nature portfolio

https://doi.org/10.1038/s41586-022-04971-z

Supplementary information

Supplementary Information for

Membrane-anchored HDCR nanowires drive hydrogen-powered CO_2 fixation

Helge M. Dietrich[1†], Ricardo D. Righetto[2,3†], Anuj Kumar[1,4], Wojciech Wietrzynski[2,3], Raphael Trischler[1], Sandra K. Schuller[4], Jonathan Wagner[5], Fabian M. Schwarz[1], Benjamin D. Engel[2,3]*, Volker Müller[1]*, Jan M. Schuller[4]*

[1] Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University; Frankfurt am Main, Germany

[2] Helmholtz Pioneer Campus, Helmholtz Munich; Neuherberg, Germany.

[3] Biozentrum, University of Basel; Basel, Switzerland

[4] CryoEM of Molecular Machines, SYNMIKRO Research Center and Department of Chemistry, Philipps-University, Marburg, Germany

[5] Department of Molecular Structural Biology, Max Planck Institute of Biochemistry; Martinsried, Germany

† Equal contribution

*Correspondence to: ben.engel@unibas.ch, vmueller@bio.uni-frankfurt.de, jan.schuller@synmikro.uni-marburg.de

Supplemental Information Table of Contents

Supplementary Information:

Interaction studies of HDCR subunits

Genetic complementation of the Δ*hdcr* strain was used to probe the connectivity of the HDCR complex. When HycB4 was omitted from the overexpression (HDCR_ΔHycB4), only HydA2 was found in the eluate after purification with HydA2-His_6 (Fig. 2C). However, addition of a second His_6-tag to the HDCR_ΔHycB4 construct (HydA2-His_6 & His_6-FdhF) led to copurification of monomeric FdhF and the protein pair HycB3-HydA2, although there was no functional interaction between FdhF and HycB3-HydA2. This verifies HycB4 as the connecting subunit between HydA2 and the protein pair of FdhF and HycB3. When HycB3 was omitted from the overexpression (HDCRΔHycB3), HydA2-His_6 purification yielded a complex containing HydA2 and HycB4, but not FdhF. This proves that HycB3 is the subunit linking HycB4 to FdhF, revealing the chain of protein interactions to be FdhF-HycB3-HycB4-HydA2. This conclusion is in complete accordance with the cryo-EM structure (Fig. 1) and is also supported by the genetic organization of the *hdcr* operon, where the genes for the enzyme complex are arranged in the same order[1].

Catalytic centers operate independently, but HycB3 is vital for FdhF activity

Hydrogenase activity was independent of HycB3 and HycB4. When HydA2 was omitted from overexpression (HDCR_ΔHydA2) or the H-cluster coordinating Cys387 was substituted with alanine (HDCR_HydA2 C387A), there was no hydrogenase activity (Extended Data Fig. 7F). Formate dehydrogenase activity was marginally affected by removal of HycB4 and HydA2, but HycB3 was required for FdhF activity (Extended Data Fig. 7F).

Supplementary Methods 1: Additional methods for Extended Data Fig. 7.

For methylviologen-dependent hydrogenase activity, 1.1 x 10^5 Pa H_2 was used as electron donor. For methylviologen-dependent formate dehydrogenase assay and analysis of hydrogen production from formate, 150 mM formate was used as electron donor. Formate production from H_2 + CO_2 was determined with a substrate gas composition of H_2 + CO_2 (80:20 [v:v], 1.1 x 10^5 Pa). The reaction buffer for pH-optima analysis (Extended Data Fig. 7a,b) composed of 50 mM MES, 50 mM MOPS, 50 mM HEPES, 50 mM EPPS, 50 mM CHES, 20 mM $MgSO_4$, 4 µM Resazurin, 2 mM DTE, pH as indicated. The reaction buffer for Extended Data Fig. 7c-h was 100 mM HEPES, 20 mM $MgSO_4$, 4 µM Resazurin, 2 mM DTE, pH 7.0.

