

Revealing the selective interactions of fibronectin with lipid bilayers

D. Nordin, O. Yarkoni, N. Savinykh, L Donlon and D. Frankel

SUPPLEMENTARY MATERIAL

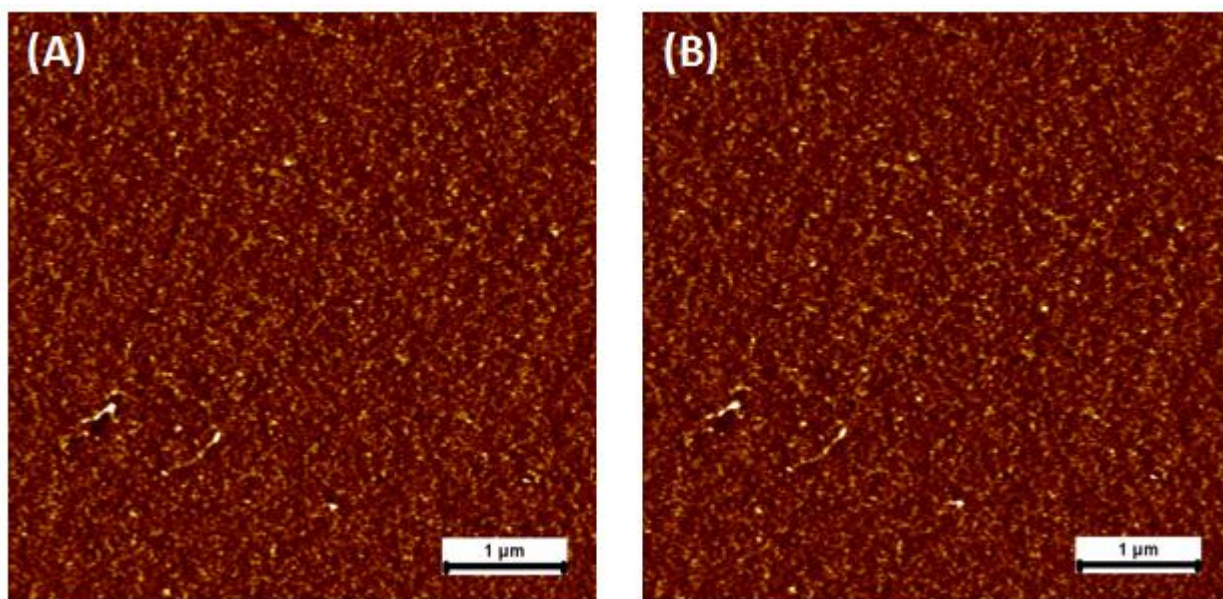


Fig. 1 Experimental protocol to determine the effect of potential tip contamination by fibronectin on a mica surface. Atomic force microscope images of fibronectin (50 $\mu\text{g/mL}$) adsorbed onto a mica surface prior (A) and subsequent (B) to force spectroscopic analysis. The images are identical and sharp illustrating that the protein remains on the surface upon completion of unfolding and does not contaminate the tip.

