Electrochemical characterization of *C. pasteurianum* hydrogenase II and four site-directed variants: catalysis and inhibition

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Biochemical methods

CpI

Holo-*Cp*I FeFe-hydrogenase from *Clostridium pasteurianum* W5 (WP_004455619.1), carrying a C-terminal Strep-tag, was heterologously produced in *Escherichia coli* BL21(DE3) Δ*iscR* cells alongside the HydEFG maturases from *Shewanella oneidensis*, as reported recently[1] and based on an earlier method[2–4]. Briefly, *E. coli* BL21(DE3) Δ*iscR* containing pET21(b)-*hydA* (for *Cp*I) and pACYCDuet-1-*hydEF-hydGX* (for the HydEFG maturases) was cultivated aerobically at 37°C in 3 L of Lysogeny Broth (LB) medium containing 100 mM MOPS buffer (pH 7.4), along with 25 mM glucose, 2 mM ferric ammonium citrate and the necessary antibiotics (kanamycin, ampicillin, chloramphenicol, standard working concentrations). Upon reaching an optical density of ~$0.5_{(600\ nm)}$ the culture was transferred to a COY anoxic chamber (~3% H_2, balance N_2) where cysteine (2 mM), sodium fumarate (25 mM) and IPTG (0.5 mM) were added and the culture was incubated at 18°C for 16 h. Following gene expression, the cells were harvested by centrifugation and resuspended at 2:1 v/w in Tris:HCl

buffer (50 mM, pH 8) containing NaCl (300 mM) and sodium dithionite (2 mM). The cells were lysed by sonication on ice in the presence of a few µg of DNAse and the cell debris was pelleted by centrifugation at 30,000 x *g* for 1 h at 4 °C. The C-terminally strep-tagged *Cp*I protein was subsequently purified by FPLC over a StrepTrapXT column (5 mL column volume, Cytiva, 1 mL/min flow-rate) in the same buffer and 50 mM biotin was used to elute the column-bound *Cp*I protein. Biotin was removed by desalting over a HiPrep 26/10 column (Cytiva) and the *Cp*I sample was concentrated over a stirred-cell concentrator (Millipore), flash-frozen in liquid N_2 and stored in the same until use. H_2 production specific activity assays (~70 µmol min^{-1} $mg^{-1}_{protein}$) were performed in MOPS buffer (pH 7, 100 mM) at 37 °C containing methylviologen (1 mM), sodium dithionite (100 mM) and 58 µg of *Cp*I using a GC-TCD (SRI Instruments) over a molecular sieve 5 Å column with Ar as the carrier gas.

CpII

Gene synthesis

The accession numbers of CpII hydrogenase sequence is WP_003446424.1.
The DNA sequence was optimized for production in E.coli, and synthesised in the MCS1 of the pET-Duet1 between NcoI and NotI with a C-terminal Streptag .

Mutagenesis of CpII : S73A, S99A, T223M, T377A

The mutagenesis were performed using the Q5® High-Fidelity DNA Polymerase (NEB), and the user protocol. We used the following primers:
CpII S73A forward : 5' TGTGCCCGGTGTACGCTATTACCGAAG 3'
CpII S73A reverse : 5' GCTCACTTCGGTAATAGCGTACACCGGG 3'
CpII S99A forward : 5' GTGATTTTTCAGACCGCACCGAGCGTGC 3'
CpII S99A reverse : 5' GCACGCTCGGTGCGGTCTGAAAAATCAC 3'
CpII T223M forward : 5' TTGTGAACGTGGCGGTGATGCCGTGCA 3'
CpII T223M reverse : 5' TGCACGGCATCACCGCCACGTTCACAA 3'
CpII T377A forward : 5' GATTTTGTGGAAGTGATGGCATGCCGCG 3'
CpII T377A reverse : 5' GCCGCCGCGGCATGCCATCACTTCCACA 3'

WT, S99A and T223M

Protein production

We produced WT CpII and the S99A and T223M mutants of CpII as described in ref. [5]. The yield WT CpII is ≈ 400 µg/L.

T377A and S73A

Protein preparation: As described by Kuchenreuther and coauthors [4], *Cp*II T377A and S73A variants were recombinantly expressed in *Escherichia coli* BL21 (DE3) *ΔiscR [3]* in their apo state without coexpression of the maturase genes. Apo-hydrogenases only harbor the [4Fe4S]-subcluster of the H-cluster, hence missing the catalytically essential $2Fe_H$-subcluster. Minor modifications to the protocol described by Kuchenreuther and coauthors included the aerobically cultivation of the expression cultures at 37 °C and the addition of 5 mM L-cysteine instead of 2 mM L-cysteine [4]. After reaching an OD_{600} of 0.4-0.6, protein expression was induced under anoxic conditions (97.5 % N_2 and 2.5 % H_2 atmosphere) in an anoxic chamber (Coy Laboratories) by addition of IPTG (0.5 mM).

