Modelling nucleocytoplasmic transport with application to the intracellular dynamics of the tumor suppressor protein p53

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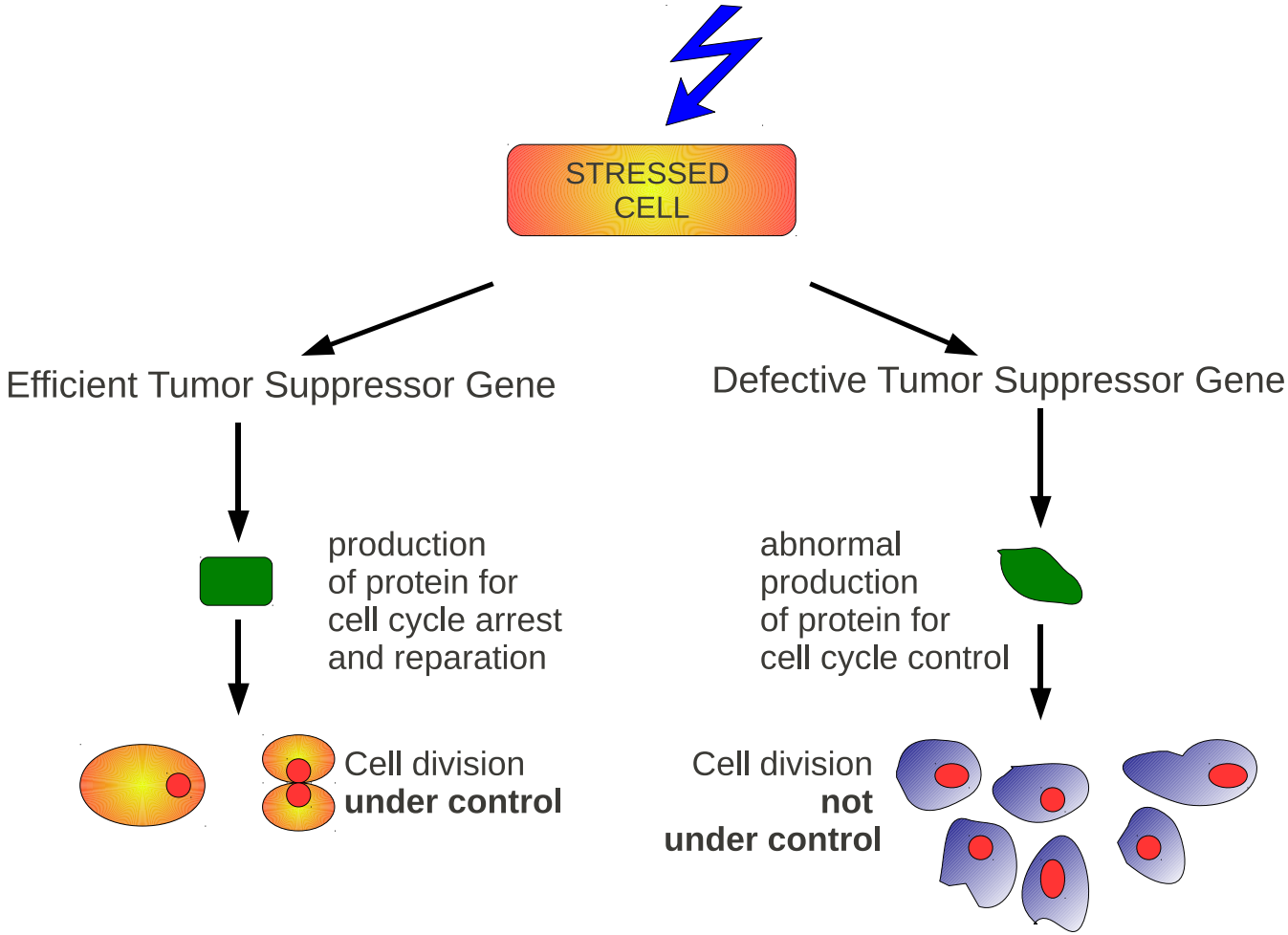
Summary

1. **A model for p53 intracellular dynamics**
   - biology of p53 - basics
   - a new model to reproduce its dynamics

2. **A model for protein transport within the cell**
   - biology of intracellular transport - basics
   - locating a single microtubule

What is p53?

In 1979 a protein of molecular mass of 53 kDa was isolated. It was named **p53**.

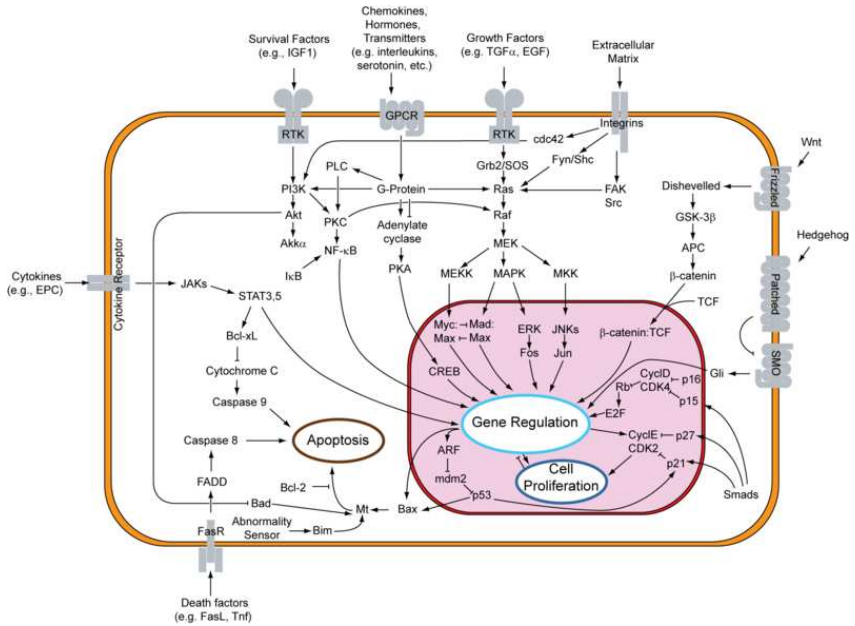


p53 roles: *the Guardian of the Genome*

After a stress p53 acts as a **transcription factor**:

- **blocks the cell cycle** progress.
- **repairs the DNA**.
- **launches apoptosis** (programmed cell death).

It has a **huge** network of interactions- hard to model!

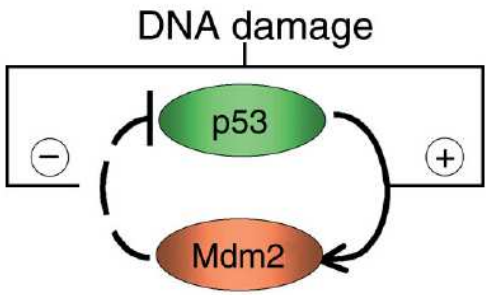


Healthy or Stressed cell

In healthy cells p53 is **dangerous**,
Mdm2 keeps a balanced cellular level of p53.

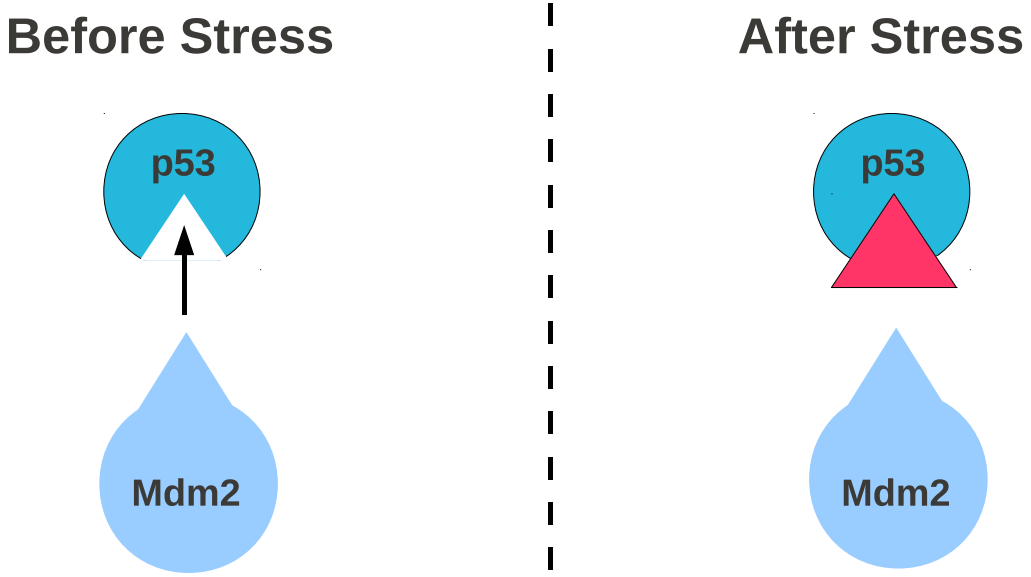
- Mdm2 induces **degradation** of p53 and **blocks** its nuclear import.
- p53 **transcribes** the mRNA of Mdm2.

In stressed cells p53 concentration rises to **prevent** the transmission of harmful **mutations**.



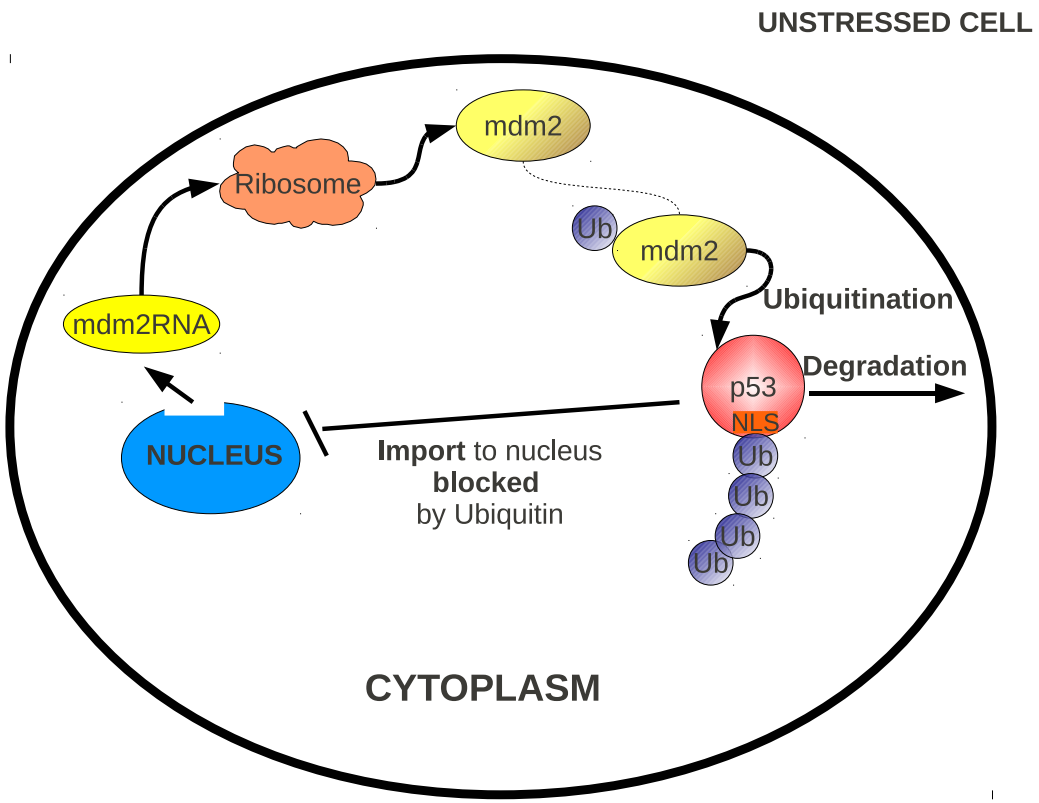
Two different "states"

