Phosphatidylethanolamine enhances amyloid fiber dependent membrane fragmentation

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Supporting Information

Materials and Methods:

Preparation of lipid vesicles. Chloroform solutions of lipids (POPC:POPS (7/3 molar ratio), POPE/POPC/POPS (3:4:3) and POPC/POPS/LysoPC (6.8/3/0.2)) were gently dried under nitrogen flow and placed under high vacuum overnight to completely evaporate any residual solvent. The resulting lipid film was rehydrated with a buffer solution (10 mM phosphate buffer solution, pH 7.4, 100 mM NaCl) to yield a final lipid concentration of 4 mg/ml and then dispersed by vigorous stirring to generate multilamellar vesicles. To make large unilamellar vesicles (LUVs), the lipid solution was then extruded 23 times through a 100 nm polycarbonate Nucleopore membrane filter (Whatman) mounted on a mini-extruder to obtain LUVs with an average diameter of 100 nm. LysoPC, when present in the LUV composition, was incorporated by adding the correct amount (2% mol) from a stock solution to a solution of preformed POPC/POPS LUVs. Dye-filled LUVs were prepared by hydrating the dry lipid film with a buffer solution containing 6-carboxyfluorescein (10 mM phosphate buffer solution, 70 mM 6-carboxyfluorescein, pH 7.4) to a final concentration of 10 mg/ml according to the procedure described above. Non-encapsulated 6-carboxyfluorescein was removed by a Sephadex G50 size exclusion column. The final concentration of lipids were checked using the Stewart assay.(*1*) All solutions were freshly prepared before each experiment.

Dye leakage assay. Membrane leakage was quantified by detecting the increase in fluorescence intensity of 6-carboxyfluorescein due to its dilution and subsequent dequenching as a consequence of membrane disruption. Samples were prepared initially by diluting the dye-filled vesicles with buffer solution (10 mM phosphate buffer solution, pH 7.4, 100 mM NaCl) to final lipid concentrations of 250 μM, 500 μM or 1000 μM and adding peptide from a 250 μM DMSO stock solution to a final peptide concentration of 2.5 μM (DMSO 1%$_{v/v}$). The presence of 100 mM NaCl in the outside buffer ensures dye release from vesicles does not occur in the absence of peptide due to the osmotic pressure exerted by the dye on the membrane bilayer. Fibers of IAPP, where present, were obtained by incubating 0.1 mg of IAPP in phosphate buffer 10 mM,

100 mM NaCl, pH 7.4 at 25^{o}C for two days. Experiments were carried out in Corning 96 well non-binding microplates, using a 1 mm glass bead to enhance shaking. Time traces were recorded using a Biotek Synergy 2 plate reader using a 494 excitation filter and a 520 emission filter at room temperature, shaking samples for 10 seconds before each read. The fraction of dye released could be calculated by:

Fraction of dye released $= (I - I_0)/(I_{100} - I_0)$ Eq. 1

where I is the emission intensity of the sample, I_0 is the emission intensity obtained in absence of peptide (baseline control) and I_{100} is the emission intensity obtained after adding Triton X-100, a detergent, which acted as a positive control to give 100% leakage. All experiments were performed in triplicate.

Lipid translocation assay. The lipid translocation assay was performed according to the method described by Muller et al.(*2*). The method is based on the dilution of a pyrene-labeled lipid probe from the outer to the inner leaflet of membrane, causing a reduction in pyrene excimer emission. The probe stock was made by dissolving the pyrene-labeled lipid in ethanol. An aliquot of this stock solution of probe was then added to a suspension of 20 μM LUV in phosphate buffer 10 mM , 100 mM NaCl, pH 7.4, so that the probe partitioned into the outer monolayer at a concentration of 3% mol of the total lipid. The ratio of the intensities of the excimer and monomer emission peaks was recorded as a function of time at 37 °C. An excitation wavelength of 344 nm was used with slits set for 4 nm bandwidths, using an emission wavelength of 397 nm for the monomer and 478 nm for the excimer. When translocation of the probe to the inner leaflet occurs, there is a reduction in the excimer-to-monomer ratio due to the effective dilution of the probe.

