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Epi-Fluorescence Imaging at the Air-Water Interface of Fibrillization of Bovine Serum Albumin and Human Insulin

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Supplemental Information

Experimental Sections

Bovine serum albumin (BSA, Catalog No. BSASG100) and human insulin (HI, Catalog No. 193900) were obtained from MP Biomedicals (Solon, OH) and were used as received. 5-(octadecanoylamino)-fluorescein (ODFL) was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were of the highest purity available.

BSA was dissolved in a phosphate buffer with 50 mM NaCl at pH 7.4 to a concentration of 20 mg/mL. HI was dissolved in HCl at pH 2.0 with 100 mM NaCl to a concentration of 1 mg/mL. BSA and HI were incubated at 65 °C for 48 and 14 hours, respectively. Solvents were prepared using deionized water from a Modulab 2020 water purification system (Continental Water Systems Corp., San Antonio, TX). The water had a resistivity of 18.2 MΩ·cm and a surface tension of 72.6 mN/m at 20.0 °C. Both solutions were diluted to 0.33 mg/mL for epi-fluorescence images and surface pressure-area isotherms. Both proteins were further diluted to 0.1 mg/mL for circular dichroism (CD) and fluorescence measurements. Monolayer experiments were carried out in a clean room class 1000, with constant temperature of 20 °C. For surface pressure-area isotherms, a KSV mini-trough (KSV Instrument Ltd., Helsinki, Finland) with an area of 225 cm^2 (7.5 cm × 30.0 cm) was used. The trough had an electronic balance, and used a Wilhelmy plate as a pressure sensor with a sensitivity of 0.02 mN/m. The protein was deposited on a 0.01 M NaCl subphase. Compression speeds of 11 mm/min were used with spreading volumes of 80 μL for both BSA and HI.

ThT in the respective buffers was prepared and used to dilute the protein samples to 0.1 mg/mL and 20 μM of ThT for fluorescence intensity measurements on a Fluorolog-3 spectrofluorometer (Horiba Scientific, Edison, NJ). Both excitation and emission slit widths were

set at 5 nm. Samples were excited at 450 nm with an emission range from 465 to 700 nm. Measurements were taken through a quartz cuvette with a path length of 10 mm.

Epi-fluorescence images were taken at the air-water interfaces on a Kibron μ-trough (Kibron Inc., Helsinki, Finland) with an area of 124.49 cm^2 (5.9 cm × 21.1 cm). A quartz window in the center allowed the light to penetrate the subphase and excite the sample. Images were taken with an Olympus IX-FLA microscope (image size: 895 μm × 713 μm) and captured by a thermoelectrically cooled CCD camera. 100 and 40 μL for BSA and HI, respectively, were deposited at the air-water interface to prepare a monolayer. This was followed 10 min later by a deposition, on top of the protein Langmuir monolayer, of 30 μL of 10^{-6} M 5-(octadecanoylamino)-fluorescein (ODFL) dissolved in $CHCl_3$/ MeOH (9:1, v/v). Circular dichroism (CD) spectra were taken on a JASCO-810 spectropolarimeter using quartz cells with 0.2 cm optical pathlength. Spectra were recorded between 190 and 260 nm at room temperature. Data were analyzed using Dichroweb.[1]

Atomic force microscopy (AFM) images were obtained using tapping mode with an Agilent 5420 AFM instrument (Agilent, Santa Clara, CA). The cantilever used in experiments had a typical force constant of 40 N/m with a resonance frequency of 300 ~ 400 kHz. A few (5 ~ 10) μL of responding diluted protein solution was deposited first on a freshly cleaved mica surface and dried for 2 h in air. After that, the mica was washed carefully using pure water to remove the salt residues. Finally, the washed mica was left for 2 h in the air before scanning. All images were taken at a resolution of 512 × 512 pixels.

(1) Whitmore, L.; Wallace, B. A. *Biopolymers* **2008**, *89*, 392.