Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Nicotinamide adenine dinucleotide detection based on silver nanoclusters stabilized by a dumbbell-shaped DNA template

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Table S1 All sequences used in the method

Name	Sequence* (5'-3')
DNA1	ATATCGACCCCCCTCGATATATATCGACCCCCCTCGATAT
DNA2	TATATCGACCCCCCTCGATATATATATCGACCCCCCTCGATATA
DNA3	ATATATCGACCCCCCTCGATATATATATATCGACCCCCCTCGATATAT
DNA4	TATATATCGACCCCCCTCGATATATATATATATCGACCCCCCTCGATATATA
DNA5	ATATATATCGACCCCCCTCGATATATATATATATATCGACCCCCCTCGATATATAT
DNA6	TCGATATATATATATATATCGACCCCCCTCGATATATATATATATATCGA
DNA7	AGCTATATATATATATATAGCTCCCCCCAGCTATATATATATATATAGCT
Probe1	Pi-ATATCGACCCCCCTCGATATATATCGACCCCCCTCGATAT
Probe2	Pi-ATATATCGACCCCCCTCGATATATATATATCGACCCCCCTCGATATAT
Probe3	Pi-ATATATATCGACCCCCCTCGATATATATATATATATCGACCCCCCTCGATATATAT

* The blue color in cytosine represents AgNCs forming domain, and the green color represents stem domain.

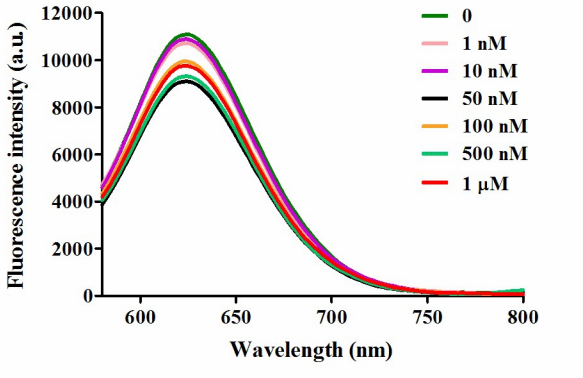

Fig. S1. The effect of NAD^+ with different concentrations on the formation of Probe3/AgNCs.

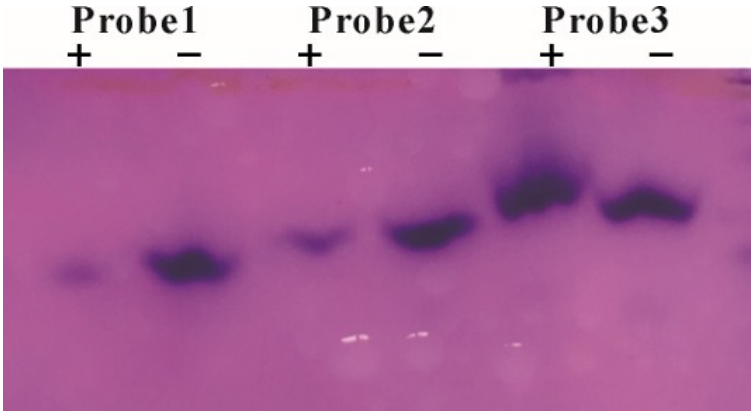

Fig. S2. Non-denaturing polyacrylamide gel electrophoresis of Probe1, Probe2, and Probe3 with (＋) and without (－) three enzymes (*E.coli* ligase, Exo III, and Exo I) in the presence of NAD^+, respectively.

