# SI: Molecular mechanism of α-synuclein aggregation on lipid membranes revealed.

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## Detailed examination of reasons for lack of lipid dependence of nucleation

Since the initial α-synuclein concentration is kept constant, the lack of lipid dependence of nucleation in Fig. 4**A** could alternatively be explained by the reduction in free protein monomer concentration upon increased initial lipid concentration due to binding to vesicles in the very early stages of the reaction. However, as shown above, this depletion effect is small, with the reduction in $m_f(0)$ going from $r(0) = 4$ to $r(0) = 8$ being $0.86m(0) \rightarrow 0.71m(0)$. For the rate of primary nucleation to be unchanged, as required by the perfect overlap of the kinetic curves for these initial lipid:protein ratios, would require $(0.86m(0))^{n_c} = 2(0.71m(0))^{n_c}$. In turn this requires $n_c \simeq 4$, i.e. a very strong scaling of the rate of aggregation with free protein concentration. However, this is far too large, with multiple previous studies reporting instead very weak scaling [1–3].

## Secondary processes are at most a minor contributor to formation of lipidic fibrils

To test whether secondary processes play a role in lipid-induced α-synuclein aggregation under our assay conditions, aggregation experiments were performed in which 10, 20, 30 and 40 μM monomeric protein were incubated with DMPS vesicles at 5x and 8x lipid to protein molar ratios, and fibril formation was monitored by ThT fluorescence. Global fits to the model Eqs. (1), (2) and (3) were performed with $k_2 = 0$ (Fig. S1**a-b**), or with $k_2$ and $n_2$ fitted alongside the other parameters (Fig. S1**c-d**). As expected, given the higher number of free fitting parameters, a non-zero secondary process rate constant yielded improved fits. However, the difference in fit quality was only modest, and not significant enough to positively confirm that a secondary process is present, given that an extra free parameter has been introduced. (The fitted reaction order of $n_2 = 0$ implies that this secondary process, if present, is either fibril fragmentation or fully saturated secondary nucleation [4].) Moreover, the fitted $k_2$ value is relatively small in all datasets analyzed, with the rate of the secondary process never much larger than that of primary nucleation. This indicates that even if present, secondary processes are not a critical part of the mechanism.

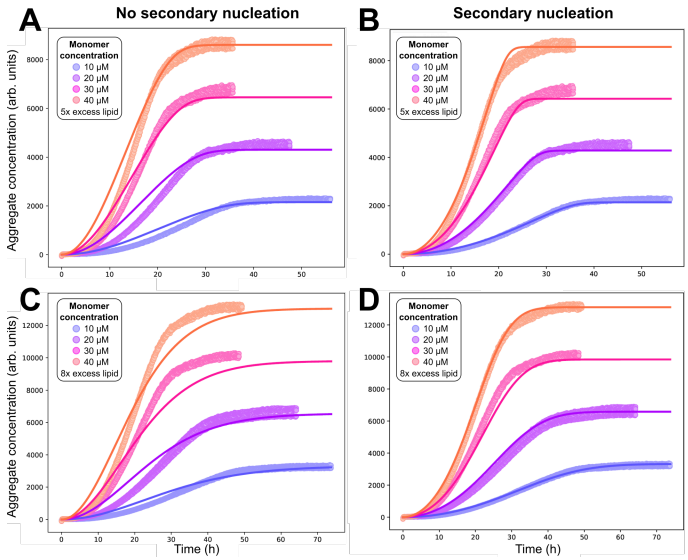


FIG. S1. **Kinetic model fitting to experimental data provides tentative evidence for weak fragmentation or secondary nucleation. A**,**B**: 5x molar ratio of monomeric lipid to α-synuclein. **C**,**D**: 8x molar ratio of monomeric lipid to α-synuclein. **A**,**C**: setting $k_2 = 0$ yields acceptable global fits of Eqs. (1), (2) and (3) to the data. **B**,**D**: freely fitting $k_2$ yields improved fits, but only slightly, implying a relatively slow secondary process may be present. Parameters: as in top 2 rows of Fig. 5.

