A model to study mechanical and equilibrium properties of biopolymeric networks through a statistical approach

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Abstract: Mechanical properties of cells are dominated by the cytoskeleton, an interconnected network of long elastic filaments. The connections between these filaments are provided by a very important mechanical element of the network: cross-linking proteins, which, due to their dynamic nature, allow remodeling in response to external cues. The reversible nature of crosslink binding/unbinding is an important mechanism that underlies these dynamical processes. Here, we develop a simple theoretical model to come close to what recent studies aim to do: study the dynamics of these molecular motors introducing some flexible conditions into the considered network, a first approach in this field.

I. INTRODUCTION

The mechanical response of cells depends in large part on the structure and elasticity of their cytoskeleton. In particular, its elasticity governs many physiological activities, including cell motility, growth and division. The cytoskeleton is composed of a variety of biopolymer networks, including filamentous actin microtubules. Beyond their role in providing structural integrity, the networks are also the basis for inter- and intra- cellular communication. If we can understand the mechanics of biopolymers, we will be able to gain a deeper understanding of many biological processes [1]. This is why the comprehension of networks mesoscale behaviors', in terms of the underlying non-equilibrium processes, such as cytoskeletal remodeling, motor activity or reversible crosslink binding or folding, remains an important theme in current biomechanical research [2]. In addition to their biological importance, studying biopolymers from a physical point of view highlights the importance of their material properties: these networks form elastic systems. Besides this linear elasticity, one of the most remarkable features of biopolymer networks is their nonlinear behavior in the elastic response; these networks are initially compliant, but beyond a critical stress, they are able to stiffen by a factor over 1000. The origin of nonlinear elasticity in these networks is regulated by an array of actin binding and cross-linking proteins. This proteins form static nodal points in the network; they bind actin filaments together to form isotropic networks.

In recent years, reconstituted F-actin networks have become a popular prototype system in which this can be studied in detail. Much of the previous research in this field focused on the frequency-dependent rheology of *permanently crosslinked* filament networks, but this won't be the purpose of our study, our motivation goes in another direction. However, it is yet to be resolved how transiently cross-linking proteins affect the frequency response of cross-linked actin networks in the elasticity dominated intermediate frequency regime [3]. Key questions revolve around how frequency affects the nature of network deformations (affine versus non-affine) and the nonlinear response properties of the network. Different theoretical models and simplified simulations schemes have been proposed, aiming to explain one or the other of these non-trivial features. In these studies, the filaments and their mechanical and thermal properties are assumed to dominate the effective rheology of the system [4], [5].

This may be different in F-actin networks crosslinked with the rather compliant cross-linking protein filamin. Experimental and theoretical work suggest a crosslink-dominated regime where network rheology is set by the crosslink stiffness, while filaments effectively behave as rigid, undeformable rods. Theoretical modeling in this field is only beginning to emerge, and precisely this is the main motivation of this work. In contrast with previous *in vitro* studies, where the ability of binding and unbinding is not allowed, we will try to present and reproduce qualitatively a simple model proposed by C. Heussinger, of the Deutsche Physikalische Gesellschaft (Institute for Theoretical Physics, Georg-August-Universitt Gttingen), which is a fully thermodynamic treatment, where crosslink binding is equilibrated for a given network deformation. This approach gives lead to a statistical study in the canonical ensemble of the dynamics of the molecular motors. It accounts for the individual crosslinks, their mechanical properties as well as their binding state, where no rate effects are considered.

Supporting this line of work, this paper will be structured as follows. In the second section, we will present and explain the model we have developed. We will introduce a Hamiltonian that describes the properties of a single filament inside an effective medium. In the third section, we will show the results of our metropolis Monte Carlo (MC) simulations for the Hamiltonian introduced. Finally, in the last section, we will discuss how a further theoretical approach can lead us to a better understanding of the obtained results.

II. THE MODEL

In this work, we will consider the properties of a single test filament crosslinked into a network. Therefore, our model is a kind of *mean field model*, where the complex interactions between the filaments are approximated by a single average effect, thus reducing the many-body problem to a one-body problem. As we want to discuss the effect of network deformation on the test filament, we need to know how a macroscopically applied strain field couples to the single filament. If the effective medium can be thought of as strictly homogeneously elastic, then the local strain felt by the filament will be identical to the macroscopic strain γ. With this assumption of "affine" deformations any network deformation couples to the end-to-end distance of the filament and leads to its extension or compression. Rheological properties are then governed by the resistance of filaments to stretching deformations. In contrast, if we consider local structural heterogeneity and we assume that filaments are anisotropic elastic objects, where bending is a much softer deformation mode than stretching, this may in fact lead to a highly non-affine response where network properties are determined by the resistance of filaments to bending deformations.