Healthy cells or **Stressed cells**



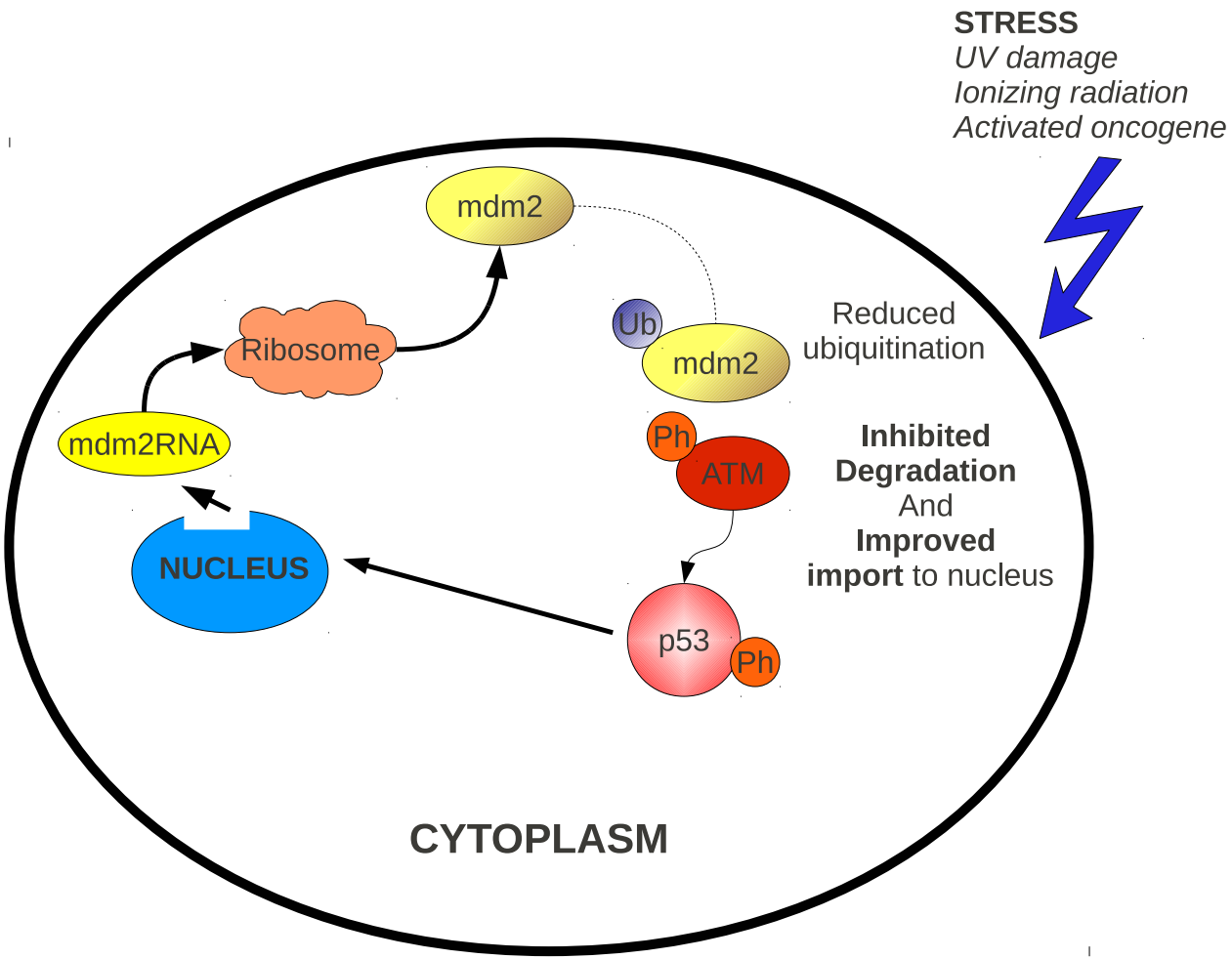
How to switch from a state to the other?

Healthy cells: **blocked import + increased degradation**



How to switch from a state to the other?

Stressed cells: modifications block p53-Mdm2 interactions.
Principal factor in case of DNA damage: **ATM**



p53 dynamics

the p53-Mdm2 network has an **oscillatory** behavior

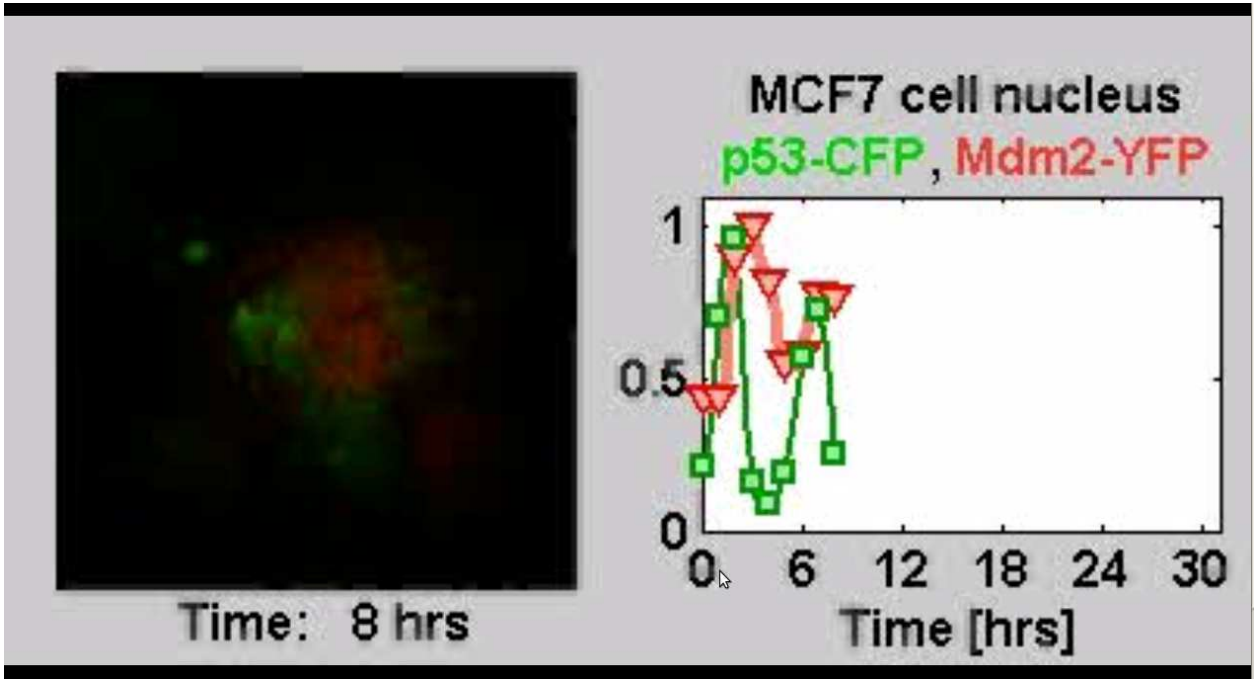


Figure: *in vitro* experiments

A time-lapse movie of one cell nucleus after exposure to a $5Gy$ gamma dose of a MCF7 breast cancer cell line

Oscillations and variability in the p53 system *Geva-Zatorsky et al.*, Molecular Systems Biology 2006

doi : 10.1038/*msb*4100068

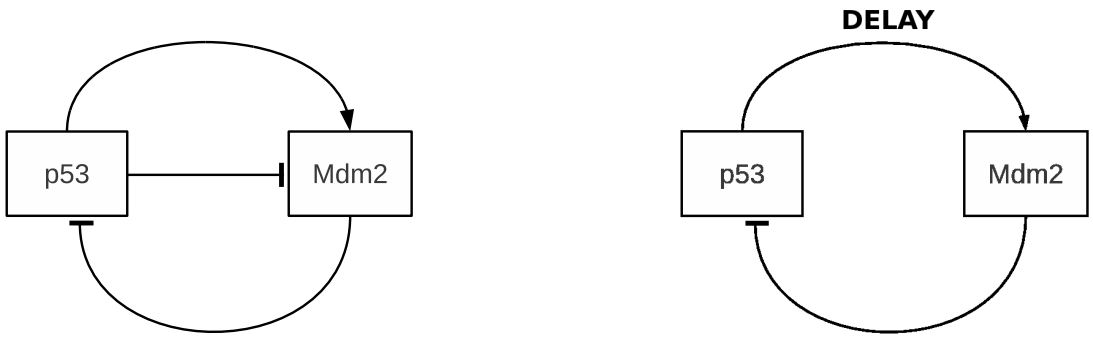
Mathematical models of p53

Why study p53?

- explain oscillations (which mechanism): **HOW?**
- understanding its behaviour: **WHY?**

Literature **ODE** models $\rightsquigarrow$ **mean** concentrations - depend on **time**

- Use **delay**: $\frac{du}{dt}(t) = f(t - \tau)$
- Use negative and positive **feedback**.

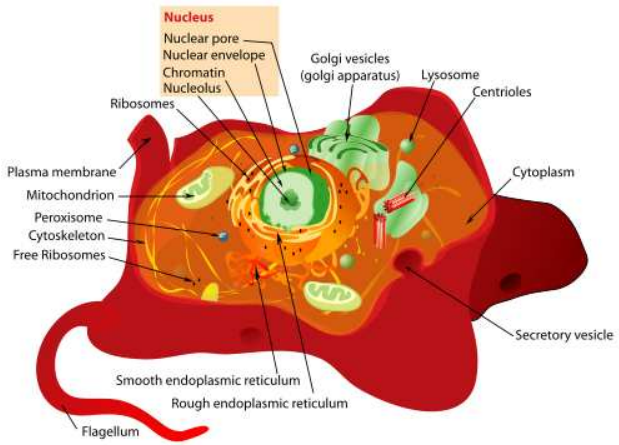


Lev-Bar-Or et al. 2001, *Monk et al.* 2003, *Ma et al.* 2005, *Ciliberto et al.* 2005, *Chickarmane et al.* 2007, *Ouattara et al.* 2010

