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# Alzheimer's peptide amyloid- $\beta$ , fragment 22-40, perturbs lipid dynamics†

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### S1 Electronic Supplementary Information

#### S1.0.1 Sample Preparation

1,2-dimyristoyl -*sn*-glycero -3-phosphocholine (DMPC) and 1,2dimyristoyl -*sn*-glycero -3-phospho -L-serine (DMPS) were purchased from Avanti Polar Lipids in lyophilized state and used as recieved. The peptide amyloid- $\beta$  consisting of residues 22-40 (H-Glu- Asp- Val- Gly- Ser- Asn- Lys- Gly- Ala- Ile- Ile- Gly- Leu- Met-Val- Gly- Gly- Val- Val- OH) was purchased from Anaspec, USA (purity>95%) also in lyophilized form. The purity of the peptide was determined by high performance liquid chromatography and mass spectrometry as guaranteed by the certificate of analysis from Anaspec.

The zwitterionic lipid DMPC undergoes a main phase transition at ~23°C, and the anionic lipid DMPS at 35°C<sup>S1</sup>. This DMPC/DMPS mixture is well suited for biophysical experiments because the phase transition temperatures fall into a range easily realizable in the laboratory. Our group has previously shown that a DMPC/DMPS (92:8 mol%) lipid mixture undergoes a phase transition at ~ 29°C, 98% relative humidity and the addition of 3 mol% A $\beta$ (25-35) shifts this phase transition up to ~ 32°C<sup>S2</sup>. In previous measurements, a peptide concentration of only ~3 mol% has been shown to change membrane dynamics and also influence the phase transitions of the samples<sup>S2</sup>.

The composition of each sample is listed in Table S1. The lipids were dissolved into 2 mL of solvent solution (80% chloroform, 20% methanol by volume). For the sample containing amyloid- $\beta$ , the peptide was dissolved into 1 mL of trifluoroacetic acid

sample	DMPC	DMPS	Αβ (22-40)
lipid	92 mg	8 mg	-
	92 mol%	8 mol%	-
amyloid-β	92 mg	8 mg	4 mg
	91 mol%	7.5 mol%	1.5 mol%

Table S1 Composition of samples.

(TFA) which renders the peptide monomeric and prevents preaggregation before interaction with the membrane<sup>S3</sup>. The TFA was evaporated in an Argon atmosphere for ten minutes. Following the TFA evaporation in the  $A\beta$  sample, the lipid solution was added. The solutions were sonicated for two minutes in a bath sonicator and vortexed to ensure complete mixing. This 2 mL solution containing either lipids or lipids and peptide was split into two 1 mL aliquots and deposited slowly onto 25 mm x 55 mm x 0.3 mm quartz or silicon slides using an artist's airbrush, resulting in bilayers oriented parallel to the supporting solid wafer. The mosaicity was determined to be  $\sim 1^{\circ}$  for the pure lipid sample, and  $\sim 4^{\circ}$  for the sample doped with the peptide fragment. A larger mosaicity was also observed by Dante et al. for a membrane doped with  $A\beta(25-35)^{S4}$ . After the central region of both sides of the solid supports were coated with 1 mL of solution ( $\sim$ 50 mg of sample material per side), the slides were placed into vacuum dessicator overnight ( $\sim$ 12 h, pressure of  $\sim$ 1 mbar) to allow solvents to evaporate. The use of quartz as a solid support material worked for higher resolution scans, but resulted in a large elastic peak centered around 1.4  $Å^{-1}$ . For the measurements exceeding 1.4  $Å^{-1}$ , silicon proved to be a more suitable material. The elastic contributions from quartz were fit from blank slide data, and subtracted from the sample data.

Past membrane diffraction results involving the amyloid- $\beta$ (25-35) peptide fragment have shown that the position of the peptide in the membrane is comparable for samples prepared by the method described above<sup>S5</sup> and also by an external peptide application<sup>S4</sup>, an approach which may be more physiologically rel-





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evant. The assumption has been made that the intercalation of amyloid- $\beta$ (22-40) peptide, and thus the dynamic changes to the system, will be the same for both sample preparation techniques, and thus the more straight-forward procedure was used.

