A mechanism for the force-velocity relation of fish keratocytes

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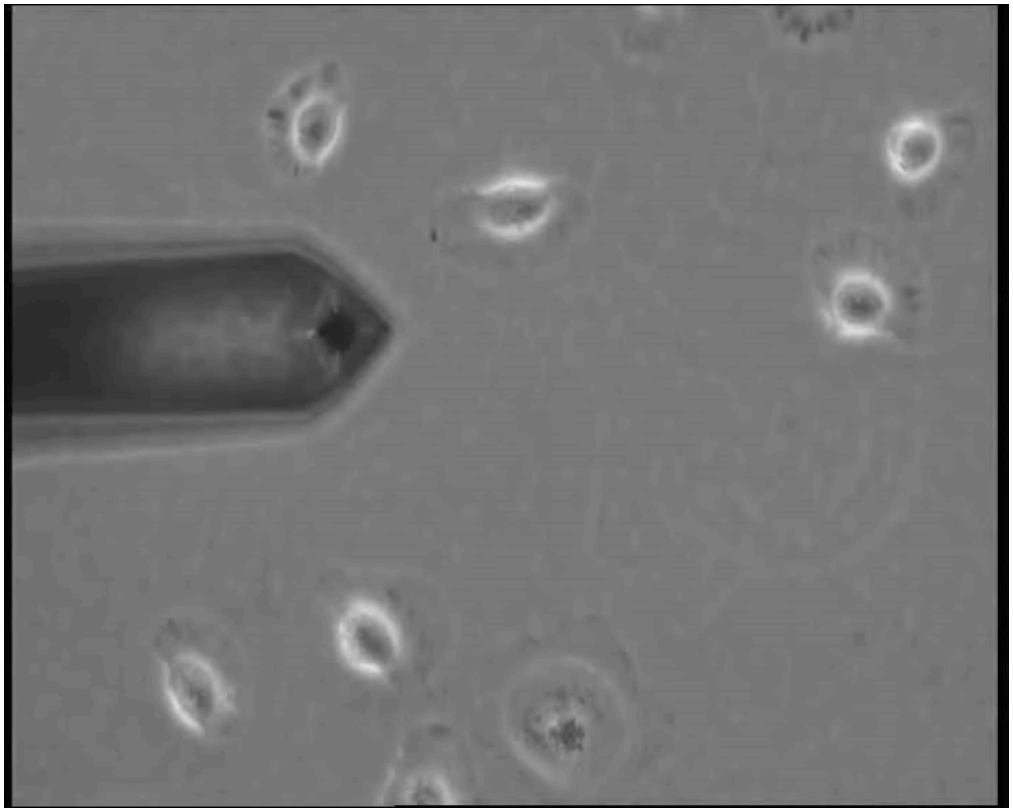
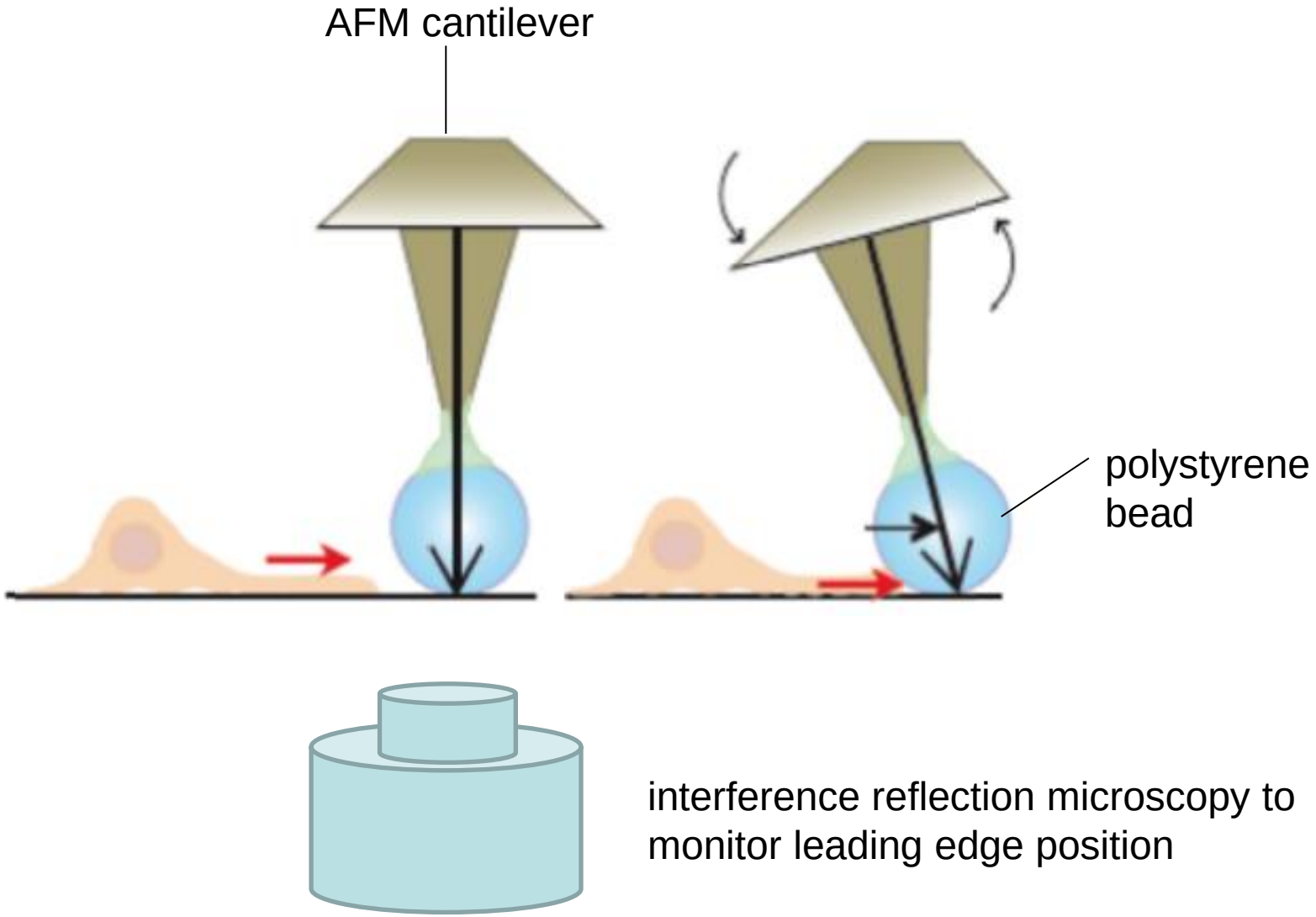
Theory: Juliane Zimmermann, Mihaela Enculescu, MDC

Measuring the force velocity relation of fish keratocytes with an AFM cantilever

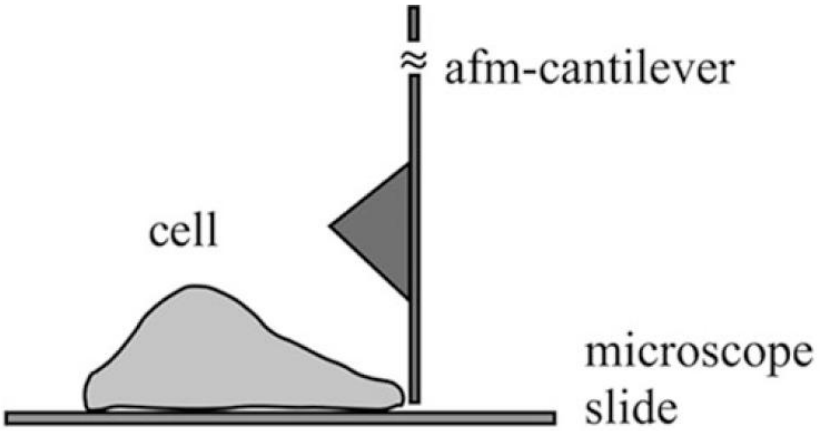


laboratory Josef Käs, University Leipzig

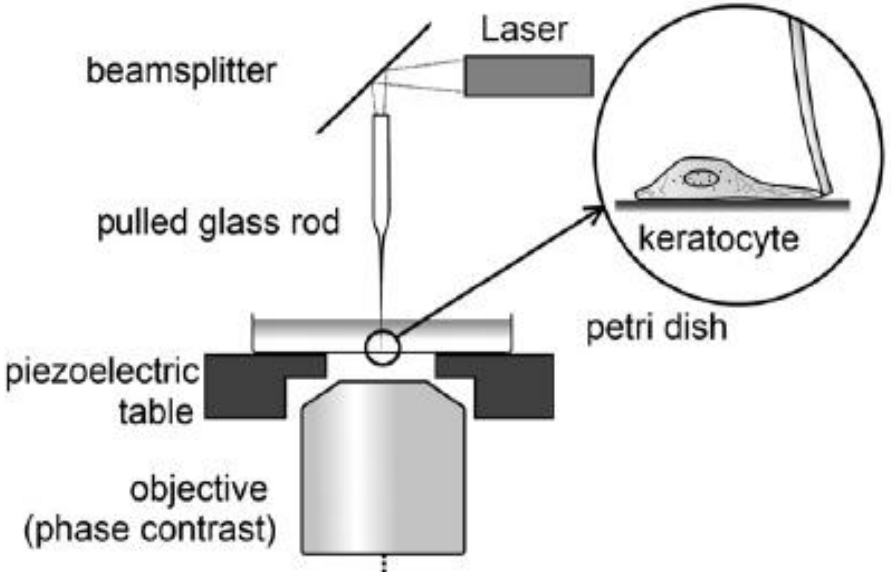
Measuring the force velocity relation of fish keratocytes with an AFM cantilever



