Supporting Online Material for

Nonequilibrium Mechanics of Active Cytoskeletal Networks

Daisuke Mizuno, Catherine Tardin, C. F. Schmidt,* F. C. MacKintosh*

*To whom correspondence should be addressed. E-mail: cfs@nat.vu.nl (C.F.S.); fcm@nat.vu.nl (F.C.M.)

Published 19 January 2007, *Science* **315**, 370 (2007)
DOI: 10.1126/science.1134404

This PDF file includes:

Supporting Online Materials

Materials and Methods

Sample preparation

Actin and myosin II were prepared from rabbit skeletal muscle according to published methods (*1*). Actin was stored at -80°C in G-buffer (2 mM Tris-Cl, 0.2 mM $CaCl_2$, 0.5 mM DTT, 0.2 mM ATP, pH 7.5) and myosin at -80°C in high salt buffer (0.6M KCl, 50 mM KH_2PO_4, PH 6.5). Actin was biotinylated following a standard procedure (*2*). Unlabeled actin, and biotinylated actin were incubated with neutravidin (Molecular Probes, cross-linker) for at least 1 h and mixed with probe particles. Then, monomeric actin was co-polymerized by diluting into F-buffer (2 mM HEPES, 2 mM $MgCl_2$, 50 mM KCl,1 mM EGTA, pH 7.5), which was pre-mixed with myosin and ATP (3.5 mM). The ratio of normal actin: biotinylated actin: neutravidin was 180:5:2. The total concentration of actin was 1 mg/ml and of myosin 170 nM. 5 μm diameter polystyrene beads (Duke Scientific) were used for laser interferometry and 1.1 μm diameter polystyrene beads were used for video microscopy. After mixing, samples were immediately infused into sample chambers (inner dimensions 15 mm x 3 mm x 100 μm) made from a microscope slide, a cover slip and double-stick tape. Measurements were started after drift, probably caused by the cross-linking process, had settled (typically ~ 30 min).

In order to stabilize low concentrations of ATP in the presence of the actin/myosin ATPase activity, ATP regeneration was used. Specifically, we used the reaction

$$\text{phosphocreatine} + \text{ADP} \rightarrow \text{ATP} + \text{creatine} + \text{phosphate}.$$

We started with an initial concentration of ~60 μM ATP, which is hydrolyzed to ADP by actin polymerization and by the myosin motors. Under steady consumption of ATP, adding a rate-limiting amount of enzyme (creatine phosphokinase) with an excess amount of substrate (phosphocreatine) leads to a steady-state concentration of ATP. To qualitatively test the

properties of the active networks at different ATP concentrations, we chose a maximal enzyme concentration (0.5 mg/ml) that was still low enough to produce a shear modulus that was measurably different from the one measured at saturating ATP concentration. From this concentration we further diluted the enzyme by factors of 10 (50 μg/ml, 5 μg/ml). We used F-buffer with 150 mM KCl, in which non-processive myosin mini-filaments switch to a processive mode at low ATP concentration.

Microrheology

The motion of probe particles was measured using laser interferometry (at high frequencies) or video-microscopy (at low frequencies). Laser interferometry was also used to measure the displacement response to an applied force. In the case of laser interferometry, two independent laser beams (diode laser, λ= 830 nm, IQ1C140, Laser 2000, and ND:YVO4, λ=1064 nm, Compass, Coherent) were focused into a custom-built inverted microscope (*3*, *4*). The two diffraction limited laser foci were superimposed in the specimen plane and formed two optical traps. The 1064 nm laser was operated at 2 W and typically attenuated to a power of 30 mW (in the specimen plane) to form a strong trap, while the 830 nm laser (operated at 150 mW and attenuated to 5 mW) served mainly for displacement detection by back-focal-plane interferometry (*5*), forming a negligibly weak trap. The driving laser could be deflected by an acousto-optic deflector (DTD 276HB6, IntraAction), in order to oscillate the trapped particle. The instantaneous trapping force exerted on the particle was measured from the relative displacement of the probe particle with respect to the oscillating trap by interferometry. The position of the particle $u(t)$ with respect to the probe laser focus was determined with a spatial resolution better than 0.1 nm. The output signal from the photodiode was fed into a lock-in amplifier (SR830, Stanford

Research Systems) that measured the amplitude and phase compared to the driving signal for the AOD as a reference signal.

For the passive measurements (PMR), the 1064 nm trapping laser was turned off and the output signal generated by the probe laser was directly recorded through an A/D board (16 bit, chicoPlus, Innovative Integration). From the displacement of the probe particle sampled at 195kHz sampling rate, we calculated the displacement power spectral density (PSD) by Fast Fourier Transform.

