Microtubule Assembly Stochastic Model

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1 Abstract

Microtubules are formed by alpha and beta tubulin dimer subunits, guanosine tri-phosphate, which we will call GTP for shorthand. These dimers can be hydrolyzed into guanosine di-phosphate, which we will call GDP for shorthand. These subunits form strings of units called protofilaments. To form a microtubule, thirteen protofilaments align to form a cylindrical shape. This structure then grows upwards with the addition of GTP units. GTP units may also fall off. These GTP units can be hydrolyzed to form GDP. GDP requires much higher energy to maintain position in the microtubule when exposed to the tip of the structure. To represent this, we used a kinetic Monte Carlo algorithm to create a stochastic model of microtubule assembly.

2 States

In the lattice, each coordinate can exist in one of four states, empty, filled with GTP, filled with GDP, or tagged GTP. A coordinate can change states through the attachment or detachment of a tubulin subunit, the hydrolysis of itself, or the hydrolysis of the tubulin subunit below.

Empty Sites: The coordinates in the lattice above the template (as described below) begin the simulation in the empty state. This is represented as a -1 in the program. GTP may only be attached in an empty state, with the requirement that there must be a filled coordinate in the row below it. Our assumption is that empty states that are directly above a filled state are the only sites where attachment is allowed.

GTP: The next state is a GTP-filled coordinate. GTP is simulated with a lattice value of 0. GTP can either undergo hydrolysis or fall off. Only the top most GTP can detach, so the coordinate above must be in the empty state.
Template: The first row of the lattice is titled the template strand. The template strand is comprised of GTP subunits. The subunits in this strand can never be removed or altered. This exists so that the microtubule is always able to grow, even if it loses all other subunits.

GDP: If GTP is hydrolyzed, then it exists as GDP. This is represented with a lattice value of 1. Once hydrolyzed, any lateral bonds lose stability, increasing the propensity for the subunit to detach if it is the highest filled coordinate.

Tagged: If GTP is attached onto a GDP subunit, it is tagged and is represented with a lattice value of 2. The attachment of GTP onto GDP decreases the stability of the GTP, causing its thermodynamic properties to match that of a GDP subunit, being that the lateral bond energy decreases, making it more unstable. To demonstrate this, a tagged GTP is treated as GDP in terms of stability in the lattice. GTP may become untagged if it has two neighbors that are both GTP, as the stability from those neighbors makes up for the lessened of stability from the GDP longitudinal neighbor.

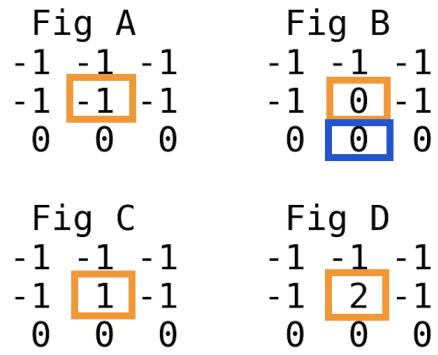

Figure 1: Representation of states in the lattice. Fig A: Empty site highlighted in orange box. Fig B: GTP filled site highlighted in orange box. Template site highlighted in blue box. Fig C: GDP filled site highlighted in orange box. Fig D: Tagged GTP filled site highlighted in orange box.

3 Events

There are 8 possible reactions in this model, attachment, detachment of GTP and GDP with 0, 1, 2 neighbors, and hydrolysis of GTP.

Attachment: Attachment must occur at the lowest empty coordinate in each column of the lattice. We assume that only GTP can attach to the lattice. If GTP is attached to GDP, then the GTP is tagged. All attachments have the same rate, regardless of number of neighbors.

Detachment: Detachment must occur at the highest filled coordinate in each column of the lattice. Detachment can occur in six different situations.

The number of neighbors affects the affinity to detach. A subunit with fewer neighbors is more likely to detach due to decreased stability from less lateral bonds, so detachment of a subunit with 0, 1, and 2 neighbors are considered 3 different events. Both GTP and GDP can detach from the lattice, so long as they are the highest filled coordinate. GTP is less likely to detach, as it has stronger lateral bonds, making it less favorable to detach. GDP falls off rather quickly. Due to longer time in the lattice, hydrolysis tends to occur from the bottom of the lattice up. As a result, there is a cap-like quality where the top few rows of subunits tend to be GTP, with GDP existing in high frequency below this cap. If GTP stochastically falls off fast enough to expose the GDP to the top layer, the microtubule will quickly fall apart due to GDP's high affinity for detachment, with this occurrence being titled a catastrophe. This causes the peak-like structure we see in the graph representing the microtubule growth. With 3 neighbor events for both GTP and GDP, this causes 6 different events in detachment.

Hydrolysis: Hydrolysis can occur to GTP anywhere in the lattice. Hydrolysis occurs at a static rate, however the lower GTP subunits tend to exist in the hydrolyzed state more than the higher units due to their increased time in the lattice. Hydrolysis decreases the stability of the subunit because of its effect on lateral bond energy.

4 Parameters

This model requires physical parameters to be input. These values were taken from the Van Buren paper [5]. These values were selected due to the similar nature of the model presented in this paper to the model we wrote.

