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# α-Synuclein Amyloid Fibrils Formed of Two Protofibrils.

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α-Synuclein (αS) is a natively unstructured, 140 amino acid protein, expressed in the cytosol of neurons. Normally, it binds to and remodels membranes at the presynaptic terminal, becoming α-helical in the process [1 and 2]. Following a nucleation event, the protein polymerizes into amyloid fibrils that are the main component of intraneuronal protein aggregates called Lewy Bodies. The presence and location of Lewy Bodies correlates with the presence and clinical presentation of a series of chronic, neurodegenerative diseases including Parkinson's disease [3]. Due to the lack of prominent surface features and sample heterogeneity, structural information has been limited.

Recombinant αS was expressed, purified and assembled into fibrils, which were observed by cryo-EM on a Philips CM200-FEG microscope and by dark-field STEM of unstained frozen-dried specimens at the Brookhaven STEM facility. From the resulting cryo-EM images and STEM micrographs, segment-averages and 2D cross-sections were calculated, using *Bsoft* [4]. Mass-per-length measurements were made from the STEM data, using *PCmass32*.

In cryo-EM (Figure 1A), the majority of fibrils have a periodic undulation in width, 8.2 - 11 nm, over an axial repeat distance of approximately 77 nm, which we take to represent a rotation through 180°. In STEM (Figure 1B), fibrils are seen to have a pair of thin threads of heightened density that we interpret to be tightly bound metal ions scavenged from buffer during the protein purification. These threads oscillate between a maximum center-to-center separation of 5 nm and a minimum separation of 0 nm (crossover points, Figure 1B inset), with the same periodicity as was observed in cryo-EM. 2D reconstruction of fibrils from cryo-EM revealed two protofibrils with elongated cross-sections, each protofibril being ~ 7.5 nm by ~ 2.5 nm (Figure 2A). The protofibril can be further divided into two ellipsoidal sub-domains (Figure 2D). The protofibrils are asymmetrically disposed about the fibril axis, giving a fibril cross-section shaped like the Greek letter Nu (Figure 2A), implying a non-equivalent mode of association. Reconstruction from the STEM data showed the two thread-like features symmetrically disposed at a radius ~ 2.5 nm from the fibril axis (Figure 2B). After the two reconstructions were aligned, the threads localized near the middle of each protofibril (Figure 2C), at the interface of the two subdomains (Figure 2D). The STEM mass-per-length measurements yielded a mean linear density corresponding to two subunits per 4.7 Å axial rise, which indicates that each protofibril has one subunit per 4.7 Å axial rise (the inter-strand spacing in cross-β structures). This value is consistent with a parallel superpleated β-structure [6]. These data, taken in conjunction with pre-existing data from other sources, form a basis for formulating specific cross-β models for these amyloid fibrils. [7]

References:
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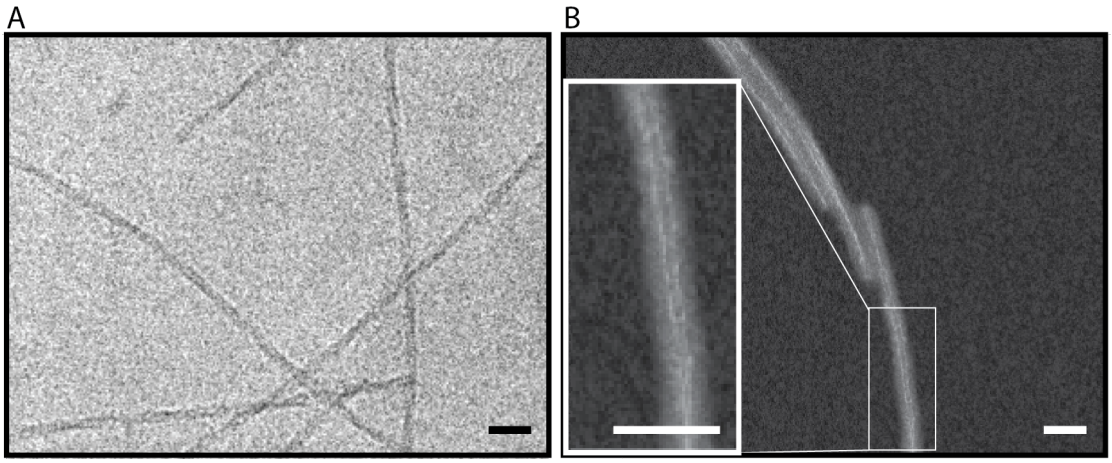


Figure 1: Micrographs of α Synuclein fibrils. Cryo-EM (A) and STEM (B). (B) Inset, magnified region with entwined thread-like feature. Scale bars are 30 nm.

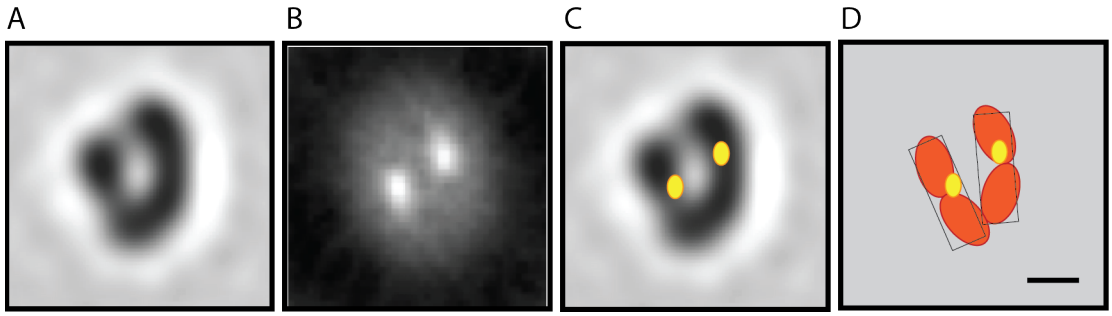


Figure 2: Cross-sectional density maps of α Synuclein fibrils. Cryo-EM (A) and STEM (B) 2D reconstructions, showing ν-shaped cross-section (A) and high-density thread positions (B), respectively. (C) Localization of high-density thread positions (yellow ovals) in the fibril cross-section. (D) Interpretive diagram of fibril cross-section formed of two protofibrils (black rectangles), each with two subdomains (orange ovals) and a high-density thread (yellow ovals). Scale bar is 4 nm.