Supporting Information: Chemical properties of lipids strongly affect the kinetics of the membrane-induced aggregation of α-synuclein

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SI Methods

1 Lipid dispersion preparation

The stocks of the lipids were purchased either as solutions or as powder. Lipid films were prepared by transferring the desired volume of lipid stock solution with a Hamilton syringe into a round bottom flask and the solvent was evaporated using a gentle flow of nitrogen gas. The flasks were then incubated for $\geq$ 1 h under vacuum to remove any residual traces of solvent. The lipid films or powders were dissolved in 20 mM phosphate buffer ((Na_2HPO_4/NaH_2PO_4), pH 6.5, 0.01%NaN_3), and stirred at a temperature above their respective melting temperature (T_m) for 2 h. The solutions were then frozen and thawed five times using dry ice and a water bath at a temperature above their T_m values, respectively. Lipid dispersions were prepared using sonication (3 $\times$ 5 min, 50 % cycles, 10 % maximum power) on ice. After centrifugation (13k rpm, 30 min), the sizes of the vesicles were measured with dynamic light scattering (Zetasizer Nano ZSP, Malvern Instruments, Malvern, UK) and found to consist of a distribution centered at 20 nm diameter.

2 Determination of the concentration of fibrils formed by α-synuclein in the presence of model membranes

For each P:L ratios, we incubated two types of samples in the micro-well plate: one that contains ThT and that was used to follow the kinetics of amyloid formation in real time and another that did not contain ThT. The rationale behind this dual incubation is that protein concentrations are easier to determine in the absence of ThT, due to the absorption band that ThT has around 280 nm. Once the increase in ThT fluorescence reached the plateau, we centrifuged (90 krpm, 30 min, 20°C) each reaction mixture which did not contained ThT and determined the concentration of soluble monomeric protein remaining in solution in the supernatant ($[\alpha - synuclein_{supernatant}]$) using absorbance at 275 nm and an extinction coefficient of 5,600. The concentration of aggregates formed ($[\alpha - synuclein_{fibrils}]$) were then calculated using the following equation:

$$[\alpha - synuclein_{fibrils}] = [\alpha - synuclein_{initial}] - [\alpha - synuclein_{supernatant}] \tag{S1}$$

with $[\alpha - synuclein_{initial}]$, the concentration of protein in the reaction mixture at time 0.

3 Circular dichroism

3.1 Data Acquisition

Quartz cuvettes with path lengths of 1 mm were used and CD spectra were obtained by averaging five individual spectra recorded between 250 and 200 nm with a bandwidth of 1 nm, a data pitch of 0.2 nm, a scanning speed of 50 nm/min, and a response time of 1 s. Each value of the CD signal intensity reported at 222 nm corresponds to the average of five measurements, each acquired for 10 s. For each protein sample, the CD signal of the buffer used to solubilize the protein was recorded and subtracted from the CD signal of the protein.

3.2 Data Analysis

The observed CD signal (CD_{obs}) consists of the sum of the signals of the lipid-bound and free α-synuclein:

$$CD_{obs} = x_{\alpha-syn_B} CD_B + x_{\alpha-syn_F} CD_F \tag{S2}$$

where $x_{\alpha-syn_B}$ and $x_{\alpha-syn_F}$ are the fractions of α-synuclein bound to the membrane and free in solution, as implied from the protein conformational change upon membrane association. CD_B and CD_F are the CD signals of the bound and free forms of α-synuclein, respectively. By assuming that $x_{\alpha-syn_B} + x_{\alpha-syn_F} = 1$, and that the signals of α-synuclein in the presence of buffer, or in the presence of model membranes under saturating conditions, correspond to CD_F and CD_B, respectively, the fraction of α-synuclein bound to SUV for each sample can be expressed as:

$$x_{\alpha-syn_B} = \frac{CD_{obs} - CD_F}{CD_B - CD_F} \tag{S3}$$

We used the following model: $\alpha - \text{syn} + \text{lipid}_\text{L} \rightleftharpoons \alpha - \text{syn(lipid)}_\text{L}$, which corresponds to a non-cooperative binding Langmuir-Hill adsorption model, and the following equation to fit the measured CD signal:

$$x_B = \frac{([\alpha - syn] + \frac{[lipid]}{L} + K_D) - \sqrt{([\alpha - syn] + \frac{[lipid]}{L} + K_D)^2 - \frac{4[lipid][\alpha - syn]}{L}}}{2[\alpha - syn]} \tag{S4}$$

where K_D (in M) is the dissociation constant and L is the number of lipid molecules interacting with one molecule of α-synuclein.

4 Differential Scanning Calorimetry - data analysis

The enthalpy associated with the transition at 25°C was analyzed using the following assumptions. The protein:lipid ratios used in Fig. 1*B* are between 1:200 and 1:5. At these protein:lipid ratios and below 25°C, all the lipid molecules are in contact with monomeric protein molecules whereas the protein molecules, being in excess, are present both in solution and bound to DMPS. When the temperature reaches 25°C, there is a strong heat effect that includes contribution from several processes, including adsorption of additional protein (corresponding to differences between 1:350 and 1:30 P:L ratios), protein conformational changes and a phase transition of the membrane from the gel to the fluid phase. Each one of these enthalpy contributions is to a first approximation proportional to the number of lipid molecules involved, and the overall change in enthalpy measured at 25°C can therefore be described as follows:

$$\Delta H_{cal,25^\circ C} = x_{lipid_B}(\Delta H_{cal,m} + \Delta H_{cal,b} + \Delta H_{cal,f}) \tag{S5}$$

where $\Delta H_{cal,m}$, $\Delta H_{cal,b}$ and $\Delta H_{cal,f}$ are, respectively, the enthalpy of melting of the lipids, the binding of α-synuclein to the lipids and the folding of the protein into an α-helix, respectively, and x_{lipid_B} is the fraction of lipid molecules bound to the protein. Using the same binding model ($\alpha - \mathrm{syn} + \mathrm{lipid_L} \rightleftharpoons \alpha - \mathrm{syn(lipid)_L}$) as that used to analyze the CD data, the fraction of lipids bound to the protein in the PL complex for any given $[\alpha - syn] : [lipid]$ ratio was calculated using the following equation:

$$x_{lipid_B} = \frac{(K_D L + [\alpha - syn]L + [lipid]) - \sqrt{(K_D L + [\alpha - syn]L + [lipid])^2 - 4[lipid][\alpha - syn]L}}{2[lipid]} \tag{S6}$$

5 Estimation of the surface area per DMPS molecule in the fluid phase

Since the surface area per DMPS molecule (A_l) in the fluid phase was not available from the literature, we estimated this value using the following equations:

$$A_l = \frac{2V_l}{D_B} \tag{S7}$$

$$V_l = V_C + V_H \tag{S8}$$

where V_l, V_H, V_C are the molecular volume, the head group volume and the hydrocarbon chain of a lipid molecule, respectively, and D_B is the bilayer thickness[1, 2]. Using the value of V_C and D_B measured for DMPC molecules in the fluid phase, 782 Å^3 and 36.9 Å[1], respectively, and taking V_H for the PS head group, 244 Å^2 [1], we can estimate the A_l value of DMPS in the fluid phase to 55.6 Å^2.

