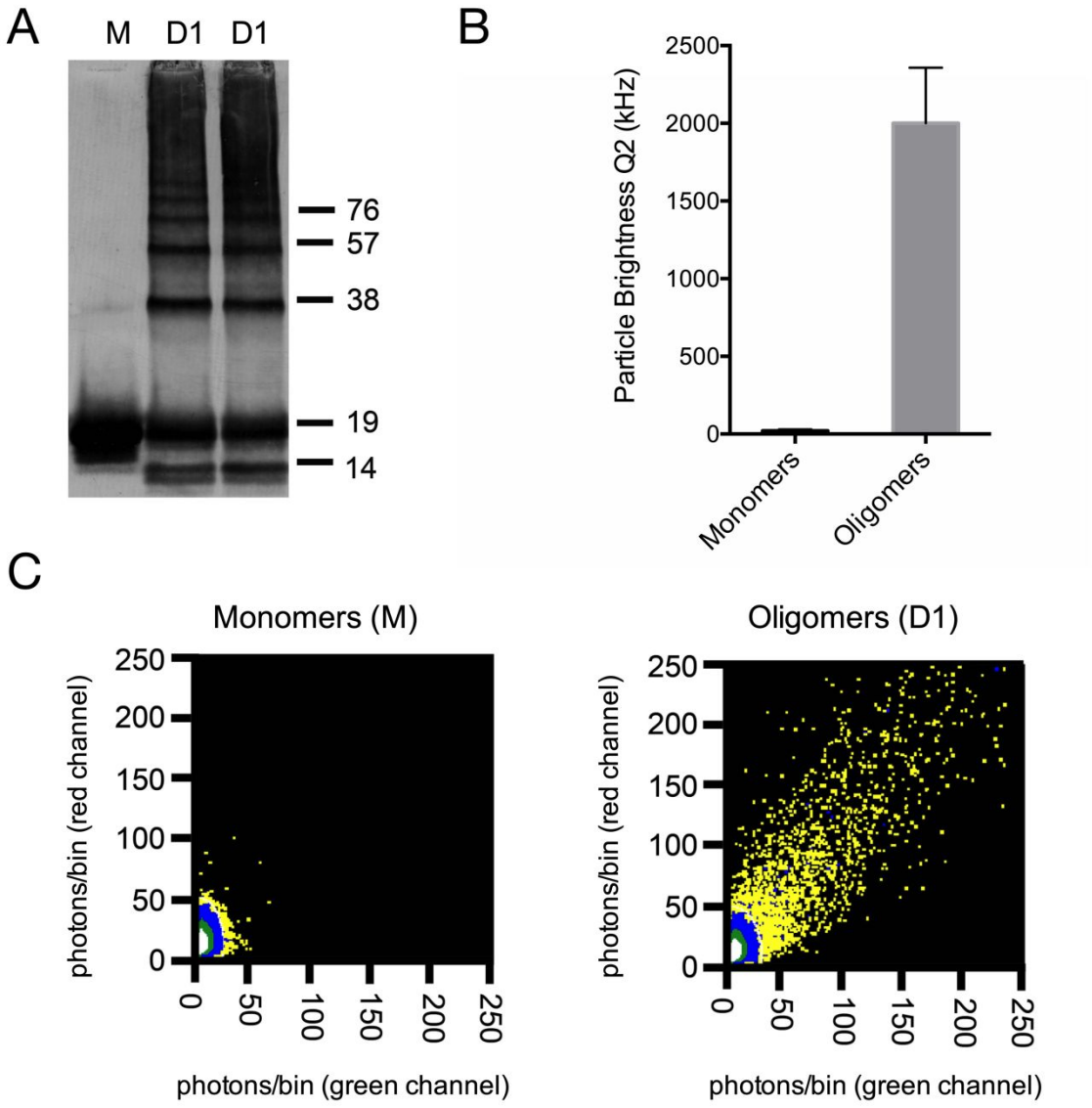


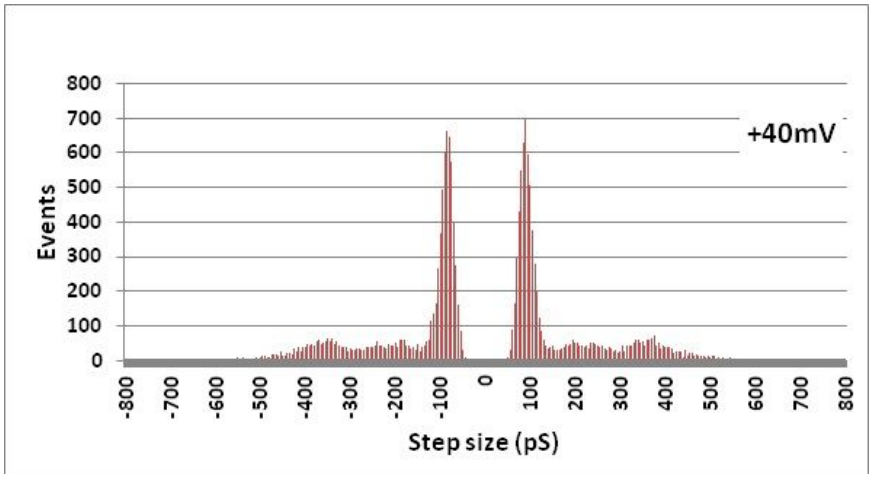
Supporting Figure S1. Formation of higher-order αS oligomers by ferric ions. Alpha-synuclein was aggregated using a standardized protocol involving the synergistic action of DMSO and Fe^{3+} (ferric) ions (refer to 'Methods'). Immunoblotting and single-particle confocal analysis were carried out on the same aggregate preparations. In the immunoblot (A), monomeric (native) αS is visible as a predominant band at 14 kDa (M), while a ladder of bands at higher molecular weight levels consistent with αS oligomers such as dimers, trimers, tetramers, pentamers and hexamers, are seen in the aggregated samples (D1). In 1D-FIDA analysis of Fe^{3+} induced αS oligomers (B), the particle brightness (*Q2*) is related to the size of the oligomers. In accordance with previous findings,[49, 65] oligomer size ranged up to 115-156 monomers per oligomer. This is also reflected in the 2D-SIFT

