Cytoplasmic diffusion: molecular motors mix it up

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Random motion within the cytoplasm gives rise to molecular diffusion; this motion is essential to many biological processes. However, in addition to thermal Brownian motion, the cytoplasm also undergoes constant agitation caused by the activity of molecular motors and other nonequilibrium cellular processes. Here, we discuss recent work that suggests this activity can give rise to cytoplasmic motion that has the appearance of diffusion but is significantly enhanced in its magnitude and which can play an important biological role, particularly in cytoskeletal assembly.

The cytoplasm of eukaryotic cells is a highly dynamic and out-of-equilibrium material that undergoes continual restructuring. This is largely driven by active processes such as polymerization of cytoskeletal filaments and forces generated by molecular motors. Such activity usually results in directed movement within cells; examples include the slow retrograde flow at the leading edge during cell crawling (Fisher et al., 1988; Waterman-Storer and Salmon, 1997; Cai et al., 2006) and the transport of motor-bound vesicles along cytoskeletal filaments (Vale, 2003). Given the intrinsically small, micrometer scales involved, the cytoplasm is also subject to the thermal agitation of Brownian motion (Brown, 1828; Einstein, 1905). Random though these thermal fluctuations may be, they are implicated in force generation by polymerization (Peskin et al., 1993; Mogilner and Oster, 1996), as well as the elastic response of cytoskeletal networks (MacKintosh et al., 1995; Gardel et al., 2004; Storm et al., 2005). Moreover, thermal fluctuations give rise to the diffusive transport of small molecules throughout the cell, without which molecular signaling would be impossible. However, it is becoming increasingly clear that nonthermal forces, such as those resulting from motor protein activity, can also lead to strongly fluctuating intracellular motion; this motion differs significantly from the directed motion commonly associated with motor activity (Caspi et al., 2000; Lau et al., 2003; Bursac et al., 2005). These active fluctuations remain poorly understood, and the relative contributions of thermal fluctuations compared with active fluctuations in living cells are only now being fully explored.

Particle-based probes of intracellular motion

To elucidate the nature of fluctuating motion in cells, many studies have analyzed the "passive" fluctuating motion of micrometer-sized spherical probe particles. If such particles were embedded in a viscous liquid driven by thermal Brownian fluctuations, they would exhibit random, diffusive motion, as shown schematically in Fig. 1 a (blue particle). Quantitatively, this means that the distance the particle has moved, Δx, after some time interval, τ, is described by $\left\langle \Delta x^2 \right\rangle = 2D\tau$, where the angled brackets indicate an average over many particles, and the diffusion coefficient, D, is given by the Stokes-Einstein equation:

$$D = \frac{k_B T}{6\pi\eta a},$$

which depends on the viscosity η and particle radius a (Einstein, 1905). This reflects the fundamentally thermal origin of diffusion in an equilibrium liquid, depending as well on the temperature (T) and Boltzmann's constant (k_B). This diffusive time dependence is shown schematically by the blue line in Fig. 1 a. Such motion is in stark contrast with steady particle motion in one direction with constant velocity v, illustrated by the black curve in Fig. 1 a; because $\Delta x = v\tau$, this motion is described by $\left\langle \Delta x^2 \right\rangle = v^2\tau^2$. In principle, one can track the motion of inert tracer particles in cells to determine whether their motion is diffusive with the appropriate diffusion coefficient. However, inside cells this picture is complicated by the fact that the cytoplasm is generally not a simple viscous liquid but rather a structured viscoelastic material (Luby-Phelps et al., 1987; Fabry et al., 2001). In a viscoelastic material, thermal fluctuations do not lead to ordinary diffusion, but rather "subdiffusive" motion, characterized by a different time-dependence: $\left\langle \Delta x^2 \right\rangle \propto \tau^{\alpha}$, where $\alpha < 1$, as shown schematically by the red curve in Fig. 1 a. Thus, studies showing diffusive, or even "superdiffusive" motion ($\alpha > 1$), within the viscoelastic cytoplasm suggested this random motion

