

SUPPLEMENTARY MATERIAL

SPECTROSCOPIC INSIGHTS INTO THE OXYGEN-TOLERANT MEMBRANE-ASSOCIATED [NiFe]-HYDROGENASE OF *RALSTONIA* *EUTROPHA* H16

Miguel Saggu¹, Ingo Zebger^{1†}, Marcus Ludwig², Oliver Lenz², Bärbel Friedrich², Peter Hildebrandt¹
and Friedhelm Lenzian^{1†}

From the ¹Technische Universität Berlin, Institut für Chemie, PC14, Straße des 17. Juni 135, D-10623 Berlin, Germany, and the ²Institute of Biology, Department of Microbiology, Humboldt-Universität zu Berlin, Berlin, Germany.

Running title: Spectroscopy of membrane associated *Re* H16 MBH

†To whom correspondence should be addressed: Tel.: +49-30-314-22489; Fax: +49-30-314-21122;

Email: f.lenzian@tu-berlin.de, ingo.zebger@tu-berlin.de

TABLE S1

Spin-quantification of the signal intensity of the [3Fe4S] and Ni_r-B center in *Re* H16 dimer in different preparations, obtained by double integration of the spectra.

| Preparation | [3Fe4S] ⁺ ^{a)} | Ni _r -B ^{b)} |
|---------------------------------------|------------------------------------|----------------------------------|
| H16 WT mainly narrow signal | 0.8 | 0.13 |
| H16 WT mixed narrow / split signal | 1.82 | 0.26 |
| | 1.47 | 0.35 |
| | 1.21 | 0.32 |

^{a)} The quantification refers to the complete [3Fe4S]⁺-signal (“narrow” plus “split” signal)

^{b)} The amount of Ni_r-B is within the experimental error in agreement with the FTIR data

TABLE S2

EPR-detected paramagnetic centers in *Re* H16 (this work) compared with those from other [NiFe] hydrogenases and known or postulated magnetic interactions between these centers

| Organism | <i>Re</i> H16 (this work) | <i>Rm</i> CH34 (39) | <i>Re</i> H16 old work (38) | <i>A. aeolicus</i> (62) | <i>A. vinosum</i> (7;63;64) | <i>Dv</i> Miyazaki F (18) |
|---------------------------|---|---|--|--|---|---|
| [FeS] clusters | split [3Fe4S] ⁺ | split [3Fe4S] ⁺ | split [3Fe4S] ⁺ | split [3Fe4S] ⁺ | split [3Fe4S] ⁺ | unsplit [3Fe4S] ⁺ |
| | coupled [4Fe4S] ¹⁺ | coupled [4Fe4S] ¹⁺ | coupled [4Fe4S] ¹⁺ | coupled [4Fe4S] ¹⁺ | coupled [4Fe4S] ¹⁺ | coupled [4Fe4S] ¹⁺ |
| paramagnetic Ni states | Ni _r -B, Ni _a -C, Ni _a -L | Ni _r -B, Ni _a -C, Ni _a -L | Ni(III) (Ni _r -B) | Ni _r -B, Ni _a -C | Ni _u -A, Ni _r -B, Ni _a -C, Ni _a -L | Ni _u -A, Ni _r -B, Ni _a -C, Ni _a -L |
| magnetic interactions | [3Fe4S] ⁺ + X ^{ox} ^{a)} | Ni _r -B + [3Fe4S] ⁺ | - | - | [3Fe4S] ⁺ + X ^{ox} ^{b)} | - |
| | Very weak X ^{ox} ^{a)} + Ni _r -B | Ni _a -C/Ni _a -L + [3Fe4S] ⁰ | - | Ni _a -C + [4Fe4S] ¹⁺ | Ni _a -C + [4Fe4S] ¹⁺ | Ni _a -C + [4Fe4S] ¹⁺ |
| | [3Fe4S] ⁰ + [4Fe4S] ¹⁺ | [4Fe4S] ¹⁺ + [4Fe4S] ¹⁺ | [4Fe4S] ¹⁺ + [4Fe4S] ¹⁺ | [4Fe4S] ¹⁺ + [4Fe4S] ¹⁺ | [4Fe4S] ¹⁺ + [4Fe4S] ¹⁺ | [4Fe4S] ¹⁺ + [4Fe4S] ¹⁺ |
| | | | | | | |

^{a)} X^{ox} is postulated to be a proximal high potential [4Fe4S]³⁺ cluster or a yet unknown additional high potential paramagnetic species (e.g. Fe³⁺) in the proximity of the proximal cluster.

