Electronic Supplementing Information

Counteracting the inhibitory effect of proteins towards lung surfactant substitutes: A fluorocarbon gas accelerates displacement of albumin by phospholipids at the air/water interface

Phuc Nghia Nguyen,[a] Mariam Veschgini,[b] Motomu Tanaka,[b] Gilles Waton,[a] Thierry Vandamme[c] and Marie Pierre Krafft*[a]

[a]Systèmes Organisés Fluorés à Finalités Thérapeutiques (SOFFT). Institut Charles Sadron (CNRS UPR 22). University of Strasbourg. 23 rue du Loess. 67034 Strasbourg Cedex 2. France.

[b]Physical Chemistry of Biosystems, Institute of Physical Chemistry - University of Heidelberg. Im Neuenheimer Feld 253. 69120 Heidelberg. Germany.

[c] Laboratoire de Conception et Application de Molécules Bioactives (CNRS UMR 7199). University of Strasbourg. 74 route du Rhin, 67401 Illkirch Cedex. France.

Materials and experimental methods

Materials. L-*α*-1,2-dipalmitoyl-*sn*-3-glycero-phosphatidylcholine (DPPC, 99% purity) and bovine serum albumin (BSA, 66.43 kD) were purchased from Sigma and used without further purification. Perfluorohexane (*F*-hexane, purity >99%) was from Sigma. Texas Red-conjugated Bovine Serum Albumin was purchased from Molecular Probes. A solution of Hepes buffer (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid, from Sigma) (20 mM) in 150 mM NaCl was prepared and its pH adjusted to 7.4 with 0.1 N NaOH. The buffer's equilibrium surface tension was 69.5 ± 0.2 mN m^{-1} at 37°C. Water was obtained from a MilliQ (Millipore) system (surface tension: 71.7 ± 0.2 mN m^{-1} at 20°C; resistivity 18.2 MΩ cm). All measurements were made at 37°C and repeated three to five times. The errors bars represent the standard deviations of the data.

Preparation of phospholipid dispersions. For bubble profile analysis tensiometry experiments, dispersions of DPPC in the Hepes buffer (10^{-3} mol L^{-1}, 50 mL) were sonicated (30 min) until they became transparent. For adsorption experiments in the Langmuir trough, more concentrated dispersions of DPPC in Hepes buffer (26 10^{-3} mol L^{-1}, 20 mL) were sonicated (2 h) until the dispersions became transparent.

Dynamic light scattering (DLS). A Malvern Zetasizer Nano ZS was used at 25°C for DLS measurements at a scattering angle of 90°. The *z*-averaged hydrodynamic mean diameter of the DPPC vesicles used for the tensiometry experiments was determined using the Malvern software to be 60-80 nm and the polydispersity was 20%.

Profile analysis tensiometry. Axisymmetric bubble shape analysis was applied to a rising bubble of air formed in the DPPC dispersion. Care was taken to only use vesicle dispersions having close mean diameters (~80 nm) and narrow size distributions. The time dependence of the interfacial tension during adsorption of the phospholipid and protein at the gas/liquid interface was measured using a Tracker® tensiometer (Teclis, Longessaigne, France, see J. Benjamins, A. Cagna, E.H. Lucassen Reynders, *Colloids Surf. A* 1996, *114*, 245). The bubble (5 µL) was formed at the end of a steel capillary with a tip diameter of 1 mm. Since the experiments lasted for up to 25 h, a lid was fitted on the measuring glass cell (10 mL) to prevent evaporation of water during the long equilibration times. It

was carefully determined that the systems had reached equilibrium at the end of each experiment.

Saturation of the bubble with *F*-hexane in the bubble configuration. A 1 mL syringe was purged three times with the *F*-hexane-saturated air that surmounts liquid *F*-hexane at 25°C (*F*-hexane saturated vapour pressure and concentration at 25°C are 2.9 10^4 Pa and 11.66 mol m^{-3}, respectively; water solubility at 25°C: 2.7 10^{-4} mol m^{-3} (A. Kabalnov *et al. Ultrasound Med. Biol.* 1998, *24*, 739.). The syringe was mounted on the injection cell of the tensiometer and the rising bubble formed.

