Cryo-EM studies of the structure of Alzheimer's Tau-amyloid filaments elucidate the disease nature of PCA and suggest novel approaches of *in vitro* filament generation.

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A1. Introduction

One of the key characteristics of most neurodegenerative diseases is the build-up of abnormal protein filaments in the brain. In a diverse range of neuropathies, from Alzheimer's to CTE to Pick's disease, these filaments are formed of Tau protein aggregations. Different disease types have different filament structures (Figure 1), suggesting reasons for their slightly different clinical presentations, but also that they form in different ways.

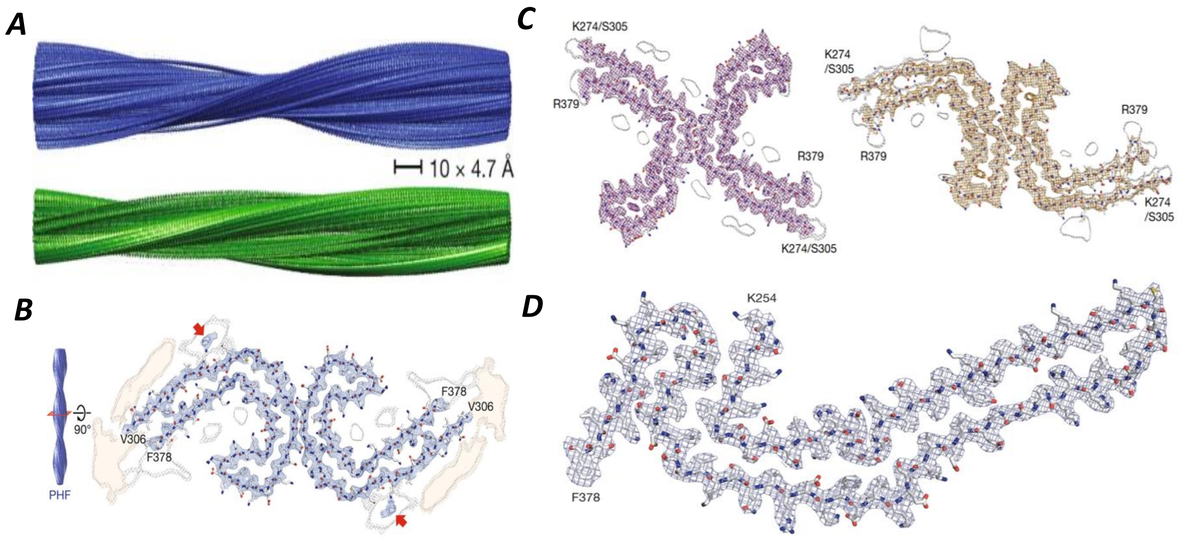

Figure 1: A comparison of the Tau filament structures of (A)(B) Alzheimer's (blue: PHF; green: SF) [1], (C) CTE[2], (D) Pick's disease[3].

A2. Posterior Cortical Atrophy structure

Posterior Cortical Atrophy (PCA) was thought to be a subtype of Alzheimer's, but its filament structure was unknown. In this study cryo-EM images of filament purified brain tissue from PCA presenting patients were collected and analysed (see table 1).

Magnification	x 105,000		Box Size (Å)	280
Defocus (μm)	-1 to -3		Extracted segments	781, 876
Microscope, camera	Krios, K2		Segments in model	80, 541
Electron dose ($e^-/Å^{-2}$)	60		Resolution (Å)	3.45
Pixel size (Å)	1.15		Helical rise (Å)	2.36
Frame Exposure (s)	0.2		Helical twist (°)	179.4

Table 1: PCA data collection (left column) and model reconstruction (right column) statistics.

Paired-Helical Filaments (PHF) and Straight Filaments (SF) were found with occupance of about 85% and 15% respectively, matching the ratio found in studies of classical Alzheimer's. The structure of the PHF was solved to 3.4 Å, but the small dataset for the SFs limited their model's resolution to around 6 Å (Figure 2). The structures and data collected here support the view that PCA is a subtype of Alzheimer's disease.

