Supporting material.

Nanoscale electrostatic domains in cholesterol-laden lipid membrane create a target for amyloid binding

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Experimental Details.

Supported lipid bilayers and amyloid binding: Phospholipid bilayers were supported on freshly cleaved mica (ruby, ASTMV-2 quality; Asheville-Schoonmaker Mica, Newport News, VA) by method of vesicle fusion (25) via alternating sonication and stirring procedures. Amyloid β(1-42) peptide (rPeptide, Bogart GA) was pretreated according to Fezoui procedure (26) to ensure the monomerinc form and was suspended in HEPES buffer (50 mM, pH 7.8) at a concentration of 0.5 mg amyloid / mL of buffer. 100 µL of amyloid solution was then added to the supported lipid membrane and incubated for 1 hour. Gentle rinsing ensured removal of excess amyloid solution, and then samples were imaged using MAC Mode AFM (Agilent) in water.

Supported lipid monolayers: Phospholipid monolayers were supported on freshly cleaved mica by Langmuir-Blodgett deposition. A mica slide was placed in a dipper arm of the Langmuir-Blodgett trough (LB) and lowered into the subphase (Nanopure water) of an LB trough from NIMA Technology (Coventry, United Kingdom). Solutions of lipid dissolved in chloroform were added to the subphase of the trough and allowed to spread and equilibrate on the subphase-air interface for 10 minutes. The monolayer was compressed at a speed 10 cm^2/min to a pressure of 45 mN/m and the pressure kept constant as the dipper arm raised the mica through the interface at a speed of 2 mm/min. The mica slide was allowed to air-dry for 10 minutes before being placed in a desiccator for a 24-hour period, after which it was affixed on a conductive plate for AFM/KPFM imaging.

Atomic force microscopy: The AFM imaging of DOPC supported bilayers with and without cholesterol and Amyloid β(1-42) was performed in a liquid cell in Milli-Q water at room temperature using MAC mode imaging with an AFM/SPM-5500, Agilent Technologies, with type II MAC mode cantilevers with tip radius of ~5nm and spring constant of ~2.8N/m. AFM imaging of monolayers was simultaneously performed with FM-KPFM mode using SmartSPM (AIST-NT) in air at normal humidity (section FM-KPFM).

Frequency Modulated Kelvin probe force microscopy (FM-KPFM): FM-KPFM is a Kelvin probe force microscopy technique for mapping the local electrostatic surface potential simultaneously with AFM topography images with superior resolution and sensitivity (few nm and few 10 mV)[21]. Previously developed KPFM methods have limited application in biological research, whilst FM-KPFM has proven to be advantageous to study the surface potential maps in

complex self-assembled biological films [1]. The FM-KPFM imaging was performed using the NC-DFM mode with a SmartSPM 1000 system provided by AIST-NT. A standard PPP-EFM (Nanosensors) cantilever with a radius of curvature (< 30 nm) and spring constant ~ 2.8N/m was used for imaging in ambient air at normal humidity

*Data Processing software and statistical analysis***:** Data collected using Agilent and AIST-NT AFM's was processed using Gwyddion v.2.25. The topography images were leveled by mean plane subtraction, corrected for line jumps and horizontal scar to nullify AFM artifacts caused while scanning with high resolution Z-scale (picometers). The images were processed to remove any polynomial background. The FM-KPFM images were not processed with any filters, to ensure the proper potential measurements; the raw data were used for cross section analysis using Gwyddion software. Statistical analysis of AFM topography images and surface roughness was done using SPIP software (v 5.1.6). In order to estimate the damaging effect of Aβ deposits on the lipid membranes we evaluated the roughness parameters of the surface for both samples. We calculated the core roughness depth, S_k, which is a measure of the nominal roughness (peak to valley) and the reduced valley depth, S_{vk}, which is a measure of the valley depth below the core roughness [2]. Data was collected on 2 μm × 2 μm high-resolution images of the membranes.
The three dimensional AFM images (surface roughness) in Figures 1c and 1d show representative regions highlighting the contrast in surface roughness between the two types of sample. An enlarged version of Figure 1c and 1d - the 3D cross section of pure DOPC membrane after 1hr incubation with Aβ (1-42) and DOPC membrane with 20% cholesterol after 1hr incubation with Aβ (1-42) is shown here, below. The S_k parameter is slightly lower for pure DOPC membrane (0.7±0.1 nm) than for cholesterol enriched DOPC membrane (0.8± 0.1 nm), which correlates with smoother surface of the membrane when there is no cholesterol present in the membrane. The S_{vk} parameter is significantly higher for cholesterol enriched DOPC membrane (0.5±0.1 nm) than for pure DOPC membrane (0.24±0.03 nm), which corresponds to a higher damage (deeper holes in the membrane) that Aβ □deposits produce in the membrane domains saturated with cholesterol. All experiments were repeated at least 3 times, 4-5 images were used for statistical analysis with no less than 100 measurements in each case.

