Supporting Material

Amyloids of alpha synuclein affect the structure and dynamics of supported lipid bilayers

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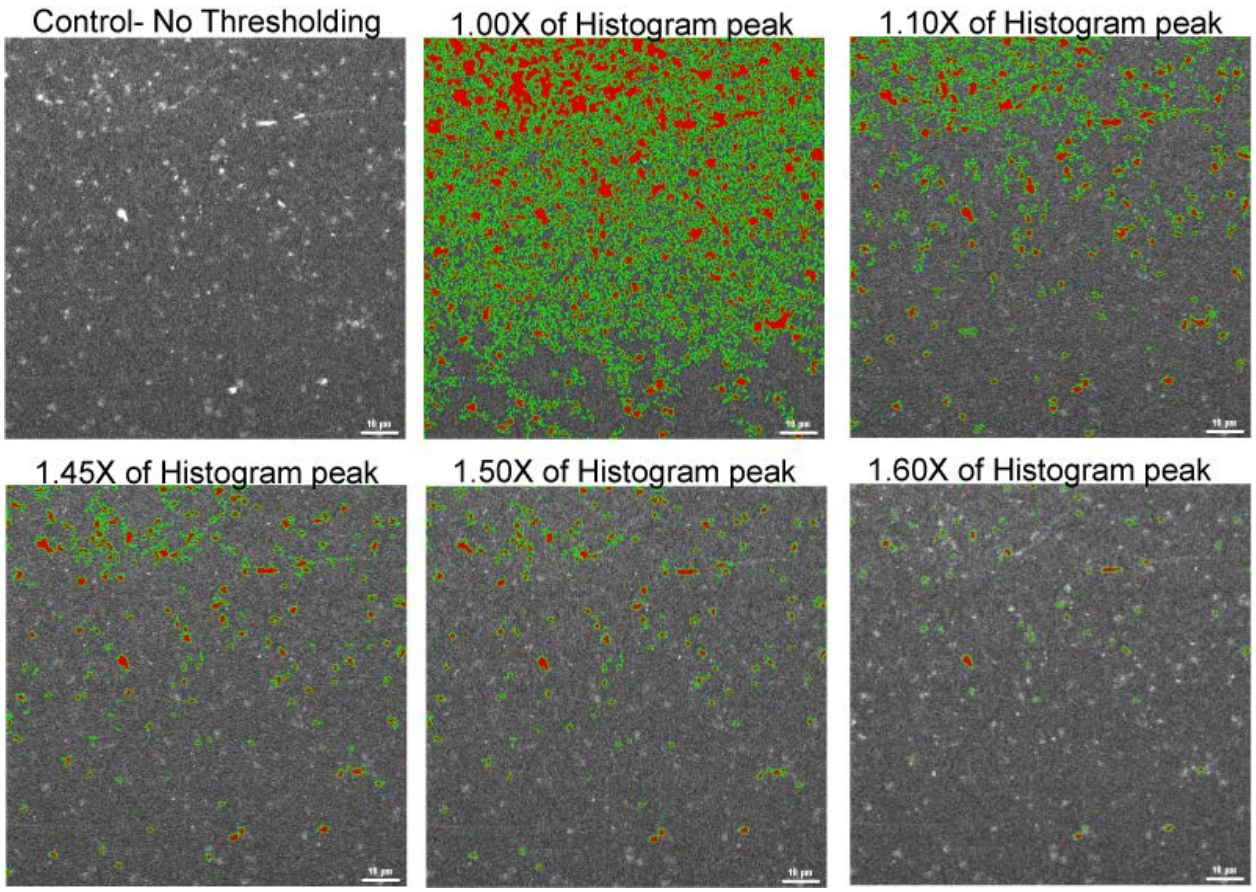

Fig S1: Optimizing thresholding parameters for cluster analysis. Using the Nikon NIS Elements software, the *ObjectCount* plugin was used to set an intensity threshold. To choose an optimum threshold, the thresholding point was varied along the intensity histogram in the protein channel. As seen in the figure, a thresholding value of 1.60X leads to exclusion of certain aggregates and a value up to 1.45X leads to over-estimation of cluster sizes. Thus 1.50X was chosen as a thresholding value for all images. All images were taken at room temperature in 50 mM HEPES, 0.1 mM EDTA, pH 7.4 buffer.