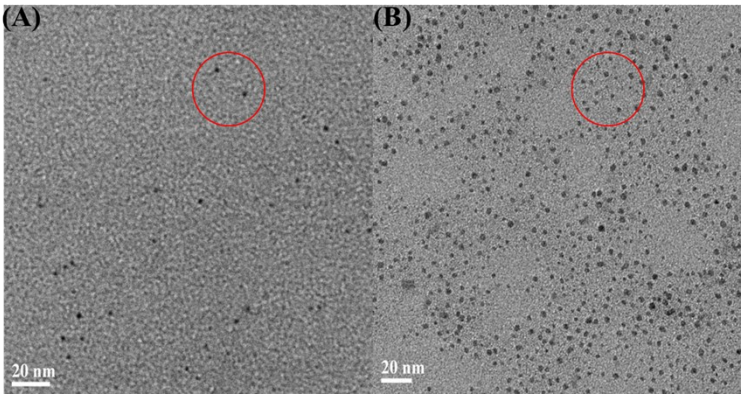

Fig. S3. TEM images of DNA/AgNCs from the reaction system before (A) and after (B) adding NAD^+. Concentration of Probe3 was 2 μM and NAD^+ was 500 nM

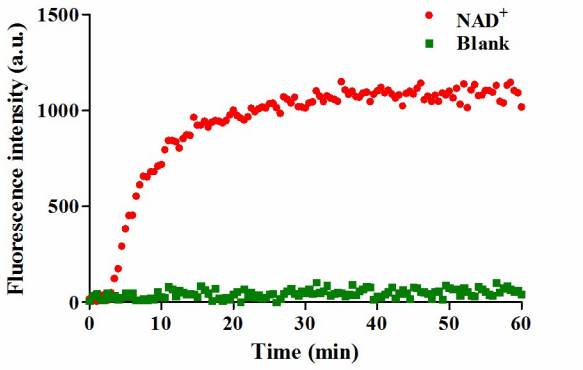

Fig. S4. Fluorescence intensity as a function of time for DNA/AgNCs in the absence (green dots) and presence (red dots) of 500 nM NAD^+, respectively.

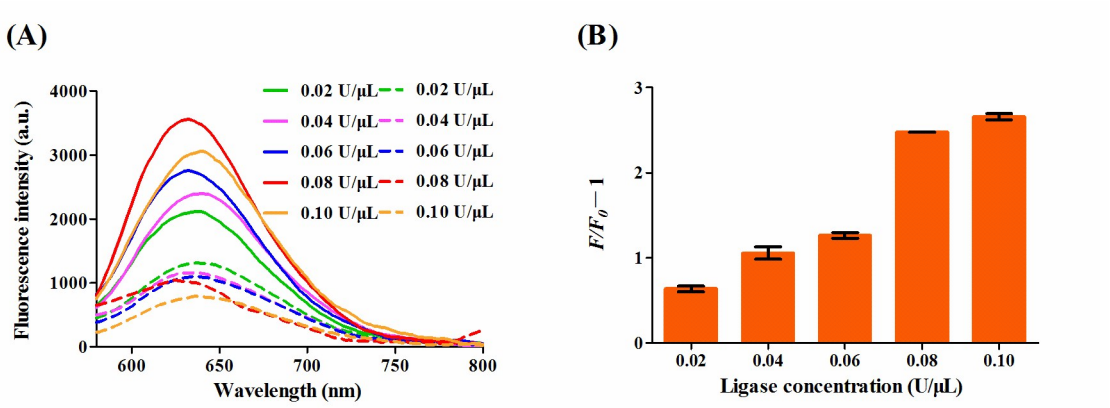

Fig. S5. Optimization of the concentration of *E.coli* DNA ligase. (A) Fluorescence emission responses of the proposed system carried out using different concentrations of *E.coli* DNA ligase. The solid and dashed lines represent fluorescence spectra of the system with and without NAD^+, respectively. (B) Relative fluorescence enhancements (F/F_0-1) for different concentrations of *E.coli* DNA ligase. F and F_0 are the fluorescence intensities at 636 nm in the presence and absence of NAD^+, respectively. The concentration of NAD^+ was 500 nM. The error bars were calculated from three independent experiments.

