CHEMPHYSCHEM

Supporting Information

Direct Visualization of Model Membrane Remodeling by α-Synuclein Fibrillization

Himanshu Chaudhary,[a] Vinod Subramaniam,[b] and Mireille M. A. E. Claessens*[a]

cphc_201700050_sm_miscellaneous_information.pdf

Direct visualization of model membrane remodeling by α-synuclein fibrillization

H. Chaudhary,[a] V. Subramaniam[a,b] and Mireille M.A.E. Claessens[a]

[a] *Nanobiophysics group. Mesa+ Institute for Nanotechnology Department of Science and Technology, University of Twente, Enschede, The Netherlands.*
[b] *Vrije Universiteit Amsterdam, De Boelelaan 1105, 1081 HV Amsterdam, The Netherlands.*

Supplementary Information

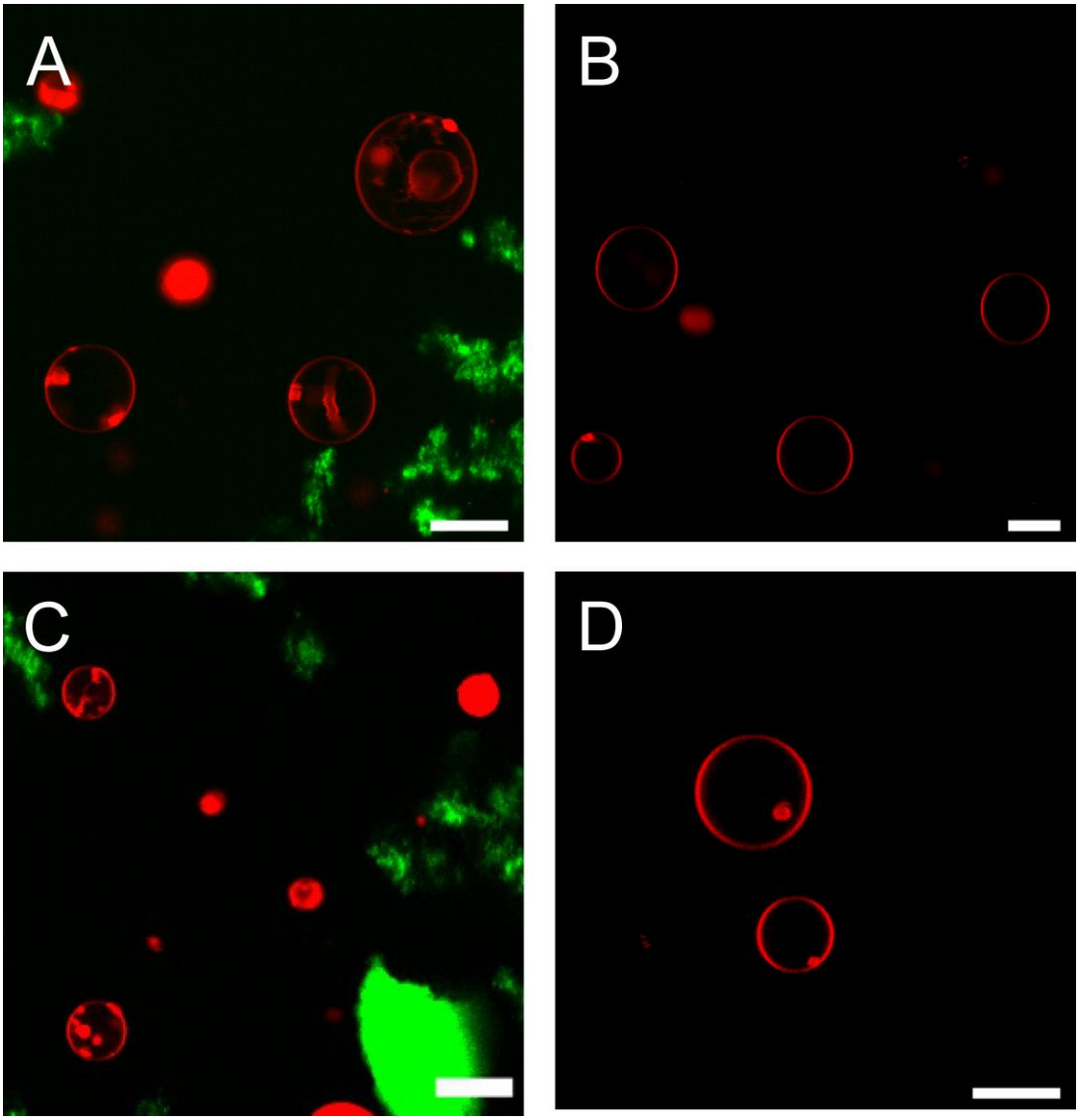

Figure S1: Preformed and growing fibrils do not disturb POPC GUV morphology. A) POPC GUVs incubated with pre-formed fibrillar αS aggregates. Fibrils were not observed to bind and accumulate at the vesicle