Scale dependence of mechanics and dynamics of active gels with increasing motor concentration

Adar Sonn-Segev, Anne Bernheim-Grosawasser, and Yael Roichman

1Raymond & Beverly Sackler School of Chemistry, Tel Aviv University, Tel Aviv 6997801, Israel
2Department of Chemical Engineering, Ilse Kats Institute for Nanoscale Science and Technology, Ben Gurion University of the Negev, Beer-Sheva 84105, Israel

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The cytoskeleton protein actin assembles into large bundles when supporting stresses in the cell, but grows into a fine branched network to induce cell motion. Such self-organization processes are studied in artificial networks of cytoskeleton proteins with thick actin bundles and large motor protein aggregates to enable optical observation. The effect of motor aggregate size on the cytoskeleton mechanical properties is studied here in networks comprised of much smaller motor assemblies. Large motor protein clusters are known to increase the stiffness of actin based networks by introducing tension and additional cross-linking cites. We find that these effects are universal to actin gels regardless of actin bundle thickness and motor aggregate size and are relevant, therefore, to a wide range of cytoskeleton based cellular processes. In contrast, motor induced active fluctuations depend significantly on motor assembly size, featuring unique non-Gaussian statistics at high concentrations of small assemblies.

Active gels inspired from the cell’s skeleton are a paradigmatic model used to understand the complex mechanisms governing key cellular processes such as motion and mechanics. These active gels are also attractive as model systems to study active matter and non-equilibrium statistical mechanics. A well studied example of such materials are networks constructed from the structural protein actin and its associated molecular motors myosin II. Both actin and myosin II are key components in cell motility and muscle contraction. Myosin motor domains (heads) generate active motion by hydrolysis of chemical fuel in the form of adenosine triphosphate (ATP). The hydrolysis process promotes a configurational change in the myosin’s structural organization, which results in stepwise walking along the actin filament. One important and unique feature of myosin II is its ability to form multimeric bipolar structures (termed mini-filaments) containing between tens to hundreds of myosin molecules. The number of myosin heads within such a mini-filament can be tuned by the salt concentration in the self-assembly buffer. This structure allows the myosin mini-filaments to connect multiple actin filaments simultaneously and move them relative to each other, thereby generating contractile forces inside the network. The ability of these proteins to self-organize into structures of different characteristic scales is of fundamental importance to their various functions, hence their remarkable diverse dynamical behaviors including reorganization, structural evolution, global compression and rupture.

The active organization and resulting mechanical properties of these gels have been studied extensively in-vitro, with and without the presence of motor proteins. It was shown that in order to withstand the internal forces applied by the myosin II motors on the actin gel an addition of a cross-linking protein is necessary, the type of cross-linker determines the structural organization of the actin into a mesh of fine or thick filaments and in turn affects the gel’s mechanical properties. In order to observe the rich dynamics of these networks directly, commonly studied networks involve large motor assemblies (hundreds of myosin heads per aggregate) and thick actin bundles which can be observed in fluorescent microscopy. While enabling direct characterization of the networks’ dynamics these structures represent only a subset of cytoskeleton networks found in living cells.

The fluctuation spectrum of in-vitro actin cytoskeleton networks was characterized through the motion of tracer beads and lone microtubules. A Gaussian distribution superimposed with fat exponential tails, which is prevalent in active systems, was observed in reconstituted active cytoskeleton networks. This athermal distribution was shown to break the fluctuation-dissipation relation. The statistics of a single active event have been studied in a bottom-up approach looking, for example, at the dynamics of a single motor molecule stepping along a filament, or at the detachment dynamics of a single motor protein from its substrate filament. These measurements are highly informative and provide insights into the operation mechanism of single motors. However, in order to attest to the coordinated activity of motors in the highly correlated systems mentioned above, one should study these active events by looking at their collective dynamics in gels.

The simultaneous investigation of motor-induced mechanics and fluctuation spectrum is challenging due to the continuous evolution of these gels ending with catastrophic collapse or rupture. Previous studies of networks with large myosin mini-filaments concentrated therefore on transient properties, or on ATP depleted networks that reach steady-state after several hours. Here we present long-lived active gels which reach active steady-state dynamics due to the small size of the myosin mini-filaments incorporated in them. These unique conditions allow us to
decouple the effect of motor proteins on the mechanical properties and structure of the gels from the random stochastic events induced by the same motor proteins. The decoupling is achieved by creating gels with fine structural features, i.e., single actin filaments locally cross-linked (without bundling agents) and small motor protein aggregates, similar to their aggregation state in the cell cortex. We characterize their structural, mechanical and statistical properties using microrheology of tracer beads, and find that the mechanical effects of myosin mini-filaments on an actin network are universal, namely, the stiffening of an actin network due to motor induced tension and cross-linking exhibits the same dependence on motor protein concentration regardless of myosin mini-filament size and actin bundle thickness. However, the statistics of motor-induced active events depends qualitatively on the motor assembly size.