Two different methods provide qualitatively the same results but with some quantitative differences

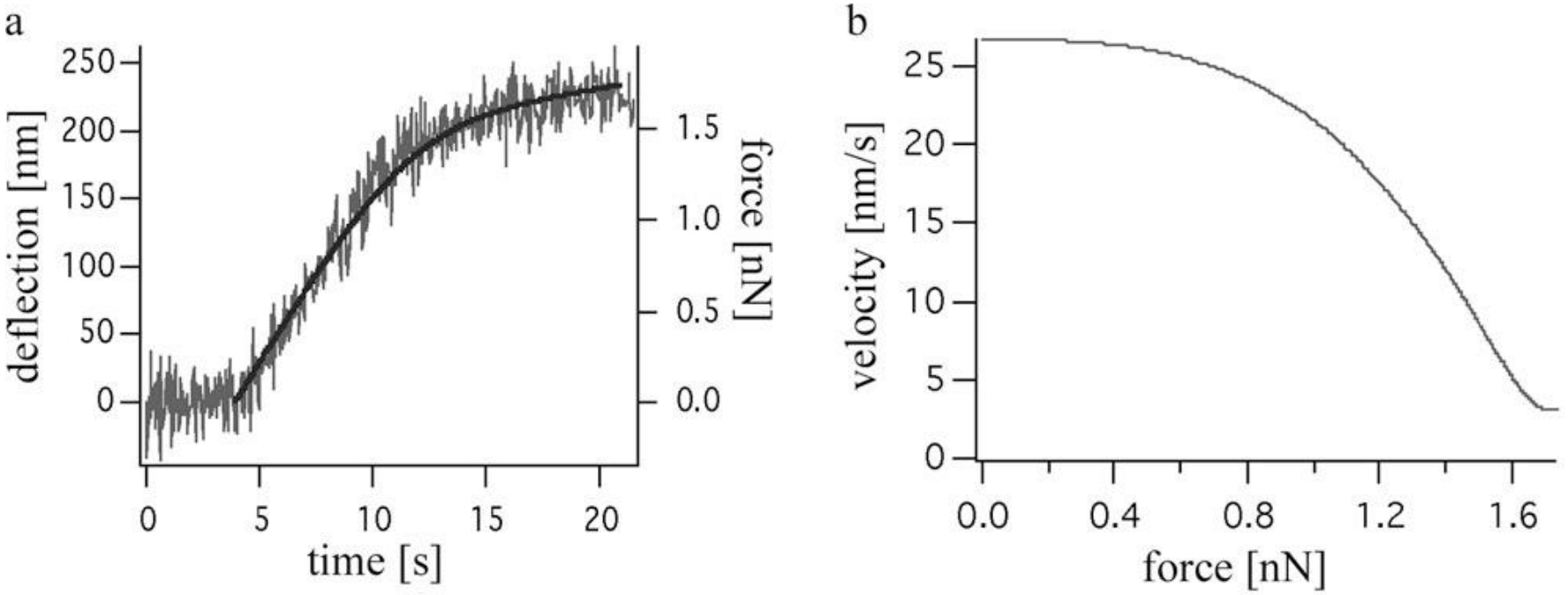


Prass, Jacobson, Mogilner, Radmacher (2006) *J. Cell Biol.* 174(6):767-772



Heinemann, Doschke, Radmacher (2011), *Biophys J* 100(6):1420-1427.

What is there to be understood?

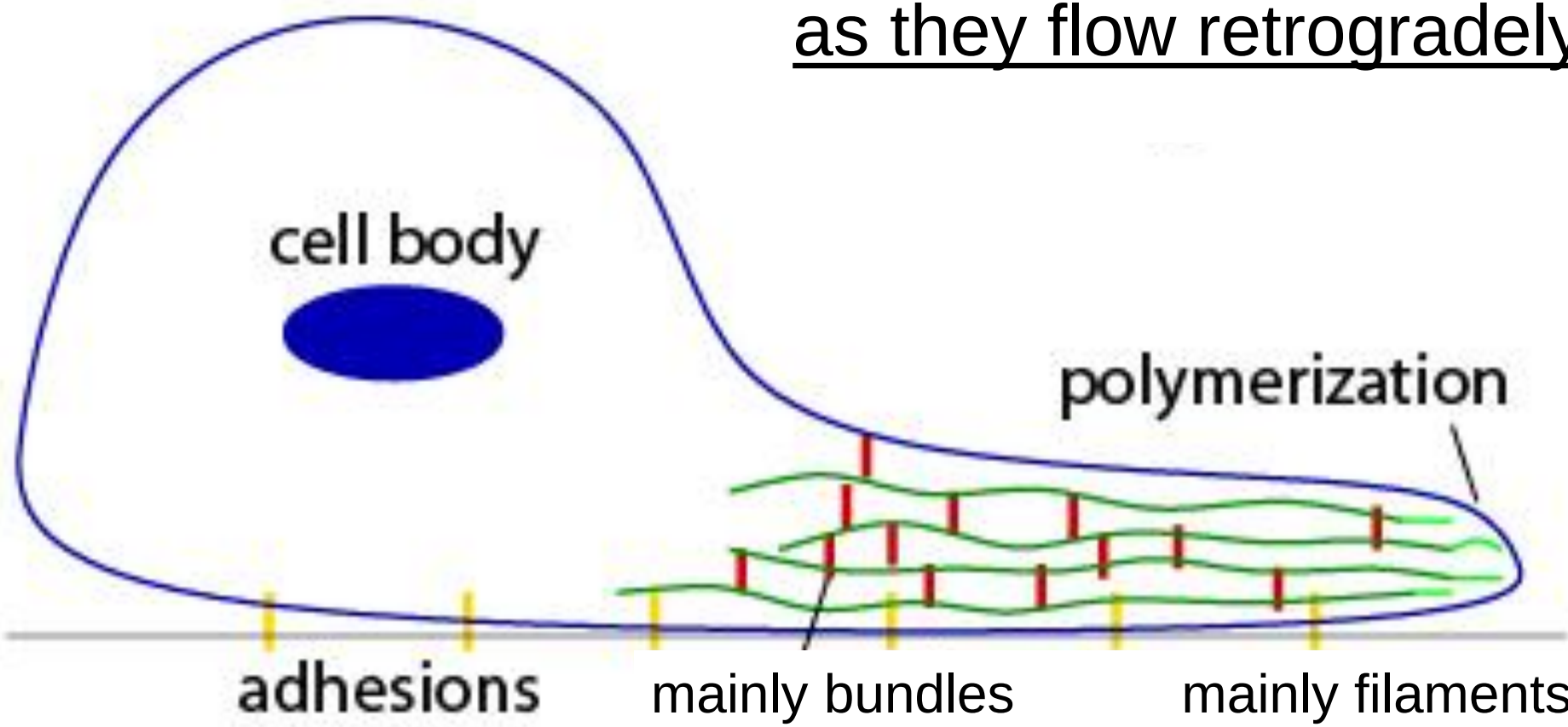


- Why do we not observe the signature of force generation by actin polymerization in the force-velocity relation?
- What explains the velocity values?
- What does it tell about lamellipodial mechanics?

M. Prass, K. Jacobson, A. Mogilner, M. Radmacher, *J. Cell Biol.* **174**, 767 (2006)

Model concept

Newly polymerized filament parts are cross-linked as they flow retrogradely



- Keren,Theriot, in *Cell Motility*, edited by P. Lenz (Springer 2008), pp. 31.
- E. Urban *et al.*, Nat Cell Biol **12**, 429 (2010).
- B. Verkhovsky, T. M. Svitkina, and G. G. Borisy, Current Biology **9**, 11 (1999).

Barnhart, Lee, Keren, Mogilner, Theriot (2011). *PLoS Biol* 9(5):e1001059

Zimmermann, Enculescu, Falcke. 2010 Phys Rev E 82:051925

What determines the value of the cross-linking velocity v_g during steady motion?