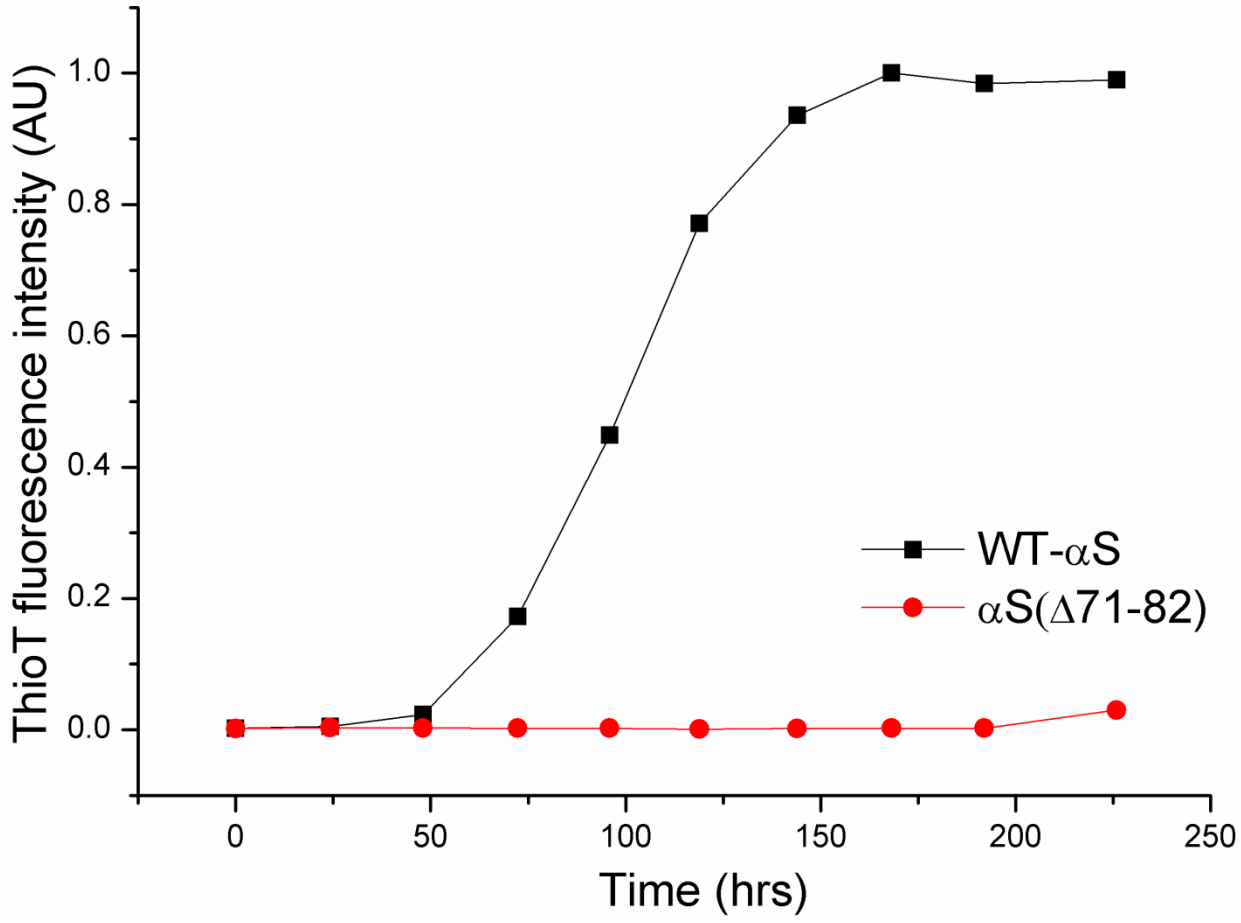

Fig S2: Aggregation kinetics of αS variants at 37^{o}C monitored by ThT fluorescence.

