Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Visible-light driven 3-hydroxybutyrate synthesis from CO_2 and acetone with the hybrid system of photocatalytic NADH regeneration and multi-biocatalysts

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1. Acetone carboxylase (AC) and 3-hydroxybutyrate dehydrogenase (HBDH) expression in *Rhodobacter capsulatus* SB1001 (*Rb. capsulatus* SB1001)

Acetone carboxylase (AC) and 3-hydroxybutyrate dehydrogenase (HBDH) were expressed in purple nonsulfur photosynthetic bacterium, *Rhodobacter capsulatus* SB1001 (*Rb. capsulatus* SB1001) by the following method. *Rb. capsulatus* SB1001 was grown photosynthetically on RCVB medium[1)] with malate as the carbon source in anaerobic conditions. The RCVB medium composition is shown in Table S1.

Table S1. The composition of RCVB medium

Component	Volume (mL)
10 % DL-Malate	40
10 % $(NH_4)_2SO_4$	10
0.64 M KPO_4	15
150 μg mL^{-1} (+)-Biotin	0.1
Super salts solution[*1]	50
Total volume	1000

[*1] Super salts solution (in 1 L): 1.0 % Na_2EDTA (20 mL), 20 % $MgSO_4 \cdot 7H_2O$ (10 mL), 7.5 % $CaCl_2 \cdot 2H_2O$ (10 mL), 0.5 % $FeSO_4 \cdot 7H_2O$ (24 mL), 1.0 g L^{-1} thiamine-HCl (1.0 mL) and trace elements[*2] (10 mL).

[*2] Trace elements (in 1 L): Na_2EDTA (2.5 g), $CoCl_2 \cdot 6H_2O$ (0.02 g), $MnCl_2 \cdot 4H_2O$ (0.2 g), H_3BO_3 (0.1 g), $CuCl_2 \cdot 2H_2O$ (0.01 g), $ZnCl_2$ (0.05 g), $Na_2MoO_4 \cdot 2H_2O$ (0.1 g), $NiCl_2 \cdot 6H_2O$ (0.05 g), Na_2SeO_3 (0.005 g) and $NaVO_3$ (0.005 g).

For the growth of *Rb. capsulatus* SB1001 on acetone to express AC, 50 mM acetone and 20 mM $NaHCO_3$ were added instead of DL-malate as the carbon sources.[2)] The RCVB medium not including malate was adjusted to pH 7.0 with KOH before autoclaving. Acetone was added to autoclaved medium and $NaHCO_3$ was also added with filter sterilization. Cultures were grown photosynthetically in 1 L bottles. The culture vessels were incubated in darkness for 16 to 20 h at 30 °C to make anaerobic conditions by respiration of *Rb. capsulatus* SB1001, then illuminated by an LED light source (TAITEC, LC-LED-450 W, illumination intensity: 3000 Lux) for 7 days at 30 °C. Cells were harvested by centrifugation (7,460 g, r.t., 2 h) and cell pastes were stored at -80 °C. Frozen cell pastes (21 g) were resuspended in an equal volume of buffer A (25 mM 3-(*N*-morpholino)propanesulfonic acid, 1.0 mM dithiothreitol, pH 7.6), then were homogenized by a glass homogenizer.[3)] The resuspended cells were treated with

ultrasonic crushing to gain the cell extract including AC and HBDH after the addition of 8.0 mg lysozyme and DNase I.[3] The cell lysate was ultracentrifuged (227,000 g, 4 °C, 1 h) using a P50AT2 rotor (Eppendorf Himac Tech. Co., Ltd., Japan) and the supernatant solution was precipitated with saturated $(NH_4)_2SO_4$ solution,[4] followed by ultracentrifugation to obtain concentrated proteins including AC and HBDH. The protein was dissolved in 500 mM 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)-NaOH pH 7.0 for preservation and used in subsequent reactions.

2. The enzyme activities of AC and HBDH from *Rb. capsulatus* SB1001

The enzyme unit for enzyme is defined a catalytic activity. The one unit (U; μmol min^{-1}) is defined as the amount of the enzyme that catalyzes the conversion of 1.0 μmol of substrate per minute. For determination of enzymatic activity of AC, the reaction was started by adding cell extract (0.2 mL) to the solution of acetone (2.5 μmol), sodium bicarbonate (0.25 mmol), ATP disodium salt hydrate (10 μmol) and magnesium chloride (25 μmol) in 5.0 mL of 500 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (pH 8.2) thermostatic chamber set at a temperature of 30.5 °C. The amount of acetoacetate produced was detected by ion chromatography (Metrohm, Eco IC; electrical conductivity detector) with ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 × 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). Under this condition, the amount of acetoacetate produced per minute of incubation time was estimated to be 51~63~ nmol. Thus, the enzyme activity of AC in 0.2 mL of cell extract was calculated to be 0.051~0.063 U.

