Present challenges of mathematics in oncology and biology of cancer
Marseille, CIRM, December 7-11th 2015

Mathematical models of the transportation of anti-tumoral genetic vaccines

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Cancer therapies

The cancer therapies currently available include

- surgery,
- radiation therapy,
- chemotherapy,

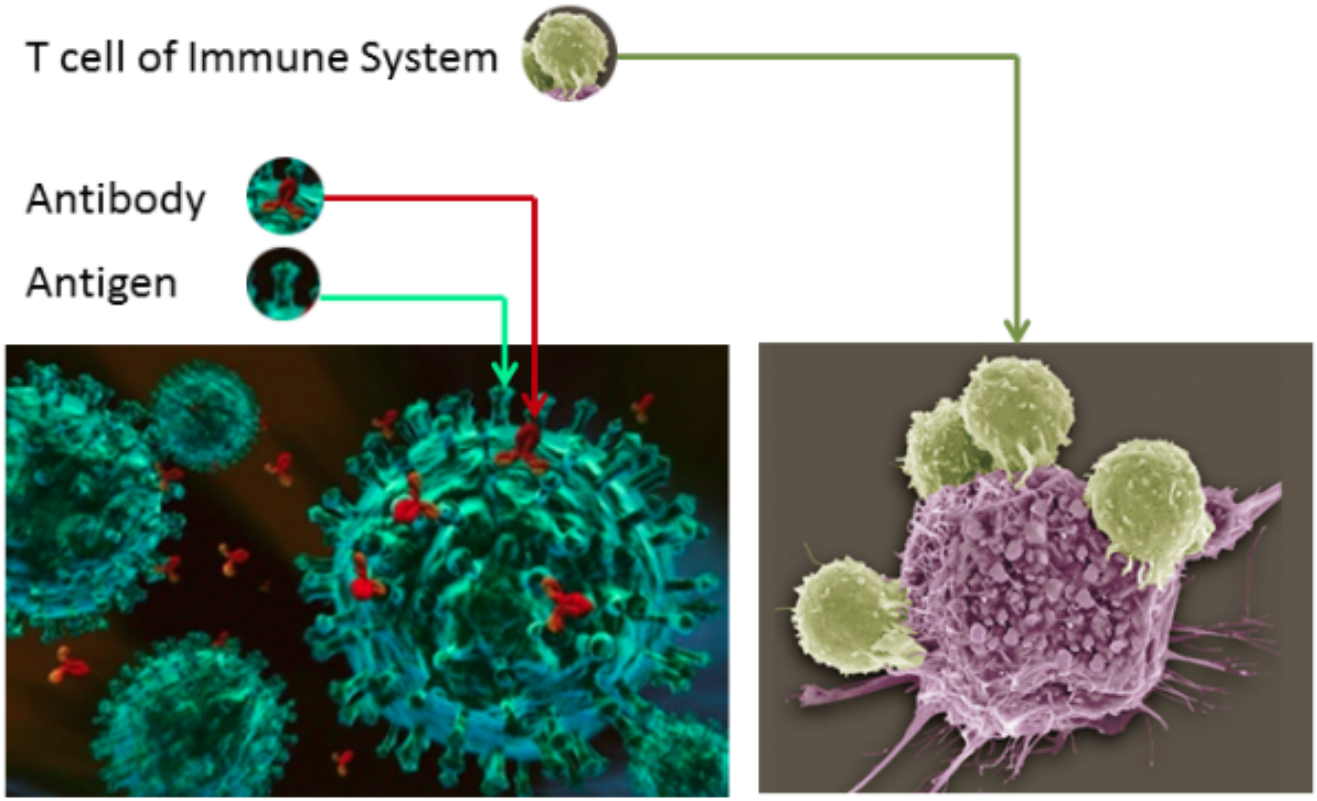
Used for a long time, they are very invasive.