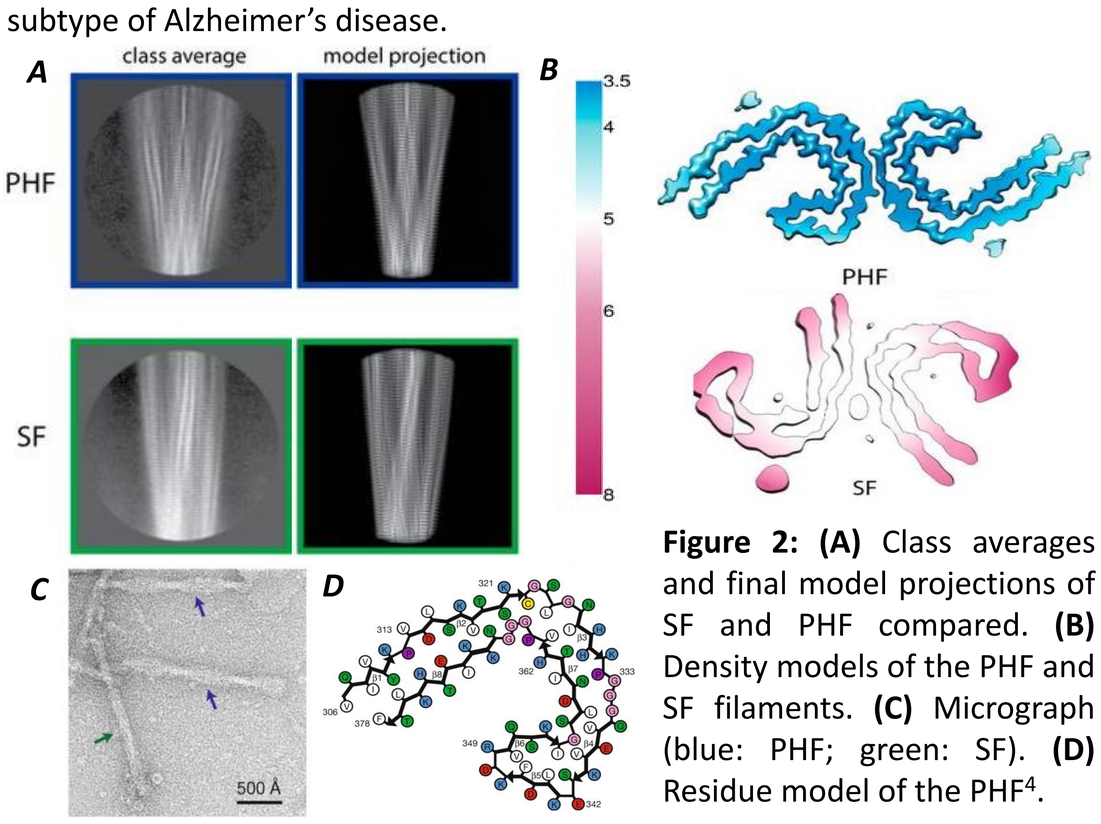

Figure 2: (A) Class averages and final model projections of SF and PHF compared. **(B)** Density models of the PHF and SF filaments. **(C)** Micrograph (blue: PHF; green: SF). **(D)** Residue model of the PHF[4].

B2. Introduction

Due to the lack of available brain tissue, filaments made *in vitro* are often studied as models of a wide variety of neurodegenerative diseases. However, in vitro filament formation has recently been shown to generate highly non-brain-typical Tau filaments (Figure 3). Thus *in vitro* models can no longer be considered as good models for neuropathies, making their study highly challenging. There is thus a need to develop methods of artificial filament formation that forms natural-type filaments.

