Power-law rheology and mechano-sensing in a cytoskeleton model with forced protein unfolding

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We describe a model of cytoskeletal mechanics based on the force-induced conformational change of protein cross-links in a stressed polymer network. Slow deformation of simulated networks containing cross-links that undergo repeated, serial domain unfolding leads to an unusual state—with many cross-links accumulating near the critical force for further unfolding. Thermal activation of these links gives rise to power-law rheology resembling the previously unexplained mechanical response of living cells. Moreover, we hypothesize that such protein cross-links function as biochemical mechano-sensors of cytoskeletal deformation.

The importance of mechanical cues for understanding cell behavior is increasingly recognized. Stem cell differentiation [1], tissue morphogenesis [2] as well as cell growth [3] and death [4] are known to be affected by cell shape or the stiffness of the surrounding extra-cellular matrix. The molecular mechanisms by which such mechanical cues produce biochemical responses, however, remain essentially unknown [5]. Similarly, the anomalous mechanical response of cells defies explanation despite the identification of the cytoskeleton’s major structural constituents and considerable modeling effort [6, 7, 8]. Rheological measurements on living cells yield a frequency dependent shear modulus that scales as a weak power-law, \(G(\omega) \sim \omega^\beta\), with \(0.1 < \beta < 0.25\), spanning many decades of frequency [9, 10, 11]. Such a mechanical response is rather unusual [12], having only been observed previously in seemingly unrelated materials such as foams and pastes. While networks of purified filamentary biopolymers (e.g. F-actin) generally have a frequency-independent plateau elasticity [13], studies of networks containing the cross-link proteins filamin [14, 15] or \(\alpha\)-actinin [16] have reproduced the weak power-law rheology as well as other aspects of the cell response, such as stress-induced stiffening. These results suggest that the power-law rheology of cells may be due to cross-link conformational change or unbinding.

In this Letter, we propose a simple cytoskeletal architecture that may explain both cell rheology and mechano-sensing, based on the force-induced serial unfolding of domains in protein cross-links. We show, using simulation, that shearing a simplified model network leads spontaneously to the accumulation of many cross-links at tensions on the cusp of unfolding. Thermally activated unfolding of these cross-links readily reproduces cells’ power-law frequency dependent shear modulus with physically plausible model parameters. Comparable models based on forced unbinding (e.g. of cross-links’ actin binding domains from actin) rather than unfolding do not produce power-law rheology. Moreover, this unusual, near-critical arrangement of cross-links is suggestive of a mechano-sensory function. We hypothesize that, by modulating the binding of signaling species, unfolding cross-link domains function as the fundamental biochemical transducers of cytoskeletal deformation.

Many cross-linking proteins (e.g. filamin, \(\alpha\)-actinin, spectrin, plakin, etc.) have a structure consisting of similar, repeated domains that can be serially unfolded by applied force [17, 18, 19], see Figure 1(a). The unfolding time is typically described by the Bell model:

\[
\tau_B(F) = \tau_a \exp\left[E_B\left(1 - \frac{F}{F_c}\right)\right], \quad E_B = \frac{F_o r_o}{k_B T}
\]

where \(\tau_a\) is a molecular attempt time, \(F_c\) is a critical force, \(r_o\) is a characteristic bond length-scale, and \(k_B T\) is the thermal energy. The exponential form causes small changes in tension to yield rather large changes in unfolding time. As domains serially unfold, the molecule becomes progressively longer and longer. The entropic elasticity of the unfolded protein causes a spring-like re-

FIG. 1: Schematic representation of serial unfolding and the cytoskeleton. (a) As cross-links are extended, domains serially unfold with typical force vs. extension curves having abrupt transitions at a critical force, \(F_c\), corresponding states are labeled. (b) Our model of the cytoskeleton consisting of a network of generic semi-flexible polymers and partially unfolded, extensible cross-links.
sponse between unfolding events, producing a ‘sawtooth’ force-extension profile, Figure 1.

We hypothesize that some cross-links unfold under physiological stresses, as sketched in Figure 1(b), and that such unfolding is the source of microscopic stress relaxation causing weak power-law rheology. Simple estimates [21] suggest that physiologically plausible stresses of order $\sim 100$ Pa yield tensions sufficient to unfold some crosslink species. While the exponential sensitivity of the Bell model naturally gives rise to very broadly distributed characteristic unfolding and relaxation times, a broad distribution alone is not sufficient. As we shall see, the power-law form of the rheology requires an exponential distribution of cross-link tension. Just such force distributions emerge spontaneously when simulated model networks are slowly sheared.

