

Supplementary Information for

Membrane Packing Defects are Necessary and Sufficient for α -Synuclein Binding

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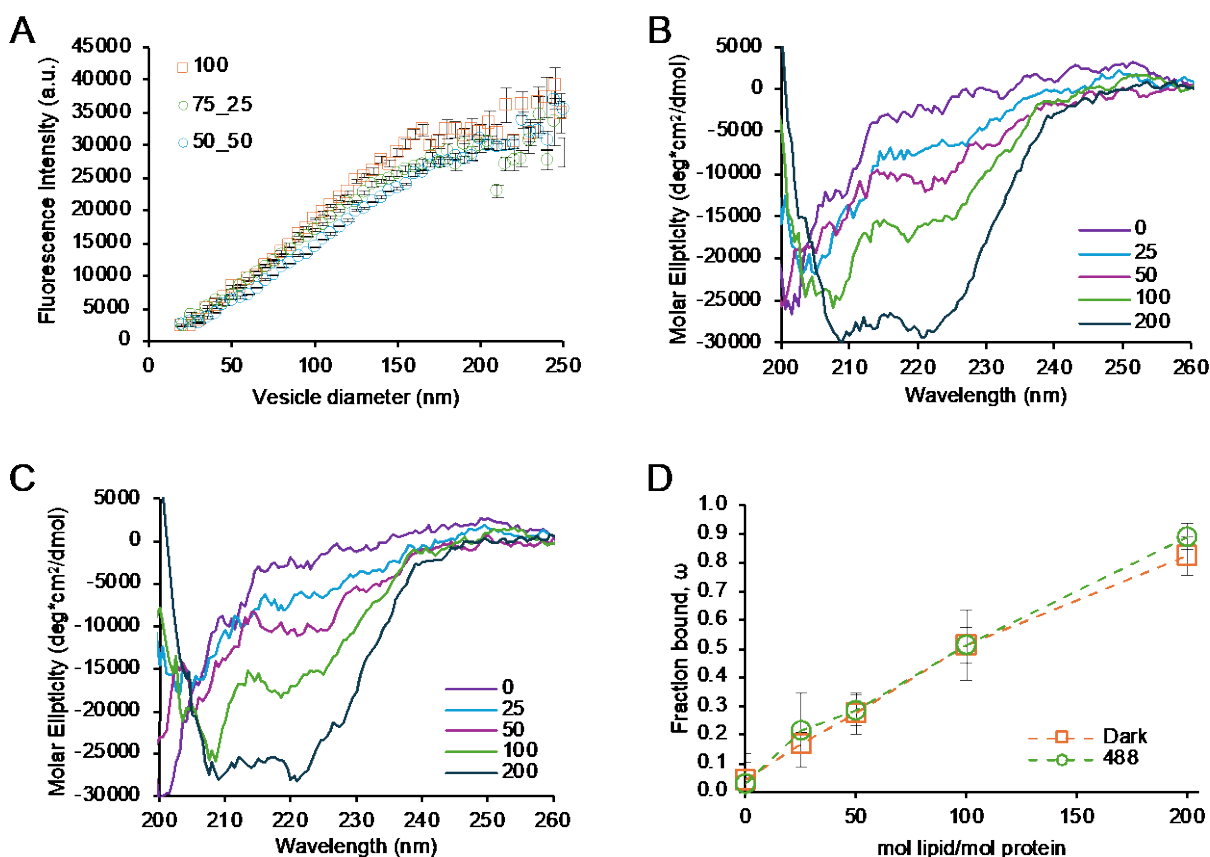
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Supplementary Discussion

