Non-equilibrium fluctuations of a semi-flexible filament driven by active cross-linkers

I. Weber1,2, C. Appert-Rolland2, G. Schehr3 and L. Santen1

1 Department of Physics, Saarland University - D-66123 Saarbrücken, Germany
2 Laboratoire de Physique Théorique, CNRS (UMR 8627), Univ. Paris-Sud, Univ. Paris-Saclay F-91405 Orsay, France
3 Laboratoire de Physique Théorique et Modèles Statistiques, CNRS (UMR 8626), Univ. Paris-Sud, Univ. Paris-Saclay - F-91405 Orsay, France

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Abstract – The cytoskeleton is an inhomogeneous network of semi-flexible filaments, which are involved in a wide variety of active biological processes. Although the cytoskeletal filaments can be very stiff and embedded in a dense and cross-linked network, it has been shown that, in cells, they typically exhibit significant bending on all length scales. In this work we propose a model of a semi-flexible filament deformed by different types of cross-linkers for which one can compute and investigate the bending spectrum. Our model allows to couple the evolution of the deformation of the semi-flexible polymer with the stochastic dynamics of linkers which exert transversal forces onto the filament. We observe a $q^{-2}$ dependence of the bending spectrum for some biologically relevant parameters and in a certain range of wave numbers $q$, as observed in some experiments. However, generically, the spatially localized forcing and the non-thermal dynamics both introduce deviations from the thermal-like $q^{-2}$ spectrum.

Introduction. – In recent years many studies were performed on active gels to investigate their complex and dynamic structure, which shows generic non-equilibrium behavior. The cytoskeleton, an important example of an active gel, is able to form self-organized structures that are the basis of many fundamental processes within cells [1–3]. The cytoskeleton is composed of actin and intermediate filaments, as well as microtubules, that take important roles for example in cell motility, cell division and intracellular transport [1,4]. It has been shown that these cytoskeletal filaments can cross-link via static [5] and dynamic interactions [6,7].

Mechanically the cytoskeletal filaments are semi-flexible filaments with very different persistence lengths. In vitro measurements estimated a thermal persistence length of the order of 17 $\mu$m for actin and a few millimeters for microtubules [8–10]. By contrast, much smaller persistence lengths are observed for microtubules in vivo (≈30 $\mu$m in [11]). These strong deformations are interpreted to be the result of non-thermal forces of the order of 1–10 pN, which is in the range of individual motors’ strength. While in some experiments it was surprisingly observed that the bending spectrum exhibits the same shape $q^{-2}$ as thermal ones [11], other observations [12] reported strong deviations from this thermal behavior.

Some continuous theoretical descriptions of active networks exist [13–15], which allow to study the deformation of an embedded filament [16]. However, it is of great interest to understand how deformations originate from microscopic discrete forcing [17–22].

In this paper we consider an idealized system (fig. 1) in which a set of cross-linkers impose transverse deformations to a semi-flexible filament (SFF). To couple the dynamics of SFF and linkers, we determine at each instant the equilibrium shape of the SFF under the constraints imposed by the cross-linkers. The method also provides the forces exerted by the deformed filament on each cross-linker, allowing to implement some feedback of the SFF onto the stochastic linkers dynamics.

We shall consider two types of linkers, having thermal or non-thermal dynamics. This will allow us to disentangle the geometrical effects from those due to the non-thermal dynamics of active linkers. We apply our algorithm to explore the dependence of the persistence length $L_p$ and of
the bending spectrum upon various parameters, including the properties of linkers and of the surrounding network.

Model. –

Semi-flexible filament. The bending energy $E$ of a SFF of length $L$ with bending rigidity $k$ is given by [23]

$$E = k \int_0^L \left( \frac{\partial \theta}{\partial s} \right)^2 \, ds \quad (1)$$

and depends on the local curvature $\partial \theta / \partial s$, where $\theta(s)$ is the tangent angle and $s$ the contour length along the filament. We shall express the value of $k$ in units of $k_{\text{MT}}$, the experimentally measured bending rigidity of microtubules (see table 1). In the following, we assume periodic boundary conditions and connect the SFF’s ends to form a ring, in order to focus on the bulk effects of motor-induced transversal filament fluctuations.

Cross-linkers. We consider two types of cross-linkers which are connected to some static background filaments and induce SFF shape fluctuations. In our model geometry, the background filaments are all perpendicular to the SFF, with a lattice spacing $d_{\text{mesh}}$ (see figs. 1 and 2). Both, the geometry as well as the assumption of a static network differ from the real structure of the cytoskeleton.

