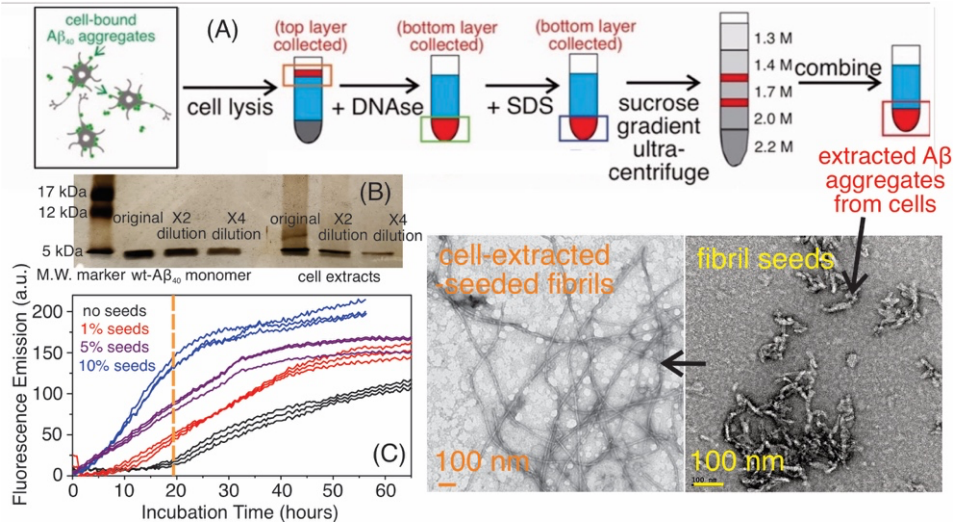
Preliminary Results and Figures



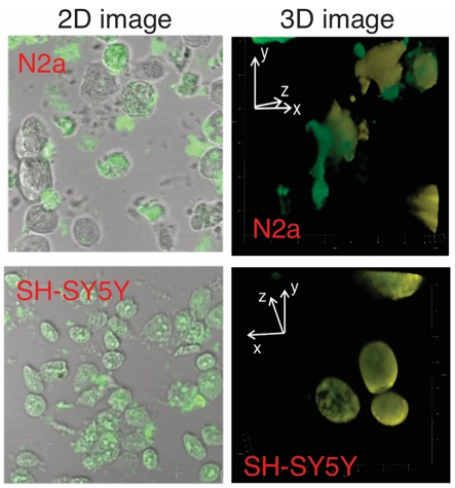


Figure 1(Left) (A) Scheme of the *ex-vivo* seeding and fibril sample preparation, including representative negatively stained TEM images of seeds and seeded fibrils. (B) Representative SDS-PAGE to determine the Aβ concentration in the extracted seeds. (C) Representative thioflavin-T (ThT) kinetics assay to determine the optimized seeding concentration and time.

Figure 2 (Right) Representative 2D and 3D confocal imaging of the Aβ deposits in N2a (top) and SH-SY5Y (bottom) cells. 5,6-FAM-conjugated Aβ (green) and DAPI (yellow) were visualized. While Aβ showed mostly intracellular aggregation in SHSY5Y cells, the deposition in N2a cells are more heterogeneous.

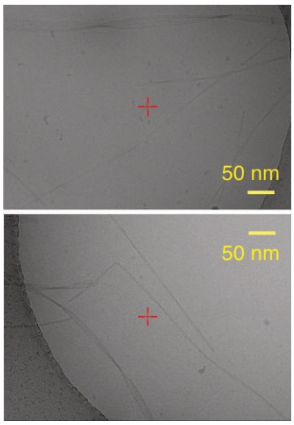


Figure 3 Representative cryo-TEM images of the parent pS8-Aβ fibrils, acquired on a 200 kV Arctica microscope with a K3 camera (CCMR, Cornell University). The optimized grid condition include: 100 μM Aβ fibril solution with a fresh addition of 2.0 mM SDS before deposition, 3.0s blot time, 10.0s wait time, blot force 4.

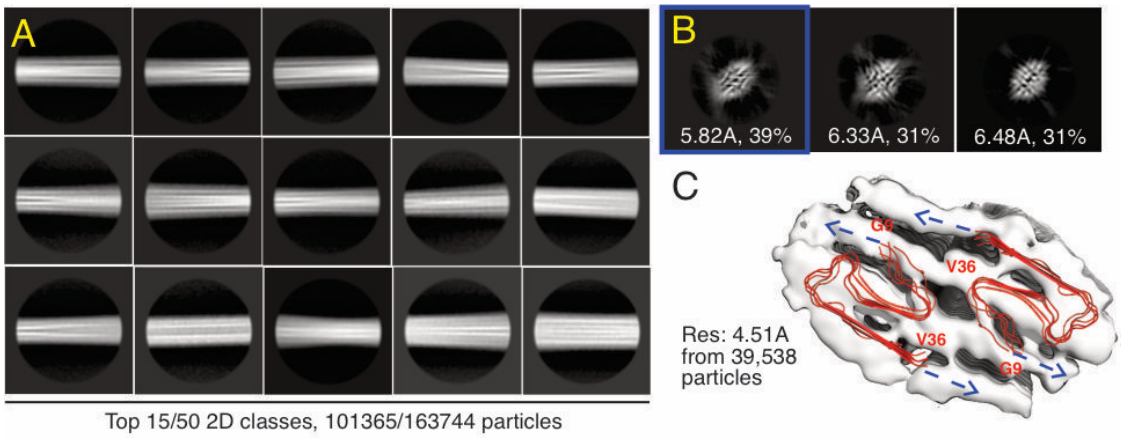


Figure 4 A preliminary test of RELION processing of a set of micrographs (~ 3,600 images with ~ 20% usable ones) collected for the parent pS8-Aβ fibril on a 300 kV Krios microscope. The dataset was collected on samples **before optimization of grid preparation conditions**, and therefore, a large fraction of micrographs contain overlapped filaments where particles cannot be extracted. A manual picking was performed to maximize the quality of particles, and a final 163,744 particles were picked. A set of 50 2D classes were acquired, where the top 15 classes with the highest resolution were selected for further processing (**Panel A**). These classes contain ~ 62% of the particles. The initial map was generated using the relion_helix_inimodel2d package, with an estimate 150 nm crossover distance from previous negatively stained TEM imaging. 3D classification (3 classes acquired) was done on a set of particles extracted based on this initial map and the subgroup with top resolution (~ 5.82 angstrom, 39,538/101,327 particles) was selected (**Panel B,** blue rectangle). The final 3D auto-refine, mask creation and post processing using RELION build-in functions resulted in a low-resolution model at ~ 4.51 angstrom (**Panel C**). The density map fits roughly to our preliminary ssNMR-derived architectural unit of the similar pS8-Aβ fibril with a C2 symmetry, which agrees with the tilt-beam TEM results in our published work (Hu et al, PNAS, 2009). We anticipate further improvement of resolution using a larger set of micrographs with improved grid conditions (Note: the current set only contains ~ 40K particles, while typical high-resolution fibril structural reconstructions use ~ 130K particles.