Lipid vesicles trigger α-syn aggregation by stimulating primary nucleation

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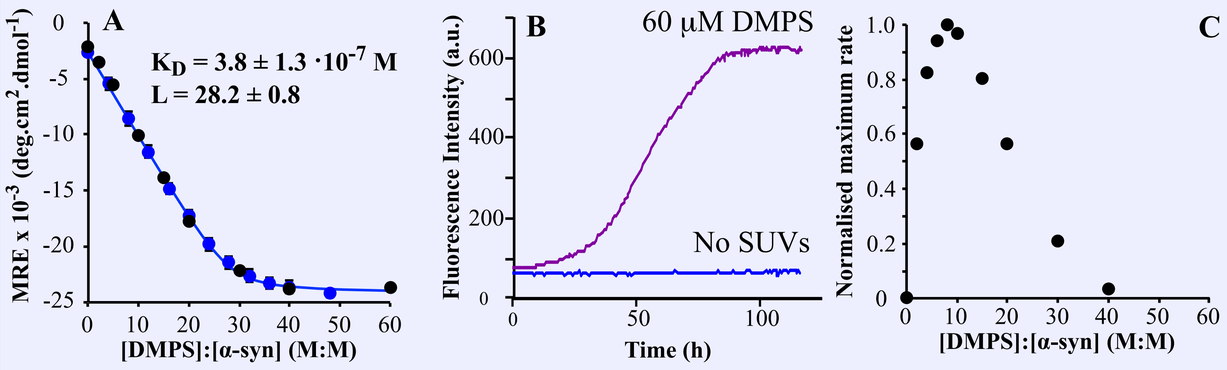
Introduction:

α-synuclein (α-syn) is a small (14 kDa) intrinsically disordered protein proposed to be involved in neuronal / synaptic vesicle plasticity. Amyloid formation of α-syn is the hallmark of Parkinson's disease. α-syn is natively unstructured in solution and adopts an α-helical fold upon binding to membranes. The interaction between α-syn and lipid vesicles has been shown to modulate its kinetics of amyloid fibril formation.

Here, we use a systematic approach to determine the mechanism and the kinetics of amyloid formation of α-syn in the presence of small unilamellar vesicles (SUVs).

Results and discussion:

Lipid vesicles promote α-syn aggregation at high protein:lipid ratio



C ***For high [α-syn]:[DMPS] ratios, the presence of SUVs strongly enhances the rate of aggregation of α-syn.***

No amyloid formation was observed when α-syn was incubated

- in the absence of SUVs
- in the presence of a large excess of SUVs.

Figure 1: A. Evolution of the CD signal of α-syn (25 μM (blue) and 50 μM (black)) measured at 222 nm as a function of [DMPS]:[α-syn] (M:M). B. Evolution of the fluorescence signal of ThT when 140 μM α-syn was incubated in the absence (blue) and in the presence of 60 μM DMPS (purple). C. Variation of the maximum rate of aggregation of α-syn with changes in the DMPS:α-syn ratio.

When pre-formed fibrils were incubated with an excess of DMPS SUVs, the protein was found to dissociate from the fibrils and populate the lipid-bound monomeric α-helical state.

The lipid-bound α-helical state of α-syn is thermodynamically more stable than the fibrillar state under these conditions.

