Mathematical modeling of the microtubule dynamic instabilities.

Florence HUBERT
Aix-Marseille University

Control of PDE and Applications, CIRM

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Collaborators

I2M

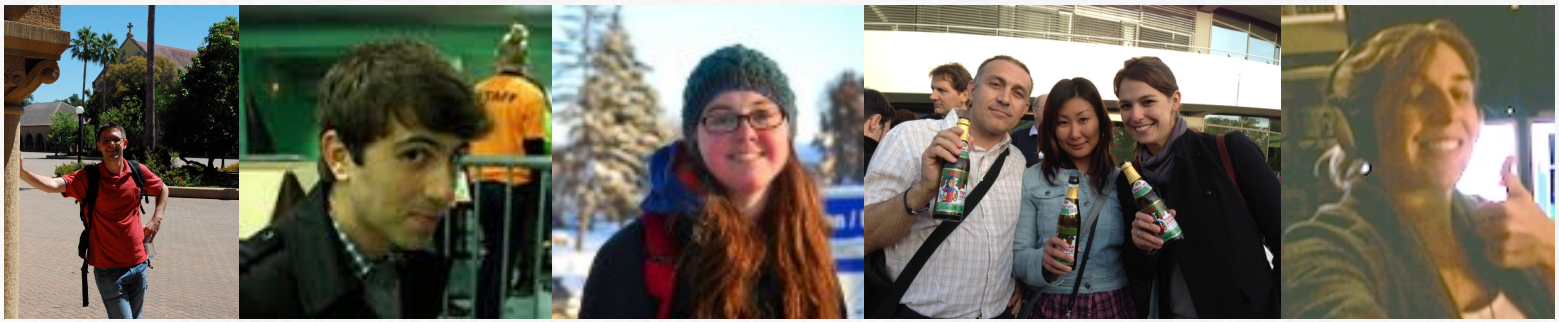
Institut de Mathématiques de Marseille

- Ayuna Barlukova (PhD student)
- Christophe Gomez (MCF)
- Rémi Tesson (PhD student)
- Diana White (Post-doct)

CRO2

Center of Oncology and onco-pharmacology

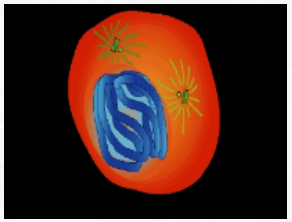
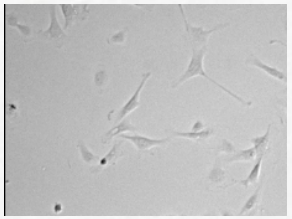
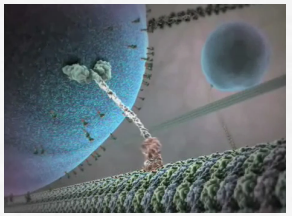
- Stéphane Honnoré (MCF-PH)
- Marie Petit (PhD student)
- Sarah Oddoux (Post-doct)



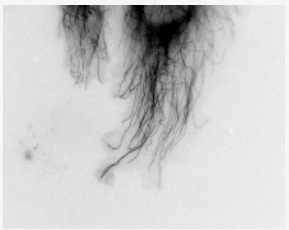
Microtubules

A therapeutic target in oncology

- MTs play a crucial role in
  - **cell division**
  - **cell migration**
  - **intracellular transport**

- MTs are a favorite target of **Microtubule Targeting Agents (MTAs)**
- **MTAs** (taxanes, vinca alkaloids) are successfully used as **antimitotic and antiangiogenic agent** in cancer treatments but also in neurodegenerative diseases.
- MTs are **highly dynamic**.
  - The dynamics is complex
  - The dynamics is mandatory to cell division and cell migration.



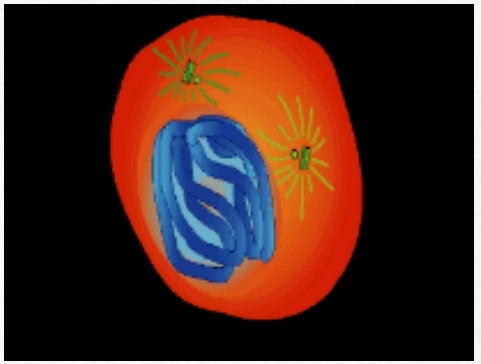
MT instabilities and cell division

The role of MTs

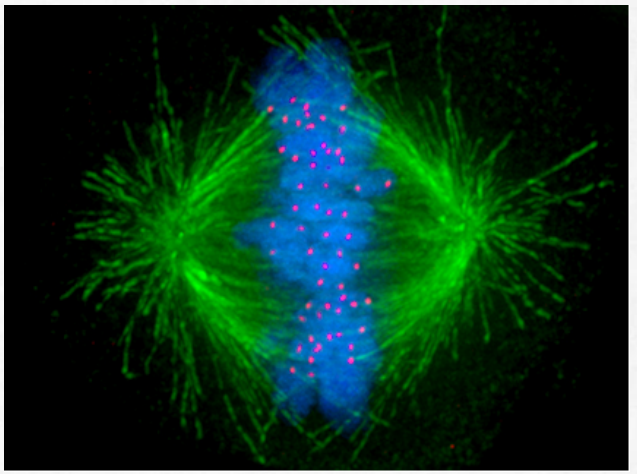
- **Prometaphase and metaphase.**
  MTs dynamics is increased.
  - Find and capture kinetochore.
  - Chromosome's congression.
- **Anaphase**
  Stabilization of MTs
  - Chromosome's separation.

Roles of MTAs

- Increase or reduction of the dynamics induce mitotic abnormalities and thus apoptose.



