Electronic Supplementary Material (ESI) for Soft Matter

Supplementary information

Dynamics of force generation by confined actin filaments

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Experimental Set up

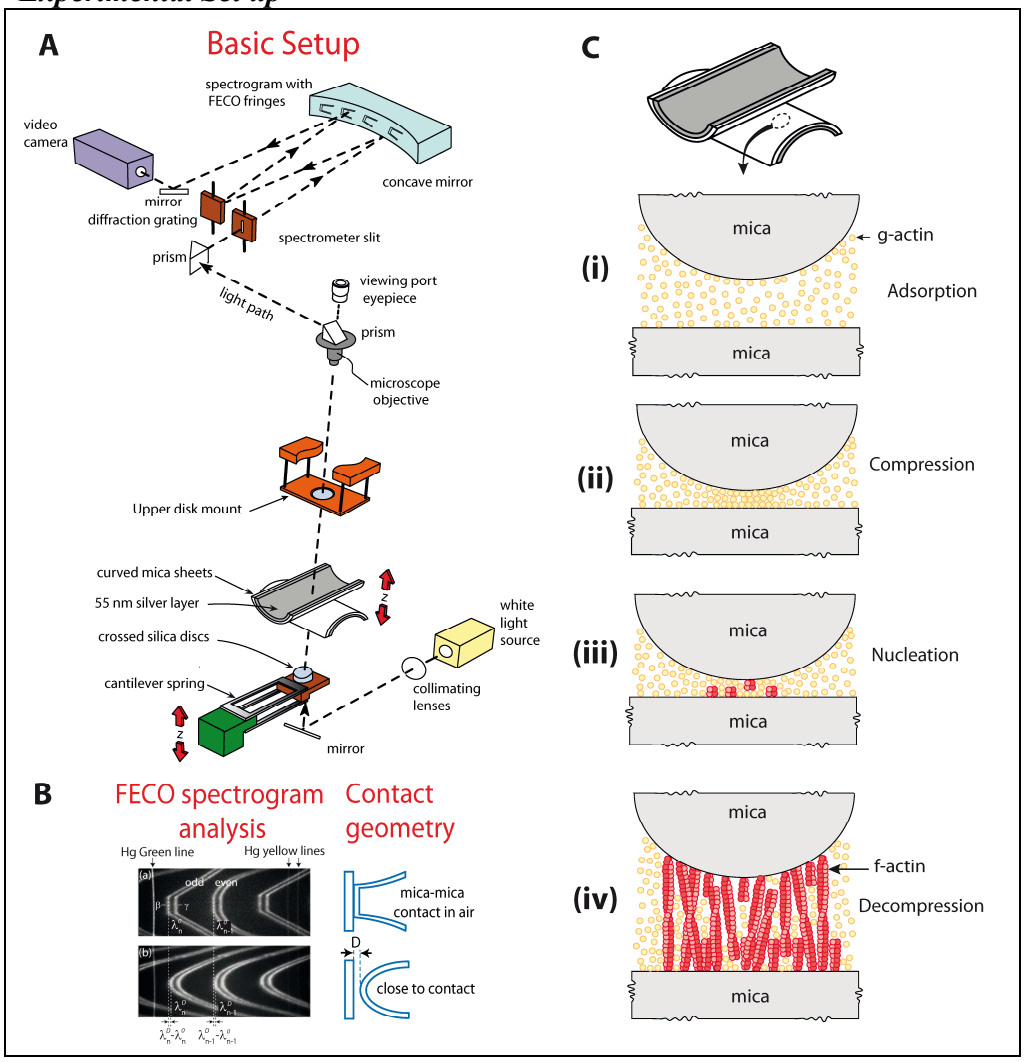


Fig. SI-1 (A) Schematic representation of the experimental set up. The actin monomer solution is injected between the two cylindrical surfaces, the lower one being supported by a double cantilever spring of known stiffness. Measurement of the separation distance between the surfaces is performed by shinning a beam of white light through the surfaces and recording the interference fringes in a spectrometer. (B) Picture of the interference fringes from two surfaces in contact in air and close to contact in buffer. The separation distance between two surfaces in an actin solution is obtained by measuring the wavelength of the tip of the contact fringe (C) Schematic representation of the "force run" experiment performed in Fig. 1 of the manuscript. First, a droplet of actin monomers in polymerization buffer was introduced between the two surfaces (i). Then the two surfaces were slowly approached until slightly compressing the protein film adsorbed on the mica surfaces (ii). The surfaces were let in contact for a few minutes (iii) and then separated a constant velocity (iv). During this process, interference fringes were recorded which allows the separation distance and the applied force to be calculated as a function of time.

The actin filaments nucleated between the surfaces are a priori randomly oriented. For clarity, only the filaments that have a significant "mechanical contribution" are represented in the figure.

Two state model for the force generation of growing actin filament[1]

In a previous report[2] we suggested that actin filaments can exist in two different states, one of them being mechanically more rigid. Based on this assumption, we consider that the addition of an actin monomer to a growing filament under an external applied force F follows a two states process. Let us call state 0 the state corresponding to a monomer A being at the tip of the filament. For a new monomer B to be added to the filament, A must change to state 1 to which B can bind in the state 0. Between each cycle, the filament grows by a distance of d = 2.7 nm.

