PNAS

Supporting Information for

Tau Filaments from Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex (ALS/PDC) adopt the CTE Fold

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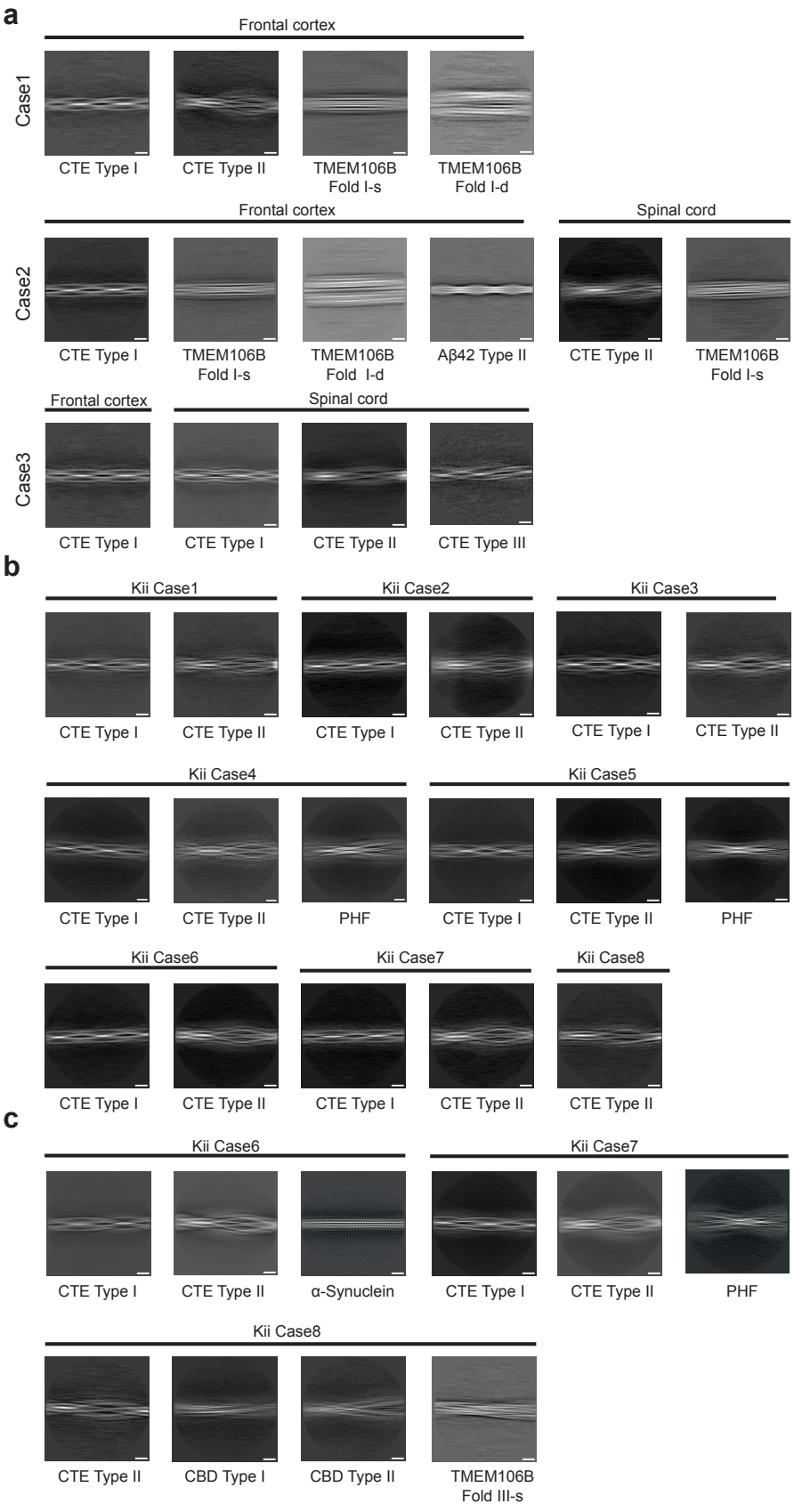
This PDF file includes:

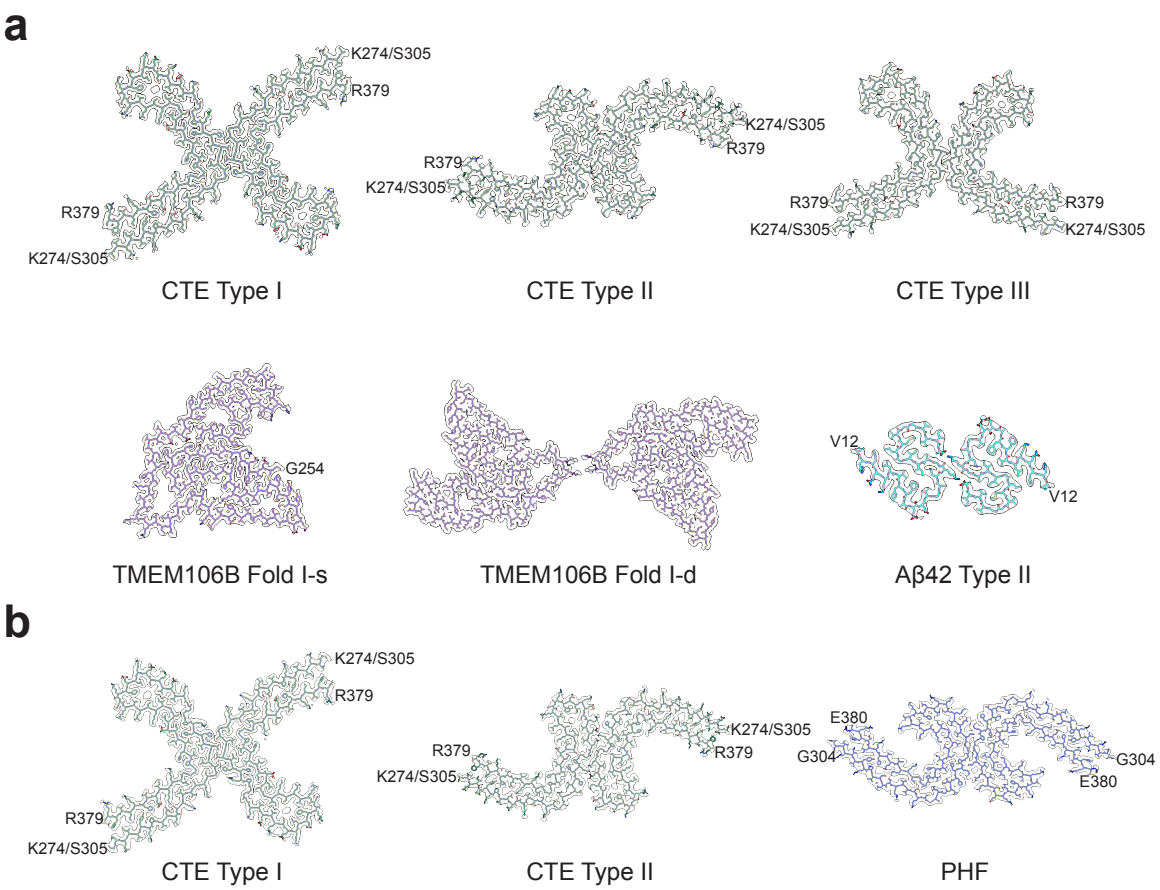
Figures S1 to S7

Tables S1 to S3

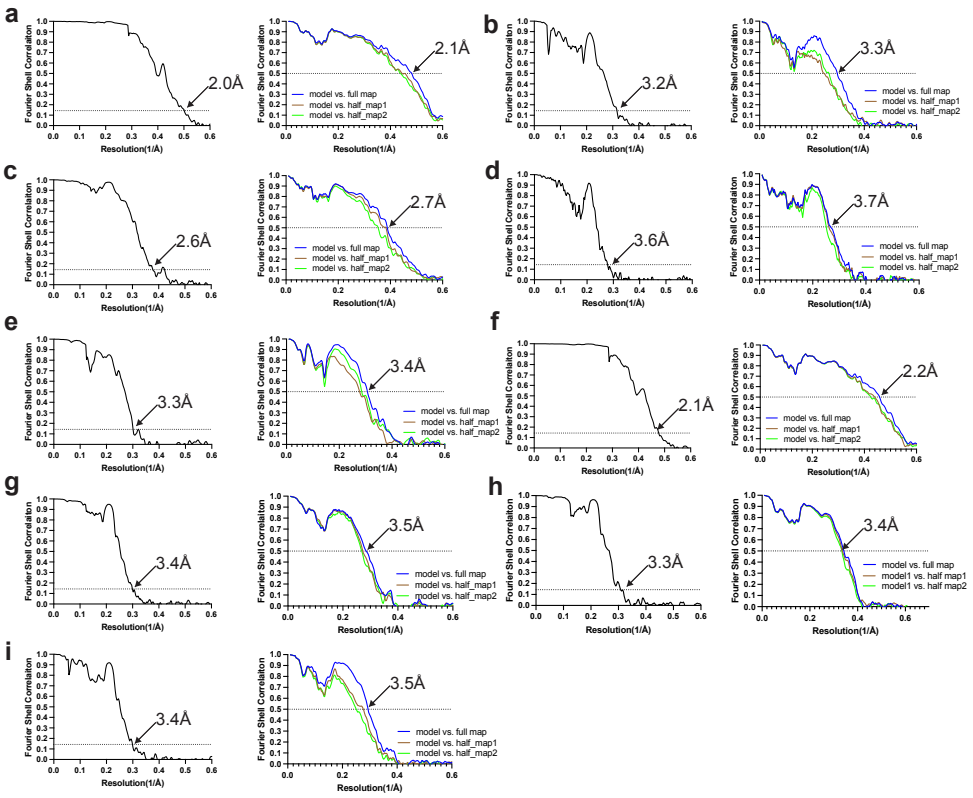
SUPPLEMENTARY FIGURES



