

## DynaPro Characterization of $\alpha$ -synuclein Oligomerization

The aggregation of the presynaptic protein  $\alpha$ -synuclein is believed to play a critical role in Parkinson's disease. It is suspected that pre-fibrillar oligomers, rather than fibrils themselves, may be the cytotoxic form. In the present experiment we used dynamic light scattering to monitor buildup and decay of oligomeric intermediates during the early stages of  $\alpha$ -synuclein fibrillation in vitro.

$\alpha$ -Synuclein (1 mg/ml) was incubated at 37°C, pH 7.4, 20 mM Tris buffer, 100 mM NaCl, with agitation. Under these conditions fibrils begin to form after 20 h. DLS measurements were performed at 20°C using a DynaPro-MSXTC instrument. Aliquots of 10  $\mu$ l of the incubation solution were centrifuged before measuring DLS. 1 ml of the supernatant was diluted with 10  $\mu$ l-filtered buffer, and measurements were taken within 3-7 min. Scattering peaks less than 0.1 nm radius and more than 1000 nm were ignored.

The results show that only monomer (with  $R_s = 3.1 \pm 0.2$  nm) was detected initially; however, by 3 h, oligomeric species with  $R_s = 21 \pm 3$  nm were observed. The concentration of these oligomers reached a maximum around 15 h, and then decreased.

The DLS results clearly show the transient buildup of an oligomer during the lag phase, which subsequently decays. Smaller-sized oligomers are observed during and after fibril formation. The figures show the plots for mass and scattering intensities after 10 h of incubation and show predominantly monomer, with a small amount of oligomer.

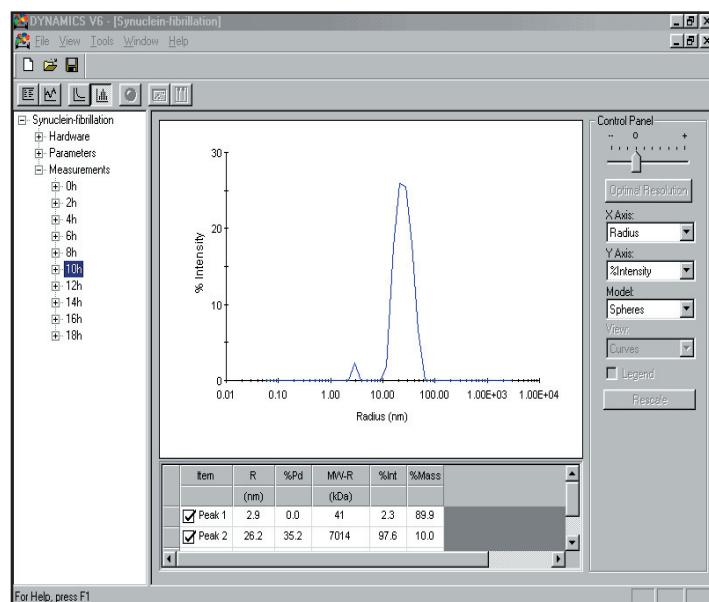


Figure 1. DynaPro dynamic light scattering profile for the aggregation of  $\alpha$ -synuclein showing monomeric and oligomeric species as a function of radius.

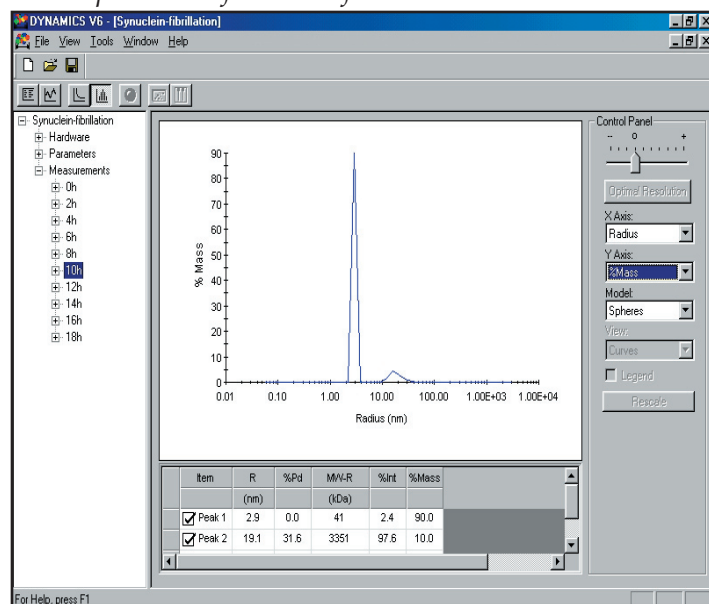


Figure 2. DynaPro dynamic light scattering profile for the aggregation of  $\alpha$ -synuclein showing monomeric and oligomeric species as a function of mass.