The excimer to monomer ratio was normalized by the following equation:

Normalized $(I_e/I_m) = [(I_e/I_m) - (I_e/I_m)_{sym})]/[(I_e/I_m)_{max}-(I_e/I_m)_{sym}]$ Eq. 2

where I_e/I_m is the excimer to monomer ratio measured at a given timepoint, $(I_e/I_m)_{sym}$ is the excimer to monomer ratio for the symmetrically labeled sample, and $(I_e/I_m)_{max}$ is the excimer to monomer ratio observed before the addition of the peptide. A comparison of the spectra of asymmetrically and symmetrically labeled LUVs in the absence of peptide is shown in Fig. S2. The I_e/I_m ratio did not change in the absence of peptide for the length of the experiment (30 minutes).

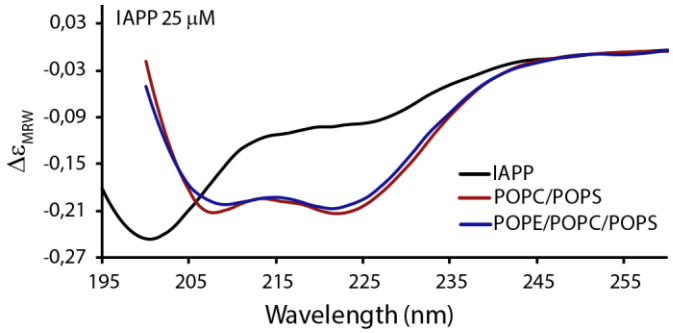
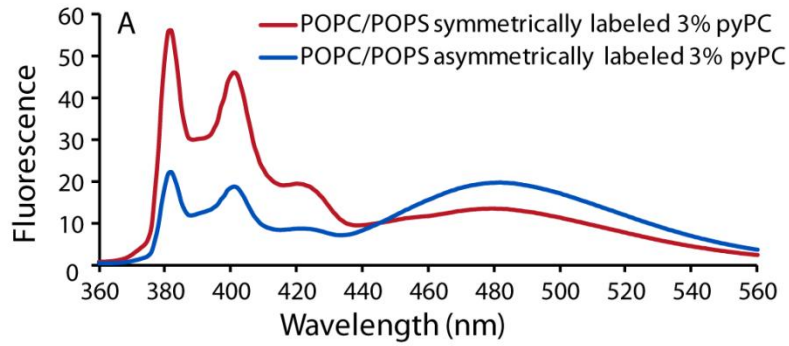


Figure S1. CD spectra of hIAPP. CD spectra of 25 µM hIAPP in the absence of membranes (black curve), and at a saturating concentration (1600 µM) of 7/3 POPC/POPS LUV (red curve) and (3/4/3) POPE/POPC/POPS (blue curve). The similarity of the CD spectra in POPC/POPS and POPE/POPC/POPS membranes suggests IAPP is fully bound in an α-helical conformation at saturating lipid concentrations that is similar in both types of membranes.



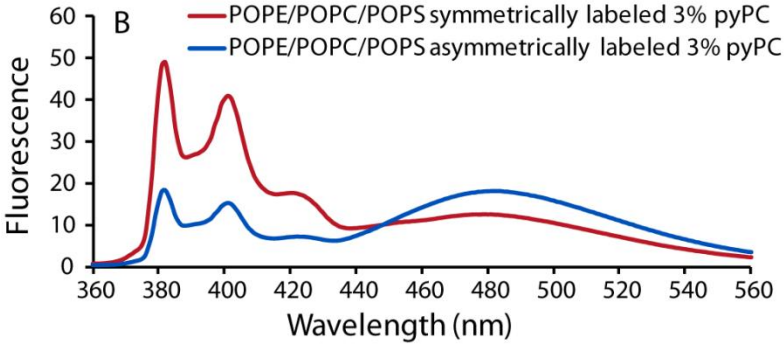


Figure S2. Pyrene emission spectra from LUVs labeled symmetrically and asymmetrically with Py-PC. Fluoresence emission spectra of (A) (7/3) POPC/POPS and (B) (3/4/3) POPE/POPC/POPS symmetrically (red curve) and asymmetrically (blue curve) labeled with 3% PyPC. The decrease in I_E/I_M ratio apparent in the red curve shows the effect of loss of asymmetry in the bilayer on the pyrene emission spectrum.

