Supplementary Data

Assembly of α-Synuclein Aggregates on Phospholipid Bilayers

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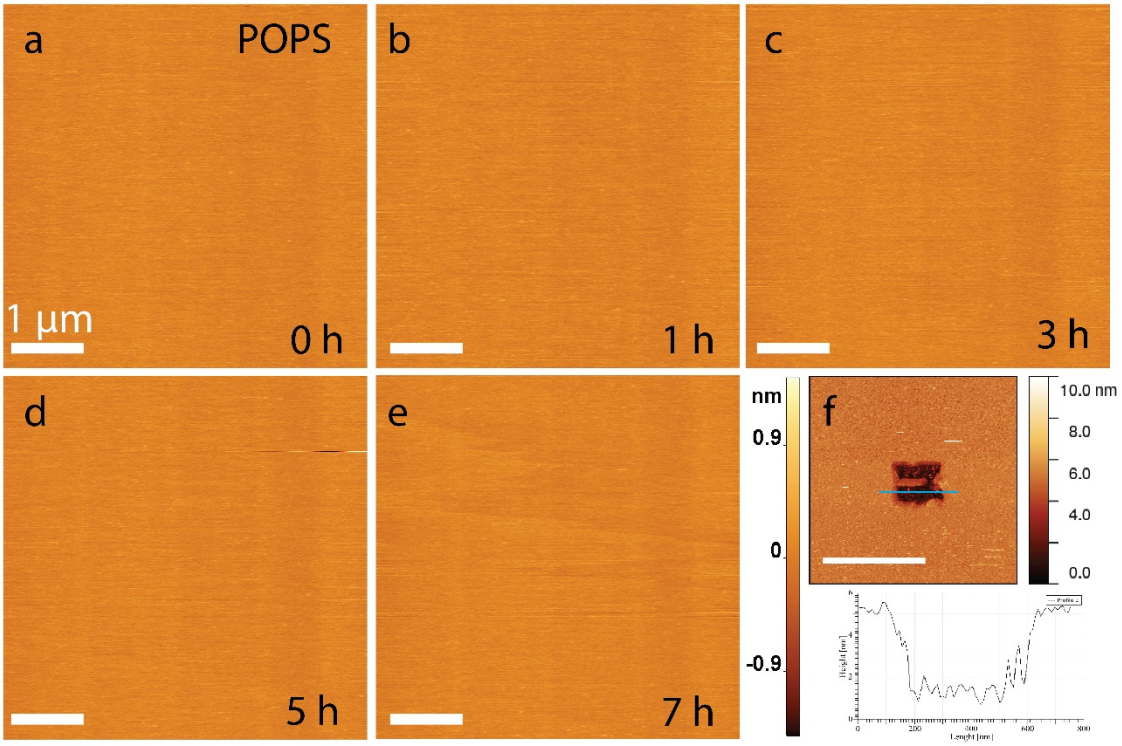
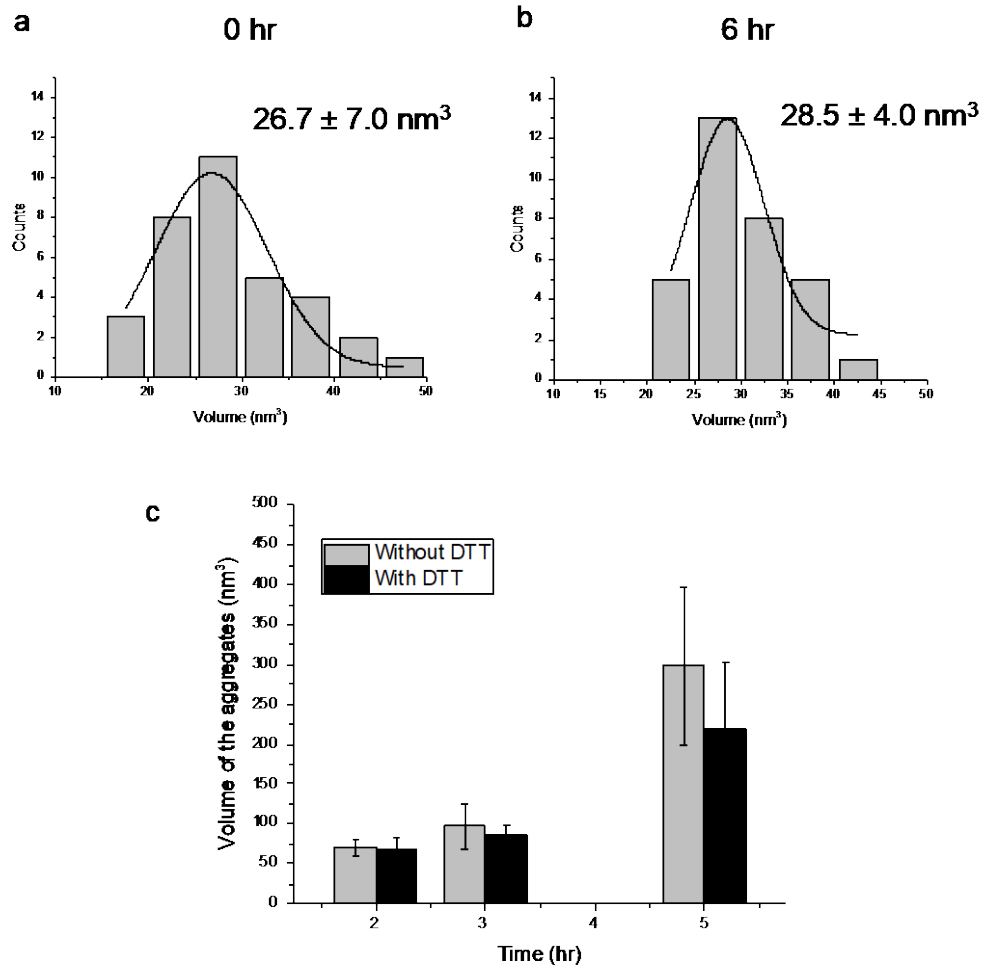
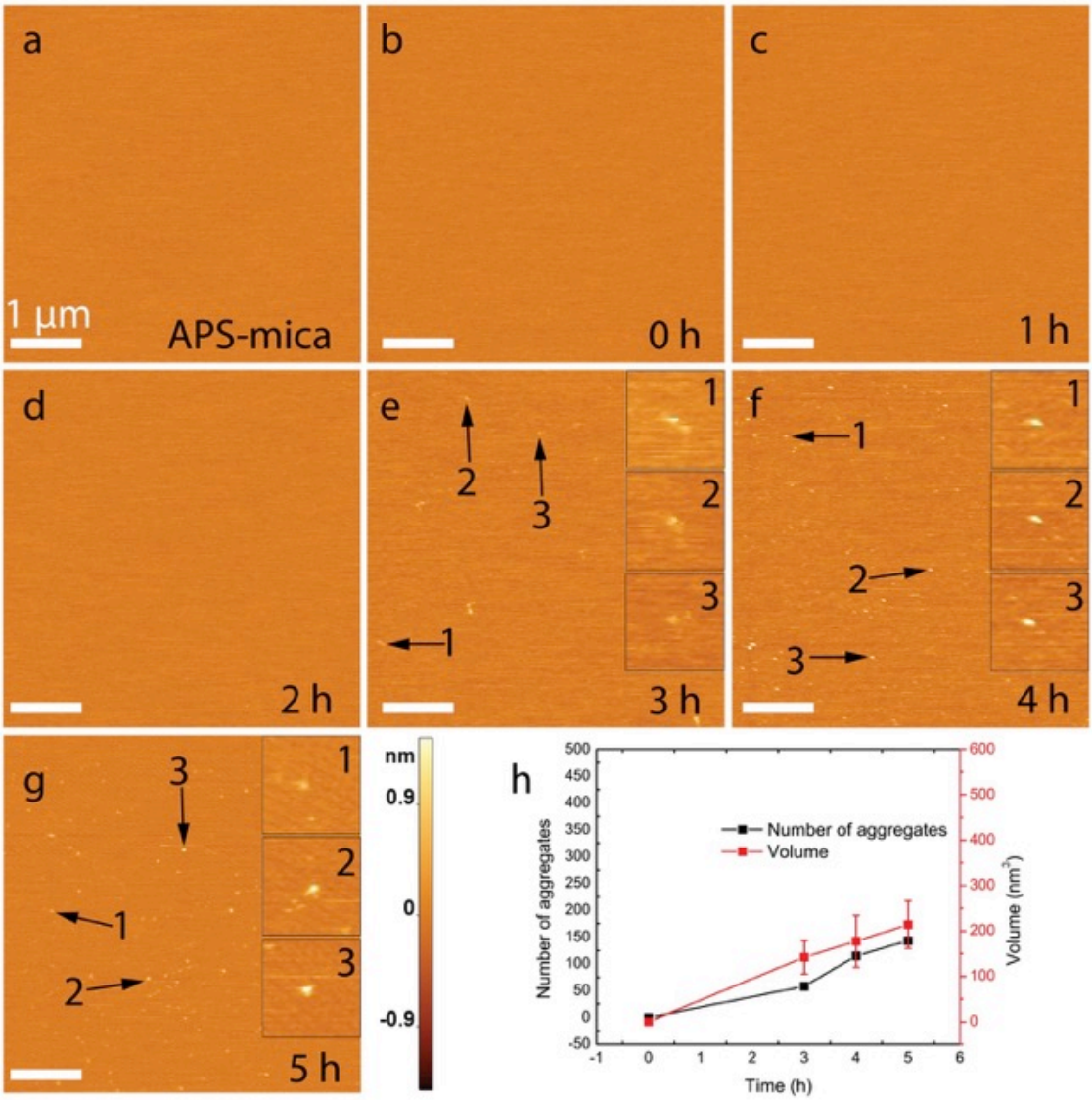
Keywords: α-synuclein, amyloid aggregation, lipid bilayer, Parkinson's disease, time-lapse AFM



