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Force-Velocity Relation for Growing Microtubules

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ABSTRACT Forces generated by microtubule polymerization and depolymerization are important for the biological functioning of cells. The mean growth velocity, V , under an opposing force, F , has been measured by Dogterom and Yurke (1997; *Science* 278:856–860) for single microtubules growing in vitro, but their analysis of the data suggested that V decreased more rapidly with F than equilibrium (or “thermodynamic”) theory predicted and entailed negative values for the dissociation rate and undefined (or unreasonable) values for the stall force, at which V vanishes. By contrast, considering the mean work done against the external load and allowing for load-distribution factors for the “on” and “off” rates, we find good agreement with a simple theory that yields a plausible stalling force. Although specific numerical results are sensitive to choice of fitting criteria, about 80% of the variation with load is carried by the “off” (or dissociation) rate, but, since that is small (in accordance with independent observations), the dominant force dependence comes from the “on” rate, which is associated with a displacement length, d_1 , significantly longer than $d_0 \approx 1/13(8.2 \text{ nm})$, the mean length increase per added tubulin dimer. Measuring the dispersion in length of the growing microtubules could provide a check. The theory implies that the stationary stall state (at $V = 0$) is not one of simple associative thermal equilibrium, as previously supposed; rather, it appears to be dissipative and kinetically controlled.

INTRODUCTION

Microtubules (MTs) are rigid, hollow cylindrical structures, each consisting of 10 to 15 parallel protofilaments arranged circumferentially. Each protofilament is a linear polymer of α - and β -tubulin heterodimers, each dimer being of length $d \approx 8.2 \text{ nm}$, when assembled in the MT. Typically, $n = 13$ protofilaments are associated laterally with an adjacent, longitudinal offset $a = hd/n = 1.5d/13 \approx 0.95 \text{ nm}$ to make a “B-lattice” sheet that is rolled up and joined helically with a single seam (and helix factor $h = 3/2$), to form a complete MT (Desai and Mitchison, 1997). The process of assembly of MTs from tubulin is accompanied by guanine triphosphate (GTP) hydrolysis and can generate forces which are important for mitosis and cellular motility. Ingenious experiments by Dogterom and Yurke (1997) have recently provided measurements of the velocity of single MTs growing in vitro as a function of the force F (evaluated parallel to the MT axis), that was generated when the MTs encountered a rigid microfabricated barrier. From analysis of the subsequent buckled shapes, and using independent measurements of the flexural rigidity, yielding $\kappa = 34 \pm 7 \text{ pN}/\mu\text{m}^2$, the load, F , and the length of the MTs could be determined as a function of time.

Dogterom and Yurke (hereafter DY) analyzed their results using a simple, thermodynamically based theoretical model (following Hill, 1987) and concluded that the observed mean velocity of growth, V , decayed with F significantly faster than predicted. Although DY claimed to fit

their data satisfactorily, in all cases their optimal parameters entailed negative kinetic rates for the “off” or dissociation process, in which a tubulin dimer leaves a protofilament. As a consequence, their fits predicted no value for the stalling force, F_S , at which the mean velocity goes to zero and growth stops. (More correctly, if one allows for the uncertainty range in the fitted off-rates, a minimum stall force, $F_S \geq 21.3 \text{ pN}$ can be predicted, but this seems excessively large.) However, naive examination of their data, as reproduced in Fig. 1, would suggest $F_S = 4.2\text{--}4.8 \text{ pN}$ and, in fact, DY observed MTs stalled with stationary ends for periods $>20\text{--}30 \text{ s}$ at forces $F \approx 4.1 \text{ pN}$.