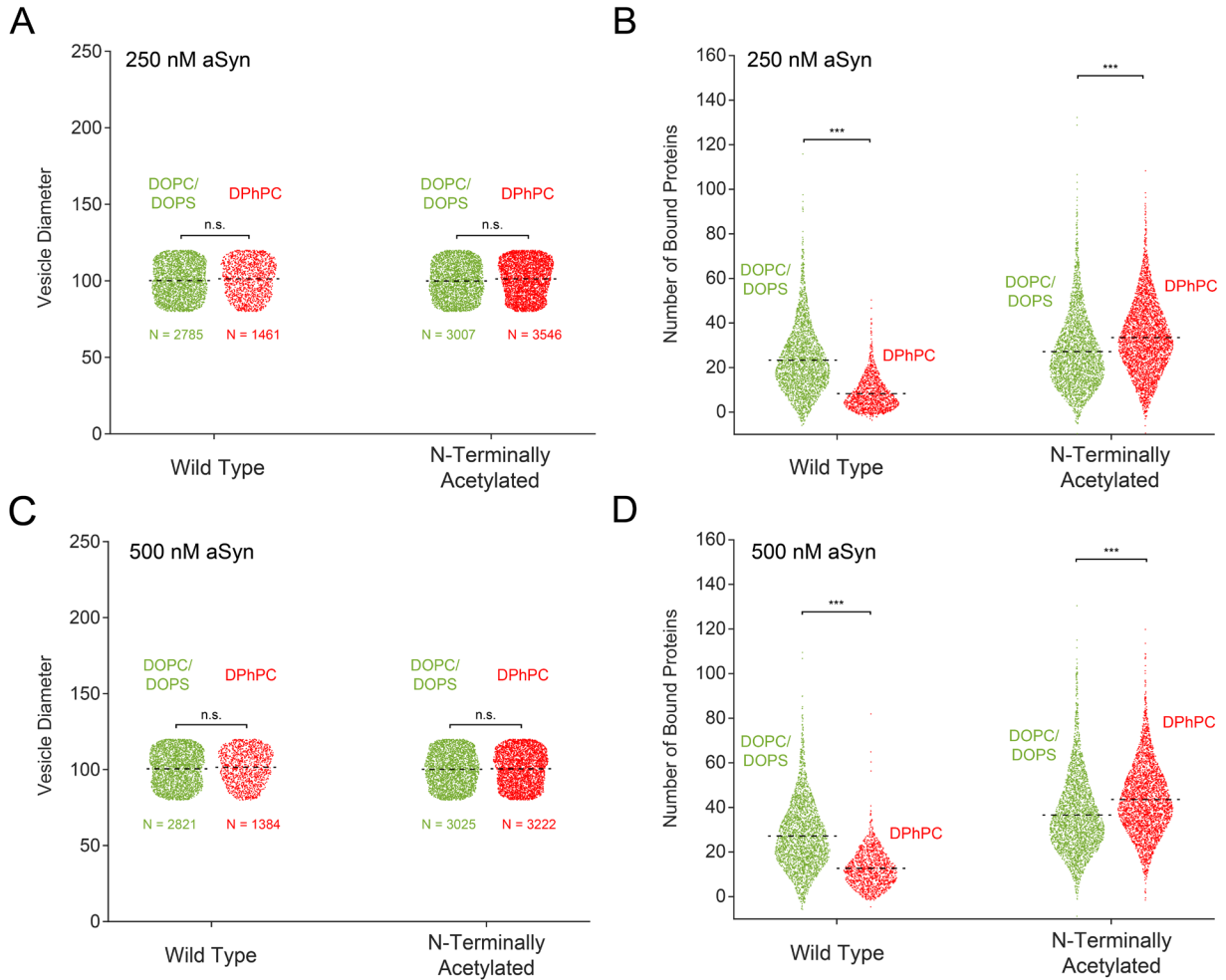
For the volume labeling assay, the following fluorescence intensity scaling behavior must be true for hollow spheres (i.e., unilamellar vesicles): (*lumen intensity or ATTO488*) $\sim \text{diameter}^3$ and (*membrane intensity or ATTO647N*) $\sim \text{diameter}^2$. Therefore, when the cube root of ATTO488 intensity is plotted vs the square root of ATTO647N intensity, the plot must exhibit linearity. Linear correlation was indeed observed between the two quantities, indicating the presence of hollow spheres or unilamellar vesicles.

For the PLL adsorption assay, there was negligible adsorption to the membranes containing only PC lipids and strong adsorption to membranes containing PS lipids. Furthermore, the level of adsorption was comparable between membranes containing DLPS and DOPS, indicating that DLPS leaching was negligible in the tethered vesicle assay used.