During the experiment, both the lipid and amyloid- $\beta$  samples were placed vertically into sealed (air-tight) aluminum cylinders. Teflon troughs were added below the samples. These troughs were partially filled with 100% D<sub>2</sub>O and K<sub>2</sub>SO<sub>4</sub> saturated salt solution, providing 98% relative humidity. Tissue paper saturated with this solution was also added to the troughs to increase the exchange surface area between the water and the air, decreasing equilibration time.

#### S1.0.2 Quasi-Elastic Neutron Scattering

Quasi-elastic neutron scattering spectra were obtained from the instruments outlined in the main text, with four energy resolutions, allowing access to dynamics in the picosecond and nanosecond range. A visual description of the experimentally accessible ranges (from Table S2) is given in Figure 8.

Scans of both the lipid and amyloid- $\beta$  samples were performed with the membrane normal oriented at  $45^{\circ}$  and  $135^{\circ}$  relative to the incoming neutron beam (see Figure 2 in the main text). These two sample orientations allowed for the observation of the out-ofplane motions of the sample (45° - with scattering vector  $\vec{Q}$  parallel to the membrane normal) and the in-plane motions ( $135^{\circ}$ - with scattering vector  $\vec{Q}$  perpendicular to the membrane normal), as seen in Figure 2 in the main text. An effect of partial powder-averaging is expected, since the exact in-plane and outof-plane scattering strictly holds only for one particular Q-value. The lipid and amyloid- $\beta$  samples were scanned at both angles at two different temperatures, 15°C and 30°C. Blank solid support data to determine background and vanadium data to determine instrumental resolution were also collected. The contribution of the amyloid- $\beta$  peptides to the scattering is less than 2%, due to the low molar presence of the peptide. Thus, the data points obtained are primarily incoherent scattering from the hydrogen atoms in the lipid molecules  $(>85\% \text{ of total signal})^{56}$ .

The data from each of the four experiments were reduced and treated using the standard procedures for each spectrometer using LAMP<sup>S7</sup>, which includes corrections for detector efficiency. The data obtained at each resolution were Fourier transformed from the energy (frequency) domain into the time domain using DAVE <sup>S8</sup>. The intermediate scattering function was divided by the transformed instrumental resolution. The normalized intermediate scattering function relating to the various instrumental resolutions were then scaled, to account for instrumental effects between various detectors, and plotted together. The complete theoretical intermediate scattering function for a sample which undergoes various relaxation processes on well separated time scales can be described by the product of the individual intermediate scattering function of each dynamical process <sup>S9</sup>. It was necessary to use the product of three intermediate scattering function contributions to fit the entire time-range of the data, as shown in Equation 1 of the main text. The intermediate scattering functions each possess an elastic contribution and a quasi-elastic contribution. The elastic contribution arises from immobile scatterers in the system, or from motions which occur with relatively long relaxation times which fall outside of the observable time window of the measurement. Within the constraints of the present experimental accuracy, the quasi-elastic contribution of the complex behaviour of a lipid model can be modeled by an exponential decay.

The accessible Q-range of different instruments is not necessarily the same, due to geometrical factors. This treatment of the data in the time domain for all four resolutions was thus only possible for an intermediate Q-range, with information for Q=0.8 Å<sup>-1</sup> and Q=1.0 Å<sup>-1</sup>. For this reason, the analysis of the intermediate scattering function does not allow for information on the Q-dependence of the relaxations, but does allow for a precise construction of theoretical fit functions to the individual data sets in the energy (frequency) domain.

The next step of the analysis involves fits to the incoherent scattering function for each particular energy resolution. Equation S1 describes the total theoretical scattering function. The  $\Gamma$  term is the full width at half maximum (FWHM) of a Lorentzian function, and is related to the relaxation times from the intermediate structure factor in the time-domain by  $\Gamma = \hbar/\tau$ . Depending on the energy resolution used, only certain relaxations contribute to the measured spectra. The observable contributions are described in Equations 3 and 4 in the main text.

