Alpha Synuclein Research Tools

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Introduction

Research into α-synuclein - a key drug target in Parkinson's Disease that has the ability to aggregate into oligomers and fibrils - has progressed rapidly in recent years. Much of the work has been completed using pre-formed recombinant fibrils (PFFs). However - it has now become apparent that there are different fibril preparations that can have quite different properties. Here we list and examine some of fibril and oligomer preparations, as well as other useful tools for alpha synuclein research.

Monomers (Types 1 and 2)

α-Synuclein monomers are preparations that are made in two ways, yielding Type 1 and 2 materials. Both are filtered to remove any fibrillar or oligomeric material larger than 30kD. Type 1 monomers (SPR-321) generate fibrils with a high Thioflavin T response, whereas Type 2 monomers (SPR-316) do not (Figure 1), although both form insoluble fibrils upon aggregation. Monomeric α-synuclein can also be used as a control for fibrillar or oligomeric α-synuclein containing experiments. Type 2 monomers (SPR-316) are being studied for use in RT-QuIC assays (Figure 2).

Figure 1.

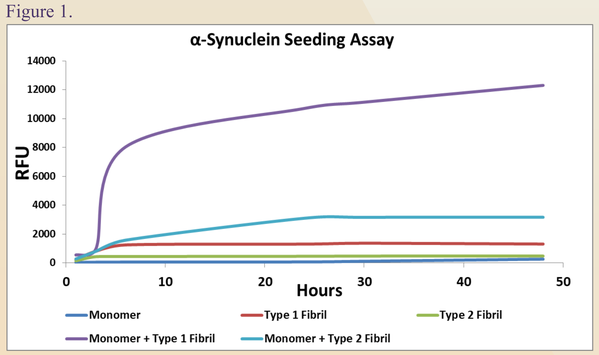
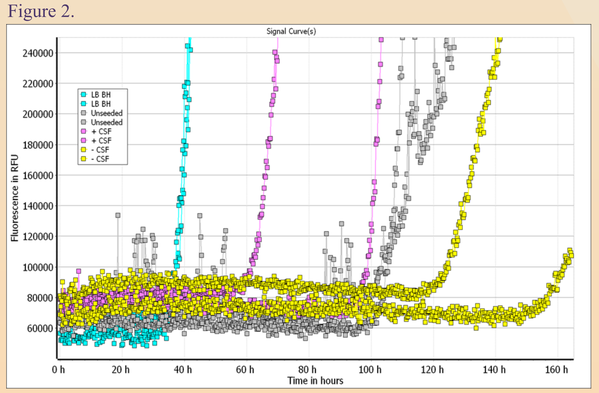


Figure 2.



Fibrils (Types 1, 2 and 3) and Filaments

Type 1 (SPR-322) and 2 (SPR-317) fibrils are generated by aggregating Type 1 and 2 monomers, respectively, under specific conditions. However, due to the endotoxin content of Type 1 fibrils (approximately 10-20 EU/ml), Type 3 fibrils (SPR-448) have been developed using an artificial, removable scaffold that allows the building of high Thioflavin T-absorbing fibrils. These have the same essential properties as Type 1 fibrils, but lower endotoxin content, (2 EU/ml or less). Filaments (SPR-450) are likely a mixture of soluble proto-fibrils. Figure 3 shows EM images of Type 1-3 fibrils and filaments. Type 1 human and mouse fibrils have been shown to induce phospho-serine129 pathology in rat neurocortical primary cells (Figure 4) and *in vivo* (Figure 5). Additionally, fluorescently-conjugated PFFs were taken up, transported into the soma, and induced α-synuclein aggregation in mouse neurocortical primary cells (Figures 6 and 7).

Figure 3. TEM of Type 1 PFFs (SPR-322) (TL); Type 2 PFFs (SPR-317) (TR); Type 3 PFFs (SPR-448) (BL); Filaments (SPR-450) (BR)

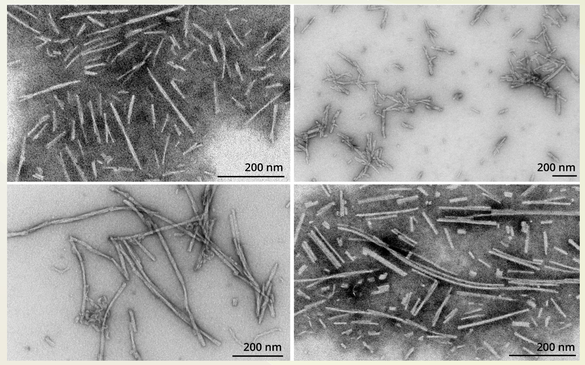


Figure 4. Type 1 PFFs (SPR-322) Type 2 PFFs (SPR-317)

