Supplementary Information: Lipid vesicles trigger α-synuclein aggregation by stimulating primary nucleation

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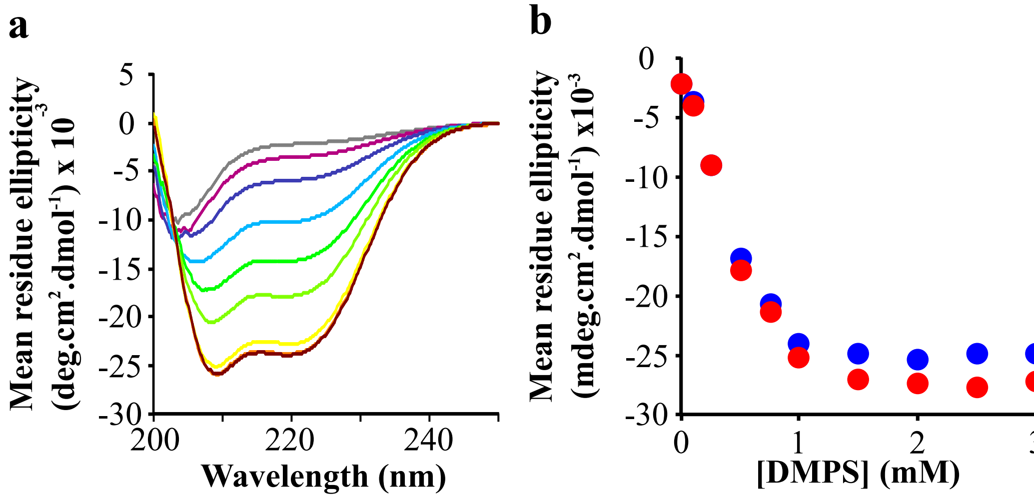
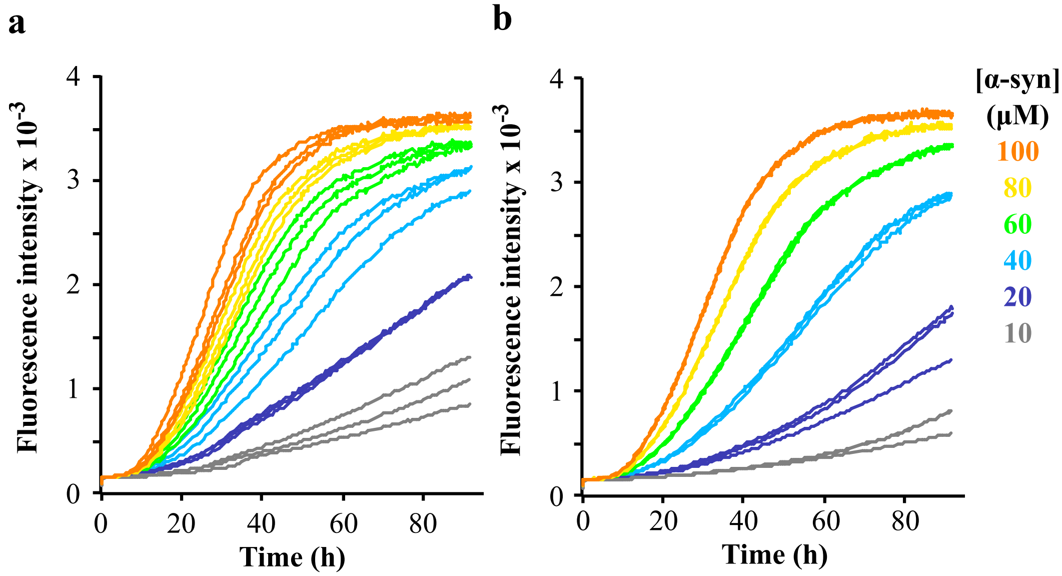
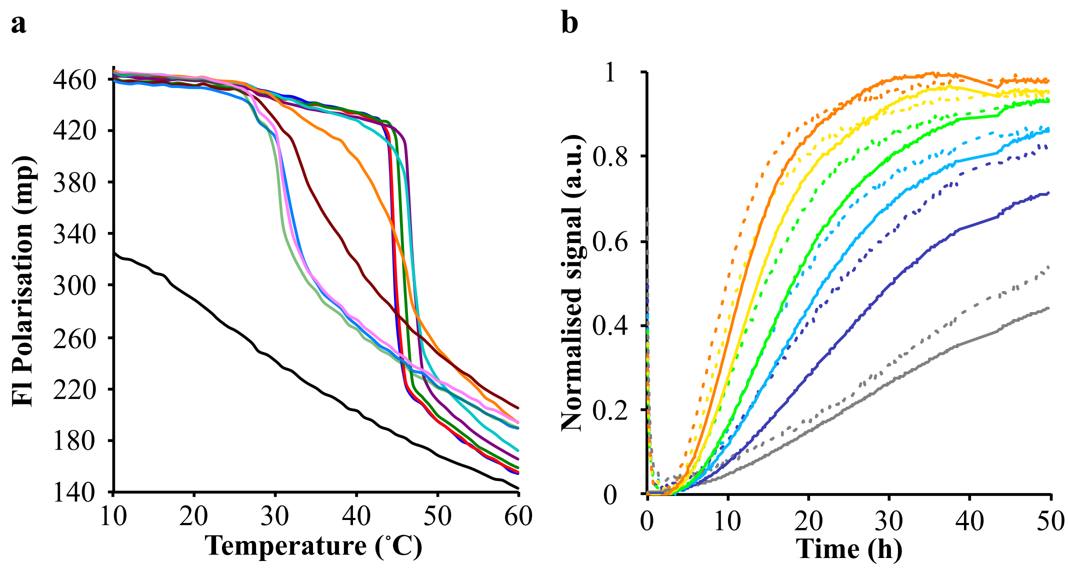
Supplementary Results



