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Supporting Material

X-Ray Diffraction and Reflectivity Validation of the Depletion Attraction in the Competitive Adsorption of Lung Surfactant and Albumin

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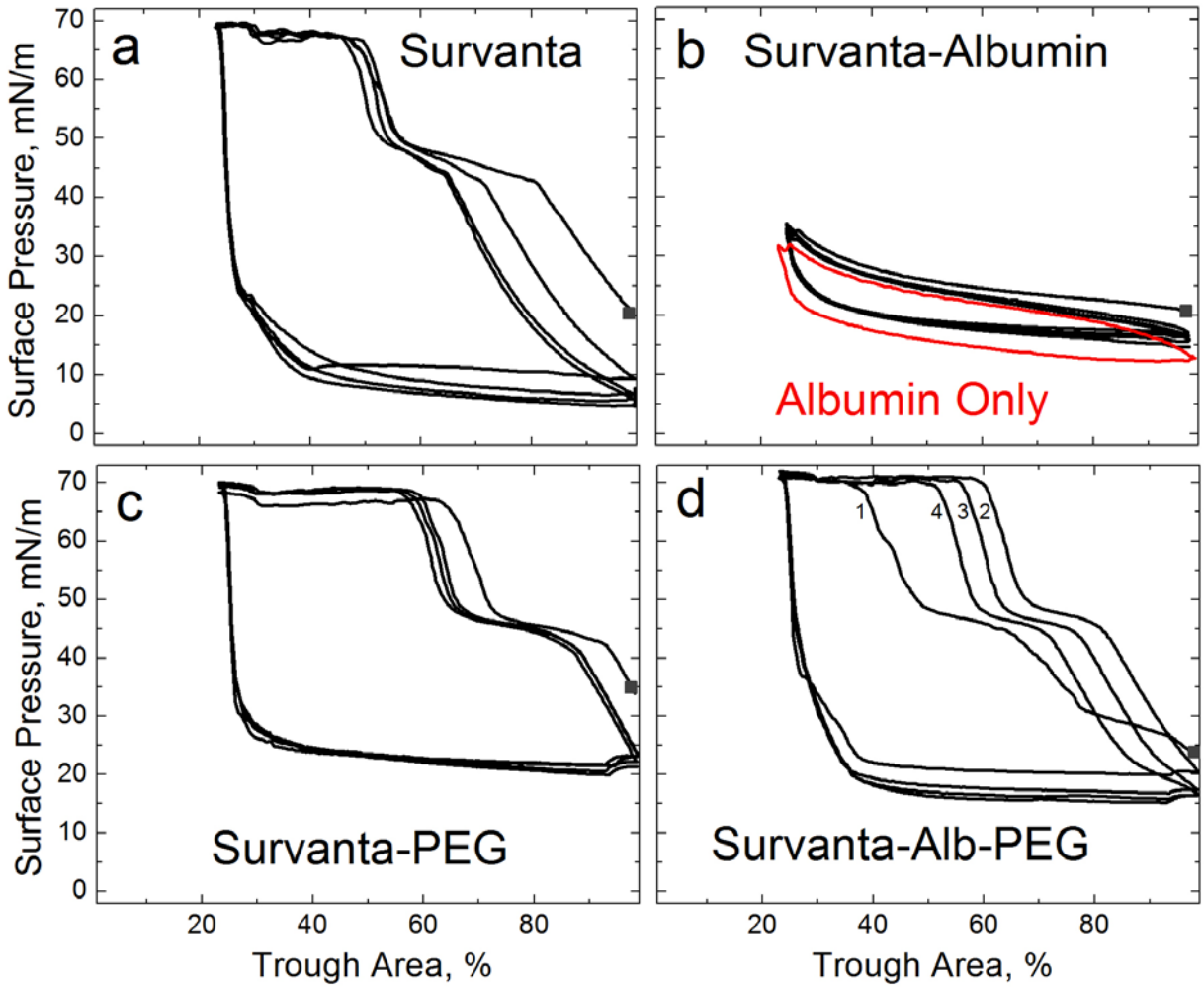
Supplemental Information

The surface pressure-area isotherms in Fig 1. were recorded at 20°C using a stainless steel ribbon trough equipped with a Wilhelmy plate (Nima, Coventry, England). To initiate each experiment, an albumin and/or polymer containing buffer (150 mM NaCl, 2 mM $CaCl_2$, 0.2 mM $NaHCO_3$, pH 7) in the Langmuir trough was allowed to equilibrate for 10 minutes; for albumin-containing subphases, Π increased to ~18 mN/m. Survanta was diluted in the same buffer to a lipid concentration of 2 mg/mL and was deposited as microliter drops from a syringe by touching the drop to the air/water interface of the open trough. The subphase was not stirred and the first compression began 20 minutes after deposition.

Fig. 1a shows a typical compression-expansion cyclic isotherm for the control subphase (180 μg of Survanta with no albumin or PEG in the solution). The collapse plateau at Π_{max} ~ 68 mN/m determines the minimum surface tension (~ 4 mN/m) of the film. When the same amount of Survanta is deposited onto a subphase containing 2 mg/mL albumin (Fig. 1b, black curve), the surface pressure does not increase above 35 mN/m even after repeated expansion and compression cycles over several hours. Both the compression and expansion isotherms are indistinguishable from albumin alone (Fig. 1b, red curve). In comparison, Fig. 1c shows that the Survanta isotherm on a subphase containing 5% wt. 10 kDa PEG has the same features as the control (Fig. 1a). PEG does not alter the collapse pressure or characteristic shape of the isotherm, but it does increase surfactant adsorption to the interface; the collapse pressure of 68 mN/m is reached at 60% trough area compared to 50% for the control (Fig. 1a). As the relationship between surface pressure and area/molecule is fixed for a given surfactant composition and temperature (See Table 1), this means that the total amount of surfactant at the interface has increased. After two compression-expansion cycles, the isotherm of 180 μg Survanta deposited onto a subphase containing 2 mg/mL albumin and 5% wt. 10 kDa PEG (Fig. 1d) resembles that in Fig. 1c, showing that PEG helps Survanta displace albumin from the interface (compare to Fig. 1b).

Figure 1 Caption

Cyclic isotherms of 180 μg Survanta. For all plots, the beginning of the first compression cycle is denoted with a solid square. (**a)** Survanta on the control subphase (no albumin or PEG). **(b)** Black curve: 180 μg Survanta deposited onto a saline buffer subphase containing 2 mg/mL albumin. Red Curve: The isotherm for the albumin subphase, with no Survanta. **(c)** Survanta on a subphase containing 5% wt. PEG. **(d)** 180 μg Survanta on a subphase containing 2 mg/mL albumin and 5% wt. 10 kDa PEG. Compression cycles are labeled 1-4 in chronological order.


a
Survanta
b
Survanta-Albumin
Albumin Only
c
Survanta-PEG
d
Survanta-Alb-PEG
1
4
3
2
Surface Pressure, mN/m
Trough Area, %
0
10
20
30
40
50
60
70
20
40
60
80