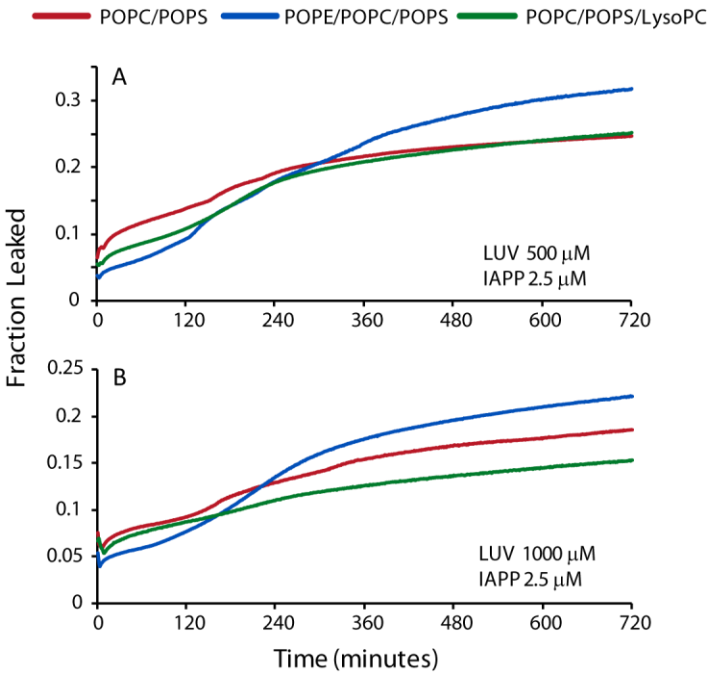


Figure S3. Effect of lipid concentration on membrane disruption induced by IAPP. Release of 6-carboxyfluorescein induced by 2.5 µM IAPP from 500 µM (A) or 1000 µM (B) 7/3 POPC/POPS (red line), 3/4/3 POPE/POPC/POPS (blue line) or 6.8/3/0.2 POPC/POPS/LPC (green line) membranes. Curves represent the average of three measurements. Experiments were performed at room temperature in 10 mM phosphate buffer, 100 mM NaCl, pH 7.4. Membrane disruption strongly decreases when the lipid concentration was increased, but the qualitative effects of membrane composition on the peptide-induced membrane disruption remain constant.