Thermal cross-linkers are permanently bound to the SFF (see fig. 2). They step in both directions along the background filament in the direction determined above. The stepping rate $p(F_{\text{SFF}})$ depends strongly on the load force $F_{\text{SFF}}$ exerted by the SFF on the linker once its chain is maximally extended. If the force $F_{\text{SFF}}$ is in opposition to the linker movement, the stepping rate is reduced in comparison to the load free stepping rate $p_0$

$$p(F_{\text{SFF}}) = \max \left[ 0, p_0 \left( 1 - \frac{F_{\text{SFF}}}{F_s} \right) \right]. \quad (2)$$

The linker stops when the load force exceeds the linker’s stall force $F_s$. On the contrary, the stepping rate increases between the SFF and one background filament) with constant rate $\omega_a$. Their binding is not direct but via a small infinitely flexible chain with maximum length $l_{\text{max}}$, allowing for an attachment of the cross-linker at discrete sites on the background filament.

The linker can exert a force on the SFF only when its chain is extended. For each attachment event, the stepping direction (+) or (−) of the cross-linker is randomly chosen and kept fixed until it detaches again.

Once attached, the active cross-linker stochastically takes discrete steps along the background filament in the direction determined above. The stepping rate $p(F_{\text{SFF}})$ depends strongly on the load force $F_{\text{SFF}}$ exerted by the SFF on the linker once its chain is maximally extended. If the force $F_{\text{SFF}}$ is in opposition to the linker movement, the stepping rate is reduced in comparison to the load free stepping rate $p_0$

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Fig. 1: (Colour online) Sketch of the model in the case of active cross-linkers. Cross-linkers (black dots) are connected to the SFF (green) through rope-like chains, which can be fully extended (straight segment) or not (wavy line). With rate $\omega_a$, cross-linkers can attach at empty intersections (○) between the SFF and the background filaments. The SFF exerts some forces $F_{\text{SFF}}$ (red arrows) on fully extended cross-linkers, which are located at positions $(x_i, h_i)$ and impose the vertical positions $z_i(x_i)$ of the SFF. Cross-linkers move upwards or downwards ((+) or (−) labels) in discrete steps along the polarized vertical background filaments, with stepping rate $p \equiv p(F_{\text{SFF}})$. Cross-linkers detach with rate $\omega_d \equiv \omega_d(F_{\text{SFF}})$.

Fig. 2: (Colour online) Sketch of the model in the case of thermal cross-linkers. Cross-linkers (black dots) are permanently bound to the SFF (green) and step with probability $\min(1, \exp(-\Delta E/k_B T))$.

Table 1: System parameters and single active cross-linker characteristics.

| Semi-flexible filament parameters | Value |
|-----------------------------------|-------|
| Bending rigidity in $k_{\text{MT}}$ units | $k$ | $k_{\text{MT}}$ |
| Background mesh size | $d_{\text{mesh}}$ | $1 \mu m$ |
| Filament contour length | $L$ | $100 \mu m$ |

| Cross-linkers parameters | Value |
|--------------------------|-------|
| Binding rate | $\omega_a$ | $0.05 \text{s}^{-1}$ |
| Unbinding rate | $\omega_d$ | $0.01 \text{s}^{-1}$ |
| Hopping rate | $p_0$ | $1 \text{s}^{-1}$ |
| Detachment force | $F_d$ | $3 \text{pN}$ |
| Stall force | $F_s$ | $6 \text{pN}$ |
| Step size | $\delta$ | $10 \text{nm}$ |
| Length of coupling chain | $l_{\text{max}}$ | $10$ steps |
for a load force in the same direction as the stepping
\[ p(F_{\text{SFF}}) = \min \left[ 2p_0, p_0 \left( 1 + \frac{|F_{\text{SFF}}|}{F_s} \right) \right]. \] (3)

The active linkers stochastically detach with rate \( \omega_d(F_{\text{SFF}}) = \omega_d \exp\left(\frac{|F_{\text{SFF}}|}{F_d}\right) \), where \( F_d \) gives the detachment force scale.

Bound cross-linkers with an extended chain can exert a force and possibly deform the SFF, which in turn will apply a restoring force on the linkers. For some deformations of the SFF the restoring force may induce sudden detachments of cross-linkers or even initiate detachment cascades.