We show here that a more systematic analysis of the original, simple DY approach leads to a fully satisfactory description of the data with rate constants in accord with independent evidence (Desai and Mitchison, 1997) and a modest stall force in the anticipated range. The theory amounts simply to the limiting $N = 1$ case of our general treatment of processive molecular motors such as kinesin (Fisher and Kolomeisky, 1999a,b). The essential issue turns out to be a consideration of the load-distribution factors for the “on” and “off” rates and of the associated displacement of the “tip” of the MT. The data provide fairly firm guidance and the results are not inconsistent with a general picture in which an open sheet of n parallel protofilaments (Desai and Mitchison, 1997) pushes against a barrier with the load, F , carried, stochastically, by the longest protofilament (Mogilner and Oster, 1997) (hereafter MO). Our treatment also predicts the dispersion of the mean contour length, say $\langle l(t) \rangle$, of the growing MT at time t : this provides a potential experimental cross-check. However, the value we find for the stall force F_S (in manifest agreement with the experimental evidence) does not accord with the thermodynamic arguments of DY and Hill (1987), discussed further by van

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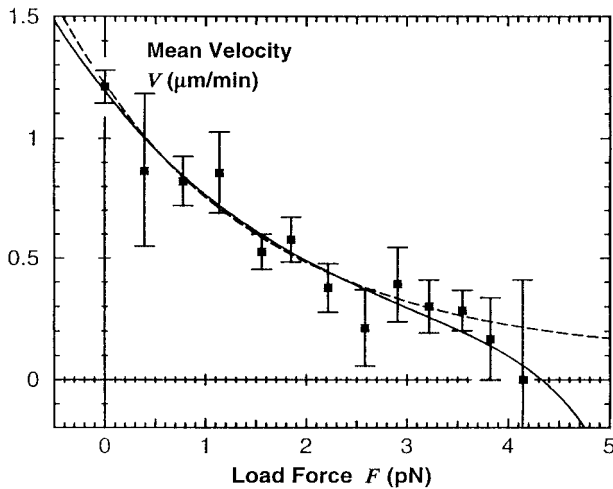


FIGURE 1 Data of Dogterom and Yurke (DY, 1997) relating mean growth velocity, V , to the load force, F , for MTs encountering a barrier. The *solid line* represents the fit developed here on the basis of Eq. 1 with the rates and load-distribution factors given in Eqs. 6. The *dashed curve* represents a fit by DY that entails a negative off rate (and an unbounded stall force).

Doorn et al. (2000). This leads us to conclude that the stationary stalled state (with $V = 0$) is not necessarily one of simple, MT-tubulin associative equilibrium. Instead, the stall state may well be dissipative, possibly entailing GTP hydrolysis, etc., and controlled overall by more detailed kinetics.

It should be noted that the failure of the DY theory to account for their experimental findings has previously led MO to generalize an earlier Brownian ratchet model. The resulting, fairly elaborate but mechanically more detailed theory (postulating n associated but statistically independent protofilaments), required numerical integration. Using an approximation to handle the disassembly process in their model, MO were led to the general stall force prediction $F_S \propto \sqrt{n}$ and were also able to obtain tolerable agreement with the DY data. (The stall force predicted by the fit in Fig. 4 of MO is, unfortunately, not clear, and their representation of the DY data lacks accuracy in the spacings along the force axis.) Subsequently, however, van Doorn et al. (2000) (hereafter vD) showed that the MO treatment of the off-process was inadequate. By simulation of a discrete version of the MO model with a special symmetry, vD concluded that F_S should, instead, be proportional to n . This was supported by theoretical arguments which, although adequate for their special case, do not suffice for more realistic generalizations that we discuss in the Appendix. Van Doorn et al. also obtained fits to the DY data that we consider unsatisfactory in that they entail excessive rates for the disassembly process and large stall forces of magnitude 9–19 pN.

An underlying issue here is the general question of how elaborate a model is needed to understand or explain a given

set of data (see also Hille (1992), pp. 31, 52, 66, 387, and 502 et seq.). It seems appropriate, in the first instance, to explore carefully the simplest models that are consistent with other experimental knowledge and evidence and that respect basic physico-chemical principles. If a good description is provided by a simple, albeit phenomenological, approach, more elaborate pictures or models can be convincingly validated only by more extensive or searching experiments.

