Supporting Information

Protein Assembly at the Air-Water Interface Studied by Fluorescence Microscopy

Zhengzheng Liao,[†] Joshua W. Lampe,[‡] Portonovo S. Ayyaswamy,[§] David M. Eckmann,[,⊥] and Ivan J. Dmochowski[*,†]*

[†]Department of Chemistry, University of Pennsylvania, [‡]Center for Resuscitation Science, Department of Emergency Medicine, Hospital of the University of Pennsylvania, [§]Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, [⊥]Department of Anesthesiology and Critical Care, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104.

Corresponding authors: eckmanndm@uphs.upenn.edu, ivandmo@sas.upenn.edu

Table of Contents

Sample Chamber and Surface Modification. The sample chamber for fluorescence microscope imaging was assembled by placing a thin piece of polydimethylsiloxane (PDMS, 1 cm × 1 cm, ~0.5 mm thick) in between two pieces of cover glass. PDMS is fabricated using a commercially available PDMS kit (Sylgard 184, Dow Corning), the elastomer and curing reagent are mixed at weight ratio of 10:1. After thorough mixing and degassing, the liquid PDMS was poured into a Petri dish and cured at 80 °C for 2.5 h. The thin slab of PDMS after curing was peeled off the dish and cut into 1 cm × 1 cm pieces. A 0.7-cm diameter cylindrical hole was punched through the middle of the PDMS, which served as the sample holder. Leaking was prevented by conformal contact between the PDMS and the bottom cover glass. Inner-chamber surface of the bottom cover glass was modified with mPEG-silane (Laysan Bio, Inc., MW 5000) in order to reduce protein adsorption.[1] And inner-chamber PDMS surfaces were oxidized and subsequently modified with the same mPEG-silane, following the literature method.[2] Sample chambers were washed three times with ethanol and deionized water (DI water, Mar Cor Premium Grade Mixed Bed Service Deionization, 18.2 megohm-cm resistivity at 25 °C), blown dry with argon and stored at –20 ºC until used for imaging.

Dye-to-Protein Ratio (DPR) Measurements. DPR was measured by both UV-Vis spectrometer and MALDI-TOF MS. For UV-Vis measurement, labeled proteins were diluted to appropriate concentration and the absorbance values at 280 nm and 595 nm were measured. DPR was calculated from the following equation:

$$DPR = \left(\frac{A_{595}}{\varepsilon_{595}^{TR}} \right) \Bigg/ \left(\frac{A_{280} - A_{595} \times 0.18}{\varepsilon_{280}^{HSA}} \right) \quad (1)$$

where A_{595} and A_{280} are the absorbance at 595 nm and 280 nm measured using a diode-array Agilent 89090A spectrophotometer, ε is the extinction coefficient of TR or HSA provided by manufacturer

(Invitrogen) and from literature,[3] and 0.18 is a correction factor to account for the contribution of the dye absorbance at A_{280}.[4] The DPR calculated following equation (1) was 1.3.

MALDI-TOF MS measurements were performed with a Bruker Daltonic Ultraflex III MALDI-TOF-TOF mass spectrometer, in a mass range from m/z 10,000-160,000. Labeled proteins were diluted with HPLC grade water before loading onto sample plate. The protein solution (1 μL) was mixed with 1 μL of matrix solution (saturated sinapinic acid in 50% CH_3CN, 49.9% HPLC grade H_2O and 0.1% TFA, in volume percentage) by repeated pipetting. After sample droplets were dried, on-plate washing was performed by spotting 0.1% TFA solution on dried sample spot for 10 s then removing excess liquid.

Circular Dichroism (CD) Spectroscopy. CD signal was converted into mean residue ellipticity [θ] following equation (2).[5]

[θ], in deg·cm^2·dmol^{-1} = (millidegrees × mean residue weight)/(pathlength in millimeters × concentration in mg·mL^{-1}) (2)

Melting temperature T_m was obtained by converting data points in thermal denaturation curves following equation (3) and (4) and fitting into equation (5) [6]:

$$K = \frac{[\theta]_N - [\theta]}{[\theta] - [\theta]_D} \quad (3)$$

$$\Delta G(T) = -RT \ln K \quad (4)$$

$$\Delta G(T) = \Delta H_m (1 - T/T_m) + \Delta C_p [(T - T_m - T \ln(T/T_m)] \quad (5)$$

where $[\theta]_N$ is ellipticity value of the protein under native conditions, $[\theta]_D$ is the ellipticity value of the protein under fully denatured conditions and $[\theta]$ is the measured ellipticity value at temperature T. K is the equilibrium constant, $\Delta G(T)$ is the Gibbs free energy at temperature T, R is the gas constant, T_m (melting temperature) is the midpoint of the thermal denaturation, and ΔH_m is the enthalpy change at $T = T_m$.