Mathematical models of p53

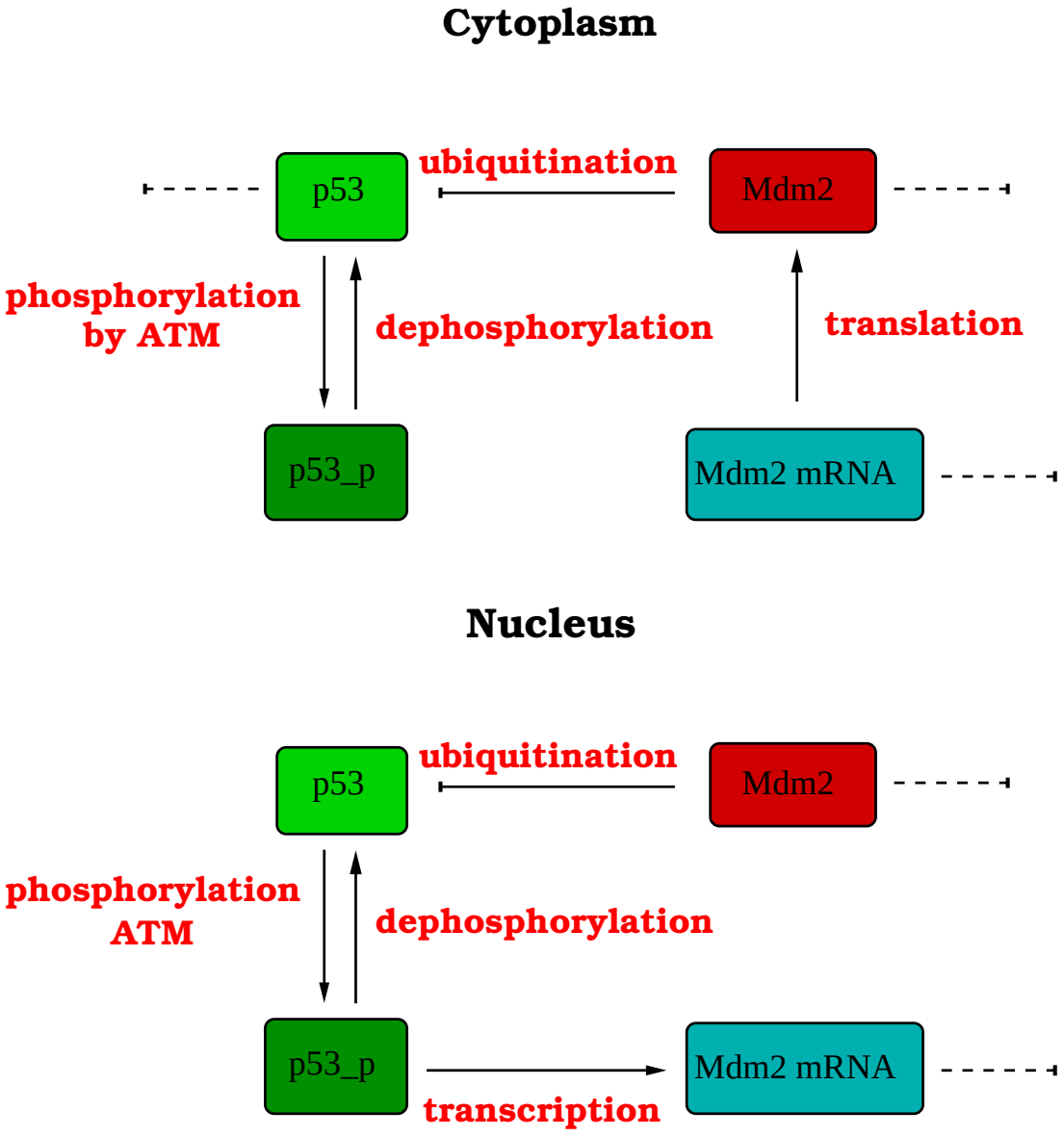
Introducing **space**:

- "Operations" in Nucleus and Cytoplasm are not homogeneous (transcription-translation-degradation depends on compartment).
- Temporal dynamics: different space scales (p53's "radius" is 2,4 nm - diameter of a cell can be $30\mu m$)



Sturrock et al. - JTB 2011, *Sturrock et al.* - Bull Math Biol. 2012

Model: biological hypotheses

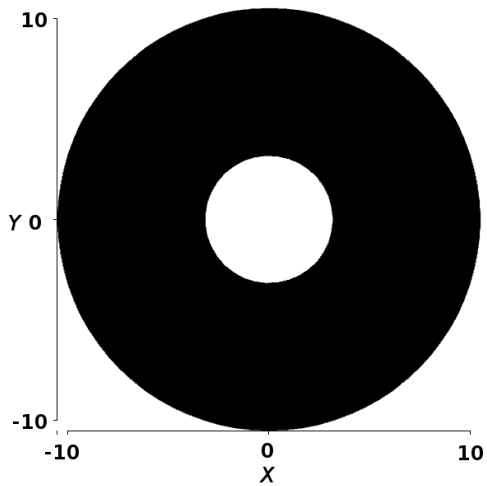


Mathematical Model

Model variables (nuclear and cytoplasmic concentrations)

- $[p53]^{(n)}$ and $[p53]^{(c)}$
- active p53: $[p53_p]^{(n)}$ and $[p53_p]^{(c)}$
- $[Mdm2]^{(n)}$ and $[Mdm2]^{(c)}$
- $[mdm2_R NA]^{(n)}$ and $[mdm2_R NA]^{(c)}$

All variables diffuse within each compartment



The Model: Nucleus

$$
\begin{cases}
\dfrac{\partial[p53]}{\partial t} = \overbrace{k_{dph}\dfrac{[p53_p]}{K_{dph}+[p53_p]}}^{\text{dephosphorylation}} + \overbrace{d_p\Delta[p53]}^{\text{diffusion}} - \overbrace{k_1[Mdm2]\dfrac{[p53]}{K_1+[p53]}}^{\text{ubiquitination}} \\
\qquad\qquad - k_3 ATM\dfrac{[p53]}{K_{ATM}+[p53]} \\
\dfrac{\partial[Mdm2]}{\partial t} = d_m\Delta[Mdm2] - \delta_m[Mdm2] \\
\dfrac{\partial[mdm2_{RNA}]}{\partial t} = k_{Sm} + \overbrace{k_{Sp}\dfrac{([p53_p])^4}{([p53_p])^4+K_{Sp}}}^{\text{p53-dependent synthesis}} + d_{mRNA}\Delta[mdm2_{RNA}] \\
\qquad\qquad - \delta_{mRNA}[mdm2_{RNA}] \\
\dfrac{\partial[p53_p]}{\partial t} = \overbrace{k_3 ATM\dfrac{[p53]}{K_{ATM}+[p53]}}^{\text{phosphorylation by ATM}} + d_{p'}\Delta[p53_p] - k_{dph}\dfrac{[p53_p]}{K_{dph}+[p53_p]}
\end{cases}
$$

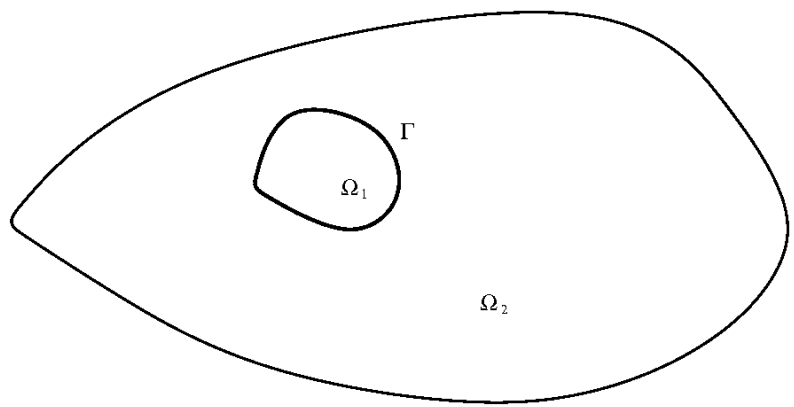
The Model: Cytoplasm

$$
\begin{cases}
\dfrac{\partial[p53]}{\partial t} = k_S + k_{dph}\dfrac{[p53_p]}{K_{dph}+[p53_p]} + d_p\Delta[p53] - k_1[Mdm2]\dfrac{[p53]}{K_1+[p53]} \\
\qquad\qquad - k_3 ATM\dfrac{[p53]}{K_{ATM}+[p53]} - \delta_{p53}[p53] \\
\dfrac{\partial[Mdm2]}{\partial t} = d_m\Delta[mdm2] + \overbrace{k_{tr}[mdm2_{RNA}]}^{\textbf{translation}} - \delta_m[mdm2] \\
\dfrac{\partial[mdm2_{RNA}]}{\partial t} = d_{mRNA}\Delta[mdm2_{RNA}] - k_{tr}[mdm2_{RNA}] \\
\qquad\qquad - \delta_{mRNA}[mdm2_{RNA}] \\
\dfrac{\partial[p53_p]}{\partial t} = k_3 ATM\dfrac{[p53]}{K_{ATM}+[p53]} + d_{p'}\Delta[p53_p] - k_{dph}\dfrac{[p53_p]}{K_{dph}+[p53_p]}
\end{cases}
$$

Kedem-Katchalsky boundary conditions

$$
\begin{cases}
d_p \dfrac{\partial [p53]^{(n)}}{\partial \mathbf{n}} = p_{p53}([p53]^{(c)} - [p53]^{(n)}) = -d_p \dfrac{\partial [p53]^{(c)}}{\partial \mathbf{n}} \\
d_{p'} \dfrac{\partial [p53_p]^{(n)}}{\partial \mathbf{n}} = p_{pp}[p53]_p^{(c)} = -d_{p'} \dfrac{\partial [p53_p]^{(c)}}{\partial \mathbf{n}} \\
d_m \dfrac{\partial [Mdm2]^{(n)}}{\partial \mathbf{n}} = p_{mdm2}([Mdm2]^{(c)} - [Mdm2]^{(n)}) = -d_m \dfrac{\partial [Mdm2]^{(c)}}{\partial \mathbf{n}} \\
d_{mRNA} \dfrac{\partial [mdm2_{RNA}]^{(n)}}{\partial \mathbf{n}} = -p_{mRNA}[mdm2_{RNA}]^{(n)} = -d_{mRNA} \dfrac{\partial [mdm2_{RNA}]^{(c)}}{\partial \mathbf{n}}
\end{cases}
$$

on the common boundary Γ.



A. Cangiani and R. Natalini. A spatial model of cellular molecular trafficking including active transport along microtubules. Journal of Theoretical Biology, 2010.

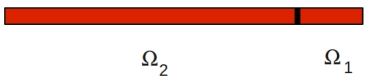
The Spatial Environment(s!)

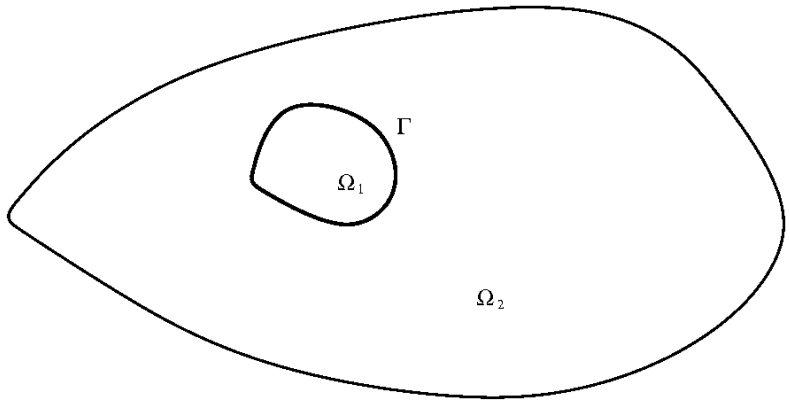
The spatial environment is the cell

- compartmental model (ODE system)

NUCLEUS $\longleftrightarrow$ CYTOPLASM

- spatial model (PDE system): 1D and 2D domains





Where Ω_1 =Nucleus, Ω_2 =Cytoplasm and Γ the common boundary, $\Gamma = \Omega_1 \cap \Omega_2$.