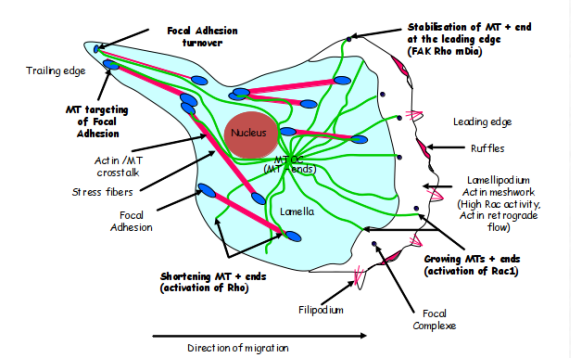
http://www.wadsworth.org



Wikipedia

MT and migration

MTs dynamics and protein activation

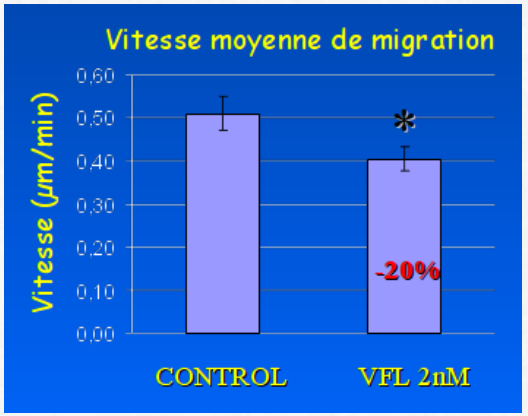


- Growing of MTs induces activation of RAC. $\rightsquigarrow$ High RAC activity promotes the actine retrograd flow in the lamellipodium.
- Shortening of MTs induces activation of RHO. $\rightsquigarrow$ Presence of RHO promotes contraction of stress fiber at the back of the cell.

MTAs and migration

- MTAs reduce endothelial migration even at non-cytotoxic concentration.

$\Rightarrow$ Antiangiogenic effect at low dose.



Pourroy & al 2006

Objectives

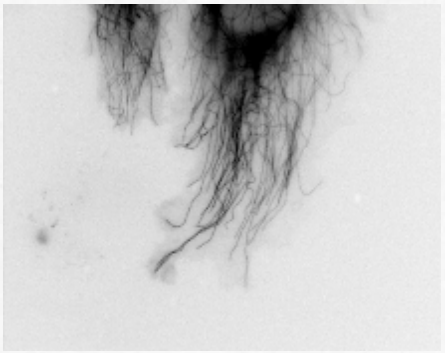
Main issues of our collaboration

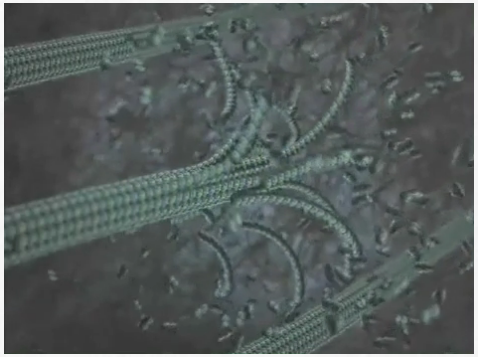
- To describe the dynamics thanks to a mathematical model.
- Better understand the role of each reaction in the dynamics and their synergy.
- Better understand the mechanism of action of each faily of MTas.

Microtubule structure

MT in the cell

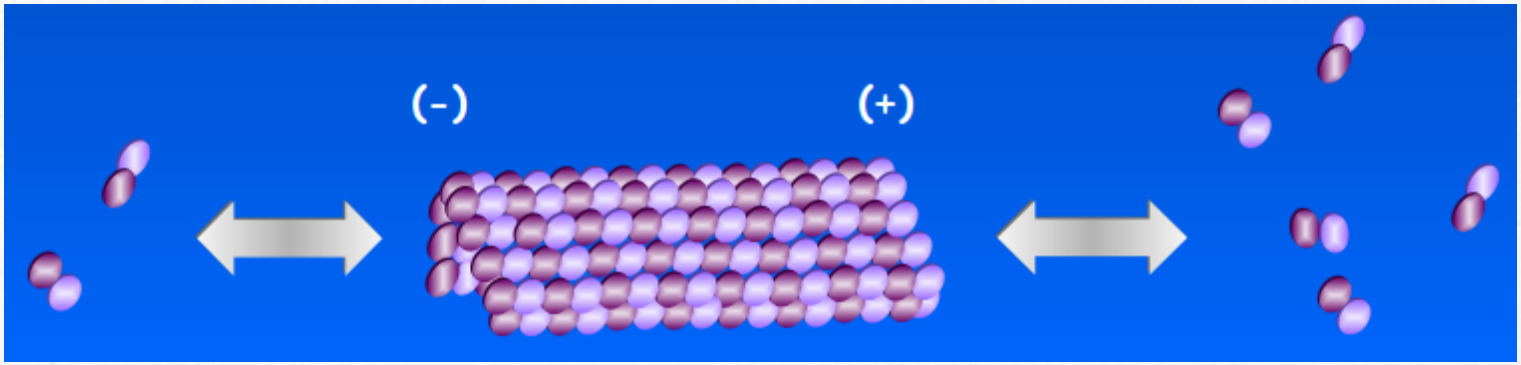
- MTs are part of the cytosqueleton.
- MTs are caracterized by their instabilities.



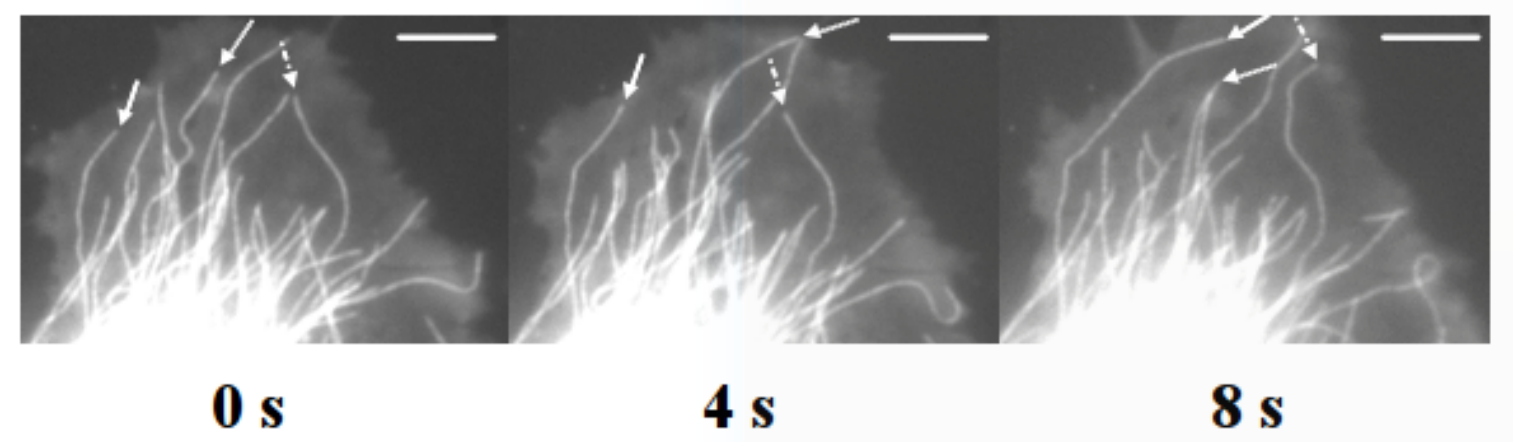


Protein structure

- Each MT is a long (up to 50μm) hollow cylinder of 25nm diameter built from about 13 protofilaments.
- Each protofilament is composed by an assembly of $\alpha|\beta$ tubulin dimers.
- The assembly is polarized with different dynamics at the + end or - end.