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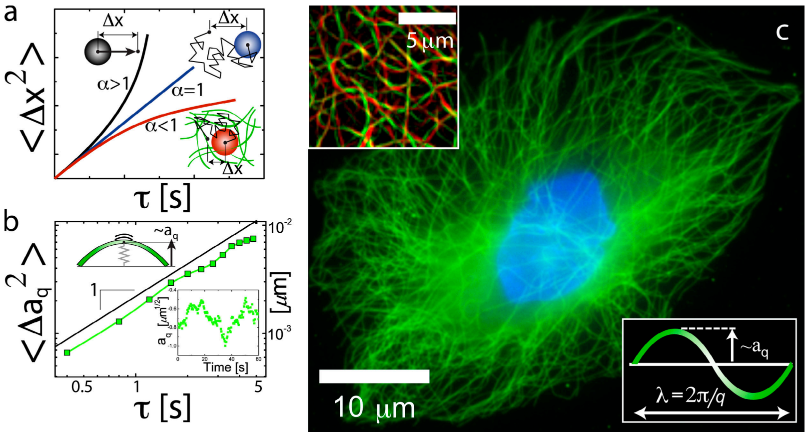


Figure 1. **Quantifying fluctuating motion.** (a) Schematic showing different types of particle motion, characterized by the mean square displacement $\langle \Delta x^2 \rangle \propto \tau^\alpha$. The particle can exhibit directed ("ballistic") motion, as shown by the black particle; here $\alpha = 2$, as shown by the black curve. Particles can also exhibit random diffusive-like behavior, as shown by the blue particle, with $\alpha = 1$ (blue line). Particles constrained by a viscoelastic network (red particle) often exhibit subdiffusive behavior, with $\alpha < 1$ (red curve). (b) Data showing the bending motion of a fluctuating intracellular microtubule. The amplitude of a bend, $\propto a_q$, randomly fluctuates in time, as shown in the bottom inset. The mean squared amplitude difference as a function of lag time displays diffusive-like behavior ($\alpha = 1$). (c) A CHO cell fixed and stained to reveal the nucleus (blue) and microtubules (green). Bottom inset shows schematically the variables used in the Fourier analysis of microtubule bending. Top inset shows fluctuations in a GFP-tubulin–transfected Cos7 cell over a time difference of 1.6 s. Microtubules that have fluctuated to a new position are in red and the earlier position is in green.

is not thermally induced (Caspi et al., 2000). However, other studies assume that random intracellular motion is thermally induced, and use this assumption to extract the mechanical properties of the cell from tracer particle motion (Tseng et al., 2002; Panorchan et al., 2006). To quantitatively clarify this, several recent studies have analyzed both measurements of the "passive" random motion of tracer particles, as well as direct, active measurements of the viscoelastic properties of the cytoplasm, for example, using a magnetic tweezer to pull on cells (Lau et al., 2003; Bursac et al., 2005). These studies have concluded that the random motion not only has an unexpected time dependence but is also significantly larger in magnitude than would be expected for purely thermal fluctuations, suggesting that biological activity can indeed give rise to random fluctuating motion that dominates over thermal fluctuations.

Considerable new insight into the underlying physical origin of these motor-driven fluctuations was obtained from studies of a reconstituted actin network incorporating myosin II motors (Mizuno et al., 2007), oligomerized into processive motor assemblies similar to those found in the cellular cytoskeleton (Cai et al., 2006). The mechanical resistance of the actin network was precisely characterized using optical tweezers to actively pull on particles within the network. The fluctuating motion of the particles was also measured, both with and without motors present. By comparing these two measurements, the contribution of the thermal motion could be distinguished from that of the nonthermal motion, showing clearly that myosin motors can give rise to strong random fluctuating motion within the network. Interestingly, these random myosin-generated forces can also lead to a pronounced stiffening of the actin network, increasing the rigidity by as much as 100-fold (Mizuno et al., 2007; unpublished data).

