Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2014

Electronic Supplementary Information

Nanomechanical Spectroscopy of Synthetic and Biological Membranes

Junhong Lü,[a,b] Ju Yang[c], Mingdong Dong[a,d] and Ozgur Sahin*[c,e]

[a] *The Rowland Institute at Harvard, Harvard University, Cambridge, MA, USA.*

[b] *Laboratory of Physical Biology, Shanghai Institute of Applied Physics, Shanghai 201800, China.*

[c] *Department of Biological Sciences, Columbia University, New York, NY 10027,USA*

[d] *Centre for DNA Nanotechnology (CDNA), Aarhus University, Aarhus C DK-8000, Denmark.*

[e]*Department of Physics, Columbia University, New York, NY 10027,USA; E-mail: sahin@columbia.edu*

1. Assigning elastic modulus values to the lipid components

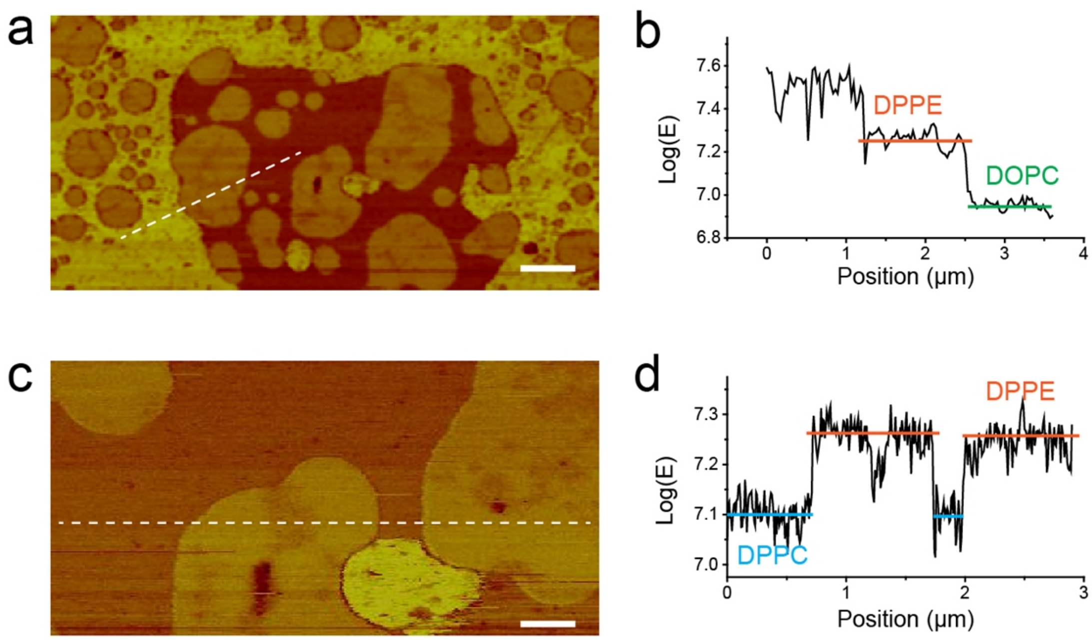

Figure S1. Effective elastic modulus maps of binary lipid bilayer systems. Color coded effective elastic modulus values correspond to DPPE:DOPC system in (a,b) and DPPE:DPPC system in (c,d). Scale bars correspond to 1 µm in (a) and 300 nm in (c). Elastic modulus maps are logarithmically scaled (Elastic modulus values are calculated in the units of Pa, and the logarithm of the numerical values in base 10 are color coded in the image. Numerical values across the dashed lines are plotted in (b) and (d) for images in (a) and (c) respectively.