We assume a time scale separation of the linker dynamics and SFF relaxation, which allows the simulation of single linker activity and consecutive instantaneous SFF relaxation. This could be realized in an *in vitro* assay.

*Equilibrium shape of a constrained SFF.* The semi-flexible filament’s shape is chosen to minimize the bending energy under the constraints imposed by the pulling cross-linkers. This implies relaxation of the filament between two steps, which can be realized at low viscosities, low ATP concentrations and for short filaments [8,24]. Between two consecutive pulling cross-linkers located in \( x_i \) and \( x_{i+1} \) the SFF shape is given by a profile \( u_i(x) \), that minimizes the energy (1) of this portion of the SFF
\[ E_i = k \int_{x_i}^{x_{i+1}} \left[ \frac{\partial^2 u_i(x)}{dx^2} \right]^2 dx \] (4)
assuming no overhang and \( |\partial_x u_i(x)| \ll 1 \).

The force per unit length is \( F \sim \partial_x^2 u_i(x) \), which vanishes at equilibrium between two attachment points. Thus the equilibrium is given by (see for instance ref. [25])
\[ u_i(x) = a_i(x - x_i)^3 + b_i(x - x_i)^2 + c_i(x - x_i) + d_i \] (5)
for \( x_i \leq x \leq x_{i+1} \). Let \( z_i \) be the vertical displacement of the SFF imposed at position \( x_i \) and \( v_i \) the local slope. The global SFF shape is given by the sequence of single segments respecting the boundary constrains to ensure the differentiability of the global polynomial:
\[ u_i(x_i) = z_i, \quad u_i(x_{i+1}) = z_{i+1}, \] (6)
\[ \partial_x u_i(x_i) = v_i, \quad \partial_x u_i(x_{i+1}) = v_{i+1}. \] (7)

Now if we consider that several of these segments are put end-to-end, we have to minimize the global energy. This minimization will determine the slopes at attachment points —and thus the whole shape.

We now present a detailed calculation of the polynomial’s coefficients. Using (7) the polynomial’s coefficients are given by
\[ a_i = -2 \frac{\Delta z_i}{\Delta x_i^3} + \frac{1}{\Delta x_i^2} (2v_i + \Delta v_i), \]
\[ b_i = 3 \frac{\Delta z_i}{\Delta x_i^2} - \frac{1}{\Delta x_i} (3v_i + \Delta v_i), \]
\[ c_i = v_i, \]
\[ d_i = z_i. \]

Here \( \Delta x_i = x_{i+1} - x_i \) is the distance between two consecutive pulling cross-linkers, \( \Delta z_i = z_{i+1} - z_i \) their vertical displacement difference and \( \Delta v_i = v_{i+1} - v_i \) the difference of slopes. The energy of the SFF is given by
\[ E = z' \tilde{B} z + v' \Lambda + v' \tilde{A} v, \] (8)
where the matrices \( \tilde{A} \) and \( \tilde{B} \) are given by
\[ \tilde{A}_{ij} = 4k \left( \frac{1}{\Delta x_i} + \frac{1}{\Delta x_{i-1}} \right), \quad \text{if} \ i = j, \]
\[ = \frac{2k}{x_{\text{max}(i,j)} - x_{\text{min}(i,j)}}, \quad \text{if} \ i = j \pm 1, \]
\[ = 0, \quad \text{else} \] (9)
and
\[ \tilde{B}_{ij} = 12k \left( \frac{1}{\Delta x_i} + \frac{1}{\Delta x_{i-1}} \right), \quad \text{if} \ i = j, \]
\[ = -\frac{12k}{(x_{\text{max}(i,j)} - x_{\text{min}(i,j)})^3}, \quad \text{if} \ i = j \pm 1, \]
\[ = 0, \quad \text{else}. \] (10)

while the components of the vector \( \Lambda \) in eq. (8) are given by
\[ \Lambda_i = 12 k \left[ \frac{\Delta z_i}{(\Delta x_i)^2} + \frac{\Delta z_{i-1}}{(\Delta x_{i-1})^2} \right]. \] (11)

The gradient \( v \) is chosen in such a way that it minimizes the total energy in (8), which allows us to write
\[ \Lambda_i = 2 \sum_j \tilde{A}_{ij} v_j. \] (12)

