Supporting Information

Amyloid-β oligomers have a profound detergent-like effect on lipid membrane bilayers, imaged by atomic force and electron microscopy

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Supplemental Experimental procedures

Supplementary Figures:

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Atomic Force Microscopy (AFM)

Atomic force microscopy was performed using a Bruker Dimension Icon atomic force microscope with 'ScanAsyst-AIR' AFM probes, with a spring constant of 0.4 N/m were purchased from Bruker. Samples deposited onto mica were imaged under ambient air within a temperature-controlled room at 22 ^{o}C. Images were acquired in Scanasyst (PeakForce^{TM}) mode at a resolution of 512 samples/line with a scan rate of 1 Hz. Image analysis was performed using Nanoscope analysis software (version 1.5) and images were typically subjected to 1st order flatten, plane fit, and in some cases a low-pass filter. Image height scales are adjusted accordingly to maximise visibility of the lipid bilayer on the mica.

AFM Data Quantification – Detergent Effect

ImageJ was used to quantify the dispersion of lipid away from the bilayer. Areas of lipid bilayer were selected by colour thresholding the micrograph to a height of 4 nm or over, and these areas were then converted into a binary image whereby bilayer was represented in black. The 'analyze' tool was then used to measure the percentage area of ≥4 nm bilayer coverage within a 16 segment (each 0.1 x 0.5 μm) sampling area, perpendicular to the lipid islands. The edge of the lipid bilayer was defined as 50% surface lipid coverage, see Supplemental Figure S5. This was repeated for between 11 to 32 regions measured from multiple preparations for each Aβ40/42 monomer, oligomer and fibre conditions.

AFM data was typically obtained from 3 or more mica samples per Aβ preparation (Aβ40/42 monomer, oligomer and fibre). Typical lipid coverage was calculated in four or more positions per mica sample. Importantly, a marked detergent effect by Aβ42 oligomer was observed in 5 out of 5 experimental repeats. However, this effect was not observed in membrane exposed to Aβ42 monomer (n = 5) or Aβ42 fibres. The detergent effect was also consistently observed on application of Aβ40 oligomers (n = 2), but not for Aβ40 monomer (n = 4) or Aβ40 fibre (n = 4). Control experiments also revealed minimal defects on bilayer incubation with 160 mM NaCl, 30 mM HEPEs buffer, pH 7.4 for the same length of time (n = 4).

Quantification of bilayer hole diameters

ImageJ software was used to quantify the range of holes generated in the supported lipid bilayer. Dark circular holes seen within the micrograph were selected by colour thresholding areas at 4 nm above the mica; just below the surface of the membrane. A 'count mask' overlay was then applied to detect the holes, and the Feret's diameter of each hole was calculated.

AFM is known to under estimate the diameter of a hole on any surface. In first approximation, a correction can be applied to offset this underestimation by correcting the measured widths for the broadening effect, using the following equation:

$$2\Delta = 2\sqrt{h(2R-h)}$$

Where h = lipid bilayer height (4.5 nm) and R = probe tip radius (12 nm). (41)

A standard correction of +18.7 nm was therefore applied to the hole diameter values reported. A total of 2,140 holes were measured for Aβ42, over a 25 μm^2 area; 86 holes per μm^2. For Aβ40, a total of 908 holes were measured over an area of 80 μm^2.

Lateral Fibre Embedding Measurements

Quantitative analysis of fibres was performed using data collected from multiple (≥ 4) experimental repeats; making 8 height measurements along the axis of single fibre filaments adhered to both mica, and membrane. Samples of 232 measurements across 29 fibres were made for each Aβ40 fibres resting on mica, and Aβ40 fibres resting on membrane. For Aβ42, 168 measurements were taken across 21 Aβ42 fibres resting on mica; and 208 measurements across 26 Aβ42 fibres resting on membrane. The thickness of the bilayer was also recorded, with 160 height measurements made across all membranes in which a fibre was resting.

Transmission Electron Microscopy (TEM)

Preparations of LUVs were imaged by TEM in the presence and absence of Aβ (10 μM monomer-equivalent). These preparations were aliquoted (5 μl) onto glow-discharged carbon-coated 300 mesh grids (Agar Scientific Ltd) using the droplet method and washed with UHQ water. A negative stain of phosphotungstic acid (2% (w/v)) was then applied before a final wash step and air-drying. Images were captured at 80,000x magnification using a JEOL model JEM-1230 electron microscope (JEOL, Ltd., Japan) operating at 80 keV, paired with a Morada 2k CCD camera system and its iTEM software package (Olympus Europa, UK).

Single particles present on each micrograph were automatically picked using the boxer module of the software EMAN2.(42) All subsequent image analyses on the two-dimensional data set - filtering, alignments, multi-variate statistical classification, averaging and iterative refinements - were performed within the Imagic-5 graphics processing environment.(43)

Aβ Assembly Kinetics

Aβ assembly kinetics were monitored by addition of 10 μM of the fluorescent dye, Thioflavin T (ThT). ThT fluoresces when bound to amyloid fibres to give a fluorescent signal proportional to the amount of amyloid fibre present, whilst ThT fluorescence for monomer and oligomeric assemblies is minimal. (39) ThT fluorescence upon fibre formation was measured using BMG-Galaxy and BMG-Omega FLUOstar fluorescence 96-well plate readers. Corning Falcon 96-well polystyrene plates were subjected to mild double orbital shaking for 30 seconds every 30 min followed by a fluorescence reading, 20 flashes per well, per cycle with 4 mm orbital averaging. Fluorescence excitation and emission detection were at 440 nm and 490 nm respectively. It has been shown that ThT does not markedly effect the formation or kinetics of Aβ-fibres. (39)

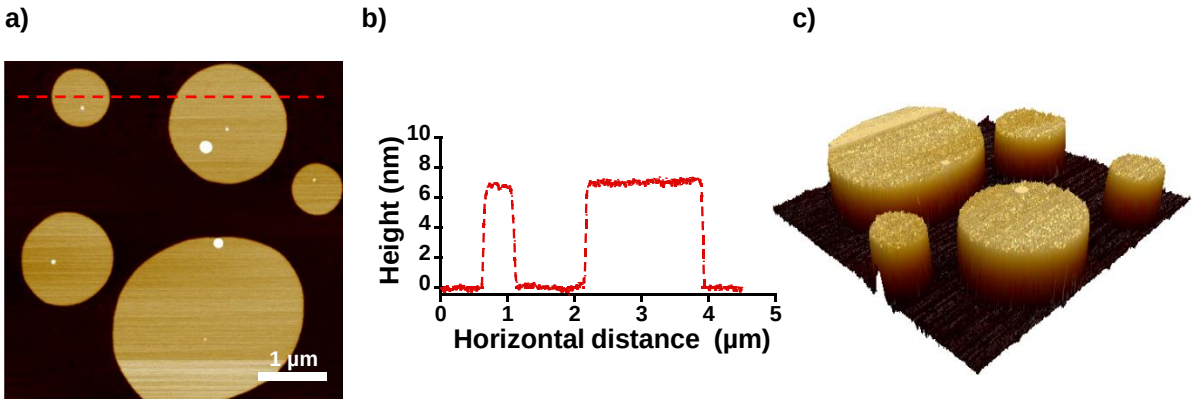


Figure S1: Characterisation of mica supported lipid bilayers:
Lipid bilayers were generated from a mixture of PC:GM1:Cholesterol (68:2:30 by weight). Lipid was deposited onto mica in aqueous buffer containing NaCl (160 mM), HEPEs (30 mM) at pH 7.4. (a) AFM topographical images of supported lipid bilayer formed by incubation of 0.6 mg/ml lipid vesicle suspension on a mica surface. Height colour scale range = 12 nm. (b) A cross-sectional graph is presented to display surface height of the sample. (c) A 3D image of the sample displays the island-like nature of the supported lipid bilayers with a 'cliff-edge' transition to mica. The location of height cross-section is represented by a dotted red line in (a).

