Supplementary Information

Model Membrane Size-dependent Amyloidogenesis of Alzheimer's Amyloid-β peptides

Misaki Kinoshita\textsuperscript{a}, Erina Kakimoto\textsuperscript{a}, Mayu S. Terakawa\textsuperscript{a,b}, Yuxi Lin\textsuperscript{a}, Tatsuya Ikenoue\textsuperscript{a,c}, Masatomo So\textsuperscript{a}, Toshihiko Sugiki\textsuperscript{a}, Ayyalusamy Ramamoorthy\textsuperscript{d,e}, Yuji Goto\textsuperscript{a}, Young-Ho Lee\textsuperscript{a}

\textsuperscript{a}Institute for Protein Research, Osaka University, Yamadaoka 3-2, Suita, Osaka 565-0871, Japan.
\textsuperscript{b}Department of Biochemistry, Weill Cornell Medical College, NY, USA.
\textsuperscript{c}Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK.
\textsuperscript{d}Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA.
\textsuperscript{e}Biophysics Program, University of Michigan, Ann Arbor, MI 48109, USA.
\textsuperscript{‡}These authors contributed equally.

Corresponding Author

\textsuperscript{*}mr0505@protein.osaka-u.ac.jp
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Figure S1. Characterization of two types of POPC vesicles with distinct sizes. (A, B) Distribution of the diameters of SUVs (A) and LUVs (B) at various POPC concentrations measured by dynamic light scattering. Average values of the hydrodynamic diameter and the lipid concentration are indicated. (C) Fluorescence spectra of ANS in the presence of SUVs (blue) or LUVs (red) and the absence of vesicles (black). (D) One-dimensional proton NMR spectra of SUVs (blue line) and LUVs (red line) are shown with numbering of the peak assignment. The chemical structure of POPC is also displayed.
Figure S2. Investigation of intermolecular interactions between Aβ1-42 and vesicles by isothermal titration calorimetry (ITC). (A-C) An ITC thermogram of the SUV (syringe) titration to Aβ1-42 solution (cell) at 37 °C (A) or 10 °C (B) and LUV titration (syringe) at 10 °C (C). The far-UV CD spectra of Aβ1-42 solution taken from the ITC cell after reactions are shown on the right. The large exothermic peak in A indicates the formation of amyloid fibrils based on our previous study,¹,² which was further supported by the far-UV CD spectrum of the cross-β structures of amyloids. The inset in the ITC thermogram shows the real-time monitoring of fast (black curve) and slow Aβ1-42 amyloid formation (cyan curve) at 37 °C and 10 °C, respectively, using ThT fluorescence. The far-UV CD spectra in B and C indicate that Aβ1-42 remained unfolded even after being incubated in an ITC cell.
Figure S3. Effects of SUVs on amyloid formation of Aβ_{1-40} at various POPC concentrations and 37 °C. (A-C) Far-UV spectra of Aβ_{1-40} at the initial (A) and final states (C) and fibrillation kinetics monitored by thioflavin T (ThT) fluorescence (B) in the presence of SUVs. The concentration of POPC is represented by the following color code: 10 (cyan), 20 (blue), 100 (green), 200 (orange), 500 (pink), and 1000 μM (red). Averages and error values were obtained from the measurement run in replicate. (D-F) AFM images of Aβ_{1-40} solution after kinetic measurements at 0 (D), 10 (E), and 1000 μM (F) are shown with scale bars indicating 1 μm.
Figure S4. Effects of LUVs on amyloid formation of Aβ1-40 at various POPC concentrations and 37 °C. (A-C) Far-UV spectra of Aβ1-40 at the initial (A) and final states (C) and fibrillation kinetics monitored by ThT fluorescence (C) in the presence of POPC LUVs. The concentration of POPC is represented by the following color code: 10 (cyan), 20 (blue), 100 (green), 200 (orange), 500 (pink), and 1000 μM (red). Averages and error values were obtained from the measurement run in triplicate as shown in the inset of B. (D-F) AFM images of Aβ1-40 solution after kinetic measurements at 0 (D), 10 (E), and 1000 μM (F) are shown with scale bars indicating 1 μm.
Figure S5. Kinetics of Aβ1-42 amyloid formation on SUVs or LUVs observed by ThT fluorescence. (A-F) Amyloid formation on SUVs (A-C) or LUVs (D-F). The raw kinetic data of amyloid formation on SUVs (A-C) or LUVs (D-F) were obtained with the three independent measurements. Each data point in A-F, shown with markers, indicates average values of three data points. Distinct colors indicate the concentration of POPC: 10 (cyan), 20 (blue), 100 (green), 200 (orange), 500 (pink), and 1000 μM (red). Data in A and D correspond to those in Figure 2A and E, respectively.
Figure S6. Conformational stability of Aβ1-42 amyloid fibrils on vesicles. (A-D) The far-UV CD spectra of amyloid fibrils of 5 μM Aβ1-42 formed without vesicles (A), with SUVs (B, C), and with LUVs (D). The concentrations of POPC used were 10 (B, D) and 1000 μM (C). The spectra at 37 °C before (black curve) and after (green curve) the heat treatment at 110 °C (orange curve) are shown. (E, F) The real-time monitoring of the ThT fluorescence of the amyloid fibrils of Aβ1-42 at 485 nm was performed in the presence of SUVs (E) and LUVs (F) at POPC concentrations of 0 (black), 10 (cyan), and 1000 μM (red).
Figure S7. Amyloid formation of Aβ1-42 on SUVs with high concentrations of POPC. Real-time monitoring of the ThT fluorescence intensity was performed in the presence of 5 μM Aβ1-42 and 1 (green), 5 (orange), and 10 mM POPC (red).
Figure S8. Fitting analyses of Aβ1-42 amyloid fibrils on SUVs. (A) Representative fit curves (solid lines) of raw kinetic data (spheres) of the Aβ1-42 aggregation, which were averaged values of three data points. (B-D) The primary (B) and secondary nucleation rate (C and D) are plotted against the concentration of POPC. Averaged and error values were calculated using data of three independent dataset which consisted of three data points. The fitting analysis was performed based on the equation suggested by Knowles, Linse, and their colleagues.\(^{3}\) \(k_n\), \(k_2\), and \(k_+\) indicate the rate constant for primary nucleation, secondary nucleation, and elongation, respectively. The concentration of POPC is as follows: 10 (cyan), 20 (blue), 100 (green), 200 (orange), 500 (pink), and 1000 µM (red).
**Figure S9.** Seeded amyloid fibrillation of $\text{A}\beta_{1-42}$ on SUVs. (A-B) ThT-based observation of formation of $\text{A}\beta_{1-42}$ amyloid fibrils without SUVs (A) and with SUVs at the lipid 20 $\mu$M. (B). Amyloid formation in the absence (black in A and blue in B) and presence of seeds (gray in A and light blue in B). (C) Elongation rates with SUVs at the lipid concentration of 0 (left two bars) and 20 $\mu$M (right two bars) in the absence (black) and presence (gray) of seeds. Average values with errors of three data points are shown.
Supplementary Reference

