Electronic Supplementary Information

Label-free biosensor based on dsDNA-templated copper nanoparticles for highly sensitive and selective detection of NAD^+

Jia Ge,* Zhen-Zhen Dong, Lin Zhang, Qi-Yong Cai, Dong-Mei Bai, and Zhao-Hui Li*

*Corresponding author.

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

Tel.: +86-371-67780037.

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

Fig. S1. Typical TEM image of CuNPs templated by extended Probe 1.

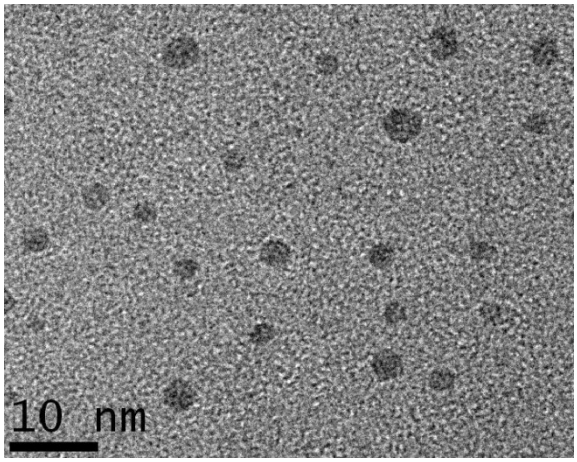

Fig. S2. Agarose gel electrophoresis demonstration of the detection of NAD^+ based on dsDNA-templated CuNPs and DNA ligation reaction. lane 1, Probe 1; lane 2, Probe 1 + Probe 2; lane 3, Probe 1 + *E. coli* ligase + KF polymerase + dNTPs; lane 4, Probe 1 + *E. coli* ligase + NAD^+ + KF polymerase + dNTPs. (Probe 1 , 1 μM; Probe 2, 1 μM; *E. coli* ligase, 25 U/mL; NAD^+, 400 nM; KF polymerase, 10 U/mL; dNTPs, 100 μM).

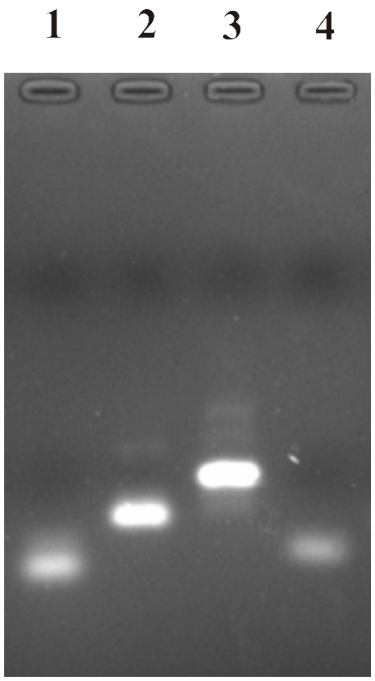

Fig. S3. Effect of the length of AT-TA on the formation of dsDNA-templated fluorescent CuNPs. F and F_0 represent the fluorescence intensities of dsDNA-templated CuNPs platform in the presence and absence of NAD^+, respectively. (Probe 1, 500 nM; Probe 3, 500 nM; Probe 4, 500 nM; Probe 5, 500 nM; Probe 6, 500 nM; *E. coli* ligase, 25 U/mL; KF polymerase, 10 U/mL; NAD^+, 400 nM; dNTPs, 100 μM; ascorbate, 5 mM; Cu^{2+}, 200 μM).