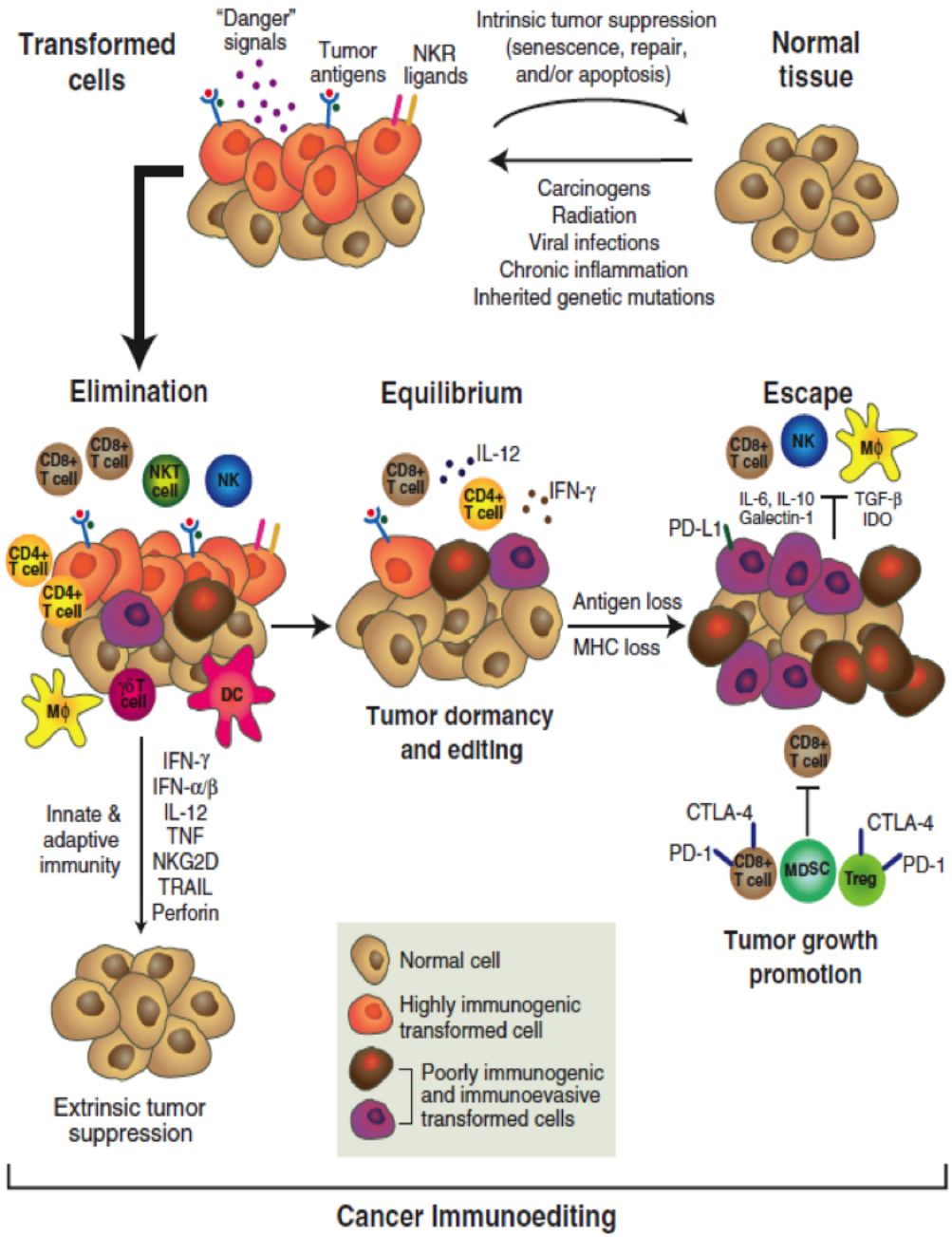
A (relatively) new approach: vaccination against cancer.

The combined treatments based on chemotherapy, radiation therapy and vaccines appear more effective in the therapy of cancer.

Cancer cells

The cancer cells present some antigens on their surface.
An antigen (**anti**bodies **gene**rator) is any substance that triggers the production of antibodies when it is introduced into the body or when it is appear on the surface of the cancer cells .
Antigens are usually attacked by the immune systems. When the antigens are produced by our organism (as in a cancer), they elude the immune system which does not attack them.




