High Throughput Phage Display Screening Identified Peptide Inhibitors Targeting the α-Synuclein for Parkinson's Disease Treatment

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Introduction

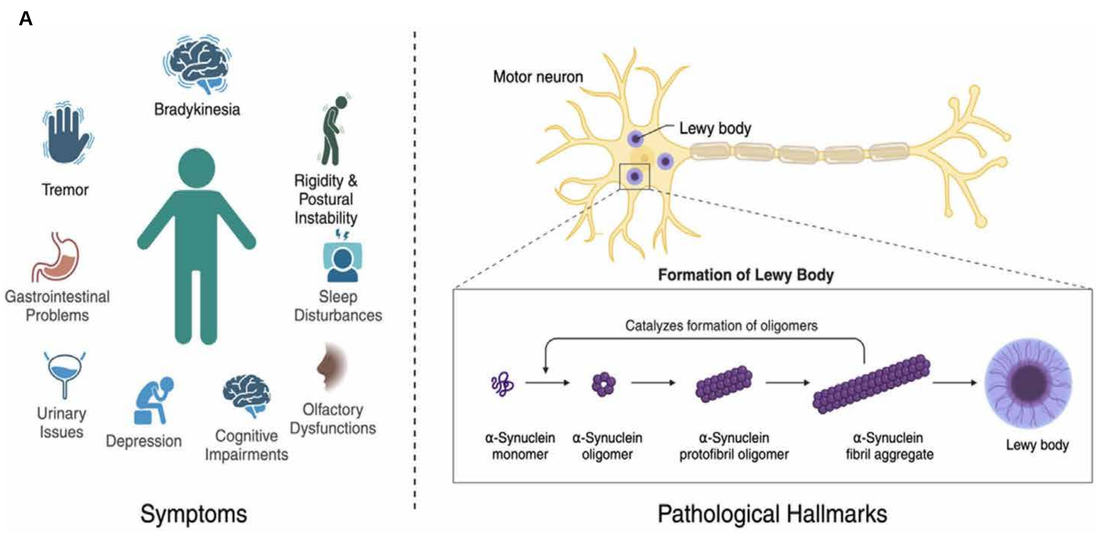

Fig. 1. Peptide inhibitor discovered for inhibition α-syn aggregation in PD. (A) α-Syn aggregation in Parkinson's disease. **(B)** Schematic illustration of SUMO1-derived peptide inhibitors suppresses early-stage α-syn aggregation.

Parkinson's disease (PD) is a chronic neurodegenerative disorder affecting 10 million worldwide. α-Syn aggregation is a key driver of PD pathogenesis and a therapeutic target. Previously we have discovered a SUMO-derived peptide inhibitor, which effectively suppresses α-syn aggregation. To enhance binding affinity, we aim to develop a SUMO-biased phage-displayed peptide library to identify peptides with enhanced binding affinity to α-syn, providing a promising avenue for developing novel therapeutics for PD.

