Supplementary Information for:

Nanorheology of living cells measured by AFM-based force-distance curves

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Coefficients for a conical tip

These coefficients were deduced in previously for a cylinder (flat punch), a sphere and a conical tip[1,2]. Here, we summarize the values for a conical tip of half angle θ that indents a layer of thickness h. These coefficients are independent of the viscoelastic model used to describe the layer.

Table S1. Coefficients for a conical tip of half-angle θ.		
j	α_j	β_j
0	$\frac{8\tan\theta}{3\pi}$	2
1	$0.721\frac{8\,tan^2\theta}{3h\pi}$	3
2	$0.650\frac{8\,tan^3\theta}{3h^2\pi}$	4
3	$0.491\frac{8\,tan^4\theta}{3h^3\pi}$	5
4	$0.225\frac{8\,tan^5\theta}{3h^4\pi}$	6

Complete derivation of the analytical expressions for power-law rheology and Kelvin-Voigt viscoelastic models

The general expression to determine the force exerted on a finite-thickness viscoelastic material is[2] (eq 3) in the main text

$$F(t) = \sum_{j=0}^{N} \alpha_j \int_0^t \varphi_E\,(t - t')\frac{d}{dt'}\left(I(t')^{\beta_j}\right)dt' \qquad \text{S1}$$

Kelvin-Voigt expressions. By replacing the φ_E for the KV relaxation function (eq 4 of the main text), we obtain

$$F = \sum_{j=0}^{N} \alpha_j \int_0^t [E + \eta_E \delta(t-\tau)] \frac{d[I^{\beta_j}]}{d\tau} d\tau \qquad \text{S2}$$

The solution is divided in two time intervals, $(0, t_{max}]$ and $(t_{\max}, t_f]$ approach and retraction

The integration gives for the interval $(t_0, t_{max}]$ (approach section of the FDC)

$$F = \sum_{j=0}^{N} \alpha_j I(t)^{\beta_j - 1} \left[3\beta_j \eta_G \dot{I}(t) + EI(t)\right] \qquad \text{S3}$$

The explicit expression for triangular indentation waveform $I=vt$, is obtained by introducing the coefficients given in the table S1,

$$F(t) = \frac{8\tan(\theta)}{3\pi} v^2 t[6\eta_G + Et] + 0.721 \frac{8\tan^2\theta}{3h\pi} v^3 t^2 [9\eta_G + Et)] + O[\frac{v^2 t^2}{h^2}] \qquad \text{S4}$$

For obtaining the expression for the interval $(t_{\max}, t_f]$ (retraction section), we modify the upper limit in the eq S2

$$F = \sum_{j=0}^{N} \alpha_j \int_0^{t_1(t)} [E + \eta_E \delta(t-\tau)] \frac{d[I^{\beta_j}]}{d\tau} d\tau \qquad \text{S5}$$

for $t_1(t) < t$ the integral of the term including the Dirac delta function is zero, then

$$F = \sum_{j=0}^{N} \alpha_j EI(t_1(t))^{\beta_j} \qquad \text{S6}$$

By introducing the coefficients for the conical tip, the definition of $t_1(t)$ (eq 9 of the main text) and assuming a linear indentation, we deduce

$$F(t_1) = \frac{8\tan(\theta)}{3\pi} E v^2 {t_1}^2 + 0.721 \frac{8\tan^2\theta}{3h\pi} E v^3 {t_1}^3 + O[\frac{v^2 t^2}{h^2}] \qquad \text{S7}$$

Power-law rheology expressions. To obtain the analytic expression of the force for the PLR model, we substitute in S1 the relaxation function given in the eq 5

$$F = \sum_{j=0}^{N} \alpha_j \int_0^t \left[E_0 \left(\frac{(t-\tau)}{t_0} \right)^{-\gamma} \right] \frac{d\left[I^{\beta_j} \right]}{d\tau} d\tau \qquad \text{S8}$$

which for an indentation performed at constant velocity gives

$$F = \sum_{j=0}^{N} \beta_j \alpha_j v^{\beta_j} \int_0^t \left[E_0 \left(\frac{(t-\tau)}{t_0} \right)^{-\gamma} \right] \tau^{\beta_j - 1} d\tau \qquad \text{S9}$$

