Electronic Supplementary Information

Visible light driven selective NADH regeneration with the system of water-soluble zinc porphyrin and homogeneously polymer dispersed rhodium nanoparticles

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Experimental section

Materials

β-Nicotinamide adenine dinucleotide oxidized form (NAD^+) and L-lactate dehydrogenase from pig heart (LDH, EC 1.1.1.27) were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Tetraphenylporphyrin tetrasulfonic acid (H_2TPPS) was purchased from Dojindo Laboratories (Kumamoto, Japan). Triethanolamine (TEOA), zinc acetate dihydrate and sodium pyruvate were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from NACALAI TESQUE, INC. Sodium borohydride was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Rh nanoparticles dispersed by polyvinylpyrrolidone (Rh-PVP) was purchased from Renaissance Energy Research (Osaka, Japan). The particle size of Rh-PVP was estimated to be c.a. 4.0 nm using TEM Image shown in the analysis table published by Wako.[S1,S2] All the materials were of analytical grade or the highest grade available and used as received without further purification. The Ar gas of ultrahigh purity (≧99.9999 %, Grade 1) was supplied by TAIYO NIPPON SANSO CORPORATION (Toyko, Japan). Water was purified with a Milli-Q purification system. Zinc tetraphenylporphyrin tetrasulfonate (ZnTPPS) was synthesized by refluxing H_2TPPS with about 5 times the molar equivalent of zinc acetate dihydrate in 50 mL methanol at 50 °C for 3 hrs according to previously reported method.[S3,S4]

Transmission electron microscopy (TEM) measurement

For the transmission electron microscopy (TEM) measurements, a drop of the sample solutions was mounted on a carbon-covered copper mesh. The TEM images of Rh-PVP were recorded with a JEM-2100F (JEOL) electron microscope operated at 200 kV.

Visible light driven NADH reduction

The visible-light-driven NADH regeneration was carried out as follows. A sample solution containing TEOA (0.20 M), ZnTPPS, Rh-PVP, NAD in 5 mL of 50 mM HEPES-NaOH buffer (pH 7.4) was de-aerated by freeze-pump-thaw cycles repeated 6 times and then flushed with Ar gas for 10 min. The sample solution in the cell equipped with a magnetic stirrer was irradiated with a 250 W halogen lamp (TOSHIBA) with a light intensity of 200 J m^{-2} s^{-1} at 30 °C. The production of 1,4-NADH was determined by absorption change at 340 nm ($\varepsilon = 6.3 \times 10^3$ cm^{-1} M^{-1}) by using a UV-Vis absorption spectroscopy system (SHIMADZU, MultiSpec-1500 spectrophotometer).

Bioactivity assay of regenerated NADH

The enzymatic activity of regenerated NADH was tested as follows. First, a sample containing pyruvate (4.0 mM) and LDH (4.0 U) in 0.4 mL of 50 mM HEPES-NaOH buffer (pH 7.4) was added into 0.4 mL of regenerated NADH in a UV cell. The absorbance of the solution was monitored using a UV-

Vis absorption spectroscopy system (SHIMADZU, MultiSpec-1500 spectrometer).

The determination of the regenerated NADH by HPLC

The reduced products of the NAD^+ were analyzed using a HPLC system with a TOSHO ODS column (4.6 × 250 mm, 5μm particle size), a Shimadzu LC-20AD SP pump, and a Shimadzu SPD-20A UV/Vis detector (detected wavelength; 260 nm). Mixed solutions of methanol/100mM potassium phosphate buffer pH 7.1 (9:1 v/v) were used as eluent.

Fluorescence quenching behavior of ZnTPPS by Rh-PVP and NAD^+

Quenching of photoexcited state of ZnTPPS by Rh-PVP was investigated using steady state fluorescence spectroscopy. The sample solution containing ZnTPPS (1.0 μM) and Rh-PVP in 50 mM HEPES-NaOH buffer (pH 7.4). The concentration of Rh-PVP was varied from 0 to 100 μM. The excitation wavelength was 422 nm due to the Soret band of ZnTPPS. The fluorescence emission spectrum of ZnTPPS was measured using a fluorescence spectrophotometer (SHIMADZU, RF-5300PC) with a 150 W Xenon lamp as a visible excitation light source. Excitation and emission band-passes were 5.0 nm. In addition, quenching of photoexcited state ZnTPPS by NAD^+ also was studied.