The coupling matrices for slopes \( \tilde{A} \) and for the local displacements \( \tilde{B} \) are both cyclic-tridiagonal. With the gradient \( v_i = \frac{1}{2} \sum_k \tilde{A}_{ik} \Lambda_k \) a simple form for the global SFF energy is
\[ E = z' \tilde{B} z - \frac{1}{4} \Lambda' \tilde{A}^{-1} \Lambda. \] (13)

Our approach also enables us to calculate the local forces exerted on the pulling cross-linkers which determine their dynamics:
\[ F_k = \frac{\partial E}{\partial z_k} = 24k \left( \frac{\Delta z_{k-1}}{\Delta x_{k-1}^3} - \frac{\Delta z_k}{\Delta x_k^3} \right) \]
\[ -12k \left\{ v_k \left( \frac{1}{\Delta x_k^2} - \frac{1}{\Delta x_{k-1}^2} \right) \right\} \]
\[ -12k \left\{ v_{k-1} \left( \frac{1}{\Delta x_{k-1}^2} - \frac{1}{\Delta x_{k-2}^2} \right) \right\}. \] (14)

The calculation above requires that the vertical displacements \( z_i \) at positions \( x_i \) are known. When a cross-linker is pulling, its chain is elongated. Thus, if \( h_i \) is the vertical position of the cross-linker, we have \( z_i = h_i \pm l_{\text{max}}, \) the sign depending on the direction of the pulling force. However, the semi-flexibility causes non-trivial response
in terms of SFF geometry and forces. As the action of a single linker may change the SFF’s global shape, we need to check after each relaxation of the SFF shape whether there is a change in the number of pulling cross-linkers, and in that case reevaluate the SFF shape that minimizes the bending energy. An iterative procedure alternatively adjusting the coupling chains and relaxing the SFF shape allows to converge towards the full equilibrium of the system.

For concreteness we now briefly summarize the update procedure, where we assume to start in a state for which we know the force applied on each bound cross-linker. This implies that we know all the transition rates at this initial time. Then an update of the filament shape consists of the following steps:

- We update the system of linkers with a tower sampling algorithm and perform stochastic events until the occurrence of an event that modifies the force exerted on the SFF.

- Then the new equilibrium shape of the SFF is calculated as explained in the previous section. Please note, that the contour length \( L \) is kept constant throughout the simulation\(^1\).

- The new forces exerted on the linkers are obtained and the new value of force-dependent rates is calculated for each linker.

This procedure is repeated to update the system. (For an illustration of the filament dynamics at different bending rigidities see the supplementary material \textit{Supplementarymaterial.pdf} as well as movies \textit{L50\_k001.mov, L50\_k1.mov} and \textit{L50\_k100.mov}.)

\textbf{Results. –}

\textit{Avalanches.} A characteristics of SFFs is that small changes in the applied forces may lead to large deformations of the SFF. For our model this means that a single motor step may change considerably the shape of the SFF, and therefore the restoring forces, which may become so high that a subset of motors will instantaneously detach. Such a detachment avalanche is done iteratively beginning with the linker that bears the largest restoring force. The SFF shape and the forces are re-estimated after each detachment event.

Now that our model is defined, we shall present some numerical results on the SFF’s shape characteristics under coupled SFF-linkers dynamics.

\(^1\)Since the optimal shape of the filament does not conserve the microtubule’s contour length it has to be readjusted after every update that changes the filament shape. For that purpose the system is equally rescaled in horizontal and vertical direction. In order to keep the average mesh size constant, the number of available background filaments for a given contour is evaluated as a function of the new projection length. The added (removed) filaments are randomly chosen from the reservoir of passive (active) filaments.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3}
\caption{(Colour online) Variance of the amplitudes \( a(q) \) for a SFF pulled by thermal linkers (symbols), for various bending rigidities (or, equivalently, various persistence lengths). The straight lines give the purely thermal bending spectra for the same persistence lengths (see eq. (15)).}
\end{figure}
one indeed observes the expected $q^{-2}$ spectrum. As long as the persistence length is at least of the order of the system size, we are in the regime of small deviations and one obtains the expected value of the persistence length.

In the context of biological applications it is interesting to notice that the mesh size of the background lattice and the typical length of the SFF determine the range of the $q^{-2}$ spectrum.

