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Electron Cryo-Microscopy of Amyloid-β Fibrils

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The amyloid-β (Aβ) fibrils formed by Aβ(1-40) and Aβ(1-42) peptides, which are associated with Alzheimer's disease, have eluded X-ray diffraction structure determination since the 1960's. Because of this, our understanding of the structural bases for fibril formation and aggregation has been significantly hindered. Fifty years on, this paucity of direct structural data has become an unwelcome hurdle in our attempts to understand pathologies that involve protein misfolding and/or aggregation into amyloid fibrils.

Electron microscopy of unstained, vitreous ice-embedded specimens (cryo-EM; Fig. 1A) followed by micrograph image processing makes three-dimensional (3D) structure determination possible for specimens which are not amenable to other structural biology techniques such as X-ray crystallography or solution-state NMR. Cryo-EM presents the advantage that small quantities of relatively low-concentration samples are sufficient for structure determination, and recent advances in image processing techniques have started to relieve the strict requirement for a homogenous sample.

Our group has used these techniques to characterize the structure of fibrils formed by Aβ(1-40) and Aβ(1-42) [1-3]. These studies provide direct 3D insights into the polymorphism of fibrils in solution [2], and suggest intriguing structural similarities between seemingly disparate fibril structures (Fig. 1) [3].

However, current methods are limited in one crucial aspect: the attainable resolution is only ~8 Å in the most favorable cases [1]. This is in contrast with the quality of the cryo-EM images themselves, which demonstrably contain structural information up to at least 4.7 Å (Fig. 2B). This discrepancy calls for an improvement in image processing methods.

The main challenge in analyzing cryo-EM images is to accurately align numerous copies of identical protein assemblies so that they may be averaged together, thereby mitigating the noise level in the micrographs – inaccurate alignments of images fundamentally limit the attainable 3D resolution. Images of Aβ(1-40) or Aβ(1-42) fibrils are particularly challenging in this respect because of the low molecular weight (and thus electron scattering) of any given section of a fibril. We are overcoming this intrinsic limitation by developing image alignment methods tailored to these types of filaments. We will describe results using our optimized image processing methods for structure determination of filamentous amyloid assemblies.

References

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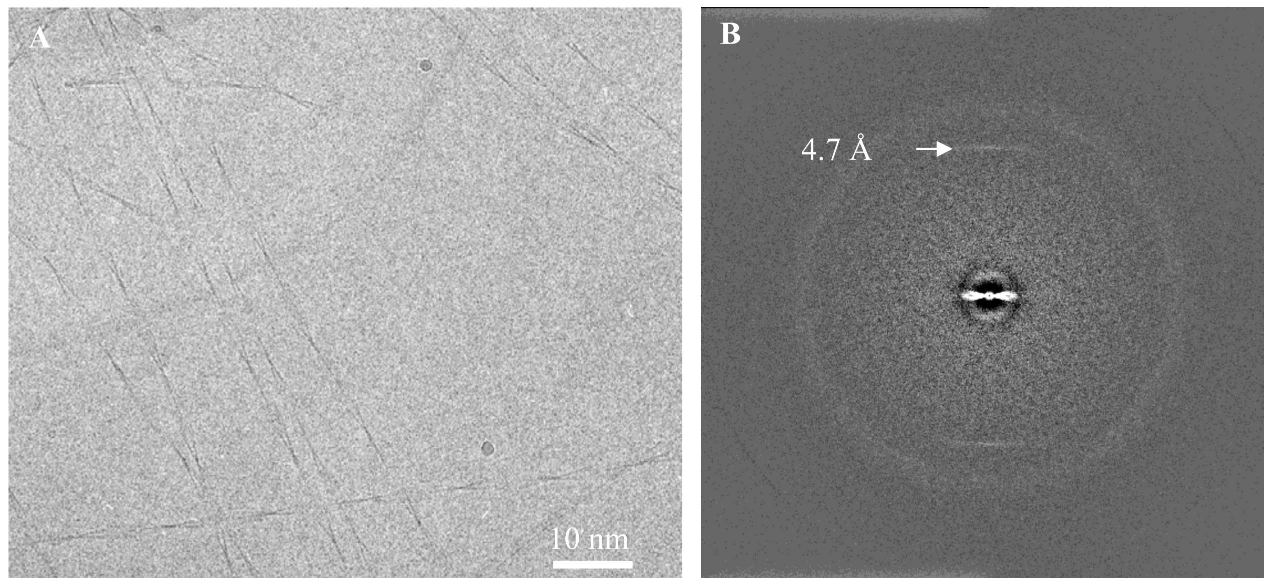

FIG. 1. Cryo-EM of Aβ(1-40) fibrils. (A) Cryo-EM micrograph of Aβ(1–40) fibrils. **(B)** Fibril segments were aligned and their power spectra averaged. The layer line at 1/4.7 $Å^{-1}$ is a characteristic of cross-β structures.