Supporting online text

S1 – Crosslink estimate

Following Refs. 6, 7, the linear elastic shear modulus is predicted to be

$$G = 6\rho k_B T l_p^2 / l_c^3 , \qquad \text{(S1)}$$

where ρ is the length density of actin filaments per unit volume, $l_p \cong 17$ μm is the persistence length, and l_c is the mean distance between crosslinks. For our system, $\rho \cong 6\times10^{12}$ m^{-2}, leading to $G' \cong$ 1-10 Pa for $l_c \cong$ 1.5 - 3.5 μm. This is similar to the moduli found by Gardel et al. (*8*).

S2 – Pre-stressed theory of G(ω)

From the shear modulus theoretically derived in Ref. 7, the single-particle response function is expected to be

$$\alpha = \frac{5}{2\pi a \rho l_c} \frac{1}{k_B T q_1^4 l_p^2} \sum_{n=1}^{\infty} \frac{1}{n^4 - i\omega / 2\omega_1} \cong \frac{5}{4\sqrt{2}\pi a \rho k_B T l_p^2} \left(\frac{2\kappa}{-i\zeta\omega} \right)^{3/4} , \qquad \text{(S2)}$$

where $q_1 = \pi / l_c$, $\omega_1 = (\kappa / \zeta) q_1^4$, and ζ is a transverse friction coefficient per unit length of a filament. Apart from the mean separation between crosslinks l_c, this depends only on known parameters, such as the persistence length/stiffness $l_p = \kappa / k_B T$, ρ, ζ and the bead radius a. In the limit of high frequencies, the response is insensitive to the distance between crosslinks l_c, as shown in the final expression in (S2). For frequencies above 100Hz, the predicted $\omega^{-3/4}$ behavior agrees well with our results for relaxed solutions/networks, for which filament tension $\sigma = 0$ (see Fig. 3a).

For $\sigma > 0$, however, one expects a reduced compliance or response (i.e., an increased modulus). In addition, a weaker frequency dependence is also expected. Both of these effects are consistent with Fig. 3a. For large enough tension σ, or low enough frequencies, this regime is expected to show an approximate $\sim \omega^{-1/2}$ dependence (*9*). The full dependence for $\sigma > 0$ can be calculated by a generalization of the calculation in Ref. 7:

$$\alpha = \frac{5}{2\pi a \rho l_c} \sum_q \frac{2k_B T q^4}{\omega_q \zeta^2 \left(2\omega_q - i\omega\right)}, \qquad \text{(S3)}$$

where $\omega_q = (\sigma q^2 + \kappa q^4)/\zeta$ accounts for both stretching and bending energy of filaments, $q = n\pi / l_c$, and n = 1, 2, 3, …. The resulting compliance for σ = 0.1 pN and $l_c = 2.6\mu$m is shown as the green line in Fig. 3a. The black curve is the calculated compliance for the same l_c, but without tension. Thus, our observed increase in network stiffness is consistent with a pretension of the filaments making up the network. However, given the localized stress exerted by the myosin motors, we do not expect this tension to be spatially uniform. In fact, some filaments will be under a much larger tension, and the value of $\sigma \cong 0.1$ pN represents an average over the sample.

S3 – Correlated motion of particle pairs

The correlated motion of particle pairs can be measured using video microscopy to track many probe particles simultaneously (*10*). We have measured the fluctuating displacements of pairs of particles (1 and 2), $u_x^{(1)}$ and $u_x^{(2)}$, along the line connecting them. (Here, for simplicity, we take the x-axis to lie along the line separating the

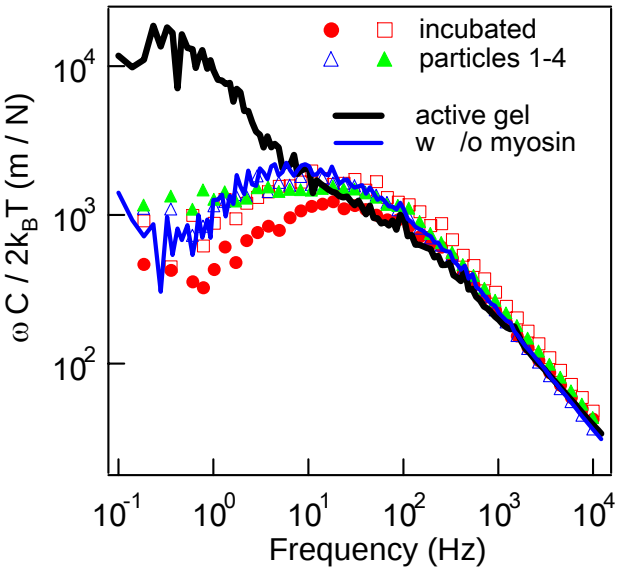
particles.) From the observed motion, we calculate the cross-correlation function, $C_{\parallel}(\omega) \equiv \int \left\langle u_x^{(1)}(t)\, u_x^{(2)}(0) \right\rangle \exp(i\omega t) dt$.