^{b)} Ni_r-B, or a proximal [4Fe4S]³⁺ cluster or an additional Fe³⁺ were discussed as origin of X^{ox}.

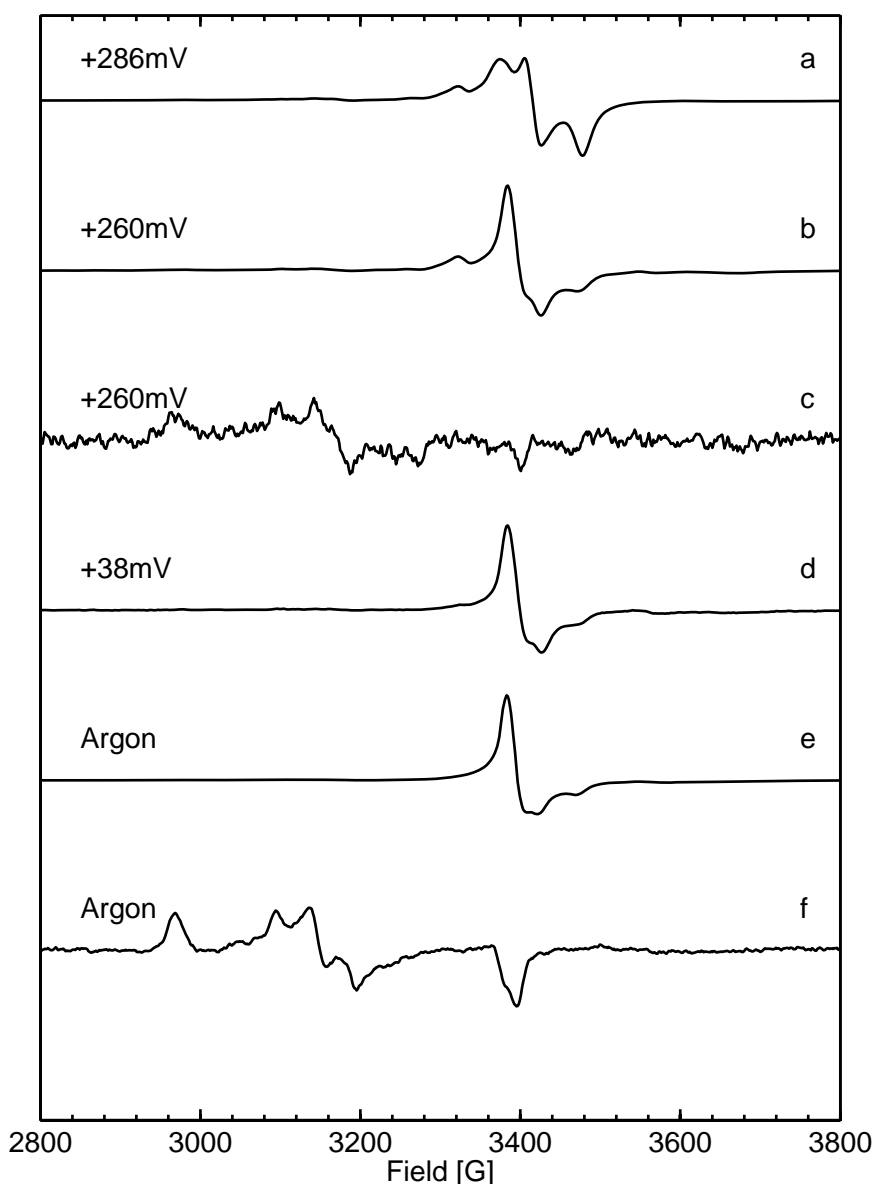


Figure S1: EPR spectra of solubilized dimeric MBH. Redox potentials had been adjusted in the samples, prior to freezing (see main paper).

(a) treated with DCIP, showing more “split” $[3\text{Fe}_4\text{S}]^+$ signal, $T = 20\text{ K}$; (b) as isolated, showing superimposed narrow and “split” $[3\text{Fe}_4\text{S}]^+$ signal, $T = 20\text{ K}$; (c) as isolated, $T = 60\text{ K}$, showing only $\text{Ni}_r\text{-B}$; (d) treated with mercapto-EtOH, showing reduced “split” $[3\text{Fe}_4\text{S}]^+$ signal intensities; $T = 20\text{ K}$; (e) treated with argon, $T = 20\text{ K}$; (f) treated with argon, $T = 80\text{ K}$, showing strong $\text{Ni}_r\text{-B}$ signals.

Signals of $\text{Ni}_r\text{-B}$ are only pronounced at higher temperatures (80 K) when the additional paramagnetic center, which gives rise to the “split” $[3\text{Fe}_4\text{S}]^+$ signal, is reduced (trace f).