Supplementary Discussion

Relevance of HDCR filamentation to its activity in different organisms

To date, there are two organisms identified to produce an HDCR, *T. kivui* and the closely related *Acetobacterium woodii*, and characterization of these enzymes showed that both HDCRs form large filaments[2,3]. In previous studies, filamentation of *A. woodii* HDCR was reported to be dependent on the presence of divalent cations, and in accordance with our findings, the filamentous form was the most active state of the enzyme[4]. While similar experiments with the *T. kivui* HDCR did not show a divalent cation dependency on filamentation, it must be noted that the shortened protomers reported in the study of Schuchmann *et al*.[4] showed an elution profile corresponding to a molecular mass of about 3500 kDa. This indicates the reported enzyme still forms roughly a 14-mer (assuming the *A. woodii* HDCR forms hexamers as in *T. kivui*) or a 20-mer (tetrameric form of the HDCR) and makes comparison of the depolymerized states of the two enzymes difficult. However, *A. woodii* HDCR in the 3500 kDa state showed a ~30 % reduction in activity compared to a similarly treated control of the filamentous form (>5000 kDa), and therefore, is consistent with our findings of a stepwise reduction in HDCR activity, depending on the number of connected active centers.

Supplementary Table 1. Coordination of the [4Fe4S]-clusters in HDCR subunits.

Cys, cysteine; HC, hydrogen cluster.

Subunit	[4Fe4S] -Number	Coordinating amino acids
FdhF	[4Fe4S] I	Cys13, Cys16, Cys20, Cys48
HycB3	[4Fe4S] I	Cys13, Cys16, Cys19, Cys150
	[4Fe4S] II	Cys23, Cys134, Cys137, Cys146
	[4Fe4S] III	Cys52, Cys55, Cys61, Cys93
	[4Fe4S] IV	Cys64, Cys83, Cys86, Cys89
HycB4	[4Fe4S] I	Cys34, Cys37, Cys40, Cys180
	[4Fe4S] II	Cys44, Cys165, Cys168, Cys176
	[4Fe4S] III	Cys83, Cys86, Cys91, Cys124
	[4Fe4S] IV	Cys95, Cys114, Cys117, Cys120
HydA2	[4Fe4S] I	Cys15, Cys18, Cys21, Cys55
	[4Fe4S] II	Cys25, Cys45, Cys48, Cys51
	[4Fe4S] (HC)	Cys184, Cys239, Cys383, Cys387

Supplementary Table 2. Conserved W- bis-pterin guanine dinucleotide (W-bisPGD) coordinating residues. Amino acids interacting with the W-bisPGD cofactor in the closest-related formate dehydrogenase (DgW-FDH from *Desulfovibrio gigas*, PDB: 1H0H) are mostly conserved in *T. kivui* FdhF. *, conserved amino acid.

W-bisPGD coordinating amino acid in DgW-FDH	Related amino acid in *T. kivui* FdhF
C20	C16 *
C54	C48 *
K56	K50 *
Q154	C135
CSe158	C139 (*)
E197	E179 *
N198	C180
N194	N176 *
D220	D202 *
R222	R204 *
G239	G221 *
D241	N223
M371	M297 *
W373	V299
N518	N403 *
Q517	E402
S522	S407 *
V542	Q427
N543	D428
K578	K450 *
D605	missing
T854	T600 *
R856	R602 *
V857	R603
T858	V604
H860	H606 *
Q862	H608
T863	T609 *
N951	N682 *

K968	K699 *

Supplementary Table 3. *T. kivui* HDCR variants used in this study.

Cys, cysteine; Ala, alanine.

