The selective detection of α-synuclein oligomers to analyse small molecule inhibitors of their formation for the treatment of Parkinson's disease

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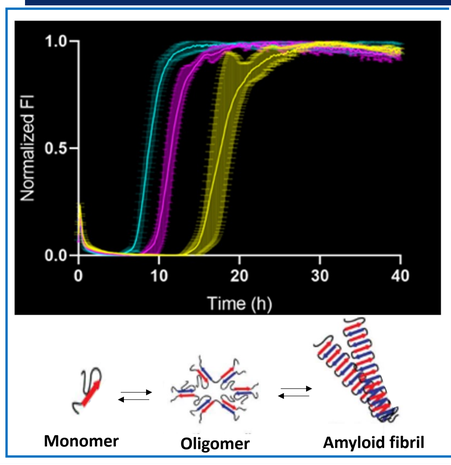
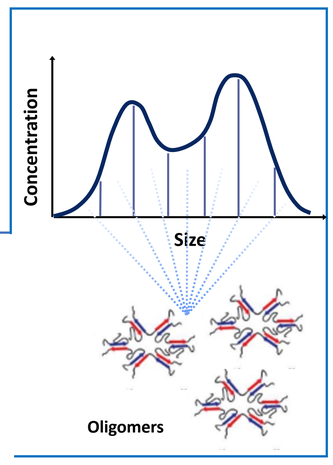
Background and Objective



Background: Aggregation of α-synuclein is a key event in Parkinson's disease and other synucleinopathies. Soluble oligomers on the aggregation pathway are widely recognized as the toxic species, as opposed to monomers or insoluble fibrils. A chemical kinetics approach is used to quantify the molecular mechanisms in the generation of oligomers. A small molecule approach is employed to target these mechanisms and suppress oligomer generation.

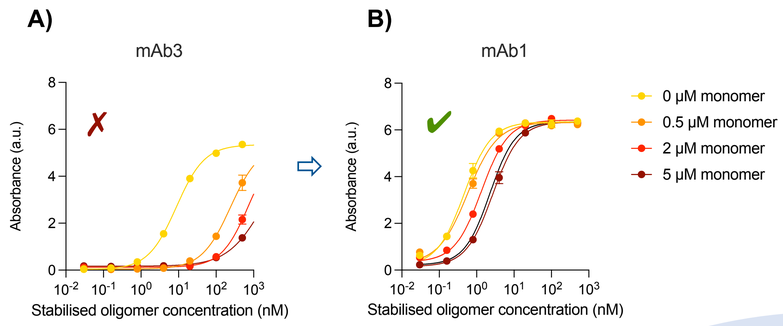
Objective: In this work, our objective was to develop a biomarker assay for the selective detection of α-synuclein oligomers enabling biomarker-driven clinical development of small molecule therapeutics. The assay aimed to achieve selectivity for a broad range of α-synuclein oligomers and sufficient sensitivity for preclinical studies, with enhanced sensitivity for clinical readout. Independent validation of oligomer reduction was confirmed by orthogonal readouts of aggregates.

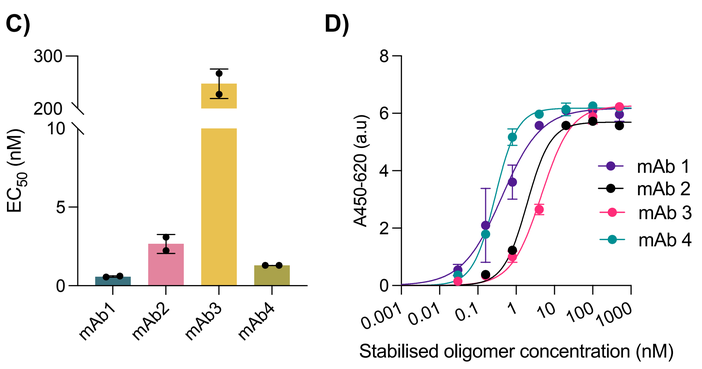


In vitro assay validation

Specificity and selectivity for oligomers

Representative graphs demonstrating antibodies which can selectively bind oligomers when competing with increasing concentrations of monomer (B) as opposed to those antibodies which are subject to interreference from high monomer levels (A).