Supplemental Figures S1-S5

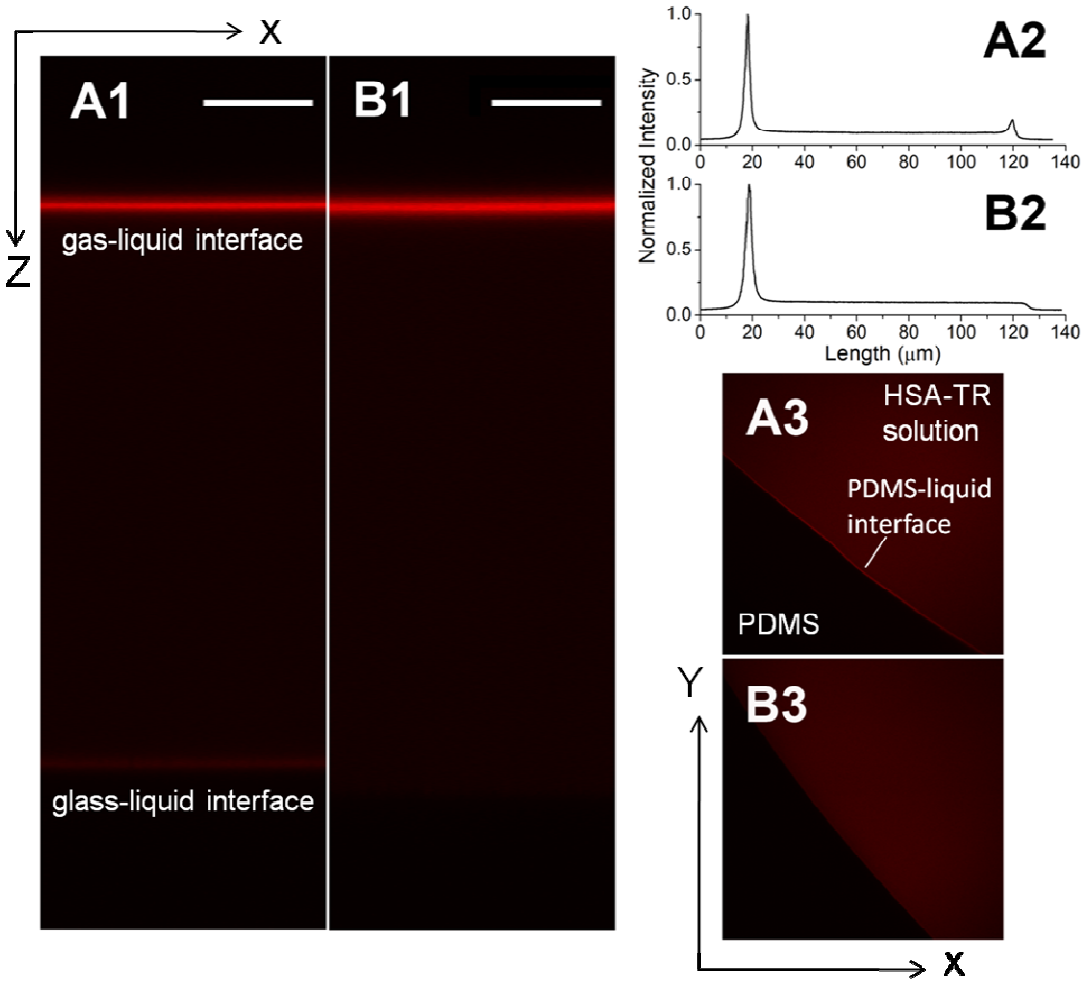

Figure S1. Confocal fluorescence micrographs of HSA-TR in sample chamber before (A) and after (B) modification of chamber with PEG. XZ-plane images of HSA-TR solution ([HSA-TR] = 0.10 mg/mL in PBS buffer) in unmodified (A1) and modified (B1) sample chamber. (A2, B2) Intensity plot along z-axis of Figure S1.A1-B1. XY-plane images focused at the edge of unmodified (A3) and modified (B3) chamber ([HSA-TR] = 0.50 mg/mL in PBS buffer). Before surface modification with PEG, adsorption of HSA-TR on solid-liquid interface was observed, which was shown as