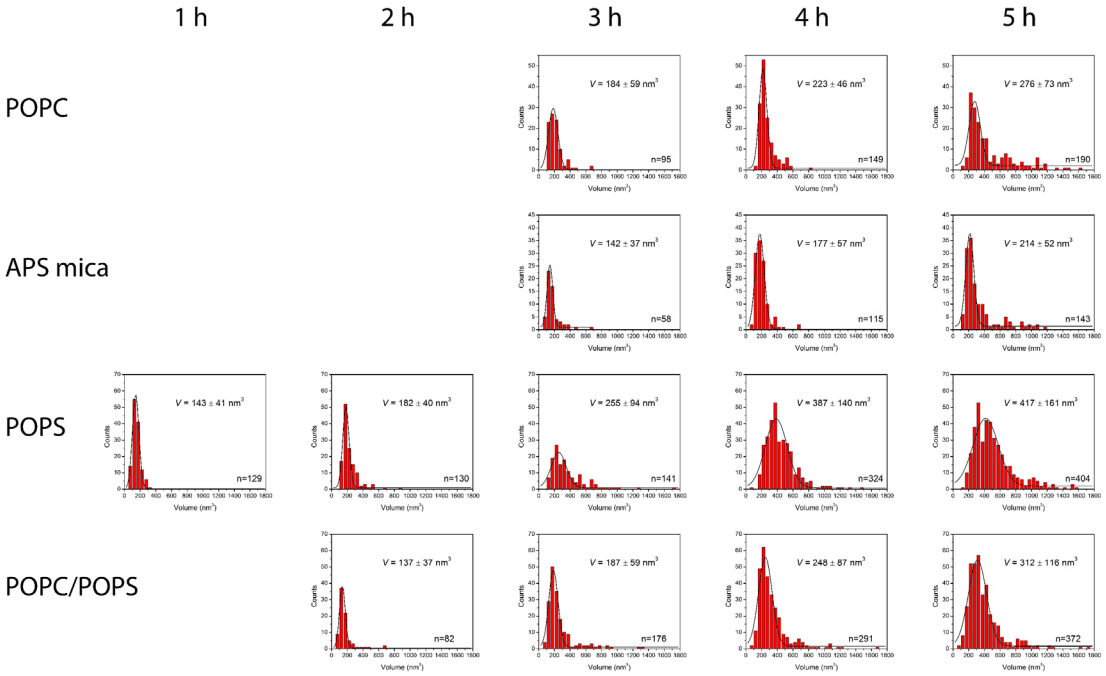
Supplementary Figure 1. Smoothness and stability of a POPS SLB. The smoothness of a POPS SLB was surveyed immediately after preparation **(a)**. There were no visible aggregate-like features. The SLB was then imaged using *in situ* time-lapse AFM for 7 h **(b-e)**, acquiring images with 1 h time interval. The SLB remained stable throughout the experiment. (**f**) Top, POPS SLB immediately following the completion of 400x400nm nanolithography using the AFM probe; bottom, line profile (blue line, top) showing the bilayer thickness. The scale bar in all panels is 1 µm, and the Z-scale for panels **a-f** is shown to the right of panel **e**.



