nature chemical biology

Article

https://doi.org/10.1038/s41589-022-01229-7

A structural basis for prion strain diversity

In the format provided by the authors and unedited

Table of Contents:

Supplementary Table 1. Determination of prion infectivity titre in cell culture

Dilution	Positive Wells (w/w_0)			Percentage Positivity		
	RML BH I6200*	ME7 BH I21487	Purified ME7 (1X)†	RML BH I6200*	ME7 BH I21487	Purified ME7 (1X)†
1.10^{-4}	24 / 24	24 / 24	24 / 24	100.0%	100.0%	100.0%
3.10^{-5}	24 / 24	24 / 24	24 / 24	100.0%	100.0%	100.0%
1.10^{-5}	20 / 24	24 / 24	15 / 24	83.3%	100.0%	62.5%
3.10^{-6}	5 / 24	16 / 24	5 / 24	20.8%	66.7%	20.8%
1.10^{-6}	2 / 24	9 / 24	1 / 24	8.3%	37.5%	4.2%
3.10^{-7}	0 / 24	2 / 24	0 / 24	0.0%	8.3%	0.0%
1.10^{-7}	0 / 24	1 / 24	0 / 24	0.0%	4.2%	0.0%
NI	0 / 24	0 / 24	0 / 25	0.0%	0.0%	0.0%

log TCIU ml^{-1} $	**5.8**	**6.1**	**5.5**

Measurement of total protein and PrP content:

Total protein (mg ml^{-1})	**10.0**	**10.0**	**0.0027**
PrP content (µg ml^{-1})	**5.4**	**11.0**	**2.7**

Calculation of specific infectivity:

log TCIU mg^{-1} protein	**4.8**	**5.1**	**8.1**
log TCIU mg^{-1} PrP	**8.1**	**8.1**	**8.1**

(w/w_0), the number of prion infected wells (w) versus the total number of inoculated wells (w_0); BH, brain homogenate; TCIU, tissue culture infectious units; NI, non-prion inoculated

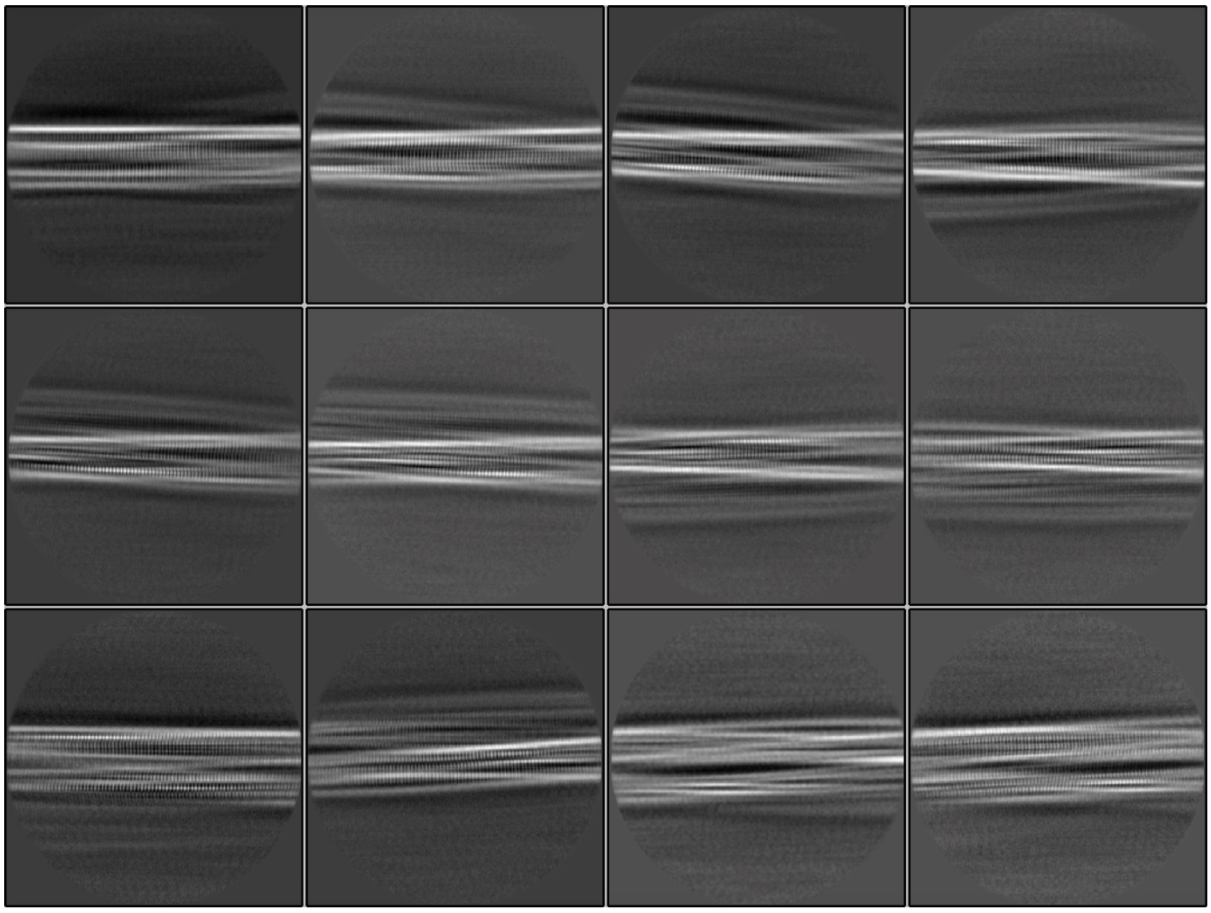
* Prion titre of $10^{7.3 + 0.5}$ (mean + s.d., n=6) intracerebral LD_{50} units/ml in Tg20 mice[1]

