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Response of biopolymer networks governed by the physical properties of cross-linking molecules

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Abstract

In this study, we examine how the physical properties of cross-linking molecules affect the bulk response of bio-filament networks, an outstanding question in the study of biological gels and the cytoskeleton. We show that the stress–strain relationship of such networks typically undergoes linear increase – strain hardening – stress serration – total fracture transitions due to the interplay between the bending and stretching of individual filaments and the deformation and breakage of cross-linkers. Interestingly, the apparent network modulus is found to scale with the linear and rotational stiffness of the crosslinks to a power exponent of 0.78 and 0.13, respectively. In addition, the network fracture energy will reach its minimum at intermediate rotational compliance values, reflecting the fact that most of the strain energy will be stored in the distorted filaments with rigid cross-linkers while the imposed deformation will be “evenly” distributed among significantly more crosslinking molecules with high rotational compliance.

Introduction

It is well-known that the mechanical response of live cells is largely determined by the cytoskeleton, a network composed of different bio-polymers such as F-actin, intermediate filaments and microtubules.¹ Because of its essential roles in processes like cell migration² and division,³ extensive effort has been made in the past few decades trying to understand the load bearing and force generation capabilities of such a bio-filament network both experimentally^{4–13} and theoretically.^{14–22} Interestingly, accumulating evidence has demonstrated that the presence and physical properties of crosslinking proteins, interconnecting and bundling individual filaments together, significantly influence how the entire network behaves. For example, it was found that variations in the concentration of crosslinkers will not only affect the bulk modulus of an F-actin network⁷ but also alter its micro-architectures.²³ In addition, switching crosslinkers from rigid proteins such as scruin to compliant ones (like filamin A) will completely change the stress–strain characteristics¹¹ of the network as well as negate the hardening effect introduced by myosin motors.^{24,25}

Despite these intriguing findings, a comprehensive understanding of exactly how the properties of individual crosslinking molecules regulate the bulk network behavior is still lacking. Specifically, the angle between two filaments at the crosslinking point was treated as either fixed or free to vary in most existing modeling investigations.^{17,19} In reality, it is

likely that a cross-linking protein will provide finite resistance against changes in the filament angle. Indeed, several recent studies have measured this quantity for different crosslinkers.^{26–29} The only theoretical attempts considering this factor, to the best of our knowledge, were made by Sharma *et al.*³⁰ and most recently by Žagar and co-workers.³¹ However, either the deformability of filaments was totally neglected³⁰ or different spring constants of the cross-linkers were assumed to be coupled with each other³¹ (*i.e.* they cannot vary independently) in those studies. Furthermore, possible breakage of crosslinking points was also not taken into account in most previous investigations and hence the rupture behavior of networks has never been carefully examined. Given that more than 23 classes of crosslinking proteins with distinct properties have been identified in cells,¹ finding answers to these questions could be critical for our fundamental understanding of how cells regulate their cytoskeleton to perform different biological duties as well as for the development of biomimetic materials in the future.

Here we report a computational investigation where a combined finite element – Langevin dynamics (FEM-LD) method was employed to capture the shear response of randomly cross-linked bio-polymer networks. In addition to bending and stretching, thermal fluctuations of individual filaments along with the deformability and breakage of cross-linkers (modeled as a combination of linear and rotational springs) have been taken into account in the present study. We show how the linear and rotational stiffness of cross-linkers regulate the load transmission, as well as strain distribution, within the network and eventually dictate their bulk mechanical properties. The dependence of network fracture energy on the compliance of crosslinking molecules is also systematically examined. Finally, connections between our results and various experimental findings are discussed.

Computational model

Simulations were conducted on two-dimensional networks constructed by randomly placing 200 filaments, each with length L and a random orientation, inside a periodic unit cell of dimensions $W \times W$ ($W = 4L$), with a representative example shown in Fig. 1. Notice that, the normalized line density of our network (defined as $\bar{\rho} = NL^2/W^2$, where N is the total number of filaments in the unit cell) is $\bar{\rho} = 12.5$, which is well above the rigidity percolation threshold of $\bar{\rho} = 5.7$.¹⁷ Within the physiological range, most bio-polymers are semi-flexible and hence the elastic properties of filaments can be characterized by two parameters: the stretching rigidity EA and the bending rigidity EI . We proceed by choosing $EI = k_B T \times 12 \mu\text{m}$ and $EA = 34 \text{ nN}$, typical values for F-actin,^{32,33} in all our simulations where $k_B T$ stands for thermal energy.