Following this scheme, it can be shown that V_e for a single filament is given by

$$V_e = d\frac{u_0u_1 - w_0w_1}{u_0 + u_1 + w_0 + w_1} \tag{1}$$

Where u_0 and u_1 are forward transition rates from state 0 and 1 respectively and w_0 and w_1 are the corresponding backward transition rates. u_i and w_i depend on F as

$$u_i = u_i(0)\exp\left(-\theta_i^+\frac{Fd}{k_BT}\right) \tag{2}$$

$$w_i = w_i(0)\exp\left(-\theta_i^-\frac{Fd}{k_BT}\right)$$

Where $\vartheta_i^{\pm}$ are load distribution factors that specify how F modifies the free energy profile of the system. These parameters are related by $\sum_i \vartheta_i^+ + \vartheta_i^- = 1$ (see note).

Therefore, the final expression of V_e as a function of F is

$$V_e = d\frac{u_0u_1\exp\left(-\left(\vartheta_0^+ + \vartheta_1^+\right)f\right) - w_0w_1\exp\left(-\left(\vartheta_0^- + \vartheta_1^-\right)f\right)}{u_0\exp\left(-\vartheta_0^+f\right) + u_1\exp\left(-\vartheta_1^+f\right) + w_0\exp\left(\vartheta_0^-f\right) + w_1\exp\left(\vartheta_1^-f\right)} \tag{3}$$

where $f = Fd/k_BT$.

In order to test the model we used the following parameters. The value of u_1 can be estimated considering that the concentration of actin in the system is 47 μM and the on rate of polymerization is approximately 11 $\mu M^{-1}s^{-1}$ [3] which gives $u_1 = 517$. The value for u_0 can be estimated to be close to 15. In the working framework we established, the stalling force f_s can be easily derived as a function of the different rates and has the form:

$$f_s = \frac{k_B T}{d} \ln\left(\frac{u_0 u_1}{w_0 w_1}\right) \qquad (4)$$

The stalling force of actin filaments has been estimated to be 9 pN which allows the product $w_0 w_1$ to be estimated. Considering that the sum of the load distribution factors $\theta_i^{\pm}$ should equal to one, the number of free parameters in equation (3) is therefore decreased from 8 to 4.

