Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting information

Role of phosphatidylserine in amyloid-beta oligomerization at asymmetric phospholipid bilayers

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Fabrication of Gold-microcavities array and bilayer formation

Briefly, polystyrene microspheres of 1 µm size were drop cast onto pieces of silicon wafer (1.5 cm × 0.8 cm) onto which a 100 nm thick layer of gold had been vapor deposited. The polystyrene spheres were allowed to dry at 4°C in a refrigerator overnight, (Figure S1, 1). Using the gold coated silicon as a working electrode, gold was then electrodeposited onto the wafer by holding the electrode at a potential of −0.6 V (vs Ag/AgCl) in a commercial gold electrodeposition solution until the metal had grown to the equator of the polystyrene spheres. (Figure S1, 2) The resulting array was then rinsed with water to remove any remaining gold salt or electrolyte from the substrate surface. To selectively modify the top surface and leave the interior cavity surface bare, the arrays were placed in a solution of 10 mM 6-mercaptohexanol in ethanol overnight while the templating spheres were left in the cavities, (Figure S1, 3).To remove PS the substrates were sonicated in THF for 10 minutes and rinsed with ethanol, (Figure S1, 4) To pre-aqueous fill the cavities before lipid bilayer deposition, functionalized gold arrays were sonicated in HEPES buffer at pH 7.4 for 30 min. Microcavity Supported Lipid Bilayers (MSLBs) were then assembled across the microcavity array using a combination of the Langmuir-Blodgett (LB) and vesicle fusion (VF) methods (LB-VF). 1

mg/mL lipid stock in chloroform solution was deposited onto the air-water interface of an NIMA 102D Langmuir-Blodgett trough. The chloroform was left to evaporate then the remaining lipids monolayer at the aqueous interface was compressed to a surface pressure of 32 mN/m. The array was then immersed in the LB trough and withdrawn at a rate of 5 mm/min while a surface pressure of 32 mN/m was maintained to form a monolayer of lipids. The array was removed from the trough, ensuring it remained wet and was then placed in a 0.5 mL solution of Large Unilamellar Vesicles (LUVs) for 1.5 hours to form the distal leaflet of the resulting symmetric or asymmetric bilayer, where the proximal LB deposited leaflet had the same (symmetry) or different (asymmetric) composition to the LUV.