Transformed cells
"Danger" signals
Tumor antigens
NKR ligands
Intrinsic tumor suppression (senescence, repair, and/or apoptosis)
Normal tissue
Carcinogens
Radiation
Viral infections
Chronic inflammation
Inherited genetic mutations
Elimination
Equilibrium
Escape
CD8+ T cell
NKT cell
NK
CD4+ T cell
γδT cell
DC
Mϕ
IL-12
IFN-γ
Antigen loss
MHC loss
IL-6, IL-10
Galectin-1
TGF-β
IDO
PD-L1
CTLA-4
PD-1
MDSC
Treg
Tumor dormancy and editing
Tumor growth promotion
Innate & adaptive immunity
IFN-γ
IFN-α/β
IL-12
TNF
NKG2D
TRAIL
Perforin
Extrinsic tumor suppression
Normal cell
Highly immunogenic transformed cell
Poorly immunogenic and immunoevasive transformed cells
Cancer Immunoediting

Production and delivery of cancer vaccine

Aim:

Produce the large amount of antibody to eliminate the cancer cells when they appear and/or they have not been killed by other forms of treatment. On the contrary of the classic vaccination, here it is injected vectors to be express the antigens.

Step1:

Identificate the antigens (Specific databases: SYFPEITHI, BIMAS, RANKPEP)

Step2:

Find better vectors

- Viral vectors
- Non-viral vectors (naked DNA plasmids)

Step3:

Insert the audjuvant sequences.

