Supplementary Information

Visible light-driven, external mediator-free H_2 production by a combination of a photosensitizer and a whole-cell biocatalyst of *Escherichia coli* expressing [FeFe]-hydrogenase and maturase genes

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Results and Discussion

The termination of light-driven H_2 formation and the photobleaching of EY

The termination of light-driven H_2 formation coincides with the photobleaching of EY (Figs. 3a, S4a and S4b). Fig. S5 suggested that the increase in the initial amount of EY extended the duration of light-driven H_2 formation by EY/whole-cell system. Indeed, neither decrease in light-driven H_2-formation rate nor EY photobleaching were observed in EY/whole-cell system with 0.5 mM EY with total 12 h of light irradiation (Figs. S4c and S5). This was further investigated by successive addition of EY and TEOA to the EY/whole-cell system during light irradiation.

Fig. S6 shows the amounts of H_2 formed by EY/whole-cell system with successive addition of EY or TEOA under 530-nm light irradiation. This measurement consisted of six sets of light irradiation periods. Reaction conditions in each light irradiation period are listed in Table S1. Table S1 also indicates the amount of H_2 formed in a control experiment (a light-driven H_2 formation by EY(0.1 mM)/whole-cell system without additional EY shown in Fig. 4).

Fig. S7 shows the UV-Vis absorbance measured before and after each reaction period. These absorbance spectra were measured with 20-times diluted suspensions.

Fig. S8 shows the H_2-formation activity with chemically-reduced MV of 0.1 ml aliquot sampled from the reaction of EY/whole-cell at **i**, **iv** and **x** in Fig. S6.

These results clearly supported that the termination of light-driven H_2 formation by EY/whole-cell system was caused by the photobleaching of EY. Comparing the H_2 formation during 1st and 2nd periods (total 688 μmol) with that of the control experiment (464 μmol), the addition of EY before the EY photobleaching occurred (See **ii** in Fig. S7a) increased the amount and the duration of light-driven H_2 formation. This seemed to be similar to the experiment in which an increased amount of EY was added initially to the system (Fig. S5). In contrast, the light-driven H_2 formations were partially revived by the addition of EY after the reaction almost stopped and the EY photobleaching occurred (3rd, 5th and 6th period). The termination of light-driven H_2 formation with the EY photobleaching was also reported by EY/cobalt-based catalyst by Lazarides et al.[31]. Whereas this cobalt-based catalyst system could be revived by addition of both EY and cobalt-catalyst, EY/whole-cell system could be partially recovered by only EY addition. This can be explained by the residual H_2-formation activities by HydA in the reaction mixture after light-driven reaction was stopped (Fig. S8). HydA was not deactivated during light-driven H_2 formation (Fig. S8), suggesting the EY photobleaching is a main reason for the termination of H_2 formation and the low stability of the EY/whole-cell system. However, the EY addition did not recover the H_2 formation completely. A detailed understanding of the EY photobleaching during light-driven H_2 formation contributes to further improvement of the durability of EY/whole-cell system.