surface. **B)** Control experiment showing stability of the vesicles used in A in the absence of fibrils. **C)** POPC GUVs incubated with 5% fibrillar seeds + 95% αS monomers. Growing fibrils did not affect the GUVs and POPC vesicle remain intact at 37 °C for at least 26 hours after the start of the experiment. **D)** Control experiment showing the stability of the vesicles used in C in the absence of fibrils, seeds and monomers. The GUVs contain a small fraction of Rh-DOPE (red) and the fibrillar aggregates are stained with ThT (green). Scale bar: 10 µm.

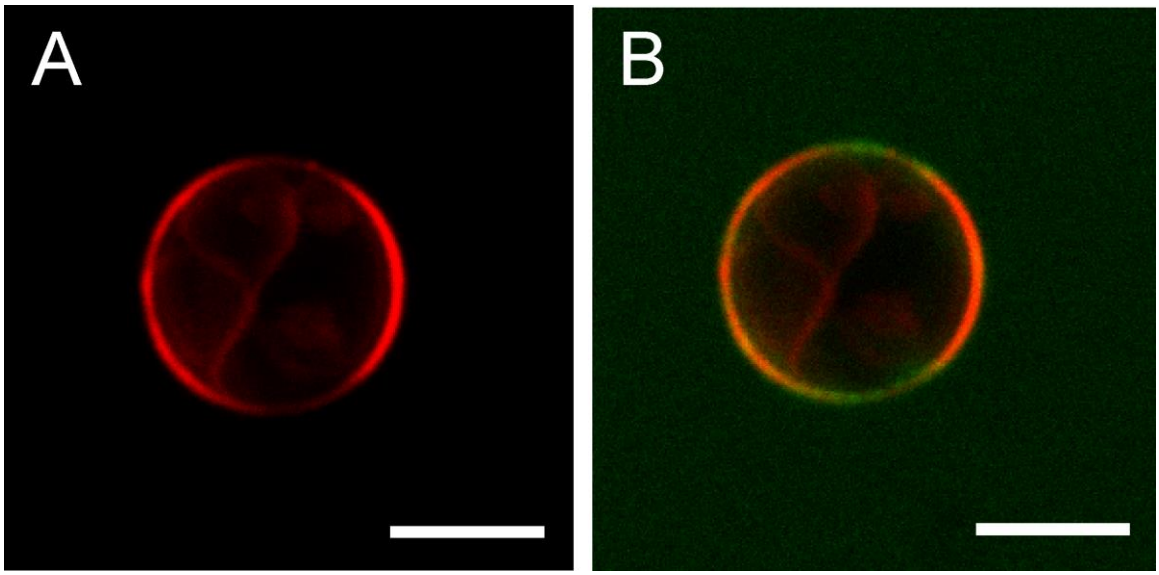

Figure S2: Binding of αS monomers to POPC/POPG GUVs. A) The vesicle membrane is made visible by doping PC/PG GUVs with RH-DOPE (red). **B)** Overlay of the fluorescence from the membrane (red) with the fluorescence from the AlexaFluor488 labelled αS monomers (green) 6 hours after monomer addition. Scale bar: 5 µm.

