SUPPORTING INFORMATION

Iron-Mediated Interaction of Alpha Synuclein with Raft-like Model Membranes

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Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of AS molecules

SDS-PAGE was performed to discriminate between monomeric and oligomeric species of the proteins. 7.5 µg of monomeric protein (WT and A53T αS) and of the corresponding aggregates obtained in presence of iron were mixed with loading sample buffer (250 mM Tris-HCl pH 6.8, 30% glycerol, bromophenol blue, without SDS and without any denaturing agent), boiled for 5 minutes and separated on a 1 mm 15% SDS-PAGE gel. Proteins were then visualized using Coomassie staining. Preformed fibrils of human synuclein WT produced in absence of iron were loaded as positive control for aggregates identification. Fe^{2+}-αS oligomers of both proteins showed a smear similar to the one observed for the WT-fibrils pointing out the presence of protein aggregation (**Figure S1**).

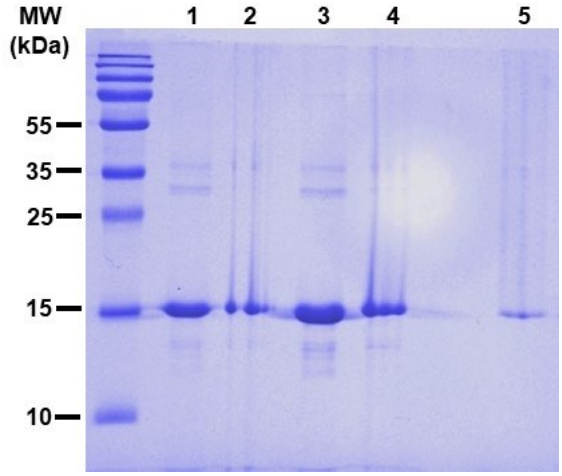


Figure S1. SDS-PAGE of αS molecules. 1). WT monomers, 2) WT iron-oligomers, 3) A53T monomers, 4) A53T iron-oligomers, 5) WT fibrils.

Dynamic light scattering (DLS) of iron-induced αS oligomers

Hydrodynamic diameter analysis of 35 µM Fe^{2+}-mediated αS oligomers were performed on a Zetasizer Nano ZS (Malvern Instruments) Dynamic Light Scattering instrument equipped with a Peltier temperature controller set at room temperature. Disposable micro-cuvettes for size measurements at a scattering angle of 173° were used. Each sample was measured three times using 10 accumulating scans to give averaged intensity-diameter and averaged volume-diameter distributions. In the case of WT αS a 98.5% monodisperse population of globular aggregates with a hydrodynamic diameter of 5.6 ± 1.5 nm (**Figure S2A-B**) was observed. On the other side, in the case of the A53T mutant we observed the formation of a 62.3% protein aggregates with a hydrodynamic dimension of 28.2 ± 5.0 nm (**Figure S2C-D**). In this second case we observed also the presence of bigger aggregated products, probably to indicate the formation of amorphous aggregates.

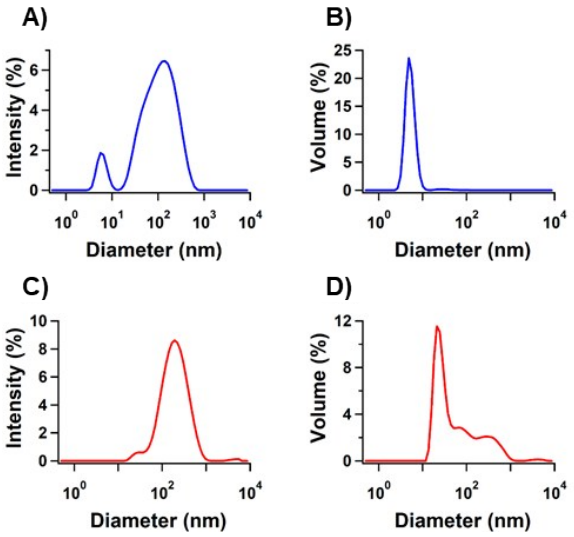


Figure S2. DLS measurements of Fe^{2+}-mediated αS oligomers. Intensity vs. diameter and volume vs. diameter graphs are reported for A-B) WT αS and C-D) A53T αS after 1 h treatment with 2 mM $FeCl_2$.

