

# Role of GTP remnants in microtubule dynamics

Sumedha, Michael F Hagan, and Bulbul Chakraborty  
*Martin Fisher School of Physics, Brandeis University, Waltham, MA 02454, USA\**

We study a model of microtubule assembly/disassembly in which GTP bound to tubulins within the microtubule undergoes stochastic hydrolysis. In contrast to models that only consider a cap of GTP-bound tubulin, stochastic hydrolysis allows GTP-bound tubulin remnants to exist within the microtubule. We find that these buried GTP remnants enable an alternative rescue mechanism, and enhances fluctuations of filament lengths. Our results also show that in the presence of remnants microtubule dynamics can be regulated by changing the depolymerisation rate.

PACS numbers: 87.12.Ka,87.17.Aa,02.50.Ey,05.40.-a

Microtubules are semiflexible polymers that serve as structural components inside the eukaryotic cell and are involved in many cellular processes such as mitosis, cytokinesis and vesicular transport [1, 2, 3]. In order to perform these functions, microtubules (MTs) continually rearrange through a process known as dynamic instability (DI), in which they switch from a phase of slow elongation to rapid shortening (catastrophe), and from rapid shortening to growth (rescue)[1]. Despite extensive experimental efforts over several decades, it has not been possible to discriminate between models that have been proposed to explain DI [4, 5, 6, 7, 8]. In this letter, we study a minimal model of DI that involves stochastic hydrolysis (SH), a mechanism that has received relatively little attention compared to interfacial hydrolysis (IH) that forms the basis of cap models[4]. With recent advances in experimental techniques[9, 10], it has become possible to quantify MT dynamics at nano-scale and, thereby, provide more stringent tests of models. We make predictions that clearly distinguish between SH and IH mechanisms, and experimental tests of these predictions should clarify the nature of hydrolysis in MT dynamics.

MTs are formed by assembly of  $\alpha - \beta$  tubulin dimers, which are polar and impart polarity to MTs. MTs grow mainly from the end that has exposed  $\beta$  tubulin, and are composed of (typically) 13 linear protofilaments.[1]

While a free tubulin dimer has a GTP molecule bound to each monomer, incorporation into a MT activates the  $\beta$ -tubulin monomer for hydrolysis of its associated GTP. GDP-bound tubulin is less stable within the MT lattice [11] and hence a GDP-bound tubulin at the tip of a MT has a higher rate of detachment (depolymerization) than a GTP-bound tubulin. While it is established that GTP hydrolysis is essential to DI, the mechanism of the process is not fully understood. Models in the IH class assume that all hydrolysis occurs at a sharp interface between GDP-bound and GTP-bound tubulins [4, 6], whereas in SH-based models, hydrolysis occurs stochastically, anywhere in the MT [7, 8, 12, 13]. Since IH models do not

allow for any GTP-bound tubulin within the GDP domain, rescue depends solely on the probability of a free GTP tubulin attaching to a depolymerising MT with a GDP-tubulin at the tip.

In SH models, GTP-monomers are located throughout the MT, with a concentration that decays exponentially with distance from the growing end[13]. These models allow for a different rescue mechanism, i.e as the MT depolymerises by detachment of GDP-tubulins, GTP tubulins in the interior of the MT, or GTP ‘remnants’ get exposed and the MT starts polymerising again. Support for presence of GTP tubulins inside the MT has been provided by recent experiments [9].

We study a particular SH model[8, 13]. In contrast to a SH model that takes into account all thirteen protofilaments of a MT[7], our model depicts the MT as a  $1-d$  sequence with rates that prescribe polymerization, depolymerization and hydrolysis. The appeal of this model is that there are only a few parameters, and the parameters can be controlled independently to understand the role that different processes play in the DI of MTs.