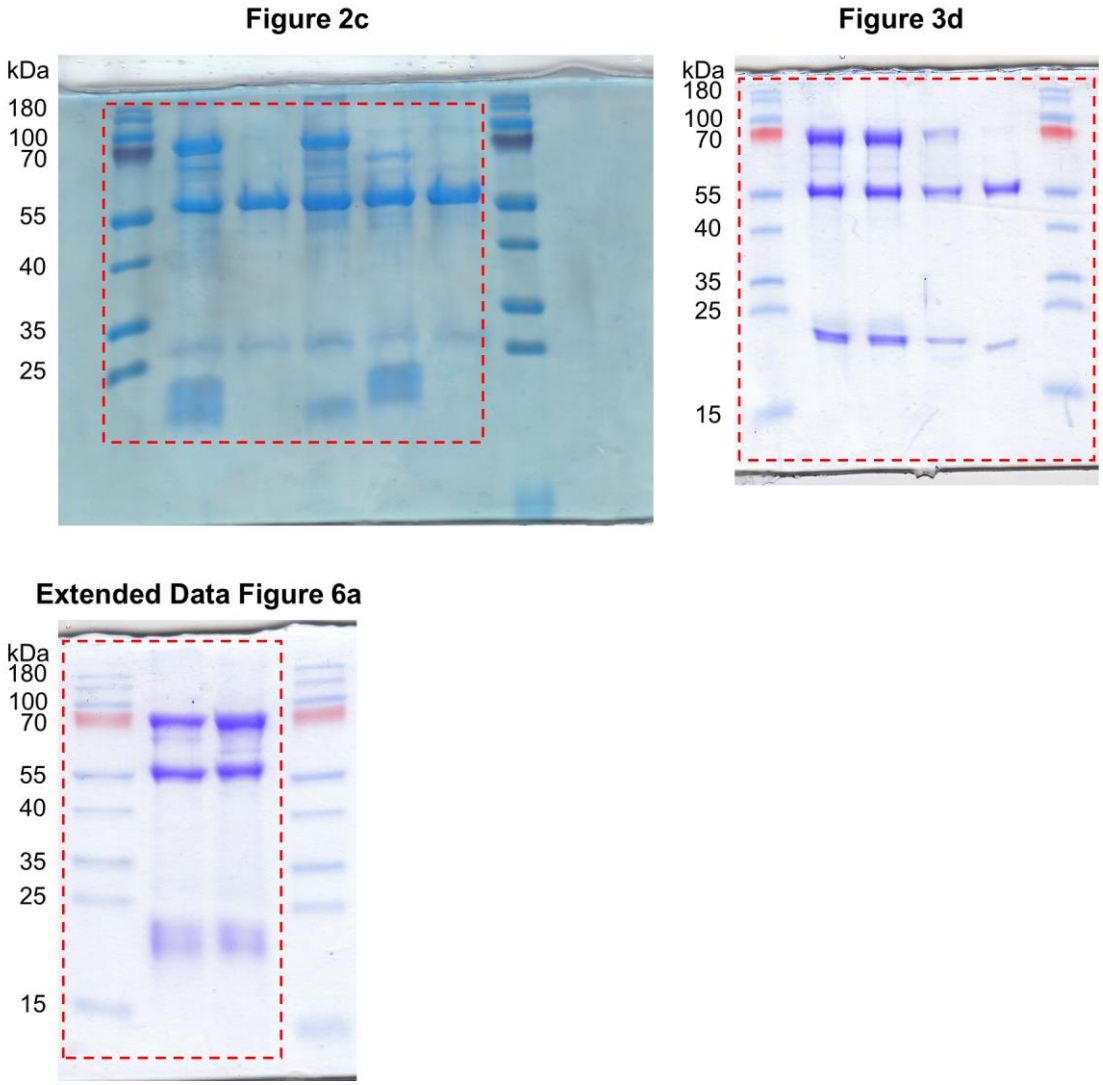
Strain	Genotype	His_6-tag location	Reference
Δ*hdcr*	*hdcr* operon deleted	none	([5])
HDCR_His	Δ*hdcr* strain + HDCR operon	*hydA2* C-term.	This study
HDCRΔHydA2	Δ*hdcr* strain + HDCR operon without *hydA2* gene	*fdhF* N-term.	This study
HDCRΔHycB4	Δ*hdcr* strain + HDCR operon without *hycB4* gene	*hydA2* C-term.	This study
HDCRΔHycB3	Δ*hdcr* strain + HDCR operon without *hycB3* gene	*hydA2* C-term.	This study
HDCRΔHycB3 ΔHycB4	Δ*hdcr* strain + HDCR operon without *hycB3* and *hycB4* genes	*hydA2* C-term.	This study
HDCRΔHycB4, His@Hyd & His@FdhF	Δ*hdcr* strain + HDCR operon without *hycB4* gene	*hydA2* C-term. and *fdhF* N-term.	This study
HDCR_HydA2 C387A	Δ*hdcr* strain + HDCR operon, *hydA2*_Cys387 substituted with Ala	*hydA2* C-term.	This study
HDCR_HycB4 Δ[4Fe4S] IV	Δ*hdcr* strain + HDCR operon, *hycB4*_Cys114, Cys117, Cys120 substituted with Ala	*hydA2* C-term.	This study
HDCR_HycB3ΔC	Δ*hdcr* strain + HDCR operon, deletion of *hycB3* amino acids 160-184	*hydA2* C-term.	This study
HDCR_HycB4ΔC	Δ*hdcr* strain + HDCR operon, deletion of *hycB4* amino acids 190-210	*hydA2* C-term.	This study