† Purified material was resuspended to the same volume as the 10% (w/v) BH from which it was derived

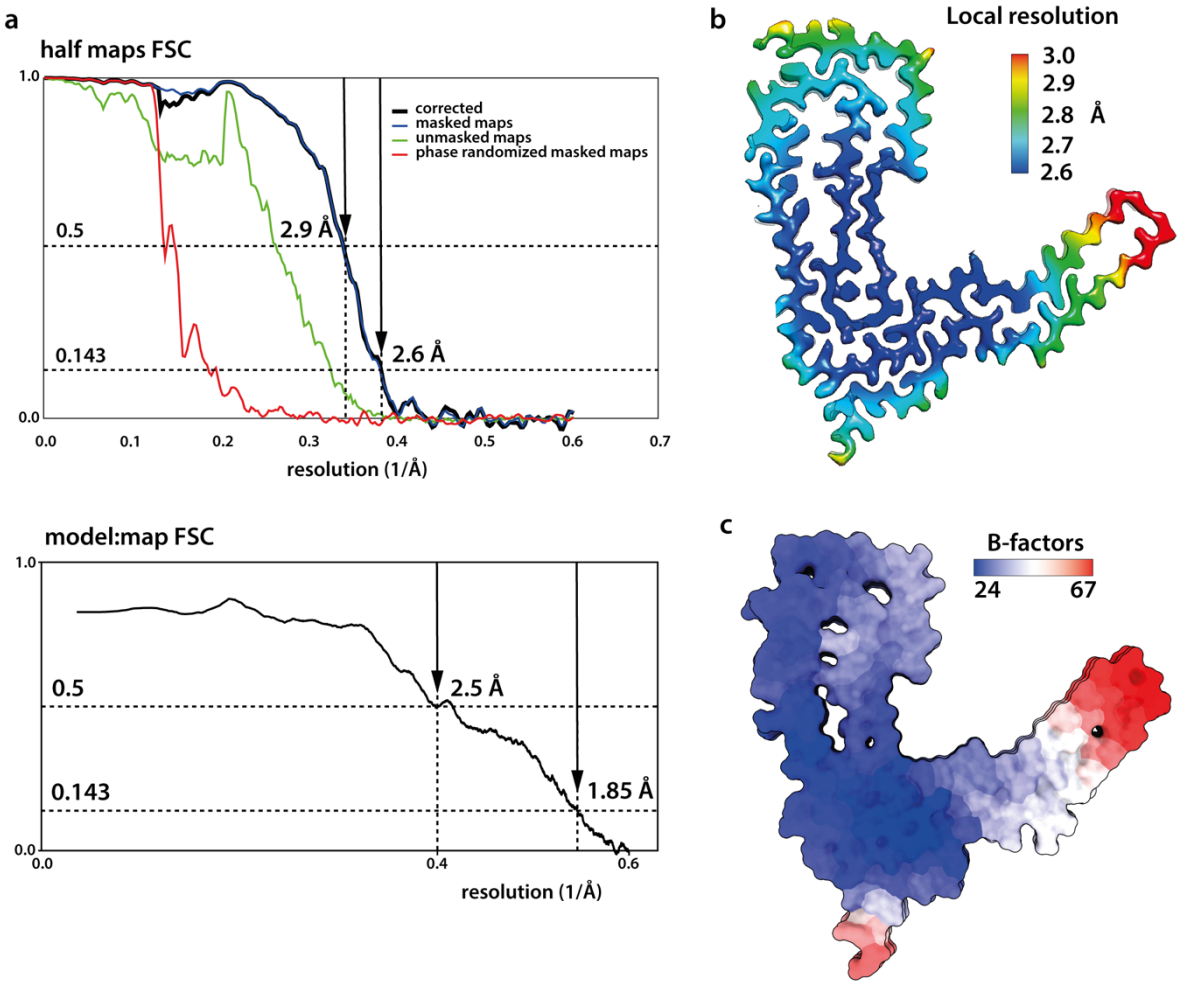
$ TCIU calculated according to the Poisson distribution[2]