The model represents the MT by a linear sequence of two species of monomers, which correspond to GTP-bound tubulin (denoted by  $+$  in rest of the paper) and GDP-bound tubulin (denoted by  $-$ ). We assume that the MT undergoes attachment and detachment only at one end, which we call the growing end (sometimes called the  $+$  end in the literature). A MT evolves via the following rules:

1. *Attachment:* If the growing tip is a  $+$  monomer, it grows with rate  $\lambda$  by addition of a  $+$  subunit.
2. *Detachment:* A  $-$  monomer at the growing end detaches with rate  $\mu$ , causing its shrinkage.
3. *Hydrolysis:* With rate 1 any  $+$  monomer in the MT can undergo hydrolysis to yield a  $-$  monomer.
4. *Non-remnant Rescue:*  $+$  subunits could attach to a growing end with a  $-$  monomer at the tip with rate  $p\lambda$  ( $p \leq 1$ ).

A previous study of the model [13] for  $p > 0$ , demonstrated a transition from a phase of bounded to unbounded growth of the MTs. The present study will focus on low  $p$  and fluctuations in the bounded growth region of the phase diagram.

---

\*Electronic address: sumedha,chakraborty,hagan@brandeis.edu

Recent experiments monitored [10] the distribution of lengths of growing and shortening excursions in *in vitro* systems of MTs. These experiments were able to resolve fluctuations at the monomer level, and the distributions were found to be exponential. In simulations of our model we find that for small values of  $p$  the growth excursions are independent of  $\mu$  and can be fitted well by  $\exp(-i/\lambda)/\lambda$  ( $i$  is the length of the excursion). The form of the distribution of shortening excursions changes non-trivially with  $\mu$  (Fig. 1).

We can fit the experimental data on the distribution of excursions [10] with our model by taking  $\lambda = 12; \mu \approx 0.1; 0 \leq p \leq 0.001$  (Fig. 1). The fitting provides tight bounds on the parameters, if we demand good fits to both positive and negative excursions simultaneously. For the low values of  $p$  obtained from the fits, rescue due to remnants dominates DI. Rare non-remnant rescues do influence the steady state but have very little effect on the statistics of fluctuations. Hence, in order to highlight the effect of remnants on DI we analyze in detail the  $p = 0$  model, which we call the GTP remnants model since the only mode of rescue is via remnants. The presence of remnants and the correlation of their location with rescue events is consistent with recent experiments. In particular, Perez et al. [9] observe GTP-bound tubulin within MTs and find that the location of these remnants correlate to locations at which rescue events occurred during MT growth.

In the remnants model, if the number of GTP monomers fluctuates to 0, rescue is no longer possible and the MT eventually shrinks completely. Any process that exposes remnants promotes rescues and makes the MT remain active for longer. In particular, increasing  $\mu$  at fixed  $\lambda$  leads to longer times ( $t_N$ ), and higher maximum lengths ( $L(t_N)$ ) of MTs before they disassemble due to loss of all GTP.

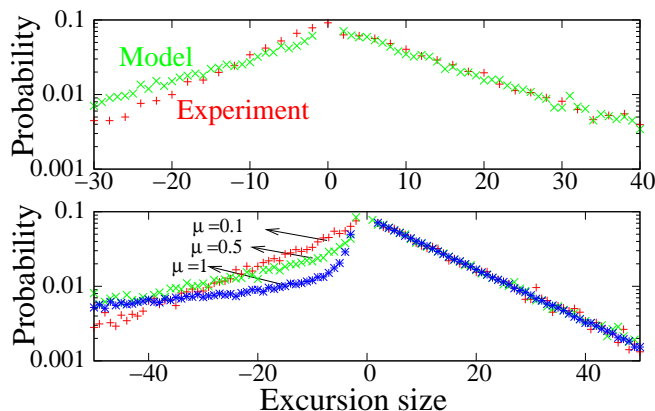


FIG. 1: *Top*: Comparison of excursions predicted by the GTP remnants model (x) with  $\lambda = 12; \mu = 0.1$ , and experimental measurements (+) [10]. *Bottom*: The distribution of excursions with  $\lambda = 12$  for  $\mu = 0.1, 0.5$  and  $1$ . While growth excursions have the distribution  $\exp(-x/\lambda)/\lambda$ , the distribution of shortening excursions changes from being exponential to non exponential and broader distributions with increasing  $\mu$ .

