AFM-based force-clamp monitors lipid bilayer failure kinetics

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Supporting Information

Supported lipid bilayers under force-clamp force spectroscopy

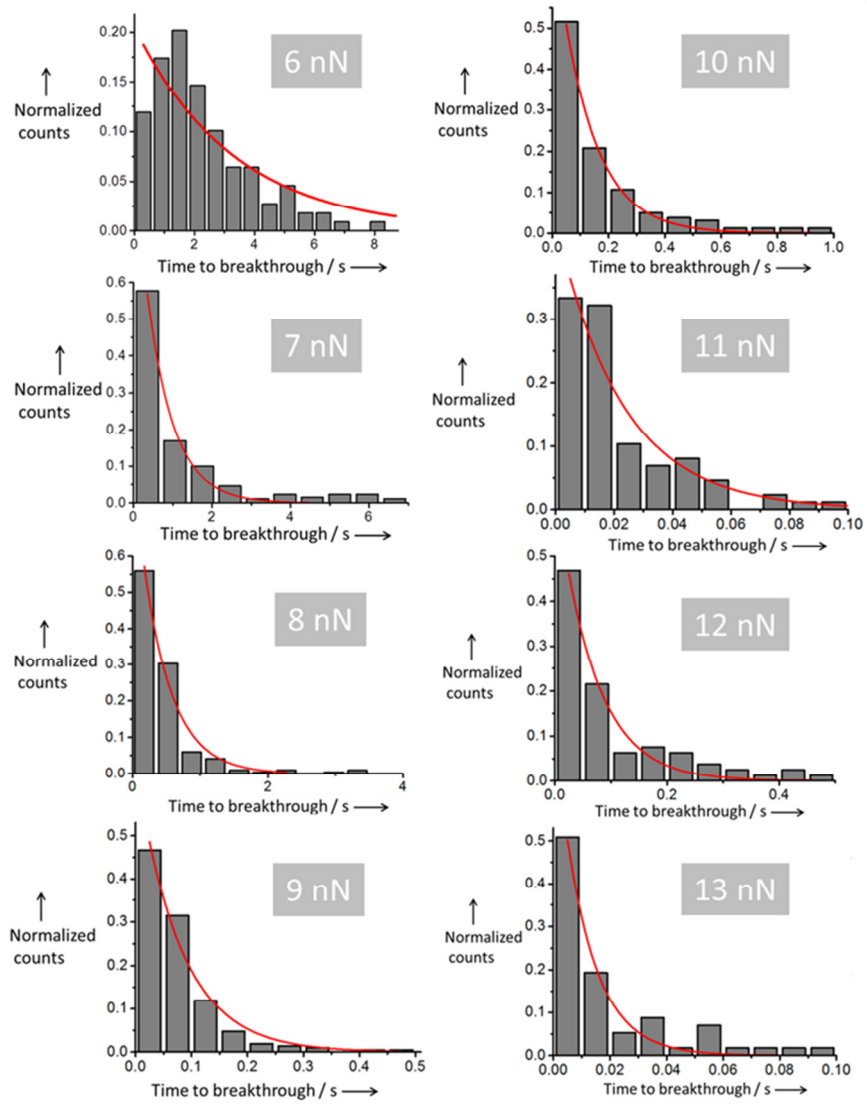

Figure S1. Normalized histogram plots of the time to breakthrough, t_b, of DPPC SLBs under force-clamp conditions, for each particular F_c (6-13 nN). The histograms are fitted to an exponential decay (continuous line).

Table S1. Parameters from the exponential decay fitting to the normalized histograms of the t_b, of DPPC SLBs under force-clamp conditions, for each particular F_c (6-13 nN); (shown in Figure S1). Standard errors of τ are only a minor contribution to the total error of the rupture rate, including the experimental error, and were not considered in the Arrhenius-Bell fitting (Figure 3a).