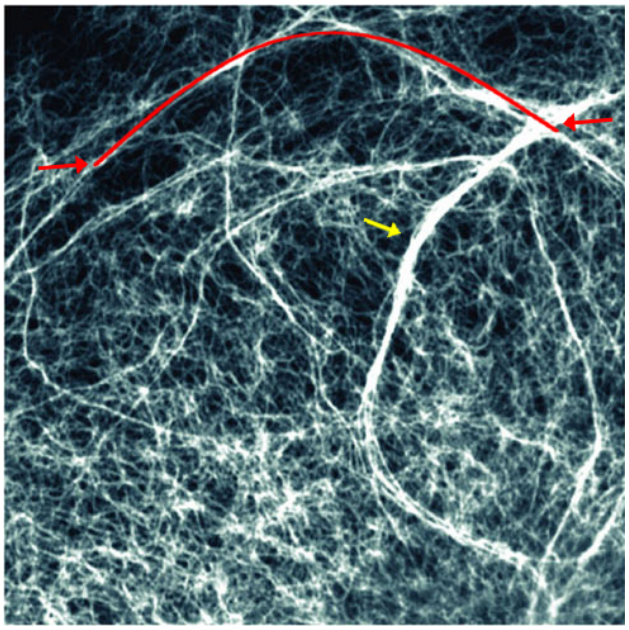

FIG. 1: Vimentin filaments in a rat-kangaroo PtK2 cell. This figure illustrates how the intermediate-filament network truly pervades all areas of the cytoplasm and can orchestrate many cellular events. The image was captured using a Zeiss LSM 510 confocal laser scanning microscope. (Figure courtesy of Lynne Chang et al., from *Intermediate Filaments Mediate Cytoskeletal Crosstalk* [6]).

The effect of the surrounding network is to confine the test filament to a tube-like region in space. This effective elastic medium around the filament has been modeled with a sine curve (shown in red in figure 1). Red arrows indicate the macroscopic strain γ applied to the medium, which is accounted for by a shift in the tube center line, $\bar{y} \propto \gamma L$. The yellow arrow shows another bundle of filaments that can easily be thought of as this effective medium modeled in a sinusoid form. The filament length L plays the role of a non-affinity length scale, which can also be understood as a lower cut-off at which the affine assumption breaks down. This breakdown is due to inextensibility and geometric correlation that develop along essentially straight filaments. The deformations on the smaller scale of the crosslinks then follow from the local network structure. Adopting this point of view, we assume that macroscopic and homogeneous network deformations locally lead to an inhomogeneous distortion of the effective medium. Non-affine deformations of the tube increase with network strain γ and are slaved to the local network structure. In other words, the filament (with its bending stiffness κ_b) resists the tube deformation and leads to a frustration effect between filament bending and crosslink deformation.

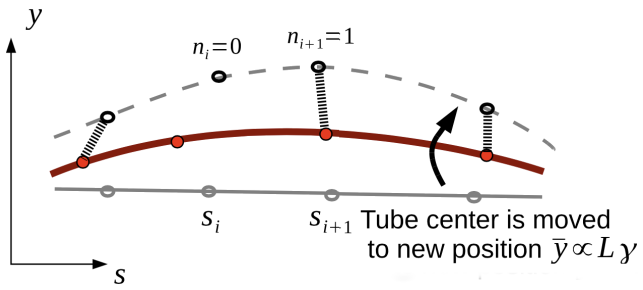

FIG. 2: Schematic representation of the test filament. In a densely crosslinked network, the tube potential mainly acts at the crosslinking sites $i = 1, ..., N_\times$, which are located at discrete points s_i along the filament axis and which may be bound ($n_i = 1$) or unbound ($n_i = 0$). The scheme shows the test filament's (red) modifications produced by the surrounding network (tube center represented by the dashed line) from its initial straight state (continuous grey line). (Figure courtesy of C. Heussinger et al., from *Stress relaxation through crosslink unbinding in cytoskeletal networks* [2]).

