

# Computational Study of influence of Microtubule Binding Proteins on Dynamic Instability through Modulation of GTPase Activity

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Microtubules (MTs) play an important role in many cellular processes, mitosis being one central example. Understanding their Dynamic Instability (DI; individual MTs have large length fluctuations over time) has many implications for medicine, such as cancer treatments. The interaction between the MTs and various Microtubule Associated Proteins (MAPs), found *in vivo*, remains an area in which much has yet to be fully understood. We use a computational model to address these problems. One finding of the present study is that DI can be quite sensitive to Microtubule Binding Proteins (MTBPs) which alter the hydrolysis rate (i.e., affect the GTPase activity) of tubulin hetero-dimers – the building blocks of MT polymer - to which they bind.

## INTRODUCTION

Microtubules (MTs) are long tubular polymers found in all eukaryotic cells. They are one of the most important components of the cytoskeleton. MTs are commonly referred to as cellular train tracks. MTs allow for intra cellular transport of chromosomes, mitotic spindles and other organelles. As well as this, MTs are important to cell motility, and play a key role in mitosis and meiosis. Irreversible elimination of microtubules causes cell death (Unger E.). The key to MTs cellular importance is their Dynamic Instability (DI) - MTs switch randomly between periods of growth (polymerization) and periods of rapid shortening (depolymerization) (Note: Some pauses (periods of negligible growth or shrinkage) have been experimentally observed, but are comparatively quite rare). If a MT changes from a period of growth to one of shortening, this transition is known as catastrophe. Meanwhile, a MT that has

transitioned from shortening to growth is said to have undergone rescue. Research suggests that DI makes MTs powerful cellular operators capable of generating forces as high as up to 4 pN—which indicates that microtubule dynamics can generate as much force as motor proteins (Hyman).

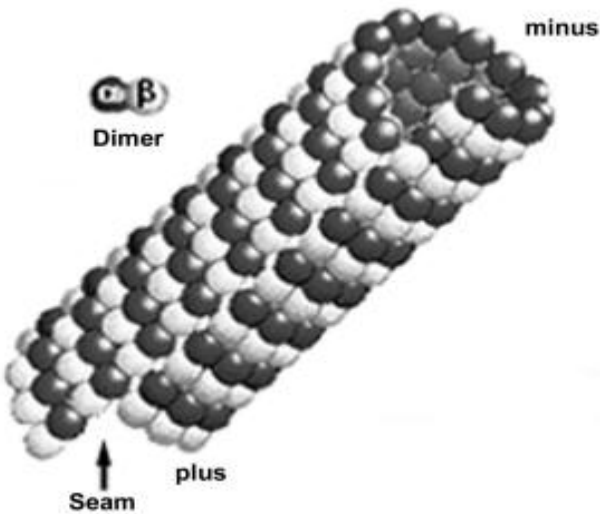
MTs consist of a chain of tubulin heterodimers. The tubulin heterodimers are made up of an  $\alpha$ -tubulin monomer and a  $\beta$ -tubulin monomer. The bond between these monomers is so strong that the heterodimer could be considered as one unit, as the bond is never broken. MTs almost always consist of 13 (certain microtubules in the neurons of nematode worms contain 11 or 15 protofilaments (Harvey Lodish, 2000)) parallel chains of heterodimers, known as protofilaments. These protofilaments curl into each other to form a near cylindrical shape. The last protofilament is 1.5 dimers (3 monomers) above the first protofilament, this is known as the seam shift. MTs have a diameter of 25nm and length varying from 200nm to 25 $\mu$ m. The most important feature of MTs is their polarity. One end of the MT terminates with the  $\alpha$  monomer (known as the minus end), while the other end terminates with an exposed  $\beta$  monomer (known as the plus end). The minus end is much more stable than the plus end, and it is usually attached to some cellular structure, such as the centrosome. We will be primarily interested in the plus end, as this end experiences DI.

