Supplementary Online Content


eFigure 1. Characterization of αSyn Aggregation In Vitro

eFigure 2. Effect of Freeze/Thaw on αSyn-PMCA

eFigure 3. Performance of αSyn-PMCA With CSF Coming From Patients Affected by Other Neurodegenerative Diseases and Alzheimer’s Disease

eFigure 4. Values of αSyn-PMCA for a Small Number of Samples From Patients Affected by DLB and MSA

This supplementary material has been provided by the authors to give readers additional information about their work.
eFigure 1. Characterization of αSyn Aggregation In Vitro

Purified seed-free αSyn (1 mg/ml) was incubated in PBS, pH 7.4 at 37°C with vigorous shaking for different times. **A**: Aggregation was monitored over time by thioflavin T (ThT) fluorescence and expressed as a percentage of the maximum ThT fluorescence. **B**: Representative values of ThT fluorescence for αSyn incubated for 0 (unaggregated) or 240h (aggregated) to show that fluorescence readings are 3 orders of magnitude those of background.

**C**: Silver-stained SDS-PAGE gel of unaggregated or aggregated preparations, showing the presence SDS-resistant aggregates of ~135KDa. **D**: electron microscopic pictures of structures obtained at different times of incubation.
eFigure 2. Effect of Freeze/Thaw on αSyn-PMCA

Human CSF (40 µl) from a PD patient was snap frozen in liquid nitrogen and thawed one or two times and added to a 96-well plate. The aggregation assay was done as described in Fig. 2 and aggregation measured by ThT fluorescence. Assay was done in duplicate and error bars represent SD.
Human CSF samples from patients affected by other neurodegenerative disorders (as indicated in the X-axis) (A) and Alzheimer’s disease (AD) (B) were analyzed by αSyn-PMCA, using the conditions described in Fig 3. The graphs show the ThT fluorescence at 314 h of incubation (which for most of the samples corresponded to the plateau of fluorescence). Bars correspond to average and SD of individual samples ran in duplicate. Black dotted line indicates the cut off for the assay set at 50 fluorescence units.
Human CSF samples from 10 patients affected by multiple system atrophy (MSA) (A) and dementia with Lewy bodies (DLB) (B) were analyzed by αSyn-PMCA, using the same conditions as for Fig 3. The graphs show the ThT fluorescence at 314h of incubation (which for most of the samples corresponded to the plateau of fluorescence). Bars correspond to average and SD of individual samples ran in duplicate. Black dotted line indicates the cut off for the assay set at 50 fluorescence units.