Oscillating bubble measurements. Oscillations were produced by a position-encoded motor and transmitted to the bubble through a piston coupled to the syringe carrying the capillary. The oscillatory regime was applied from the beginning of the experiment on, once the intended bubble volume had been attained. The bubble was set under sinusoidal oscillations with a period T of 10 s and surface variation amplitude ΔA of 15%. Temperature was regulated at 37°C. These conditions were kept constant throughout the experiments.

Adsorption in the Langmuir trough. Surface pressure/area isotherms were measured using a KSV NIMA Langmuir Minitrough made of Teflon and equipped with two moving barriers and a glass window for microscopy. A water bath circulator was used to regulate the temperature at (37 ± 1)°C. The evaporated water was replaced continuously during the experiments. The Langmuir trough was enclosed in a Plexiglas chamber, equipped with gas in- and outlet. The barriers were subjected to sinusoidal oscillations with a period T of 33 s and surface area variation amplitude ΔA of 15%. For all experiments highly concentrated solutions of BSA (6.7 10^{-5} mol L^{-1}, 2 mL) and DPPC (26 10^{-3} mol L^{-1}, 20 mL) were prepared and injected into the sub-phase yielding a final concentration of 7.5 10^{-7} mol L^{-1} for BSA and 3 10^{-3} mol L^{-1} or 1 10^{-3} mol L^{-1} for DPPC. For BSA and DPPC isotherms, an equivalent volume of the sub-phase was removed before injection of the concentrated sample solutions to keep the sub-phase volume constant. To investigate the competitive adsorption of BSA and DPPC at the interface, first BSA was injected into the Hepes sub-phase and allowed to adsorb to the interface. DPPC was added 1.5 h after the injection of BSA. Surface pressure was measured by means of a Wilhelmy-plate balance.

Saturation of the atmosphere with *F*-hexane in the Langmuir trough configuration. A flow of nitrogen was led into the chamber through three subsequent bubbling into liquid *F*-hexane. *F*-hexane was allowed to adsorb at the interface for 0.5 h before BSA was injected into the sub-phase.

Fluorescence microscopy. The Langmuir trough was mounted on an Eclipse TE2000-U Nikon microscope. A X-Cite 120 Metal Halide lamp was used as illumination source. A BSA solution containing 2 mol% Texas Red-labeled BSA was used for fluorescence microscopy.

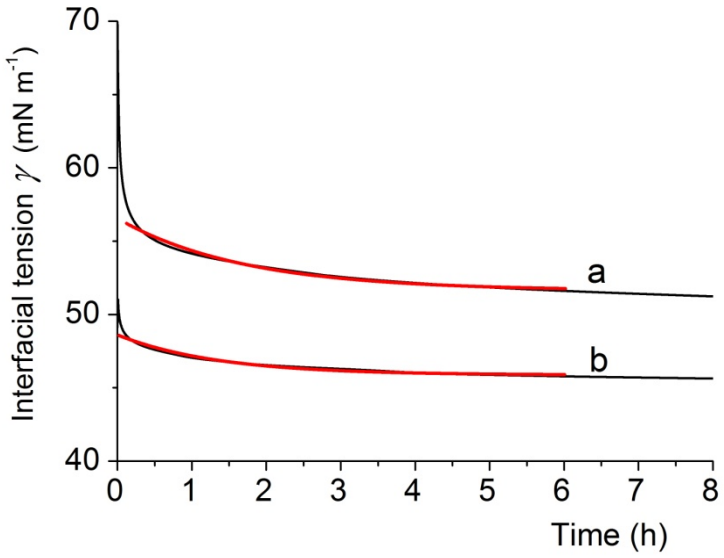

Figure S1. Kinetics of adsorption at 37°C of BSA (7.5 10^{-7} mol L^{-1}) at the surface of an oscillating bubble (T = 10 s, ΔA = 15%) of a) air and b) *F*-hexane-saturated air. The characteristic times for the transfer of the BSA molecules from the buffer solution to the interface, t_1, were obtained by fitting the curves with an exponential decay function $y = y_0 + Ae^{-t/t_\alpha}$ in the region of interest (in red).