Experimental conditions: 1 mW microwave power; microwave frequency 9.56 GHz; 1 mT modulation amplitude, 12.5 kHz modulation frequency.

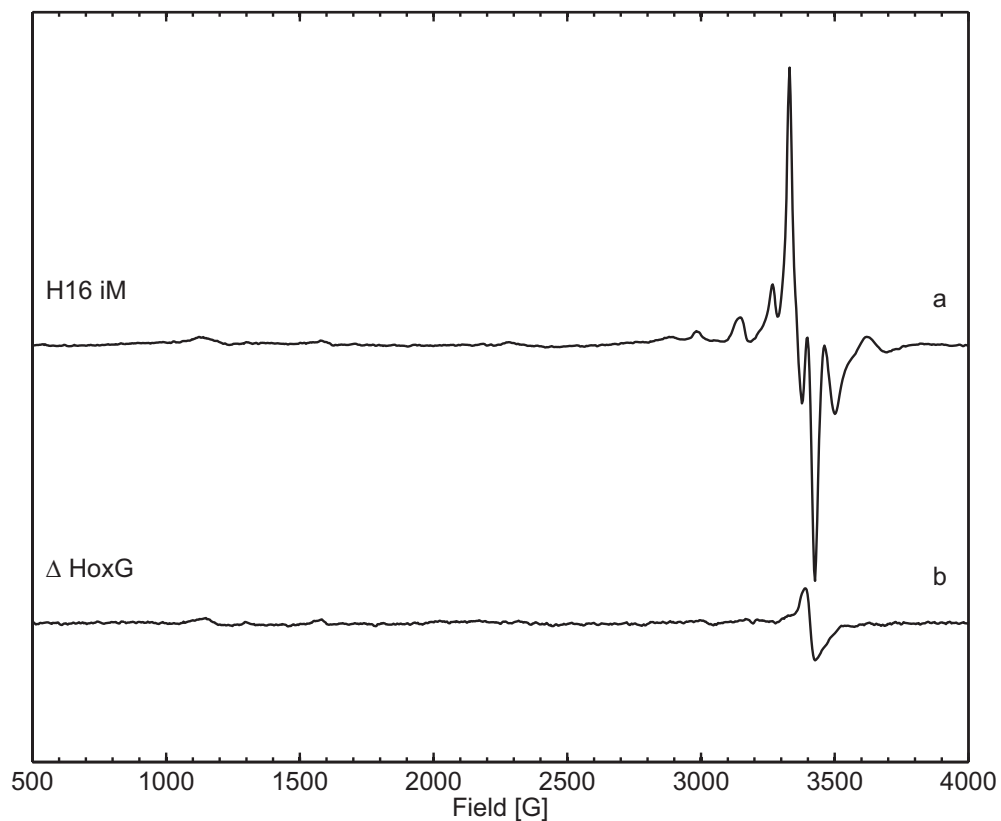


Figure S2: EPR spectra of inner membrane preparations from *Re* H16 at $T = 20$ K. The signal intensity is normalized to the $g = 6$ signal at 1100 Gauss, which might result from a heme-containing protein.

(a) as-isolated inner membrane containing the wild-type MBH trimer

(b) as-isolated membranes lacking MBH due to a genetic deletion of the gene *hoxG* for the MBH large subunit