ODE system: exchange between compartments

Let $\mathbf{S}$ be one of the species $\mathbf{S} = p53, Mdm2, mdm2RNA,$ or $p53_p$, $\mathbf{S}^{(n)}$ its nuclear concentration, $\mathbf{S}^{(c)}$ its cytoplasmic concentration.

$$\frac{d\mathbf{S}^{(n)}}{dt} = \text{Nuclear Reactions} - \rho_{\mathbf{S}} V_r(\mathbf{S}^{(n)} - \mathbf{S}^{(c)})$$
$$\frac{d\mathbf{S}^{(c)}}{dt} = \text{Cytoplasmic Reactions} + \rho_{\mathbf{S}}(\mathbf{S}^{(n)} - \mathbf{S}^{(c)})$$

$$\text{where } V_r = \frac{\text{cytoplasmic volume}}{\text{nuclear volume}}$$

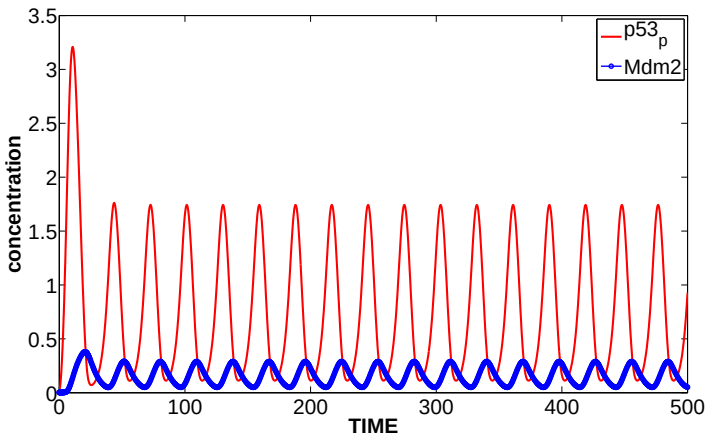
ODE system: positivity of solutions and sustainend oscillations

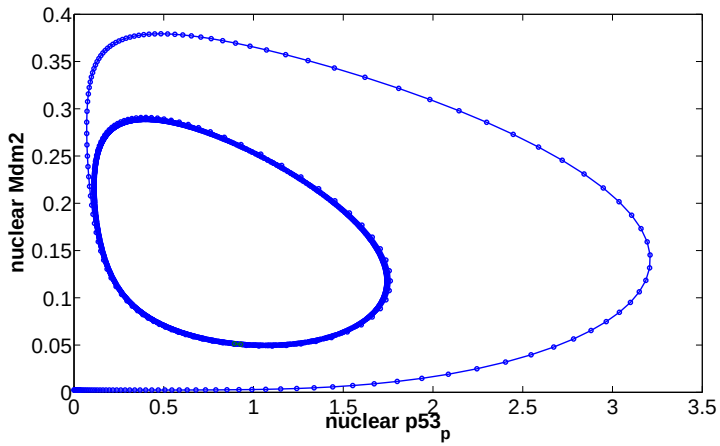
Proposition

The positive quadrant is invariant for the flow of the system if $ATM > 0$.

Numerics

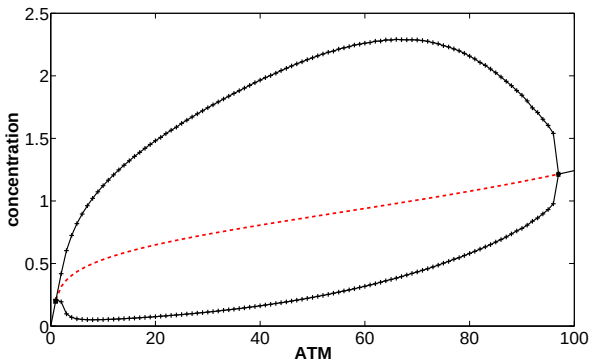
Sustained oscillations appear for $ATM_{min} < ATM < ATM_{max}$.

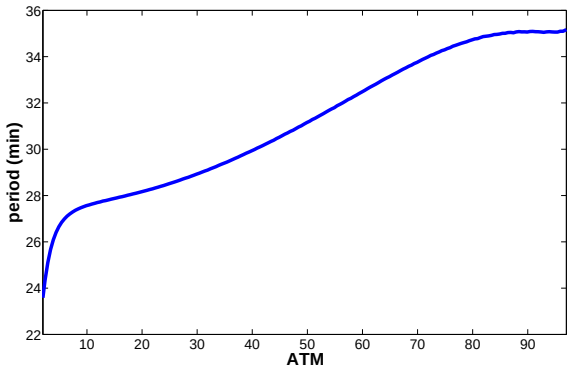




Supercritical Hopf bifurcation and oscillations

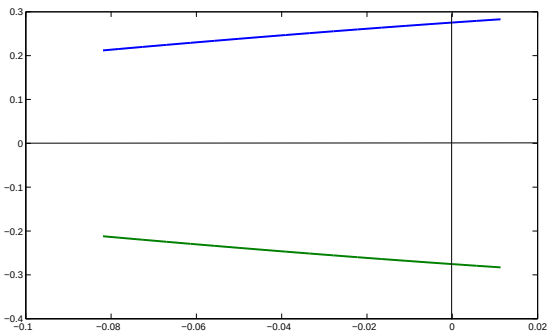
- **ATM** and oscillations: existence of a supercritical Hopf Bifurcation





red dotted curve: unstable equilibrium point
+ **marked** curve: amplitude of oscillations
blue curve: period of the oscillations (minutes)

- Hypothesis of the Hopf bifurcation theorem satisfied by our model -numerical proof



Simulations in a 1-dimensional PDE system

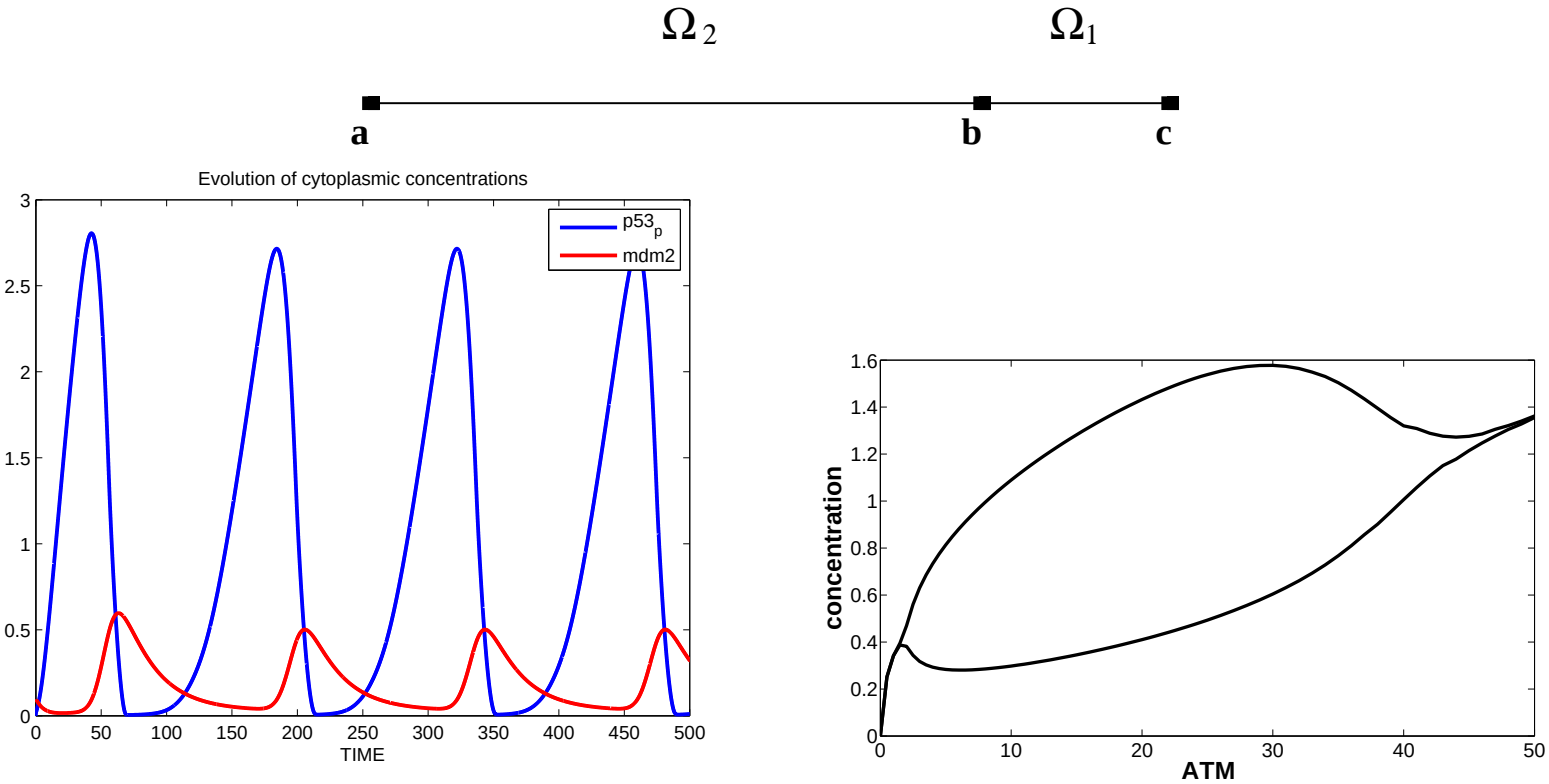


Figure: Simulations of the 1-dimensional PDE system; **Left**: temporal evolution of p53 nuclear concentrations. **Right**: 'Bifurcation diagram' over **ATM**

The 1-dimensional environment does not permit a 'spatial' analysis

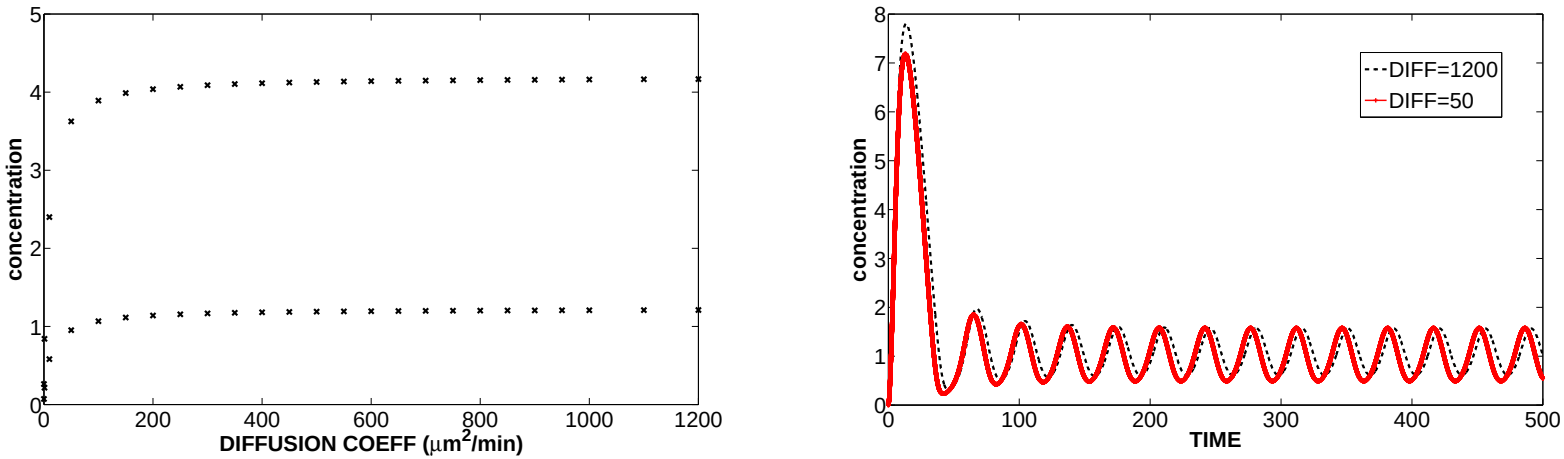
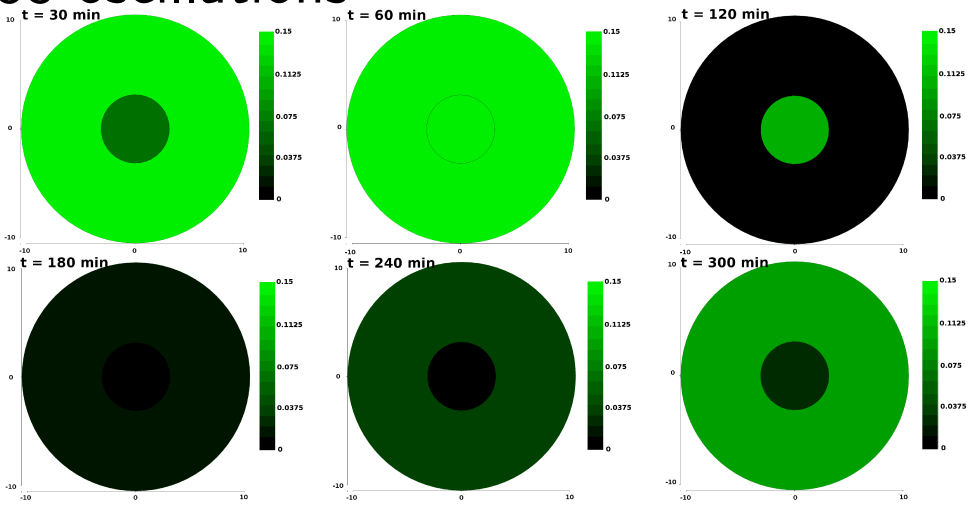