Solving this integral gives us

$$F = \sum_{j=0}^{N} \beta_j \alpha_j v^{\beta_j} \frac{E_0 \ t^{\beta_j - \Upsilon} \Gamma[\beta_j] \Gamma[1-\Upsilon]}{{t_0}^{-\gamma} \ \Gamma[1+\beta_j - \Upsilon]} \qquad \text{S10}$$

For a conical tip (coefficients of table S1) and by keeping only the two first three terms we get the expression of the force during the approach (eq.7 of the main text)

$$F(t) = \frac{8\tan(\theta)}{3\pi} 2 v^2 t^2 \left(\frac{t}{t_0} \right)^{-\gamma} \frac{\Gamma[2]\Gamma[1-\Upsilon]}{\Gamma[3-\Upsilon]} + 0.721 \frac{8\tan^2\theta}{3h\pi} 3 v^3 t^3 \left(\frac{t}{t_0} \right)^{-\gamma} \frac{\Gamma[3]\Gamma[1-\Upsilon]}{\Gamma[4-\Upsilon]} + \cdots] \qquad \text{S11}$$

To get the expression of the force during the retraction, we change the upper limit of the integral from t to t_1 in eq S9 and use the definition of t_1 given by eq 9 of the main, then

$$F = \sum_{j=0}^{N} \beta_j \alpha_j v^{\beta_j} \int_0^{t_1} \left[E_0 \left(\frac{(t-\tau)}{t_0} \right)^{-\gamma} \right] \tau^{\beta_j - 1} d\tau \qquad \text{S12}$$

by solving the integral,

$$F = \sum_{j=0}^{N} \beta_j \alpha_j v^{\beta_j} \frac{E_0 \; t_1^{\beta_j - \Upsilon} \Gamma[\beta_j]\Gamma[1-\Upsilon]}{t_0^{-\gamma} \quad \Gamma[1+\beta_j-\Upsilon]} \qquad \text{S13}$$

By introducing the coefficients for a conical tip and the constant velocity hypothesis, we get the eq 12 of the main text,

$$F(t_1) = \frac{8\tan(\theta)}{3\pi} 2v^2 t_1^2 \left(\frac{t_1}{t_0}\right)^{-\gamma} \frac{\Gamma[2]\Gamma[1-\Upsilon]}{\Gamma[3-\Upsilon]} + 0.721 \frac{8\,tan^2\theta}{3h\pi} 3v^3 t_1^3 \left(\frac{t_1}{t_0}\right)^{-\gamma} \frac{\Gamma[3]\Gamma[1-\Upsilon]}{\Gamma[4-\Upsilon]} - O[\frac{v^2t^2}{h^2}] \qquad \text{S14}$$

where $t_1(t)$ is given by eq 10 of the main text.

Correspondence between velocities and equivalent indentation frequencies

To find a correspondence between the velocities used in FDC-nanorheology and the frequencies applied in oscillatory microrheology experiments[3–5] we propose the following protocol. First, we define an average velocity for a sinusoidal displacement by multiplying the frequency of the oscillatory movement f by the total distance travelled in a single cycle d_{cycle},

$$v_{av} = d_{cycle} * f \qquad \text{(S.14)}$$

By applying this analogy to a typical value of the amplitude used in AFM-oscillatory microrheology (15 nm), we get that d_{cycle} would be of 60 nm. The linear relationship between the equivalent sinusoidal frequency and the FDC velocity would be determined by a multiplicative factor of 0.06. Here, a velocity of 300 µm/s corresponds to an equivalent frequency of 5 kHz while a velocity of 10 µm/s corresponds to a frequency of 166 Hz.

Statistical analysis of the experimental data

The FDCs were obtained on 20 different NIH 3T3 fibroblast cells. On each of them the tip's velocity was varied from 10 to 300 μm/s range. For each velocity, we acquired 64 FDCs on each cell. Each experimental point plotted in the Figure 6 (panels a-b) represents the median value of the 64 FDCs.

A box-plot representation has been used to show the distribution of E_0 and γ obtained for each velocity. In a box plot, the line corresponds of the distribution, respectively. The top and bottom of the box represent, respectively, the 75th and 25th percentiles. The whiskers out of the box denote the range of outer-most data that fall within 1.5 x interquartile range.