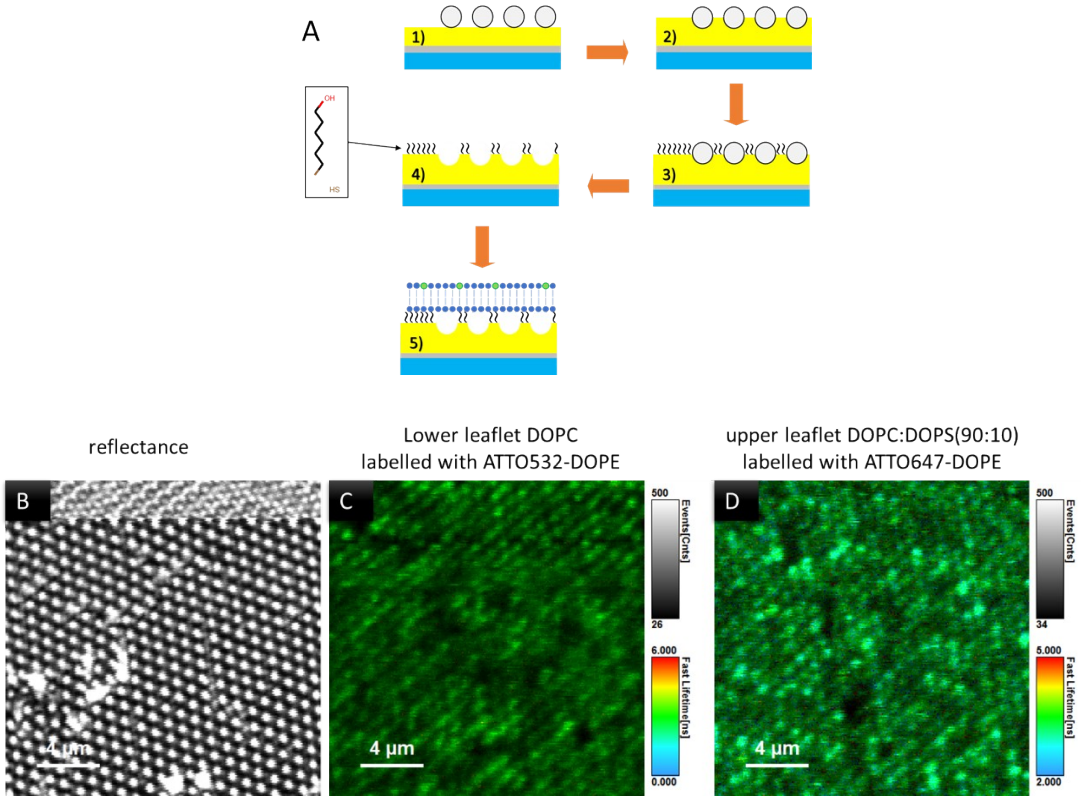


Figure S1: A) Schematic representation of the stepwise fabrication of gold microcavity array and lipid bilayer formation over gold-MSLBs. B) Reflectance image showing the buffer filled cavities (white circular feature) over which a DOPC:DOPS(90:10)//DOPC MSLB was assembled. Fluorescent life time imaging microscopy (FLIM) image of C) the lower DOPC leaflet stained with ATTO532-DOPE (0.01mol%) and D) FLIM image of the upper DOPC:DOPS(90:10) leaflet stained with ATTO655-DOPE (0.01 mol%) showing all the cavities are filled and subsequently lipid bilayers are spanned. In each panel, scale bar are 4 µm.

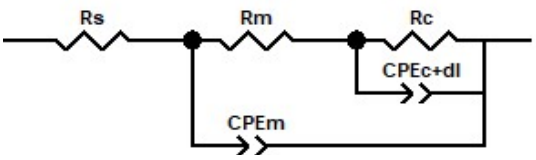


Figure S2: The equivalent circuit model used to fit the AC impedance data of the MSLB. Rs, Rm and Rc are the resistance of the solution, membrane, and cavity array, respectively. CPEm and CPEc+dl are the constant phase element of the membrane and the electrode double layer constant phase element.