DI originates in conformational changes that occur in the tubulin heterodimers after polymerization. The tubulin subunits bind to the nucleotides GTP and GDP to form GTP-Tu and GDP-Tu, respectively. A crucial observation is that tubulin polymerizes in the presence of non-hydrolysable GTP to form stable microtubules (Hyman A. A., 1992). The transformation of GTP-Tu to GDP-Tu through hydrolysis generates sufficient

energy for depolymerization to occur. Thus, a growing MT can develop what is known as GTP cap; the end of MT consists primarily of GTP-Tu dimers, but following hydrolysis, the GTP-Tu dimers convert to GDP-Tu dimers, and these exposed dimers will depolymerize rapidly, thus implementing catastrophe.

Proteins that modulate microtubule dynamics have been known traditionally as microtubule-associated proteins or MAPs (Anderson, 2000). A particular type of MAP that binds to the MTs is called a Microtubule Binding Protein (MTBP). Cells, such as neurons, maintain long processes, called axons, and thus require a stable system of MTs, as any depolymerization could have disastrous consequences, neuron retraction for example. MTs in these cells are believed to be stabilized by MTBPs such as Tau, Map1A, and Map1B. Tau is believed to encourage stability in axonal cells by cross-linking microtubules into thick bundles (Harvey Lodish, 2000). So far, studies of MTBPs have told us little about the mechanisms by which proteins modulate the dynamics of the microtubule ends. The reason is that they bind all along the microtubule lattice, yet we expect that their effect on dynamics should take place only at the microtubule end (Hyman J. H.). Other important MTBPs include Stathmin and Op18, as these tubulin binding proteins promote depolymerization and catastrophe.

Due to MTs' importance in many cellular processes, they are a subject of intense scientific study. Drugs, such as Taxol, interfere with mitosis by stabilizing MT ends, and thus preventing dangerous (e.g. cancerous) cells from dividing and spreading (Jordan, 2004). However, tubulin is a very complicated compound, and while experimental techniques have allowed us to improve our understanding of the MT structure, they are quite static. As a result, we know little about the dynamics of MTs.



**Figure 1:** A microtubule of 13 protofilaments forming a near cylindrical shape. The  $\alpha$ -tubulin dimers and  $\beta$ -tubulin dimers are coloured black and white, respectively. The minus end, with an exposed  $\alpha$ -monomer, is located at the top of the image, while the plus end, the end associated with Dynamic Instability, with an exposed  $\beta$ -monomer, is located at the bottom. Also, notice at the seam, there's a shift of 3 monomers from the first protofilament to the last.

## METHODS

M. Alber et al have developed a model which accurately models the dynamic instability experienced in MTs. This is the model that I worked with over the summer. In preparation, I had read up on alternative approaches to modeling MTs, ranging from the Mechanochemical approach used by V. Van Buren et al, to I.V. Maly et al's method of modeling DI as a diffusive process. To be able to use the model, it was necessary for me to learn some basic Unix commands, as well to reacquaint myself with Matlab and C++. The model we discuss here consists of only two dimers: GTP-Tu and GDP-Tu. The model has 5 processes: Protofilament Growth, Protofilament Shortening, Inter-Protofilament Bond Growth, Inter-Protofilament Bond Shortage, and Transition of each GTP-Tu dimers into GDP-Tu dimers (i.e. GTPase). Protofilament growth is the addition of one tubulin subunit onto the plus end of the MT, and is dependent on the tubulin concentration and the GTP/GDP value at the tip. Shortening depends on the lateral bonds between subunits. More than one subunit can depolymerize at one time. The rate

of bond breakage/shortening is asymmetric, that is, they depend on the identity of the left and right neighbor i.e. GTP|GDP interaction differs from GDP|GTP interaction. The inter-protofilaments bond can be thought of as a zipper: A bond can only be removed if the highest bond between two protofilaments, and can only be added onto the top on the highest existing bond. Hydrolysis is modeled as a first order stochastic process.

That is, a dimer is selected randomly. The GTP dimer may change to GDP according to some probability. This occurs on all dimers. The most important aspect of the model to me was the config files. They allowed me to alter the tubulin concentration, seam shift, hydrolysis rate, interaction strengths between various dimers, etc. As a result, I was able to run the model for different values and observe the effects these changes had on the MTs and Dynamic Instability in particular.