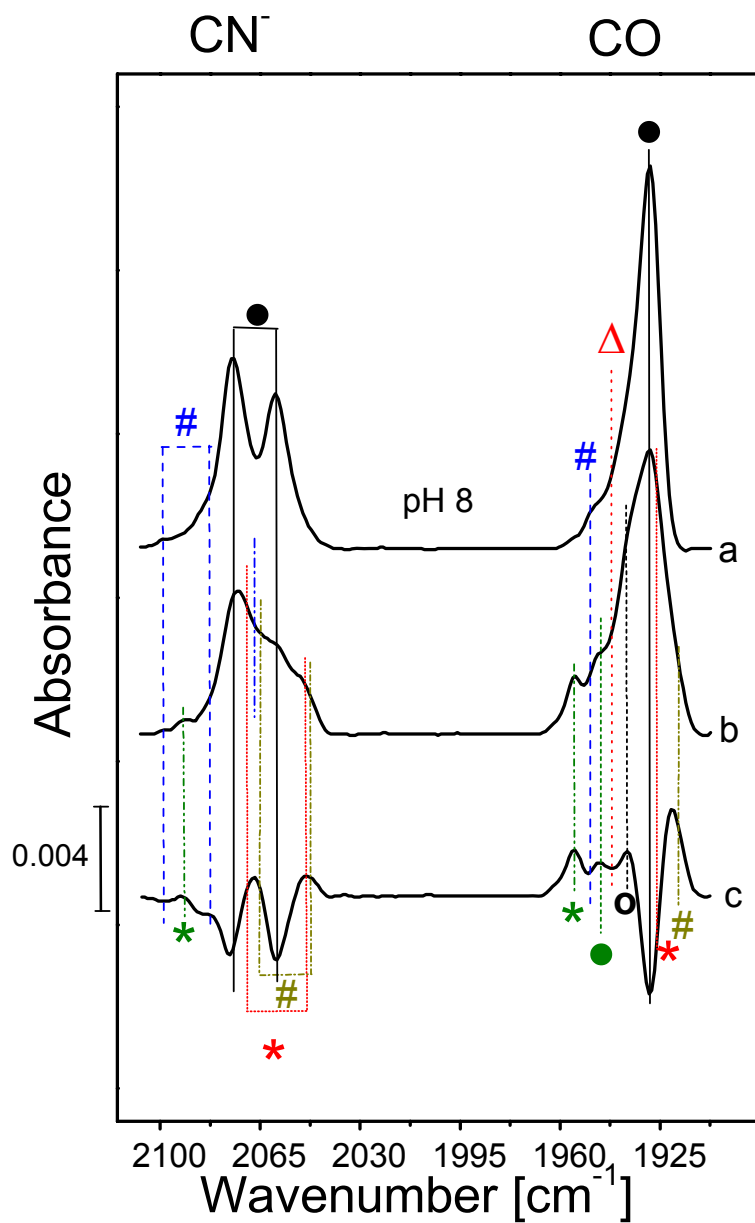


Figure S3 FTIR spectra of the *Re* MBH, oxidized (as isolated) enzyme (a), the H₂-reduced samples (1 bar H₂) (b), and, the corresponding difference spectra (H₂-reduced minus oxidized) (c) at pH 8. Definition of the symbols: Δ (red): Ni_{r/u}-S; # (blue): Ni_r-B; o: Ni_{r/a}-S; • (black): Ni_{ia}-S (only 20% active); * (green): Ni_a-C; • (green): Ni_a-SR; * (red): Ni_a-SR'; # (yellow): Ni_a-SR''

| | | |
|--------------|---|--|
| Ral_eut_HoxK | 1 | -----MVFETFYEVRRQGISRRSFLKYCSLTATSLGLGPSFLPQIAHAMET-KPRTPV |
| Alc_hyd_HoxK | 1 | -----MIETFYEVRRQGISRRSFLKYCSLTATSLGLSPVFPVKIVHAMET-KPRTPV |
| Ral_met_HoxK | 1 | -----METFYEIMRRKGISRRSFMKYCSLTATSLGLAPSFPVQIAHAMEN-KPRTPV |
| Rho_cap_HupS | 1 | -----MSDIETFYDVMRRQGITRRSFMKFCSLTAAALGLGPSFVPKIAEAMET-KPRTPV |
| Rhi_leg_HupS | 1 | -----MATAETFYDVIRRQGITRRSFTHKFCSLTAASLGFGPGAATAMAAEALET-KERVPV |
| Bra_jap_HupS | 1 | -----MGAATETFYSVIRRQGITRRSFHKFCSLTATSLGLPLAASRIANALET-KPRVPV |
| Thi_ros_HupS | 1 | -----MPTTETYYEVRRQGITRRSFLKFCSLTATALGLSPTFAGKIAHAMET-KPRIPV |
| Aqu_aeo_HyaA | 1 | -----METFWEVFKRHGVSRRDFLKFAITITGLMGLAPSMVPEVVRAMET-KPRVPV |
| Esc_col_HyaA | 1 | -----MNNEETFYQAMRRQGVTRRSFLKYCSLAATSLGLGAGMAPKIAWALEN-KPRIPV |
| Esc_col_HybO | 1 | -----MTGDNTLIHSHGINRRDFMKLCAALAATMGLSSKAAAEAESVTN-PQRPPV |
| All_vin_HynS | 1 | -----PSV |
| Thi_ros_HynS | 1 | -MAARNPTDKTLGESLRERGVSRRGFLKFCATASMMALPPSMAPAIAAALEQ-AKRPSV |
| Des_gig_HydA | 1 | -----MKFCTAVAVAMGMGPAFAPKVAEALTA-KKRPSV |
| Des_vul_HydA | 1 | MKISIGLGKEGVEERLAERGVSRDFLKFTAIAVTMGMGPAFAPEVARALMG-PRRPSV |
| Des_bac_HysS | 1 | -----MSLSRREFVKLCSAGVAGLGISQIYHPGIVHAMTEGAKKAPV |

