Supporting Information:

Lipid dynamics and phase transition within

α-synuclein amyloid fibrils

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Materials and methods

Materials

1,2-Dimyristoyl-*sn*-glycero-3-phospho-L-serine (sodium salt; DMPS) and 1,2-dilauroyl-*sn*-glycero-3-phospho-L-serine (sodium salt: DLPS) were purchased from Avanti Polar Lipids (Alabama, USA). Sodium phosphate monobasic (NaH$_2$PO$_4$, BioPerformance Certified, > 99.0%), sodium phosphate dibasic (Na$_2$HPO$_4$, ReagentPlus, > 99.0%) and sodium azide (NaN$_3$, ReagentPlus, > 99.5%) were purchased from Sigma Aldrich (Poole, UK).

Protein and lipid preparation

α-synuclein was expressed and purified using the same protocol as that reported previously.[S1–S3] Lipid vesicles used for the aggregation experiment and differential scanning calorimetry measurement were prepared via extrusion and sonication, respectively. Briefly, lipids (DMPS or DLPS) in powder form were suspended in phosphate buffer and the solution was stirred for 4 h at 45°C (a temperature above the melting temperature of both DLPS and DMPS). Then the lipid suspensions were sonicated on ice or extruded through 100nm pore membranes (Avanti Polar Lipids, Alabama, USA) at 45°C.

Cryo-electron Microscopy (Cryo-EM)

50 μM α-synuclein was incubated in phosphate buffer in the presence of lipid vesicles made with DMPS or DLPS (100 μM) under quiescent conditions and at 30°C for 4 d. The grids were then prepared as described previously.[S4] Briefly, reaction mixtures containing α-synuclein proto-fibrils were deposited on lacey carbon filmed copper grids, which were then plunged into liquid ethane at -180°C. The resulting grids were stored under liquid nitrogen until imaged using an electron microscope (Philips CM120 BioTWIN Cryo) equipped with a post-column energy filter (Gatan GIF100) (acceleration voltage: 120 kV). The images were recorded digitally with a CCD camera under low electron dose conditions.

MAS NMR

Sample preparation

The proto-fibrils were prepared by incubating 100 μM α-synuclein and 2 mM DMPS or DLPS solubilised as vesicles in phosphate buffer (20 mM NaH_2PO_4/Na_2HPO_4, 0.01 % NaN_3, pH 6.5) in Corning® 96-well half area black with clear flat bottom made of polystyrene treated with NBS™ (#3881, Corning Ltd, Corning, USA) under quiescent conditions at 30°C for 4 d. Reaction mixtures were then collected from each well, gathered and centrifuged at 90 krpm for 1 h. The pellet was then inserted into NMR rotor inserts using the following procedure: the insert was attached to a 10 μL tip using parafilm and the sample was deposited at the larger end of the tip. The insert attached to the tip was then centrifuged for 30 s at 1,000 g to let the sample slide into the rotor.

Samples of protein-free lipid lamellar phase, referred to as "pure lipid system" throughout the text, were prepared by suspending 10 mg DMPS or DLPS powder in phosphate buffer for 2 h at maximum stirring and 50°C. The samples were then centrifuged at 90 krpm for 1 h and the pellets transferred into the rotor, as described above. All experiments were performed with fully hydrated samples to avoid dehydration-induced changes in lipid self-assembly.

Data acquisition

A set of four spectra was acquired for each sample: [13]C MAS direct polarisation (DP), ^{13}C MAS cross-polarisation (CP), ^{13}C MAS Insensitive Nuclei Enhanced by Polarization Transfer (INEPT) and ^{31}P MAS NMR spectra. Measurements were performed using a Bruker Avance-II 500 spectrometer (Bruker, Karlsruhe, Germany) equipped with a 4 mm CP/MAS HX probe at a field of 11.7T, resulting in ^{1}H, ^{13}C and ^{31}P resonance frequencies of 500, 125 and 200 MHz, respectively. For temperatures below 20°C, we used a BVT-2000 temperature control and a BCU-05 cooling box unit, with sample heating induced by MAS and the radiofrequency pulses being taken into account. When temperatures were set to

