*Supporting Information for:*

# Effects of phosphatidylcholine membrane fluidity on the conformation and aggregation of N-terminally acetylated α-synuclein

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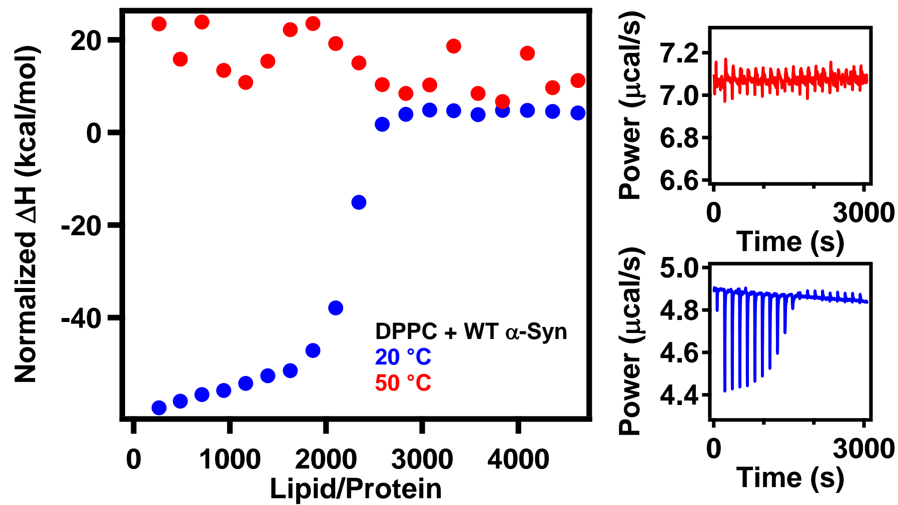



**Figure S1.** Titration of DPPC SUVs (2 µL injections of 45 mM lipid) into α-syn (2 µM in a cell volume of ~200 µL) at 20 °C (blue) or 50 °C (red) using isothermal calorimetry. Molar heat released was calculated by integrating each individual heat flow signal (see raw isotherms on the right) as a function of the lipid/protein ratio in the cell (left).

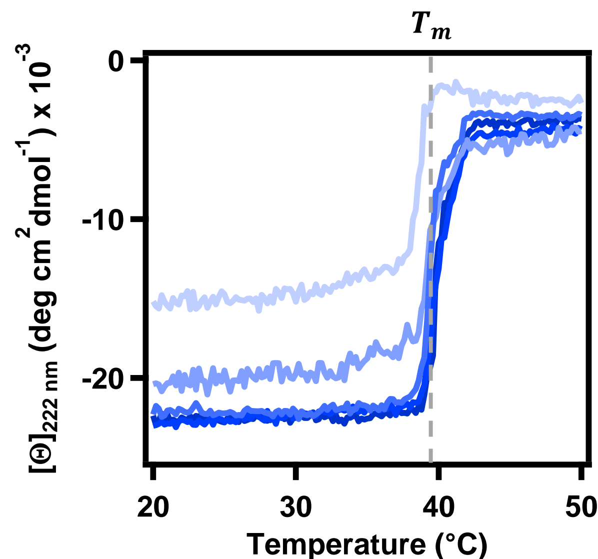



**Figure S2.** Effect of increasing temperature on the α-helical content ($[\Theta]_{222\ nm}$) of α-syn (5 µM) in the presence of DPPC vesicles (1.0 – 4.6 mM). Increasing lipid-to-protein (L/P) ratios are indicated by a light-to-dark blue color scale. Gray dashed line indicates the transition temperature, $T_m$ = 39 °C, for DPPC. Scan rate = + 20 °C/h.

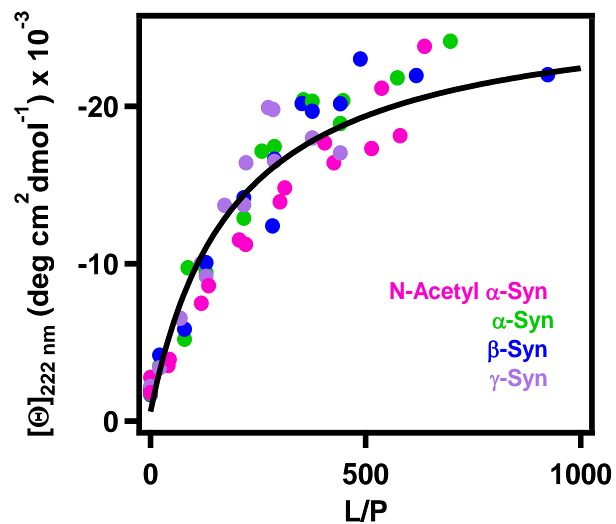



**Figure S3.** Change in α-helicity ($[\Theta]_{222\ nm}$) of N-acetyl α-syn (magenta, n = 2), α-syn (green, n = 2), β-syn (blue, n = 2), and γ-syn (purple, n = 2) to DPPC vesicles at 20 °C. CD data were globally fit and shown as a solid line ($K_p = 700 \pm 200\ M^{-1}$).