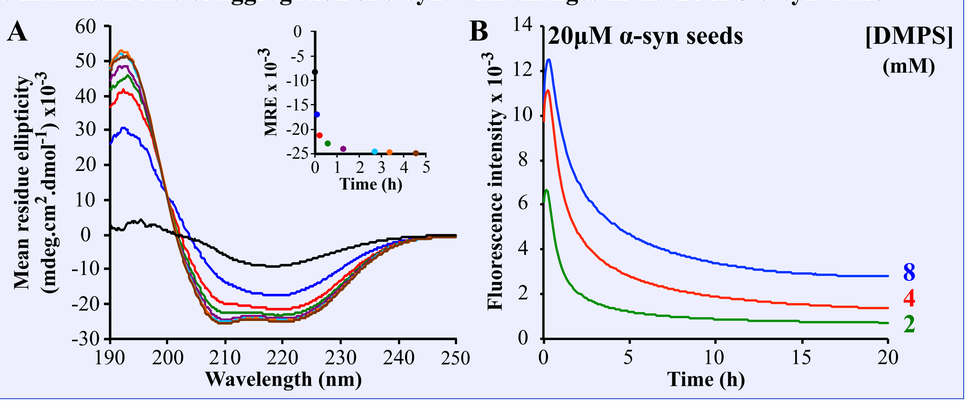
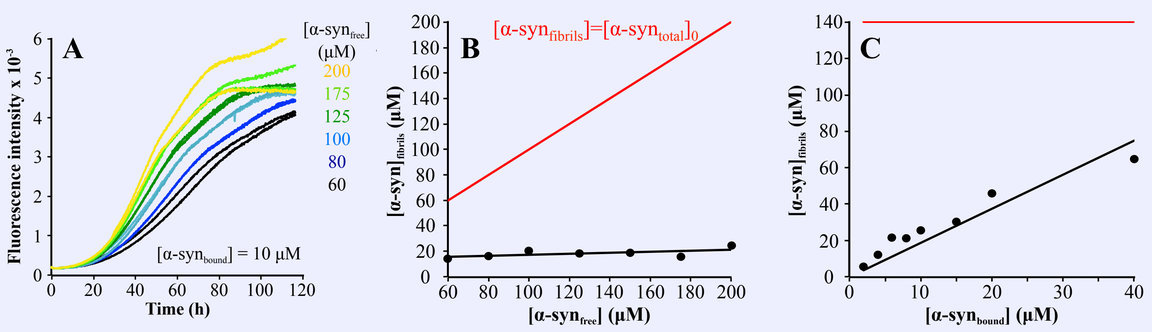


Figure 2: A. Evolution of the CD signal of α-syn when 20 μM pre-formed amyloid fibrils were incubated in the presence of 2 mM DMPS SUVs at 37°C (t = 0 (black), 5 (blue), 17 (red), 39 (green), 81 (purple), 165 (cyan), 205 (orange), 275 min (brown)). Insert: evolution of the CD signal of α-syn measured at 222nm as a function of time. B. Evolution of the ThT fluorescence with time when 20 μM preformed amyloid fibrils were incubated in the presence of an excess of DMPS SUVs at 37°C (2 mM (green), 4 mM (red) and 8 mM (blue)).

Free and membrane-bound α-syn play different roles in amyloid formation

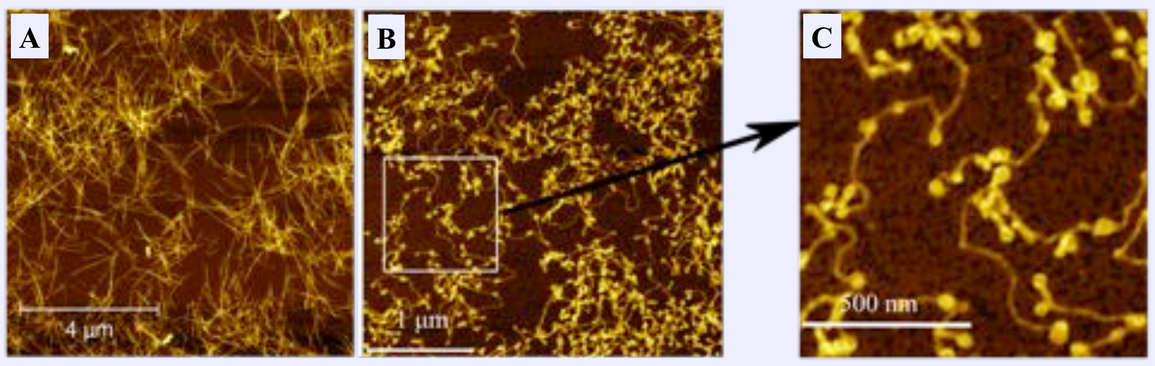


The concentration of protein molecules converted into fibrils was found to be:

- constant for all the different initial concentrations of α-syn free in solution
- proportional to the initial concentration of α-syn bound to the vesicles.