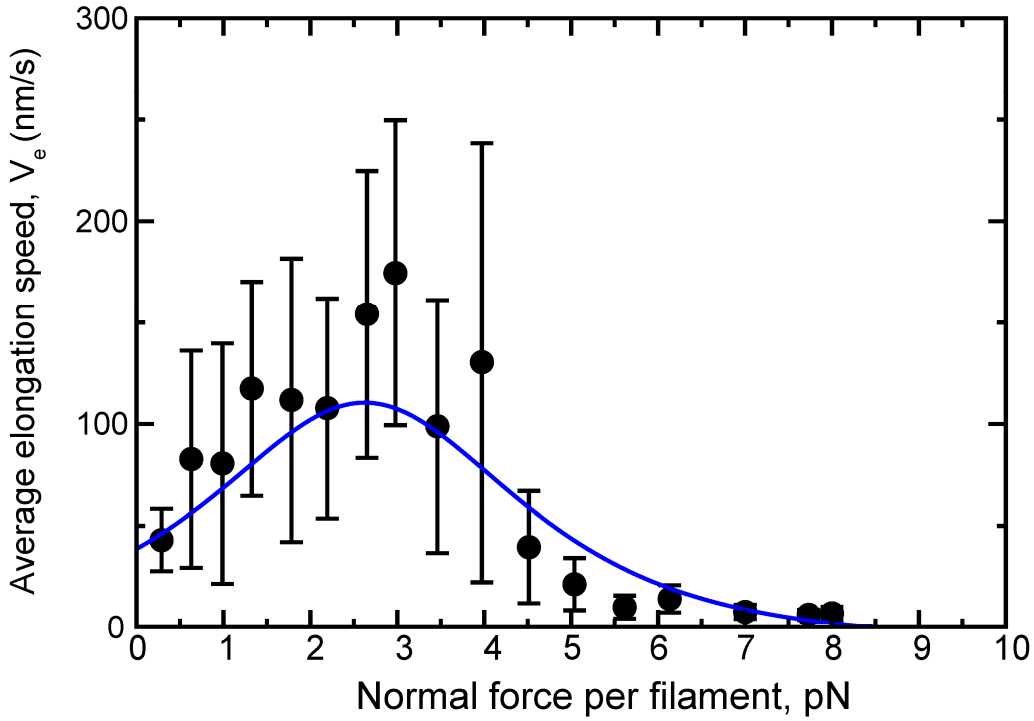


Figure SI-2 shows the experimental data reported in Figure 2 of the manuscript taking into account the total number of filament in the contact area (approximately 10^9 filaments). The blue curve represents a fit to the experimental data using Eq. 3 and using the following parameters: u_0=15; u_1=517; w_0=5; w_1=6; ϑ_0^+ =-1; ϑ_1^+ =1; $\vartheta_0^- = \vartheta_1^-$ =0.5. It is important to notice that the model can fit consistently the experimental data only if ϑ_0^+ is negative which means that the intermediate state is favorable under the applied force[1].

θ

Note: It is important to note that the direction of the force is opposite to the growth direction and in the same direction of the shrinking direction. Now let us consider only the growth pathway, and let call for this case the theta parameters θ(g). The reason why sum of θ(g)=1 in this case is explained in Fisher and Kolomeisky, Ref. 9 (after Eq. 17).

It is assumed that the different parameters θ are independent of the force, therefore they are also valid at the stall force and near the stall force. Since at the stall force (when Vg=0), mechanical equilibrium is reached, detailed force balance leads to sum θ(g)=1. Suppose that the actin filament increases its length by d. Then the external work done by the filament is Fd. At equilibrium, ΔG of the process is equal to zero. And ΔG= ΔG(0) +Fd, where ΔG(0) corresponds to the standard free energy change. Expression for ΔG involves θ – it is kT*ln(product of forward rates/product of backward rates), and one can conclude that the sum θ(g)=1.

Now consider the shrinking and define the θ(s) for this case. In this case a similar argument should give sum of θ(s)= -1, the negative sign "-" being due to the force direction. In this case the force is in the same direction as the shrinking direction. It is shown in Ref. 9 of the manuscript that identical equations describe both processes meaning that θ(s)= -θ(g).