**Actin networks in active steady state**

In our experimental system, the actin-myosin network is kept mechanically stable by the addition of passive, chemical cross-linkers (via biotin-avidin bonds). Our purpose is to achieve active gels with varying degree of activity maintaining steady-state dynamics for long durations, in order to collect sufficient statistics on motor activity. Towards this end we keep the network structural features fine, refraining from bundling the actin filaments by the choice of a suitable cross-linker [4]. In our system we use small mini-filaments of motor proteins containing only tens (19 or 32) of two-headed myosin molecules, and provide a low but constant concentration of ATP (0.1 mM). In this ATP concentration the motor clusters stay connected to the actin filaments for statistically longer durations acting both as cross-linkers and active elements. The pinching forces applied by these small motor clusters have a limited range due to the relatively small number of heads in each cluster and the shortage in ATP. This limitation ensures that, while the motors generate active forces in the network, there is limited large-scale reorganization or deformations of the network, i.e., reaching steady state dynamics. To follow the active fluctuations in our gels we use embedded tracer particles, with size on the order or larger than the network’s mesh size so that their motion will reflect the fluctuations of the network. By design the actin network’s mesh size is 0.3 µm, the average distance between the static chemical cross-links in our system is on the order of 3 µm, the tracer particle size is 1 µm in diameter, and the average distance between myosin mini-filaments embedded within the network ranges from 50 µm to 1.3 µm. A schematic illustration of our experimental model system is shown in Fig. 1a.

Our experiments consist of polymerization of the actin network in the presence of myosin mini-filaments and passivated colloidal tracer particles. Initial gelation takes place in the first few minutes of the experiment. Each gel is monitored for approximately three hours in which short movies are taken every fifteen minutes. The statistics of active events should depend on the amount of activity in the system both in the form of increase in motor concentration and in motor cluster size. To examine this we performed two series of experiments, one with mini-filaments containing 19 myosin molecules (i.e., double headed) per cluster and the other with mini-filaments containing 32 myosin molecules per cluster. In each series of experiments we varied the myosin concentration from $[\text{Myosin}]/[\text{Actin}]=0$ to $[\text{Myosin}]/[\text{Actin}]=0.02$ ($N_{\text{myo}}=19$) and $[\text{Myosin}]/[\text{Actin}]=0.01$ ($N_{\text{myo}}=32$), corresponding to a ratio of myosin mini-filaments to actin filaments $0 < F_{\text{myo}}/F_{\text{actin}} < 4$ and $0 < F_{\text{myo}}/F_{\text{actin}} < 1.5$, respectively ($F_{\text{actin}}$ is calculated by considering that there are 370 actin subunits in a 1 µm long filament). The tracer particle trajectories are then extracted using conventional video microscopy techniques [29].