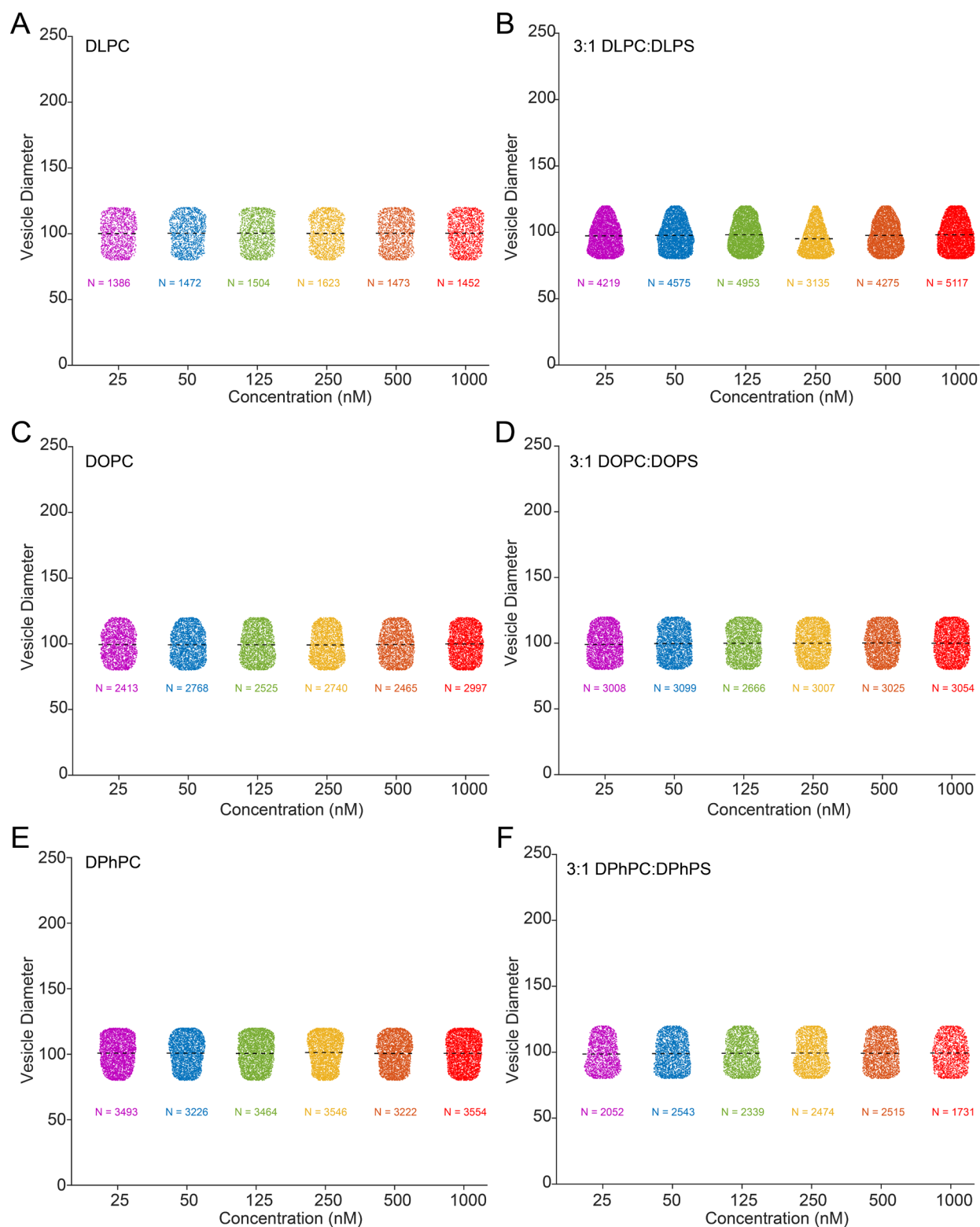


Supplementary Figure 1. Fluorescence Microscopy and CD spectroscopy reveal that ATTO 488 does not alter α Syn's binding affinity for membranes. (A) Results from fluorescence microscopy-based tethered vesicle assay. Protein fluorescence intensity is plotted as a function of vesicle diameter on SUVs composed of 3:1 DPhPC:DPhPS. NTA- α Syn was incubated with vesicles at a total protein concentration of 250nM. Among 3 separate experiments, the ratio of labeled:dark NTA- α Syn was varied from 1:0, 3:1, and 1:1. In the experiments containing dark α Syn, the protein intensities were corrected by appropriate

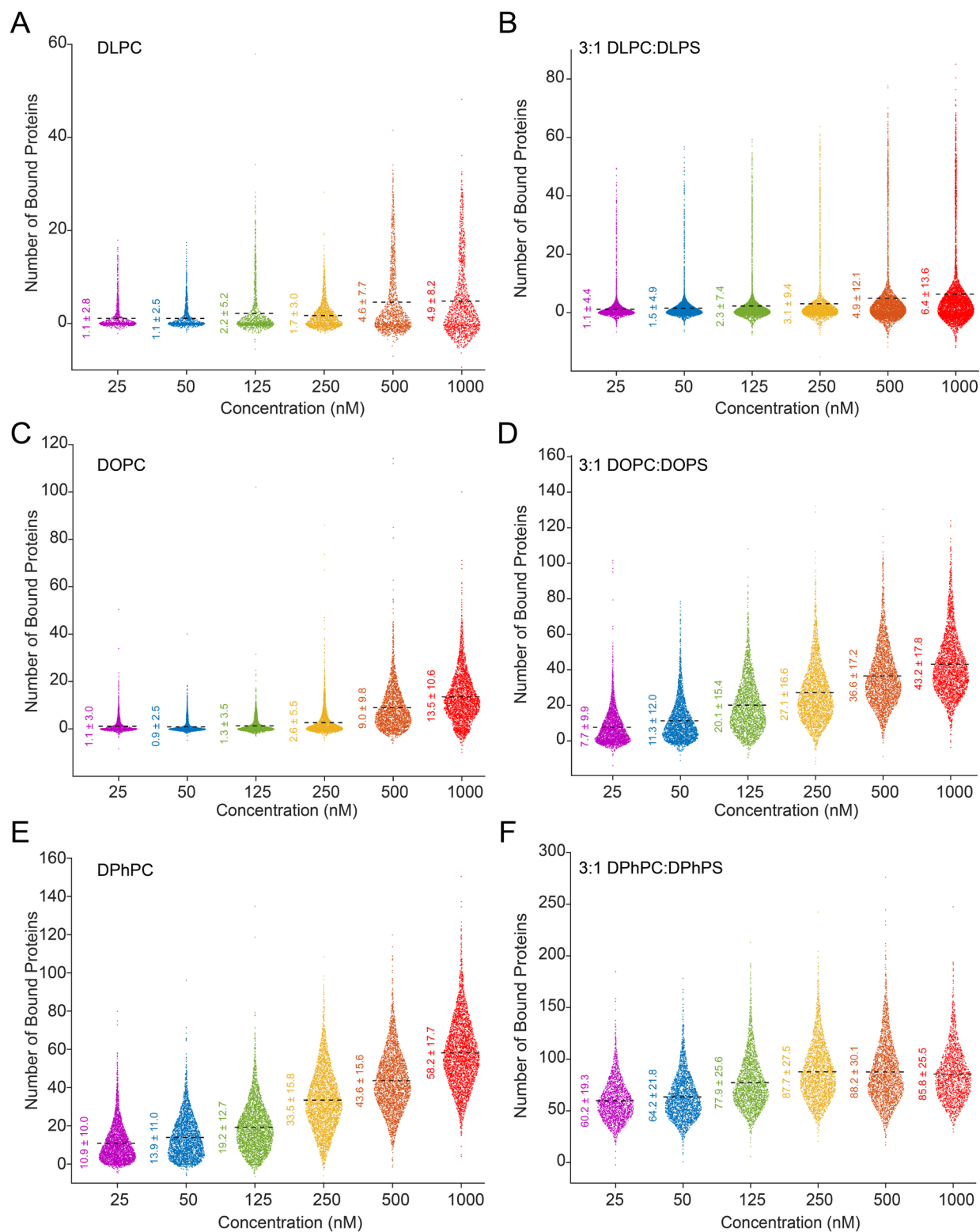
factors (i.e., either 75% or 50%) to account for the dark α Syn population. The 3 intensity profiles converge to comparable values, indicating that binding was not affected by fluorescent labeling with ATTO 488. Each data point represents a 5nm bin containing $N = 17-374$ vesicles. Error bars represent the standard error of the mean (SEM). (B-C) Results from CD spectroscopy using SUVs containing 3:1 DPhPC:DPhPS. (B) CD spectra for 1 μ M NTA- α Syn-ATTO488 incubated with SUVs ranging from 0-200 μ M lipid concentration. (C) CD spectra for 1 μ M dark NTA- α Syn incubated with SUVs ranging from 0-200 μ M lipid concentration. (D) The fraction of NTA- α Syn bound, ω , as a function of lipid concentration. The CD spectroscopy-derived binding curves for dark and fluorescent NTA- α Syn in panel D are nearly identical.