For our simulations, we constructed two-dimensional networks having periodic connectivity in the left/right direction, whose nodes are initially offset from a triangular lattice by a Gaussian-distributed amount, Figure 2(a,b). Force carrying mechanical links connect these nodes (15% of the nearest-neighbor bonds are left empty to introduce topological disorder). Networks larger than roughly 25 by 35 nodes ($\sim 10^4$ links) gave results that were essentially independent of system size and changes in network disorder. To model serial unfolding, each link has a linear ‘sawtooth’ force-extension curve: $F = k((x-x_0) \mod x_c)$, where $k$ is a spring constant, $x$ is the instantaneous link extension, $x_c \equiv F_c/k$ is the critical extension for bond rupture and $x_0$ is the link length at the beginning of the simulation (typically 5-100 $x_c$). Our network does not contain separate rod-like and cross-link elements; each of our simulated links may be considered conceptually equivalent to a single inextensible rod of length $x_0$ connected in series with a cross-link having an infinite number of unfoldable domains. Compressed links generate a compressive force (for $x < x_0$, $F = -k(x-x_0) \mod x_c$), but this is not critical to our results.

The network is sheared by translating the nodes clamped to the upper boundary in a series of small steps ($\Delta \gamma < 10^{-3}$) at a constant strain rate, $\gamma(t) = \dot{\gamma} t$. All nodes are first displaced according to an affine shear deformation, and then relaxed to mechanical equilibrium (zero total force on each node) by moving the non-boundary nodes using an ‘over-damped’ steepest descent algorithm. During relaxation, links move freely between branches of the force extension curve until an equilibrium configuration is reached. This is equivalent to assuming that domains unfold and refold instantaneously when the tension reaches $F_c$ and zero, respectively.

To model Bell-type thermally-activated unfolding a Kinetic Monte Carlo (KMC) algorithm is used. The expected unfolding rate of each tensed ($F > 0$) link is computed from its tension and the Bell model (rate $= 1/\tau_B(F)$). Consistent with the KMC algorithm, an exponentially distributed time step is generated which is inversely proportional to the total unfolding rate, and the link to unfold is selected in a rate weighted manner. To unfold the selected sub-critical link, its force extension curve is temporarily modified to be zero in the $x_c$-wide extension interval it occupies. The network is then relaxed, with the selected link typically moving into an adjacent, non-zero interval.

It is illustrative to first consider the $T = 0$ limit of the unfolding simulation (i.e. without Bell unfolding). In that case, the network configuration depends only on the strain, $\gamma$, and the network’s geometrical parameters. For small strains, a roughly uniform distribution of link tension develops. For strains, $\gamma > 0.4$, links accumulate at tensions somewhat smaller than $F_c$ (Figure 2(c)). The distribution of link tensions approximates an increasing exponential function of force over a small range, $P(F) \sim e^F/F_c$, the qualitative form required to produce power-law rheology. As the strain is increased further, the range of forces showing exponential behavior broadens, but maintains the same slope: $F_c/F_c \approx 0.4$.

In principle, the evolution of thermalized networks is more complicated, depending additionally on the Bell parameters $E_B$ and $\tau_a$ and the strain rate $\dot{\gamma}$, (assumed to be slow, $\dot{\gamma} \tau_a \ll 1$). In practice, however, the qualitative form of $P(F)$ is quite similar to the athermal case, but with a somewhat smaller critical force, see Figure 3(a). Indeed, the $P(F)$’s from all of our thermalized unfolding simulations could be accurately scaled onto one another by renormalizing their forces by an effective critical force, $F'_c$. Important for later discussion, the number of ‘unfolded’ domains in the network is a monotonic function of network strain, with little dependence on strain rate or hysteresis upon strain reversal, Figure 3(b).
ology varies quite slowly with the strain rate: 

Surprisingly, the frequency range with power-law rheology has a terminal mode at frequencies below the zero-force value, $G_\gamma^\ast \gamma |G_\gamma^\ast| = 0$ and $G_\gamma^\ast \gamma |G_\gamma^\ast| = 1$ respectively. The dotted line is from the unbinding simulation at $\gamma = 0.5$, $E_B = 25$, $\tau_a = 50$ nsec, and $\gamma = 10$ sec$^{-1}$ (b) The number of unfolded domains as a function of strain. The solid line is during strain application and the dotted line is during strain reversal. (c) Most probable unfolding force as a function of dimensionless strain rate.

