

## Supplementary Information

### Free-Labeled Fluorescent Method for ATP Detection Assisted by T4

#### DNA Ligase

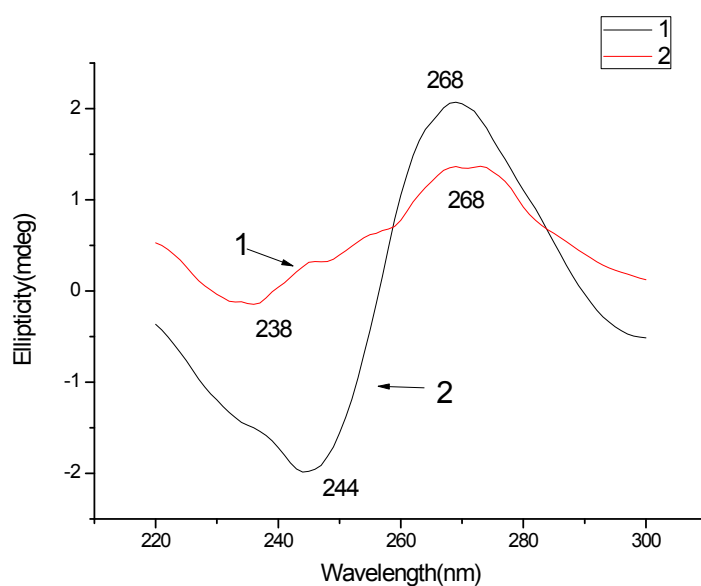
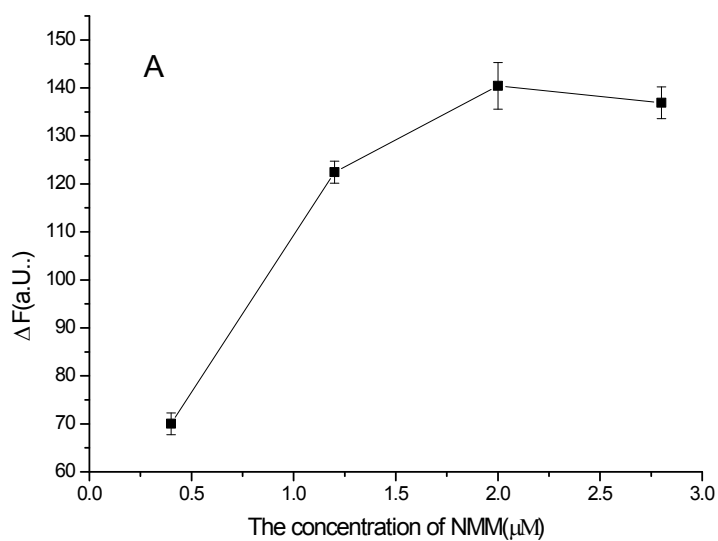
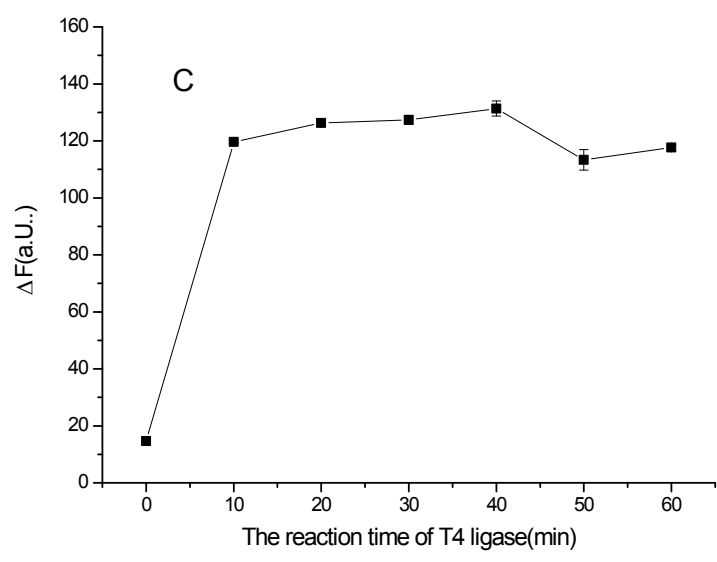
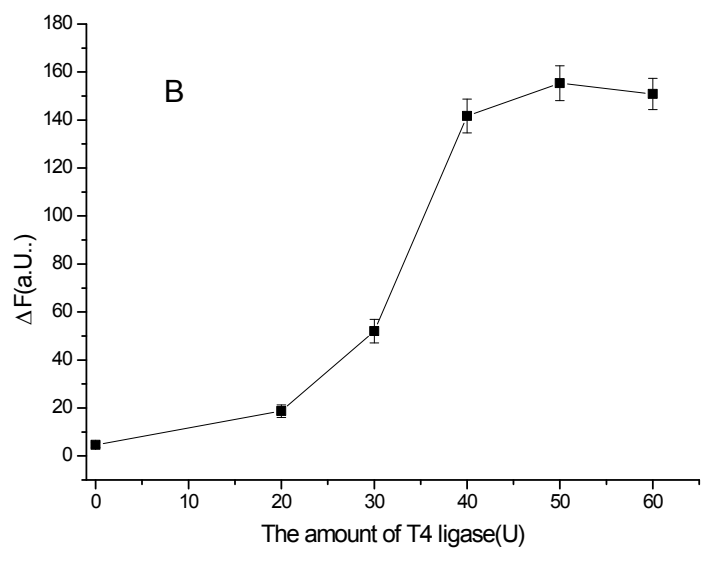


Figure S1 The CD Spectrum of Curve (1) 2  $\mu$ M G-DNA in 20 mM Tris/HCl (20 mM KCl, 70 mM NaCl, 10 mM MgCl<sub>2</sub>, pH 7.4), Curve(2) 1  $\mu$ M H1+1  $\mu$ M H2 +75 U T4+ 20 U ExoIII, in 20 mM Tris/HCl(20 mM KCl, 70 mM NaCl, 10 mM MgCl<sub>2</sub>, pH 7.4).