Measurement of H_2 production with Rh-PVP

The H_2 production was determined by a gas chromatograph (GC-2014, SHIMADZU Corporation) with a TCD detector. The activation charcoal column (column length: 3 mm I.D. × 2 m) was used for detecting the gas. The temperatures of injection, column and detector were 100.0, 70.0 and 100.0 C, respectively. Ar gas was used as the carrier gas and the flow rate was 30.0 mL min^{-1}.

Visible light driven pyruvate reduction through NADH regeneration

Visible-light-driven pyruvate reduction to malate with TEOA, ZnTPPS, Rh-PVP, NAD^+ and LDH was carried out as follows. A sample solution containing TEOA (0.20 M), ZnTPPS (19 μM), Rh-PVP (0.25 mM), NAD^+ (1.0 mM), sodium pyruvate (2.0 mM) and LDH (20 units) in 5 mL of 50 mM HEPES-NaOH buffer (pH 7.4) was deaerated by freeze-pump-thaw cycles repeated 6 times, and then flushed with Ar gas for 10 min. The sample solution in the cell equipped with a magnetic stirrer was irradiated with a 250 W halogen lamp (TOSHIBA) with light intensity of 200 J m^{-2} s^{-1} at 30 °C. Ultraviolet ray with wavelength of shorter than 390 nm were blocked with cut-off filter. The concentration of lactate production in sample cell were analyzed by an ionic chromatograph system (Metrohm Eco IC; electrical conductivity detector) with an ion exclusion column (Metrohm Metrosep Organic Acid – 250/7.8; column length: 250 × 7.8 mm; composed of a polystyrene / divinylbenzene copolymer with sulfonic acid groups; Temperature: 35 °C). The 1.0 mM perchloric acid and 50 mM lithium chloride were used as an eluent and a regenerant, respectively. The absorption spectrum of sample solution

was also monitored by UV-vis absorption spectroscopy system (SHIMADZU, MultiSpec-1500 spectrophotometer).

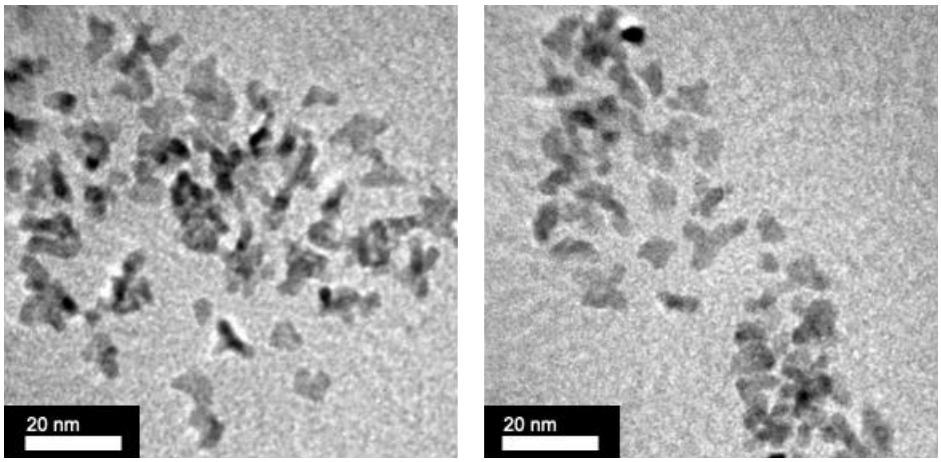

Fig. S1. Transmission electron microscopy (TEM) images of Rh-PVP.

