

SUPPLEMENTARY INFORMATION

Cryo-EM Structures Reveal Tau Filaments from Down Syndrome Adopt Alzheimer's Disease Fold

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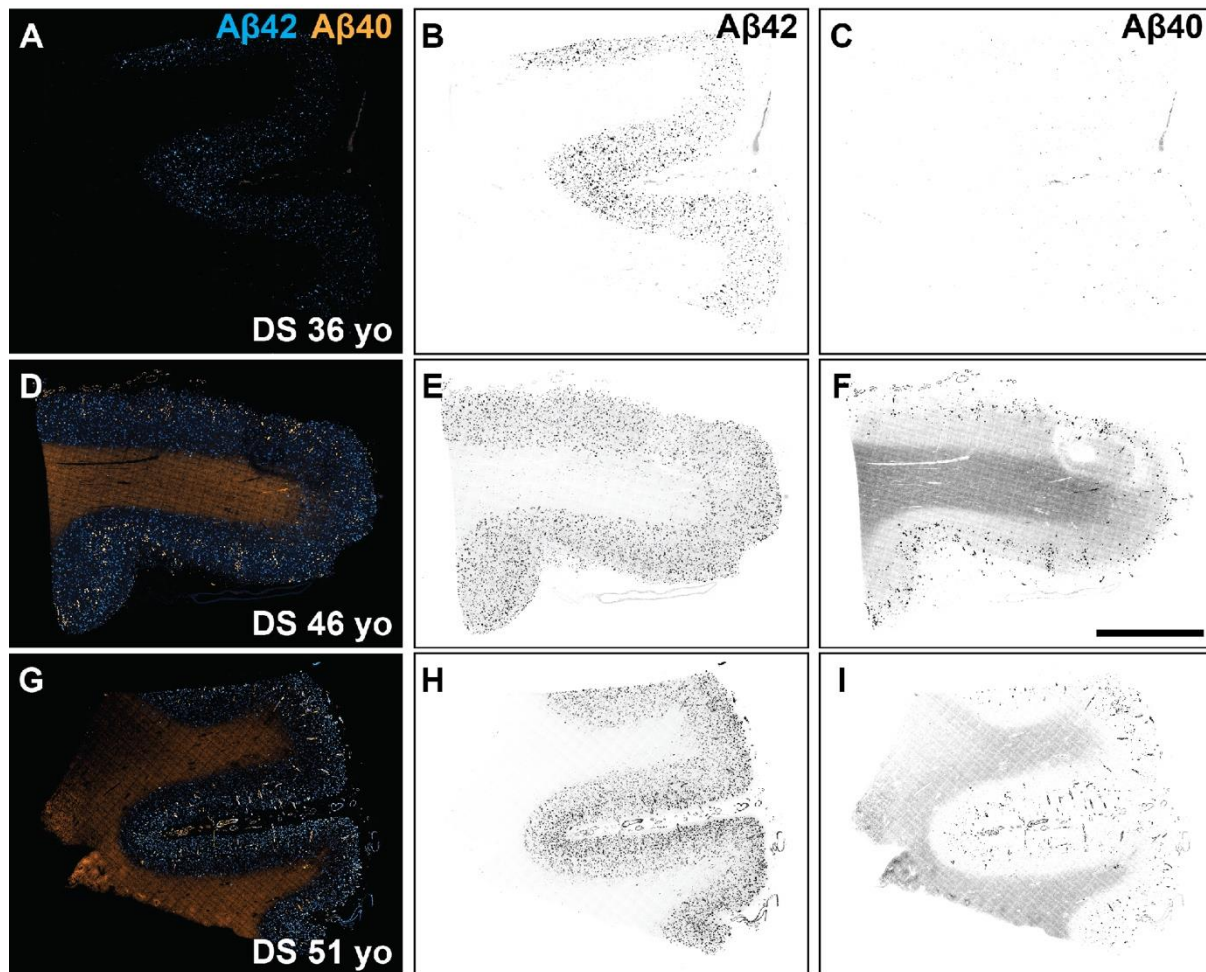
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KEY WORDS: Down syndrome, Alzheimer's disease, tauopathy, protein conformation, cryo-electron microscopy