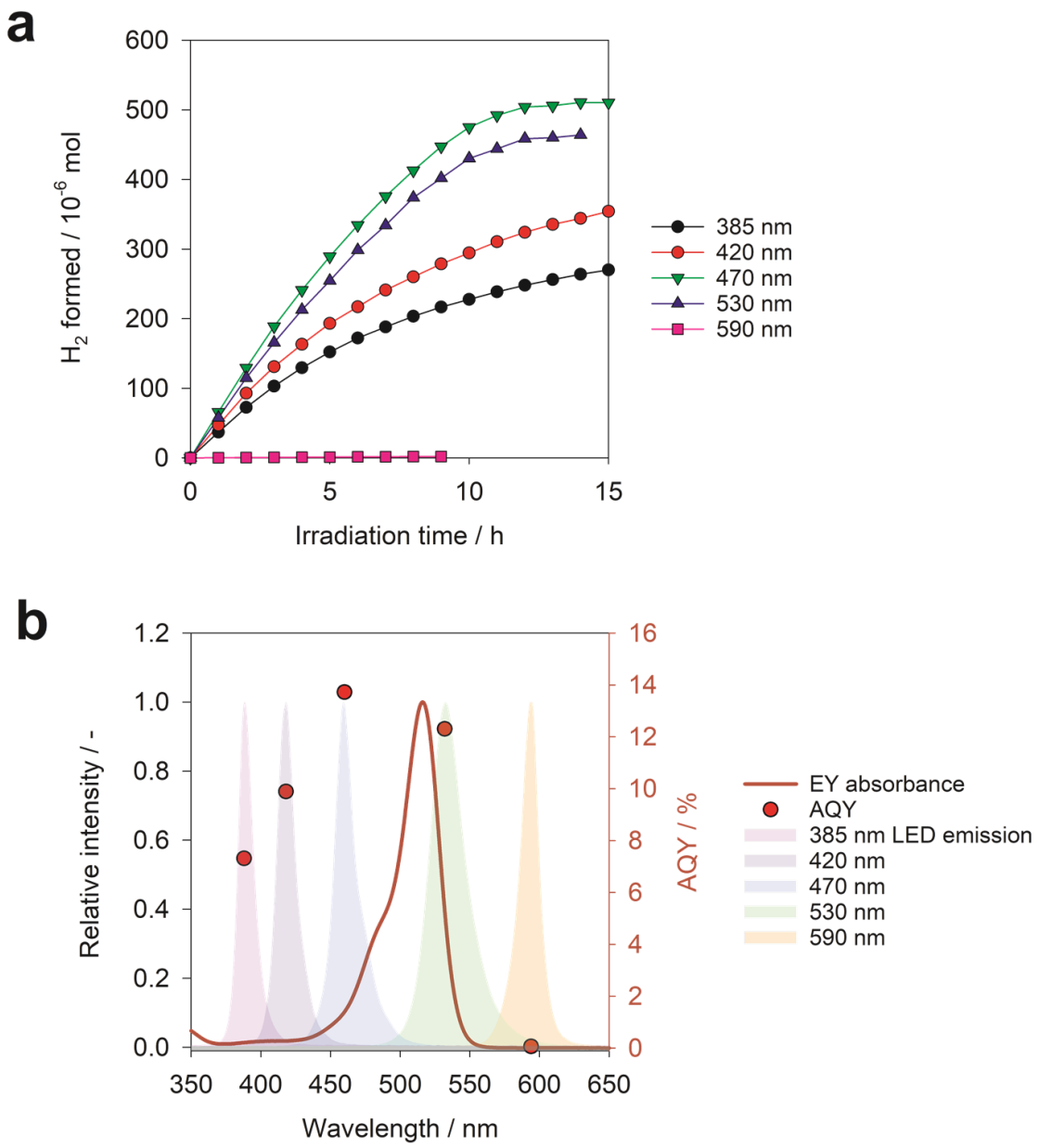

Fig. S1 Wavelength-dependent light-driven H_2 formation by the EY and whole cell of *E. coli* (Hyd+) system.

Reaction conditions: 30 ml of 100 mM TEOA-HCl (pH 8), 0.1 mM EY, and 0.1 g-wet cell of *E. coli* (Hyd+), at 25°C. Light sources: 385 nm, 420 nm, 470 nm, 530 nm and 590 nm LED; rates of incident photon are shown in Table S2.

(a) The amount of H_2 formed by EY/whole-cell system under several light sources.

(b) The values of AQY, the absorbance of EY in 100 mM TEOA-HCl (pH 8), and the emission of light sources. Emission spectra were measured using a USB2000 spectrometer (Ocean Optics).

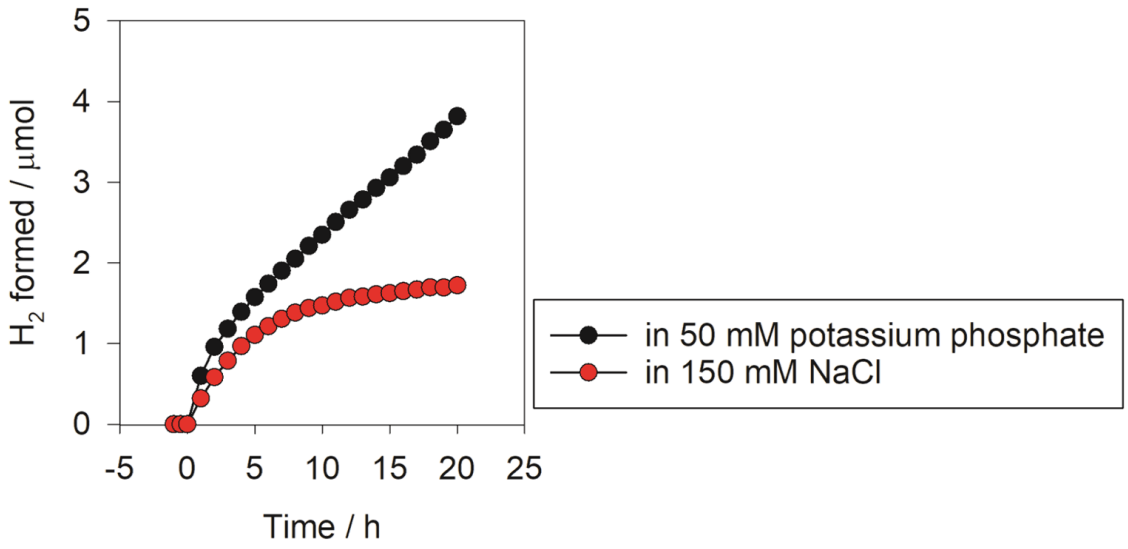

Fig. S2 Light-driven H_2 formation by the EY and *E. coli* whole cell biocatalyst system in 50 mM potassium phosphate buffer (pH 7) and in 150 mM NaCl.

