Supplemental Information

Supplemental Methods

Western blot

We incubate the differentiated PC-12 cells in the presence and absence of purified Aβ oligomer samples for 3 days and then lyse with ice-cold RIPA buffer (PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS). We then collect the lysed solution for Western blot. After homogenization, we centrifuge cell samples at 12000 rpm for 20 min and collect the supernatants. We add 2X SDS sample buffer into the supernatant solutions and heat samples at 95 ^{o}C for 10 min. We separate the samples on a 12% polyacrylamide gel then transfer to a PVDF membrane with transfer buffer (25mM Tris, 192mM Glycine, 0.1% SDS,20% Methanol, pH 8.3) at 80 V for 90 min at 4°C. We block the blots with 5% BSA in TBST (10 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.1% Tween-20) for 1 h at room temperature, then incubate with anti-Caspase 3 (Sigma AB3623) and anti-Vinculin (Sigma SAB4200729), diluted 1:100 and 1:1000, respectively, in blocking buffer (caspase-3 and vinculin) overnight at 4°C. After washing 3 times with TBST, we incubate the membrane in anti-Mouse IgG conjugated with horseradish peroxidase for 3 hr at room temperature. Before film imaging, we wash the blot 3 times with TBST and soak for 5 min in chemiluminescent substrate (Thermo Scientific catalog # 34080).

Primary neuron culture

We use postnatal day 0/1 (P0/1) CD1 pups for the generation of primary neuron cultures. CD1 breeder pairs (Charles River) are fed food and water *ad libitum* and maintained under 12 hr light/dark conditions under a protocol approved by the Penn State College of Medicine Institutional Animal Care and Use Committee (IACUC). All procedures are conducted according to NIH and approved IACUC guidelines. For the generation of primary neuron cultures, we sacrifice P0/1 pups by decapitation using surgical scissors. We place isolated brains in cold HEPES buffered Hanks' Balanced Salt solution (pH 7.8). We dissect out the cortical cap of each brain and remove the meninges. Isolated cortices are transferred to conical tubes of warm neuron plating medium: Neurobasal Plus (Gibco), 10% FBS (Gibco), 1x GlutaMAX (Gibco), 1x Penicillin-Streptomycin (10,000 U/mL, Gibco). We triturate cortices in plating medium with a cut p1000 pipette tip, followed by trituration with an intact tip. We measure the cell concentration using the Countess II automated cell counter (Invitrogen), and plate 5.47*105 cells/cm^2 on poly-D-lysine (Cultrex)-coated 96-well plates. We place the cells in the incubator at 37ºC, 5% CO_2 overnight to allow cells to attach. The next morning, we switch the medium to Neuronal Medium: Neurobasal Plus (Gibco), 1X B27 Plus supplement (Gibco), 1x GlutaMAX (Gibco), 1x Penicillin-Streptomycin (10,000 U/mL, Gibco). We

change half the medium every 5-7 days and used confluent neuron cultures for calcium and viability assays after 12-14 days in culture.

Measurement of Ca^{2+} influx in primary neurons

We solubilize human Aβ42 (Novex 75492034A) in trifluoroacetic acid to a concentration of 110 µM and vortex vigorously. We initiate Aβ42 aggregation by diluting the peptide solution to 11 µM in PBS and incubate at 37 ºC for 24 hours. As a control, we dilute the same volume of TFA vehicle in PBS and incubate at 37 ºC for 24 hours. We then dilute each of the aggregated Aβ42 and TFA control in neuronal media to the indicated concentrations. We remove all media from the neurons and replace with the Aβ42 media or TFA vehicle control media. After 72 hours, we wash cells twice with artificial cerebrospinal fluid (aCSF) (124mM NaCl, 4.4 mM KCl, 1.2mM $MgSO_4$:$7H_2O$, 1mM NaH_2PO4:H_2O, 2.175g NaHCO3, 2.5mM $CaCl_2$:$2H_2O$, and 1.825g glucose in 1L of dH2O, pH 7.4). We then add 100uL of 2.3uM Fluo-4 (diluted in aCSF) to each well and incubate at 37ºC to allow Fluo-4 to enter the cells. After 15 minutes, we aspirate the wells and wash twice with calcium-free aCSF. We add 100uL of aCSF to all wells, except a set of wells which receive 10 uM ionomycin (a membrane permeable calcium ionophore) as a positive control. We then determine fluorescence intensity using a SpectraMax i3 plate reader.