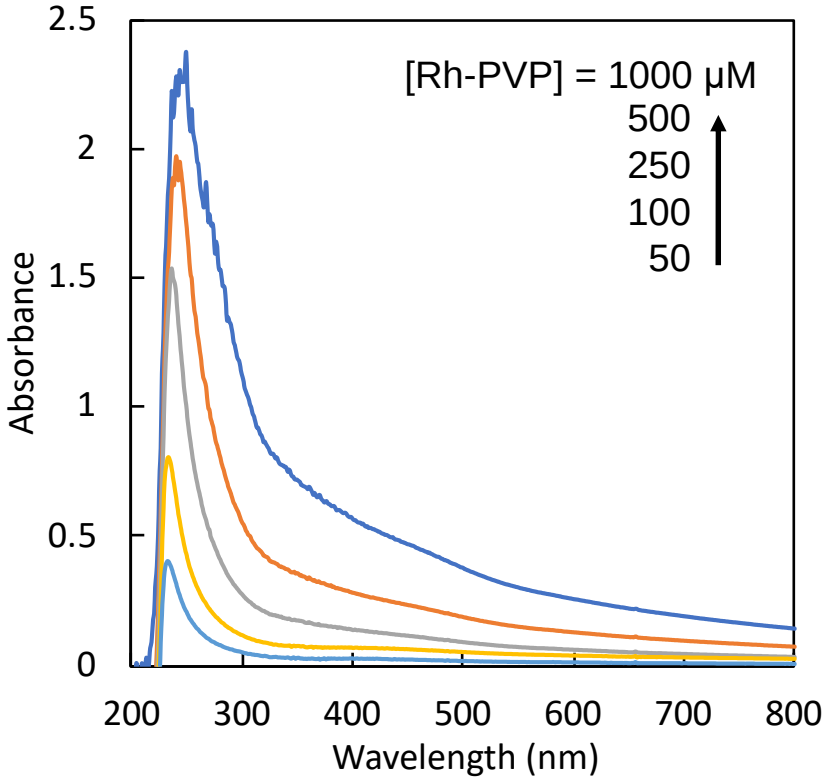

Fig. S2. UV-Vis absorption spectrum of Rh-PVP in HEPES-NaOH (pH 7.4) buffer.

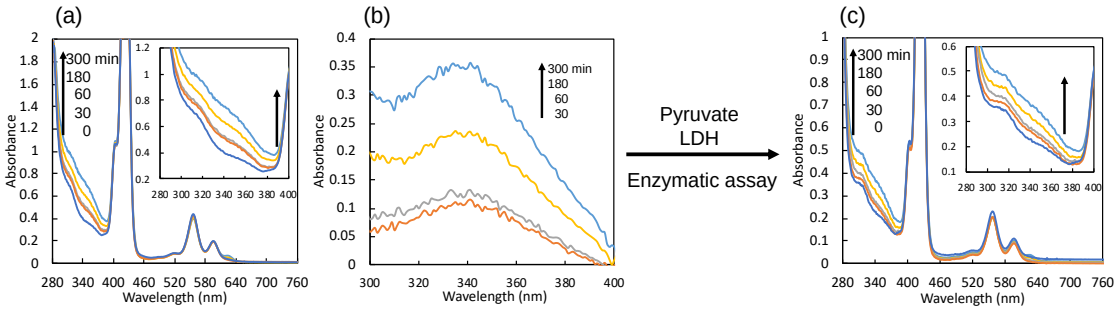

Fig. S3. Protocol for monitoring the reduction process with UV-Vis spectroscopy and enzymatic assay.

(a) Different UV-Vis absorption spectrum change of sample solution consisting of TEOA, ZnTPPS and NAD^+ with visible light irradiation time. Baseline is HEPES-NaOH buffer. (b) Baseline is 0 min irradiation. (c) Different UV-Vis absorption spectrum change of after enzymatic assay. A sample solution consisted of TEOA (0.1 M), ZnTPPS (9.5 μM), LDH (2 U) and pyruvate (2 mM).