The aggregation reaction was carried out using 50 mM HEPES, 0.1 mM EDTA at 300rpm constant orbital shaking conditions in a fluorescence microplate reader. The protein concentration was kept at 100 µM and the ThT concentration was 10 µM.

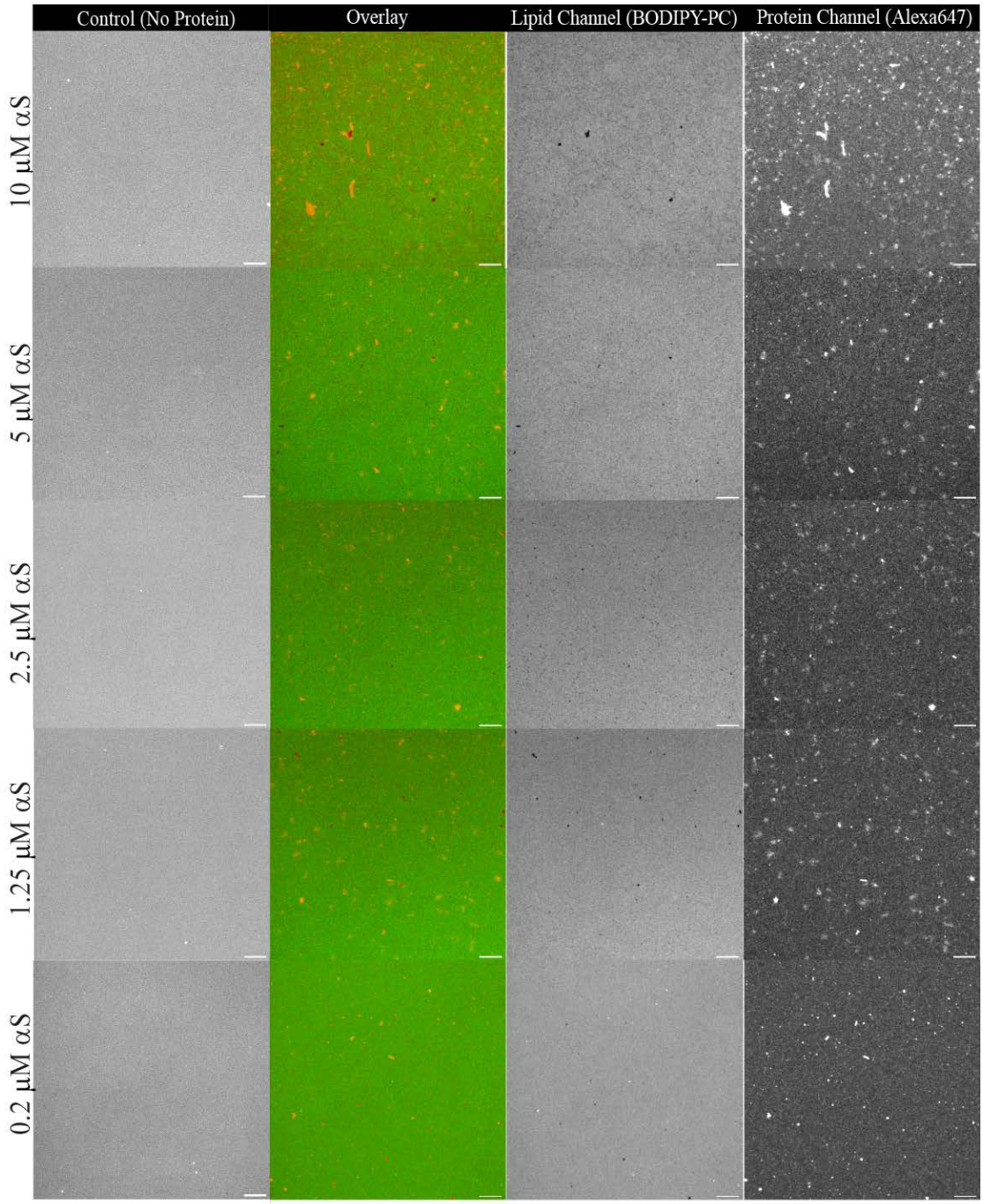

Fig S3: Adsorption of WT-αS on POPC:POPG (50:50) SLBs. Representative confocal images of SLBs before (control) and after addition of increasing amounts of WT-αS (labeled to AlexaFluor 647). 0.25 mol% of BODIPY-PC was incorporated as a fluorescent lipid probe in SLBs. The lipid channel clearly shows increasing membrane damage in form of defects and cracks as the protein concentration is increased from 0.2 μM WT-αS to 10 μM WT-αS. Correspondingly the cluster sizes also seem to increase upon increasing protein concentration. Images are contrasted to the same extent to facilitate proper comparison. All images were taken at room temperature in 50 mM HEPES, 0.1 mM EDTA, pH 7.4 buffer. The scale bar is 10 μm.