Methods

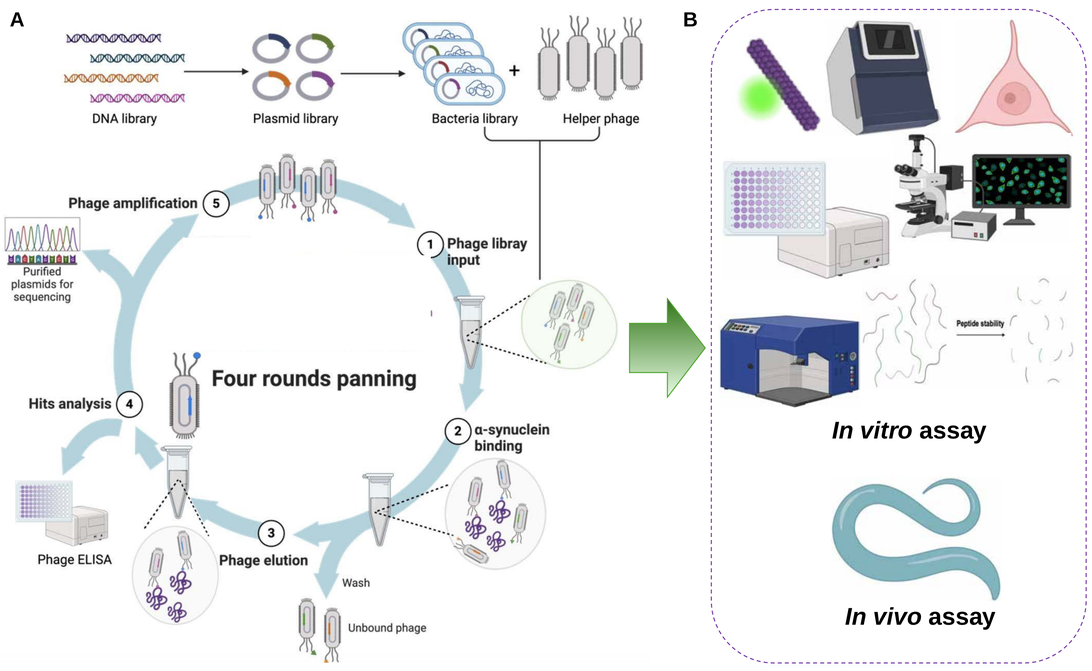

Fig. 2. Phage display reveals peptide binders of α-syn. (A) Schematic representation of the construction and screening cycle of phage display peptide library bound to α-syn. The format of the phage peptide library was marked in the cycle's center. **(B)** The biological activity evaluation of the selected peptide hits.

Conclusion & Future work

(1) We designed a phage display peptide library based on SUMO1 scaffold and identified PD-6 peptide targeting syn with a nanomolar affinity.

(2) PD-6 inhibits syn aggregation, penetrates neuronal cells, reduces toxic oligomer formation, and is resistant to protease degradation *in vitro*.

(3) PD-6 suppressed syn-induced toxicity in *C. elegans* PD models.

(4) We plan to evaluate the neuroprotective effect of PD-6 in a mouse model.