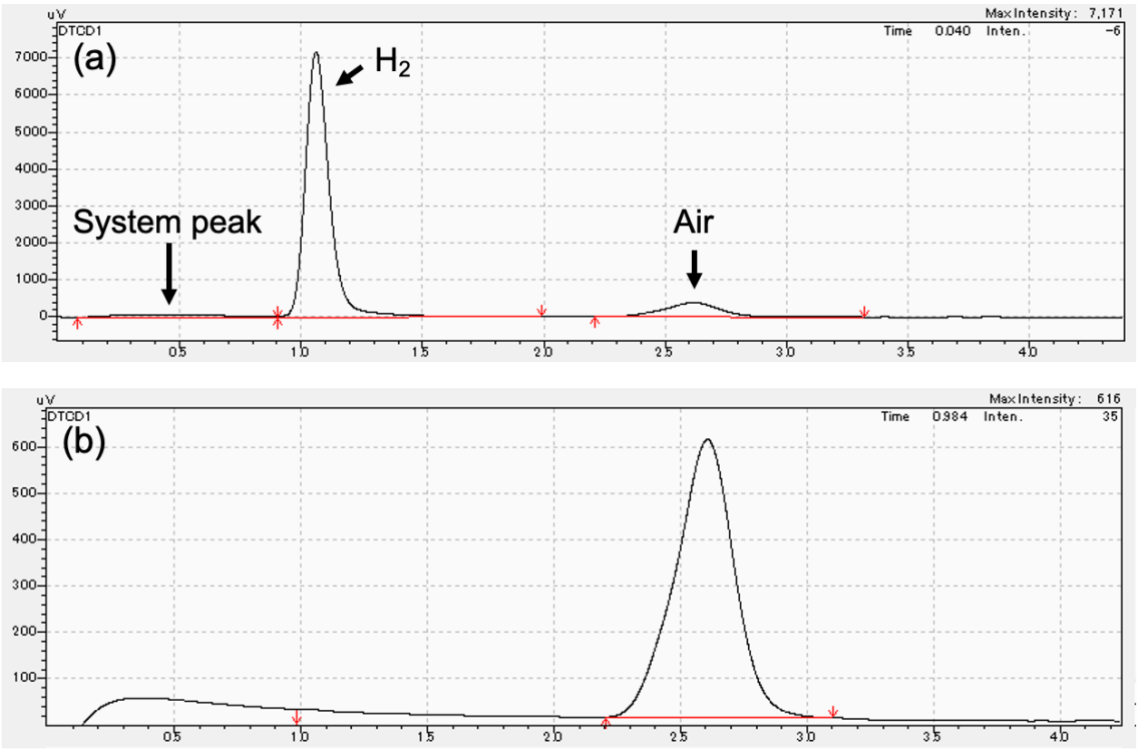

Fig. S4. The gas chromatogram of H_2 gas and Air (a), and analysis of the gas phase in the system of TEOA, ZnTPPS and Rh-PVP after 3 h irradiation (b).