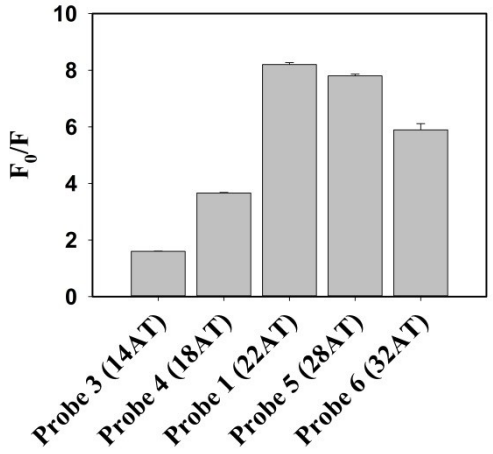

Fig. S4. Optimization of Cu^{2+} concentration on the formation of fluorescent CuNPs. The results were the average of three repetitive experiments with error bars indicating the standard deviation. (Probe 1, 500 nM; ascorbate, 5 mM).

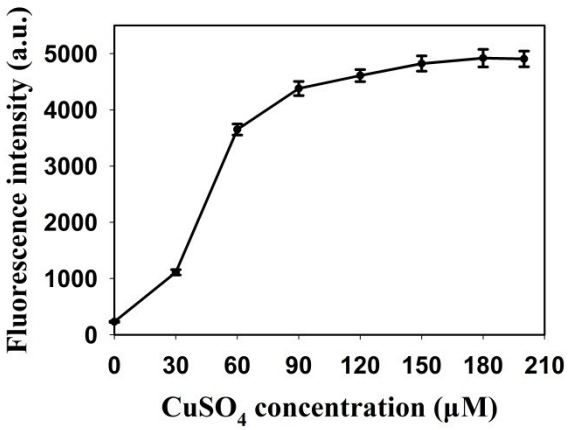

Fig. S5. Optimization of ascorbate concentration on the formation of fluorescent CuNPs. The results were the average of three repetitive experiments with error bars indicating the standard deviation. (Probe 1, 500 nM; Cu^{2+}, 200 μM).

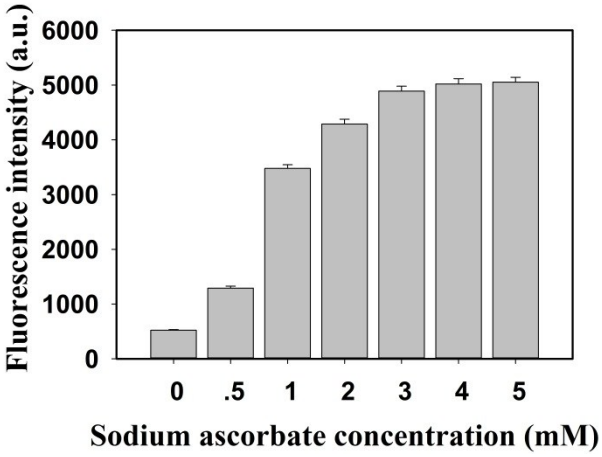

Fig. S6. (A) Optimization of dNTPs concentration. The concentration of KF polymerase was 10 U/mL. (B) Optimization of KF polymerase concentration. The concentration of dNTPs was 100 μM. F and F_0 are fluorescence intensities of dsDNA-templated CuNPs platform in the presence and absence of NAD^+. (Probe 1, 500 nM; E. coli ligase, 25 U/mL; NAD^+, 400 nM; ascorbate, 5 mM; Cu^{2+}, 200 μM).