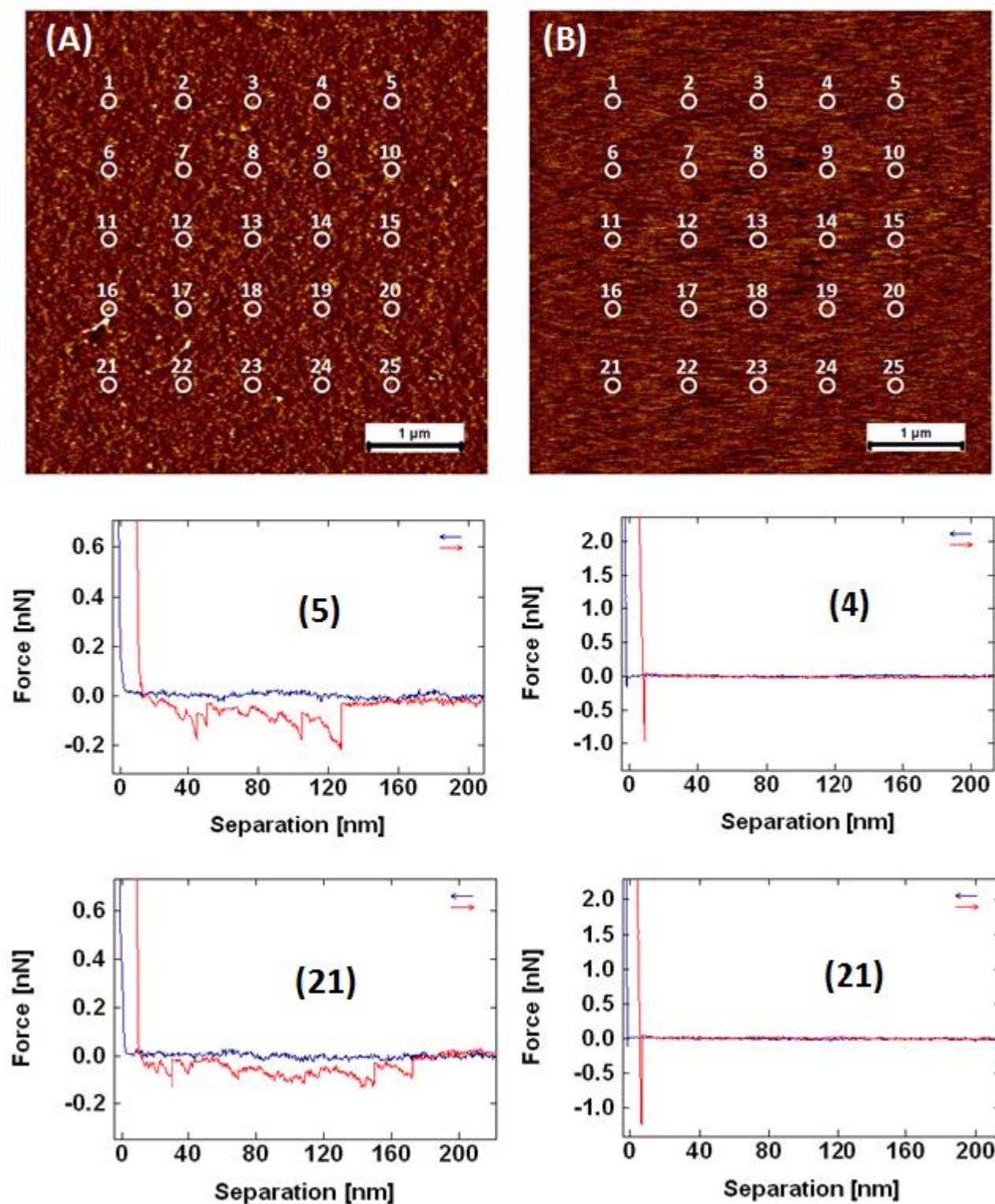


Fig. 2 Force-distance curves obtained for the forced unfolding of fibronectin on a mica surface (A). Numbered points on the image correspond to individual force distance measurements with representative spectra shown for points 5 and 21. Upon completion of force spectroscopic analysis the same tip was used to image a freshly cleaved mica surface (B). The image is sharp and force-distance curves obtained at points on the surface show only

a single adhesion event, ruling out contamination of the tip by fibronectin. Representative spectra are shown for points 4 and 21.

