Supporting Information

Reversing the course of the competitive adsorption between a phospholipid and proteins at an air/water interface

Phuc Nghia Nguyen,[a] Gilles Waton,[a] Thierry Vandamme[b] and Marie Pierre Krafft*[a]

[a] Institut Charles Sadron (CNRS UPR 22). University of Strasbourg. 23 rue du Loess. 67034 Strasbourg Cedex 2. France.

[b] 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. 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 70 ± 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. Dispersions of DPPC (10^{-3} mol L^{-1}, 50 mL) were sonicated (30 min) until they 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 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.

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 periods of 4 s to 100 s and a surface variation amplitude of 15%. Temperature was regulated at 37°C. These conditions were kept constant throughout the experiments.

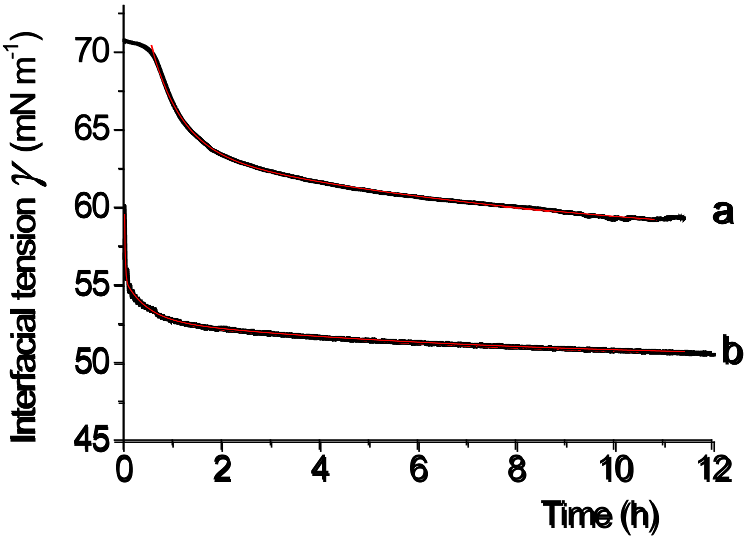

Figure S1. Kinetics of adsorption of BSA at the surface of a static air bubble at 37°C. The characteristic times for the transfer of the BSA molecules from the buffer solution to the interface were obtained by fitting the curves (in red) with a decay exponential function $y = y_0 + Ae^{-xt}$ in the region of interest. The BSA concentrations were: a) 1.5 10^{-8} mol L^{-1} and b) 7.5 10^{-5} mol L^{-1}. For the lowest concentration, the kinetic barrier due to the diffusion of the BSA molecules in the aqueous phase to the interface is clearly visible.

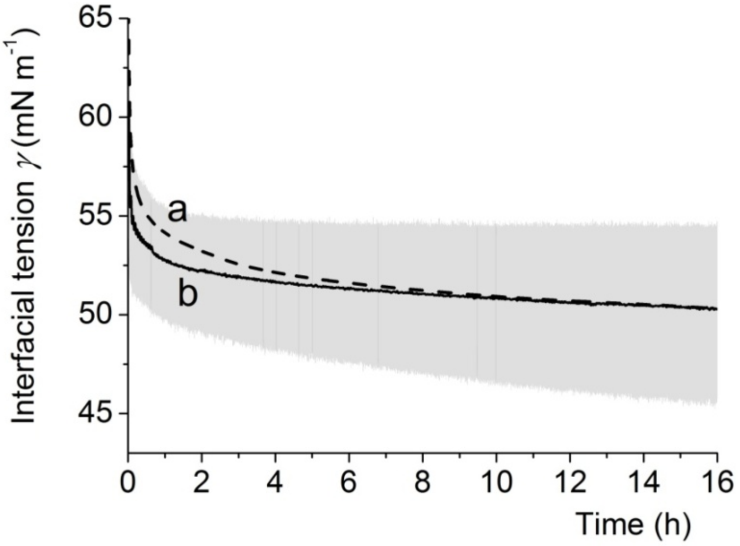

Figure S2. Kinetics of adsorption of BSA (7.5 10^{-7} mol L^{-1}) at the surface of an air bubble at 37°C. The bubble was a) static (dotted line); b) submitted to oscillations (solid line; $T = 10$ s, $\Delta A = 15\%$). The oscillatory regime was applied for 16 h. The grey area represents the fluctuations in interfacial tension associated with the oscillations. The solid line corresponds to mean values obtained by treating the data through a low-pass digital filter.

