Supporting Information

for

Amyloid-β adopts a conserved, partially folded structure upon binding to zwitterionic lipid bilayers prior to amyloid formation

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Experimental Section

Materials

Unlabeled $A\beta_{40}$ (> 95% purity) was purchased from Genscript (Piscataway, NJ, USA). Uniformly ^{15}N-labeled $A\beta_{40}$ was purchased from rPeptide (Bogart, GA, USA). HiLyte Fluor 647-$A\beta_{40}$ was purchased from Anaspec (Fremont, CA, USA). All peptide was used as received without additional purification. 1,2-Dilauroy-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were purchased from Avanti Polar Lipids *Inc*. (Alabaster, AL, USA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Vesicle preparation

Large unilamellar vesicles (LUVs) of pure DLPC, DOPC, and POPC were prepared from a chloroform solution. The resulting solution was dried under N_2 (g) and then placed under high vacuum overnight to remove the residual solvent. The resultant film was rehydrated with buffer solution (20 mM PO_4, pH 7.4, 50 mM NaCl) to yield a final lipid concentration of either 10 mg/mL (for fluorescence binding experiments) or 1 mM (for all other experiments). Lipid was resuspended by vigorous mixing and the resulting solution was extruded 23 times through a 100 nm polycarbonate Nucleopore membrane filter (Whatman) mounted on a mini-extruder (Avanti Polar Lipids *Inc*.) to obtain a homogenous solution of LUVs with an average diameter of 100

nm. The formation of vesicles was confirmed by dynamic light scattering to ensure solution homogeneity.

Peptide preparation

In order to break up preformed aggregates in the initial peptide aliquot, both unlabeled and uniformly ^{15}N-labeled peptide was dissolved in 1% NH_4OH and aliquoted to 0.1 mg/sample, and lyophilized. HiLyte Fluor 647-Aβ_{40} was similarly dissolved in 1% NH_4OH and aliquoted to 0.01 mg/sample and lyophilized. All peptide was dried under vacuum for 48 h to remove excess moisture.

Circular dichroism studies

Circular dichroism (CD) measurements were carried out at a concentration of 20 μM Aβ_{40} in the presence of 4 μM LUV in buffer (20 mM PO_4, 50 mM NaF, pH 7.4). Freshly lyophilized peptide was first dissolved in 5 μL of 1% NH_4OH prior to dilution into the buffer to prevent the formation of early aggregates. CD measurements were performed on a JASCO J-1500 CD Spectrometer using a 0.1 cm path length cell. The spectra were acquired at 10 °C at a scan rate of 100 nm/min and averaged over 10 scans. Measurements were taken immediately after mixing the sample (t = 0 h), after 4 h incubation at 4 °C, and after 24 h incubation at 4 °C.

Fluorescence anisotropy binding studies

To prepare the peptide for fluorescence experiments, HiLyte Fluor 647-Aβ_{40} was dissolved in 5 μL 1% NH_4OH and diluted into buffer (20 mM PO_4, pH 7.4, 50 mM NaCl) to a final peptide concentration of 1 μM. Fluorescence measurements were performed on a Jasco FP-6500 fluorimeter equipped with excitation and emission polarizing filters. Fluorescence polarization was used to measure the binding affinity of Aβ_{40} for 100 nm LUVs of the various lipids. 150 μL of a 1 μM solution of HiLyte Fluor 647-Aβ_{40} was titrated with LUVs from a 10 mg/mL stock solution. Lipid was titrated into peptide solution to a maximum concentration of 7 mM. The fluorescence anisotropy was monitored with an excitation wavelength of 647 nm and emission wavelength of 660 nm using a 10 nm bandwidth for both excitation and emission at each titration point. Anisotropy (<r>) was calculated using equation 1 where G is defined by equation 2. *I* is the intensity observed with excitation (first subscript) and emission (second subscript) polarizers set to either vertical or horizontal, providing four measurements which contribute to the anisotropy calculation.

$$< r > \; = \frac{I_{VV} - G * I_{VH}}{I_{VV} + 2 * G * I_{VH}} \qquad \text{(Eq. 1)}$$

$$G = \frac{I_{HV}}{I_{HH}} \qquad \text{(Eq. 2)}$$

The anisotropy values measured were then plotted against their respective lipid concentrations and the data were fit using equation 3 to identify the binding affinity (K_d) of peptide for the LUVs.

$$< r >= \frac{< r >_{inf} * [lipid]}{K_d + [lipid]} + < r >_0 \qquad \text{(Eq. 3)}$$

NMR spectroscopy

For all samples analyzed by NMR, 0.1 mg peptide (uniformly ^{15}N-labeled or unlabeled) was dissolved with 3 μL DMSO-d_6 (Cambridge Isotope, Tewksbury, MA, USA) and diluted with phosphate buffer, NaCl, D_2O, and ddH_2O to a final peptide concentration of 80 μM (20 mM PO_4, pH 7.4, 50 mM NaCl, 7% v/v D_2O). For those samples containing lipid, LUVs were also added from a 1 mM stock solution containing a single lipid for a final lipid concentration of 16 μM (0.2 equiv). For all experiments, spectral were processed using Topspin 2.1 (Bruker) while assignments and intensity fits were performed by SPARKY 3.113.

