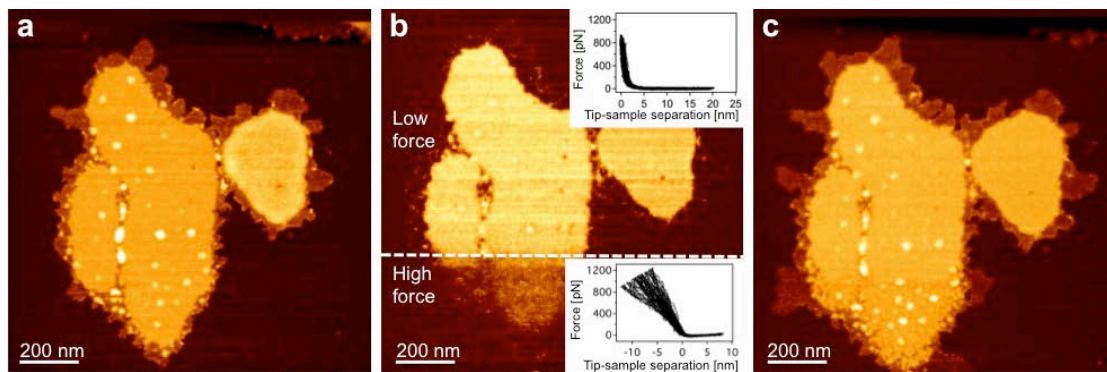


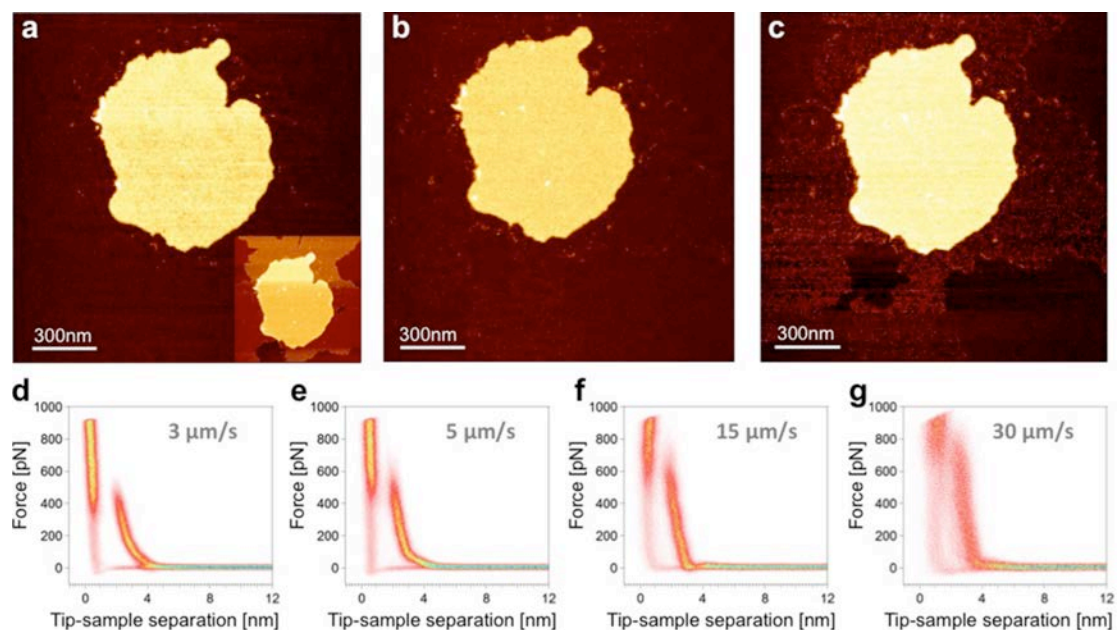
Nanomechanical Properties of Proteins and Membranes Depend on Loading Rate and Electrostatic Interactions