Light irradiation started at 0 h. Light intensity: incident photon rate = 1.65×10^{-5} mol-photon min^{-1}. Black: 30 ml of 50 mM potassium phosphate buffer (pH 7), 0.1 mM EY, and 0.1 g-wet cell of *E. coli* (Hyd+), at 25°C. Red: 30 ml of 150 mM potassium phosphate buffer (pH 7), 0.1 mM EY, and 0.1 g-wet cell of *E. coli* (Hyd+), at 25°C.

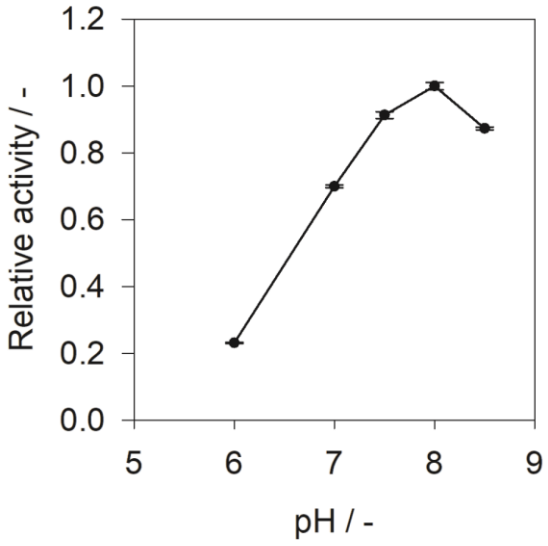

Fig. S3 Optimal pH for H_2 formation by whole cell of *E. coli* (Hyd+) with chemically reduced MV in TEOA-HCl buffer (pH 6-8.5).

The reaction mixture consisted of 2 ml of 100 mM TEOA-HCl (pH 6-8.5 adjusted using HCl), 5 mM MV^{2+}, 25 mM sodium dithionite, and a 0.01 g-wet cell of *E. coli* (Hyd+). The reaction mixture was pre-heated at 37°C, then the reaction was started by adding sodium dithionite solution, allowed to proceed for 30 s, then stopped by adding 10 wt% trichloroacetic acid. The amount of H_2 formed was analyzed by GC. Plots represent the average of two experiments; error bars show the difference between the average and maximum and minimum values.

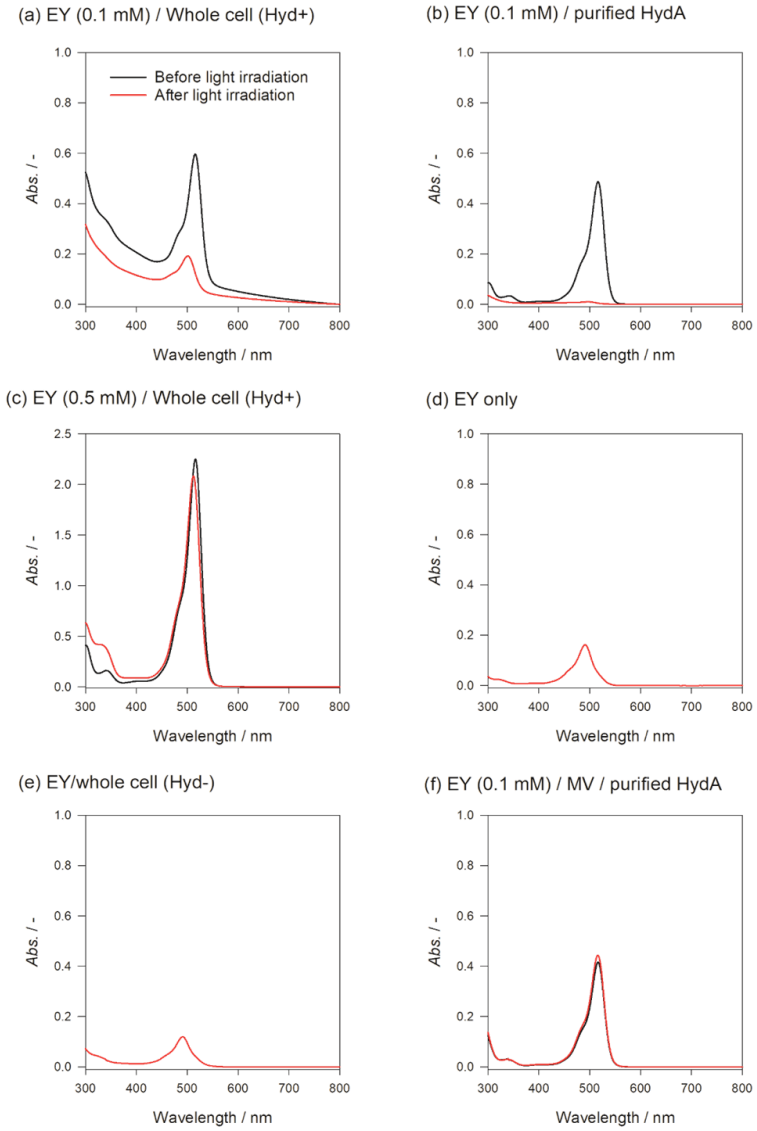

Fig. S4 UV-Vis absorbance spectra of the EY/biocatalyst system reaction solution before and after light irradiation.