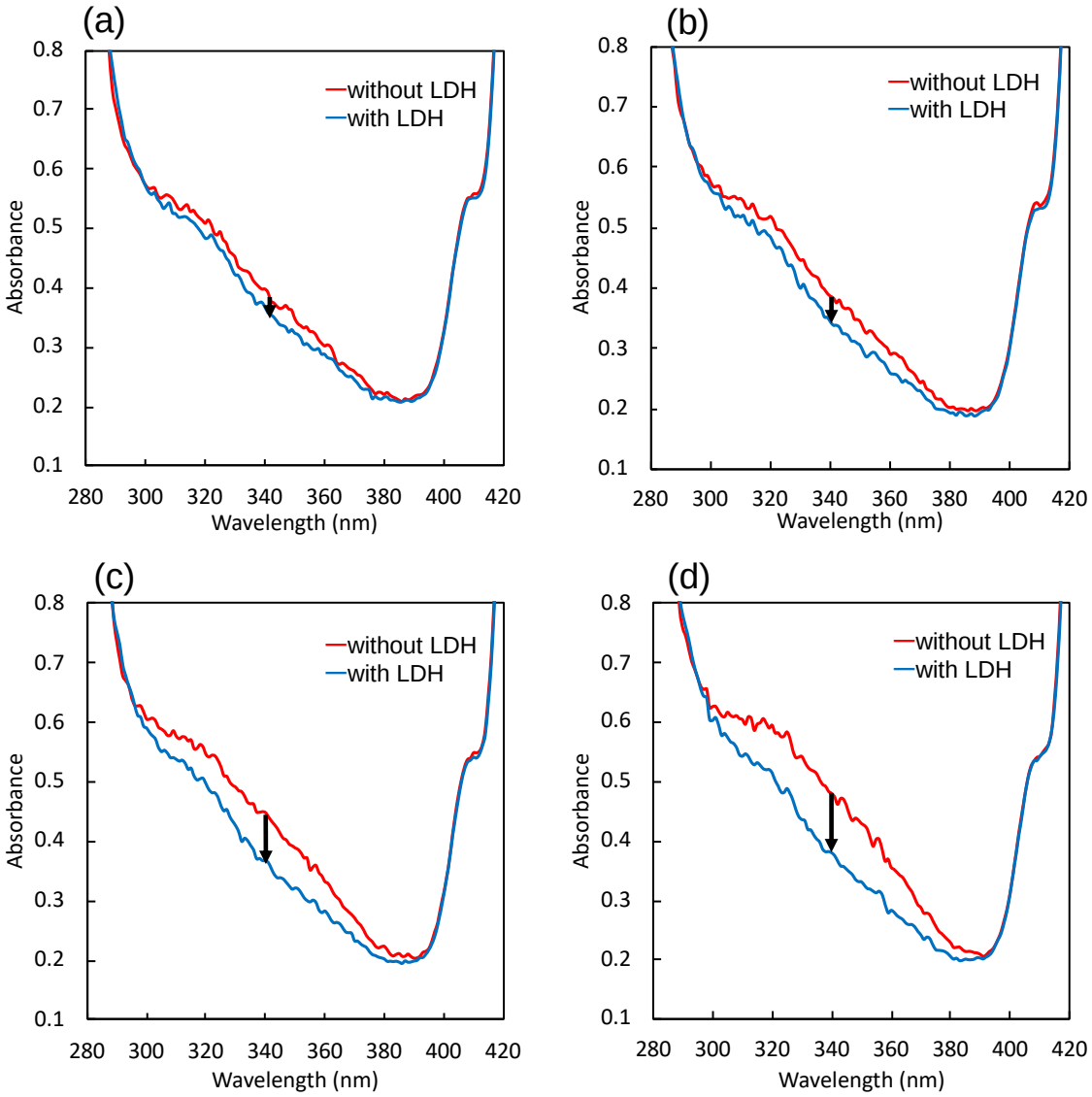

Fig. S5. Different UV-Vis absorption spectrum change of before and after enzymatic assay. A sample solution consisted of TEOA (0.1 M), ZnTPPS (9.5 μM), Rh-PVP (125 μM), LDH (2 U) and pyruvate (2 mM). (a) 30 min irradiation, (b) 60 min irradiation, (c) 180 min irradiation, (d) 300 min irradiation.