The cultures were incubated overnight at ambient temperatures. All following protein purification steps were performed under anoxic conditions (97.5 % N_2 and 2.5 % H_2 atmosphere) in an anoxic chamber (Coy Laboratories). All solutions used during protein purification were degassed prior to use. After cell harvest, the cell pellet was resuspended in 0.1 M Tris-HCl buffer (pH 8.0) supplemented with 2 mM sodium dithionite (NaDT). Cell lysis was facilitated first by the addition of lysozyme (10 mg per liter expression culture) and Triton X-100 (1 % v/v) to the resuspended cells with an incubation period of 1 h on ice and second by sonication on ice. To pellet cell debris, the samples were centrifuged at 181,000 x *g* for 1 h at 4 °C. Purification of the *Cp*II variants was conducted via affinity chromatography according to the manufacturer's instructions enabled by a C-terminal Strep-tag II, which selectively binds to the Strep-Tactin® Superflow resin (IBA Lifesciences). The samples were concentrated via spin filtration (30 kDa MWCO) and stored under anoxic conditions in 0.1 M Tris-HCl buffer (pH 8.0) supplemented with 2 mM NaDT at 4 °C. Protein concentrations were determined employing the Bradford protein assay in reference to a BSA standard calibration curve.

In-vitro Maturation: All steps were conducted under anoxic conditions (97.5% N_2 a 2.5% H_2 atmosphere) in an anoxic chamber (Coy Laboratories) as described by Esselborn and coauthors [6]. An excess of the $2Fe_H^{MIM}$ complex ($Fe_2[\mu\text{-}(SCH_2)_2NH](CN)_2(CO)_4[Et_4N]_2$), synthesized as described previously [7], was added to the apo-*Cp*II samples and incubated for 135 min on ice in the dark to ensure successful insertion of $2Fe_H^{MIM}$ into the apo-hydrogenases and its conversion to the active holo-enzyme. To prevent potential light-induced decomposition of the cofactor or the cofactor mimic, $2Fe_H^{MIM}$ and holo-hydrogenases were handled under dim light conditions. Afterwards, spin filtration (30 kDa MWCO) was employed to remove excess $2Fe_H^{MIM}$ from the holo-hydrogenases and to exchange the buffer to 0.1 M potassium phosphate buffer (pH 6.8) supplemented with 2 mM NaDT, while minimizing protein loss. Holo-*Cp*II samples were aliquoted and stored under anoxic conditions at 4 °C. Protein concentrations were determined via Bradford assay using a BSA standard calibration curve.

Electrochemistry

Methods

Protein films for CpII WT and all the variants were produced by applying a small drop (0.5 µl) of protein solution with a concentration in the nM range onto a freshly polished pyrolytic graphite edge electrode (diameter: 3 mm), and letting it dry for about 30 seconds. The electrode was inserted in the electrochemical cell filled with mixed buffer (containing 5 mM of each of MES, CHES, HEPES, TAPS, Na-acetate, and Na_2SO_4 100 mM, titrated with NaOH 1 M or H_2SO_4 1M, always chloride-free), and rotated at a high rate (3000 rpm) to prevent H_2 depletion near the electrode surface. The temperature of the electrochemical cell was set to 30°C.

We use chloride free buffers because halides inhibit some FeFe-hydrogenases[8], but the inhibition of CpI by halides is slow, and we saw no effect of chlorides on H_2-oxidation by CpII.

Voltammetric catalytic responses

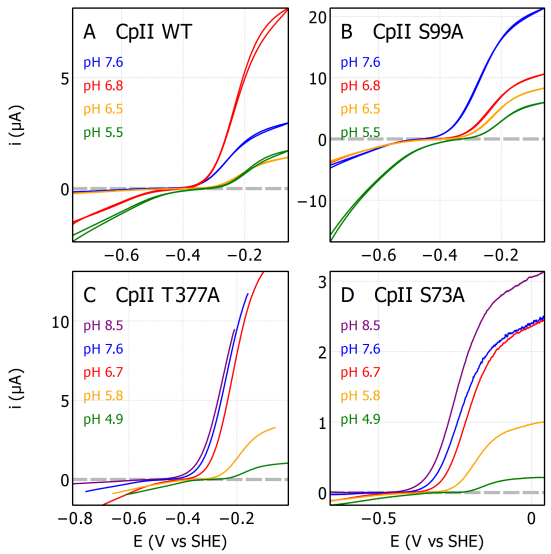


Figure S1. Cyclic voltammograms recorded with *CpII* WT and variants S99A, T377A and S73A under H_2 100%, at 30°C, with a scan rate of 20 mV/s and electrode rotation rate of 3000 rpm. The precise value of the pH was calculated from the equilibrium potential (measured thanks to a film of a reversible hydrogenase, *DdHydAB* in this case, using the same buffers) via the Nernst equation.

The following **table S1** reports, for CpII WT and its variants (except T223M), the values of the two catalytic potentials, their difference (that is a direct measure of the irreversibility of an enzyme) and the catalytic bias measured at different pHs. CpI WT is added for comparison. (*) Regarding some of the mutants, the fits of the model to the cyclic voltammetric data were not as good as for WT CpII, and the resulting values of the catalytic potentials and catalytic bias are less reliable.

	pH	**Ecat ox (*) (mV vs SHE)**	**Ecat red (*) (mV vs SHE)**	**ΔE (*) (mV)**	**bias (*)**
CpII WT	7.6	-288	-551	263	16.4
	6.8	-261	-511	250	5.8
	6.8	-256	-518	262	5.4
	6.8	-256	-502	246	10.0
	6.5	-244	-481	237	7.6
	5.5	-220	-442	222	0.9
S99A	7.6	-320	-549	229	5.2
	6.8	-283	-515	232	2.2
	6.8	-277	-515	238	2.7
	6.8	-273	-512	239	3.5
	6.8	-271	-504	233	5.2
	6.5	-257	-492	235	3.0
	5.5	-229	-453	224	0.4
T377A	8.5	-299	-615	316	58.1
	7.6	-282	-561	279	17.7
	6.7	-249	-499	250	7.9
	6.7	-245	-508	263	9.6
	5.8	-209	-461	252	3.4
	4.9	-183	-412	229	0.9
S73A	8.5	-288	undet.	/	/
	7.6	-269	-538	269	70.4

