Supporting Information

Direct Correlation of Structures and Nanomechanical Properties of Multicomponent Lipid Bilayers

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Batch Analysis Code:

The set of force curves comprising the force map were analyzed using a custom algorithm implemented in IGOR Pro (Wavemetrics, Portland, OR, USA). Individual force curves were read in as deflection force F (in N), and piezo distance z (in m), along with (x,y) position.

The piezo distance z is calculated as: $s=(z-z_0) - (d-d_0)/c$, where the location (z_0,d_0) defines the contact point of the tip with the sample, s is the separation, and c is a minor correction factor that is ideally close to unity, but can fluctuate for each force curve due to the slight change of deflection sensitivity during force map collection. The quantities calculated for each force curve were breakthrough force, indentation, elastic modulus, and adhesion force.

To calculate breakthrough force, indentation, and elastic moduli, it was necessary to locate the contact point (A), the breakthrough region (AB), and the substrate (C) of the extension curve (Figure S1).

Breakthrough Force. The breakthrough force is defined as the difference in force between the contact point (A), and the entrance point (B) in the extension curve.

An algorithm was utilized to automatically search for the breakthrough points. Briefly, the $F(d)$ curve was box-smoothed and its first derivative was found. A segment of the non-contact region was identified as the baseline and its associated standard deviation was calculated. To locate the breakthrough region, the first-derivative graph was incrementally searched for a sharp peak (arising from the abruptness of the B-C breakthrough) that exceeded a pre-defined multiple of the standard

deviation from the non-contact baseline. The minimum of this peak corresponds to point C, the breakthrough exit point. To find point B, the local maximum prior to point C is searched for. The threshold multiplier can be adjusted, depending on the quality of the entire batch of force curves, to be more strict or relaxed in searching out the breakthrough region. If a certain force curve does not meet the threshold criteria, a breakthrough region is not identified and the force curve is dropped from the analysis. This is useful for dropping curves not exhibiting a breakthrough event, or those with multiple ruptures.

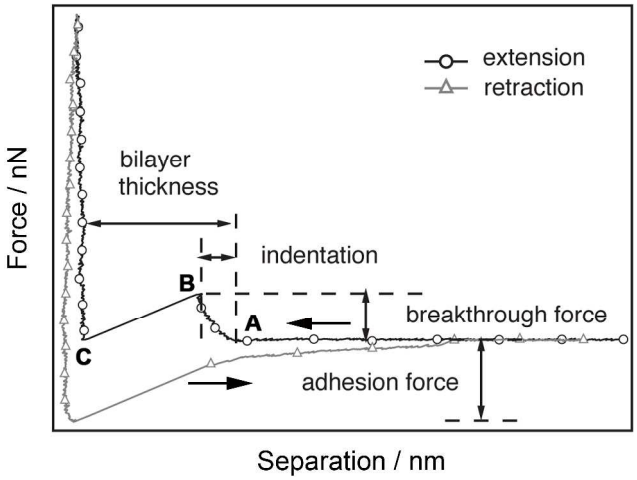

Figure S1. Force curves indicating quantities extracted: breakthrough force, indentation, and adhesion force.

A contact point finding algorithm was also implemented to facilitate the batch analysis process. The algorithm assumes that the breakthrough entrance has already been found, and searches points only prior to point B. To speed up the analysis, only a portion of the curve prior to point B is searched. Each point in the selected portion is evaluated as a candidate contact point with the following algorithm: the candidate point is the junction of a piece-wise function. Any points before the candidate contact point are considered the non-contact region, and are fit to a straight line. Points beyond this point up to (B) belong to the indentation region and are also fit to a line. Although a quick 3/2 power fit in this region may have been more accurate, use of a second linear fit is much quicker, and given the evaluation scheme to be described, was not deemed necessary. The average mean square error of the proposed piecewise function and the experimental data is calculated and the candidate point that provides the

lowest mean square error becomes the contact point to be used for subsequent calculations. To further prevent false identification, an added criterion to ensure the candidate point is sufficiently close to the y-value of the non-contact region was implemented.

Indentation. Indentation is the total distance between the contact point (A) and the onset of breakthrough (B). This portion of the Force-separation curve is extracted and re-zeroed to perform a power-law fit.

$$F = C \cdot \delta^{P} \tag{S1}$$

where the exponent P is fixed to 3/2 to match the paraboloid-shaped tip models. The coefficient C obtained from the fit is equated to coefficients in equation S2 to determine Young's modulus using values for the other parameters which are mentioned below.

Elastic Modulus. The elastic modulus is a mechanical property of the lipid layer that can be obtained by fitting the indentation region of the extension curve to the Sneddon model for a semi-infinite sample contacted by a paraboloidal-shaped tip (equation S2):

$$F = \frac{4E\sqrt{R}}{3(1-\sigma^2)}\delta^{3/2} \tag{S2}$$

where E is Young's modulus, σ is Poisson's ratio, R is the tip radius, α is half the semi-vertical angle of a conical tip, F is the load, and δ is indentation. In our calculations, a tip radius of 25 nm and a Poisson ratio of 0.5 were used. SEM images of the DNPS AFM tip (k ~ 0.25 N/m) were collected to affirm the tip radius used in the calculation.

