α-Synuclein in α-Helical Conformation at Air-Water Interface: Implication of α-Synuclein Conformation and Orientation Changes During Its Accumulation/Aggregation

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Supplemental Information

Experimental Sections

Materials. Hydrochloride, sodium dihydrogen phosphate, sodium hydroxide, trifluoroacetic acid, sodium chloride, ammonium sulfate, isopropyl β-D-thiogalactopyranoside (IPTG), Trizma base (Tris), and organic solvents were purchased from Thermo Fisher Scientific Inc. (Pittsburgh, PA). *E coli* BL21 (DE3) and dithiothreitol (DTT) were purchased from Invitrogen Corp (Carlsbad, CA) and lysozyme was from EMD Inc. (Gibbstown, NJ). Quartz slides used for Langmuir-Blodgett film deposition of α-syn with dimension of 1.0 × 4.0 cm^2 were purchased from Hellma Cells Inc. (Plainview, NY). All aqueous solutions were prepared using Millipore water (18 MΩ•cm). Tris buffer was made by mixing 20 mM Tris solution with concentrated hydrochloride to pH 7.4. Phosphate buffer was prepared by mixing 20 mM sodium dihydrogen phosphate and 20 mM sodium hydroxide with a ratio of 9/10 (V/V).

Expression and Purification of α-Synuclein. *E. coli* BL21 (DE3) were transfected with pRK172/α-synuclein plasmids kindly donated by Prof. P. Lansbury (Harvard University). The expression of α-synuclein (α-syn) was induced by IPTG. Cells were harvested, resuspended in phosphate buffer solution (pH 7.4), and lysed by lysozyme. Following sonication, the crude α-syn was separated from the precipitate by centrifuge and was mixed with 1 mM DTT after the centrifuge. The crude α-syn was purified by semi-preparative reversed-phase (RP) HPLC (Shimadzu 6AD, Columbia, MO) with a column (Jupiter-10-C18-300, 10 mm i.d. × 250 mm) from Phenomenex (Torrance, CA). The mobile phases were 0.1% trifluoroacetic acid in water (V/V, mobile phase A) and 0.1% trifluoroacetic acid in acetonitrile (V/V, mobile phase B). The elution gradient was 25–75% B for 20 min at a flow rate of 4.7 mL/min. The α-syn elution was lyophilized by a VirTis BenchTop 4 K freeze dryer (VirTis SP Scientific, Gardiner, NY) and the dried powder of α-syn was used to make fresh solutions for all the experiments. The purity and conformation of the fresh solution of α-syn were checked by HPLC and CD respectively before measurements.

Surface Chemistry Study. The surface pressure-area (π−A) isotherm and stability study of the Langmuir monolayer of α-syn were conducted in a Langmuir trough (Nima Technologies, Coventry, UK) with a dimension of 30.0 × 10.0 cm^2 at room temperature. The concentration of the α-syn aqueous solution was 0.30 mg/mL and 70 μL of the α-syn solution were spread at the air-water interface, followed by a 1 hour period for the monolayer formation. The Langmuir-Blodgett films of α-syn were made by transferring the α-syn Langmuir monolayers to quartz slides under the surface pressure 10 mN/m. The quartz slide was moved at 0.3 cm/min.

Circular Dichroism Measurement. The circular dichroism (CD) spectra were measured by a JASCO J-815 spectropolarimeter and a quartz cell of 1-mm path length was used to contain α-syn solution. The spectrum was recorded with a response time of 8 s and a scan speed of 20 nm/min with two accumulations. The CD spectrum of the Langmuir-Blodgett films on quartz slide was also measured under the same condition.