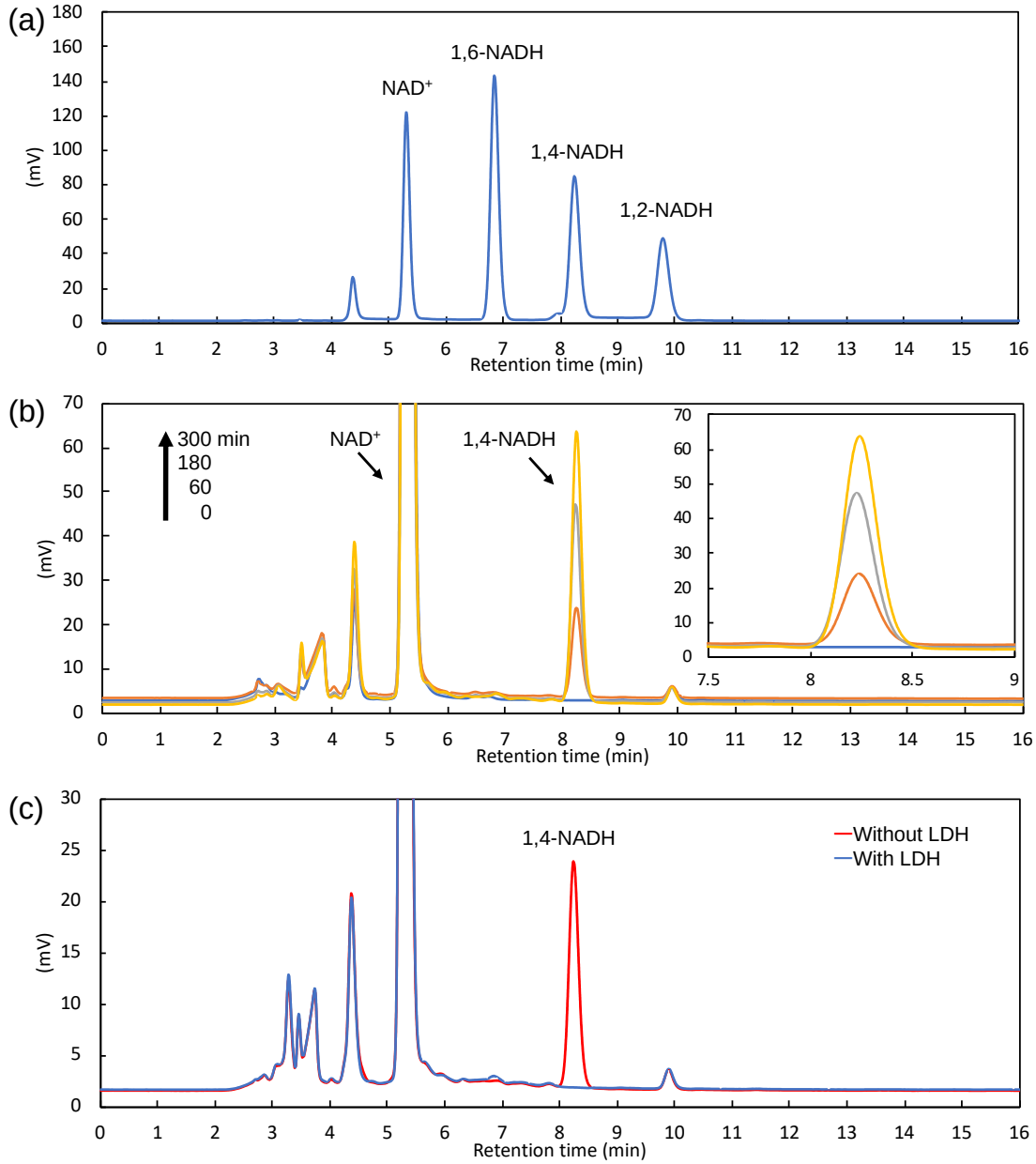

Fig. S6. Analytical HPLC chromatograms. (a) After the reduction of NAD^+ with sodium borohydride. (b) Visible light driven NAD^+ reduction with the system consisted of TEOA, ZnTPPS, Rh-PVP and NAD^+. (c) Enzymatic assay for visible light driven NAD^+ reduction after 300 min irradiation. A sample solution consisted of TEOA (0.1 M), ZnTPPS (9.5 μM), Rh-PVP (125 μM), pyruvate (2 mM) and LDH (2 U, — or 0 U, —).

The attribution of 1,2- and 1,6-NADH were referred to the previous report.[S5]

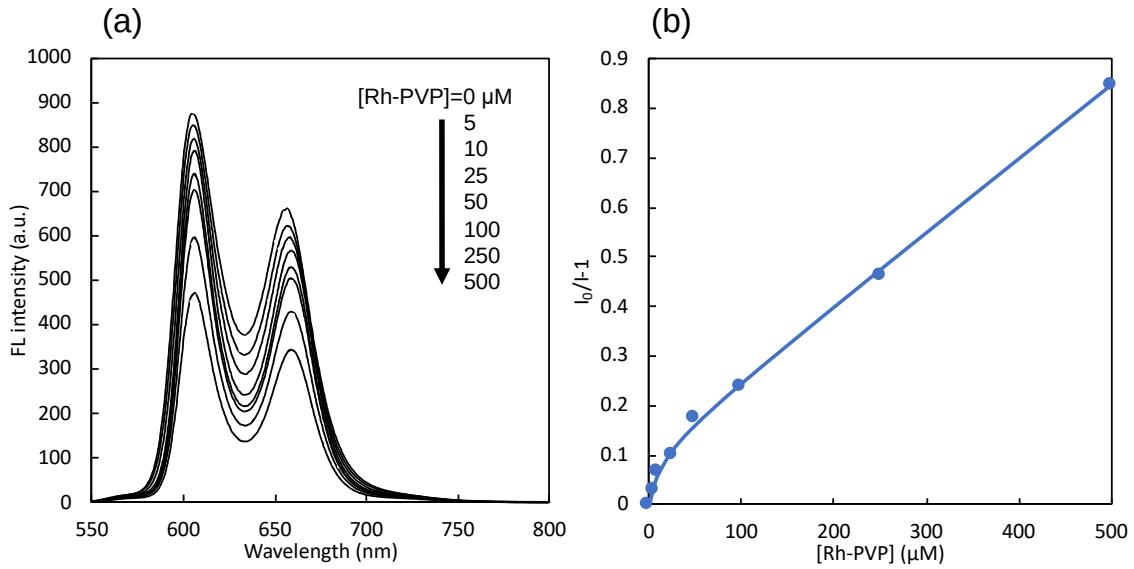

Fig. S7. (a) Fluorescence spectra change of ZnTPPS with Rh-PVP. (b) Modified Stern-Volmer plot for the fluorescence quenching of ZnTPPS by Rh-PVP. The excitation and fluorescence wavelength were 422 and 606 nm, respectively.

The analysis of quenching behavior of ZnTPPS

Here, let us focus on the electron transfer from the photoexcited state of ZnTPPS (1.0 μM) to Rh-PVP (0 - 500 μM) was investigated by measuring the fluorescence intensity at 606 and 656 nm by exciting the ZnTPPS at 422 nm. Fig. S7 (a) shows the fluorescence spectrum change of ZnTPPS with Rh-PVP. The fluorescence maximum of the ZnTPPS at 606 nm and 656 nm was decreased with increasing Rh-PVP concentration. The fluorescence of ZnTPPS was quenched by Rh-PVP. The relative fluorescence intensity (I_0/I, where I_0 is the intensity in the absence of Rh-PVP) against Rh-PVP concentration are shown in Fig. S7 (b). The I_0/I plots show a non-linear relationship as shown in Fig. S7 (b). The non-linear dependence was rationalized in terms of the existence of multi different quenching sites. These multi populations were analyzed using a modified form of the Stern–Volmer equation following eq. S1.

