molecule-labeled molecule is free in solution, it rotates fast and the ***P*** value is relatively small. However, when the fluorescent molecule-labeled molecule forms a complex with another substance, its rotational rate decreases and the ***P*** value will increase. The degree of variation in the ***P*** value depends on the strength of the binding interaction and the size of the formed complex.[4,5] From the principle described above, it is clearly that the ***P*** value will significantly increase when a fluorophore-labeled oligonucleotide binds a larger molecule (AuNP-functionalized DNA).

For amplfied enzyme assay, 2.0 μL hairpin DNA-modified AuNPs solution (corresponding to 5 μM of hairpin DNA) and 2.0 μL FAM-labeled ssDNA (5.0 μM) were added to a 1.5 mL vial containing 496 μL of 50 mM Tris-HCl solutions (10 mM $MgCl_2$, 0.1 mM ATP, 1% BSA, 12 units λ exo, pH 8.0). Subsequently, 2 μL of T4 PNK solution with different concentration was added, and the mixture was incubated for 30 min at 37 ℃ before FP measurement. 300 μL of final solution was used for FP measurements. The control experiments using non-modified hairpin DNA and FAM-labeled ssDNA were carried out under identical conditions. All experiments were repeated three times. Each sample was measured three times. FP measurements were performed on an FL3-P-TCSPC system (Jobin Yvon, Inc., Edison, NJ, USA). The FP of the sample solution was monitored by exciting the sample at 494 nm and measuring the emission at 520 nm. And slits for both the excitation and the emission were set at 5 nm.

In the inhibition experiment, to comparison of the inhibition abilities of test compounds, each

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compound (10 mM) was also contained in the 50 mM Tris-HCl solutions (10 mM $MgCl_2$, 0.1 mM ATP, 1% BSA, 12 units λ exo, pH 8.0), respectively. After mixing with hairpin DNA-modified AuNPs, FAM-labeled ssDNA, and 1 unit of PNK, the reaction was performed at 37 ℃ for 30 min. Other assay steps were the same as those described above.

References

(1) Y. Huang, S. Zhao, Z. F. Chen, Y. C. Liu and H. Liang, *Chem. Commun.*, 2011, **47**, 4763.X.

(2) Xu, M. Han and C. A. Mirkin, *Angew. Chem. Int. Ed.*, 2007, **46**, 3468.

(3) B. C. Ye and B. C. Yin, *Angew. Chem., Int. Ed.*, 2008, **47**, 8386.

(4) M. E. McCarroll, F. Haddadian Billiot and I. M. Warner, *J. Am. Chem. Soc.*, 2001, **123**, 3173.

(5) N. J. Gibson, H. L. Gillard, D. Whitcombe, R. M. Ferrie, C. R. Newton and S. Little, *Clin. Chem.*, 1997, **43**, 1336.

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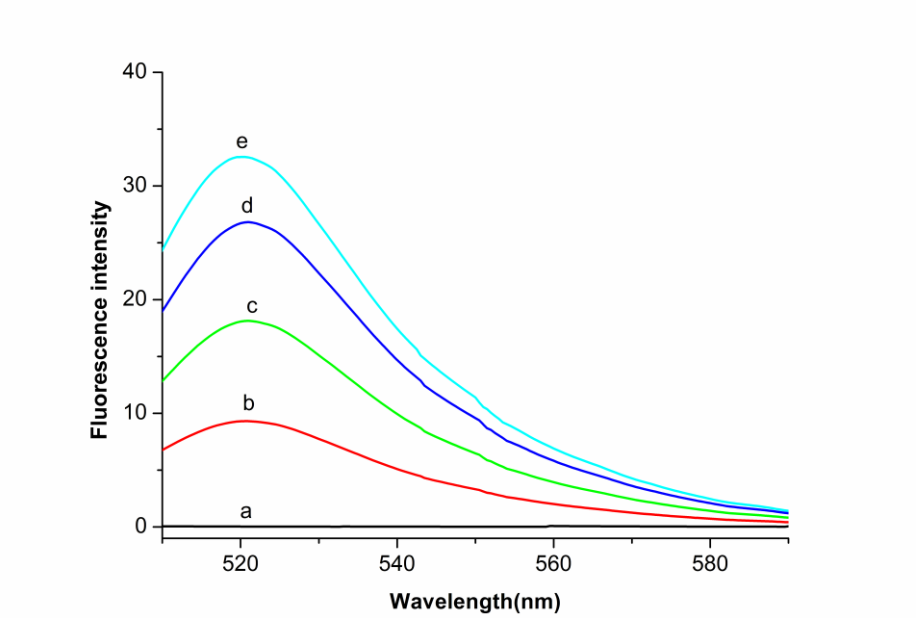

Figure S1. Fluorescence spectra of the centrifugates from the mixture of hairpin DNA-functionalized AuNPs and FAM-labeled ssDNA upon analyzing 1 U/mL T4 PNK at different reaction time (curve b-e: 5, 10, 15, 20, 30 min) or in the absence of PNK (curve a).