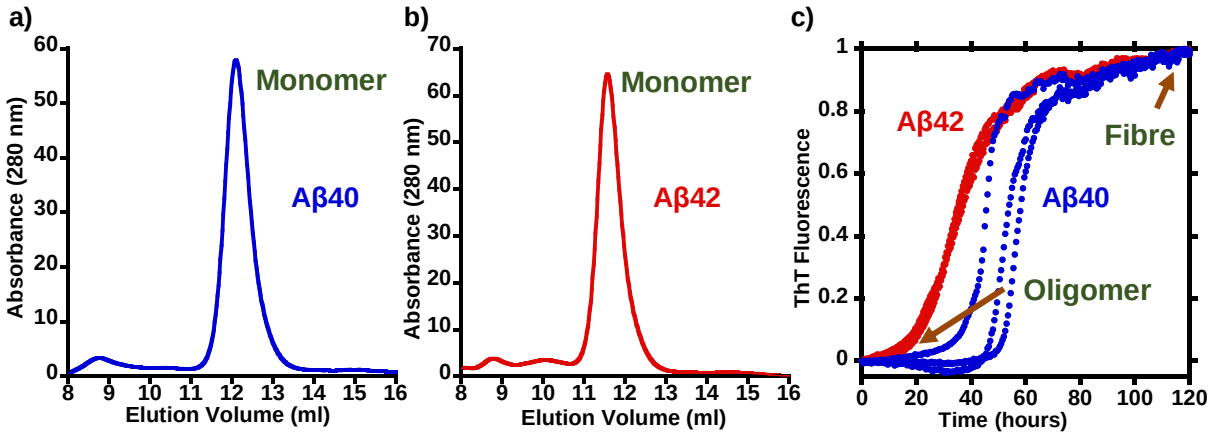
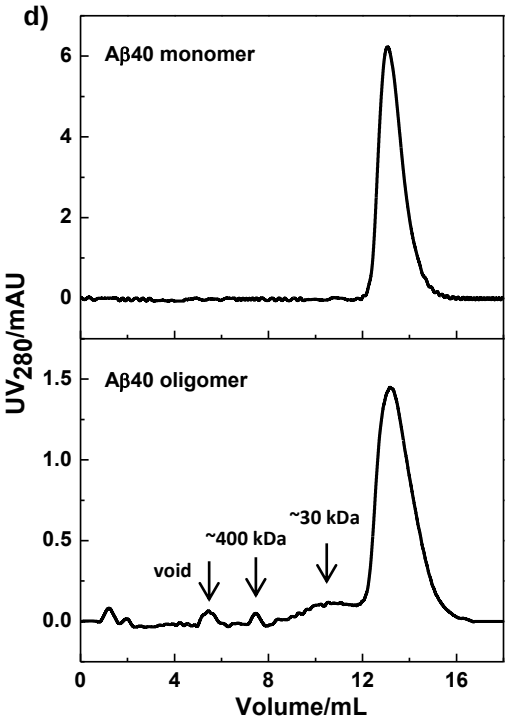


Figure S2: Isolation of Aβ40 & Aβ42 monomer, oligomer and fibre.
Aβ aggregates were removed from solution by running solubilised Aβ through size-exclusion chromatography. SEC elution profile (280 nm) indicates the elution of a single monomeric fraction of (a) Aβ40, and (b) Aβ42. The Aβ monomeric samples were taken directly from the SEC column elution. (c) ThT fluorescence fibre growth assays for Aβ40 and Aβ42 (10 μM) in aqueous buffer containing NaCl (160 mM), HEPEs (30 mM), at pH 7.4. For AFM and TEM experiments, Aβ samples (in which ThT had not been added) were taken from the same well plate at the appropriate time-points. Samples designated oligomeric Aβ were taken from wells towards the end of the lag-phase while fibre samples were taken from the well plate once ThT fluorescence signal had plateaued. (d) Comparison of SEC elution profiles of Aβ40 monomer with that of Aβ40 at the lag-phase. The heterogeneous lag-phase mixture contains a range of oligomer sizes, while appreciable monomeric Aβ is still present.



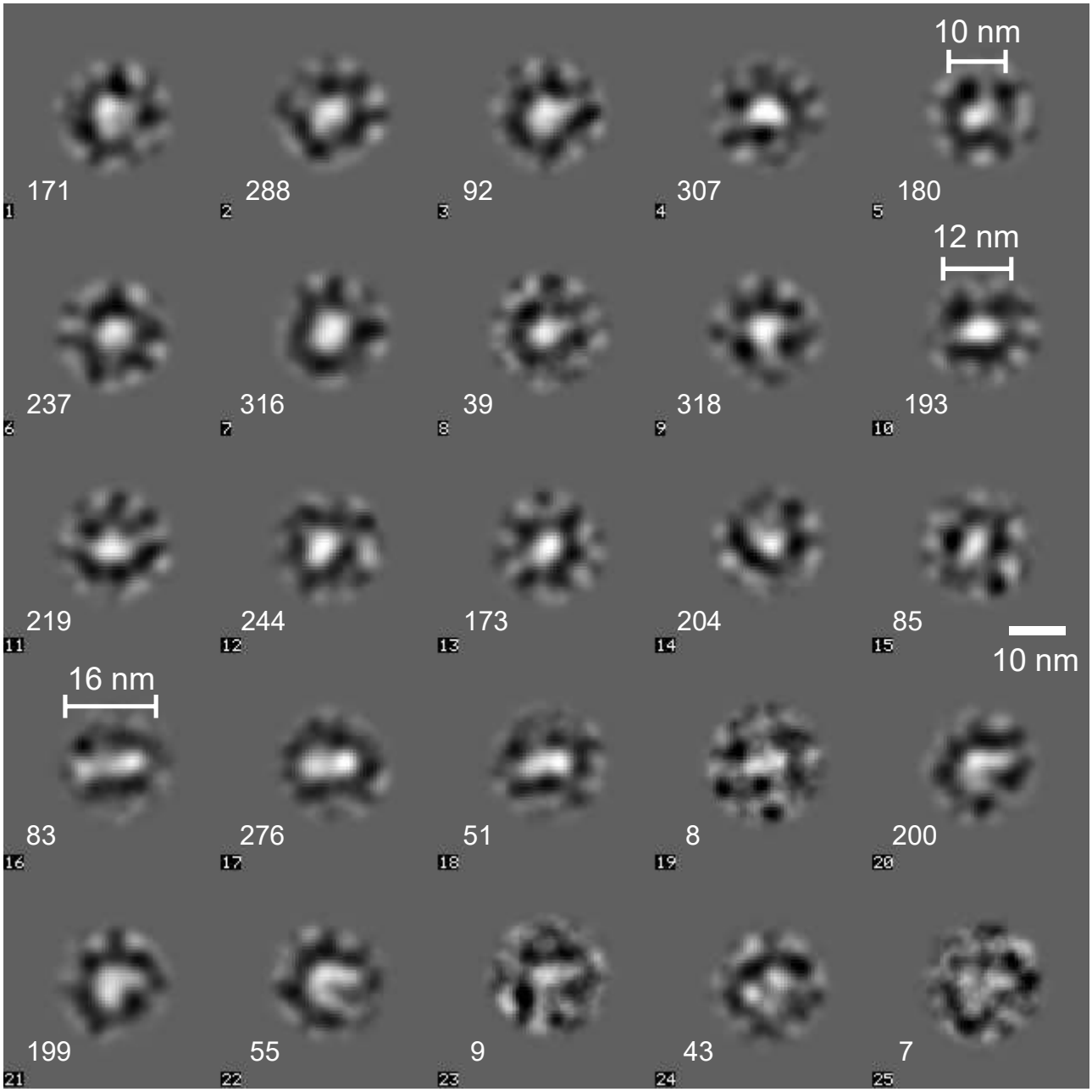


Figure S3: Single particle image averaging characterisation of Aβ preparations by TEM on negatively stained samples; heterogeneous oligomers of Aβ42. A total of 3997 single particles were classified into the 25 class averages. The number of particles in each class average is indicated. Range of oligomer sizes are also indicated. The first 15 images are largely circular in morphology and represent 70% of the particles. Aβ42 (10 μM monomer equivalent) was taken from well plates towards the end of the lag phase in NaCl (160 mM), HEPES (30 mM) at pH 7.4.