Microtubule bending dynamics reflects active fluctuations

The random fluctuating motion arising from the activity of cytoskeletal motor proteins can have important biophysical consequences. In an attempt to directly measure the underlying fluctuating forces, a different but complementary approach has recently been developed. It uses endogenous cytoskeletal microtubules as probes. Microtubules are stiff biopolymer filaments that are present in almost every animal cell and are physically linked to other components of the cytoskeleton (Rodriguez et al., 2003; Rosales-Nieves et al., 2006). Thus, as with spherical probe particles, their motion reflects forces and fluctuations of the network (Waterman-Storer and Salmon, 1997; Odde et al., 1999). However, in contrast to spherical probes, microtubules also exhibit a local bending motion, whose amplitude can, like a simple elastic spring, be used to determine the applied force (Fig. 1 b). Microtubules in cells indeed appear highly bent, as can be seen, for example, in the fluorescence image of the microtubule network within an adherent CHO cell, shown in Fig. 1 c. These bends fluctuate dynamically in time, which can be seen by subtracting sequential images, as shown in the top inset of Fig. 1 c.

This motion was studied in cells by tracking individual fluorescent microtubules, using both Cos7 and CHO cells (Brangwynne et al., 2007b). The curves defined by the microtubules are analyzed using a Fourier analysis technique developed to characterize the bending fluctuations of isolated biopolymers in thermal equilibrium (Gittes et al., 1993; Brangwynne et al., 2007a). This analysis is based on the fact that each curve can be represented as a sum of simpler sinusoidal curves, each of a different amplitude (a_q) and wavelength (λ). The wavelength is typically written in terms of its wave vector, $q = 2\pi/\lambda$, as sketched in the bottom inset of Fig. 1 c. Using this approach, intracellular motion was analyzed by calculating the mean-squared difference in amplitude using $\left\langle \Delta a_q^{\,2} \right\rangle$, as a function of lag time τ.

This quantity is analogous to that calculated for fluctuating particles, $\left\langle \Delta x^2 \right\rangle$, but here effectively measures the fluctuating motion of the microtubule at different wavelengths. In thermal equilibrium, the amplitude difference will grow with τ up to a maximum value given by

$$\left\langle \Delta a_q^{\,2} \right\rangle_{\tau \to \infty} = \frac{k_B T}{\kappa q^2},$$

where $k_B T$ is the thermal energy scale and κ is the microtubule bending rigidity. The ratio of these defines the persistence length:

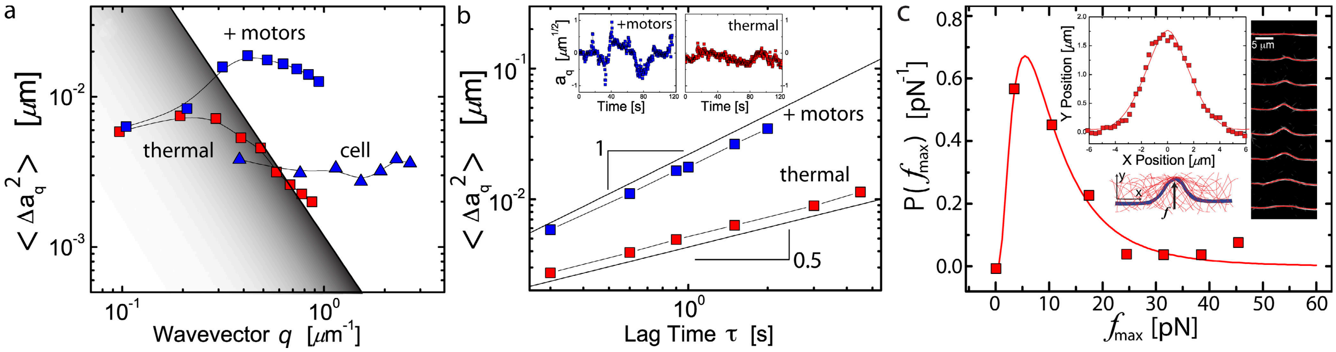


Figure 2. **Microtubule bending in vivo and in vitro.** (a) Thermal microtubules in an in vitro network of F-actin exhibit a roughly q^{-2} spectrum of fluctuations (solid line), although wave vectors smaller than $q \sim 0.4\ \mu m^{-1}$ have not reached their maximum fluctuations on this time scale (τ = 2 s; red squares). In the presence of myosin II motors (blue squares), the bending fluctuations are significantly larger than thermal on short wavelengths (high q). Curves are means of 10 filaments. Intracellular microtubule fluctuations show similar behavior (blue triangles), with amplitudes larger than thermal on short wavelengths, as shown by the mean of 23 filaments from a CHO cell (τ = 2 s). (b) Microtubules embedded in an in vitro actin network in thermal equilibrium exhibit small fluctuations (inset, red squares, $q \sim 0.3\ \mu m^{-1}$), which evolve subdiffusively, i.e., $\langle \Delta a_q^2 \rangle \propto \tau^{0.5}$, because of the elasticity of the surrounding actin network (red squares, mean of ~10 filaments). In myosin-driven networks, the fluctuations are significantly larger and steplike (inset, blue squares, $q \sim 0.3\ \mu m^{-1}$). These large nonthermal fluctuations are diffusive in character, i.e., $\langle \Delta a_q^2 \rangle \propto \tau$ (blue squares, mean of ~10 filaments). (c) The bending fluctuations of microtubules in vitro are highly localized and relax rapidly as shown in the top right inset (78 ms between each frame, top to bottom). These localized bends can be well fit to the expected shape resulting from transverse point forces (Landau and Lifshitz, 1986; Brangwynne et al., 2008). A localized bend with the fit to the theoretical form (red line) is shown in the top inset. From these fits, a distribution of localized force pulses with a mean magnitude of ~10 pN is found (main plot).

$$l_p = \frac{\kappa}{k_B T},$$

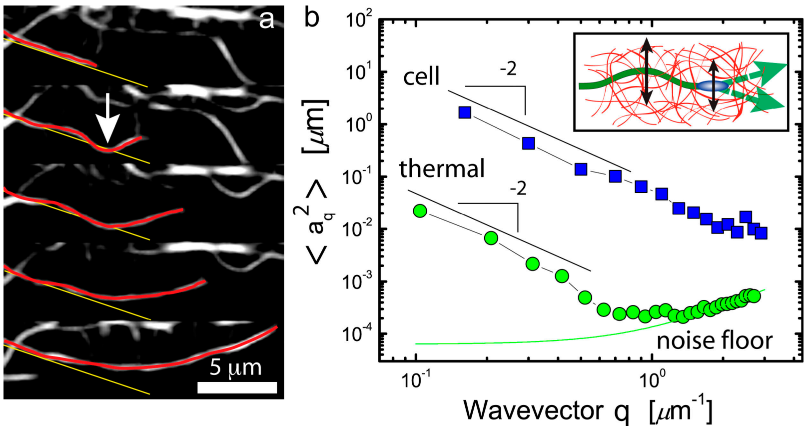
which represents the length scale at which thermal fluctuations completely change the direction of the filament; for microtubules, l_p is on the order of 1 mm (Gittes et al., 1993). This establishes the maximum amplitude of bending fluctuations that can be induced by thermal agitation and allows thermal and motor-induced fluctuations within the cell to be distinguished.

For microtubules in cells, the amplitude of the fluctuations was found to grow roughly linearly in time, the behavior expected for simple Brownian diffusion (Fig. 1 b). However, strikingly, the maximum bending amplitude in cells is much larger than that expected for thermally induced bends for $l_p \approx 1$ mm. This is most apparent for small wavelength bends, $q > 1\ \mu m^{-1}$, as shown by the blue triangles in Fig. 2 a, which are significantly above the maximum thermal amplitude shown by the solid line. Thus, although random intracellular motion can exhibit features similar to random Brownian motion, it appears inconsistent with a purely thermal origin.

In these experiments in living cells, there are several unknown variables that could play a role. For example, the persistence length of microtubules may vary within the cell, caused by a possible length dependence (Pampaloni et al., 2006) or arising from the effects of microtubule-associated proteins (Felgner et al., 1997). A simplified in vitro cytoskeleton was therefore developed, incorporating purified microtubules in a model actin network (Brangwynne et al., 2008). In the absence of motor proteins or other sources of nonequilibrium activity, microtubules embedded in the actin network are subject to only thermal forces that result in small bending fluctuations; as with the motion of spherical probe particles, the viscoelasticity of the surrounding network leads to subdiffusive behavior of the bending amplitudes, $\langle \Delta a_q^2 \rangle \propto \tau^{0.5}$, as shown in Fig. 2 b. These fluctuations display a small maximum amplitude corresponding to $l_p \approx 1$ mm, as shown by the red squares in Fig. 2 a. When myosin II motor assemblies are added to the actin network, the embedded microtubules show dramatically different behavior: they bend significantly more, and the bends are highly localized. However, although this behavior is driven by processive motor activity, the microtubule bends fluctuate randomly in time, with localized bends growing and shrinking rapidly, as illustrated by the typical time series shown in the inset of Fig. 2 b; this behavior is similar to that found using spherical probe particles (Mizuno et al., 2007). These microtubule bends appear to result from localized transverse forces (Landau and Lifshitz, 1986), as sketched in the bottom inset of Fig. 2 c. Analogous to a simple spring (force proportional to displacement, $F = k\Delta x$), the maximum force was directly determined from the amplitude of these bends, revealing force pulses on the order of 10 pN, which is consistent with that of a few myosin motors acting together (Finer et al., 1994). Interestingly, short wavelength bends can also arise from compressive forces acting within cells (Brangwynne et al., 2006).

