Nanoscale Structural Analysis of a Lipid-Driven Aggregation of Insulin

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Supporting Information

Experimental Section:

Materials: Bovine insulin was purchased from Sigma-Aldrich (St. Louis, MO, USA), 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC or PC), and 1',3'-bis[1,2-distearoyl-sn-glycero-3-phospho]-glycerol (18:0 cardiolipin (CL)) were purchased from Avanti (Alabaster, AL, USA).

Liposome preparation: DMPC and CL large unilamellar vesicles (LUVs) were prepared accordingly to the method reported by Galvagnion et al.[58] Briefly, 0.6 mg of the lipid were dissolved in 2.6ml of phosphate buffered saline (PBS) pH 7.4. Lipid solutions were heated in water bath to ~50 °C for 30 min and then placed into liquid nitrogen for 3-5 min. This procedure was repeated 10 times. After this, lipid solutions were passed 15 times through a 100 nm membrane that was placed into the intruder (Avanti, Alabaster, AL, USA). LUV sizes were determined by dynamic light scattering (DLS).

Insulin aggregation: In the lipid-free environment, 400 μM of insulin was dissolved in PBS; solution pH was adjusted to pH 3.0 using concentrated HCl. For Ins:CL and Ins:PC, Ins:PC:CL (1:0.5:0.5) 400 μM of insulin was mixed with an equivalent concentration of the corresponding lipid; solution pH was adjusted to pH 3.0 using concentrated HCl. Next, the solutions were placed in the plate reader (Tecan, Männedorf, Switzerland) and incubated at 37 °C under 510 rpm for 24 hours.

AFM imaging: AFM imaging was performed using silicon AFM probes with related parameters force constant 2.7 N/m and resonance frequency 50-80 kHz were purchased from Appnano (Mountain View, CA, USA) on AIST-NT-HORIBA system (Edison, NJ). Analysis of collected images was performed using AIST-NT software (Edison, NJ, USA).

AFM-IR: AFM-IR imaging was conducted using a Nano-IR3 system (Bruker, Santa Barbara, CA, USA). The IR source was a QCL laser. Contact-mode AFM tips (ContGB-G AFM probe, NanoAndMore, Watsonville, CA, USA) were used to obtain all spectra and maps. Treatment and analysis of collected spectra was performed in Matlab.

Attenuated total reflectance Fourier-transform Infrared (ATR-FTIR) spectroscopy: Spectra of dry lipid powders were acquired using Spectrum 100 FTIR spectrometer (Perkin-Elmer, Waltham, MA, USA). Three spectra were collected from each sample.

Cell toxicity assays: Mice midbrain N27 cells were grown in RPMI 1640 Medium (Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (FBS) (Invitrogen, Waltham, MA, USA) in 96 well-plate (5,000 cells per well) at 37 °C under 5% CO_2. After 24 hours, the cells were found to fully adhere to the wells reaching ~70% confluency. Next, 100 μL of the cell culture was replaced with 100 μL RPMI 1640 Medium with 5% FBS containing protein samples. After 48 hours of incubation, lactate dehydrogenase (LDH) assay was performed on the cell medium using CytoTox 96 non-radioactive cytotoxicity assay (G1781, Promega, Madison, WI, USA). Absorption measurements were made in plate reader (Tecan, Männedorf, Switzerland) at 490 nm. Every well was measured 25 times in different locations.