	6.7	-247	-498	251	8.9
	6.7	-239	-493	254	14.6
	5.8	-203	-449	246	6.8
	4.9	-165	-425	260	1.1
CpI	7.0	-399	-474	75	0.3

Michaelis constants

To measure the Michaelis-Menten constants, the H_2 oxidation current was measured at different potentials while the cell solution, initially saturated with H2, was flushed with argon. The value of Km expressed in atm can be converted to mM using a H_2 solubility of 0.75 mM/atm at 30 °C.
The raw data were then corrected for film loss using QSoas, by the "b" command.

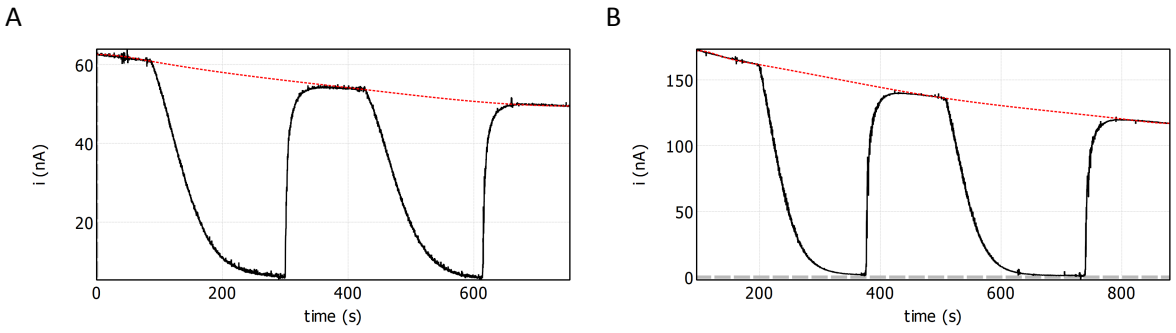


Figure S2. Raw chronoamperometric H2-oxidation data for CpII WT (panel A) and T223M (panel B), when the electrochemical cell is subsequently flushed with H_2 and Ar to measure the Michaelis constant for H_2. The fact that T223M has a K_M which is almost twice the WT value is visible in the data. Red dotted lines represent the correction of the film loss, obtained using the program QSoas[9] and its command "b".

CO inhibition

CO inhibition was tested in chronoamperometry experiments by measuring the current as a function of time at a certain potential (-260 or -160 or +40 mV), and injecting two consecutive aliquots of a CO-saturated buffer. This results in an immediate increase of the CO concentration in the open electrochemical cell, that successively decays exponentially while CO is flushed away by the continuous bubbling of H_2. The precise initial concentration of CO into the cell can be calculated knowing the injection volume, the buffer volume into the cell, and the concentration of CO into the vial (that is 1 mM at 20°C, i.e. the temperature at which the buffer was kept).
The raw data were first corrected to remove the effect of film loss using QSoas, a process that can be tricky in case the reaction with CO is not fully reversible.

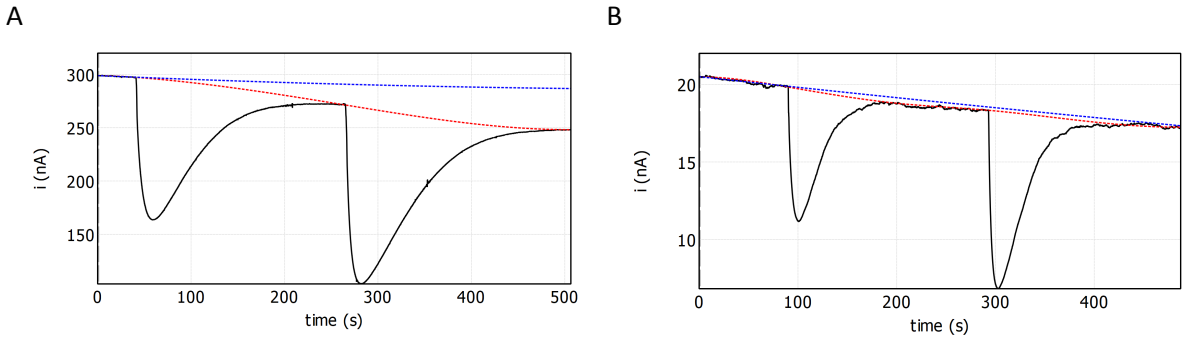


Figure S3. Panel A shows, for *CpII* WT, how to correct for the film loss assuming that the reaction is fully reversible (red baseline, obtained using the program QSoas[9] and its command “b”), or partly irreversible (blue blase line, obtained using the program QSoas[9] and its command “B”). For each of the enzymes WT, S99A, T223M and T377A it makes a difference to use either one or the other command. Panel B shows the same analysis with the S73A variant. In this case, there is no evidence that the reaction may be partly irreversible. Experimental conditions: H_2 100%, electrode potential + 40 mV vs SHE, electrode rotation rate 3000 rpm, 30°C, in chloride-free mixed buffer pH7.