The model thus describes an interplay between crosslink deformation and filament bending. In this way, the actual network is substituted by an effective potential that acts on the test filament. A convenient expression for this potential is:

$$V = \frac{1}{2}\int_0^L k(s)\,(y(s) - \bar{y}(s))^2\,ds. \qquad (1)$$

In this expression and the followings, s is the arclength of the filament, so $s = [0, L]$. $k(s)$ is the strength of the confinement and will be described forwards, and the tube center $\bar{y}(s)$ may or may not be different from the reference configuration of the filament. The tube potential is

a useful representation of many-body network effects on the test filament. However, the parameters entering this potential, here $k(s)$ and $\bar{y}(s)$, and their relation to network deformation and mechanical properties are usually unknown.

The filament, described in terms of the worm-like chain model, has a bending energy that can be written as

$$H_b = \frac{\kappa_b}{2} \int_0^L \left(\frac{\partial^2 y}{\partial s^2} \right)^2 ds, \tag{2}$$

where κ_b is the filament bending stiffness and $y(s)$ is the transverse deflection of the filament form its reference configuration, that we have supposed at $y_0(s) = 0$ (straight). The network we have assumed to be represented by an effective medium that couples to the test filament only at the crosslinking points, allows us to define the $k(s)$ as

$$k(s) = k_\times \sum_{i=1}^{N_\times} n_i \delta(s - s_i), \tag{3}$$

where $k_\times$ is the crosslink stiffness and $N_\times$ is the total number of crosslinking sites. As we are interested in the effects of reversible crosslink binding, we include an occupation variable $n_i = 0, 1$. If the crosslink is bound at site i, we'll have $n_i = 1$; if it is unbound, then $n_i = 0$.

In the continuum limit of equations (1)-(3) and assuming $n_i \to \sum_i n_i / N_\times \equiv n$, the energy of the test filament can be written as

$$H = \frac{\kappa_b}{2} \int y''(s)^2 ds + \frac{k_\times n}{2a} \int \left(y(s) - \bar{y}(s) \right)^2 ds. \tag{4}$$

where $a = L/N_\times$ is the discretization length, which will serve as the unit of length in this work, i.e. $a = 1$. What we have introduced to simulate this first approach to molecular motion is the possibility of crosslink unbinding ($n_i = 1 \to 0$), so that the frustration effect between filament bending and crosslink deformation is avoided. The price to pay is the binding enthalpy $H_{bind} = -|\mu| \sum_i n_i$, which is assumed to favor the bound crosslink state with $n_i = 1$.

III. SIMULATIONS, RESULTS AND DISCUSSION

A. Monte Carlo simulations

For the Monte Carlo simulations we represent the filament by a one-dimensional lattice, as shown in figure 2. For each lattice site ($i = 1, ..., N_\times$) we define the pair (y_i, n_i), where y_i is the local transverse displacement and n_i is the crosslink occupation variable. As the Monte Carlo moves, we use single-site displacements and crosslink binding/unbinding moves, the latter attempted with 10% probability. In each displacement move, a site i is selected randomly and the current displacement y_i is replaced by $y_i' = y_i + \delta$, with $-0.1a < \delta < 0.1a$ drawn uniformly randomly. The new transverse displacement y_i' is accepted with probability $P_{disp} = \min[1, e^{-\beta \Delta H}]$, where ΔH is the energy difference between the initial and the final states. During a crosslink move, a bond is selected randomly, and the corresponding occupation variable n_i is flipped ($n_i \to 1 - n_i$). The new state is accepted with probability $P_{xlink} = \min[1, e^{-\beta \Delta H + \beta \mu \Delta N}]$, with μ the crosslink chemical potential and $\Delta N = \pm 1$ the change in the number of crosslinks.

In the following, we show data where the filament bending stiffness $\kappa_b = 1$ serves as the unit of energy. The chemical potential is $|\mu| = 0.001$ and the inverse temperature is $\beta = 1000$, such that the persistence length[$*$] is $l_p = 1000$, measured in units of lattice sites a[$**$] . The filament length is taken to be $L = 20a$, i.e. $N_\times = 20$. We will assume the filament to have zero deflection at its ends, $y_0 = y_{N_\times} = 0$ and so the ending sites always bounded, $n_0 = n_{N_\times} = 1$. The tube center is taken as $\bar{y}(s) = \gamma L \sin(qs)$ with $q = \pi/L$, which corresponds to the longest possible wavelength compatible with the chosen boundary conditions.