ANALYSIS

In our notation (Fisher and Kolomeisky, 1999a,b; hereafter FK) the initial DY analysis can be regarded as resting on the expression

$$V(F) = d_0[u_0 e^{-\theta^+ F d_1 / k_B T} - w_0 e^{\theta^- F d_1 / k_B T}], \quad (1)$$

for the mean velocity V as a function of the load F . Here $d_0 = d/n$ represents the mean increase in MT contour length, $l(t)$, resulting from the addition of one $\alpha\beta$ -dimer to the periodic helical structure of a fully assembled MT (Desai and Mitchison, 1997). DY took $n = 13$, which we will adopt for all numerical work, in their notation $d_0 = \delta$. (However, for reasons that are unclear to us, vD appear to set $d_0 = d$ when relating V to the on- and off-rates in their Introduction and Eq. 4.)

The on-rate for the addition of a GTP-tubulin dimer is $u_0 = k_0[\alpha\beta]$. It is generally accepted that k_0 lies in the range from 2 to 10 $\mu\text{M}^{-1}\text{s}^{-1}$ (see Desai and Mitchison, 1997, p. 89). For the DY experiments with 25 μM tubulin, this indicates the range

$$u_0 = 1500 - 7500 \text{ min}^{-1}. \quad (2)$$

The off-rate for the disassembly of a protofilament under zero load ($F = 0$) by the loss of one GTP-tubulin dimer is small, but must be positive. It seems appropriate to accept

$$0 < w_0 \lesssim 6 \text{ min}^{-1}, \quad (3)$$

(Drechsel et al., 1992), although there has been some controversy (Desai and Mitchison, 1997, p. 89).

The parameters θ^+ and $\theta^- = 1 - \theta^+$ (FK) represent load distribution factors determining the degree to which the load affects the forward or on-rate vs the backwards or off-rate. (In DY's notation, $\theta^+ = q$.) The recognition and determination of the load-distribution factors is a crucial feature of this level of modeling. (For load distribution factors in the context of ion channels see chapter 14 of Hille (1992), but note that the implications of $\theta^+ \neq \theta^-$ are not considered.) In general, θ^+ and θ^- need not be positive (FK), but in the present situation it is reasonable to suppose that neither one can be negative.

Finally, $F d_1$ represents the most probable work needed to add a single tubulin dimer against the load F : the value of d_1 is an important parameter requiring careful consideration.

On the basis of near-equilibrium thermodynamic considerations (Hill, 1987) DY assumed, with little discussion, that $d_1 = d/n$ (although they briefly mentioned the possibility of larger d_1 ; see also vD). We believe this assumption is not unconditionally warranted and will analyze alternatives.

Taking $d_1 = d/13 \approx 0.63$ nm and supposing $\theta^+ = 1$ (so that $\theta^- = 0$), DY fitted their data by minimizing $\chi^2(\sigma)$ (Press et al., 1986) where $\sigma = \{\sigma_i\}$ denotes the experimentally generated standard errors (displayed in their Fig. 4 and reproduced here in Fig. 1). Their fit yielded the reduced value $\chi^2(\sigma) \approx 0.43$ (normalized by $\nu = N_{\text{data}} - N_{\text{param}} = 13 - 3 = 10$) and generated

$$u_0, w_0 = 1791, -127 \text{ min}^{-1} \quad \text{for} \quad \lambda \equiv d_1/d = \frac{1}{13}. \quad (4)$$

The value for the on-rate, u_0 , accords with Eq. 2, but the negative value of w_0 is clearly unphysical. (Although the positive values of w_0 allowed by the uncertainties $\Delta w_0 = \pm 190 \text{ min}^{-1}$ encompass the inequalities in Eq. 3, the fitted magnitude is also excessive.) Finally, DY found the fitted V - F decay rate to be

$$C \equiv \kappa d_1/k_B T = 18 \pm 4 \mu\text{m}^2, \quad (5)$$

or, equivalently (see Fig. 1), $k_B T/d_1 \approx 1.9$ pN. By contrast, accepting $\lambda = 1/13$ (i.e., $d_1 = d/13$), the unambiguous prediction is $k_B T/d_1 \approx 6.8$ pN or (using the measured value of κ) $C = 5 \pm 1 \mu\text{m}^2$. The discrepancy of a factor of 3 or more was the basis for DY's conclusion that theory failed to predict the relatively rapid decay of V as F increased.