*Infrared Reflection-Absorption Spectroscopy Measurement***.** Infrared reflection-absorption spectroscopy (IRRAS) at the air-water interface was performed on an EQUINOX 55 FTIR spectrometer (Bruker Optics, Billerica, MA) equipped with an XA-511 external reflection accessory designed for the air-water interface experiments. The IR beam was conducted out of the spectrometer and focused onto the air-water interface of the Kibron *μ*-trough (Kibron Inc., Helsinki, Finland). The reflected IR beam was diverted to a HgCdTe (MCT) detector cooled by liquid nitrogen. The bare air-water interface was used as the background for measuring spectra of the α-syn sample. The IRRAS signal was obtained from the following equation: $S = R/R_b$, where R is the reflectance of the α-syn monolayer and R_b is the reflectance of the background. The spectra of both background and sample were acquired with a resolution of 8 cm^{-1} by an average of 1200 scans. The IRRAS measurement was carried out in a clean room [class1000] where constant conditions of temperature (20.0 ± 0.5 °C) and humidity (50 ± 1%)

were maintained. The constant humidity helps to decrease the noise of IRRAS from water vapor vibration.

IRRAS of α-Synuclein on Deuterium Oxide. To exclude that the peaks shown in Figure 3 in the text are from H_2O deforming,[1] deuterium oxide (D_2O) was used as the subphase because the D_2O deforming peak[2] is at ~1210 cm^{-1}. As shown in Figure S1, due to the H/D exchange, the amide II band of α-syn is shifted to 1405 cm^{-1} in the IRRAS[3] It is worth noting that the H/D exchange can also downshift amide I band for 5 ~ 10 cm^{-1}.[1-3] Consequently, the peak of amide I at 1646 cm^{-1} in Figure S1 correlates well to that shown in Figure 3 at 1655 cm^{-1}, which is now clearly verified to be the fingerprint of α-helical conformation in α-syn Langmuir monolayer.

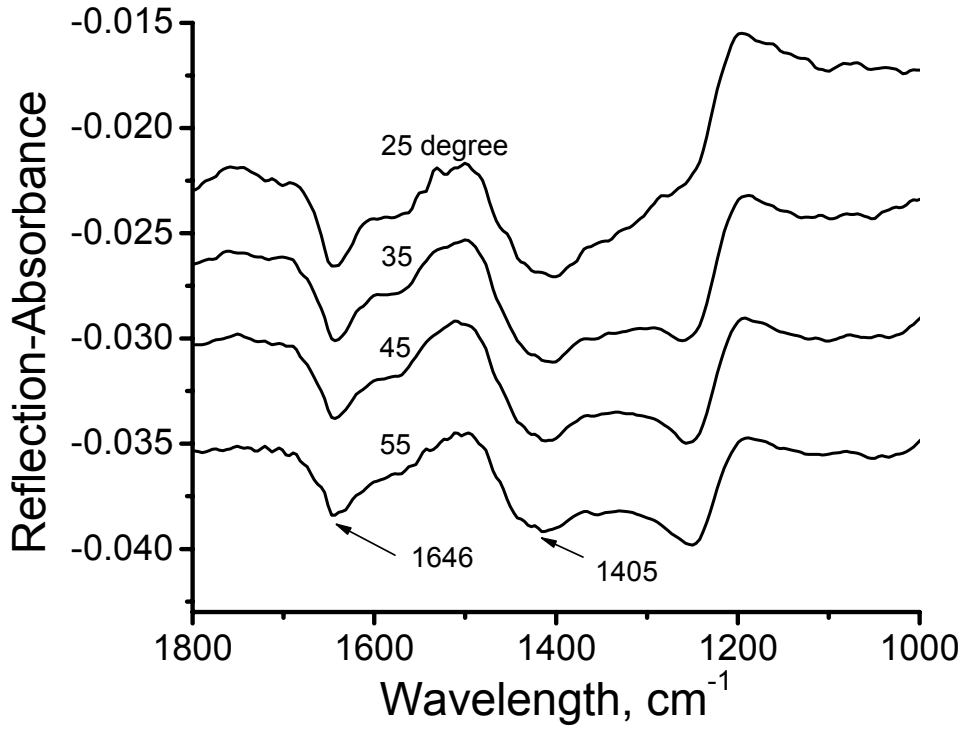

Figure S1. S-polarized IRRAS of α-synuclein Langmuir monolayer at 10 mN/m when the subphase was D_2O containing 0.159 M NaCl.

References

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