Relative to filament:

cross-linking velocity v_g = polymerization velocity

In terms of velocities in the lab frame:

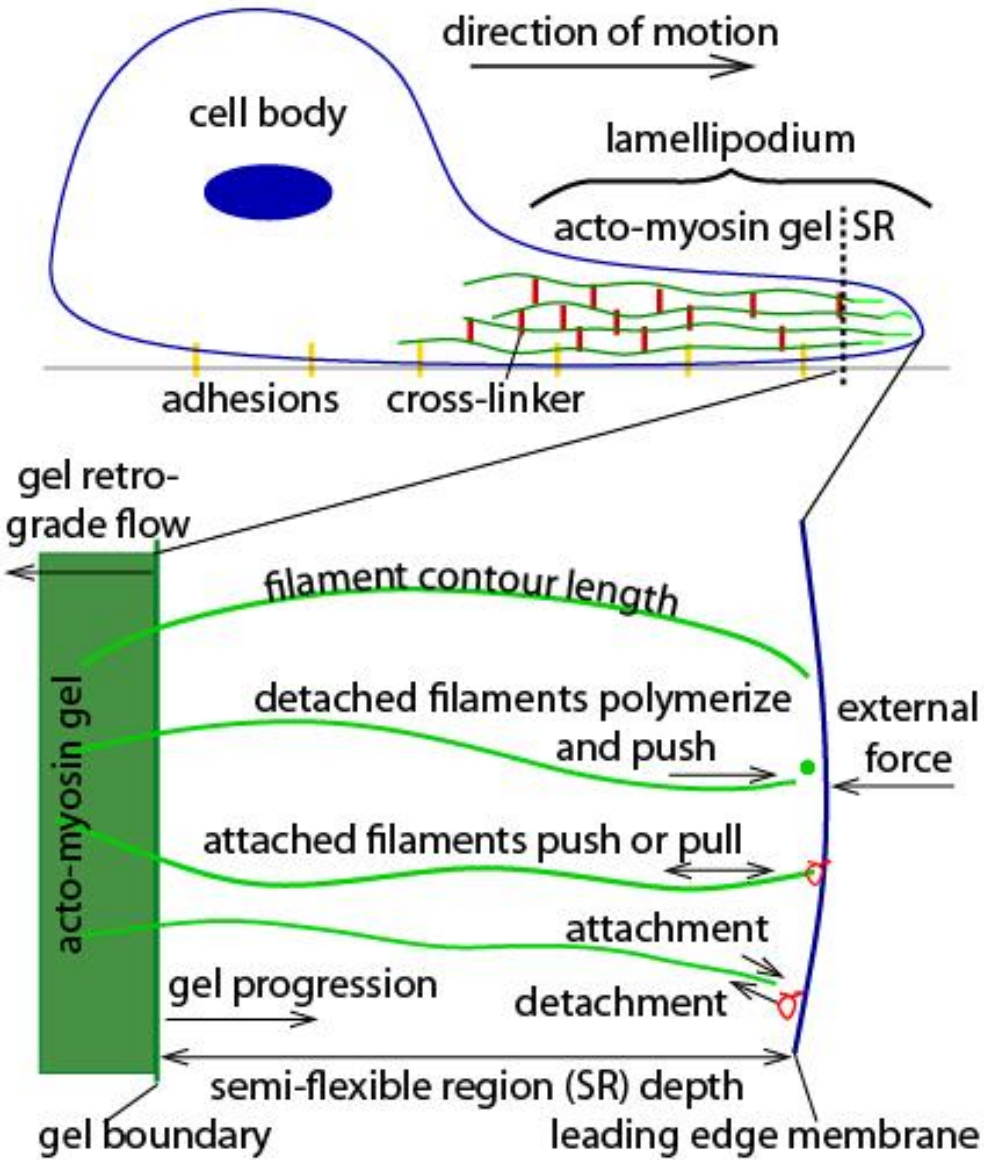
polymerization velocity = cell velocity + | retrograde flow |

cross-linking velocity v_g = cell velocity + | retrograde flow |

→ the cross-linking velocity can be measured

J. Zimmermann, M. Enculescu, M. Falcke, *Phys. Rev. E* **82**, 051925 (2010).

Model concept



- Filaments polymerize with a force-dependent rate and push the leading edge membrane.
- Filaments can attach to the membrane via linker molecules and either push or pull (turns out they push almost all the time during the force-velocity measurements).
- Attached filaments do not polymerize.
- The filament number is constant during the experiment.
- The gel boundary moves due to cross-linking and retrograde flow.
- Retrograde flow is determined by the force acting on the membrane, gel contraction, adhesion and gel viscosity.
- Adhesion is described as friction.

number of attached filaments

$$\partial_t n_a = k_a n_d - k_d(l_a, z) n_a,$$

attachment - detachment

free length of detached filaments

$$\partial_t l_d = v_p(l_d, z) - \tilde{v}_g(l_d, z) + k_d(l_a, z) \frac{n_a}{n_d}(l_a - l_d),$$

polymerization

cross-linking

detaching filaments

free length of attached filaments

$$\partial_t l_a = -\tilde{v}_g(l_a, z) + k_a \frac{n_d}{n_a}(l_d - l_a),$$

cross-linking

attaching filaments

gel boundary – membrane distance

$$\partial_t z = \frac{1}{\kappa}[n_a F_a(l_a, z) + n_d F_d(l_d, z) - f_{ext}] - u.$$

forces from attached (F_a) and detached (F_d) filaments

cantilever force

membrane velocity

-

gel boundary velocity

A. Gholami, et al., *New Journal of Physics*, 10(3) (2008) 033022
M. Enculescu, et al. *Physical Review E*, 78 (2008) 031915

Retrograde flow at the gel boundary

retro-grade flow $= -\frac{\mu L}{4\eta} g_1 - \frac{f_0}{L\xi} g_2$

- contraction μ increases retrograde flow
- force f_0 exerted on leading edge membrane increases retrograde flow

L depth of the lamellipodium
η gel viscosity
ξ friction coefficient describing adhesion
μ stress due to contraction by myosin
v_g cross-linking velocity
u gel boundary velocity
f_0 force exerted on leading edge membrane
h_0 height of lamellipodium at the leading edge
g_1, g_2 constants depending on ξ, L, η, h_0, v_g

Semi-analytic solution of the gel equations by Kruse et al., Phys. Biol. 3 (2006) 130–137

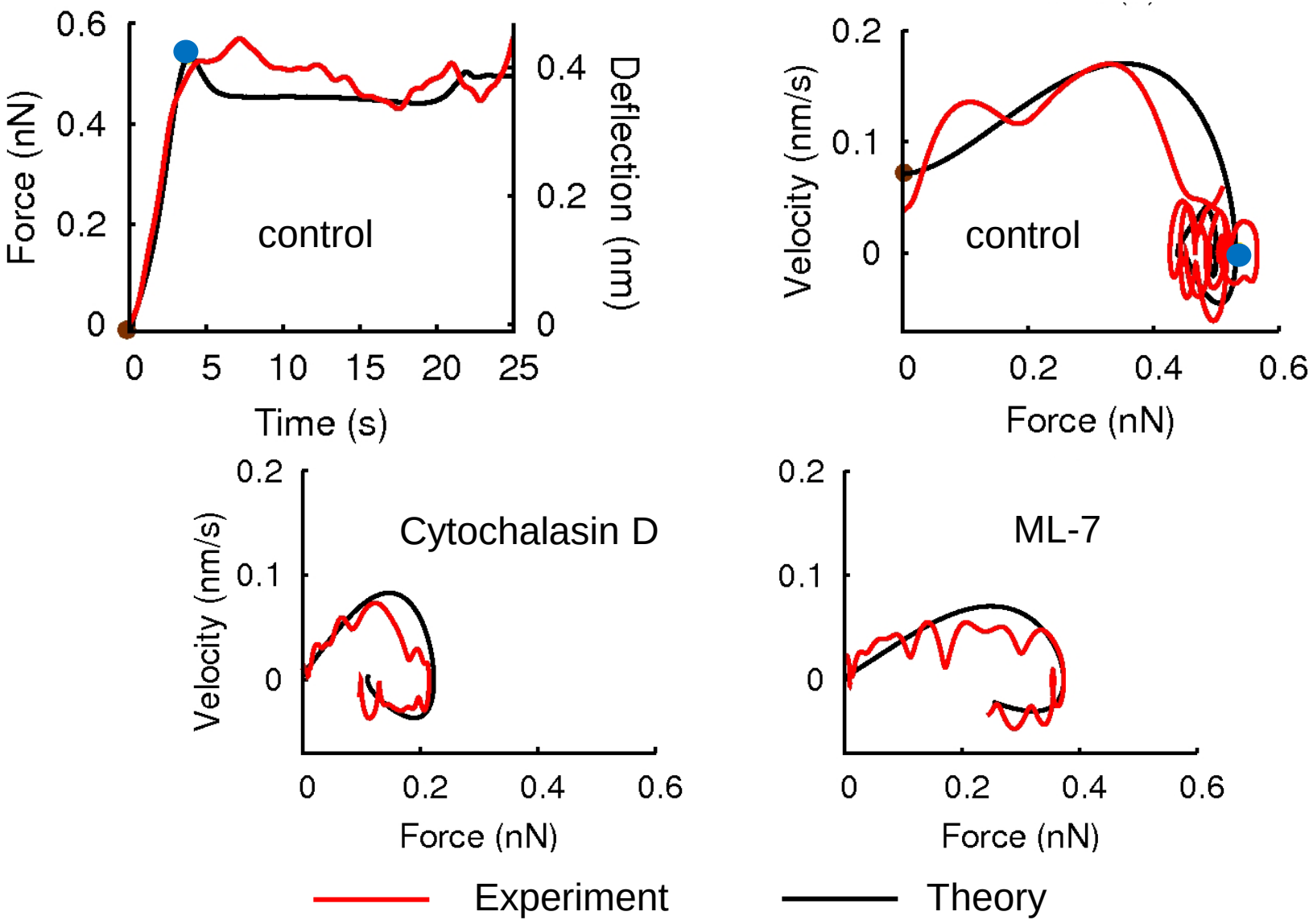
J. Zimmermann, M. Enculescu, M. Falcke, *Phys. Rev. E* **82**, 051925 (2010).