(a) 0.1 mM EY and 0.1 g wet-cell of whole cell (Hyd+) in 100 mM TEOA-HCl (pH 8).
(b) 0.1 mM EY and 30 U of purified HydA in 100 mM TEOA-HCl (pH 8).
(c) 0.5 mM EY and 0.1 g-wet-cell of whole cell (Hyd+).
(d) 0.1 mM EY only in 100 mM TEOA-HCl (pH7).
(e) 0.1 mM EY and 0.1 g wet-cell of whole cell (Hyd-) in 100 mM TEOA-HCl (pH7).
(f) 0.1 mM EY, 1 mM MV, and 30 U of purified HydA.
The spectra were measured with the solution diluted 20-times by water. Light source, 530 nm LED; irradiation time, 4 h × 3 (total 12 h) for (a)-(d), and (f). 6 h for (d). In (d) and (e), absorbance spectra after light irradiation were shown.

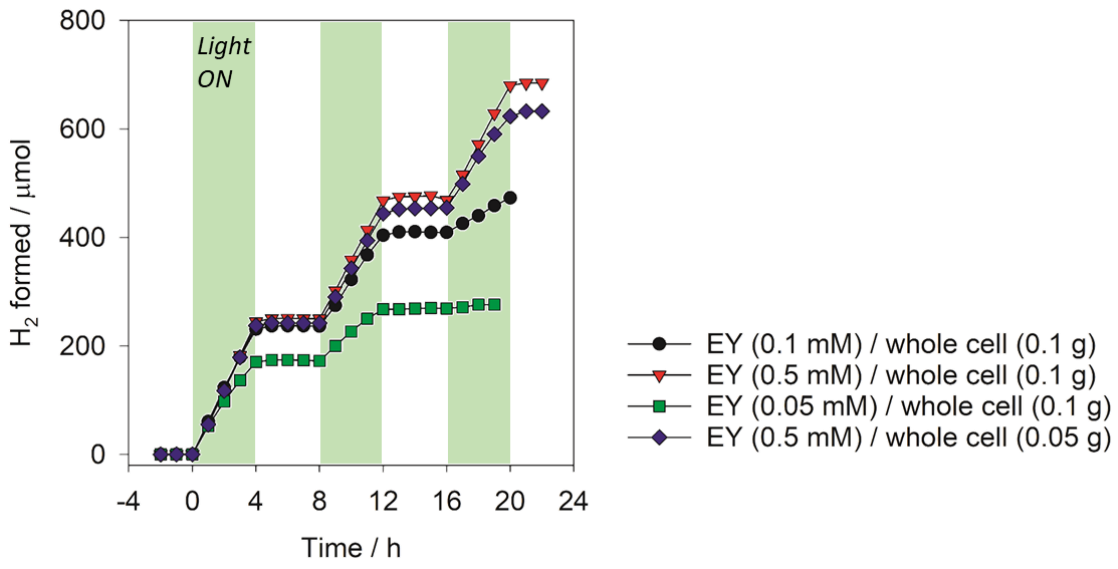

Fig. S5 Effects of the amounts of EY and whole cells on light driven H_2 formation by the EY/whole cell system.

Reaction systems consisted of 30 ml of 100 mM TEOA-HCl (pH 8), 0.05-0.5 mM EY, and a 0.1 or 0.05 g-wet cell of *E. coli* (Hyd+). Light source, 530 nm LED; irradiation time, 4 h × 3 (total 12 h).