6 Estimation of the energy of transfer of a lipid molecule from water to a bilayer

The free energy of transfer of a lipid molecule from water to a bilayer has been found to be approximately proportional to the number of aliphatic carbons in the lipid chain[2]:

$$\Delta G^\circ_{tr} = (\frac{\partial \Delta G^\circ_{tr}}{\partial n_{CH}}) n_{CH} + \Delta\Delta G^\circ_{tr,\circ} \tag{S9}$$

where $\frac{\partial \Delta G^\circ_{tr}}{\partial n_{CH}}$ and $\Delta\Delta G^\circ_{tr,\circ}$ are the gradient and intercept, respectively, of a linear plot. This linear dependence was shown to hold for lipids with zwitterionic (phosphatidyl choline (PC)) and negatively charged (phosphatidyl glycerol(PG)) head groups and chain lengths ranging from 8 to 18 aliphatic carbons[2]. We used the linear dependence of $\frac{\Delta G^\circ_{tr}}{RT}$ known for 1,2-dihexanoyl-sn-glycero-3-phospho-L-serine ($(6{:}0)_2$PS), 1,2-dioctanoyl-sn-glycero-3-phospho-L-serine ($(8{:}0)_2$PS) and 1,2-dilauroyl-sn-glycero-3-phospho-L-serine ($(10{:}0)_2$PS)[2–5], with $R = 8.314$ J.mol^{-1}.K^{-1} and $T = 303$ K to determine $\frac{\partial \Delta G^\circ_{tr}}{\partial n_{CH}}$ (PS) (-3.59 kJ.mol^{-1}) and $\Delta\Delta G^\circ_{tr,\circ}$ (PS) (2.49 kJ.mol^{-1}) for phosphatidyl serine (PS) ($R^2 = 1.0$). We then extrapolated this linear dependence to $n_{CH} > 10$, as observed for PC and PG lipids, and we estimated ΔG°_{tr} for DLPS ($(12{:}0)_2$, $n_{CH} = 12$), DMPS ($(14{:}0)_2$, $n_{CH} = 14$), DPPS ($(16{:}0)_2$, $n_{CH} = 16$), POPS ((16:0)/(18:1), $n_{CH} = 17$), DOPS ($(18{:}1)_2$, $n_{CH} = 18$) using Eq. S9, $\frac{\partial \Delta G^\circ_{tr}}{\partial n_{CH}}$ (PS) and $\Delta\Delta G^\circ_{tr,\circ}$ (PS).

SI Figures

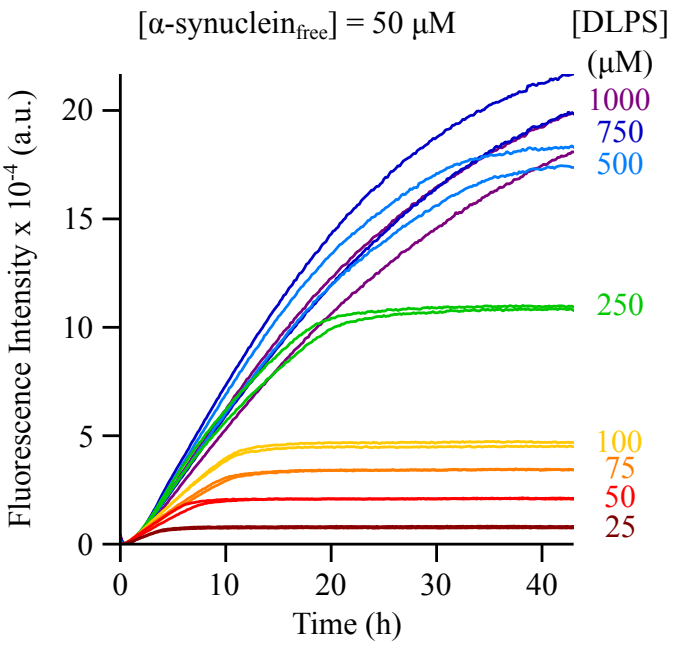

Fig. S 1: The concentration of fibrils formed by α-synuclein in the presence of DLPS is proportional to the concentration of lipid. Change in the fluorescence signal of the ThT when 50 μM α-synuclein is incubated in the presence of increasing concentration of DLPS (25 (dark red), 50 (red), 75 (orange), 100 (yellow), 250 (green), 500 (blue), 750 (dark blue) and 1000 (purple) μM).

