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Supplemental Information

The Conformation of Interfacially Adsorbed Ranaspumin-2 Is an Arrested State on the Unfolding Pathway

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Supplemental Material: The Conformation of Interfacially Adsorbed Ranaspumin-2 is an Arrested State on the Unfolding Pathway.

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Dynamic Light Scattering

Dynamic light scattering (DLS) was performed using an ALV-5000 spectrometer-goniometer equipped with an ALV/LSE-5004 digital multiple tau correlator and 632.8 nm He-Ne laser. The scattering signal was collected at a detection angle of 90°. All experiments were performed at 20°C. Data was analysed using ALV-Correlator software; the correlation function was fitted using regularization analysis to yield a size distribution of hydrodynamic radii. Size distribution plots were created using intensity as the y-axis.

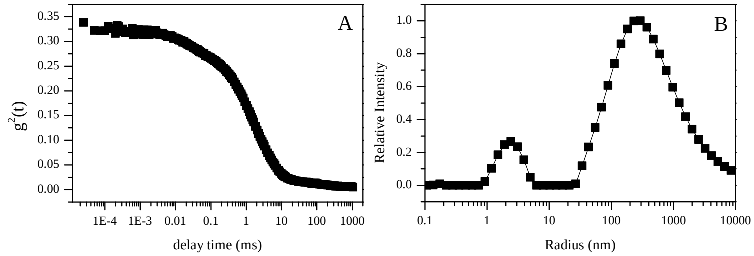

Figure 1: DLS of Rsn-2

Rsn-2 does not form an elastic layer at the interface

We investigated the surface activity of Rsn-2 by first measuring surface pressure-area (Π-A) isotherms in a Langmuir trough. Surface pressure is defined relative to a reference surface tension, $\Pi = \gamma_0 - \gamma$, where γ_0 is the surface tension of the pure subphase and γ is the surface tension of surfactant and subphase. In these experiments, movable barriers reduce the area of the subphase on which the surfactant can form a layer, thus increasing the surface concentration. An experiment with a constant compression rate is shown in Fig.2A. The transition from a high rate of surface pressure change to a lower one occurs approximately at a surface pressure value of 18 mN/m. The kink observed at this surface-pressure value is indicative of surface layer collapse. In addition we measured the area-pressure isotherms (Fig.2C) by compressing the surface layer by equal area intervals (10 cm^2), and then allowing the surface pressure to equilibrate. The slopes of the equilibrated isotherms after each equilibration step gives an indication of the elasticity of the surface layer and is given by,

$$\epsilon = -\frac{d\Pi}{d\ln A} \tag{1}$$

where ϵ is the surface dilatational modulus, Π is the surface pressure and A is the interfacial area. We find that a sizeable decrease in the elasticity, which then maintains a constant value, occurs at the area in which we find the kink in the isotherm. Again, this decrease in elasticity is indicative of surface layer failure and occurs at the same transition surface-pressure as the experiments in Fig. 2A.

To better understand the mechanism of surface layer collapse we performed successive compression and expansion cycles which are shown in Fig.2C. First, it is clear that the compression-expansion cycles show significant hysteresis. Moreover, after each complete expansion, the surface pressure value does not return to the starting value of the previous compression but is lower. Second, the surface pressure at the kink in the isotherm remains the same for each compression cycle and is the same value that we observed in Fig.2A & B. However, for each successive compression, this value occurs at smaller trough areas. For elastic surface layers where there exists connectivity between the constituents of the surface film, one finds that surface layer collapse coincides with the appearance of wrinkles and a distinct compression-expansion

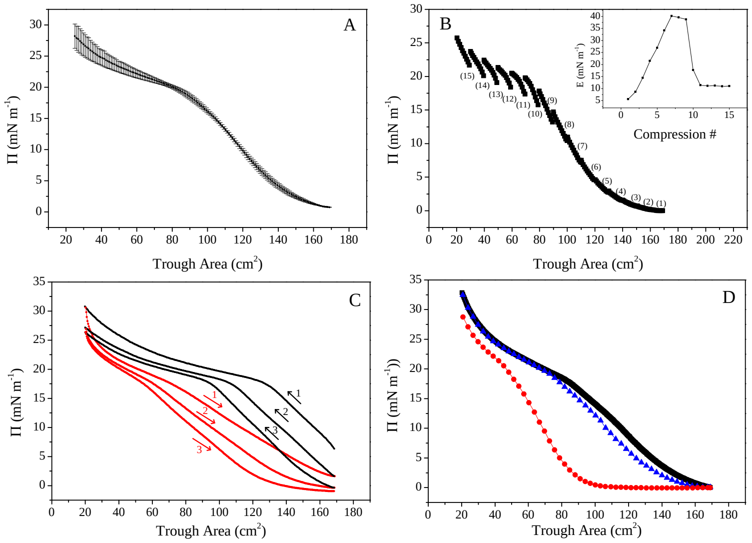

Figure 2: Properties of Rsn-2 at an air-water interface. (A) Surface pressure area isotherm for Rsn-2 under constant compression. The error bars are the standard deviation of the surface pressure for three repeats. (B)Equilibrated surface pressure isotherms, where each equilibration step is numbered. In these experiments, the surface is compressed at 10 cm^2 intervals and then allowed to equilibrate for 3 minutes. The inset shows the elasticity of the surface layer for each compression step (Eq.4). (C) Repeated continuous compression (black) and expansion (red) cycles of Rsn-2 film shows a clear hysteretic response. (D) Consecutive compressions of Rsn-2 film with varying equilibration times: first compression (black squares), 0.5 hrs after first compression (red circles), 3 hrs after second compression (blue triangles).

hysteretic response that differs from what we observe for Rsn-2 [1]. The behavior we observe for Rsn-2 is better attributed to the expulsion of protein from the interface into the subphase [1, 2, 3] and is suggestive that Rsn-2 does not form a cohesive elastic film.

Indeed, Fig. 1D is further evidence for protein loss from the interface due to compressions. After an initial compression, the barriers are immediately expanded and the system is allowed to equilibrate for 0.5 hrs. After this time, another compression is performed and we find that, as above, the isotherm has shifted to lower trough areas. However, if we then re-expand after this compression and re-equilibrate for 3 hours, the subsequent compression superimposes on the first compression. This shows that the origin of this shift can be attributed to loss of protein from the interface, which can then diffuse back to the interface. Interestingly, this result is suggestive that Rsn-2 adsorption is reversible in that it can be expelled from the interface and re-form a film with nearly identical characteristics as the initial film.

References

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