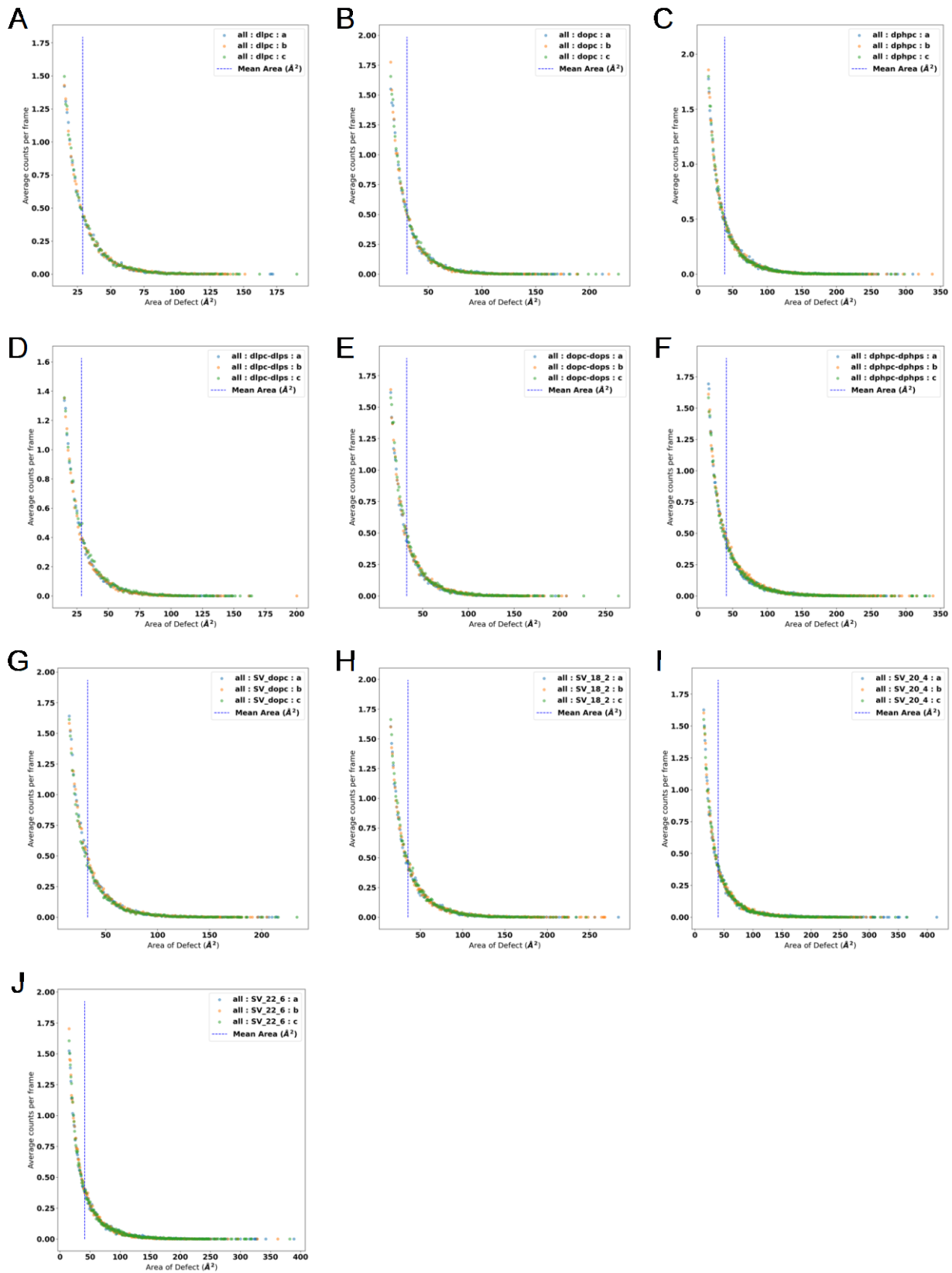
Supplementary Figure 2. Supplementary data for experiments comparing WT-αSyn and NTA-αSyn binding to DOPC/DOPS and DPhPC membranes. (A) The distribution of diameters for analyzed vesicles that were incubated with 250 nM αSyn. (B) The number of proteins bound to vesicles in B. (C) The distribution of diameters for analyzed vesicles that were incubated with 500 nM αSyn. (n.s. = not significant as determined by unpaired Student's t-test). (D) The number of proteins bound to vesicles in C. Errors in A and C represent standard deviations. n.s. = not significant as determined by unpaired Student's t-test. *** = $P < 0.0001$ as determined by Welch's t-test (WT) and unpaired Student's t-test (NTA).



