

SUPPORTING INFORMATION

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

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Figure S3: anaerobic purification of FeS-MehydA and SLS analysis.

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Figure S5: sequence alignment of MeHydA with CpHydA.

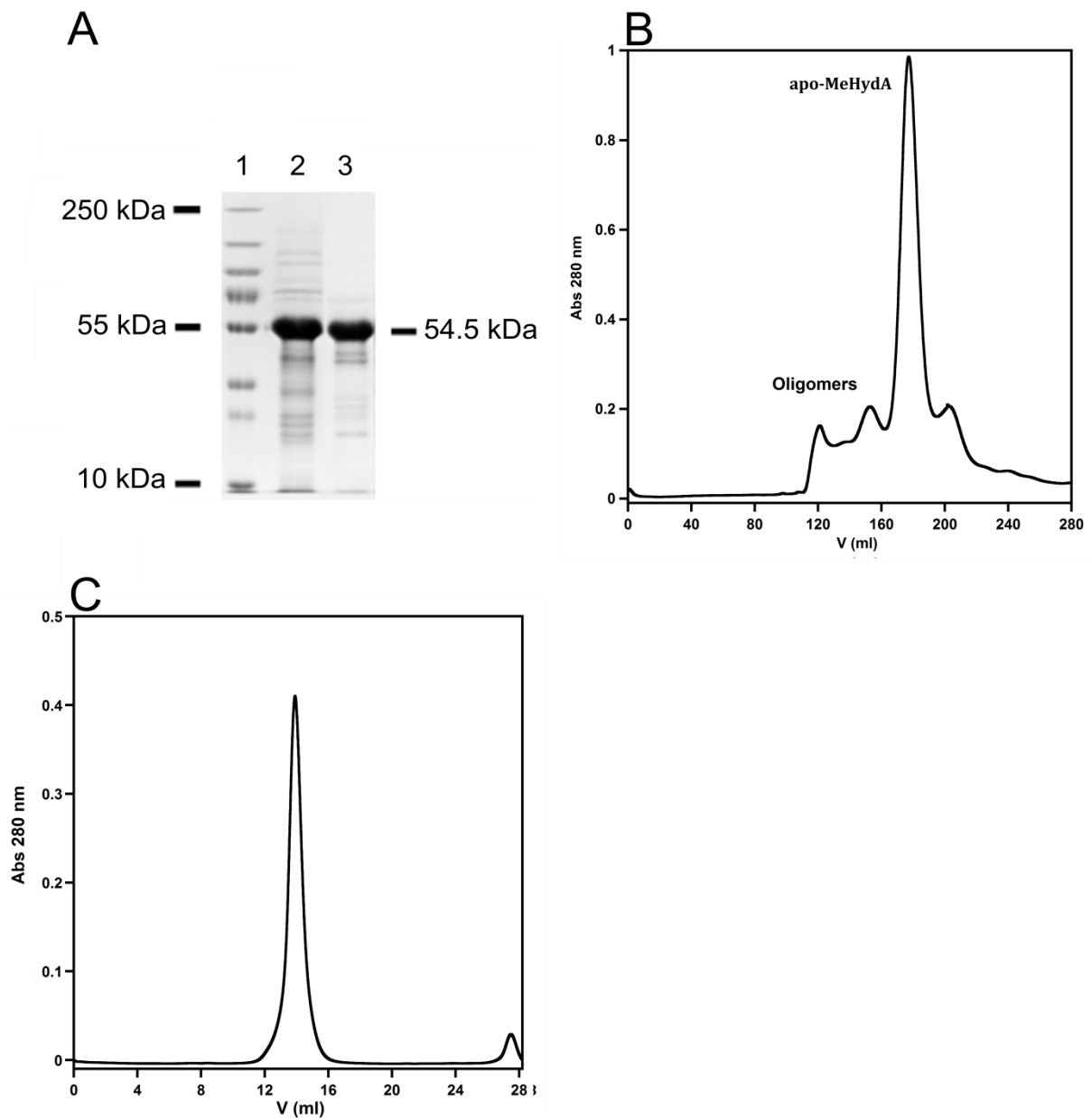


Figure S1: Apo-MeHydA was purified to homogeneity in 2 chromatographic steps. A) SDS-PAGE of the protein eluted with an imidazole gradient using a His trap column (lane 2). Protein obtained after the size exclusion chromatography step (lane 3). MW Protein Standard (lane 1). B) Size exclusion chromatography 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.