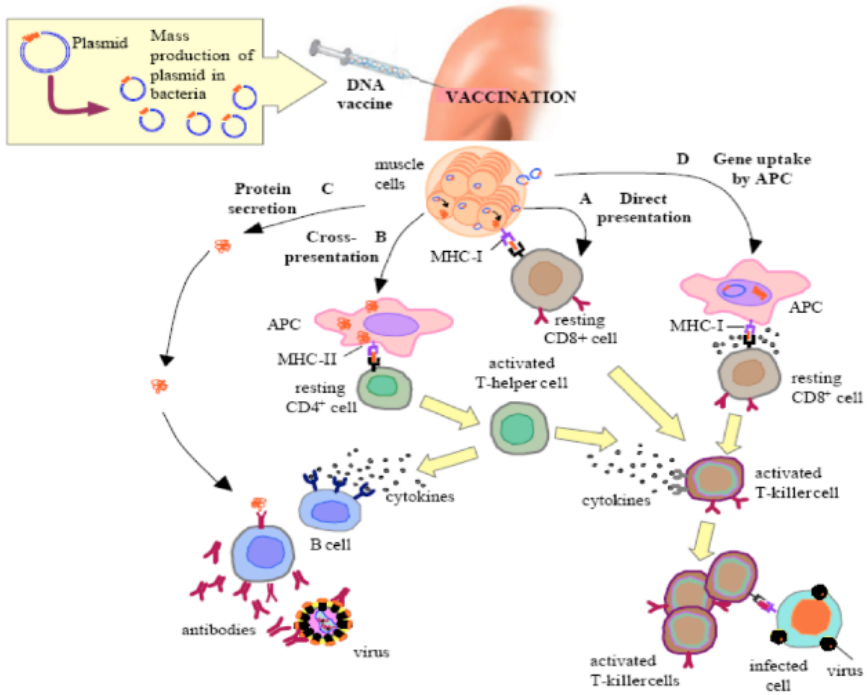


Figure : Mechanism of DNA vaccination by *Signori et al, 2010.*

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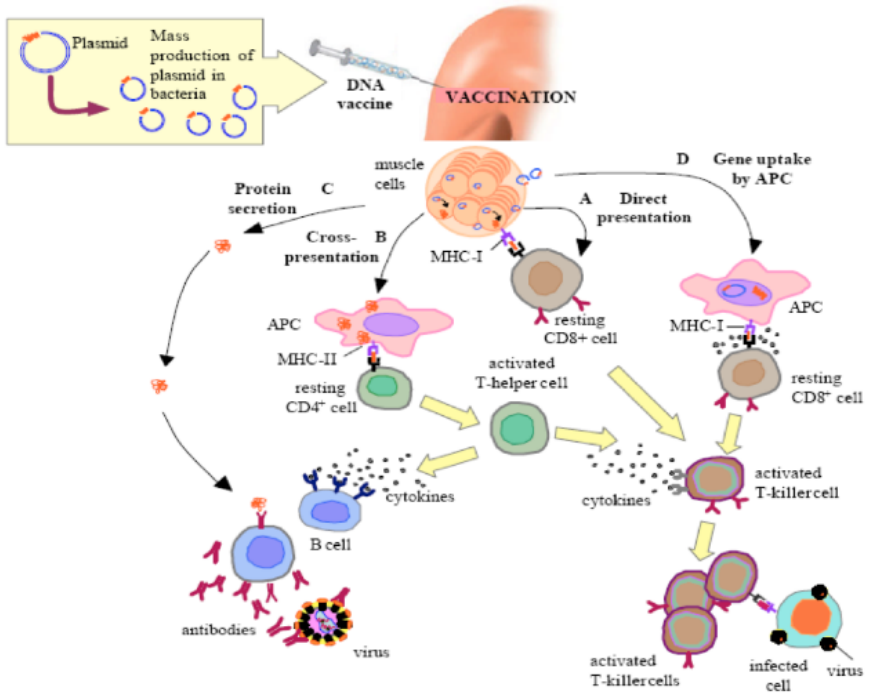

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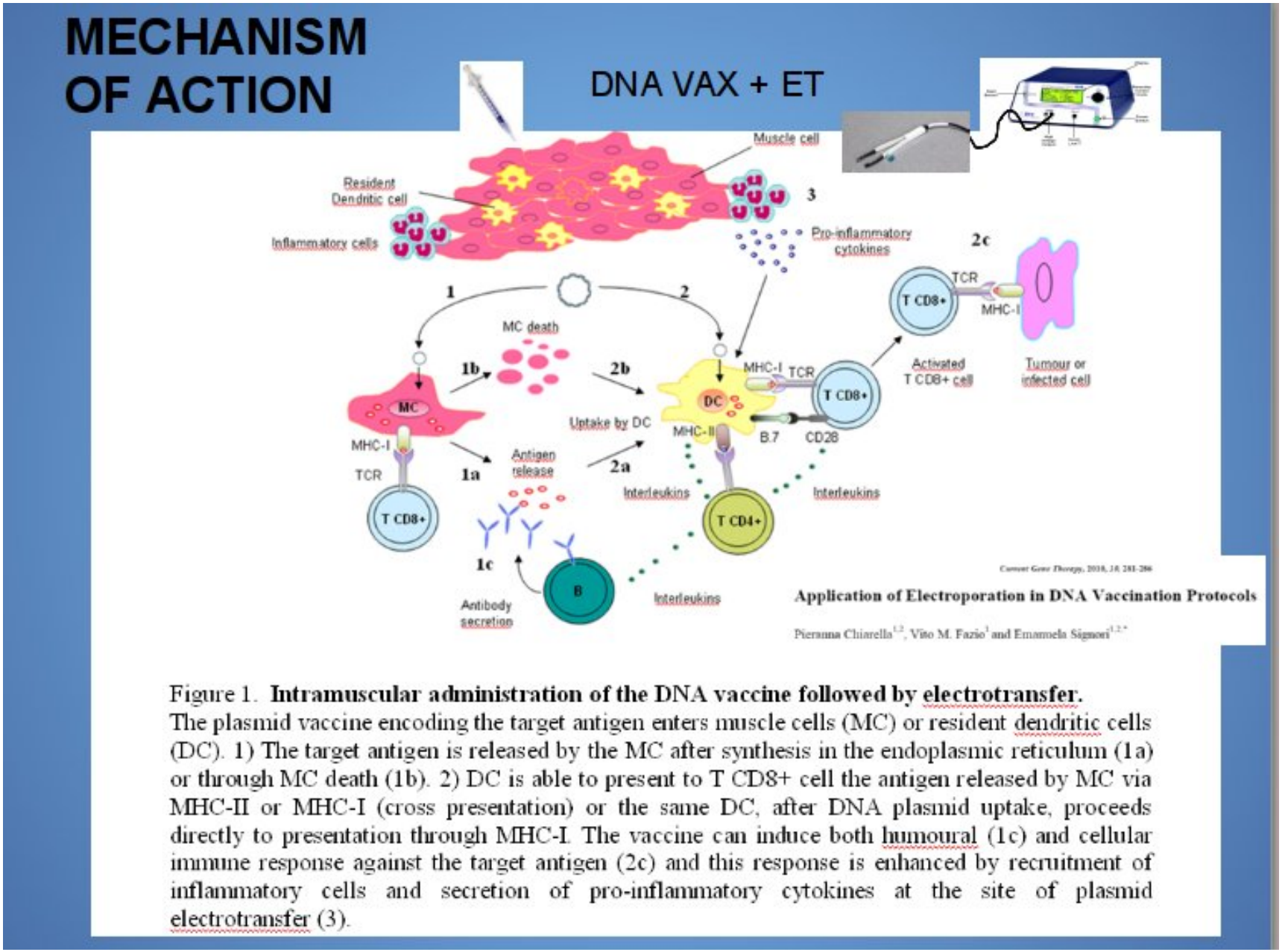