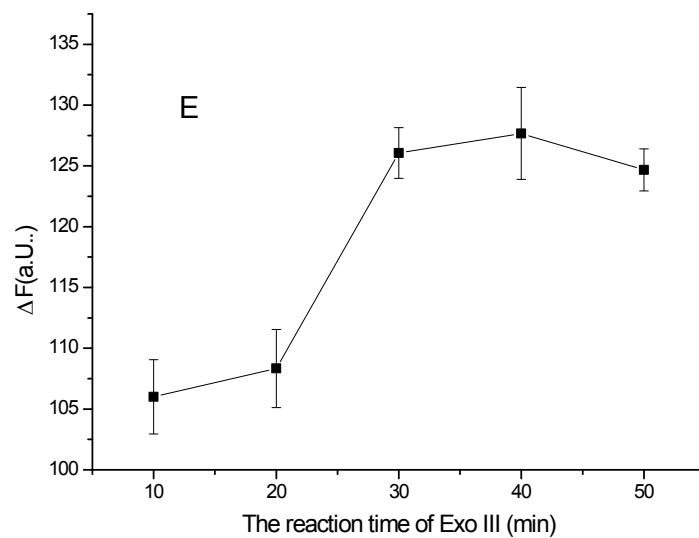
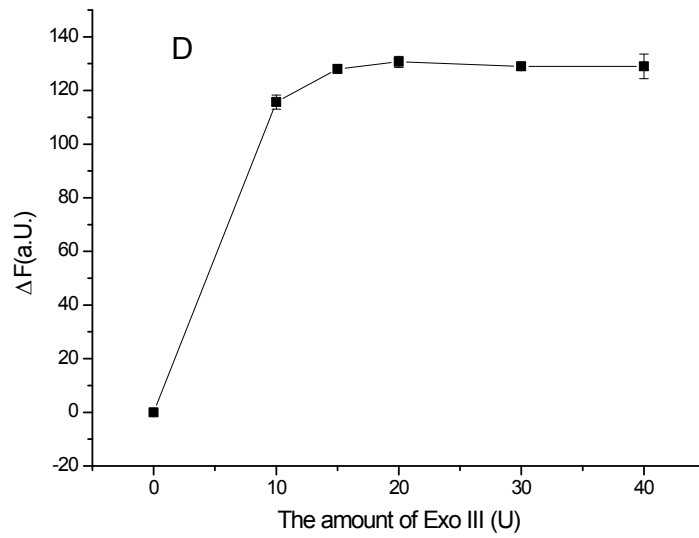


Figure S2 The effect of (A) the concentration of NMM; (B) the concentration of T4 DNA ligase; (C) the incubation time of T4 DNA ligase; (D) the concentration of T4 DNA ligase; (E) the incubation time of Exo III; on the fluorescence response of the ATP sensing system. The concentration of H1 and H2 was 100 nM and the concentration of ATP was 1.5  $\mu$ M. Error bars showed the standard deviation of three independent experiments.  $\Delta F$  was calculated by  $F_0 - F$ , where  $F$  and  $F_0$  represent fluorescence intensity in the presence and absence of ATP.

**Table S1 Comparison of the ATP analysis**

Detection method	Detection range	Detection limit	reference
Fluorescence	0.2 –50 $\mu$ M	93 nM	1
Fluorescence	0.5 –50 $\mu$ M	140 nM	2
Fluorescence	30 – 800 $\mu$ M	15 $\mu$ M	3
Fluorescence	0.01 –1 $\mu$ M	4 nM	4
Fluorescence	Not clearly mention	0.44 and 0.65 mM	5
Electrochemical	10 nM – 1 $\mu$ M	3.4 nM	6
Fluorescence	5 – 300 nM	3.75 nM	This work

**Table S2 Recovery of ATP in spiked human serum samples**

Samples	Added (nM)	Found (nM)	Recovery (%)	RSD (% , n=3)
1	40	39.8	99.4	7.2
2	60	62.9	104.9	6.2
3	120	115.0	95.9	4.8

## References

- 1 Q. W. Song, R. H. Wang, F. F. Sun, H. K. Chen, Z. K. Wang, N. N, and J. Ouyang, *Biosensors and Bioelectronics*, 2017, 87, 760–763.
- 2 Y. L. Wei, Y. X. Chen , H. H. Li , S. M. Shuang , C. Dong, and G. F. Wang, *Biosensors and Bioelectronics* 2015,63,311–316
- 3 Q. G. Chen, Q. Q. Guo, Y. Chen, J. Pang, F. F. Fu, and L. Q. Guo, *Talanta*, 2015, 138, 15–19.
- 4 X. Li, X. L. Ding, and J. Fan, *Analyst*, 2015, 140, 7918.
- 5 J. N. Xu, and C. Y. Wei , *Biosensors and Bioelectronics*, 2017, 87, 422–427
- 6 T. X. Hu, W. Wen, X. H. Zhang, S. F. Wang, *Analyst*, 2016, 141, 1506–1511