

# Electronic Supplementary Information

## CO<sub>2</sub> photoreduction at enzyme-modified metal oxide nanoparticles

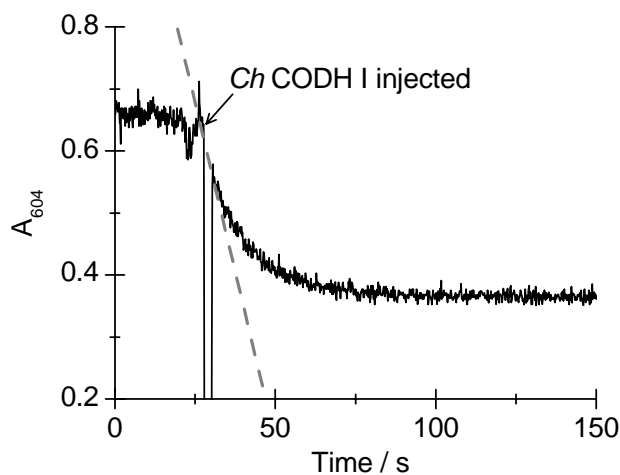
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Fraser A. Armstrong

### *Carboxydotherrnus hydrogenoformans* growth and CODH purification

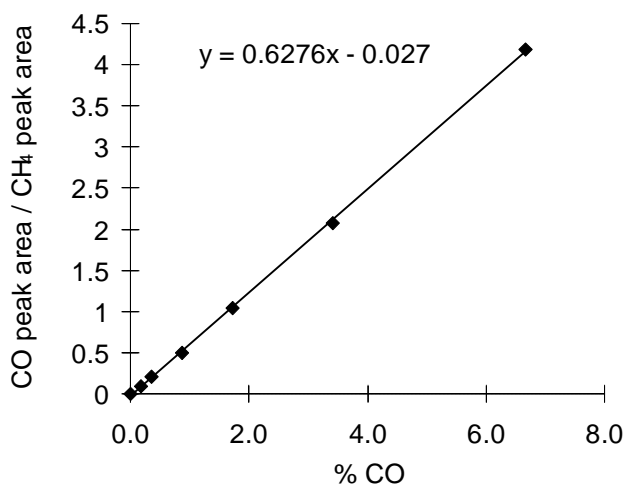
*Carboxydotherrnus hydrogenoformans* was grown in a 10 L fermentor in the medium described<sup>1</sup> except that the CO flow rate during growth was between 1 and 1.5 L min<sup>-1</sup> and the fermentor medium was supplemented with 9 mM sodium pyruvate. The fermentor was inoculated from a culture grown in 1 L of the same medium, in a sealed 2 L bottle, on 30 mM sodium pyruvate. This medium was saturated with CO before inoculation.

CODH I and II were purified mostly as described.<sup>1</sup> The procedure described was modified as follows: after suspension in lysis buffer, cells were sonicated for 15 min, and the cell lysate was centrifuged for 45 min at 120,000 x g. The Source 15 ISO column was omitted, and instead CODH I and II were loaded together on butyl sepharose after treatment with ammonium sulfate. CODH I and II were separated by the butyl sepharose column and were further purified separately. Each protein was loaded on a 75 mL high resolution Q sepharose column and eluted with an 800 mL linear gradient from 0 to 0.5 M NaCl. 1 M ammonium sulfate was added to each protein for a final purification using a 50 mL Source 15 ISO column with an 800 mL linear gradient from 1 to 0.2 M ammonium sulfate.

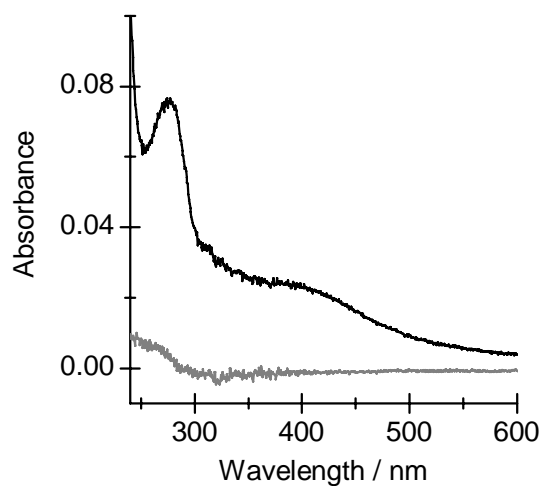
1. V. Svetlitchnyi, C. Peschel, G. Acker, and O. Meyer, O, *J. Bacteriol.*, 2001, **183**, 5134-5144