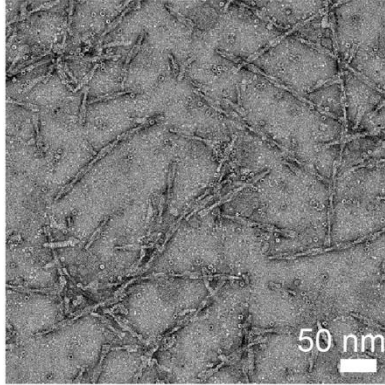
Supplementary Figure S1



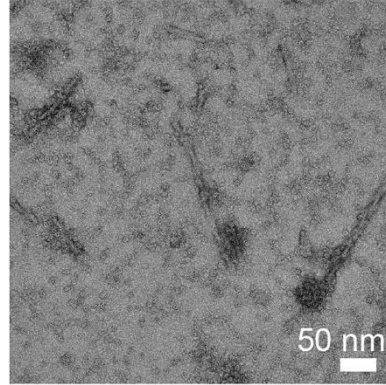
Supplementary Figure S1: Fluorescence images of immunolabeled A β 42 and A β 40 plaques in DS brain samples used for cryo-EM experiments. Formalin-fixed frontal cortex sections stained with A β isotype-specific antibodies against c-terminal length isoforms A β 42 (cyan) and A β 40 (orange) in DS case 6, 36 year old (A-C), DS case 3, 46 year old (D-F), and DS case 2, 51 year old (G-I). Individual fluorescence channels were converted to greyscale and inverted for A β 42 (B, E, H) and A β 40 (C, F, I). Scale bar = 2 mm.

Supplementary Figure S2

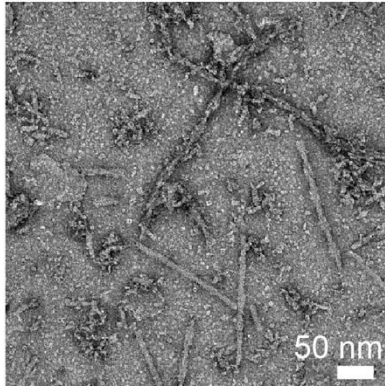
Case 1 (63y)



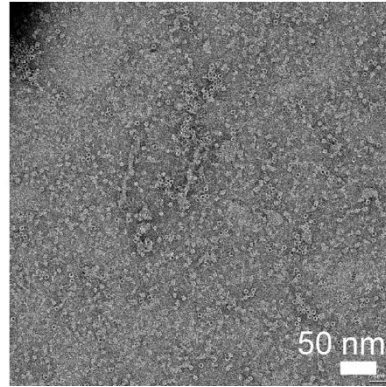
Case 2 (51y)



Case 3 (46y)