The dynamic, localized microtubule bends observed in vitro lead to fluctuations in the bending amplitudes that are large and distinctly nonthermal at short wavelengths (large q), as shown by the comparison of thermal (Fig. 2 a, red squares) and motor-driven (blue squares) fluctuations for $q \geq 0.2\ \mu m^{-1}$. At longer wavelengths, however, the fluctuations are indistinguishable from those of thermally excited filaments, as expected for microtubules whose lateral motion is restricted by the surrounding elastic environment. Surprisingly, with added myosin motors, the actively driven bends fluctuate in a diffusive-like manner, $\langle \Delta a_q^2 \rangle \propto \tau$, as shown in Fig. 2 b (blue squares). This nonthermal diffusive behavior can be understood in terms of steplike or on–off dynamics in the forces applied by individual motor assemblies as they bind and unbind to cytoskeletal filaments (Mizuno et al., 2007; MacKintosh and Levine, 2008). Thus, even processive motor activity has stochastic

Figure 3. **Nonequilibrium fluctuations affect the growth dynamics and final structure of the microtubule network.** (a) A microtubule within a GFP-tubulin–transfected Cos7 cell, highlighted in red, can be seen growing toward the bottom right, in the direction indicated by the yellow line. In the second frame, the filament experiences a naturally occurring bending fluctuation caused by internal forces, indicated by the arrow. As a result, the orientation of the microtubule tip changes and the microtubule grows upward, giving rise to a long wavelength bend. Frame times are 0, 36, 46, 53, and 92 s, top to bottom. (b) Inset schematic shows how lateral bending fluctuations of microtubules will cause fluctuations in the microtubule tip during growth, giving rise to curved polymerization trajectories. The Fourier spectrum of a single representative microtubule in thermal equilibrium is shown by the green circles, calculated from the maximum variance of the fluctuations. This microtubule has a persistence length, l_p = 4 mm. The Fourier amplitude of an ensemble of microtubules in CHO cells, $\langle a_q^2 \rangle$, is shown by the blue squares.



features that can give rise to diffusive-like motion of cytoplasmic components. Although it is likely that myosin II is not the only motor contributing to this behavior in cells, these experiments help elucidate the underlying biophysical processes.

Implications of active fluctuations for transport and cytoskeletal assembly

These random nonthermal force fluctuations appear to be a ubiquitous feature of living cells. They can, therefore, play an important role in a variety of cellular processes. For example, as a result of large microtubule bending fluctuations, the tips of growing microtubules also undergo large fluctuations in directional orientation, leading to highly curved microtubule shapes that appear to be “frozen-in” by the surrounding elastic cytoskeleton, as shown in the example in Fig. 3 a. These random fluctuations in tip orientation are analogous to random thermal fluctuations and give rise to microtubule bends with a thermal-like dependence on *q*, as shown in Fig. 3 b. However, the corresponding nonequilibrium persistence length is reduced to ~30 µm, ~100-fold less than the thermal persistence length. Thus, the microtubule network is more bent by ~100-fold in these cells as compared with isolated microtubules in solution. Although the effects of microtubule binding proteins or defects in the tubulin lattice could also contribute, tip fluctuations alone are sufficient to explain these large bends. Driven fluctuations can thus play a significant role in determining cytoskeletal architecture.