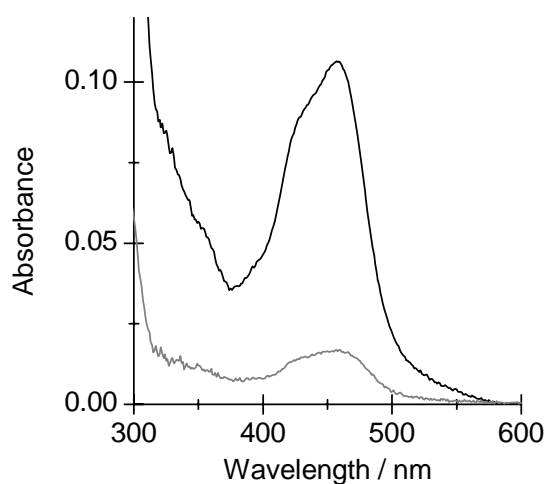
**Fig. S1** An assay for CO<sub>2</sub> reduction activity at *Ch* CODH I in aqueous solution at 20 °C, carried out anaerobically in a glove box (O<sub>2</sub> < 3 ppm). Oxidised methyl viologen (MV<sup>2+</sup>, 0.2 mM) in 3 mL CO<sub>2</sub>-saturated MES buffer solution (200 mM, pH 6) was reduced by addition of sodium dithionite (0.5 mol sodium dithionite per 1 mol MV<sup>2+</sup>) to form MV<sup>+</sup>. At the point indicated, 0.16 nmol of *Ch* CODH I was injected, and the absorbance at 604 nm monitored over time. The initial rate of MV<sup>+</sup> oxidation (grey dotted line) was used to determine the initial turnover frequency of the enzyme for CO<sub>2</sub> reduction (95 ± 36 s<sup>-1</sup>).



**Fig. S2** Typical calibration plot for GC detection of CO. Known amounts of CO were injected into the headspace of a sealed Pyrex vessel (total volume 9 mL) containing 2% CH<sub>4</sub> in CO<sub>2</sub>. The vessel also contained 5 mL MES buffer solution (200 mM, pH 6). The response to CO was measured against the CH<sub>4</sub> internal standard.



**Fig. S3** UV-vis absorption spectra of an aqueous solution of *Ch* CODH I (0.51  $\mu\text{M}$ ) before (black) and after (grey) 20 min exposure to a suspension of P25 TiO<sub>2</sub> (1 mg mL<sup>-1</sup>). Prior to collecting spectra, nanoparticles were removed by centrifugation and filtration.



**Fig. S4** UV-vis absorption spectra of an aqueous solution of RuP (11  $\mu\text{M}$ ) before (black) and after (grey) 20 min exposure to a suspension of P25 TiO<sub>2</sub> (1 mg mL<sup>-1</sup>). The particles had first been modified with *Ch* CODH I, by exposure to a 0.51  $\mu\text{M}$  enzyme solution, as in Fig. S3. Prior to collecting spectra, nanoparticles were removed by centrifugation and filtration.

Amount of RuP used in assembly of 5 mL suspension / nmol	RuP concentration / $\mu\text{M}$	$A_{455\text{ nm}}$ (before exposure to $1\text{ mg mL}^{-1}$ nanoparticles)	$A_{455\text{ nm}}$ (of supernatant, after exposure to $1\text{ mg mL}^{-1}$ nanoparticles)	Amount of RuP adsorbed on 5 mg nanoparticles / nmol
5.60	1.12	0.0104	0.0035	$3.7 \pm 0.03$
16.8	3.36	0.0302	0.0044	$14.4 \pm 0.11$
28.0	5.60	0.0505	0.0074	$23.9 \pm 0.06$
39.2	7.84	0.0681	0.0117	$32.5 \pm 0.12$
56.0	11.2	0.1059	0.0161	$47.5 \pm 0.05$
78.4	15.7	0.1406	0.0333	$59.8 \pm 2.68$
113	22.6	0.1953	0.0587	$79.1 \pm 4.89$
150	30.0	0.2546	0.0837	$101 \pm 6.16$

**Table S1** Determination of RuP loadings on 5 mg of P25 TiO<sub>2</sub>, when different amounts of dye are used in assembly of the photocatalyst. The nanoparticles were first modified with 2.56 nmol of *Ch* CODH prior to adsorption of RuP.