Figure S2 shows that for NIH 3T3 fibroblasts an increase in E_0 reduces the power-law exponent γ. In other words, the fibroblasts present lower fluidity when the apparent Young's modulus increases.

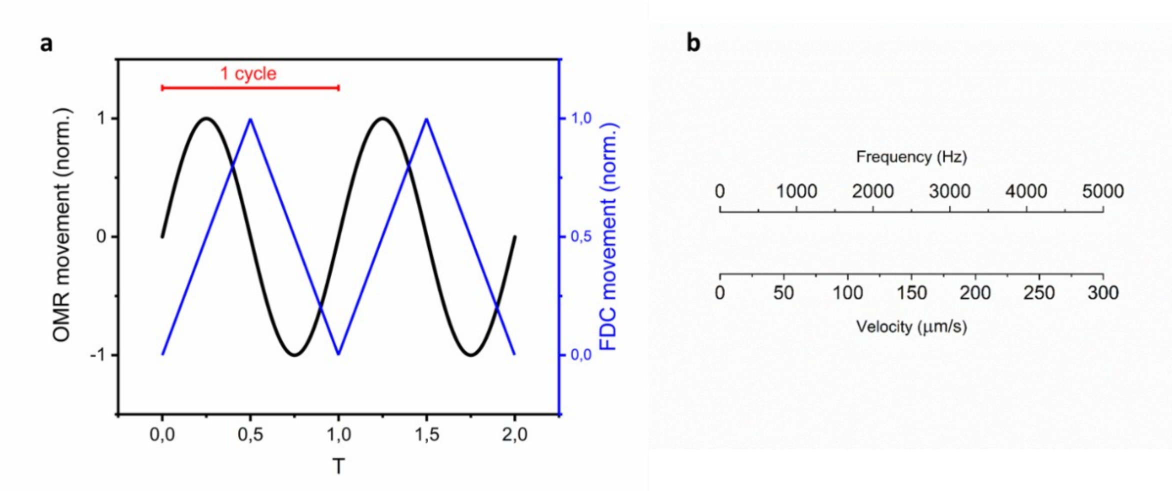

Figure S1. a. Schematic comparison between the movement executed in a FDC and the tip displacement exerted in an OMR kind experiment. b. Relation between frequencies and velocities for an amplitude of 15 nm in the OMR sinusoidal oscillation.

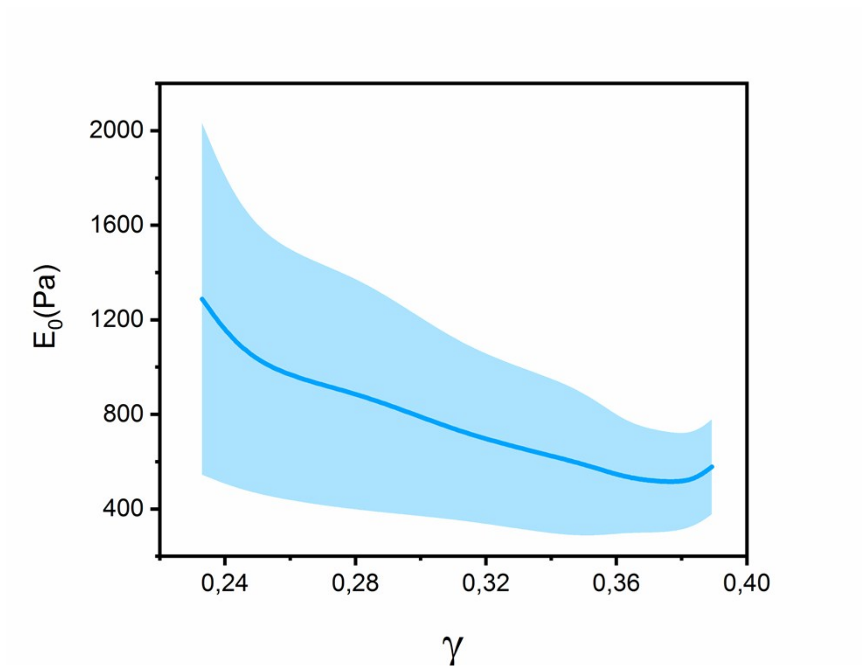

Figure S2. Plot of E_0 as function of γ for all experimental results. The blue line represents the median value and the area shaded in blue is the region delimitated by median±mean deviation.

References

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