Microtubule dynamics - a three protofilament model

Report

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28.08.2015

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1 Introduction

1.1 Context

Microtubule play an important role in many areas of biology, yet the dynamic instability is not very well understood so far. A microtubile consists of thirteen protofilamens which are built of GDP and GTP tubulin. GTP tubulin frequently hydrolize and become a GDP tubulin. GTP tubulin can only attach or detach at the end of the microtubule. The ends are much more unstable when they consist of GDP tubulins, detachment can lead to rapid shrinking of the microtubule, what we call a catastrophe.

1.2 Targets

The goal of this project is to take the existing standard model with a poison distribution and take it one step further. Instead of simulating only one protofilament, we are going to simulate three. As a simplification, we consider only blocks of tubulin, which means our protofilaments have always the same length. Nevertheless this significantly changes the probability distributions for the attachment and detachment process. This report tries to document the various steps which were undertaken to analyse, implement and test the new model.

2 Analysis

2.1 Three protofilaments model

We use a model of microtubule with multiple protofilaments, a first version will have three. The behaviour at the head is calculated for each line of dimers separately. First we have to calculate the probability distributions for each case.
If we assume the detachment of each dimer will be given by a poisson distribution with average $\lambda_1, \lambda_2, \lambda_3$. The probability that the layer decayes until time t is given by the probability that the first dimer decays until time t_1, the second until time t_2 and the third until time t. We then have:

$$\begin{aligned} P_{\lambda_1,\lambda_2,\lambda_3} &= \int_0^t dt_1 \int_{t_1}^t dt_2 \lambda_1\lambda_2\lambda_3 e^{-\lambda_3(t-t_2)} e^{-\lambda_2(t_2-t_1)} e^{-\lambda_1 t_1} \\ &= \frac{\lambda_1\lambda_2\lambda_3}{\lambda_3-\lambda_2}\left(\frac{e^{-\lambda_1 t}-e^{-\lambda_2 t}}{\lambda_2-\lambda_1} - \frac{e^{-\lambda_1 t}-e^{-\lambda_3 t}}{\lambda_3-\lambda_1}\right) \end{aligned}$$

In our case, we only have to deal with two different lambdas, one for the GTP dimer and one for the GDP, we will call those parameters λ_T and λ_D. Which leaves us with four different cases: 3xT, 2xT 1xD, 1xT 2xD and 3xD. Each case has of course different combinatorial possibilities, the case TTD can also be considered as TDT and DTT. The possibilities are listed in the table below:

λ_1	λ_2	λ_3
T	T	T
D	T	T
T	D	T
T	T	D
T	D	D
D	T	D
D	D	T
D	D	D

We use the result from above and consider the case $\lambda_1 = \lambda_T, \lambda_2 = \lambda_T, \lambda_3 = \lambda_D$:

$$P_{\lambda_T,\lambda_T,\lambda_D} = \frac{\lambda_T^2\lambda_D}{\lambda_D - \lambda_T}\left(\lim_{\lambda_1 \longrightarrow \lambda_T} \frac{e^{-\lambda_1 t} - e^{\lambda_T t}}{\lambda_T - \lambda_1} - \frac{e^{-\lambda_T t} - e^{-\lambda_D t}}{\lambda_D - \lambda_T}\right) \tag{1}$$

$$= \frac{\lambda_T^2\lambda_D}{\lambda_D - \lambda_T}\left(te^{-\lambda_T t} - \frac{e^{-\lambda_T t} - e^{-\lambda_D t}}{\lambda_D - \lambda_T}\right) \tag{2}$$

For the case $\lambda_1 = \lambda_T, \lambda_2 = \lambda_D, \lambda_3 = \lambda_T$:

$$P_{\lambda_T,\lambda_D,\lambda_T} = \frac{\lambda_T^2\lambda_D}{\lambda_T - \lambda_D}\left(\lim_{\lambda_3 \longrightarrow \lambda_T} \frac{e^{-\lambda_3 t} - e^{\lambda_T t}}{\lambda_T - \lambda_3} + \frac{e^{-\lambda_T t} - e^{-\lambda_D t}}{\lambda_D - \lambda_T}\right) \tag{3}$$

$$= \frac{\lambda_T^2\lambda_D}{\lambda_T - \lambda_D}\left(- te^{-\lambda_T t} + \frac{e^{-\lambda_T t} - e^{-\lambda_D t}}{\lambda_D - \lambda_T}\right) \tag{4}$$

$$= \frac{\lambda_T^2\lambda_D}{\lambda_D - \lambda_T}\left(te^{-\lambda_T t} - \frac{e^{-\lambda_T t} - e^{-\lambda_D t}}{\lambda_D - \lambda_T}\right) \tag{5}$$