DY also tried other values of θ^+ and θ^- , but to no avail. In particular, a fit with $\theta^+ = \theta^- = 0.5$ again yielded $\chi^2(\sigma) \approx 0.43$ but generated the still larger value $C = 34 \pm 4 \mu\text{m}^2$. This fit gave the off-rate $w_0 = -11 \pm 16 \text{ min}^{-1}$ which, though of more acceptable magnitude, is still of unphysical sign.

Why might the assumption $d_1 = d/n$ be inadequate? Consider a growing MT that faces a rigid barrier perpendicular to the axis of growth. Because of the lateral offset of $a = 0.95$ nm between filaments in the B-lattice structure of the growing protofilament sheet (see Introduction; Desai and Mitchison, 1997), one should expect that, in general, only a single filament will bear the load F . (This is also the general picture underlying the work of MO and vD.) Now suppose that the tip of a particular filament, j , is at a distance z_j from the barrier that is less than $d \approx 8.2$ nm, the MT length needed to accommodate a single tubulin dimer. (If $z_j = 0$, the filament j is bearing the load.) Then the most probable work needed to assemble a dimer onto the end of this filament, which will thereafter bear the full load, is $F(d - z_j)$. (Of course, if $z_j > d$, there should be no cost in work to add a dimer to the filament.) Conversely, we expect that the rate of depolymerization of the load-bearing protofilament (through the loss of one tubulin dimer) should be enhanced by a Boltzmann factor involving an energy of magnitude $\leq Fd$. These considerations suggest that d_1

should be related to the average $\langle d - z_j \rangle$ (for $z_j \leq d$) and is, therefore, likely to significantly exceed d/n ; indeed, $d_1 \approx d$ seems not implausible.

Accordingly, we will treat $\lambda = d_1/d$ as a parameter, bounded above by unity, and ask what information the experiments provide both as to λ and the load-distribution factors $\theta^+ = 1 - \theta^-$. For fixed λ , there are still just $N_{\text{param}} = 3$ fitting parameters: θ^+ and the two rates, u_0 and w_0 . To anticipate our analysis, we find that the parameters

$$u_0, w_0 = 1887, 0.33 \text{ min}^{-1}, \quad \theta^+ \approx 0.22, \quad \text{and} \quad \lambda = 1, \quad (6)$$

(i.e., $d_1 = d \approx 8.2$ nm) provide a very satisfactory optimal fit to the DY data; see Fig. 1.

Furthermore, via the relation (see Eq. 1 and FK)

$$F_S = (k_B T/d_1) \ln(u_0/w_0), \quad (7)$$

this fit predicts the stall force $F_S \approx 4.3$ pN, which, as mentioned, is in clear accord with the experimentally observed facts. In light of this result, we see few grounds for placing credence in the large values, $F_S = 9 - 19$ pN, found by vD within their model, where the equilibrium force yields the stall condition $V = 0$. It should also be remarked that Eq. 7 predicts no direct dependence on n in contrast to the DY assumption $d_1 = d/n$. However, taking cognizance of lateral interactions between assembling protofilaments, the subsequent hydrolysis of GTP, and the closure of the protofilament sheet to form a complete MT, could lead to a dependence of d_1 on n . If MTs of varying n could be produced in a controlled fashion, this issue could, in principle, be addressed experimentally. We return to the question in the discussion below.