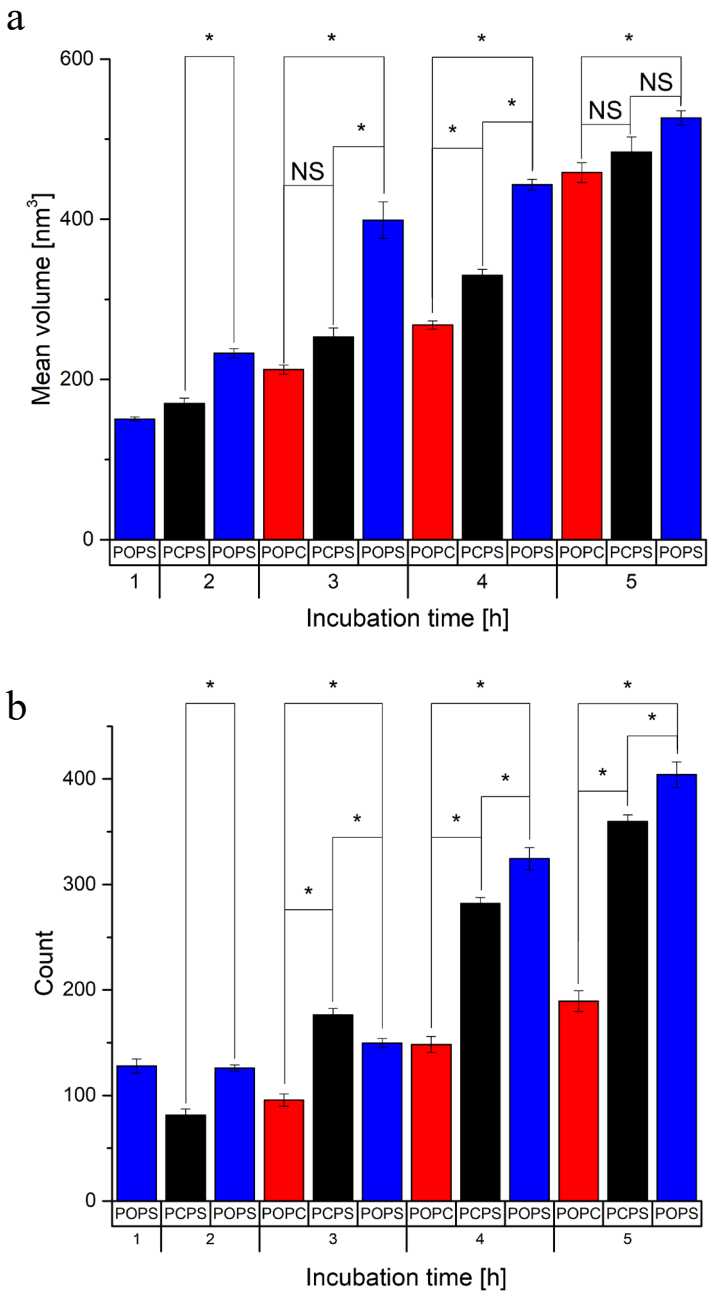
Supplementary Figure 2. Volume distribution of α-synuclein particles after being incubated in solution for **(a)** 0 hr and **(b)** 6 hr in conditions exactly as during to the time-lapse experiments. The volume of the protein indicates that α-synuclein remains as monomer even after 6 hr of incubation in solution. (**c**) volume of the aggregates formed on APS-functionalized mica surface in presence and absence of 1 mM DTT in the imaging buffer (10 mM sodium phosphate, 1 mM DTT, pH 7.4). Aggregate volume remains similar in both situation.



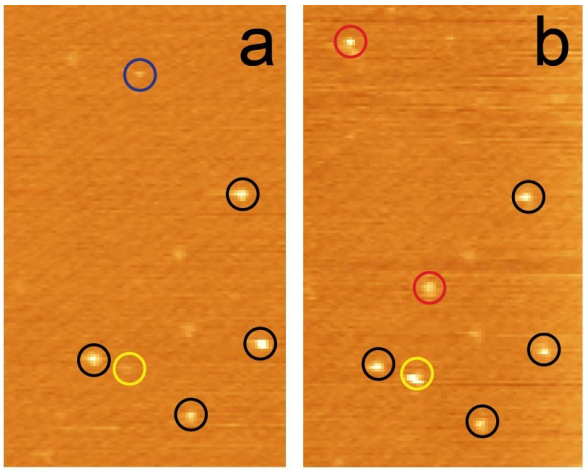
Supplementary Figure 3. α-Syn aggregation on an APS-mica surface. The homogeneity of the APS-mica surface was confirmed with AFM imaging **(a)** before exchanging the buffer with an α-syn solution. An image was acquired immediately after the buffer exchange **(b)**. The same area was then monitored by acquiring images with 1 h time interval **(c-g)**. There were no discernable protein aggregates until 3 h incubation, panel **e**. Aggregates grew on the surface in terms of both number and size, panel **f** and **g**. Insets show zoomed images of three representative aggregates aligned on the right side of each panel **e-g**. **(h)** Line plot showing the time-dependent evolution of aggregate quantity and mean volume. The data are shown as the mean ± SD. The scale bar in panels **a-g** is 1 µm, and the Z-scale for all panels is shown to the right of panel **g**.



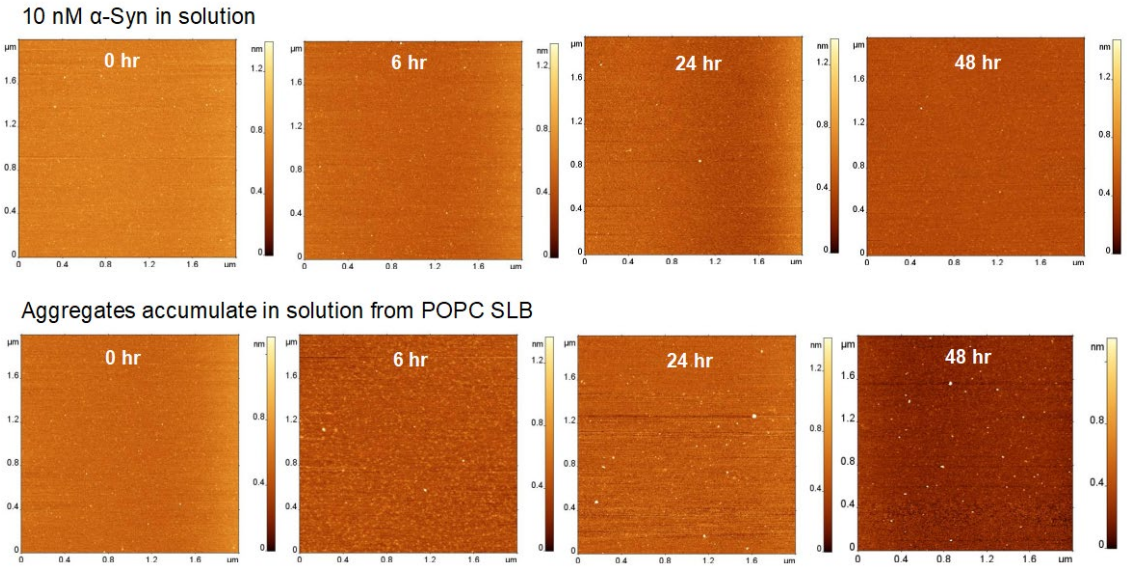
Supplementary Figure 4. Volume distributions of α-syn aggregates on different surfaces at different time points. Each row represents data from the same surface, while each column is a different incubation time-point. The black curves are Gaussian fits. The most probable volumes are shown as mean ± SD. The number of aggregates is provided in the bottom-right corner of each graph.