a small peak of fluorescence intensity from HSA-TR at solid-liquid interface (Figure S1.A2). After surface modification, no surface excess was observed at the solid-liquid interface by confocal imaging.

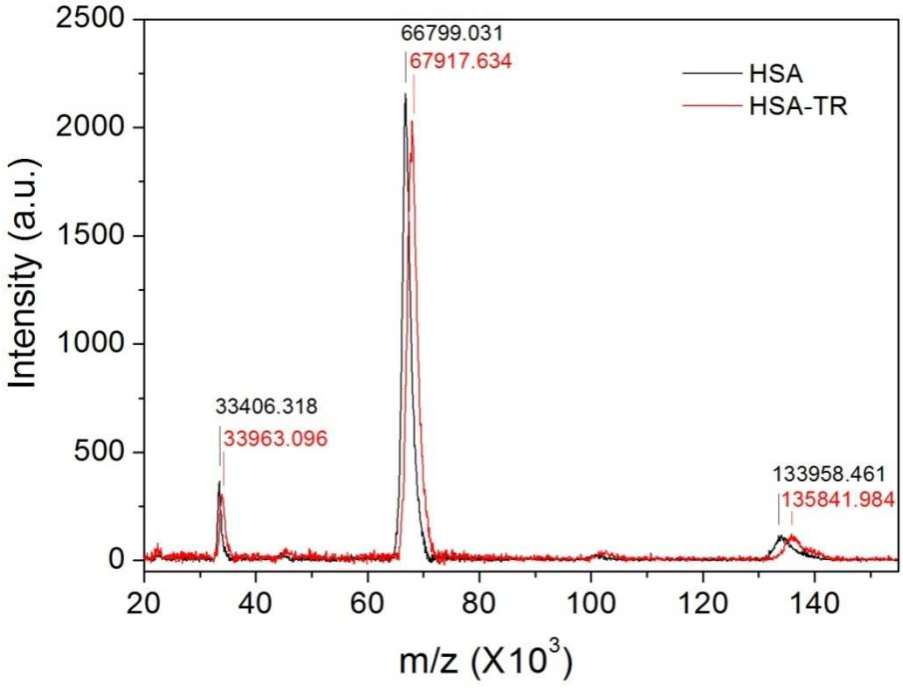

Figure S2. MALDI-MS spectra of HSA (black) and HSA-TR (red). From left to right, the peaks are assigned to $A + 2H^+$, $A + H^+$, and $2A + H^+$ for HSA and HSA-TR, respectively, where "A" represents the protein molecule. Using $A + H^+$ peaks of HSA and HSA-TR: $\triangle m = m(HSA\text{-}TR) - m(HSA) = 1119$. Because MW(TR) = 723 g/mol, DPR = $\triangle m/MW(TR) = 1.5$.