The enhanced diffusive dynamics that result from motor activity may also affect the rates of biochemical reactions that take place on the cytoskeletal scaffold (Forgacs et al., 2004), as well as those usually thought to be limited by thermal diffusion. Thus, “active” cytoplasmic diffusion could represent a kind of microscopic mixing that enables rapid diffusion of vesicles and small molecules.

Thermal fluctuations have been known to play a fundamental role in the behavior of all nonliving matter since Einstein’s seminal work in 1905 (Einstein, 1905), in a paper explaining how thermal forces give rise to the diffusive motion first observed by Brown in 1828 (Brown, 1828). We propose that active intracellular force fluctuations represent the biological analogue, controlling cell behavior while subject to biochemical regulation. Interestingly, this may actually be closer to Brown’s initial concept of a vital microscopic activity, in contrast to the nonliving, thermal motion that bears his name (Brown, 1828). This active motion is clearly a ubiquitous and important phenomenon in living cells; indeed, it may be that active processes contribute to virtually all randomly fluctuating, diffusive-like motion in cells.

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References

Brangwynne, C.P., F.C. MacKintosh, S. Kumar, N.A. Geisse, J. Talbot, L. Mahadevan, K.K. Parker, D.E. Ingber, and D.A. Weitz. 2006. Microtubules can bear enhanced compressive loads in living cells because of lateral reinforcement. *J. Cell Biol.* 173:733–741.

Brangwynne, C.P., G.H. Koenderink, E. Barry, Z. Dogic, F.C. MacKintosh, and D.A. Weitz. 2007a. Bending dynamics of fluctuating biopolymers probed by automated high-resolution filament tracking. *Biophys. J.* 93:346–359.

Brangwynne, C.P., F.C. MacKintosh, and D.A. Weitz. 2007b. Force fluctuations and polymerization dynamics of intracellular microtubules. *Proc. Natl. Acad. Sci. USA.* 104:16128–16133.

Brangwynne, C.P., G.H. Koenderink, F.C. MacKintosh, and D.A. Weitz. 2008. Nonequilibrium microtubule fluctuations in a model cytoskeleton. *Phys. Rev. Lett.* 100:118104.

Brown, R. 1828. On the particles contained in the pollen of plants; and on the general existence of active molecules in organic and inorganic bodies. *Edinburgh New Philosophical Journal.* 5:358–371.

Bursac, P., G. Lenormand, B. Fabry, M. Oliver, D.A. Weitz, V. Viasnoff, J.P. Butler, and J.J. Fredberg. 2005. Cytoskeletal remodelling and slow dynamics in the living cell. *Nat. Mater.* 4:557–561.

Cai, Y., N. Biais, G. Giannone, M. Tanase, G. Jiang, J.M. Hofman, C.H. Wiggins, P. Silberzan, A. Buguin, B. Ladoux, and M.P. Sheetz. 2006. Nonmuscle myosin IIA-dependent force inhibits cell spreading and drives F-actin flow. *Biophys. J.* 91:3907–3920.

Caspi, A., R. Granek, and M. Elbaum. 2000. Enhanced diffusion in active intracellular transport. *Phys. Rev. Lett.* 85:5655–5658.

Einstein, A. 1905. Investigations on the theory of the Brownian movement. *Annalen der Physik.* 17:549–560.

Fabry, B., G.N. Maksym, J.P. Butler, M. Glogauer, D. Navajas, and J.J. Fredberg. 2001. Scaling the microrheology of living cells. *Phys. Rev. Lett.* 87:148102.

Felgner, H., R. Frank, J. Biernat, E.-M. Mandelkow, E. Madelkow, B. Ludin, A. Matus, and M. Schliwa. 1997. Domains of neuronal microtubule-associated proteins and flexural rigidity of microtubules. *J. Cell Biol.* 138:1067–1075.

Finer, J.T., R.M. Simmons, and J.A. Spudich. 1994. Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature.* 368:113–119.

Fisher, G.W., P.A. Conrad, R.L. DeBiasio, and D.L. Taylor. 1988. Centripetal transport of cytoplasm, actin, and the cell surface in lamellipodia of fibroblasts. *Cell Motil. Cytoskeleton.* 11:235–247.

