

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 [Authors & Referees](#) 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

We did not perform data collection in this study.

Data analysis

This study is centered around new algorithms that were developed for data analysis. These algorithms are implemented in the cryoSPARC software package, and this implementation was used for validation in the study. CryoSPARC is freely available for academic use, and is available via licensing for commercial use, at www.cryosparc.com. The version of cryoSPARC used in this study will be made available to editors/reviewers according to Nature Research guidelines.

Versions of software package and tools used:

cryoSPARC v2.11

Python 2.7

Chimera 1.4 and ChimeraX 0.9

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 [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

No new datasets were created in this study. The raw datasets analyzed in this study were either downloaded from the EMPIAR repository (EMPIAR-10024, EMPIAR-10330, EMPIAR-10261) or were provided by authors of other studies, cited in the main text.

The following PDB and EMDB datasets were used: EMD-0341, PDB-6N4Q.

All other results and outputs of data analysis in this study are available from the corresponding author on 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](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	For each dataset analyzed, the sample size of particle images used in the analysis was determined simply based on how many particle images were captured during microscopy for the datasets that were analyzed. These sample sizes are sufficient as the final resolution of reconstructed 3D protein density maps is statistically validated and results are only interpreted at resolutions where the specific sample size of particle images for a given dataset produces sufficient signal-to-noise ratio.
Data exclusions	No data were excluded.
Replication	All results in the study can be replicated exactly by running the described algorithm/software with the same input data.
Randomization	Randomization was used in the statistical validation of achieved reconstruction resolution for each image dataset that was analyzed. Randomization takes the form of randomly splitting a dataset into two halves, so that each can be reconstructed independently. This process is explicitly described in the main text.
Blinding	Blinding during data analysis was not relevant as human input is not used in the analysis of the data. Blinding during data collection was not relevant as no new raw data was collected for 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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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