The case $\lambda_1 = \lambda_D, \lambda_2 = \lambda_T, \lambda_3 = \lambda_T$ differs from the first two cases, it is harder to calculate but yields the same result. We start by setting $\lambda_2 = \lambda_T + \epsilon$ and $\lambda_3 = \lambda_T - \epsilon$:

$$P_{\lambda_D,\lambda_T,\lambda_T} = \lim_{\epsilon \longrightarrow 0} \frac{\lambda_D(\lambda_T+\epsilon)(\lambda_T-\epsilon)}{-2\epsilon}\left(\frac{e^{-\lambda_D t} - e^{-(\lambda_T+\epsilon)t}}{\lambda_T + \epsilon - \lambda_D} - \frac{e^{-\lambda - Dt} - e^{-(\lambda_T-\epsilon)t}}{\lambda_T - \epsilon - \lambda_D}\right) \tag{6}$$

$$= \lim_{\epsilon \longrightarrow 0} \frac{\lambda_D(\lambda_T^2 - \epsilon^2)e^{-\lambda_T t}}{-2\epsilon((\lambda_T - \lambda_D)^2 - \epsilon^2)}\Big((\lambda_T - \lambda_D)(e^{\epsilon t} - e^{-\epsilon t}) - \epsilon(2e^{-(\lambda_T - \lambda_T)t} - e^{-\epsilon t} + e^{\epsilon t})\Big) \tag{7}$$

$$= \frac{\lambda_D\lambda_T^2 e^{-\lambda_T t}}{(\lambda_T - \lambda_D)^2}\Big(e^{-(\lambda_D+\lambda_T)t} - (\lambda_T - \lambda_D)t\Big) \tag{8}$$

$$= \frac{\lambda_T^2\lambda_D}{\lambda_D - \lambda_T}\left(te^{-\lambda_T t} - \frac{e^{-\lambda_T t} - e^{-\lambda_D t}}{\lambda_D - \lambda_T}\right) \tag{9}$$

The case where $\lambda_1 = \lambda_2 = \lambda_3 = \lambda_T$ is a gamma distribution and simplifies as follows:

$$P_{\lambda_T,\lambda_T,\lambda_T} = \frac{\lambda_T^3 t^2 e^{-\lambda_T t}}{2} \tag{10}$$

We should check that the expressions are probability distributions by integrating from 0 to ∞. Which is the case for all distributions
We can now calculate each of the four cases:

$$P_{TTT} = P_{\lambda_T,\lambda_T,\lambda_T} \tag{11}$$
$$P_{TTD} = \frac{P_{\lambda_T,\lambda_T,\lambda_D} + P_{\lambda_T,\lambda_D,\lambda_T} + P_{\lambda_D,\lambda_T,\lambda_T}}{3} = P_{\lambda_T,\lambda_T,\lambda_D} \tag{12}$$
$$P_{TDD} = \frac{P_{\lambda_D,\lambda_D,\lambda_T} + P_{\lambda_D,\lambda_T,\lambda_D} + P_{\lambda_T,\lambda_D,\lambda_D}}{3} = P_{\lambda_D,\lambda_D,\lambda_T} \tag{13}$$
$$P_{DDD} = P_{\lambda_D,\lambda_D,\lambda_D} \tag{14}$$

What we will be interested in, is the probability that a line of dimers has not yet decayed at time t. Which is the sum of the probabilities that it decays after time t. This reasoning translates into the following formulas:

$$\int_t^\infty P_{TTT} = \frac{1}{2} e^{-\lambda_T t}(\lambda_T^2 t^2 + 2\lambda_T t + 2) \tag{15}$$
$$\int_t^\infty P_{TTD} = \frac{1}{(\lambda_T - \lambda_D)^2}\Big(\lambda_T^2 e^{-\lambda_D t} + \lambda_D e^{-\lambda_T t}(-\lambda_T^2 t + \lambda_T(\lambda_D t - 2) + \lambda_D)\Big) \tag{16}$$
$$\int_t^\infty P_{TDD} = \frac{1}{(\lambda_D - \lambda_T)^2}\Big(\lambda_D^2 e^{-\lambda_T t} + \lambda_T e^{-\lambda_D t}(-\lambda_D^2 t + \lambda_D(\lambda_T t - 2) + \lambda_T)\Big) \tag{17}$$
$$\int_t^\infty P_{DDD} = \frac{1}{2} e^{-\lambda_D t}(\lambda_D^2 t^2 + 2\lambda_D t + 2) \tag{18}$$