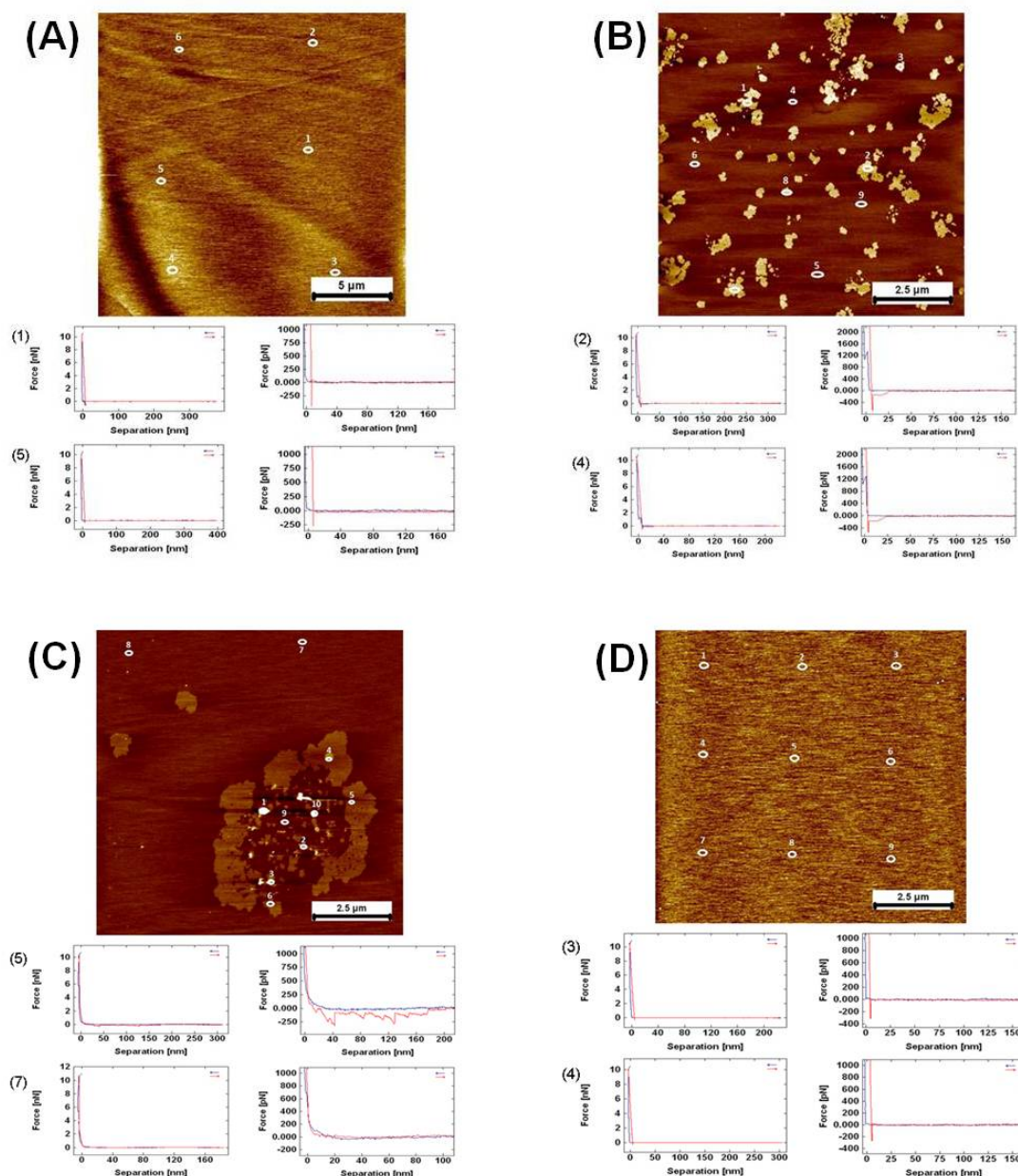


Fig. 3 Experimental protocol to determine effect of potential tip contamination by fibronectin on mixed DOPC/DPPC bilayers. Image and associated force spectra taken on mica prior to lipid bilayer experiments (A). Numbered points on the image correspond to individual force-distance measurements with representative spectra shown for points 1 and 5 which show only a single adhesion event and confirm that there is no fibronectin present. Image and associated force spectra taken on the mixed lipid bilayer prior to fibronectin adsorption representative curves taken at points 2 and 4. Again no sawtooth pattern or evidence of fibronectin contamination is observed. Image and associated force spectra of fibronectin adsorption on lipid bilayers with representative curves from points 5 and 7. Curve taken at point 5 on the DPPC gel phase domain displays both a protein breakthrough event and sawtooth pattern corresponding to fibronectin on the same curve. A lipid breakthrough

event but not sawtooth pattern is observed at point 7 taken in the DOPC liquid disordered phase (C). Image and associated force spectra taken on bare mica using the tip employed in the fibronectin/lipid bilayer experiments. No sawtooth patterns or lipid breakthroughs are visible with force curves characteristic of a clean tip. For all images many force distance curves were taken, only representative points are presented in this figure.

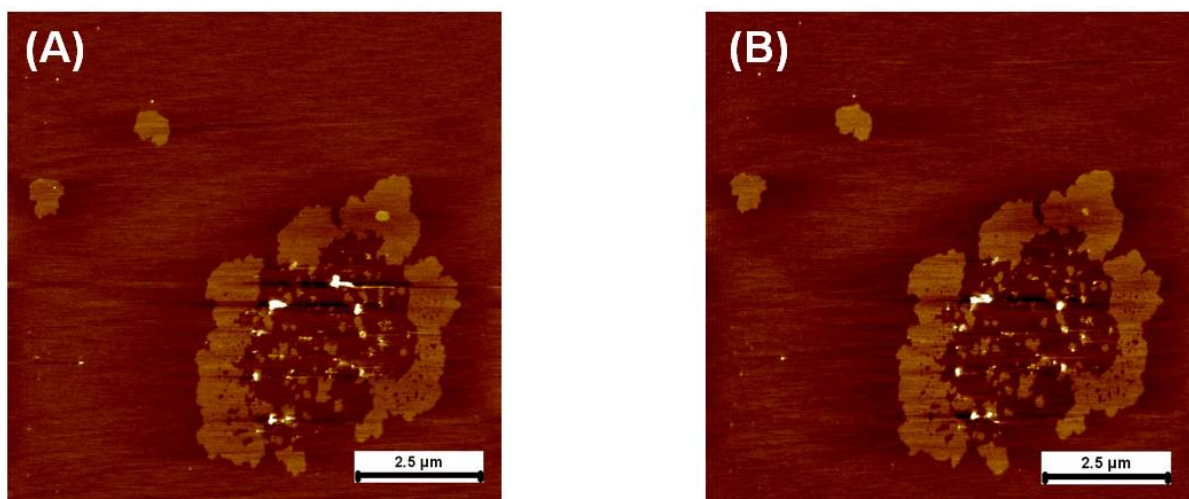


Fig. 4 Determination of the effect of drift and force spectra collection on lipid bilayer imaging. AFM image taken prior to (A) and subsequent (B) to measurement of force spectra. Drift is seen to have a negligible effect with the image position being virtually identical. A small amount of bilayer deformation is visible as would be expected for force distance measurements on a lipid bilayer.