The effective critical force behavior has a simple explanation. Bell-type molecules subjected to a constant force loading rate have a well-defined most probable force for unfolding, which is a logarthmic function of the loading rate [13]. The constant strain rate, $\gamma$, of our model slowly stretches links, leading to a constant force loading rate. We can identify the resulting most probable link unfolding force with $F_c^\prime$, which, as expected, scales logarithmically with $\gamma$, Figure 3(c). Links with tensions even slightly smaller than $F_c^\prime$ have an almost negligible Bell unfolding rate (since $E_B \gg 1$), leading $P(F)$ to evolve much as an athermal model with ‘all or nothing’ unfolding at critical force $F_c^\prime$.

The frequency-dependent mechanical response of our network can be estimated by superposing Maxwell modes [22]. Formally, such a modulus describes the network’s differential response to a small oscillatory applied stress superposed on the network’s static stress. This is compatible with the emerging view that cell measurements actually report such a differential shear modulus at a cell-generated prestress. [14]. As expected, the computed shear modulus, Figure 4(top), has a roughly power-law form, $G^\ast(\omega) \sim \omega^\beta$, over a finite frequency range, with a non-universal exponent $\beta \approx 2.5F_c/E_B$. The modulus has a terminal mode at frequencies below the zero-force unfolding rate, $\tau_a^{-1} \exp(-E_B)$, and approaches a plateau value, $G_o$ at frequencies higher than $\omega_{max} \approx 1/\tau_B(F_c^\prime)$. Surprisingly, the frequency range with power-law rheology varies quite slowly with the strain rate: $\omega_{max} \sim \gamma^b \gamma^{-b-1}$, with $b \approx 0.18$.

In a separate study of forced unbinding, (rather than unfolding), force-extension curves with a single period were used ($F = k(x - x_o)$ for $|F| < F_c$, zero otherwise). Any link at zero force in mechanical equilibrium after a time/strain step corresponds to a link that has exceeded its critical force and unbound. We then reset its $x_o$ parameter to its new equilibrium length to simulate rapid rebinding at zero force with the same network connectivity. Activated unbinding is handled as before, with the KMC-selected link having its force set to zero, followed by network relaxation and resetting of the $x_o$’s of any unbound links. Unlike the unfolding simulations, the unbinding simulations never showed any accumulation of near critical links, Figure 4(a), indicating such models do not produce power-law rheology.

Before proceeding, some discussion of the model’s strain rate, $\gamma$, is in order. We suppose that in cells $\gamma$ is caused by molecular motor sliding or filament treadmilling at a roughly constant velocity. Rather than a uniform, pure shear deformation on the cellular scale, a spatially random deformation field with a typical, mesoscopic strain rate $\gamma$ would serve just as well. We fur-
ther suppose that other active processes continuously ‘re-model’ the network with a turnover rate comparable to $\dot{\gamma}$, such that typical network segments are strained to $\gamma \sim 1$ in a dynamic steady state that replicates the non-steady behavior of our model at $\gamma \sim 1$. To be consistent with 100-1000 second estimates for cytoskeletal turnover, $\dot{\gamma}$ should thus be in the range $10^{-3}$-$10^{-2}$ sec$^{-1}$.

The computed rheology reproduces the cell response qualitatively, but falls short of quantitative replication. The experimental literature reports at least five frequency decades of power-law scaling with exponents in the range $0.10 < \beta < 0.25$, while our model only yields five decades for $\beta < 0.17$ (or higher exponents over a narrower frequency range). This is due to the modest ‘height’ of our exponential force distribution, $P(F)/P(F=0) \approx 3$, as in Figure 3(a). Recent athermal simulations using a more realistic network structure yield a more pronounced exponential $P(F)$, suggesting our limited frequency range may be an artifact of our simplified network geometry. To estimate the model parameters corresponding to the physical case, we dimensionalized our model, added a high-frequency contribution $G^* \sim \omega^{0.75}$, and compared it to literature data. Our model reproduces the response of normal HASM cells, Figure 3(bottom), with physically plausible parameter values: $E_B = 25$, $\tau_a = 70$ nsec and $G_o = 5$ kPa. The simulated strain rate, $\dot{\gamma} \approx 7$ sec$^{-1}$, is higher than our earlier estimate; smaller dimensionless strain rates were too computationally intensive to be accessible. Because of the slow variation of model rheology with $\dot{\gamma}$, however, a more realistic value $\dot{\gamma} = 10^{-2}$ sec$^{-1}$, should yield a similar response with slightly smaller exponent, $\beta \approx 0.15$, and narrow the frequency range with power law rheology by $(700)^{0.18} = 3.2 \times$, or half a decade.