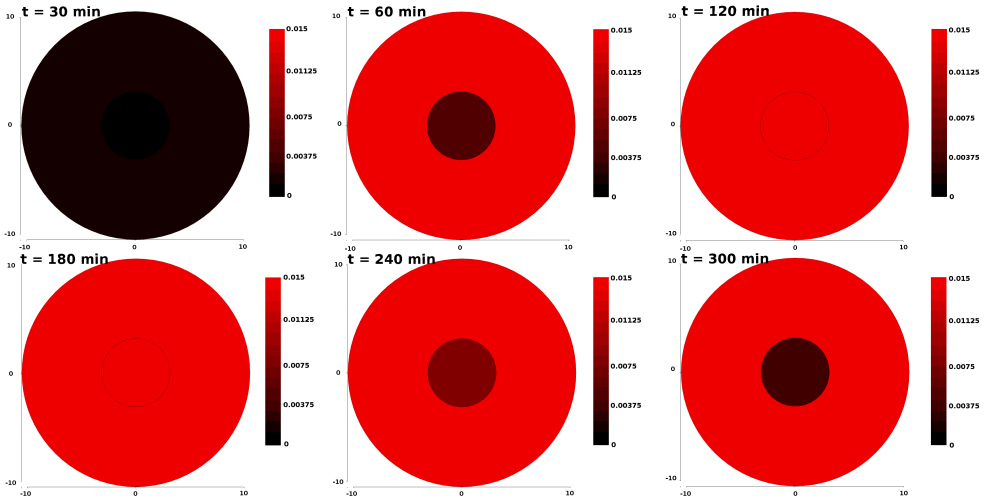
Figure: Simulations of the 1-dimensional PDE system; **Left**:'Bifurcation diagram' over the diffusion coefficients . **Right**: temporal evolution of p53 nuclear concentrations for different diffusion values

Simulations in a 2-dimensional PDE system

p53 oscillations



Mdm2 oscillations

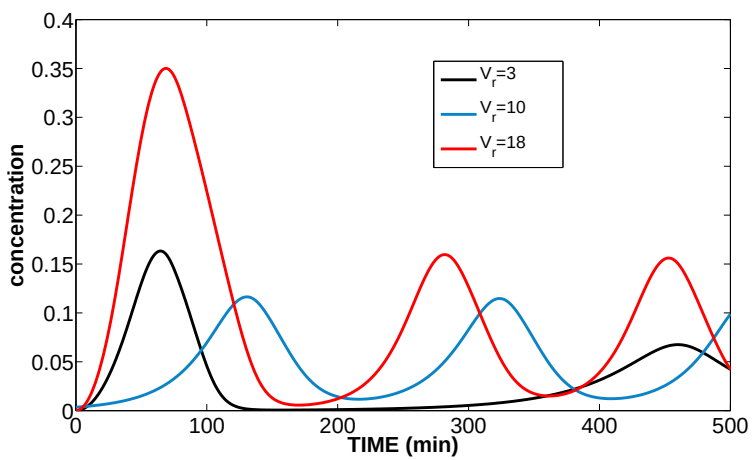


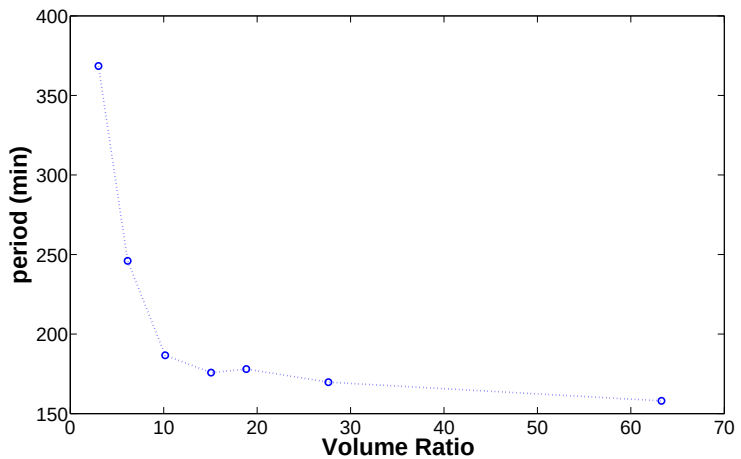
$$D_S = 10\mu m^2/s$$
$$D_{mRNA} = 0.1\mu m^2/s$$
$$p_S = 0.16\mu m/s$$
Volume ratio $(C : N) = 10 : 1$

Oscillations appear for realistic diffusion and permeability values

Parameter	Description	Ref. values	values for oscillations
Vol	Total area of the simulations domain	$300\mu m^2$	$Vol > 0(\mu m^2)$
V_r	Volume ratio Cytoplasm:Nucleus	10	$2 \leq V_r \leq 100$
p_i	Protein permeabilities	$10\mu m/\text{min}$	$5 \leq p_S \leq 5000(\mu m/\text{min})$
D_i	Protein diffusion coefficients	$600\mu m^2/\text{min}$	$10 \leq D_S \leq 1000(\mu m^2/\text{min})$

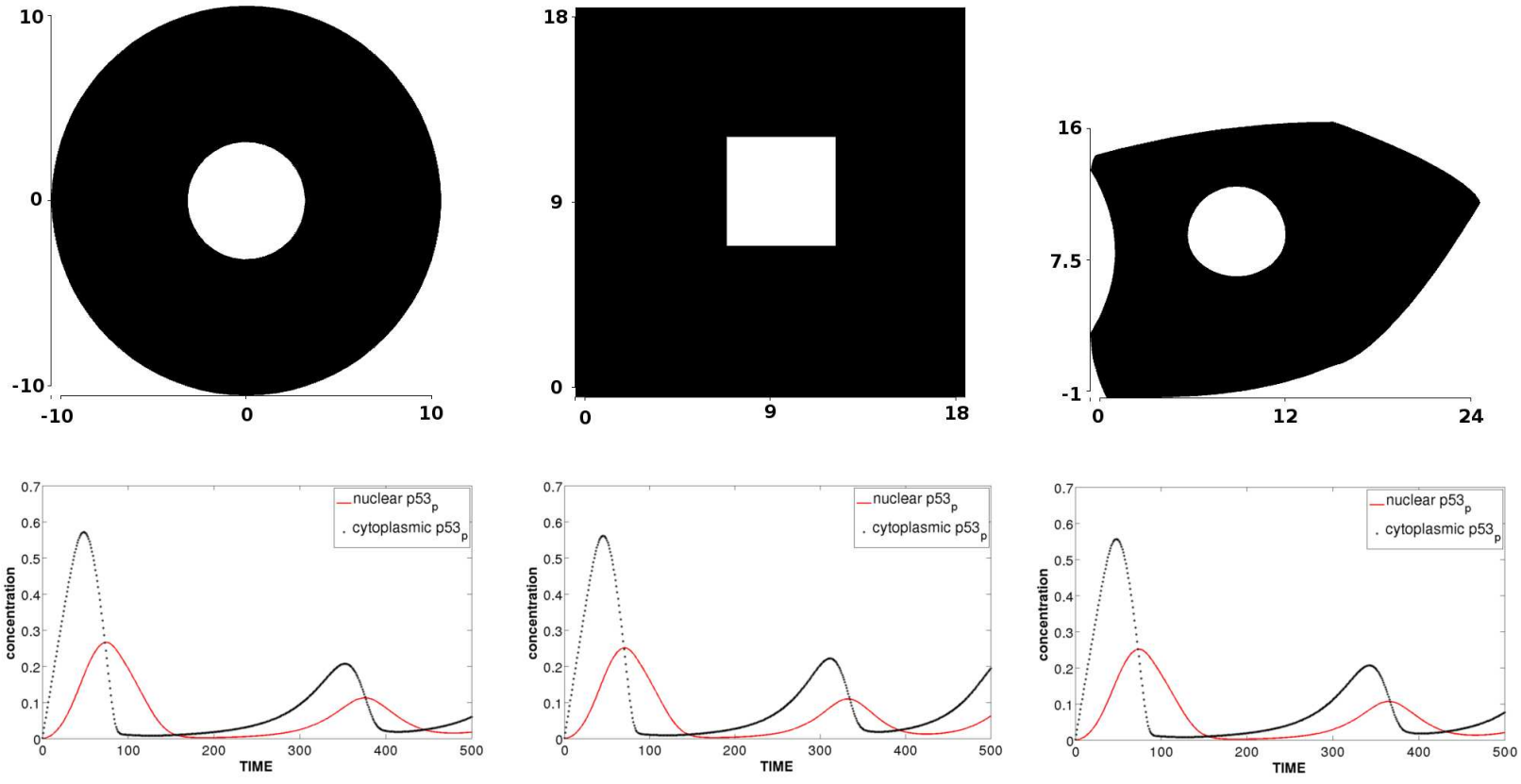
Table: Parameter ranges of spatial values for which oscillations occurs. the ratio "protein diffusion:mRNA diffusion" has been fixed to 100:1.





D. Fusco et al., Curr. Biol 2003, *Shav Tal et al.* Science 2004, *Hong et al.* J Biomater Nanobiotechnol 2010

The geometry of the domain does not influence the dynamics of the system

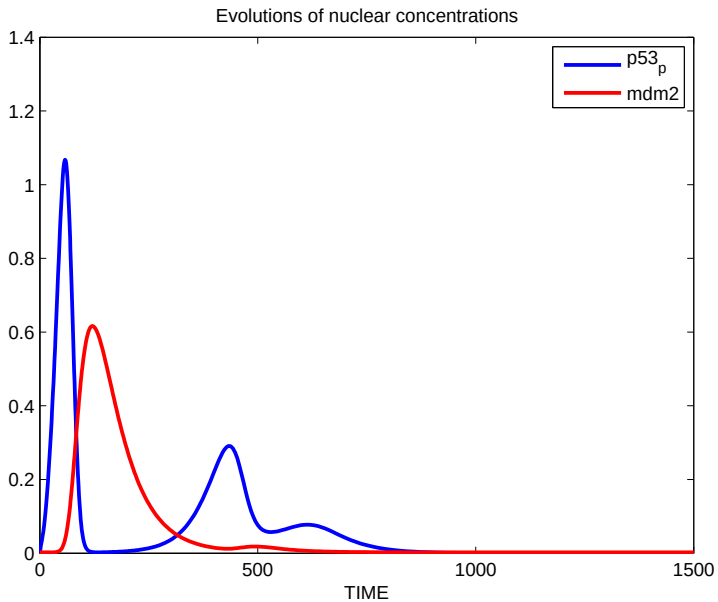


Conclusion - Part I

- Spatial physiological model that reproduces the oscillations
- ATM as a 'natural' bifurcation value
- Oscillations appear for realistic diffusion and permeability values
- The geometry of the domain does not influence the dynamics of the system

Future directions - Part I

- include the import and export pathways (NLS-NES)
- *in silico* experiments with drugs
- How the mutations act on the dynamics?
- 3D extension



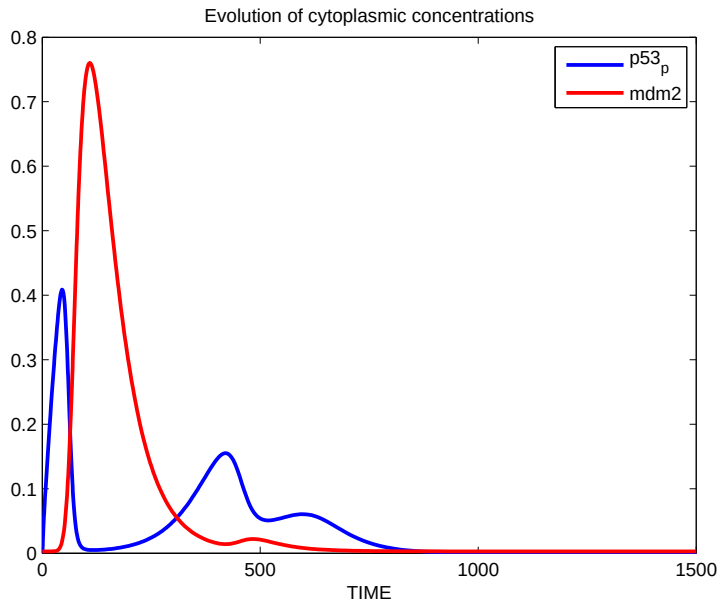


Figure: A basic example of DNA repair: a few oscillations occur. Here the bifurcation parameter ATM is a variable of the system

Also we need to compare the model with real biological data!