values ranging from 25 to 60 °C, the same procedure without the cooling box unit was used. Polarisation Transfer (PT) ^{13}C MAS NMR spectra were acquired using a spectral width of 200 ppm and an acquisition time of 50 ms, under 67 kHz TPPM ^{1}H decoupling.[S5] For each ^{13}C spectrum, 3200 scans were accumulated with a recycle delay of 4 s. ^{13}C chemical shifts were externally referenced to solid α-glycine at 43.67 ppm.[S6] ^{1}H and ^{13}C hard pulses were applied at a $\frac{\omega_1^{H/C}}{2\pi} = 80$ kHz, CP was performed with $t_{CP} = 1$ ms, $\frac{\omega_1^{C}}{2\pi} = 80$ kHz and $\frac{\omega_1^{H/C}}{2\pi}$ linearly ramped from 72 to 88 kHz, covering the $\pm \omega_R$ matching conditions, and INEPT spectra were recorded with the delay times $\tau = 1.8$ ms and $\tau' = 1.2$ ms. The experimental time-domain data were processed as described previously[S4] with a Matlab in-house code partially derived from matNMR,[S7] using line broadening of 20 Hz, and zero-filling of 8192 time-domain points. DMPS and DLPS peak assignments were made based on DMPC spectra, as described in previous studies.[S8]

^{31}P spectra were acquired using a spectral width of 200 ppm, an acquisition time of 50 ms, 2048 scans and a recycle delay of 4s. The rate of spinning in the MAS experiments was 5000Hz for the ^{13}C spectra and 1250Hz for ^{31}P spectra.

^{31}P MAS NMR - Data analysis

Isotropic chemical shift values were set using the resonance of the phosphate buffer ($\delta = 0$ ppm). ^{31}P chemical shift anisotropy values ($\Delta\sigma$) were determined using Herzfeld-Berger sideband analysis[S9] as implemented in matNMR.[S7] The anisotropic part of the $\Delta\sigma$ is defined as:

$$\Delta\sigma = \sigma_{33} - \frac{1}{2}(\sigma_{22} - \sigma_{11}) \tag{1}$$

where σ_{ii} are the principal tensor components. When the lipid bilayer is in the liquid crystalline phase, the chemical shielding tensor is averaged to an effective tensor that is axially

symmetric.[S10] The anisotropic part of this time-average tensor has been defined as:

$$\Delta\sigma = \sigma_\parallel - \sigma_\perp = \frac{3}{2}(\sigma_\parallel - \sigma_i) \tag{2}$$

where σ_i is the isotropic chemical shift, $\sigma_\parallel$ is the low intensity shoulder ($\sigma_\parallel = \sigma_{33}$), and $\sigma_\perp$ is the high intensity shoulder ($\sigma_\perp = \sigma_{11} = \sigma_{22}$) of the axially symmetric powder pattern.[S10]

Differential Scanning Calorimetry

Sample preparation

DMPS-induced α-synuclein proto-fibrils were prepared as described above in the "MAS NMR - Sample preparation" section and DMPS vesicles were prepared as described above in the "Protein and lipid preparation" section.

Data acquisition

All the samples were degassed before the acquisition of the DSC thermograms. The thermograms were acquired using a Microcal VP-DSC calorimeter (Malvern Instruments) with a scanning rate of 1°C.min^{-1} from 5 to 65°C. All of the DSC thermograms reported in this article were corrected by subtracting the thermogram of the phosphate buffer and correspond to the first scan, unless otherwise stated.