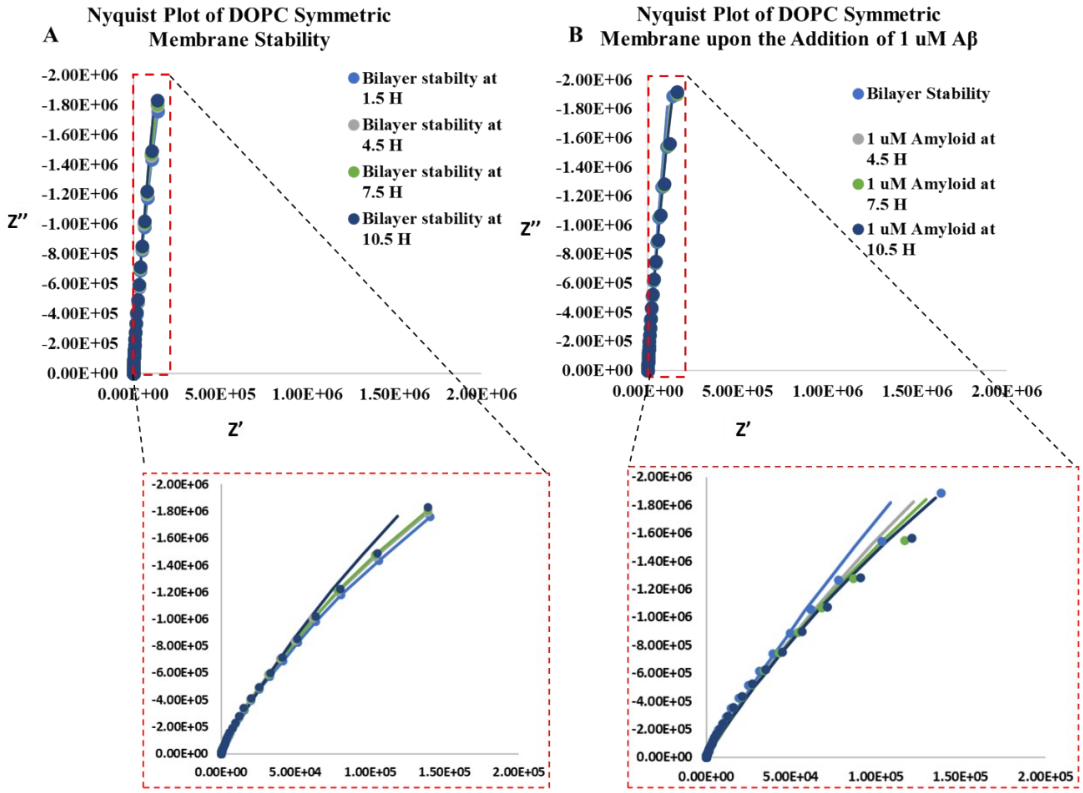


Figure S3: Representative non Faradaic Nyquist Plots of DOPC symmetric MSLB in the A) absence and B) presence of 1 µM Aβ in the contact buffer solution, HEPES pH 7.4 versus Ag/AgCl reference electrode at 0V. In each panel-coloured circles are the raw data obtained from impedance. Solid lines are the data fitted using the equivalent circuit model. The respective Nyquist graphs of in the bottom panel of A and B shows the expanded view of the selected region highlighted in rectangular box (dotted red line).

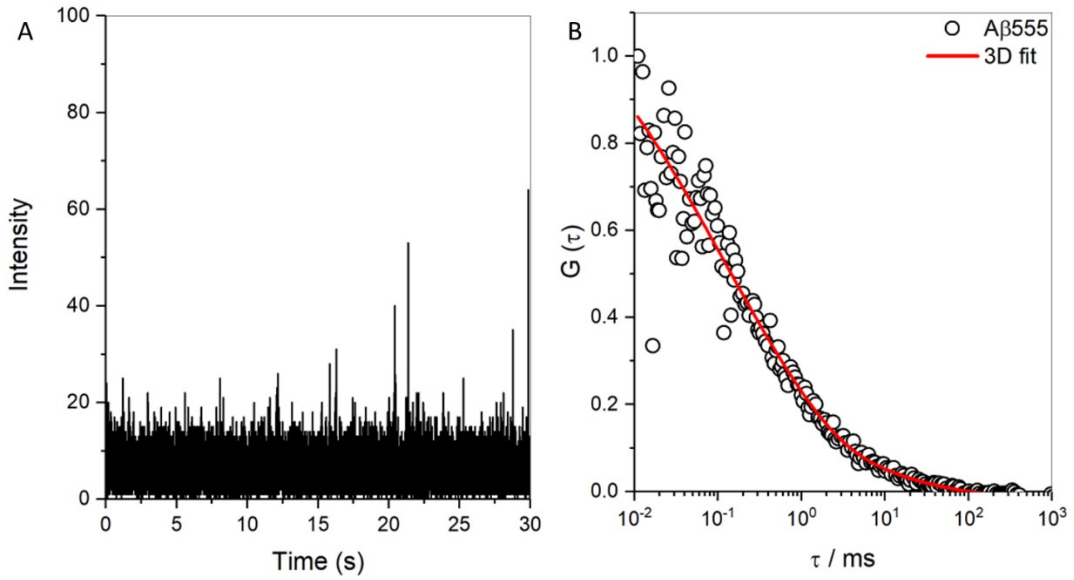
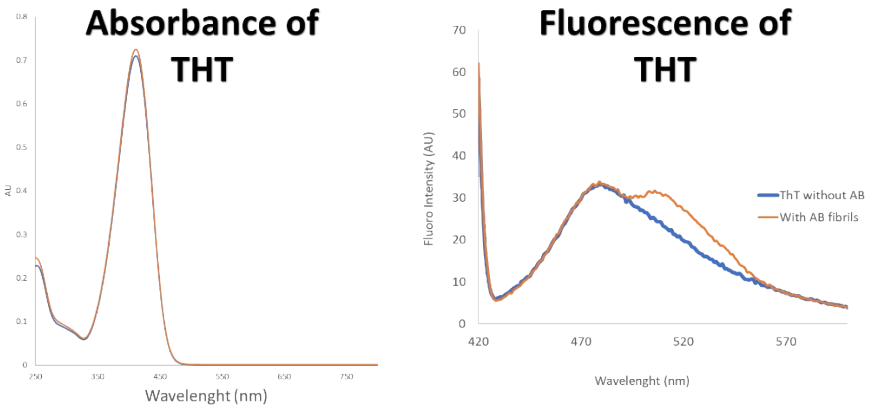


