

A continuous model for microtubule dynamics with catastrophe, rescue and nucleation processes

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Overview of the talk

- ▶ Introduction to the role of microtubules and their formation processes
- ▶ Formulation of the partial differential equation model
- ▶ Numerical simulations
- ▶ Outlook, conclusion

Microtubules

- ▶ protein polymers made of α/β -tubulin heterodimers
- ▶ essential part of the cytoskeleton of all eukaryotic cells (besides actin cytoskeleton and intermediate filaments)
- ▶ play key roles in intracellular vesicle transport and chromosome separation during mitosis
- ▶ interference with the polymerization/depolymerization of microtubules is the mechanism of action of many anticancer drugs (e.g. Taxol)

Microtubules



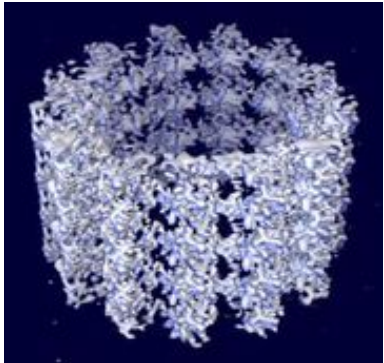
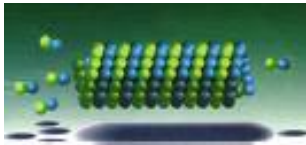
Microtubules from the mitotic spindle during cell division.

Structure of microtubules

An individual microtubule is a hollow cylinder of 25 *nm* diameter built usually from 13 protofilaments (Bolteraurer *et al.* 1999), that show a directionality.

Following common practice, we will call an α/β -tubulin heterodimer a “monomer”.

Structure of microtubules



Growth at the + and - ends of a microtubule (left) and three-dimensional lattice structure of a microtubule (right).

Structure of microtubules

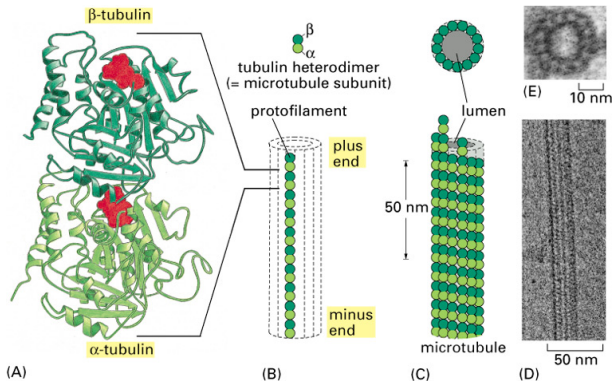


Figure 16-6. Molecular Biology of the Cell, 4th Edition.

From Alberts *et al.*, Molecular Biology of the Cell

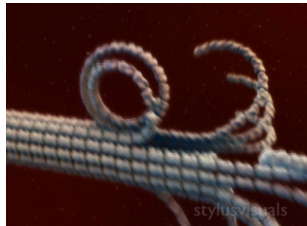
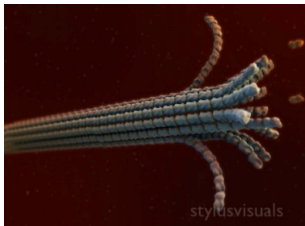
Microtubule growth

- ▶ monomers exist in two different energetic states, namely bound to a molecule of guanosine triphosphate (GTP, higher energetic state) or guanosine diphosphate (GDP, lower energetic state)
- ▶ only GTP bound monomers are able to polymerize into microtubules
- ▶ after the GTP monomers have been added to the growing microtubule, GTP bound to β -tubulin is rapidly hydrolyzed (dephosphorylated) to form a bound GDP subunit

Dynamic instability

- ▶ GTP cap on the tip of the growing microtubule gives rise to the stability of the microtubule (Mitchison & Kirschner 1984)
- ▶ a microtubule without GTP cap switches to a “collapsing” state, characterized by rapid depolymerization of the microtubule into its free (GDP) subunits
- ▶ a catastrophically shrinking microtubule can acquire a new GTP cap and return to the growing population, a “rescue” event

Dynamic instability



Conformational changes of the tubulin units cause rapid depolymerization.