Izhar D. Medalsy and Daniel J. Müller

SUPPORTING INFORMATION

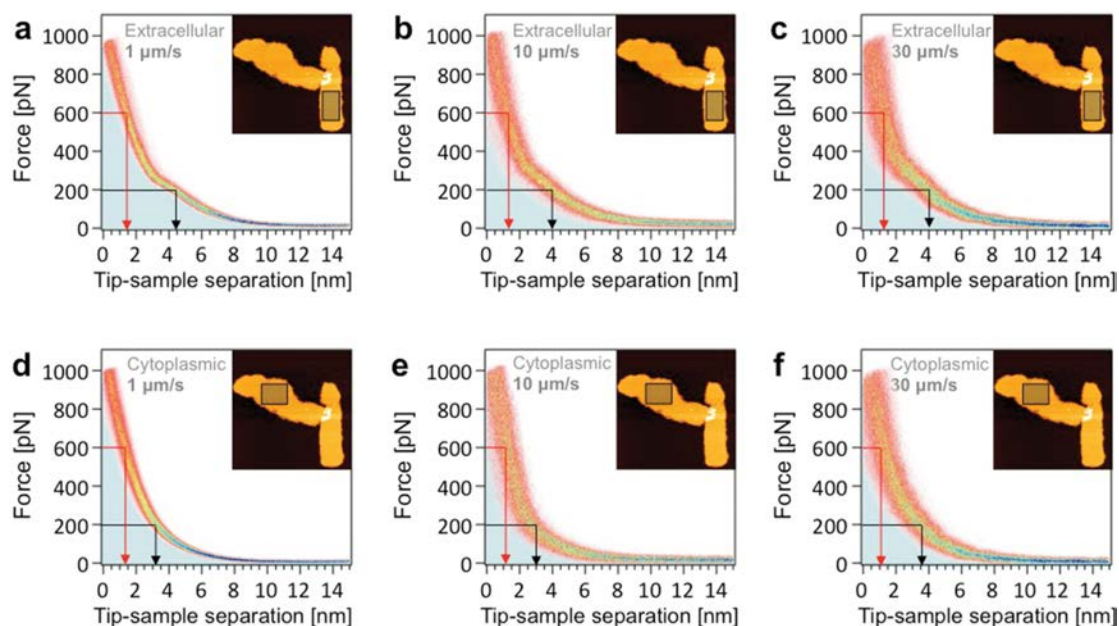


Supporting Figure S1. Probing the purple membrane stability using FV-AFM imaging. (a) FV-AFM topograph of purple membranes exposing the cytoplasmic surface (left side, with white blobs) and the extracellular surface (right side, smooth surface). The topograph was recorded in buffer solution (300 mM KCl, pH 7.4, 10 mM Tris-HCl) applying a maximal force of 200 pN. (b) When repeatedly imaging the purple membranes the imaging force was increased from 900 pN (upper third of topograph) to $\approx 1,200$ pN (lower third of topograph). At $\approx 1,200$ pN purple membrane started disintegrating. The inset at the top shows an ensemble of force-distance curves that was recorded applying a maximal force of 900 pN on the extracellular purple membrane surface. The inset at the bottom shows an ensemble of force-distance curves that was recorded applying a maximal force of $\approx 1,200$ pN on the extracellular purple membrane surface. The irregular shapes of the force-distance curves reflect the fact that the purple membrane disintegrates. (c) Purple membrane after recording nine consecutive FV-AFM topographs applying maximal forces of 1,000 pN. The topograph reveals that the lower part of the purple membrane went through nonreversible deformation when applying forces of $\approx 1,200$ pN whereas the upper part of the purple membrane remained intact. The full color scale of the topographs corresponds to a vertical range of 12 nm. The FV-AFM data was recorded in buffer solution (300 mM KCl, pH 7.4, 10 mM Tris-HCl).



Supporting Figure S2. Penetration of the AFM tip through the phospholipid bilayer depends on tip-sample velocity (e.g., loading rate). (a-c) FV-AFM topographs of purple membrane surrounded by phospholipid bilayers and recorded at AFM tip-sample velocities of 3 $\mu\text{m/s}$ (a), 15 $\mu\text{m/s}$ (b), and 40 $\mu\text{m/s}$ (c). The inset in (a) shows the intact phospholipid bilayer imaged at an AFM tip-sample velocity of 3 $\mu\text{m/s}$. All topographs were recorded applying maximal forces of 900 pN and their full color scale corresponds to a vertical range of 8 nm. (d-g) The average force-distance curves show a 'jump', which indicates that at applied forces of 900 pN the AFM tip penetrates the lipid layer (but not the purple membrane - compare suppl. Fig. S1). With increasing tip-sample velocity (d-g) the force required to push the AFM tip through the lipid bilayer increases.¹ The FV-AFM data was recorded in buffer solution (300 mM KCl, pH 7.4, 10 mM Tris-HCl). Each average force-distance curve (d-g) is a density plot from $\approx 1,000$ superimposed force-distance curves recorded on the purple membrane surface.

¹ Alessandrini, A.; Seeger, H. M.; Caramaschi, T.; Facci, P., Dynamic force spectroscopy on supported lipid bilayers: effect of temperature and sample preparation. *Biophys J.* **2012**, *103*, 38-47.



Supporting Figure S3. Density plots of force-distance curves recorded at different tip-sample velocities on the extracellular (a-c) and cytoplasmic (d-f) purple membrane surface. Compared to Fig. 3 the force-distance curves were recorded in buffer solution containing less electrolyte (100 mM KCl, pH 7.4, 10 mM Tris-HCl). All force-distance curves change slope with the tip-sample velocity increasing from 1 $\mu\text{m/s}$ to 30 $\mu\text{m/s}$. The insets outline the surface area of purple membrane from which force-distance curves were taken. Black arrows indicate the tip-sample separation at a tip-sample force of 200 pN. Red arrows indicate the region above which the slope of the force-distance curve approached linearity. Each density plot represents an average force-distance curve from $\approx 1,000$ superimposed force-distance curves either recorded on the extracellular or cytoplasmic purple membrane surface.