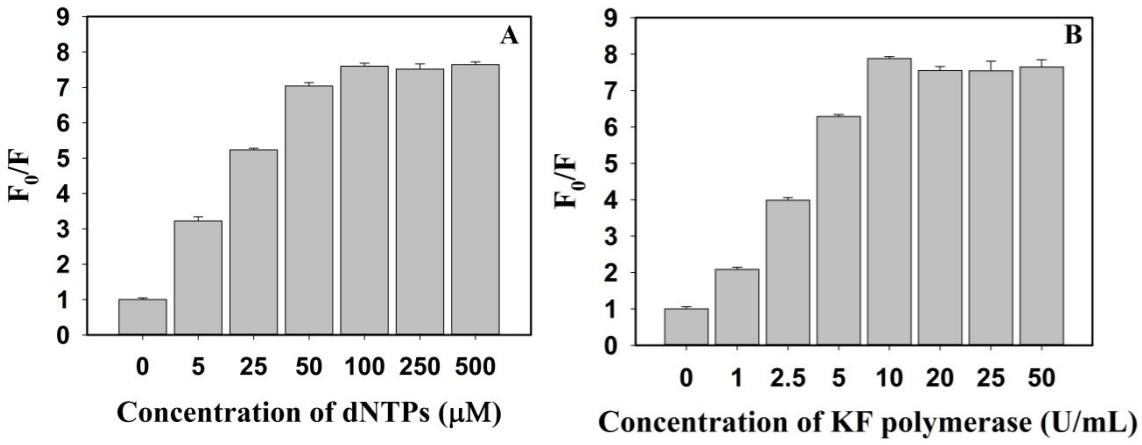

Fig. S7. Effect of E. coli ligase concentration on fluorescence intensity. The results were the average of three repetitive experiments with error bars indicating the standard deviation. (Probe 1, 500 nM; KF polymerase, 10 U/mL; NAD^+, 400 nM; dNTPs, 100 μM; ascorbate, 5 mM; Cu^{2+}, 200 μM).

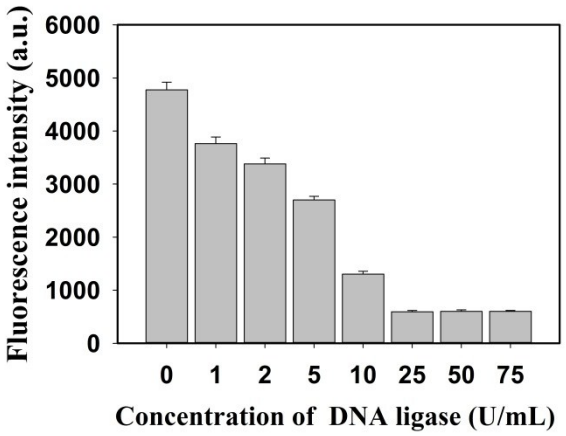

Fig. S8. The effect of the reaction time of ligase on the change of the fluorescence intensity of the NAD^+ sensing system. F and F_0 represent the fluorescence intensities of dsDNA-templated CuNPs platform in the presence and absence of NAD^+, respectively. Error bars were the standard deviation of three measurements. (Probe 1, 500 nM; *E. coli* ligase, 25 U/mL; KF polymerase, 10 U/mL; NAD^+, 400 nM; dNTPs, 100 μM; ascorbate, 5 mM; Cu^{2+}, 200 μM).

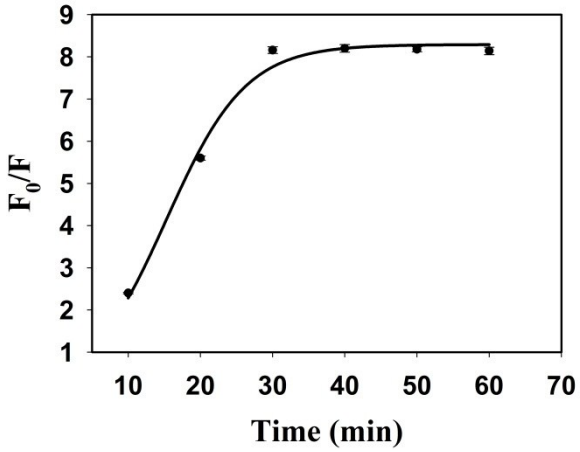

Fig. S9. Effect of extension reaction time on fluorescence intensity. The results were the average of three repetitive experiments with error bars indicating the standard deviation. (Probe 1, 500 nM; KF polymerase, 10 U/mL; dNTPs, 100 μM; ascorbate, 5 mM; Cu^{2+}, 200 μM).