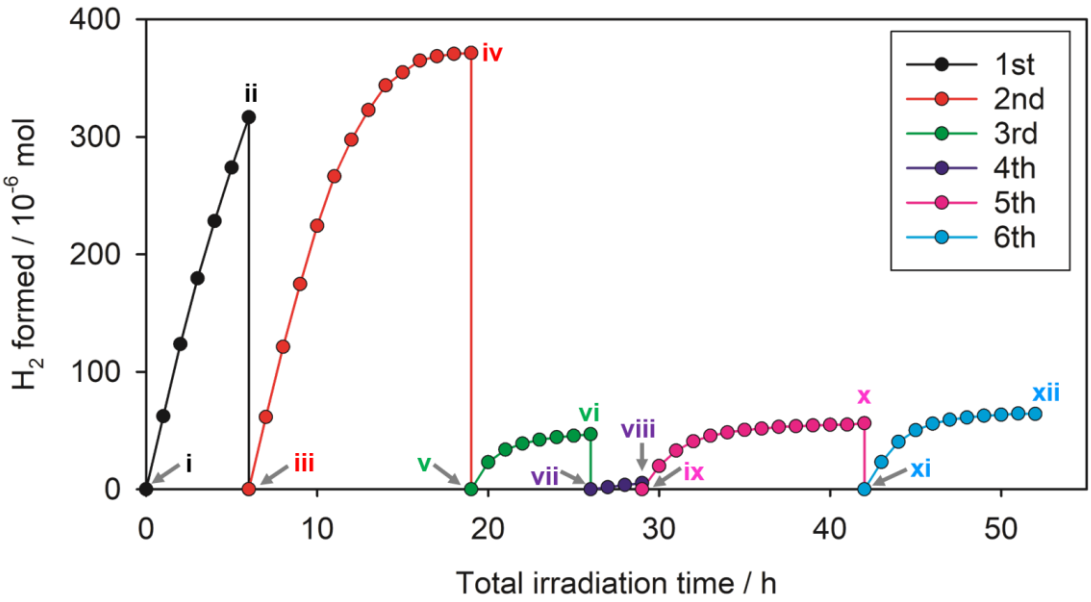

Fig. S6 Successive EY or TEOA addition to EY/whole-cell system during light-driven H_2-formation.

The light-driven H_2-formaion consisted of six light irradiation periods. Reaction condition during each period was shown in Table S1. Numbers indicated (i - xii) correspond to the absorbance spectra (Fig. S7) and the H_2-formation activity assays (Fig. S8).

Light-driven H_2 formation was carried out with the gas circulating reaction system. The system was evacuated after each reaction period and Ar gas was refilled (-50 kPa). The quartz reaction vessel with valves was disconnected from the system and was moved inside the glove box (N_2 atmosphere). After a sampling for absorbance measurements and hydrogenase activity assays, the EY and TEOA solutions were added to the reaction suspension. Then, the reaction vessel was connected again to the gas circulating system, the headspace gas was evacuated and replaced to Ar (-70 kPa), and the light irradiation was restarted.