Proteinase-K treatment

The proteinase-K digested fibrils were prepared by incubating DMPS-induced α-synuclein proto-fibrils (prepared as described in "MAS NMR - Sample preparation" section) in the presence of 3 μM proteinase-K (Ambion, Germany) under quiescent conditions for 3 h. The mixture was then placed in the calorimeter for measurements.
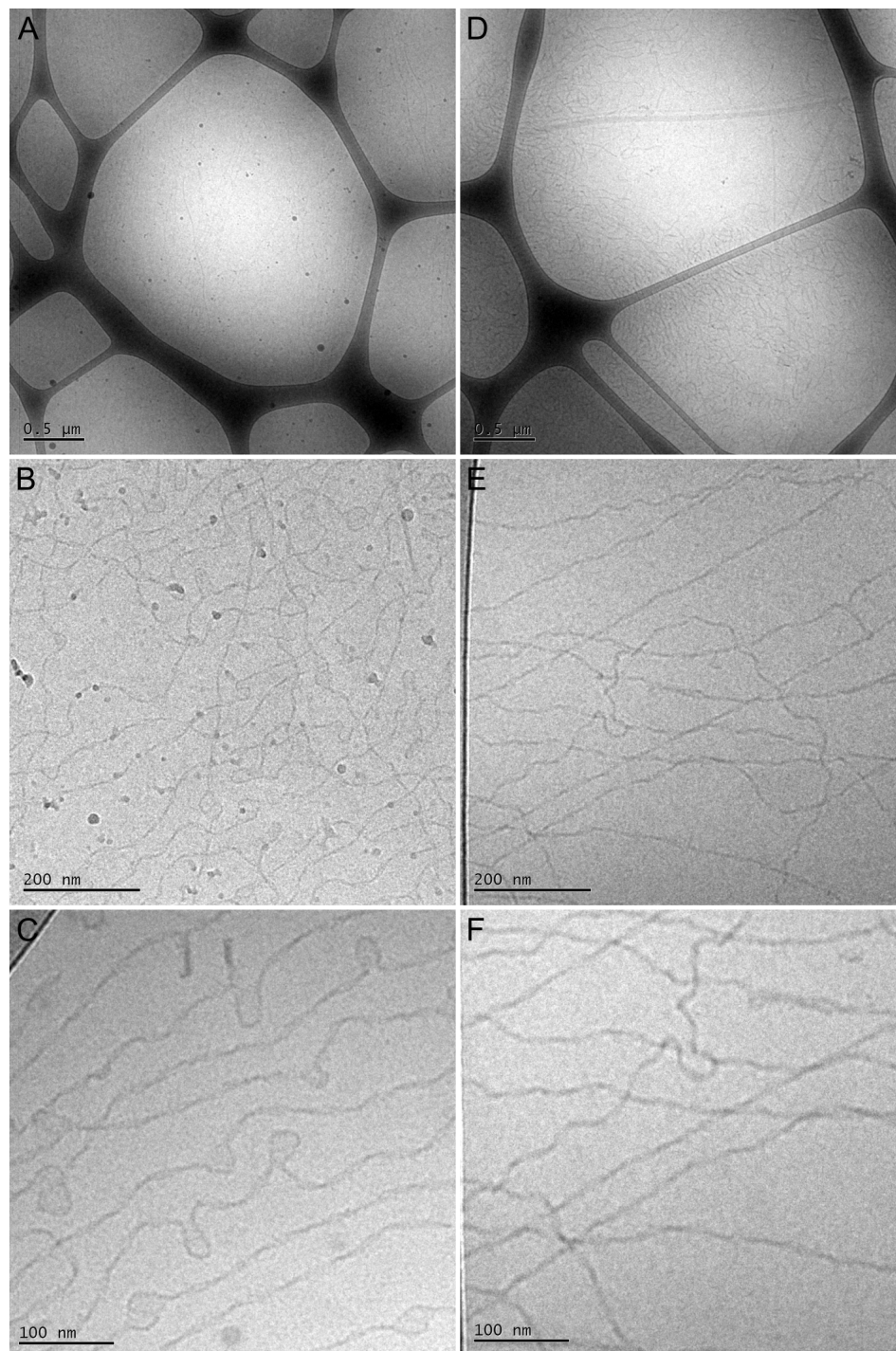
Supplementary Figures