In Fig. 1b, a typical trajectory of a tracer particle (140 s long) in an actin gel containing no myosin motors (passive gel), is compared to a trajectory of a bead in an active gel ($[\text{Myosin}]/[\text{Actin}]=0.02$, Fig. 1c) of the same duration. Both trajectories show that the tracer particle undergoes diffusive motion, however the particle embedded in the active gel seems more confined. This result is counter-intuitive, since we expect motor activity to enhance the gel’s fluctuations and therefore the tracer particles’ motion, as reported previously [12, 22]. However, motor proteins are known to stiffen actin gels due to two effects: they act as additional cross-linkers and they apply tension on the actin filaments [13]. Both processes reduce the entropy of the gel network causing it to stiffen. Therefore, we conclude that the added active fluctuations from the motor’s activity have less effect on the range of motion of tracer particles than the increase in stiffness they induce, in our experiments. We attribute the difference between our result and those reported previously to the much smaller size of our myosin mini-filaments. Nonetheless, we do observe that some of the particles in the active gels experience relatively large displacements that we attribute to motor activity, e.g. the particle trajectory in the inset of Fig. 1c and its displacement Fig. 1d. In order to verify that our gels arrive at an active steady-state we extract the time and ensemble mean square displacement (MSD) of the tracer particles. Repeating this measurement throughout the lifetime of the gel and comparing the value of the MSD at a lag time of $\tau = 7$ s we can see that gels at most motor concentration reach steady state after approximately 50 min (see Fig. 1d and supplemental material Fig S1).
FIG. 1: Description of experimental system. a, Schematic illustration of the active gels and its components. Myosin mini-filaments are embedded within a network of actin filament cross-linked by biotin-avidin bond. The fluctuations of the network are probed by the tracer particle (1 µm diameter) motion. The light yellow circle marks the range within which motors strongly affect the tracers motion, i.e. $r < r_c$. b, c, Typical trajectory of a 1 µm polystyrene particle in the cross-linked actin network: passive network, [Myosin]/[Actin]=0 (b) and active network, [Myosin]/[Actin]=0.02 (c). The concentration of actin monomers is 24 µM. Motor mini-filaments are constructed with $N_{myo}=19$ myosin molecules. The two trajectories were taken at 70 Hz for about 140 s. An example of a trajectory of a particle that experiences large steps is plotted in the inset of c and its absolute displacement is given in e. d, MSD at $\tau=7$ s along the experiment time. Colors and symbols correspond to different [Myosin]/[Actin] ratios: 0 (blue circles), 0.0017 (red squares), 0.0025 (green triangles), 0.005 (orange diamonds), 0.0083 (violet right triangles), 0.0125 (magenta stars) and 0.02 (black pluses).

Mechanical stiffening due to myosin activity

The local mechanical properties of the active gels in steady state were characterized by the MSD of tracer particles within the gels at increasing concentrations of myosin (Fig. 2a,b). The stiffening effect of the motor proteins observed above on the single trajectory level is demonstrated here in the sample averaged measurement as well, from the decreases of the MSD with the increase in myosin concentration. The longitudinal displacement correlations as a function of particle separation (Fig. 2c,d) show that stress propagation in these gels resembles that of passive actin gels with no myosin motors [30, 31], i.e. the response of these gels to mechanical perturbation decays fast at distances shorter than $r_c \sim 5$ µm (Fig. 2c,d and their insets) and slower at larger distances. As a measure of the gels’ stiffness we used the MSD at $\tau=7$ s (lag-time within the elastic plateau of the gels), after the gels reached a steady state, and plot it as a function of [Myosin]/[Actin] (Fig. 2e,f). Clearly the stiffening of the gels seen from the decrease in the MSD here and in Fig. 2a,b is non-linear. In fact, by assuming the two known contributions of motor proteins to stiffening: cross-linking and tension, we can fit our experimental data (red solid line in Fig. 2e,f) according to:

$$G' \sim c_0 + c_1[\text{Myosin}]/[\text{Actin}] + (c_2/c_{crit}^x) ([\text{Myosin}]/[\text{Actin}])^x ,$$

where the constants $c_i$ are fitting parameters, and the power-law $x$ is equal to zero below the critical concentration, $c_{crit}$ (see supplemental materials). This relation is based on two assumptions: the effect of cross-linker density on the mechanical properties of actin gels reported previously [32], and that the internal stress applied by the motors on the network increase linearly with motor concentration. We find that the critical [Myosin]/[Actin] concentration ratio for gel stiffening due to cross linking ($c_{crit}$) is 0.007 and 0.003 for $N_{myo}=19$ and 32 respectively. Surprisingly, this is in accord with the results of [32] and [18, 33] for passive gels (without motor proteins) with much larger structural features, based on the rod-like character of myosin II mini-filaments (for details see supplemental materials.).

We analyze two additional parameters characterizing the mechanical properties of active gels as suggested in [22]: the diffusion exponent, $\alpha$, MSD$\sim \tau^\alpha$, here measured for lag-times between $\sim0.01-0.1$ s, and the standard deviation of the Gaussian part of the van Hove correlation, $\sigma$, scaled by the standard deviation of the Gaussian in the passive gel, $\beta = \sigma_{active}/\sigma_{passive}$ (see Fig. 3a for clarity). In weakly cross-linked semi-flexible networks $\alpha$ usually takes values of $\sim0.75-0.85$ [34]. A decrease in its value implies that the tracer experiences a more stiffened or confined environment.
In accord with the MSD at $\tau = 7$ s we see a two stage stiffening of the gels as myosin concentration increases. This behavior holds also for $\beta$ (see insets of Fig. 3a,b), where a stiffening or confinement effect in the environment of the particles should correspond to values of $\beta < 1$, while an activity induced broadening should result in $\beta > 1$. Interestingly, our results on the stiffening pattern as a function of myosin concentration are similar to the ones reported previously using much larger myosin assemblies and larger structural features of the gels [10]. However, there are differences in the values of $\alpha$ and $\beta$ from previous reports [11, 22], namely the decrease in $\alpha$, which does not exhibit normal or super diffusion, and the narrowing of the displacements distributions ($\beta < 1$). These differences are attributed to the much smaller size of our myosin mini-filaments; due to their size the stiffening effect is more pronounced than the active displacements they can induce. To verify this observation more quantitatively we examine the fluctuation spectrum of the tracer particles.