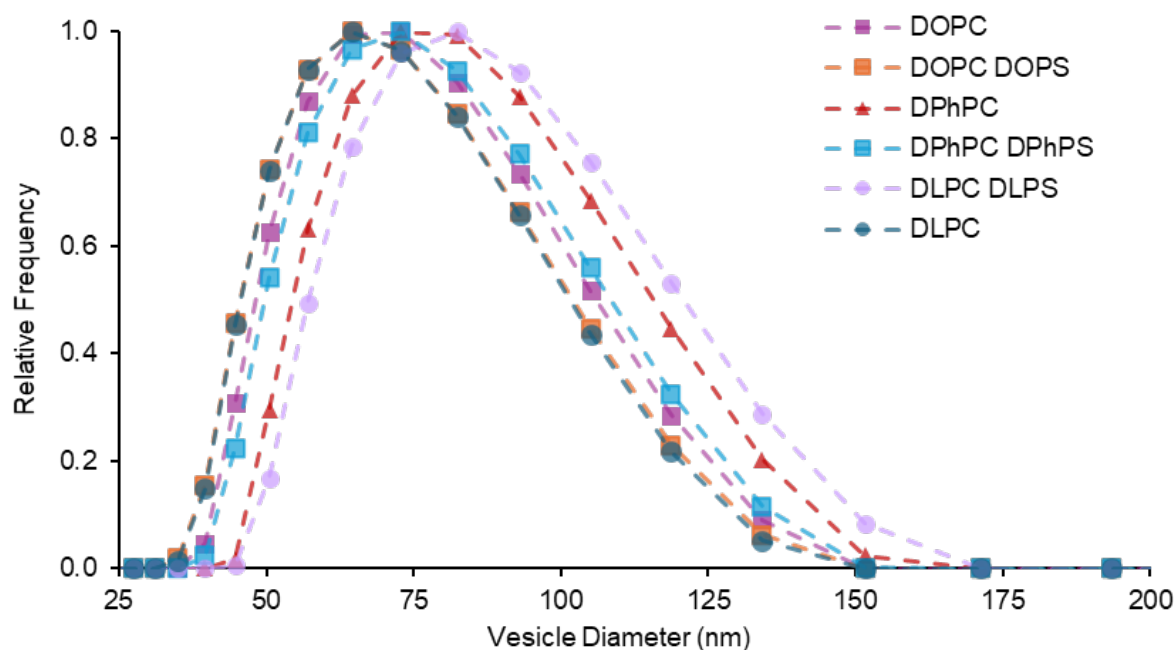
Supplementary Figure 3. Distributions of diameters for analyzed vesicles from Figures 3 and 4 of the main text. Black dashed lines indicate the average diameter of the populations. All data sets possessed an average vesicle diameter of approximately 100 nm.



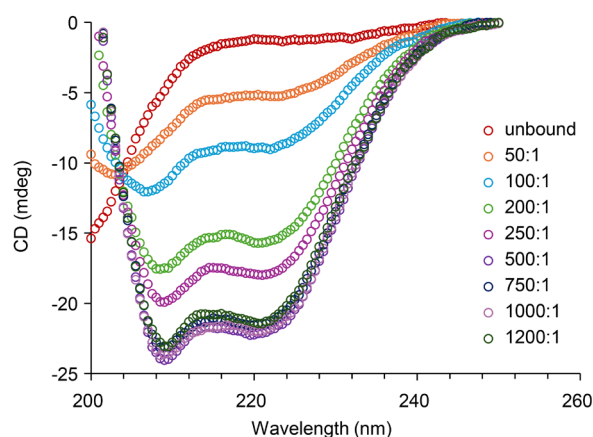
Supplementary Figure 4. Protein content on analyzed vesicles from Figures 3 and 4 of the main text. The distribution of proteins bound to individual vesicles. These distributions are coupled to the distributions in Figure S3. The black dashed line and values indicate the average number of proteins bound. Errors listed represent standard deviations.