Supplemental Figures

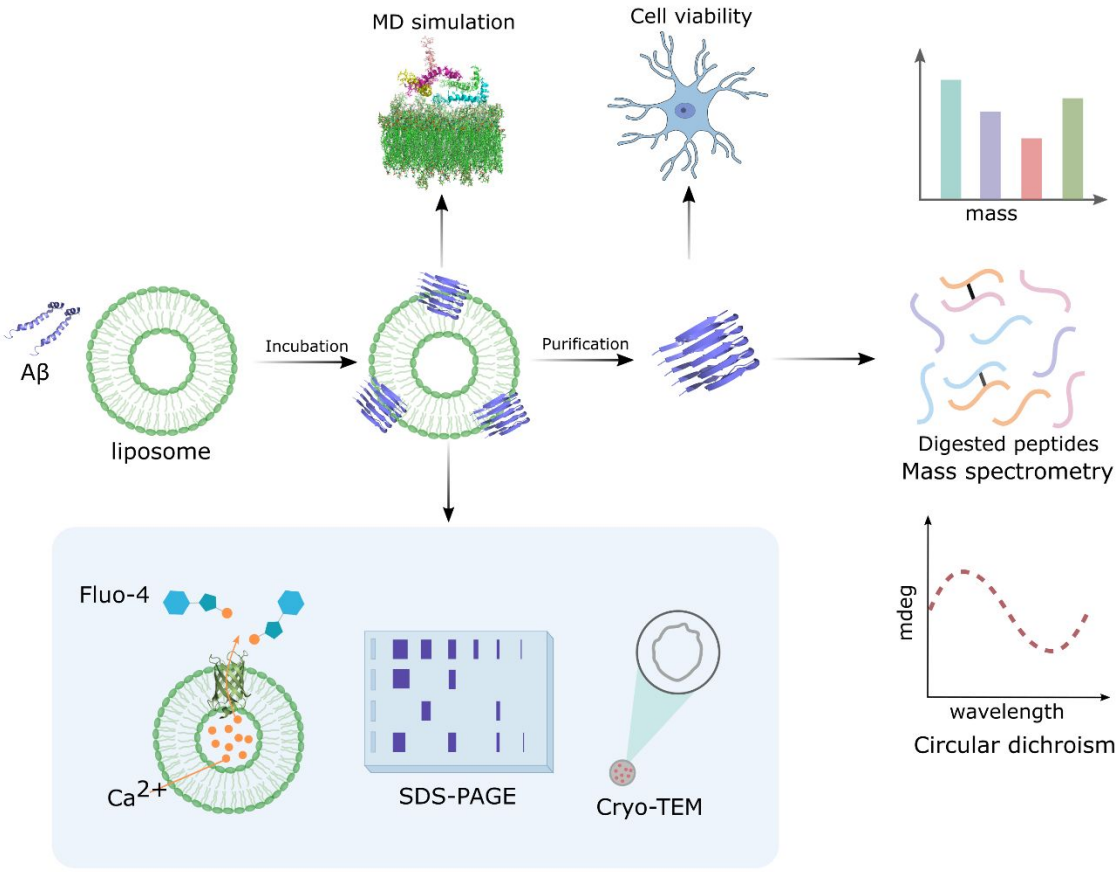

Figure S1. Schematic of the hypothesis and the design. We incubate Aβ with GM1 liposomes to determine the effect of GM1 membrane on the formation of Aβ fibrils and oligomers. We perform MD simulation to explore the interaction between Aβ and GM1 membrane. We purify the Aβ oligomers formed on liposomes membrane, determine the toxicity, and characterize the structure with CD and mass spectrometry. We design a calcium-encapsulation assay to provide new evidence for the Aβ ion channel hypothesis. We also determine the effect of Aβ on the morphology of liposomes membrane using Cryo-TEM.