Gel boundary velocity u = cross-linking velocity v_g
+ retrograde flow

$$u \approx v_g - \frac{\mu L}{4\eta} g_1 - \frac{f_0}{L\xi} g_2$$

J. Zimmermann, M. Enculescu, M. Falcke,
Phys. Rev. E **82**, 051925 (2010).

Force-velocity relation of fish keratocytes



The free cell velocity and retrograde flow are reproduced also quantitatively.

The mechanism from first contact till stall

leading edge

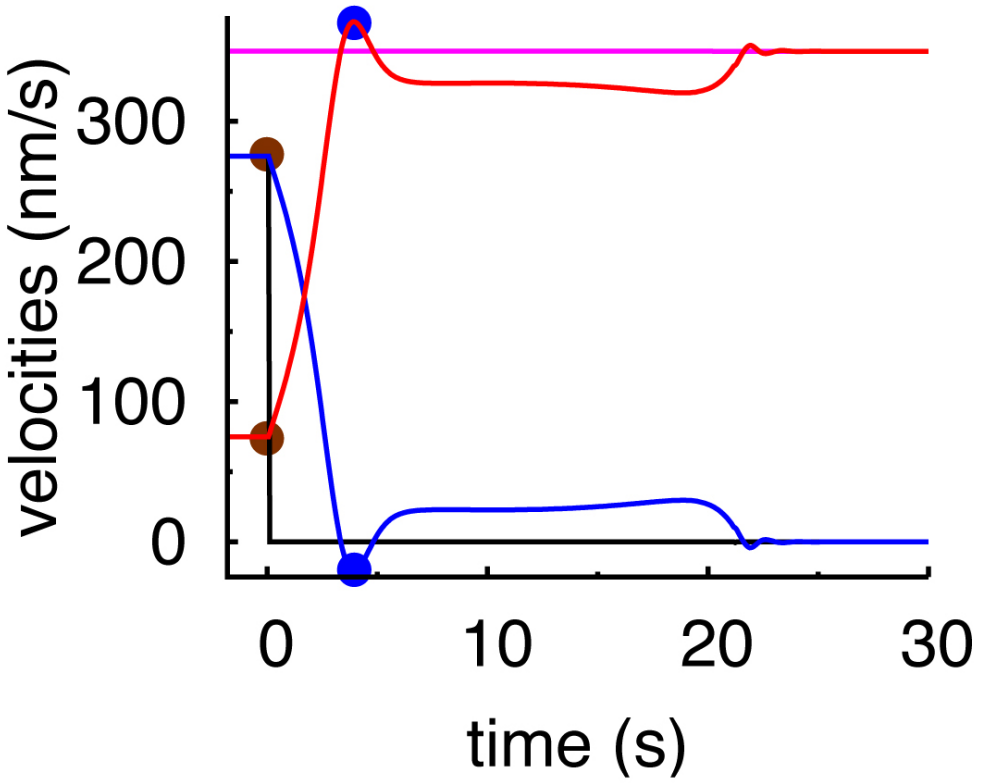
gel boundary

retrograde flow

|gel boundary velocity|+|retrograde flow velocity|=constant

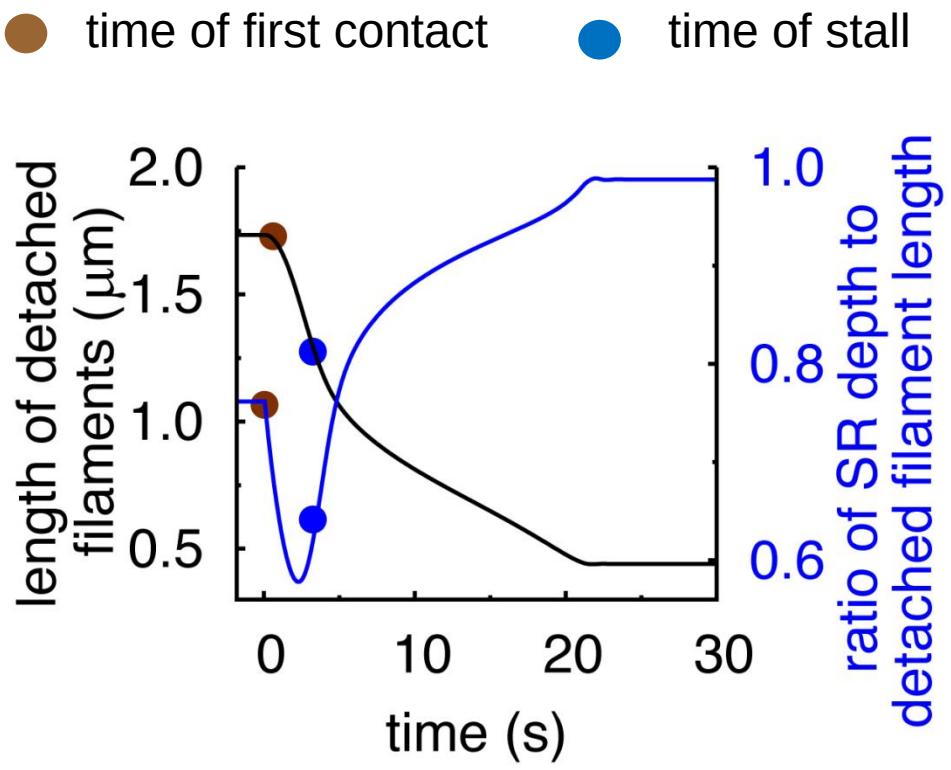
time of first contact

time of stall



- leading edge slows down immediately due to initial elastic response of the semi-flexible region
- gel boundary decelerates slower than leading edge
- retrograde flow speeds up
- stalling when retrograde flow equals polymerization velocity
- adaptation to stalled state

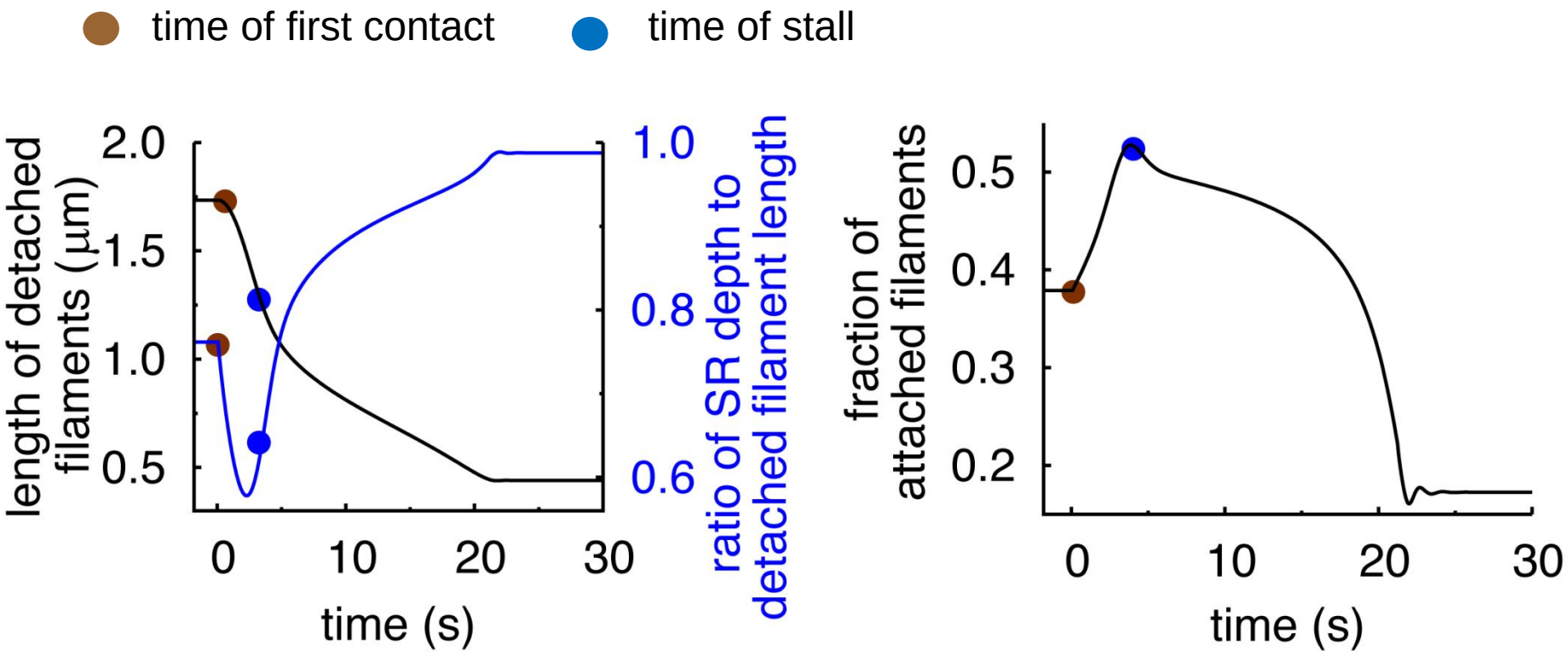
Force-velocity relation of fish keratocytes: initial filament bending and elastic response