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|--------------|----|---|
| Ral_eut_HoxK | 53 | LWLHGLECTCCSESFIRSAHPLAKDVVLSMISLDYDDTLMAAAGHQAEAILLEEIMTKYKG |
| Alc_hyd_HoxK | 53 | LWLHGLECTCCSESFIRSAHPLAKDVVLSMISLDYDDTLMAAAGHQAEAILGEVMTKYKG |
| Ral_met_HoxK | 52 | LWLHGLECTCCTEAFIRSAHPLAKDVVLSMISLDYDDTLMAAAGHQAEAILLEEIMTKYKG |
| Rho_cap_HupS | 55 | VVWHGLECTCCSESFIRSAHPLAKDVVLSMISLDYDDTLMAAAGHAAEAFAFEETIAKYKG |
| Rhi_leg_HupS | 55 | IWMHGLECTCCSESFIRSAHPLVKDVVLSMISLDYDDTIMAAAGHQAESILAETKEKYKG |
| Bra_jap_HupS | 56 | IWMHGLECTCCSESFIRSAHPLVKDAVLSMISLDYDDTIMAAAGHQAEAILLEETRAKHKG |
| Thi_ros_HupS | 55 | VWLHGLECTCCSESFIRSAHPLVSDVILSMISLDYDILIMAAAGHQAEAILLEEVHRKHAG |
| Aqu_aeo_HyaA | 52 | LWIHGLECTCCSESFIRSATPLASDVVLSMISLEYDDTLMAAAGEAVEKHRERIIKEYWG |
| Esc_col_HyaA | 55 | VWIHGLECTCCTESFIRSAHPLAKDVILSLISLDYDDTLMAAAGTQAEVFEEDIIITQYNG |
| Esc_col_HybO | 52 | IWIGAQECTGCTESLLRATHPTVENLVLETISLEYHEVLSAAFHQVEENKHNALKEYKG |
| All_vin_HynS | 4 | VWLSFQECTGCTESLTRAHPTLEDLILDFISLDYHHTLQAAASGEAAEAARLQAMDENRG |
| Thi_ros_HynS | 59 | IWLSFQECTGCTESLTRSHAPTLEDLILDVILSDYHHTLQAAAGDAAEHAREQAMAANPG |
| Des_gig_HydA | 34 | VYLHNAECTGCTESLLRTVDPYVDELILDVISM DYHETLMAGAGHAVEEALHEAIKG--- |
| Des_vul_HydA | 60 | VYLHNAECTGCTESVLRAFEPIYIDTLILDVILSDYHETIMAAAGDAAEAALQAVNSPH- |
| Des_bac_HysS | 43 | IWVQGGCTGCTSVSLLNAVHPRIKEILLDVISLEFHPTVMASEGEMALAHMYEIAEKFNK |

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| Ral_eut_HoxK | 113 | NYILAVEGNPP-LNQDGMSCIIIGG-----RPFIEQLKYVAKDAKAIISWGS ^C ASW |
| Alc_hyd_HoxK | 113 | NYILAVEGNPP-LNQDGMSCIIIGG-----KPFIDQLRHVAKDAKAIISWGS ^C ASW |
| Ral_met_HoxK | 112 | QYILAVEGNPP-LNEDGMFCIQSG-----KPFIDKLKHVAKDAKAIIAWGS ^C ASW |
| Rho_cap_HupS | 115 | NYILAVEGNPP-LNEDGMFCITGG-----KPFVEKLRHAAEGAKAIISWGA ^C ASY |
| Rhi_leg_HupS | 115 | KYILAVEGNPP-LNEGGMFCIDGG-----KPFVEKLRHAAEGAKAIIAWGA ^C ASW |
| Bra_jap_HupS | 116 | QYILAVEGNPP-LNEGGMFCIDGG-----KPFVEKLRHAAEGAKAIIAWGA ^C ASW |
| Thi_ros_HupS | 115 | NYILAVEGNPP-LNQDGMSCIIIGG-----RPFLEQLLEMADSCKAVISWGS ^C ASW |
| Aqu_aeo_HyaA | 112 | NYILAVEGNPP-LGEDGMYCIIIGG-----RPFVEILKESAEGAKAVIAWGS ^C ASW |
| Esc_col_HyaA | 115 | KYILAVEGNPP-LGEQGMFCISSG-----RPFIEKLRHAAEGAKAIIAWGS ^C ASW |
| Esc_col_HybO | 112 | QYVLVVDGSIPLKDNGIYCMVAG-----EPIVDHIRKAAEGAAAIIAIGS ^C ASW |
| All_vin_HynS | 64 | QYLVIVDGSIPGPDNPGFSTVAG-----HSNYSILMETVEHAAAVIIVGT ^C AAF |
| Thi_ros_HynS | 119 | EYLVIVDGSIPGPDNPGYSTVAG-----HSNYAMLVETVNAAVIIVGT ^C ATF |
| Des_gig_HydA | 91 | DFVCVIEGGIP-MGDGGYWGKVGR-----RNMYDICAIVAPKAKAVIAIGT ^C ATY |
| Des_vul_HydA | 119 | GFIIVVEGGIP-TAANGIYKQVAN-----HTMLDICSRLPKAQAVIAYGT ^C ATF |
| Des_bac_HysS | 103 | NFFLLVEGAIP-TAKEGRYCVGETLDAKGGHHEVTMMELIRDLAPKSLATVAVGT ^C SAY |