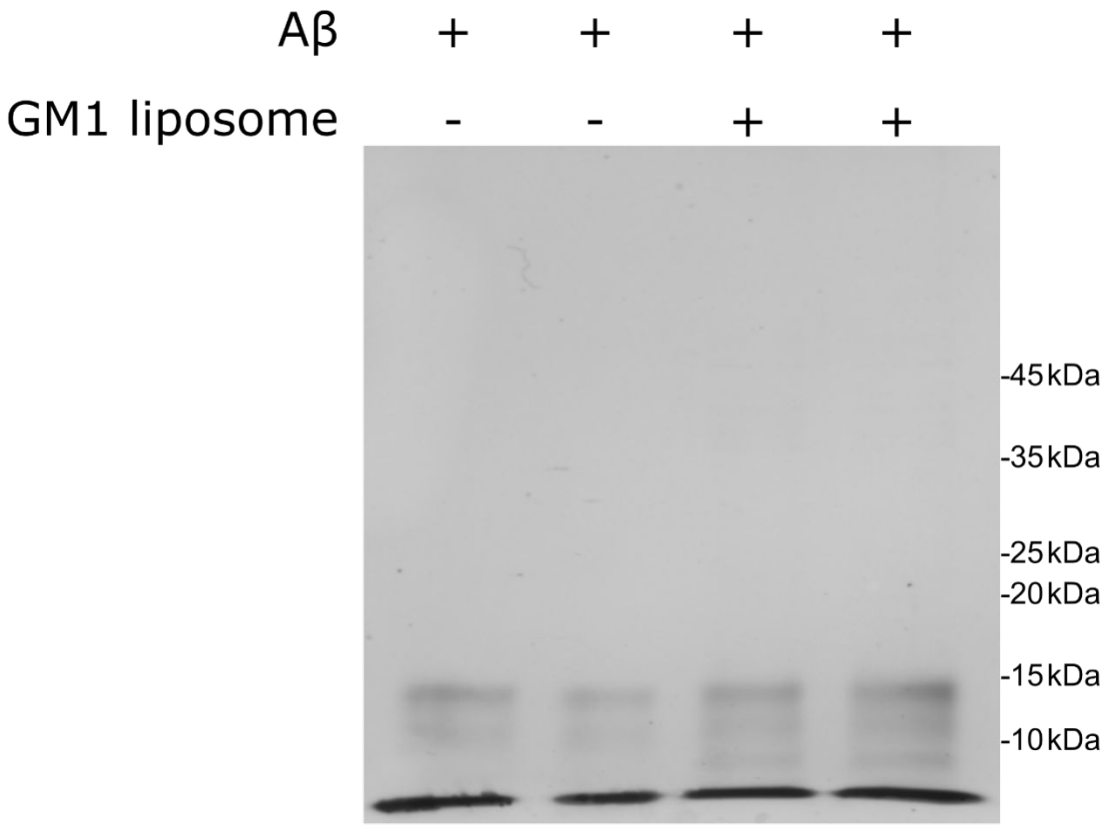

Figure S2. SDS-PAGE and silver staining results of Aβ oligomers formed in the absence and presence of GM1 liposomes at the start of the incubation.

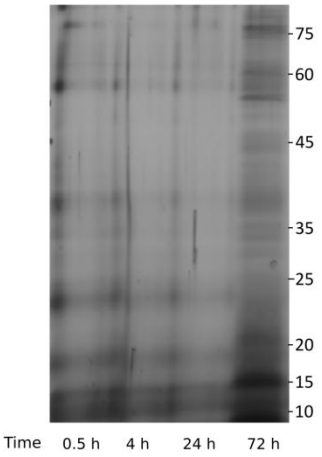

Figure S3. SDS-PAGE and silver staining results of Aβ oligomers formed in the presence of sphingomyelin-rich liposomes at different time points.