Filaments take the first blow by bending, then the width of the semiflexible region decreases, filaments become stiffer (like length^{-4}) and straight.

- Such an elastic response was also measured by Heinemann et al. Biophys. J. 2011
- The filament length corresponds to structural data by Urban et al. Nat Cell Biol 2010 and Schaub et al. J Cell Biol 2007 (especially when we take the effect of cofilin on the persistence length into account, (McCullough et al. J.Mol.Biol. 2008)).
- The differential stiffness of the SR of the freely running cell agrees very well with the values for cross-linked F-actin networks measured in the Weitz lab (Gardel et al. Science 2004).

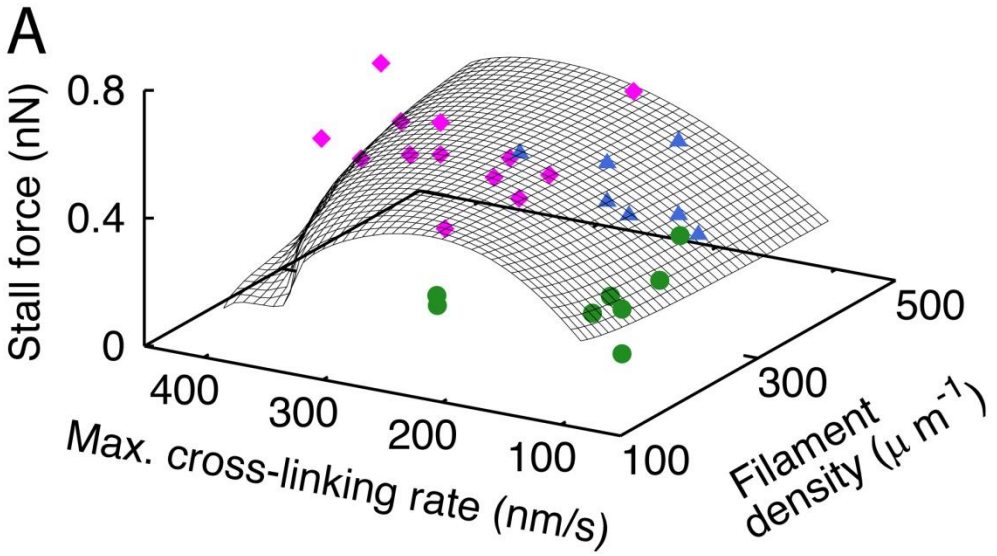
The adaptation phase starts after stalling



Details of the adaptation phase vary strongly between individual cells.

The existence of the adaptation phase demonstrates that the force-velocity relation is not stationary, does not reflect force-clamp measurements.

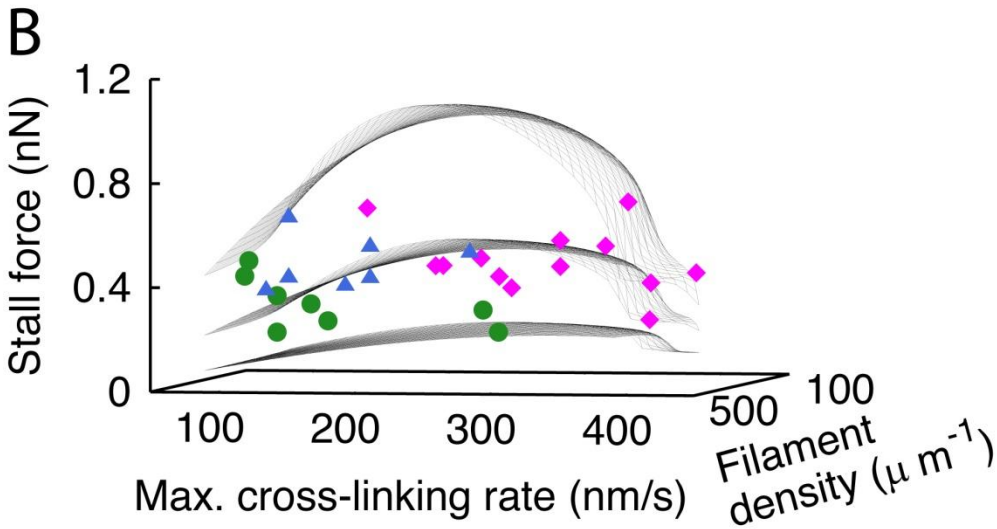
The fit of simulations to individual experiments relates experimental records to parameter values



Control

CD

ML-7



Surfaces from bottom to top:

friction coefficient ξ = 0.1, 0.23, 0.4 nNs μm^{-3}

gel viscosity η = 0.5, 0.83, 1.3 nNs μm^{-2}

Both CD and ML-7 reduce cell velocity and retrograd flow of the unhindered cell

	Control Measured Simulated	CD Measured Simulated	ML-7 Measured Simulated
Velocity of unhindered cell (nm/s)	240 ± 47 233 ± 47	98 ± 53 93 ± 47	127 ± 43 128 ± 45
Retrograde flow velocity of unhindered cell (nm/s)	68 ± 30 72 ± 35	27 ± 11 35 ± 27	42 ± 12 28 ± 8.8

Only parameters in line with the action of the drug on the actin network exhibit significantly different values between control and drug application

Parameter	Control	CD	ML-7	Units
Filament density	302 ± 42	181 ± 32	300 ± 0	μm^{-1}
Maximum value of the polymerization rate	611 ± 205	588 ± 80	613 ± 106	nm/s
Maximum value of gel cross-linking rate	306 ± 75	129 ± 73	157 ± 52	nm/s
Viscosity of the actin gel	0.91 ± 0.38	0.90 ± 0.37	1.03 ± 0.17	$nNs/\mu m^2$
Friction coefficient of actin gel with adhesion sites	0.23 ± 0.12	0.22 ± 0.11	0.243 ± 0.053	$nNs/\mu m^3$
Active contractile stress in actin gel	8.33	8.33	0	$pN/\mu m^2$

significant change

CD - capping protein → reduces filament density → reduces cross linking rate

ML7 - inhibits myosin, myosin acts mainly as cross linker in the central fish keratocyte lamellipodium → reduces cross linking rate