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| Ral_eut_HoxK | 162 | GCVQAAKNPTQATPVHKVIT----DKPIIKVPG ^C PPPIAEVMTG-----VITYMLTFDR |
| Alc_hyd_HoxK | 162 | GCVQAAKANPTQATPIHKVIT----DKPIIKVPG ^C PPPIAEVMTG-----VITYMLTFDR |
| Ral_met_HoxK | 161 | GCVQAAKNPTQATPIHKVIT----DKPIIKVPG ^C PPPIPEVMTG-----VITYMLTFDR |
| Rho_cap_HupS | 164 | GCVQAAAPNPTQATPVHKVIT----DKPIIKVPG ^C PPPIAEVMTG-----VITYMLTFDR |
| Rhi_leg_HupS | 164 | GCVQAAKNPTQATPIDKVIL----DKPIIKVPG ^C PPPIAEVMTG-----VVTFITTFGK |
| Bra_jap_HupS | 165 | GCVQAAKNPTQATPIDKVIT----NKPIIKVPG ^C PPPIAEVMTG-----VVTFITTFGK |
| Thi_ros_HupS | 164 | GCVQAARPNPTQATPVHEVIR----DKPVIKVPV ^C PPPIAEVMTG-----VLTYYILTFDR |
| Aqu_aeo_HyaA | 161 | GCVQAAKNPTTAVPIDKVIK----DKPIIKVPG ^C PPPIAEVMTG-----VIMYMLVDFDR |
| Esc_col_HyaA | 164 | GCVQAARPNPTQATPIDKVIT----DKPIIKVPG ^C PPPIPDVMSA-----IITYMVTFDR |
| Esc_col_HybO | 161 | GGVAAAGVNPTGAVSLQEVLP----GKTVINIPG ^C PPPNHNFLA-----TVAHIITYGK |
| All_vin_HynS | 114 | GGLPQARPNTGAMSVMDLVR----DKPVINVPV ^C PPPIPMVITG-----VIAHYLTFGR |
| Thi_ros_HynS | 169 | GGLPGANPNPTGAMSVMDLVK----DKPVINVSG ^C PPPIPMVITG-----VIAHYLTFGR |
| Des_gig_HydA | 140 | GGVQAAKNPTGTVGVNEALGKL--GVKAINIAG ^C PPPNPMNVFG-----TVVHLLT-KG |