This correlation function can only be positive for (visco)elastic materials in thermal equilibrium (*11*). For non-equilibrium contractile forces, as indicated in Fig. 4a, the correlation can be either positive or negative. We observe both signs (Fig. 4d) in the low-frequency range, corresponding to the non-equilibrium fluctuations in Fig. 2b.

S4 – Additional control experiments

We carried out additional control experiments to test for the possible role of myosins bound to our probe particles. We did this to confirm that the non-equilibrium fluctuations we observed were intrinsic to the *in vitro* network, and not simply the result of the motion of our probe particles due to myosins possibly bound to them.

We incubated probe particles for 4-5 hrs in a 50 mM KCl buffer with the same concentration of myosin filaments as used in the active networks. The particles were then spun down in an Eppendorf centrifuge once and resuspended into cross-linked actin gels without any additional myosin and with 1mM ATP concentration (conditions under which actin-myosin networks super-precipitate) without sonification. These gels did not exhibit any significant non-equilibrium fluctuations (violation of the FDT), as shown in the figure below. Nor did we observe super-precipitation of these samples at any ATP concentration.



Thus, even if there is significant binding of myosin (either monomeric or as filaments) to our probe particles, this effect cannot account for the non-equilibrium fluctuations observed in Figs. 2b and 3a. Furthermore, it is likely that myosin mini-filaments adsorb less easily to the probe beads than isolated myosin dimers with exposed tail domains, and thus motors directly attached to the beads would most likely act non-processively.

Movies

Movie S1 - Super-precipitation in entangled actin in the presence of myosin mini-filaments

The 25x accelerated movie shows the super-precipitation in entangled actin with myosin (KCl 50 mM). As the ATP concentration decreases, processive myosin first makes small acto-myosin bundles and then aggregates these into large clots. Field of view: 50 μm x 60 μm.

Movie S2 - Nonthermal fluctuations in cross-linked actin in the presence of myosin mini-filaments

The movie is 4x accelerated, taken for an active gel (cross-linked actin in the presence of myosin mini-filaments). Cross-linked actin is kept homogeneous by the crosslinks while under myosin-generated tension. Strong non-thermal fluctuations are observable in the motion of embedded probe particles (diameter = 1.1 μm). Field of view: 50 μm x 60 μm.

Movie S3 - Thermal fluctuations in cross-linked actin without myosin

The movie is 4x accelerated. The sample had the same composition as in movie 2, but without myosin. Because of the stiff, cross-linked network structure, fluctuations are barely apparent. Field of view: 50 μm x 60 μm.

Movie S4 – Superprecipitation in an ATP-depleted actin-myosin gel

The movie is 4x accelerated. The sample was the same as in movie S2, the movie was recorded after ATP had been consumed. Because of the increasing tension generated by processive myosin, even a cross-linked actin gel collapsed. Field of view: 50 μm x 60 μm.

Supporting references

1. L. W. Cunningham, D. W. Frederiksen, R. B. Vallee, *Structural and contractile proteins* (Academic Press, New York, 1982).

2. P. A. Janmey *et al.*, *Nature* **347**, 95 (1990).

3. M. Buchanan, M. Atakhorrami, J. F. Palierne, C. F. Schmidt, *Macromolecules* **38**, 8840 (2005).

4. D. Mizuno, F. C. MacKintosh, C. F. Schmidt, in preparation.

5. F. Gittes, C. F. Schmidt, *Opt. Lett.* **23**, 7 (1998).

6. F. C. Mackintosh, J. Kas, P. A. Janmey, *Phys. Rev. Lett.* **75**, 4425 (1995).

7. F. Gittes, F. C. MacKintosh, *Phys. Rev. E* **58**, R1241 (1998).

8. M. L. Gardel *et al.*, *Science* **304**, 1301 (2004).

9. A. Caspi, M. Elbaum, R. Granek, A. Lachish, D. Zbaida, *Phys. Rev. Lett.* **80**, 1106 (1998).

10. J. C. Crocker *et al.*, *Phys. Rev. Lett.* **85**, 888 (2000).

11. A. J. Levine, T. C. Lubensky, *Phys. Rev. Lett.* **85**, 1774 (2000).