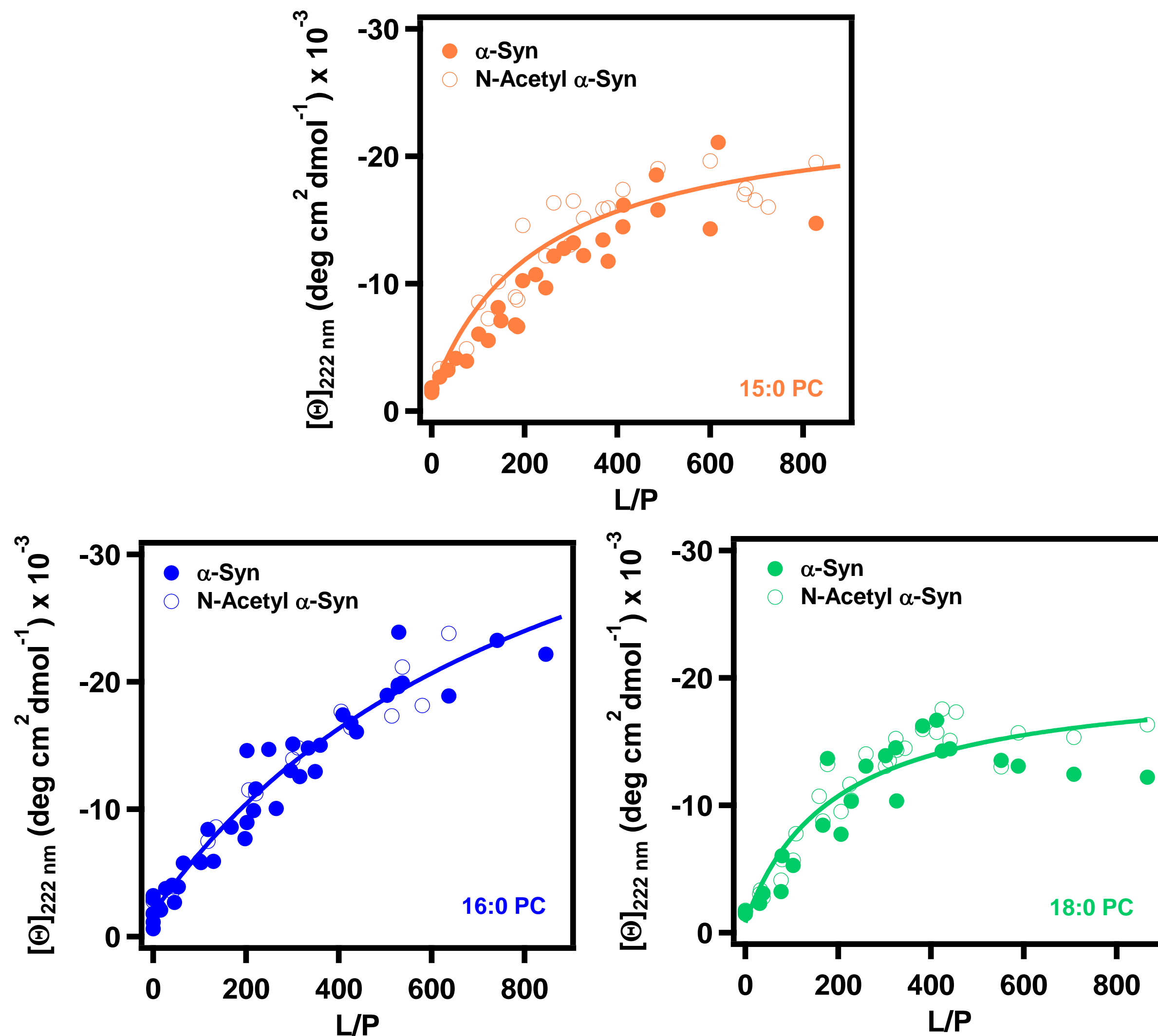



**Figure S4.** Comparison between unacetylated and N-acetyl α-syn binding to PC vesicles with varying chain lengths as assessed by CD spectroscopy. Equilibrium binding curves generated at 20 °C from $[\Theta]_{222\ nm}$ for unacetylated (filled circle, $n \geq 3$) and N-acetyl (open circle, $n \geq 3$) α-syn (5 μM) to 15:0 PC (orange), 16:0 PC or DPPC (blue), and 18:0 PC or DSPC (green). Data were globally fit to a two-state equilibrium, and $K_p$ values were 1400 ± 500 $M^{-1}$, 700 ± 200 $M^{-1}$, and 1400 ± 700 $M^{-1}$ for 15:0 PC, 16:0 PC, and 18:0 PC, respectively.