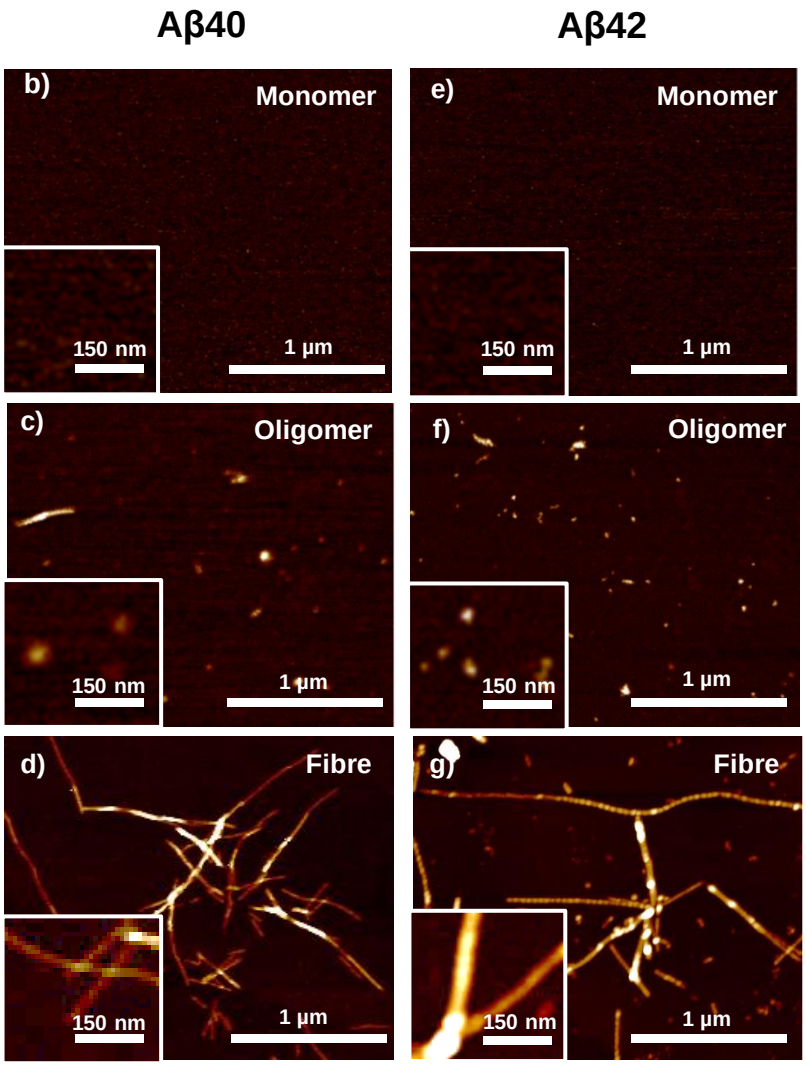


Figure S3: Characterisation of Aβ preparations on mica.
Representative AFM topographical images of Aβ monomer, heterogeneous oligomer and fibre deposited onto mica are presented for both Aβ40 (b - d), and Aβ42 (e - g). Height colour scale range = ≤12 nm. In each case, Aβ (10 µM) was deposited onto mica in buffer which contained NaCl (160 mM), HEPES (30 mM) at pH 7.4.

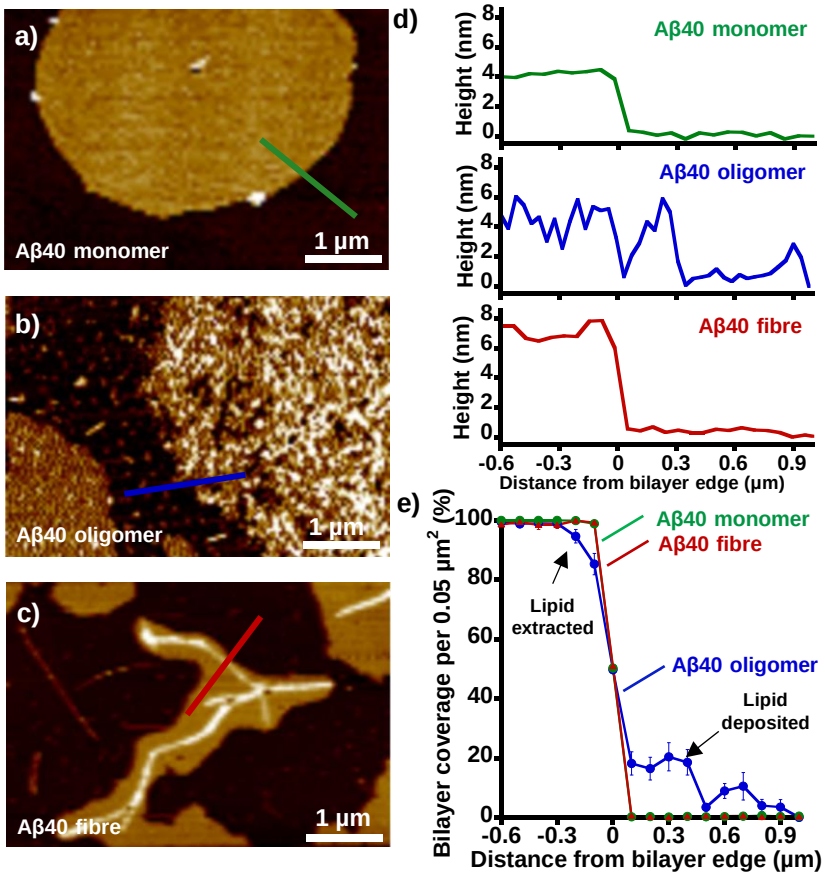


Figure S4: Aβ40 oligomers have a detergent-like effect on the bilayer which is not seen for Aβ40 monomer or Aβ40 fibre.

AFM topographical images of mica-supported lipid bilayers composed of PC:GM1:Cholesterol on exposure to; a) Aβ40 monomer, b) Aβ40 oligomer, and c) Aβ40 fibre. Typical height colour scale range = ≤12 nm. d) Height cross-sections taken from each image are presented, marked by a colour-coordinated line on each respective micrograph. e) Bilayer coverage at the edge of a lipid bilayer is presented for bilayers exposed to Aβ40 monomer (green), Aβ40 oligomer (blue) and Aβ40 fibre (red). Each data-point represents an average % bilayer coverage within a 0.1 x 0.5 μm region of interest, with 50% bilayer coverage defined as the edge of an island of lipid bilayer. Error bars represent standard error. Typically for each peptide preparation, n = 20 per data point, measured across 3 separate mica-supported lipid bilayer preparations.