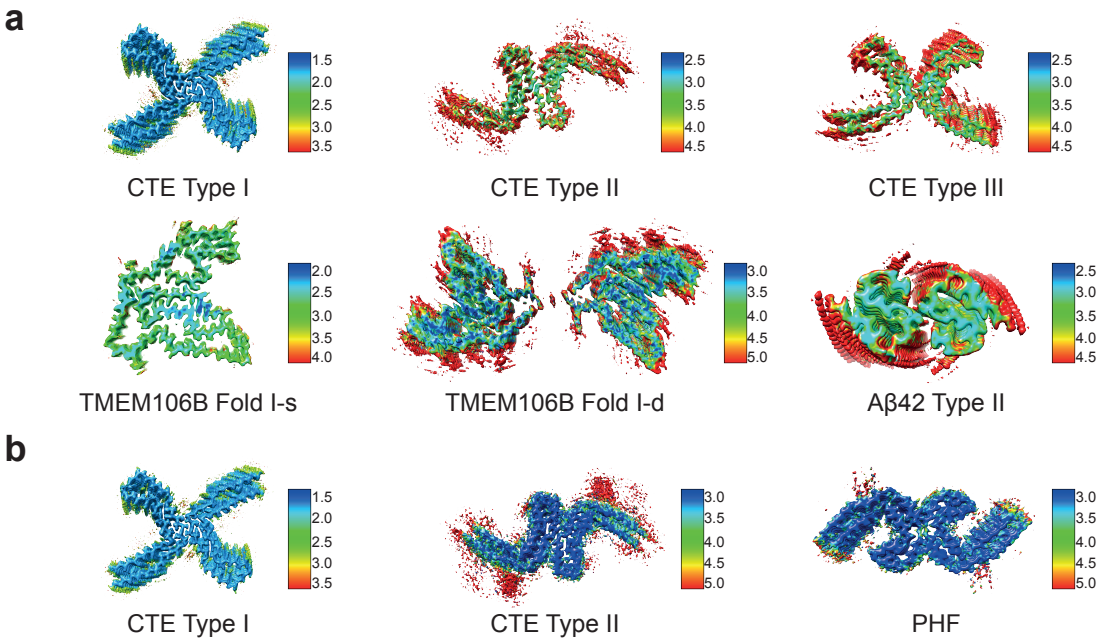
Supplementary Figure 1: Two-dimensional classification of filaments from Guam (a) and Kii (b) ALS/PDC.
Scale bar, 10 nm.