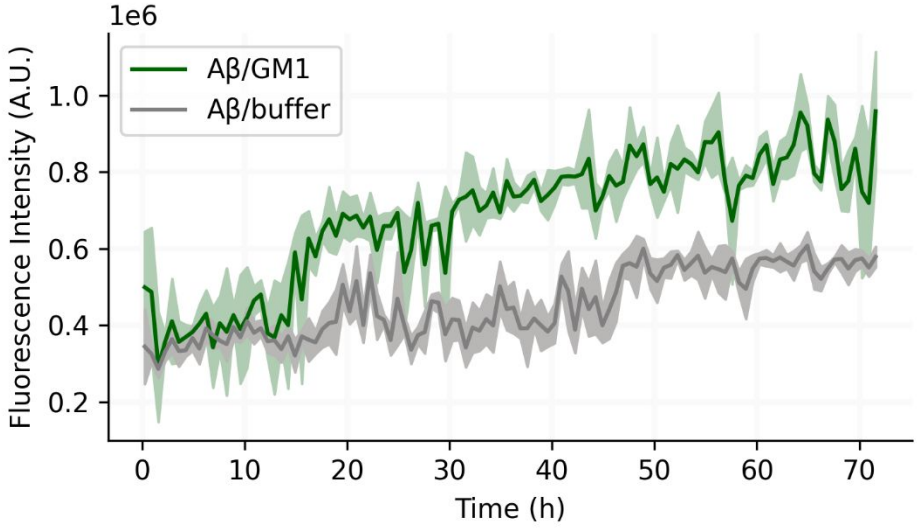

Figure S4. The formation of Aβ fibrils is identified by ThT.

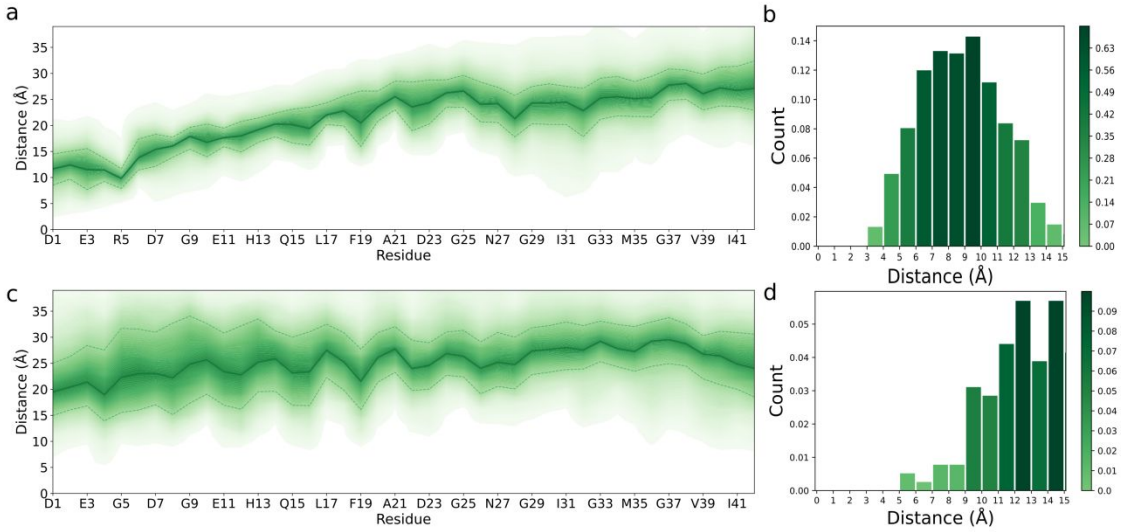

Figure S5. The distances between atoms of each residue in wild type Aβ and atoms of sphingomyelin in the membrane. The upper area edge, the upper dashed line, the solid line, the lower dashed line, and the lower area edge are the 100, 75, 50, 25, and 0 percentiles for the distance between each residue and sphingomyelin. The color corresponds to the percentile. The grey vertical bar indicates the region of 5th residue.

