Collective effects in force generation by multiple cytoskeletal filaments pushing an obstacle

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Abstract. We report here recent findings that multiple cytoskeletal filaments (assumed rigid) pushing an obstacle typically generate more force than just the sum of the forces due to individual ones. This interesting phenomenon, due to the hydrolysis process being out of equilibrium, escaped attention in previous experimental and theoretical literature. We first demonstrate this numerically within a constant force ensemble, for a well known model of cytoskeletal filament dynamics with random mechanism of hydrolysis. Two methods of detecting the departure from additivity of the collective stall force, namely from the force-velocity curve in the growing phase, and from the average collapse time versus force curve in the bounded phase, is discussed. Since experiments have already been done for a similar system of multiple microtubules in a harmonic optical trap, we study the problem theoretically under harmonic force. We show that within the varying harmonic force ensemble too, the mean collective stall force of N filaments is greater than N times the mean stall force due to a single filament; the actual extent of departure is a function of the monomer concentration.

1. Introduction

Cytoskeletal filaments are dynamic, long protein-polymers, which form the structural components of cells. Two major types of cytoskeletal filaments are microtubules and actin filaments. Actin filaments are made of G-actin monomers and microtubules are made of tubulin monomers. These monomers are typically bound to ATP/GTP molecules when they polymerize, and they hydrolyze to ADP/GDP molecules with passing time [1]. In addition to maintaining structural integrity, they also aid in several functions, such as cell motility, movement of chromosomes during cell division, and transportation within cells [2, 3]. These functional properties are aided by their ability to generate forces against barriers they encounter, by regulating their structure based on microscopic polymerization, depolymerization and hydrolysis processes. A single filament or a bundle of filaments can grow on an average, against the barrier, if the collective force generated by the system is greater than the force exerted by the barrier. If the compressive force increases, the average growth velocity of the system decreases until it vanishes at the 'stall force' of the system. This is the maximum force that can be generated by the bundle of filaments, at which the bundle, on an average, stalls [4, 5, 6]. The highly

dynamic nature of cytoskeletal filaments are observed at high external forces or low free monomer concentrations, in a process termed as dynamic instability, which is characterised by large length fluctuations of the filaments. During this, the filament can shrink to very short length by undergoing 'catastrophes' and then grow back in events called 'rescues' [7, 8]. Understanding these dynamic features of cytoskeletal filaments and their force generation capabilities are important to understand their contribution in cellular functions.

In the absence of irreversible hydrolysis of ATP/GTP-bound monomer to ADP/GDP-bound monomer in the filament, thermodynamic arguments can deduce the stall force of the system as

$$f_s = \frac{k_B T}{d} log(\frac{u_0}{w_0}) \tag{1}$$

where w_0 is the depolymerization rate and u_0 is the concentration dependent polymerization rate [9, 10, 4, 5]. For a N-filament system, these theories obtain a stall force that is proportional to N, and the constant of proportionality is equal to the stall force of a single filament:

$$f_s^{(N)} = N f_s^{(1)}. \tag{2}$$

Introduction of hydrolysis into the system introduces two different depolymerization rates for the filament, depending on the tip of the filament being in either ATP/GTP-bound or ADP/GDP-bound state. Detailed studies, taking into account the monomer level polymerization/depolymerization and hydrolysis, but on single filaments, have been carried out theoretically in the past [6, 11, 12, 13, 14]. These models incorporate the mechanism of hydrolysis as either sequential or random. In the former, an ATP/GTP subunit can hydrolyse only at its interface with an ADP/GDP subunit, thereby maintaining a 'cap' of ATP/GTP-bound monomers at the end of the filament. In random hydrolysis model, hydrolysis can happen at any random position along the polymer. Recently, we have shown that [15, 8], in the presence of hydrolysis, the collective stall force of a bundle of N filaments, $f_s^{(N)} \neq N f_s^{(1)}$, and for typical experimental parameters, $f_s^{(N)} > N f_s^{(1)}$.

