Supplementary Information

Ultrasensitive and selective detection of nicotinamide adenine dinucleotide by target-triggered ligation-rolling circle amplification

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Experimental details

Materials

E. coli DNA ligase, and deoxyribonucleoside 5'-triphosphate mixture (dNTPs) were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Phi29 DNA polymerase was obtained from Fermentas (Lithuania). DNA ladders, nicotinamide adenine dinucleotide (NAD^+) and its analogs such as NADH, nicotinamide adenine dinucleotide phosphate ($NADP^+$), reduced nicotinamide adenine dinucleotide phosphate (NADPH), adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine 5'-monophosphate (AMP) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). All other reagents were of analytical grade. All aqueous solutions were prepared using ultra-pure water (≥ 18 MΩ, Milli-Q, Millipore).

The oligonucleotides were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China), and their base sequences in detail were as follows:

cDNA: 5'-GGCGAGACACGGTGCTGCAG -3'

Padlock probe:

5'-PO_4-GTGTCTCGCCTTCTTGTTTCCTTTCCTTGAAACTTCTTTCTTTCTTCGCTGCAGCACC –OH-3'

Molecular beacon (MB):

5'-FAM-AGCTTAATCCTTGAAACTTCTTCTTAGCT-DABAYL -3'

In the padlock probe, the underlined letters at the two ends indicate complementary sequences of the cDNA.

Target-triggered ligation-rolling circle amplification (L-RCA) for NAD^+ assay

In a typical procedure, first, a 10 μL reaction mixture in solution (60 mM pH 8.0 Tris-HCl, 8 mM $MgCl_2$, 20 mM $(NH_4)_2SO_4$, and 2.4 mM EDTA), which contained 200 nM of cDNA and 600 nM of padlock probe, was denatured at 90 ^oC for 5 min and

cooled down to room temperature. Then, the reaction mixture was incubated in 25 ^{o}C for 30 min to afford the padlock probe-cDNA hybrids. Second, the padlock probes were ligated for subsequent RCA by adding 2 μL of 10×BSA (0.05%), 0.5 μL of *E. coli* DNA ligase (3 U), 2 μL different concentrations of NAD^+ and 5.5 μL water into the solution of padlock probe-cDNA duplex and allowing the NAD^+-triggered ligation reaction at 37 ^{o}C for 60 min.[1] In the presence of NAD^+, the *E. coli* DNA ligase could catalyze the ligation between 3'-OH and 5'-PO_4 end of the padlock probes, triggering the formation of circular DNA template. Third, the resulting solution was added into 200 μL of Phi29 reation buffer containing 625 μM dNTPs, and 1 U/μL phi29 polymerase for RCA reaction.[2] The polymerization reaction was carried out at 37 ^{o}C for 70 min. After ligation, the resulting solution was maintained at 90 ^{o}C for 5 min to inactivate phi29 polymerase and terminate RCA reaction. Finally, the MBs with the final concentration of 1 μM were added into the end products of RCA to perform 30 min hybridization at 37 ^{o}C prior to fluorescence measurement.

Fluorescence Measurement

All fluorescence measurements of samples were performed on FluoroMax-4 fluorescence spectrometer (Horiba Jobin Yvon) at room temperature. The emission spectra were collected from 505 nm to 580 nm with an excitation wavelength of 495 nm. The fluorescence intensity at 518 nm was used to choose the optimal experimental conditions and evaluate the performance of the fluorescent biosensor. Both the excitation and emission slit widths were set at 3 nm.

The verification of NAD^+-triggered L-RCA reaction by agarose gel electrophoresis

9 μL of L-RCA reaction products triggered by different concentrations of NAD^+ were firstly mixed with 1 μL 10×loading buffer, respectively. The mixtures were subjected to electrophoresis on 1% agarose gel containing ethidium bromide (1 μg/mL) in 1×TAE buffer (40 mM Tris, 20 mM glacial acetic acid, 1 mM EDTA, pH 8.0) at a constant potential of 56 V for 45 min. Gels were photographed by G:Box gel imaging system (Syngene, Cambridge).

Specificity Valuation

The selectivity was evaluated by measuring and comparing the response of NAD^+ to that its analogs including NADH, $NADP^+$, NADPH, ATP, ADP and AMP. The final concentrations of NAD^+ and its analogs were 50 nM and 10 μM, respectively. Fluorescence intensity was recorded at 518 nm with an excitation

wavelength of 495 nm, and fluorescence enhancement was used to evaluate the specificity of the novel NAD^+ sensing strategy.

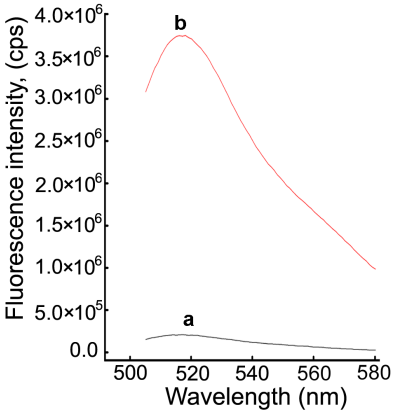

Fig. S1 Fluorescence spectra of the MBs in the NAD^+ sensing system. (a) In the absence of target NAD^+. (b) In the presence of 10 μM target NAD^+.

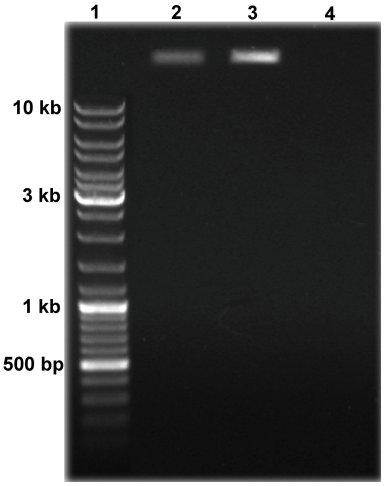

Fig. S2 Agrose Gel (1%) Electrophoresis for the verification of L-RCA reaction products triggered by different concentrations of NAD^+. Lane 1: DNA ladder marker. Lane 2: 10 nM NAD^+. Lane 3: 1 μM NAD^+. Lane 4: control experiment without NAD^+.

Reference

1. J. S. Li, T. Deng, X. Chu, R. H. Yang, J. H. Jiang, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2010, **82**, 2811.

2. S. B. Zhang, Z. S. Wu, G. L. Shen and R. Q. Yu, *Biosens Bioelectron*, 2009, **24**, 3201.