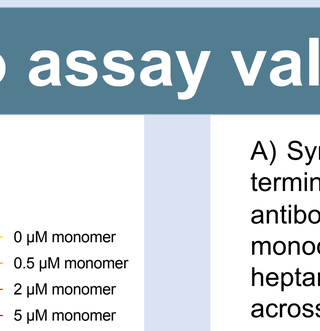




C) Summary bar plot of EC_{50} value for stabilized oligomers in a matrix containing 500nM of α-synuclein monomer.
D) Lower limit of detection of stabilized oligomers for lead candidate monoclonal antibodies for oligomer biomarker assay.

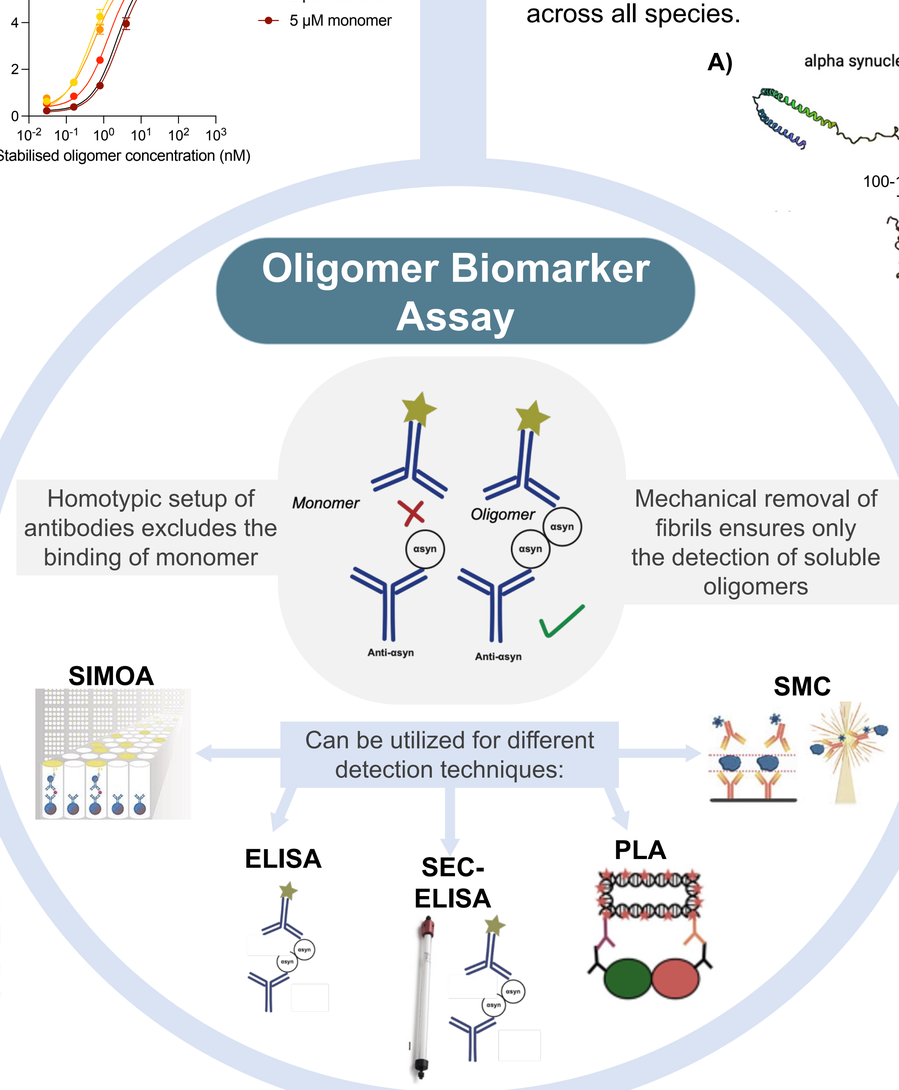
Oligomer Biomarker Assay

Homotypic setup of antibodies excludes the binding of monomer



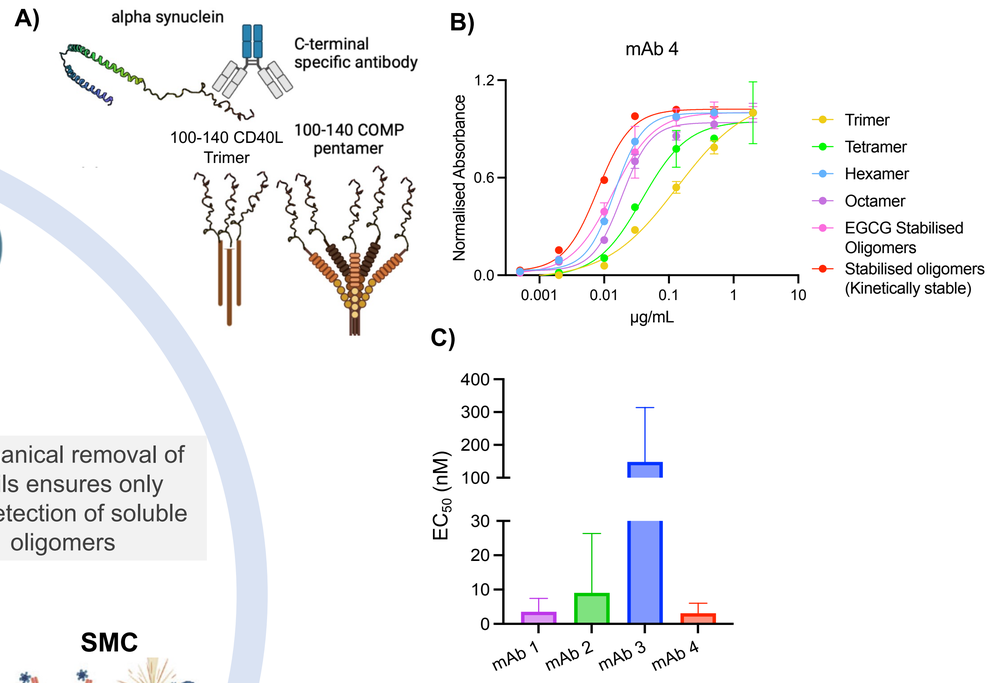
Mechanical removal of fibrils ensures only the detection of soluble oligomers

Can be utilized for different detection techniques:



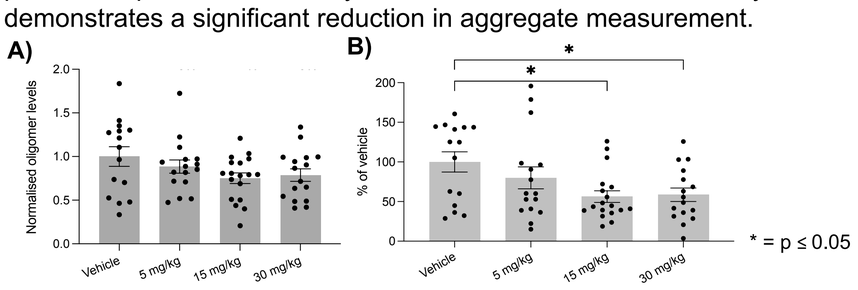
Detection of diverse oligomer species

A) Synthetic oligomer calibrators were generated by grafting repeating units of the exposed C-terminal fragment of alpha-synuclein onto scaffolds. B) Representative data for a monoclonal antibody which binds a diverse range of oligomers with similar EC_{50}. C) Summary bar plot of lead monoclonal antibodies demonstrating EC_{50} for trimers, tetramers, pentamers, hexamers, heptamers, octamers and stabilized oligomers. mAbs 1 and 4 demonstrate the smallest EC50 across all species.