For determination of enzymatic activity of HBDH, the reaction was started by adding cell extract (0.2 mL) to the solution of lithium acetoacetate (10 μmol) and NADH (25 μmol) in 5.0 mL of 500 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (pH 7.5) thermostatic chamber set at a temperature of 30.5 °C. The concentration of NADH was monitored by absorption spectrum change using UV-visible absorption spectroscopy (SHIMADZU, MaltiSpec-1500) with the molar coefficient at 340 nm (ε=6220 cm^{-1} M^{-1}).[5] The NADH consumption could be calculated as a 3-hydroxybutyrate concentration. Under this condition, the amount of 3-hydroxybutyrate produced per minute of incubation time was estimated to be 0.42~0.47 μmol. Thus, the enzyme activity of HBDH in 0.2 mL of cell extract was calculated to be 0.42~ 0.57 U.

3. Detection for acetoacetate and 3-hydroxybutyrate using ion chromatography

The amount of acetoacetate or 3-hydroxybutyrate was detected using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 x 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min^{-1}. The retention time for acetoacetate was detected at 13.5-14.5 min. The electrical conductivity changes in the various acetoacetate concentrations (0 - 1000 μM) were shown in Figure S1.

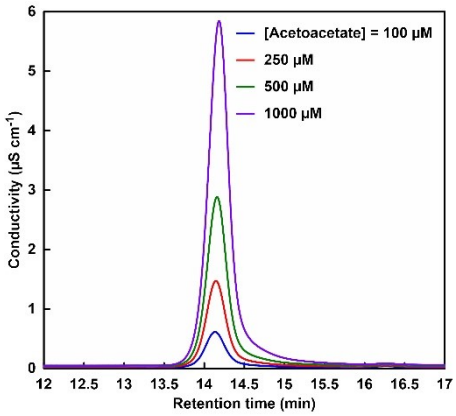

Figure S1. Chromatogram of lithium acetoacetate (0 - 1000 μM) in 50 mM-HEPES buffer (pH 7.0).

Figure S2 shows the relationship between the acetoacetate concentration and the detection peak area using ion chromatograph.

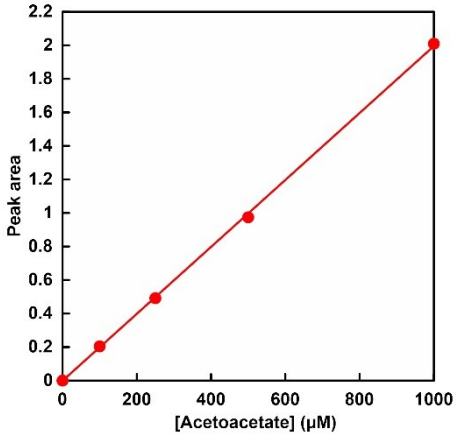

Figure S2. Relationship between the acetoacetate concentration and the detection peak area.

As shown in Figure S2, the acetoacetate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S1).

$$\text{Peak area} = 0.0020 \times [\text{Acetoacetate}](\mu M) \quad \text{(S1)}$$

The retention time for 3-hydroxybutyrate was detected at 14.58 min. The electrical conductivity changes in the various 3-hydroxybutyrate concentrations (0 - 1000 μM) were shown in Figure S3.

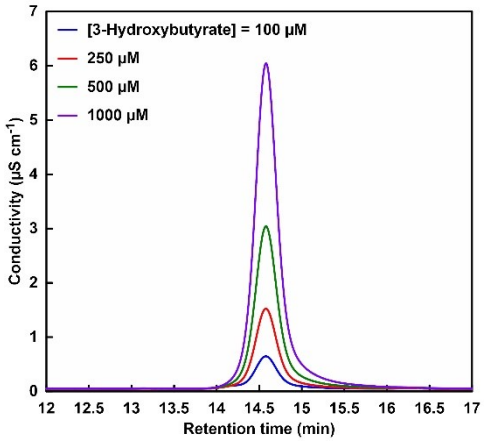

Figure S3. Chromatogram of sodium 3-hydroxybutyrate (0 - 1000 μM) in 50 mM-HEPES buffer (pH 7.0).

Figure S4 shows the relationship between the 3-hydroxybutyrate concentration and the detection peak area using ion chromatograph.