B. Results and Discussion

In figures 3 and 4 we monitor the average crosslink occupation $n = \sum_i \langle n_i \rangle / N_\times$ as well as an average energy E, which is obtained by minimizing the total elastic energy for given crosslink occupation. We vary the network strain γ as well as the crosslink stiffness $k_\times$. As expected in any metropolis Monte Carlo method, the initial conditions (n_i and y_i) of each site of the lattice are not relevant and the equilibrium final state is always reached. It can easily be seen that crosslink stiffness has very important effect on the thermodynamic state of the system.

What we see is that for mechanically weak crosslinks, network strain γ has not a very strong influence on

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[$*$] Physically, the persistence length $l_p = \kappa / k_B T$ defines the distance over which correlations in the direction of the tangent are lost. Segments of polymer that are shorter than l_p can be described as elastic rods (stiff), while those that are much longer than l_p can be characterized with a random walk (flexible). In particular, since l_p can also be thought of as the distance over which thermal energy randomizes filament orientation, entropy will play an essential role in characterizing the physics of semiflexible biopolymer networks.

[$**$] The persistence length l_p is usually much larger than the relevant filament length L.

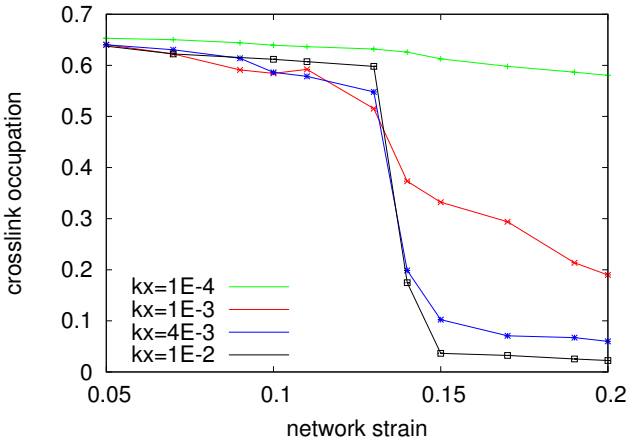

FIG. 3: The crosslink occupation n as a function of the network strain γ for different crosslink stiffnesses $k_\times$.

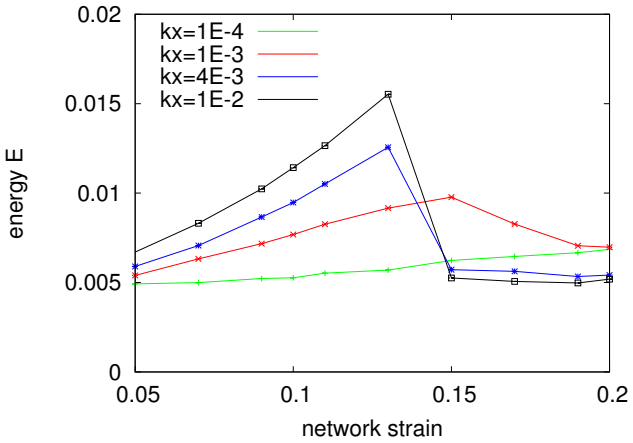

FIG. 4: The elastic energy E as a function of the network strain γ for different crosslink stiffnesses $k_\times$.

the binding state. Only a few crosslinks unbind upon increasing network deformation. In this regime, the crosslinks are not strong enough to enforce filament bending. The elastic energy is small and primarily stored in the crosslinks. As a consequence, the energy of the bound state raises and the statistical weight is shifted towards the unbound state. This is the regime discussed in studies where crosslink unbinding is not considered: the crosslinks are modeled as nonlinear elastic elements, which leads to a significant stiffening of the network under strain. We have checked that imposing nonlinear crosslink compliance in our model reproduces this behavior (see figure 5). Otherwise, with the possibility of crosslink unbinding, this stiffening due to crosslink mechanics generally competes with a softening effect. When the crosslinks are strong enough, their deformation energy starts competing with the filament bending energy. Now crosslinks can force filaments into deformation and the elastic energy is mainly stored in the filaments. At large network strains this energy is too high and unbind states become favorable. This can be seen in an evident way in the discontinuous unbinding transition, where nearly all crosslinks unbind simultaneously. Associated with this transition we can see a free-energy barrier.