Inhibition of *CpII* by oxygen

O_2 inhibition was tested in chronoamperometry experiments by measuring the current as a function of time at different potentials (ranging from -260 to +240 mV vs SHE), and injecting aliquots of an O_2-saturated buffer. This results in an immediate increase of the O_2 concentration in the open electrochemical cell, that successively decays exponentially while O_2 is flushed away by the continuous bubbling of H_2. The precise initial concentration of O_2 into the cell can be calculated knowing the injection volume, the buffer volume into the cell, and the concentration of O_2 into the vial (that is 1.4 mM at 20°C, i.e. the temperature at which the buffer was kept).
The raw data were fitted with a model that takes into account:

- A sudden drop in current right after the injection due to the dilution of H_2. This contribution is small since the Km is rather small;
- A sudden drop in current due to the reduction of some of the injected O_2 at the electrode, that happens even at high potentials;
- An irreversible loss in current due to the inactivation of the enzyme, described by the bimolecular rate constant kd (for *CpII* WT, S99A, T223M and T377A);
- A partially reversible loss in current for *CpII* S73A, described by a certain bimolecular inactivation rate constant ki and a reactivation rate constant ka.

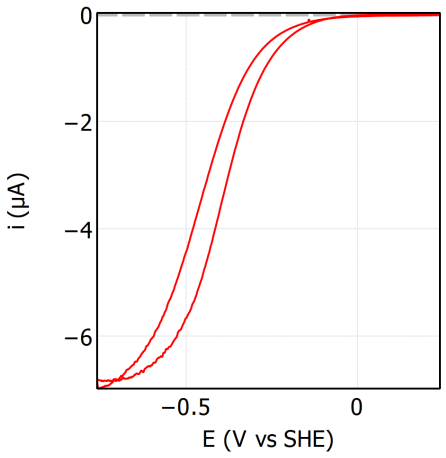


Figure S4. Direct reduction of O_2 at a rotating disc PGE electrode at T=30°C, pH=7, electrode rotation rate=2000 rpm. 40 µM O_2 was injected at high potential before sweeping the electrode potential down. The capacitive current recorded in the absence of O_2 was subtracted.

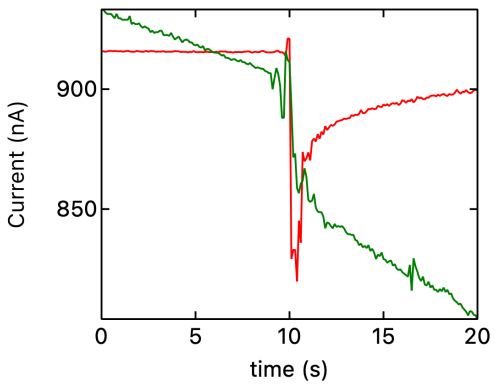


Figure S5. In green : a chronoamperometry experiment performed at +40 mV with a film of *CpII* WT, where an O_2-saturated buffer is injected at t=10s. In red, the same experiment but without any enzyme on the electrode, offsetting the two signals for better comparison. The sudden drop in current is mainly explained by the reduction of O_2 at the electrode, with a minor contribution given by the depletion of H_2.