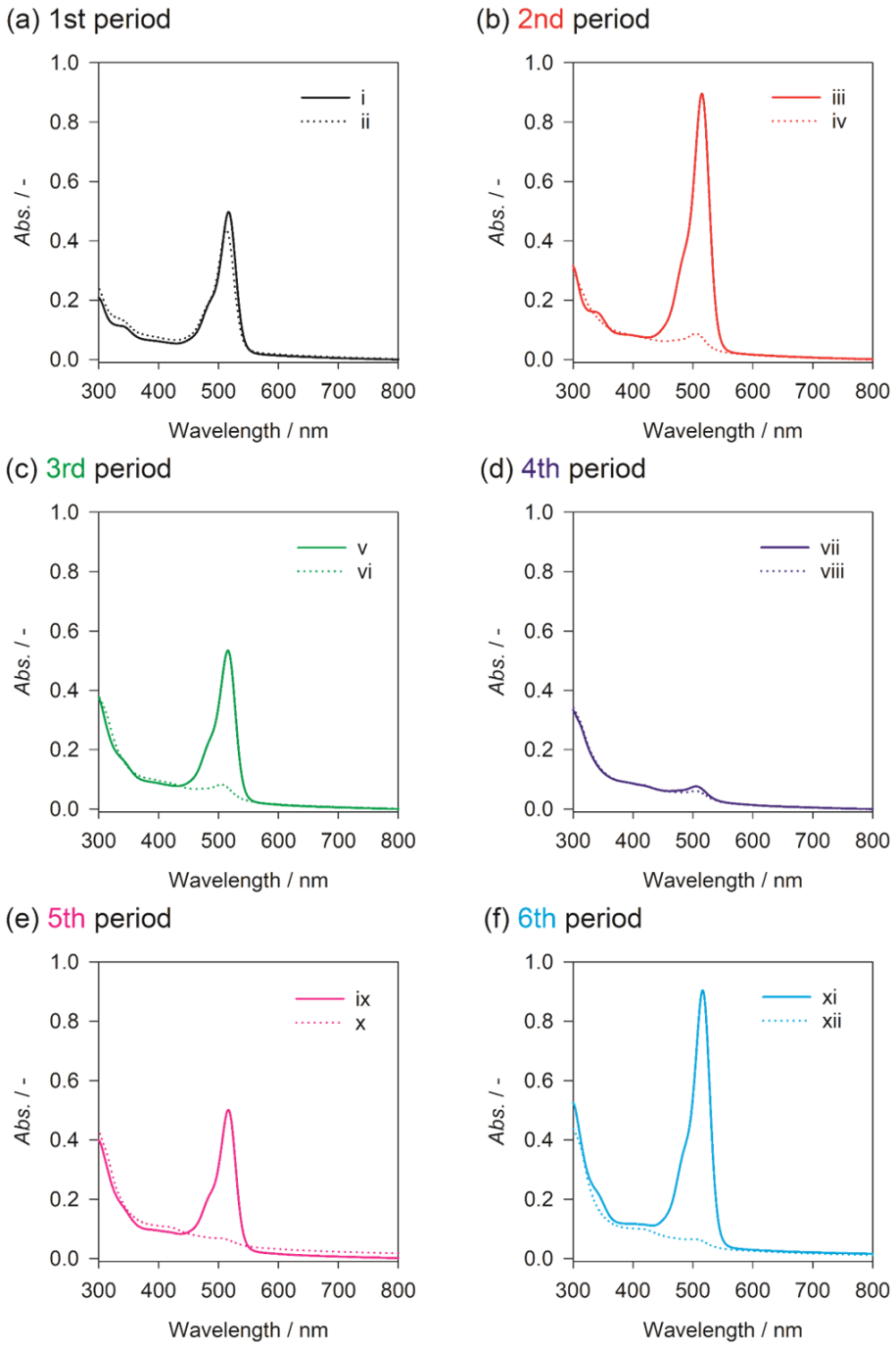

Fig. S7 UV-Vis absorbance spectra of EY/whole-cell system reaction solution before and after each light irradiation period during light-driven H_2-formation.

The absorbance spectra were measured with the solution diluted 20-times by water. The reaction periods and numbers indicated (i-xii) correspond to Fig. S6 and Table S1.

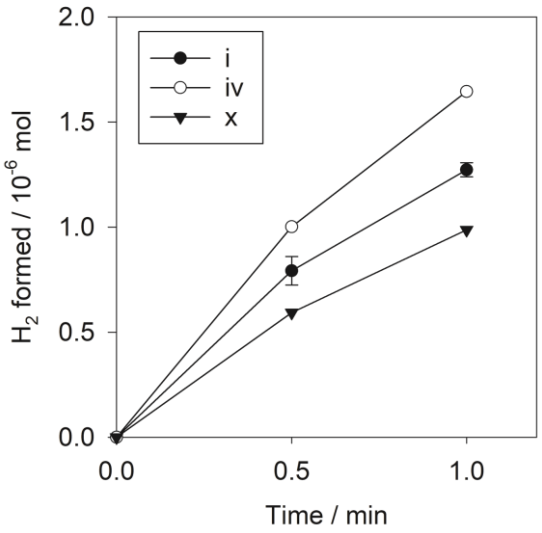

Fig. S8 H_2 formation with chemically reduced MV by 0.1-ml aliquot of the reaction mixture (at i, iv, and x in Fig. S6).

The reaction was consited of 2 ml of 100 mM TEOA-HCl (pH 8), 5 mM MV, 25 mM sodium dithionite, and 0.1-ml aliquot sampled from EY/whole-cell system at **i**, **iv** and **x** in Fig. S6. The mixture was pre-heated at 37°C for 2 min, then the reaction was started by adding sodium dithionite solution, allowed to proceed for 30 s or 1 min, then stopped by adding 10 wt% trichloroacetic acid. The amount of H_2 in the headspace was measured. For **i**, plots and bars represent the average and the standard deviation of three independent experiments.