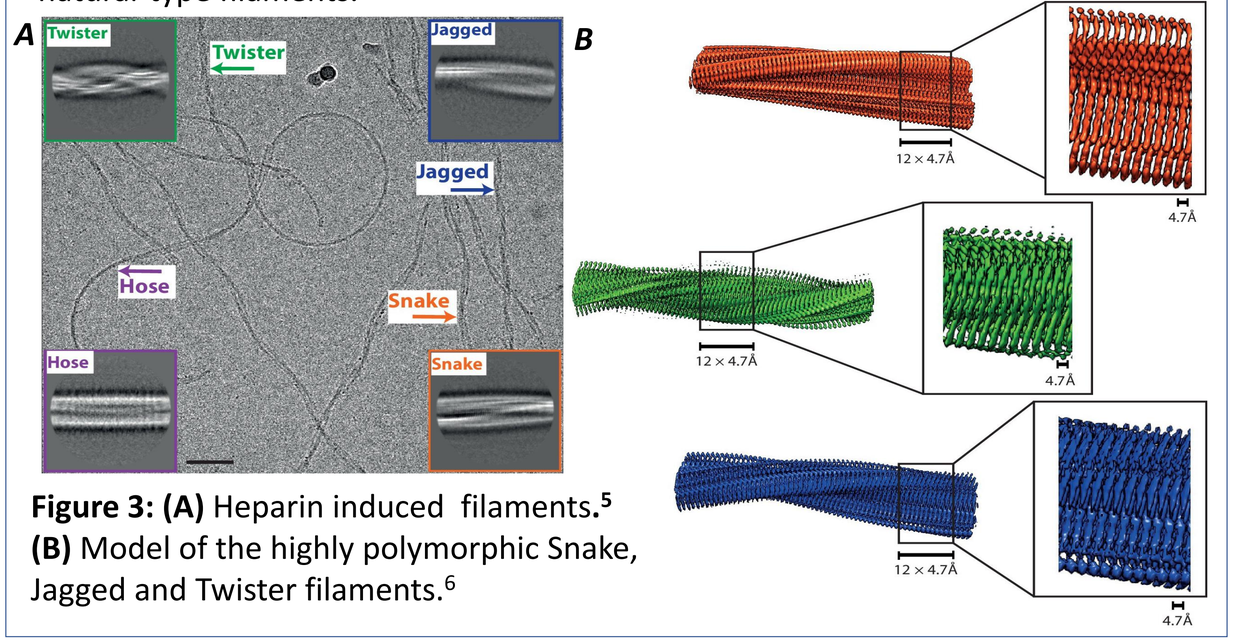

Figure 3: (A) Heparin induced filaments.[5] **(B)** Model of the highly polymorphic Snake, Jagged and Twister filaments.[6]

B2. In vitro filament formation using Arachidonic Acid

While the filaments for heparin induced aggregate structures have been shown to not resemble natural disease filaments there are a wide range of other methods of inducing filament formation. The CTE filament structure shows an as yet unidentified density within the centre of its C-shaped curl (Figure 4), suggesting a mechanism of filament formation via association and action of this small chemical. While arachidonic acid is definitely not the observed molecule, it was investigated whether it would behave in a similar way to induce filament formation. Unfortunately the cryo-EM dataset had a low contrast. However, filament shaped 2D class averages were observed and 6-8 Å 2D models were obtained with a good fit to the experimental data. Although these models showed a novel filament fold type, they are unique among artificially generated filaments in their distinctive C-shape (Table 2 and Figure 4).

Magnification	x 105,000		Box Size (Å)	190
Defocus (μm)	-1 to -3		Extracted segments	411, 282
Microscope, camera	Krios, K2		Segments in model	45, 691
Electron dose ($e^-/Å^{-2}$)	55		Resolution (Å)	6.2
Pixel size (Å)	0.85		Helical rise (Å)	2.34
Frame Exposure (s)	0.3		Helical twist (°)	179.2

Table 2: Arachidonic Acid supplemented data collection (left column) and initial model reconstruction (right column) statistics.