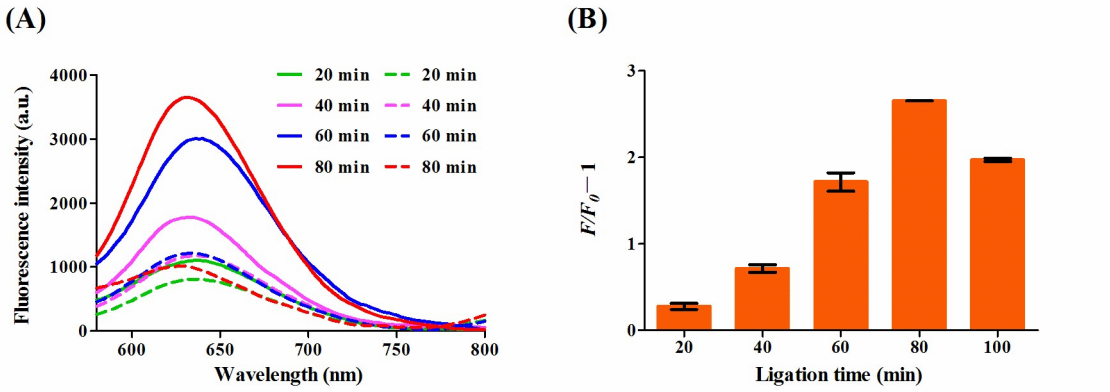

Fig. S6. Optimization of the ligation time of *E. coli* DNA ligase. (A) Fluorescence emission responses of the proposed system carried out using different ligation times of *E.coli* DNA ligase. The solid and dashed lines represent fluorescence spectra of the system with and without NAD^+, respectively. (B) Relative fluorescence enhancements (F/F_0-1) for different ligation time of *E.coli* DNA ligase. F and F_0 are the fluorescence intensities at 636 nm in the presence and absence of NAD^+, respectively. The concentration of NAD^+ was 500 nM. The error bars were calculated from three independent experiments.

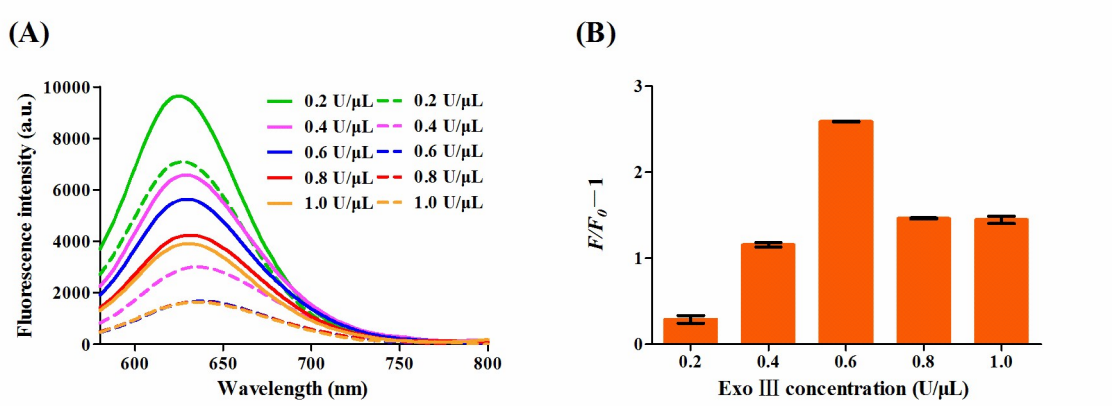

Fig. S7. Effect of Exo III concentration. (A) Fluorescence emission spectra for different concentrations of Exo III. The solid and dashed lines represent fluorescence spectra of the system with and without NAD^+, respectively. (B) Relative fluorescence enhancements (F/F_0-1) for different concentrations of Exo III. F and F_0 are the fluorescence intensities at 636 nm in the presence and absence of NAD^+, respectively. The concentration of NAD^+ was 500 nM. The error bars were calculated from three independent experiments.

