Supporting Information

Deformation of stable and toxic hIAPP oligomers by liposomes with distinct nanomechanical features and reduced cytotoxicity

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Preparation of hIAPP oligomers

The human islet amyloid polypeptide (hIAPP) with a disulfide bond between the cysteine residues in positions 2 and 7 was purchased from Science Peptide Biolog-ical Technology Co., LTD (Shanghai, China) with a purity of 98%. The fresh hIAPP was prepared as stock solution. Briefly, 2 mg of the hIAPP powder was dissolved in 1 mL of 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFIP, from Tokyo Chemical Industry, Japan). The mixed solution was treat-ed with sonication for 5 seconds, and vortexed three times for 5 seconds each in order to ensure the completion of reaction. After transferred into a thermo-shaker (PHMT, Grant instruments, England), the solution was further shaken overnight at 25 ℃ with a speed of 360 rpm. Then, the solution was stored at -20 ℃ for subsequent utilization. For the production of hIAPP oligomer, 7.8 μL of above hIAPP stock solution was taken into a 1.5 mL centrifuge tube and sealed with the parafilm. After-wards, the tube was stored into a vacuum drying oven (Jinghong Co., Ltd., China) for 1 h at room temperature for removing the solvent, followed by adding ddwater to dissolve the hIAPP film to the final concentration of 20 μM /L. Finally, the tube was placed on the thermo-shaker for 15 min, 120 min and 300 min at 37 ℃ with a speed of 360 rpm.

hIAPP Fibrillization

the similar procedure was used above, except by adding phosphate buffer solution (PBS) (10 mM, pH 7.4) to dissolve the hIAPP film to the final concentration of 20 μM /L.

Preparation of DOPC liposomes

The phospholipid DOPC was used in this experiment, which was purchased from the Avanti Polar Lipids, Inc (Alabaster, AL, USA). Large unilamellar vesicles (LUVs) were synthesized by using the methods described in previous report. In brief, DOPC powder was dissolved in chloroform solution. The organic solvent was removed in vacuum oven overnight. The obtained lipid films were hydrated with 1 mL of phosphate buffer solution (10 mM, pH 7.4) to get the multilamellar vesicles (MLVs). Then, the MLVs were subjected to 10 free-thaw cycles to equilibrate the vesicles with the buffer. Large unilamellar vesicles were prepared from MLVs by extruding the sample 21 times through a 100 nm polycarbonate filter. Size distribution of the as-synthesized DOPC liposomes above were measured by dynamic light scattering (Zetasizer Nano S90, Malvern Instruments) at 25 ℃.

Fluorescence dye leakage

Calcein was dissolved in phosphate buffer to achieve a final concentration of 30 mM. Subsequently, lipid film was hydrated with calcein solution and adequately oscillated to obtain multilamellar vesicles. The sample was frozen and thawed through 7 cycles to obtain multi-layered vesicles (MLVs). After eluted through a filter (220 nm), the suspension in the previous step was treated by a Milli-pore filter to remove the non-encapsulated calcein by high speed rotation for two hours. In the end, phosphate buffer solution was added into the remaining solution to obtain 1 mL vesicle solutions. For the measurement of fluorescence, 50 μL of calcein-liposome solution and 50 μL of phosphate buffer solution were thoroughly mixed to monitor the release of calcein from calcein-containing vesicles by a fluorescence spectrophotometer (F-4500; Hitachi). Likewise, the dye leakage due to part disruption

of lipid vesicles was examined by mixing 50 μL of hIAPP aggregates solution and 50 μL of calcein-liposome solution. All fluorescence assays were performed at room temperature with excitation at 496 nm and emission at 515 nm. Each measurement was repeated three times.

Quartz crystal microbalance (QCM) measurements

All of QCM measurements were conducted on a Q-sense E4 (Biolin Scientific, Sweden). The Au-coated QCM Chips (Au-cut, 5 MHZ) purchased from Dongwei Biotech (Hangzhou, China) were used in the experiment without any pretreatment. Different solutions including DOPC liposomes, phosphate buffer solution and hIAPP aggregates were allowed to flow through the QCM chamber by means of a peristaltic pump at a speed of 50 μL min^{-1}. Initially, the phosphate buffer solution (10 mM, pH 7.4) was injected into the chamber, the frequency shift induced by which was considered to be the baseline. After that, 200 μL of vesicles solution was injected into the flow channel of QCM at 37 ℃. After the frequency stabilizing, another 200 μL of hIAPP aggregates solution was pumped into the chamber. The interaction of liposome with hIAPP aggregates on the surface of Au-coated chip led to QCM frequency shift down. On the basis of the variation of frequency, the interaction between liposomes and hIAPP aggregates can be evaluated.