For the KPFM statistical analysis, random cross sections were taken on the Contact Potential Difference 1(CPD1/KPFM channel) to measure the potential difference between the domain and the substrate manually. For quantitative purpose, we analyzed no less than 50 cross sections on 3 images and measured potential difference of approximately 120 domains.

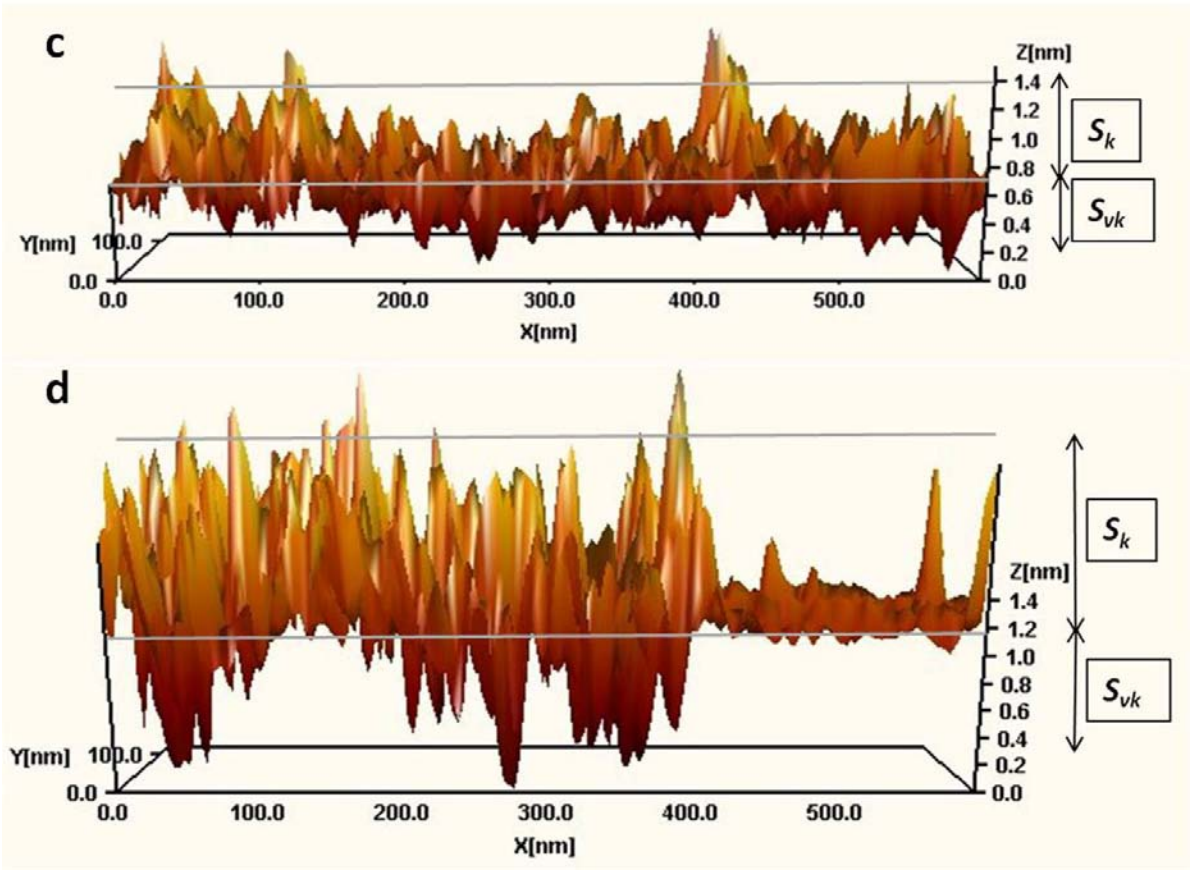

Figure S1. 3D-Cross-sectional analysis emphasizing the importance of surface roughness parameters S_k and S_{vk} of (c) pure DOPC membrane after 1hr incubation with Aβ (1-42), (d) - DOPC membrane with 20% cholesterol after 1hr incubation with Aβ (1-42)

Supporting References

[1] Moores, B., F. Hane, L. Eng, and Z. Leonenko. 2010. Kelvin probe force microscopy in application to biomolecular films: frequency modulation, amplitude modulation, and lift mode. *Ultramicroscopy*. 110: 708-711

[2] Wang, Q., X. Fan, W. Gaoa and J. Chen. 2006. Characterization of bioscoured cotton fabrics using FT-IR ATR spectroscopy and microscopy techniques, *Carbohydrate Research* 341: 2170–2175.