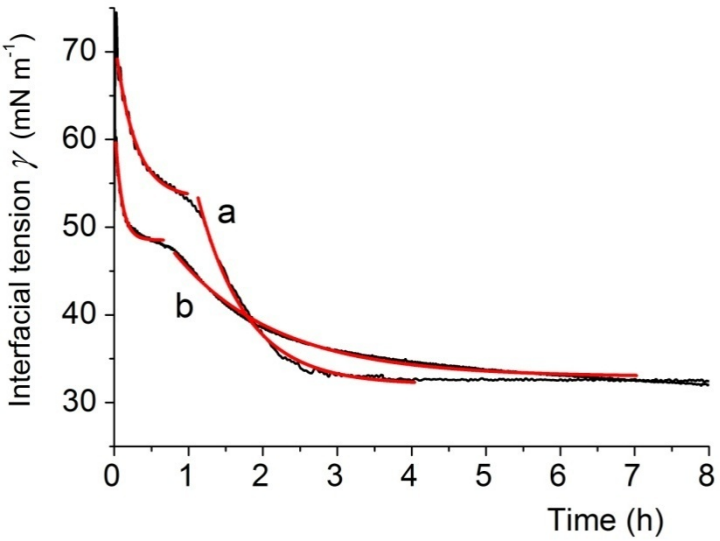

Figure S2. Kinetics of adsorption at 37°C of DPPC (10^{-3} mol L^{-1}, as a dispersion of vesicles) at the surface of an oscillating air bubble (T = 10 s, ΔA = 15%) of a) air and b) *F*-hexane-saturated air. The characteristic times of the first and second regimes (t_1 and t_2) were obtained by fitting the corresponding portions of the experimental curves with an exponential decay function $y = y_0 + Ae^{-t/t_\alpha}$ (in red).

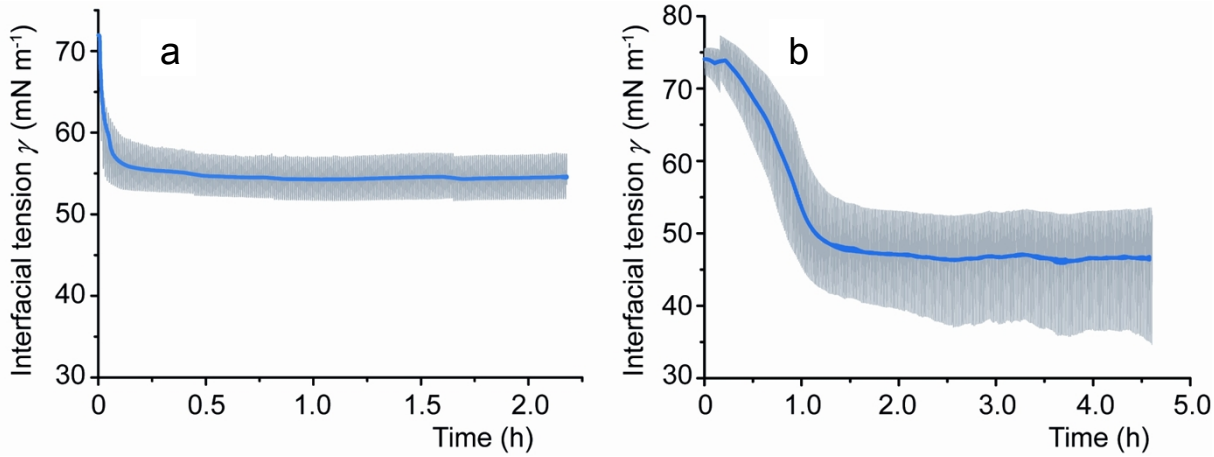

Figure S3. Adsorption kinetics of a) BSA and b) DPPC vesicles at a planar air/water interface. BSA ($7.5\ 10^{-7}$ mol L^{-1}) and DPPC ($1\ 10^{-3}$ mol L^{-1}) were injected in the Hepes buffer sub-phase of the Langmuir trough. The interfacial films were submitted to sinusoidal oscillations transmitted through the barriers of the Langmuir trough (T 33 s; ΔA 15%). The grey area represents the fluctuations in interfacial tension associated with the oscillations. The blue line corresponds to mean values obtained by treating the data through a low-pass digital filter.

