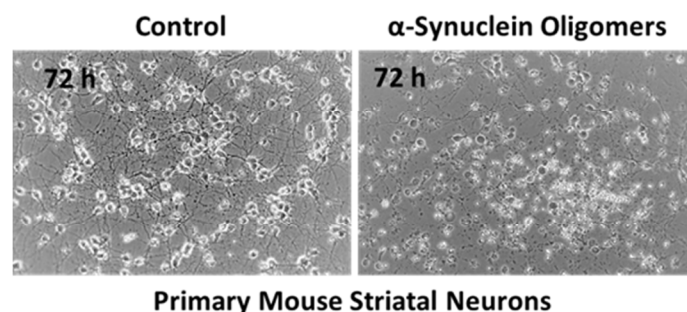


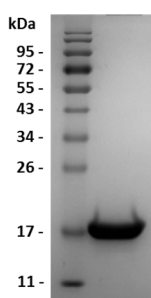
# In Vitro Neuroprotection Screen for Parkinson's Disease

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

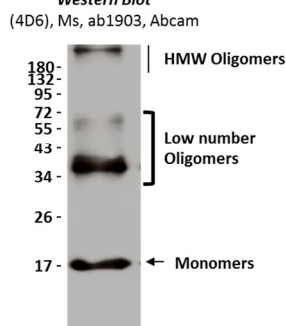
Mouse  $\alpha$ SO and  $\alpha$ SF are prepared from wild-type mouse  $\alpha$ -synuclein expressed in *E. coli* and purified to homogeneity (>95%). Monomers were checked for the absence of toxicity.



Purified Mouse  $\alpha$ -Synuclein  
Coomassie Stain



Mouse  $\alpha$ -SynO  
Western Blot



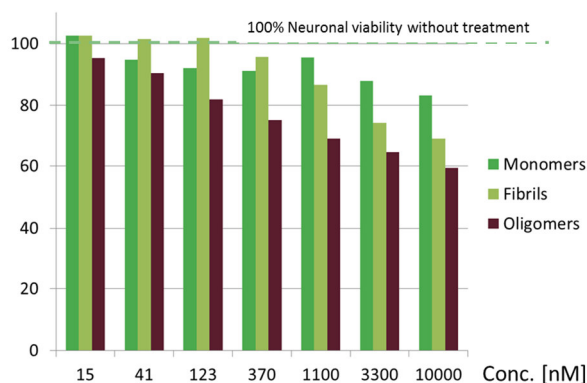
## Assay Format for Neuroprotective Compounds

Mouse primary striatal neurons are sensitive to  $\alpha$ SO and  $\alpha$ SF. Grown in 48 well plates for 10 days, neurodegeneration is induced by 1  $\mu$ M  $\alpha$ SO or 10  $\mu$ M  $\alpha$ SF, resulting in ~40 % neuronal death within 72h. Test items and  $\alpha$ SO or  $\alpha$ SF are either mixed before application, or neurons are pre-incubated with test compounds before  $\alpha$ SO addition. 72h after  $\alpha$ SO treatment, cell viability is determined by 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 /  $\alpha$ SF (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 72 h using the MTT assay. The exponential increase of the concentration shows that  $\alpha$ SO (70% at 1.1  $\mu$ M) are approximately-10 x more toxic than  $\alpha$ SF (70% at 10  $\mu$ M):

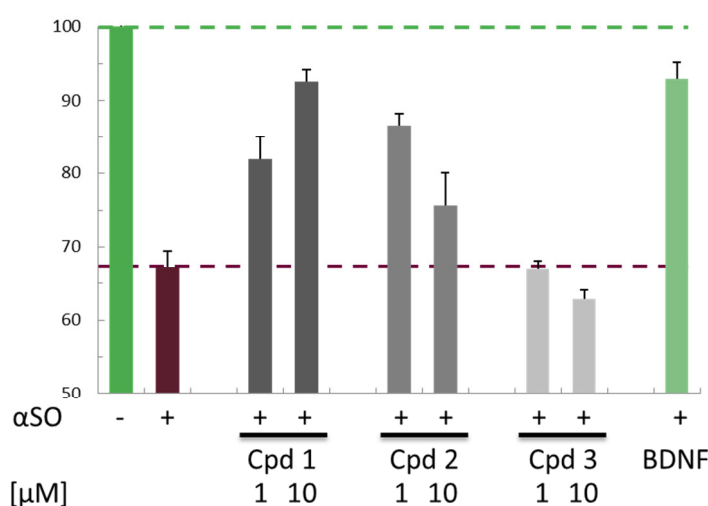


## Neuronal Changes Induced by $\alpha$ SO

$\alpha$ 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