The time  $t_N$  is an interesting characteristic of DI in the GTP remnants model, since beyond  $t_N$  the MT can only depolymerize and shrinks to zero in time of order of  $L(t_N)/\mu$ . Fig 2 shows  $\langle t_N \rangle$  as a function of  $\ln \mu$  for various values of  $\lambda$ . We see that  $\langle t_N \rangle$  increases monotonically with increasing  $\lambda$  and  $\mu$ . For a given  $\lambda$  as we increase  $\mu$ ,  $\langle t_N \rangle$  eventually saturates at a value of the order of  $\exp(\lambda)/\lambda$ . In Fig. 2, we also show numerically obtained values of the growth velocity ( $v = \langle L(t_N) \rangle / \langle t_N \rangle$ ) at time  $t_N$ . The velocity increases with increasing  $\lambda$  and  $\mu$ , and for a given  $\lambda$ , it saturates for large  $\mu$ . As illustrated in Fig. 2,  $v = (\lambda - 1)\mu/(1 + \mu)$  leads to good scaling collapse of the data.

The distributions of  $t_N$  and  $L(t_N)$  for various  $\lambda$  and  $\mu$  (Fig. 3) broaden with increasing  $\mu$ , reaching asymptotic forms for  $\mu \gg \lambda$ . The increase of fluctuations, indicated by these broadening distributions, is a consequence of remnants. Experimental measurements of these distributions should be able to distinguish between SH and IH mechanisms and provide indirect evidence for the presence of remnants. Keeping this in mind, we provide some exact results for distribution functions in the limit of (a)  $\mu = 0$ , and (b)  $\lambda \rightarrow \infty$  and  $\mu \rightarrow \infty$ . Obtaining approximate analytical expressions for distributions and averages allows experimental verifications of the predictions of the model at different values of the system parameters.

The probability distribution of total length of the MT ( $L(t)$ ) at time  $t$  follows the following equation:

$$\frac{dP(L, t)}{dt} = \lambda(1 - n_0(t))[P(L - 1, t) - P(L, t)] + \mu n_0(t)[P(L + 1, t) - P(L, t)] \quad (1)$$

where  $n_0(t)$  is the probability of having a GDP at the tip of MT and is given by:

$$\frac{dn_0(t)}{dt} = 1 - n_0(t) - \mu n_0(t)P(+ - >, t) \quad (2)$$

here  $P(+ - >, t)$  is the probability that, given the tip of the MT is a -, the second last tubulin dimer is a +. Let us first consider  $\mu = 0$  for arbitrary value of  $\lambda$ . In this limit,  $n_0(t) = 1 - \exp(-t)$ , and substituting in Eq. 2 leads to:

$$P(L, t) = e^{(-\lambda(1 - e^{-t}))} \frac{(\lambda(1 - e^{-t}))^L}{L!} \quad (3)$$

The average length at any time  $\langle L(t) \rangle = \lambda(1 - \exp(-t))$ . Similarly, the average number of GTP-bound tubulins at time  $t$  is  $\langle T(t) \rangle = \lambda t \exp(-t)$ . Hence, the average time at which the amount of GTP goes to zero scales roughly as  $\ln(\lambda)$ . This is different from the exponential dependence of  $t_N$  on  $\lambda$  for large  $\mu$  indicated by the scaling collapse in Fig. 2. As seen in Fig. 2,  $\langle t_N \rangle$  increase monotonically from a value of order  $O(\ln \lambda)$  to a value of order  $\exp(\lambda)$  as we increase  $\mu$  from 0 to  $\infty$ .

Another limit where we can obtain exact results for  $\langle L(t) \rangle$  and  $\langle T(t) \rangle$  is  $\lambda \rightarrow \infty, \mu \rightarrow \infty$ . In this limit,