Control experiments

- Interaction forces across a suspension of actin filament stabilized by phalloidin and end capped by gelsolin

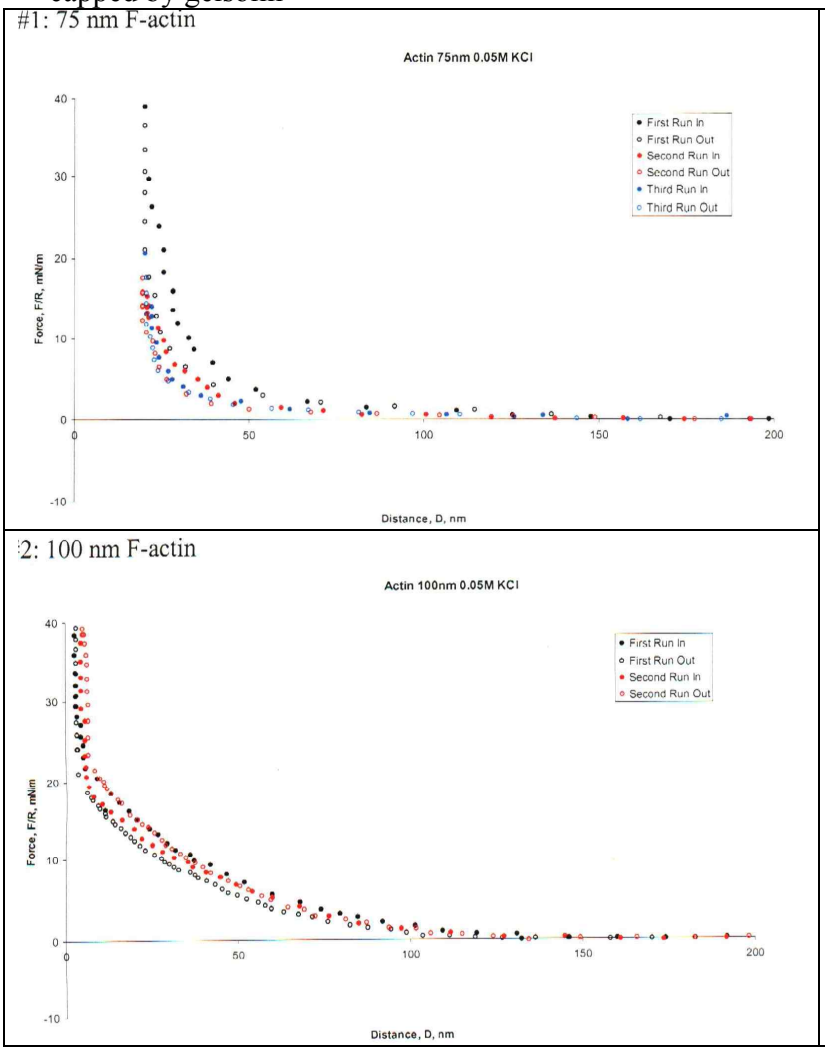
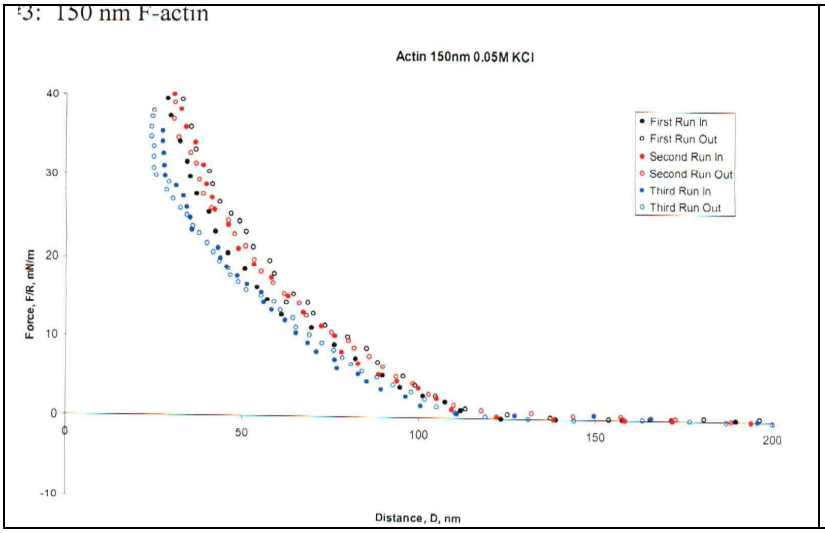