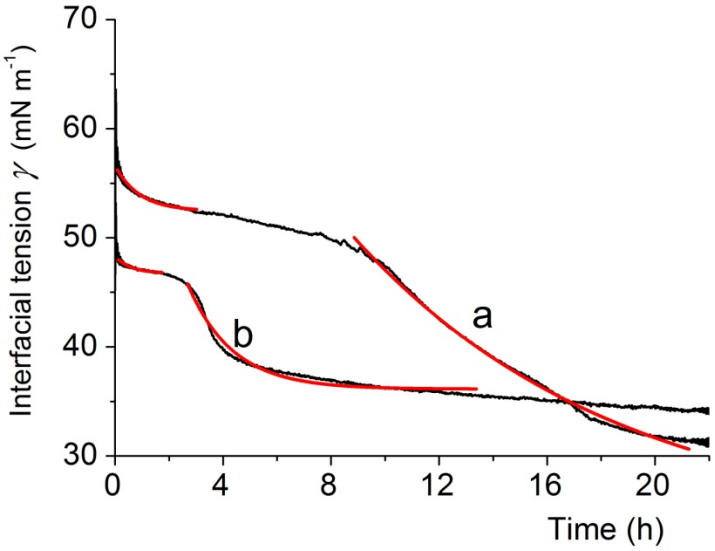

Figure S4. Competitive adsorption kinetics (37°C) of DPPC ($1\ 10^{-3}$ mol L^{-1}) and BSA ($7.5\ 10^{-7}$ mol L^{-1}) at the surface of a) an air bubble and b) an *F*-hexane-saturated air bubble. The bubble was submitted to oscillations (T 10 s, ΔA 15%). The characteristic times of the first and second regimes were obtained by fitting the corresponding portions of the experimental curves with an exponential decay function $y = y_0 + Ae^{-t/t_\alpha}$ (in red).

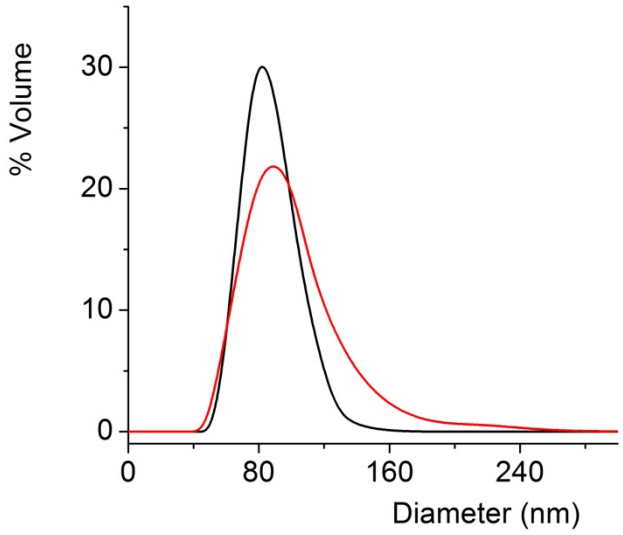

Figure S5. Size distribution of DPPC vesicles immediately after preparation (black), and after 22 h (red) at 37°C, as determined by dynamic light scattering.

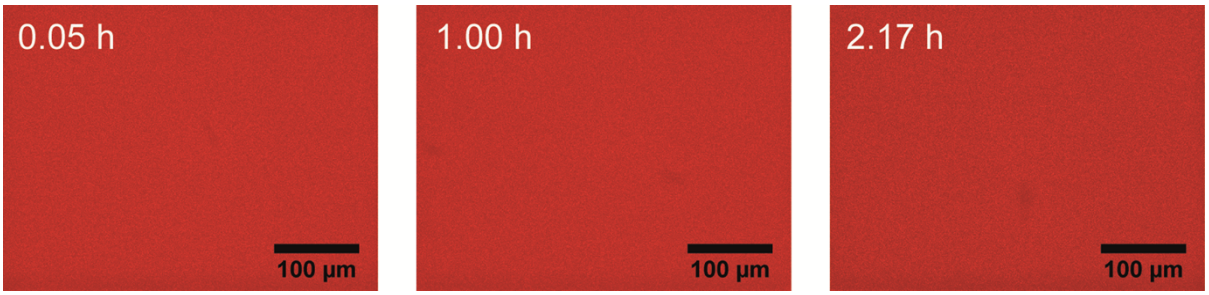

Figure S6. Fluorescence micrographs of BSA doped with 2 mol% Texas Red-conjugated BSA adsorbed at the air/buffer interface at various time points. The interfacial film was subjected to sinusoidal oscillations (T 33 s; ΔA 15%). The BSA concentration was: 7.5 10^{-7} mol L^{-1}.