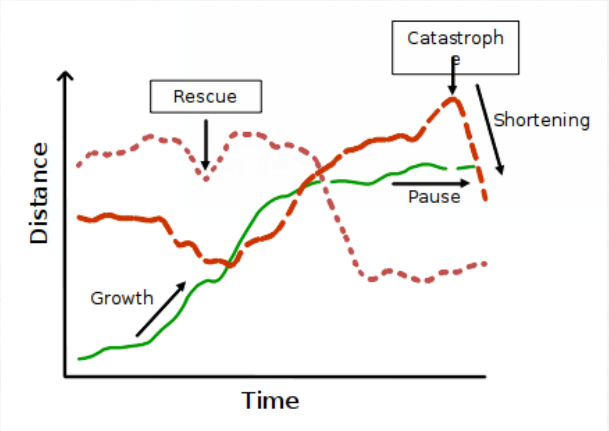


Microtubules instabilities

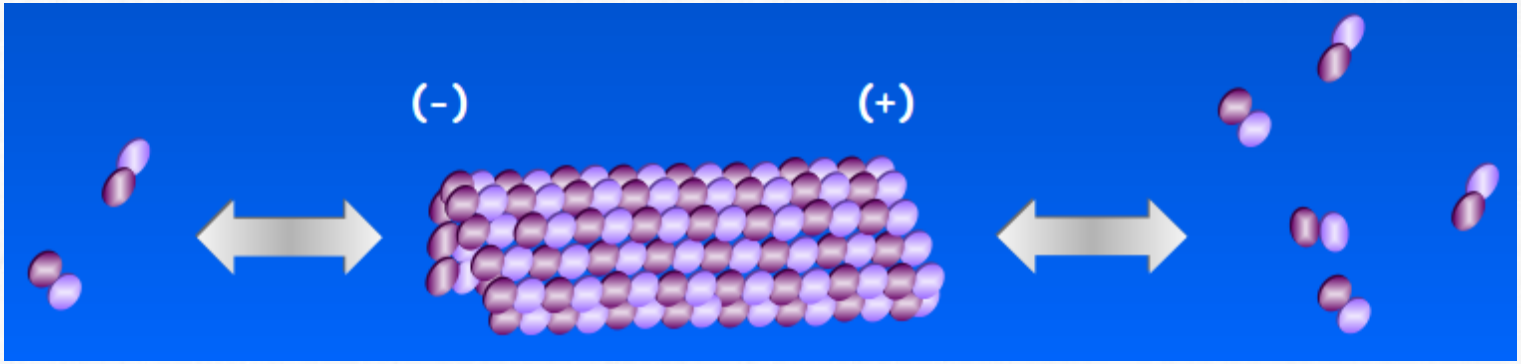


Dynamics overview

- Phase of growing are followed by phases of sudden shortening called **catastrophe.**
- **Phases of catastrophe** are followed by **phases of Rescue**



Dynamics of one MT



Protein structure

- Each MT is a long (up to 50μm) hollow cylinder of 25nm diameter built from about 13 protofilaments.
- Each protofilament is composed by an assembly of $\alpha|\beta$ tubulin dimers.
- The assembly is polarized with different dynamics at the + end or - end.
  - + End (tubulin β) : highly dynamic
  - − End (tubulin α) : link to centrosome in cells

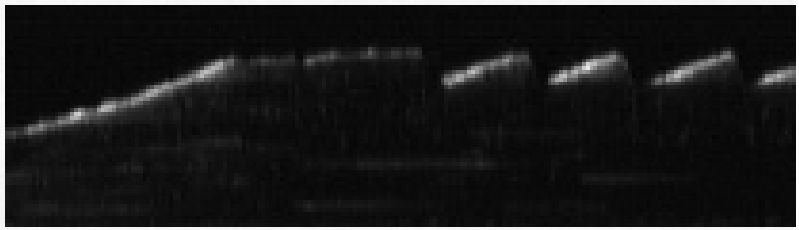
Energetic structure

- Dimers can be in two energy states :
  - GTP : Guanosine **tri**phosphate - active form
  - GDP : Guanosine **di**phosphate - inactive form