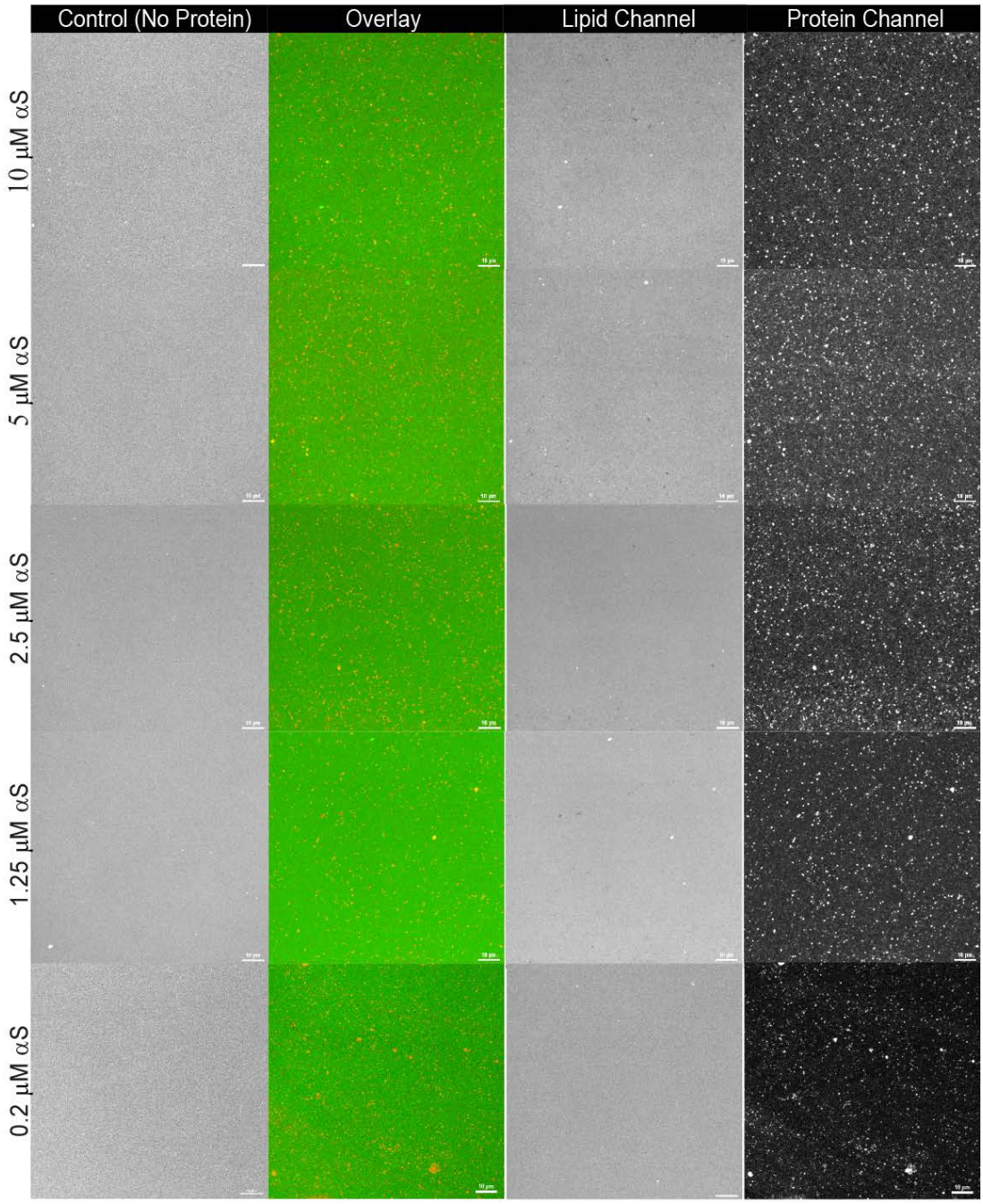

Fig S4: Adsorption of αS(Δ71-82) on POPC:POPG (50:50) SLBs. Representative confocal images of SLBs before (control) and after addition of increasing amounts of αS(Δ71-82) mutant which was labeled to AlexaFluor 647. 0.25 mol% of BODIPY-PC was incorporated as a fluorescent lipid probe in SLBs. The above images show clearly no effect of incubation of αS(Δ71-82) as the concentration is increased from 0.20 μM to 10 μM. There is hardly any significant damage seen across this concentration range. Correspondingly the cluster sizes also do not show a difference. Images are contrasted to the same extent to facilitate proper comparison. All images were taken at room temperature in 50 mM HEPES, 0.1 mM EDTA, pH 7.4 buffer. The scale bar is 10 μm.