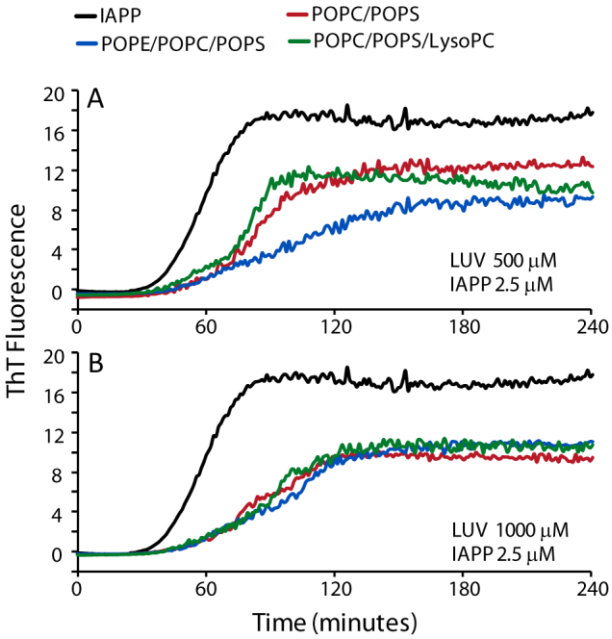


Figure S4. Effect of lipid concentration on fiber kinetics measured by ThT. Fiber formation measured by ThT fluorescence of 2.5 μM IAPP in the presence of 500 μM (A) or 1000 μM (B) 7/3 POPC/POPS (red line), 3/4/3 POPE/POPC/POPS (blue line) or 6.8/3/0.2 POPC/POPS/LPC (green line) membranes. Black lines indicate fiber formation in the absence of membranes. Curves represent the average of three measurements. Experiments were performed at room temperature in 10 mM phosphate buffer, 100 mM NaCl, pH 7.4.