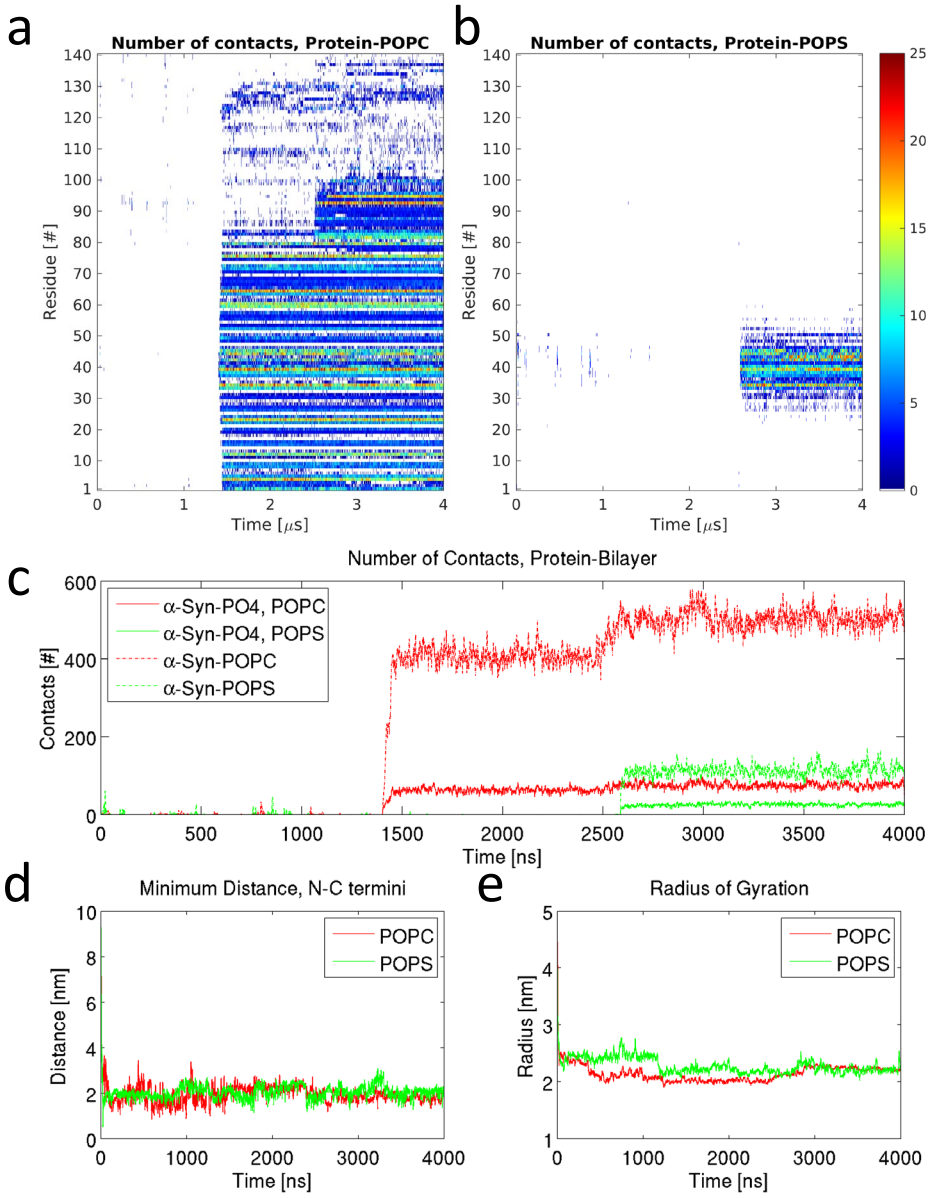
Supplementary Figure 5: The volume **(a)** and numbers of α-syn aggregates at different time intervals **(b)** on POPC, POPS and POPC/POPS lipid bilayer surface. Rapid appearance of aggregates was observed on POPS (within 1 hr). The volume of aggregates remains larger on POPS compared with other bilayers at all time points. Values for volume and aggregate numbers shown as means with SEM from pairwise ANOVA and statistical significance analyzed by Tukey at 0.05 level. Asterisk signifies significance while NS is non-significant.



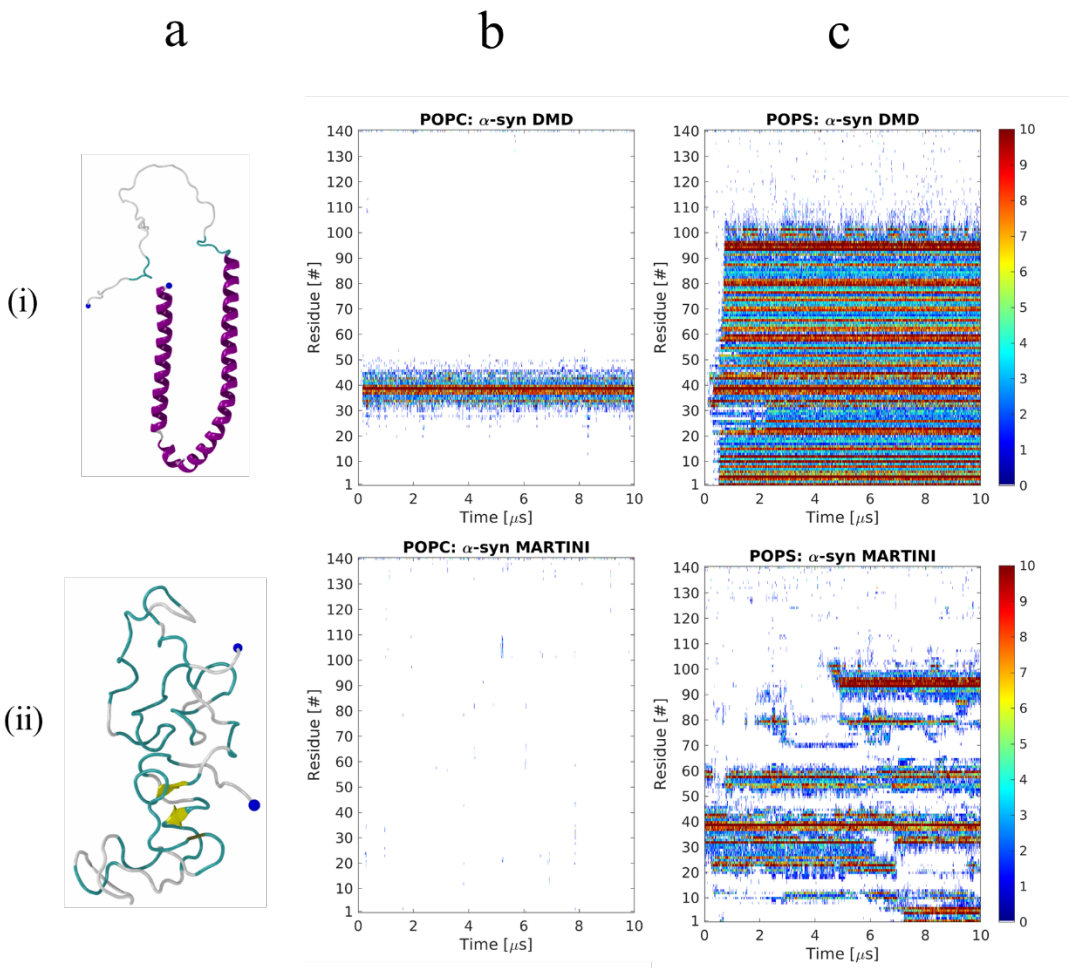
Supplementary Figure 6: Association and dissociation events of α-syn aggregates on POPC SLB captured between 4 h **(a)** and 5 h **(b)**. The aggregates highlighted with black circles stayed on the surface within the capture time frame. Blue circle in frame **a** corresponds to the aggregate that disappear in frame **b**. New aggregates that appeared in **b** are shown with red circles. A growing aggregate is circled with yellow. The images are 1.7 µm × 0.9 µm in size.