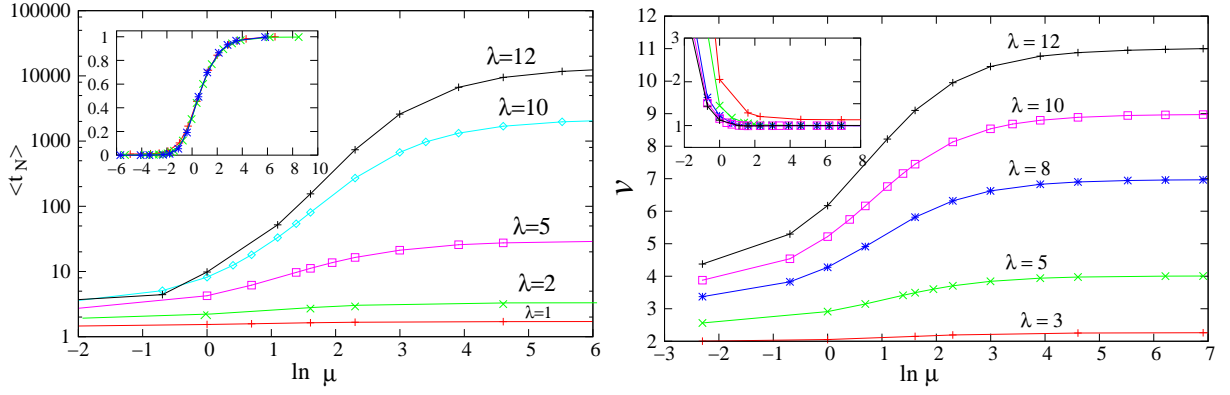


FIG. 2: *Left*: The average time at which the number of GTP-bound subunits goes to zero ( $\langle t_N \rangle$ ) as a function of  $\ln \mu$ . The inset shows a scaling collapse of the plots, with  $y$ -axis scaled by  $e^\lambda/\lambda$  and the  $x$ -axis displaced by  $\lambda$ , for  $\lambda = 8, 10, 12$ . *Right*: The average growth velocity ( $v = \langle L(t_N) \rangle / \langle t_N \rangle$ ) for indicated values of  $\lambda$  as a function of  $\ln \mu$ . In the inset, we have scaled  $v$  by  $\mu(\lambda - 1)/(\mu + 1)$  to show the scaling collapse for  $\lambda = 5, 8, 10, 12$  and  $\mu > 1$ . The dataset which does not fall on the same curve corresponds to  $\lambda = 3$ .

$P(+ - >, t)$  can be assumed to be equal to unity for all  $t$ . We found numerically that most catastrophes were of  $O(1)$  and hence this approximation provides a sensible description of the MT behaviour in the growing phase for any  $\lambda$  and  $\mu$ . With  $P(+ - >, t) = 1$ , we get,

$$P(L, t) = (\lambda)^{L/2} I_L \left( \frac{2\mu t \lambda^{1/2}}{1 + \mu} \right) \exp \left( -\frac{\mu(\lambda + 1)t}{1 + \mu} \right) \quad (4)$$

where  $I_L(x)$  is the modified Bessel function of first kind and Eq. 4 leads to:

$$\langle L(t) \rangle = \frac{\mu(\lambda - 1)}{(1 + \mu)} t \quad (5)$$

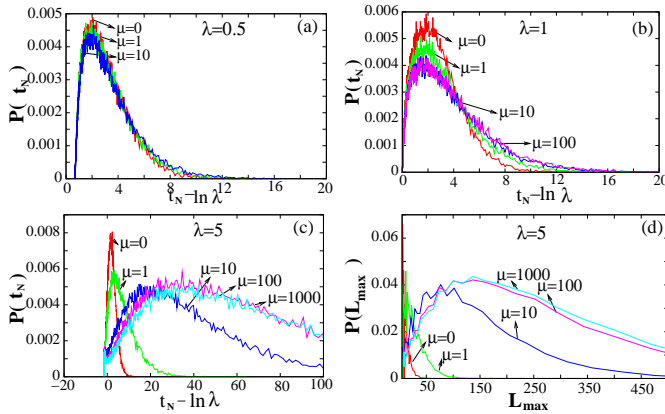


FIG. 3: (a)-(c) The distributions of times at which the amount of GTP in the MT goes to zero ( $t_N$ ). The  $x$ -axis is scaled by  $\ln \lambda$  (the average value of  $t_N$  for  $\mu = 0$ ). The distribution shifts to the right with increasing  $\mu$  until it saturates. (d) The distribution of maximum lengths for  $\lambda = 5$ . The distributions of maximum lengths and  $t_N$  have similar dependencies on  $\mu$ .