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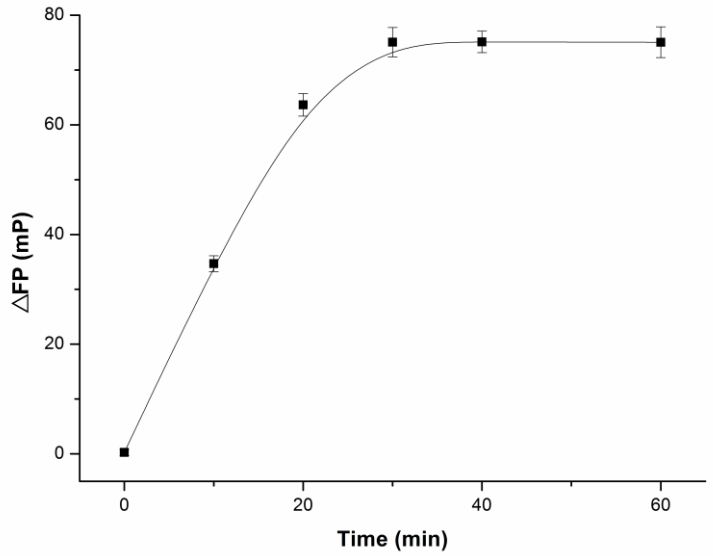

Figure S2. Optimization of the reaction time. The concentrations of T4 PNK, ATP and λ exo were 0.01 U/mL, 0.1 mM and 12 units, respectively.

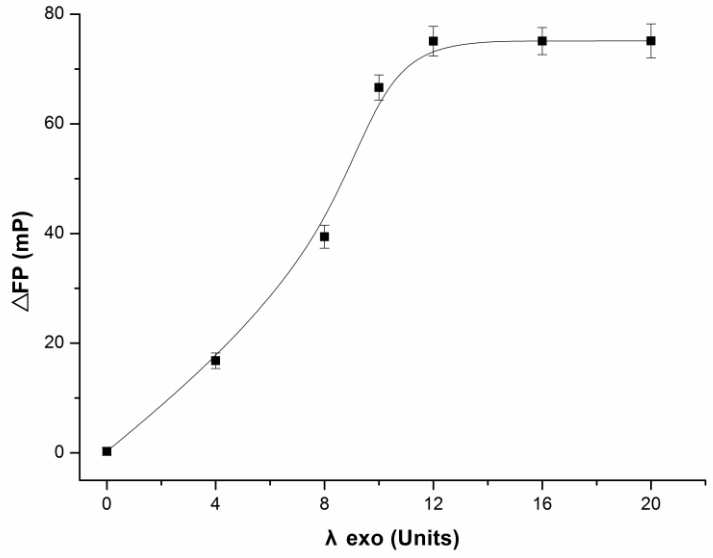

Figure S3. Optimization of λ exo concentration. The concentrations of T4 PNK and ATP were 0.01 U/mL and 0.1 mM, respectively.