Mathematical models for polymerization/depolymerization processes

discrete

- ▶ v. Smoluchowski (1916), considered coagulation and fragmentation processes
- ▶ Becker & Döring (1934), countably many ordinary differential equations, one for every n -mer

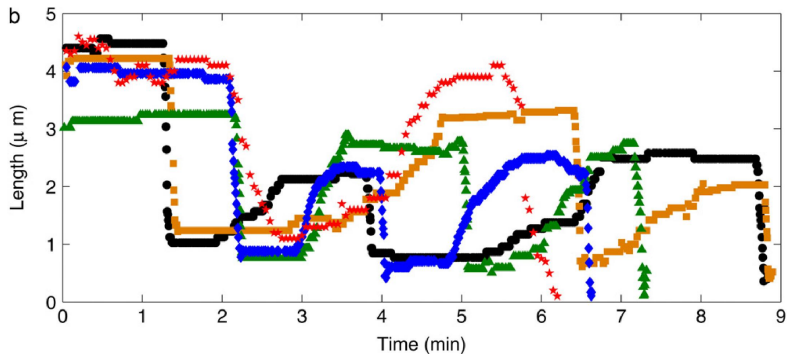
continuous (typical length of a polymer \gg length of a single monomer unit)

- ▶ H. Amann, Ph. Laurençot & S. Mischler, C. Walker & G. Simonett
- ▶ Greer *et al.* (2003), developed a model for prion formation

Mathematical models for microtubule dynamics exist at different level of details, both deterministic and stochastic, both ordinary and partial differential equations.

- ▶ Mitchison & Kirschner 1984
- ▶ Flyvbjerg *et al.* 1994, 1996
- ▶ Bolterauer, Tuszyński *et al.* 1999, Rezania *et al.* 2008

Mathematical models for microtubule dynamics



Rezania *et al.*, Biophys. J. 2008, stochastic simulations of individual microtubules

Features of our model

Our model will be fully continuous with respect to time and microtubule length and will describe a large population of microtubules, rather than individual microtubules. Hence, we will use partial differential equations for a population density.

Variables of the model (1)

Let $Y = \{(x, y) \in \mathbb{R}^2 : x > y > 0\}$, let $u(x, y, t)$ denote the density of microtubules of total length x that have a GTP cap and whose GDP domain has length y so that

$$\|u(\cdot, \cdot, t)\|_{dy dx} = \int_0^\infty \int_0^x u(x, y, t) dy dx$$

is the concentration of microtubules. The total length of GDP and GTP-bound tubulin found in microtubules with a GTP cap is given by

$$\|u(\cdot, \cdot, t)\|_{x dy dx} = \int_0^\infty \int_0^x u(x, y, t) x dy dx.$$

Variables of the model (2)

Let $v(x, t)$ denote the density of microtubules of length x without a GTP domain, their concentration is

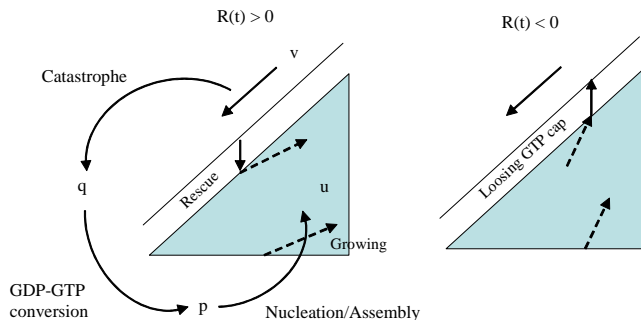
$$\|v(\cdot, t)\|_{dx} = \int_0^{\infty} v(x, t) dx,$$

and the total length of GDP-bound tubulin in collapsing microtubules is

$$\|v(\cdot, t)\|_{x dx} = \int_0^{\infty} v(x, t)x dx.$$

Finally, we need two scalar concentrations of free GTP monomers $p(t)$ and free GDP monomers $q(t)$.