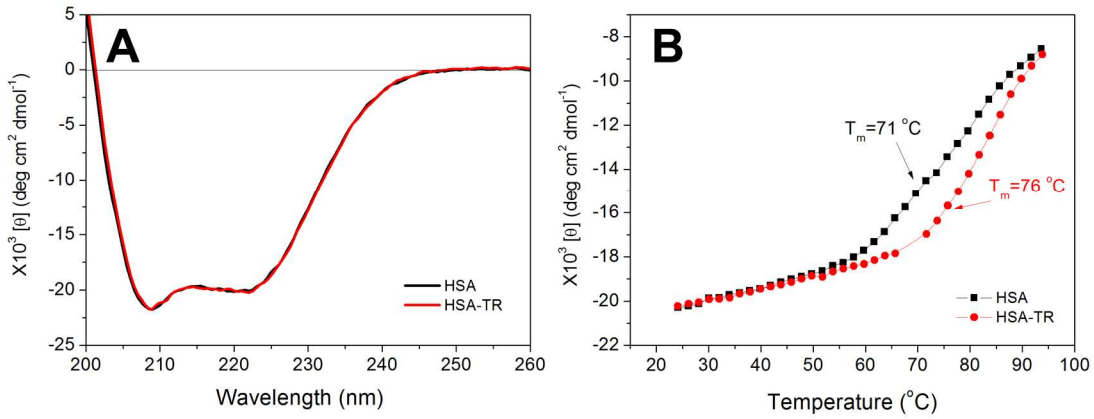

Figure S3. Characterization of HSA and HSA-TR by CD spectroscopy. (A) Far-UV CD spectra of HSA (black) and HSA-TR (red) at 25 °C. (B) Thermal denaturation curves of HSA (black) and HSA-TR (red). Far-UV CD spectra of HSA and HSA-TR overlap with each other well (Figure S3.A), indicating that TR does not cause detectable changes to the characteristic α-helical structure of HSA. In thermal denaturation curves (Figure S3.B), HSA-TR appears to be more thermally stable. The melting temperature of HSA-TR is 5 ºC higher than that of HSA. We hypothesize that Texas Red dye can interact with ligand binding sites in HSA, and thus enhance the structural stability of the protein.

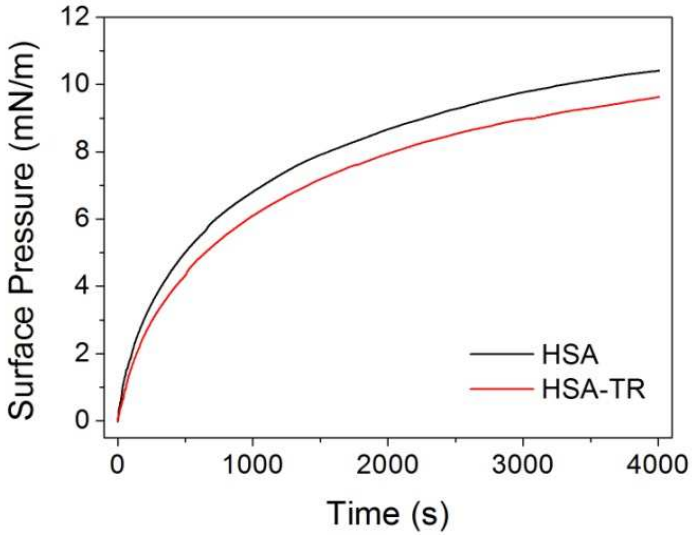

Figure S4. Surface pressure of HSA and HSA-TR (0.10 mg/mL in 10 mM PBS buffer). Each curve is the average of two parallel experiments. Surface pressure of HSA is slightly higher than that of HSA-TR, suggesting that HSA is more efficient at displacing water from the AWI. However, the difference is within 1 mN/m, which is equivalent to the experimental error.

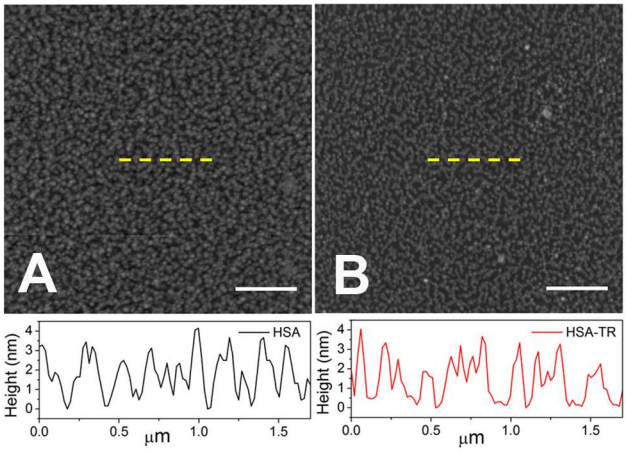

Figure S5. AFM images of HSA and HSA-TR films formed at the AWI and transferred to mica surface. Initial subphase concentrations were 0.10 mg/mL. Top row: AFM images of (A) HSA, (B) HSA-TR. Scale bar: 1.0 µm. Bottom row: height of protein films plotted along yellow dashed line drawn in (A, black) and (B, red). The thickness of the protein layer is around 3 nm, which is in accordance with the "height" of a HSA molecule.

References

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