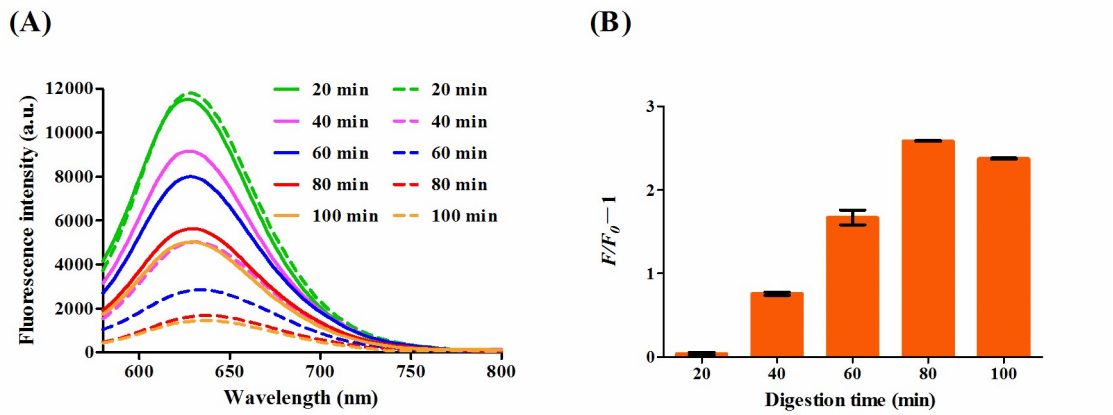

Fig. S8. Optimization of the digestion time of Exo III. (A) Fluorescence emission spectra for different digestion times of Exo III. The solid and dashed lines represent fluorescence spectra of the system with and without NAD^+, respectively. (B) Relative fluorescence enhancement (F/F_0-1) for different ligation time of Exo III. F and F_0 are the fluorescence intensities at 636 nm in the presence and absence of NAD^+, respectively. The concentration of NAD^+ was 500 nM. The error bars were calculated from three independent experiments.

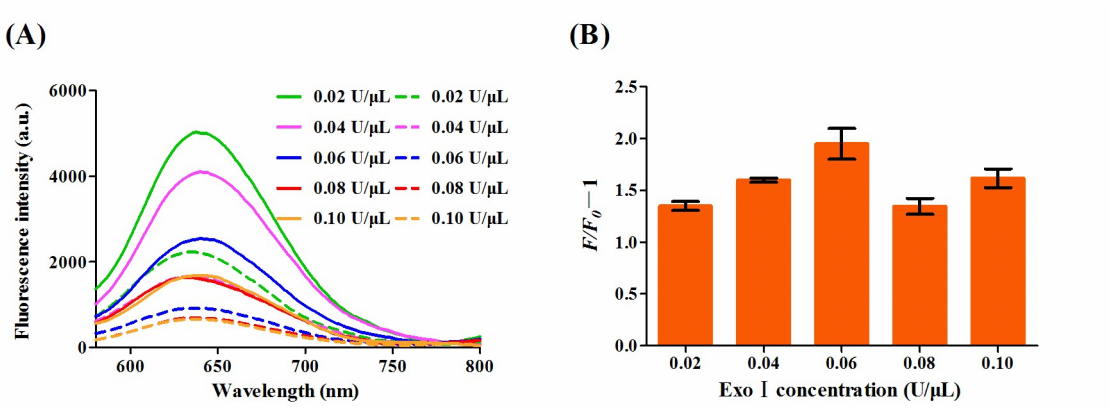

Fig. S9. Optimization of Exo I concentration. (A) Fluorescence emission spectra obtained with different concentrations of Exo I. The solid and dashed lines represent fluorescence spectra of the system with and without NAD^+, respectively. (B) Relative fluorescence enhancement (F/F_0-1) for different concentrations of Exo I. F and F_0 are the fluorescence intensities at 636 nm in the presence and absence of NAD^+, respectively. The concentration of NAD^+ was 500 nM. The error bars were calculated from three independent experiments.

