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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<u>Authors & Referees</u> and the<u>Editorial Policy Checklist</u>.

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	Сог	Confirmed		
	x	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement		
	x	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		
x		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons		
	x	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 Give <i>P</i> 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.		
~	c.			

Software and code

Policy information about availability of computer code

Data collection x-ray data was collected at the Advanced Light Source on beamlines BL 8.2.1 and BL 8.2.2 molecular dynamics simulations were conducted using Amber16

Data analysis

ImageJ 1.51j8 iMosflm 7.2.1 and 7.2.2 Phenix 1.13 CCP4 Interface 7.0.076 Coot 0.8.9.2 PyMOL 2.2.3 PyMOL 1.8.6 Gaussview 5.0.9 Avogadro 1.9.0 Amber16 VMD 1.9.4 CPPTRAJ: Version 4.14.0 (AmberTools 19.00) PYTRAJ 2.02.dev0 MDTRAJ 1.9.3 Schrödinger software release 2019-4. Amber 18 Gaussian 09 ANTECHAMBER 19.0 Ambertools 19

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/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 <u>data availability statement</u>. 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

All PDB files and MTZ files related to structures reported in this work are available under the following PDB accession codes, 60KC, 60KF, 60KG, and 60LT.

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 <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

All studies must dis	close on these points even when the disclosure is negative.
Sample size	Biochemical assays were completed using the wt FabF enzyme and 9 FabF mutants.
Data exclusions	No data were excluded.
Replication	All biochemical assays were repeated in triplicate while two mutants were repeated in sextuplicate (G399A and G402A).
Randomization	The data represented are a biochemical assay on enzyme function which require a rational approach to data collection and analysis; therefore, randomization is not applicable to our experimental set up.
Blinding	Blinding is not applicable to any biochemical assay performed in 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.

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Materials & experimental systems

- n/a Involved in the study

 Involved in the study

 Antibodies

 Eukaryotic cell lines

 Palaeontology
- **X** Animals and other organisms
- **X** Human research participants
- Clinical data

Methods

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