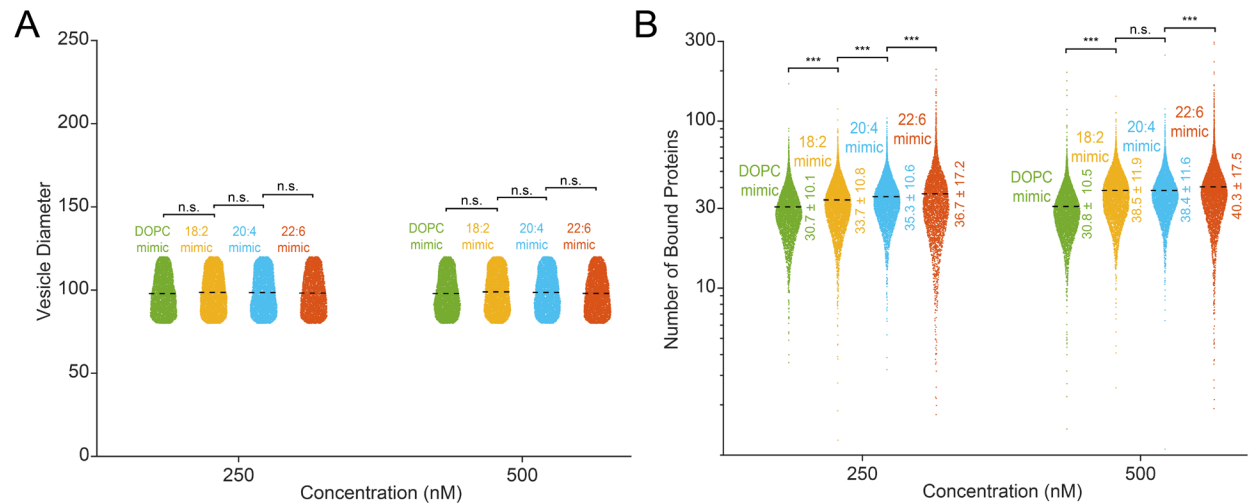
Supplementary Figure 5. Frequency distributions for defects on MD-simulated membranes. Distributions for (A) DLPC, (B) DOPC, (C) DPhPC, (D) 3:1 DLPC:DLPS, (E) 3:1 DOPC:DOPS, (F) 3:1 DPhPC:DPhPS, (G) SV mimics containing DOPC, (H) SV mimics containing 18:2 PC, (I) SV mimics containing 20:4 PC, and (J) SV mimics containing 22:6 PC. Each of the 3 distributions in each panel is normalized by the total number of simulation frames – 1000 frames.



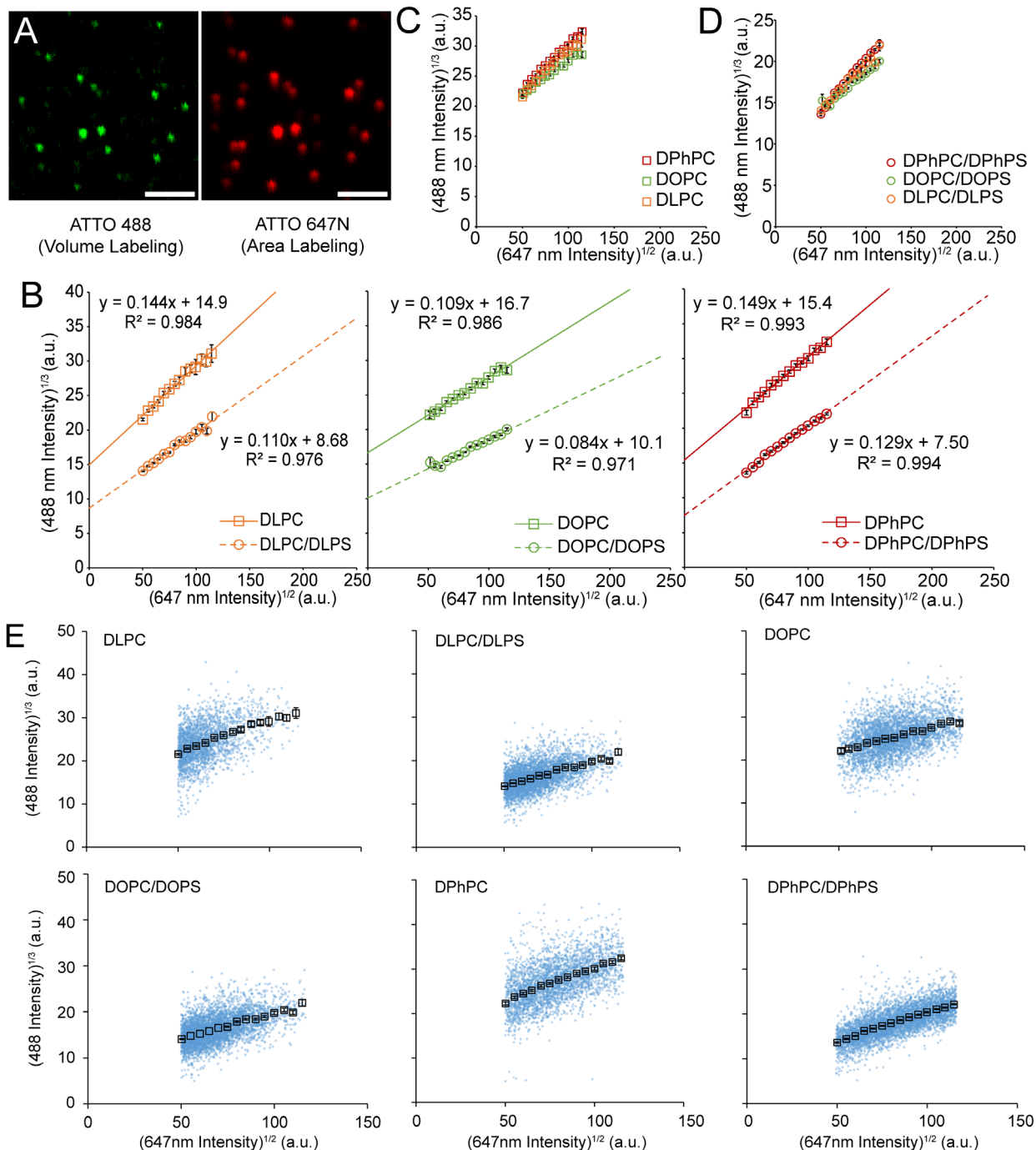
Supplementary Figure 6. DLS distributions of 50 nm extruded vesicles used for CD Spectroscopy. The distributions shown are the average of 3 measurements for each sample.