Now it transpires that recognizing λ as a trial parameter does not immediately resolve the issue of fitting the data numerically if one follows the procedures of DY. Thus on minimizing $\chi^2(\sigma)$, weighted as explained, one still finds that the fitted value of w_0 is always negative. However, when λ is increased from $1/13 \approx 0.077$ to the limit $\lambda = 1$, $\chi^2(\sigma)$ drops to a minimum of 0.424 (from 0.429); the resulting fit yields

$$u_0, w_0 = 1911, -0.165 \text{ min}^{-1}, \quad \theta^+ = 0.24, \quad \text{for} \quad \lambda = 1. \quad (8)$$

Apart from the negative value of w_0 , which, however, is now of small magnitude compared to the inequalities in Eq. 3, the accord with our fit, Eqs. 6, is quite good. Conversely, when λ is decreased w_0 increases rapidly, as does θ^+ , whereas $\chi^2(\sigma)$ increases slowly.

But although minimizing $\chi^2(\sigma)$ with weights experimentally determined via the σ_i is a popular and convenient procedure, it is not enshrined in gold! To consider alternatives (see the informative discussion in sec. 14.6 of Press et al., 1986), note first that for the experiments in question, $V(0)$, the mean velocity under zero load, was determined (as $V(0) \approx 1.22 \mu\text{m}/\text{min}$) in distinct fashion by extensive direct

observations that did not require analysis of buckled shapes. We conclude that, in any fitting procedure, this datum may warrant separate consideration and higher weighting.

Second, consider the last data point in Fig. 1 with $V_{13} \approx 0$ but a large standard deviation, σ_{13} . Experimentally, this represents, as already stressed, the observation of MTs that are in or close to a stalling state. The large deviation results in part from the natural stochastic fluctuations. These are, in fact, predicted to increase when F approaches F_S as indicated by the variation of the dispersion, $D(F)$, of the MT length that is plotted in Fig. 2 using the expression

$$D(F) = \frac{1}{2}(d^2/n)[u_0 e^{-\theta^+ F d_1/k_B T} + w_0 e^{\theta^- F d_1/k_B T}], \quad (9)$$

(see FK) and the parameter values in Eqs. 6. Thus, the observed σ_{13} does not merely represent error. Furthermore, our primary concern is with the mean value. Consequently, weighting this datum as $1/\sigma_{13}^2$ (which amounts to less than 7% of the mean weight $1/\sigma_i^2$) represents de facto neglect of a particularly important piece of evidence.

In light of these considerations, we examined the equi-weighted sum of squares, say $\chi^2(\sigma_0)$, in which we take $\sigma_i = \sigma_0$ for all i and normalize σ_0 so that the DY fit (Eq. 4) yields the same value, $\chi^2 \approx 0.429$. The results are shown in Table 1 for $\lambda = d_1/d$ spanning the full range from $\lambda = 1/13$ to 1. Note, first, that $\chi^2(\sigma_0)$ is significantly less than the DY value in all cases. Secondly, although the trends are similar to those seen when $\chi^2(\sigma)$ is used, the off-rate, w_0 , is never negative and the on-rate, u_0 , always falls within the accepted range of Eq. 2. The minimum $\chi^2(\sigma_0)$ again occurs at the limit $\lambda = 1$ and is $\sim 11\%$ smaller than at $\lambda = 1/13$. (Over the same range $\chi^2(\sigma)$ falls only $\sim 1.2\%$.) Furthermore, for $\lambda \gtrsim 0.6$, the off-rate, w_0 , satisfies the condition 3. Finally,