Fig. SI-3: Interaction forces between two mica surfaces immersed in a suspension of actin filaments of different length (75, 100 and 150 nm) that have been stabilized and capped by gelsolin in order to stop their polymerization. As can be seen no fluctuations in the forces could be detected. Instead, a smooth interaction profile was measured on the approach and separation of the surfaces.



The interaction force profiles show not oscillations independently of the length of the filaments demonstrating that the oscillations reported in the manuscript come from the polymerization of the filaments only.

- Fluctuation of the separation distance between two surfaces immersed in polymerization buffer

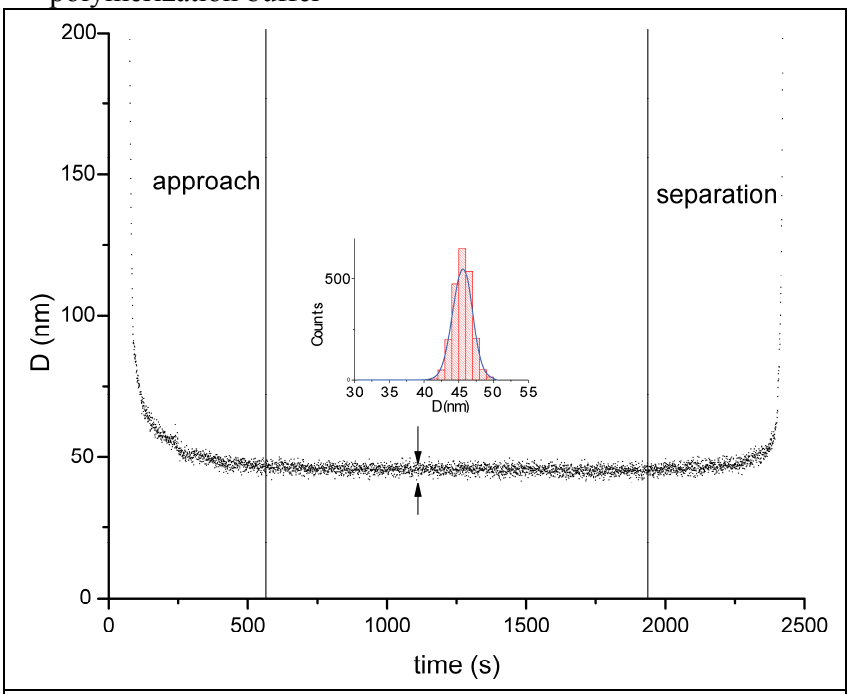


Fig. SI-4: Time evolution of the separation distance between two surfaces immersed in polymerization buffer. Approach and separation phases are separated by a phase were the surfaces were hold still during approximately 20 minutes. No significant mechanical oscillations could be observed.

