SUPPORTING INFORMATION

Chemical assembly of multiple metal cofactors: the heterologously expressed multidomain [FeFe]-hydrogenase from *Megasphaeraelsdenii*.

*Giorgio Caserta^a, Agnieszka Adamska-Venkatesh^b, Ludovic Pecqueur^a, Mohamed Atta^c, Vincent Artero^c, Roy Souvik^c, Edward Reijerse^b, Wolfgang Lubitz^b and Marc Fontecave^a**

^a Laboratoire de Chimie des Processus Biologiques, Collège de France,
Université Pierre et Marie Curie, CNRS UMR 8229, 11 place Marcelin Berthelot,
75005 Paris, France

^b Max-Planck-Institut fürChemischeEnergiekonversion, Stiftstrasse 34-36,
45470 Mülheim an der Ruhr, Germany.

^c Laboratoire de Chimie et Biologie des Métaux, Université Grenoble Alpes, CEA/BIG, CNRS, 17 rue des martyrs, 38000 Grenoble, France

Figure List

Figure S1: Aerobic protein purification.

- Figure S2: *in vitro* [4Fe-4S] clusters reconstitution of apo-MehydA.
- Figure S3: anaerobic purification of FeS-MeHydA and SLS analysis.
- Figure S4: progressive reduction of maturated MeHydA(adt) observed by FTIR.

Figure S5: sequence alignment of MeHydA with CpHydA.

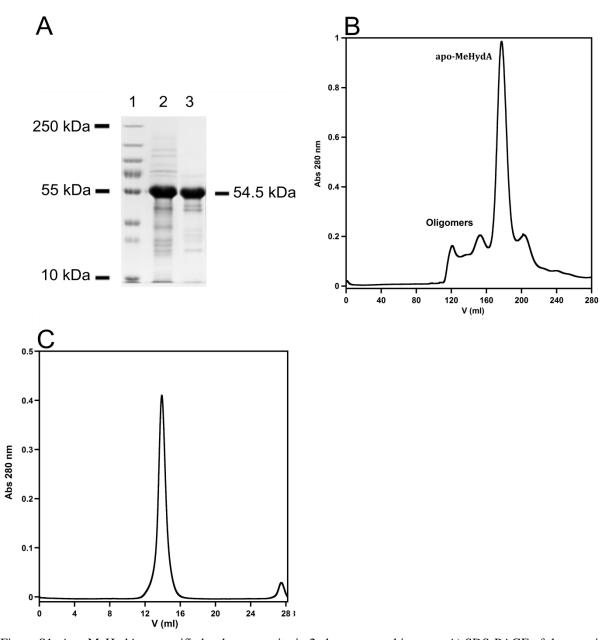


Figure S1: Apo-MeHydAwas purified to homogeneity in 2 chromatographic steps. A) SDS-PAGE of the protein eluted with an imidazole gradient using a His trap column (lane 2). Protein obtained after the size exclusion chromatography step (lane 3). MW Protein Standard (lane 1). B) Size exclusion chromatography elution profile of apo-MeHydA obtained after the His trap purification step.C) Size exclusion chromatography elution profile of apo-MeHydA used for chemical reconstitution of the [4Fe-4S] clusters. The elution volume of the apo-MeHydA is 13.7 mL.

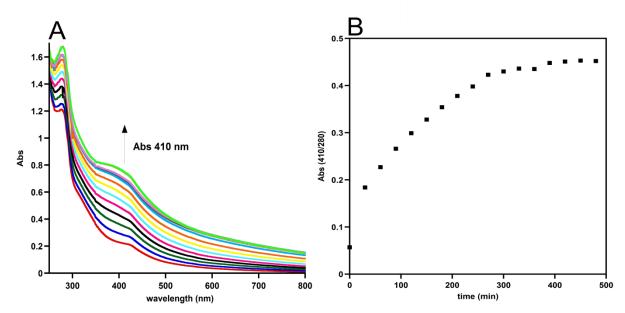


Figure S2: *In vitro* reconstitution of the [Fe-S] clusters in MeHydA. A) UV-visible absorption spectra of 100 μ M MeHydA in 50 mMTris pH 8, 300 mMNaCl, 10% v/v glycerol, 5 mM DTT using a 1 mm pathlength cuvette recorded every 30 minutes.B) Plot of A_{410/280} absorption ratioas a function of time. The [4Fe-4S] cluster reconstitution is completed after 300 minutes.