The effective elastic modulus values measured in Figure 1 can be uniquely assigned to the three lipid components (DOPC, DPPC, and DPPE) by preparing binary lipid bilayers and looking for the missing values in the measured spectrum of elastic moduli. Figure S1a and Fig. S1c shows effective elastic modulus maps recorded on binary lipid bilayers (a- DPPE:DOPC; c- DPPE:DPPC). The numerical values (logarithm of the elastic modulus) across the dashed lines (Fig. S1b,d) show that values around 7.1 are missing in the DPPE:DOPC sample, and values around 6.8 are missing in the DPPE:DPPC sample. This immediately allows assigning 7.1 to DPPC and 6.8 to DOPC. Then, the remaining value, 7.3, gets assigned to DPPE.

2. Differentiating extracellular and cytoplasmic sides of the purple membrane

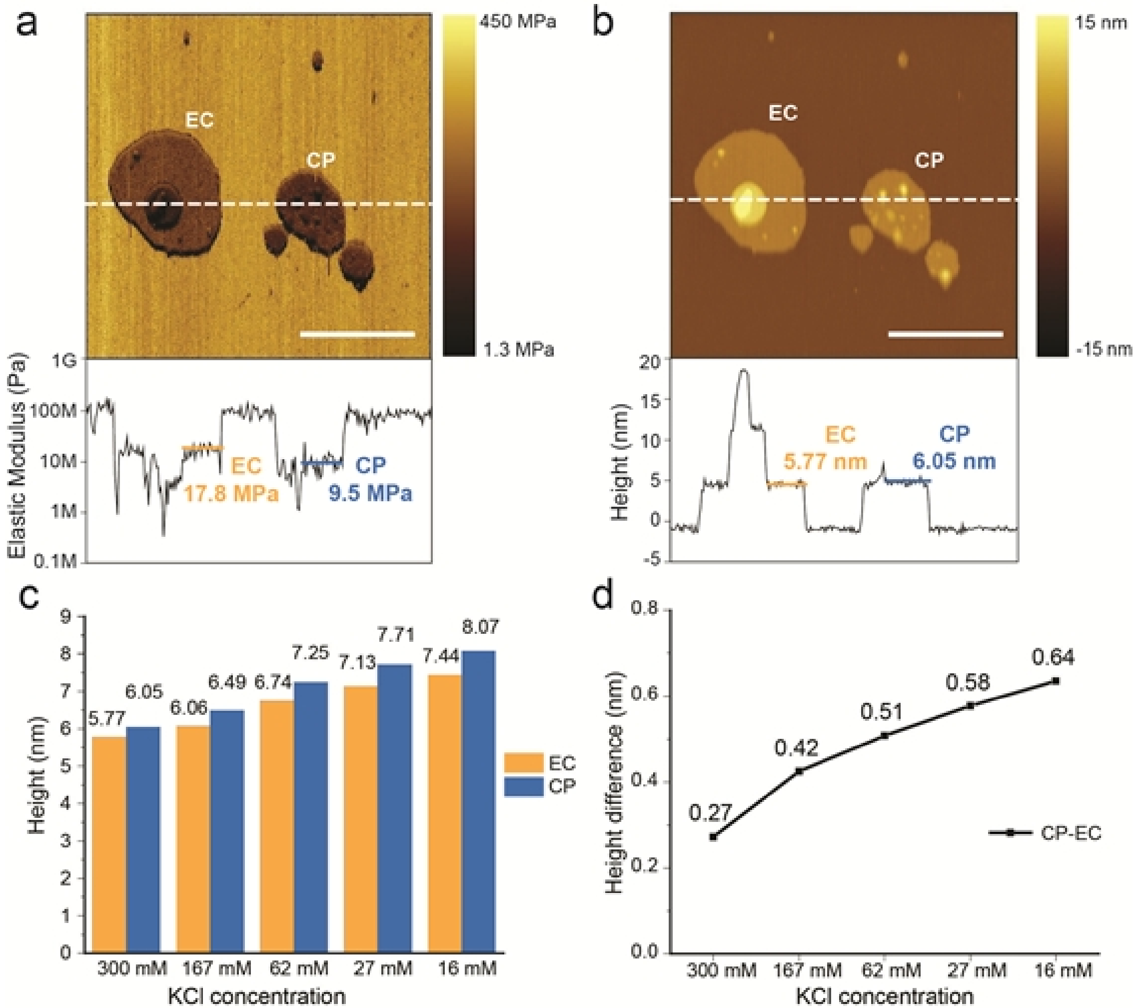

Figure S2. Purple membranes investigated at different ionic strengths. A representative elastic modulus image (a) of purple membrane patches is recorded simultaneously with the height image (b) Scale bars: 1µm, Scan direction: top to bottom. EC: extracellular, CP: cytoplasmic. (Buffer conditions: 300 mM KCl, 10 mM Tris/HCl, pH 9.0). Numerical values are plotted from left to right along the dashed lines. Heights of the same membrane patches were measured at different ionic concentrations (10 mM Tris/HCL is fixed, KCl concentration is varied). Measured height values (c) and height differences between the CP labeled patch and EC labeled patch (d) increase with reduced KCl concentrations. Also note that the relative increase in the heights of the two sides (between 300mM KCl and 16 mM KCl) is 33.4% for the CP side and 23.7% for the EC side.

In the elastic modulus map of purple membranes measured with microsecond force spectroscopy, we repetitively observed clear contrasts among different membrane patches, as displayed in Figure S2a. The right membrane patch has a lower effective modulus of 9.5MPa than the left one with 17.8MPa. Previous studies suggested that such lower elastic modulus results from more flexible bacteriorhodopsin structures on the cytoplasmic (CP) side of purple membranes than on the extracellular (EC) side[1]. Definitive evidence of the sidedness of purple membranes can be provided by high-resolution topography which reveals the different substructures of bacteriorhodopsin lattices between CP