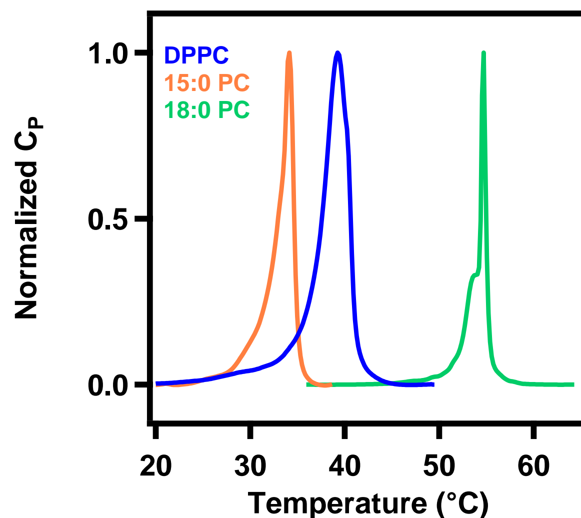



**Figure S5.** DSC thermograms of PC phospholipids with varying acyl chain lengths. Lipid stocks were degassed and injected into the calorimeter. DSC measurements were taken using MOPS buffer (20 mM MOPS, 100 mM NaCl, pH 7) as a reference over a given temperature range. Scan rate = + 20 °C/h. Data were buffer subtracted and normalized to the lipid concentration. The transition melting temperature ($T_m$) was determined by fitting the data to a 2-state model: 15:0 PC ($T_m$ = 34 °C), DPPC ($T_m$ = 39 °C), and 18:0 PC ($T_m$ = 55 °C).

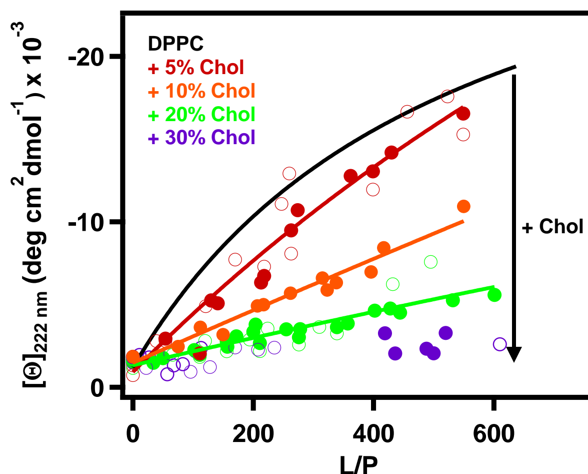



**Figure S6.** Impact of cholesterol (Chol) on the binding of unacetylated and N-acetyl α-syn to DPPC vesicles at 20 °C as determined by CD. Equilibrium binding curves from $[\Theta]_{222\ nm}$ for unacetylated (open circle) and N-acetyl (closed circle) α-syn (5 µM) to DPPC (black, global fit) and DPPC with 5% (red, $n$ = 4), 10% (orange, $n$ = 2), 20% (green, $n$ = 5), and 30% Chol (dark purple, $n$ = 3). Data for N-acetyl α-syn are also shown in Figure 7A. Global fits to a two-state equilibrium are shown as solid lines, and $K_p$ values were 400 ± 200 $M^{-1}$, 200 ± 100 $M^{-1}$, and 80 ± 60 $M^{-1}$ for 5%, 10%, and 20% Chol, respectively.