Inhibition of *CpI* by oxygen

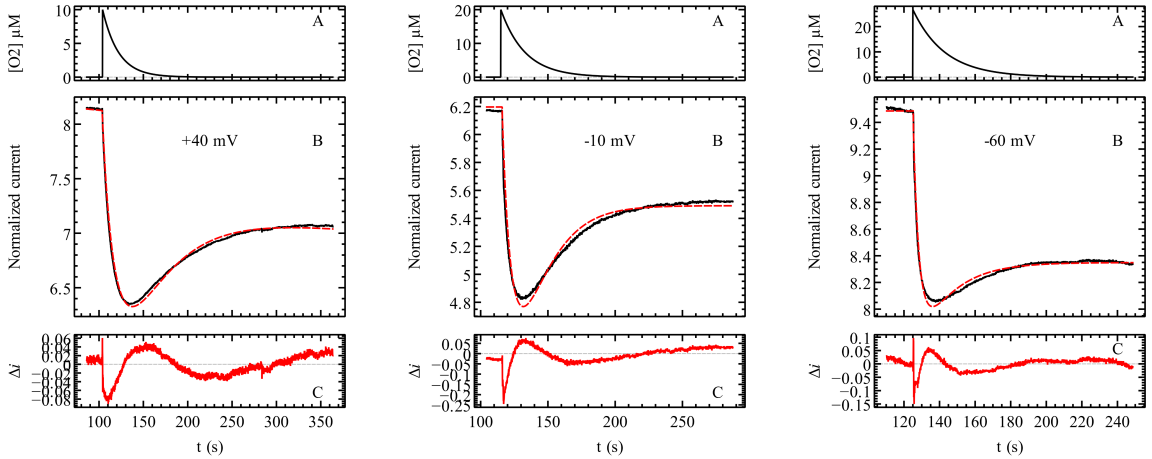
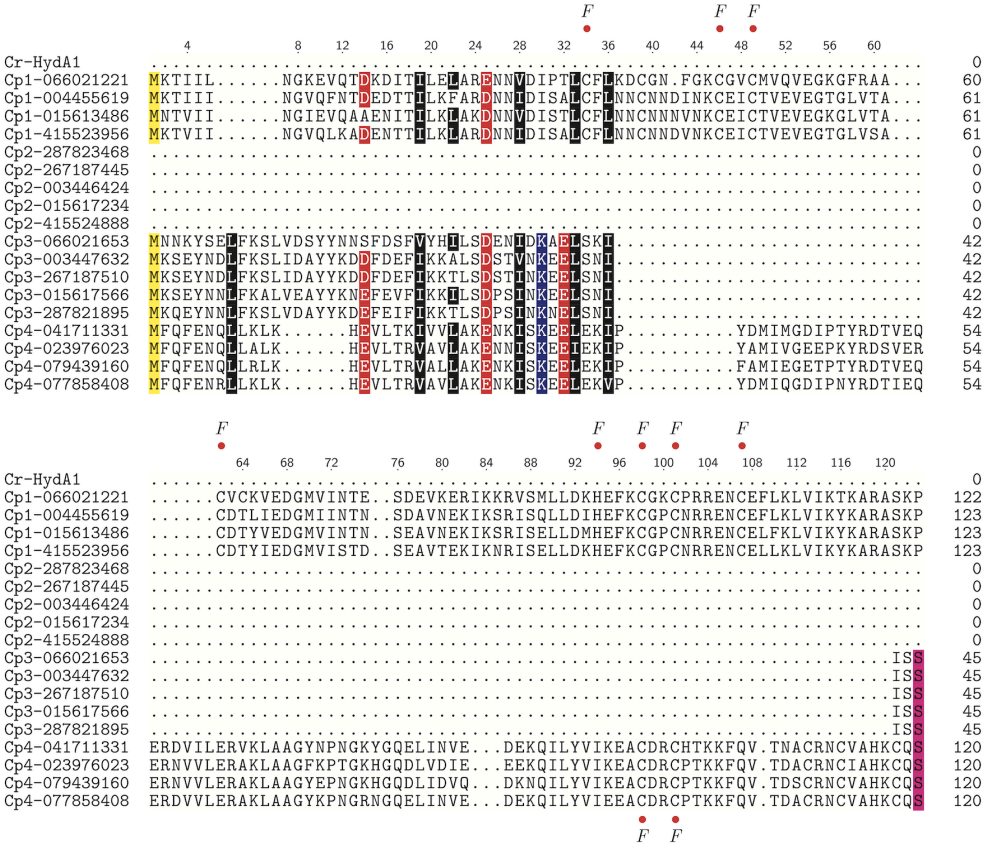


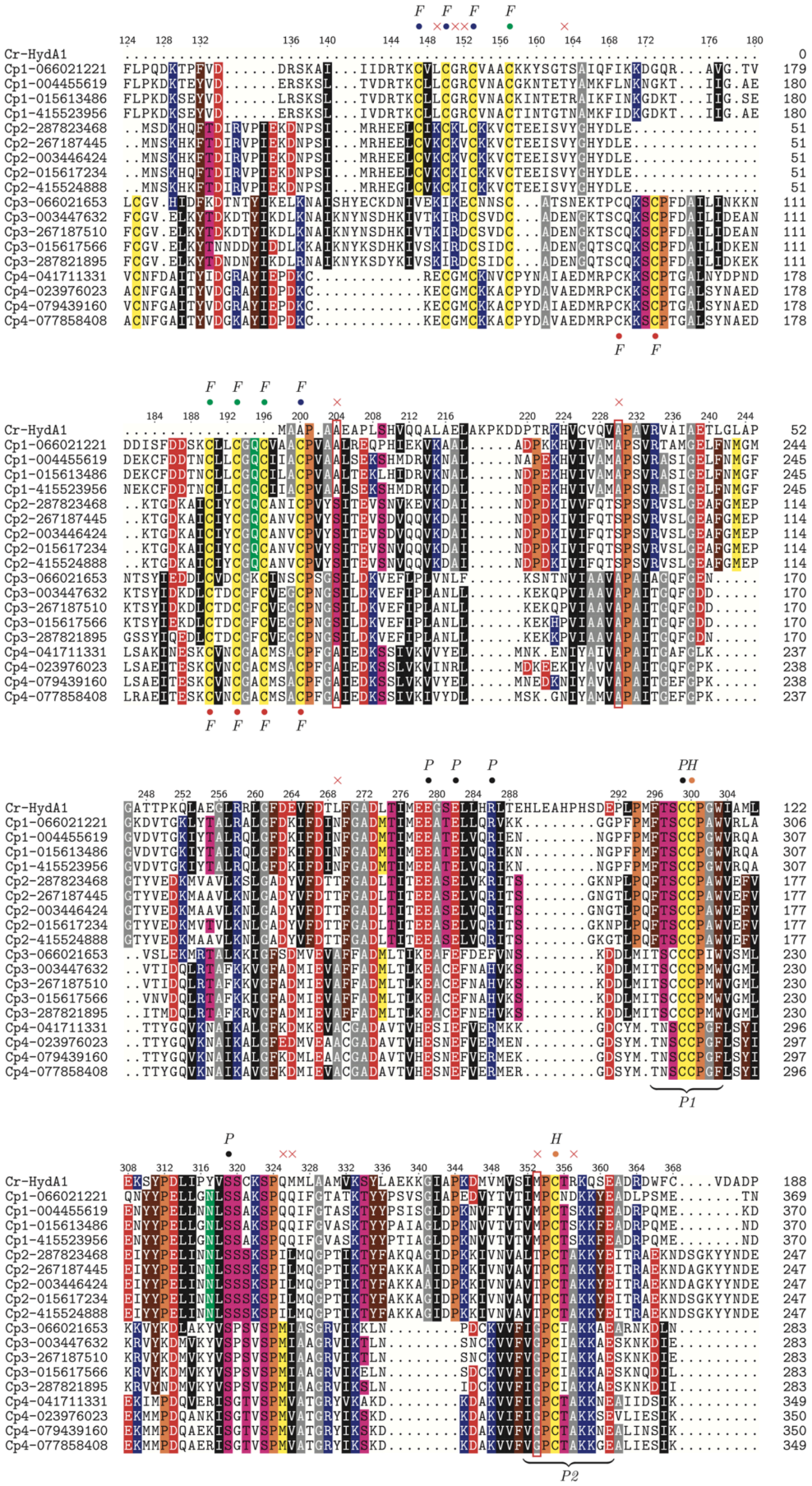
Figure : fits of the two-step model to three selected O_2 inhibition experiments with CpI WT at different potentials. Panel A: O_2 concentration profile. Panel B: data in black solid line and fit of the model described by eq 4 and 5 in dash red line. Panel C: difference between the fit and the data. Conditions: pH 7; 12°C; 3k rpm.