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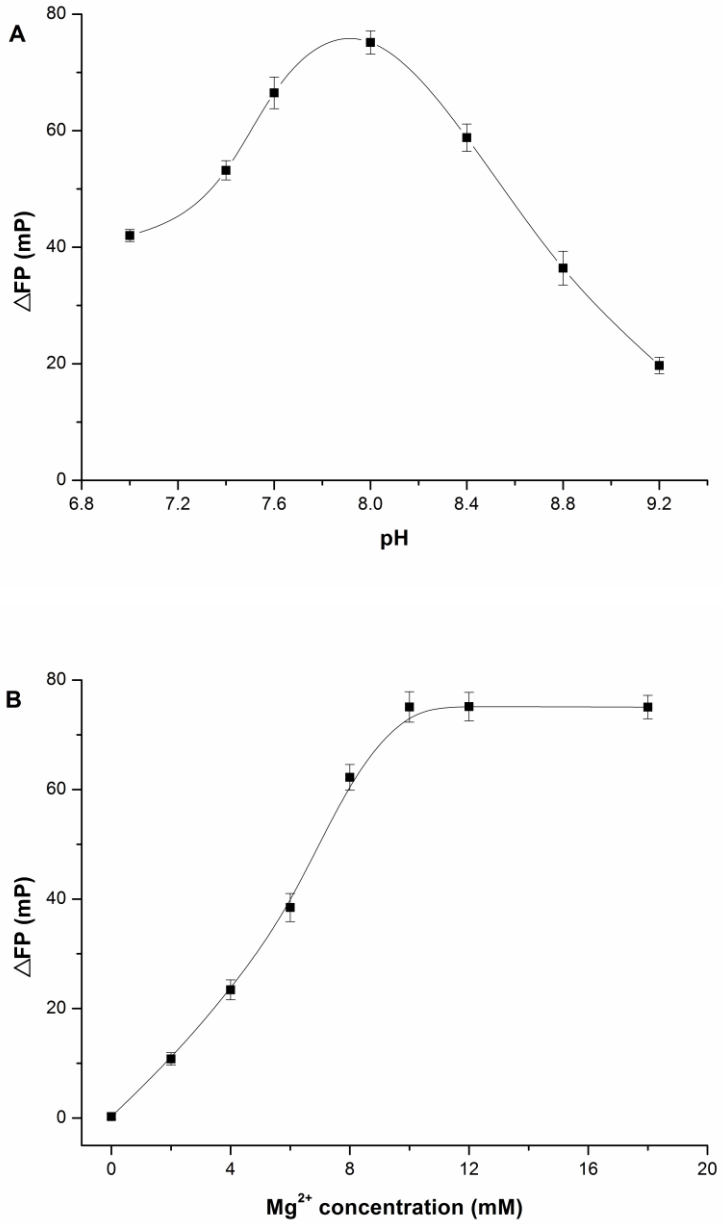

Figure S4. Optimization of pH (A) and Mg^{2+} concentration (B). The concentrations of T4 PNK, ATP, and λ exo were 0.01 U/mL, 0.1 mM, and 12 units, respectively.

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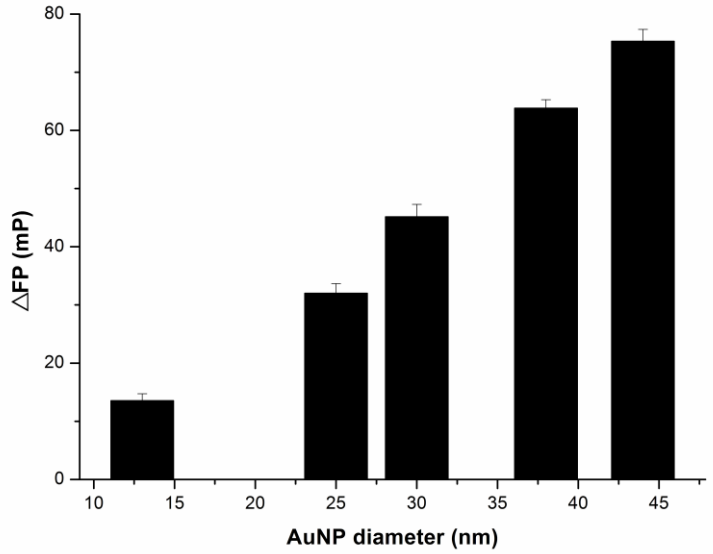

Figure S5. Fluorescence polarization changes from the AuNP-DNA system using different diameter size of AuNPs upon analyzing 0.01 U/mL T4 PNK.

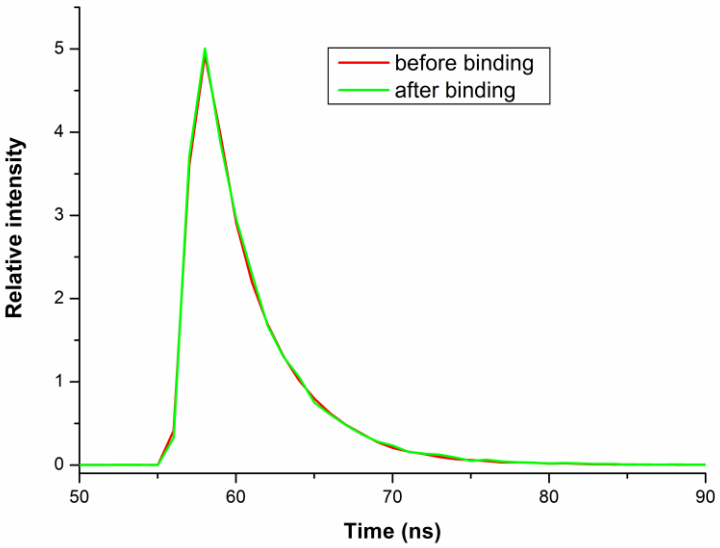

Figure S6. PL decay of FAM-labeled DNA before (A) and after (B) binding with the functionalized AuNPs.