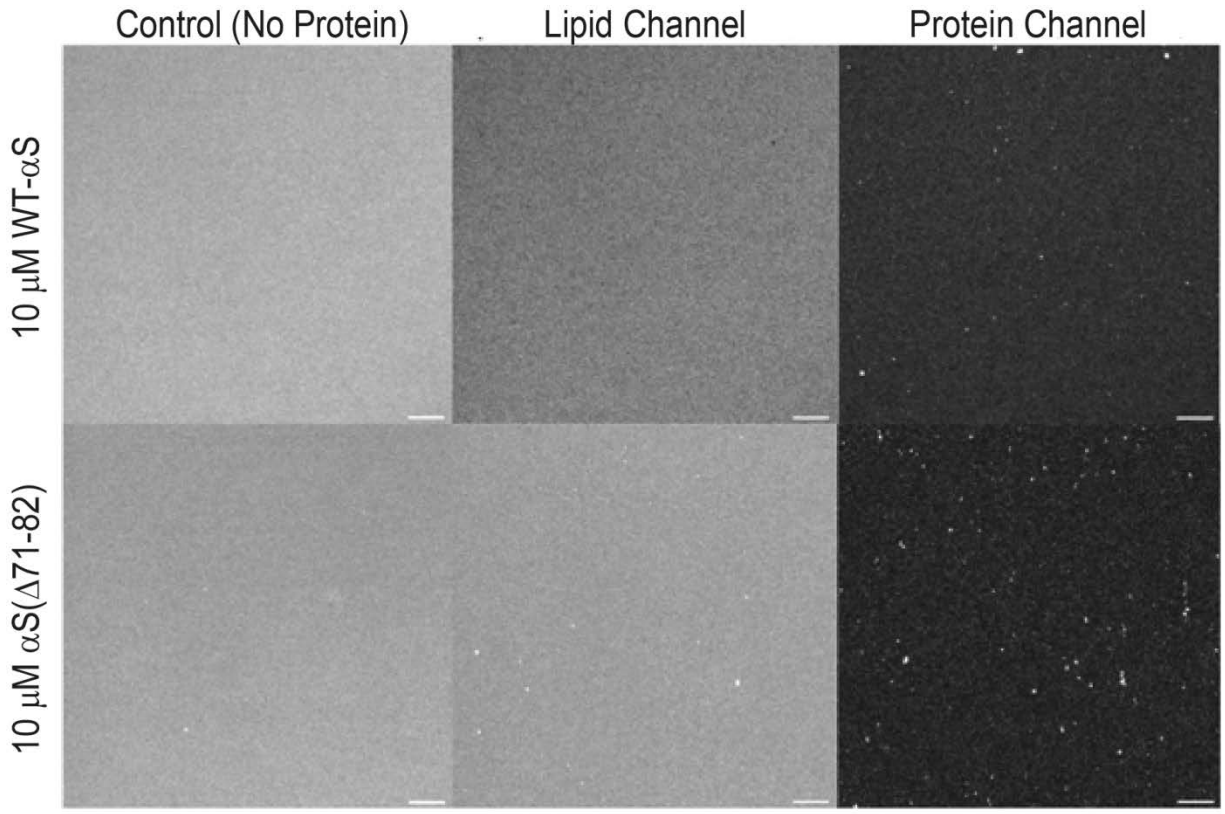

Fig S5: Adsorption of αS on POPC : POPG (75:25) SLBs. Representative images of adsorption of 10 μM αS after an 18 hour incubation period. The lipid channel shows very small defects and fluorescence intensity loss with the WT-αS (top panels), whereas no such effects are seen with αS(Δ71-82) (lower panels). Images are contrasted to the same extent to facilitate proper comparison. All experiments were performed at room temperature in 50 mM HEPES, pH 7.4, 0.1 mM EDTA buffer. The scale bar is 10 μm.