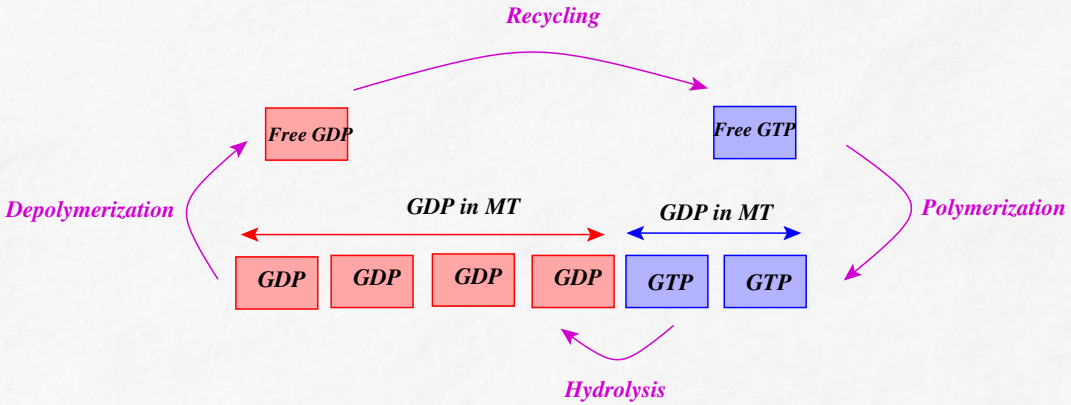
Dynamics of one MT at its + end

Dimers of tubulin

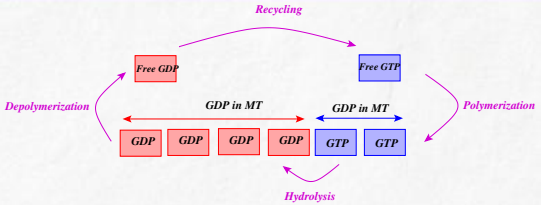
- Dimers can be in two energy states :
  - GTP : Guanosine **tri**phosphate - active form
  - GDP : Guanosine **di**phosphate - inactive form
- Dimers can be polymerized or not. In fine,
  - GTP polymerized in MTs
  - GDP polymerized in MTs
  - Free GTP
  - Free GDP
- Biological observations obtained by the use of End Binding GTP proteins :
  - Existence of a GTP-stabilizing cap
  - Disparition of the cap at the catastrophe



- Four reactions

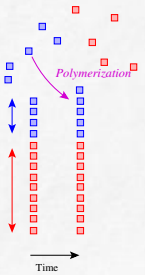


Dynamics of one MT at its + end



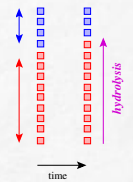
Polymerization

- Free GTP can polymerized at the + end.
  - It creates a GTP stabilizing cap at the + end of the MT.
  - The velocity of polymerization is an increasing function of the free GTP available.



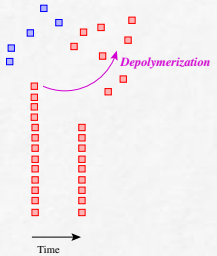
Hydrolysis

- GTP cap can be hydrolyzed into GDP .
  - This reaction induces shortening of the cap.
  - Aging of MTs plays a role in hydrolysis.



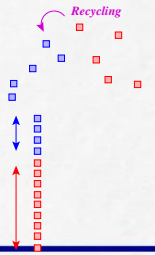
Depolymerization (Catastrophy)

- Once the cap is lost the MT depolymerizes suddenly.
  - Free GDP is reintroduced in the media.



Recycling

- Free GDP can be recycled into free GTP .
  - This free GTP is then available for polym.
  - Resumption of polymerization is called Rescue.

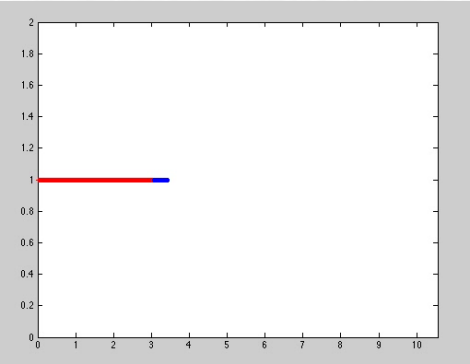


Some mathematical models

Stochastic approach

- Enable to reproduce the dynamics of one protofilament

Hinow & al 2011



Approach followed by C. Gomez.

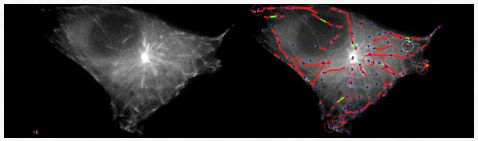
Possibility to take into account MTAs.

Deterministic approach

- Used to follow the mean behaviour of a MTs family.

Hinow & al 2009

- Example in cell :



Oddoux & al, Mean parameters obtain thanks to PlusTiptracking software

- Example in vitro : oscillations observed.

Petit & al, experiment in progress

.

Mathematical challenges

Improve Hinow & al 2009 approach

1. Change polymerization velocity profile.
2. To take into account **adging of MTs** and better represent Catastrophy's frequencies as evaluated by our pharmacologist.
   - This would enable us to model MTAs.

Phd work of A. Barlukova

3. To take into account the influence of End Binding Protein (EB1 and EB3) on MTAs efficiency.

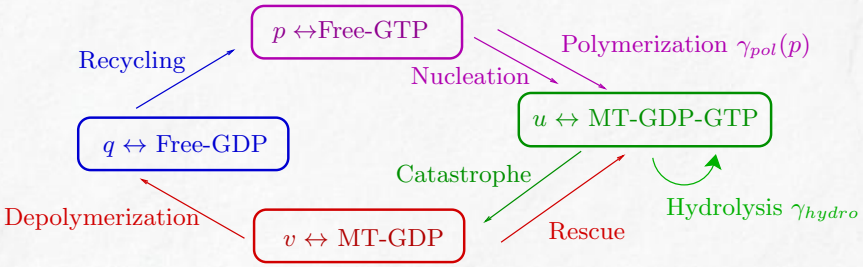
Postdoctoral work of D. White

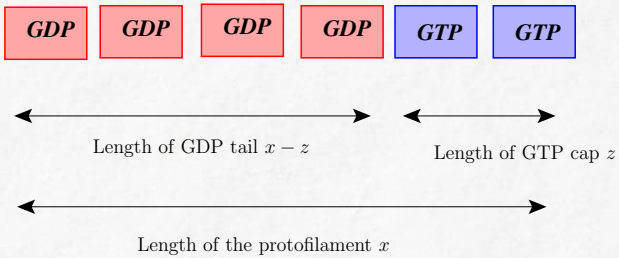
MTs and migration

3. Take into account MT impact on cell migration.

PhD work of R. Tesson

Hinow & al 2009 approach





The unknowns

1. $u(t, z, x)$ density of MTs with a cap at time t with a length x and a cap of length z.
   - Domain : $\{(t, z, x)$ such that $t \geq 0,\ 0 \leq z \leq x\}$.
   - Boundaries :

$$\begin{aligned} \Gamma_{nucl} &= \{(t, z, x) \text{ such that } t \geq 0,\ 0 \leq z = x\} \\ \Gamma_{cata} &= \{(t, z, x) \text{ such that } t \geq 0,\ 0 = z \leq x\} \\ \Gamma_{init} &= \{(t, z, x) \text{ such that } t = 0,\ 0 \leq z \leq x\} \end{aligned}$$

2. $v(t, x)$ density of MT in depolymerization at time t with a length x.
   - Domain : $\{(t, x)$ such that $t \geq 0,\ 0 \leq x\}$.
3. $p(t)$ free GTP tubulin available at time t.
4. $q(t)$ free GDP tubulin available at time t.