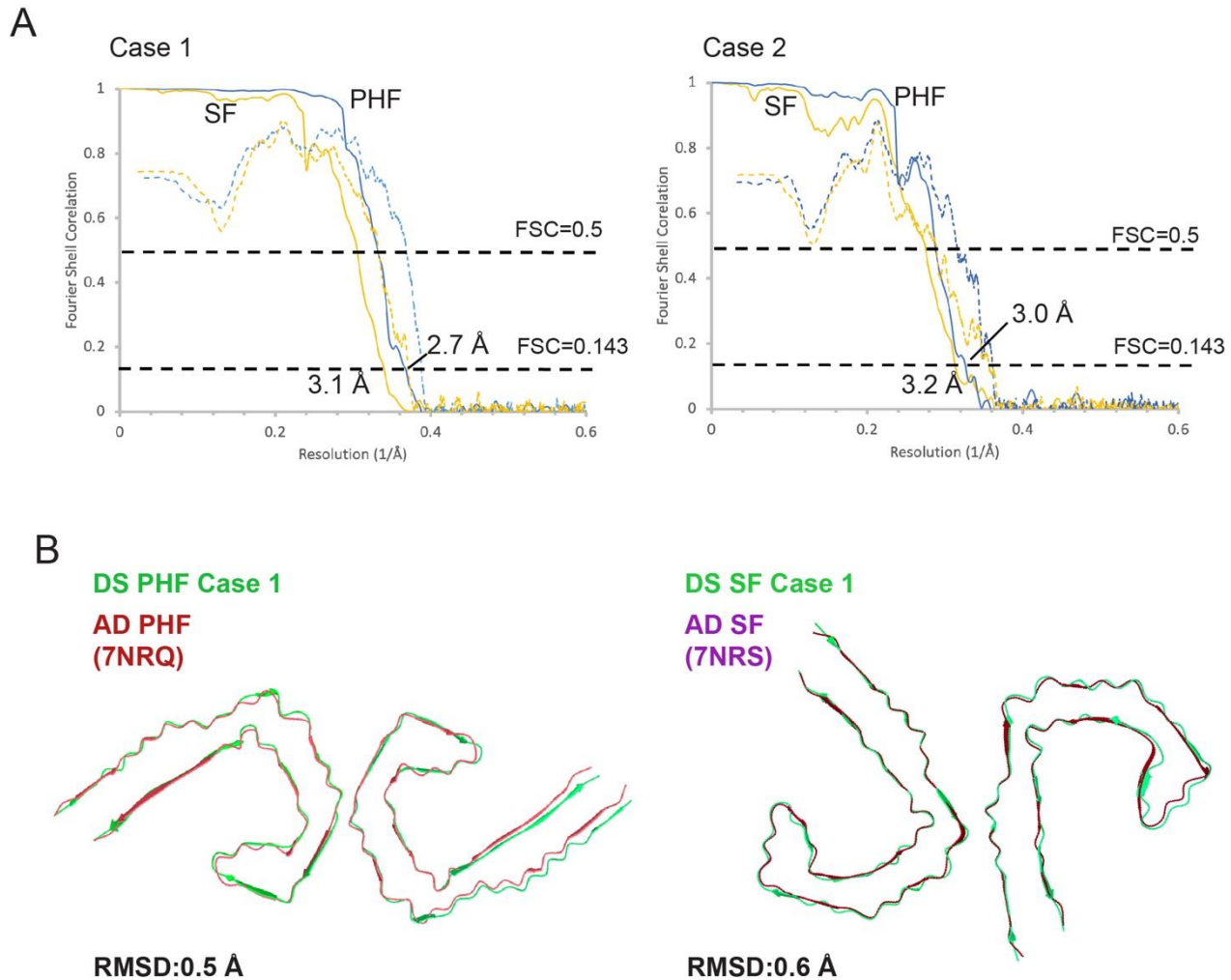


Case 4 (36y)



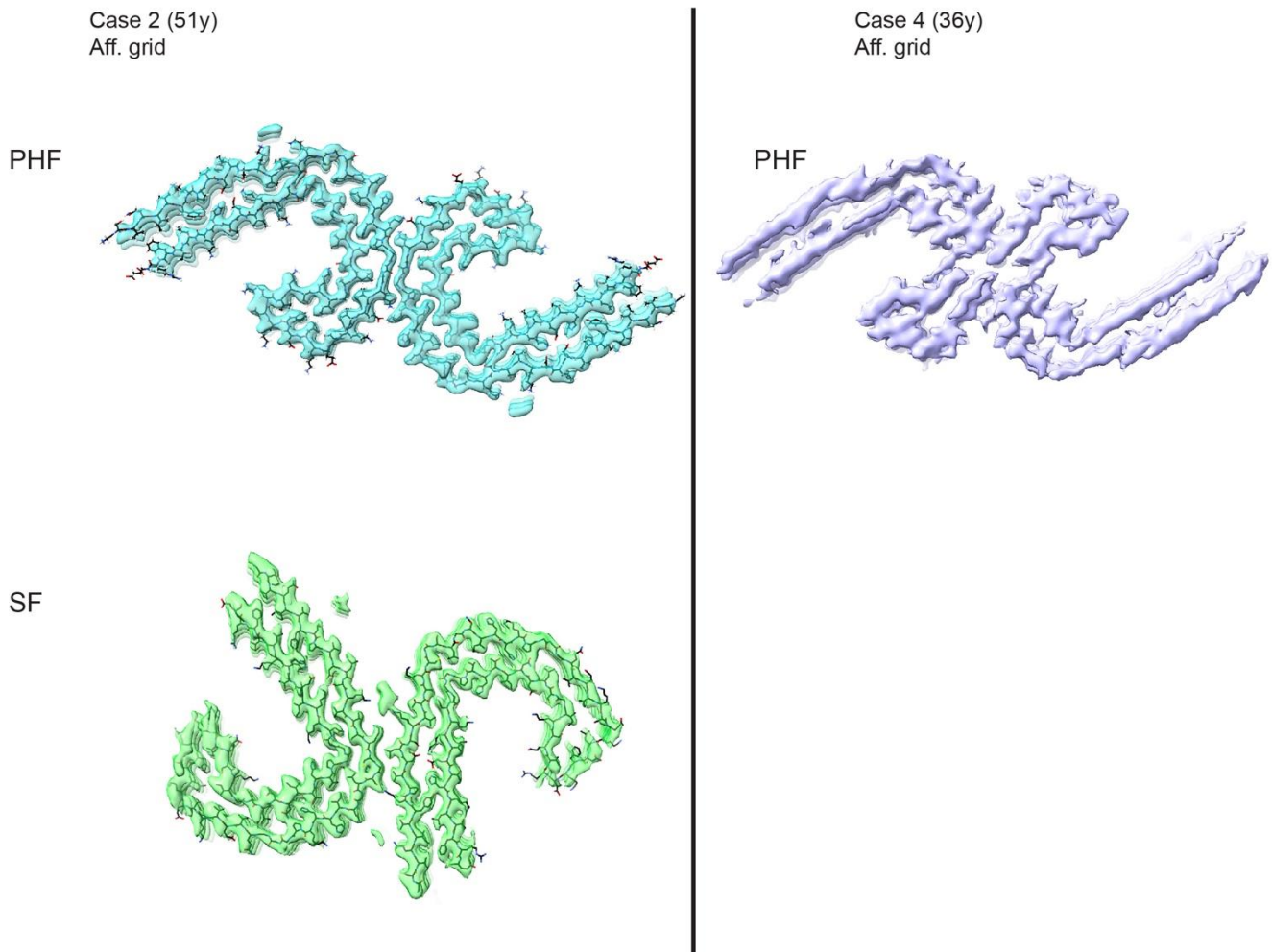
Supplementary Figure S2. Negative stain EM images of sarkosyl insoluble tau following isolation from frontal cortex of DS cases (indicated) to show fibrils and other background contaminants.