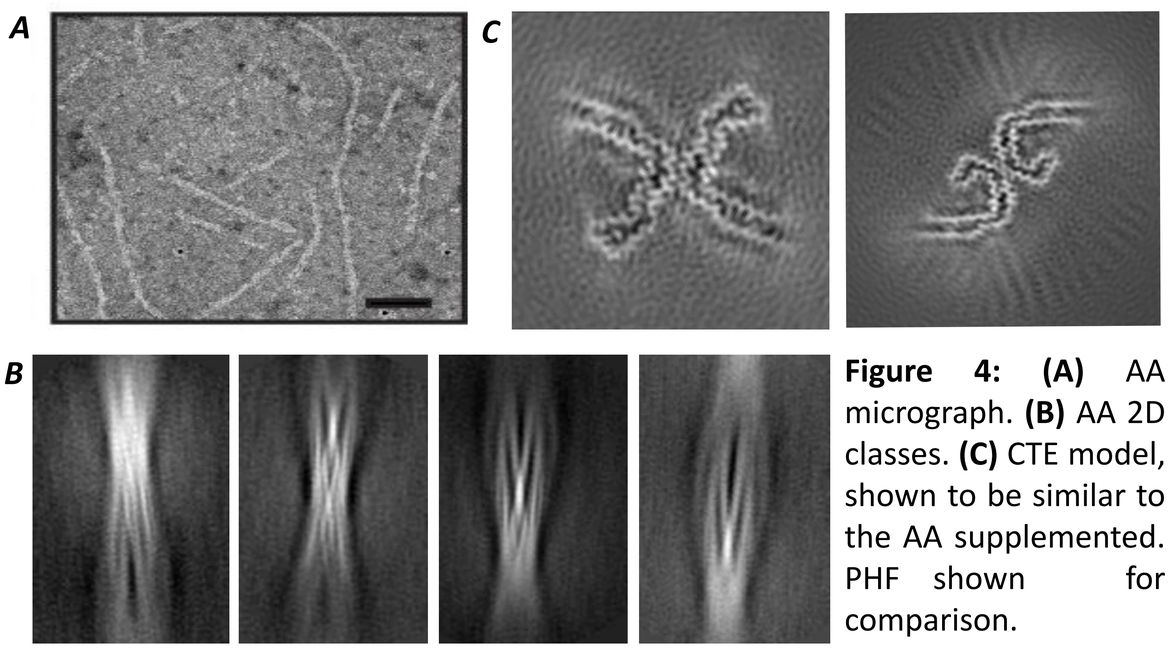

Figure 4: (A) AA micrograph. **(B)** AA 2D classes. **(C)** CTE model, shown to be similar to the AA supplemented. PHF shown for comparison.

References:

[1] A Fitzpatrick *et al. Nature* **547**: 1 (2017). 'Cryo-EM structures of tau filaments from Alzheimer's disease.'

[2] B Falcon *et al. Nature* **568**: 420 (2019). 'Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules.'

[3] B Falcon *et al. Nature* **561**: 137 (2018). 'Structures of filaments from Pick's disease reveal a novel tau protein fold.'

[6] Fitzpatrick *et al. Nature* **547**: 1 (2017). 'Cryo-EM structures of tau filaments from Alzheimer's disease (ext. data).'

[5] W Zhang *et al*. *eLife* 8:e43584 (2018). 'Heparin-induced tau filaments are polymorphic and differ from those in Alzheimer's and Pick's diseases.'

[6] W Zhang *et al*. *eLife* 8:e43584 (2018). 'Heparin-induced tau filaments are polymorphic and differ from those in Alzheimer's and Pick's diseases (ext. data).'

Perspective and Future Directions

This study elucidated a number of novel results:

- It was shown that PCA is a subtype of classical Alzheimer's, although with a slightly different clinical presentation.
- The PCA patient studied had filaments representative of both Alzheimer's and CTE, showing that neurodegenerative diseases can overlap and multiple neuropathies can be present in an individual.
- A novel potential approach to generate *in vitro* filaments has been shown; while it needs additional work to verify their exact shape, it suggests that this could be a better approach to artificially generate filaments.
- The likely positioning of arachidonic acid in the filament supports the view that small molecule binding to Tau can induce filament formation. A higher resolution structure is needed to confirm the placement of the acid.

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