Figure S1: Cryo-EM images of proto-fibrils formed by α-synuclein in the presence of DLPS and DMPS vesicles. The proto-fibrils were formed after mixing 50 μM monomeric α-synuclein with 100 μM DMPS (A-C) or 200 μM DLPS (D-F) solubilised as small unilamellar vesicles in phosphate buffer at pH 6.5 and 30°C, and incubating this mixture for 4 d under quiescent conditions (see Methods for further details).
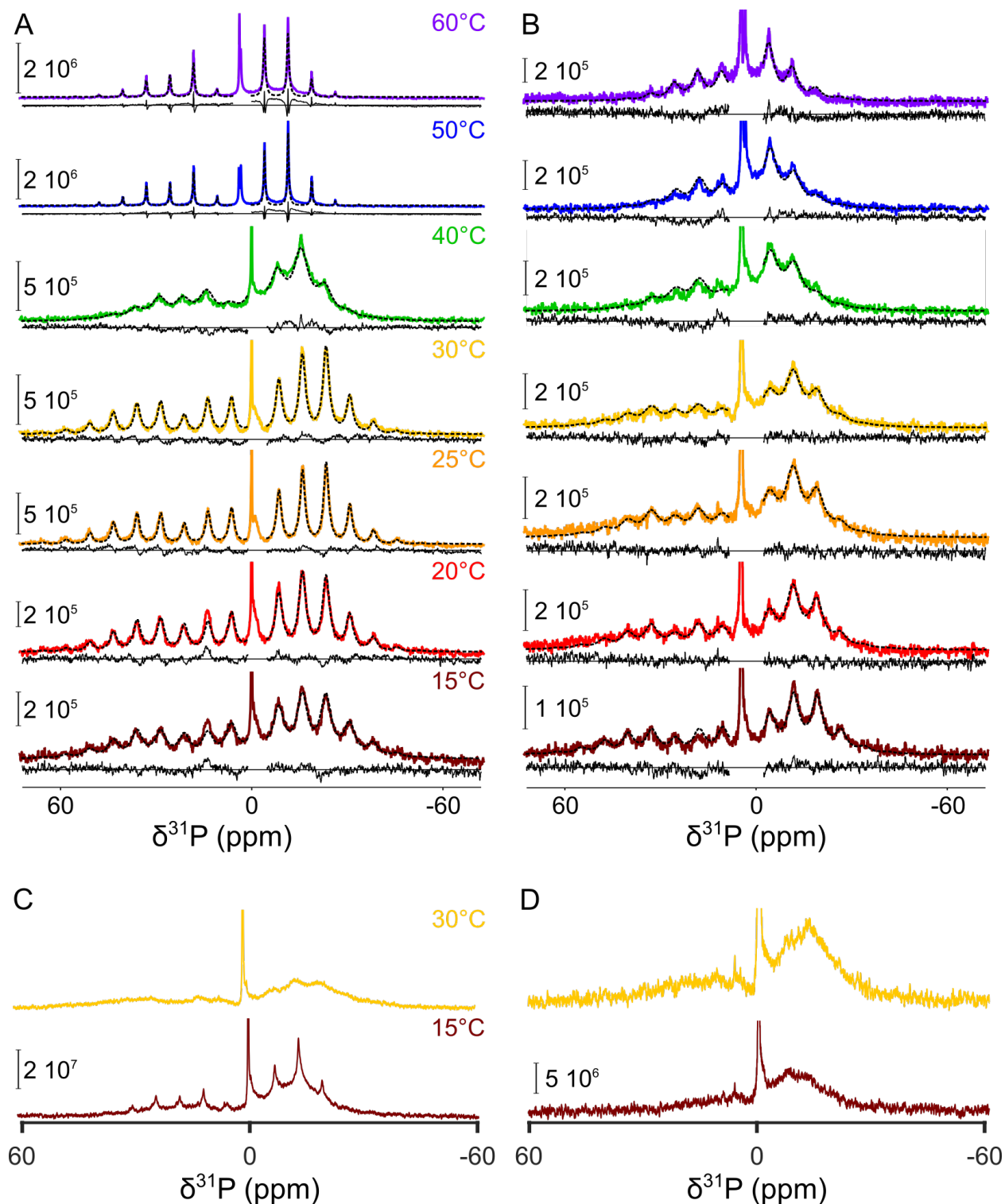
Figure S2: ^{31}P MAS NMR spectra of pure lipid systems (A, DMPS, C, DLPS) and lipid-induced α-synuclein proto-fibrils (B, DMPS, D, DLPS) measured at different temperatures. The proto-fibrils were formed by incubating 100 μM α-synuclein in the presence of 2mM DMPS or DLPS dispersed as vesicles in phosphate buffer at pH 6.5 and 30°C, and incubating this mixture for 4 d under quiescent conditions.

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