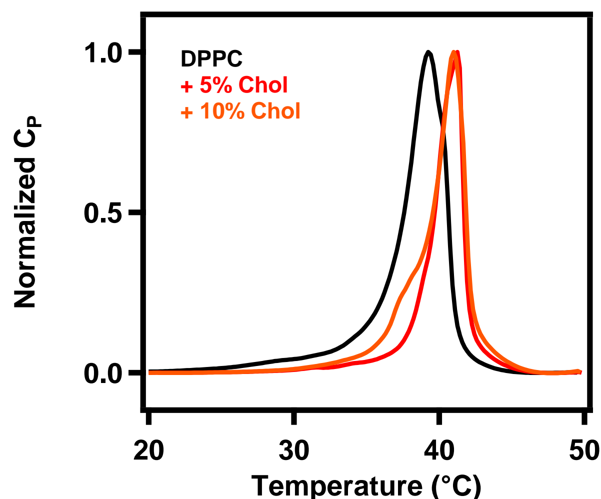



**Figure S7.** DSC thermograms of PC phospholipids with varying acyl chain lengths. Lipid stocks were degassed and injected into the calorimeter. DSC measurements were taken using MOPS buffer (20 mM MOPS, 100 mM NaCl, pH 7) as a reference from 20 to 50 °C. Scan rate = + 20 °C/h. Data were buffer subtracted and normalized to the lipid concentration. The transition melting temperature ($T_m$) was determined by fitting the data to a 2-state model: DPPC alone ($T_m$ = 39 °C) and DPPC + 5% (red) and 10% (orange) Chol ($T_m$ = 41 °C).

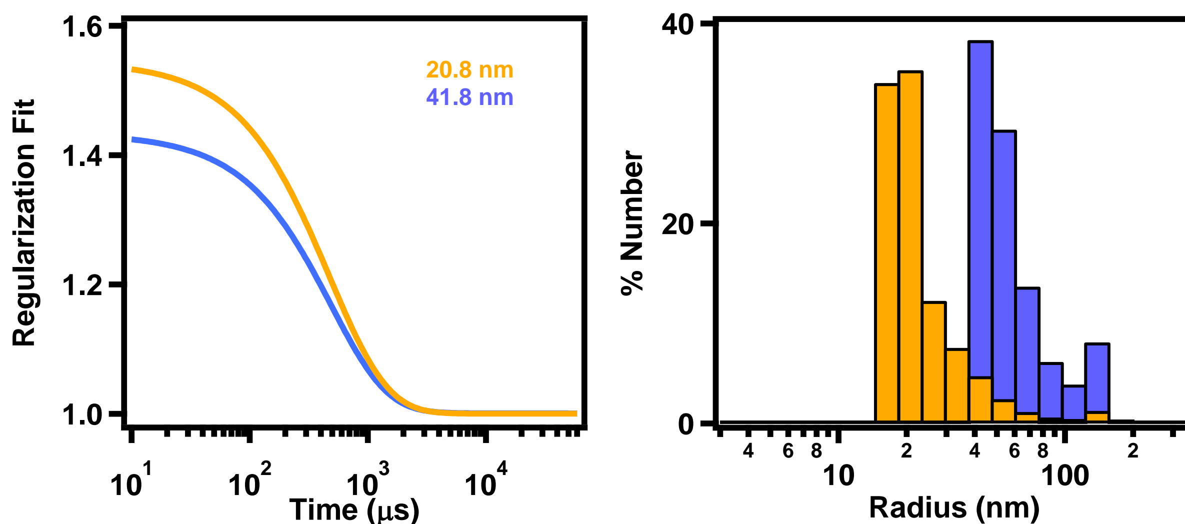



**Figure S8.** Representative DLS of DOPC/SM/Chol (2:2:1) vesicles at 20 °C. Average hydrodynamic radii (right) were estimated using a regularization fit based on correlation curves (left) and the Rayleigh sphere model: $r_{avg}$ = 20.8 nm (orange) and $r_{avg}$ = 41.8 nm (blue).