Figure S4: Representative fluorescence A) counts per second (CPS) and B) autocorrelation function (ACF) curve of 20 nM fluorescently labeled Aβ555 mixed with 1 μM unlabelled $Aβ_{1-42}$ in HEPES buffer at pH 7.4. (A) Fluorescence fluctuation intensity shows no aggregation or photobleaching during this 30 s measurement window, indicating its monomeric in nature. (B) Corresponding normalized ACF curve of Aβ555 (open symbol) with 3D-diffusion model fit (red solid line). The diffusion coefficient estimated to be 96±6 $μm^2/s$ and was obtained from the transit time and a known confocal volume.



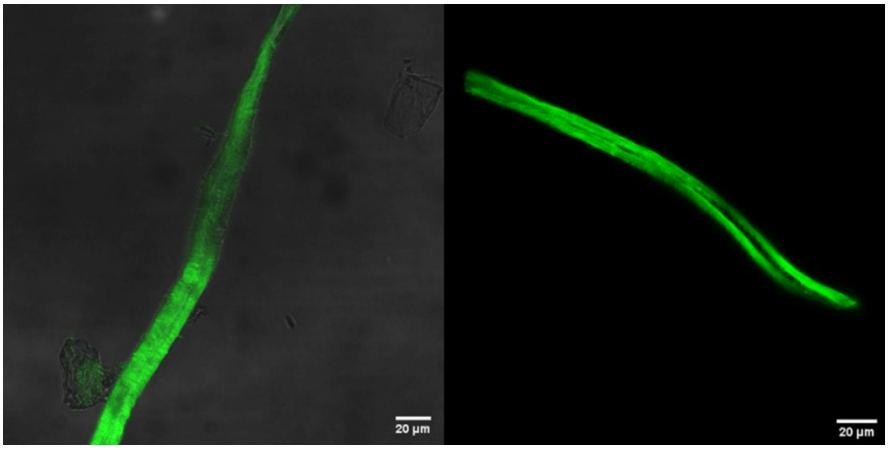


Figure S5: Absorbance (left) and emission (right) of Tht with (orange) and without (blue) the contact solution form electrochemistry containing Aβ (HEPES buffer solution containing Aβ after EIS run at pH 7.4). For emission, the contact solution was excited at absorbance maximum. The contact solution was then analyzed with confocal imaging with excited at 405 nm and detection window 430 – 580 nm.

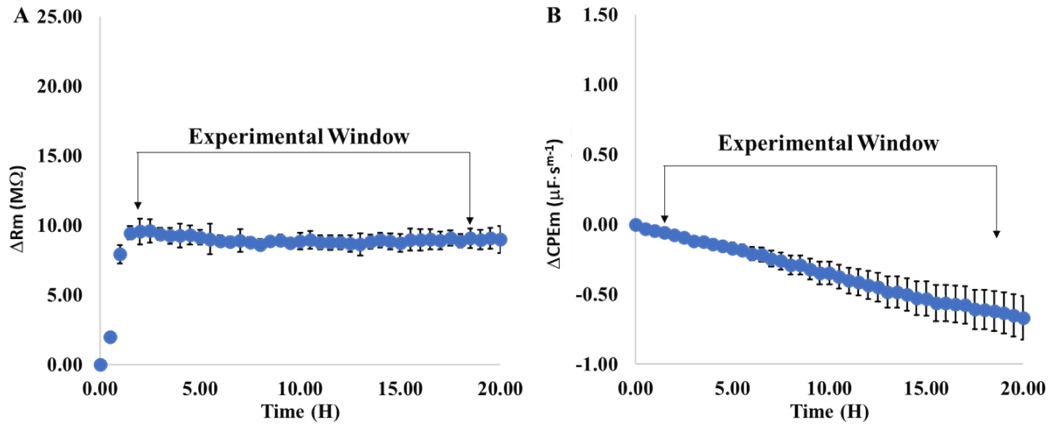


Figure S6: A) Relative resistance changes and B) capacitance of a DOPC:DOPS(95:5)//DOPC asymmetric bilayer, reflecting stability in HEPES buffer at pH 7.4 over 24 hours. Equilibration of lipid bilayer over the initial 1.5 hours and then experimental window is shown between 2 and 18 hours. N=3 and error bars the standard error.