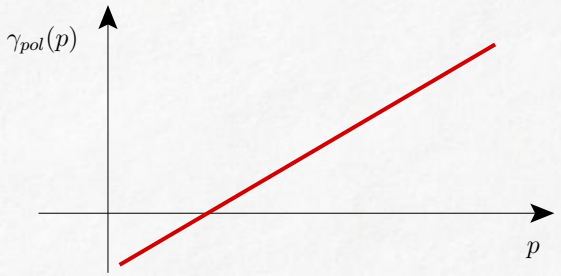
Hinow & al 2009 approach

Equation for u

$$\partial_t u + (\underbrace{\gamma_{pol}(p(t))}_{\text{Polymerization rate}} - \underbrace{\gamma_{hydro}}_{\text{Hydrolysis rate}})\partial_z u + \underbrace{\gamma_{pol}(p(t))}_{\text{Polymerization rate}} \partial_x u = 0$$

This equation reflects :

- Polymerization of MTs with a velocity γ_{pol} depending on $p(t)$:



- Hydrolysis where γ_{hydro} is assumed to be constant.

Hinow & al 2009 approach

Equation for u

$$\partial_t u + (\underbrace{\gamma_{pol}(p(t))}_{\text{Polymerization rate}} - \underbrace{\gamma_{hydro}}_{\text{Hydrolysis rate}})\partial_z u + \underbrace{\gamma_{pol}(p(t))}_{\text{Polymerization rate}} \partial_x u = 0$$

This equation reflects :

- Polymerization of MTs with a velocity γ_{pol} depending on $p(t)$:
- Hydrolysis where γ_{hydro} is assumed to be constant.

Boundary conditions for u

- On Γ_{nucl}, The sign of the entrance flux $B \cdot \begin{pmatrix} -1 \\ 1 \end{pmatrix} = \gamma_{hydro} > 0$ is positive

$$u(t, x, x) = \mu \Psi(x) p(t)^2.$$

- On Γ_{cata}, the sign of the entrance flux $B \cdot \begin{pmatrix} 0 \\ 1 \end{pmatrix} := R(t)$ depends on the sign of $R(t) = \gamma_{pol}(p(t)) - \gamma_{hydro}$

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

Hinow & al 2009 approach

Equation for u

$$\partial_t u + \gamma_{pol}(p(t))\partial_x u + (\gamma_{pol}(p(t)) - \gamma_{hydro})\partial_z u = 0$$

Equation for v

$$\partial_t v - \gamma_{depol}\partial_x v = -\lambda v(R(t) > 0) - R(t)^- u(t, 0, x)$$

This equation reflects :

- Depolymerization of MTs with a velocity γ_{depol} assumed to be constant.
- Catastrophy/Rescue events

Equation for p

$$\frac{d}{dt}p = -\gamma_{pol}(p(t)) \int_0^\infty \int_0^x u(t, z, x)\, dz dx + \kappa q - \mu p^2$$

Equation for q

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

Hinow & al 2009 approach

Conservation of total tubulin

$$\frac{d}{dt}\left(L_u(t) + L_v(t) + p(t) + q(t)\right) = 0$$

where

- Total length of MTs with cap : $L_u(t) = \int_0^\infty \int_0^x xu(t,z,x)dz\,dx$
- Total length of MTs in depol : $L_v(t) = \int_0^\infty xv(t,x)dz\,dx$

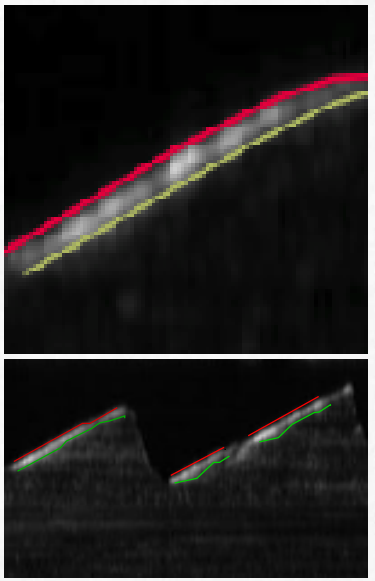
Adging of MTs

Frequence of catastrophe in vitro increases with age of MT

Gardner & al , Cell 2011

Kymograph of a MT

Visuation of time evolution of the cap of a MT marked thanks to EB protein .



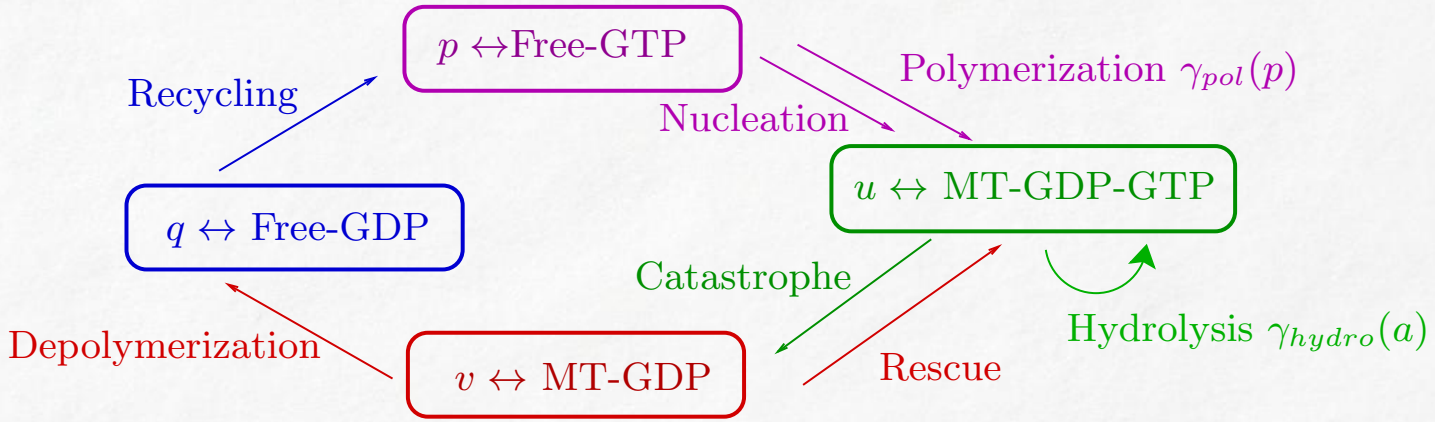
- Stable growth speed away from catastrophe
  - $\rightsquigarrow$ Change the profile of γ_{pol}.
- Presence of alterations in the cap
  - $\rightsquigarrow$ Change the profile of γ_{hydro}.