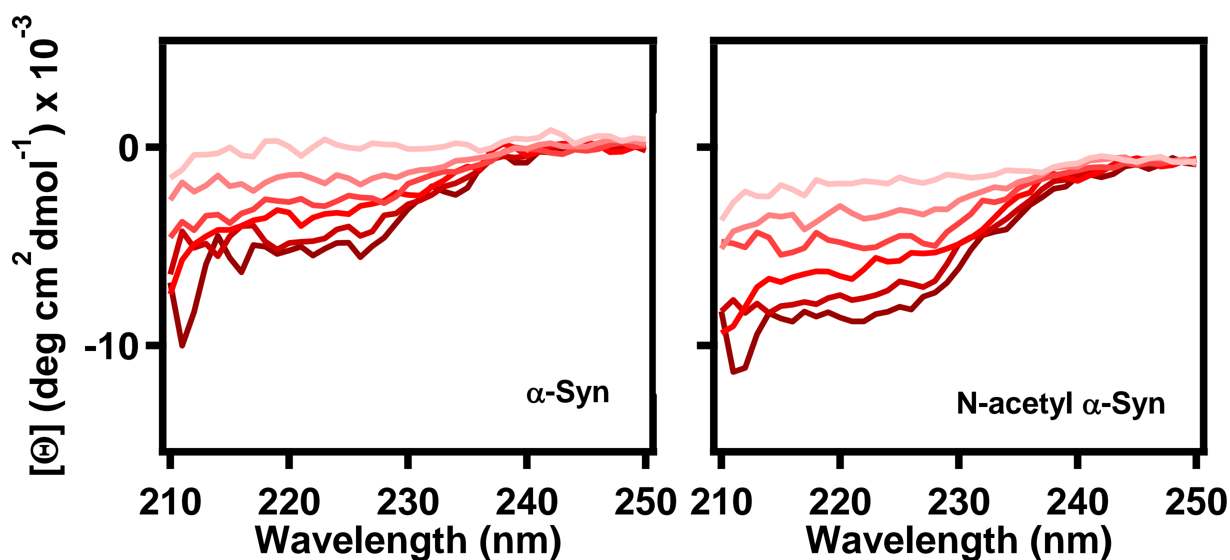



**Figure S9.** Effect of N-terminal acetylation on α-syn helical content in the presence of DOPC/SM/Chol (2:2:1) vesicles. CD spectra of unacetylated (left) and N-acetyl (right) α-syn (5 μM) as a function of increasing lipid raft vesicles (0 – 1.9 mM) at 20 °C are shown as light-to-dark red traces. The average hydrodynamic radius was 16.3 nm by DLS.

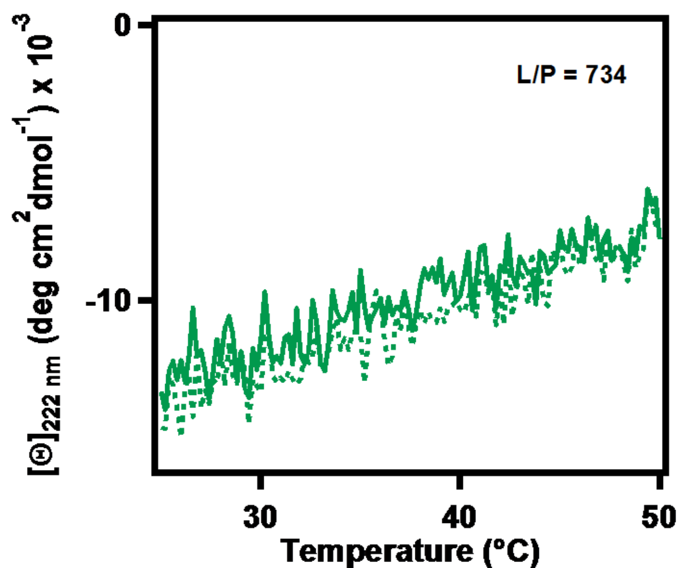
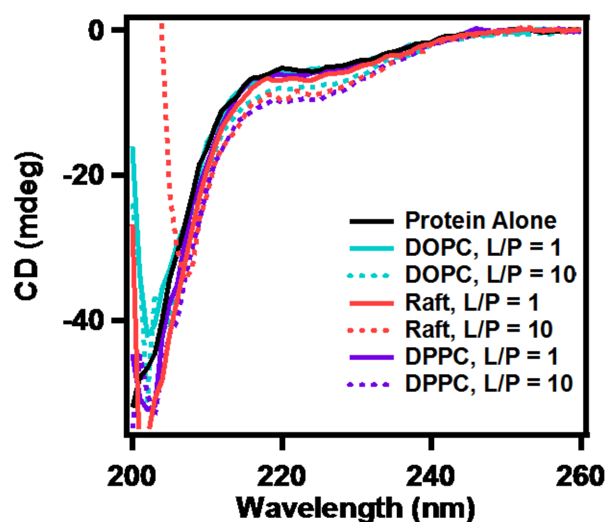



**Figure S10**. Effect of increasing (dotted trace) and decreasing (solid trace) temperature on the α-helical content ($[\Theta]_{222\ nm}$) of α-syn (5 μM) in the presence of DOPC/SM/Chol (2:2:1) vesicles (3.7 mM). Scan rate = + 20 °C/h.