The result that the MT is always in the growing phase for  $\lambda > 1$  is a consequence of the assumption

$P(+ - >, t) = 1$ , which breaks down at some finite time  $t$ , for arbitrary values of  $\lambda$  and  $\mu$ . From Fig. 2(Right), however, it is seen that the growing stage is very well approximated by Eq. 5 as the velocity obtained from simulations matches well with the expression of average velocity in Eq. 5. In fact, for a given  $t$ , the distribution of lengths (Eq. 4) broadens with  $\mu$  in a manner similar to that seen in the simulations (Fig. 3)). The variance of distribution of  $P(L, t)$  from Eq. 4 comes out to be  $\frac{2\mu t}{1 + \mu}$ , which depends only on  $\mu$  and  $t$ .

Similarly, one can solve for the distribution and mean of  $T(t)$ :

$$\langle T(t) \rangle = \frac{\lambda \mu}{1 + \mu} - \frac{\lambda \exp(-(1 + \mu)t)}{\mu(1 + \mu)} - \frac{\lambda(\mu - 1) \exp(-t)}{\mu}$$

This equation also matches the simulation results, as we found that the average amount of GTP in the MT during the growing phase fluctuates around  $\frac{\lambda \mu}{(1 + \mu)}$ .

A number of other predictions can be made in this limit of large  $\lambda$  and  $\mu$ , which applies to the growing phase,  $t \ll \langle t_N \rangle$ . For example, the average number of catastrophes at time  $t$ , to leading order is given by  $\langle C(t) \rangle = \frac{\mu^2 t}{(1 + \mu)^2}$  and the average number of GTP islands in the MT is predicted to be:  $\frac{\lambda \mu (1 - \exp(-2t))}{2(1 + \mu)}$ .

The predictions, presented above, reflect the sensitivity of dynamical properties to the depolymerization rate  $\mu$ , and are a fingerprint of remnants. Experimental tests of these predictions can, therefore, provide insight into the nature of hydrolysis and rescue mechanisms in DI.

The above analysis was restricted to  $p = 0$  in order to highlight the effect of remnants, and because the distribution of growth and shrinkage excursions in *in vitro* experiments[10], could only be fitted with small values of  $p$ . As  $p$  is increased, the dynamics changes from rescues primarily due to remnants at small  $p$  to non-remnant rescues that are also present in IH models at  $p \simeq 1$ . Fig. 4 shows the time trace for MT length for a representative

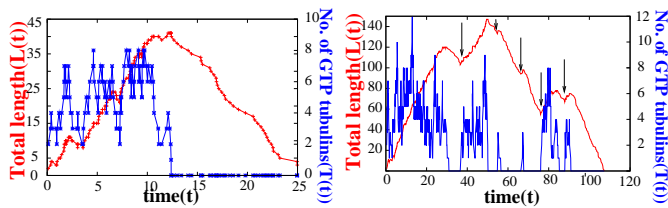


FIG. 4: MT length  $L(t)$  (red lines) as a function of time for  $\lambda = 5, \mu = 4$  and  $p = 0$  (Left);  $p = 0.01$  (Right). The blue lines show the total amount of GTP as a function of time for the same runs. Arrows in the second plot indicate the rescues due to  $p > 0$ .

run for  $p = 0$  and  $p = 0.01$ . Analysis of these trajectories shows that a small, non-zero value of  $p$  introduces rare rescue events (indicated by arrows in the figure). These events change the overall length of MT, but the statistics of growth, rescue and catastrophes remain similar to  $p = 0$ . Measurements of these statistics should, therefore, provide tests of the remnants model of DI and provide clear distinction between IH models that do not have remnants and SH models which do.

Our model can be easily extended to accommodate more detailed features of MTs while keeping the basic mechanism of remnant-dominated rescues. For example, spatially varying hydrolysis rates due to the structure of MTs[7] can be modeled by quenching some GTP-bound sites in our 1-d model. Similarly, the effect of motors that mechanically depolymerize MTs without dependence on GTP-states [14] can be modeled by assuming a depolymerizing rate  $\mu$  for both GTP and GDP-bound tubulins. Preliminary studies of the model with quenched disorder indicate that, although the time for which MT grows changes and there is a transition to unbounded growth as a function of percent of quenched sites, the distribution of excursions and velocity of growth remains

unchanged, and therefore is a robust feature of the remnant model. Studies of the model mimicking motors also indicate that the basic features of the remnant model remain unchanged as long as  $\lambda > \mu + 1$ .