Points where two filaments intersect are treated as cross-links. As pointed out earlier, the crosslinking protein will prevent two filaments from separating and rotating from each other. To describe such constraints in the simplest manner, two filaments are assumed to be connected by a linear as well as a rotational spring, with spring constants κ_s and κ_T respectively. Consequently, the energy stored in each cross-linker is

$E_C = \frac{1}{2}\kappa_s\delta^2 + \frac{1}{2}\kappa_T(\theta - \theta_0)^2$, where δ is the separation of the intersecting points and $(\theta - \theta_0)$ is the change in the relative angle between two filaments. We can then define that two

filaments will disengage from each other once E_C reaches a threshold value E_{lim} and never interact again afterward. The bulk network response under simple shear (induced by displacing the top of the unit cell horizontally while keeping the bottom fixed as illustrated in Fig. 1(A)) was simulated *via* the finite element-Langevin dynamics (FEM-LD) method developed by us previously.³⁴ Specifically, each filament was discretized into numerous equal-sized Euler–Bernoulli beam elements and a total Lagrangian finite strain formulation³⁵ was employed to take into account its large bending and stretching deformations.

Results and discussion

To quantitatively reveal how the physical properties of cross-linking molecules affect the bulk behavior of the network, we present simulation results according to two dimensionless

parameters defined as $\alpha = \frac{\kappa_s L}{EA}$ and $\beta = \frac{\kappa_T}{EAL}$. Obviously, a smaller α (or β) corresponds to a linearly (or rotationally) more compliant cross-linker. Choosing $L = 120$ nm, typical length of F-actins in lamellipodium of motile cells,³⁶ the representative response of the network is given in Fig. 1(B) where the stress–strain curves clearly exhibit linear increase – strain hardening – stress serration – total fracture transitions, similar to those observed in biological gels.⁷ It is conceivable that hardening is caused by the shift of filament deformation from bending to stretching²² while load serrations correspond to the breakage of individual cross-linkers; refer to the inset of Fig. 1(B). It can also be seen that changing the stiffness of crosslinking molecules can completely alter the network behavior, with increasing α or β leading to a higher bulk modulus. In addition, no apparent strain hardening will be observed if very compliant cross-linkers are used, which can be understood by realizing that most imposed deformation in this case will be absorbed by soft crosslinking molecules rather than causing the filaments to bend/stretch. These features have indeed been reported from recent experiments on *in vitro* actin networks cross-linked by rigid scruin or compliant flaminin A or α -actinin.^{7,12}

Based on simulations conducted on 8 different random networks with the same line density, the initial shear modulus G as a function of α is illustrated in Fig. 2(A). Essentially, irrespective of the value of β , G increases with α when it is relatively small. However, G becomes saturated and approaches the limiting value G_0 (the modulus corresponding to rigid cross-linkers, *i.e.* $\alpha = \beta = \infty$) when α is very large. Interestingly, we found that the dependence of G on α and β (in the regime where α is not too high) can be well-fitted by the following scaling law:

$$G/G_0 \sim \alpha^{0.78} \beta^{0.13} \quad (1)$$

which is represented by the solid line in Fig. 3(A). The validity of eqn (1) has also been tested (and confirmed) on networks with $L = 1.2 \mu\text{m}$ (*i.e.* the approximate F-actin length in the main cortex of cells³⁶), refer to Fig. 2(B). Note that a slightly different scaling relation has also been proposed by a recent study,²⁹ where filaments were assumed to be rigid and hence their elastic deformations (*i.e.* bending and stretching) are totally neglected.