The free GTP tubulin concentration was defined as 10 µM, being held constant as the reactions proceed, assuming an external supply to the system maintaining this concentration.

The rate of addition is defined to be constant throughout the lattice, regardless of the presence or state of neighbors. It is also assumed that only GTP can attach to the lattice and only at the tip of the microtubule. It is assumed that there cannot be a hole between two longitudinal subunits, so addition can occur only at the tip. This rate is a second-order rate defined as $k_+ = 2M^{-1}s^{-1}$.

The off rates are calculated using the free energy change of longitudinal bonds and lateral bonds, $\Delta G_{Lat} = -5.7 * k_B T$ J, $\Delta G_{Long} = -6.8 * k_B T$ J. Hydrolysis of GTP in the lattice increases the free energy change of lateral bonds, $\Delta G_{Kink} = 2.5 * k_B T$ J. Since the calculations divide out the Boltzmann constant * temp, they are excluded in the code. The off rates are first-order with units of s^{-1} and are calculated using the following equation:
$\Delta G = -k_B T * ln(\frac{k_+}{k_-}[tubulin])$

The hydrolysis rate is a first-order rate defined as $k_h = .95s^{-1}$.

k_+	ΔG_{Long}	ΔG_{Lat}	ΔG_{Kink}	k_h
$2M^{-1}s^{-1}$	$-6.8 * k_BT$	$-5.7 * k_BT$	$2.5 * k_BT$	$.95s^{-1}$

Table 1: Parameter values based on values from [5]

5 Algorithm

The Gillespie algorithm, a kinetic Monte Carlo algorithm is implemented to model this microtubule assembly. The model loops until the current time reaches the maximum time. Time is calculated as each unit being one second and the overall time being 2 minutes. At the beginning of each loop, the different states are stored using helper functions.

Propensities: The propensities of each event occurring is calculated by multiplying the defined rates with the tubulin concrentration, if applicable, and the number of sites this reaction can occur at.

Time and Selecting reaction: The sum of the propensities is calculated as the total rate. If this value is 0, this means no more reactions are possible, so the simulation ends. If not, the reaction time is selected by the equation $-ln(random(0-1))/total\_rate$. This selects a time value that correlates to the overall rate. The reaction is selected by finding a random value between 0 and 1 and multiplying by the total rate. This creates a weighted chance for each reaction being selected based on their propensity. Next, the value is associated with an integer correlating to its reaction.

Reaction: A random site within the pool of possible sites for the previously selected reaction is selected and updated to reflect the effects of the reaction. After the reaction occurs, the lattice is iterated through to check to see if a tagged GTP subunit now has two GTP neighbors, untagging that unit.

Recording values: Finally, the time for the event is added to the current time to update this value. Additionally, the column growth history appends the heights of the columns after the latest event and the time array appends the current time.

6 Results

Our aim was to develop a model that followed the same pattern in a graph of growth vs time as the Chaaban paper [2]. This involves peaks with a rising slope

of approximately 1.5 µm / minute followed by a quick fall. There are multiple peaks of different heights, but all follow the similar pattern. We were able to achieve this. Our slope is approximately 1.2 µm / min, with the same general trends. We did encounter a problem where our off rates required a multiplied factor. The off rates for GTP were required a factor of 10000 and for GDP a factor of 100000000. At this moment, we are not entirely sure where this discrepancy is, however, with the factor, the model appears to be functioning correctly.

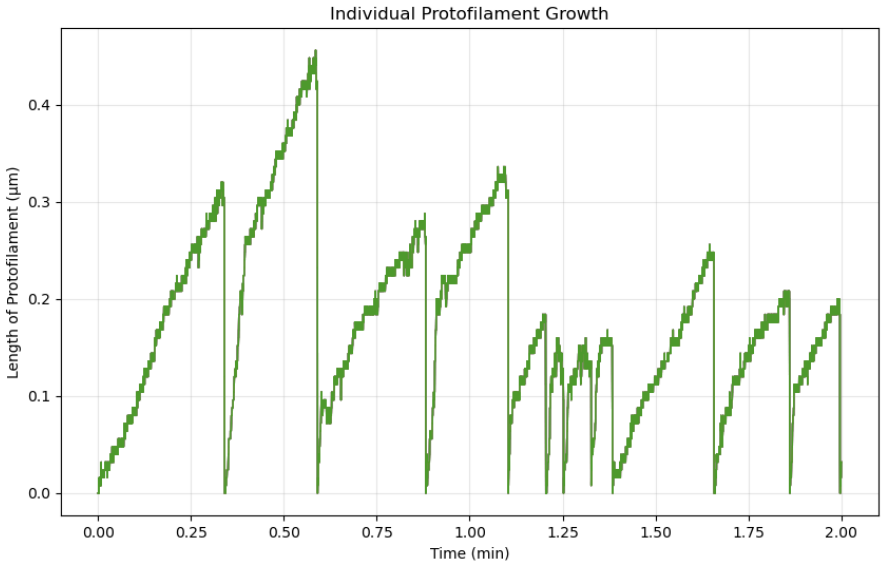

Figure 2: Graph of microtubule growth for 1 protofilament versus time to demonstrate the growth of the microtubule

7 References

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