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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	EPU 2 (version 2; Thermo Fisher), MetaMorph (Molecular Devices), iControl (Tecan), ImmunoSpot (Immunospot)
Data analysis	Relion 5.0-beta (MRC-LMB, Cambridge), crYOLO 1.8.2. (Wagner et al., 2019), CTFFIND4.1 (Rohou and Grigorieff, 2015), Coot 0.9.6 (Emsley et al., 2010), PHENIX 1.19.2 (Afonine et al., 2018), REFMAC5 5.5 (Murshudov et al., 2011), ISOLDE 1.2 (Croll, 2018), MolProbity 4.5.1 (Williams et al., 2018), UCSF Chimera 1.15 (Pettersen et al., 2004), UCSF ChimeraX 1.1.1 (Pettersen et al., 2021) MS data: MaxEnt1 (Waters) Image and spectral analysis: Fiji / ImageJ, RStudio (posit), Origin2025b (Origin Labs), python scripts developed for this paper are deposited in: www.github.com/ioannachatzitheodosiou/Optosplit_Analysis

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

Cryo-EM maps were deposited into the Electron Microscopy Data Bank (EMDB) (https://www.ebi.ac.uk/pdbe/emdb) under the following accession numbers: EMD-57062. Corresponding atomic coordinates were deposited in the Protein Data Bank (PDB) (https://www.rcsb.org) under the following PDB ID code: 29BR. Uncropped and unprocessed western blots and Scrapie Cell Assay data are provided as Source Data with this paper.

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

Life sciences study design

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

Sample size	cryo-EM: Sample size (the number of particle images or segments) was not predetermined. We aimed to collect at least 6,000 multi-frame movies as this was expected to provide sufficient particles for high resolution 3D reconstruction. This was achieved. Satisfactory sample size is reflected in the final near-atomic and isotropic resolution of the 3D reconstruction, sufficient for building and refining an atomic model. imaging: N = 55 to 140 particles from 15 images were analyzed for each data set. Data from 8 independent cell culture wells were analyzed for each biological sapmle in Scrapie Cell Assays.
Data exclusions	In single-particle cryo-EM data processing so called 'bad particles' are excluded due to their obvious poor quality, which precludes their alignment with the consensus data. The sparse regions of micrographs where sample image quality is poor (for example, due to grid surface contamination giving rise to local noise in particle image or due to sample heterogeneity) would ideally be not selected for processing, but this cannot be avoided, especially when using automated particle picking, as in this study. Image processing algorithms reveal such poor particles as not classifiable under objective computational criteria into any biologically relevant class, which objectifies exclusion. No particles were excluded from analysis in imaging. Microplate wells without identifiable cells were excluded from SCA analysis.
Replication	The purification methods used was first reported in 2015 (Wenborn et al Sci Rep 2015) and with minor modifications in 2022 and 2023 (Manka et al 2022; 2023). The method is robust and we have had no replication failures after >100 repetitions. The method has also been independently used and replicated in other laboratories. Novel cryo-EM structures reported in this study were reproduced in independent cryo-EM data sets. Serial prion amplfication assays were replicated in three independent experiments from independent biological samples.
Randomization	Randomization is not relevant to cryo-EM structure determination, which was targeted for each prion sample that was analyzed. Fields of view in image analysis were randomly selected.
Blinding	Classifications of particle images were performed computationally and therefore objectively. Blinding was not relevant for prion purification and sample characterization. Scrapie cell assays were performed blinded to sample identity.

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

Antibodies

Antibodies used	8H4 (Merck Life Science, Cat no P0110-200UL), ICSM18 and ICSM35 (D-Gen Ltd.) mouse monoclonal antibodies were used for Western blotting (at 0.2 µg/ml concentration) in conjunction with alkaline-phosphatase-conjugated goat anti-mouse IgG secondary antibody (Sigma-Aldrich, Cat No A2179 at 1:10,000 dilution). ICSM 35 and ICSM 18 mouse anti-PrP monoclonal antibodies were used for immunohistochemistry and Scrapie Cell Assay, respectively. ICSM 35 and ICSM 18 antibodies were supplied by D-Gen Ltd, London and details of their production and characterization are provided in Khalili-Shirazi et al. Biochim Biophys Acta. 2007;1774:1438-50. Numerous other anti-PrP antibodies from commercial or academic sources could be used for these purposes and there is no reliance on the particular properties of ICSM 35 and ICSM 18.
Validation	Validation is provided in Wenborn et al 2015 and references cited therein.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Prion infectivity in brain homogenates was measured using the Scrapie Cell Assay (Klohn et al. 2003) using four cell lines as described previously, Mahal et al. 2007. The cell lines used were; PK1/2 cells (an established cell line; D-Gen Ltd London, originally derived from N2a cells that were obtained from, and authenticated by, the American Type Culture Collection (ATCC)), R33 cells (an established cell line; that was a gift from Professor Charles Weissmann, originally derived from N2a cells obtained and authenticated by the ATCC), LD9 cells (an established cell line; that was a gift from Professor Charles Weissmann, originally derived from murine L929 fibroblasts supplied and authenticated by the ATCC), CAD5 cells (an established cell line; that was a gift from Professor Charles Weissmann, originally derived from Cath.a-differentiated (CAD) cells Qi et al. 1997).
Authentication	Original cell lines were authenticated as described above. Cells used in this study were not authenticated by us.
Mycoplasma contamination	Cells used in this study have been tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	none

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Brains from C57BL/6JOlaHSd mice terminally-affected with RML, 22L and ME7 mouse-adapted prion strains were sourced from a previously published study, Wenborn et al 2015. PrP-KO mice line F10 with the FVB genotype (Harlan) were generatedand characterized in a previous study (Malucci, 2002). 50 mice (m/f) were bred in house and ethically culled to generate brain homogenates for PMCA experiments.
Wild animals	none
Reporting on sex	RML, 22L and ME7 scrapie brain homogenates originated from female mice. Brains from male and female F10 mice were pooled for PMCA brain homogenates.
Field-collected samples	none
Ethics oversight	Brains from mice with clinical prion disease were used for Western blotting, Scrapie Cell Assay, immunohistochemistry and to isolate purified prion fibrils. Brain samples originating from prion transmissions performed at Imperial College London between 1995 and

2003 were generated in accordance with licences approved and granted by the UK Home Office (Project Licences 70/2045, 70/3679, 70/4039 and 70/5064) and conformed to institutional guidelines. Brain samples from prion transmissions performed at UCL were generated in accordance with licences approved and granted by the UK Home Office (Project Licences 70/6454, 70/7274, 70/9022 and PP3260075) and conformed to institutional and ARRIVE guidelines. All protocols and procedures at UCL involving animals were approved by the MRC Prion Unit at University College London Animal Welfare and Ethical Review Body.

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

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a