The rates of secondary nucleation and fragmentation are negligible.

Figure 3: A. Duplicates of the evolution of the fluorescence signal of ThT when increasing concentrations of α-syn were incubated in the presence of a constant concentration of DMPS (300 μM) B,C. Evolution of the concentration of α-syn that is converted into fibrils as a function of the concentration of α-syn free in solution (B), or bound to the SUVs (C).



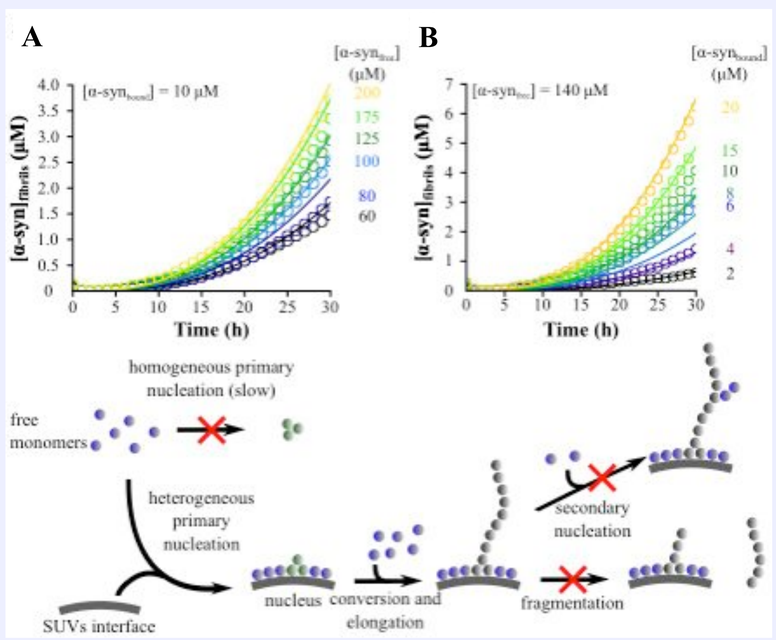
Two distinct types of structures are present:

- small spherical species with a diameter of 50 nm → SUVs coated with α-syn
- thin filaments that appear to be attached to the SUVs → amyloid fibrils.

The average length of the fibrils observed indicates that on average only a single nucleation event had occurred at each vesicle.

Figure 4: A,B. AFM images of aggregates of α-syn formed after incubation of 200 μM monomeric α-syn (A) in the presence of pre-formed seed fibrils, and (B) in the presence of 600 μM DMPS SUVs. C. Expanded region of the image in B.

Lipid vesicles can enhance the rate of heterogeneous primary nucleation by over six orders of magnitude



- Substantial evidence point towards the fact that heterogeneous primary nucleation (nucleation happening at the air/water interface or the lipid/water interface) rather than homogeneous primary nucleation (nucleation involving only free monomers) is the initial step in α-syn aggregation.
- n = 0.2 → the dependence of the nucleation rate on the concentration of free protein is almost negligible
- The rate of conversion between the two different nuclei is remarkably close to the one observed in the single molecule experiments in the absence of SUVs.

The rate of formation of nuclei is three orders of magnitude greater than the upper limit of the nucleation rate estimated for quiescent conditions in the absence of SUVs.

Figure 5: A,B. Global fits of the early time-points of the α-syn aggregation curves obtained for the different monomer and DMPS concentrations using a two-step nucleation mechanism ($k_n k_+ = 1.2\ 10^{-5}\ M^{-(n+1)}\ s^{-2}$, $K_M = 125$ μM , n = 0.2, $k_b = 1.9\ 10^{-5}\ s^{-1}$).

Conclusions:

- Taken together, our results provide a self-consistent explanation for the observed modulation of α-syn amyloid formation by lipid vesicles.
- At low α-syn:lipids ratios: all the protein molecules are bound to the surface of the vesicles, in a predominantly helical conformation, no fibril formation is observed.
- At high α-syn:lipids ratios: the rate of heterogeneous primary nucleation of α-syn can be enhanced by at least three orders of magnitude relative to that occurring in bulk solution and at the air/water interface.

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