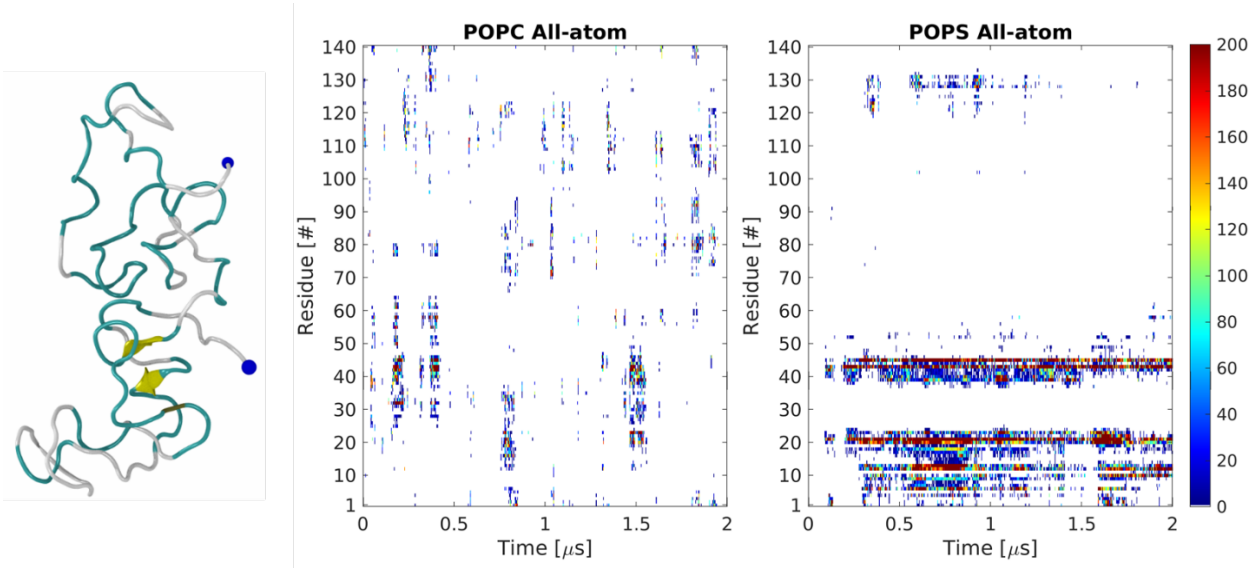
Supplementary Figure 7: AFM images of α-syn aggregates in bulk solution (absence of SLB, **top** row) and aggregates in bulk solution above the POPC bilayer surface (**bottom** row). Significant difference is observed after 24 hr, where larger and more numerous aggregates are found in the solution above the POPC bilayer surface.



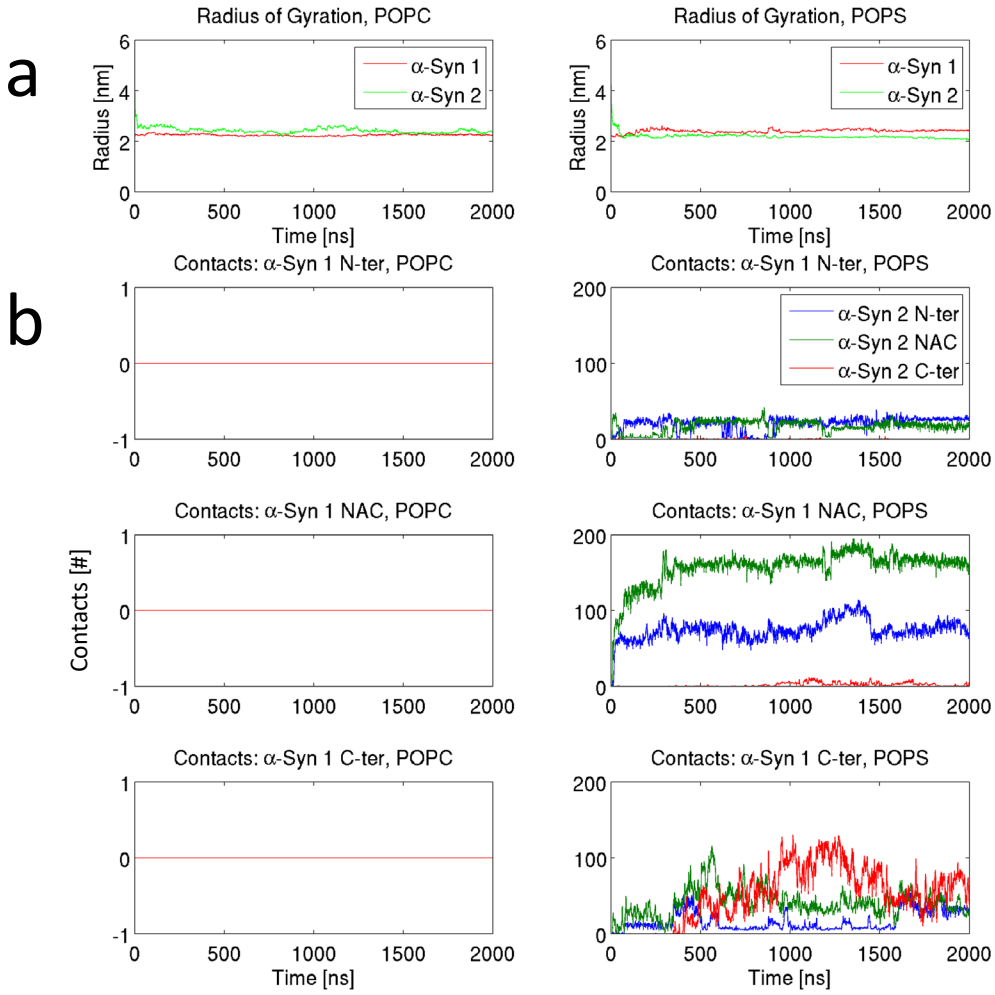
Supplementary Figure 8. Molecular dynamics simulation of α-syn interaction with lipid bilayers. Kymographs showing the time dependent interaction of α-syn residues with lipid bilayers of POPC **(a)** and POPS **(b)**, respectively. **(c)** Graphical representation of α-syn total contacts (distance <0.6 nm, dashed lines) and contacts with PO_4 groups (solid lines) of the membranes. **(d)** Minimum distance between the N- and C-termini of α-syn vs time. **(e)** Time-dependent change in radius of gyration of α-syn. The color bar for panels **a** and **b** represents the number of contacts that each residue of the protein makes with the bilayer.



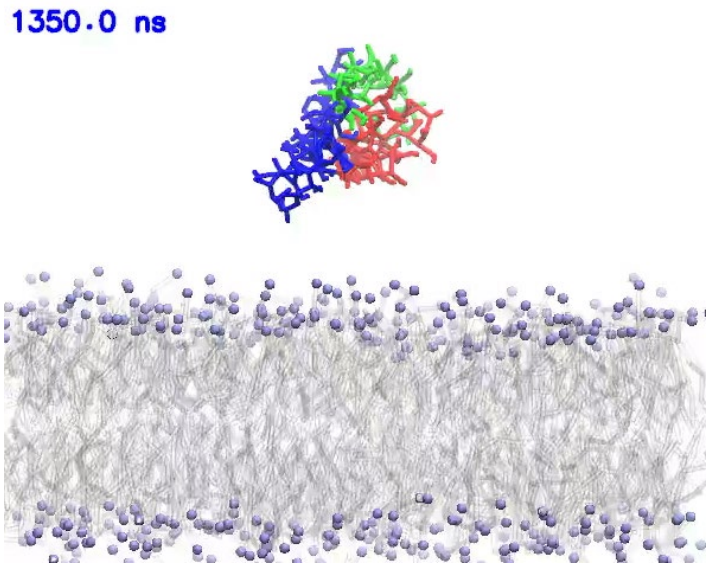
Supplementary Figure 9. Molecular dynamics simulation of two different α-syn conformations interacting with lipid bilayers. (**a**) The conformations of α-syn were obtained from (i) previous DMD simulations and (ii) 2 μs coarse-grained simulation with backbone assigned as random coil. Kymographs show the time dependent interaction of α-syn residues with PO_4 groups of the lipid bilayers on POPC **(b)** and POPS **(c)**, respectively. For all conformations, interactions with the bilayer initially occur through the N-terminus of the α-syn, specifically residues 30-50. The color bars from panel **c** represent the number of contacts that each residue of the protein makes with the bilayer.