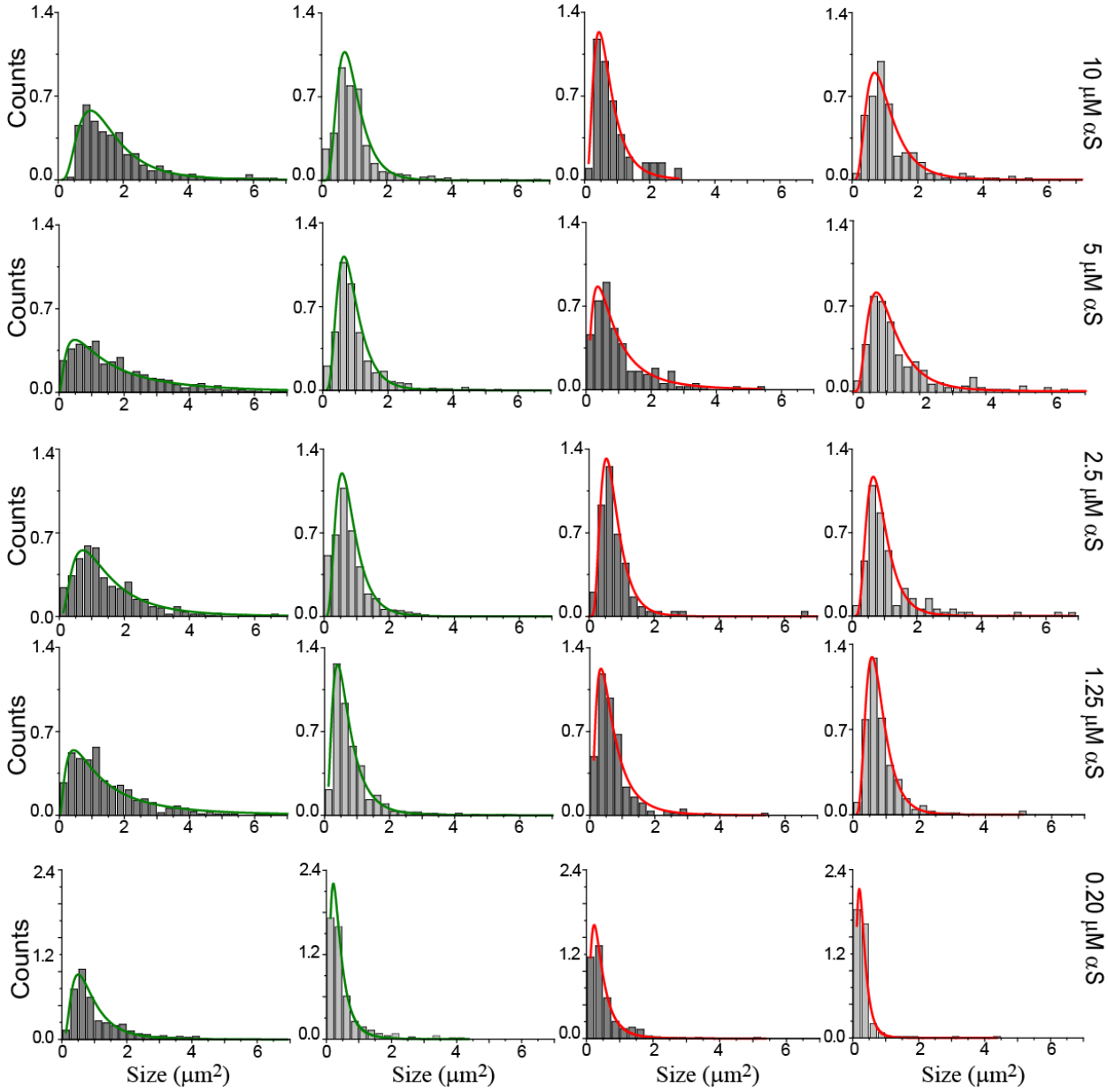

Fig S6: Aggregate area histograms of WT-αS and αS(Δ71-82) formed on POPC:POPG SLBs. Using the ObjectCount plugin in the Nikon NIS Elements software, a distribution of the aggregate areas was obtained. These distributions were area normalized to 1 and were fitted to a log-normal distribution. The WT-αS aggregate area distributions are represented by dark grey bars and the αS(Δ71-82) aggregate area distributions are represented by light gray bars. The aggregates of WT-αS, show a wider distribution (green fits) in 50% POPG SLBs as compared to the WT-αS in 25% POPG SLBs (red fits).