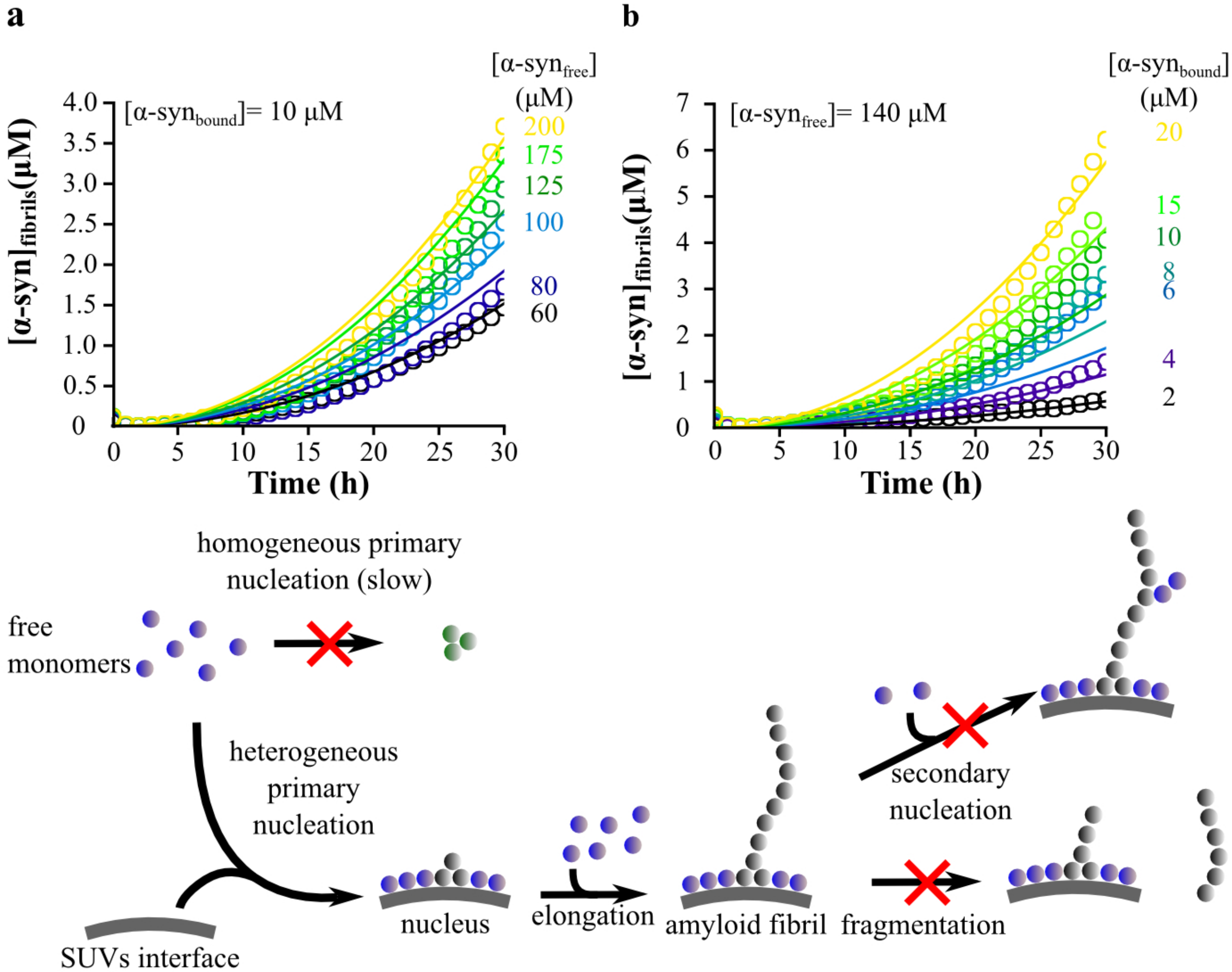
Supplementary Figure 1: α-syn binding to DMPS SUVs and LUVs monitored by Circular Dichroism (CD). a) CD spectra of α-syn (50 μM) measured in the absence (grey) and in the presence of 0.1 (purple), 0.25 (blue), 0.5 (cyan), 0.75 (green), 1 (light green), 1.5 (yellow), 2 (orange) and 3 mM (brown) DMPS. b) Change in the CD signal of α-syn measured at 222 nm in the presence of increasing concentration of DMPS in the form of either SUVs (blue) or LUVs (red).