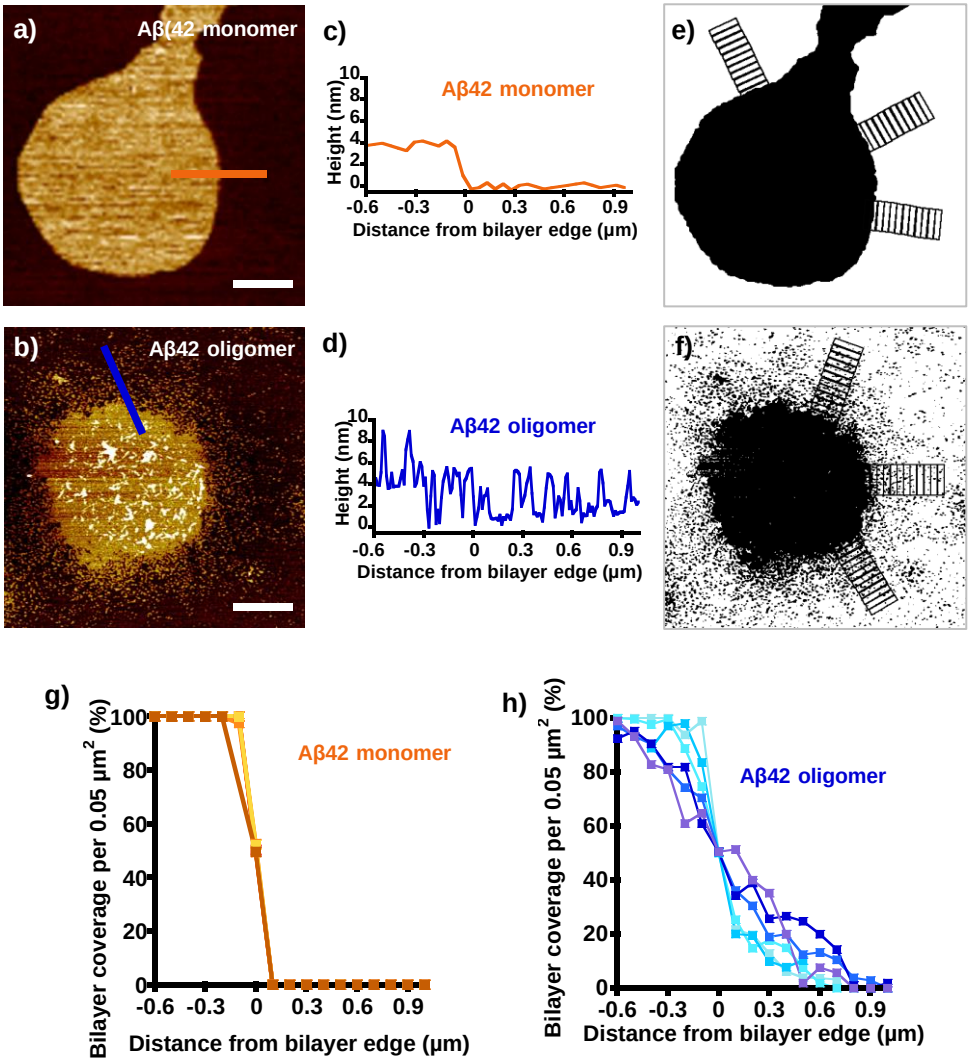


Figure S5: Segmented analysis of bilayer mica coverage to measure lipid dispersion.

AFM topographical images of lipid bilayer exposed to (a) Aβ42 monomer and (b) Aβ42 oligomer are presented alongside accompanying height cross-sections (c) and (d). Typical height colour scale range = ≤12 nm. Binary masks generated by application of a colour threshold (4 nm) to the micrographs show areas of lipid bilayer in black, and areas of mica in white for both (e) Aβ42 monomer and (f) Aβ42 oligomer. Each binary image displays 3 segmented ladders which each contain 16 segments, each segment being 0.1 x 0.5 μm in size. ImageJ was used to measure the percentage bilayer coverage in each segment. The results of 6 separate representative ladders are presented for both (g) Aβ42 monomer and (h) Aβ42 oligomer. Scale bar = 1 μm.

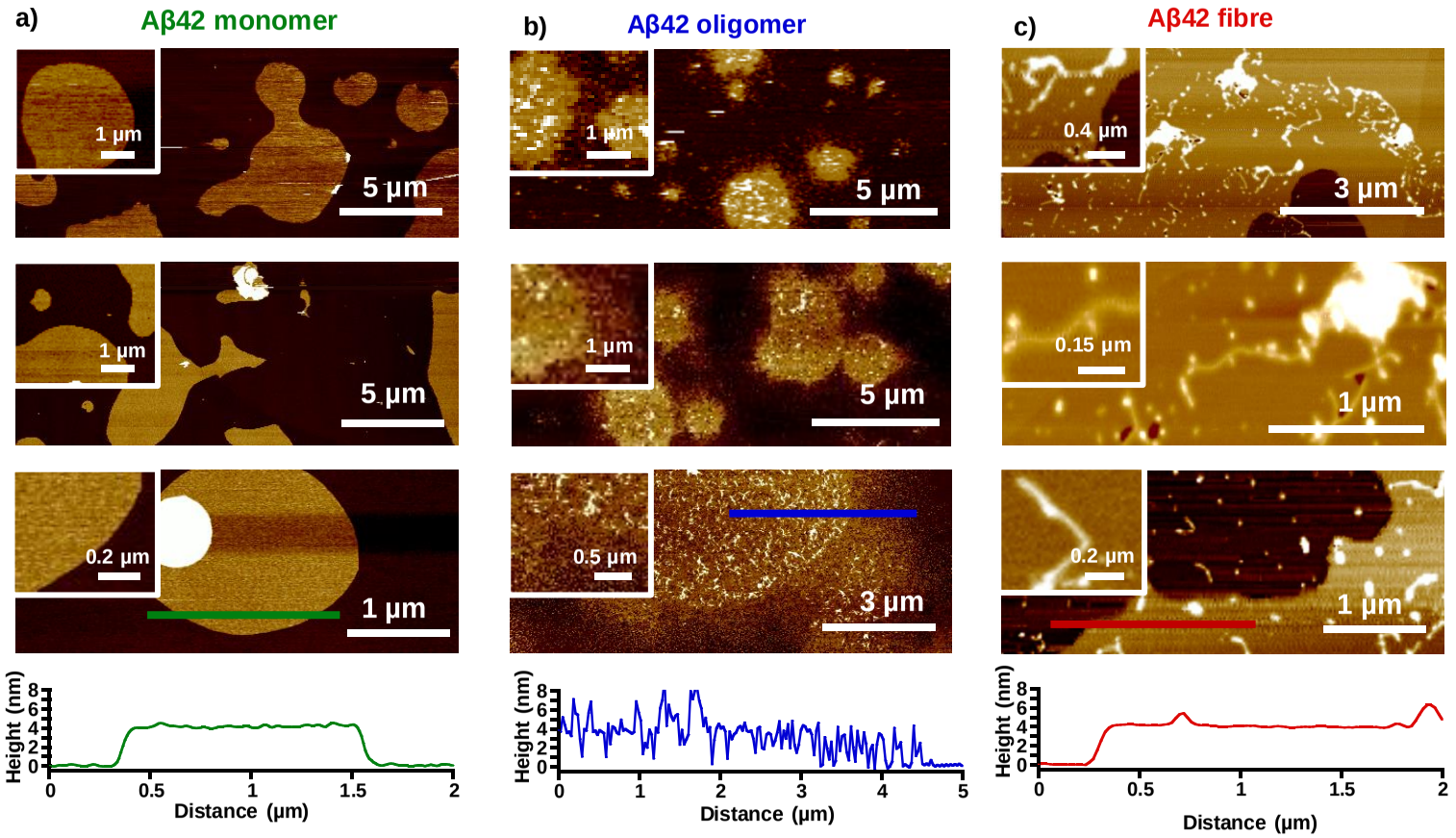


Figure S6: Impact of Aβ42 monomer, oligomer, and fibre on supported lipid bilayer

Three AFM topographical images, and an accompany height cross-section, are presented for mica-supported lipid bilayer exposed to (a) Aβ42 monomer, (b) Aβ42 oligomer, and (c) Aβ42 fibre. . Typical height colour scale range = ≤12 nm. The location of the cross-section is represented by a solid colour-coded line. Aβ (10 µM monomer equivalent) was deposited onto mica-supported lipid bilayer in aqueous buffer which contained NaCl (160 mM) , HEPES (30 mM) at pH 7.4. Only oligomers cause a profound detergent effect on the lipid bilayer with an observed extraction and deposition of lipid.