Transfer-NOESY (tr-NOESY)

Line broadening was first observed by 1D ^{1}H NMR upon the addition of 0.2 equiv (16 μM) lipid, suggesting exchange between unbound and bound species; this peptide:lipid ratio was utilized throughout for lipid containing NMR samples and was observed to be stable over the course of the experiments. Therefore, transfer-NOESY (tr-NOESY) experiments were carried out on Bruker TCI 900 spectrometer, equipped with a cryo-probe to investigate the membrane bound state of unlabeled Aβ_{40} to the three LUVs.[1-3] Two-dimensional (2D) ^{1}H-^{1}H TOCSY and ^{1}H-^{1}H tr-NOESY spectra were acquired at 80 and 150 ms mixing times, respectively at 10 °C. Tr-NOESY spectra were acquired using 2 K data points in t_2 and 512 data points in t_1 and a spectral width of 12 ppm in both dimensions. H$^\alpha$ chemical shifts were then calculated relative to the random coil shift values maintained by the Biological Magnetic Resource Bank (BMRB, University of Wisconsin).

Paramagnetic relaxation enhancement titration

A solution of $MnCl_2$ was titrated into a solution of uniformly ^{15}N-labeled Aβ_{40} in the presence of LUVs described above and monitored by SOFAST-HMQC on a Bruker 600 MHz spectrometer equipped with a cryoprobe at 10 °C. Spectra of each sample in the presence of 0 equiv (0 μM), 0.5 equiv (40 μM), and 1 equiv (80 μM) $MnCl_2$ were obtained from 128 t_1 points and a 100 ms recycle delay. Relative intensities for all spectra were obtained by a comparison of spectra in the presence of spectra acquired in the presence of both 0.5 and 1.0 equiv $MnCl_2$ to spectra in the absence of the paramagnetic reagent. Peak assignments for the spectra were based on previously published spectra.[4]

Relaxation measurements

The ^{15}N transverse relaxation rate constants ($R_2 = 1/T_2$) for the residues of $A\beta_{40}$ were determined by collecting a series of ^{15}N-^{1}H HSQC spectra under a variety of relaxation delays on a Bruker 600 MHz spectrometer equipped with a cryoprobe at 17 °C using the uniformly ^{15}N-labeled $A\beta_{40}$ samples described above; R_2 values were measured for samples in the presence of all three different bilayers, as well as in the absence of lipids. The echo delays of 8, 16, 32, 48, 64, 80, 96, 128, 160, 194, 240, and 320 ms were used for the measurement of R_2. ΔR_2 values were then calculated as the difference between the R_2 values of peptide in the presence of the three LUVs (DLPC, DOPC, and POPC) and the R_2 values of peptide in the absence of lipid. Positive ΔR_2 values are indicative of a protein population, which is more rigid than the peptide population in the absence of lipid, while negative ΔR_2 values are indicative of a less rigid population.

References:

1. G. M. Clore, Gronenborn, A. M., *J. Magn. Reson.*, 1982, **48**, 402-417.
2. A. Bhunia, H. Mohanram and S. Bhattacharjya, *Biopolymers*, 2009, **92**, 9-22.
3. Z. Wang, J. D. Jones, J. Rizo and L. M. Gierasch, *Biochemistry*, 1993, **32**, 13991-13999.
4. S. Vivekanandan, J. R. Brender, S. Y. Lee and A. Ramamoorthy, *Biochem. Biophys. Res. Commun.*, 2011, **411**, 312-316.