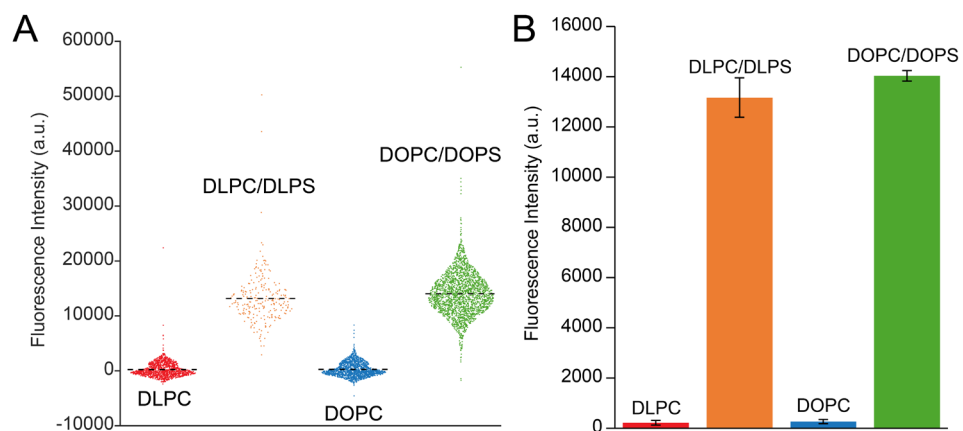
Supplementary Figure 7. Circular dichroism spectra of NTA- α Syn for 3:1 DPhPC:DPhPS.



Supplementary Figure 8. Supplemental NTA- α Syn binding data for the SV mimics. (A) The distributions of diameters for analyzed vesicles, which all possessed an average diameter of approximately 100 nm. The number of vesicles (N) analyzed within each condition are as follows: DOPC mimic with 250 nM α Syn (N = 4756), 18:2 PC mimic with 250 nM α Syn (N = 5660), 20:4 PC mimic with 250 nM α Syn (N = 5078), 22:6 PC with 250 nM α Syn (N = 4050), DOPC mimic with 500 nM α Syn (N = 5184), 18:2 PC mimic with 500 nM α Syn (N = 5431), 20:4 PC mimic with 500 nM α Syn (N = 4896), and 22:6 PC with 500 nM α Syn (N = 5047). (B) Number of proteins bound to vesicles in C that were incubated with 250 or 500 nM NTA- α Syn. The black dashed line and values on jitter plots in C and D indicate the average vesicle diameter and number of proteins bound, respectively. n.s. = not significant as determined by unpaired Student's t-test. *** = $P < 0.001$ as determined by unpaired Student's t-test.



Supplementary Figure 9. Volume labeling assay. (A) Fluorescence micrographs of SUVs encapsulating ATTO 488 dye for volume labeling and containing ATTO 647N in the bilayer for area labeling. Scale bars represent 2 μm . (B) Scaling relationship between SUV volume and surface area for PC membranes and 3:1 PC:PS membranes. The intensity values correspond to SUVs with an average diameter of 100 nm. (C) Overlay of the cube root of 488 nm intensity versus the square root of 647 nm intensity for DPhPC, DOPC, and DLPC. (D) Overlay of the cube root of 488 nm intensity versus the square root of 647 nm intensity for DPhPC/DPhPS, DOPC/DOPS, and DLPC/DLPS. (E) Raw data and moving averages for results in panels B-D. Error bars represent the standard error of the mean. For DLPC N = 7-545; DLPC/DLPS N = 10 – 727; DPhPC N = 93 – 321; DPhPC/DPhPS N = 243 – 442; DOPC N = 41 – 464; DOPC/DOPS N = 15 – 803.



Supplementary Figure 10. Vesicle surface charge assay. (A) All ATTO-488 intensities for all measured vesicles with the black dashed line is the average plotted in panel B. (B) The average fluorescence intensity of ATTO 488-labeled PLL adsorbed to DLPC (N = 1290), 3:1 DLPC:DLPS (N = 241), DOPC (N = 1506), and 3:1 DOPC:DOPS (N = 2254) SUVs with diameters ranging from 90-110 nm. Error bars represent the 99% confidence interval.