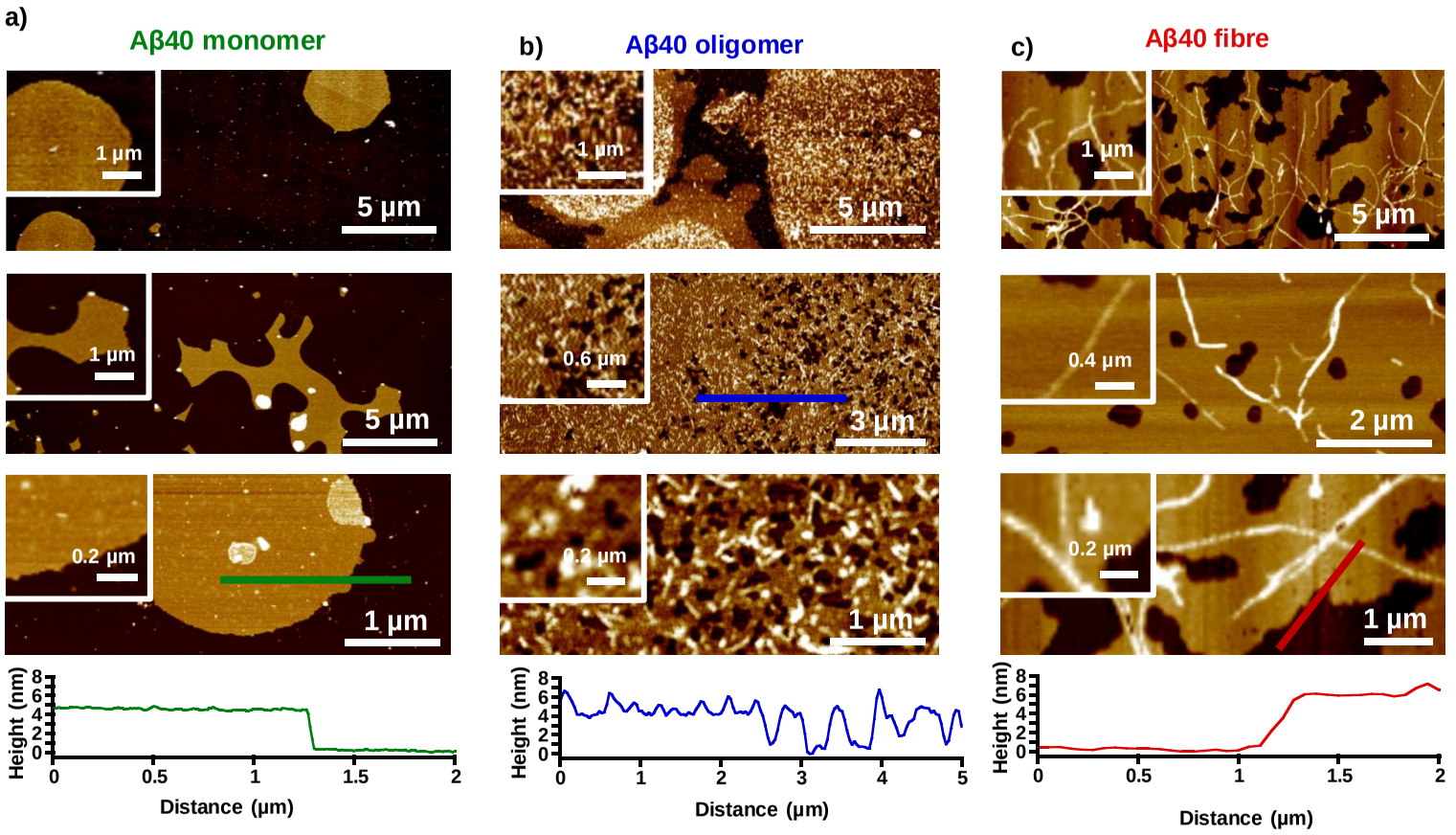


Figure S7: Impact of Aβ40 monomer, oligomer, and fibre on supported lipid bilayer

Three AFM topographical images, and an accompany height cross-section, are presented for mica-supported lipid bilayer exposed to (a) Aβ40 monomer, (b) Aβ40 oligomer, and (c) Aβ40 fibre. . Typical height colour scale range = ≤12 nm. The location of the cross-section is represented by a solid colour-coded line. Aβ was deposited onto mica-supported lipid bilayer in aqueous buffer which contained NaCl (160 mM), HEPES (30 mM) at pH 7.4. Only oligomers cause a profound detergent effect on the lipid bilayer with an observed extraction and deposition of lipid.

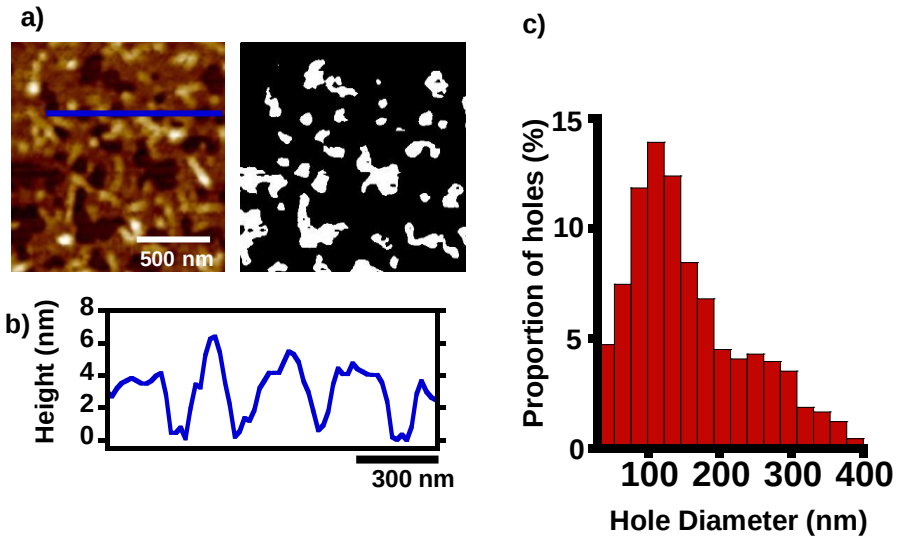


Figure S8: Aβ40 oligomer induced holes within supported lipid bilayers.
(a) Left: AFM topographical images showing a region of supported lipid bilayer which has a concentrated region of holes formed after incubation with Aβ40 oligomer. *Right:* Binary map of holes, generated by colour thresholding of the atomic force micrograph. Height colour scale range = 12 nm. (b) Height cross-section taken from the atomic force micrograph (section location indicated on image by solid blue line). Typically both the upper and lower leaflets of bilayer are extracted. (c) Range of hole diameters observed with a modal value of 100 nm.