Assumption (A new approach of hydrolysis)

- *MTs undergo degradations that stimulates hydrolysis.*
  - $\rightsquigarrow$ γ_{hydro} *may depend on an age of MT.*
- *Existence of a delay between incorporation in MT and hydrolysis (decoration time).*

A new model of MT instabilities

A. Barlukova PhD work



MTs in polymerization

- Density of the population of MT in polymerization $u = u(t, a, z, x)$
  - t time, a age, x length, z length of the cap.
- Density of the population of MT in depolymerization $v = v(t, x)$
  - t time, x length.
- Amount of Free GTP tubulin $p = p(t)$
- Amount of Free GDP tubulin $q = q(t)$

A new model of MT instabilities

A. Barlukova PhD work

Balance equation for MT in Polymerization u

$$\partial_t u + (\underbrace{\gamma_{pol}(p(t))}_{\text{Polymerization rate}} - \underbrace{\gamma_{hydro}(a)}_{\text{hydrolysis rate}})\partial_z u + \underbrace{\gamma_{pol}(p(t))}_{\text{Polymerization rate}} \partial_x u + \partial_a u = 0$$

Boundary conditions for u

- **Nucleation**,

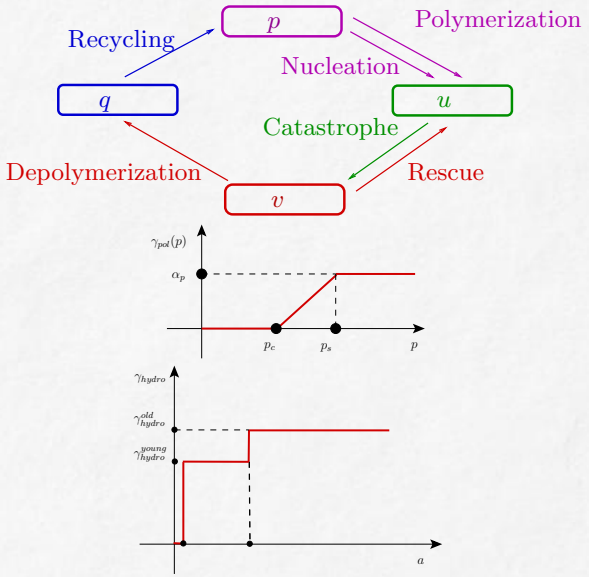
$$u(t, a, x, x) = \psi(x)\Psi(a)\mathcal{N}(p(t)).$$

- **Rescue event**, if the entrance flux
  $R(t, a) = \gamma_{pol}(p(t)) - \gamma_{hydro}(a) > 0$

$$R(t, a)u(t, a, 0, x) = \lambda\Theta(a)v(t, a)$$

- **Age boundary**

$$u(t, 0, z, x) = 0$$



A new model of MT instabilities

A. Barlukova PhD work

Equation for MT in depolymerization v

$$\partial_t v \underbrace{-\gamma_{depol}\partial_x v}_{\text{Depolymerization}} = \underbrace{-\lambda v \int_a (R(t,a) > 0)\Theta(a)\, da}_{\text{Rescue event}} + \underbrace{\int_a R(t,a)^{-} u(t,a,0,x)\, da}_{\text{Catastrophe event}}$$

Equation for free GTP p

$$\frac{d}{dt}p = -\gamma_{pol}(p(t)) \int_0^\infty \int_0^x \int_0^\infty u(t,a,z,x)\, dadzdx + \underbrace{\kappa q}_{\text{Recycling}} \underbrace{-\mu\mathcal{N}(p)}_{\text{Nucleation}}$$

Equation for free GDP q

$$\frac{d}{dt}q = \underbrace{\gamma_{depol} \int_0^\infty v(t,x)\, dx}_{\text{Depolymerization}} \underbrace{-\kappa q}_{\text{Recycling}}$$

Properties of the new model

Conservation of total tubulin

$$\frac{d}{dt}\left(L_u(t) + L_v(t) + p(t) + q(t)\right) = 0$$

where

- Total length of MTs with cap : $L_u(t) = \int_0^\infty \int_0^x \int_0^\infty x u(t,a,z,x) da\, dz\, dx$
- Total length of MTs in depol : $L_v(t) = \int_0^\infty x v(t,x) dz\, dx$

Frequence of catastrophe

$$F_{cat}^{temp}(t) = \frac{\int_0^\infty \int_0^\infty \chi \frac{1}{a} u(t,a,0,x)\, da\, dx}{\int_0^\infty \int_0^\infty \chi u(t,a,0,x)\, da\, dx},\ F_{cat}^{spa}(t) = \frac{\int_0^\infty \int_0^\infty \chi \frac{1}{x_a} u(t,a,0,x)\, da\, dx}{\int_0^\infty \int_0^\infty \chi u(t,a,0,x)\, da\, dx},$$

$$x_a = \int_0^a \gamma_{pol}(p(t-a+s))\, ds,\ \chi = (R(t,a,x,0) < 0)$$

Numerical approximation

- Finite volume approach in z, x
- Semi-lagragian in a
- Adequat approximation of integral terms to preserve tubulin at the discrete level.

