Electronic Supplementary Information

Converting Molecular Information of Redox Coenzymes via Self-Assembly

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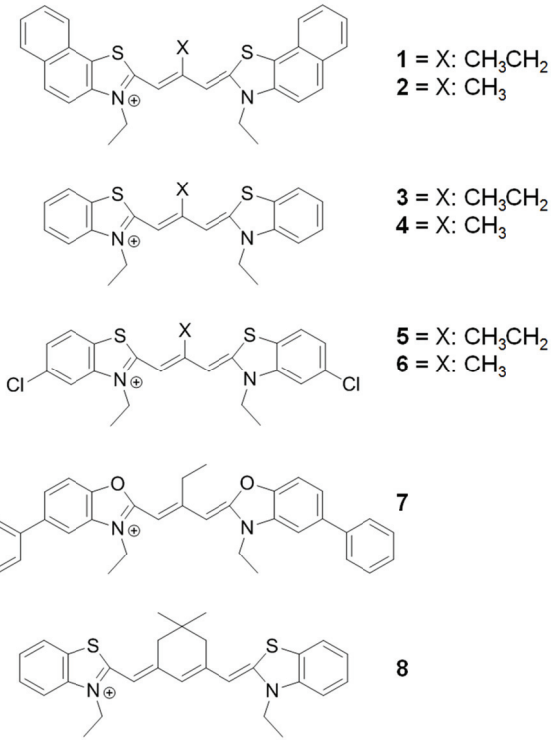
1. General methods

Materials. Cationic cyanine dye, naphtho[2,1-d]thiazolium, 3-ethyl-2-[2-[(3-ethyl-naphtho[2,1-d]thiazol-2(3H)-ylidene)methyl]-1-butenyl]-, iodide (**1**) (Hayashibara Biochemical Laboratories, Inc.), β-nicotinamide adenine dinucleotide (NAD^+) and β-dihydronicotinamide adenine dinucleotide (NADH, Wako Pure Chemical Industries, Ltd.) were used as received. Stock solutions of the dye were prepared in methanol, and their concentration was spectroscopically determined by using the extinction coefficient of ε_{577}= 1.23 × 10^5 $M^{-1}cm^{-1}$. Aqueous stock solutions of NAD^+ and NADH were prepared in pure water (Direct-Q system, Millipore, Co.) and concentration of these solutions were determined by using ε_{260}= 1.77 × 10^4 $M^{-1}cm^{-1}$ and ε_{340}= 6.2 × 10^3 $M^{-1}cm^{-1}$, respectively. By adding aqueous sodium hydroxide, pH of the aqueous mixtures of the dyes and these coenzymes was adjusted to 9 (methanol content; 2 volume %).

Measurements. UV-vis absorption and circular dichroism (CD) spectra were recorded on a JASCO V-670 spectrophotometer and JASCO J-820 spectropolarimeter at 20 °C, respectively. Quartz cell with 1-cm path length was used. Emission and excitation spectra were recorded on PerkinElmer LS55 spectrophotometer with 1-mm path length cell. Confocal laser scanning microscopy (CLSM) images were taken with a Carl Zeiss LSM510 META equipped with a 40x objective lens. Fluorescence with longpass filter (615 nm, λ_{ex} 514 nm) was monitored. Transmission electron microscopy (TEM) was conducted on a JEOL JEM-2010 (acceleration voltage, 120 kV).

Preparation of TEM samples. A drop of aqueous mixtures of cyanine dye (**1**) and coenzymes was placed on carbon-coated Cu grids. After standing for ca. 1 minute, these droplets were removed by adsorbing to filter paper. These specimens were dried in vacuo, and then aqueous uranyl acetate (2 %) solution was carefully dropped on them. Attention was paid not to evaporate the aqueous uranyl solutions on TEM grids, and they were also removed by using filter paper. The stained samples were then dried in vacuo and observed by electron microscopy.

Screening of dyes. To screen cyanine dyes that specifically co-assemble with NAD^+ and NADH, cationic cyanine dyes in methanol were systematically added to aqueous solutions of each coenzyme (pH 9, 2 vol % methanol). Among the eight dyes tested, the butterfly-shaped cyanine dye (**1**, Chart S1) showed most remarkable color changes in aqueous NAD^+ and NADH, respectively.



Dyes	Spectral Shift	
	NADH	NAD^+
1	Yes (dark pink)	Yes (blue)
2	Yes (dark pink)	Yes (dark pink)
3	No	No
4	No	No
5	No	No
6	Yes (dark pink)	No
7	No	No
8	No	No

Chart S1. Chemical structures of cyanine dyes tested in this study and their spectral shifts by mixing with NADH and NAD^+.