Overview of the interactions



Schematic cycle of tubulin. Phases of growth and shrinking occur, depending on the present amount of free GTP tubulin $p(t)$.

Equations of the model (1)

$$\frac{\partial}{\partial t} u(x, y, t) + (\alpha p(t) - \beta) \frac{\partial}{\partial x} u(x, y, t) + \gamma \frac{\partial}{\partial y} u(x, y, t) = 0$$

- ▶ new monomers are added at rate $\alpha p(t)$
- ▶ occasionally a GTP bound monomer can be lost from the microtubule at rate β
- ▶ $\gamma > 0$ is the progression rate of the GDP zone

Characteristic curves

$$\frac{dx}{dt} = \alpha p(t) - \beta, \quad \frac{dy}{dt} = \gamma$$

The variable y can also be interpreted as the “age” of the microtubule (prior to the first catastrophe event), since hydrolysis starts immediately upon nucleation.

Boundary conditions

On $\Gamma_1 = \{(x, y) \in \mathbb{R}_{\geq 0}^2 : y = 0\}$: nucleation of microtubules without a GDP domain.

Let $\psi(x)$ be the length distribution of freshly nucleated microtubules and $L^* = \int_0^\infty x\psi(x) dx$ the average length of freshly nucleated microtubules, then

$$\gamma u(x, 0, t) = \frac{\mu}{L^*} p^n(t) \psi(x),$$

where μ is the rate of nucleation and n the order of the nucleation reaction.

Boundary conditions

On $\Gamma_2 = \{(x, y) \in \mathbb{R}_{\geq 0}^2 : x = y\}$: exit to the collapsing state or return from there. Hence Γ_2 may be part of the outflow or inflow boundary of Y , depending on whether

$$R(t) = \alpha p(t) - \beta - \gamma \begin{cases} > 0 \\ < 0 \end{cases} .$$

If $R(t) > 0$, then the system is in growth phase and microtubules without a GTP cap are rescued. The boundary condition on Γ_2 is then given by

$$R(t)u(x, x, t) = \lambda v(x, t), \quad \text{if } R(t) > 0,$$

where $\lambda \geq 0$ is the propensity of shrinking microtubules to be rescued.

Boundary conditions

If $R(t) < 0$, then the system is in shrinking phase and Γ_2 is part of the outflow boundary of the domain Y . Microtubules reaching the boundary Γ_2 are transferred to the population of microtubules without a GTP cap $v(x, t)$.

Equations of the model (2)

$$\frac{\partial}{\partial t} v(x, t) - \delta \frac{\partial}{\partial x} v(x, t) = \begin{cases} -R(t)u(x, x, t), & \text{if } R(t) < 0 \\ -\lambda v(x, t), & \text{if } R(t) > 0 \end{cases}$$

- ▶ microtubules without a GTP cap are shrinking at a rate $\delta > 0$
- ▶ if $R(t) < 0$ (the system is in a state of shrinking), microtubules that lose their GTP cap enter the population of microtubules without a GTP cap
- ▶ if $R(t) > 0$ (the system is in a state of growth), microtubules without a GTP cap are rescued and re-enter the class u

Equations of the model (3)

$$\frac{d}{dt}q = \delta \int_0^{\infty} v(x, t) dx - \kappa q$$

- ▶ GDP-bound monomers are gained by catastrophic depolymerization, and
- ▶ are converted to GTP-bound monomers at rate κ (we assume there is always an unbounded amount of GTP available)