Supplementary Figure S3



Supplementary Figure S3. Cryo-EM structure analysis. (A) Fourier shell correlation (FSC) curves of two independently refined half maps for the PHF (Blue) and SF (Orange) showing resolution at FSC=0.143 and corresponding map vs. model curves (dashed) and FSC=0.5 line. (B) Overlay and α -carbon RMSD values for the PHF and SF structures from case 1 compared to published structures (PDB: 7NRQ and 7NRS, respectively).

Supplementary Fig. S4



Supplementary Figure S4. Final cryoEM refined maps of tau PHF and SF from data acquired using GO-AT8 affinity grids to isolate tau filaments. For DS case 2 both PHF and SF structures were solved to 3.0 and 3.2 Å resolution, respectively (left), and for case 4 the PHF map was solved to 5 Å resolution (right).

Brain Bank	ID	Disease	Region	UMAP name	Age at death	Sex	APOE	Postmortem interval (hours)	Xtau neuropath score	XA β neuropath score
KCL	A035/03	AD	Frontal cortex	AD 1	59	F	ND	36	4	4
KCL	A061/03	AD	Frontal cortex	AD 2	55	M	ND	18	4	4
OXF	NP177-2013	AD	Frontal cortex	AD 3	90	F	$\epsilon 3/\epsilon 3$	48	4	3
UCI	14-08	AD	Frontal cortex	AD 4	86	M	$\epsilon 3/\epsilon 3$	4	2	2
UCI	37-15	AD	Frontal cortex	AD 5	87	F	$\epsilon 3/\epsilon 4$	4	2	3
UCI	4-02	AD	Frontal cortex	AD 6	83	M	$\epsilon 3/\epsilon 4$	3	4	3
BCN	907	DS	Frontal cortex	DS 1	63	M	$\epsilon 3/\epsilon 3$	6	4	3
BCN	714	DS	Frontal cortex	DS 2	36	F	$\epsilon 3/\epsilon 3$	12	2	2
UMD	4335	DS	Frontal cortex	DS 3	28	M	$\epsilon 4/\epsilon 4$	26	2	2
UMD	4870	DS	Frontal cortex	DS 4	51	F	$\epsilon 2/\epsilon 3$	4	4	4
UMD	5510	DS	Frontal cortex	DS 5	65	M	$\epsilon 3/\epsilon 3$	10	4	4
UMD	5600	DS	Frontal cortex	DS 6	57	M	$\epsilon 3/\epsilon 3$	6	4	3
UCI	29-06	DS	Frontal cortex	-	45	F	$\epsilon 3/\epsilon 3$	3	4	4
UCI	29-06	DS	Temporal cortex	-	45	F	$\epsilon 3/\epsilon 3$	3	-	-
UCI	3-17	DS	Frontal cortex	-	57	M	$\epsilon 3/\epsilon 3$	4	3	4
UCI	3-17	DS	Temporal cortex	-	57	M	$\epsilon 3/\epsilon 3$	4	-	-
UCI	30-00	DS	Frontal cortex	-	61	M	$\epsilon 3/\epsilon 3$	11	4	4
UCI	30-00	DS	Temporal cortex	-	61	M	$\epsilon 3/\epsilon 3$	11	-	-
UCI	46-94	DS	Frontal cortex	-	62	F	$\epsilon 3/\epsilon 3$	3	4	4
UCI	46-94	DS	Temporal cortex	-	62	F	$\epsilon 3/\epsilon 3$	3	-	-
UCI	30-05	DS	Frontal cortex	-	57	F	$\epsilon 3/\epsilon 3$	3	4	4
UCI	30-05	DS	Temporal cortex	-	57	F	$\epsilon 3/\epsilon 3$	3	-	-