Supplementary Table 1. Molecular membrane compositions for all bilayer simulations.

System	Composition (number of molecules)
<i>DPHPC</i>	288 DPhPC
<i>DPHPC/PS</i>	216 DPhPC, 72 DPhPS
<i>DLPC</i>	288 DLPC
<i>DLPC/PS</i>	216 DLPC, 72 DLPS
<i>DOPC</i>	288 DOPC
<i>DOPC/PS</i>	216 DOPC, 72 DOPS
<i>SV DOPC</i>	100 DOPC, 100 DOPE, 40 DOPS, 160 Cholesterol
<i>SV 18:2</i>	100 D(18:2)PC, 100 DOPE, 40 DOPS, 160 Cholesterol
<i>SV 20:4</i>	100 D(20:4)PC, 100 DOPE, 40 DOPS, 160 Cholesterol
<i>SV 22:6</i>	100 D(22:6)PC, 100 DOPE, 40 DOPS, 160 Cholesterol

Supplementary Table 2. Details of the simulation equilibration and production steps. These steps were repeated for each membrane composition.

Step	Thermostat (K)	Barostat (bar)	Sim. Length (ps)	Timestep (fs)	Restraints	Strength (kJ/mol/nm)
<i>Equil. 1</i>	V-rescale, 303.15	None	125	1	Head Groups, Dihedrals	1000, 1000
<i>Equil. 2</i>	V-rescale, 303.15	None	125	1	Head Groups, Dihedrals	400, 400
<i>Equil. 3</i>	V-rescale, 303.15	C-rescale, 1.0	125	1	Head Groups, Dihedrals	400, 200
<i>Equil. 4</i>	V-rescale, 303.15	C-rescale, 1.0	250	2	Head Groups, Dihedrals	200, 200
<i>Equil. 5</i>	V-rescale, 303.15	C-rescale, 1.0	500	2	Head Groups, Dihedrals	40, 100
<i>Equil. 6</i>	V-rescale, 303.15	C-rescale, 1.0	500	2	None	N/A
<i>Production</i>	V-rescale, 303.15	Parrinello-Rahman, 1.0	400,000	2	None	N/A

Supplementary Table 3. Calculated fraction bound (ω) of NTA- α Syn from CD Spectroscopy. Each lipid composition and lipid:protein ratio tested is shown. The errors listed are the standard error of the mean.

Lipid Composition	Fraction Bound (ω)							
	mol lipid/mol protein							
	50:1	100:1	200:1	250:1	500:1	750:1	1000:1	1200:1
DLPC	0.010 $\pm$ 0.002	0.016 $\pm$ 0.002	-0.005 $\pm$ 0.002	0.018 $\pm$ 0.002	0.001 $\pm$ 0.005	-0.010 $\pm$ 0.004	-0.010 $\pm$ 0.007	0.015 $\pm$ 0.004
DOPC	0.046 $\pm$ 0.006	0.048 $\pm$ 0.002	0.068 $\pm$ 0.003	0.029 $\pm$ 0.009	0.149 $\pm$ 0.010	0.178 $\pm$ 0.012	0.215 $\pm$ 0.017	0.295 $\pm$ 0.017
DPhPC	0.069 $\pm$ 0.004	0.150 $\pm$ 0.002	0.299 $\pm$ 0.002	0.367 $\pm$ 0.003	0.633 $\pm$ 0.003	0.795 $\pm$ 0.002	0.911 $\pm$ 0.002	0.926 $\pm$ 0.002
DLPC/DLPS (3:1)	-0.010 $\pm$ 0.002	0.016 $\pm$ 0.001	-0.003 $\pm$ 0.005	0.009 $\pm$ 0.002	0.033 $\pm$ 0.001	0.044 $\pm$ 0.002	0.037 $\pm$ 0.005	0.032 $\pm$ 0.005
DOPC/DOPS (3:1)	0.069 $\pm$ 0.004	0.114 $\pm$ 0.008	0.263 $\pm$ 0.005	0.328 $\pm$ 0.005	0.607 $\pm$ 0.003	0.759 $\pm$ 0.004	0.806 $\pm$ 0.011	0.814 $\pm$ 0.010
DPhPC/DPhPS (3:1)	0.175 $\pm$ 0.007	0.359 $\pm$ 0.005	0.691 $\pm$ 0.003	0.811 $\pm$ 0.002	1.024 $\pm$ 0.001	0.989 $\pm$ 0.002	1.015 $\pm$ 0.002	0.972 $\pm$ 0.002

Supplementary Table 4. Apparent dissociation constant (K_d) and cooperativity (n) determined by Langmuir-Hill fit to binding curves. K_d and cooperativity values were determined by fitting data in Figures 3E and 4B to Equation 2 from the main text. The errors listed are the standard error of the mean.

Lipid Composition	K_d	n
DOPC	3376 $\pm$ 897	0.97 $\pm$ 0.17
DPhPC	330 $\pm$ 13	1.69 $\pm$ 0.10
DOPC/DOPS	386 $\pm$ 13	1.49 $\pm$ 0.07
DPhPC/DPhPS	125 $\pm$ 8	1.99 $\pm$ 0.21

Supplementary Reference

- 1 Marsh, D. (CRC Press, 2013).