Experiments have studied the dynamics of single filament in presence of a barrier (exerting force) [16], and multiple filaments in harmonic traps [17, 18]. Laan *et al.* [18] in particular, studied multiple microtubules in the presence of a harmonic force (an optical trap), as opposed to a constant applied force, as considered in the papers discussed above. They observed a linear variation of the collective stall force of the filament bundle with the number of filaments.

In this paper, we discuss the cooperativity of a bundle of N filaments growing against a constant compressive force similar to what is discussed in [15]. This result is further corroborated by studying catastrophe timescales of the filaments, under high forces [8]. We then proceed to present some new results for filaments in the presence of a harmonic force. Even in the latter case, we find that, additivity does not hold for average forces generated by the bundle.

2. The model
The model we study consist of N parallel filaments, growing against a barrier, which exerts a compressive force against their growth. First we would consider a constant force as in Figure 1 [15], and then a harmonic force as in Figure 4. A filament here is supposed to represent an entire microtubule with all 13 protofilaments – thus with the addition of each monomer, the system effectively moves a distance equal to 0.6 nm (=8 nm/13), where 8 nm is the actual subunit length of a tubulin monomer [6, 14]. The filaments are independent, except that they interact with each other through their contact with the barrier. Filaments are allowed to grow only from one end, and the other end acts as a reflecting boundary. Filaments, in contact with the barrier, grow with a polymerization rate $u = k_0 C exp(-\frac{fd}{k_B T})$, in accordance with Kramer's theory[15, 19], where k_0 is the intrinsic polymerization rate and C is the free

Table 1: Rates corresponding to microtubules.

Parameter	k_0 $(\mu M^{-1} s^{-1})$	w_T (s^{-1})	w_D (s^{-1})	r (s^{-1})
Value	3.2	24	290	0.2

GTP-bound monomer concentration in the solution, k_B is the Boltzmann constant and T is the absolute temperature. Filaments away from the barrier polymerizes with a force independent rate $u_0 = k_0 C$. The GTP-bound monomer can undergo random hydrolysis, i.e., a GTP-monomer at any random position along the filament can undergo hydrolysis with rate 'r'. This 'chemical switching' of monomers from the GTP-bound to GDP-bound state is irreversible in nature, forcing the dynamics to be out of equilibrium. The tip of the filament can now be in either GTP or GDP-bound state, accordingly there are different depolymerization rates, w_T and w_D, respectively, for GTP-monomer and GDP-monomer. The GDP-monomer is found to have a much higher depolymerization rate than their GTP counterpart [1], .i.e., $w_D \gg w_T$. This suggests that the filament is more prone to depolymerization when the tip is in the GDP-bound state rather than the GTP-bound state. Hence, a filament with a cap structure (successive T-monomers at the tip) is more stable against depolymerization than those without a cap. The depolymerization rates w_T and w_D are considered to be force independent.
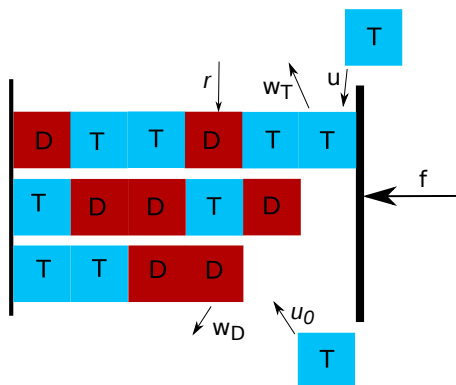

Figure 1: Schematic diagram of a multifilament system of parallel filaments growing against a constant force. Filaments, in contact with the barrier, grow with a polymerization rate $u = k_0 C exp(-\frac{fd}{k_B T})$, where as those away from the barrier polymerize with a rate $u_0 = k_0 C$, where k_0 is the intrinsic polymerization rate and C is the free GTP-bound monomer concentration in the solution. Depolymerization rates are w_T and w_D, depending on whether the tip of the filament is in GTP-bound and GDP-bound states, respectively. At any position along the filament, a GTP-bound monomer can hydrolyse into a GDP bound monomer with rate r.