Once I had accustomed myself with the model (after running tens of different simulations), it was time to study the importance of MAPs, or MTBPs to be more specific. MTBPs bind to GTP-Tu and GDP-Tu dimers, and modulate dynamic instability. One is able to adjust the model to include MTBPs. You can set the rate at which the MTBPs binds to GTP and GDP dimers (called  $k_{on}$  GTP and  $k_{on}$  GDP respectively), as well as its freeing rate (called  $k_{off}$  GTP and  $k_{off}$  GDP). You can also set the rate at which a bounded GTP dimer hydrolyzes to GDP, called  $b_{hyd}$  here. My first task was to test the model for some kind of bug. For all my work, I set the rate at which GTP hydrolyzes to GDP at 0.7. Thus, I had to add a MBTP to the model which attach would itself to the dimers, but had no effect on the model. I set  $k_{on} = k_{off} = 1$ , and  $b_{hyd} = 0.7$ . It should be noted that given the stochastic nature of the model, a change in the random seed will produce an ultimately different plot. Thus, it was necessary to run the model 5

times with a MAP included and to compare this model's mean rescue velocity, mean catastrophe velocity, rescue frequency, and catastrophe frequency with those of the original model where MTBPs were excluded. With the inevitable variation in mind, the figures were relatively close to each other. Thus, the inclusion of a MTBP did not corrupt the model.

Next, I had to run the model using different values for the 3 important variables i.e.  $k_{on}$ ,  $k_{off}$  and  $b_{hyd}$ , with all other variables (except seed random) held constant. As the model must check each dimer to see if the MBTP will bind to it, this model is much more computationally expensive than the model ignoring MTBPs. It takes roughly 1.5days to complete the MTBP-included simulation. After trying various values, I identified 4 fundamental consequences of including a MBTP:

1. Little or no difference from original model.
2. Increased Dynamic Instability.
3. Reduced Dynamic Instability.
4. Bounded growth