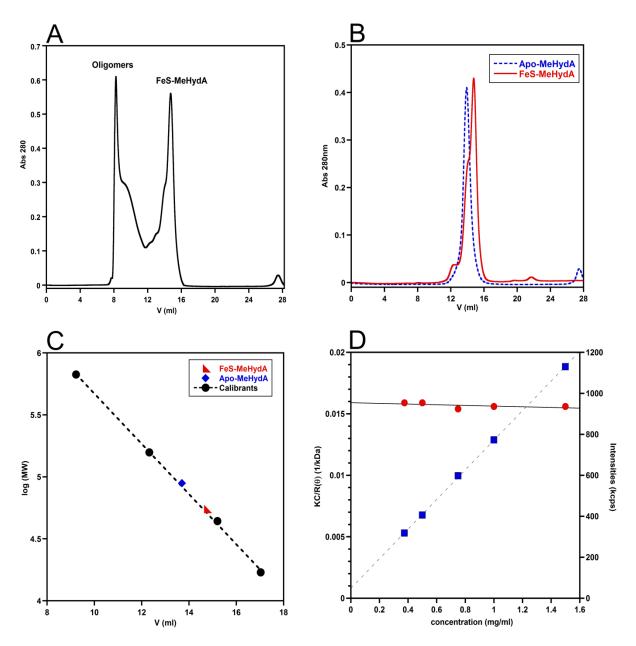


Figure S3: Purification of reconstituted FeS-MeHydA. A) SEC elution profile on a S200 10/300 GL of MeHydA after overnight [4Fe-4S] clusters reconstitution. The peaks at low elution volumes (8-10 mL) correspond to oligomers. B) S200 elution profile of apo-MeHydA (dashed blue) and FeS-MeHydA (red). C) Superdex S200 10/300 GL calibration curve using Thyroglobulin (670 kDa, 9.22 mL), γ -globulin (158 kDa, 12.32 mL), Ovalbumin (44 kDa, 15.2 mL) and Myoglobin (17 kDa, 17.03 mL). The molecular weights derived for apo-MeHydA and FeS-MeHydAare 90 kDa and 54.5 kDa respectively, suggesting apo-MeHydA might be a dimer. D) Debye plot of ofapo-MeHydA at different concentrations in 50 mMTris pH 8, 300 mMNaCl (red filled circles). The intensity scattered for each concentration of apo-MeHydA (blue squares) is shown as a control for sample quality. The estimated molecular weight extracted from the linear fit with the Zimm equation of the Debye plot is 62.7 \pm 0.8 kDa. Therefore apo-MeHydA is a monomer and becomes more compact upon [4Fe-4S] clusters incorporation.

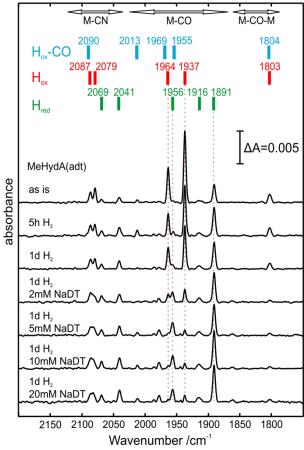


Figure S4. Progressive reduction of maturated MeHydA(adt) observed by FTIR. 0.5 mMholo-MeHydA in 50 mM TRIS pH 8.0, 300 mMNaCl were flushed with H_2 for 24 h, followed by adding different amounts of sodium dithionite to obtain the H_{red} state.