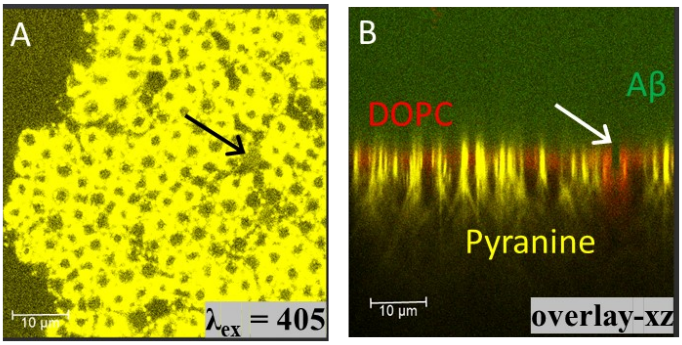


Figure S7: A) Confocal microscopy image of pyranine (5 umol) filled PDMS cavities spanned with DOPC MSLB labelled with Atto 655. Pyranine was excited at 405 nm and emission detected over the range 440 to 470 nm

B) Overlay (x-z plane) image obtained from the identical spatial regime as shown in Fig. 3 (main text) showing the DOPC MSLB (red) spanning the pores, Aβ555 (green) and pyranine (yellow) from within the wells where it is seen in solution and adsorbed onto the side walls of the pores. The arrow indicates an unfilled pore where pyranine and Atto signal are overlaid and both emission signals can be seen to be coincident. The bilayer is expected to collapse along the unfilled pore walls and the coincident signal confirms the most intense emission signal from the pyranine is from its adsorption on the interior of the pore walls. The tendency of pyranine to adsorb reversibly at hydroxylated surfaces is well known.

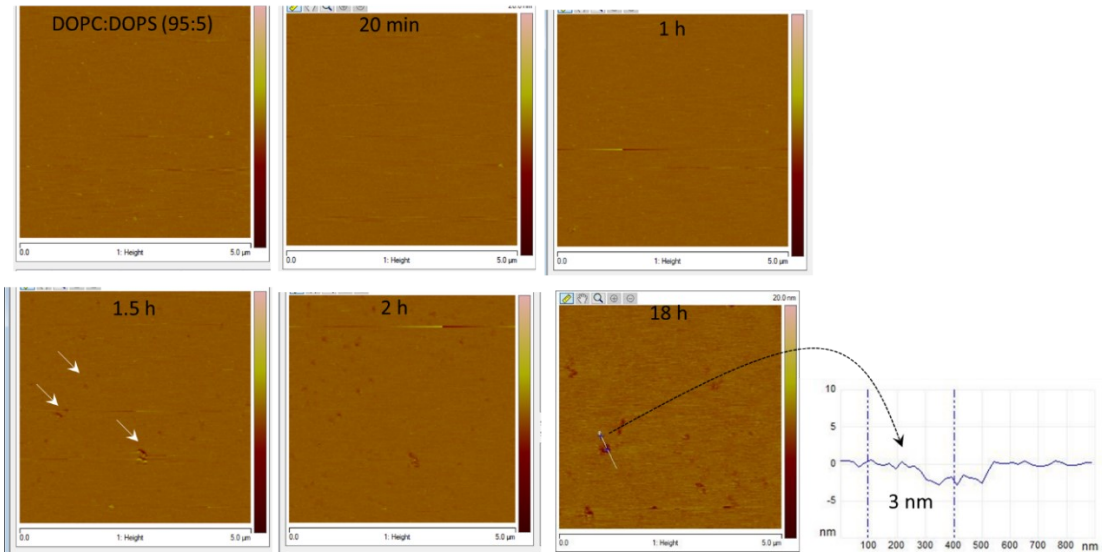


Figure S8: Temporal evolution of change in DOPC:DOPS (95:5) membrane topography upon incubation of 1 μM $A\beta_{1-42}$. The pore like features can be visualized at or above 1.5 hours of incubation window. All images are taken under HEPES at 20±1°C. The image size was 5 μm ×5 μm.