How to calibrate parameters ?

$$\alpha_{pol},\ p_c,\ p_s,\ \gamma_{hydro}^{young},\ \gamma_{hydro}^{new}, \gamma_{depol},\ \kappa,\ \lambda,\ \mu, a_s \ldots$$

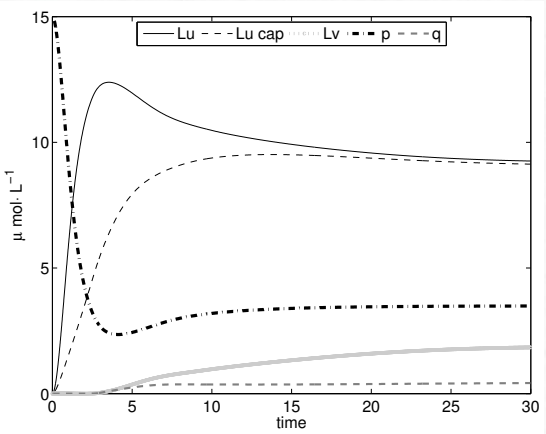
Observed data

- Mean growth speed $\rightsquigarrow$ mean value of γ_{hydro}
- Mean shortening speed $\rightsquigarrow$ γ_{depol}
- Frequence of catastrophe (temporal or spacial)
- Frequence of rescue (temporal or spacial)
- Mean size of the cap
- Mean value of the size of MT
- On kymograph : $\gamma_{pol}(p)$, γ_{hydro}^{young}, γ_{hydro}^{new}
- ....

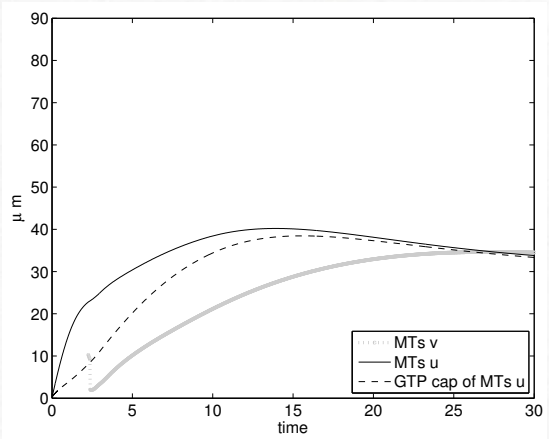
	Growth rate (µm/min)	Shortening rate (µm/min)	Catastrophe Fr. (per min)	Rescue Fr. (per min)	Catastrophe Fr. (per µm)	Rescue Fr. (per µm)	N (MTs)
Control	3.87 ± 1.00	19.09 ± 16.03	1.72 ± 0.12	2.12 ± 0.29	0.47 ± 0.03	0.12 ± 0.01	62
Patupilone 1 nM	3.47 ± 0.71	36.36 ± 23.44	1.60 ± 0.12	4.03 ± 0.47	0.47 ± 0.03	0.23 ± 0.02	55
Patupilone 10 nM	3.67 ± 1.16	21.04 ± 15.95	1.89 ± 0.14	3.44 ±0.44	0.55 ± 0.04	0.19 ±0.02	53
Patupilone 100 nM	2.92 ± 1.42	17.93 ± 12.86	2.07 ± 0.19	3.46 ± 0.58	0.85 ± 0.08	0.22 ± 0.03	30
Paclitaxel 1 nM	4.13 ± 1.47	17.87 ± 11.24	2.10 ± 0.17	3.79 ± 0.49	0.58 ± 0.04	0.23 ± 0.03	37
Paclitaxel 10 nM	4.89 ± 1.56	21.30 ± 15.85	2.62 ± 0.20	5.77 ± 0.63	0.58 ± 0.045	0.33 ± 0.03	38
Paclitaxel 100 nM	5.99 ± 1.54	28.12 ± 25.85	2.96 ± 0.19	4.57 ± 0.50	0.50 ± 0.033	0.18 ± 0.02	50

Numerical output of the model

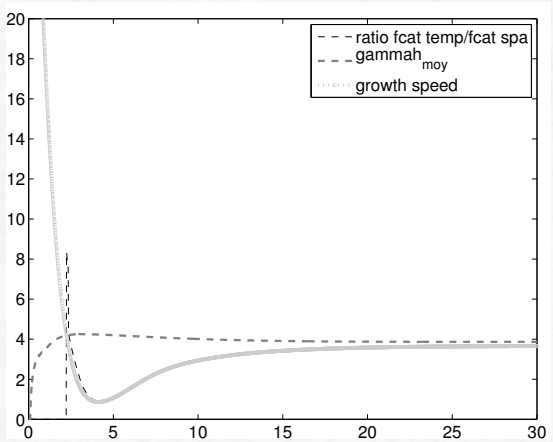
A control test



Global behaviour



In silico kymograph



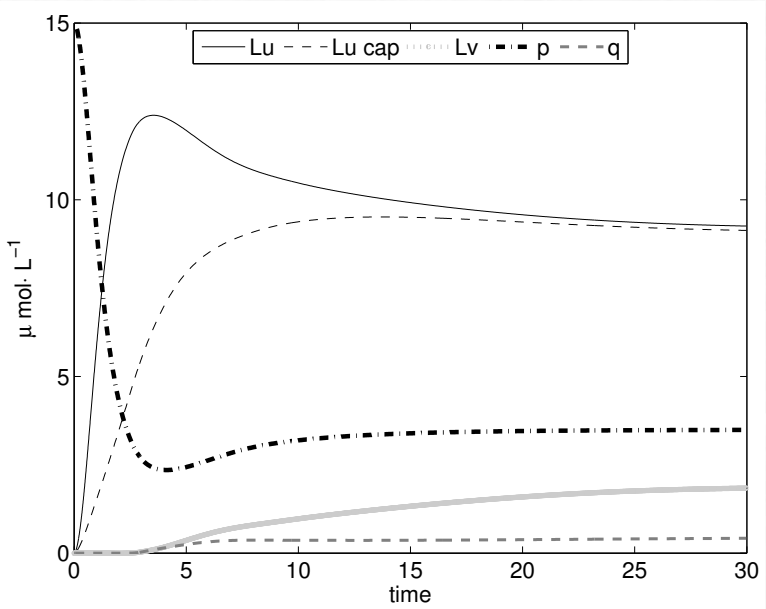
Link between growth speed, γ_h, Freq of cata

	Growth rate	Shortening rate	Catastrophe Fr (per min)	Catastrophe Fr (per μm)
Pagano & al	3.87±1	19.09 ±16	1.72 ±0.12	0.47 ±0.03
In silico	3.86	19 (= δ, fixed)	1.49(F)-1.85(M)	0.4(F)-0.78(M)