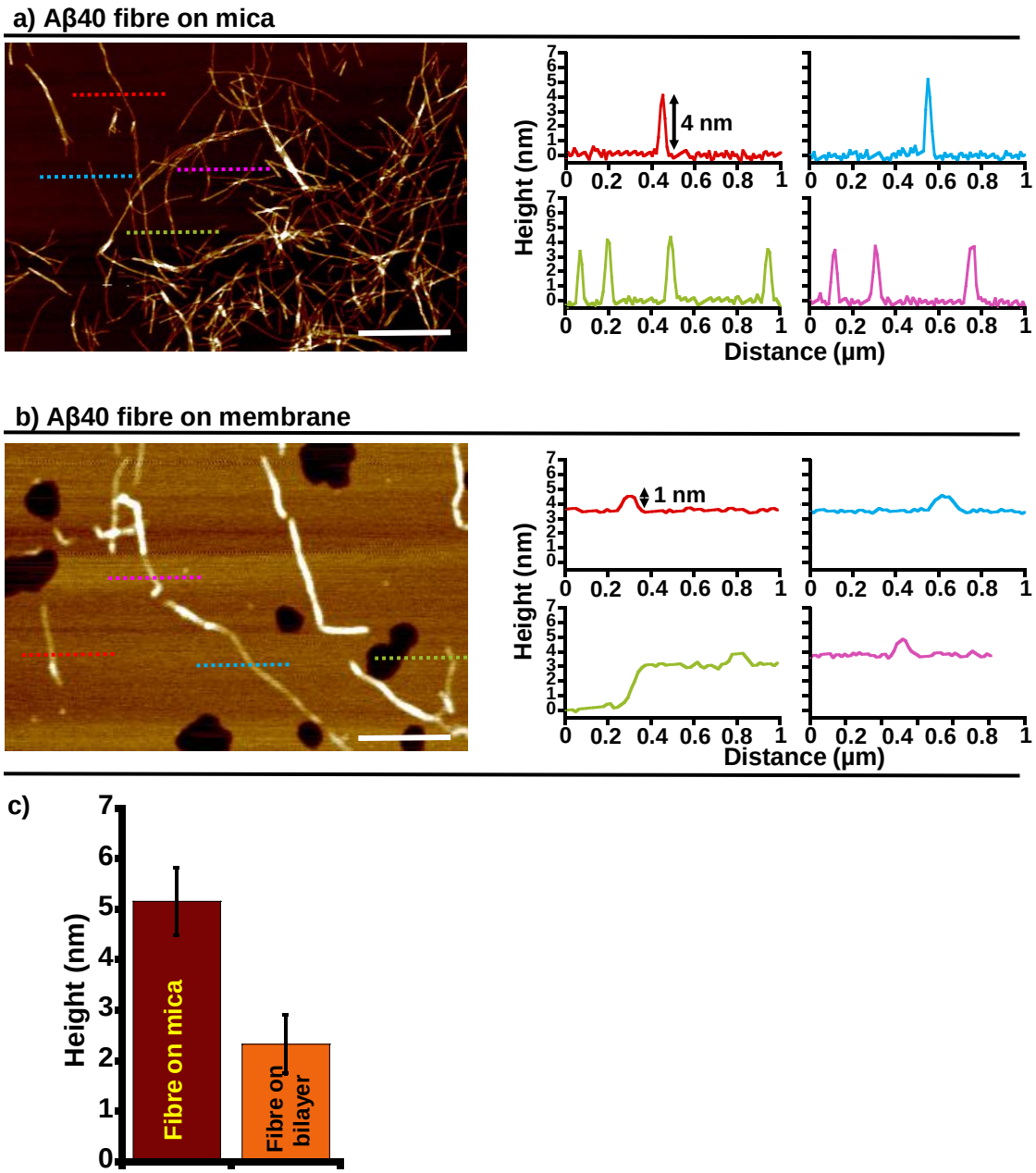


Figure S9: Aβ40 fibres adhere laterally and embed into the upper leaflet of lipid bilayers

AFM height micrographs are presented with four accompanying cross-sections of Aβ40 fibres imaged on both (a) mica, and (b) on the surface of a membrane. Typical height colour scale range = ≤12 nm. Scale bar = 1 μm. The locations of the cross-sections are depicted by colour co-ordinated dashed lines. (c) Mean Aβ40 fibre height recorded on both mica and above the membrane. Variation in height is presented by standard deviation error bars. Fibre height measurements were taken only from areas in which a single fibre filament was visible, with no evidence of multiple fibre periodicity. Aβ (10 μM monomer equivalent) was deposited onto mica-supported lipid bilayer in aqueous buffer which contained NaCl (160 mM), HEPES (30 mM) at pH 7.4. Note that there is no evidence of lipid extraction or detergent effect.

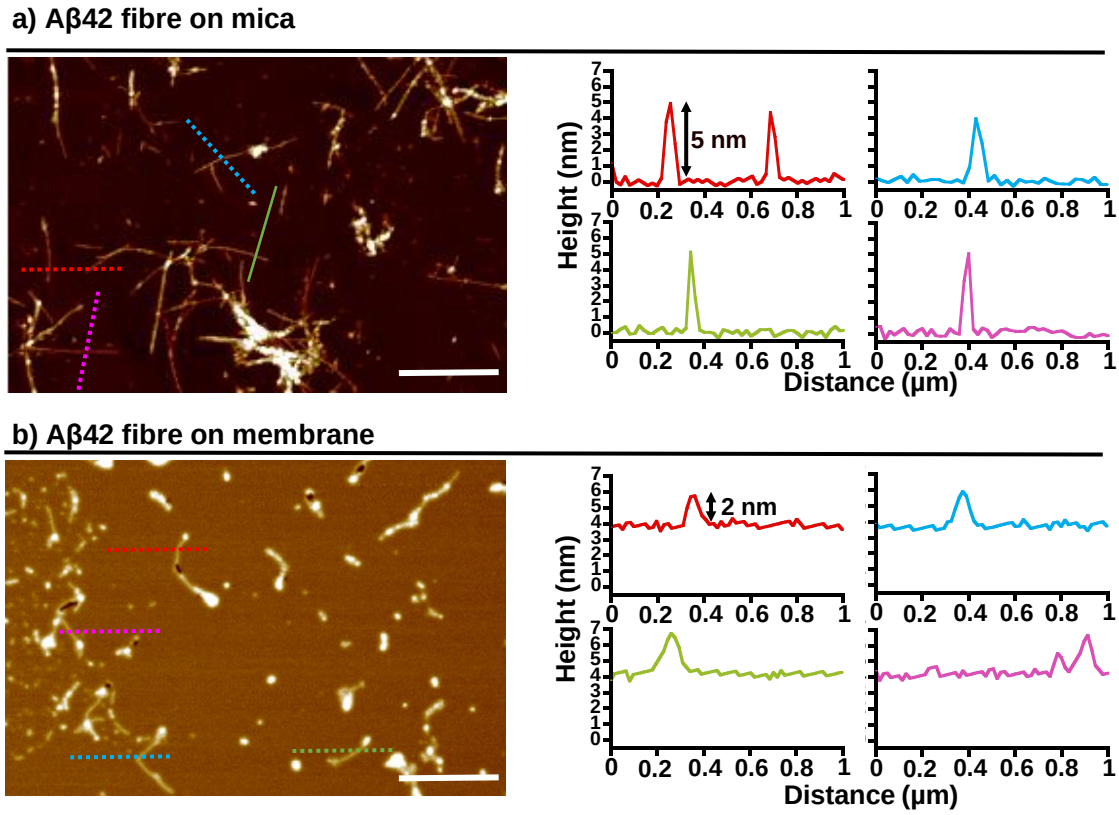


Figure S10: Aβ42 fibres laterally embed into lipid bilayer.
Further examples of AFM topographical images are presented with four accompanying cross-sections of Aβ42 fibres imaged on both (a) mica, and (b) on the surface of a membrane. Typical height colour scale range = ≤12 nm. Scale bar = 1 μm. The locations of the cross-sections are depicted by colour co-ordinated dashed lines. Fibre height measurements were taken only from areas in which a single fibre filament was visible with no evidence of multiple fibre twisting periodicity. Aβ (10 μM monomer equivalent) was deposited onto mica-supported lipid bilayer in buffer which contained NaCl (160 mM), HEPES (30 mM) at pH 7.4. Note there are no examples of lipid extraction or detergent effect for fibre interactions with the bilayer.