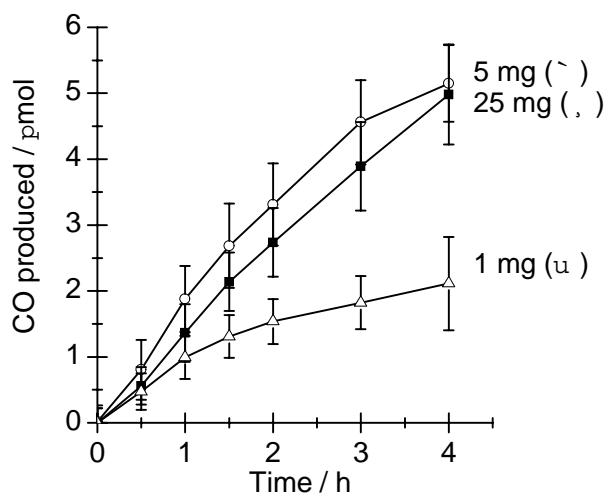
Amount of <i>Ch</i> CODH I used in assembly of 5 mL suspension / nmol	<i>Ch</i> CODH I concentration / $\mu\text{M}$	$A_{280\text{ nm}}$ (before exposure to $1\text{ mg mL}^{-1}$ nanoparticles)	$A_{280\text{ nm}}$ (of supernatant, after exposure to $1\text{ mg mL}^{-1}$ nanoparticles)	Amount of <i>Ch</i> CODH I adsorbed on 5 mg nanoparticles / nmol
0.51	0.10	0.0154	0.0016	$0.47 \pm 0.06$
1.28	0.26	0.0388	0.0061	$1.08 \pm 0.16$
1.54	0.31	0.0478	0.0080	$1.28 \pm 0.04$
1.97	0.39	0.0535	0.0000	$1.97 \pm 0.03$
2.56	0.51	0.0766	0.0047	$2.40 \pm 0.04$
3.28	0.66	0.0935	0.0075	$3.02 \pm 0.36$
5.12	1.02	0.1483	0.0095	$4.79 \pm 0.11$

**Table S2** Determination of *Ch* CODH I loadings on 5 mg of P25 TiO<sub>2</sub>, when different amounts of enzyme are used in assembly of the photocatalyst.

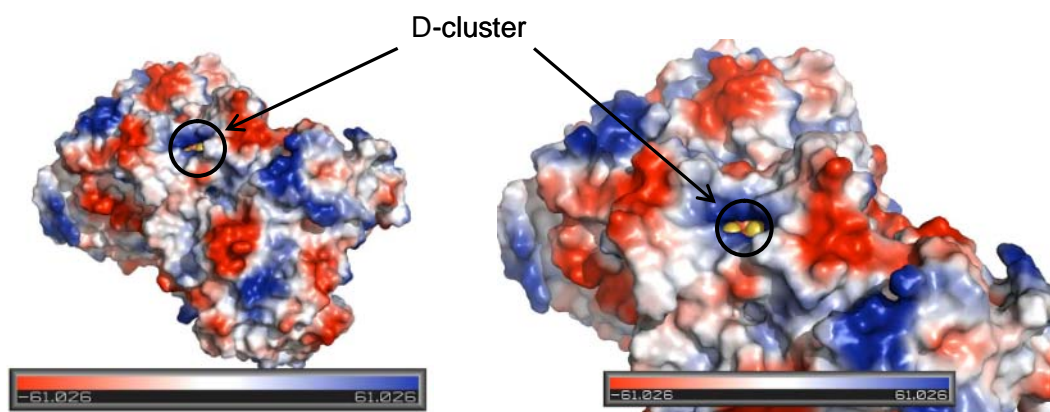
#### Calculation of amount of *Ch* CODH I required for monolayer coverage on 5 mg P25 TiO<sub>2</sub>

