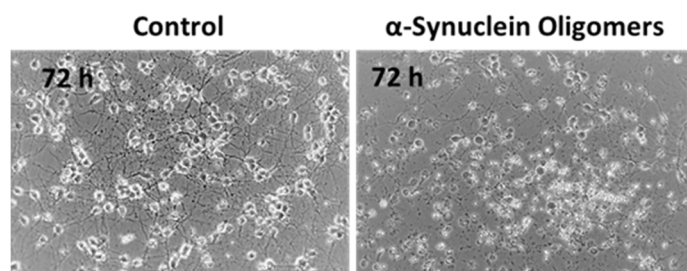
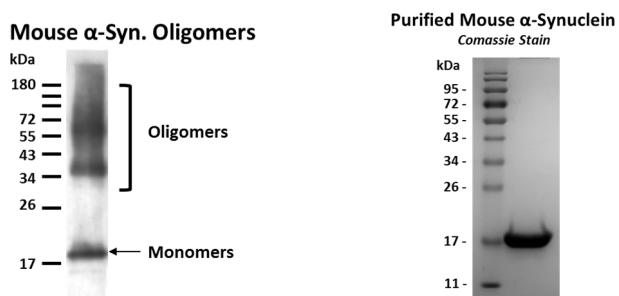


# In Vitro Neuroprotection Screen for Parkinson's Disease

**SynAging uses proprietary mouse  $\alpha$ -synuclein oligomer preparations ( $\alpha$ SO) to model Parkinson's disease in mouse primary striatal neurons.**

Mouse  $\alpha$ SO are prepared from wild-type mouse  $\alpha$ -synuclein expressed in *E. coli* and purified to homogeneity (>95%). Monomers were checked for the absence of non- $\alpha$ SO toxicity. Oligomers are prepared over 24h according to SynAging's proprietary procedure.



**Primary Mouse Striatal Neurons**

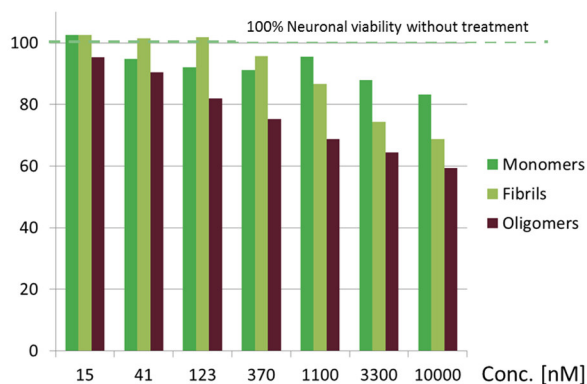
## Assay Format for Neuroprotective Compounds

Mouse primary striatal neurons are sensitive to  $\alpha$ SO and  $\alpha$ -synuclein fibrils ( $\alpha$ SF). Grown in 48 well plates for 10 days, neurodegeneration is induced by 300 nM  $\alpha$ SO, resulting in ~40 % cell death within 72h. Test items and  $\alpha$ SO/ $\alpha$ SF are either mixed or neurons are pre-incubated with test compounds before  $\alpha$ SO addition. 72h after  $\alpha$ SO treatment, cell viability is determined with the MTT assay. All experiments are performed in triplicate and the following controls are on every multi well plate:

- vehicle
- $\alpha$ SO/  $\alpha$ SF (negative control)
- BDNF (positive control)
- test item only, no  $\alpha$ SO (compound control)

## $\alpha$ SO are the Most Toxic Form of $\alpha$ -Synuclein

$\alpha$ -Synuclein was added to primary mouse striatal neurons in the form of: monomers, fibrils and oligomers at various concentrations. Neuronal viability was measured after 72h using the MTT assay. The logarithmic plot of the results clearly shows that  $\alpha$ SO are ~10 x more toxic than the fibrillar form:

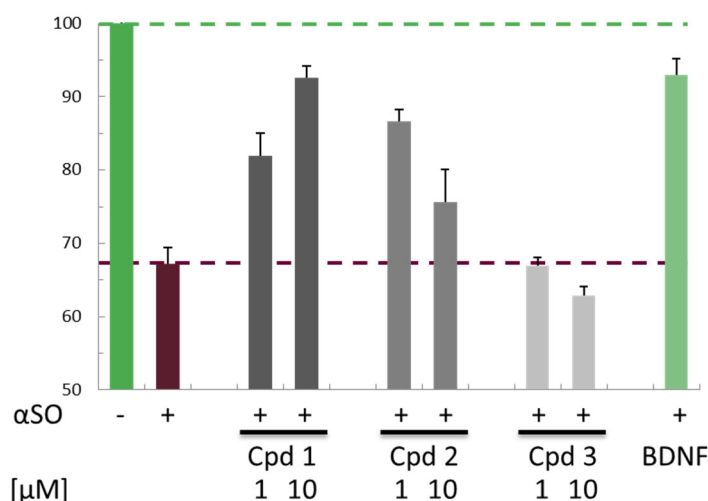


## Neuronal Changes Induced by $\alpha$ SO

$\alpha$ SO induce time-dependent neurotoxicity in primary mouse neurons (DIV 11):

- severe cell shrinkage
- destruction of the neuronal network
- phenotype different from A $\beta$ O
- slower damage than A $\beta$ O

Study results are available within five weeks. Typical results are shown below:



**SynAging SAS: Your partner in naturally induced phenotypic models, accelerating drug discovery for proteopathic CNS diseases**