Supplementary Table 4. Plasmids used in this study. The plasmids used in this study were either used for PCR (20 ng per 25 µl reaction) or for transformation of *T. kivui* (1 mg). C, cysteine; A, alanine; aa, amino acids.

Plasmid	Purpose	Reference
pMU131	Shuttle vector	([6])
pPB5	Cloning vector for HDCR operon with S-layer promoter	([7])
pHD001	Overproduction of His_6-tagged HDCR	This study
pHD015	Overproduction of His_6-tagged HDCRΔHydA2	This study
pHD020	Overproduction of His_6-tagged HDCRΔHycB3	This study
pHD024	Overproduction of His_6-tagged HDCRΔHycB4, His_6-tag at FdhF and His_6-tag at HydA2	This study
pHD026	Overproduction of His_6-tagged HDCR, HydA2 C387A	This study
pHD028	Overproduction of His_6-tagged HDCR, HycB4 C114A, C117A, C120A	This study
pLR002	Plasmid containing HDCR without HydA2, His_6 at *hycB4*	This study
pLR002c	Plasmid containing HDCR without HydA2, His_6 at *hycB4* and *fdhF*	This study
pLR003b	Overproduction of His_6-tagged HDCRΔHycB4	This study
pLR004	Overproduction of His_6-tagged HDCRΔHycB3ΔHycB4	This study
pRT8	Overproduction of His_6-tagged HDCR, HycB3Δaa160-184	This study
pRT9	Overproduction of His_6-tagged HDCR, HycB4Δaa190-210	This study

Supplementary Table 5. Primers used in this study.