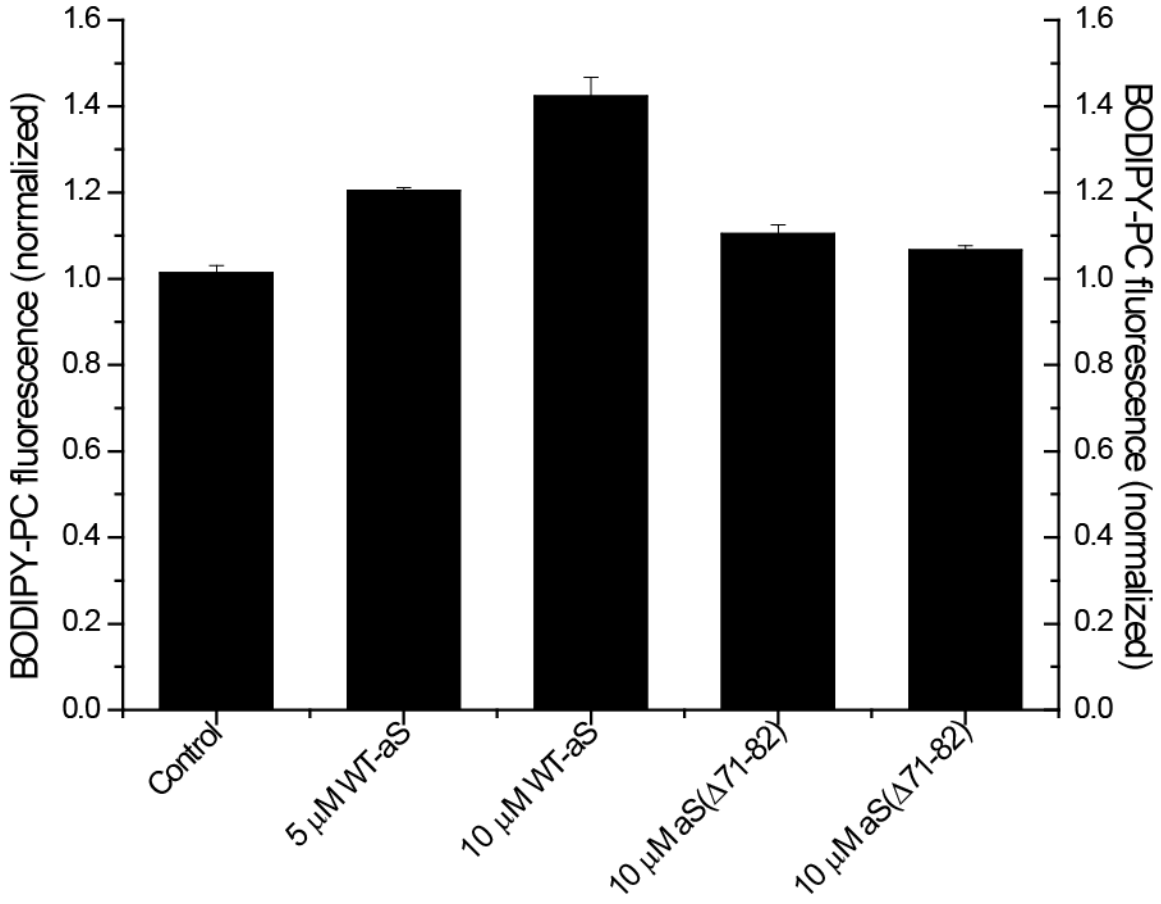

Fig S7: Lipid fluorescence in buffer after αS incubation on POPC:POPG SLBs. Relative increase in BODIPY-PC fluorescence seen in the buffer above the SLBs after incubation of αS variants over POPC: POPG (50:50) SLBs. All values were obtained by normalizing against the fluorescence obtained from buffer before incubation of αS variant. All experiments were performed at room temperature in 50 mM HEPES, 0.1 mM EDTA, pH 7.4 buffer.

	Protein Concentration (μM)	Cluster area (μm^2)	Number of clusters analysed	Width (s) of curve	Adjusted R- square
WT-αS on POPC:POPG (50:50) SLBs	10	1.41 ± 0.02	589	0.58	0.96
	5	1.50 ± 0.08	440	1.09	0.95
	2.5	1.27 ± 0.07	447	0.75	0.91
	1.25	1.21 ± 0.05	415	0.99	0.93
	0.20	0.78 ± 0.03	394	0.66	0.94
WT-αS on POPC:POPG (75:25) SLBs	10	0.77 ± 0.06	85	0.60	0.97