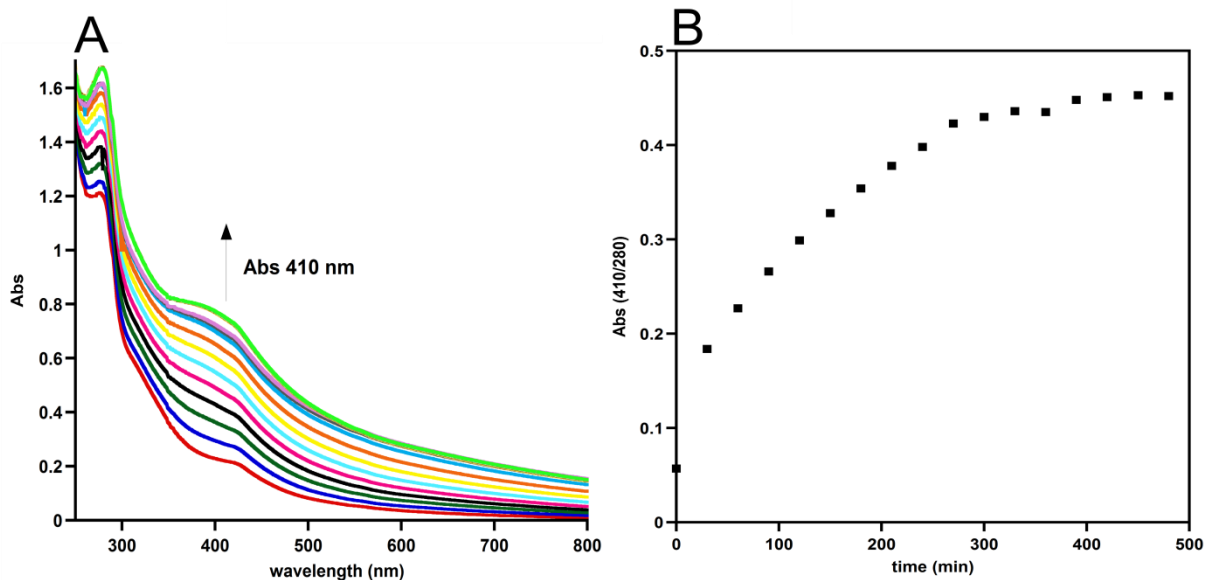


Figure S2: *In vitro* reconstitution of the [Fe-S] clusters in MeHydA. A) UV-visible absorption spectra of 100 μ M MeHydA in 50 mM Tris pH 8, 300 mM NaCl, 10% v/v glycerol, 5 mM DTT using a 1 mm pathlength cuvette recorded every 30 minutes. B) Plot of $A_{410/280}$ absorption ratios as a function of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