2. Spectroscopic and morphological studies of aqueous 1/NAD^+ and 1/NADH mixtures.

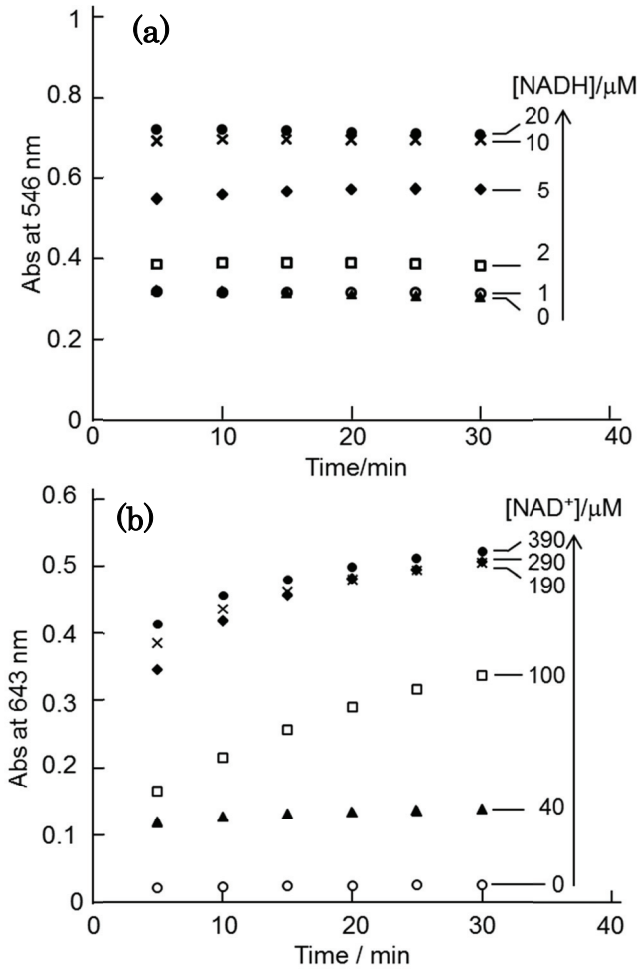


Fig. S1. Time courses of absorption intensity at (a) 546 nm and (b) 643 nm respectively, upon adding methanol solutions of cyanine dye (**1**) to aqueous NADH (a) or NAD^+ (b). [**1**] = 10 μM, [NADH] = 0-20 μM, [NAD^+] = 0-390 μM, pH 9, methanol content; 2 vol %.

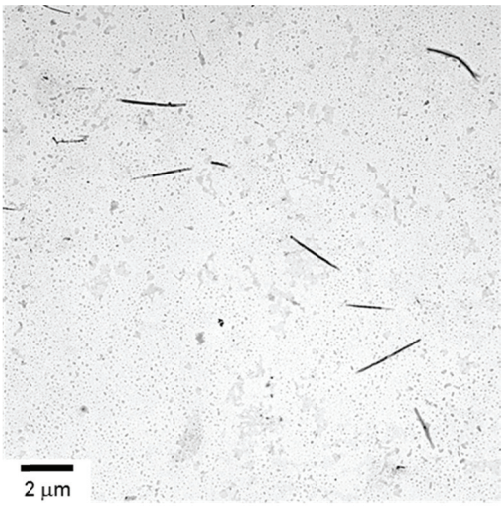


Fig. S2. TEM image of cyanine dye (**1**) dispersed in water. [**1**] = 10 μM, methanol content; 2 vol %. The sample was post-stained by 2% uranyl acetate.

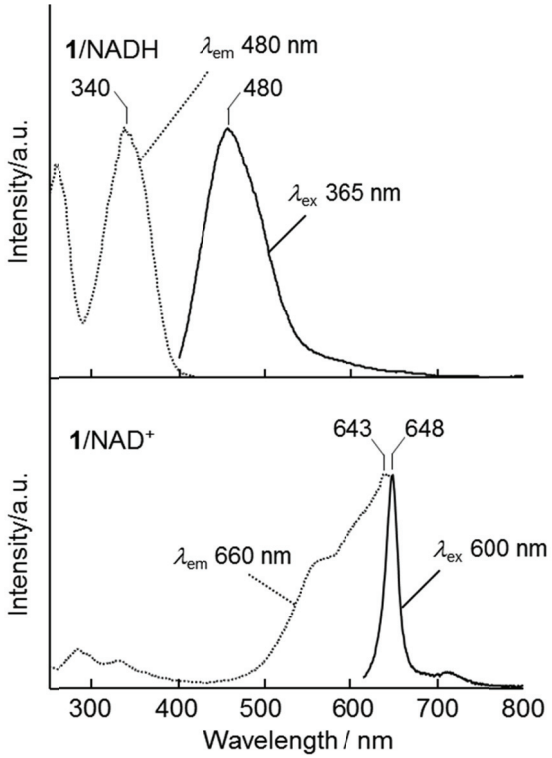


Fig. S3. Excitation (dotted line) and emission (solid line) spectra obtained for aqueous mixtures of **1**/NADH and **1**/NAD^+. [**1**] = 10 μM, [NADH] = 100 μM, [NAD^+] = 390 μM, pH 9, methanol content; 2 vol %.

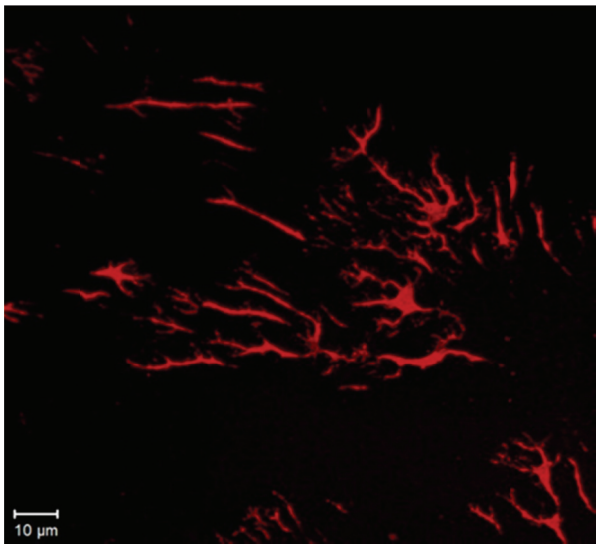


Fig. S4. Confocal laser scanning microscopic (CLSM) image of aqueous dispersion of **1**/NAD^+. The fluorescence image was obtained by using Ar laser (λ_{ex} = 514 nm) with longpass filter (615 nm), [**1**] = 10 μM, [NAD^+] = 390 μM.