Initial velocity drop and cantilever stiffness

leading edge

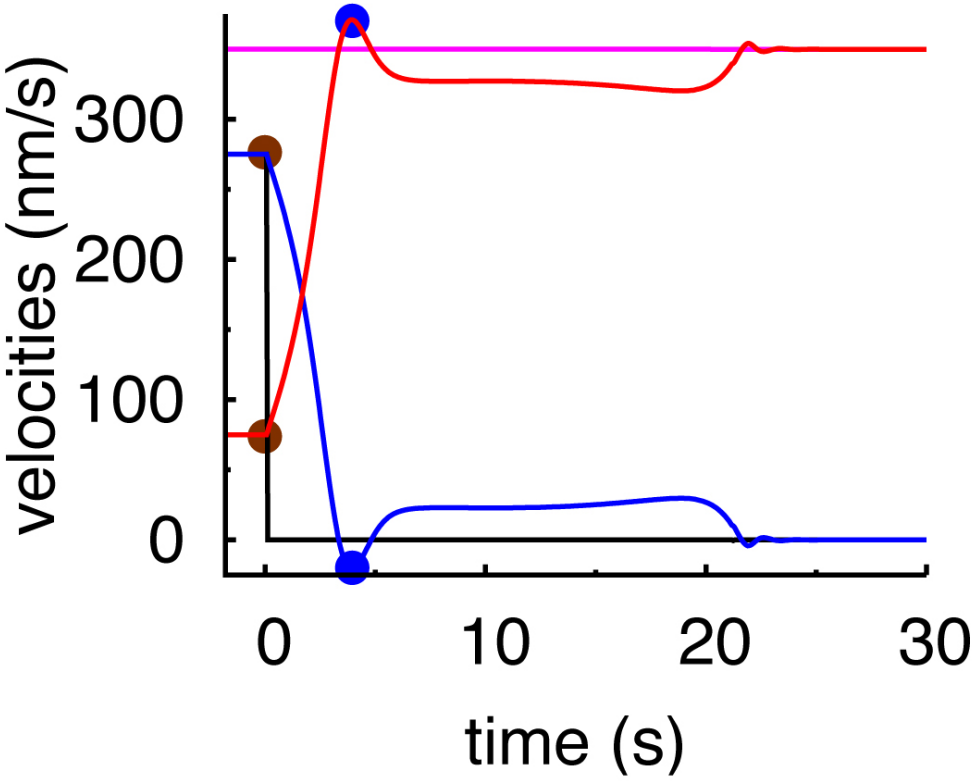
gel boundary

retrograde flow

|gel boundary velocity|+|retrograde flow velocity|=constant

time of first contact

time of stall

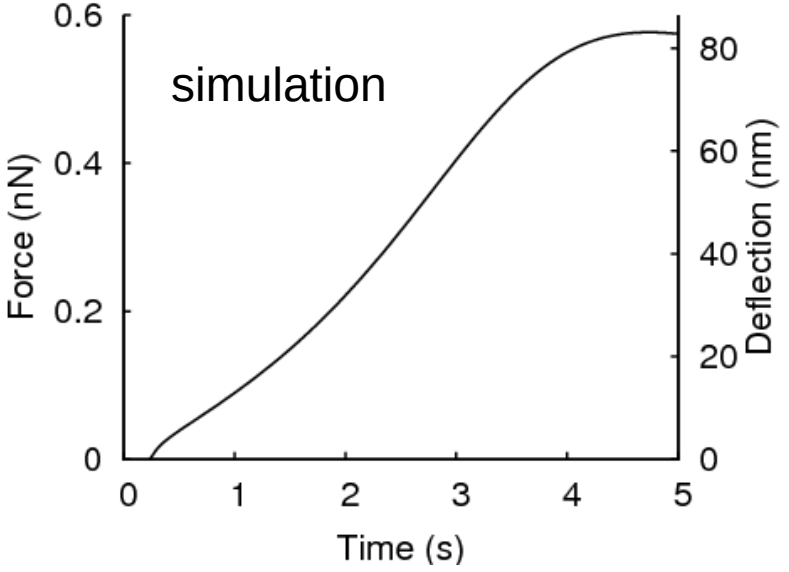


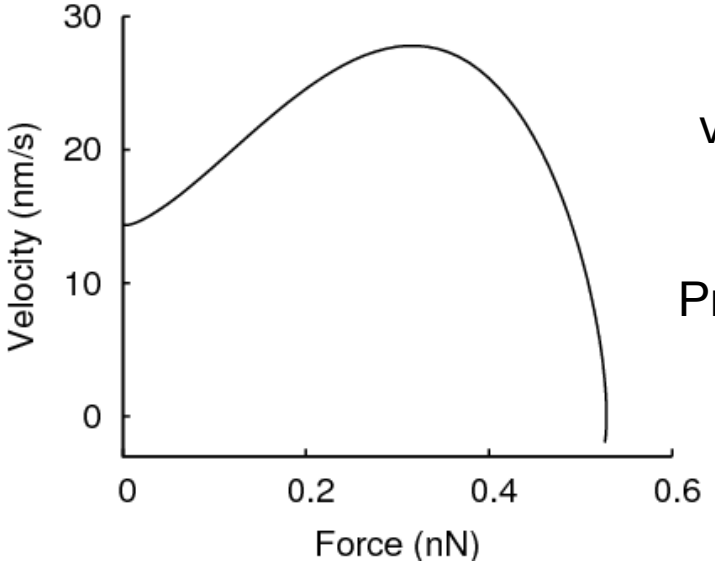
In our experiments and simulations, the ratio of free cell velocity to the velocity after the initial drop is about 2600.

In the experiments by Prass et al. 2006, it is about 7 only.

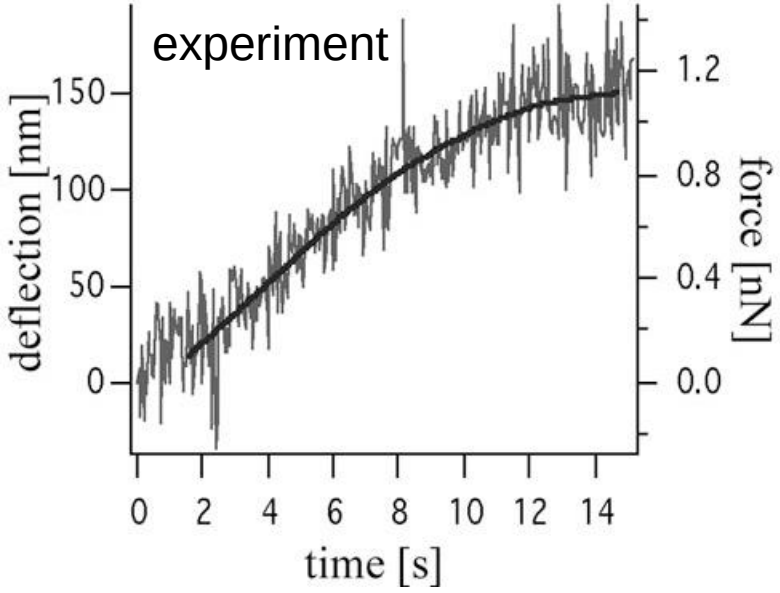
How can that be explained?

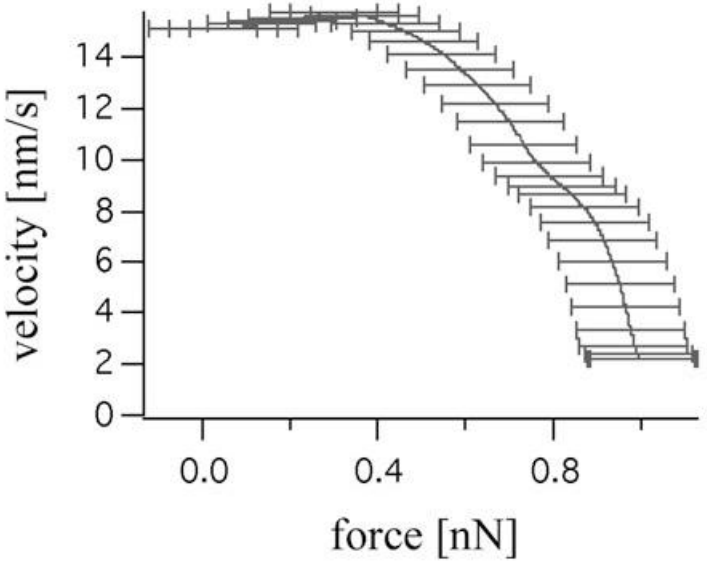
Cantilever stiffness determines the magnitude of the initial velocity drop