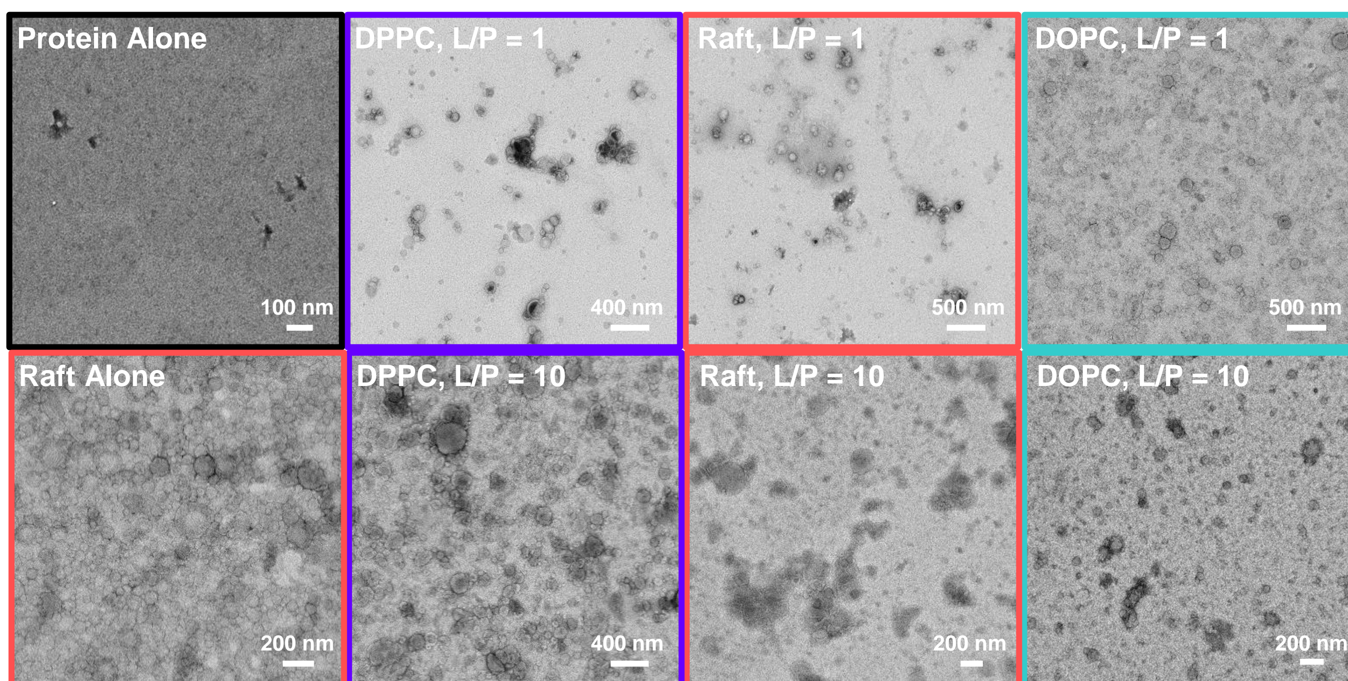




**Figure S11.** CD spectra and TEM images of pre-aggregation samples of N-acetyl α-syn with DOPC, DPPC, or DOPC/SM/Chol (2:2:1) SUVs. CD spectra at 20 °C of N-acetyl α-syn (50 μM) in the absence (black) or presence of DOPC (teal), DPPC (purple), and DOPC/SM/Chol (2:2:1) (pink) vesicles at L/P = 1 (solid traces) and L/P = 10 (dotted traces). TEM images of pre-aggregation N-acetyl α-syn in the absence or presence of DPPC, DOPC/SM/Chol (2:2:1), and DOPC vesicles at L/P = 1 (top) and L/P = 10 (bottom). Raft vesicles alone (500 μM) are also shown. Scale bars are as indicated.

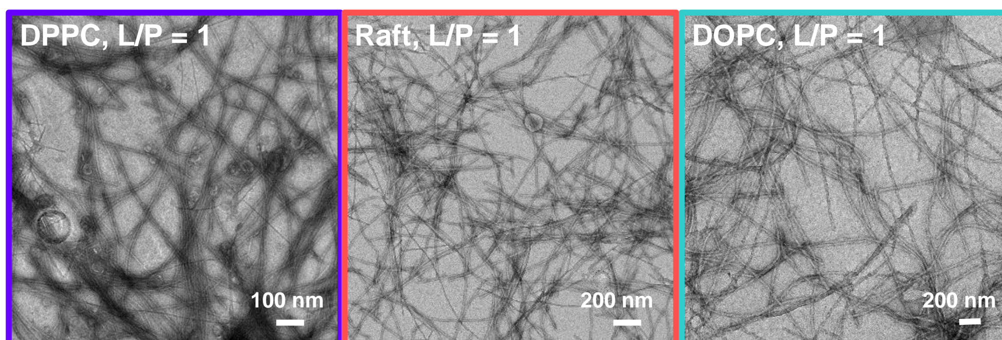



**Figure S12**. TEM images of N-acetyl α-syn (50 μM) in the presence of DPPC (purple), DOPC/SM/Chol (2:2:1) (pink), and DOPC (teal) vesicles after aggregation at L/P = 1. Scale bars are as indicated.