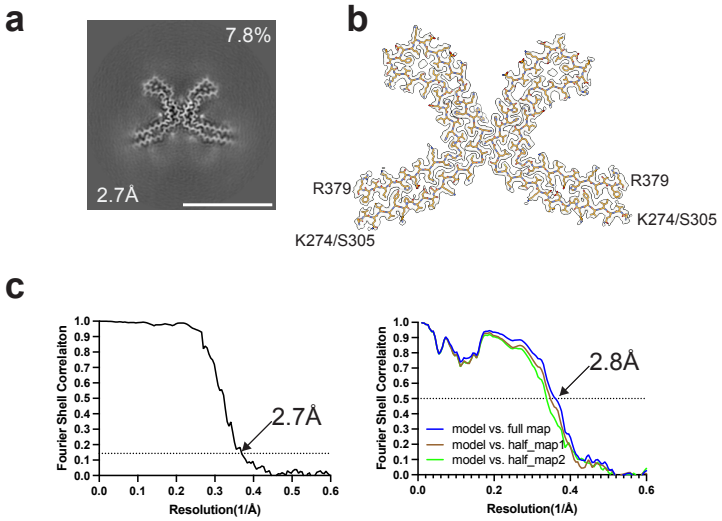
Supplementary Figure 2: Cryo-EM density maps of filaments from Guam and Kii ALS/PDC.
(a), In Guam ALS/PDC, Type I, Type II and Type III tau filaments (green), singlets and doublets of TMEM106B filaments (fold I) (purple) and Type II Aβ42 filaments (cyan) were present.
(b), In Kii ALS/PDC, Type I and Type II tau filaments (green), as well as tau paired helical filaments (PHFs) (blue), were present.



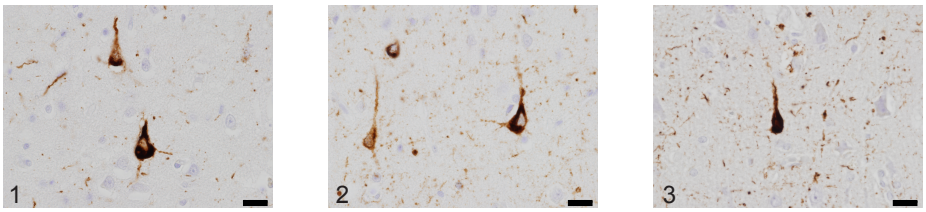
Supplementary Figure 3: Fourier shell correlation (FSC) curves. FSC curves of cryo-EM maps (left panel) and model to map validation (right panel). **(a)**, Guam ALS/PDC CTE tau Type I. **(b)**, Guam ALS/PDC CTE tau Type II. **(c)**, Guam ALS/PDC TMEM106B fold I-s. **(d)**, Guam ALS/PDC TMEM106B fold I-d. **(e)**, Guam ALS/PDC CTE tau Type II Aβ42. **(f)**, Kii ALS/PDC CTE tau Type I. **(g)**, Kii ALS/PDC CTE tau Type II. **(h)**, Kii ALS/PDC tau PHF. **(i)**, Guam ALS/PDC CTE tau Type III.