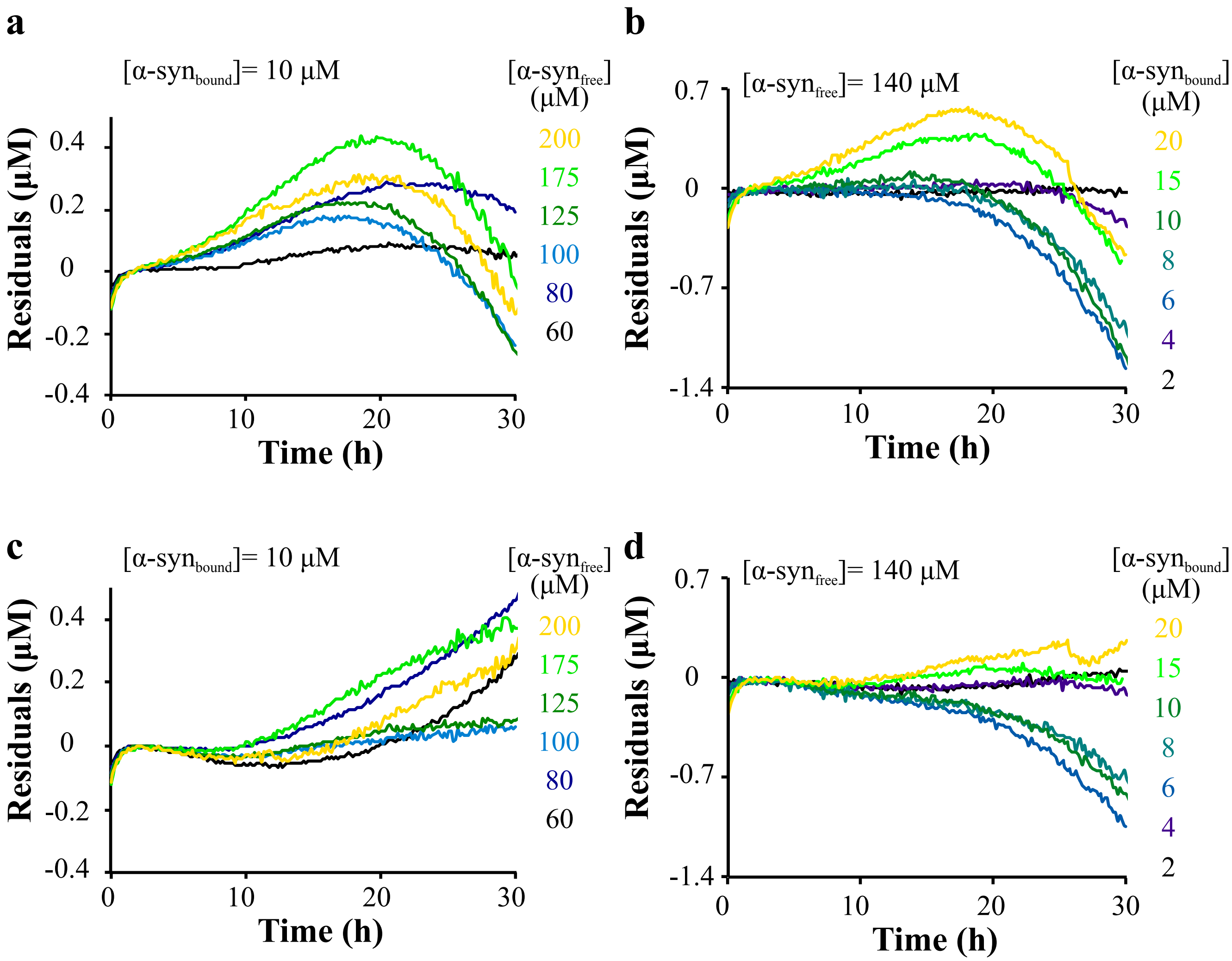
Supplementary Figure 2: Stimulation of α-syn aggregation by DMPS SUVs and LUVs. Triplicates of the change in the fluorescence signal of ThT when increasing concentrations of α-syn (10 (grey), 20 (blue), 40 (cyan), 60 (green), 80 (yellow), 100 μM (orange)) were incubated in the presence of a constant concentration of DMPS (100 μM) in the form of either SUVs (a) or LUVs (b).