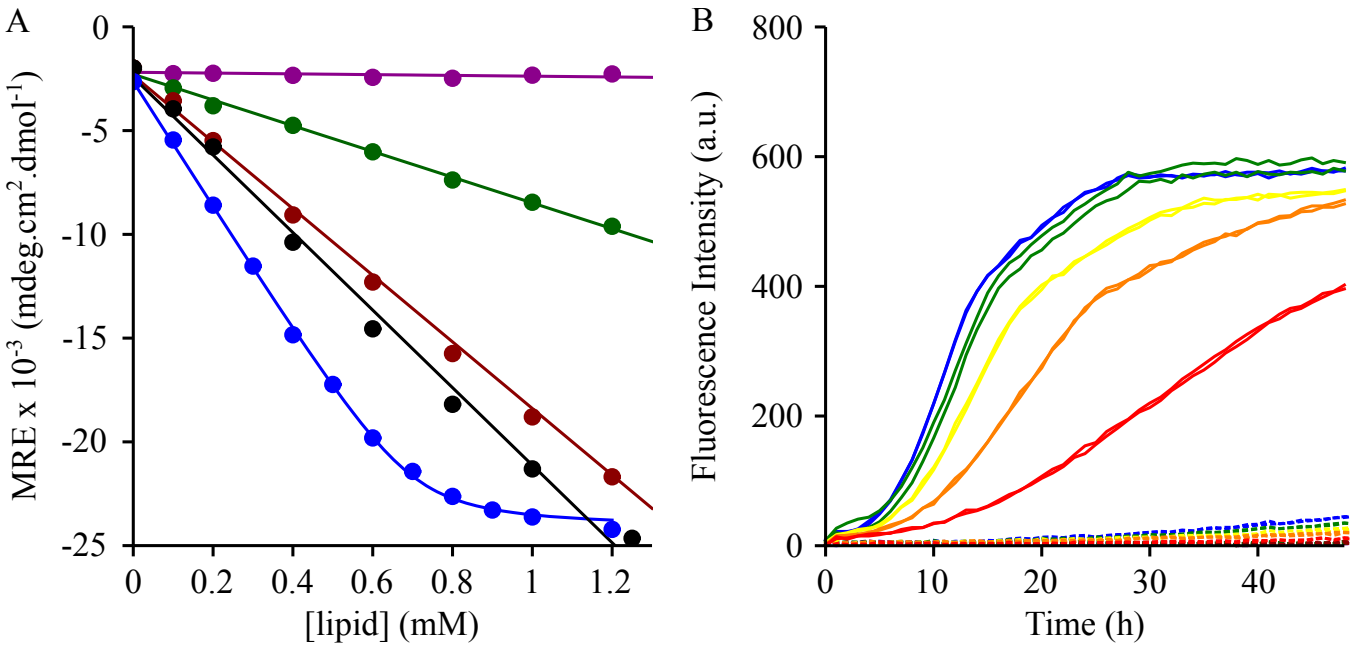

Fig. S 2: Effects of a change in the charge of the model membranes on the binding of α-synuclein and the kinetics of amyloid formation. (*A*) Change in the Mean Residual Ellipticity at 222 nm of α-synuclein (20 μM) incubated at 30°C in the presence of increasing concentrations of DMPC:DMPS (M:M) model membranes (DMPC:DMPS = 0:100 (blue), 25:75 (black), 50:50 (dark red). 75:25 (dark green), 100:0 (dark purple). (*B*). Evolution of the ThT fluorescence signal when 20 (red), 40 (orange), 60 (yellow), 80 (green) and 100 (blue) μM α-synuclein is incubated in the presence of 100 μM DMPS (solid line) or DMPC:DMPS (25:75) (dotted lines) and when 100 μM α-synuclein is incubated in the presence of 100 μM DMPC:DMPS (50:50, M:M) (dark red), DMPC:DMPS (75:25, M:M) (dark green) or DMPC (dark magenta).

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