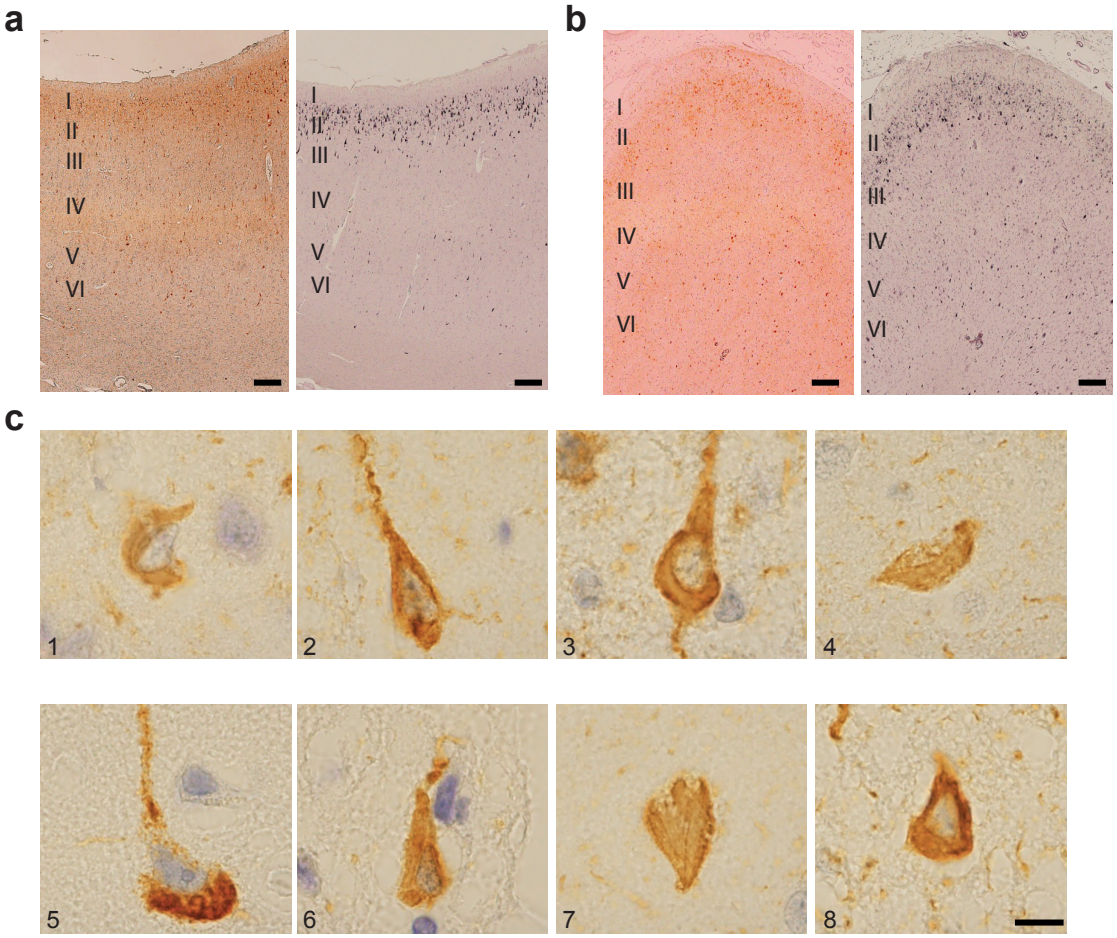
Supplementary Figure 4: Local resolution estimation of filaments from Guam (a) and Kii (b) ALS/PDC.



Supplementary Figure 5: Type III filaments from CTE.
(a), Cross-section perpendicular to the helical axis of the cryo-EM structure of Type III filaments from temporal cortex of CTE case 2 (30), with a projected thickness of approximately one rung along the helical axis. Scale bar, 10 nm. **(b)**, Cryo-EM density map and model of Type III filament. **(c)**, Fourier shell correlation curves of cryo-EM maps of Type III filaments (left panel) and model to map validation (right panel).



Supplementary Figure 6: Immunostaining of tau inclusions from Guam ALS/PDC.
Sections from the frontal cortex of cases 1-3 stained with anti-tau antibody AT8. Scale bar, 20 μm.



Supplementary Figure 7: Immunostaining of tau inclusions from Kii ALS/PDC.
(a,b), Temporal cortex from cases 3 and 4 stained with anti-tau antibody AT8 (left) and with Gallyas-Braak silver (right). Tau inclusions are concentrated in layers II/III. Scale bar, 200 μm.
(c), Sections from the temporal cortex of cases 1-8 stained with AT8. Scale bar, 10 μm.

SUPPLEMENTARY TABLES

Supplementary Table 1: Cases of ALS/PDC.

	Case number	Sex	Age at death (year)	Duration of illness (year)	Clinical subtype
Guam	1	M	68	11	PDC
	2	M	73	13	PDC
	3	F	73	9	PDC
Kii	1	F	63	5	ALS
	2	M	77	2	ALS
	3	F	60	9	PDC
	4	F	70	12	PDC
	5	F	76	1	ALS
	6	F	74	14	PDC
	7	M	74	7	PDC
	8	F	71	14	PDC

*Guam cases (1-3) have not been published before. Kii cases 1-8 correspond to cases 1,8,9,10,7,14,15 and 12 in (11).

Supplementary Table 2: Cryo-EM data collection, refinement and validation statistics for Guam ALS/PDC.