Equations of the model (4)

$$\frac{d}{dt}p = -(\alpha p - \beta) \int_0^\infty \int_0^x u(x, y, t) dy dx + \kappa q - \mu p^n$$

- ▶ GTP-bound monomers are replenished by the conversion of GDP monomers, and
- ▶ lost due to growth and nucleation of microtubules

Conservation property

The total amount of bound and free tubulin (bound to either GDP or GTP) is conserved,

$$\frac{d}{dt} \left(\|u(t)\|_{x dy dx} + \|v(t)\|_{x dx} + q(t) + p(t) \right) = 0.$$

Proof. simple calculation, using the first equation written in divergence form

$$u_t + \nabla \cdot (\mathbf{b}(t)u) = 0,$$

with

$$\mathbf{b}(t) = \begin{pmatrix} \alpha p(t) - \beta \\ \gamma \end{pmatrix},$$

and the divergence theorem.

Parameterization

parameter	value	reference
α	$0.5 - 11.5 \mu\text{m min}^{-1} \mu\text{M}^{-1}$	Walker 1988
β	$1.6 - 35 \mu\text{m min}^{-1}$	Walker 1988
γ	$0.25 \mu\text{m min}^{-1}$	Flyvbjerg 1996
δ	$44 - 50 \mu\text{m min}^{-1}$	Walker 1988
n	1 - 12	Bolterauer 1999
μ	$5.9 \times 10^3 \text{M}^{-1} \text{min}^{-1}$	Jackson 1980
κ	3 - 120 min^{-1}	Bolterauer 1999

Experimental and/or computational estimates for parameters published in the literature (we tried to use as few sources as possible).

Numerical simulations

We use an upwind scheme with adaptive time step for the partial differential equations and the explicit Euler method for the ordinary differential equations.

We discretize Y into 500×500 cells where each cell has a dimension of $200 \text{ nm} \times 200 \text{ nm}$.

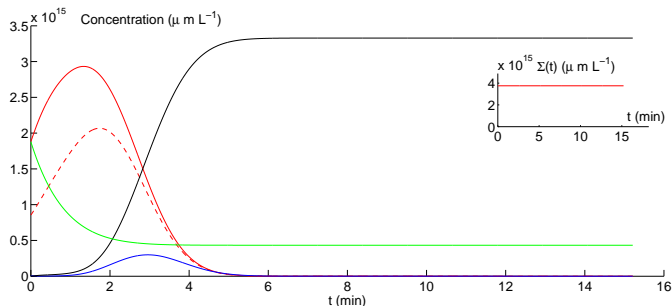
Initial conditions

Unless stated otherwise, initial data are

$$u^0(x, y) = c \exp\left(-\frac{(x - 10)^2}{5^2} - \frac{(y - 5)^2}{2.5^2}\right),$$

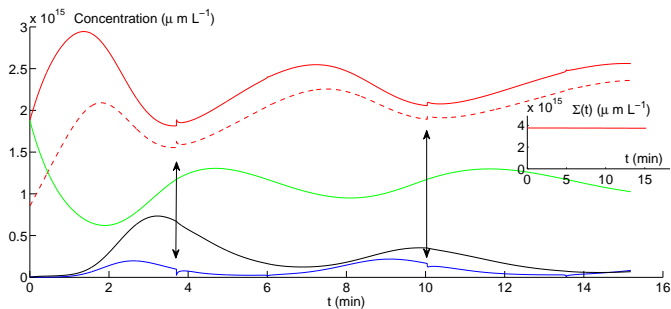
where the constant c is chosen such that $\|u^0\|_{x dy dx} \equiv 5 \mu M$, this is half the concentration of the total bound tubulin. The initial concentration of free GTP-bound tubulin is $p^0 \equiv 5 \mu M$. The remaining two initial data are chosen to be 0.