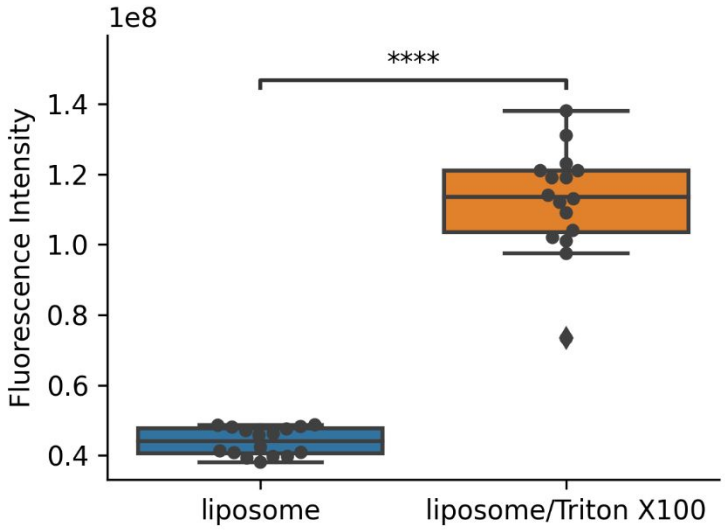

Figure S6. **Validation of the encapsulation of Ca^{2+} in liposomes.** The y-axis is the ratio of the fluorescence intensity of Ca^{2+}-encapsulated liposomes treated by Triton X-100 to the fluorescence intensity of Ca^{2+}-encapsulated liposomes not treated by Triton X-100. Triton X-100 could dissolve lipid and destroy liposomes, thereby releasing Ca^{2+} encapsulated in the liposomes. The fluorescence intensity after adding Triton X-100 is significantly higher than that before adding Triton X-100, suggesting that Ca^{2+} are successfully encapsulated in liposomes. P-value: NS ($0.05 < p <= 1$), * ($0.01 < p <= 0.05$, ** ($0.001 < p <= 0.01$, *** ($0.0001 < p <= 0.001$, **** ($p <= 0.0001$).

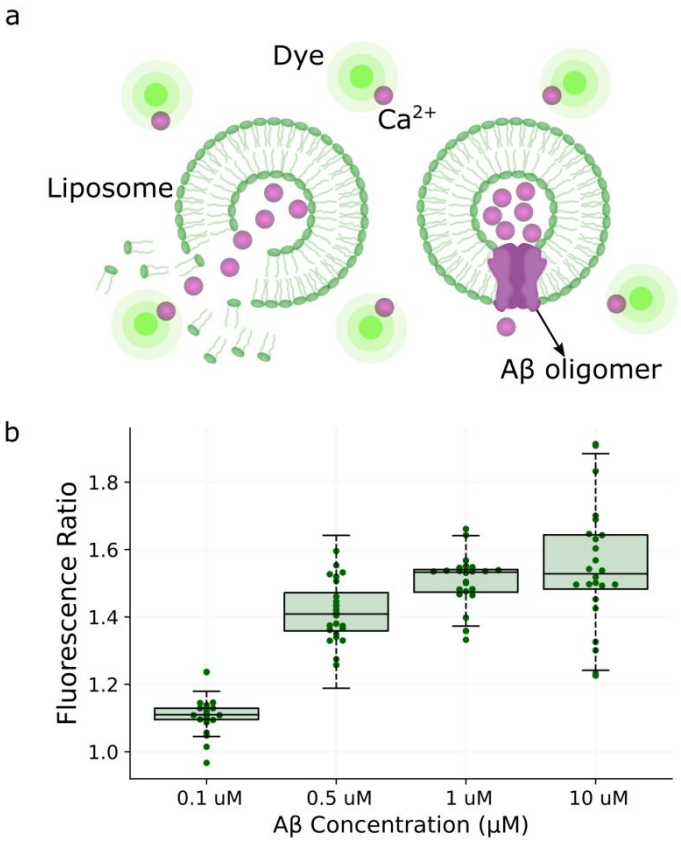

Figure S7. **Validation of the disruption effect of Aβ oligomers on liposome membranes.** (a) Schematic of the experimental. We encapsulate Ca^{2+} in liposomes and incubate them with Aβ of different concentrations. (b) The ratio of the fluorescence intensity of Ca^{2+}-encapsulated liposomes incubated with Aβ to that without Aβ.

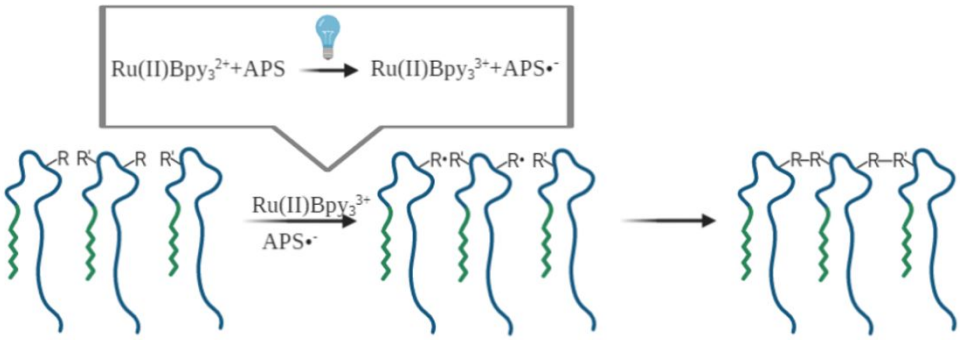

Figure S8. **The schema of PICUP reaction mechanism**. R is the reactive group in Aβ. R' is the adjacent reactive side-chains in Aβ.