Stability of supported planar lipid bilayers in liquid environment

Before incubating biomolecules with the prepared membranes, the stability of our planar three component model membrane mixture (DOPC/SM 66:33 + 5% cholesterol) in Milli-Q water has been assessed. We focused on its structural integrity and on the possible morphological changes in the coexisting two lipid phases after a prolonged incubation in liquid environment. After 24 hours in Milli-Q H2O, the membrane maintains almost the 95% of its structural integrity, remaining clean and with very flat domains (**Figure S3A-B**). The topographic height variation ΔZ between the So and Lα phase (1.2 ± 0.3 nm) does not change with respect of the same value of the as-prepared membrane (see for reference the image in Figure 3 of the manuscript), as well as the relative ratio between Lα and So phase (66.5/33.5). However, there is clear evidence for the formation of defect sites (d), in the form of damaged areas (black holes in the figure) which seem to be essentially located at the level of the Lα phase. This phase is usually more prone to destabilization by environmental factors, being less packed and less ordered than the ordered domains. The black circular holes have a height distribution of 2.7 ± 0.3 nm, which agrees with the extraction of the upper fluid monolayer from the lipid bilayer membrane (**Figure S3C**). We supposed that the unfavorable exposure of lipid acyl chains to water leads to an unstable situation with the lack of an optimal membrane packing and the consequent increase of the roughness.

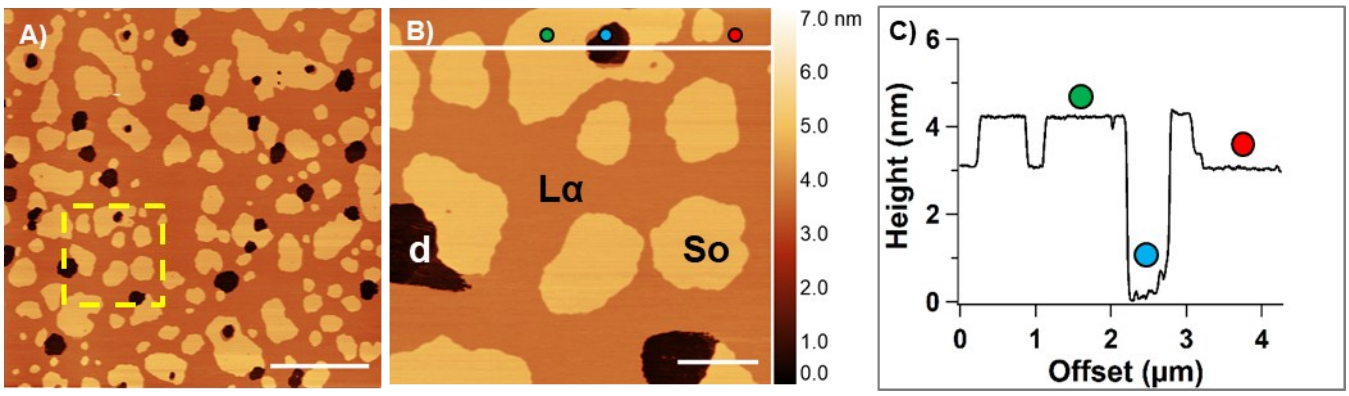


Figure S3. AFM morphological analysis of raft-like ternary SLB composed by DOPC, SM and cholesterol after 24 hours in Milli-Q water. A) AFM topography of the membrane surface (20 x 20 μm^2). The bilayer has only 5% of defects (d). B) Zoomed image (5 x 5 μm^2) to highlight structural details. C) Height profile shows the three different lipid areas on the membrane (red ●, Lα; green ●, So; light blue ●, d). Scale bar: A) 5.0 µm, B) 1.0 µm. Images were acquired in aqueous buffer in dynamic AC-mode. Lα=liquid-disordered, So=solid-ordered, d=defects.

