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α Synuclein Amyloid Fibrils With Two Entwined Protofibrils Each With One Subunit Per 4.7 Å Axial Rise.

Altaira D. Dearborn[1], Naiqian Cheng[1], J. Bernard Heymann[1], Jobin Varkey[2], Ralf Langen[2], and Alasdair C. Steven[1].

[1.] Laboratory of Structural Biology Research, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, USA.
[2.] Zilkha Neurogenetic Institute, University of Southern California, Los Angeles, USA.

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disease affecting motor function. PD is characterized by dopaminergic neuronal cell death, particularly in the substantia nigra pars compacta, and by the presence of Lewy bodies. α Synuclein (αS) fibrils are the main component of Lewy bodies, and previous research suggests that its fibrillation is part of the disease pathology. Normally, the 140 aa long protein has a membrane remodeling function in the presynaptic terminal [1]. It is alpha-helical when associated with lipid and a random coil in the cytosol [2]. In fibril formation, the protein polymerizes into a cross-beta structure [3]. Owing to the lack of prominent surface features and potential heterogeneity within the sample, high-resolution structural information has been elusive.

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 at the Brookhaven STEM facility [4]. The resulting cryo-EM images were processed using *Bsoft*. STEM measurements were made using PCmass32 and fit to a Gaussian distribution. To investigate internodal distance in real space, we looked closely at the modest variation in fibril width. To reduce noise, each fibril was segmented and those segments were averaged, using various segment lengths (112 nm and 140 through 169 nm). Average images of lengths at or near the node periodicity exhibit regular broadening of the fibril width between nodes with higher contrast than the original fibril. Fibril width was measured from 76 Å-long segments, and plotted as a function of position along the fibril.

Fibrils assembled *in vitro* (Figure 1A) are generally similar in morphology to fibrils isolated from diseased human tissue; they are roughly 8 to10 nm in diameter and more or less straight [5]. We noted a faint long-range periodicity along the fibrils, both in the cryo-micrographs (Figure 1A) and in the STEM data (Figure 1B). Mass-per-length measurements made from the latter data gave a unimodal distribution with a mean density equivalent to two subunits per 4.7 Å axial rise (Figure 1C). The cryo-EM images exhibit a periodic thinning and thickening with an inter-node distance of about 75 nm. This suggests that the fibril is in fact a pair of protofibrils wrapping around a common axis. This inference is strongly supported by STEM images that show an unusual feature in the form of two thin threads of enhanced density. This enhanced density is likely to be a small amount of specifically bound residual salt – not enough to affect the mass-per-length data significantly but enough to clearly delineate the two-stranded nature of the fibril (Figure 1B). It follows that each protofibril has one subunit per 4.7 Å axial rise consistent with a parallel superpleated β-structure [6]. The diffraction patterns of some fibrils contained reflections indicative of the regular periodicity (Figure 1D). Because the periodicity is of long-range, it cannot be measured precisely from the position of the reflection; it is, however, between 70 and 80 nm, consistent with the direct estimates of the inter-node spacing. Our ongoing analysis is directed toward determining this repeat distance as precisely as possible by real-space methods (Figure 1E & F) [7].

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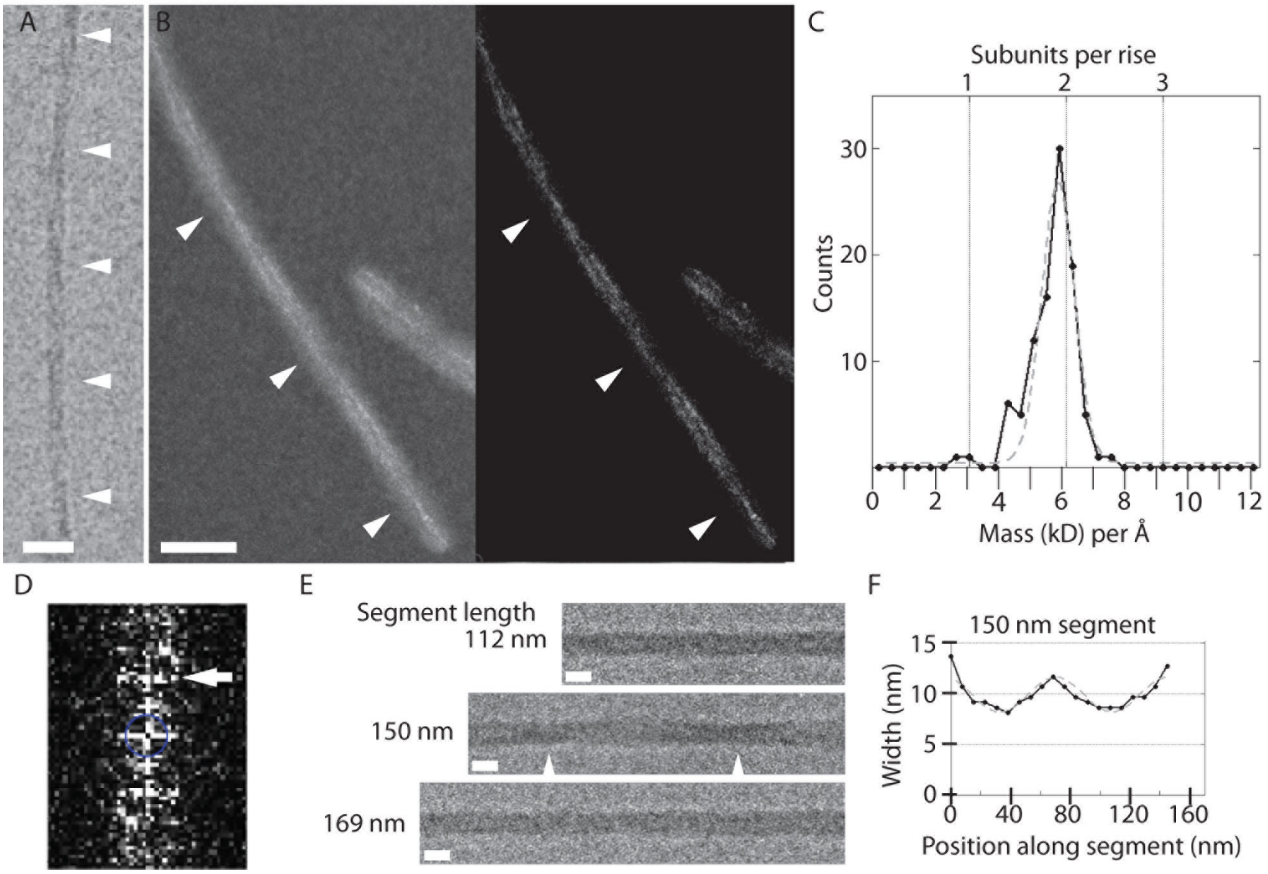

Figure 1: Helical twist of α Synuclein fibrils. Cryo EM (A) and STEM (B) of α Synuclein fibrils, scale bars equal 30 nm and arrowheads indicate nodes. STEM image shown at two levels of contrast. (C) Histogram of STEM subunits per length measurements and Gaussian fit (grey line) centered at 1.92 ± 0.17 subunits per 4.7Å of rise (n = 96). (D) Single fibril diffraction pattern, arrow indicates position of a layer line at 72 ± 6 nm. (E) Average images of segments of lengths as labeled, scale bars equal 10 nm and arrowheads indicate nodes (F) Width of averaged fibril segments as a function of position along that average image, fit to a sine curve (grey line).

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