Dimensions for enzyme molecule <sup>1</sup>	$88\text{ \AA} \times 63\text{ \AA} \times 60\text{ \AA}$
Assuming 'footprint' dimensions of $88\text{ \AA} \times 63\text{ \AA}$ , 'footprint' area	$5.28 \times 10^{-17}\text{ m}^2\text{ molecule}^{-1}$
BET surface area of P25 TiO <sub>2</sub> <sup>2</sup>	$50\text{ m}^2\text{ g}^{-1}$
Surface area of 5 mg P25 TiO <sub>2</sub>	$50\text{ m}^2\text{ g}^{-1} \times 5 \times 10^{-3}\text{ g} = 0.25\text{ m}^2$
<b>Upper limit for monolayer coverage</b>	$0.25\text{ m}^2 / 5.28 \times 10^{-17}\text{ m}^2\text{ molecule}^{-1} = 4.73 \times 10^{15}\text{ molecules} = \mathbf{7.9\text{ nmol}}$

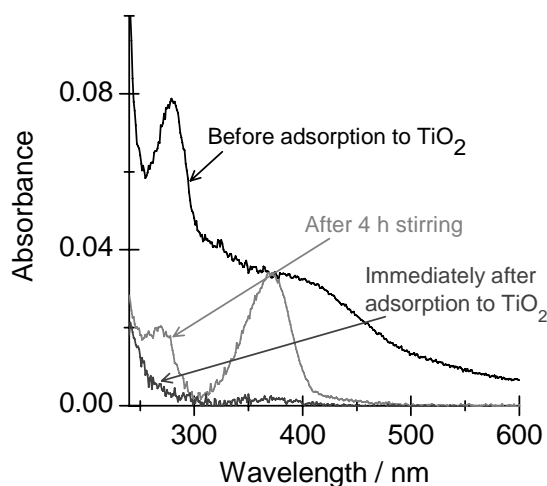
1. H. Dobbek, V. Svetlitchnyi, L. Gremer, R. Huber and O. Meyer, *Science*, 2001, **293**, 1281-1285.
2. Degussa, *Manufacturer Technical Information*, 2005.



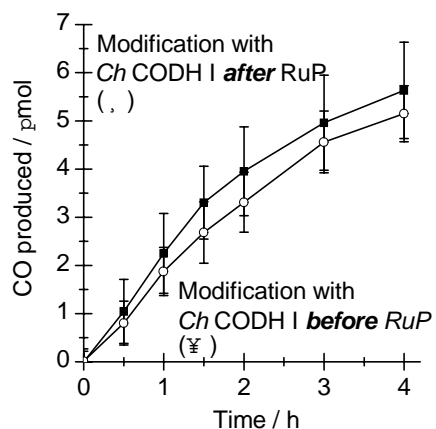
**Fig. S5** Visible light-driven CO<sub>2</sub> photoreduction at P25 TiO<sub>2</sub> nanoparticles (different total masses, as indicated) modified with 2.56 nmol *Ch* CODH I and 56 nmol RuP. Other conditions/components: 20 °C, 5 mL buffer (200 mM MES, pH 6).



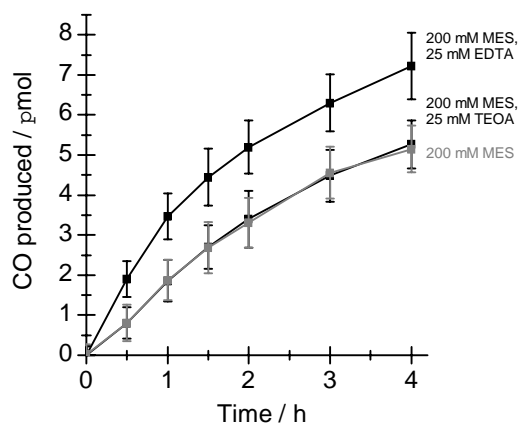
**Fig. S6** Surface charge distribution of *Ch* CODH II, created using PyMOL.



