

Reporting Summary

Nature Portfolio 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 Portfolio 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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Spectrophotometer, Cary 60 UV-Vis Spectrophotometer Agilent technologies. Synchrotron data collection are all stated in the Table 1. Mass spectrometry, Exploris 480 instrument connected to an Ultimate 3000 RSLC nano and a nanospray flex ion source (all ThermoFisher Scientific, USA).
Data analysis	Microsoft Office Excel Professional Plus 2016 (16.0.5448.1000) Microsoft Office Powerpoint Professional Plus 2016 (16.0.4266.1001) SPAdes v. 4.0.0 prokka v. 1.14.6 GTDB-Tk v2.4.0 SingleM against the GTDB-Tk database version 2.4.0 FASTQC v0.11.9 software SemiBin (v. 2.1.0) MUSCLE v3.8.1551 RAxML v.8.2.12 autoPROC 1.0.5 XDS and aimless from CCP4 (v. 1.12.14) PHENIX v. 1.19.2-4158 AlphaFold 2

COOT version 0.9.8.3 EL
MAFFT 7 and ESPrnt 3.0
Open-source Pymol Version 2.2.0
Byonic or Proteomediscoverer Sequest

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The metagenomic sequences from the Amon Mud Volcano enrichment generated in this study were deposited in the NCBI Bioproject database under accession code PRJNA1248948. The metagenomic sequences for ANME-2d samples were deposited in the NCBI Bioproject database under accession code PRJNA1289043. The structural data generated in this study (structure factors and models) have been deposited in the Protein Data Bank under the accession codes 9QR3 (as isolated MCR ANME-2c, <https://doi.org/10.2210/pdb9QR3/pdb>), 9QM5 (Krypton-pressurized MCR ANME-2c, <https://doi.org/10.2210/pdb9QM5/pdb>), 9QR1 (MCR ANME-2dO, <https://doi.org/10.2210/pdb9QR1/pdb>), and 9QQT (MCR ANME-2dV, <https://doi.org/10.2210/pdb9QQT/pdb>). The mass spectrometry data generated in this study have been deposited on the Zenodo server under accession record 15784324 (doi 10.5281/zenodo.15784324).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender n.a.

Reporting on race, ethnicity, or other socially relevant groupings n.a.

Population characteristics n.a.

Recruitment n.a.

Ethics oversight n.a.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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](https://www.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	As all samples were obtained through enrichment, biomass was limited and processed as a whole entity without biological replicates except for ANME-2dO (three purification, stated in the manuscript).
Data exclusions	No data was excluded from the analysis.
Replication	Due to the restrictions in available biomass all purification procedures were only performed once. UV/Vis spectra were recorded in technical triplicates and duplicates as indicated in the manuscript.
Randomization	Randomization was applied for Rfree generation during protein structure refinement.
Blinding	n.a.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.