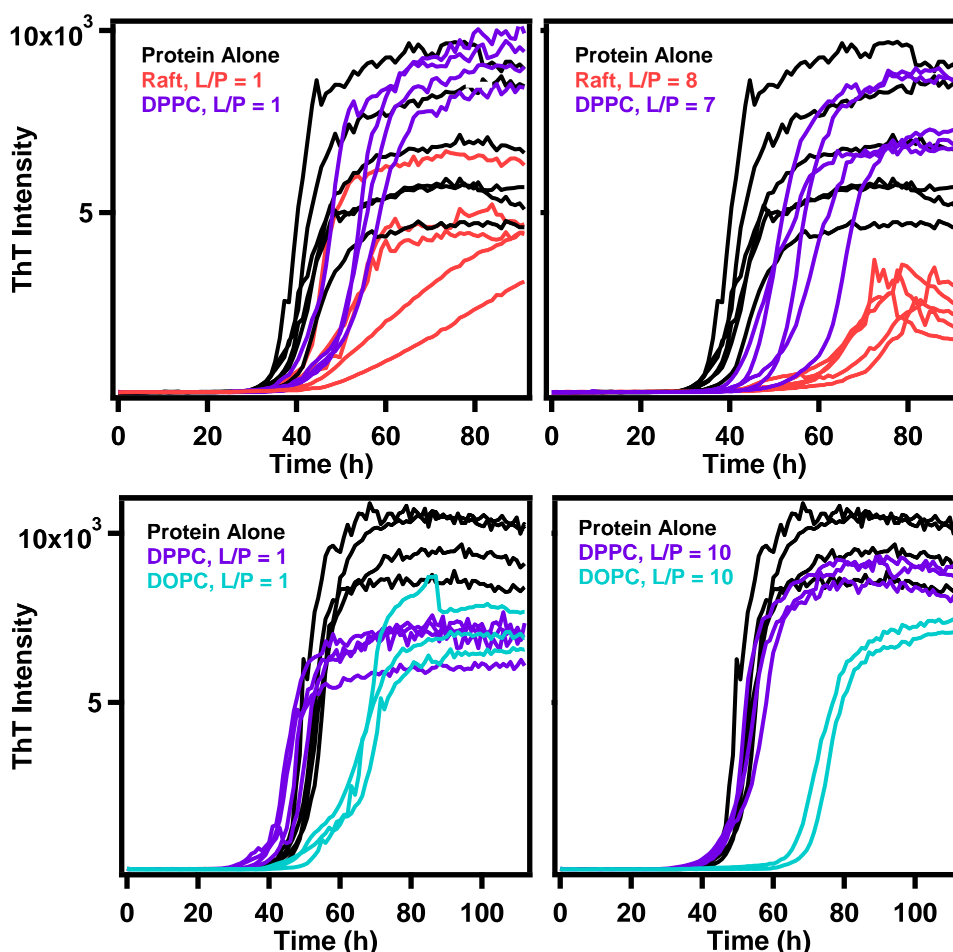



**Figure S13.** Aggregation kinetics of N-acetyl α-syn in the presence of DOPC, DPPC, and DOPC/SM/Chol (2:2:1). Amyloid formation of N-acetyl α-syn (50 μM) monitored by ThT fluorescence (37 °C with shaking) at pH 7 in the absence (black) or presence of DOPC (teal), DPPC (purple), and DOPC/SM/Chol (2:2:1) (pink) vesicles at L/P = 1 (left) or L/P ~ 10 (right). Data were collected from two independent plates.

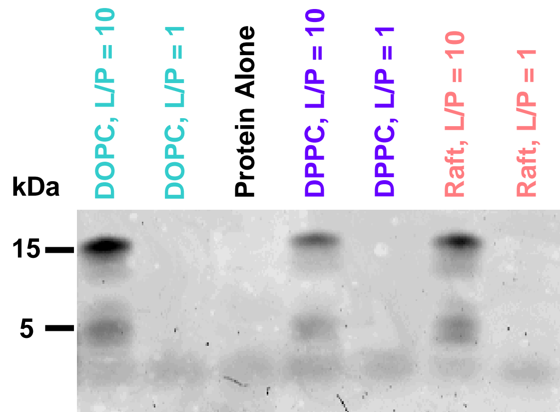



**Figure S14**. SDS-Gel electrophoresis analysis of the soluble fraction of post-aggregation N-acetyl α-syn samples (50 μM) at L/P = 1 and 10. Samples (200 μL) were ultracentrifuged at 100,000 rpm (TLA100 rotor) for 30 min at 4 °C. Supernatant was removed and separated by SDS/PAGE (NuPAGE 4-12% Bis-Tris, Invitrogen) and stained for 1 h with Coomassie (SimplyBlue SafeStain, Invitrogen).