At this point, it is informative to consider the corresponding α and β values for typical crosslinking proteins. Direct measurements indicated that, when under small stretching, flaminin A behaves like a worm-like chain (WLC) with a contour and persistence length of $l_0 \approx 160$ nm and $l_p = 20$ nm, respectively.^{24,37} As such, the effective linear stiffness of such protein can be approximately calculated as $k_s = \frac{3k_B T}{2l_p l_0} \approx 2 \times 10^{-6} \text{ N m}^{-1}$,³⁸ translating to an α value of 7×10^{-6} (or 7×10^{-5}) for $L = 120$ nm (or $L = 1.2 \mu\text{m}$). However, successive unfolding of flaminin A can take place as the stretching magnitude increases. In this case, the force will elevate rapidly with the strain before undergoing a sudden drop. From the force–extension curve³⁷ recorded, k_s at this stage is estimated to be around $6.2 \times 10^{-3} \text{ N m}^{-1}$, corresponding to $\alpha = 2.2 \times 10^{-2}$ or 2.2×10^{-1} for F-actin networks with $L = 120$ nm or $1.2 \mu\text{m}$. In consistent with these estimations, a k_s value of the order of $2 \times 10^{-4} \text{ N m}^{-1}$ was adopted in recent Brownian dynamics simulations^{39–41} where flaminin A was modeled as having two actin-binding arms³⁹ that possess an overall rotational stiffness of ~ 7 nN nm (*i.e.* $\beta \approx 1.7 \times 10^{-4}$ to 1.7×10^{-3}). Evidently, all these values are within the parameter ranges examined here. Interestingly, in direct contrast to flaminin A, it was found that α -actinin can link two actin filaments with arbitrary orientations together,²⁷ suggesting that very little (if any) rotational constraint will be provided by such molecules (that is, $\beta \rightarrow 0$). On the other hand, recent experiment also indicated that the linear stiffness of α -actinin is approximately 50% of that of flaminin A.⁴²

Besides bulk modulus, another interesting question to ask is how much energy a network can absorb before catastrophic rupture occurs. By calculating the area under the stress–strain curves, this quantity U_f (often referred to as the fracture energy) as a function of α and β is shown in Fig. 3 where the maximum strain energy E_{lim} any cross-linker can sustain is taken as $6k_B T$ (a value suggested by direct measurements on flaminin A²⁶). Surprisingly, although U_f increases monotonically with α , this quantity was found to reach its minimum at certain intermediate β values, a trend that becomes more and more obvious when α is relatively large (say >10).

To understand this rather perplexing finding, we examine exactly how the load is transmitted within the network. Specifically, choosing $\alpha = 10$, the number of cross-linkers with strain energy stored inside higher than $1/2 E_{\text{lim}}$, along with the apparent stress, is plotted as a function of the applied strain in Fig. 3(C) and (D). Locations of these heavily deformed cross-linking points are highlighted in the insets of Fig. 3(D) showing the deformed networks. It can be seen that the stress level increases rapidly with strain when β is large while very few cross-linkers are subjected to severe deformation. This means most energy will be stored in the deformed filaments instead of the stiff cross-linking molecules, eventually leading to a large value in fracture energy. On the other hand, significantly more cross-linkers will be highly strained when β is very small, meaning that the load will be homogeneously distributed among crosslinking points in this case which allows the network to store more energy before fracture. These observations explain why U_f reaches its minimum at intermediate β values where both the overall stress level and the number of highly distorted cross-links remain moderate.

Finally, the influence of thermal fluctuation is investigated. Insets in Fig. 4 show the amplified stress–strain plots at small strains and near the breakage points of cross-linkers. Clearly, the entropy effect is significant only when the macroscopic strain is less than ~1%, in agreement with our previous findings.³⁴ In addition, it can be seen that the presence of thermal excitations will always advance the rupture of cross-linking molecules, a finding that can be understood by realizing that random undulations of filaments can momentarily increase the load level in a cross-linker beyond the threshold value and cause its early breakage.

Conclusion

In this paper we report a computational investigation of the mechanical response of bio-polymer networks where, in addition to bending and stretching, thermal fluctuations of individual filaments along with the deformability and breakage of cross-linkers have also been taken into account. We show that when subjected to shear, the stress–strain relationship of a random network will typically undergo linear increase – strain hardening – stress serration – total fracture transitions, a feature that has indeed been observed in bio-polymer gels.⁷ A scaling law demonstrating the heavy influence of the linear and angular compliances of crosslinking molecules on the bulk modulus of the network was also identified which explains our simulation results very well. Findings here are consistent with recent experiments showing that switching cross-linkers from angularly rigid proteins such as scruin to compliant ones (like filamin A) will completely change the stress–strain characteristics of *in vitro* actin networks.^{7,12}

Interestingly, the network fracture energy is also significantly affected by the physical properties of crosslinking molecules. In particular, this quantity was found to reach its minimum at intermediate angular compliance values, which reflects the fact that most applied strain will be accommodated by filament bending/stretching when the crosslinking molecules are very stiff while the imposed deformation will be distributed among significantly more cross-linkers when they become very soft, both leading to a high strain energy stored in the network before its eventual rupture. Finally, we show that thermal fluctuations of filaments will affect the network response significantly at small strains (less than ~1%), and advance the breakage of individual crosslinks.