Supplementary Table 2. Cryo-EM data collection, refinement and validation statistics

	ME7 (EMD-15043) (PDB 8A00)
Data collection and processing	
Magnification	105,000 x
Voltage (kV)	300
Electron exposure (e–/Å^2)	49.4
Defocus range (μm)	from -2.4 to -0.9
Pixel size (Å)	0.828
Symmetry imposed	C1
Initial particle images (no.)	223,065
Final particle images (no.)	40,239
Map resolution (Å)	2.6
FSC threshold	0.143
Map resolution range (Å)	2.6-3
Refinement	
Initial model used (PDB code)	7QIG
Model resolution (Å)	2.6
FSC threshold	0.143
Model resolution range (Å)	121.7 - 2.6
Map sharpening *B* factor (Å^2)	-26.75
Model composition	
Non-hydrogen atoms	6456
Protein residues	408
Ligands	none
***B* factors (Å^2)**	
Protein	24.18-67.46
r.m.s. deviations	
Bond lengths (Å)	0.002
Bond angles (°)	0.580
Validation	
MolProbity score	1.49
Clashscore	2.79
Poor rotamers (%)	0
Ramachandran plot	
Favored (%)	93.53
Allowed (%)	6.47
Disallowed (%)	0



Supplementary Figure 1. 2D classification of ME7 prion fibrils. Gallery of representative 2D classes (box size: 397.5 x 397.5 Å).



Supplementary Figure 2. Quality of the ME7 map and model. a Top, Fourier Shell Correlation (FSC) plots for independent half-reconstructions, determined in Relion 4.0-beta. The final plot (black line) is corrected for overfitting with high-resolution noise substitution. Bottom, FSC plot for the model:map fit. **b** Local resolution of the protein density calculated with Relion 4.0-beta LocRes. **c** Atomic B-factor values colour-coded on the solvent-excluded model surface.

Supplementary references

1 Wenborn, A. et al. A novel and rapid method for obtaining high titre intact prion strains from mammalian brain. *Sci Rep* **5**, 10062 (2015).

2 Edgeworth, J. A., Jackson, G. S., Clarke, A. R., Weissmann, C., & Collinge, J. Highly sensitive, quantitative cell-based assay for prions adsorbed to solid surfaces. *Proc Natl Acad Sci USA* **106**, 3479-3483 (2009).