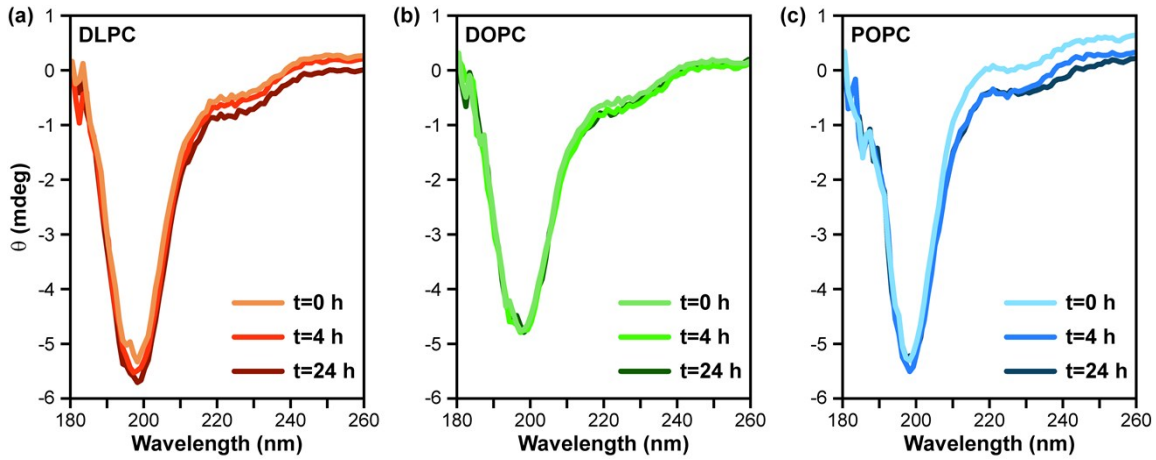


Figure S1. Conformational analysis of $A\beta_{40}$ upon incubation with lipids for at least 24 h by CD. When $A\beta_{40}$ (20 μM) was co-incubated with 0.2 equiv (4 μM) LUVs composed of (a) DLPC, (b) DOPC, or (c) POPC at different time intervals. The random coil structure persisted in the presence of all three bilayers over the experimental timescale of 24 h.

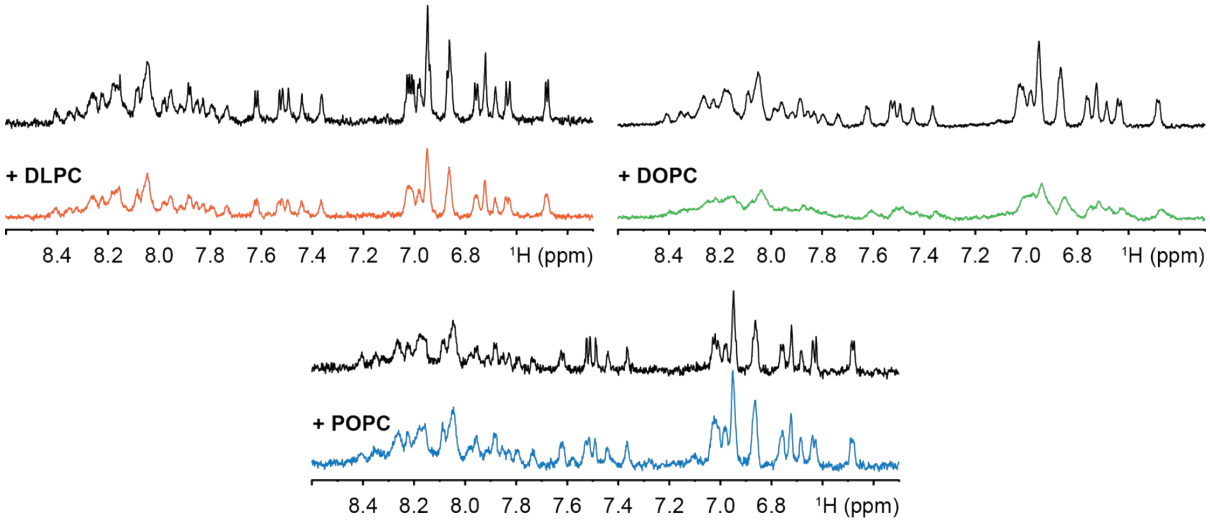


Figure S2. Effect of LUVs on lipid-bound and –unbound states of peptide. $A\beta_{40}$ undergoes a fast-mediate exchange in the presence of substoichiometric lipid concentrations. The amide and aromatic regions of the ^{1}H region of $A\beta_{40}$ (80 μM) NMR spectra broaden in the presence of low concentrations of LUVs (14 μM lipid; colored spectra) relative to the lipid-free spectra (black), indicative of an exchange between lipid-bound and -unbound states of the peptide.