The structure factor measured is the convolution of the theoretical structure factor with the instrumental resolution, as presented in Equation S2. With F a normalization factor.

$$\begin{split} S_{theo}(\vec{Q},\hbar\omega) &= S_1(\vec{Q},\hbar\omega) \otimes S_2(\vec{Q},\hbar\omega) \otimes S_3(\vec{Q},\hbar\omega) \eqno(S1) \\ & \text{with} \end{split}$$

$$S_i(\vec{Q},\hbar\omega) = [A_i\delta(\hbar\omega) + (1-A_i)L_i(\Gamma_i,\hbar\omega)]$$

and

$$L_i(\Gamma_i,\hbar\omega) = rac{1}{\pi}rac{\Gamma_i/2}{(\hbar\omega)^2 + (\Gamma_i/2)^2}$$

When observed in the energy domain, the individual intermediate scattering functions found in Equation 1 of the main text are transformed to a sum of the  $A_i$  factor multiplied by the Dirac delta function and a Lorentzian function,  $L_i$ . This produces the theoretical structure factor shown in Equation S1. The three terms, representing three time-separated relaxations present in the sample are convoluted rather than multiplied together, according to convolution theorem <sup>S10</sup>. The approach to analyze the data by a convolution of structure factors has been used recently in the literature to describe lipid dynamics as studied by quasi-elastic neutron scattering, with the number of dynamical processes visible depending on the instrumental resolution of the measurement <sup>S11,S12</sup>.

$$S_{measured}(\vec{Q},\hbar\omega) = F \cdot \left[ S_{theo}(\vec{Q},\hbar\omega) \otimes S_{resol}(\vec{Q},\hbar\omega) \right]$$
(S2)

The wide range of instrumental resolutions used in the measurements (from 1  $\mu$ eV to 100  $\mu$ eV) was chosen to allow the ob-



**Fig. S1** Exemplary QENS spectra and fits. For comparison, the Q~1 Å<sup>-1</sup> data from the four experimental resolutions are shown. a)IN16:  $\Delta E=1 \mu eV$ , b)IN5:  $\Delta E=15 \mu eV$ , c)ToFToF 8Å:  $\Delta E=25 \mu eV$  and d)ToFToF 5Å:  $\Delta E=100 \mu eV$ . Data are shown on a logarithmic intensity axis with respective error values in green circles. This data set was fit using Equation S2, with Equations 3 and 4 of the main text as the theoretical scattering function. The data collected using IN16 were fit using Equation 3 with a single Lorentzian peak (pink), whereas two Lorentzian peaks (pink and red solid lines) as in Equation 4 were used to fit the IN5 (b) and ToFToF (c, d) data. The theoretical scattering functions were constructed by comparing the instrumental observation range to the relaxation times found using Equation 1.

servation of the range of lipid dynamics occurring over multiple orders of magnitude in time.

To determine the Q-dependence of the dynamical processes ob-

served, fits to the broadening of the incoherent structure factor,  $S_{inc}(\vec{Q},\omega)$ , were performed. The reduced data were binned (to increase statistics) and fit using the "lsqcurvefit" fitting procedure in MATLAB<sup>S13</sup>. The vanadium curves were fit to Gaussians to yield the instrumental resolution function. Example fits for each energy resolution used are presented in Figure S1. The widths of the fitted Lorentzian contributions were plotted against the scattering vector Q<sup>2</sup>, which can be interpreted with dynamical models. In particular, the backscattering data from IN16 (1 µeV resolution) describe a long-range diffusion, and the time-of-flight data (15, 25 and 100 µeV resolution) describe restricted local lipid dynamics <sup>S14</sup>.

A statistical analysis was performed to test whether the differences between the samples with and without peptide were statistically significant. This was performed as an unpaired t-test (also known as Welch's unequal variances t-test <sup>S15,S16</sup>). The statistic t and the degree of freedom of the test were determined for each diffusion coefficient and its respective uncertainty and evaluated at the 95% confidence limit.