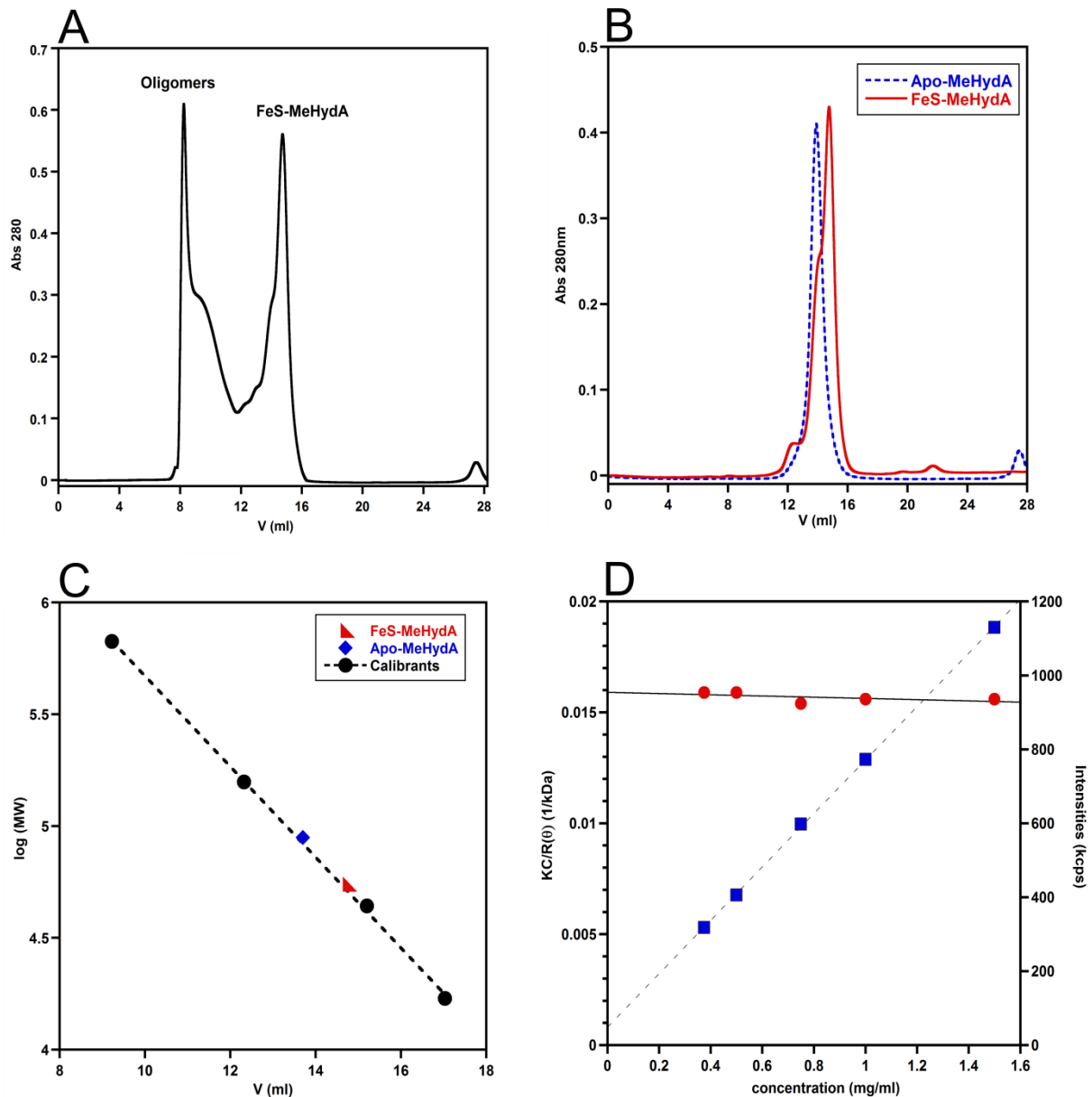


Figure S3: Purification of reconstituted FeS-MeHydA. A) SEC elution profile on a S200 10/300 GL of MeHydA after overnight [4Fe-4S] clusters reconstitution. The peaks at low elution volumes (8-10 mL) correspond to oligomers. B) S200 elution profile of apo-MeHydA (dashed blue) and FeS-MeHydA (red). C) 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-MeHydA are 90 kDa and 54.5 kDa respectively, suggesting apo-MeHydA might be a dimer. D) Debye plot of apo-MeHydA at different concentrations in 50 mM Tris pH 8, 300 mM NaCl (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 ± 0.8 kDa. Therefore apo-MeHydA is a monomer and becomes more compact upon [4Fe-4S] clusters incorporation.