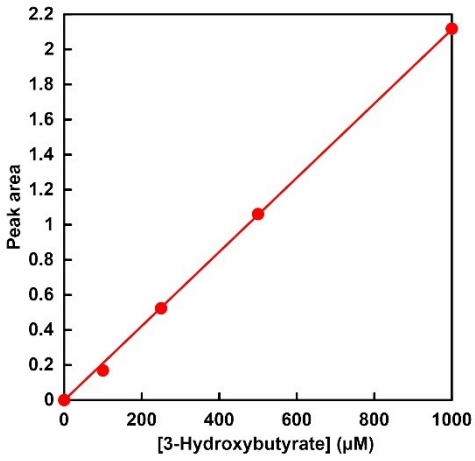

Figure S4. Relationship between the 3-hydroxybutyrate concentration and the detection peak area.

As shown in Figure S4, the 3-hydroxybutyrate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S2).

$$\text{Peak area} = 0.0021 \times [\text{3-Hydroxybutyrate}]\ (\mu M) \quad \text{(S2)}$$

4. Acetoacetate production from acetone and bicarbonate with AC from *Rb. capsulatus* SB1001

Acetoacetate production from acetone and bicarbonate with AC contained in the cell extract from *Rb. capsulatus* SB1003 was investigated. The reaction was started by adding cell extract (AC 0.063 U, HBDH 0.42 U) to the solution of acetone (0.5 mM), sodium bicarbonate (50 mM), ATP disodium salt hydrate (2.0 mM) and magnesium chloride (5.0 mM) in 5.0 mL of 500 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (pH 8.2) thermostatic chamber set at a temperature of 30.5 °C. The amount of acetoacetate produced was detected by ion chromatography (Metrohm, Eco IC; electrical conductivity detector) with ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 × 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups).

5. Visible-light driven NADH regeneration system of TEOA, ZnTPPS and $[Cp^*Rh(bpy)(H_2O)]^{2+}$

$[Cp^*Rh(bpy)(H_2O)]^{2+}$ was synthesized from $[Cp^*RhCl_2]_2$, purchased from Tokyo Chemical Industry Co., Ltd., and 2,2′-bipyridine according to a reported literature.[6] A sample solution containing TEOA (0.2 M), ZnTPPS, obtained from Frontier Scientific Inc, (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM) and NAD^+ purchased from Oriental Yeast Co., Ltd, (2.0 mM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.0) was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon gas for 10 min. The sample solution was irradiated with a 250 W halogen lamp (Panasonic) as a visible-light source at 30.5 °C. The concentration of NADH produced was monitored by absorption spectrum change using UV-visible absorption spectroscopy (SHIMADZU, MaltiSpec-1500) with the molar coefficient at 340 nm (ε=6220 cm^{-1} M^{-1}).

Figure S5 shows the time dependence of NADH concentration in the sample solution containing TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$and NAD^+ in HEPES-NaOH buffer (pH 7.0) with visible light irradiation. As shown in Fig. S5, the NADH production rate is higher at the beginning of the reaction within 60 min, and is slightly slower at the end. At the initial period of irradiation, the concentration of the accumulated reduced

species $[Cp^*Rh(bpy)H]^+$ increased and the NADH production rate increased, and then, it is predicted that catalytically NADH was produced by the redox of the $[Cp^*Rh(bpy)(H_2O)]^{2+}$.

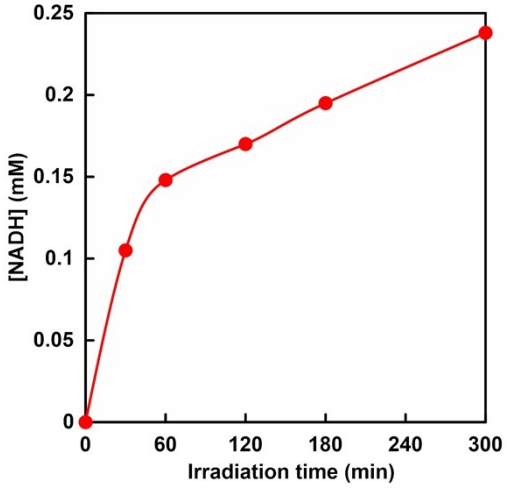

Figure S5. The time dependence of NADH concentration in the sample solution containing TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$ and NAD^+ in HEPES-NaOH buffer with visible light irradiation.