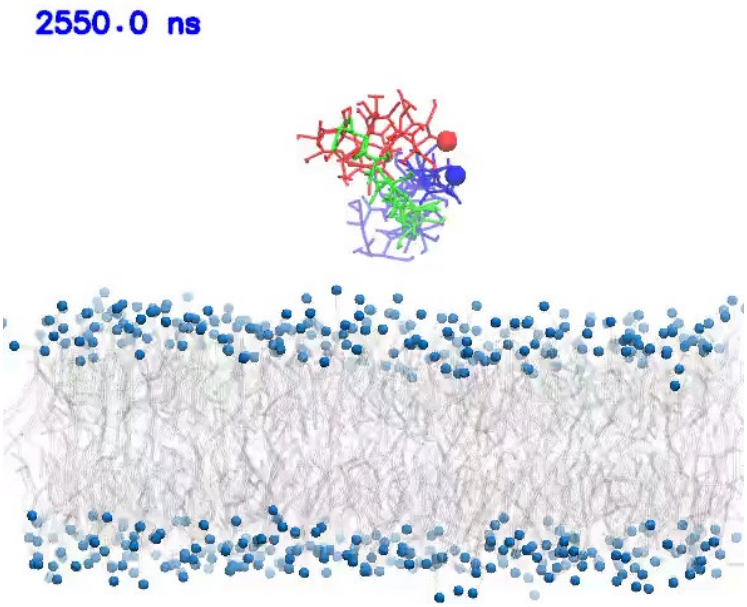
Supplementary Figure 10. All-atom molecular dynamics simulation of α-syn interacting with lipid bilayers. Initial conformation of the α-syn protein was obtained by reverse mapping a coarse-grained structure (after 2 μs simulation, same as Figure S9a (ii)). Kymographs show the time dependent interaction of α-syn residues with PO_4 groups of the lipid bilayers on POPC **(a)** and POPS **(b)**. Interactions primarily occur through the residues in the N-terminus of the protein, with initial interactions being through the same segment as was observed in coarse-grained simulations. The color bar represents the number of contacts that each residue of the protein makes with the bilayer.



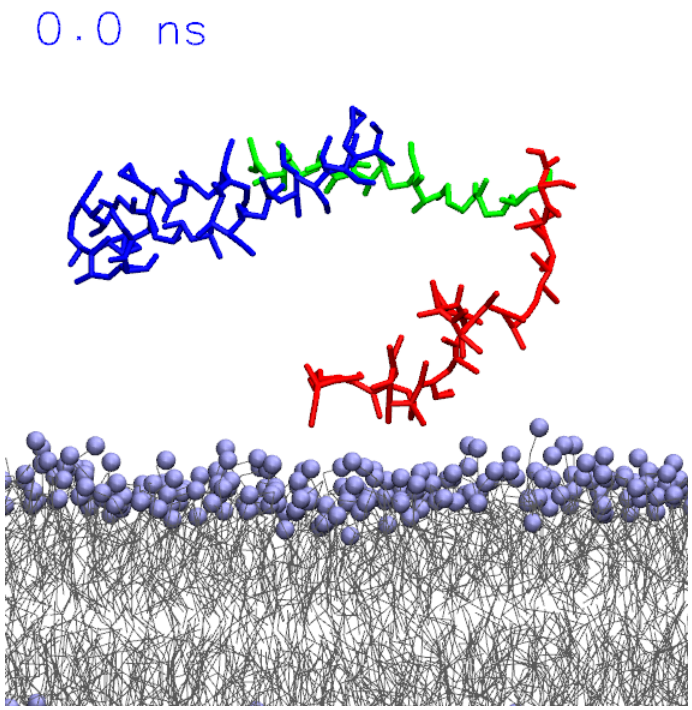
Supplementary Figure 11. MD simulation of a free α-syn molecule interacting with membrane-bound α-syn. **(a)** Radius of gyration for membrane-bound (α-Syn 1) and free (α-Syn 2) α-syn molecules in the POPC (left) and POPS (right) systems. **(b)** Inter-peptide contacts for the α-syn molecules in the POPC (left) and POPS (right) systems. Number of contacts (distance <0.6 nm) between membrane bound α-syn (1 N-ter, 1 NAC, and 1 C-ter) and segments of the free α-syn (denoted 2) molecules are plotted versus time.



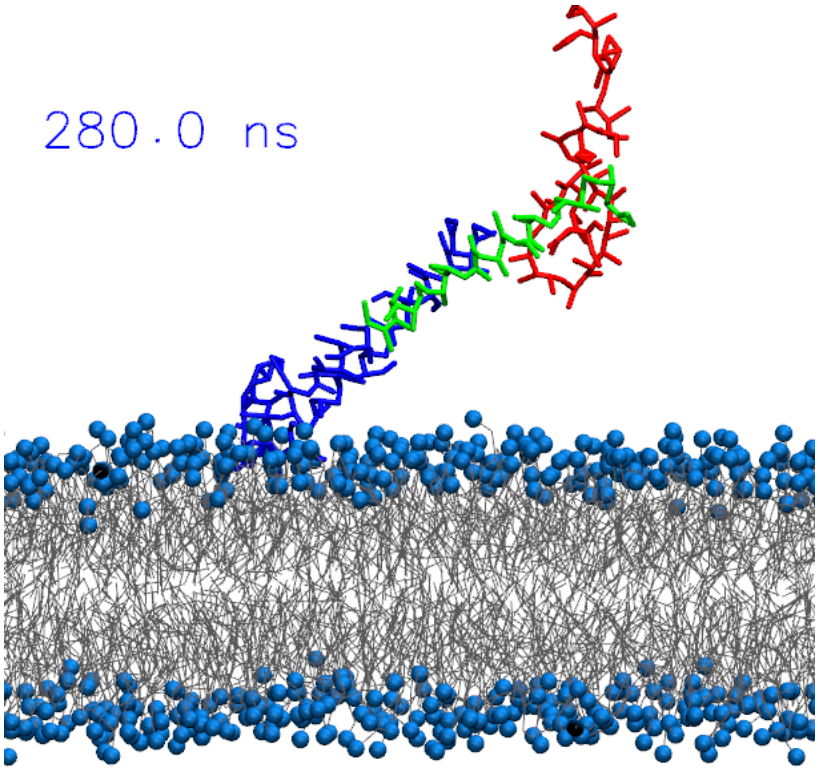
Supplementary Movie 1. Coarse-grained MD simulation of α-syn interaction with a POPC lipid bilayer. Stable binding of the α-syn protein to the bilayer is observed. The α-syn N-terminal segment is colored blue, NAC region is in green, and the C-terminal segment is in red. N- and C-terminal residues are highlighted with a sphere. Lipid tails are in grey, while the POPC head groups are in purple.