histograms (C), wherein αS oligomers are detected as high-intensity signals in the scanned measurements.

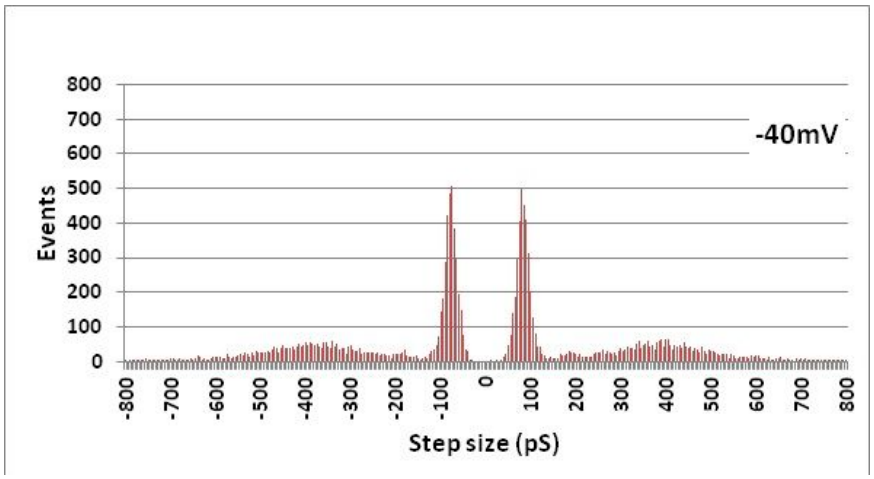
Supporting Table S2. Latency to first opening after addition of αS oligomeric preparation. Shown is the time (in min) elapsed before insertion/opening of the first pore, with each trial lasting 120 min. (ND = no pore detected)

Trial	Latency to first opening (min)		
	IM-Type	**L-Type**	**C-Type**
1	15	ND	ND
2	8	11	ND
3	ND	21	95
4	20	ND	8
5	ND	ND	7
6	25	ND	ND
7	ND	ND	10
8	30	12	ND
9	45	ND	ND
10	ND	ND	50
11	100	85	ND
12	45	ND	ND
Mean ± SD	36 ± 29	32 ± 35	34 ± 38
Median	27	16	10

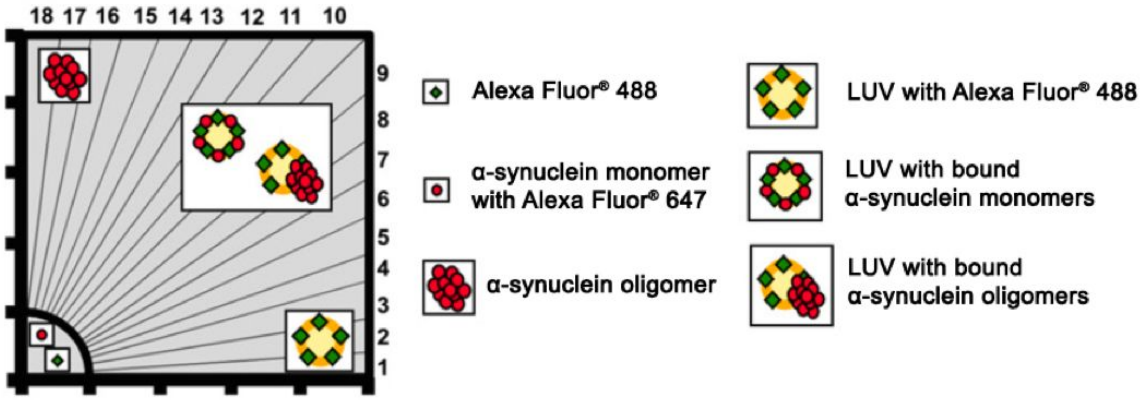
A



B



Supporting Figure S3. Histogram of conductance steps of αS pores in IM-type bilayers. Histograms of conductance step values at (A) +40 mV and (B) -40 mV in IM-type bilayers shows clear evidence of quantization, with the first peak corresponding to ~100 pS value for a single synuclein pore conductance, and subsequent peaks at ~200 pS and ~400 pS.



Supporting Figure S4. Segment representation in 2D-FIDA histograms. Binding of αS to lipid vesicles (LUVs) was analyzed using the SIFT-2D software package and for each of the two channels (red and green), photons per bin were plotted in a 2D-FIDA histogram. Data points of monomers are situated near the origin, while the red-labeled oligomers result in data points along the *y*-axis (segments 16-18) and the green-labeled vesicles result in data points along the *x*-axis (segments 1-3). Vesicle-bound proteins are represented along the bisectrix of the histogram (segments 4-15).