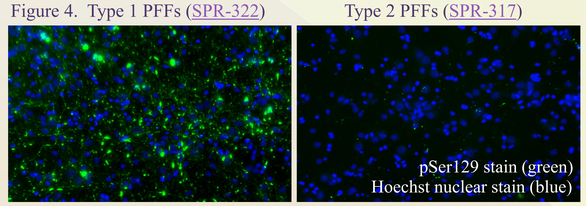


Figure 5. IHC analysis of rat brain injected with Type 1 mouse alpha synuclein PFFs (SPR-324) shows α-synuclein pathology

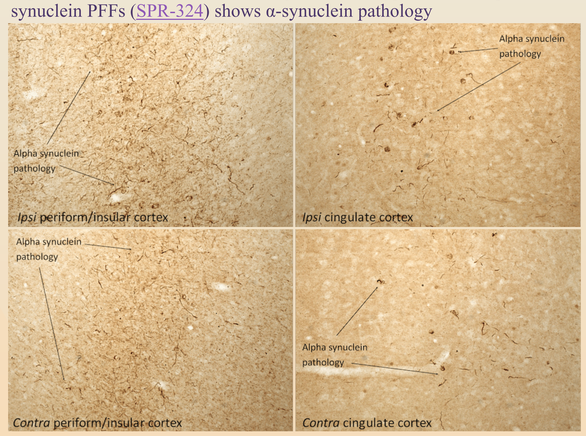


Figure 6.ICC of primary mouse cortical neurons seeded with ATTO633-labelled α-synuclein PFFs (SPR-322)

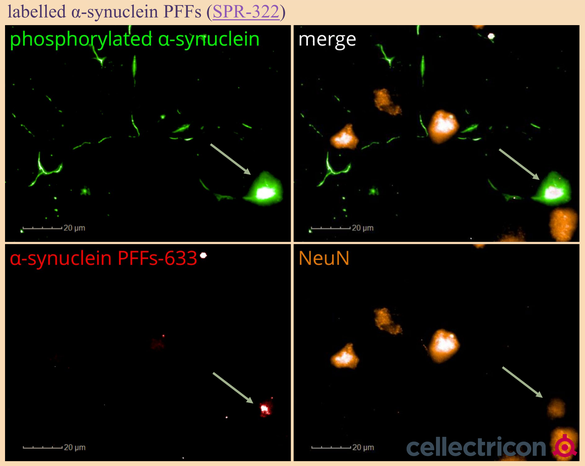
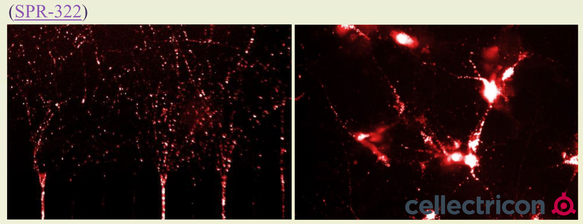


Figure 7.ICC of primary mouse cortical neurons cultured in a microfluidic co-culture system seeded with ATTO633-labelled α-synuclein PFFs (SPR-322)



α-Synuclein Mutants

Several mutants of human α-synuclein have been studied. Probably the best studied is the A53T mutant, which makes the resultant protein more prone to aggregation and fibrillization (Figure 8). Both total ELISA-based α-synuclein assays and FRET-based aggregation assays can detect the PFFs as well as the aggregation process (Figure 9). A53T PFFs also induce phospho-serine129 pathology in rat neurocortical primary cells (Figure 10).

Figure 8. TEM of A53T human α-synuclein PFFs. (SPR-326)

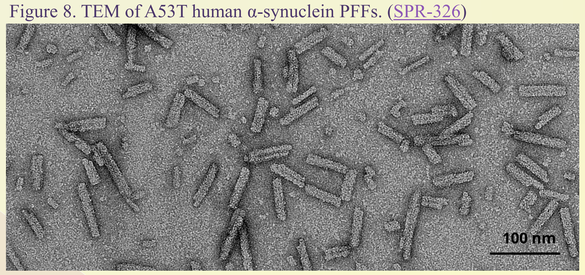


Figure 9. Cisbio Aggregation (top) and Total α-synuclein assay kits of A53T PFFs (SPR-326)