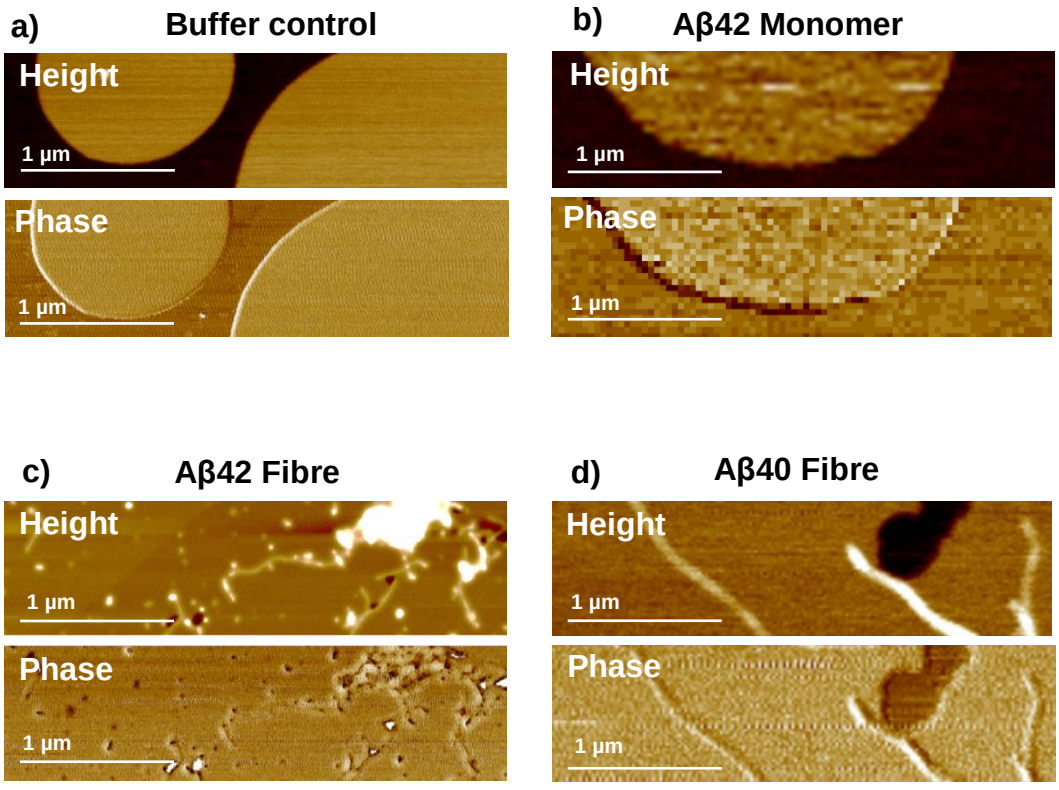


Figure S11a-d: Aβ42 oligomers can insert and rest beneath the surface of a flat lipid bilayer.

A single representative AFM is displayed with an accompanying phase-contrast image of the same sample area for membrane exposed to (a) buffer control, (b) Aβ42 monomer, (c) Aβ42 fibre, (d) Aβ40 fibre, Typical height colour scale range = ≤12 nm. Scale bar = 1.0 µm. 10 µM Aβ was deposited onto mica-supported lipid bilayer in buffer which contained NaCl (160 mM) , HEPES (30 mM) at pH 7.4.

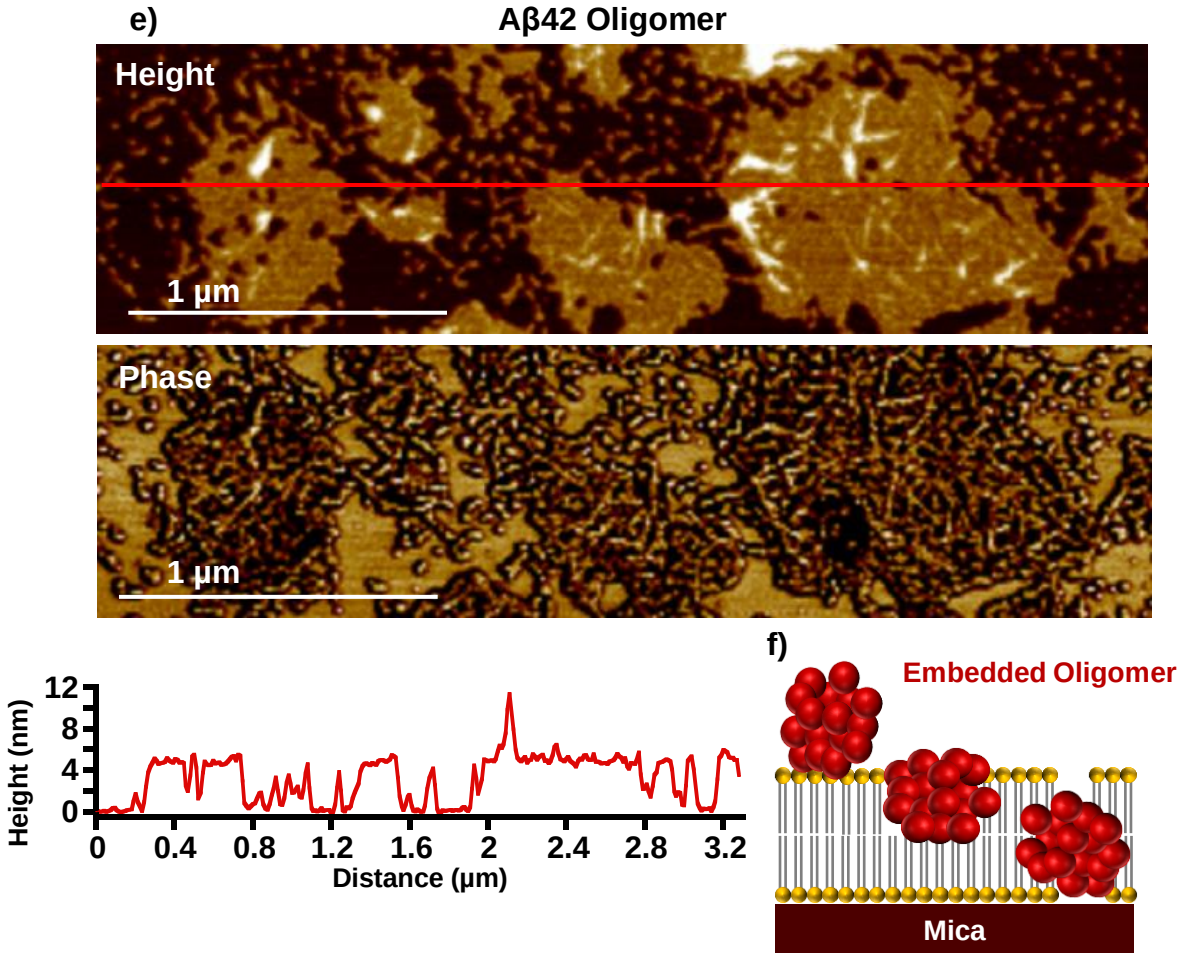


Figure S11e-f: Aβ42 oligomers can insert and rest beneath the surface of a flat lipid bilayer. A single representative AFM topographical image is displayed with an accompanying phase-contrast image of the same sample area for membrane exposed to (e) Aβ42 oligomer. Typical height colour scale range = ≤12 nm. Scale bar = 1.0 µm. A cross-section has also been drawn across the height micrograph for Aβ42 oligomer (red line) and a cross-sectional topography is presented below. (f) A schematic diagram summarising three observed states of Aβ42 oligomer residing within the membrane. 10 µM Aβ was deposited onto mica-supported lipid bilayer in buffer which contained NaCl (160 mM) , HEPES (30 mM) at pH 7.4.