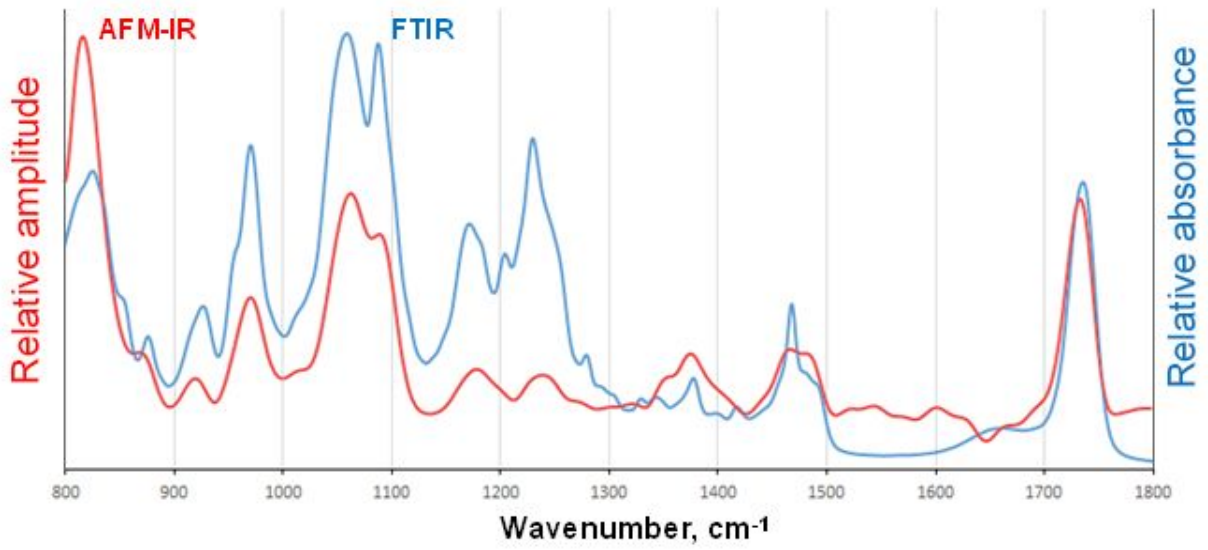

Figure S1. FTIR (blue) and corresponding AFM-IR (red) spectra of PC.

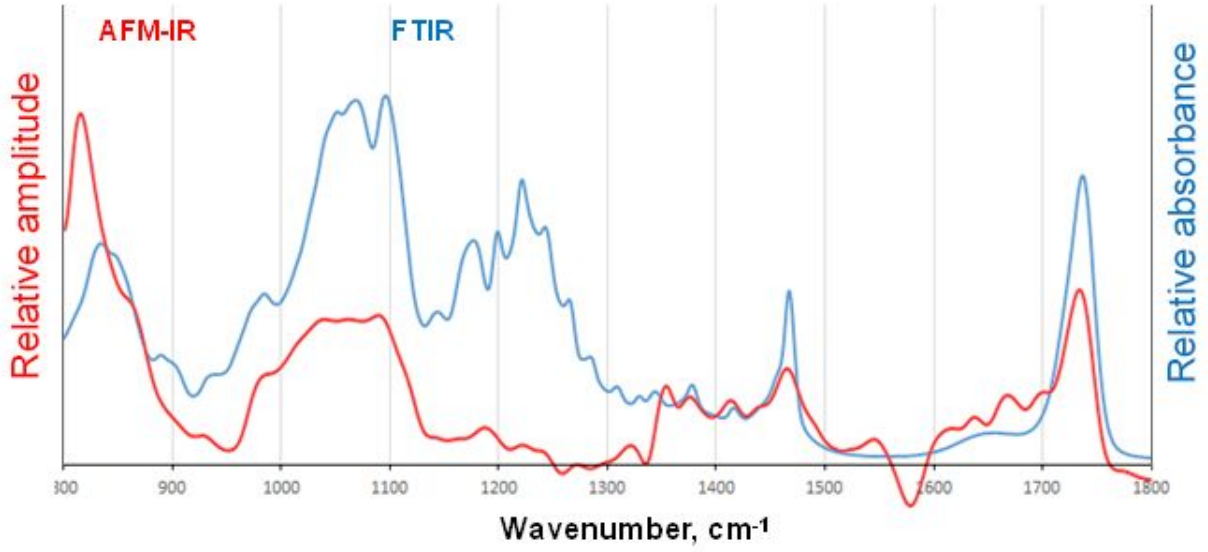

Figure S2. FTIR (blue) and corresponding AFM-IR (red) spectra of CL.