Figure 1. **Intramuscular administration of the DNA vaccine followed by electrotransfer.** The plasmid vaccine encoding the target antigen enters muscle cells (MC) or resident dendritic cells (DC). 1) The target antigen is released by the MC after synthesis in the endoplasmic reticulum (1a) or through MC death (1b). 2) DC is able to present to T CD8+ cell the antigen released by MC via MHC-II or MHC-I (cross presentation) or the same DC, after DNA plasmid uptake, proceeds directly to presentation through MHC-I. The vaccine can induce both humoural (1c) and cellular immune response against the target antigen (2c) and this response is enhanced by recruitment of inflammatory cells and secretion of pro-inflammatory cytokines at the site of plasmid electrotransfer (3).

Strategies to improve DNA vaccine and its efficacy

Use non-viral vectors, as Naked DNA plasmid

- advantage: very safe, expression of different antigens in the same construct
- disadvantage: **low gene expression and immune response**

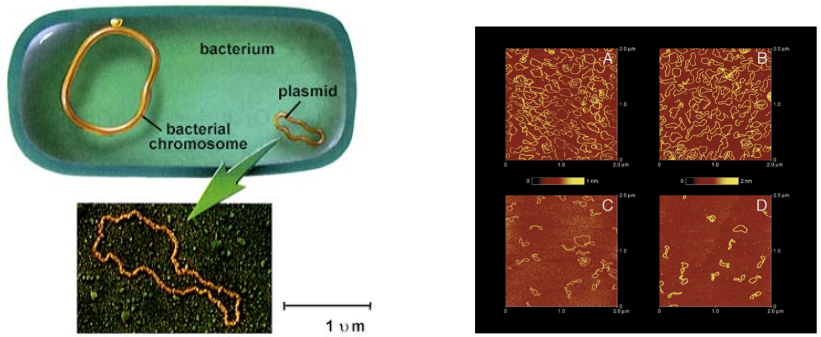
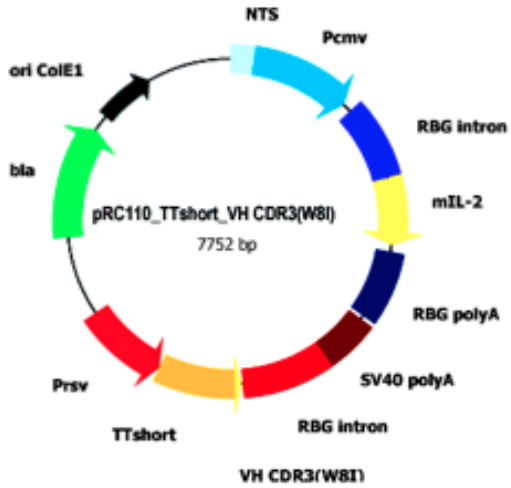


Figure : Plasmids: at left, bacteria plasmid; at right, colture of plasmids.