Forgacs, G., S.H. Yook, P.A. Janmey, H. Jeong, and C.G. Burd. 2004. Role of the cytoskeleton in signaling networks. *J. Cell Sci.* 117:2769–2775.

Gardel, M.L., J.H. Shin, F.C. MacKintosh, L. Mahadevan, P. Matsudaira, and D.A. Weitz. 2004. Elastic behavior of cross-linked and bundled actin networks. *Science.* 304:1301–1305.

Gittes, F., B. Mickey, J. Nettleton, and J. Howard. 1993. Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. *J. Cell Biol.* 120:923–934.

Landau, L.D., and E.M. Lifshitz. 1986. Theory of Elasticity. Pergamon Press, Oxford. 187 pp.

Lau, A.W.C., B.D. Hoffman, A. Davies, J.C. Crocker, and T.C. Lubensky. 2003. Microrheology, stress fluctuations, and active behavior of living cells. *Phys. Rev. Lett.* 91:198101.

Luby-Phelps, K., P. Castle, D.L. Taylor, and F. Lanni. 1987. Hindered diffusion of inert tracer particles in the cytoplasm of mouse 3T3 cells. *Proc. Natl. Acad. Sci. USA.* 84:4910–4913.

MacKintosh, F.C., J. Kas, and P. Janmey. 1995. Elasticity of semiflexible biopolymer networks. *Phys. Rev. Lett.* 75:4425–4429.

MacKintosh, F.C., and A.J. Levine. 2008. Nonequilibrium mechanics and dynamics of motor-activated gels. *Phys. Rev. Lett.* 100:018104.

Mizuno, D., C. Tardin, C.F. Schmidt, and F.C. Mackintosh. 2007. Nonequilibrium mechanics of active cytoskeletal networks. *Science.* 315:370–373.

Mogilner, A., and G. Oster. 1996. Cell motility driven by actin polymerization. *Biophys. J.* 71:3030–3045.

Odde, D.J., L. Ma, A.H. Briggs, A. Demarco, and M.W. Kirschner. 1999. Microtubule bending and breaking in living cells. *J. Cell Sci.* 112:3283–3288.

Pampaloni, F., G. Lattanzi, A. Jonas, T. Surrey, E. Frey, and E.L. Florin. 2006. Thermal fluctuations of grafted microtubules provide evidence of a length-dependent persistence length. *Proc. Natl. Acad. Sci. USA.* 103:10248–10253.

Panorchan, P., J.S. Lee, T.P. Kole, Y. Tseng, and D. Wirtz. 2006. Microrheology and ROCK signaling of human endothelial cells embedded in a 3D matrix. *Biophys. J.* 91:3499–3507.

Peskin, C.S., G.M. Odell, and G.F. Oster. 1993. Cellular motions and thermal fluctuations: the Brownian ratchet. *Biophys. J.* 65:316–324.

Rodriguez, O.C., A.W. Schaefer, C.A. Mandato, P. Forscher, W.M. Bement, and C.M. Waterman-Storer. 2003. Conserved microtubule-actin interactions in cell movement and morphogenesis. *Nat. Cell Biol.* 5:599–609.

Rosales-Nieves, A.E., J.E. Johndrow, L.C. Keller, C.R. Magie, D.M. Pinto-Santini, and S.M. Parkhurst. 2006. Coordination of microtubule and microfilament dynamics by *Drosophila* Rho1, Spire and Cappuccino. *Nat. Cell Biol.* 8:367–376.

Storm, C., J.J. Pastore, F.C. MacKintosh, T.C. Lubensky, and P.A. Janmey. 2005. Nonlinear elasticity in biological gels. *Nature.* 435:191–194.

Tseng, Y., T.P. Kole, and D. Wirtz. 2002. Micromechanical mapping of live cells by multiple-particle-tracking microrheology. *Biophys. J.* 83:3162–3176.

Vale, R.D. 2003. The molecular motor toolbox for intracellular transport. *Cell.* 112:467–480.

Waterman-Storer, C.M., and E.D. Salmon. 1997. Actomyosin-based retrograde flow of microtubules in the lamella of migrating epithelial cells influences microtubule dynamic instability and turnover and is associated with microtubule breakage and treadmilling. *J. Cell Biol.* 139:417–434.