Thioflavin T (ThT) assay

ThT fluorescence assay was employed to monitor the dynamics of amyloid peptide aggregation and fibrillation. The fluorescence intensity was recorded by using a Hitachi F-4500 fluorescence spectrophotometer (Japan) with excitation at 450 nm and emission at 485 nm, respectively. 7.8 μL of prepared hIAPP solution was desiccated in vacuum and then dissolved in ddwater or PBS to form polypeptide aggregates solution. A total of 150 μL sample solution com-posed of solvent, polypeptide solution and 1 mM ThT solution was added into a 0.1 cm quartz cell for the final measurement. The volume ratio of solvent, polypeptide and ThT was 4:1:1. Meanwhile, the fluorescence intensity of mixed solution of liposomes and oligomers was also tested as described above. All the measurements were repeated in triplicate and averaged the intensity value of every sample.

Circular dichroism (CD) spectra

CD spectra measurements were performed on a spectropolarimeter (JASCO, Hachioji City, Japan) with a model No. PTC-348W1 (JASCO), which is a typical way to characterize the secondary structure of peptides. All experiments were proceeded at room temperature and collected the spectra within a region of 190-250 nm. The slit-width was set at 2 nm and scan speed was 50 nm min^{-1}. For all the samples, the signal of ddwater and phosphate buffer solution were subtracted as the baseline. Each sample was held in the 0.1 cm quartz cuvette with a volume of 300 μL. We tested mixed solution of DOPC liposomes and oligomers as described above. Each CD experiment was repeated three times.

Atomic force microscopy (AFM)

A sample of the hIAPP aggregates solution about 20 μL was deposited on freshly cleaved muscovite mica, and then the residue liquid on the surface was removed after dried in ambient conditions for 10 min. Subsequently, the sample was rinsed three times with ddwater and further dried in air before the measurement. All AFM measurements were performed with a commercial AFM MultiMode VIII (Bruker, Santa Barbara, USA) in a PeakForce-tapping mode with ultrasharp silicon cantilevers (OMCL-AC160TS-R3, Olympus) and a nominal spring constant of 26 Nm^{-1} under ambient conditions. The AFM images were collected with a resolution of 512 × 512 pixels under optimized feedback parameters at a scan frequency of 1 Hz.

On the other hand, quantitative nanomechanical mapping (QNM) primarily focused on the measurements of

nanostructure and nanomechanical properties of the biological samples, mainly including Young's modulus map and adhesion map, etc, which were recorded in such PeakForce QNM mode and performed under atmospheric conditions at a scan rate of 1 Hz (Multimode SPM and Nanoscope V controller, Bruker). The ultra-sharp silicon tips, with a standard spring constant of 200 Nm^{-1} and a typical tip radius of 2 nm, were used for morphology, modulus and adhesion imaging. The AFM cantilevers were calibrated by using a calibration sample of polystyrene film (PSFILM-12M), whose standard Derjaguin-Mueller-Toporov (DMT) modulus was 2.7 GPa. The analysis of DMT modulus and adhesion were accomplished by the Nanoscope Analysis software. In the experiments, a rigorous AFM probe calibration process was performed, and all samples were tested with the same probe under the same conditions.

Transmission electron microscopy (TEM)

The morphology and size of hIAPP aggregated were analyzed by using a Tecnai12 transmission electron microscope (Philips, Netherlands) that operated at an acceleration voltage of 120 KV. In detail, the hIAPP aggregates were deposited on copper rhodium 100 mesh grids with continuous carbon films. Before adding samples, the grids were charged via a glow discharger for 15 s at 30 mA negative charge. Samples were adsorbed to the grids for 2 min prior to rinsing with two drops of 10 µL water for 10 s. In the end, samples for TEM were then stained with a drop of 10 µL freshly filtered 2% uranyl acetate for 30 s and then rinsed twice to re-move the excess uranyl acetate before the measurement.

Cell viability assay

Insulinoma cell line (INS-1 cell) purchased from the Cell Resource Center of Chinese Academy of Medical Sciences (Beijing, China) was cultured in SeraFree Cryopreservation Media medium (RPMI 1640) supplemented with 100 UI mL^{-1} penicillin, 0.1 mg mL-1 streptomycin, 10% fetal bovine serum (FBS), sodium pyruvate (1 nM/L), and β-mercapto-ethanol (50 µM/L)) at 37℃ in humidified (95% air, 5% CO2) atmosphere. The cells were planted into a 96-well microplate with a density of about 8000 cells per well. After being incubated for 48 h, the cells were exposed to the different concentration solutions of PBS as control, hIAPP oligomers and fibril induced by liposomes, and followed by incubating at 37 °C for another 48 h. The cytotoxicity was determined using CCK-8 kits (Dojindo Molecular Technologies, Tokyo, Japan). Absorbance was measured at 450 nm with a Tecan Infinite M200 microplate reader (Tecan, Durham, USA). Each experiment was repeated three times under the same conditions.