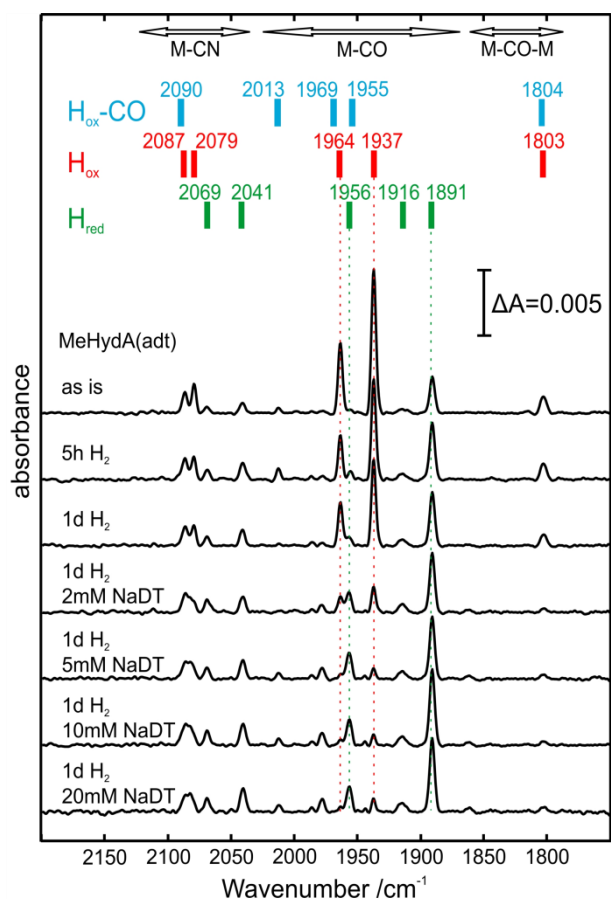


Figure S4. Progressive reduction of matured MeHydA(adt) observed by FTIR. 0.5 mMholo-MeHydA in 50 mM TRIS pH 8.0, 300 mMNaCl were flushed with H₂ for 24 h, followed by adding different amounts of sodium dithionite to obtain the H_{red} state.

Sequence alignment

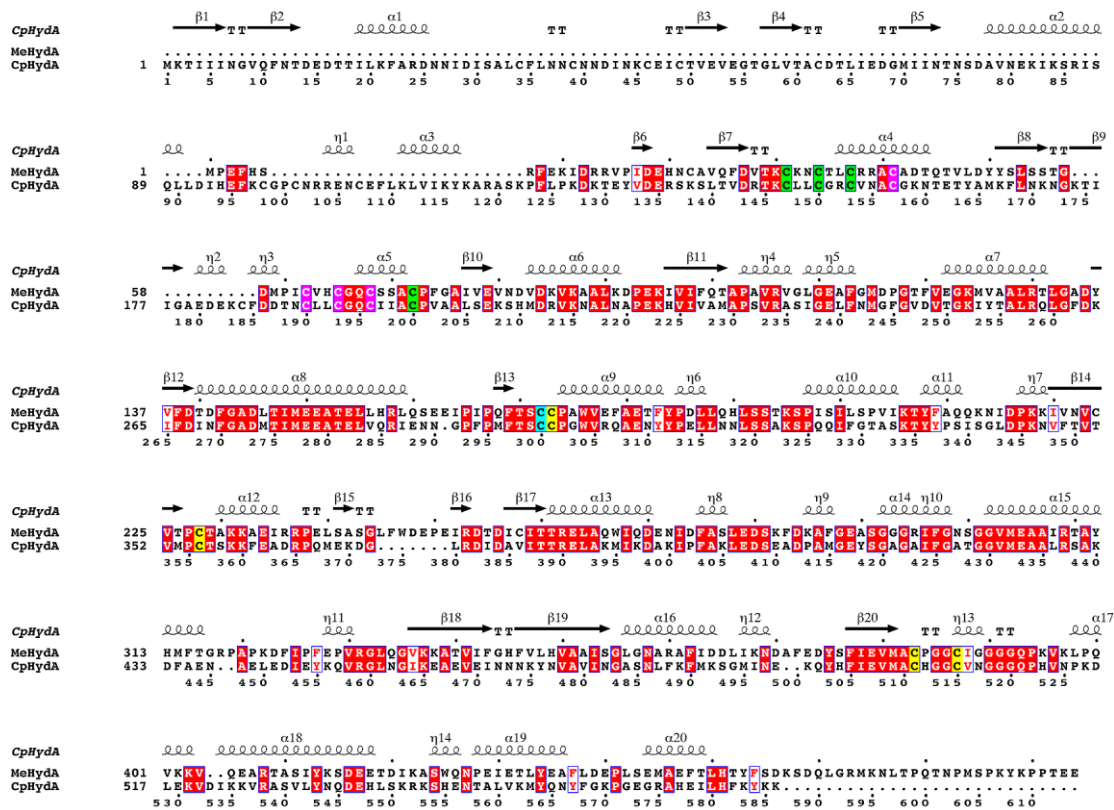


Figure S5: Sequence alignment from clustalO of *Megasphaera elsdenii* and *Clostridium pasteurianum* HydA. Conserved amino acids are boxed in red. Cysteines boxed in green and magenta are ligands of one FeS cluster respectively. Cysteines boxed in yellow coordinate the FeS cluster of the H-domain with 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.