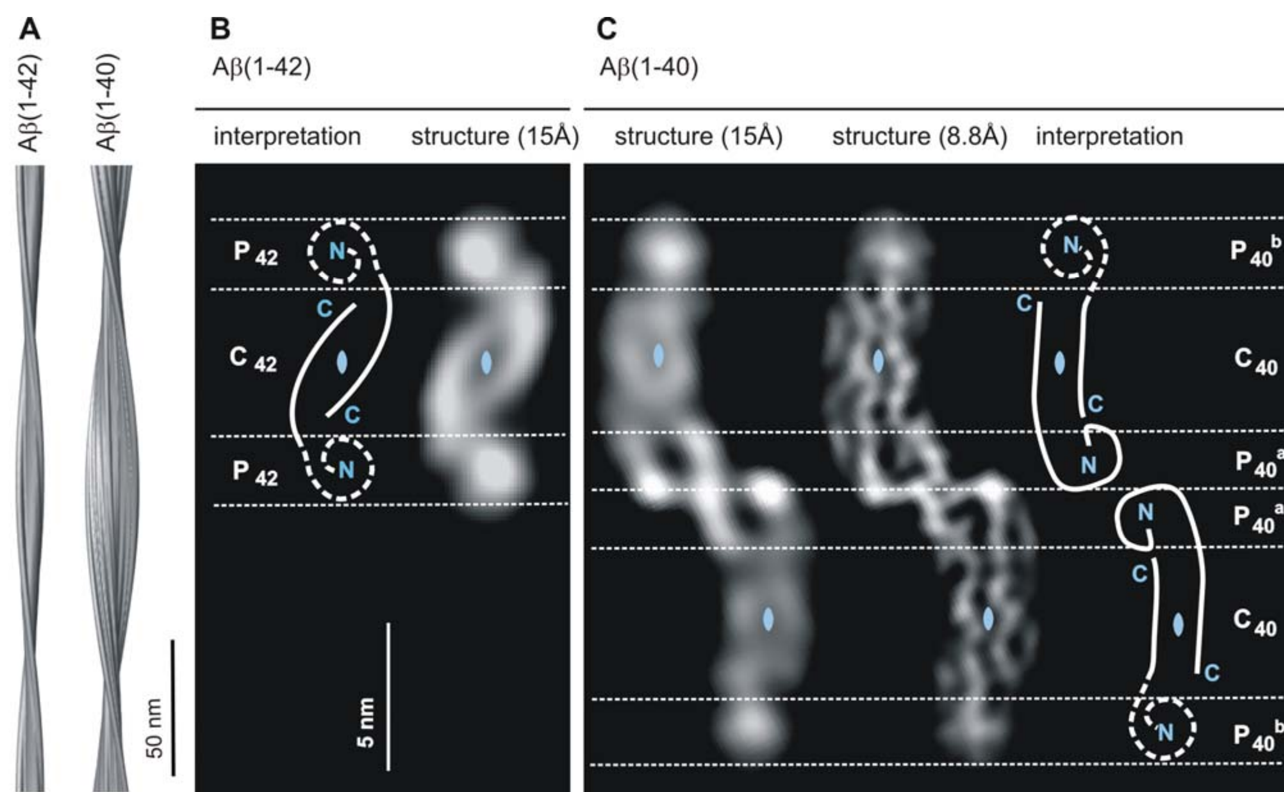

FIG. 2. Reconstructed densities of Aβ(1–40) and Aβ(1–42) fibrils. (A) Side views of reconstructions of Aβ(1–42) and Aβ(1–40) fibrils. **(B)** Section across the density map of Aβ(1–42) fibril (right) and hypothesized peptide arrangement (left). **(C)** Cross-sections of an Aβ(1–40) fibril density map filtered at 15 Å (left) and 8 Å (middle) resolution [1] and interpretation of density map in terms of peptide arrangement (right). Blue ovals indicate local pseudo-twofold symmetry axes.

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