For which we will need the inverse to calculate a random time from a random number in $[0, 1]$

3 Implementation

3.1 Parameter Fitting

There are multiple variables which influence the microtubule dynamics in our model:

1. The rate of hydrolization k_h
2. The rate of detachment for GTP k_-^T
3. The rate of detachment for GDP k_-^D

4. The rate of attachment k_+

While we fixed the rate of hydrolizion at $k_h = 0.33$ and $k_-^T = \frac{k_-^D}{k_f}$ where k_f is a factor to be chosen.
The rate of attachment is defined as follows:

$$k_+ = k^{eq} \cdot d \cdot N_p$$

Where k^{eq} is the rate of attachment at equilibrium, it will be treated as a variable in the fitting process. The variable d stands for the concentration of free tubulin in micro Molars (μM), which will vary between 5 and 20. Finally N_p is the number of protofilaments, and will be fixed to 3 for this project. This means we have four variables to play with: k_-^D, k_f, d, k^{eq}

Our goal for the fitting process is a behaviour similar to the one measured in the walker paper. Which means we want to see an elongation rate of $\frac{dL}{dt} = 1.71\frac{\mu m}{min}$ at a concentration of $d = 10\mu M$ and $\frac{dL}{dt} = 3.36\frac{\mu m}{min}$ for $d = 15\mu M$. We fixed $k_f = 900$ for this part of the fitting. First we looked for combinations of k_-^D and k^{eq} which allow for the given growth rate at $d = 10$. And then checked for the rate at $d = 15$

k^{eq}	k_-^D	$\frac{dL}{dt}\|_{d=10}$	$\frac{dL}{dt}\|_{d=10}$
4.44	100'000	1.71	8.5
2.375	50'000	1.71	8.5
1.34	25'000	1.71	4.9
0.86	12'500	1.71	3.38
0.852	12'250	1.71	3.36
0.66	5'000	1.71	2.82

The growth rate curve of the resulting process looks like in the following image:

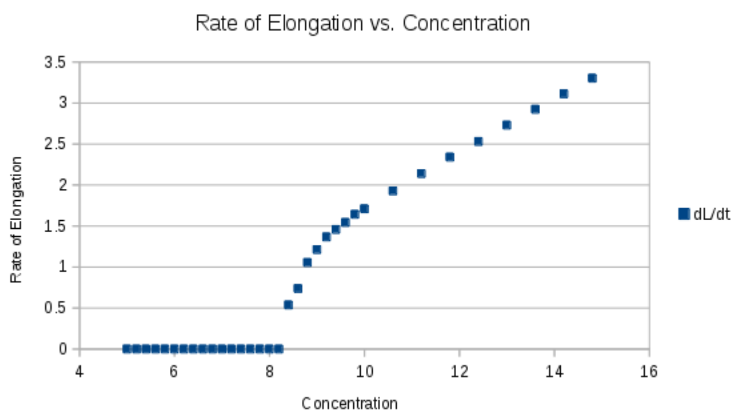

Figure 1: Growth rate at $k_f = 900, k^{eq} = 0.852, k_-^D = 12'250$

The problem with those values is that they do not produce the expected and measured behaviour between the values 7 and 10 micro molar. Therefore the $k^{eq}0$ parameter had to be adapted to fit the behaviour at $d = 7$ and exhibit the same increase in growth between $d = 10$ and $d = 15$ as before. The value of k^{eq} which lead to the desired behaviour was 1200. The growth rate graph now looks as following:

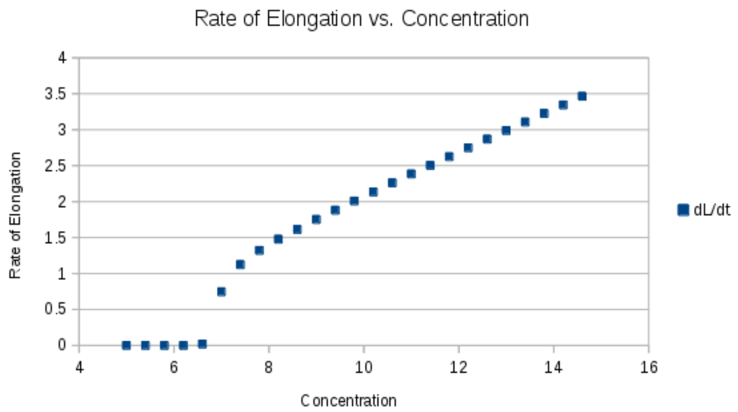

Figure 2: Growth rate at $k_f = 1200, k^{eq} = 0.852, k_-^D = 12'250$

As the model now behaves according to the data in the Walker paper, the next step is to measure the catastrophes and their properties. The following values were used for the rest of the project:

$$k_f = 1200, k^{eq} = 0.852, k_-^D = 12'250$$

3.2 Detection of catastrophes

The detection of catastrophes is done by monitoring the last ten detachment or attachment actions. As soon as there are more detachment than attachment operations in the log, the catastrophe is said to have started after the last attachment event. If then again the last ten action contain more attachment than detachment events, the catastrophe is said to have stopped at the last detachment event.

4 Results

As in the Walker paper we calculated two different measures for catastrophes and compared the outcome to the measured values. The simulations were run over 1'000'000 time units for each concentration. The results are only for the plus end of the microtubule, as our model does not cover the minus end.

4.1 Frequency of catastrophes

According to the Walker paper the frequency of catastrophes should decrease linearly with an increase of free tubulin in the solution. The frequency of catastrophes starts at 0.005 for $7\mu M$ and decreases linearly to 0 for a concentration of $15\mu M$. Our measures were decreasing but not as expected.

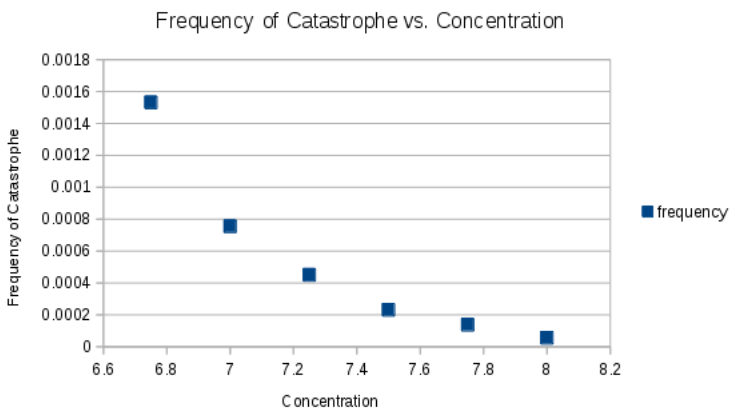

Figure 3: Rate of catastrophes for various concentrations

The rate of catastrophes starts much lower at approx. a quarter of those in the Walker paper, and decreases exponentially to become zero at $8\mu M$.

4.2 Frequency of Rescue

The Walker paper measured a linear increase of the frequency of rescue starting from 0 at $5\mu M$ and ending at 0.05 at $15\mu M$. Our measures were increasing as well, but from another level.

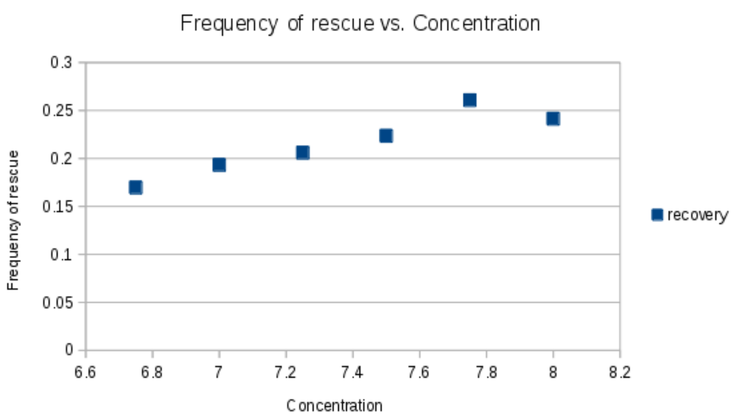

Figure 4: Rate of rescue for various concentrations

The image shows a linear increase as well, but starting from a much higher level and also with a steeper slope. As the frequency of rescue is only measurable when catastrophes appear, we can only display values for the same concentrations as before.