Force-clamp value (F_c, nN)	**Mean lifetime (τ, s)**	**Standard Error**	**Adjusted R^2**	**# counts**
6	3.309	0.687	0.760	109
7	0.670	0.060	0.983	263
8	0.424	0.050	0.975	223
9	0.079	0.007	0.976	212
10	0.124	0.007	0.991	166
11	0.023	0.004	0.905	95
12	0.068	0.008	0.963	86
13	0.011	0.001	0.954	66

Activation energy calculation by means of constant-velocity force spectroscopy (Dynamic Force Spectroscopy)

In a dynamic force spectroscopy experiment on indentation of SLBs, it has been well-established that the mean breakthrough force F_b increases linearly with the logarithm of the loading rate (r) [1,2] (Equation S1). In this case, the rupture activation energy of the bilayer rupture in absence of force can be calculated taking into account the universal relation between the force dependence of the activation energy ΔE and the force dependence of the loading rate proposed by Butt *et al.*[1] (Equation S2).

$$F_0 = a + b \ln r \qquad \text{Equation S1}$$

$$\Delta E(F_0) = -k_B T \ln\left[\left(\frac{0.693K}{A}\right)\frac{dr}{d\,F_0}\right] \qquad \text{Equation S2}$$

In the present work, we use the data obtained by means of Dynamic Force Spectroscopy at different loading rates to compare the activation energy values with those obtained by means of AFM-FC. Figure S3 shows the experimental mean F_b values measured as a function of three different r (0.1, 1 and 10 μm·s^{-1}). We provide this data for DPPC in the same experimental conditions that AFM-FC experiments performed in this work. The dash line corresponds to the fitting to the Equation S1. The constants a and b were determined from the fit (a = 16.61 ± 0.30 nN, b = 1.68 ± 0.14 nN). All numbers given were obtained with loading rates in units of μm·s^{-1}.

Taking into account Equation S2, the activation energy in Equation S2 is expressed as:

$$\Delta E(F_0) = -k_B T \ln\left(\frac{1.60K}{Ab} r\right) = k_B T \left[2.30\frac{a-F_0}{b} - \ln\left(\frac{1.60K}{Ab}\right)\right] \qquad \text{Equation S3}$$

When this relation is extrapolated to zero mean breakthrough force (F_0 = 0), it provides the intrinsic activation energy of the lipid bilayer rupture in absence of applied force (ΔE_0). For A = 6 kHz and a, b obtained from the experimental results, we obtain for DPPC mica-SLB a ΔE_0 value of 11.8 k_BT (that is 29.4 kJ mol^{-1} at 300K).

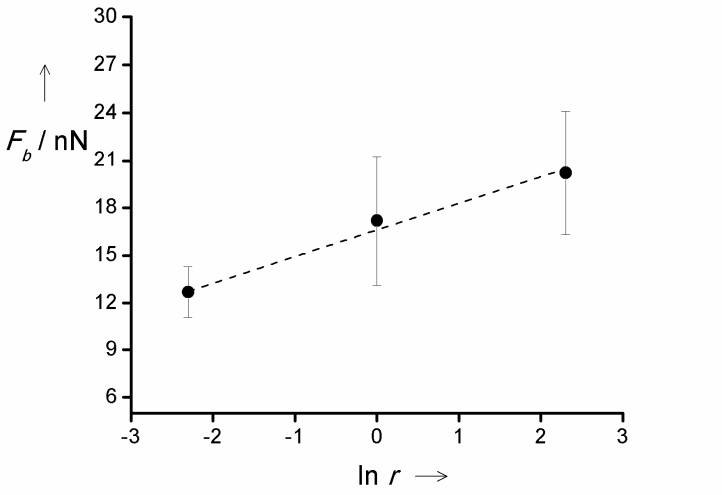

Figure S2. Dependence of the F_b on loading rate for DPPC SLB in buffer solution consisting of 20 mM HEPES and 150 mM NaCl (pH = 7.4) at room temperature. The dashed line corresponds to the fitting to equation S1.

Dynamics of the deforming surface: model, determination of d_s

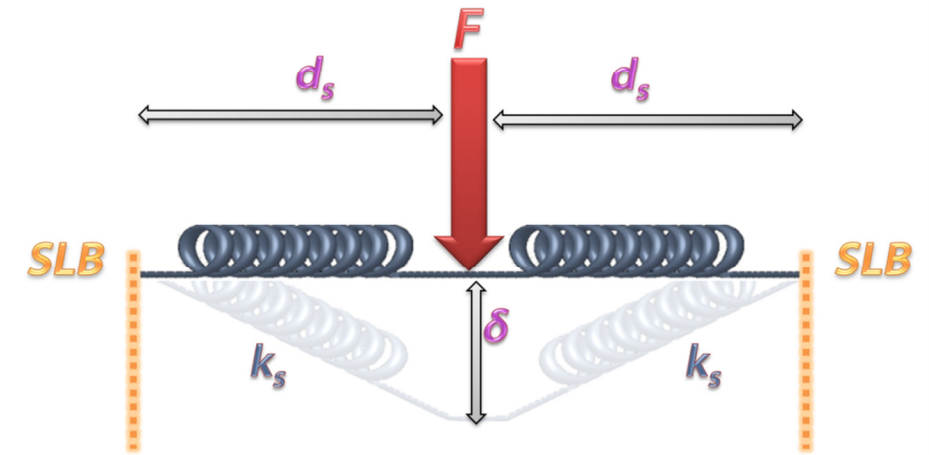

Figure S3. Schematic diagram of the F(δ) model described in the text and represented by Equation 4.

