Supporting Information

NMN Sensor Cocktail: Selective Sensing of Nicotinamide Mononucleotide over Citric Acid

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Materials and general methods

All reagents and solvents employed including nicotinamide mononucleotide (NMN), citric acid (CA), nicotinamide (N), adenosine triphosphate (ATP), nicotinamide riboside (NR), nicotinamide adenine dinucleotide (NAD^+), and dimethyl sulfoxide (DMSO) were obtained from commercial sources (mainly Sigma-Aldrich/Merck) and used as supplied without further purification. NBD-B2 and Styryl-51F were synthesized following the procedure in references.[1-3] Deionized water was obtained from Merck Milli-Q purification system. Fluorescence measurements were performed on a microplate reader. (TECAN Infinite M1000 Pro).

Screening of NBD and Styryl library for NMN.

The NBD and Styryl library, composed of 80 NBD based compounds and 80 styryl based compounds individually, are used for 96-well based screening.[1-3] For the screening, NBD or Styryl compounds (10 μL, 100 μM in 50% DMSO) were mixed with NMN solution (90 μL, 5 mg mL^{-1}) or DIW (90 μL) as a control. The fluorescence intensities of the mixtures were analyzed under λ_{ex} = 470 nm and λ_{em} = 520 – 750 nm conditions. The mixtures showing stronger fluorescence intensity than control were selected as hit compounds.

Preparation of NBD-B2 and Styryl-51F mixture and fluorescence measurement

NBD-B2 (5.6 mg, 10 μmol) and Styryl-51F (5.3 mg, 10 μmol) were dissolved in DMSO (1 mL) individually, to make 10 mM of NBD-B2 and Styryl-51F solution. NMN (500 mg, 1.5 mmol) and CA (500 mg, 2.6 mmol) were dissolved in DIW (10 mL) to make 50 mg mL^{-1} NMN and CA solution. The NBD-B2, Styryl-51F, NMN, and CA solutions were used for the fluorescence measurements with additional dilution on purpose. The NBD-B2 or Stryryl-51F solutions (1 μL) was mixed with the NMN or CA solutions (10 μL) and DIW (90 μL). After

waiting 10 mins, the fluorescence intensities of the mixtures were analyzed under λ_{ex} = 470 nm and λ_{em} = 520 – 750 nm conditions. (Figure 2 a, b) NBD-B2 and Styryl-51F mixtures were prepared by mixing NBD-B2 and Styryl solutions with various mixing ratio: 1:0, 10:1, 1:1, 1:5, 1:10 (v/v NBD-B2 : Styryl-51F). The NBD-B2 and Styryl-51F mixtures (6 μL) were mixed with the NMN or CA solutions (10 μL) and DIW (90 μL). After waiting 10 mins, the fluorescence intensities of the mixtures were analyzed under λ_{ex} = 470 nm and λ_{em} = 520 – 750 nm conditions. (Figure 2c, d, S1 a-c) For the quantitative analysis of the ratiometric fluorescence of the NBD-B2 and Styryl-51F (1:5) mixture, the NMN and CA solutions were prepared by varying the concentration from 0 to 10 mg mL^{-1} with an interval of 1 mg mL^{-1} and 0 to 1 mg mL^{-1} with an interval of 0.2 mg mL^{-1} and mixed with the NBD-B2 and Styryl-51F (1:5) mixture (6 μL). After waiting 10 mins, the fluorescence intensities of the mixtures were analyzed under λ_{ex} = 470 nm and λ_{em} = 520 – 750 nm conditions. (n=3) (Figure 3 a-d, S2)

Fluorescence measurement with various substrates

Nicotinamide (50 mg, 400 μmol), adenosine triphosphate (50 mg, 98 μmol), nicotinamide riboside (50 mg, 200 μmol), nicotinamide adenine dinucleotide (50 mg, 75 μmol) were dissolved in DIW (1 mL) individually. Each of the substrate solutions (20 μL) were mixed with the NBD-B2 and Styryl-51F (1:5) mixture (6 μL) and DIW (80 μL). After waiting 10 mins, the fluorescence intensities of the mixtures were analyzed under λ_{ex} = 470 nm and λ_{em} = 520 – 750 nm conditions. (Figure 4 a, b)