$$I_0/I=[\Sigma(f_n/(1+K_{SVn}[Q]))]^{-1} \quad \text{(eq. S1)}$$

f_n is the fractional contributions to each quencher accessible site. K_{SVn} is the quenching constant for each site. Q is quencher. When n was equal to 2, the best-fit curve was obtained as shown in Fig. S7 (b). From results using a modified form of the Stern–Volmer equation, the K_{SV1} and K_{SV2} values were

estimated to be 1.3×10^{-3} and 8.7×10^{-4} μM^{-1}, respectively. The f_1 and f_2 values were estimated to be 0.898 and 0.102. The K_{SV} value of methylviologen (MV), widely used as an electron mediator, is 0.12 μM.[S6] The K_{SV} values of Rh-PVP are much lower than that of MV. Thus, the Rh-PVP weakly interacts with ZnTPPS than that of MV. Thus, we estimated that it is a static quenching due to the interaction between ZnTPPS and Rh-PVP without energy transfer. Therefore, we concluded that the electron transfer from the photoexcited singlet state of ZnTPPS (^{1}ZnTPPS*) to Rh-PVP did not occur and photoexcited triplet state of ZnTPPS (^{3}ZnTPPS*) to Rh-PVP occurred.

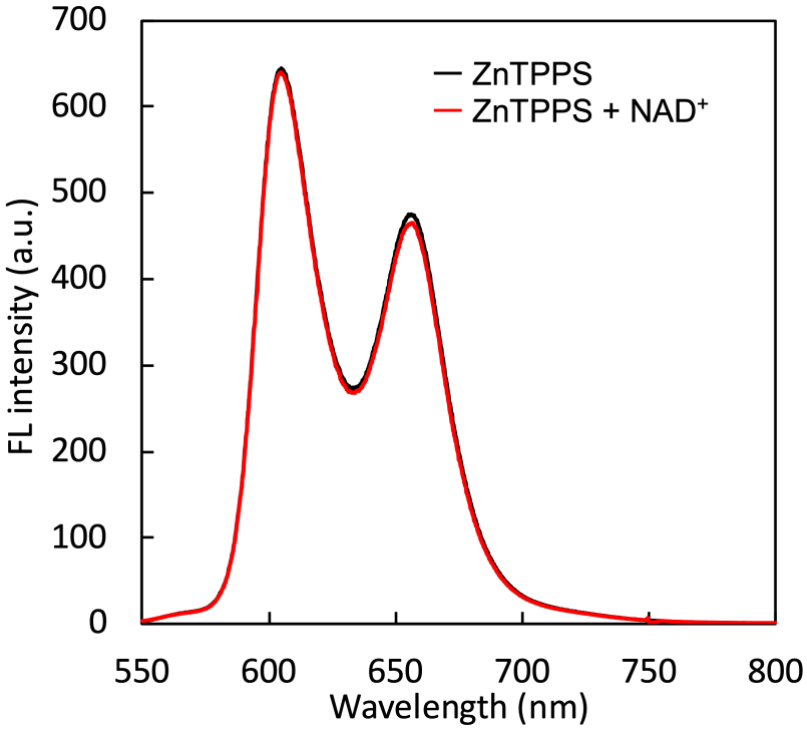

Fig. S8. Fluorescence spectra of ZnTPPS (1.0 uM) in the presence and absence of NAD^+ (250μM, —red) in HEPES-NaOH buffer (pH 7.4). The excitation wavelength is 422 nm.

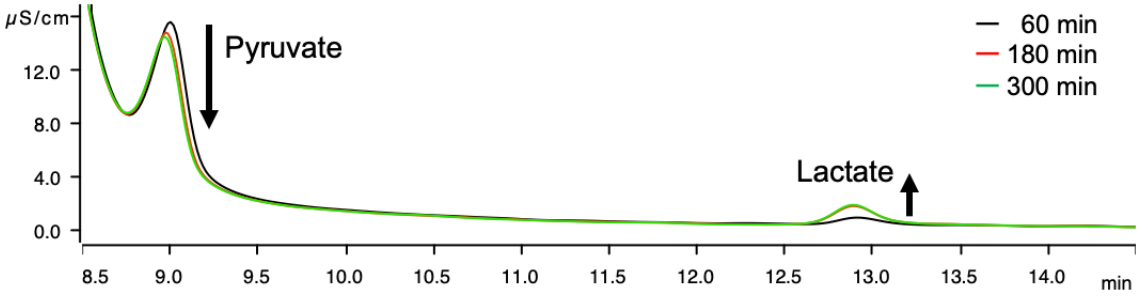

Fig. S9. The chromatogram for visible light driven pyruvate reduction with the system consisted of TEOA, ZnTPPS, Rh-PVP, NAD^+, LDH and pyruvate.