3. Results under constant force

We present here, the results on the collective behaviour of a system of N filaments growing against a constant force. The graphs presented are for microtubules, although similar results can be obtained for a system of actin filaments as well [15]. The parameters used for simulations are listed in Table 1.

3.1. Stall force measurement using growth velocity

Using kinetic Monte-Carlo, we have simulated the growth trajectories of a 2-filament system, in order to study their growth dynamics. The system of filaments grow on an average, as long

as the force exerted is less than the stall force that can be generated by them. As the applied force increases, the average growth velocity of the filament decreases, until it vanishes at the stall force of the system. Figure 2 shows various traces of filament length (equivalently, position of the wall from the reflecting boundary) as a function of time, for a system of two microtubule filaments. The top curves, corresponding to various realisations, represent the situation where we apply a force equal to $2f_s^{(1)} = 14.48pN$. It can be seen that the two filament system does not stall at this force, but grows with a positive average velocity. The force at which the filament growth could be stalled occurs at a slightly higher force ($= 15.45$ pN), for which the resulting growth trajectories are shown in the bottom curves. Hence, the stall force, when the average filament velocity vanishes, is greater than $2f_s^{(1)}$. Similar results can be obtained for higher number N of filaments (data available in [15]), and it is observed that the excess stall force $\Delta^{(N)} = f_s^{(N)} - Nf_s^{(1)}$, increases almost linearly with the number N.
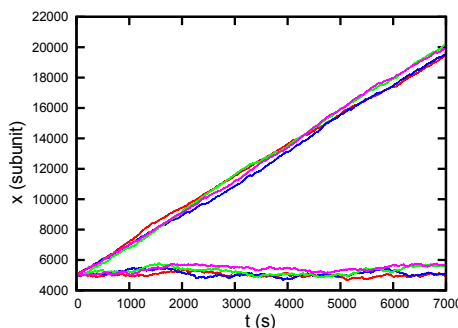

Figure 2: Different traces of the length of a two filament system, growing against a constant compressive force (f), is plotted as a function of time. The top and bottom curves correspond to $f = 2f_s^{(1)}$ and $f = f_s^{(2)}$, respectively. Concentration of GTP-bound monomers in the solution is 25μM.

3.2. Stall force measurement using collapse times of catastrophes

If the system of filaments is subjected to a force larger than its stall force, the steady state system velocity would be negative, but due to the reflecting boundary wall, the system stays bounded in length [6]. In the bounded state, the filaments undergo repeated length fluctuations known as catastrophes and rescues. Stall force of a multi-filament system can be measured by studying these catastrophes and rescues, at high forces [8]. As the applied compressive force decreases, filaments undergo catastrophes with decreasing frequencies. This can be quantified using average collapse time (T_{coll}) as discussed in [8], which is defined as the average time taken for the bundle of filaments to shrink from a maximum length to zero length during a cycle of rescue and catastrophe. As the catastrophes occur less frequently, the collapse time increases with decreasing force. With force approaching the stall force from above, the collapse time diverges. The collective behaviour of multiple filaments can be inferred by studying the collapse time of the system as one approaches the stall force. Figure 3 shows the average collapse time of microtubules, for two, three and four filaments, as a function of the ratio of applied force to the stall force of a single filament. The two curves in each panel correspond to the case with no hydrolysis (red) and with random hydrolysis (blue). Red curves diverge as the force ratio becomes equal to the number of filaments, indicating that the stall force of the N-filament system is equal to $Nf_s^{(1)}$. But, it is evident from the plot that the blue curves show divergence before the applied force is reduced to $Nf_s^{(1)}$. This point towards a cooperative behaviour of the N-filament system, where the stall force of N-filaments $f_s^{(N)}$ is greater than $Nf_s^{(1)}$, corroborating the analysis via force-velocity relation, as discussed in the previous section.
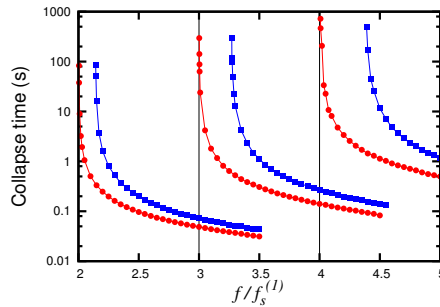
Figure 3: Average collapse time as a function of scaled force $f/f_s^{(1)}$ for a system of two, three and four microtubules, at a free monomer concentration of 25μM. The curves with symbols ● and ■ correspond to the cases without hydrolysis and with hydrolysis, respectively.