**Kinetics in the presence of preformed aggregates**

To further verify our model, and show its validity also in data where the primary nucleation step is bypassed, we monitored the aggregation kinetics in the presence and absence of preformed seed fibrils. These seeded experiments are less straightforward than in pure protein systems, because of the sensitivity of lipidic fibrils. With agitation, for example caused by pipetting a solution, the conversion of lipidic fibrils to pure protein fibrils can easily be triggered [2]. Thus, adding seed fibrils to a solution of monomeric protein and SUVs, runs the risk of introducing pure protein fibrils, whose aggregation will then dominate the system. Therefore the standard seeding protocols cannot be used in this system. Instead, we employed the following protocol: seed fibrils are formed by letting a small volume of monomeric protein and SUVs aggregate under the standard conditions in a multi-well plate, giving an effective seed concentration of approximately 10%. To initiate the seeded reaction, the protein + SUV mix at the desired concentration is then carefully added to the well containing the seed solution. This leaves the seed fibrils relatively undisturbed and avoids formation of pure protein fibrils (as evident by the aggregation kinetics consistent with formation of lipidic fibrils alone). Our model was able to fit the data well, globally at both the seeded and unseeded conditions, see Fig. S2.

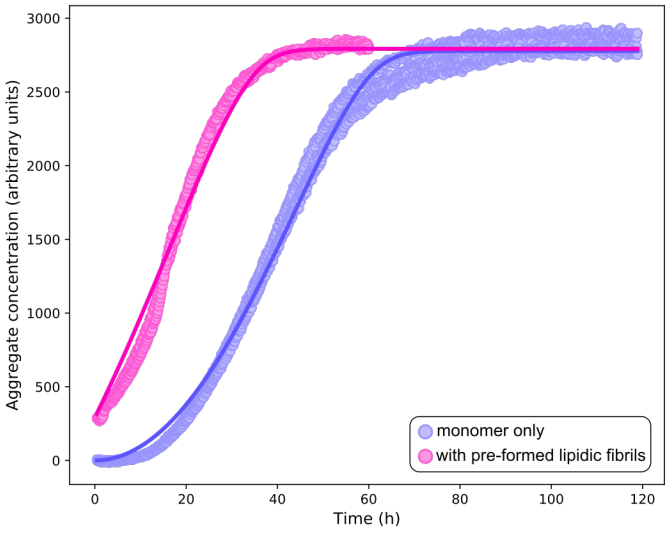


FIG. S2. **Model fitting to kinetic data of aggregation with and without preformed lipidic fibrils.**Eqs. (1), (2) and (3) are able to globally fit the aggregation with and without preformed seed fibrils, providing further evidence for the validity of our model. Parameters: $k_+k_n = 9.8 \times 10^{-5}$, $k_+k_2 = 2.3 \times 10^{-4}$ (fitted), $n_c = 0.6$, $n_2 = 0$, $\chi = 10.5$, $k_{\rm on}/k_+ = 8.3$ (determined a priori). Protein concentration: 20 µM; lipid concentration: 100 µM.