Primer	Sequence (5´-3´)
P1	GTGTAATTTTTTTATACAAATAATATTTCAATTCG
P2	CCTGTTTACCATCTTTCATACAGTCAATCCTCCTCC
P3	GAGGAGGATTGACTGTATGAAAGATGGTAAACAGGAAAAG
P4	GATTTTTAATGGTGATGGTGATGGTGTACTTTTTTTCTCGGTGTATATTTAG
P5	AGTACACCATCACCATCACCATTAAAAATCAAAAATTTTGTTGGTAGTG
P6	GGGTTTATCGACCTGCAGC
P11	ATCCTCTAGAGTCGACCTG
P12	GGTACCGAGCTCGAATTG
P39	TAAGAGTGGTAAGATAAAAATCAAAAATTTTGTTGGTAGTG
P40	ATTTTTGATTTTTATCTTACCACTCTTAAAAAACTC
P47	GGGAGGAAAATGAATGATTTTTGCTAATATTTCTATCTATTTGAG
P48	AATATTAGCAAAAATCATTCATTTTCCTCCCTTTTCCTTTGC
P55	TCCAGAAGGAGCAATAAGCGGTGGTG
P56	CAAGTCATCACTTCAACG
P57	TAAGAGTGCTTTATTGGCATGTCCATTTG
P58	GCGCCTATTGCCAACTTTTCATCTACTACG
PLR3	GTAAGACACCATCACCATCACCATTAAAAATCAAAAATTTTGTTGGTAG
PLR4	TGATTTTTAATGGTGATGGTGATGGTGTCTTACCACTCTTAAAAAACTC
PLR7	TCTATCTATTTGAGATTTTCATGTCTGCAAATAAAGCTA
PLR8	TTATTTGCAGACATGAAAATCTCAAATAGATAGAAATATTAGC
PLR11b	GTATGCACCATCACCATCACCATAAAGATGGTAAACAGGAAAAGGTT
PLR12b	CTTTATGGTGATGGTGATGGTGCATACAGTCAATCCTCCTCCTTGTA
PLR15	AAAGGGAGGAAAATGATTTATTAATTAATAATATTGAAAAGAAAGGGATGTG
PLR16	ATATTATTAATTAATAAATCATTTTCCTCCCTTTTCCTTTG
PRT7	TAATGATTTTTGCTAATATTTCTATCTATTTGAG
PRT8	ACCATCAACCAATTTCAAAGCC
PRT9	TAATTTATTAATTAATAATATTGAAAAGAAAGGGATG
PRT10	CATAACCAAAGTCAACGCATTTTC
Pseq5	GAATCTTCAAATTCAGGCAATAAGC
Pseq6	TAGCCGACCCAAGAATGAAC
Pseq7	TTCAGCCAAATAGCCGAGAG
Pseq8	AGTAGCGGCTGCTAAAGTTG
Pseq9	AGGTGCGACGGTTGGTACTG
Pseq10	GCTCCGGCTATTAGAGTTTC
Pseq11	ATGTATAAGCGGTGGTGGAC
Pseq12	GCGTTATGCCTACCTATATCTTC
Pseq13	TTCCGGAGAAGGCGCTACA



Supplementary Figure 1. Uncropped gel images.

Supplementary Video 1. Overall presentation of the segmented HDCR cryo-EM density and corresponding atomic model.

Colors are used as in Fig. 1.

Supplementary Video 2. *In situ* visualization of bundled HDCR filaments inside a *T. kivui* cell.

Sequential slices back and forth through the cryo-ET volume, followed by reveal and tour of the cellular segmentation. Segmentation colored as described in Fig. 5.

Supplementary References:

1 Hess, V., Poehlein, A., Weghoff, M. C., Daniel, R. & Müller, V. A genome-guided analysis of energy conservation in the thermophilic, cytochrome-free acetogenic bacterium *Thermoanaerobacter kivui*. *BMC Genomics* **15**, 1139 (2014).

2 Schuchmann, K. & Müller, V. Direct and reversible hydrogenation of CO_2 to formate by a bacterial carbon dioxide reductase. *Science* **342**, 1382-1385 (2013).

3 Schwarz, F. M., Schuchmann, K. & Müller, V. Hydrogenation of CO_2 at ambient pressure catalyzed by a highly active thermostable biocatalyst. *Biotechnol. Biofuels* **11**, 237 (2018).

4 Schuchmann, K., Vonck, J. & Müller, V. A bacterial hydrogen-dependent CO_2 reductase forms filamentous structures. *FEBS J.* **283**, 1311-1322 (2016).

5 Jain, S., Dietrich, H. M., Müller, V. & Basen, M. Formate is Required for Growth of the Thermophilic Acetogenic Bacterium *Thermoanaerobacter kivui* Lacking Hydrogen-Dependent Carbon Dioxide Reductase (HDCR). *Front. Microbiol.* **11**, 59 (2020).

6 Shaw, A. J., Hogsett, D. A. & Lynd, L. R. Natural Competence in *Thermoanaerobacter* and *Thermoanaerobacterium* Species. *Appl. Environ. Microbiol.* **76**, 4713-4719 (2010).

7 Benner, P. *Proteinproduktion im thermophilen, acetogenen Bakterium Thermoanaerobacter kivui.* Bachelor thesis, Goethe University Frankfurt am Main, (2016).