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|--------------|-----|--|----------|----------------------------|-------------------------------------|--------------------------------------|---|------------------|
| Des_vul_HydA | 168 | GGVQAAKPNPTGAKGVNDALKHL--GVKAINIAG | C | PPNPYNLVG-----TIVYYLKNKA | | | | |
| Des_bac_HysS | 162 | GGIPAAEGNVTGSKSVRDFFADEKIEKLLVNVPG | C | PPHPDWMVGTLVAAWSHVLPNTEHP | | | | |
| | | | D | D | | | | |
| Ral_eut_HoxK | 212 | IPELDRQGRPKMFYSQRI | HDK | CYRRPHFDAGQFVEEWDDESARKGF | C | LYKMG | C | KGPTTYN |
| Alc_hyd_HoxK | 212 | FPELDRQGRPKMFYSQRI | HDK | CYRRPHFDAGQFVESWDDDESARKGY | C | LYKVG | C | KGPTTYN |
| Ral_met_HoxK | 211 | IPELDRQGRPKMFYSQRI | HDK | CYRRPHFDAGQFVEAWDDDSARKGY | C | LYKVG | C | KGPTTYN |
| Rho_cap_HupS | 214 | MPELDRQGRPAMFYSQRI | HDK | CYRRPHFDAGQFVEHWDDDENARKGY | C | LYKMG | C | KGPTTYN |
| Rhi_leg_HupS | 214 | LPELDRQGRPKMFYSQRI | HDK | CYRRPHFDAGQFVEEWDDEGARKGY | C | LYKMG | C | KGPTTYN |
| Bra_jap_HupS | 215 | LPELDRQGRPKMFYSQRI | HDK | CYRRPHFDAGQFVEEWDDEAARKGY | C | LYKMG | C | KGPTTYN |
| Thi_ros_HupS | 214 | LPELDRQGRPLMFYQRI | HDK | CYRRPHFDAGQFVESWDDDEGARRGY | C | LYKVG | C | KGPTTYN |
| Aqu_aeo_HyaA | 211 | IPPLDSQGRPKMFYGNRI | HDT | CYRRSFFNAGQFVEQFDDEGAKKGW | C | LYKVG | C | RGPTTYN |
| Esc_col_HyaA | 214 | LPDVDRMGRPLMFYQRI | HDK | CYRRPHFDAGQFVQSWDDDAARKGY | C | LYKMG | C | KGPTTYN |
| Esc_col_HybO | 211 | PPKLDKRNPTFAYGRLI | HEH | CERRPHFDAGRFAGEFGDEGHREGW | C | LYHLG | C | CKGPETYG |
| All_vin_HynS | 164 | LPEVDGYGRPLAFYQSI | HDR | CYRRPFYDKGLFAESFDDEGAKQGW | C | LYRLG | C | CKGPPTYN |
| Thi_ros_HynS | 219 | LPELDAYNRPMAFFGQSI | HDR | CYRRPFYDKGLFAKTFDDEGARLGW | C | LYELG | C | CKGPPTYN |
| Des_gig_HydA | 191 | MPELDRQGRPVMFFGETV | HDN | CPRPKHFEAGEFATSFGSPEAKKG | C | LYELG | C | CKGPPTYN |
| Des_vul_HydA | 220 | APELDSLNRPTMFFGQTV | HEQ | CPRLPHFADAGEFAPSFESEEARKGW | C | LYELG | C | CKGPVTMN |
| Des_bac_HysS | 222 | LPELDDDRPLLFFGDNI | HEN | CPLYLDKYDNSEFAETFTKPG----- | C | KAELG | C | CKGPSTYA |
| | | | M | M | M | M | | |
| Ral_eut_HoxK | 272 | ACSTTRWNEGT-SFPIQSGHG | C | IG | C | SED--GFWDKGSFYDRLT | G | ISQFG--VEANADKIG |
| Alc_hyd_HoxK | 272 | ACSTTRWNGGT-SFPIQSGHG | C | IG | C | SED--GFWDKGSFYSRLTNIHQFG-- | I | EANADSVG |
| Ral_met_HoxK | 271 | ACSTVQWNEGT-SFPIKAGHG | C | IG | C | SED--GFWDKGSFYDRLTDIHAFG-- | I | EANADQIG |
| Rho_cap_HupS | 274 | ACSTVPLERRR-HFPIQSGHG | C | IG | C | SED--GFWDQGSFYDRLTTIKQFG-- | I | EATADQIG |
| Rhi_leg_HupS | 274 | ACSTVRWNGGV-SFPIQSGHG | C | IG | C | SED--GFWDNGSFYDRLTNIHQFG-- | I | EANADKVG |
| Bra_jap_HupS | 275 | ACSTVRWNGGV-SFPIQSGHG | C | IG | C | SED--GFWDKGSFYDRLTNIKQFG-- | I | EKNADQIG |
| Thi_ros_HupS | 274 | ACSTIRWNGGV-SFPIQSGHG | C | IG | C | SED--GFWDKGSFYQHVTDTHAFG-- | I | EANADRTG |
| Aqu_aeo_HyaA | 271 | SCGNMRWYNGL-SYPIQSGHG | C | IG | C | CAEN--NFDNGPFYERIGIIPVPG-- | I | ESKADKVG |
| Esc_col_HyaA | 274 | ACSSTRWNDGV-SFPIQSGHG | C | IG | C | CAEN--GFWDRGSFYSRVVDIPQMG-- | T | HSTADTVG |
| Esc_col_HybO | 271 | NCSTLQFCDVGGVWPVAIGHPC | Y | C | NEEGIGFHKGIHQLANVENQTPRSQKPDVNAKEGG | | | |
| All_vin_HynS | 224 | ACATMKWNDGT-SWPVEAGHP | C | IG | C | SEP--QFWDAGGFYEPVSVPLTLGPATLLGAGAAG | | |
| Thi_ros_HynS | 279 | ACATMRWNDGT-SWPVEAGHP | C | IG | C | SEP--RFWDAGGFYNTVSVVPTSASGVNVLAAGAAG | | |
| Des_gig_HydA | 251 | NCPKQLFNQVN--WPVQAGHP | C | IG | C | SEP--NFDWLYSPFYSA----- | | |
| Des_vul_HydA | 280 | NCPKIKFNQTN--WPVDAGHP | C | IG | C | SEP--DFWDAMTPFYQN----- | | |
| Des_bac_HysS | 277 | DCAKRRWNGI-NWCVEN-AV | C | IG | C | VEP--DFPDGKSPFYVAE----- | | |
| Ral_eut_HoxK | 327 | GTASVVVGAAVTAHAAASAIKRASKK | | | | NETSGSEH----- | | |
| Alc_hyd_HoxK | 327 | VTAVGVVGAATAAHAASAIKRARHKDAAQDTAATQK | | | | ----- | | |
| Ral_met_HoxK | 326 | GTAAGVVGAAVAHAASAIKRVKRDNNARKPDASTPANH | | | | ----- | | |
| Rho_cap_HupS | 329 | WTATGLVGAAVAHAASVVKRAQKK--NEEA | | | | ----- | | |
| Rhi_leg_HupS | 329 | MTAAGVVGGATAAHAAVTAVKRLITK--REKADA | | | | ----- | | |
| Bra_jap_HupS | 330 | MVAAGAVGAAVAHAAVTAVKRLATK--REDADHNS | | | | ----- | | |
| Thi_ros_HupS | 329 | IAVATRRGAHRAHAASVVKRVQOK--KEEDQS | | | | ----- | | |
| Aqu_aeo_HyaA | 326 | AIAAAAAAGGAIIHGIASKIRKSGEK--EE | | | | ----- | | |
| Esc_col_HyaA | 329 | LTALGVVAAAVGVHAVASAVDQRRRHNOQPTETEHQPGNEDKQA | | | | ----- | | |
| Esc_col_HybO | 331 | NVSAGAIIGLLGGVGLVAGVSMVAVRELGRQKKDNADSRGE | | | | ----- | | |
| All_vin_HynS | 281 | AVVGGGLAALSRRKGRDAAATRPVT--VDELEQKL | | | | ----- | | |
| Thi_ros_HynS | 336 | AIVGGAVAALAKKQTKTAVAHROPVT--VEELEAKL | | | | ----- | | |
| Des_gig_HydA | | ----- | | | | ----- | | |
| Des_vul_HydA | | ----- | | | | ----- | | |
| Des_bac_HysS | | ----- | | | | ----- | | |