Complementary scatter plot and bar diagram of Figure 2 and 3

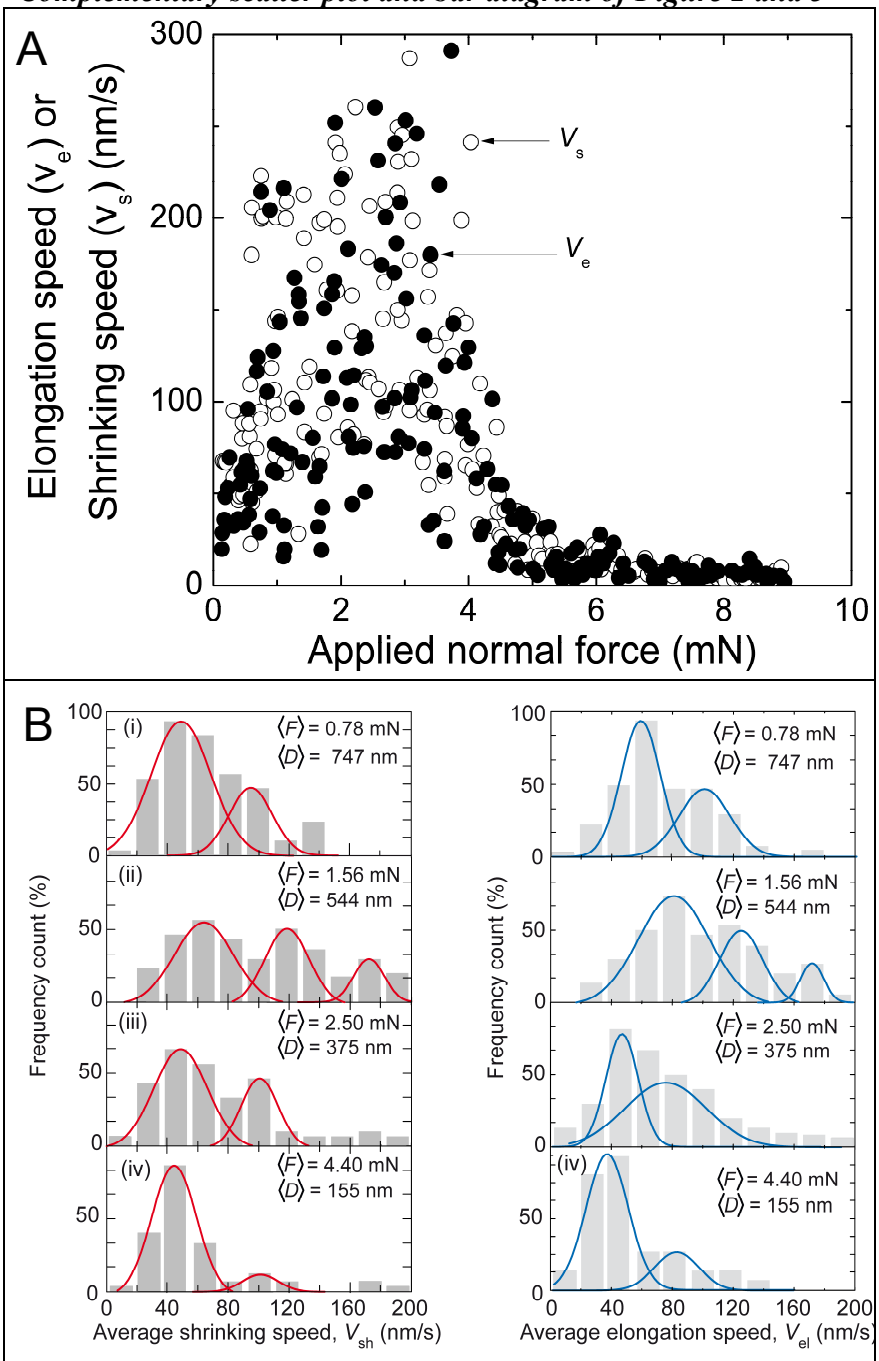


Fig. SI-5: A Scatter plot of V_{el} and V_{sh} as a function of the applied load. Error bars in load and velocity are smaller than the symbol. Data from Fig.2 were constructed by averaging 11 time consecutive velocity values. **B** Bar diagram of V_{sh} and V_{el} distribution obtain from "static experiments" during which the applied load was maintained constant on average and the fluctuations of the separation distance *D* were recorded as a function of time.

References

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2. G. W. Greene, T. H. Anderson, H. Zeng, B. Zappone and J. N. Israelachvili, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 445-449.
3. T.D. Pollard J. Cell Biol. 1986, **103**, 2747-2754