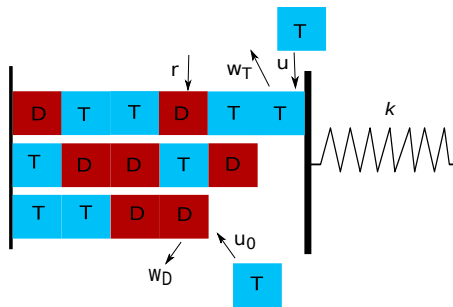## 4. Results under a harmonic force

Figure 4: Schematic diagram of a multifilament system of parallel filaments growing against a harmonic force. The kinetic rates here are similar to the ones mentioned in Figure 1.

In vitro experiments [18], conducted on multiple parallel microtubules, growing against a harmonic force, have indicated an additive nature of stall force. These results are contrary to the theme we have built up so far, for constant force. So, this requires careful theoretical study. In the experiment, multiple filaments were allowed to grow from seeds, and one end the filament bundle was attached to a bead in an optical trap. A rigid barrier was held at the growing end of the bundle of filaments. Thus, as the filaments grow against the barrier, the position of the beam in the trap changes, which results in a restoring force, which is a linear function of the filament length. It was observed that the most probable forces produced by the multi-filament system occur at integral multiples of some unit.

Comparison of a theoretical model with the above in vitro study is needed. In this section, we theoretically model the growth of a system of N-filaments against a harmonic force, similar to that in [18]. Here, multiple filaments grow against a barrier which is attached to a spring with a stiffness constant k, as shown in Figure 4. The other end of the filament acts as a reflecting boundary as before. As the filament grows, it pushes against the spring, which in turn applies a restoring force on the filament, which is proportional to the length of the filament in contact with the spring. The important difference with the previous section is that, force now varies, and we would have a distribution of forces.

The forces generated by the system are obtained from filament lengths (x), as $f = -k(x-x_0)$, where x_0 is the reference length. The distribution of forces, related to the distribution of lengths x, is plotted in the insets of Figure 5 (a) and 5 (b), for two different free monomer concentrations C. It is evident from the main figures (see caption of Figure 5 (a) and 5 (b)) that the system
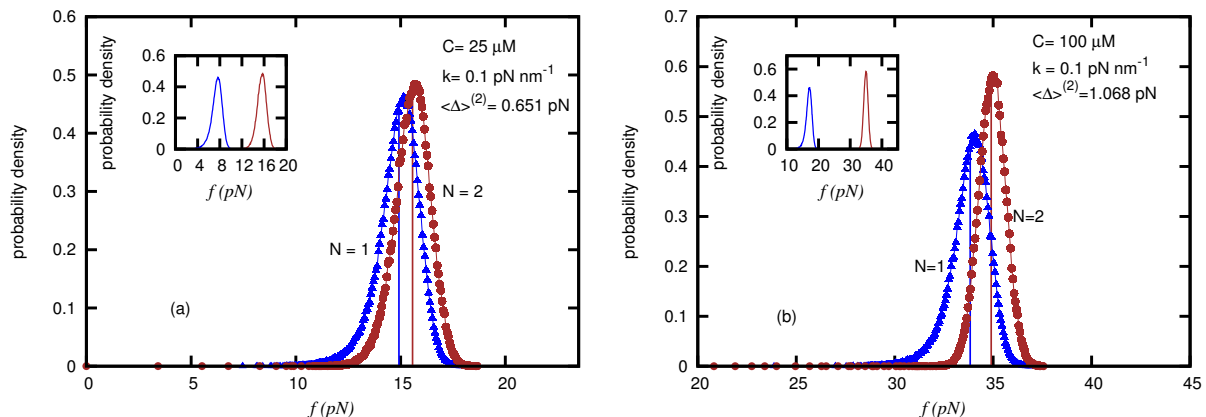
Figure 5: The two insets show the distributions of forces (f) generated by a single microtubule (at lower forces) and two microtubules (at higher forces), at two values of $C = 25\mu$M (a), and 100μM (b). The stiffness constant of the trap is k=0.1 pN nm^{-1}. In the main figures of (a) and (b), the distribution for the single-filament system is plotted against $f + \langle f_s^{(1)}\rangle$ (curves with ▲), for comparison with the distribution of the 2-filament system plotted against f (curves with ●). The point is that they do not coincide, implying that the mean stall forces shown by the vertical lines follow the relation $2\langle f_s^{(1)}\rangle \neq \langle f_s^{(2)}\rangle$.

