Electronic Supplementary Information (ESI)

A label-free fluorescence DNA probe based on ligation reaction with quadruplex formation for highly sensitive and selective detection of nicotinamide adenine dinucleotide

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1. Experimental Section

Chemicals and Materials

The synthetic oligonucleotide, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and nicotinamide adenine dinucleotide phosphate (NADP) were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). *Escherichia coli* DNA ligase was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Klenow Fragment (exo-) and dNTPs were obtained from New England Biolabs, Inc. (Beverly, MA, USA). Nicotinamide adenine dinucleotide (NAD^+), the reduced formof nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate hydride (NADPH) were purchased from Sigma-Aldrich Chemical Co.. N-methyl mesoporphyrin IX (NMM) was purchased from Porphyrin Products Inc (Logan, UT), and its

concentration was measured by absorbance at 379 nm assuming an extinction coefficient of $1.45\times10^{5}m^{-1}cm^{-1}$. All the other chemicals were of analytical reagent grade and used without further purification. All solutions were prepared in Milli-Q water (resistance>18 MΩ·cm) from a Millipore system. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter.

Fluorescence Measurements

All fluorescence measurements were carried out on an F-7000 spectrometer (Hitachi, Japan). The instrument settings were chosen as follows: λ ex = 399 nm (bandpass 5 nm), λ em = 610 nm (bandpass 5 nm), PMT detector voltage = 800 V. All measurements were performed in a buffer solution containing 30 mM Tris-HCl, 4 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$ and 1.2 mM EDTA (pH=8.0).

Assay Process

A mixture containing 500 nM oligonucleotide, 0.09 U/μL E.coli DNA ligase was prepared in a buffer (pH=8.0) of 50 μL volume in the presence of varying concentrations of NAD^+. The mixture was incubated at 37℃ for 30 min. Then, 1 μL dNTPs (10 mM/μL) solution and 0.5 μL Klenow Fragment (exo-) (5 U/μL) polymerase solution were added into the mixture. After incubated again for 30 min, 1.2 μL NMM (10 μM) solution, 0.8 μL KCl (100 mM) solution and appropriate buffer were added and made the last volume of the solution was 80 μL. Finally, the mixture solution was incubated at 37°C for 20 min. The fluorescence intensity of the mixture solution was measured in a 200 μL quartz cuvette at room temperature.

2. Supplenmentary Figures

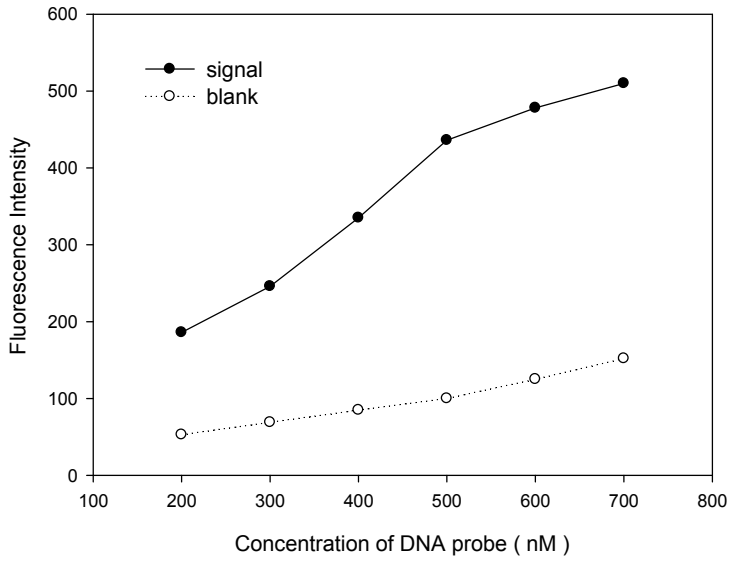

Figure S1. The effect of the concentration of oligonucleotide on the fluorescence response of the NAD^+ sensing system for blank (dotted line) and 500 nM NAD^+ (solid line).

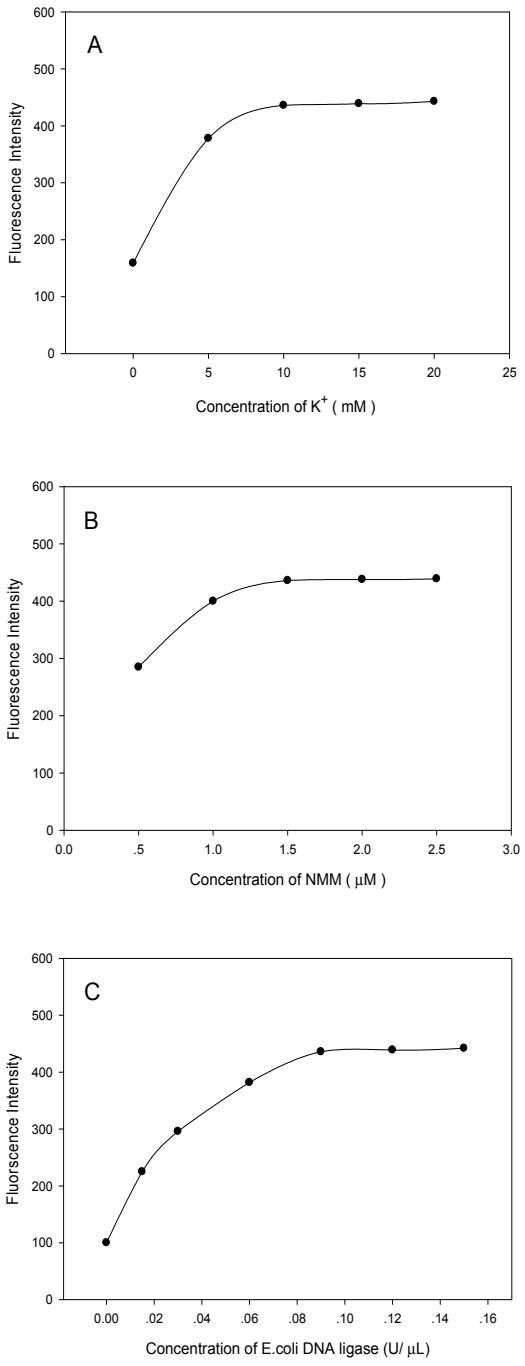

Figure S2. The effect of (A) the concentration of K^+, (B) the concentration of NMM and (C) the concentration of *E. coli* DNA ligase on the fluorescence response of the NAD^+ sensing system. The concentration of NAD^+ is 500 nM.

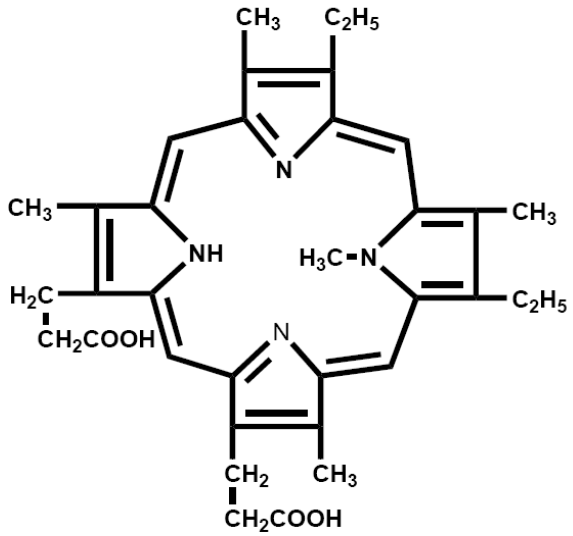

Figure S3. Molecular structure of NMM.