Figure S4 Amino acid sequence alignment of the small subunits of aerobic and anaerobic [NiFe] hydrogenases. The cysteine and histidine residues involved in coordination of the proximal (P), medial (M) and distal (D) iron-sulfur clusters are highlighted in red and green. Additional cysteine residues that are in close proximity to the proximal [4Fe4S] cluster are shown in green. The grey shaded signal sequences are cleaved off during the process of Tat-mediated membrane translocation. The blue regions are involved in the connection of the hydrogenase small subunit to

the membrane-integral cytochrome *b*. These amino acid stretches are absent in the periplasmic hydrogenases from *Desulfovibrio/Desulfomicrobium* species that interact with *c*-type cytochromes. Strain abbreviations (database accession numbers of the protein sequences are given in brackets): Ral_eut, *Ralstonia eutropha* H16 (NP_942643); Alc_hyd, *Alcaligenes hydrogenophilus* M50 (P33375); Ral_met, *Ralstonia metallidurans* CH34 (YP_583452); Rho_cap, *Rhodobacter capsulatus* (CAA31869); Rhi_leg, *Rhizobium leguminosarum* (P18637); Bra_jap, *Bradyrhizobium japonicum* (P12635); Thi_ros, *Thiocapsa roseopersicina* (AAA27409); Aqu_aeo, *Aquifex aeolicus* VF5 (NP_213454); Esc_col, *Escherichia coli* K12 (NP_415491, BAE77058); All_vin, *Allochromatium vinosum* (AAU93828); Des_gig, *Desulfovibrio gigas* (P12943); Des_vul, *Desulfovibrio vulgaris* 'Miyazaki F' (YP_002434696); Des_bac, *Desulfomicrobium baculatum* (P13063).