Sequence alignment

<i>СрНуд</i> А Менуда Срнуда	1	→TT MKTIIINGVQF i 5 10			TT T NNCNNDINKCEI 40 45	$\begin{array}{c} & & & & & & & \\ \mathbf{r} & & & & & & \\ \mathbf{r} & & \\ $	T TT CDTLIEDGMIINTN 65 70 75	
<i>СрНуд</i> А МеНудА СрНудА	1 89	QQ MPEFHS. QLLDIH <mark>EF</mark> KCG 90 95 10	nl 222 20 PCNRRENCEFLKL 0 105 110	R 🔽	EKIDRRVPIDEH! LPKDKTEYVDER:	N C A V Q F <mark>D</mark> V <mark>T K C</mark> K N	CTLCRRACADTQTV GRCVNACGKNTET 0 155 160 1	LDYYS <mark>I</mark> SST <mark>C</mark>
<i>СрНуд</i> А МеНудА СрНудА	58 177		TNCLLCGQCIIAC	P F G A I V E V N D V D P V A A L S E K S H M D	K V K A A L K D P E K I R V K N A L N A P E K H	VIFQTAPAVRVGL VIVAM <mark>AP</mark> SVRASI	GEAFGMDPGTFVEG GELFNMGFGVDVTG	a7 00000000 - KMVAALRTLGADY KIYTALEQLGFDK 255 260
<i>СрНуд</i> А МеНудА СрНудА	137 265 2	VFDTDFGADLT	IMEEATELVORIE	SEEIPIPOFTSC	C P G W V R Q A E N Y Y I	DLLOHLSSTKSP	al0 al1 00000000 0000 ISILSPVIKTYFAQ QQIFGTASKTYYPS 330 335 34	QKNI <mark>DPK</mark> KIVNVC ISGL <mark>DPK</mark> NVFTVT
<i>СрНуд</i> А МеНудА СрНудА	225 352	V T P C T A K K A E I VM P C T S K K F E A	RRPELSASCLFWD	EPEIRDTDICIT	TRELAQWIQDEN TRELAKMIKDAK	I D F A S L E D S K F D K I P F A K L E D S E A D P	η9 α14 η10 00 000000 AFGEASCGCRIFCN AMGEYSCACAIFCA 15 420 425	SGGVMEAAIRTAY
<i>СрНуд</i> А МеНудА СрНудА	313 433	0000 HMFTGRPÅPKD DFAENAELE 445 450	F I P F E P V R G L Q G V D I E Y K Q V R G L N G I	KKATVIFGHFVL	H V A A I S G L G N A R I N V A V I N G A S N L F I	A 🔽 I D D L I K N D A F E	→ TT 2 DYSFIEVMACPGGC So5 510 515	I G <mark>G G G Q P K V</mark> K L P Q V N <mark>G G G Q P</mark> H <mark>V</mark> N P K D
<i>СрНуd</i> А МеНуdА СрНуdА	517	VK <mark>KV</mark> QEA <mark>R</mark> T	SVLYNQDEHLSKR		E A F L D E P L S E M A Q N Y F G K P G E G R A	2	QLGRMKNLTPQTNP 0 595 600 6	MSPKYKPPTEE 05 610

Figure S5: Sequence alignment from clustalO of *Megasphaeraelsdenii*and Clostridium *pasteurianum*HydA. Conserved amino acids are boxed in red. Cysteines boxed in green and magenta are ligands of one FeS cluster respectively. Cysteines boxed in yellow coordinate the FeS cluster of the H-domain with the conserved cysteine C501 of CpHydA (corresponding to C387 of MeHydA) involved in the diiron coordination. **The cysteine boxed in cyan is assumed to be involved in proton transfer**. The secondary structure elements of CpHydA derived from the crystallographic structure (pdb 3c8y) are displayed. The figure was generated with ESPript3[1].

References

[1] X. Robert, P. Gouet, Deciphering key features in protein structures with the new ENDscript server, Nucleic Acids Res., 42 (2014) W320-324.