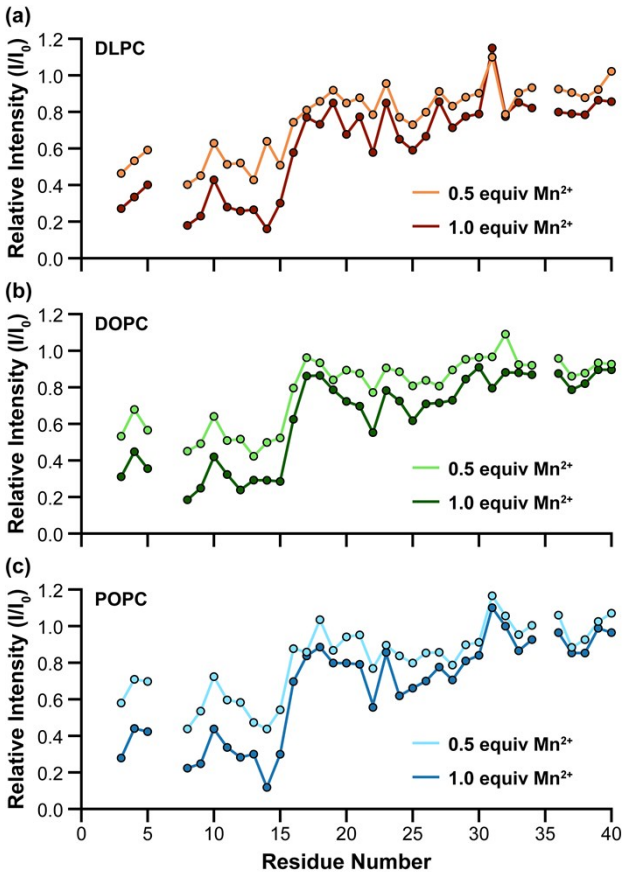


Figure S3. Paramagnetic quenching of the peptide signal. A solution of uniformly ^{15}N-labeled Aβ_{40} (80 μM) in the presence of LUVs (0.2 equiv, 16 μM) containing either (a) DLPC, (b) DOPC, or (c) POPC was titrated with a solution of $MnCl_2$ and the residue signal intensity was monitored by 2D SOFAST-HMQC (Bruker 600 MHz spectrometer, equipped with cryoprobe at 10 °C). The quenching of residue specific signal is indicative of exposure to buffer while maintenance of the initial intensity suggests the membrane interaction and protection from paramagnetic quenching.