Adhesion Force. Adhesion is the minimum value of the retract portion of the force curve relative to the non-contact region, that is, when the tip is lifted sufficiently far away from the sample and experiences no tip-sample force.

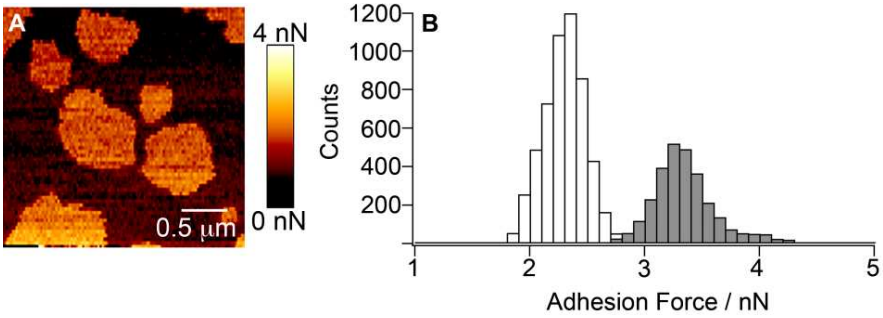

Figure S2. Adhesion force map (A) and corresponding histogram (B) of a DEC bilayer. Solid bars correspond to the liquid ordered domains while the hollow bars to the fluid disordered phase.

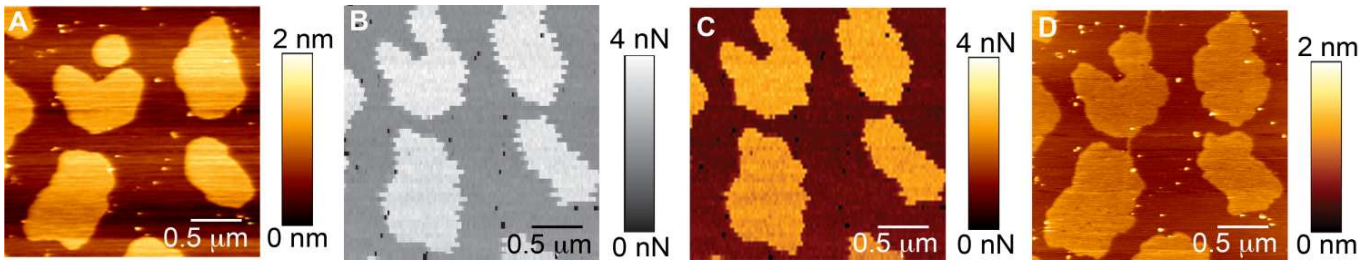

Figure S3. AFM height images of a DEC bilayer *before* (A) and *after* (D) force mapping and the corresponding breakthrough force (B) and adhesion (C) maps.

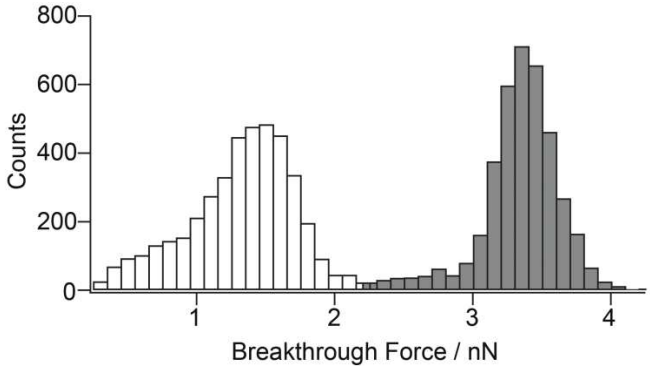

Figure S4. Histogram of the breakthrough forces of a DEC 111 lipid bilayer. Solid bars correspond to the liquid ordered domains while the hollow bars to the fluid disordered phase.

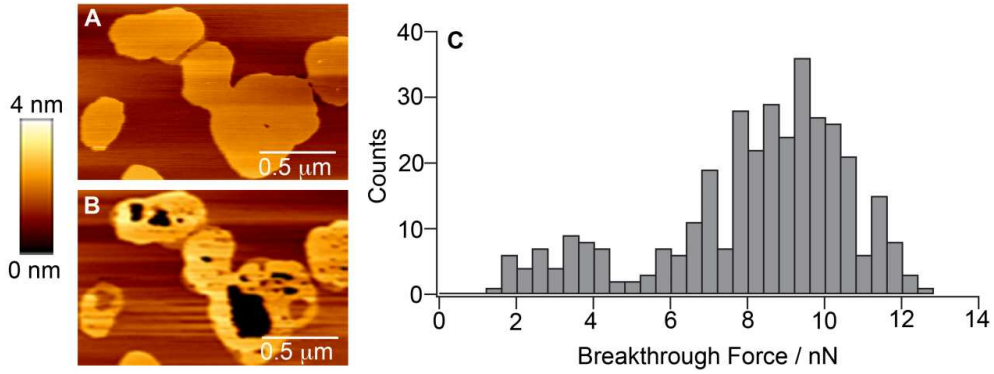

Figure S5. AFM height images *before* (A), *after* (B) force mapping, and histogram of the breakthrough forces of a DPPC gel phase in a DOPC/DPPC (1:1 molar ratio) lipid bilayer (C).