Given that more than 23 classes of crosslinking proteins, with distinct properties, have been identified in cells,¹ findings from this study could greatly help us understand how cells regulate their cytoskeleton to perform different biological duties. In addition, quantitative predictions regarding the influence of cross-linker behavior on the apparent modulus and fracture toughness of bio-filament networks may also provide critical insights for the development of biomimetic materials in the future. Finally, it is conceivable that more features can be incorporated into the present computational platform to examine important issues such as the role of binding/dissociation kinetics of cross-linking molecules in the remodeling and rheological response of bio-polymer gels^{43–45} as well as the transmission and sensing of forces within the cytoskeleton of cells.^{46,47} Studies along these lines are currently underway.

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References

- Kreis, T., Vale, R. Cytoskeletal and motor proteins. Vol. 2. Oxford Univ Press; Oxford: 1999.
- Lauffenburger DA, Horwitz AF. Cell. 1996; 84:359–369. [PubMed: 8608589]
- Schroeder TE. Proc Natl Acad Sci U S A. 1973; 70:1688–1692. [PubMed: 4578441]
- Janmey PA, Euteneuer U, Traub P, Schliwa M. J Cell Biol. 1991; 113:155–160. [PubMed: 2007620]
- MacKintosh FC, Janmey PA. Curr Opin Solid State Mater Sci. 1997; 2:350–357.
- Xu J, Wirtz D, Pollard TD. J Biol Chem. 1998; 273:9570–9576. [PubMed: 9545287]
- Gardel ML, Shin JH, MacKintosh FC, Mahadevan L, Matsudaira P, Weitz DA. Science. 2004; 304:1301–1305. [PubMed: 15166374]
- Chaudhuri O, Parekh S, Fletcher D. Nature. 2007; 445:295–298. [PubMed: 17230186]
- Janmey PA, McCormick ME, Rammensee S, Leight JL, Georges PC, MacKintosh FC. Nat Mater. 2007; 6:48–51. [PubMed: 17187066]
- Liu J, Koenderink GH, Kasza KE, MacKintosh FC, Weitz DA. Phys Rev Lett. 2007; 98:198304. [PubMed: 17677669]
- Wagner B, Tharmann R, Haase I, Fischer M, Bausch A. Proc Natl Acad Sci U S A. 2006; 103:13974. [PubMed: 16963567]
- Gardel ML, Nakamura F, Hartwig JH, Crocker JC, Stossel TP, Weitz DA. Proc Natl Acad Sci U S A. 2006; 103:1762–1767. [PubMed: 16446458]
- Dalhaimer P, Discher DE, Lubensky TC. Nat Phys. 2007; 3:354–360.
- Gardel ML, Shin JH, MacKintosh FC, Mahadevan L, Matsudaira P, Weitz DA. Phys Rev Lett. 2004; 93:188102. [PubMed: 15525211]
- Storm C, Pastore J, MacKintosh F, Lubensky T, Janmey P. Nature. 2005; 435:191–194. [PubMed: 15889088]
- Bausch A, Kroy K. Nat Phys. 2006; 2:231–238.
- Wilhelm J, Frey E. Phys Rev Lett. 2003; 91:108103. [PubMed: 14525511]
- MacKintosh FC, Ka's J, Janmey PA. Phys Rev Lett. 1995; 75:4425. [PubMed: 10059905]
- Broedersz CP, Storm C, MacKintosh FC. Phys Rev Lett. 2008; 101:118103. [PubMed: 18851336]
- Head DA, Levine AJ, MacKintosh FC. Phys Rev Lett. 2003; 91:108102. [PubMed: 14525510]
- Huisman EM, Van Dillen T, Onck PR, Van der Giessen E. Phys Rev Lett. 2007; 99:208103. [PubMed: 18233190]
- Onck PR, Koeman T, Van Dillen T, Van der Giessen E. Phys Rev Lett. 2005; 95:178102. [PubMed: 16383874]
- Lieleg O, Claessens MMAE, Bausch AR. Soft Matter. 2010; 6:218–225.
- Koenderink GH, Dogic Z, Nakamura F, Bendix PM, MacKintosh FC, Hartwig JH, Stossel TP, Weitz DA. Proc Natl Acad Sci U S A. 2009; 106:15192–15197. [PubMed: 19667200]
- Chen P, Shenoy VB. Soft Matter. 2010; 6:3548–3561. [PubMed: 21113355]
- Lee H, Pelz B, Ferrer JM, Kim T, Lang MJ, Kamm RD. Cell Mol Bioeng. 2009; 2:28–38.
- Courson DS, Rock RS. J Biol Chem. 2010; 285:26350–26357. [PubMed: 20551315]
- Nakamura F, Osborn TM, Hartemink CA, Hartwig JH, Stossel TP. J Cell Biol. 2007; 179:1011–1025. [PubMed: 18056414]
- Stossel TP, Condeelis J, Cooley L, Hartwig JH, Noegel A, Schleicher M, Shapiro SS. Nat Rev Mol Cell Biol. 2001; 2:138–145. [PubMed: 11252955]