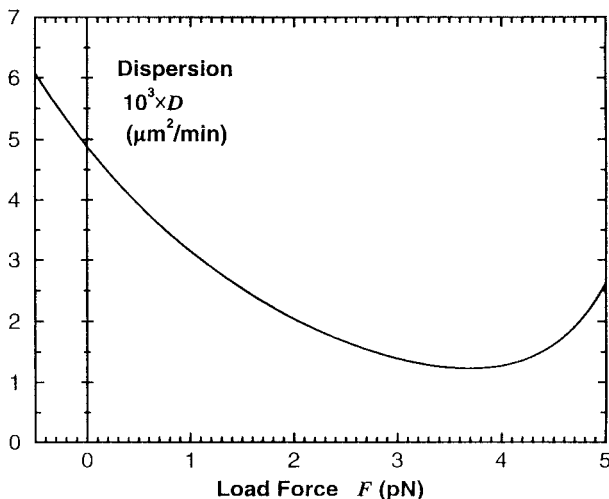


FIGURE 2 Dispersion $D \approx \langle [l(t) - \langle l(t) \rangle]^2 \rangle / 2t$ of the contour length, $l(t)$, of MTs growing with time t under a load force F according to Eq. 9 plotted with the parameters used in Fig. 1; see Eqs. 6.

TABLE 1 Results of the equiweighted least-squares fitting for different values of $\lambda = d_1/d$ with χ^2 normalized to 0.429 for the favored fit of DY (see Eq. 4)

λ	u_0, min^{-1}	w_0, min^{-1}	θ^+	χ^2
1	1830	0.34	0.21	0.322
$3/4$	1828	2.40	0.28	0.332
$1/2$	1838	17.7	0.40	0.346
$1/4$	1978	162	0.69	0.353
$1/13$	3282	1468	1.67	0.356

the load distribution factor θ^+ (≈ 0.21) is close to the value found from $\chi^2(\sigma)$; see Eqs. 8.

We could well adopt the $\lambda = 1$ values from Table 1 as acceptable. However, as discussed above, we believe it is appropriate to enhance the weight of the observations made under zero load. Accordingly, we supplemented the $\chi^2(\sigma_0)$ criterion by using $\sigma_1 = 2\sigma_0$ for the $F = 0$ datum. The corresponding set of fits closely parallels those displayed in Table 1 with little change in χ^2 (as renormalized) but, as anticipated, a better fit to the central value of $V(0)$. The values of u_0 , v_0 , and θ^+ found this way are those recorded in Eqs. 6 and displayed graphically in Fig. 1. The fit is clearly very satisfactory and, in particular, by comparison with DY and MO, the marked downward curvature of $V(F)$ seen in the data for $F \gtrsim 3$ pN is well represented.

DISCUSSION

We have successfully fitted the velocity-force data obtained by DY for MT growth under load, with a simple theory, embodied in Eq. 1, that yields a stall force of $F_S \approx 4.3$ pN in manifest agreement with the observations: see Fig. 1. Furthermore, our fitted on- and off-rates, u_0 and w_0 , are fully consistent with the constraints arising from independent experiments; see Eqs. 6 and 2 and the inequalities in Eq. 3. Crucial features of the theory are consideration of the load-distribution factors, θ^+ and $\theta^- = 1 - \theta^+$, for the on- and off-rates, and allowance for a force displacement length, d_1 , that may be as long as a single tubulin dimer, namely, $d = 8.2$ nm. In fact, the optimal fits (see, e.g., Table 1) yield $\lambda \equiv d_1/d \approx 1$.

For the load-distribution factors, however, the fits indicate $\theta^+ \approx 0.22$; this value may be converted to a mean on-displacement of a protofilament tip of $d^+ \equiv \theta^+ d \approx 1.8$ nm. In heuristic terms, this might measure the typical distance by which one of the $n = 13$ protofilament tips has to withdraw further from the barrier surface (under a thermal fluctuation) in order to make room for a tubulin dimer, in accord with the picture of MO and vD. In light of the longitudinal offset, $a \approx 0.96$ nm, between adjacent protofilament tips at the edge of an otherwise complete B-lattice sheet, one might then guess naively that d^+ would be given roughly by $d - \langle z \rangle = d - 1/2(n - 1)a \approx 2.4$ nm. The

agreement is tolerable, but, at best, these considerations should be regarded as speculative suggestions.