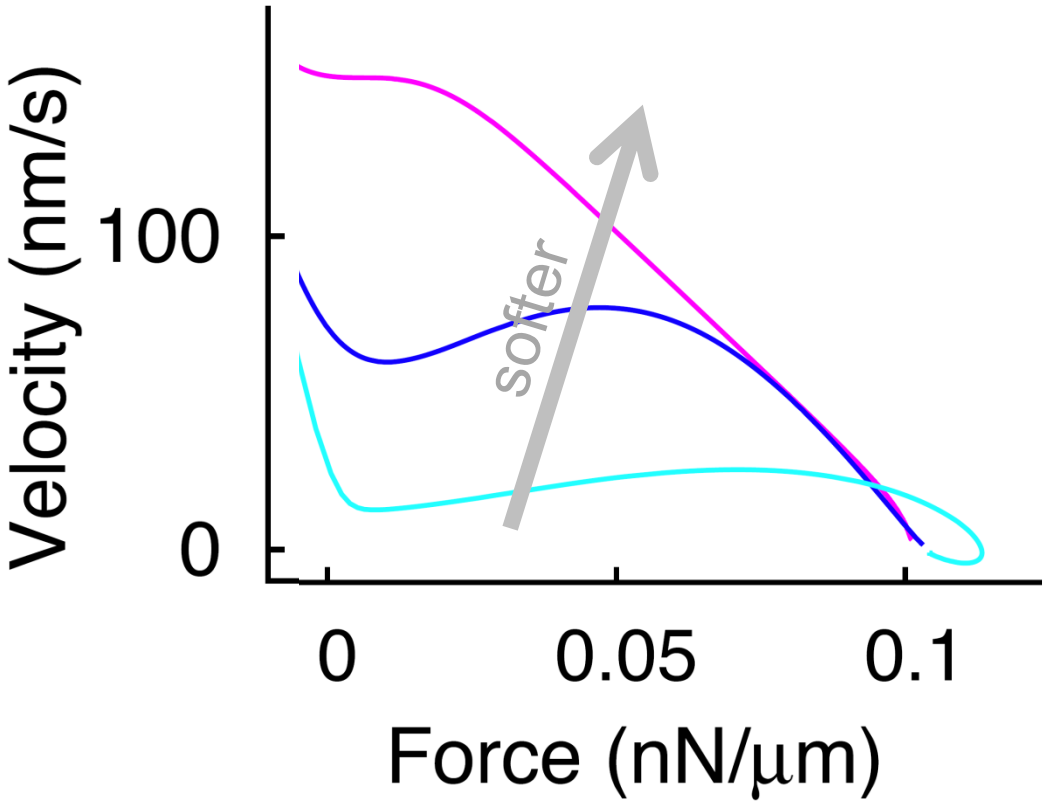
The initial velocity drop is much smaller with Prass's softer cantilever.





Prass, M., K. Jacobson, A. Mogilner, M. Radmacher. 2006. J. Cell Biol. 174:767-772.

Cantilever stiffness determines the magnitude of the initial velocity drop



The F-actin persistence length l_p: published values (*in vitro*)

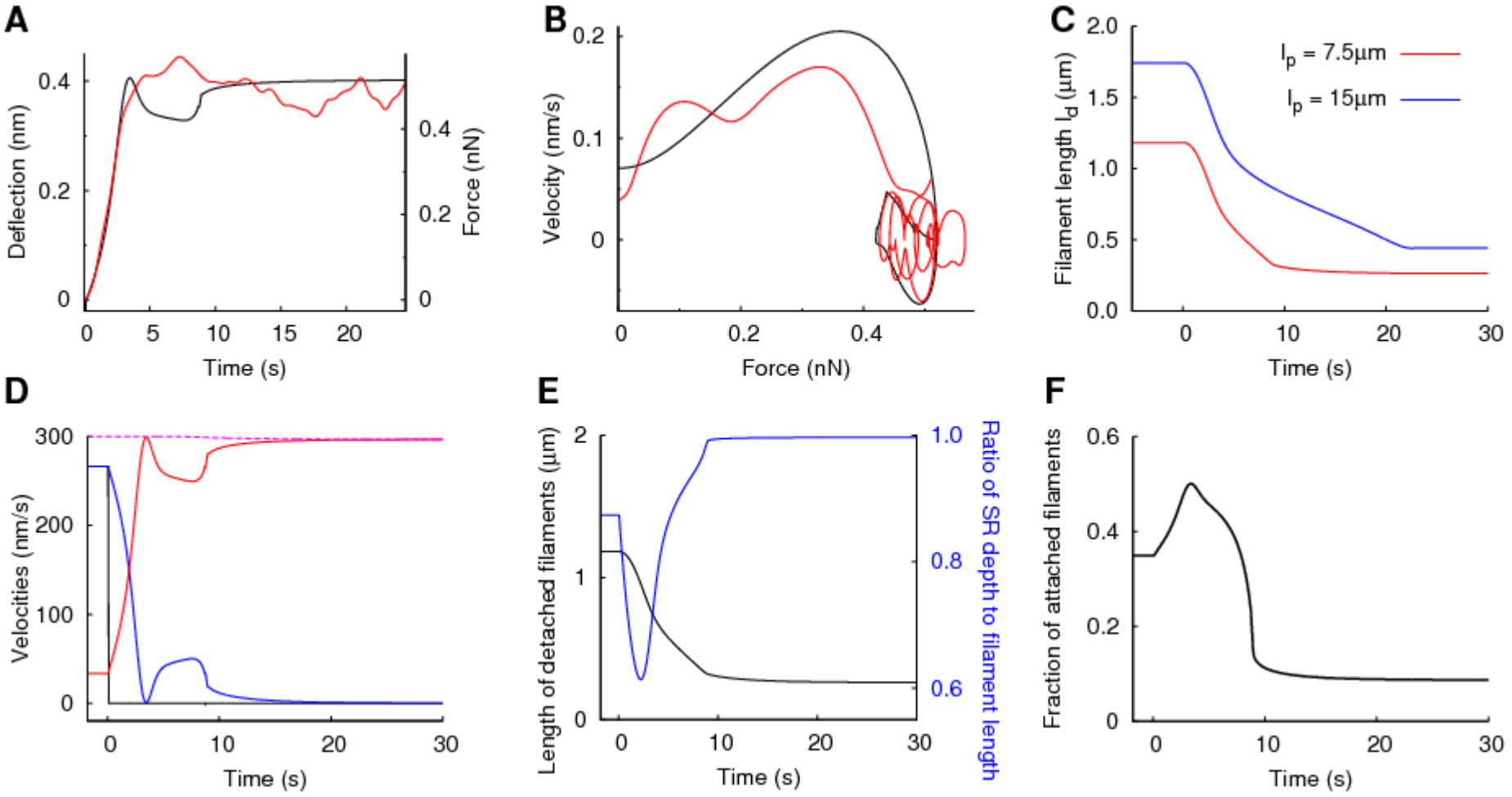
1. l_p = 17 µm — Gardel ML, *et al.* (2004) *Science* 304(5675):1301
2. l_p = 15 µm — Le Goff L, et al. (2002) *Phys Rev Lett* 89(25):258101
3. l_p = 9-13.5 µm — Isambert H, *et al.* (1995) *J Biol Chem* 270(19):11437

With cofilin

4. l_p = 2.2-9.8 µm — McCullough BR, et al. (2008) *J Mol Biol* 381(3):550
   Pfaendtner J, et al. (2010) *PNAS* 107(16):7299

We used l_p = 15 µm. How sensitive are results to the value of l_p ?

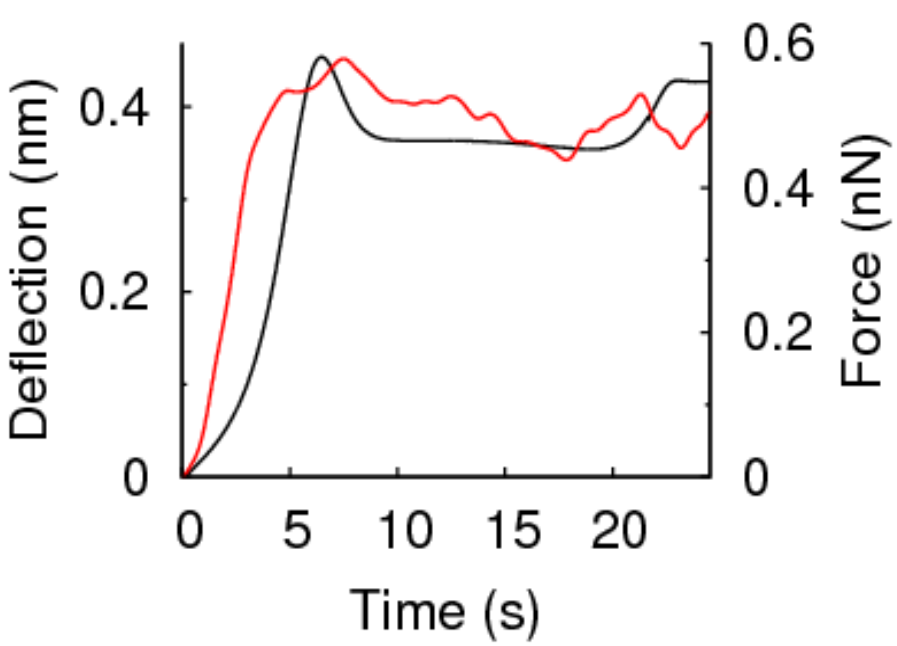
The F-actin persistence length determines the free polymer length

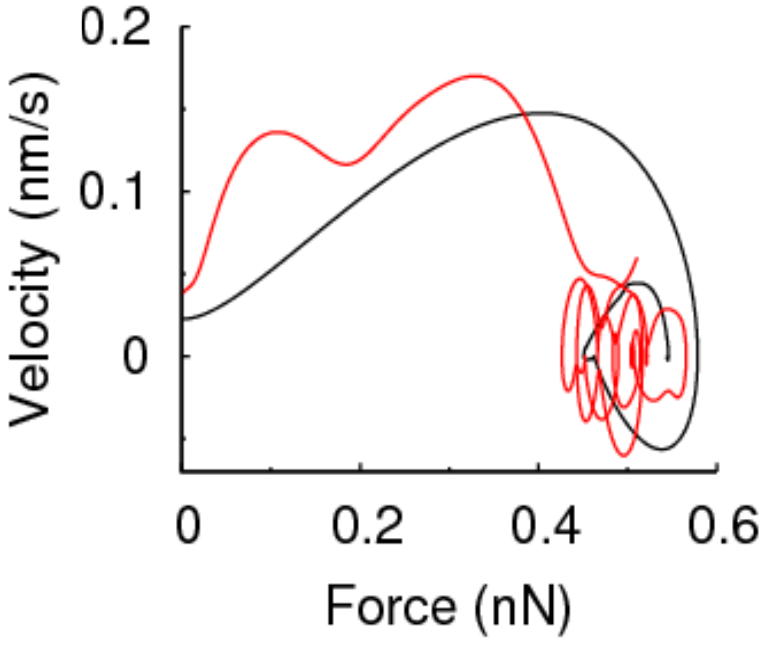


Persistence length of F-actin l_p= 7.5 µm (instead of 15 µm).