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Results

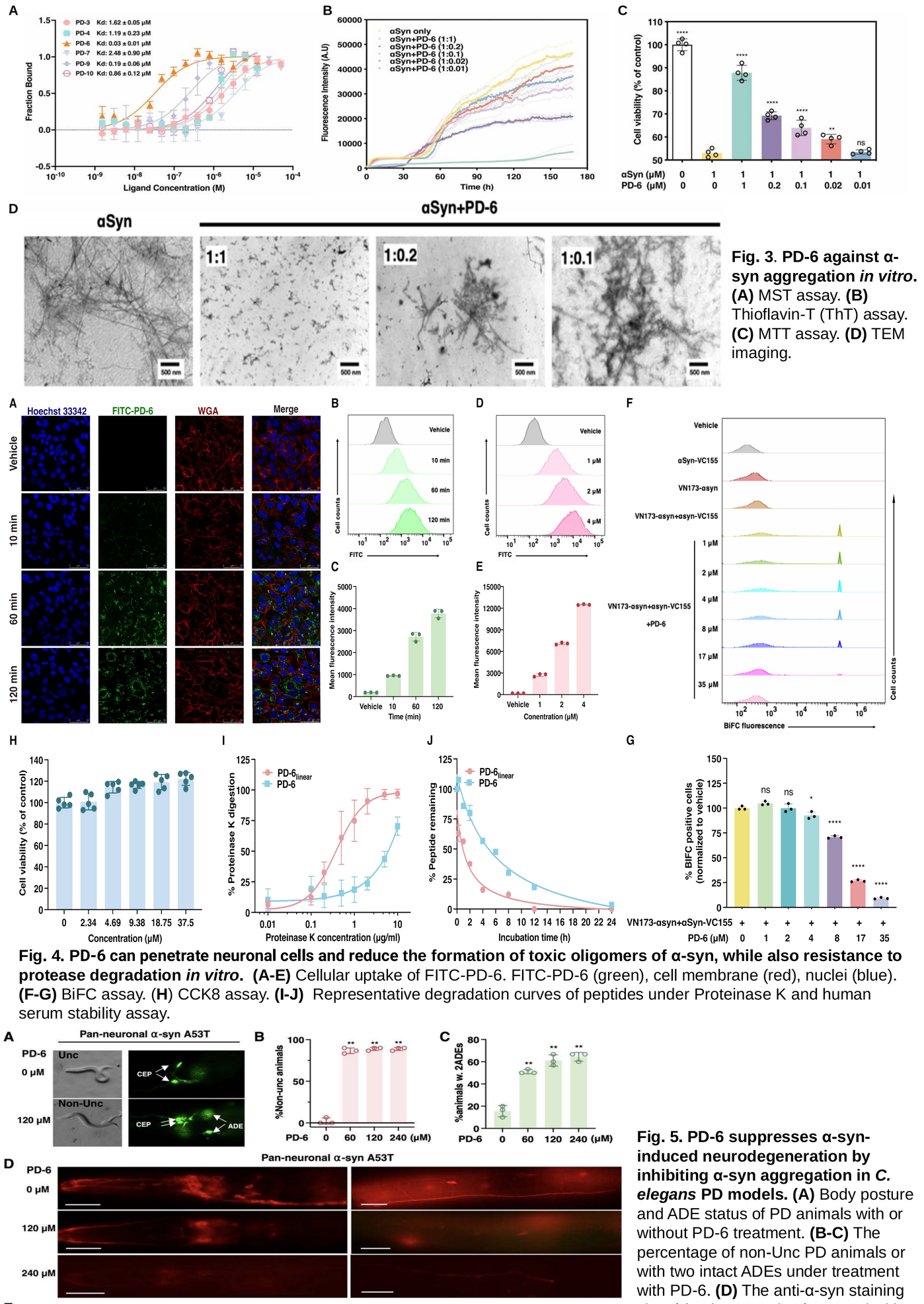

Fig. 3. PD-6 against α-syn aggregation *in vitro*. (A) MST assay. **(B)** Thioflavin-T (ThT) assay. **(C)** MTT assay. **(D)** TEM imaging.

Fig. 4. PD-6 can penetrate neuronal cells and reduce the formation of toxic oligomers of α-syn, while also resistance to protease degradation *in vitro*. (A-E) Cellular uptake of FITC-PD-6. FITC-PD-6 (green), cell membrane (red), nuclei (blue). **(F-G)** BiFC assay. (**H**) CCK8 assay. **(I-J)** Representative degradation curves of peptides under Proteinase K and human serum stability assay.