Supported lipid multibilayers (SLMs) under force-clamp force spectroscopy: statistical analysis of the separation steps.

AFM indentation on an SLM under constant force conditions (AFM-FC) yields a staircase-like penetration, with each step in the separation-time trace marking the lipid failure of a single bilayer (as shown in Figure 4b). The height of the different steps (separation) observed was analyzed and the average values obtained are displayed in Figure S6 and summarized in Table S3. We applied the Student's t test, considering that the mean separation is 3.53 ± 0.47 nm. For a p = 0.05, the population mean of both the layer facing the mica substrate and the layer towards the liquid interface are significantly different for the test mean (3.53). For the intermediate layers, the population means are not significantly different with the test mean.

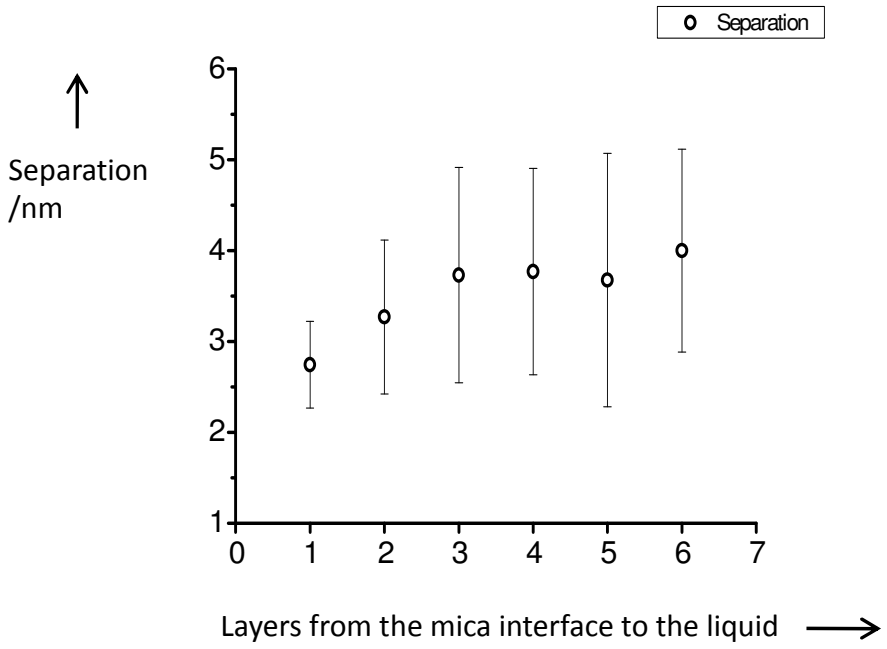

Figure S4. Separation step average from the separation-time graph obtained by AFM-FC as a function of the layer number (from the one in direct contact with the mica substrate (1) towards the one facing the liquid (6)) for a DPPC SLM system in 20 mM HEPES and 150 mM NaCl, pH = 7.4.

Table S2. Descriptive statistics of the separation steps from the separation-time graph obtained by AFM-FC as a function of the layer number for DPPC SLMs in 20 mM HEPES and 150 mM NaCl, pH = 7.4

Bilayer number	Counts	Mean separation (nm)	Standard deviation (nm)
Mica +1	51	2.75	0.48
+2	27	3.27	0.85
+3	43	3.73	1.19
+4	51	3.77	1.14
+5	51	3.67	1.40
Facing the liquid	52	4.00	1.12