Supplementary Table S2: Fixed postmortem donor tissues used for EMBER analysis.

	Case 1		Case 2		Case 3		Case 4	
Data collection	PHFs	SFs	PHFs	SFs	PHFs	SFs	PHFs	CTE/SSPE-II-like
Magnification	x105,000	x105,000	x105,000	x105,000	x105,000	x105,000	x105,000	x105,000
Defocus range (mm)	-0.8 to -1.8	-0.8 to -1.8	-0.8 to -1.8	-0.8 to -1.8	-0.8 to -1.8	-0.8 to -1.8	-0.8 to -1.8	-0.8 to -1.8
Voltage (kV)	300	300	300	300	300	300	300	300
Microscope	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios
Detector	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3
Frame exposure time (s)	2.024	2.024	2.024	2.024	2.024	2.024	2.024	2.024
Dose rate (e-/physical pixel/sec)	16	16	16	16	16	16	16	16
Total dose (e-/Å ²)	46	46	46	46	46	46	46	46
Pixel size (Å)	0.834	0.834	0.834	0.834	0.834	0.834	0.834	0.834
Movies collected	9160		5902		6949		1293	
Grids & sample	Conventional grids & Pronase		Affinity grid & no Pronase		Conventional grid & no Pronase		Affinity grid & no Pronase	
Reconstruction								
Box size (pixel)	280	280	280	280	280	280	280	280
Inter-box distance (Å)	17	17	17	17	17	17	17	17
Total segments*	1127462	329537	7488898	287943	271767	86388	197928	17473
Final Particles (no.)	215640	47801	79599	26964	47264	35177	32831	N/A**
Resolution (Å)	2.7	3	3.1	3.2	2.9	3.1	5	7.8
B-factor (Å ²)	-83.71	-81.34	-89.69	-71.29	-68.79	-90.05	-262.95	-415.37
Helical rise (Å)	2.39	4.81	2.37	4.77	2.4	4.81	2.3	2.38
Helical twist (°)	179.45	-1.08	179.5	-1.04	179.48	-1.06	179.45	179.55
*after initial 2D classification								
**no subsequent classification								

Supplementary Table S3: Cryo-EM data collection and structure determination.

Model	Case 1		Case 2	
	PHFs	SFs	PHFs	SFs
model resolution	2.7	3.0	3.1	3.5
atoms	35910	39501	40698	39513
Residues	2310	2541	2618	2541
Bonds (RMSD)				
Length (Å) (# > 4 σ)	0.010 (0)	0.010 (0)	0.010 (0)	0.011 (0)
angles (°) (# > 4 σ)	1.717 (92)	1.791 (70)	1.871 (38)	1.914 (95)
MolProbity score	2.21	2.32	2.53	2.63
Clash score	18.87	32.66	40.97	46.84
Ramachandran plot (%)				
Outliers	0.00	0.00	0.00	1.33
Allowed	6.67	4.69	6.67	6.67
Favored	93.33	95.31	93.33	92
Rotamer outliers (%)	0.00	0.00	0.00	0.00

Supplementary Table S4: Cryo-EM Model Building.