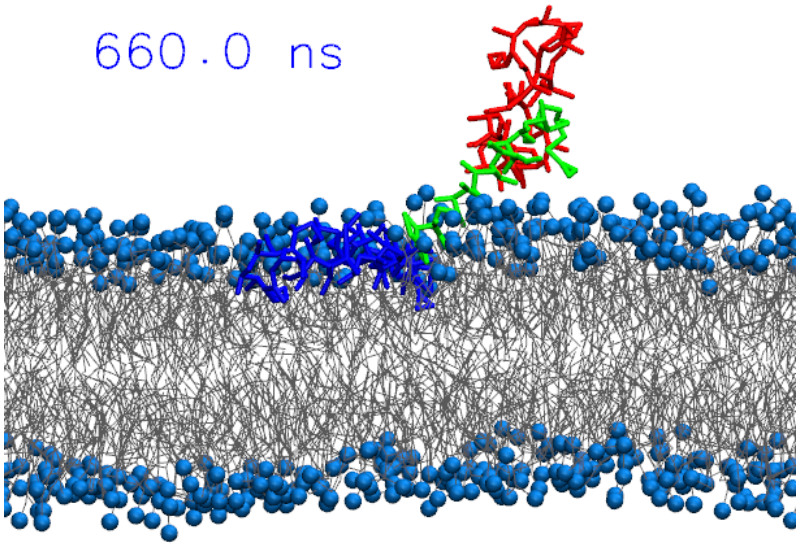
Supplementary Movie 2. Coarse-grained MD simulation of α-syn interaction with a POPS lipid bilayer. The stable binding event of α-syn to the bilayer is shown. α-syn N-terminal segment is colored blue, NAC region is in green, and the C-terminal segment is in red. N- and C-terminal residues are highlighted with a sphere. Lipid tails are in grey, while the POPS head groups are in blue.



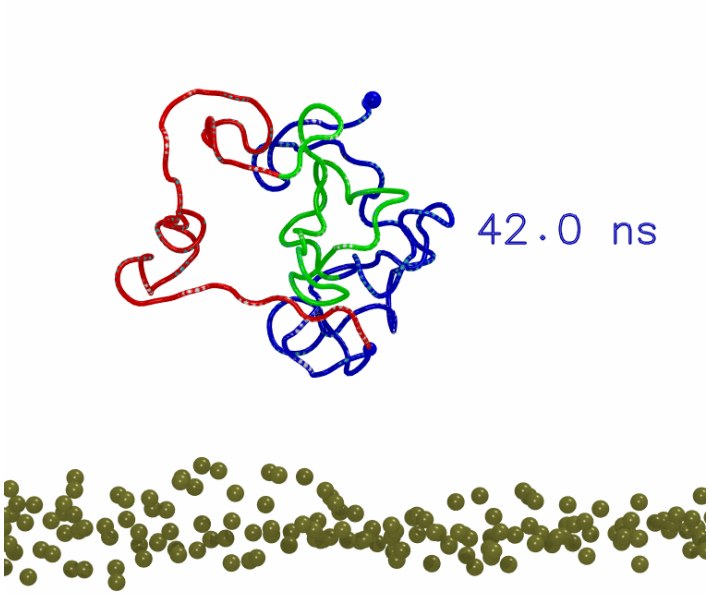
Supplementary Movie 3. Coarse-grained MD simulation of interaction of α-syn monomer, initial conformation from DMD simulations, and POPC bilayer. Interaction with and binding to the POPC surface is observed. Blue backbone denotes the N-terminal region, green backbone the NAC region, and red backbone the C-terminal region of the α-syn molecule. Lipid tails are in grey, while the POPC head groups are in purple.



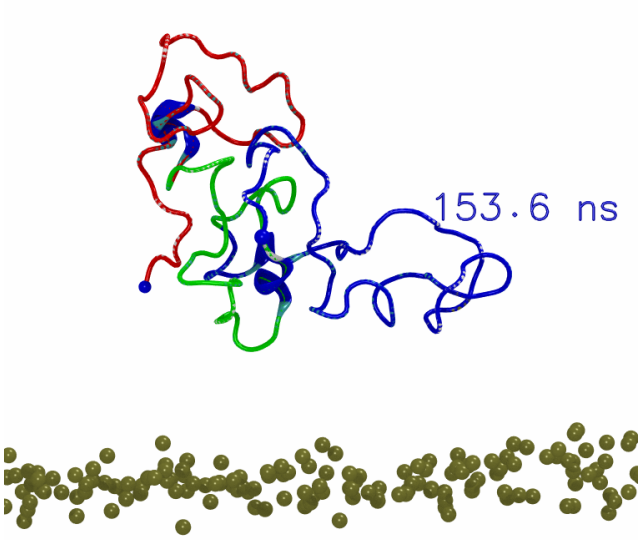
Supplementary Movie 4. Coarse-grained MD simulation of interaction of α-syn monomer, initial conformation from DMD simulations, and POPS bilayer. Conversion from a bound extended orientation to a parallel (to the bilayer surface) orientation is observed, with majority of N-terminal region going to the surface of the bilayer. Blue backbone denotes the N-terminal region, green backbone the NAC region, and red backbone the C-terminal region of the α-syn molecule. Lipid tails are in grey, while the POPS head groups are in blue.



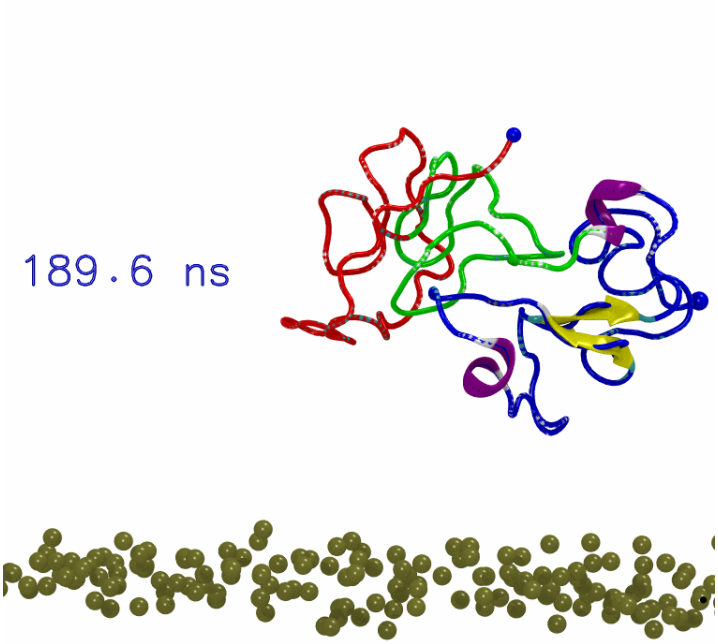
Supplementary Movie 5. Coarse-grained MD simulation of interaction of α-syn monomer, initial conformation from DMD simulations, and POPS bilayer. Stable interaction and insertion of the N-terminal and NAC regions in the bilayer interfacial region is observed while the C-terminal region stays off the surface and flexible. Blue backbone denotes the N-terminal region, green backbone the NAC region, and red backbone the C-terminal region of the α-syn molecule. Lipid tails are in grey, while the POPS head groups are in blue.