## References

- 1 J. R. Silvius, *Lipid-Protein Interact.*, John Wiley & Sons, Inc., New York, 1982.
- A. Buchsteiner, T. Hauβ, S. Dante and N. Dencher, *Biochim. Biophys. Acta*, 2010, **1798**, 1969–1976.
- 3 S. Jao, K. Ma, J. Talafous, R. Orlando and M. Zagorski, *Amyloid Int. J. Exp Clin. Invest*, 1997, 4, 240–252.
- 4 S. Dante, T. Hauss and N. A. Dencher, *Biochemistry*, 2003, **42**, 13667–13672.
- 5 S. Dante, T. Hauss and N. A. Dencher, *Biophys. J.*, 2002, **83**, 2610–2616.
- 6 V. Sears, Neutron news, 1992, 3, 26-37.
- 7 D. Richard, M. Ferrand and K. G.J., J. Neutron Res. 4, 1996, 33–39.
- 8 R. Azuah, L. Kneller, Y. Qiu, P. Tregenna-Piggott, C. Brown, J. Copley and R. Dimeo, *Res. Natl. Inst. Stan. Technol.*, 2009, 114, 341–358.
- 9 R. Lechner, Phys. B Condens. Matter, 2001, 301, 83-93.
- 10 R. Bracewell, *The Fourier Transform and Its Applications*, McGraw-Hill, New York, 3rd edn, 1999, pp. 108–112.
- 11 U. Wanderlingh and G. D'Angelo, J. Chem. Phys, 2014, 140, 174901.
- 12 V. K. Sharma, E. Mamontov, D. Anunciado, H. M. O. Neill and V. S. Urban, J. Phys. Chem. B., 2015, **119**, 4460–4470.
- 13 MathWorks, *MATLAB and Statistics Toolbox Release 2012b*, 2012.
- 14 M. Bee, Quasielastic Neutron Scattering, Hilger, Adam, Bristol, 1988.
- 15 B. Welch, Biometrika, 1947, 34, 28–35.
- 16 G. D. Ruxton, Behav. Ecol., 2006, 17, 688-690.
- 17 R. Zorn, Nucl. Instruments Methods Phys. Res. Sect. A Accel. Spectrometers, Detect. Assoc. Equip., 2009, 603, 439–445.

Spectrometer	$\Delta E$ (µeV)	λ(Å)	$Q_{range}$ (Å $^{-1}$ )	t <sub>window</sub> (ps)
IN16 (ILL)	1	6.3	0.6-1.9	400-1240
IN5 (ILL)	15	10	0.3-1.1	10-83
ToFToF (FRM-II)	25	8	0.5-1.3	4-50
ToFToF (FRM-II)	100	5	0.7-1.7	1-12.4
	Spectrometer IN16 (ILL) IN5 (ILL) ToFToF (FRM-II) ToFToF (FRM-II)	Spectrometer         ΔE (μeV)           IN16 (ILL)         1           IN5 (ILL)         15           ToFToF (FRM-II)         25           ToFToF (FRM-II)         100	$\begin{tabular}{ c c c c c c c } \hline Spectrometer & $\Delta E$ ($\mu eV$) $$\lambda$ (Å) \\ \hline IN16$ (ILL) $$1 $$6.3 \\ IN5$ (ILL) $$15 $$10 \\ \hline ToFToF$ (FRM-II) $$25 $$8 \\ \hline ToFToF$ (FRM-II) $$100 $$5 $$5 $$100 \\ \hline ToFToF$ (FRM-II) $$100 $$$100 \\ \hline ToFToFTOF$ (FRM-II) $$100 $$$100 \\ \hline ToFTOFTOF$ (FRM-II) $$100 $$$100 \\ \hline ToFTOFTOFTOFTOFTOFTOFTOFTOFTOFTOFTOFTOFTOF$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$

**Table S2** Spectrometers used and their respective elastic energy resolution ( $\Delta E$ ), wavelength ( $\lambda$ ), Q-range and resolution time ( $t_{window}$ ). Conversion from energy to time was calculated using  $t_{resolution}$ =1.24  $\mu$ eV·ns/ $\Delta E_{FWHM}$ <sup>S17</sup>. An estimate of the lower limit of the time window was given by the dynamic range, determined from the spectrometer's characteristics, the upper limit is estimated from the instrumental resolution, characterized with a vanadium standard.