On the other hand, it must be pointed out that our fits violate the conclusions of the thermodynamic considerations of DY, vD, and Hill (1987) for the stall force. If the stationary, stalled state observed when $F = F_S$ and $V = 0$ can be regarded as being in full thermochemical equilibrium (in a process of GTP-tubulin dimer association and dissociation) these arguments yield the previous expression for F_S , namely Eq. 7, but with d_1 replaced by $d_0 = d/n$, the mean increase in MT contour length on addition of a single dimer. Since we find $d_1 \approx d$ there is, formally, a 13-fold discrepancy! Indeed, it was on the basis of this thermodynamic view that DY took $d_1 = d_0$ or, equivalently, $\lambda = d_1/d = 1/13$. That, in turn, as we have seen, was the root cause of the difficulty they encountered fitting their data to theory, leading to unphysical, negative off-rates and undefined or excessively large stall force values. Furthermore, the constraints on the on- and off-rates (u_0 and w_0 in Eqs. 2 and 3) yield a lower boundary on the thermodynamic prediction for F_S of 35.9 pN. This value exceeds by a factor of 8 the observations of MTs stalled for 1 min or so at forces $F \approx 4.4$ pN (see Fig. 1).

It seems necessary to conclude that, unless there are serious deficiencies in some of the experimental observations, the fluctuating stalling state, albeit stochastically stationary and in apparent equilibrium, does not actually represent full thermochemical equilibrium. Rather, the state would seem to be dissipative and under kinetic control. Perhaps GTP hydrolysis is playing some active role, even though this does not seem to be directly in accord with the picture of a catastrophe-avoiding “cap” of GTP-tubulin on the end of the MT (Desai and Mitchison, 1997) as it attempts to grow against the barrier. Possibly the situation is modified when the MT is buckled and strained as in the DY experiments.

Finally, we make some comments on the treatment of van Doorn et al. (2000) who, by simulating a discretized version of the MO model of n independently growing parallel protofilaments associated in a sheet, verified the thermodynamic prediction for F_S . Furthermore, they presented an analytical argument yielding this result for general n , but, as we indicate in the Appendix, their probabilistic reasoning is not generally valid. Indeed, one may reasonably object that their discrete model is atypically simple in that it imposes a special lateral offset, namely, $a = d/n$ (≈ 0.63 nm for $n = 13$) in place of the more appropriate value $a = 1.5 d/n \approx 0.96$ nm. This results in a special symmetry. Nevertheless, we have explicitly verified the thermodynamic result for F_S in the vD model for the case $n = 2$ extended to allow a general offset a ($< d$) and arbitrary load-distribution factors. (In the notation of vD, one has $a \equiv \sigma$, and all protofilament tips must then reside at sites that are an integral multiple of a from the barrier; for $a \neq d/n$ that is no longer the case (see Appendix). However, vD state that they simulated various

extensions of their model, which may have included other values of a , always finding the same result for F_S .) Our analysis yields, in addition, explicit expressions for V as a function of F (which vD do not obtain; see Appendix). Thus we have no reason to doubt the claim of vD regarding the n -dependence of F_S in their model. Indeed, the result is probably valid also when a general lateral offset and load-distribution factors, $\theta^+ + \theta^- = 1$, are allowed for. Note, however, a paradoxical defect of the MO-vD models that appears when $F \rightarrow 0$: specifically, as pointed out in the Appendix, Eq. 1 is not matched in this limit, as the definitions of u_0 and w_0 would seem to demand.

In applying the simulations of their model vD also generated apparently plausible fits to the original DY data. However, these fits entail what, as explained, we regard as unphysically large values of F_S , namely, in the examples vD present, 9.2 and 18.5 pN. Moreover, even these values are obtained at the cost of violating the constraints both for the on-rates (by accepting values that are too small by a factor of 7 or more; see Eq. 2) and for the off-rates (by employing values exceeding the limit imposed by the inequality 3). Indeed, their values of F_S violate the lower bound, 35.9 pN, obtained above for a consistent prediction using the thermodynamic expression. We might also remark that the fits of vD, like those of DY, are concave (i.e., d^2V/dF^2 is positive) for $F \gtrsim 1$ pN and so fail to represent the definite convexity (or downward curvature) suggested by the data for $F \gtrsim 3$ pN (see Fig. 1).

