Electronic Supplementary Information

A sensitive and label-free T4 polynucleotide kinase/phosphatase detection based on poly(thymine)-templated copper nanoparticles coupled with nicking enzyme-assisted signal amplification

Jia Ge,*[ab] Lin Zhang,[a] Zhen-Zhen Dong,[a] Qi-Yong Cai,[a] and Zhao-Hui Li*[a]

*Corresponding author.

[a] College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450001, P. R. China.

[b] State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, P. R. China

Tel.: +86-371-67780037; Fax: +86-371-67781556.

E-mail address: jiage0630@hnu.edu.cn ; zhaohui.li@zzu.edu.cn.

Table S1. Comparison of analytical performance of different methods for T4 PNKP activity detection

Methods	Detection limit (U/mL)	References
^{32}P-labeling	0.17	1
Fluorescence	0.04	2
Fluorescence	0.05	3
Fluorescence	0.01	4
Fluorescence	0.02	5
Fluorescence	0.49	6
Fluorescence	0.1	7
Colorimetric	0.06	8
Luminescence	0.05	9
Electrochemistry	0.01	10
Fluorescence	0.02	Our method

Table S2. The relative standard deviations of Fig. 3 and Fig. 5.

	Concentration of T4 PNKP (U mL^{-1})	**RSD(%), n=3**
Fig. 3	0.02	6.26
	0.05	3.40
	0.1	4.64
	0.2	2.80
	2	1.50
	5	3.28
	10	2.50
	20	2.65
Fig. 5	**Concentration of T4 PNKP (U mL^{-1})**	**RSD(%), n=3**
	0.02	5.96
	0.05	3.75
	0.1	3.60
	0.2	4.06
	2	3.94
	5	3.68
	10	2.76
	20	2.86

Fig. S1. Investigation of the fluorescence intensity of obtained CuNPs as a function of amplification time. (Probe 1, 50 nM; T4 PNKP, 25 U/mL; KF polymerase, 25 U/mL; Nb.BbvCI, 50 U/mL; dNTPs, 200 μM; ascorbate, 5 mM; Cu^{2+}, 200 μM).

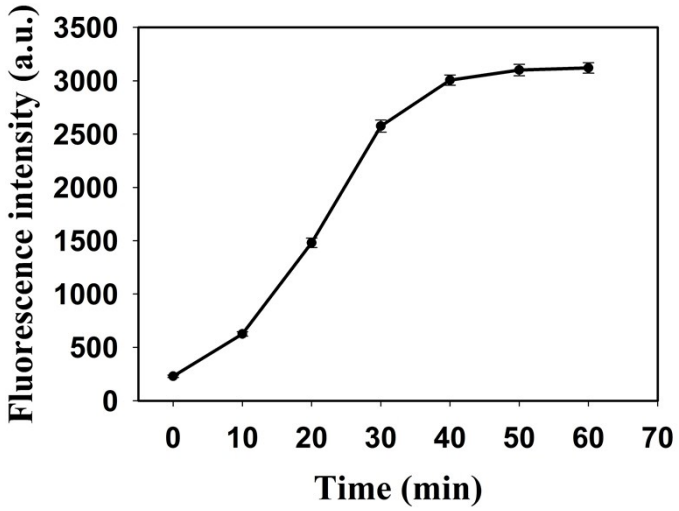


Fig. S2. Effect of Nb.BbvCI concentration on fluorescence intensity. The results were the average of three repetitive experiments with error bars indicating the standard deviation. (Probe 1, 50 nM; T4 PNKP, 25 U/mL; KF polymerase, 25 U/mL; dNTPs, 200 μM; ascorbate, 5 mM; Cu^{2+}, 200 μM).

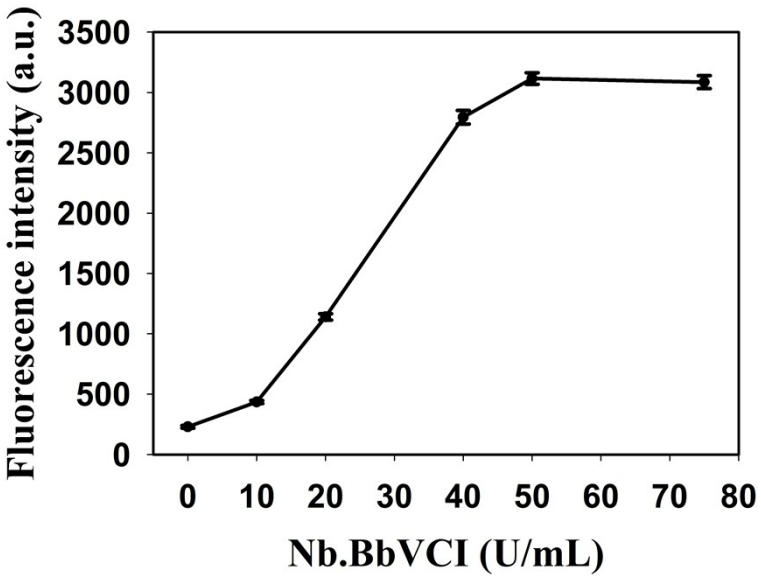


Fig. S3. (A) Optimization of dNTPs concentration. The concentration of KF polymerase was 25 U/mL. (B) Optimization of KF polymerase concentration. The concentration of dNTPs was 200 μM. (Probe 1, 50 nM; T4 PNKP, 25 U/mL; Nb.BbvCI, 50 U/mL; ascorbate, 5 mM; Cu^{2+}, 200 μM).