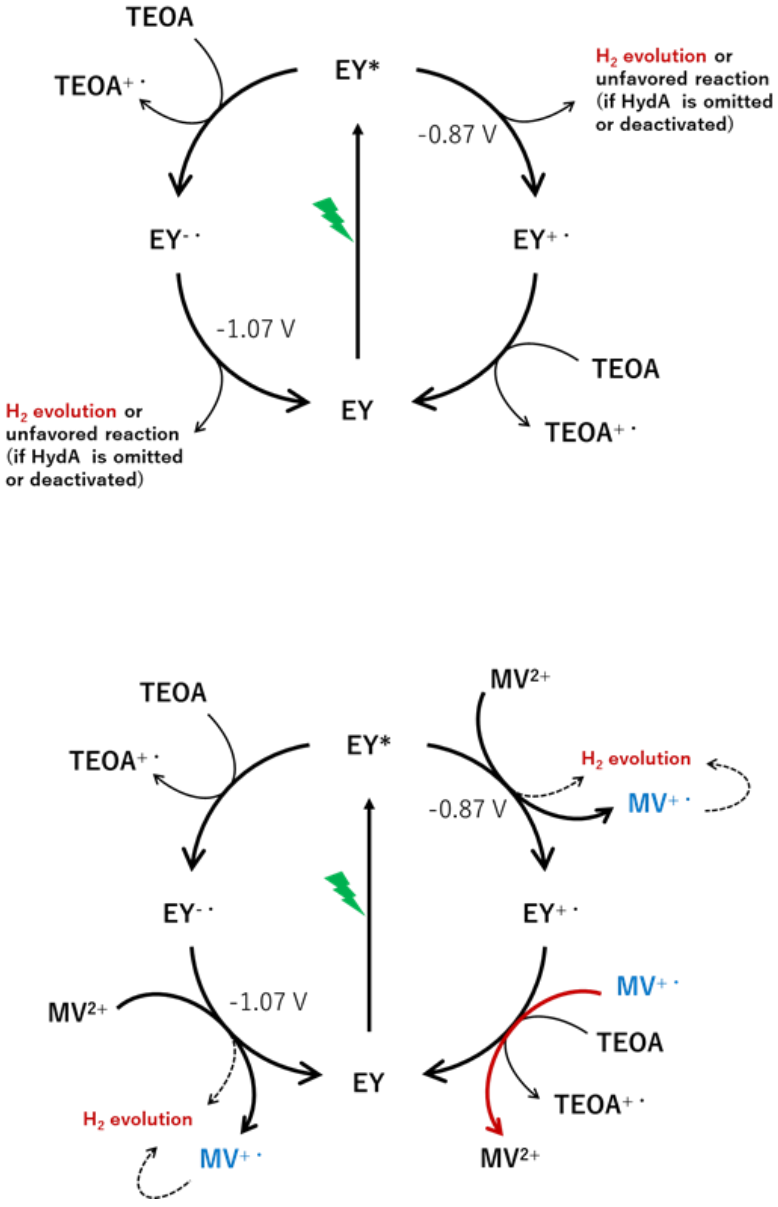

Fig. S9 Quenching routes of excited EY with TEOA and MV.
Reductive and oxidative quenching routes of photoexcited EY (EY*) are shown. Redox potentials are shown vs NHE. (Ref. 31)