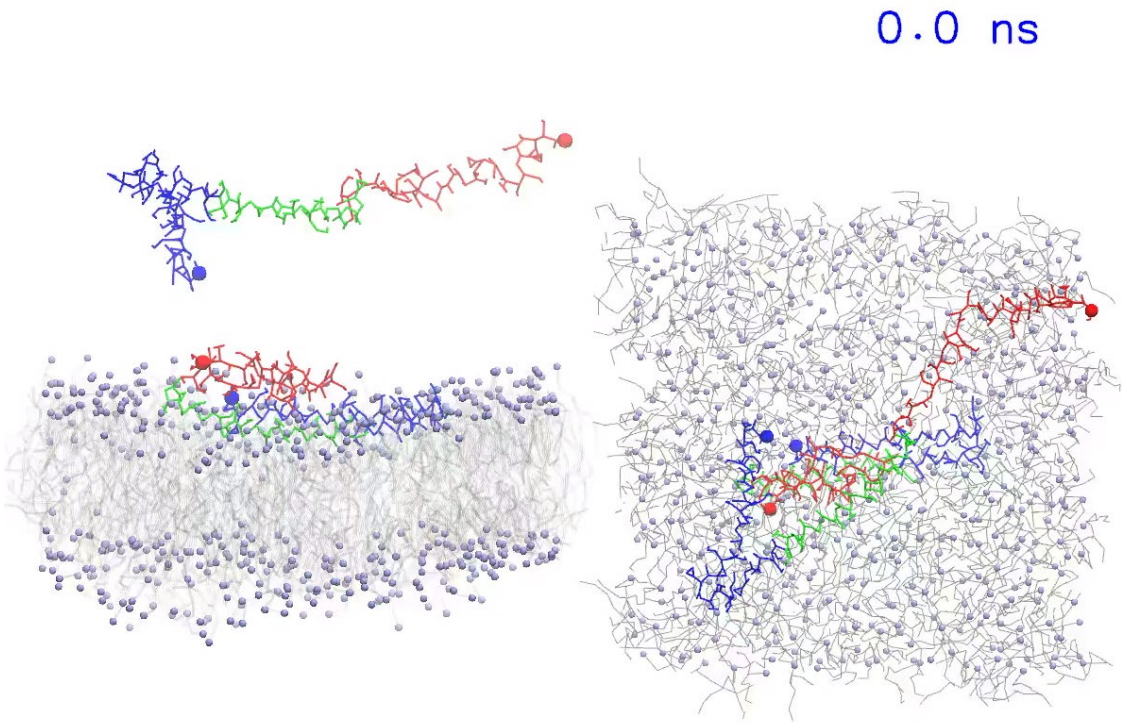
Supplementary Movie 6. All-atom MD simulation of interaction of α-syn monomer, initial conformation from coarse-grained simulations, and POPC bilayer. Initial interactions happen through N-terminal region followed by residues in the NAC- and C-terminal regions. Large blue sphere denotes the N-terminus Cα, the small blue sphere the C-terminal Cα, blue backbone the N-terminal region, green backbone the NAC region, and red backbone the C-terminal region of the α-syn molecule. Gold spheres mark the P atoms of the lipid heads. Interacting protein residues and lipid head groups are depicted in licorice style, with protein residues being in a wider shape.



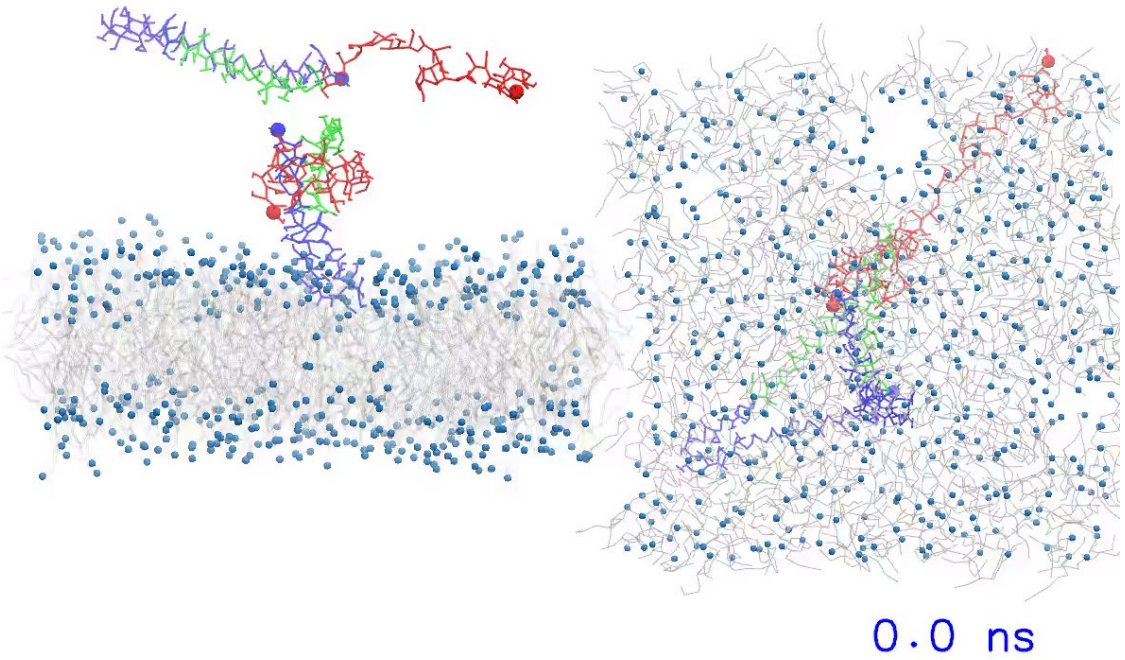
Supplementary Movie 7. All-atom MD simulation of interaction of α-syn monomer, initial conformation from coarse-grained simulations, and POPC bilayer. Interaction with the bilayer is observed, initially through the N-terminal region followed by the NAC-region; which is then converted to an extended conformation with only N-terminal region interacting, as observed in coarse-grained simulations. Large blue sphere denotes the N-terminus Cα, the small blue sphere the C-terminal Cα, blue backbone the N-terminal region, green backbone the NAC region, and red backbone the C-terminal region of the α-syn molecule. Gold spheres mark the P atoms of the lipid heads. Interacting protein residues and lipid head groups are depicted in licorice style, with protein residues being in a wider shape.



Supplementary Movie 8. All-atom MD simulation of interaction of α-syn monomer, initial conformation from coarse-grained simulations, and POPS bilayer. Stable interaction with the bilayer is observed, through the N-terminal region; initially in a compact conformation which then extends with C-terminal region moving away from the bilayer surface, similar to observations from coarse-grained simulations. Large blue sphere denotes the N-terminus Cα, the small blue sphere the C-terminal Cα, blue backbone the N-terminal region, green backbone the NAC region, and red backbone the C-terminal region of the α-syn molecule. Gold spheres mark the P atoms of the lipid heads. Interacting protein residues and lipid head groups are depicted in licorice style, with protein residues being in a wider shape.



Supplementary Movie 9. Coarse-grained MD simulation of a free α-syn interacting with a membrane bound α-syn on a POPC bilayer. The binding of α-syn to the bilayer is shown. α-syn N-terminal segment is colored blue, NAC region is in green, and the C-terminal segment is in red. N- and C-terminal residues are highlighted with a sphere. Lipid tails are in grey, while the POPC head groups are in purple.



Supplementary Movie 10. Coarse-grained MD simulation of a free α-syn interacting with a membrane bound α-syn on a POPS bilayer. The binding of α-syn to the membrane-bound α-syn is shown. α-syn N-terminal segment is colored blue, NAC region is in green, and the C-terminal segment is in red. N- and C-terminal residues are highlighted with a sphere. Lipid tails are in grey, while the POPS head groups are in blue.