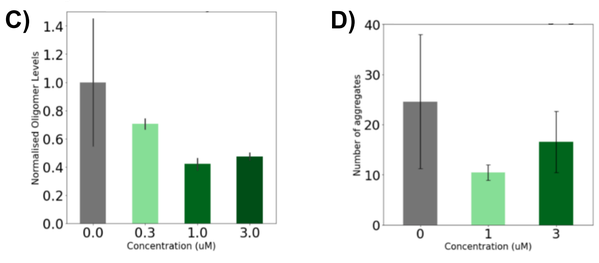


WTX significantly reduces α-synuclein oligomers & aggregates

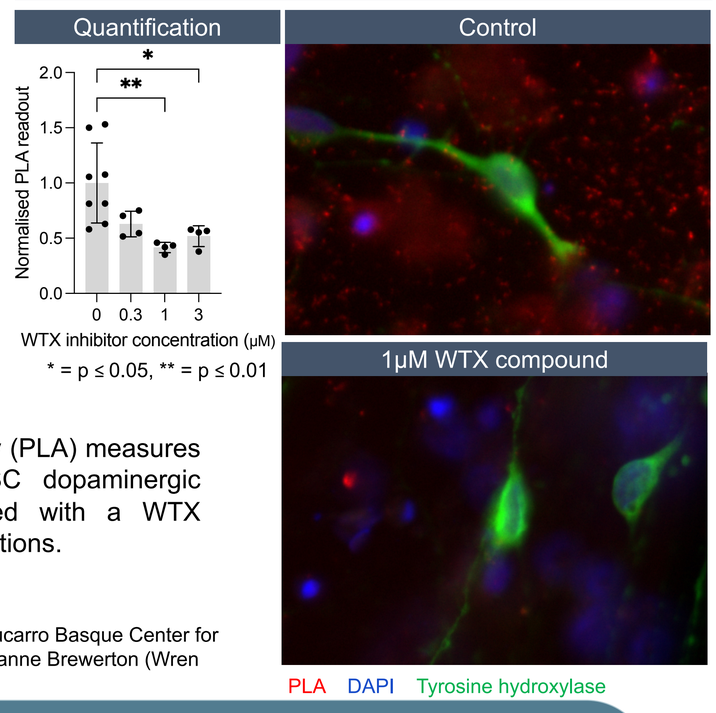
A) A homotypic ELISA measures a reduction in oligomers in Line 61 alpha-synuclein mouse model treated with a WTX compound for 8 weeks. B) Corresponding aggregate measurement using a ps129 specific antibody from immunohistochemistry demonstrates a significant reduction in aggregate measurement.



C) A homotypic ELISA measures a reduction in oligomers in iPSC dopaminergic neurons treated with a WTX compound.
D) Corresponding aggregate measurement using TIRF microscopy demonstrates a reduction in aggregate measurement.



WTX significantly reduces α-synuclein oligomers in iPSC dopaminergic neurons



A homotypic proximity ligation assay (PLA) measures a reduction in oligomers in iPSC dopaminergic neurons which have been treated with a WTX compound under high seeding conditions.

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Demonstration of oligomer reduction in pre-clinical studies

Conclusions

Wren Therapeutics is developing a new class of small molecule therapeutics for Parkinson's Disease that target the source of α-synuclein oligomer generation. These molecules have been designed to disrupt the aggregation chain of production, which begins with toxic α-synuclein oligomers and culminates with α-synuclein aggregates. We are also advancing the development of oligomer biomarker technologies to measure both on-target engagement of these therapeutics and to monitor disease progression to enable biomarker driven clinical development.