**Force spectroscopy on lipid bilayers: a combined nanomechanical and Chemical Force Titration study**

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1. INTRODUCTION

**Force Spectroscopy** with AFM is a powerful tool to study the nanomechanical properties of supported planar lipid bilayers (SLBs). Force plots on lipid bilayers show a discontinuity in the approaching curve that is interpreted as the penetration of the AFM tip through the lipid bilayer. The force at which this discontinuity occurs is the maximum force the bilayer is able to withstand before breaking and it can be regarded as a `fingerprint` of the bilayer stability. In addition, lipid bilayer stability and compactness depend on ionic strength and temperature.

**Chemical Force Microscopy** enables us to titrate a complex surface such as a lipid bilayer composed of a phospholipid with a phosphatidylcholine head with a COOH group attached to the tip. The local pH of the phospholipid head has been measured, and results have been related to those obtained through zeta potential measurements.

2. EFFECT OF TEMPERATURE ON THE NANOMECHANICS OF ILBs (ref. 1)

AFM contact mode images showing the phase transition for a DMPC supported bilayer upon heating the sample (a) 19.0°C, (b) 24.4°C, (c) 27.2°C, (d) 28.3°C, (e) 29.3°C, (f) 30.3°C, (g) 31.3°C, (h) 32.9°C, (i) 37.3°C. All images have been acquired by applying a constant force of 1.5-2 nN.

**FORCE SPECTROCOPY**

- Force-distance curve. The discontinuity corresponds with the rupture of the bilayer. The force at which this rupture takes place is called yield threshold and it is a mechanical fingerprint of every bilayer at a certain temperature.

- Dependence of the yield threshold with temperature for bilayers of DMPC. Dark areas stand for the temperature range (∆Tₜ) in which phase transitions are observed in supported planar bilayers through AFM images.

- Histograms of the yield threshold value for DLPC, DMPC, DPPC and DOPC measured in water and in high ionic strength conditions (150 mM NaCl + 20 mM MgCl₂).

3. EFFECT OF IONIC STRENGTH ON THE NANOMECHANICS OF ILBs (ref. 2)

Yield threshold values vs. ionic NaCl concentration for all the obtained force plots on DMPC bilayers. Inset: Force vs. z-piezo displacement plot for a DMPC bilayer. The width of the jump, ~4.5 nm, corresponds well with the bilayer height measured using contact mode AFM.

4. TITRATION FORCE MICROSCOPY (ref. 3)

(A) Approaching force curves between a chemically -COOH probe and a DLPC mica-supported lipid bilayer as a function of bulk pH. A repulsion between probe and surface is observed for 3<pH<4.5, no repulsion or attraction is observed for pH 0.02-4.50, and attraction between probe and surface is observed for 3.51<pH<4.1. (B) Scheme of the way of quantitatively measuring attractive and repulsive forces.

(B) Qualitative representation of the surface charges of a -COOH chemically modified probe and a DLPC supported lipid bilayer as a function of pH. Red line stands for tip charge evolution with pH. Blue line stands for surface charge evolution with pH. Black line stands for direct multiplication of blue and red lines. Experimental interaction forces between a -COOH chemically modified AFM probe and a supported DLPC membrane. Positive forces stand for repulsion and negative forces stand for attraction (jump to contact). Note the high degree of accordance between black curve in (A) and curve (B).

REFERENCES