In conclusion, although a simple theory, consistent with distinct information regarding the on- and off-rates, fits the force-velocity data of Dogterom and Yurke for MTs growing against a load, the concept that the stalled condition is one of thermal equilibrium is called into question on various counts.

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APPENDIX

Velocity versus Force for the Generalized $n = 2$ MT Model of van Doorn et al.

Consider the MT growth model of vD with just $n = 2$ protofilaments in the sheet but with a general lateral offset $a < d$ when the tip of protofilament $i = 1$ pushes on the barrier, and a complementary offset, $b = d - a$, when tip-2 bears the load. In addition, we suppose that the load is distributed so that the on-rate for a tubulin dimer to fasten to a tip at a distance z from the barrier is $u_0 \phi^{\theta^+ \max\{d-z, 0\}}$ while the off-rate from a tip which can then move to a distance z from the barrier is $w_0 \phi^{-\theta^- \max\{d-z, 0\}}$, where $\phi^x = \exp(-Fx/k_B T)$ and $\theta^+ + \theta^- = 1$. The first step is to construct the master equations governing the probabilities for the full set of distinct protofilament con-

figurations, namely, those with tip-1 and those with tip-2 on the barrier. By solving the master equations we find that the mean velocity for steady state growth of the longest protofilament of an indefinitely long MT can be expressed as

$$V(F) = d(u_0\phi^{\theta^+d} - w_0Q\phi^{-\theta^-d}) + aS(u_0R\phi^{\theta^+a} - w_0\phi^{-\theta^-a}) \\ + bS(u_0\phi^{\theta^+b} - w_0R\phi^{-\theta^-b}), \quad (\text{A1})$$

where $Q = (u_0\phi^{\theta^+d} + w_0)/(u_0 + w_0\phi^{-\theta^-d})$, $R = (u_0\phi^{\theta^+b} + w_0\phi^{-\theta^-a})/(u_0\phi^{\theta^+a} + w_0\phi^{-\theta^-b})$, and $S = (1 - Q)/(1 + R)$. It is also feasible to compute the asymptotic dispersion in length.

One can now check that each of the three terms in Eq. A1 vanishes identically when $\phi^d = (w_0/u_0)^2$. This serves to confirm the stall-force expression, Eq. 7, with the ‘‘thermodynamic’’ value $d_1 = d/2$. On the other hand, when $F \rightarrow 0$, so that $\phi = Q = R = 1$ and $S = 0$, one finds $V = d(u_0 - w_0)$ instead of the expected $n = 2$ result $V = 1/2d(u_0 - w_0)$: see Eq. 1. This appears to be a general defect of the MO-vD models (for all n) associated, it would seem, with neglect of binding between adjacent protofilaments.

In the special case considered by vD where $a = d/n$ (so that $a = b$ when $n = 2$) the only allowed distances of a protofilament tip from the barrier are $z_j = ja$ where $j = 0, 1, 2, \dots$. Then, at most one tip can occupy each position z_j and, because of the associated tip relabeling symmetry (a figure is helpful to see this) all the occupation probabilities for the positions are independent of which protofilament tip, $i = 1, 2, \dots, n$, is in contact with the barrier. Although not explained by vD, this independence is essential to the probabilistic argument they use to derive the overall off rate. By contrast, when $a \neq b$ in the case $n = 2$ discussed above, there are two distinct sets of positions, $z_j^{(1)} = jd + a$ when tip 1 is on the barrier, and $z_j^{(2)} = jd + b$ when tip 2 is in contact. Furthermore, the total probabilities

for these two sets are, in general, different so the line of reasoning used by vD fails.

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