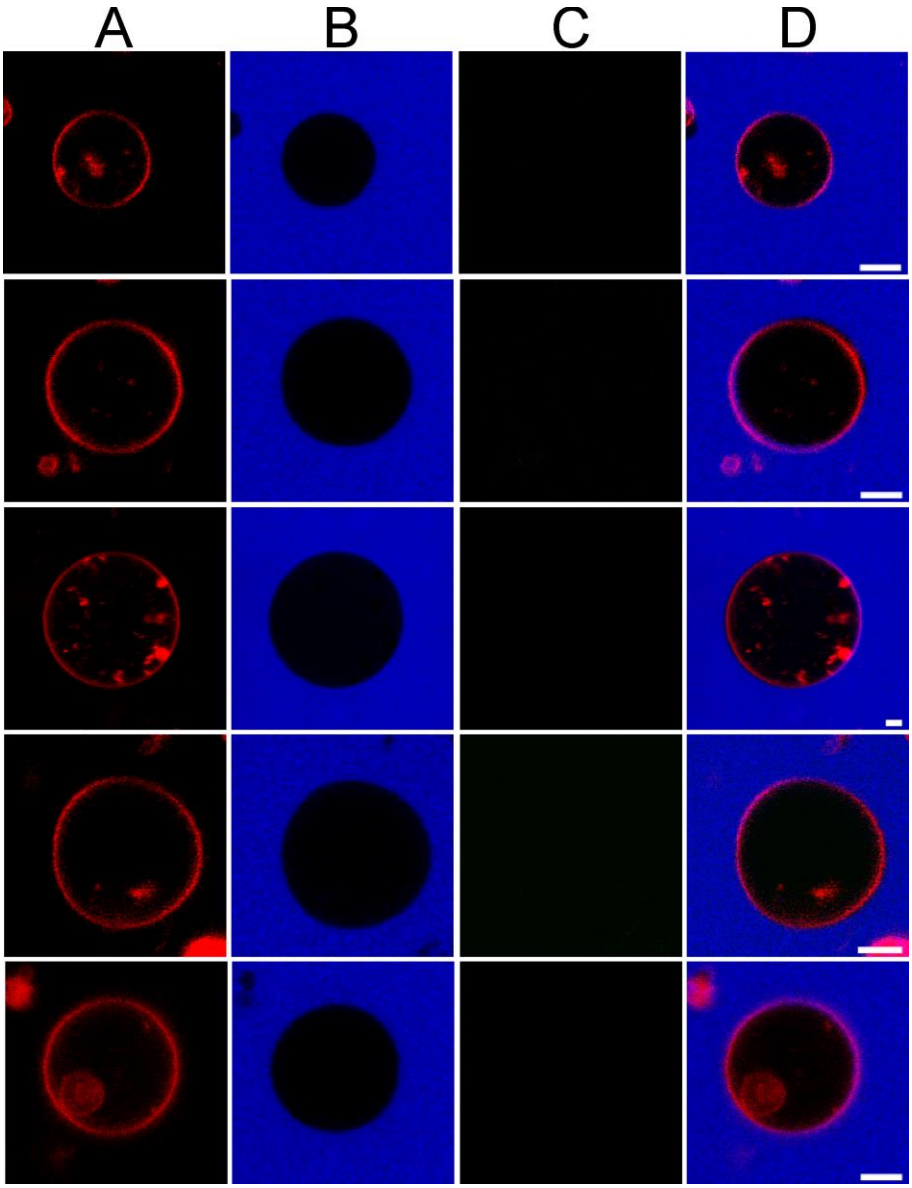

Figure S3: Vesicles remain intact during with incubation αS monomers. POPC/POPG vesicles were incubated with 100 μM αS monomers (1 mol% Alexa 647 labelled 140C αS) at 37 °C in quiescent conditions for 3 hours. To be able to follow possible amyloid formation 10 μM of dye ThT was added to the mixture. **A)** Rh-DOPE fluorescence (red). **B)** αS-Alexa647 fluorescence (blue). **C)** ThT fluorescence. **D)** Overlay of A, B and C. The absence of ThT fluorescence in panel C) indicates that at the timescale of the experiment αS was not able to aggregate into amyloid fibrils. Monomeric protein was also not able to cross the membrane. The vesicle interior stayed devoid of labelled protein (B). The presence of monomers did not result in the polygonal structures observed in the presence of both monomers and seeds. The sphericity of the vesicles from top to bottom was determined to be S=0.922, S=0.928, S=0.958, S=0.907 and S= 0.909. Scale bar: 5 μm.