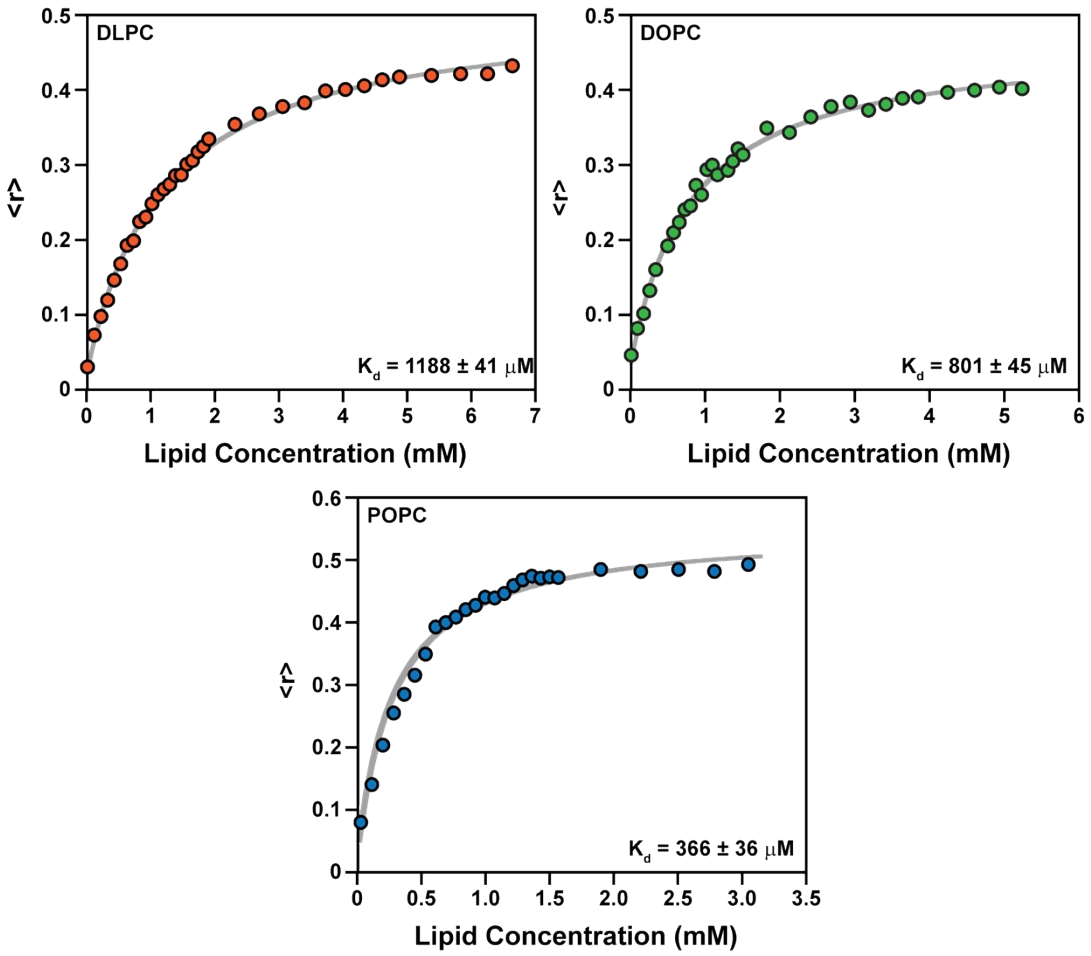


Figure S4. Binding affinities of $A\beta_{40}$ to lipid bilayers based on fluorescence polarization. 100 nm LUVs (DLPC, DOPC, and POPC) were titrated into 1 μM HiLyte 647-$A\beta_{40}$ and the fluorescence polarization was monitored. Anisotropy values were converted to fraction bound and fit to obtain binding affinities of $A\beta_{40}$ for the various bilayers.

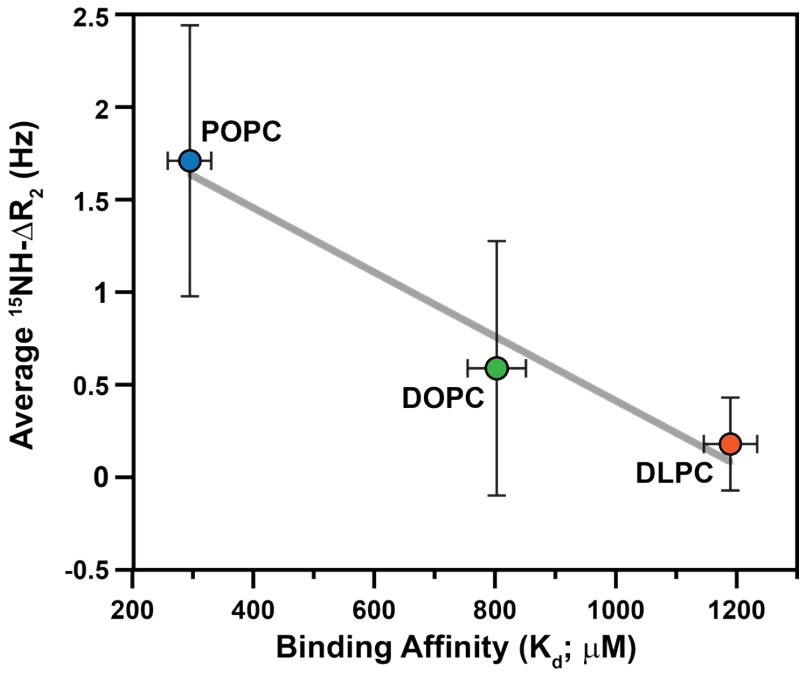
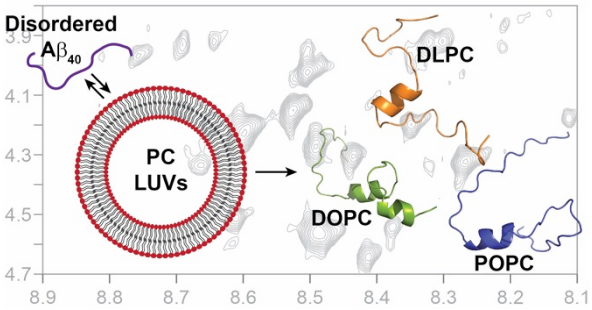


Figure S5. Correlation between backbone flexibility and membrane affinity. There is a linear correlation between the average ΔR_2 and the binding affinity of the $A\beta_{40}$ peptide in the presence of each bilayer. The R_2 experiments of $A\beta_{40}$ with and without LUVs were performed on a Bruker 600 MHz spectrometer at 10 °C. The tighter the binding affinity (K_d) indicates the larger the ΔR_2, indicative of the reduced ensemble flexibility.

TOC


Disordered
Aβ40
PC
LUVs
DLPC
DOPC
POPC
4.1
4.3
4.5
4.7
8.9
8.8
8.7
8.6
8.5
8.4
8.3
8.2
8.1