Supported lipid multibilayers under force-clamp force spectroscopy

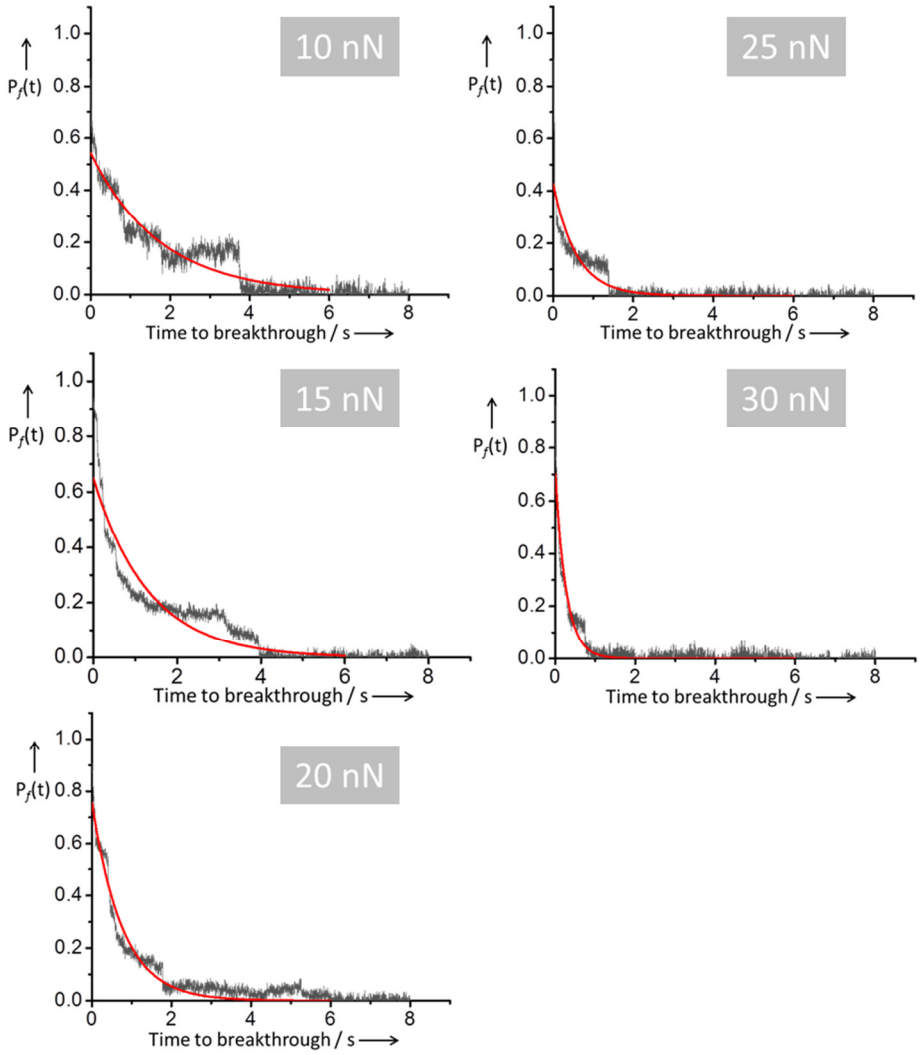

Figure S5. Normalized average time course (ATC) of lipid failure for DPPC SLMs under force-clamp conditions for each particular F_c (10-30 nN). Each ATC corresponds to the summation of 4-5 individual separation-time recordings. The ATCs are fitted to an exponential decay (continuous line).

Table S3. Parameters from the exponential decay fitting to the normalized ATCs for DPPC SLMs under force-clamp conditions, for each particular F_c (10-30 nN) shown in Figure S2. Standard errors of τ are only a minor contribution to the total error of the rupture rate, including the experimental error, and were not considered in the Arrhenius-Bell fitting (Figure 3b).

Force-clamp value (F_c, nN)	**Mean lifetime (τ,s)**	**Standard Error**	**Adjusted R^2**
10	1.757	0.012	0.882
15	1.325	0.010	0.873
20	0.759	0.004	0.943
25	0.583	0.006	0.823
30	0.274	0.002	0.932

References

1. H. J. Butt, V. Franz, *Phys. Rev. E* **2002**, *66,* 031601.
2. a) S. Loi, G. Sun, V. Franz, H.-J. Butt, *Phys. Rev. E-Stat. Nonlin. Soft Matter Phys.* **2002**, *66*, 031602; b) V. Franz, S. Loi, H. Muller, E. Bamberg, H. H. Butt, *Coll. Surf. B* **2002**, *23*, 191; c) R. M. A. Sullan, J. K. Li, C. C. Hao, G. C. Walker, S. Zou, *Biophys. J.* **2010**, *99*, 507.