Summary

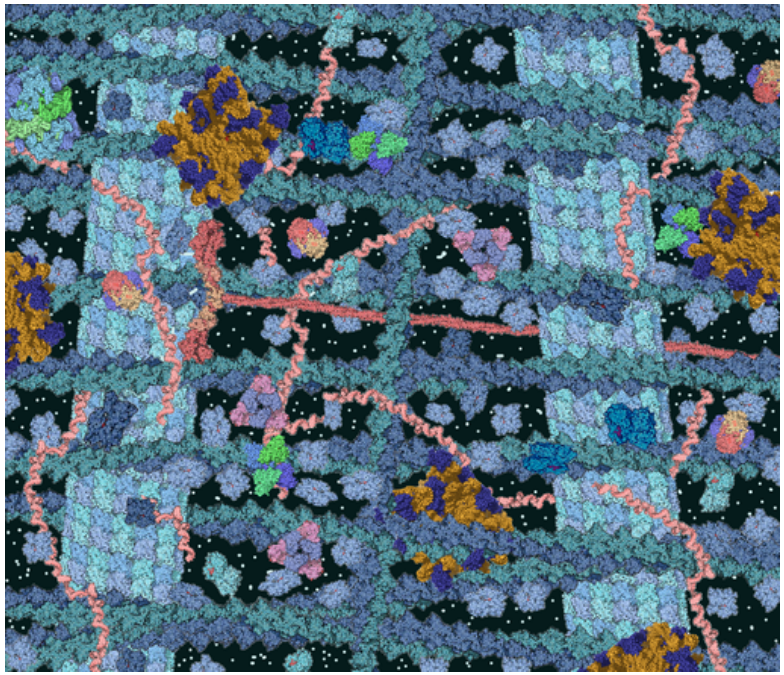
1. A model for p53 intracellular dynamics
   - biology of p53 - basics
   - a new model to reproduce its dynamics

2. **A model for protein transport within the cell**
   - **biology of intracellular transport - basics**
   - **locating a single microtubule**

Diffusion to model transport

Transport of proteins is modeled by **DIFFUSION**.

Diffusion alone can be an efficient mechanism...



in such a crowded environment?

Diffusion means **average**
Direction is random
Is this mechanism **always** efficient?

Transport a signal

Approach **faster** to the nucleus $\Longrightarrow$ use **MICROTUBULE** structure.

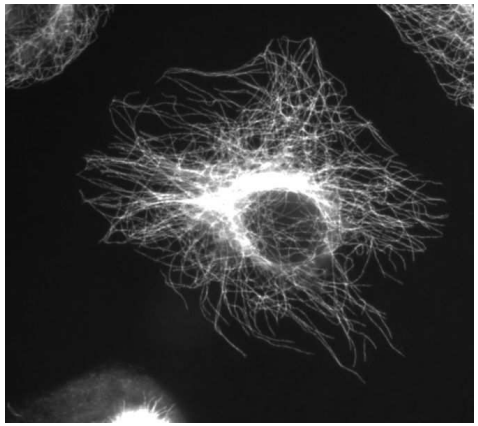


Fig: Wikimedia commons.

Microtubules (**MTs**) are filaments that carry out several activities (motility of the cell, distribution of vescicles and organelles within the cell).
Microtubule Structure

- Filaments of α and β tubulin dimer anchored at the centrosome (MTOC-Microtubule Organizing Centre).
- MTOC is near the nucleus.
- They have a polarity (plus and minus end) $\Longrightarrow$ **direction**
- Radial Structure

Transport a message

Some proteins such as the pRb (a **TUMOR SUPPRESSOR** protein) use MTs to accumulate **efficiently** in the nucleus.
MTs integrity and dinamicity is **not** a **REQUIREMENT** but still useful for **efficient** accumulation.

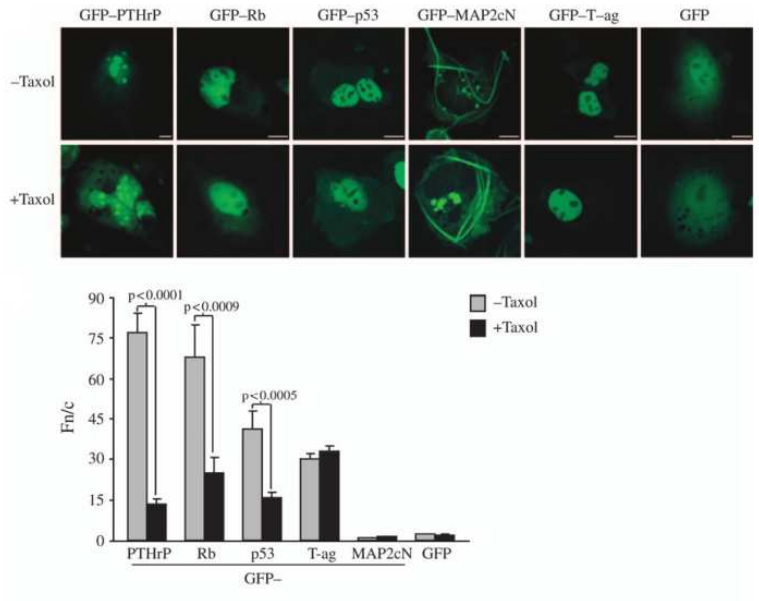
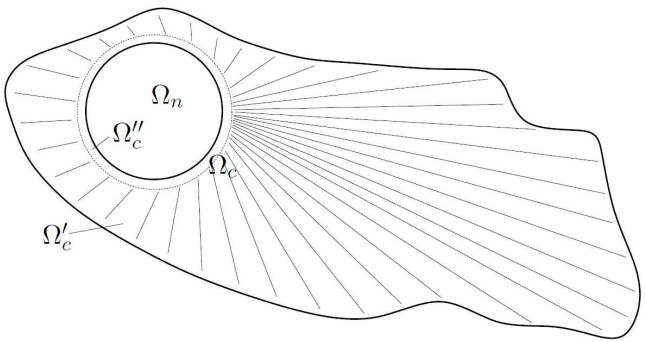


Figure: Quantitative analysis of nuclear import in cells treated with TAXOL.*Roth et al, Traffic 2007; 8: 673686*

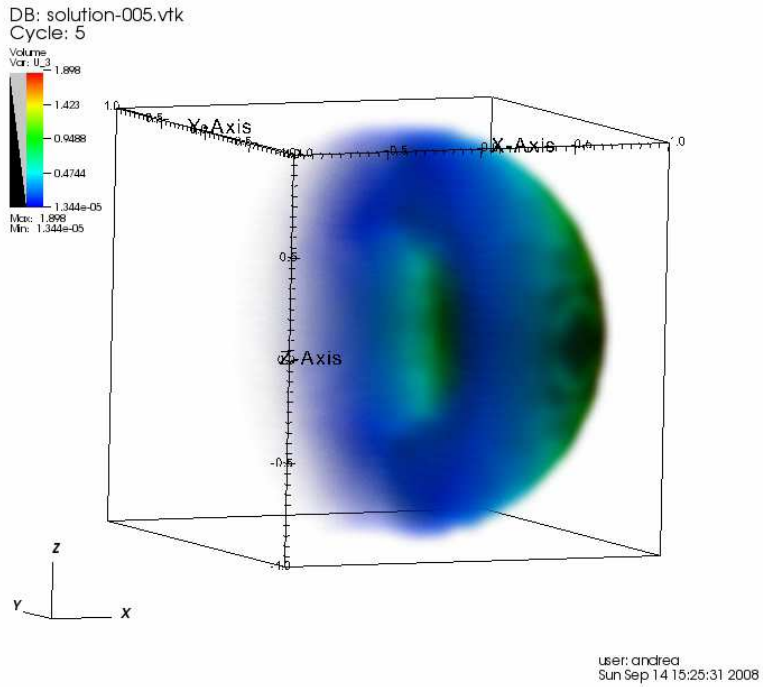
Here's a CARTOON of how it works...

Previous works

Point out the importance of MT for efficient transport within the cell



Cangiani-Natalini,J Theor Biol. 2010 Dec 21;267(4):614-25



Smith one dimensional model: lateral diffusion is supposed to homogenize any concentration gradient. *Smith-Simmons,Biophys J. 2001 Jan;80(1):45-68.*

The model

We introduce a simplified 2-dimensional model

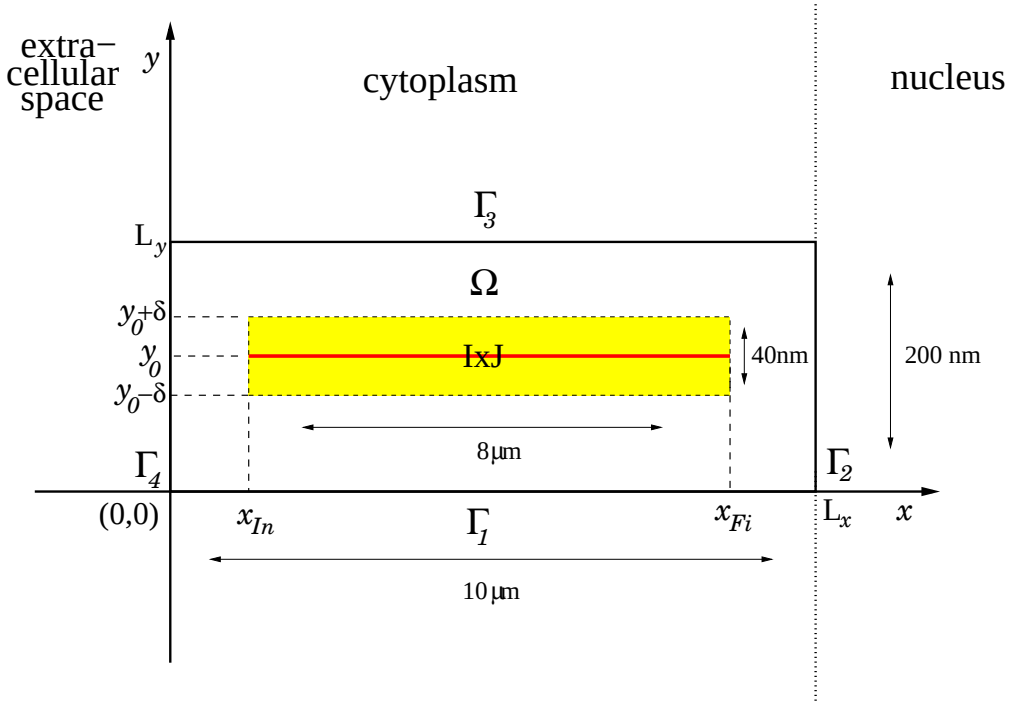


Figure: Considered area of the cytoplasm: $\Omega = [0, L_x] \times [0, L_y]$. **The yellow area** ($I \times J = [x_{In}, x_{Fi}] \times [y_0 - \delta, y_0 + \delta]$) is the attraction area of the microtubule filament, the **red strip** is the MT in y_0.