Figure 2: Original Model excluding MAPs

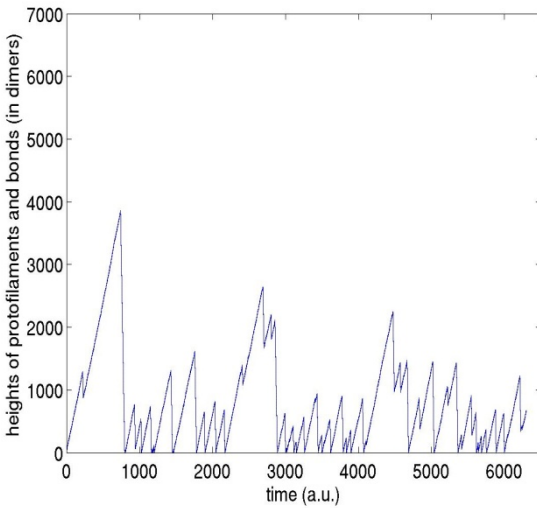


Figure 3: kon= 0.8, koff = 0.8, bhyd =0.82

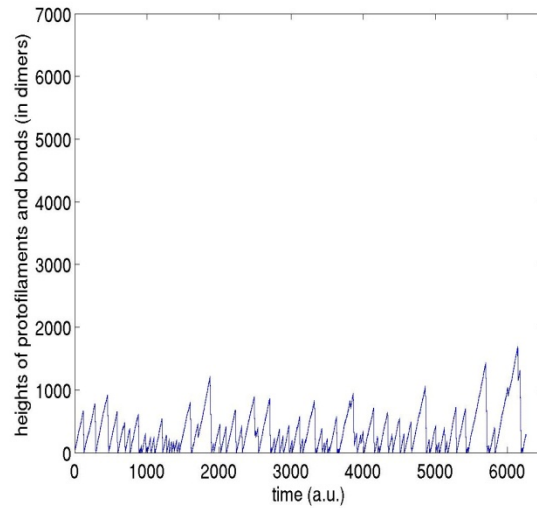


Figure 4: kon= 0.2, koff = 1, bhyd =0.35

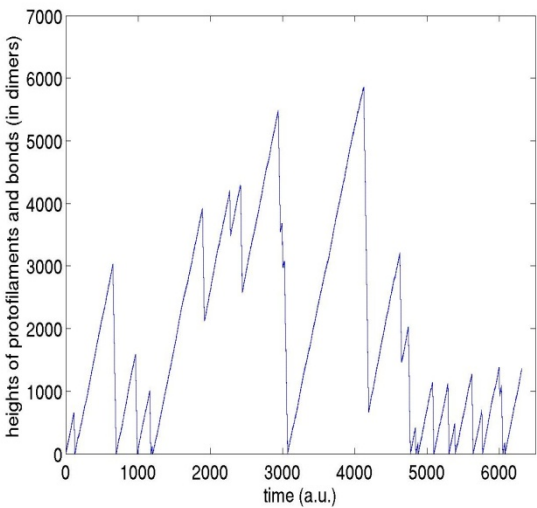


Figure 5: kon= 0.8, koff = 0.2, bhyd =0.2

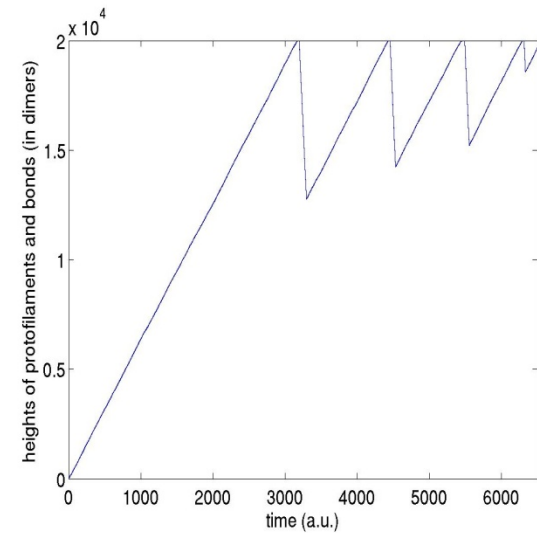


Fig 2: The 4 above length-history graphs demonstrate the 4 different effects MBTPs may have on our model. Length is given in dimers, while time is given in arbitrary unit. Above each graph are its associated kon, koff and bhyd figures. Figure 2 is the graph of a typical MT excluding MAPs, used here for illustrative purposes. Figure 3 has an MTBP included; this MT is much more dynamic as a result. Figure 4 shows a graph that has become much less dynamic due to the addition of an MBTP. Figure 5 contains a graph that has an MBTP attached which makes the MT grow to the cell wall (you can set the max MT length in the configuration file). Note: Figure 5 is not to the same scale as the other figures. It displays a max height of 20,000 dimers, compared with 7,000 dimers in the other figures.

## Conclusions

By adjusting  $k_{on}$ ,  $k_{off}$ , but most importantly,  $b_{hyd}$ , one is able to modulate dynamic instability. Figure 3 has a MBTP which promotes depolymerisation and catastrophe and recreates the role of MTBPs such as Stathmin and Op18. MTBPs such as MAP1A bind to the ends of a MT and inhibit depolymerisation. Figure 4 has a MBTP which has a similar effect on our MT. One is able to achieve any desired effect on MTs' dynamic instability by attaching an MBTP with appropriate  $b_{hyd}$ ,  $k_{on}$  and  $k_{off}$  values. Thus, our model suggests that MTs are hugely sensitive to MTBPs. But more specifically, MTBPs which will alter the rate at which a bounded GTP-Tu dimer hydrolyzes.

## Future Work

The ultimate goal is to produce a model of a cellular system of MTs. When MAPs are included, it can take up to 2days to run the model. Even with parallel computing, we are still some way from creating an efficient cellular model. As for MAPs, I assumed that a bounded dimer is identical to an unbounded dimer, except for a difference in the hydrolysis rate. This assumption may be slightly simplistic. It's necessary to continue to work with biologists to test the model with biologically relevant parameter values.

## Acknowledgements

Thanks to my advisor Dr. Mark Alber for his guidance, Dr. Gennady Margolin for his assistance throughout the summer and to Dr. Holly Goodson for helping me with the biological aspect of the project. I'd also like to thank the Tyndall National Institute and Science Foundation Ireland for providing me with this tremendous opportunity to do

research at Notre Dame. Simulations were performed on the Notre Dame Biocomplexity Cluster supported in part by NSF MRI Grant No. DBI-0420980.

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