nature portfolio

Corresponding author(s): Wenqing Chen, Binju Wang, Zhengyu Zhang

Last updated by author(s): Aug 11, 2025

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 (*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	HPLC data were obtained on Shimadu LC-20AT instrument. LC-HRMS data were acquired on a Thermo Fisher Scientific ESI-LTQ Orbitrap. NMR data were collected on Bruker AVANCE III HD 400 or Bruker AVANCE NEO 600. The diffraction data of the protein crystals were collected at the BL19U1/BL02U1/BL10U2 beamlines of the Shanghai Synchrotron Radiation Facility. All MD simulations were performed with the Amber 20 package. Hybrid QM/MM Hamiltonian calculations resemble the most populated one from MD. All hybrid QM/MM Hamiltonian calculations were performed using ChemShell software combining Turbomole for the QM region and DL_POLY for the MM region.
Data analysis	Chemical structure: ChemDraw (20.0) was used for describing the chemical structures of compounds. NMR: MestreNova (14.0.1) was used for anaysis of NMR data. LC-HRMS: Xcalibur (2.2.0) was used for analysis of LC-HRMS data. Crystallography: X-ray Detector Software(XDS)package(version 20240712)was used for indexing,intergrating and scaling. The following programs in the CCP4 package(8.0.012)and Phenix package(version 1.20.1-4487)were used for crystal structure determination. CRANK2 for experimental Se-Met SAD phasing,phaser_MR for molecular replacement,Buccaneer for model building, Refmac5 and phenix.refine for refinements. Coot(version 0.9.8.92)was used for structural model building. PyMOL(version 2.4.0)was used to produce structural figures. Bioinformatics: Sequence alignment was performed using online version of CLUSTALW(http://www.genome.jp/tools-bin/clustalw). Sequence alignment figures were generated using online tool ESPript3.0 (http://espript.ibcp.fr/ESPript/ESPript/). Others software: CorelDRAW (2020), Origin (2023b).

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

Data that support the finding of this study are available within the paper and Supplementary Information. The Genbank accession numbers of SnPolE, SmPolE, PolF, SaPolF and SzPolF are PZH16170.1, TXS29575.1, AFP55317.1, AXK31912.1, and RLL69652.1. Atomic coordinates and structure factors for the crystal structures reported in this work have been deposited to the Protein Data Bank(PDB) under accession numbers as follows: apo SnPolE, 9J2E; SnPolE-BH4, 9J2M; SnPolE-BH4-L-isoleucine, 9J2P; SnPolE-F140A, 9J33; SnPolE-Y157A, 9J2R; SnPolE-Y280A, 9J30; apo SmPolE, 9J3G; Fe(II)-SzPolF-L-isoleucine, 9J2C; Fe2(II/II)-SzPolF-L-isoleucine, 9J2B; Fe(II)-SzPolF-2, 9J28; Fe2(II/II)-SzPolF-2, 9J2A; Fe2(II/II)-SzPolF-1, 9J29; Fe2(II/II)-PolF-L-isoleucine, 9J3V; Fe(II)-SaPolF-L-isoleucine, 9J27. Source data are provided with this paper.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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

Life sciences study design

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

Sample size	In this study, the fermentation data and enzymatic activity analyses were determined with the sample of n=3.
Data exclusions	No data were excluded from the analyses.
Replication	Apart from the determination of X-ray crystallographic structure, all enzymatic assays were carried out in at least 3 independent experiments (N = 2) with replicates (n = 3).
Randomization	No randomization was required because genetically and biochemically identical samples were used.
Blinding	This study does not involve human/animal subjects and group allocation, therefore, blinding was irrelevant to this study .

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

Methods

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