The arrangement of structural molecules on the cusp of conformational change suggests an optimal configuration for a chemical mechano-sensor—suggesting that cells may maintain a metabolically costly dynamic cytoskeleton as much for its sensory as its structural functions. Unlike other proposed sensor mechanisms, that transduce molecular stress, the earlier noted correspondence of unfolding and network strain indicates the sensing of deformation on the supramolecular scale. Many cross-linking species specifically bind a number of signaling proteins, including heat shock proteins, protein kinase C, RapA, Pip2, Pip3, P33-kinase, and M6K1 (for reviews). Any of these proteins that specifically binds (or unbinds) cross-link domains upon forced unfolding would then transduce the shear or extensional strain of the network, which is presumably a prerequisite for shape or matrix compliance sensing. Furthermore, the myriad, multi-domain cross-link proteins and their isoforms localized to different parts of the cell suggest that each different cell sub-structure could have its own mechano-sensing capability.

Our cross-link unfolding model reproduces the cell response and makes biochemically testable predictions: that some cross-link species should be partially unfolded under normal physiological conditions, and that their unfolding should increase with cell deformation. Such biochemical studies, combined with rheology simulations having more realistic network structure, hold the prospect of a cytoskeleton model grounded in polymer and single-molecule biophysics, one that can be integrated with mechano-sensory signaling pathways.

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[1] R. McBeath, D. M. Pirone, C. M. Nelson, K. Bhadriraju, and C. S. Chen, Dev Cell 6, 483 (2004).
[2] M. J. Paszek et al., Cancer Cell 8, 241 (2005).
[3] H. B. Wang, M. Dembo, and Y. L. Wang, Am J Physiol Cell Physiol 279, C1345 (2000).
[4] C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, and D. E. Ingber, Science 276, 1425 (1997).
[5] P. A. Janmey and D. A. Weitz, Trends Biochem Sci 29, 364 (2004).
[6] M. L. Gardel et al., Science 304, 1301 (2004).
[7] D. H. Wachsstock, W. H. Scharz, and T. D. Pollard, Biophysical Journal 66, 801 (1994).
[8] N. Wang et al., Proc Natl Acad Sci U S A 98, 7765 (2001).
[9] B. Fabry et al., Phys Rev Lett 87, 148102 (2001).
[10] W. Feneberg et al., Biophys J 87, 1338 (2004).
[11] J. Alcaraz et al., Biophys J 84, 2071 (2003).
[12] P. Sollich, Physical Review E 58, 738 (1998).
[13] M. L. Gardel et al., Phys Rev Lett 91, 158302 (2003).
[14] M. L. Gardel et al., PNAS (USA) 103, 1762 (2006).
[15] M. L. Gardel et al., Phys Rev Lett 96, 088102 (2006).
[16] Y. Tseng and D. Wirtz, Biophysical Journal 81, 1643 (2001).
[17] S. Furni et al., FEBS Lett 498, 72 (2001).
[18] M. Rief et al., Science 276, 1199 (1997).
[19] M. Rief et al., J Mol Biol 286, 553 (1999).
[20] G. I. Bell, Science 200, 618 (1978).
[21] A stress of 100 Pa = 100 pN/µm$^2$. In a gel of short filaments with $\sim 30$ cross-links/µm$^2$, tension would be carried through $\sim 10$ (or $30^{3/2}$) cross-links/µm$^2$. The resulting 10 pN tension can unfold spectrin domains, and is about one quarter that required to unfold IgG domains.
[22] H. H. Winter, Journal of Non-Newtonian Fluid Mechanics 68, 225 (1997).
[23] C. A. Otey and O. Carpen, Cell Motil Cytoskeleton 58, 104 (2004).