Active cross-linkers. As a second step of our analysis, we now consider a model with active cross-linkers. For active cross-linkers one observes generically strong deviations of the bending spectrum from $q^{-2}$ even in the regime $1/L < q < 1/d_{\text{mesh}}$. The actual form of the spectrum depends strongly on the model parameters. In fig. 4 we show bending spectra for SFF with $k = 1$, i.e., the bending rigidity of microtubules. For this value of $k$ and realistic biological parameters for the linker forces and mesh size, we find a thermal like $q^{-2}$ spectrum for small wave vectors while deviations arise for larger wave numbers. The extension of the $q^{-2}$ regime is even larger if $F_D$ and $F_s$ are scaled up by a factor of $n = 10$, i.e., if linkers are made stronger. Using the $q^{-2}$ part of the bending spectrum we obtain an apparent persistence length of 26 μm, surprisingly close to the experimentally obtained value of 30 μm for in vivo microtubule fluctuations [11]. Considering the much more complex cellular environment and the fact that the actual density of cross-linkers may differ from our choice of the mesh size, this coincidence should not be overrated. However, the order of magnitude we obtained for the filament’s persistence length shows that the transverse deformations imposed by active cross-linkers may have a strong influence on the filament’s shape. As mentioned above, generically we observe rather strong deviations from the $q^{-2}$ regime. Therefore, from now on, the persistence is estimated via the tangent angle correlation (16) to ensure reliable persistence length estimation for all bending rigidities. In the case of purely thermal fluctuations, the persistence length is proportional to the bending rigidity. Figure 5 reveals that the active linker-driven SFF’s persistent length evolves in a more complex way. For small $k$ we observe only a weak dependency of the apparent SFF stiffness on the bending rigidity, as the deformations are limited by the mesh size. An increase of the bending rigidity to $k \geq 1$ leads to a super-linear increase of the persistence length up to, and beyond the SFF length.

Finally we also studied the effect of varying the mesh size of the underlying network, for various bending rigidities of the SFF and a fixed number of active linkers. As seen in fig. 5, for low bending rigidities, the persistence length slightly decreases with $d_{\text{mesh}}$ (This dependence is linear in $d_{\text{mesh}}$, as can be seen in fig. 6). Though we use here the model beyond the limit of small deformations, we
expect this conclusion to hold. Indeed, closer linkers can enforce deformations at smaller scales.

Surprisingly this behavior is inverted for large bending rigidities, where one observes larger persistence lengths for higher densities of active linkers. Indeed, when $d_{\text{mesh}}$ decreases, the curvature induced by a single linker step is more pronounced. At high bending rigidities, this strong local deformation will result into strong load forces, which most likely the linker will not be able to sustain. Therefore, it is difficult to deform the stiff SFF at all, if the density of cross-linkers is too high.

**Discussion.** – In this paper we have proposed a modeling approach to describe the dynamics of a semi-flexible filament subject to fluctuations generated by a finite set of cross-linkers. For any configuration of the linkers, we are able to compute the equilibrium shape of the semi-flexible filament, using a semi-analytical method, and also to calculate the feedback on the linkers dynamics due to the SFF rigidity. This allows us to study quantitatively the effect of cross-linker induced deformations on the shape of the SFF for various types of linker dynamics. We are in particular able to describe avalanches events.

In this work we considered fluctuations generated by thermal and active linkers, where the dynamics of the latter is based on the dynamics of typical kinesin motors at low ATP concentration [26]. In both cases linkers step perpendicular to the SFF. For thermal linkers we observe a $q^{-2}$ regime in the bending spectrum, whose range is limited by the length of the SFF for small $q$ and by the distance between two linkers for large $q$.

In the case of active cross-linkers, one observes typically strong deviations from the $q^{-2}$ bending spectrum, as a signature of non-thermal fluctuations, in agreement with experimental observations [12]. For biologically relevant parameters, however, a $q^{-2}$ spectrum has been observed in a certain interval of wave numbers, as in some other experiments [11]. Interestingly, using this part of the bending spectrum we obtain an estimate of the persistence length which is very close to the experimental value found in [11]. This agreement is remarkable, in view of the fact that we only assumed fluctuations perpendicular to the filament and motor-based microscopic dynamics.

Studying the variations of the persistence length with various parameters, we found in particular that at high bending rigidities, the persistence length increases with the density of cross-linkers.

Regarding the global vertical dynamics of the motor-driven SFF, our simulation results show the absence of bidirectional persistent displacement, which could be expected from a tug-of-war scenario [27]. These results are in agreement with explicit transport models [28,29].

Our approach can be generalized to other types of non-thermal forcing and boundary conditions and thus could be used in order to describe a large range of experimental settings.

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