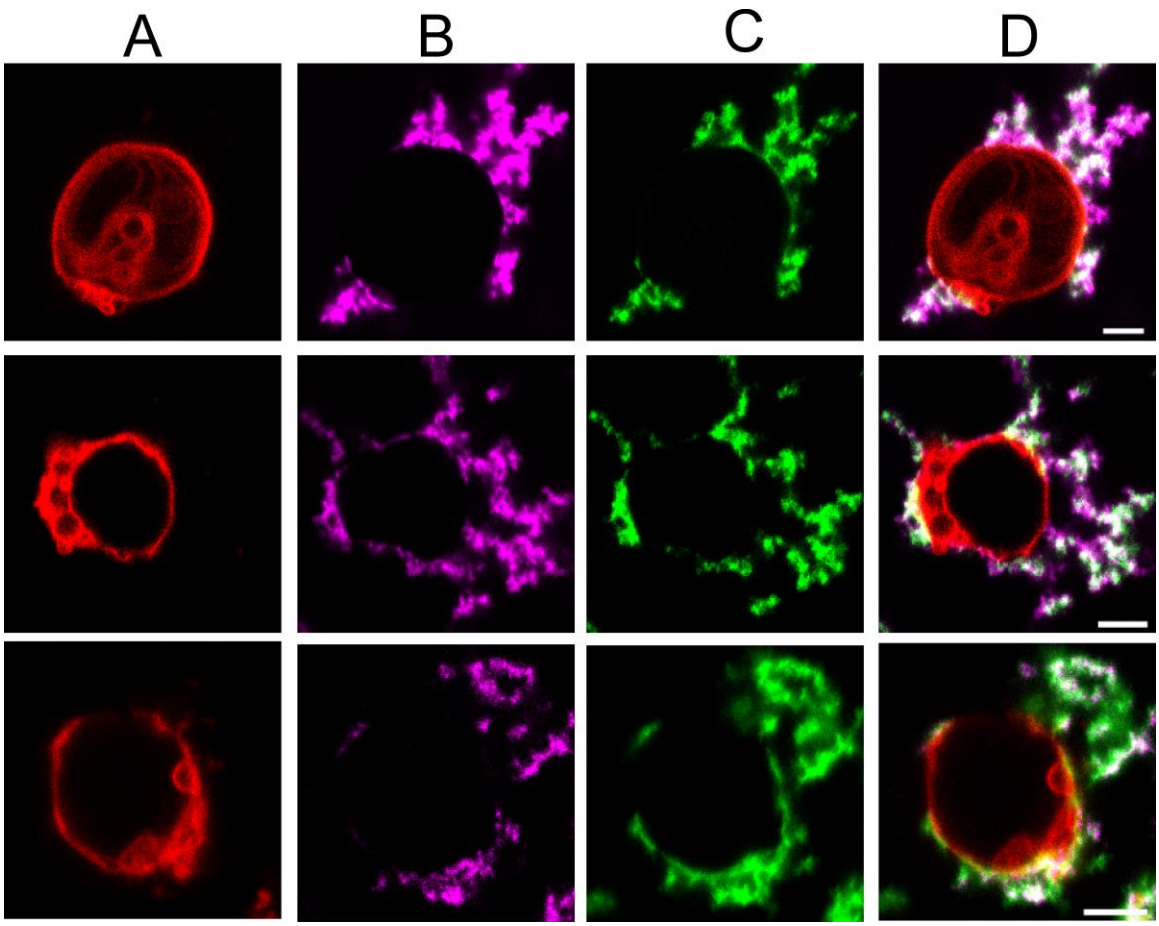

Figure S4: Formation of membrane facets on multilamellar GUVs induced by αS fibril formation at the membrane surface. POPC/POPG vesicles were incubated with 20 μM seeds and 80 μM αS monomers (1 mol% Alexa 647 labelled 140C αS) at 37 °C in quiescent conditions. The images show changes in vesicles morphology induced by αS fibrillization on the membrane surface. **A)** Rh DOPE fluorescence (red), **B)** αS-AlexaFluor 647 fluorescence (magenta), **C)** fluorescence of amyloid fibrils stained with ThT (green) and **D)** overlay of all 3 channels. Scale bar: 5 μm.

Vesicle shape transformation versus fibril binding: Below we give a rough, order of magnitude estimation of the minimum fibril binding energy required for the observed changes in vesicle shape.

For our calculation we consider only the bending energy costs for the shape transformation from a sphere to an icosahedron. The 20 faces of the icosahedron are flat, the edges are formed by half cylinders of 50 nm radius. The bending costs are primarily associated with the high curvature of the edges, the energy cost associated with the flat surfaces is negligible.

The surface area of the vesicle remains constant upon reshaping $4\pi R^2 = 5\sqrt{3}a^2$, with *R* being the radius of the initial vesicle and *a* being the length of one of the 30 edges of the icosahedron. Modeling the edges of the icosahedron as half cylinders gives $G_{bend} = 30(\frac{\pi}{2}K_b\frac{a}{r}) = 15\pi K_b\frac{\sqrt{\frac{4\pi}{5\sqrt{3}}R^2}}{r}$.

Typical vesicle radii in our studies were *R*~6 µm, using K_b~20 k_BT, we obtain G_{bend}~$1.4\cdot10^5$ k_BT per icosahedral vesicle. This is the energy that is required for the shape transformation from spherical to icosahedral vesicles and needs to be gained from fibrils binding the membrane surface.

Amyloid fibrils have a typical width of 10 nm, assuming a 10% coverage of the vesicle with fibrils we calculate 4500 µm of adsorbed fibril per vesicle which sets the minimal adhesion energy to be of the order of $1.4\cdot10^5$ k_BT /4500 µm= 30 k_BT /µm fibril.