6. Visible-light driven 3-hydroxybutyrate production system of TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+, cell extract and acetoacetate

A sample solution containing TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+ (2.0 mM), lithium acetoacetate (0.5 mM), and cell extract (AC 0.051 U, HBDH 0.47 U) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.0) was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon gas for 10 min. The sample solution was irradiated with a 250 W halogen lamp as a visible-light source at 30.5 °C. The concentration of acetoacetate or 3-hydroxybutyrate was analyzed by ionic chromatograph system.

7. Enantioselectivity of HBDH in the cell extract for 3-hydroxybutyrate production from acetoacetate

There are L- and D-enantiomers in 3-Hydroxybutyrate. The enantioselectivity of HBDH in the cell extract was investigated by the oxidation of D- or L-3-hydroxybutyrate to acetoacetate with HBDH in the cell extract in the presence of NAD^+. The sample solution consisted of 0.2 mL cell extract (AC 0.045 U, HBDH 0.31 U), sodium D-hydroxybutyrate or L-3-hydroxybutyric acid (0.2 μM) and NAD^+ (1.0 mM) in 5.0 mL of 500mM HEPES buffer (pH 7.0) at 30 °C. D- or L-3-Hydroxybutyrate and acetoacetate were detected by the ion chromatograph system. Figure S6 shows the ion

chromatograms of the sample solution consisted of 0.2 mL cell extract, sodium D-3-hydroxybutyrate and NAD^+ in HEPES buffer during the incubation. Although there is a discrepancy between the retention times observed in Figures S1 and S3, calibration is performed with a standard sample for each measurement. As shown in Figure S6, the peak due to the acetoacetate increased with the incubation time.

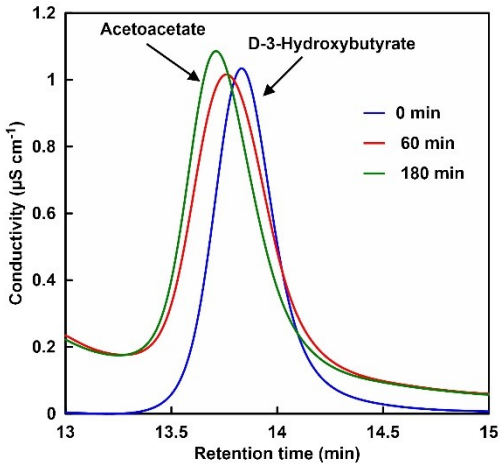

Figure S6. The ion chromatograms of the sample solution consisted of 0.2 mL cell extract, sodium D-3-hydroxybutyrate and NAD^+ in HEPES buffer during the incubation.

From the result in Figure S6, acetoacetate production was observed due to the oxidation of D-3-hydroxybutyrate with HBDH in the cell extract in the presence of NAD^+. Figure S7 shows the ion chromatograms of the sample solution consisted of 0.2 mL cell extract, L-3-hydroxybutyric acid and NAD^+ in HEPES buffer during the incubation.

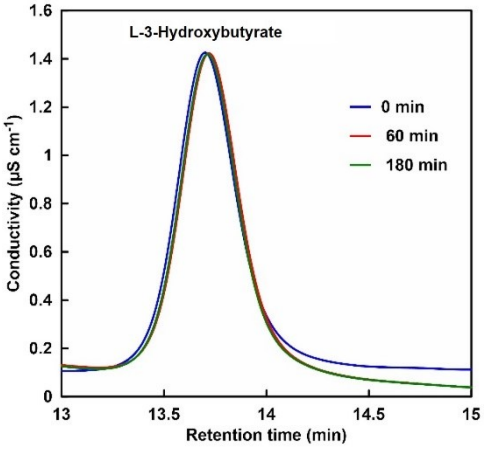

Figure S7. The ion chromatograms of the sample solution consisted of 0.2 mL cell extract, L-3-hydroxybutyric acid and NAD^+ in HEPES buffer during the incubation.

As shown in Figure S7, no peak due to the acetoacetate was observed with the incubation time. From the result in Figure S7, no acetoacetate production was observed with HBDH in the cell extract in the presence of NAD^+.

8. Visible-light driven 3-hydroxybutyrate production from acetone and bicarbonate with the system of TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+, ATP and cell extract

A sample solution containing acetone (0.5 mM), TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+ (2.0 mM), sodium bicarbonate (50 mM), ATP disodium salt hydrate (2.0 mM), magnesium chloride (5.0 mM) and cell extract (AC 0.051 U, HBDH 0.47 U) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.0) was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon gas for 10 min. The sample solution was irradiated with a 250 W halogen lamp as a visible-light source at 30.5 °C. The concentration of acetoacetate or 3-hydroxybutyrate was analyzed by ionic chromatograph system. Control experiments are as follows. A sample solution containing acetone (0.5 mM), TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+ (2.0 mM), sodium bicarbonate (50 mM), ATP disodium salt hydrate (2.0 mM), magnesium chloride (5.0 mM) and cell extract (AC 0.061 U, HBDH 0.57 U) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.0) was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon gas for 10 min. The sample solution was incubated under the dark condition.