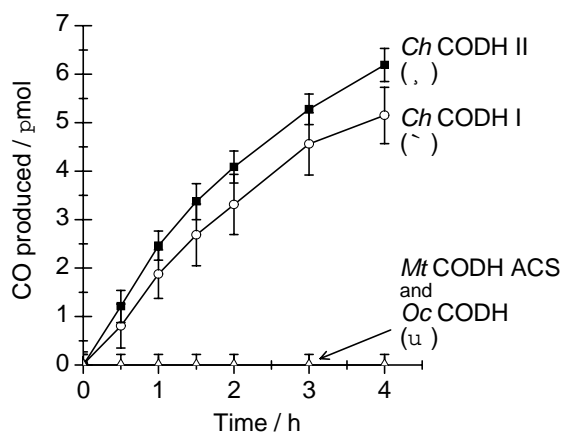
**Fig. S7** UV-vis absorption spectra of an aqueous solution of *Ch* CODH I (0.51  $\mu\text{M}$ ) before exposure to P25 TiO<sub>2</sub> (black), immediately after adsorption to P25 TiO<sub>2</sub> (dark grey), and after 4 h stirring with P25 TiO<sub>2</sub> (light grey). P25 TiO<sub>2</sub> concentration is 1 mg mL<sup>-1</sup>. Protein absorbance is at 280 nm; the absorbance at 370 nm is thought to be due to small fragments of TiO<sub>2</sub> which are broken off larger nanoparticles over several hours.



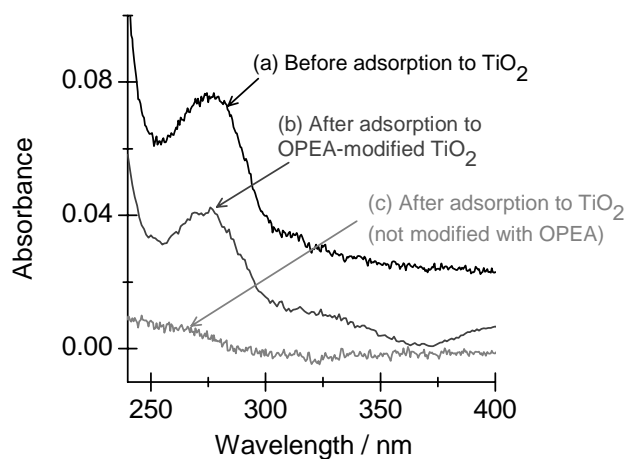
**Fig. S8** Visible light-driven CO<sub>2</sub> photoreduction at P25 TiO<sub>2</sub> nanoparticles (5 mg) modified with 2.56 nmol *Ch* CODH I and 56 nmol RuP. Grey trace: nanoparticles are first modified with enzyme, and then photosensitiser. Black trace: nanoparticles are first modified with photosensitiser, and then enzyme. Other conditions/components: 20 °C, pH 6, 5 mL buffer (200 mM MES, pH 6).



**Fig. S9** Visible light-driven CO<sub>2</sub> photoreduction at P25 TiO<sub>2</sub> nanoparticles (5 mg) modified with 2.56 nmol *Ch* CODH I and 56 nmol RuP. The suspension buffer was either 200 mM MES, 200 mM MES and 25 mM TEOA, or 200 mM MES and 25 mM EDTA (as indicated). Other conditions/components: 20 °C, pH 6, 5 mL buffer (pH 6).



**Fig. S10** Visible light-driven CO<sub>2</sub> photoreduction at P25 TiO<sub>2</sub> nanoparticles (5 mg) modified with 56 nmol RuP and 2.56 nmol of either *Ch* CODH I, *Ch* CODH II, *Mt* CODH ACS or *Oc* CODH. Other conditions/components: 20 °C, 5 mL buffer (200 mM MES, pH 6).



**Fig. S11** UV-vis absorption spectra of aqueous solutions of *Ch* CODH I (0.51 μM), (a) before 20 min exposure to a 1 mg mL<sup>-1</sup> suspension of P25 TiO<sub>2</sub>; (b) after exposure to a 1 mg mL<sup>-1</sup> suspension of P25 TiO<sub>2</sub> that has been modified with OPEA (0.3 mg per 5 mg of nanoparticles); and (c) after exposure to a 1 mg mL<sup>-1</sup> suspension of P25 TiO<sub>2</sub> that has *not* been modified with OPEA. Prior to collecting each spectrum, nanoparticles were removed by centrifugation and filtration.