**FIG. 2: Mechanical properties as a function of myosin concentration.** a,b. Time and ensemble-averaged MSD of probe particles as a function of lag-time $\tau$ approximately 100 min after polymerization. Mini-filaments are constructed with $N_{\text{myo}} = 19$ (a) or $N_{\text{myo}} = 32$ (b) myosin molecules. Colors and symbols correspond to different [Myosin]/[Actin] ratios: 0 (blue circles), 0.0017 (red squares), 0.0025 (green triangles), 0.0083 (violet right triangles), 0.01 (maroon down triangles), 0.0125 (magenta stars) and 0.02 (black pluses).

**c,d** Longitudinal displacement correlations as a function of particle separation at lag time $\tau = 0.014$ s at [Myosin]/[Actin]=0.0025 with $N_{\text{myo}} = 19$ (c) and $N_{\text{myo}} = 32$ (d). The cross-over distance (orange dashed line) is defined at the intersection of the fitted bulk ($r_1$) and intermediate ($r_3$) power-laws decays of $D_\parallel$. Inserts show the cross over distance dependence on [Myosin]/[Actin].

**e,f** MSD at $\tau = 7$ s as a function of [Myosin]/[Actin] reflects the two mechanisms of gel stiffening.

**Fluctuation spectrum in the presence of myosin activity**

The statistics of tracer particles’ motion is characterized by the probability distribution of their displacements as a function of lag time $\tau$, $P(\Delta x, \tau)$, known as the van Hove correlation function (Fig. 3a,b). By examining these distributions, the statistics of particle fluctuations can be resolved, and should provide insight into the active events influencing the particle’s motion. As expected in passive gels, the van Hove correlations are Gaussian-like, since the fluctuations of particles are purely thermal with only small deviations due to the network’s heterogeneous cross-linked structure. At low myosin concentrations the displacement distributions are also Gaussian-like, with widths that decrease gradually with the increase in myosin concentration, in accord with the stiffening effect discussed above. However, above a certain motor concentration threshold we observe the appearance of shoulders ($N_{\text{myo}} = 19$, Fig. 3a) and peaks ($N_{\text{myo}} = 32$, Fig. 3b) in the displacement distribution. This threshold concentration is [Myosin]/[Actin]=0.015 for the small clusters and [Myosin]/[Actin]=0.004 for the large clusters. This displacement distribution is unique and different from the Gaussian distribution superimposed with fat exponential tails which is found most frequently in active matter, e.g., active granular materials [35], active DNA gels [36], suspensions of eukaryotic microorganisms [37], within living cells [38], and most importantly in acto-myosin networks [22, 23]. Looking at the non-Gaussian
parameter, NGP = (⟨Δx(τ)⟩^4/β(⟨Δx(τ)⟩^2)^2 − 1 of these distributions, which quantifies the non-equilibrium contribution to the van-Hove distribution (insets of Fig. 3), we observe a threshold myosin concentration above which it increases. Importantly, the threshold concentrations observed here in the three parameters: α, β and NGP, coincide with the threshold concentration above which the shoulders or peaks appear in the displacement distributions discussed above. It is obvious that the onset of the increase in NGP coincides with appearance of shoulders or peaks in the displacement distributions. The threshold concentration above which a dramatic stiffening is observed is not straightforward 40.