	5	0.78 ± 0.03	156	0.87	0.93
	2.5	0.68 ± 0.02	99	0.50	0.98
	1.25	0.58 ± 0.02	237	0.72	0.97
	0.20	0.38 ± 0.01	332	0.71	0.99
αS(Δ71-82) on POPC:POPG (50:50) SLBs	10	0.95 ± 0.02	880	0.47	0.94
	5	0.85 ± 0.01	656	0.49	0.96
	2.5	0.72 ± 0.03	676	0.51	0.85
	1.25	0.61 ± 0.08	1202	0.64	0.99
	0.20	0.34 ± 0.02	307	0.70	0.99
αS(Δ71-82) on POPC:POPG (75:25) SLBs	10	0.93 ± 0.02	303	0.55	0.93
	5	0.95 ± 0.05	283	0.57	0.96
	2.5	0.81 ± 0.02	139	0.46	0.93
	1.25	0.68 ± 0.04	277	0.47	0.98
	0.20	0.27 ± 0.02	208	0.41	0.94

Table S8: Cluster sizes of αS on POPC: POPG supported lipid bilayers.

The above table depicts cluster sizes obtained by fitting the area histograms by a log normal distribution. At least 10 images were obtained and used for calculating the cluster areas. The error bars indicate standard errors for each measurement.