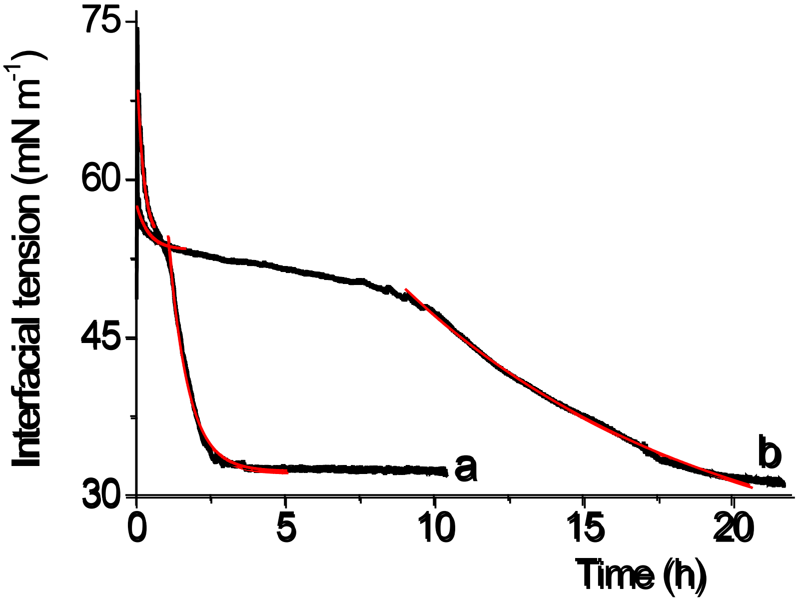

Figure S3. Kinetics of adsorption (37°C) at the surface of an air bubble of a) DPPC alone (10^{-3} mol L^{-1}); and b) a DPPC/BSA (1 10^{-3}:7.5 10^{-7} mol L^{-1}) combination. DPPC was provided as an aqueous dispersion of vesicles. The bubble was submitted to oscillations (*T* 10 s, Δ*A* 15%) for 10 to 22 h throughout the experiment. The characteristic times of the first and second regimes were obtained by fitting the corresponding portions (red) of the curves with a decay exponential function $y = y_0 + Ae^{-xt}$.

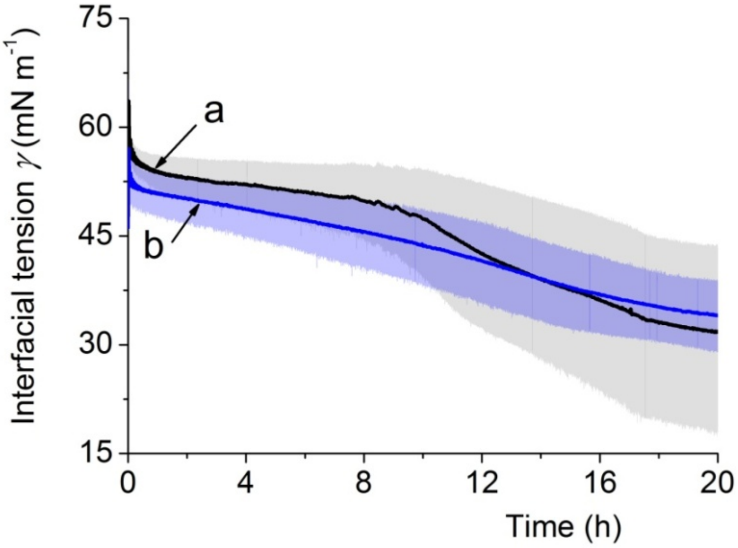

Figure S4. Kinetics of adsorption of a DPPC/BSA mixture at the surface of an air bubble at 37°C. The DPPC/BSA concentrations were: a) 1 10^{-3}:7.5 10^{-7} mol L^{-1}, and b) 1 10^{-3}:1.5 10^{-6} mol L^{-1}. The bubble was submitted to oscillations (*T* = 10 s, Δ*A* = 15%) for 20 h. The lightly colored areas represent the fluctuations in interfacial tension associated with the oscillations. The solid lines correspond to mean values obtained by treating the data through a low-pass digital filter.