In thermal equilibrium, the average step size of a bead, \( σ \), further support this view by providing rough estimations of the forces required to produce such discrete displacements. To investigate the temporal dependence of the active events we looked at the distributions of particle displacements in the networks with the highest myosin concentration ([Myosin]/[Actin]=0.02, \( N_{\text{myo}} = 32 \)) at different lag-times (note that Fig. 3a,b was calculated for the shortest lag-time, \( τ = 0.014 \) s). In Fig. 4a,b the distributions are plotted for lag-times between \( τ = 0.014 - 0.14 \) s. These plots reveal that (i) the locations and heights of the shoulders do not change as the lag-time increases, and (ii) as lag-time increases the central Gaussian broadens and masks the shoulders, resulting in a Gaussian like shape of the distributions. These observations imply that the duration of active events taking place in the network is shorter than 0.014 s (the shortest lag-time), and therefore do not produce directed motion for long durations as was reported previously 11, 22, 23. We further support this view by providing rough estimations of the forces required to produce such discrete displacements. In thermal equilibrium, the average step size of a bead, \( σ \), is related to the network’s stiffness \( k \), which opposes its motion, through the equipartition theorem with \( k_b T = k_0 \sigma^2 \). We use the equilibrium relations to obtain a lower bound estimation of \( k \). The forces applied in a single active event, calculated by this approach \( f = \Delta x_k \), are between 0.7-1.4 pN (Fig. 4). These estimations are on the order of the force produced by a single motor head during one hydrolysis cycle (approximately 1.4 pN 39), and correspond to up to a few successive steps of heads before the motor cluster is detached from the network.

The distinct shoulders observed in the tracers displacement distributions in the gels with high motor content indicate discrete active events. Remarkably, a recent theoretical study of the dynamics of a tracer particle in an active gel obtained similar features for their particle displacement distributions appearing only at sufficiently high network stiffness 40, in accord with our results (Fig. 3). The active force in this model arises from the independent and random action of motors in the gel with a given finite displacement due to the elastic response of the network.

The probability distribution of the tracer particles’ motion can be modeled statistically as a linear combination of thermal and active displacements, \( Δx_t = Δx - Δx_a, Δx_a \) respectively: \( P(Δx) = \int_{−∞}^{∞} P_{\text{active}}(Δx_a) \cdot P_{\text{thermal}}(Δx − Δx_a) dΔx_a \) (see supplementary). \( P_{\text{active}} \) can be expanded in a series in the number of active perturbations \( n \), leading to the given active displacement: \( P_{\text{active}}(Δx_a) = \sum_n P_l(n) \cdot P_\sigma^n(Δx_a) \), with \( P_l(n) \) the probability of \( k \) active events to occur at the same temporal window. Choosing \( P_{\text{active}} \) to be exponential recovers the previously reported results of a probability distribution with a central Gaussian part and exponential tails (see supplementary) 11, 22, 23. In order to fit our experimental data we are required to make two assumptions: (i) there is a cutoff on the maximal size of an active step, and (ii) there is higher probability to have two consecutive active events than expected from...
FIG. 4: Characterization of active events. a, b, Ensemble-averaged van Hove correlation functions for different lag times for networks with [Myosin]/[Actin] = 0.02, \(N_{\text{myo}} = 19\) (a) and [Myosin]/[Actin] = 0.01, \(N_{\text{myo}} = 32\) (b). Colors correspond to different lag times: \(\tau = 0.014\) (blue), 0.028 (cyan), 0.042 (violet), 0.056 (green), 0.07 (red), 0.084 (grey), 0.098 (indigo), 0.112 (turquoise), 0.126 (orange) and 0.14 s (black). c, Ensemble-averaged van Hove correlation functions at \(\tau = 0.014\) s showing peaks or shoulders at large displacements. Different colors correspond to different networks; [Myosin]/[Actin]=0.02, \(N_{\text{myo}} = 19\) (blue), [Myosin]/[Actin]=0.005, \(N_{\text{myo}} = 32\) (green) and [Myosin]/[Actin]=0.01, \(N_{\text{myo}} = 32\) (red). Arrows point to displacements of higher probability (\(\Delta x_{\text{peak}}\)), i.e., the appearance of peaks/shoulders. The brown oval highlights that at the same \(N_{\text{myo}}\), some of the peaks appear at the same displacement. d, Using the values of \(\Delta x_{\text{peak}}\) extracted from c (see inset) the force applied by the motors at a single active event is estimated (see text for explanation). e, f, Fitting of the ensemble-averaged van Hove correlation functions for networks with [Myosin]/[Actin] = 0.02, \(N_{\text{myo}} = 19\) without (e) and with (f) temporal correlations between active events.