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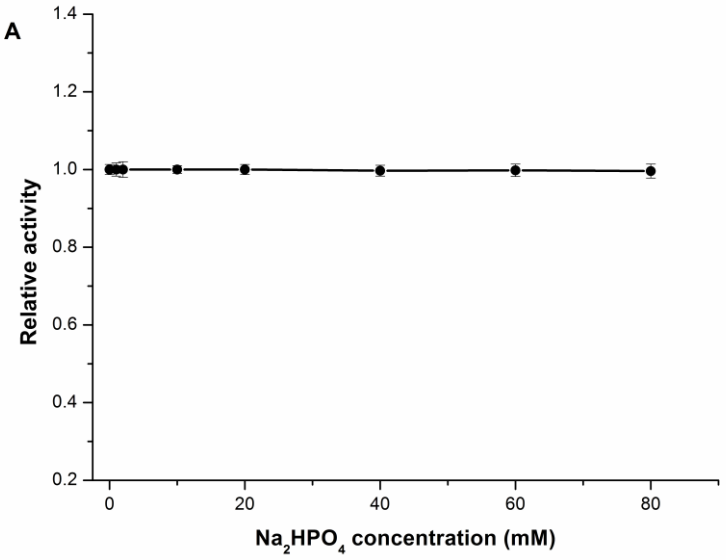

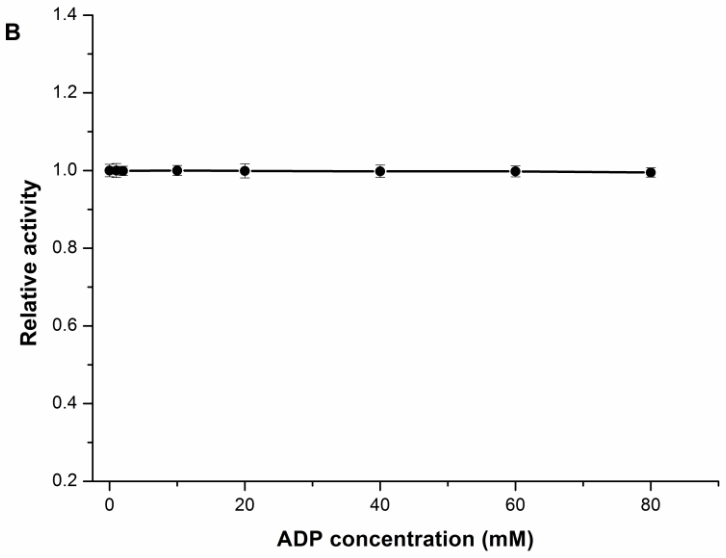

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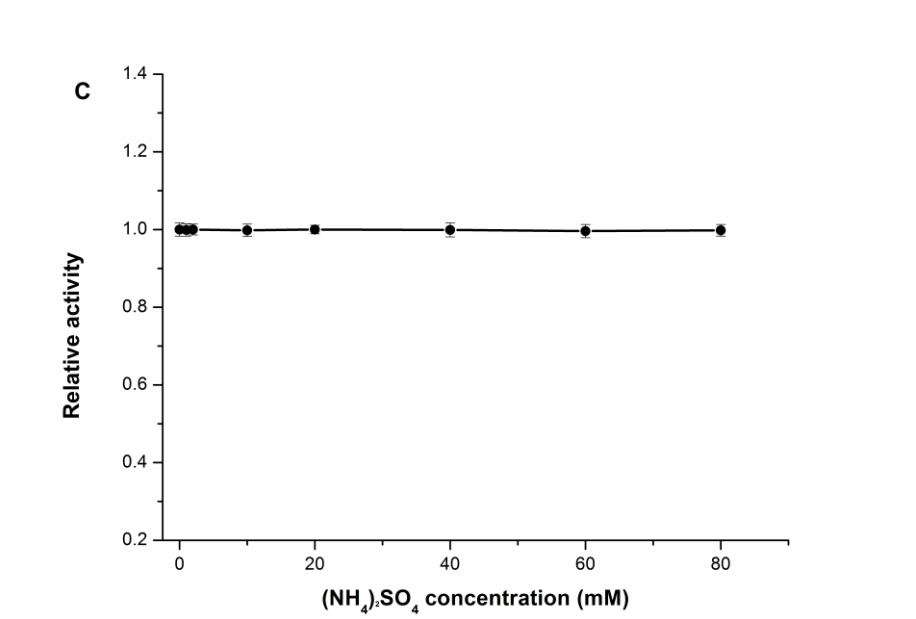

Figure S7. The effect of different concentrations of inhibitors on the activity of λ exo. (A) Na_2HPO_4; (B) ADP; (C) $(NH_4)_2SO_4$.