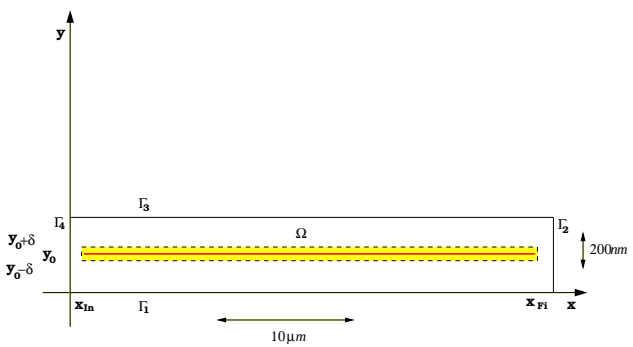
Main features:

- positioning the microtubule
- considering one way motor protein
- cargo and cargo + protein representation: bi-dimensional and 1-dimensional equations

The Mathematical Model

We define: u, v and W (free cargo, cargo+motor and transported cargo).
Applying **Fick**'s law of diffusion and **Mass Action Law** for kinetic Reactions we get:

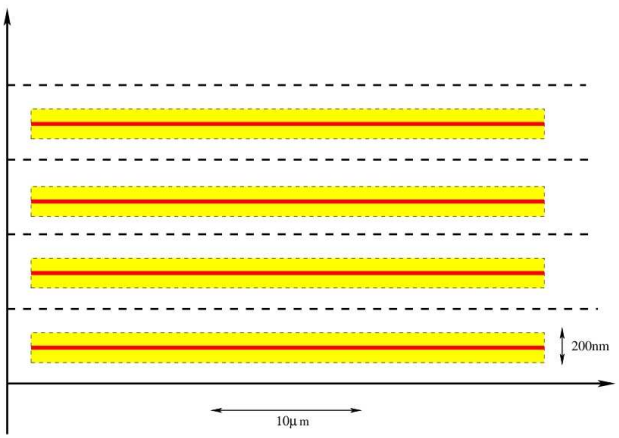
$$\begin{cases} \frac{\partial u}{\partial t} = d_u \Delta u - ku + k_- v, & \text{in } \Omega_c, \\ \frac{\partial v}{\partial t} = d_v \Delta v + ku - k_- v - k_1 v \mathbb{1}_{\mathbf{I}\times\mathbf{J}} + k_{-1} W \frac{\mathbb{1}_{\mathbf{I}\times\mathbf{J}}}{|\mathbf{J}|} \\ \qquad\qquad + cW(x_{Fi})\delta_0(x - x_{Fi}, y - y_0), & \text{in } \Omega_c, \\ \frac{\partial W}{\partial t} + c\frac{\partial W}{\partial x} = -k_{-1} W + k_1 \int_{\mathbf{J}} v dy, & \text{in }]x_{In}, x_{Fi}[. \end{cases}$$

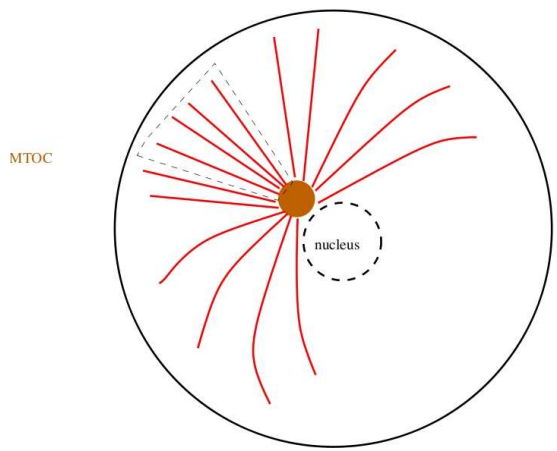


Boundary conditions

We suppose the microtubules homogeneously distributed within the cell $\Longrightarrow$ on the long side of the domains we use periodic boundary conditions.

$$\begin{cases} \frac{\partial u}{\partial n} = 0, & \\ \frac{\partial v}{\partial n} = 0, & \text{on } \Gamma_4, \\ d_u \frac{\partial u}{\partial n} + p_u u = 0, & \\ d_v \frac{\partial v}{\partial n} + p_v v = 0, & \text{on } \Gamma_2, \\ w(x_{In}) = 0. & \end{cases}$$





Left: **Neumann** homogeneous boundary conditions no crossing of the membrane (cytoplasmic side).

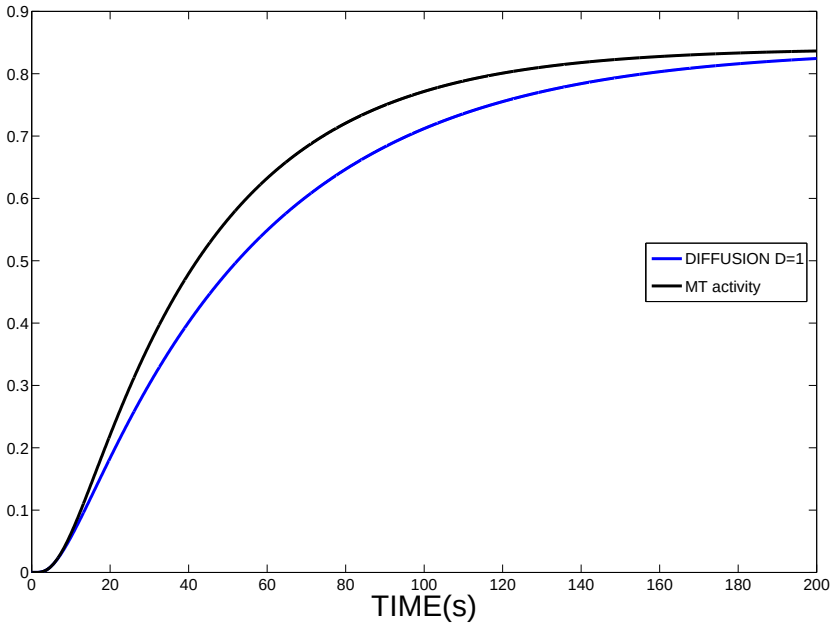
Right: outgoing flow proportional to the species concentration (**Robin** boundary condition).

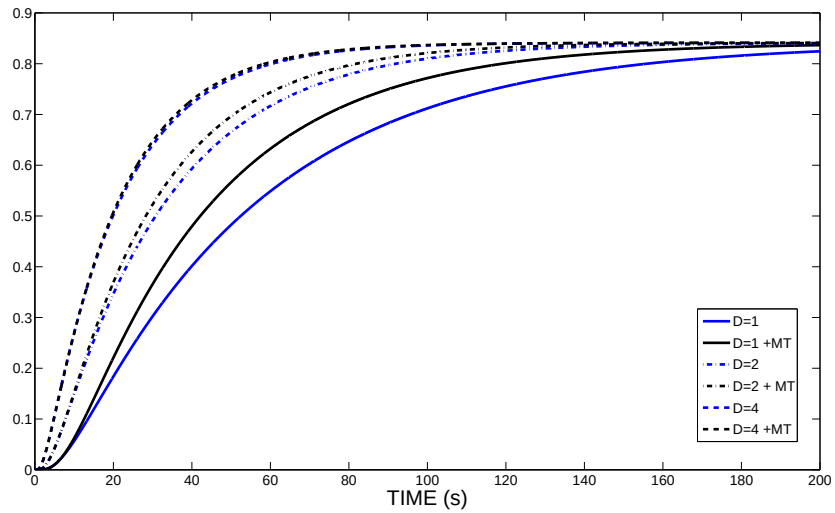
Results

$$\phi_u(t) = -d_u \int_0^{L_y} \nabla u(\bar{x}, y) \cdot \mathbf{n}(\bar{x}, y) dy,$$
$$\phi_v(t) = -d_v \int_0^{L_y} \nabla v(\bar{x}, y) \cdot \mathbf{n}(\bar{x}, y) dy,$$

integrating over time we define:

$$F_u(t) = \int_0^t \phi_u(t) dt \quad \text{and} \quad F_v(t) = \int_0^t \phi_v(t) dt\,.$$