Optimize the plasmid transport in a cell and in the muscle tissue

- Injection of the plasmid in the muscle tissue (Enhancing permeability with hyaluronidase)
- Opening the pores of the cell membrane (Electroporation)
- transport inside of the cytoplasm (Using microtubules)
- entering the nucleus (RAN cycle and Importin)



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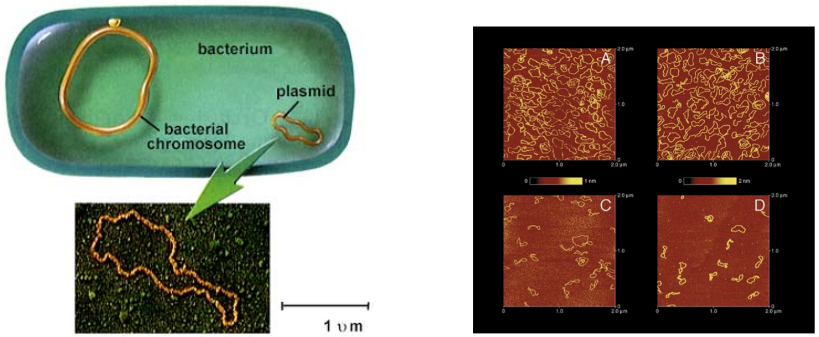

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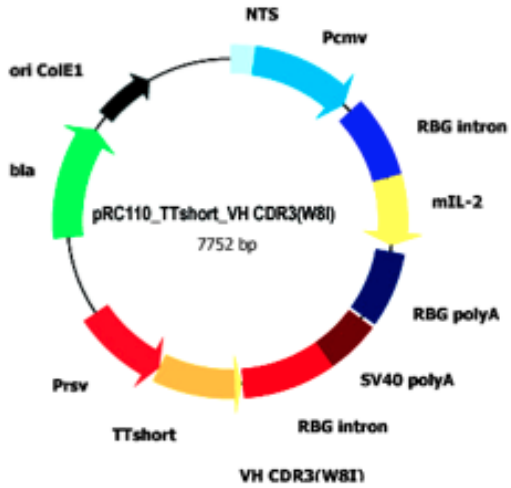

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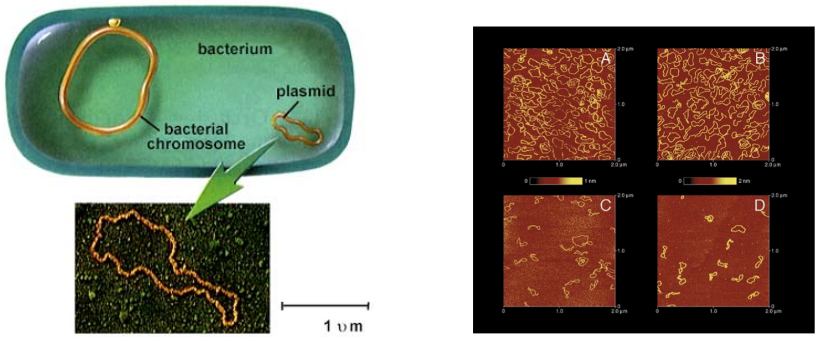

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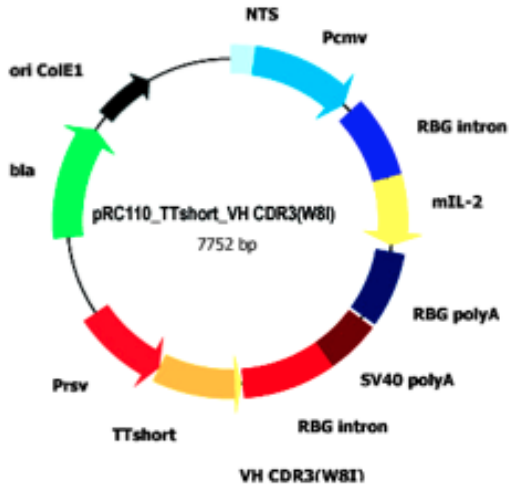