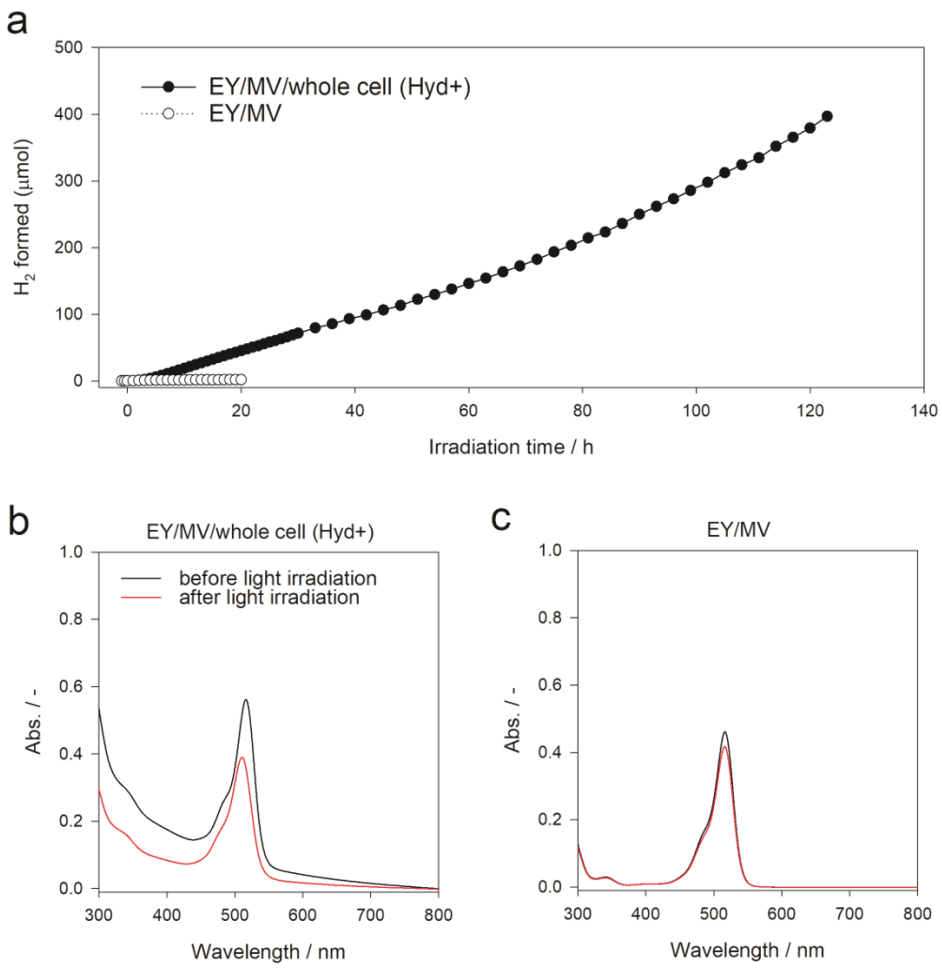

Fig. S10Sustained H_2 formation by EY/whole cell system supplemented with MV.
(a) H_2 formed under light irradiation. Reaction systems consisted of 30 ml of 100 mM TEOA-HCl (pH 8), 0.1 mM EY, 1 mM MV, and a 0.1 g-wet cell of *E. coli* (Hyd+) (EY/whole cell) or 30 ml of 100 mM TEOA-HCl (pH 8), 0.1 mM EY, 1 mM MV (EY/MV). Light source, 530 nm LED (intensity, 1.65×10^{-5} mol-photon min^{-1}).
(b), (c) Absorbance of reactions before and after light irradiation.

Table S1 Description of each reaction period in the experiment of successive EY addition to the EY/whole cell system (Fig. S6)

Irradiation period	Addition	Volume of the reaction suspension / ml	Sampling volume before and after the period / ml	Irradiation time [a] (Total) / h	Amount of H_2 formed during first 1-h of the period /μmol	Amount of H_2 formed during the period (Total) / μmol
1st (i → ii)	-	30.0 [b]	0.1	6 (6)	62.2	316.6 (316.6)
2nd (iii → iv)	1.5 ml of 2 mM EY	31.4	0.5	13 (19)	61.6	371.3 (687.9)
3rd (v → vi)	1.5 ml of 2 mM EY	32.4	1.0	7 (26)	23.1	46.9 (734.8)
4th (vii → viii)	3 ml of 100 mM TEOA (pH 8)	34.4	1.0	3 (29)	1.77	5.3 (740.1)
5th (ix → x)	1.5 ml of 2 mM EY & 1.5 ml of 100 mM TEOA (pH 8)	36.4	2.0	13 (42)	19.9	56.2 (796.3)
6th (xi → xii)	3 ml of 2 mM EY & 1.5 ml of 100 mM TEOA (pH 8)	38.9	-	10 (52)	23.2	64.2 (860.5)
Control [c]	-	30.0 [b]	-	14	58.0	464.0

a) 530 nm LED light (1.56×10^{-5} mol photon min^{-1}).
b) a 30-ml reaction solution containing 0.1 mM EY and a 0.1-g wet cell of *E. coli* (Hyd+) in 100 mM TEOA (pH 8.0, adjusted by HCl) at 25°C.
c) The light-driven H_2 formation by EY/whole-cell system under 530 nm light (1.55×10^{-5} mol photon min^{-1}) shown in Fig. 4.

Table S2 Calculation of incident photon for AQY analysis

Wavelength [a] / nm	Light power / W cm^{-2}	Area / cm^2	E_{total} [b] / J s^{-1}	E_p [c] / J	Np [d] / s^{-1}	Rate of incident photon [e] / mol s^{-1}	Rate of H_2 formation [f] / μmol h^{-1}	AQY [g] / %
385	7.12×10^{-3}	12.25	0.0872	5.146×10^{-19}	1.695×10^{17}	2.81×10^{-7}	37.1	7.32
420	8.28×10^{-3}	9.08	0.0752	4.717×10^{-19}	1.594×10^{17}	2.65×10^{-7}	47.1	9.89
460 [h]	7.63×10^{-3}	9.08	0.0693	4.316×10^{-19}	1.605×10^{17}	2.67×10^{-7}	65.8	13.72
530	5.75×10^{-3}	10.18	0.0585	3.738×10^{-19}	1.566×10^{17}	2.60×10^{-7}	57.6	12.30
590	4.86×10^{-3}	10.75	0.0522	3.358×10^{-19}	1.556×10^{17}	2.58×10^{-7}	0.300	0.06

a) The emission spectra of LED light sources are shown in Fig. S1b.

b) The total energy of incident photon, E_{total} (W or J s-1) = Power (W cm^{-2}) × Area (cm^2)

c) The energy of one photon, E_P (J) = hc/λ,

where h is the Planck constant (6.63×10^{-34} J s), c is the speed of light (2.998×10^8 m s^{-1}) and λ is wavelength (m).

d) The number of incident photon, N_P (s^{-1}) = E_{total} (J s^{-1}) / E_P (J).

e) Rate of incident photon (mol s^{-1}) = N_P / Avogadro number (6.022×10^{23} mol^{-1})

f) Rate of H_2 formation was calculated based on the amount of H_2 formed in first 1-h in Fig. S1a.

g) Apparent quantum yield, AQY (%) = 100 × (2 × Rate of H_2 formation) (Rate of incident photon)$^{-1}$

h) Because the emission peak by the 470-nm LED light source was observed at 460 nm (Fig. 1b), the calculation was based on the actual value.