To summarize, we have studied the role of rescue due to remnants in MT dynamics, and shown that remnants give rise to features of DI that are very different from IH models. These features are robust, and with recent progress in experimental techniques [9, 10], should provide tools for resolving the mechanism of hydrolysis inside a MT. Some particularly notable features of SH models, where remnants play an important role, that distinguish them from IH models are: 1) The distribution of MT lengths is not exponential but instead depends on  $\lambda$  and  $\mu$ , broadening as  $\lambda$  and  $\mu$  are increased. Measurements of rates of growth and shortening of individual MTs, both *in-vivo* and *in-vitro*, indeed exhibit variability that cannot be explained by exponential distributions[15]. 2) The distribution of times for which a MT grows before a complete collapse also has a broad, non-exponential distribution in SH models. 3) The velocity of growth, besides depending on the free tubulin concentration (through  $\lambda$ ), also depends on depolymerisation and hydrolysis rates. Interestingly, at the same tubulin concentration, MTs exhibit much higher growth rates *in-vivo* in comparison to *in-vitro*[17]. In conclusion, our study of a minimal model of MT DI, leads to predictions that provide sensitive tests of the mechanism of hydrolysis, and of the presence of GTP remnants in MTs. Experimental tests of these predictions should lead to a better understanding of how DI is controlled through various agents both *in-vivo* and *in-vitro*.

We thank the authors of [10] for providing us with their data for MT excursions *in-vitro*. S and MFH acknowledge support by NIH grant R01AI080791, and S, MFH and BC were supported in part by the Brandeis NSF-MRSEC.

- 
- [1] E. Karsenti, F. Nedelec and T. Surrey, *Nature Cell Biology* **8**, 1204 (2006).  
 [2] A. Desai and T. J. Mitchison, *Annu. Rev. Cell. dev. Biol.* **13**, 83-117 (1997).  
 [3] J. Howard and A. A. Hyman, *Nature* **422** 753 (2003).  
 [4] M. Dogterom and S. Leibler, *Phys. Rev. Lett.* **70**, 1347 (1993); H. Flyvbjerg, T.E. Holy and S. Leibler, *Phys. Rev. Lett.* **73**, 2372 (1994)  
 [5] T.L. Hill, *Biophys. J.* **49**, 981 (1986)  
 [6] E.B. Stukalin and A. B. Kolomeisky, *Bio. Phys. J.* **90**, 2673 (2006).  
 [7] V. Van Buren, D. J. Odde, and L. Cassimeris, *PNAS* **99** 6035-6040 (2002).  
 [8] B. Chakraborty and R. Rajesh, unpublished.  
 [9] A. Dimitrov, M. Quesnolt, S. Moutel, I. Cantaloube, C. Pous and F. Perez, *Science* **322** 1353-1356 (2008).  
 [10] H. T. Schek, M. K. Gardner, J. Cheng, D. J. Odde and A. J. Hunt, *Current Biology* **17** 1445-1455 (2007).  
 [11] Y. Gebremichael, J. W. Chu and G. A. Voth, *Biophysical Journal* **95** 2487-2499 (2008).  
 [12] H. Flyvbjerg et al, *Phys. Rev. E* **54**, 5538 (1996).  
 [13] T. Antal, P.L. Kaprivsky, S. Redner, M. Mailman and B. Chakraborty, *Phys. Rev. E* **76**, 041907 (2007).  
 [14] V. Varga, J. Helenius, K. Tanaka, A. A Hyman, T. U. Tanaka and J. Howard, *Nature Cell Biology* **8**, 957-962 (2006).  
 [15] R. F. Gildersleeve, A. R. Cross, K. E. Cullen, A. P. Fagen and R. C. Williams, *J. Bio. Chem.*, **267**, 7995-8006 (1992); S. Predigo and R. C. Williams Jr., *Biophysical Journal* **83**, 1809-1819 (2002).  
 [16] A. W. Hunter and L. Wordeman, *Journal of Cell Science* **113**, 4379-4389 (2000).  
 [17] Cassimeris L, *Cell Motil. Cytoskelet.* **26** 275-281 (1993).