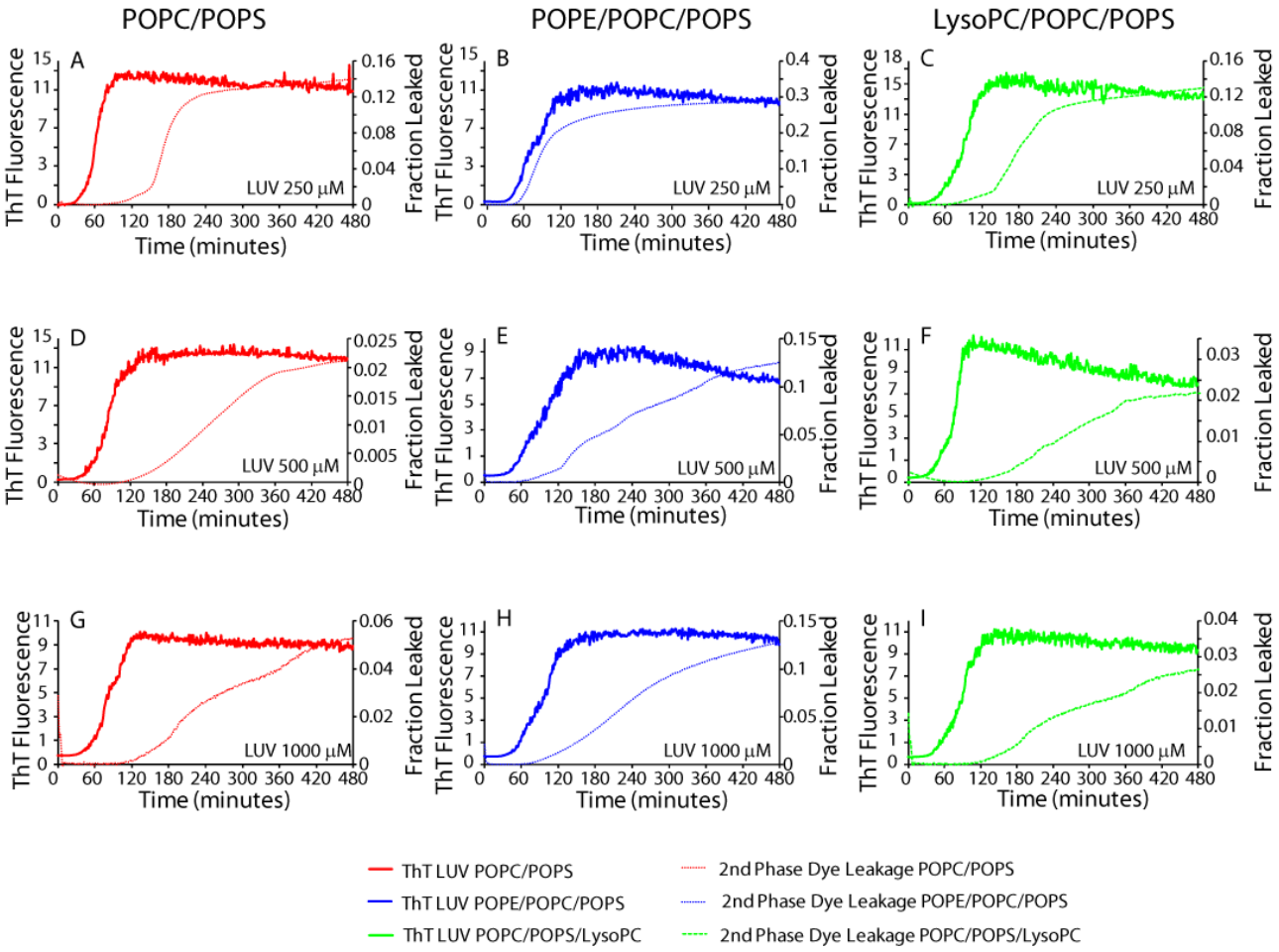


Figure S5. Comparison between fiber formation kinetics and the second step of dye release. ThT and the second phase of dye release curves from 7/3 POPC/POPS (A,D,G), 3/4/3 POPE/POPC/POPS (B,E,H) and 6.8/3/0.2 POPC/POPS/LysoPC (C,F,I) membranes at 250 μM (A,B,C), 500 μM (D,E,F) and 1000 μM (G,H,I) lipid concentration. The second phase of dye release was obtained by subtracting the double exponential fit calculated from the first 60 minutes of membrane disruption from the total membrane disruption curve.

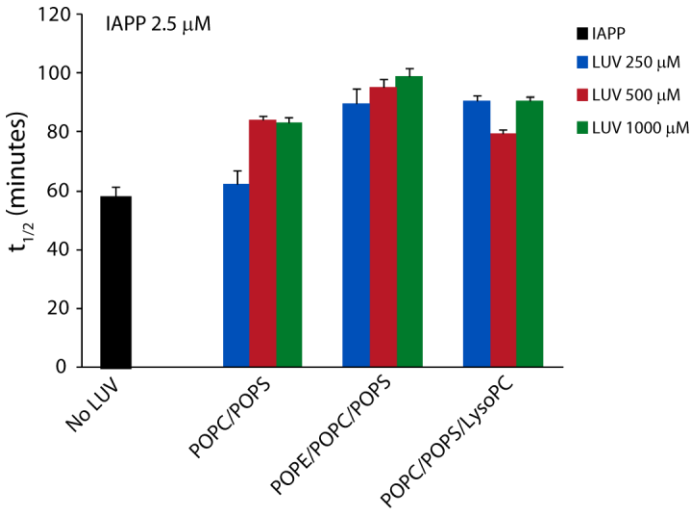


Figure S6. Time to half of amyloid fiber formation of hIAPP. Time to half ($t_{1/2}$) values of hIAPP fiber formation calculated by fitting the ThT curves with a sigmoidal curve.

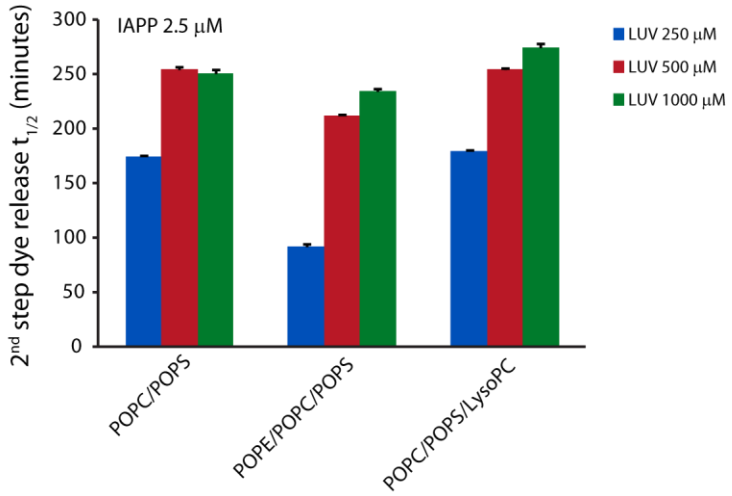


Figure S7. Time to half of second step of dye release induced by amyloid fiber. Time to half ($t_{1/2}$) value during the fiber-dependant step of membrane disruption calculated by fitting the second phase of dye leakage curves with a sigmoidal curve. The second phase of dye release was obtained by subtracting the double exponential fit calculated from the first 60 minutes of membrane disruption form the total membrane disruption curve.