The origin of the cutoff in the active displacements distributions can be understood from the small size of our motors. Since our motors are constructed from 19 or 32 myosin molecules, the motors are able to produce a processive movement of only a few (1-3) steps before the cluster is detached from the filament and the build-up tension is released moving the tracer particle. In large mini-filaments which contain hundreds of molecules, there is a much lower probability of all molecules to detach, and the cluster can produce tens and hundreds of steps before detaching completely from the filament.

In this paper we have shown that myosin motors affect in-vitro actin networks in three distinct ways: they stiffen the network by the addition of cross-linking points and by adding tension to the network, and produce active, non-thermal, fluctuations in the gels. The first two processes were reported also for networks with much larger structural features, i.e. actin bundle thickness and motor mini-filament size, and are expected to apply for cytoskeleton dynamics in living cells in general. Working with small myosin cluster allows us to avoid the catastrophic rupture and compression usually seen in in-vitro networks, suggesting the motor cluster size as a control parameter for structural stability within cells. From the active fluctuations studied here, we conclude that consecutive active events are correlated, i.e., the resulting quick stress release from an active event increases the probability of a second release thereafter. It will be interesting to look for such correlations in active events in-vivo and in systems with large motor mini-filaments.

METHODS

Active actin-myosin networks were reconstituted in vitro by polymerizing G-actin in the presence of biotin-avidin cross-linkers and myosin II mini-filaments. G-actin was purified from rabbit skeletal muscle acetone powder, with a gel filtration step, stored on ice in G-buffer (5 mM Tris HCl, 0.1 mM CaCl2, 0.2 mM ATP, 1 mM DTT, 0.01% NaN3, pH 7.8) and used within two weeks. Purification of myosin II skeletal muscle was done according to stan-
The concentration of the G-actin and myosin II was determined by absorbance measured using UV/Visible spectrophotometer (Ultraspec 2100 pro, Pharmacia) in a cuvette with a 1 cm path length and the extinction coefficients: G-actin - \( \varepsilon_{280} = 26,460 \text{ M}^{-1} \text{cm}^{-1} \) and two-headed myosin II - \( \varepsilon_{280} = 268,800 \text{ M}^{-1} \text{cm}^{-1} \). Myosin II dimers were stored at \(-80^\circ\text{C}\) in high salt buffer (0.5 KCl) that preserves them in a monomeric form. Biotinylated actin (Cytoskeleton, Inc.) and neutravidin (Invirogen, used as a cross-linker) were premixed with unlabeled actin and left over at least one hour on ice. The ratios were unlabeled: biotinylated: neutravidin = 5000:5:2 giving a total actin concentration of 24 \( \mu \text{M} \) and an average distance between cross-linkers of \( \approx 3 \mu \text{m} \). To initiate the formation of processive myosin II mini-filaments, the myosin solution was diluted with G-buffer to the desired KCl concentration, 0.13 M or 0.1 M, corresponding to motor clusters, each composed of \( \approx 19 \) or 32 myosin domains, respectively.

Polystyrene colloids with radius of 0.55 \( \mu \text{m} \) (Invitrogen, Lot # 742530) were pre-incubated with a 10 mg/ml BSA solution to prevent non specific binding of protein to the bead surface. We set the average filament length to be \( \approx 13 \mu \text{m} \) by addition of capping protein.

Active network formation was initiated by adding the actin solution, myosin mini-filaments in various concentrations, capping protein and beads to motility buffer (10 mM HEPES, 1 mM MgCl\(_2\), 0.1 mM MgATP, 0.5 mg/mL creatine kinase, 5 mM creatine phosphate, 0.2 mM EGTA, and 0.1 or 0.13 M KCl). Creatine kinase and phosphate were used as an ATP regenerating system. Immediately after polymerization the sample was infused into a glass cell, 150 \( \mu \text{m} \) high, and sealed with grease. The glass surfaces were coated with methoxy-terminated polyethylene glycol to prevent binding of the proteins to the glass. Shortly after cell loading, samples were fluorescently imaged at \( \lambda = 605 \text{ nm} \) with 40 \( \times \) air objective. Each sample is monitored for approximately 160 min, in which short movies are taken every fifteen minutes, starting from \( \approx 5 \) min after polymerization. The initial gelation process of the samples takes place in the first few minutes of the experiment. To avoid wall effects imaging was done at a plane distanced at least 80 \( \mu \text{m} \) from the cell walls. Particle motion was recorded using a CMOS video camera (Gazelle, Point Gray) at a frame rate of 70 Hz and was tracked with accuracy of at least 13 nm using conventional algorithms.

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* Electronic address: roichman@tau.ac.il

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