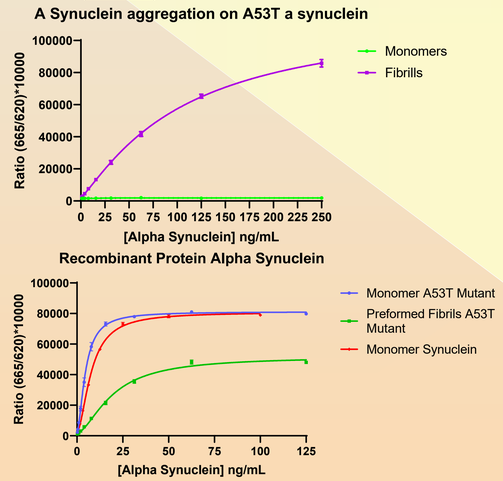
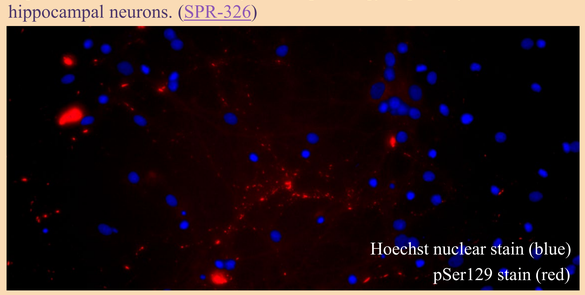


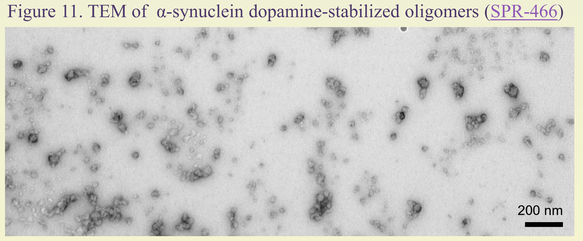
Figure 10. A53T PFFs seed α-synuclein pathology in primary rat hippocampal neurons. (SPR-326)



α-Synuclein Oligomers

It has become apparent that α-synuclein goes through numerous steps to become insoluble PFFs. These intermediates include a number of large and small oligomers. Interest has increased in these as they appear to have different properties to PFFs and may represent the toxic element associated with these proteins. Dopamine and its oxidized byproducts appear to be instrumental in the formation of stabilized and toxic oligomers (Figure 11), that have clear morphological differences to soluble filaments or insoluble PFFs being generally circular with a diameter of about 25nm.

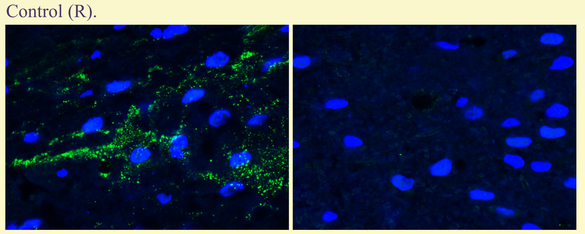
Figure 11. TEM of α-synuclein dopamine-stabilized oligomers (SPR-466)



α-Synuclein Antibodies

Ninety percent of α-synuclein in Lewy bodies is phosphorylated at Ser129, making antibodies against it useful tools for detecting α-synuclein inclusions.

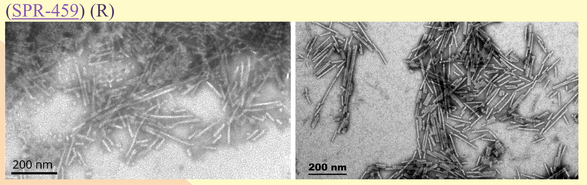
Figure 12. IHC analysis using alpha synuclein pSer129 antibody (SPC-742) of mouse brain injected with alpha synuclein AAV vector (L). Control (R).



β- and γ-Synuclein, Monomers and Fibrils

α-Synuclein has a high degree of homology with both β- and γ-synuclein, although neither has been well studied. Although γ-synuclein can aggregate and seed reactions, β-synuclein lacks the NAC domain and cannot readily fibrillize or seed reactions unless chemically forced to do so, making it a potential valuable control for α-synuclein. Both monomeric and fibrillized (Figure 13) versions of these proteins have been generated.

Figure 13. TEM of β-synuclein PFFs (SPR-457) (L) and γ-synuclein PFFs (SPR-459) (R)



Acknowledgments

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