Comparison of the protein sequences of clostridial hydrogenases

Alignement

Figure S7 Alignment of the sequences of the proteins listed in main text table 1. The numbering above the sequences is that of W5 *CpI*. The red, blue and green dots on top indicate the cysteines binding the accessory clusters (F), the black dots point to the proton transfer residues in *CpI*, the orange dots show the residues binding the H cluster. Red crosses indicate the residues listed in ref [10]. The red dots *below* the sequences point to the cysteines binding the accessory clusters in *CpIV*. The positions of the three motifs that characterize FeFe-hydrogenases are also shown using braces. The alignment was drawn with TeXshade.

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Cp1-004455619 FLPKDKTEYVD.....ERSKSL...TVDRTKCLLCGRCVNACGKNTETYAMKFLNKNGKT...IIG.AE 180
Cp1-015613486 FLPKDKSEYVD.....LRSKSL...TVDRTKCLLCGRCVNACKINTETRAIKFINKGDKT...IIG.SE 180
Cp1-415523956 FLPKDKSEYVD.....ERSKSL...IVDRTKCLLCGRCVNACTINTGTNAMKFIDKGDKT...IIG.AE 180
Cp2-287823468 ..MSDKHQFTDIRVPIEKDNPSI...MRHEELCIKCKLCKKVCTEEISVYGHYDLE..... 51
Cp2-267187445 ..MNSKHKFTDIRVPIEKDNPSI...MRHEELCVKCKVCKKVCTEEISVYGHYDLE..... 51
Cp2-003446424 ..MNSKHKFTDIRVPIEKDNPSI...MRHEELCVKCKVCKKVCTEEISVYGHYDLE..... 51
Cp2-015617234 ..MNSKHQFTDIRVPIEKDNPSI...MRHEELCVKCKICKKVCTEEISVYGHYDLE..... 51
Cp2-415524888 ..MNSKHKFTDIRVPIEKDNPSI...MRHEGLCVKCKICKKVCTEEISVYGHYDLE..... 51
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F F
F F F F
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Cp1-015613486 DEKCFDDTNCLLCGQCILACPVAALTEKLHIDRVKNAL.....NDPEKHVIVAMAPSVRASIGELFNMGF 245
Cp1-415523956 NEKCFDDTNCLLCGQCIIACPVAALSEKSHMDRVKDAL.....NDPEKHVIVAMAPSVRASIGELFNMGF 245
Cp2-287823468 ..KTGDKAICIYCGQCANICPVYSITEVSNVKEVKDAI.....NDPDKIVVFQTSPSVRVSLGEAFGMEP 114
Cp2-267187445 ..KTGDKAICIYCGQCANVCPVYSITEVSDVQQVKDAI.....NDPDKIVIFQTSPSVRVSLGEAFGMEP 114
Cp2-003446424 ..KTGDKAICIYCGQCANVCPVYSITEVSDVQQVKDAI.....NDPDKIVIFQTSPSVRVSLGEAFGMEP 114