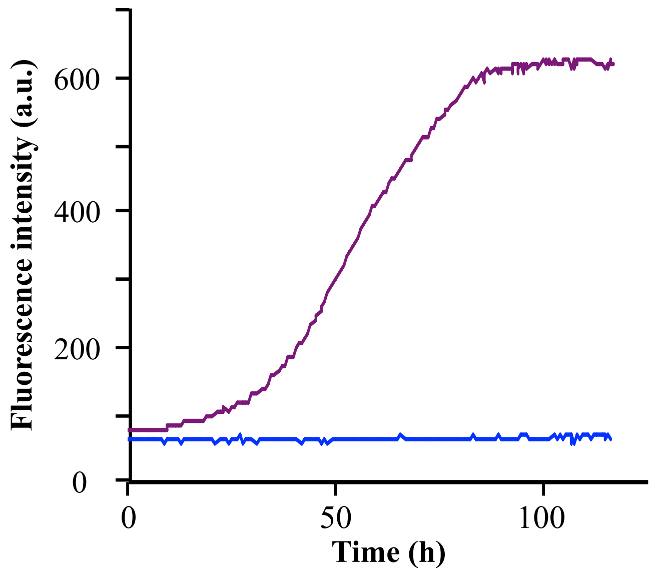
Supplementary Figure 3: Membrane fluidity monitored via fluorescence polarisation of membrane bound 1,6-diphenyl-hexa-1,3,5-triene (DPH). a) Change in the fluorescence polarisation (FP) of DPH embedded in the membrane of DMPS SUVs with temperature monitored in the absence (blue), the presence of 0.5 (red), 1 (green), 2.5 (purple), 5 (cyan), 10 (orange), 20 (brown), 40 (light blue), 60 (pink) and 80 μM (light green) α-syn and in the presence of 10% Triton X-100 (black) (used to solubilise the DMPS lipids). b) Change in both the ThT fluorescence (continuous line) and the FP of DPH (dotted line) monitored as a function of time when increasing concentrations of α-syn (25 (grey), 50 (blue), 75 (cyan), 100 (green), 150 (yellow), 200 μM (orange)) were incubated in the presence of a constant concentration of DMPS (600 μM).



Supplementary Figure 4: Global fits of the early time-points of the α-syn aggregation curves obtained for the different monomer and DMPS concentrations to the one-step nucleation mechanism ($k_n k_+ = 4.7\ 10^{-6}\ M^{-(n+1)}\ s^{-2}$, $K_M = 125$ μM , n = 0.2). The data set a and b correspond to the early time-points of the aggregation curves of α-syn when increasing concentrations of α-syn (60 (black), 80 (blue), 100 (light blue), 125 (dark green), 175 (green), 200 μM (yellow)) were incubated in the presence of a constant concentration of DMPS (300 μM) (a, see Fig. 4a of the main manuscript for the full time curves) and when free monomeric α-syn (140 μM) was incubated in the presence of increasing concentrations of DMPS (60 (black), 120 (purple), 180 (dark blue), 240 (light blue), 300 (dark green), 450 (light green), 600 (yellow), 1200 (orange) μM) (b, see Fig. 4b of the main manuscript for the full time curves), respectively. The "one-step" nucleation model is shown under the curves.



Supplementary Figure 5: Residuals of the fits of the early time-points of α-syn aggregation curves obtained for the different monomer and DMPS concentrations to the one-step nucleation (a,b) and two-step nucleation (c,d) models. The mean squared error of the fits to a single step and a two-step nucleation models are $6.14 \cdot 10^{-14}$ and $3.72 \cdot 10^{-14}$, respectively, and illustrate that the two-step nucleation model better describe our entire set of data. The corresponding fits are displayed in Fig. 6 of the main text and Supplementary Fig. 4, respectively.



Supplementary Figure 6: Change in 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).