Force extension relation of semi-flexible polymers suggests scaling of the free polymer length like $l_p^{1/2}$, which approximately applies here.

Fast feedback from force to adhesion (catch bonds) appears not to have an essential role in shaping the force-velocity relation

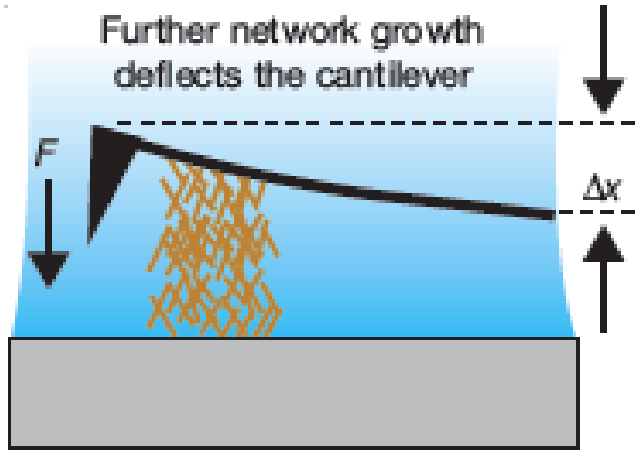




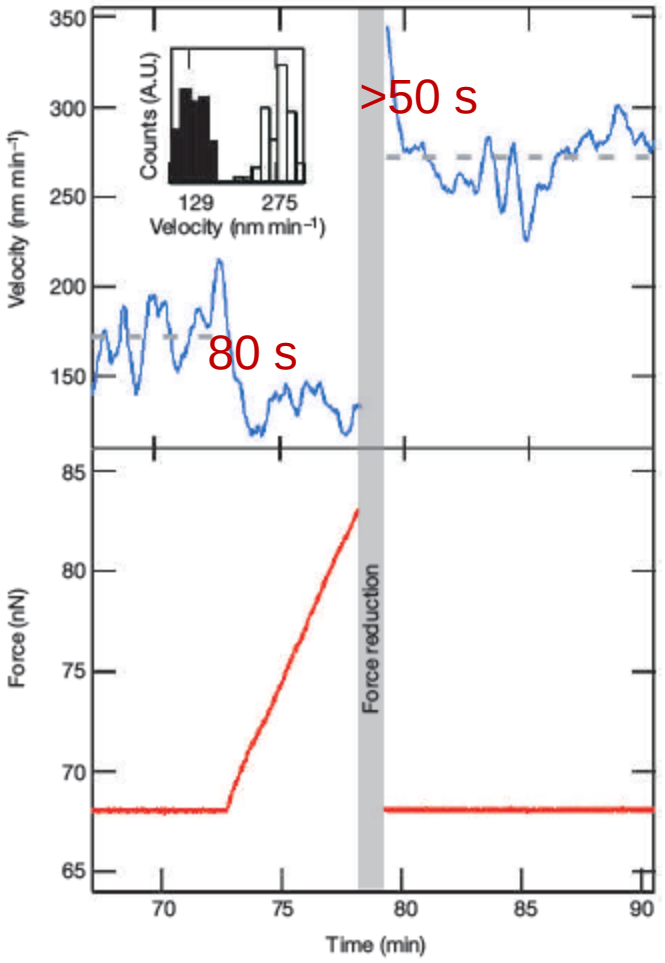
$$\text{adhesion } \xi = \xi_0 + 1.38 \frac{\text{s}}{\mu\text{m}^3} f_0, \quad \xi_0 = 0.13 \frac{\text{nN s}}{\mu\text{m}^3}$$

$$\text{at stall force } \xi = 0.93 \frac{\text{nN s}}{\mu\text{m}^3}$$

Dependence on load history

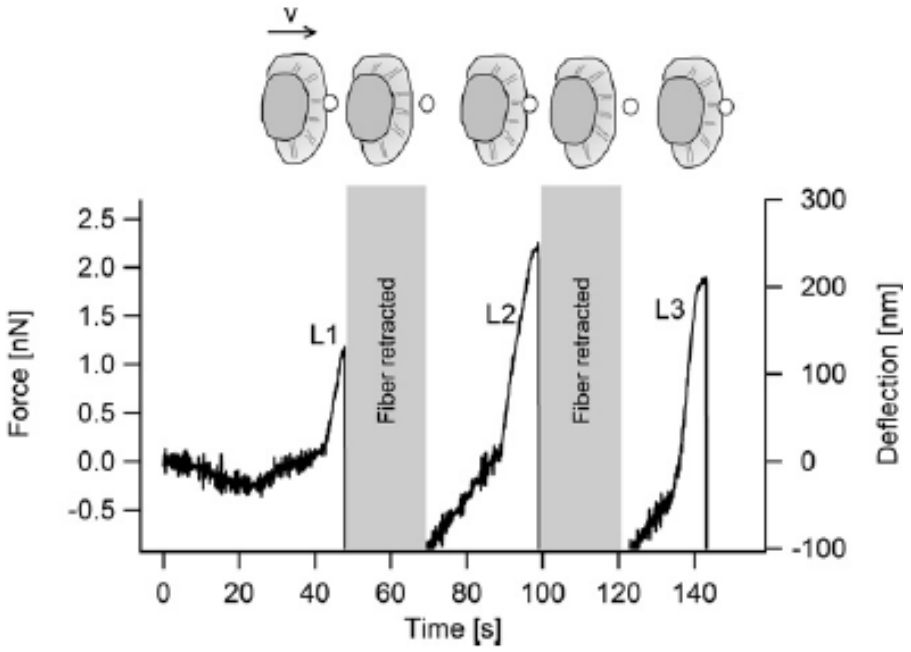


- the time scale is minutes, including the transients
- dependence on load history: „increasing filament density with force is a plausible explanation for our observations“
- high velocity state is maintained for minutes

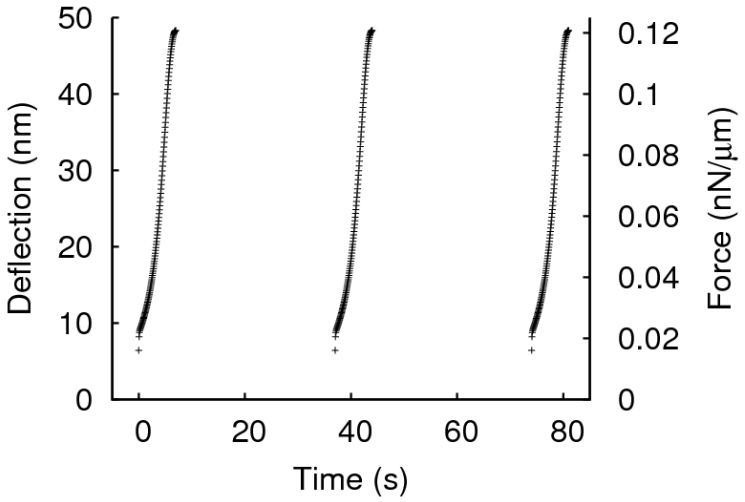


Parekh, Chaudhuri, Theriot, Fletcher (2005) *Nat Cell Biol* 7(12):1219-1223

Dependence on load history



No dependence on load history in repetitions with about 40 s interval.



Simulations neither show a dependence on load history on that time scale.

Heinemann, Doschke, Radmacher (2011), *Biophys J* 100(6):1420-1427.

Conclusion force-velocity relation

- The force velocity relation exhibits an initial leading edge velocity drop, motion against rising force till stalling and adaptation to the stalled state.

- The suggested mechanism explains it quantitatively by a transient elastic response of the lamellipodium region close to the leading edge (semi-flexible region) and slower increase of retrograde flow.

- It explains also the action of CD, ML-7, the effect of changing cantilever stiffness, and repetition experiments.

- The force-velocity relation is not stationary.