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**Sample-size estimation**

* You should state whether an appropriate sample size was computed when the study was being designed
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Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

Sample-size estimation is not appropriate for the experiments in this manuscript. Replicate information is provided below.

**Replicates**

* You should report how often each experiment was performed
* You should include a definition of biological versus technical replication
* The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
* If you encountered any outliers, you should describe how these were handled
* Criteria for exclusion/inclusion of data should be clearly stated
* High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

-Sample size for compartment diameter quantification via negative-stain TEM provided in results and Supplementary Figure legend 1-4. Experiment was performed with 2 biological replicates.

-Absorbance spectra measurements for *S. elongatus* sulfate starvation experiments were performed with 3 technical replicates of 2 biological replicates.
-SDS-PAGE gel of lysates for sulfur-starved *S. elongatus* is representative of 2 biological replicates.

-Liquid chromatography-mass spectrometry data of excised gel bands is from a single experiment.

-All *in vivo* and *in vivo* SDS-PAGE data are representative of 2 biological replicates.

-Replicate information for cysteine desulfurase activity data is provided in the Figure 7 figure legend. Data points are the mean of 3-6 technical replicates. Error bars are SD. Data was fitted with the Michaelis-Menten equation using GraphPad Prism 8 as noted in the methods section.

**Statistical reporting**

* Statistical analysis methods should be described and justified
* Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
* For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
* Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

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-Provided “PDB EM Map/ Model Validation Reports” cover all expected statistics for CryoEM data reported in this manuscript.

-Data points for cysteine desulfurase activity experiments are the mean of 3-6 technical replicates. Error bars are SD. Data was fitted with the Michaelis-Menten equation using GraphPad Prism 8 as noted in the methods section.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

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* Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
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Group allocation was not necessary for this manuscript.

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* Where provided, these should be in the most useful format, and they can be uploaded as “Source data” files linked to a main figure or table
* Include model definition files including the full list of parameters used
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PDB and MRC files for the structures reported here are available in the supplementary zip file.