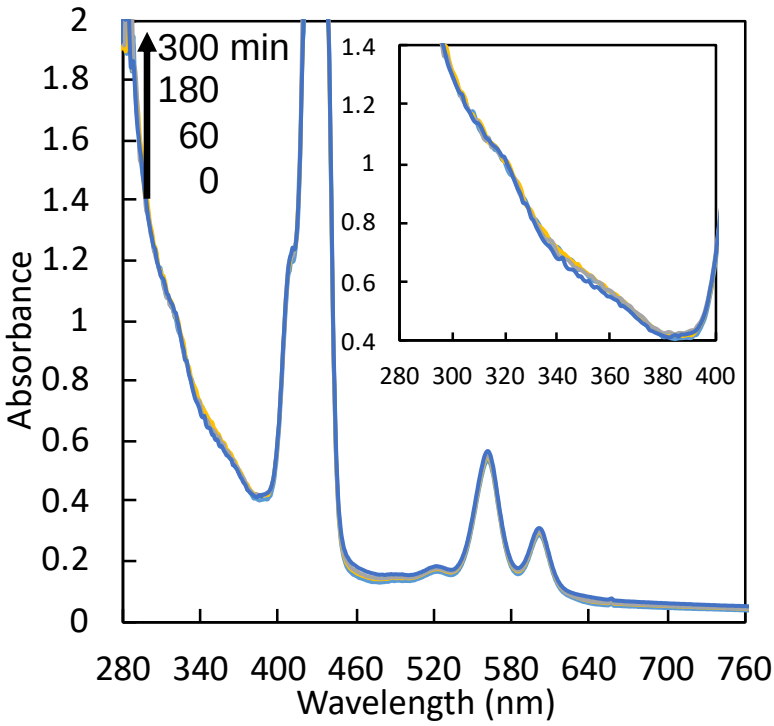

Fig. S10. The UV-Vis absorption spectrum changes of the reaction mixture for visible-light-driven pyruvate reduction. The system consisted of TEOA, ZnTPPS, Rh-PVP, NAD^+, LDH, and pyruvate.

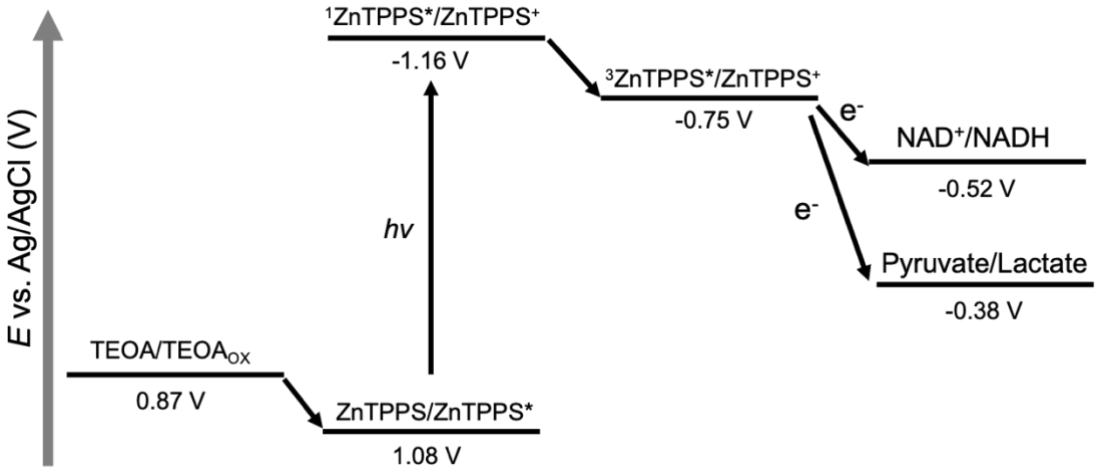

Fig. S11. The energy diagram for visible light driven pyruvate reduction with the system of TEOA, ZnTPPS.

References

S1 Wako Organic Square No.47, p10

S2 Wako Organic Square No.54, p5 (DECEMBER,2015)

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