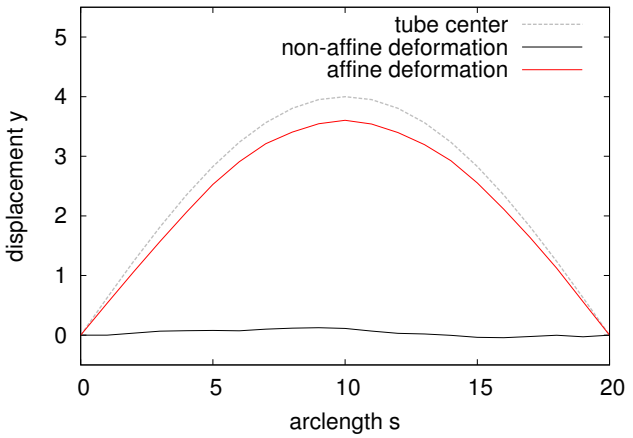

FIG. 5: Conditions: $k_\times = 10^{-2}$ and $\gamma = 0.20$. Red line indicates filament's behavior supposing affine deformations, where unbinding is not permited. Black line represents the filament supposing non-affine deformations (our model). Grey dashed line represents the tube center, $\bar{y}$. Notice that non-affine filament's behavior corresponds with the unbind state reached after the transition in figure 3.

IV. CONCLUSIONS

This work's aim goes beyond previous models in considering the filament and the crosslink stiffness both as factors for the rheological properties of crosslinked filament networks. As compared to earlier studies, the unrealistic assumption of affine deformations is abandoned in favor of a model that incorporates the filament length as the fundamental non-affinity scale. As mentioned above, the nature of this model is purely thermodynamic, so it could have been studied and analyzed analytically through the canonical ensemble, as the system is supposed to be in equilibrium with a thermal bath (β is taken constant). We have applied a mean field theory to approximate the effect of the surrounding network on the filament. These theories can be viewed as the "zeroth-order" expansion of the Hamiltonian fluctuations, i.e. physically, we assume that the system has no fluctuations (which coincides with the idea of replacing all interactions with a "mean field"). It might be worth mentioning that, while mean field theories arose primarily in the field of statistical mechanics, they have recently been applied in inference, graphical models theory, neuroscience, and artificial intelligence [8].

The discontinuous transition in the crosslink occupa-

tion variable $n(\gamma)$ that we see in figure 3 is firstly understood as a first-order phase transition, and experimental results in some *in-vitro* systems show consistent results (as mentioned in C. Heussinger's work). However, a more exhaustive analysis taking into account the size of the system and its fluctuations would have lead us to a different conclusion: the discontinuity on the variable n turns out to be a second-order transition. This evaluation may be the first thing to consider doing in a possible extension of the present work. Also focusing on the study of statistics and phase transitions, critical exponents could be calculated so as to reinforce the obtained results. Another possible way to proceed in order to extent our study could go on the direction of analyzing how modifications on the filament affect and change the surrounding network. Aiming to go a little bit further, a next-step model could consider how binding/unbinding are reflected as a change in the tube potential. The conditions and considerations here assumed are just a simple and primary approach to the reality observed in true cytoskeletal filaments, where little modifications lead to an obvious impact in the surrounding neighbors and network. Anyway, we are yet far to the fully understanding of these systems' behavior.

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[1] Norman Y. Yao. *Nonlinear Mechanics of Biopolymer Networks* (thesis). Harvard University (2009).
[2] C. Heussinger. *New Journal of Physics* **14**, 095029 (2012).
[3] O. Lieleg, M. M. A. E. Claessens, Y. Luan and A. R. Bausch. *Physical Review Letters* **101**, 108101 (2008).
[4] Chase P. Broedersz, Martin Depken, Norman Y. Yao, Martin R. Pollak, David A. Weitz and Frederick C. MacKintosh. *Physical Review Letters* **105**, 238101 (2010).
[5] D. Cole, D. Ringelberg, and C. Reynolds. *Journal of Geotechnical and Geoenvironmental Engineering* **138**, 9 1063-1074 (2012).
[6] L. Chang and R. D. Goldman. *Nature Reviews. Molecular Cell Biology* **5**, 601-613 (2004).
[7] L. M. Sander, *Equilibrium Statistical Physics: With Computer simulations in Python*, (Createspace, Michigan 2013). Chapter 4, 108-115
[8] *Mean field theory.* (2015). Consulted on May 2015, from `https://en.wikipedia.org/wiki/Mean_field_theory`.