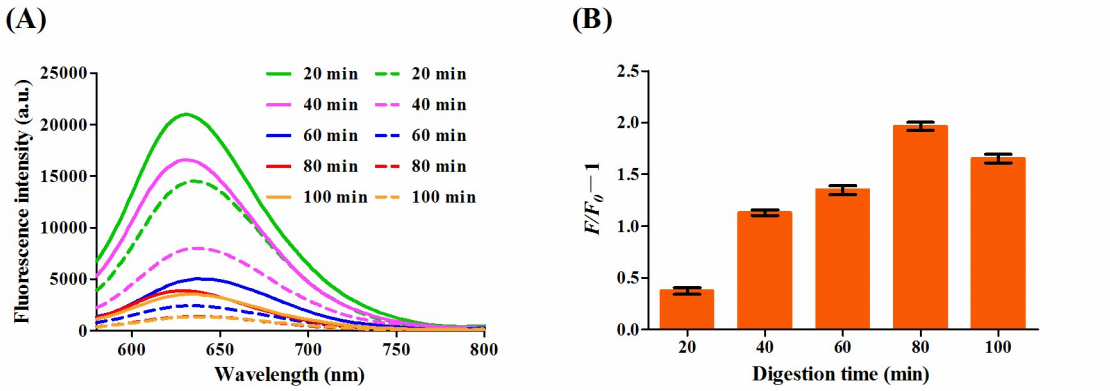

Fig. S10. Optimization of the digestion time of Exo I. (A) Fluorescence emission spectra for different digestion times of Exo I. The solid and dashed lines represent fluorescence spectra of the system with and without NAD^+, respectively. (B) Relative fluorescence enhancement (F/F_0-1) for different digestion time of Exo I. F and F_0 are the fluorescence intensities at 636 nm in the presence and absence of NAD^+, respectively. The concentration of NAD^+ was 500 nM. The error bars were calculated from three independent experiments.

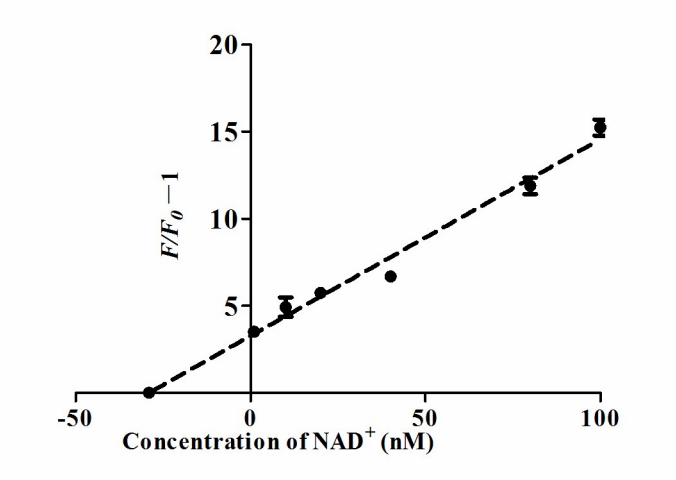

Fig. S11. Standard additions plot for detection of NAD^+ spiked in cell lysate. The absolute value of the x-intercept is 29.08 nM. F and F_0 are the fluorescence intensities at 636 nm in the presence and absence of NAD^+, respectively. The error bars were calculated from three independent experiments.

Table S2. Comparison of our method and other methods for NAD^+ detection

Method	Detection limit	Reference
Molecular beacon-based method	0.3 nM	1
Ligation-triggered DNAzyme cascade	50 pM	2
Ligation-triggered rolling circle amplification	1 pM	3
Ligation-induced quadruplex formation	0.5 nM	4
Inhibition of G-quadruplex assembling by DNA ligation	10 nM	5
Ligase-mediated inhibition on strand displacement amplification with G-quadruplex	50 pM	6
Electrochemical method	1.8 nM	7
Our method	0.25 nM	-

Reference

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