30. Sharma A, Sheinman M, Heidemann KM, MacKintosh FC. *Phys Rev E: Stat, Nonlinear, Soft Matter Phys.* 2013; 88:052705.
31. Žagar G, Onck PR, van der Giessen E. *Biophys J.* 2015; 108:1470–1479. [PubMed: 25809259]
32. Boal, D. *Mechanics of the Cell.* Cambridge University Press; 2002.
33. Liu X, Pollack GH. *Biophys J.* 2002; 83:2705–2715. [PubMed: 12414703]
34. Lin Y, Wei X, Qian J, Sze KY, Shenoy VB. *J Mech Phys Solids.* 2014; 62:2–18.
35. Reddy, JN. *An Introduction to Nonlinear Finite Element Analysis: With Applications to Heat Transfer, Fluid Mechanics, and Solid Mechanics.* Oxford University Press; 2014.
36. Fletcher DA, Mullins RD. *Nature.* 2010; 463:485–492. [PubMed: 20110992]
37. Furukawa S, Ito T, Yamazaki M. *FEBS Lett.* 2001; 498:72–75. [PubMed: 11389901]
38. Marko J, Siggia E. *Macromolecules.* 1995; 27:981.
39. Kim T, Hwang W, Lee H, Kamm RD. *PLoS Comput Biol.* 2009; 5:e1000439. [PubMed: 19609348]
40. Kim T, Hwang W, Kamm RD. *Exp Mech.* 2009; 49:91–104.
41. Kim T, Gardel ML, Munro E. *Biophys J.* 2014; 106:526–534. [PubMed: 24507593]
42. Ferrer J, Lee H, Chen J, Pelz B, Nakamura F, Kamm R, Lang M. *Proc Natl Acad Sci U S A.* 2008; 105:9221–9226. [PubMed: 18591676]
43. Müller KW, Bruinsma RF, Lieleg O, Bausch AR, Wall WA, Levine AJ. *Phys Rev Lett.* 2014; 112:238102. [PubMed: 24972229]
44. Ehrlicher AJ, Krishnan R, Guo M, Bidan CM, Weitz DA, Pollak MR. *Proc Natl Acad Sci U S A.* 2015; 112:6619–6624. [PubMed: 25918384]
45. Wang X, Hong W. *Soft Matter.* 2012; 8:8171–8178.
46. Wang H, Abhilash AS, Chen CS, Wells RG, Shenoy VB. *Biophys J.* 2014; 107:2592–2603. [PubMed: 25468338]
47. Liu AP, Richmond DL, Maibaum L, Pronk S, Geissler PL, Fletcher DA. *Nat Phys.* 2008; 4:789–793. [PubMed: 19746192]

Fig. 1.

(A) A representative random network (with periodic boundary conditions) used in the present study. A cross-linker, modeled as a combination of linear and rotational springs, is inserted at every intersection point between two filaments. (B) Stress-strain curves of the network shown in (A) with different types of crosslinking molecules. The green dashed line corresponds to the perfectly linear response of the network with rigid cross-linkers with G_0 being the initial modulus. Snapshots of the deformed network (at points a and b) are also provided where the red line indicates two points that were originally crosslinked together (*i.e.* the cross-linker was broken).

Fig. 2.

The apparent modulus of networks as a function of the linear and rotational stiffness of cross-linkers: (A) $L = 120 \text{ nm}$ and (B) $L = 1.2 \mu\text{m}$.

Fig. 3.

The fracture energy of networks as a function of the linear (A) and rotational (B) compliance of crosslinking molecules. (C) Evolution of the number of highly strained cross-linkers, with energy stored inside higher than 50% of the breakage value $-6k_B T$, under three rotational stiffness values. (D) Stress-strain curves corresponding to the cases shown in (C). Snapshots of the deformed network are given in the insets where locations of the highly strained cross-linkers are indicated by red dots.

Fig. 4.
Influence of thermal fluctuations on the network response.