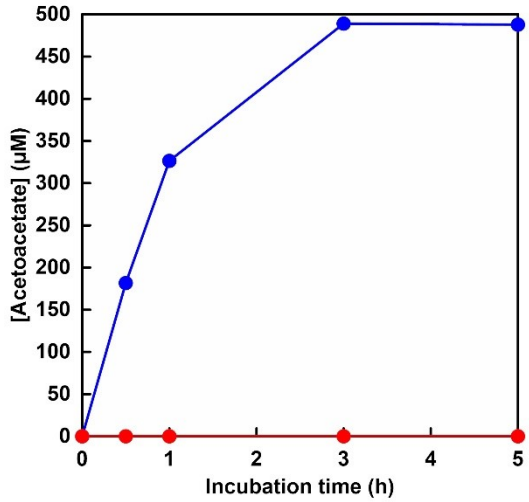

Figure S8. Time dependence of the acetoacetate and 3-hydroxybutyrate concentrations in the sample solution containing acetone, TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+, sodium bicarbonate, ATP disodium salt hydrate, magnesium chloride and cell extract in HEPES-NaOH buffer (pH 7.0) with

incubation under the dark condition.

Figure S8 shows the time dependence of the acetoacetate and 3-hydroxybutyrate concentrations in the sample solution containing acetone, TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+, sodium bicarbonate, ATP disodium salt hydrate, magnesium chloride and cell extract in HEPES-NaOH buffer (pH 7.0) with incubation under the dark condition. As shown in Figure S8, only acetoacetate production was observed under the dark condition.

A sample solution containing TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+ (2.0 mM), sodium bicarbonate (50 mM), ATP disodium salt hydrate (2.0 mM), magnesium chloride (5.0 mM) and cell extract (AC 0.061 U, HBDH 0.57 U) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.0) (without acetone) was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with argon gas for 10 min. The sample solution was irradiated with a 250 W halogen lamp as a visible-light source at 30.5 ^oC. The concentration of acetoacetate or 3-hydroxybutyrate was analyzed by ionic chromatograph system. Figure S9 shows the ion chromatograms of the sample solution containing TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+, sodium bicarbonate, ATP disodium salt hydrate, magnesium chloride and cell extract in HEPES-NaOH buffer (pH 7.0) with irradiation.

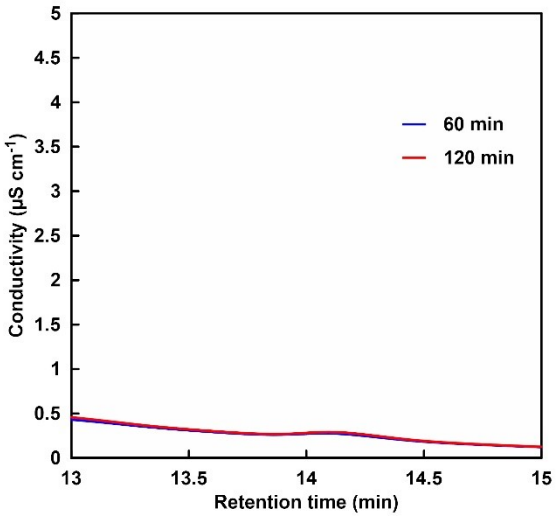

Figure S9. The ion chromatograms of the sample solution containing TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+, sodium bicarbonate, ATP disodium salt hydrate, magnesium chloride and cell extract in HEPES-NaOH buffer (pH 7.0) with irradiation.

As shown in Figure S9, no peaks due to the acetoacetate and 3-hydroxybutyrate were observed without acetone.

9. Visible-light driven 3-hydroxybutyrate production from acetone and CO_2 with the system of TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+, ATP and cell extract

A sample solution containing acetone (0.5 mM) TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+ (2.0 mM), ATP disodium salt hydrate (2.0 mM), magnesium chloride (5.0 mM) and cell extract (AC 0.061 U, HBDH 0.57) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.2) was deaerated by freeze-pump-thaw cycles repeated 6 times and then flushed with CO_2 gas for 10 min. The sample solution was irradiated with a 250 W halogen lamp as a visible-light source at 30.5 °C. The concentration of acetoacetate or 3-hydroxybutyrate was analyzed by ionic chromatograph system.

Reference

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