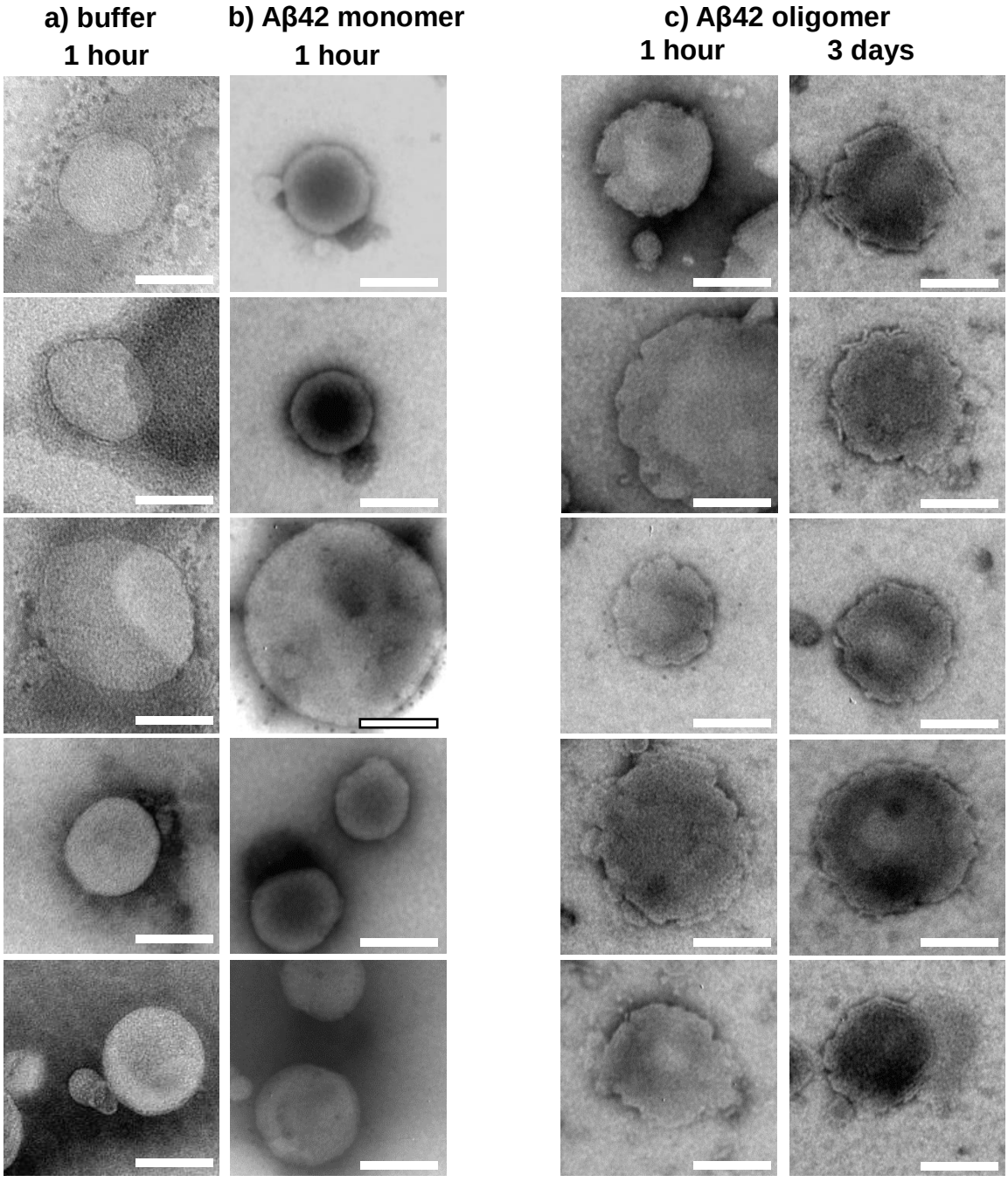


Figure S12: Aβ42 oligomer induces lipid vesicle bilayer curvature
TEM micrographs of lipid vesicles (LUVs) composed of PC:GM1:Cholesterol (68:2:30 by weight). Vesicles appear unperturbed after 1 hour incubation with (a) aqueous buffer, and (b) Aβ42 monomer. Curved bilayer perturbations and discontinuities are associated with Aβ42 oligomer (c) similar for both 1 hour and 72 hours incubation with Aβ. Aβ (10 μM monomer equivalent) was incubated with lipid vesicles in aqueous buffer which contained NaCl (160 mM), and HEPES (30 mM) at pH 7.4. Scale bars = 100 nm

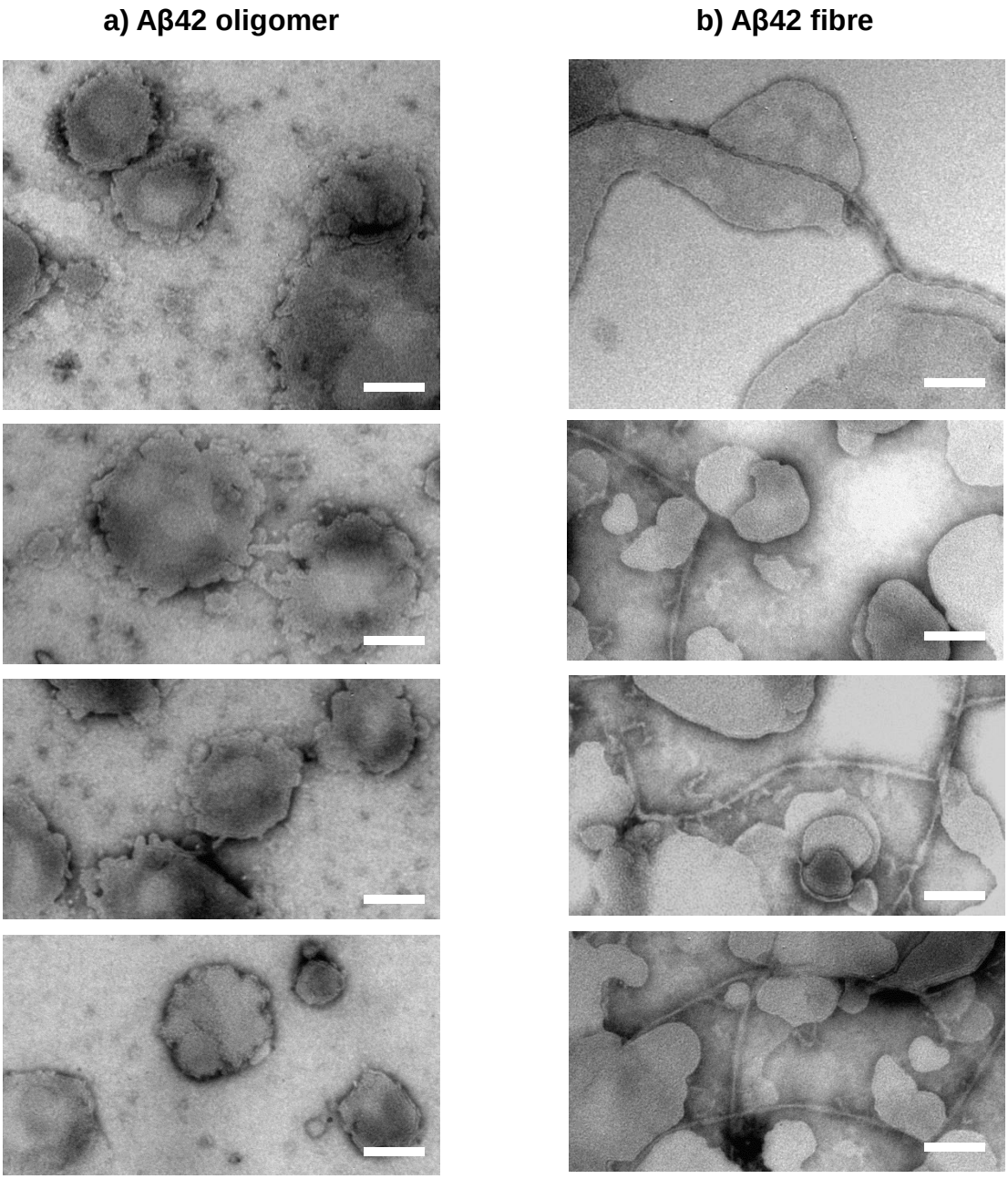


Figure S13: Aβ42 oligomer induces lipid vesicle bilayer curvature
TEM micrographs of lipid vesicles (LUVs) composed of PC:GM1:Cholesterol (68:2:30 by weight). Severe defects and perturbations of the lipid bilayer is evident in the presence of (a) Aβ42 oligomer, but not seen in the presence of Aβ42 fibre (b), where the membrane are smooth and continuous in appearance. Aβ (10 μM monomer equivalent) was incubated with lipid vesicles in aqueous buffer which contained NaCl (160 mM), and HEPES (30 mM) at pH 7.4. Scale bars = 100 nm