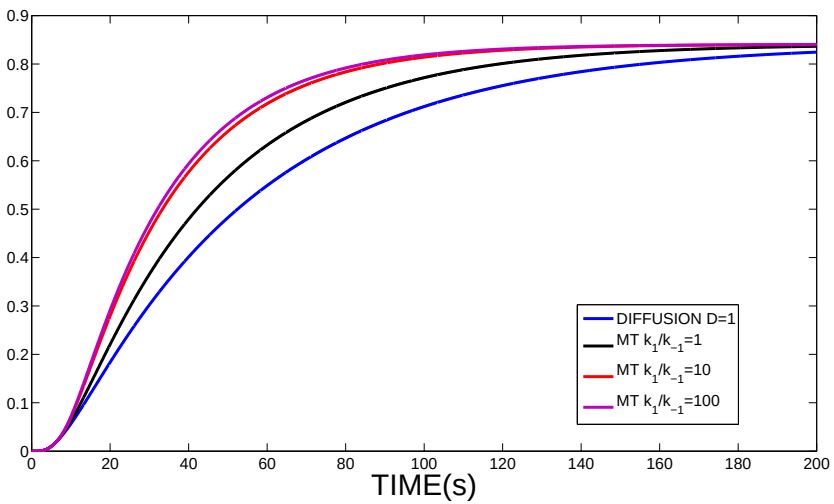


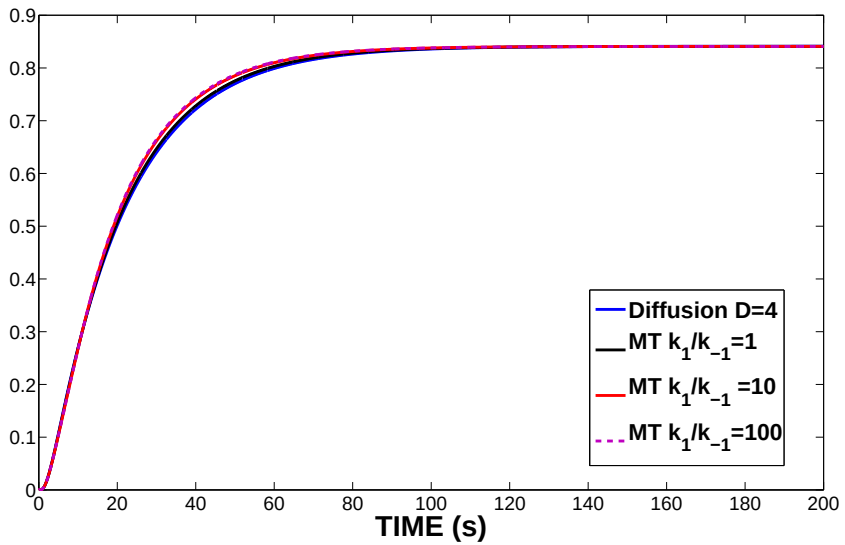


Detachment and attachment rates from the MT increase the total flow

k_1 : attachment rate, k_{-1} : detachment rate

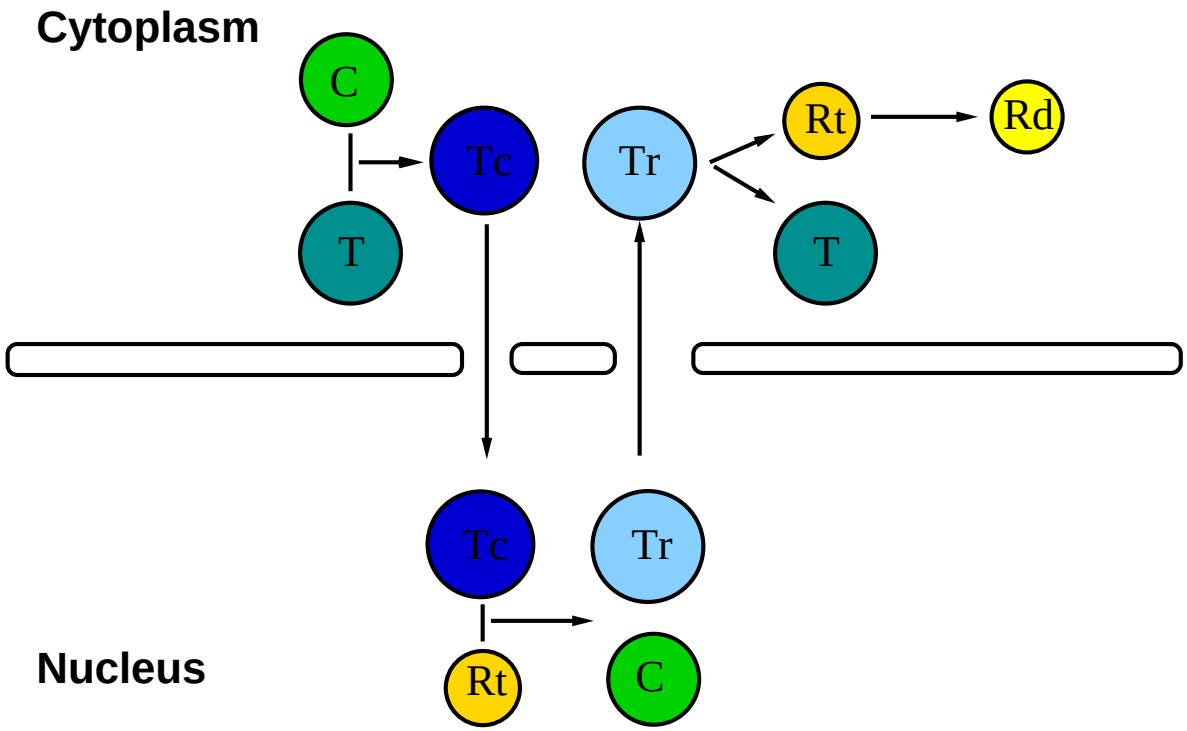
$$\tau_{on} = \frac{1}{k_{-1}} \qquad \tau_{off} = \frac{1}{k_1}$$



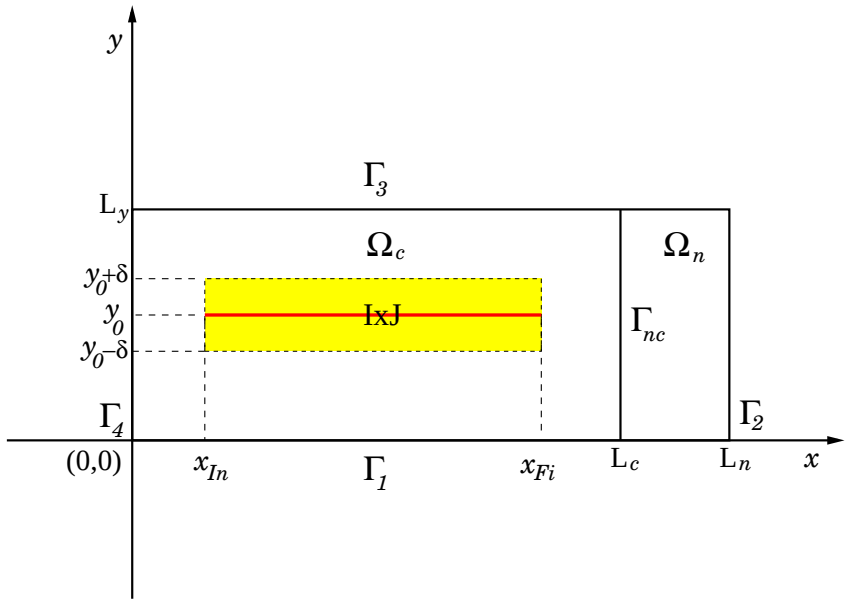


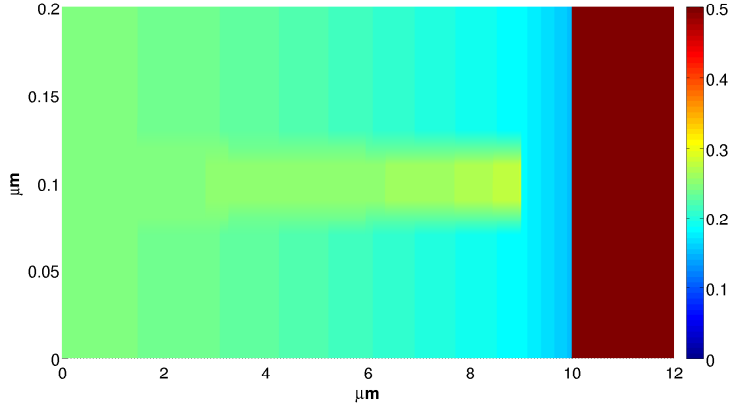
The import pathway

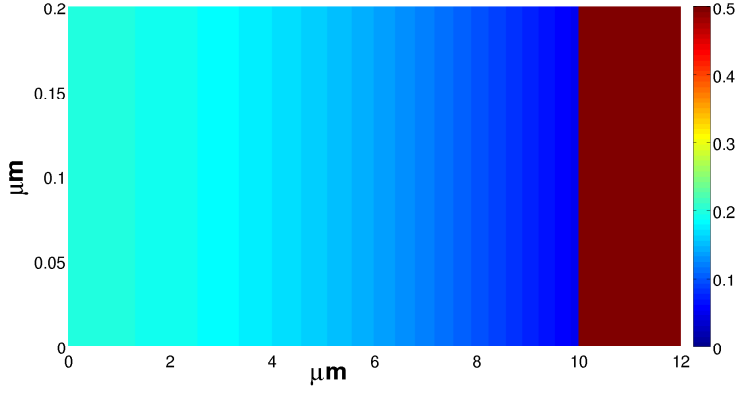
We couple our model with a model of import pathway and we add a nuclear compartment.



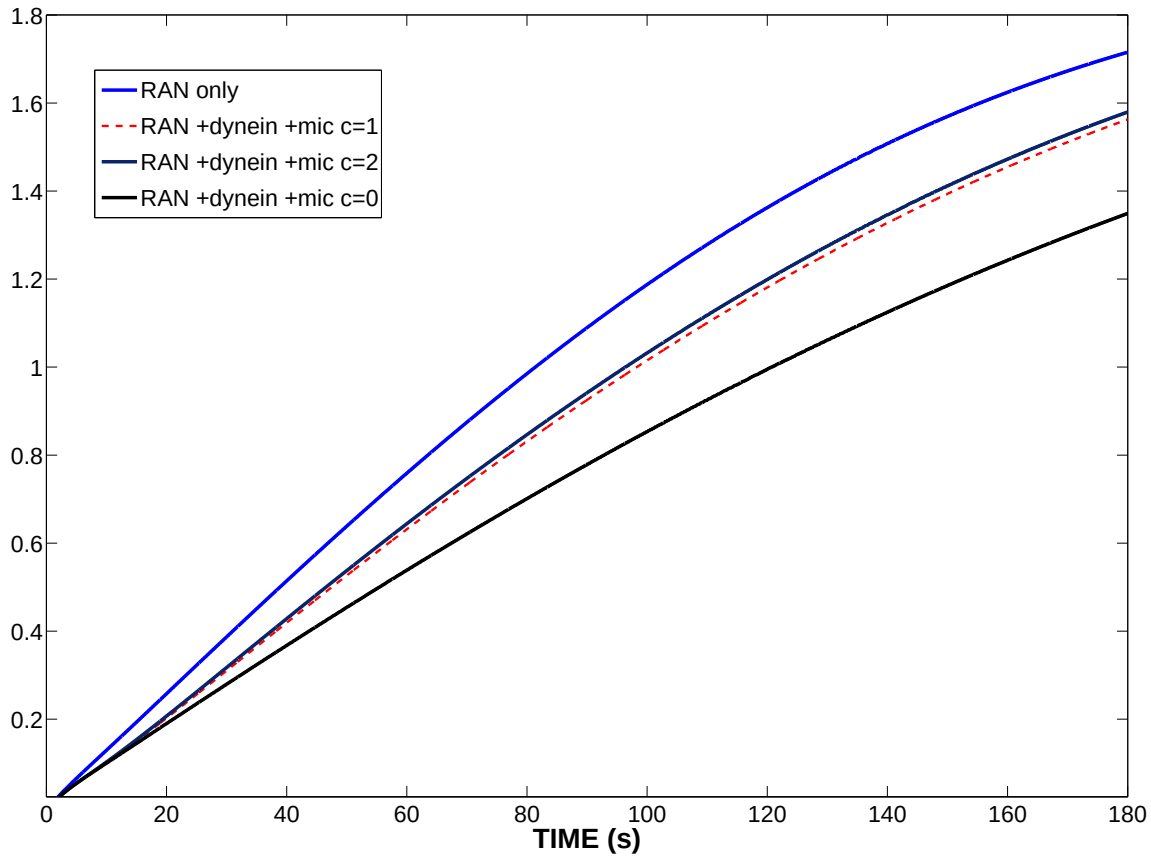
Adding the nuclear compartment







The microtubule attracts the cargo and its import is slowed down

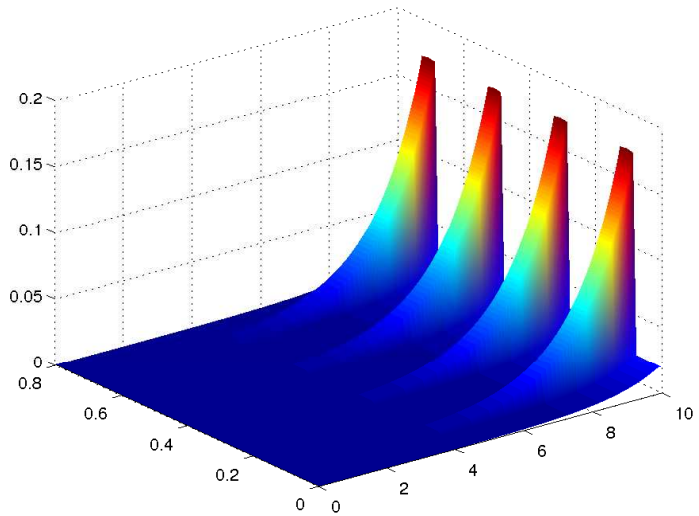


Conclusion - Part II

- No import pathway: the macromolecules flow increases with MT activity (diffusion coeff up to $6\mu m^2/s$)
- No import pathway: detachment and attachment rates from the MT increase the total flow
- Import pathway: the competition between importin and microtubule subtracts free cargo to import

Future Directions - Part II

- Highlight the importance of microtubule activity in NLS proteins transport (Ran Pathway).



- Various geometries
- basis for studying the transmission of DNA vaccines (Maria Grazia Notarangelo Ph.D Thesis)

Modelling nucleocytoplasmic transport with application to the intracellular dynamics of the tumor suppressor protein p53

Luna Dimitrio