	Guam Case3 spinal cord		Guam Case1 frontal cortex		Guam Case2 frontal cortex	
Data collection						
Microscope	Titan Krios		Titan Krios		Titan Krios	
Voltage (kV)	300		300		300	
Detector	Falcon4		Falcon4		Falcon4	
Magnification	96,000		96,000		96,000	
Electron exposure (e–/Å^2)	40		40		40	
Defocus range (μm)	-1.0 to -2.0		-1.0 to -2.0		-1.0 to -2.0	
Pixel size (Å)	0.824		0.824		0.824	
Data processing	CTE TypeI	CTE TypeIII	CTE type II	TMEM106B Fold I-s	TMEM106B Fold I-d	Aβ42 TypeII
Box size (pixel)	400	400	400	400	400	400
Symmetry imposed	C1	C1	C1	C1	C1	C2
Initial particle images (no.)	256,999		130,240		281,921	
Final particle images (no.)	140,124	13,456	3,105	57,802	11,131	20,363
Map resolution (Å) FSC threshold 0.143	2.0	3.4	3.2	2.6	3.6	3.3
Helical rise (Å)	2.37	4.74	2.38	4.81	4.79	4.79
Helical twist (°)	179.41	-1.19	179.4	-0.4	-0.42	-2.99
Refinement						
Model resolution (Å) FSC threshold 0.5	2.1	3.5	3.3	2.7	3.7	3.4
Map sharpening *B* factor (Å^2)	-26	-54	-30	-28	-69	-43
Model composition						
Non-hydrogen atoms	2870	3444	3444	4340	8680	1356
Protein residues	375	450	450	540	1080	186
Ligands	0	0	0	0	0	0
B factors (Å^2)						
Protein	104.2	196.3	312.4	141.1	222.2	156.8
R.m.s. deviations						
Bond lengths (Å)	0.004	0.007	0.0056	0.0056	0.0071	0.0073
Bond angles (°)	1.006	1.541	1.11	1.367	1.537	1.369
Validation						
MolProbity score	0.95	2.06	1.39	2.21	2.54	2.17
Clashscore	0.51	3.56	1.99	4.15	5.19	4.71
Poor rotamers (%)	1.52	6.82	1.52	4.76	5.95	4.35
Ramachandran plot						
Favored (%)	97.26	95.89	95.89	91.73	84.21	93.1
Allowed (%)	2.74	4.11	4.11	8.27	15.79	6.9
Disallowed (%)	0	0	0	0	0	0
PDB	8OT6	8OT9	8OTC	8OTD	8OTE	8OTF
EMDB	EMD-17171	EMD-17173	EMD-17174	EMD-17175	EMD-17176	EMD-17177

Supplementary Table 3: Cryo-EM data collection, refinement and validation statistics for Kii ALS/PDC and CTE.

	Kii Case5			CTE Case2
Data collection				
Microscope	Titan Krios			Titan Krios
Voltage (kV)	300			300
Energy filter slit (eV)	20			20
Detector	K3			Falcon4
Magnification	105,000			96,000
Electron exposure (e–/Å^2)	40			40
Defocus range (μm)	-1.0 to -2.0			-1.2 to -2.4
Pixel size (Å)	0.826			0.824
Data processing	CTE typeI	CTE typeII	PHF	CTE Type III
Box size (pixel)	400	400	400	256
Symmetry imposed	C1	C1	C1	C1
Initial particle images (no.)	414,439			538,420
Final particle images (no.)	181,144	33,337	39,424	41,955
Map resolution (Å) FSC threshold 0.143	2.1	3.4	3.3	2.7
Helical rise (Å)	2.39	2.39	2.38	4.78
Helical twist (°)	179.39	179.36	179.45	-1.14
Refinement				
Model resolution (Å) FSC threshold 0.5	2.2	3.5	3.4	2.8
Map sharpening *B* factor (Å^2)	-31	-84	-73	-55
Model composition				
Non-hydrogen atoms	2870	2870	4109	3444
Protein residues	375	375	539	450
Ligands	0	0	0	0
B factors (Å^2)				
Protein	101.6	247.6	238.6	199.6
R.m.s. deviations				
Bond lengths (Å)	0.0063	0.0065	0.0067	0.0103
Bond angles (°)	0.91	1.378	1.593	1.517
Validation				
MolProbity score	1.3	2.15	2.53	1.81
Clashscore	1.02	8.88	7.78	3.27
Poor rotamers (%)	3.03	1.52	4.48	5.30
Ramachandran plot				
Favored (%)	97.26	90.41	87.62	97.26
Allowed (%)	2.74	9.59	12.38	5.30
Disallowed (%)	0	0	0	0
PDB	8OTG	8OTH	8OTJ	8OTI
EMDB	EMD-17178	EMD-17179	EMD-17181	EMD-17180