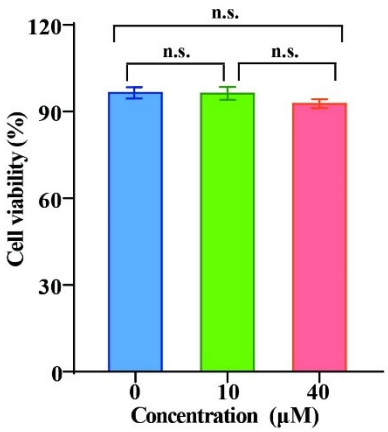

Fig. S1. The cell viability of the hIAPP fibrils in PBS. INS-1 cells were treated by hIAPP fibrils for 48 hours (one-way analysis of variance (ANOVA) with Tukey's correction, not significant (n.s.); error bars are standard deviation, s.d.). 0 □M means the cell treated by PBS solution.

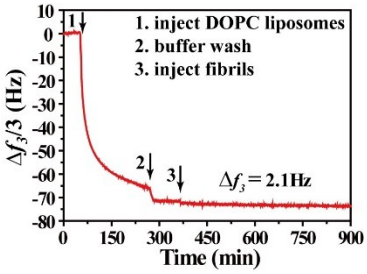

Fig. S2. Real-time QCM diagrams for the interaction between hIAPP fibrils and DOPC liposomes.

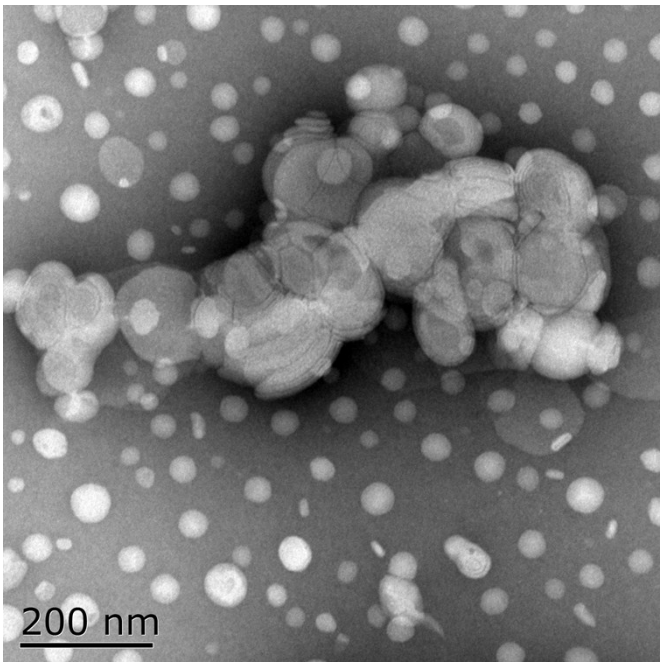

Fig. S3. TEM images of large area DOPC nanoliposome.

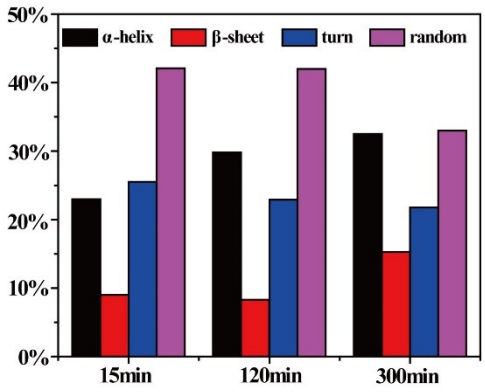

Fig. S4. Detailed secondary structure analysis of hIAPP oligomers with different incubation time.

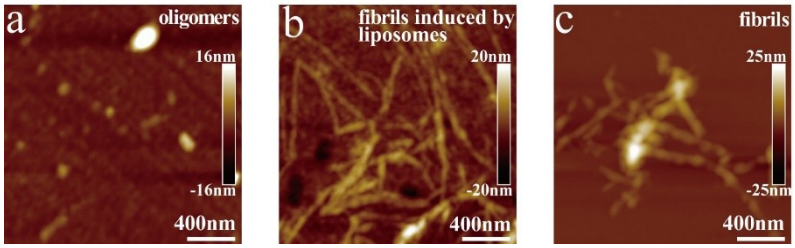

Fig. S5. AFM morphology images of various hIAPP aggregates according to the ones in nanomechanical measurements.