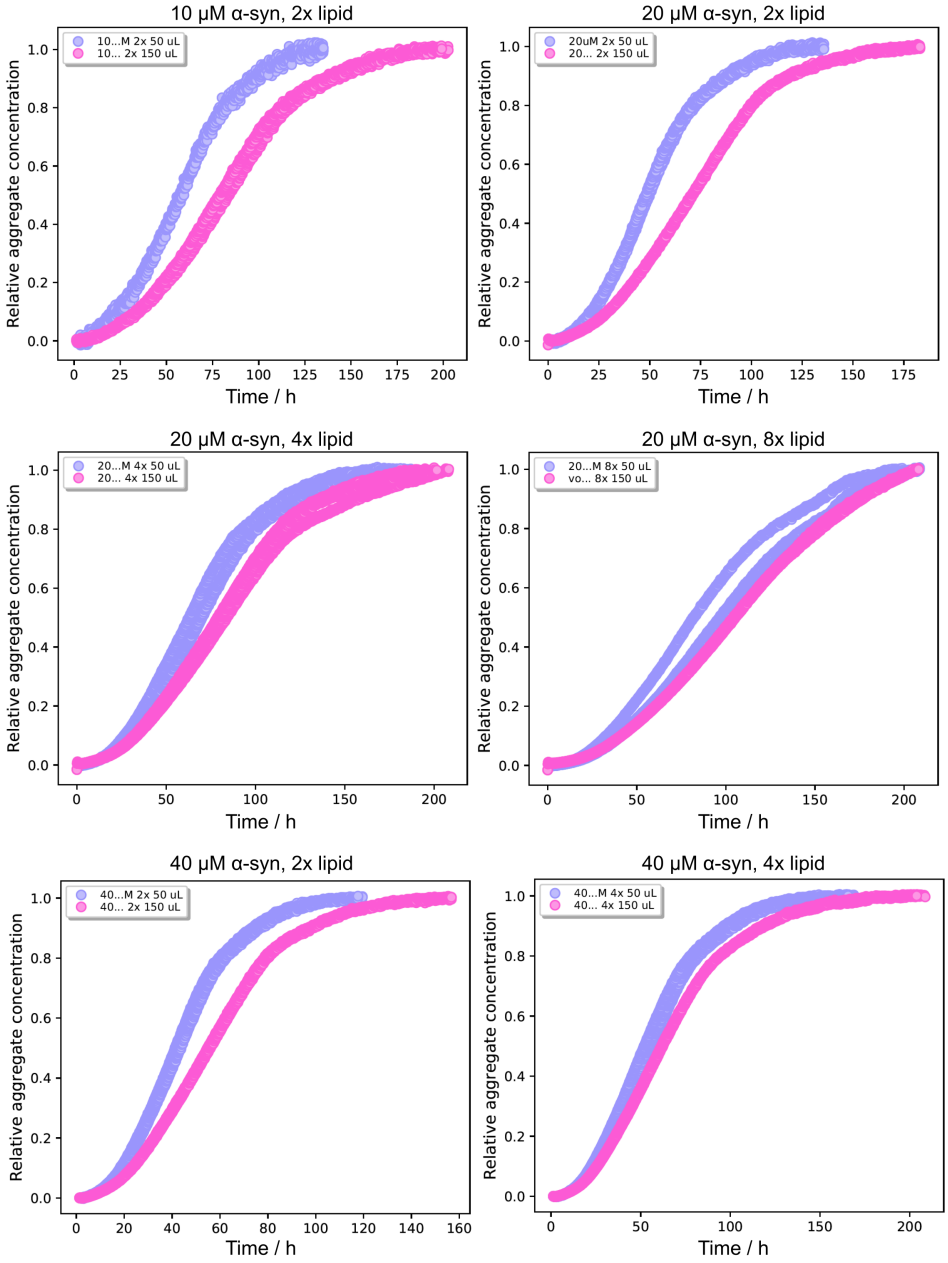


FIG. S3. **Additional data at varying volume.** Additional data, to supplement the data shown in Fig. 4B of the main text, of aggregation at different volumes. These data show that the effect of varying volume is present across lipid and monomer concentrations. Data at 50 µl (purple) and 150 µl (pink) are shown. The aggregation kinetics at lower volumes are faster, consistent with surface catalysed nucleation.

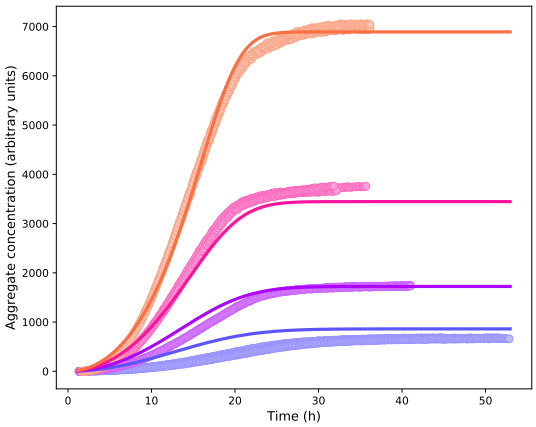


FIG. S4. Fits to the data in Fig. 3A. Parameters: $k_+k_n = 9.3 \times 10^{-5}$, $k_+k_2 = 1.2 \times 10^{-3}$, $n_c = 0.6$, $n_2 = 0$ (fitted globally), $\chi = 10.5$, $k_{\rm on}/k_+ = 8.3$ (determined a priori).

