CryoEM preliminary data for
Probing the three dimensional structure of TDP-43 amyloid fibril

We optimized several factors for the cryogenic sample preparation, such as: sample concentration, blotting time and buffer conditions and verified them by screening on TFS Glacios at NYSBC/SEMC (Figure 1). We also collected a preliminary data set of ~1000 images on a TFS Krios and processed the data using Cryosparc. From 2D classification of picked filaments we can see that the filaments are highly heterogeneous but display local order (see Figure 2, classes 1,2 & 3). We estimate that only about 10% of the filaments are suitable for further processing and requires a larger dataset on the order of ~20000 images. 172,000 particles were picked using helix tracing module in Cryosparc, which we used to do 2D classification. Although we can see several classes showing fine features, but the particle number was not sufficient to do obtain a reliable 3D initial model or to estimate helical parameters.

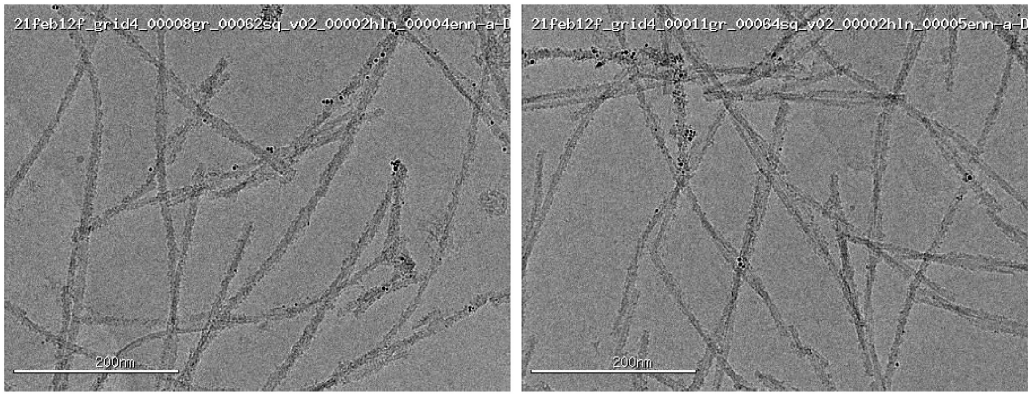

Figure1. TDP-43 in vitrified ice. Micrographs were taken on Krios with 64,000x magnification, -1.2 µm defocus range, 1.076Å pixelsize and 51.69 e^{-}/Å^2 electron dose. Most of the fibrils tend to bundle together, but there is a sufficient individual strands for particle picking and downstream processing.

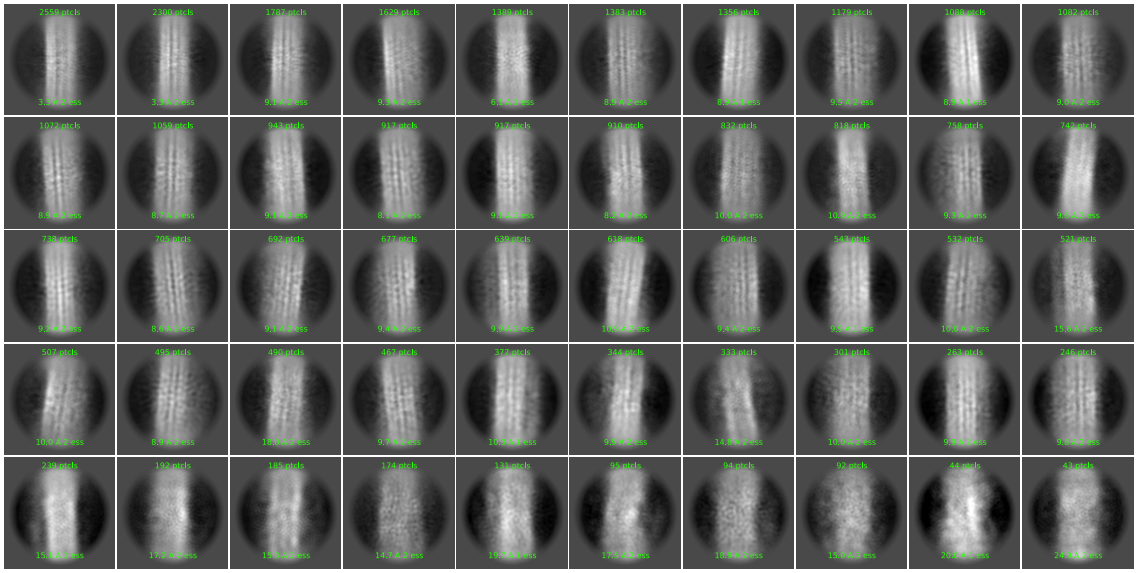

Figure 2. TDP-43 fibrils 2D classification.
Particles were picked using filament picker, extracted with a box size of 192 and 2D classified in Cryosparc. Local order can be seen in several classes, but more particles per class is needed for a better classification