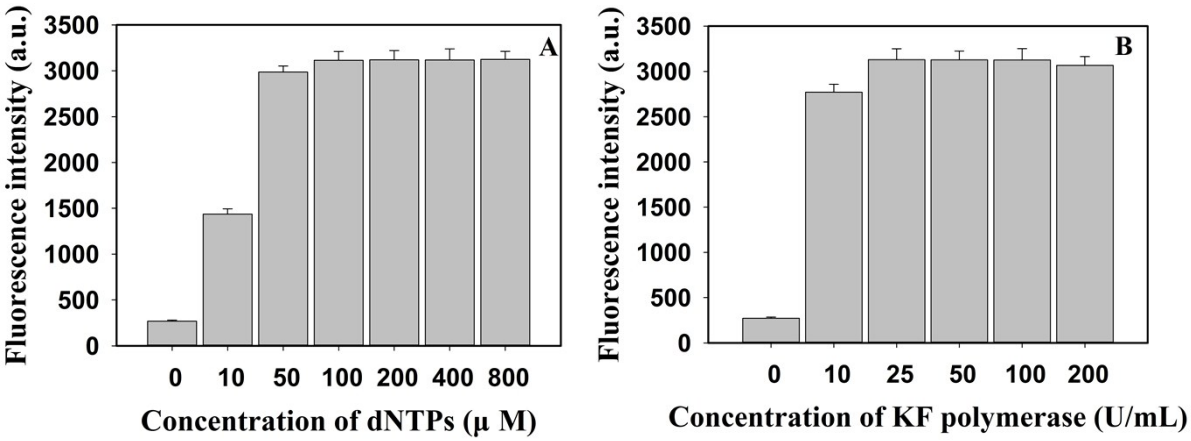
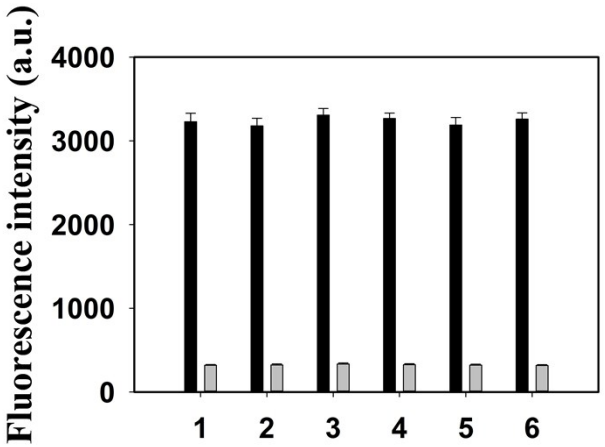


Fig. S4. Fluorescence spectra of the obtained CuNPs with T4 PNKP (black histogram) and without T4 PNKP (gray histogram) in the presence of different orders of addition of three enzyme : (1) T4 PNKP + KF polymerase + Nb.BbvCI; (2) T4 PNKP + Nb.BbvCI+ KF polymerase; (3) KF polymerase + T4 PNKP + Nb.BbvCI; (4) KF polymerase + Nb.BbvCI + T4 PNKP; (5) Nb.BbvCI + T4 PNKP + KF polymerase; (6) Nb.BbvCI+ KF polymerase + T4 PNKP. (Probe 1, 50 nM; T4 PNKP, 25 U/mL; KF polymerase, 25 U/mL; Nb.BbvCI, 50 U/mL; dNTPs, 200 μM; ascorbate, 5 mM; Cu^{2+}, 200 μM).



References:

1 F. Karimi-Busheri, G. Daly, P. Robins, B. Canas, D. J. Pappin, J. Sgouros, G. G. Miller, H. Fakhrai, E. M. Davis, M. M. Le Beau and M. Weinfeld, *J. Biol. Chem.*, 1999, **274**, 24187–24194.

2 C. Song and M. P. Zhao, *Anal. Chem.*, 2009, **81**, 1383-1388.

3 L. Lin, Y. Liu, X. Zhao and J. H. Li, *Anal. Chem.*, 2011, **83**, 8396–8402.

4 L. Lin, Y. Liu, J. Yan, X .S. Wang and J. H. Li, *Anal.Chem.*, 2013, **85**, 334–340.

5 C. X. Song, X. H. Yang, K. M. Wang, Q. Wang, J. B. Liu, J. Huang, L. L. He, P. Liu, Z. H. Qing and W. Liu, *Chem. Commun.*, 2015, **51**, 1815–1818.

6 L. L. Zhang, J. J. Zhao, H. Zhang, J. H. Jiang and R. Q. Yu, *Biosens. Bioelectron.*, 2013, **44**, 6–9.

7 Z. Z. Dong, L. Zhang, M. Qiao, Jia. Ge, A. L. Liu and Z. H. Li, *Talanta*, 2016, **146**, 253-258.

8 C. Jiang, C. Y. Yan, J. H. Jiang and R. Q. Yu, *Anal. Chim. Acta*, 2013, **766**, 88–93.

9 H. Z. He, K. H. Leung, W. Wang, D. S. H. Chan, C. H. Leung and D. L. Ma, *Chem. Commun.*, 2014, **50**, 5313–5315.

10 Y. H. Wang, X. X. He, K. M. Wang, X. Q. Ni, J. Su and Z. F. Chen, *Biosens. Bioelectron.*, 2011, **32**, 213–218.