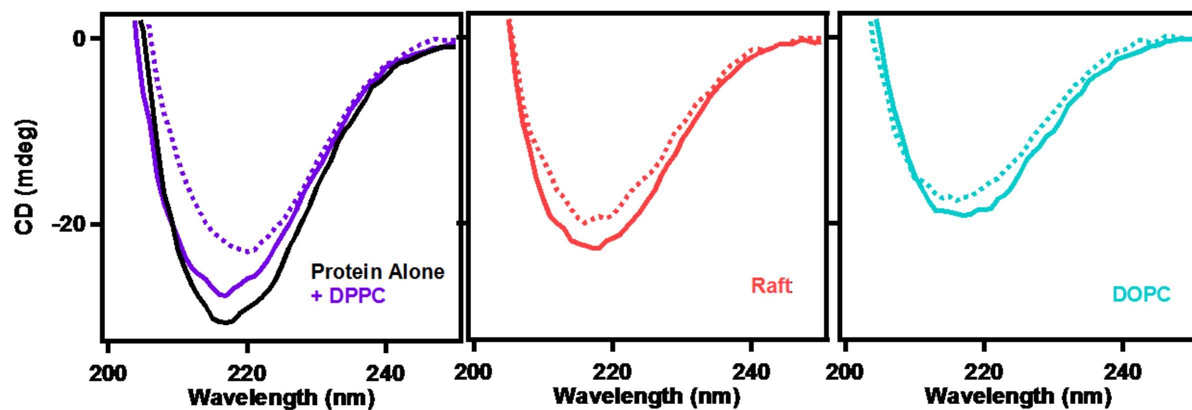



**Figure S15**. CD spectroscopy of N-acetyl α-syn fibril samples aggregated in the absence of ThT. CD spectra at 20 °C of N-acetyl α-syn (50 μM) in the absence (black) or presence of DPPC (purple), DOPC/SM/Chol (2:2:1) (pink), and DOPC (teal) vesicles at L/P = 1 (solid) and L/P = 10 (dotted).

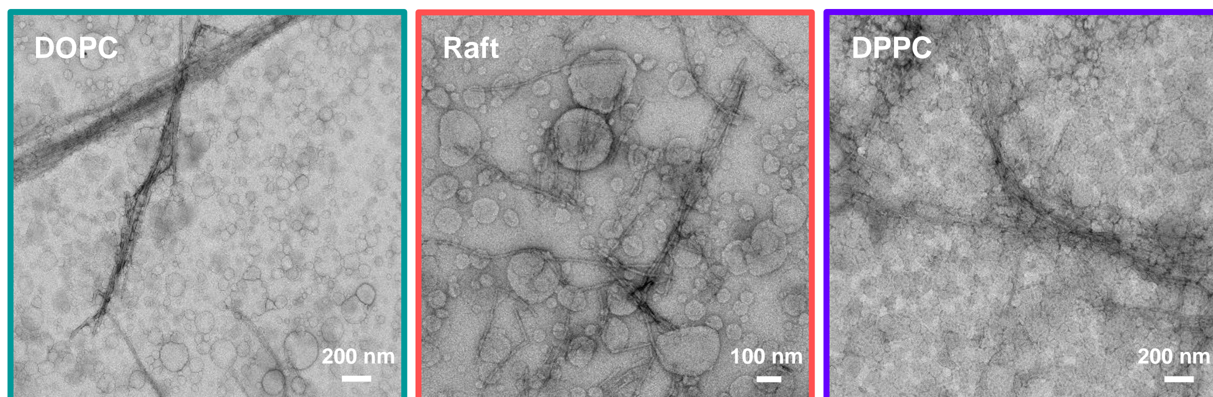



**Figure S16.** TEM images of post-aggregation samples of N-acetyl α-syn (50 μM) with DOPC (teal), DOPC/SM/Chol (2:2:1) (pink), and DPPC (purple) SUVs at L/P = 100. Scale bars are as indicated.