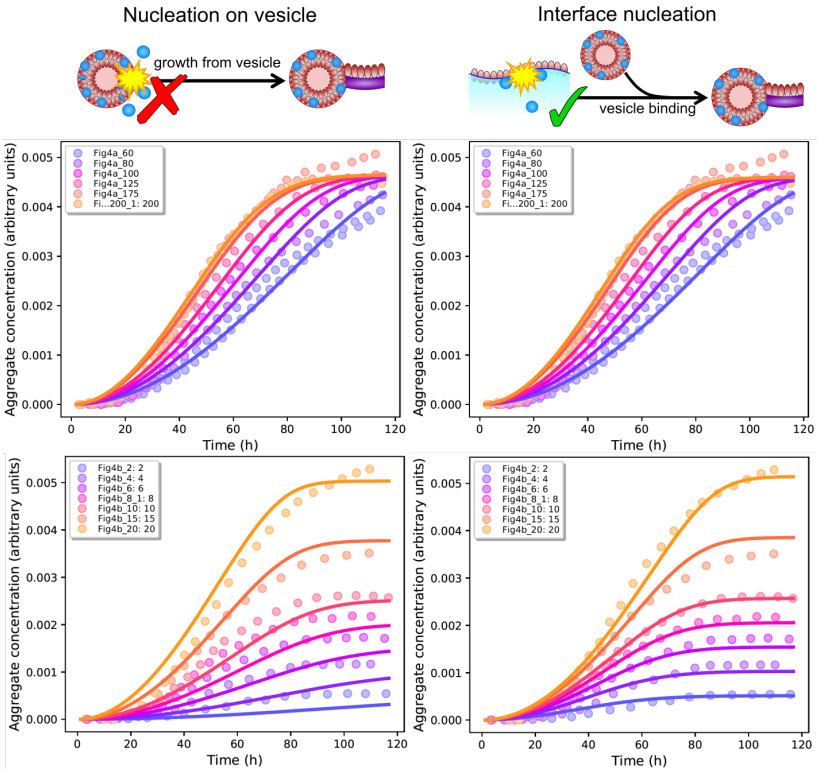


FIG. S5. **Fits of interface and bulk nucleation to data from Galvagnion et al.[1].** Data were fitted globally to a model assuming nucleation on vesicles in bulk solution (left column) or on interfaces (right column). Although interface nucleation model fits the data substantially better, the bulk nucleation model has more success than with other datasets. This is because the data with strongly varying lipid concentration (bottom row) feature much lower lipid:protein ratios than in Figs 4-5. At these ratios the elongation rate begins to acquire lipid dependence. This mathematically resembles primary nucleation rate lipid dependence, if $k_{\rm on}/k_+$ is not known. Parameters: $k_+k_n = 4.0 \times 10^{-5}$, $k_+k_2 = 6.6 \times 10^{-6}$ (top row), $k_+k_n = 4.1 \times 10^{-5}$, $k_+k_2 = 1.9 \times 10^{-5}$ (bottom row), $n_c = 0.3$, $n_2 = 0$ (fitted globally across both rows), $\chi = 10.5$, $k_{\rm on}/k_+ = 8.3$ (determined a priori). $k_+k_n$ and $k_+k_2$ were fitted separately for each row because the data in each were collected in separate experiments; however, their values are found to be the same to within expected levels of error (typically at least a factor of 3 between separate plate reader experiments).

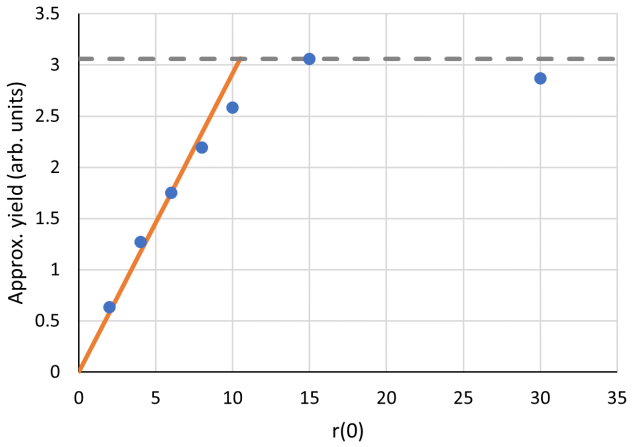


FIG. S6. Plots of relative lipidic fibril yield vs initial lipid:protein ratio $r(0)$ as inferred from maximum ThT signal observed in Fig. 1 of Galvagnion et al. [1] (constant initial protein concentration $m(0) = 50$ μM). Yield reaches a maximum as $r(0)$ approaches 15. This implies the optimal stoichiometry of lipid:protein within fibrils is approximately 15. Yield below $r(0) = 15$ is convex in $r(0)$, implying stoichiometry in fibrils is flexible and reduces when lipid is limiting to promote yield. Linearity is approximately restored below $r(0) = 8$ (orange line), implying fibril stoichiometry is fixed below $r(0) = 8$ (i.e. further reductions in fibril stoichiometry to boost yield are no longer thermodynamically favourable). Extrapolating this linear relationship to the maximum yield possible implies the stoichiometry in this regime to be $\chi \simeq 10.5$.

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