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Cp3-003447632 KTSYIDKDLCTDCGFCVEGCPNGSLLDKVEFIPLANLL.....KEKQPVIAAVAPAITGQFGDD..... 170
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Cp4-079439160 ..TTYGQVKNAIKALGFKDMLEAACGADAVTVHESNEFVERMEK.....GDSYM.TNSCCPGFLSYI 297
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P1
P H
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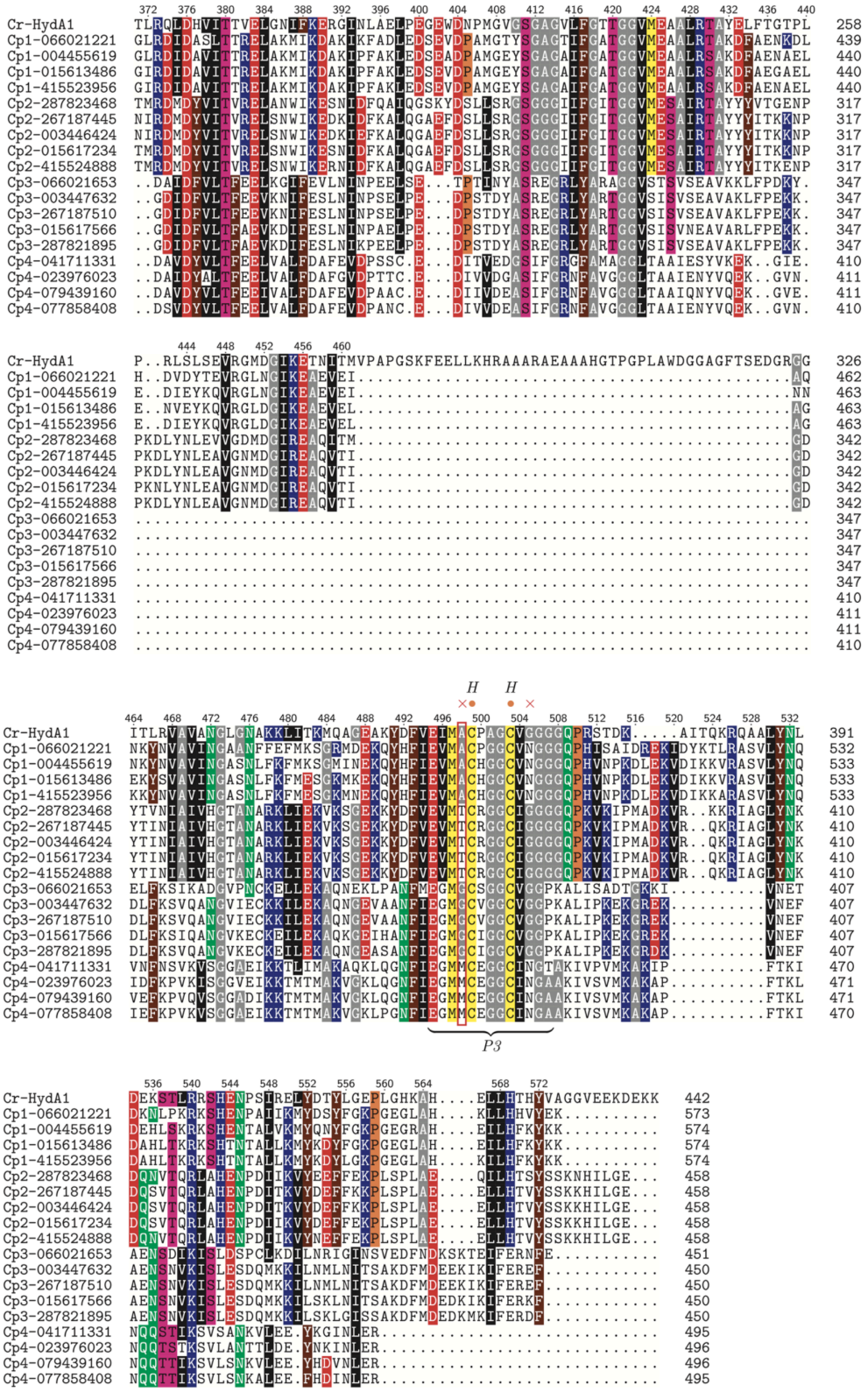
Cp1-066021221 QNYYPELLGNLSSAKSPQQIFGTATKTYYPSVSGIAPEDVYTVTIMPCNDKKYEADLPSME.....TN 369
Cp1-004455619 ENYYPELLNNLSSAKSPQQIFGTASKTYYPSISGLDPKNVFTVTVMPCTSKKFEADRPQME.....KD 370
Cp1-015613486 ENYYPELLGNLSSAKSPQQIFGVASKTYYPAIAGLDPKNVVTVTVMPCTSKKFEADRPQME.....ND 370
Cp1-415523956 ENYYPELLGNLSSAKSPQQIFGVASKTYYPTIAGLDPKNVVTVTVMPCTSKKFEADRPQME.....ND 370
Cp2-287823468 EIYYPELINNLSSSKSPILMQGPTIKTYFAKQAGIDPKKIVNVALTPCTAKKYEITRAEKNDSGKYYNDE 247
Cp2-267187445 EIYYPELINNLSSSKSPILMQGPTIKTYFAKKAADPKKIVNVAVTPCTAKKYEITRAEKNDAGKYYNDE 247
Cp2-003446424 EIYYPELINNLSSSKSPILMQGPTIKTYFAKKAIDPKKIVNVAVTPCTAKKYEITRAEKNDAGKYYNDE 247
Cp2-015617234 EIYYPELINNLSSSKSPILMQGPTIKTYFAKKAGIDPKKIVNVAVTPCTAKKYEITRAEKNDSGKYYNDE 247
Cp2-415524888 EIYYPELINNLSSSKSPILMQGPTIKTYFAKKAGIDPKKIVNVAVTPCTAKKYEITRAEKNDSGKYYNDE 247
Cp3-066021653 KKVYKDLAKYVSPSVSPMIASGRVIKKLN.....PDCKVVFIGPCIAKKAEARNKDLN..... 283
Cp3-003447632 KRVYKDMVKYVSPSVSPMIAAGRVIKTLN.....SNCKVVFVGPCIAKKAESKNKDIE..... 283
Cp3-267187510 KRVYKDMVKYVSPSVSPMIAAGRVIKTLN.....SNCKVVFVGPCIAKKAESKNKDIE..... 283
Cp3-015617566 KRVYKDMVKYVSPSVSPMIAAGRVIKELN.....SDCKVVFIGPCIAKKAESKNQDIL..... 283
Cp3-287821895 KRVYNDMVKYVSPSVSPMIAAGRVIKSLN.....IDCKVVFIGPCIAKKAESKNKDII..... 283
Cp4-041711331 EKIMPDQVERISGTVSPMVATGRYVKAKD.....KDAKVVFIGPCTAKKNEAIIDSIK..... 349
Cp4-023976023 EKMMPDQANKISGTVSPMVATGRYIKSKD.....KDAKVIFIGPCTAKKSEVLIESIK..... 350
Cp4-079439160 EKMMPDQAEKISGTVSPMVATGRYIKSKD.....KDAKVVFVGPCTAKKNEALINSIK..... 350
Cp4-077858408 EKMMPDQAERISGTVSPMVATGRYIKSKD.....KDAKVVFVGPCTAKKGEALIESIK..... 349
P2