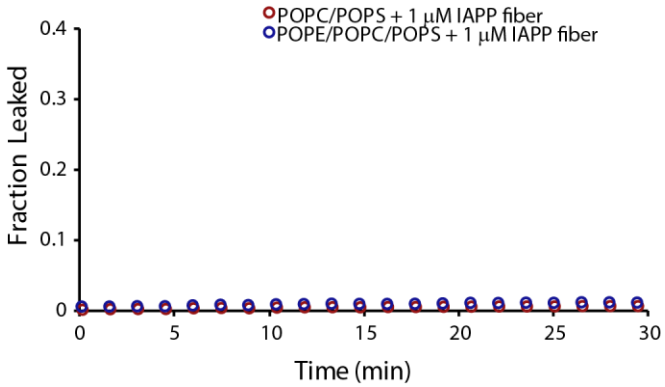


Figure S8. Preformed fibers of IAPP do not cause loss of membrane asymmetry. Lipid translocation in 20 μM POPC/POPS (7/3) or POPE/POPC/POPS (3/4/3) large unilamellar vesicles labeled with 3% PyPC in the outer leaflet caused by 1 μM preformed IAPP fibers in the absence of monomeric IAPP. All experiments were performed at 37°C in 10 mM phosphate buffer, 100 mM NaCl, pH 7.4.

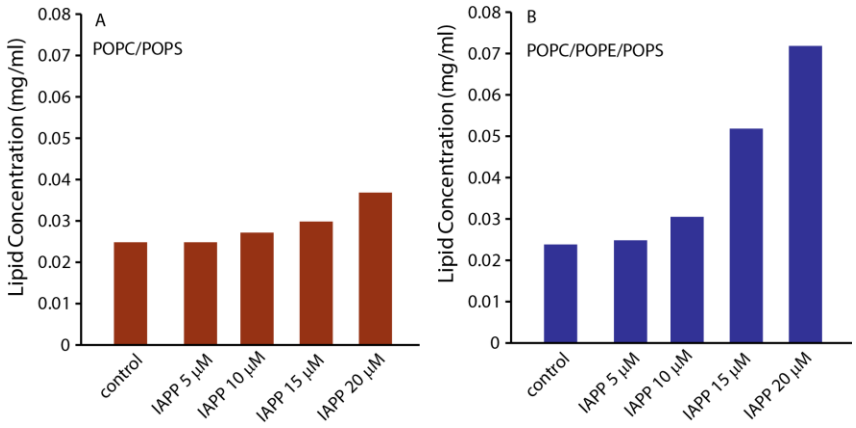


Figure S9. Concentration dependence of membrane fragmentation induced by IAPP**.** IAPP at the indicated concentrations was incubated for 5 hours at 25 °C with 1 mg/ml large unilamellar vesicles containing POPC/POPS (7/3) or POPE/POPC/POPS (3/4/3). Following incubation, samples were centrifugated at 14,000 rpm and lipid concentrations in the supernatant were measured by the Stewart assay. All experiments were performed in 10 mM phosphate buffer, 100 mM NaCl, pH 7.4.

References

1. Stewart, J. C. (1980) Colorimetric determination of phospholipids with ammonium ferrothiocyanate, *Anal. Biochem. 104*, 10-14.

2. Muller, P., Schiller, S., Wieprecht, T., Dathe, M., and Herrmann, A. (2000) Continuous measurement of rapid transbilayer movement of a pyrene-labeled phospholipid analogue, *Chem. Phys. Lipids 106*, 89-99.