Simulation results (1)



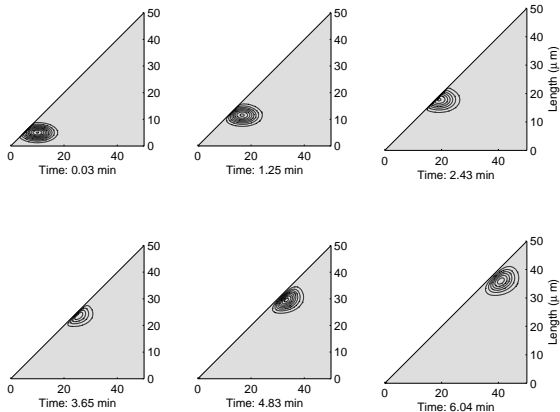
Time evolution of microtubules in u (solid red curve) and v (blue curve) pools, and tubulin dimers in p (green curve) and q (black curve) pools for the parameter set $\alpha = 2.5 \mu\text{m min}^{-1} \mu\text{M}^{-1}$, $\beta = 2.4 \mu\text{m min}^{-1}$, $\gamma = 5.4 \mu\text{m min}^{-1}$, $\delta = 50 \mu\text{m min}^{-1}$ and $\mu = \kappa = \lambda = 0$ (no nucleation, recycling and rescue).

Simulation results (2)



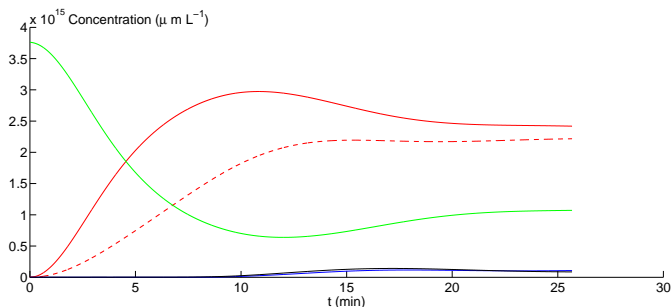
As before, but for the parameter set $\mu = 5.9 \times 10^{-4} \mu\text{M}^{-1} \text{min}^{-1}$, $\kappa = 1 \text{min}^{-1}$ and $\lambda = 0.136 \text{min}^{-1}$. Arrows indicate rescue events, the conservation of the total amount of tubulin is shown in the inset plot.

Simulation results (3)



Time evolution of the population density u (equidensity contours beginning from a Gaussian profile).

Simulation results (4)



Same parameter values as before but with initial conditions $u^0 \equiv 0$ and $p^0 = 10\mu\text{M}$ (all growth starts from nucleation).

We have proposed a deterministic model at the population level that includes *all* processes taking place during microtubule polymerization/depolymerization, namely: growth, nucleation, catastrophic shrinkage and rescue events.

We are able to reproduce commonly seen dynamical behaviors, such as

- ▶ complete depolymerization in case of lacking recycling of GDP-monomers, and
- ▶ damped oscillations in a growing population.

Open questions (future work)

- ▶ What is the width of the GTP zone? Is it constant, regardless of the growth of the microtubule? Is hydrolyzation of bound GTP tubulin a catalyzed reaction?
- ▶ How do the dynamics change *in vivo*? How to incorporate microtubule associated proteins (MAPs) into the model?
- ▶ Mathematical challenges: we have a nonlinear transport equation with nonlinear boundary conditions. Does it admit periodic solutions?

Another mathematical challenge

Our model is hyperbolic in nature, without a “length diffusion” term $\frac{\partial^2 u}{\partial x^2}$. However, such a term has been included in earlier partial differential equations models (Flyvbjerg *et al.* 1994, 1996).

Collet *et al.* (2002) and Laurençot and Mischler (2002) discussed convergence of the solutions of the discrete Becker-Döring system to solutions of the continuous Lifshitz-Slyozov equation (without diffusion) under certain scaling assumptions.

Acknowledgments

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Thank you for your attention

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