and EC sides[2]. However, we found it difficult to achieve this with the particular cantilever we were using in this study. An alternative and independent approach to identifying the sidedness relies on the fact that CP sides have higher negative charge densities than EC sides[3], and thus show stronger repulsive electrostatic interactions between the tip and the sample[4].

Because repulsive electrical interactions result in a higher apparent height and these interactions are affected by ionic concentrations[5], the apparent heights of both sides of purple membranes should increase with decreasing ionic strength of the imaging buffer. The increase in height can be expected to be more dramatic for the CP side due to its higher surface charge density. Therefore, we hypothesize that if the compliant membrane patch belongs to the cytoplasmic side, its apparent height should increase more significantly with decreasing ionic strength compared to the membrane match exhibiting a higher elastic modulus.

To test this hypothesis, we measured the heights of purple membrane patches with distinct apparent elastic moduli at gradually decreasing ionic strengths. Figure S2b shows a typical height image of the same membrane patches as in Figure a. The corresponding height profile reveals apparent heights for the two patches of 5.77 nm and 6.05 nm, respectively. Figure S2c shows that the apparent heights of both patches increase with reduced KCl concentration, so does the difference between them as presented in Figure S2d. Furthermore, the percentage change in heights of the CP and EC sides (measured between 300mM KCl and 16 mM KCl) is 33.4% for the CP side and 23.7% for the EC side. This observation provides strong support that the membrane patches with lower elastic moduli correspond to the cytoplasmic sides of purple membranes, while those with higher moduli correspond to the extracellular sides.

References:

1. Dong, M. D.; Husale, S.; Sahin, O. *Nature Nanotechnology* **2009,** 4, (8), 514-517.
2. Muller, D. J.; Engel, A. *Nat Protoc* **2007,** 2, (9), 2191-2197.
3. Zhong, S.; Li, H.; Chen, X. Y.; Cao, E. H.; Jin, G.; Hu, K. S. *Langmuir* **2007,** 23, (8), 4486-4493.
4. Muller, D. J.; Engel, A. *Biophysical Journal* **1997,** 73, (3), 1633-1644.
5. Israelachvili, J. N. *Intermolecular and Surface Forces, 3rd Edition* **2011**, 1-674.