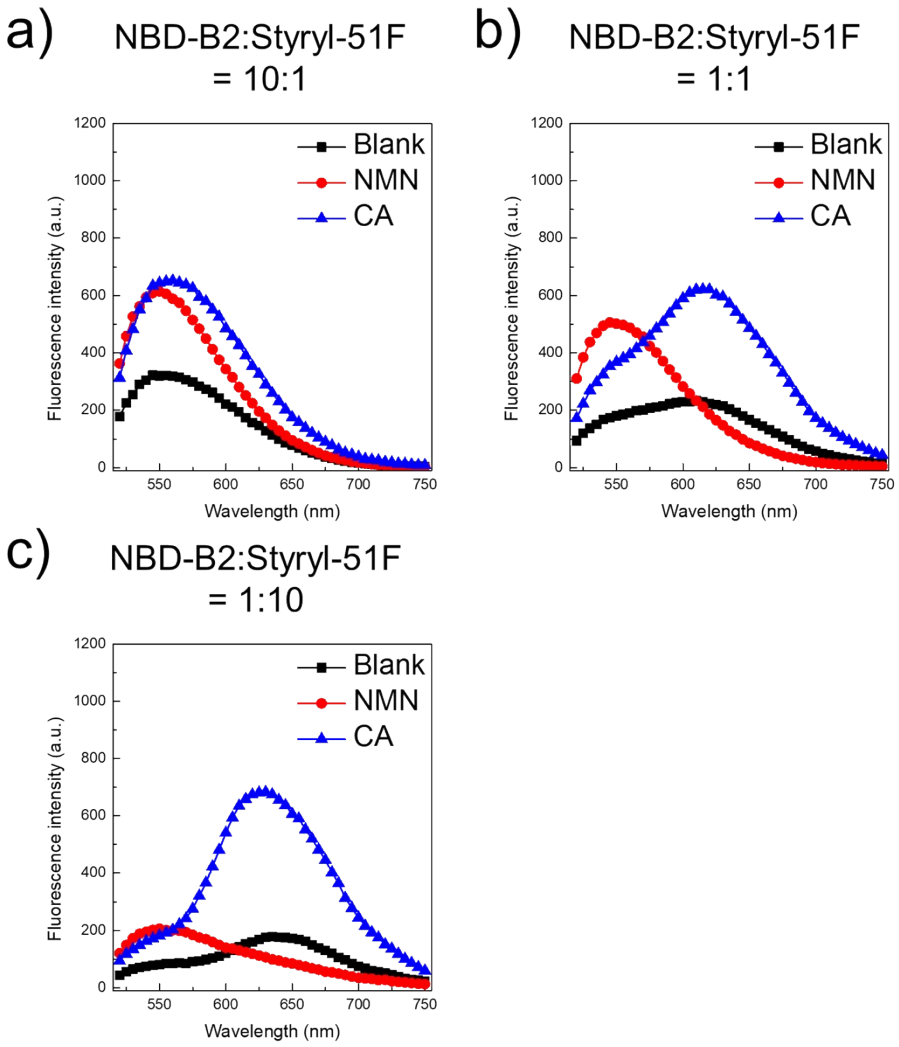

Figure S1. Fluorescence spectra of the mixture of NBD-B2 and Styryl-51F with various mixing ratio, (a) 10:1, (b) 1:1, and (c) 1:10.

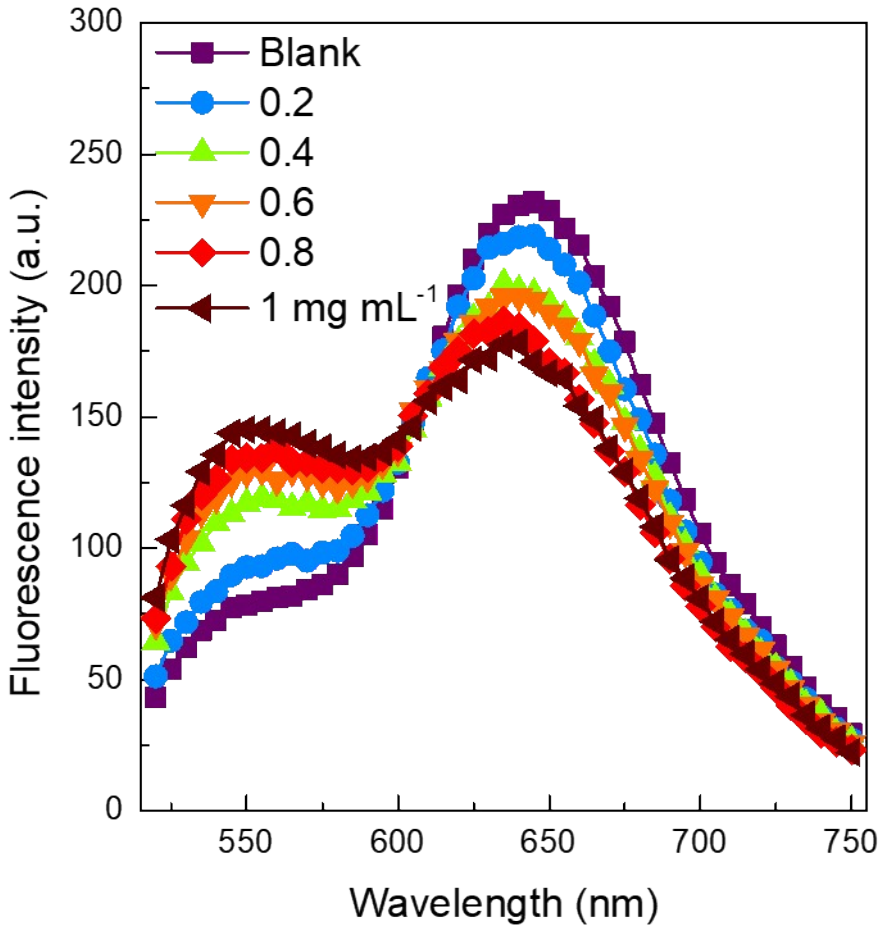

Figure S2. A change of fluorescence spectra of the NBD-B2 and Styryl-51F (1:5) mixture along with the change of NMN concentration from 0 to 1 mg mL^{-1} with an interval of 0.2 mg mL^{-1}.

References

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