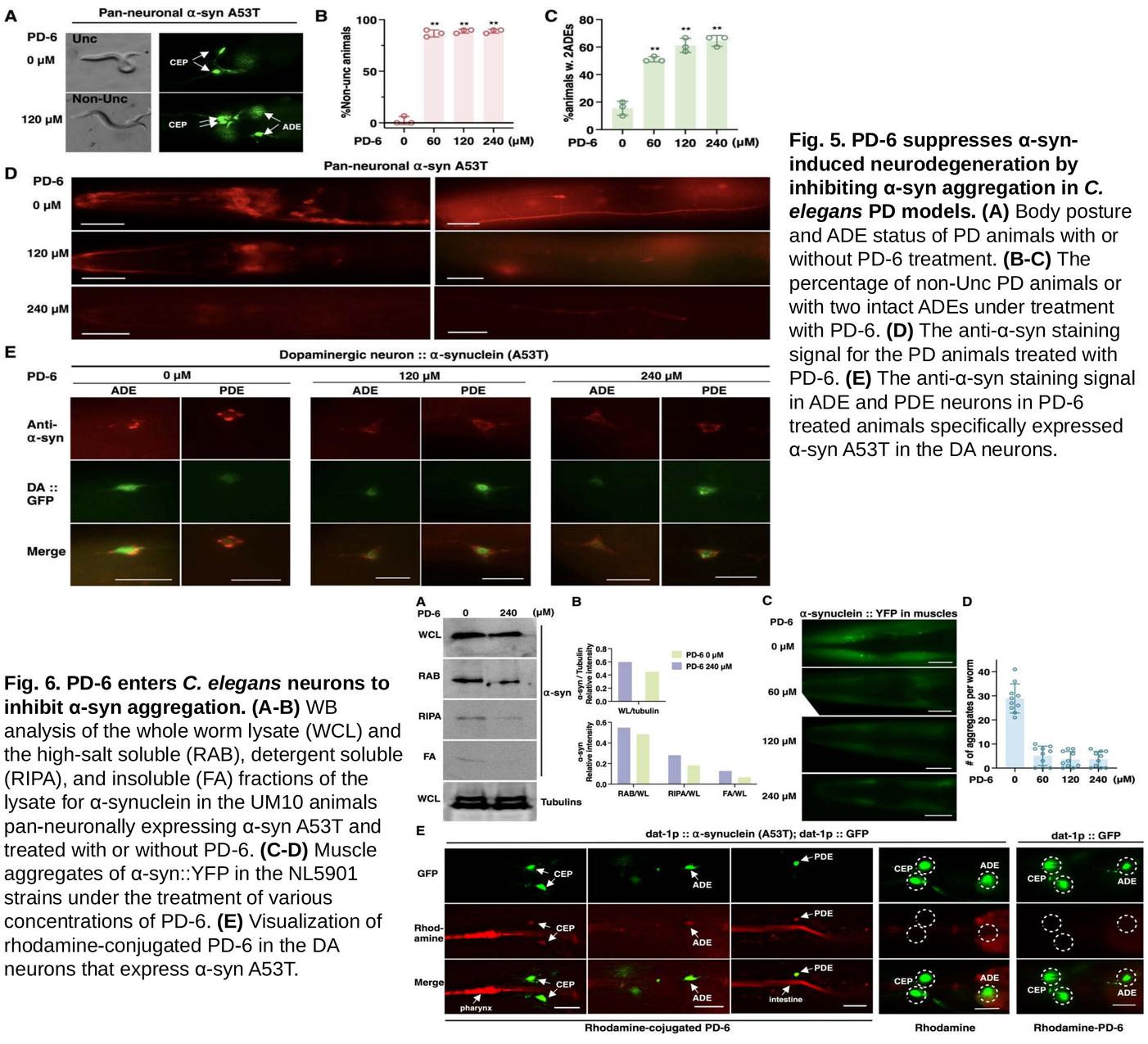

Fig. 5. PD-6 suppresses α-syn-induced neurodegeneration by inhibiting α-syn aggregation in *C. elegans* PD models. (A) Body posture and ADE status of PD animals with or without PD-6 treatment. **(B-C)** The percentage of non-Unc PD animals or with two intact ADEs under treatment with PD-6. **(D)** The anti-α-syn staining signal for the PD animals treated with PD-6. **(E)** The anti-α-syn staining signal in ADE and PDE neurons in PD-6 treated animals specifically expressed α-syn A53T in the DA neurons.

Fig. 6. PD-6 enters *C. elegans* neurons to inhibit α-syn aggregation. (A-B) WB analysis of the whole worm lysate (WCL) and the high-salt soluble (RAB), detergent soluble (RIPA), and insoluble (FA) fractions of the lysate for α-synuclein in the UM10 animals pan-neuronally expressing α-syn A53T and treated with or without PD-6. **(C-D)** Muscle aggregates of α-syn::YFP in the NL5901 strains under the treatment of various concentrations of PD-6. **(E)** Visualization of rhodamine-conjugated PD-6 in the DA neurons that express α-syn A53T.