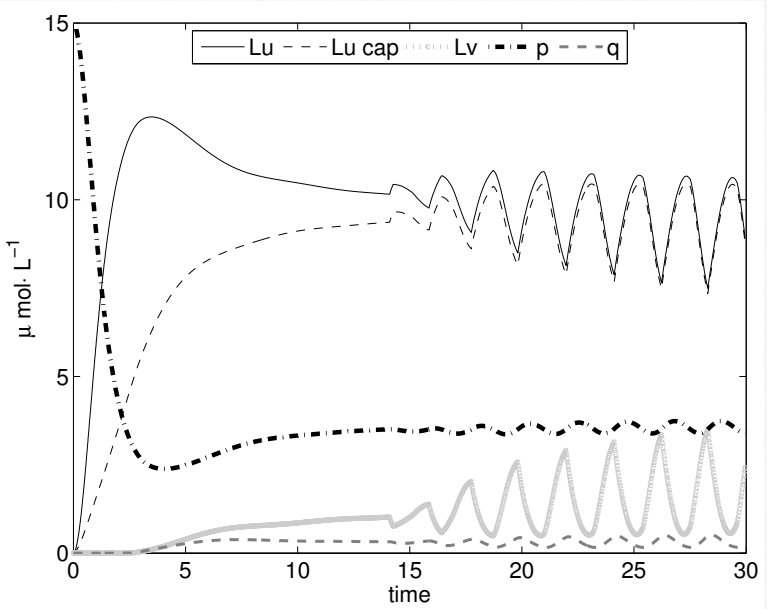
Impact of MTAs

Modification of the decoration time

parameter	α_p	p_c	p_s	γ_h^{young}	γ_h^{old}	μ	λ	κ	a_s
Control	32	2	15	3.7	4.3	$1.5e-18$	2	2.4	1



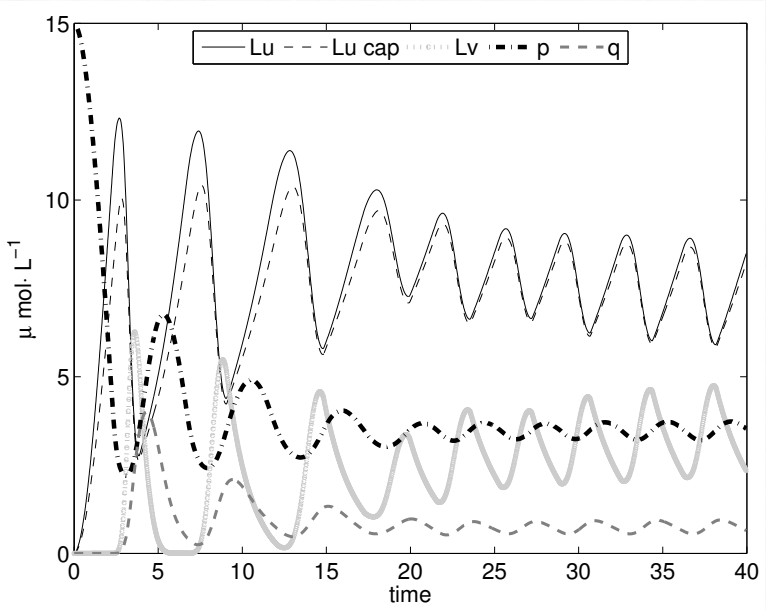
Control $a_{deco} = 0.1$



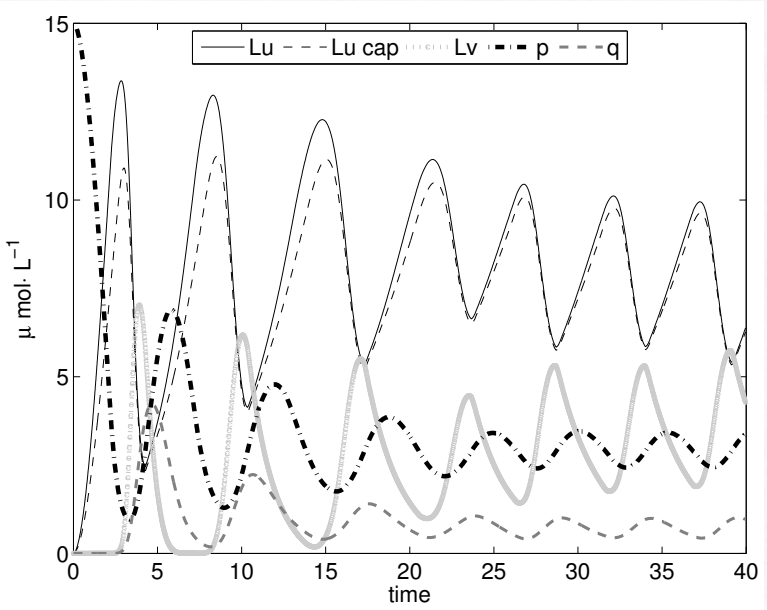
$a_{deco} = 0.02$

Impact of MTAs

Examples with high growth speed



Test 1



Test 2

parameter	α_p	p_c	p_s	γ_h^{young}	γ_h^{old}	μ	λ	κ	a_s
Test 1	10	2	4	7.4	7.6	$1.5e-18$	0.136	1.2	1
Test 2	11	0.5	4	7.5	8	$1.5e-18$	0.136	1.2	1

Conclusion/Perspectives

MTas

- At high dose, MTas have a cytotoxic effect.
  - ⇝ To do : Go further in the calibration of parameters.

Catastrophe model

- Weakness of the model : depolymerizations are not sudden enough
  - ⇝ In progress : Taken into account catastrophes via fragmentation models.

End binding proteins

- EB seems to promote MTas efficency.
  - ⇝ In progress : Take into account EB in the deterministic model. or in the stocchastic model. D. White Post doct work, C. Gomez work with M. Petit CRO2

Migration

- Cell migration modeling

CEMRACS 15 work with J. Olivier, O. Theodoly, A. Trescases, M. Jedouaa, I. Khames

- Control the migration of the cells thanks to MT dynamics.

Rémi Tesson PHD work

Resistance

- Chimiotherapies are known to induce resistence phenomenon.
  - A new hypothesis is to say that sensible cells may control resistent cells.

PhD work of C. Carrère, advised by A. Benabdallah, G. Chapuisat, coll. with M. Carré

Thank you very much

Some advertisement !

Workshop math-cancer in CIRM December 7th-11th 2015

`http://math-cancer-cirm2015.math.cnrs.fr/`

Inscriptions are still open !