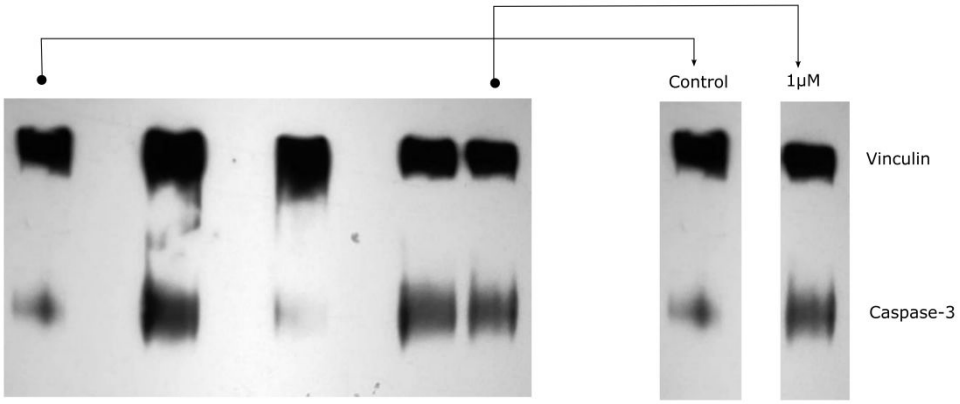

Figure S9. The levels of the apoptotic marker cleaved caspase-3 and vinculin in PC-12 cells. Vinculin is used as the control. PC-12 cells are incubated in the presence (1 μM) or absence of membrane Aβ oligomers. The level of Caspase-3 is measured by western blot.

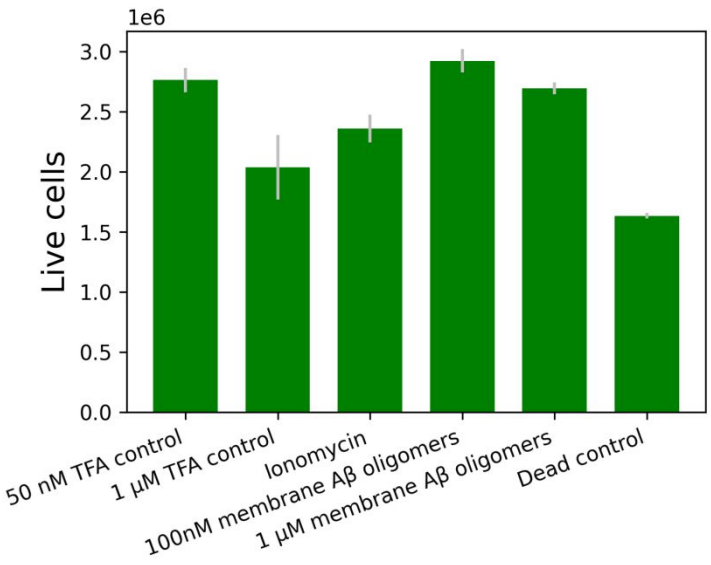

Figure S10. Cell viability and calcium efflux in primary neurons. Characterization of the cell viability of primary neuron cells. The treatment of TFA vehicle and methanol serve as the live control and dead control.

Table S1. The analysis results of the MS data of digested Aβ fibrils and oligomers. Aβ are incubated with or without GM1 liposomes and are digested by pepsin. The analyses are conducted by the Byos software and then exported by the ProteinPilot software. The "liposome fibrils" sheet includes the results of Aβ fibrils formed in the presence of GM1 liposomes; the "solution fibrils" sheet includes the results of Aβ fibrils formed in the absence of GM1 liposomes; the "liposome oligomers" sheet includes the results of Aβ oligomers formed on GM1 liposomes; the "solution oligomers" sheet includes the results of Aβ oligomers formed in the absence of GM1 liposomes.