H
H
P3

Identity and similarity

Table S2. Identity and similarity between the protein sequences listed in main text table 1, calculator by TeXshade

	Cr	Cp1-066021221	Cp1-004455619	Cp1-015613486	Cp1-415523956	Cp2-287823468	Cp2-267187445	Cp2-003446424	Cp2-015617234	Cp2-415524888	Cp3-066021653	Cp3-003447632	Cp3-267187510	Cp3-015617566	Cp3-287821895	Cp4-041711331	Cp4-023976023	Cp4-079439160	Cp4-077858408
Cr	—	58.4	59.5	59.5	59.8	60.5	61.0	60.7	61.0	61.3	44.9	46.6	46.6	46.3	46.0	45.0	45.6	46.2	46.1
Cp1-066021221	43.6	—	82.0	81.3	82.0	55.8	56.3	56.3	56.7	56.3	37.6	38.6	38.6	39.1	38.6	38.8	38.3	38.8	38.8
Cp1-004455619	43.3	71.5	—	93.5	93.7	58.6	58.3	58.3	58.8	58.3	38.3	39.8	39.8	39.3	38.6	39.5	39.0	39.4	39.3
Cp1-015613486	43.6	71.2	87.9	—	95.6	58.8	58.6	58.6	59.0	58.3	38.0	38.8	38.8	38.8	37.9	39.5	38.3	39.6	39.1
Cp1-415523956	43.6	70.5	89.8	92.3	—	59.3	59.3	59.3	59.7	59.0	37.3	39.3	39.3	39.1	38.1	39.7	38.5	39.4	38.8
Cp2-287823468	43.6	39.5	41.6	42.5	42.7	—	94.5	94.5	96.5	95.6	39.1	39.7	39.7	39.7	38.9	39.7	40.2	40.5	39.4
Cp2-267187445	44.7	40.9	42.5	43.2	43.4	89.7	—	100.0	97.5	98.0	38.8	39.7	39.7	39.4	38.6	38.6	39.9	39.9	38.9
Cp2-003446424	44.7	40.9	42.5	43.2	43.4	89.5	99.7	—	97.5	98.0	38.5	39.4	39.4	39.2	38.6	38.6	39.9	39.9	38.9
Cp2-015617234	45.2	41.3	42.5	42.9	43.2	92.3	96.2	96.0	—	98.0	39.6	40.2	40.2	40.0	39.2	38.9	39.9	39.9	38.9
Cp2-415524888	45.2	40.9	42.2	42.7	42.9	91.9	96.7	96.5	97.5	—	39.1	40.0	40.0	39.7	38.9	38.9	40.2	40.2	39.2
Cp3-066021653	26.4	21.9	22.4	21.9	21.9	22.8	22.8	22.8	23.6	23.6	—	77.7	77.7	76.2	77.7	52.8	52.9	53.4	53.3
Cp3-003447632	26.5	24.1	24.1	23.4	24.3	23.9	24.2	24.2	24.7	25.0	65.5	—	99.7	94.4	93.3	50.8	50.4	50.7	50.6
Cp3-267187510	26.5	23.8	24.3	23.1	24.5	23.9	24.2	24.2	24.7	25.0	65.7	99.5	—	94.4	93.5	50.8	50.4	50.7	50.6
Cp3-015617566	26.5	23.6	22.7	22.2	23.1	22.6	22.6	22.6	23.1	23.1	64.8	89.1	89.3	—	94.8	50.3	50.0	51.4	50.6
Cp3-287821895	27.4	23.8	22.2	22.0	22.7	23.1	23.1	23.1	23.6	23.6	65.1	87.7	88.2	90.2	—	49.8	49.2	50.2	49.6
Cp4-041711331	28.1	22.6	22.8	23.5	23.5	24.0	23.5	23.2	23.5	23.5	35.6	34.2	34.4	33.7	34.7	—	87.8	91.1	90.3
Cp4-023976023	27.0	22.1	23.4	22.6	22.8	22.9	22.6	22.3	22.6	22.9	35.1	33.1	33.4	33.4	34.3	77.9	—	92.9	92.9
Cp4-079439160	27.7	22.3	23.4	23.2	23.4	24.3	24.3	24.0	24.3	24.3	34.3	33.4	33.6	33.1	34.3	82.4	86.4	—	95.5
Cp4-077858408	26.7	21.7	22.4	22.8	22.6	22.6	22.6	22.4	22.6	22.6	35.4	33.9	34.2	33.7	34.4	82.4	86.0	88.8	—

% identity

% similarity

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