of two microtubules generate a mean force $\langle f_s^{(2)}\rangle$, greater than twice the individual mean stall force $\langle f_s^{(1)}\rangle$:

$$\langle f_s^{(2)}\rangle > 2\langle f_s^{(1)}\rangle. \tag{3}$$

For $C = 25\mu M$, average excess force, $\langle\Delta\rangle^{(2)} = \langle f_s^{(2)}\rangle - 2\langle f_s^{(1)}\rangle$=0.651 pN, and for $C = 100\mu M$, $\langle\Delta\rangle^{(2)}$=1.068 pN. The deviations are small, but if looked for carefully, forces of ~ 1 pN may be detected in experiments.

5. Conclusion
Cytoskeletal filaments show highly dynamic behaviour, constantly regulating their structures, and in the presence of an obstacle, can generate resistive forces [3, 16]. Force generation capabilities of cytoskeletal filaments have been studied by various theoretical models. But the cooperative behaviour of a bundle of filaments are not well understood, since previous studies were done on either single filament or on multiple filaments in the absence of hydrolysis. The role of irreversible hydrolysis of ATP/GTP-monomer to ADP/GDP monomer has been carefully probed in [15]. It is observed that by introducing hydrolysis, the resulting non-equilibrium system has an enhanced collective stall force, as compared to the sum of individual stall forces, i.e. in a N-filament system, $f_s^{(N)} > Nf_s^{(1)}$. The robustness of this result has been checked using a variety of models (with both sequential and random hydrolysis), over broad experimentally relevant parameter regimes, and for an analytically solvable toy model. The relation of this result with the violation of detailed balance is discussed in [15]. This work was further extended in [8], where the fluctuation properties of multiple filaments in the bound phase were studied. The study demonstrates a cooperation among the filaments against catastrophes, quantified by an average collapse time, "T_{coll}"– it is found to increase with the number of filaments, rendering more stability to multi-filament systems as compared to single filaments, against an applied force. Variation of T_{coll} as a function of scaled force $f/f_s^{(1)}$ shows that the forces at which T_{coll} diverges, which are the stall forces of the corresponding system, are not integral multiples of the single filament stall force.

Experimentally, Laan *et al.*[18] inferred that a force dependent collective instability exists in microtubules, by considering the growth of multiple microtubules in a harmonic force. They observe that the most probable forces that the system of microtubules produce are integral multiples of some unit. In order to probe whether the additivity of stall forces shown in [18] is a particularity of harmonic force, we have extended the multifilament model of [15, 8], by replacing the constant force ensemble by a harmonic force ensemble. In this paper, a comparison of force distributions of single filaments with those of multiple filaments within our model show that the mean force exerted by multiple filaments is greater than the sum of mean force of individual filaments. The deviation is small, and likely to be ignored within experimental errors, but strictly speaking, is non-zero. Hence, even in the presence of harmonic force, as opposed to constant force, we see that the cooperativity observed in [15] and [8] still hold. A further detailed study of multiple filaments in the presence of harmonic force, by considering various reaction rates and other parameters is necessary, to understand how the interesting deviation from additivity may be enhanced and brought within detectable limits of experiments.

Acknowledgments

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