

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☒ ☐ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection Bruker OPUS spectroscopy software (version 7.5) was used for IR data collection

Data analysis We used APE (A-Plasmid-Editor) to plan cloning steps, designing primer sequences and verifying DNA sequences; Western blots and SDS page gels were analyzed using the software FluorChem FC2 (version 3.2.2, Cell Biosciences); Protein concentrations were analyzed using SoftMax Pro 7.0.3 (Molecular Devices); IR data were analyzed using Bruker OPUS (version 7.5) and Win-IR Pro (Version 2.6, Bio-Rad Laboratories); EPR spectra simulation was performed with the Matlab toolbox Easyspin 5.2.25; Mössbauer data were processed and simulated using the WMOSS4 program (www.wmoss.org); Raw NRVs data were converted to single-phonon 57Fe partial vibrational density of states (PVDOS) using the PHOENIX software package via the "NRVS Spectra Processing Tool" web interface (<https://www.spectra.tools/>); XAS data were processed using Athena, part of the Demeter software package (<https://bruceravel.github.io/demeter/>); Theoretical EXAFS models were calculated using Artemis by using the multiple scattering FEFF6 code; the PyMOL Molecular Graphics System (Version 2.2.0) was used for preparation of protein structures in Figure 1; Spectroscopy plots were generated by Origin 2018b (64 bit, b9.5.5.409 Academic); high-resolution (>600 dpi) figures were prepared using Inkscape (1.0.1, 3bc2e813f5).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data supporting the findings of this study are available within the article and the Supplementary Information. Source data are provided with this paper. Raw XAS data were obtained at the SSRL synchrotron. Raw NRVS data were generated at the synchrotron facilities Petra III and SPring-8, and are available in their processed form upon request. Additional reagents will be made available by the corresponding authors upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	IR spectroscopic experiments on the hydrogenase intermediates were performed three times with three independent samples. Iron and nickel quantitations by ICP-OES were performed on three independent samples. No statistical methods were used to predetermine sample size. A sample size of $n = 3$ is routinely used to monitor significant deviations, to calculate standard errors, and to allow meaningful interpretation of the data.
Data exclusions	No data were excluded.
Replication	Biochemical, biological and spectroscopic data were replicated. SDS PAGE gels, ICP-OES and IR measurements were performed three times ($n = 3$) with independent samples, revealing consistent results. Synchrotron XAS and NRVS measurements were not replicated because they require expensive and time-consuming measurements, which are possible only upon peer-review of research proposals. Mössbauer samples were not replicated because of expensive sample preparation with ^{57}Fe . All XAS, NRVS and Mössbauer samples were quality-checked before the measurements by IR spectroscopy and ICP-OES.
Randomization	Randomization was not relevant because no experimental groups were used in this study.
Blinding	Blinding was not applicable since the experiments did not involve group allocation.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Self made polyclonal antibodies raised against the <i>Cupriavidus necator</i> proteins HoxG (AK88, 1:10,000) and HoxK (AK87, 1:5000) were used as primary antibodies. Alkaline phosphatase-labelled goat-anti-rabbit IgG (Dianova/Jackson ImmunoResearch, product code nr.: 111-055-003, Lot 85152), Hamburg, Germany) was used as secondary antibody (1:10,000).
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