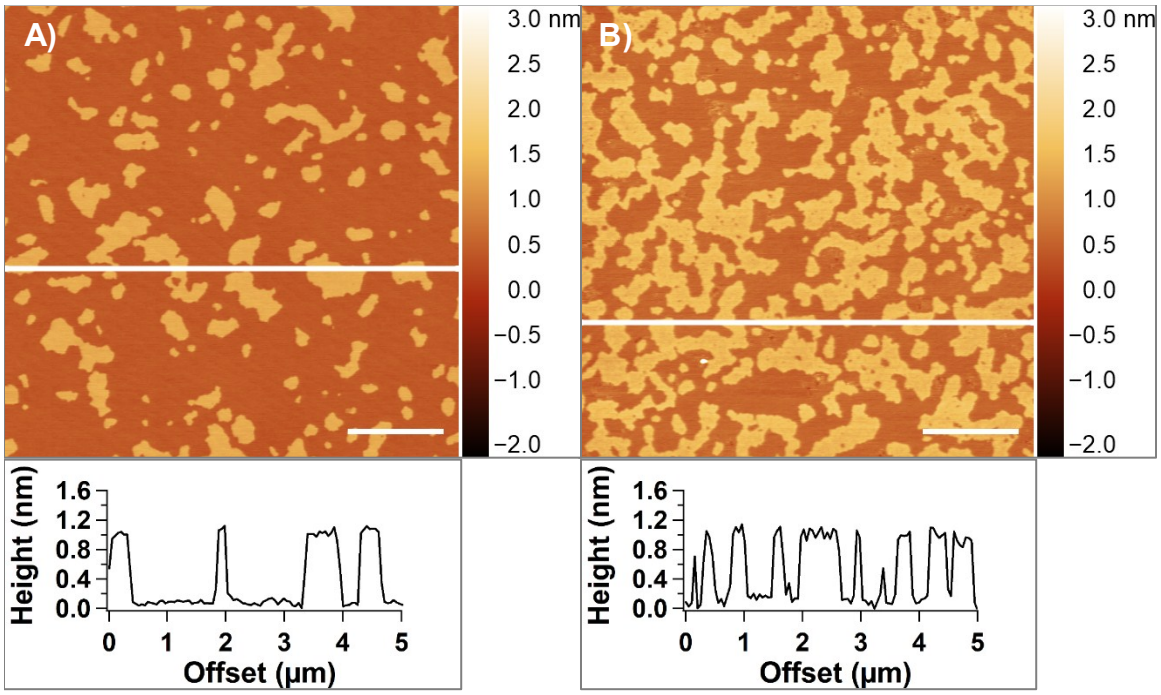
Effect of cholesterol on lipid phase separation of raft-like model membrane

We increased the concentration of cholesterol to 20% maintaining the same DOPC:SM ratio (DOPC/SM (66:33) + 20%Chol) in order to investigate possible morphological changes within the different lipid phases induced by cholesterol. 20% of cholesterol is a reasonable physiological value for plasma cell membrane [1]. The membrane characterized by 5% of cholesterol shows single raft-like domains that protrude from the fluid phase with an average height of 1.2 ± 0.2 nm (**Figure S4 A**). These lipid islands have heterogeneous lateral size distribution in the range of 0.2 – 1.0 μm. Even in this case the surface ratio of the two coexisting phases (Lα:So) derived from AFM images is 67:33 and matches well with the initial lipid composition. This ratio can be rationalized assuming that cholesterol is mostly sequestered by the SM in the solid ordered phase, increasing its fluidity and promoting a So to Lo transition [2].

The membrane with 20% of cholesterol displayed a different morphology having raft domains with a more dendritic shape and being characterized by a higher raft surface coverage (Lα:Lo=57:43) compared to the membrane with lower cholesterol content (**Figure S4 B**).

Assuming the same occupied surface per molecule (which is not exactly true since since there are not reference values for the intrinsic volume of cholesterol [3]) we calculated the excess surface that is due to 20% cholesterol-induced lipid ordering from the relative molar percentage, finding DOPC/SM+Chol = 54:47, not far from the measured one. Indeed, the entire volume occupied by the different phases changes by changing cholesterol content. The hydrophobicity of cholesterol (contains only one OH group per molecule), normally screened by sequestering adjacent, polar lipids, introduces to the membrane a certain level of viscosity and dynamic clustering of chol-SM aggregates, reducing the rafts height with respect to the fluid, disordered lipid phase around. This explanation is supported by the 20% decrease of the average ΔZ (Lo-Lα) value (0.9 ± 0.3 nm)

measured and by the slight increase of the ordered phase roughness when moving from 5% to 20% chol. Finally, lipid-raft domains appear more dendritic when the level of cholesterol is increased to 20%. This is a sign of diffusion-limited aggregation, which is in agreement with the dynamic molecular clustering described above. Further studies are required to corroborate this hypothesis.



C)

	ΔZ (nm)	Lα/So (% area)
5% chol	1.2 ± 0.3	67/33
20% chol	0.9 ± 0.3	54/46

Figure S4. AFM images of raft-like membranes (DOPC/SM (66:33)) in presence of A) 5% and B) 20% of cholesterol, respectively. C) The membrane with higher cholesterol content shows a higher lipid raft coverage and a decrease in the ΔZ (So-Lα) value compared to the membrane with 5% of cholesterol. Scale bar: 1.0 µm.

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