Considering that monomeric protein binds the membrane with ~12 k_BT, and that not the whole membrane binding site of the monomer is available in the fibril, the estimated 30 k_BT /µm fibril seems feasible, confirming the suggested vesicle reshaping mechanism.

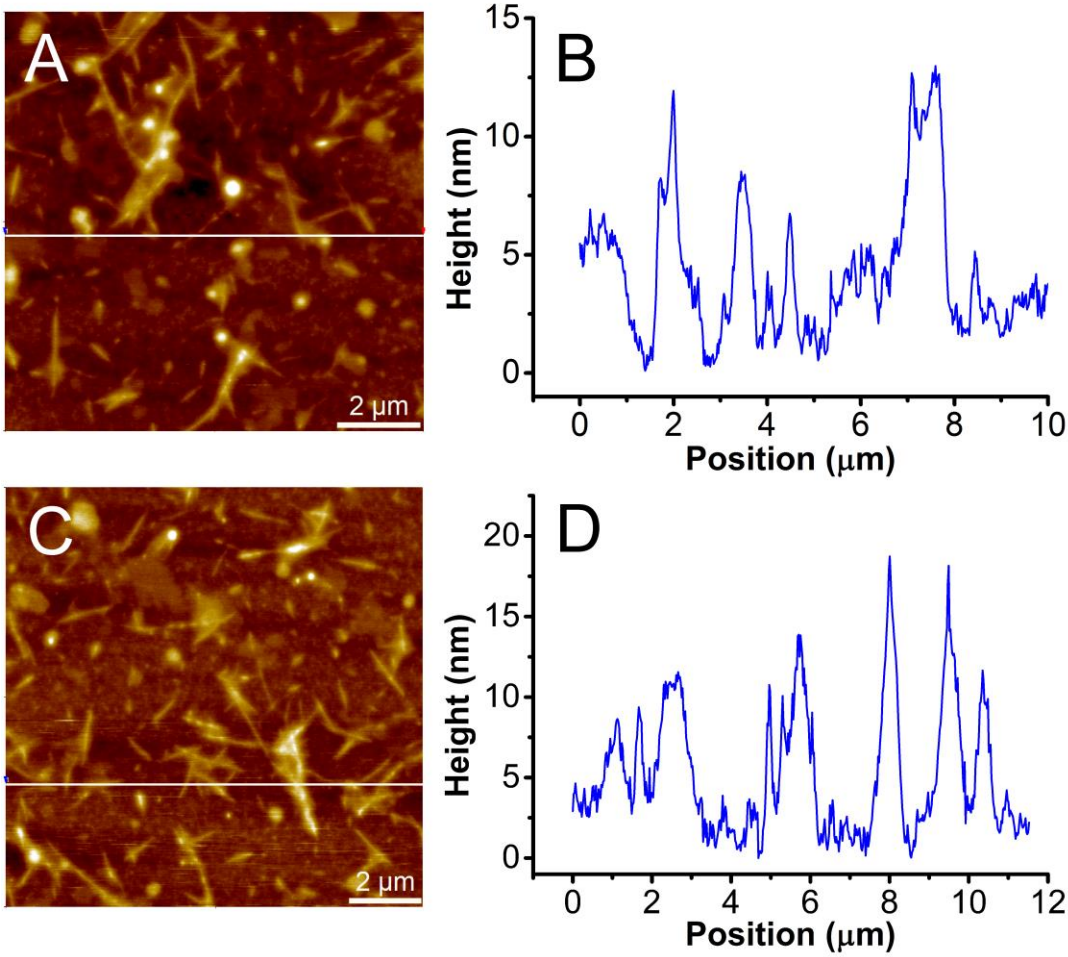

Figure S5: AFM images of fibrillar aggregates formed during seeded aggregation in presence of GUVs. In **A**) and **B)** images of fibrillar aggregates formed during aggregation in the presence of GUVs. In graph **C)** and **D)** cross-sections through these images indicate that the observed fibrillary structures typically have a height of <10 nm. The experiments were performed as mentioned above except that the aggregation was performed on mica surface in a closed chamber to avoid evaporation. After 3 hours of incubation, the samples were rinsed gently with 300µl of deionized water and dried under gentle stream of nitrogen gas. The sample was imaged in tapping mode on bioscope catalyst instrument (Bruker Santa Barbara CA, USA). All measurement were made with non-conductive silicon nitride probes (MSCT tip F, K= 0.60 N/m Bruker Camarillo)