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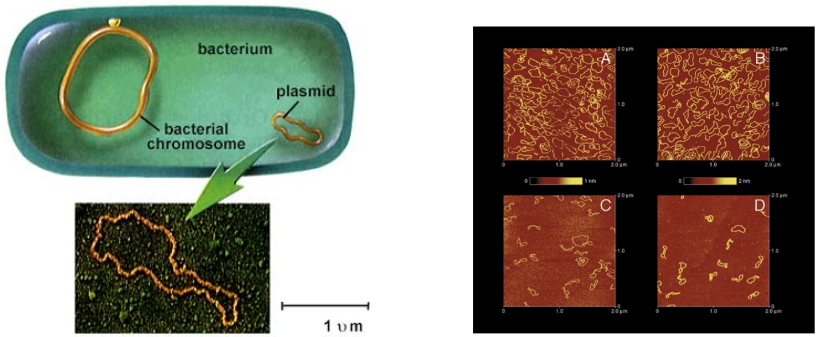

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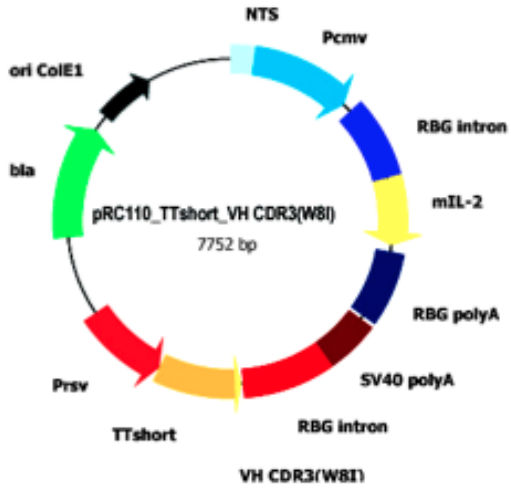

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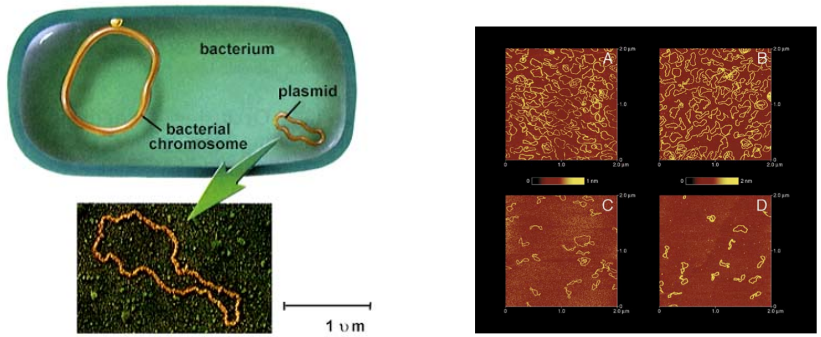

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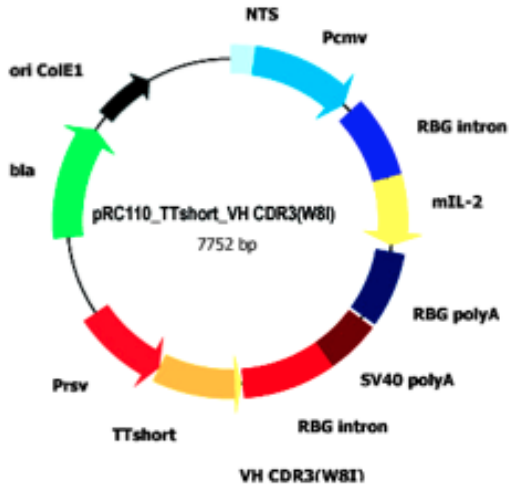
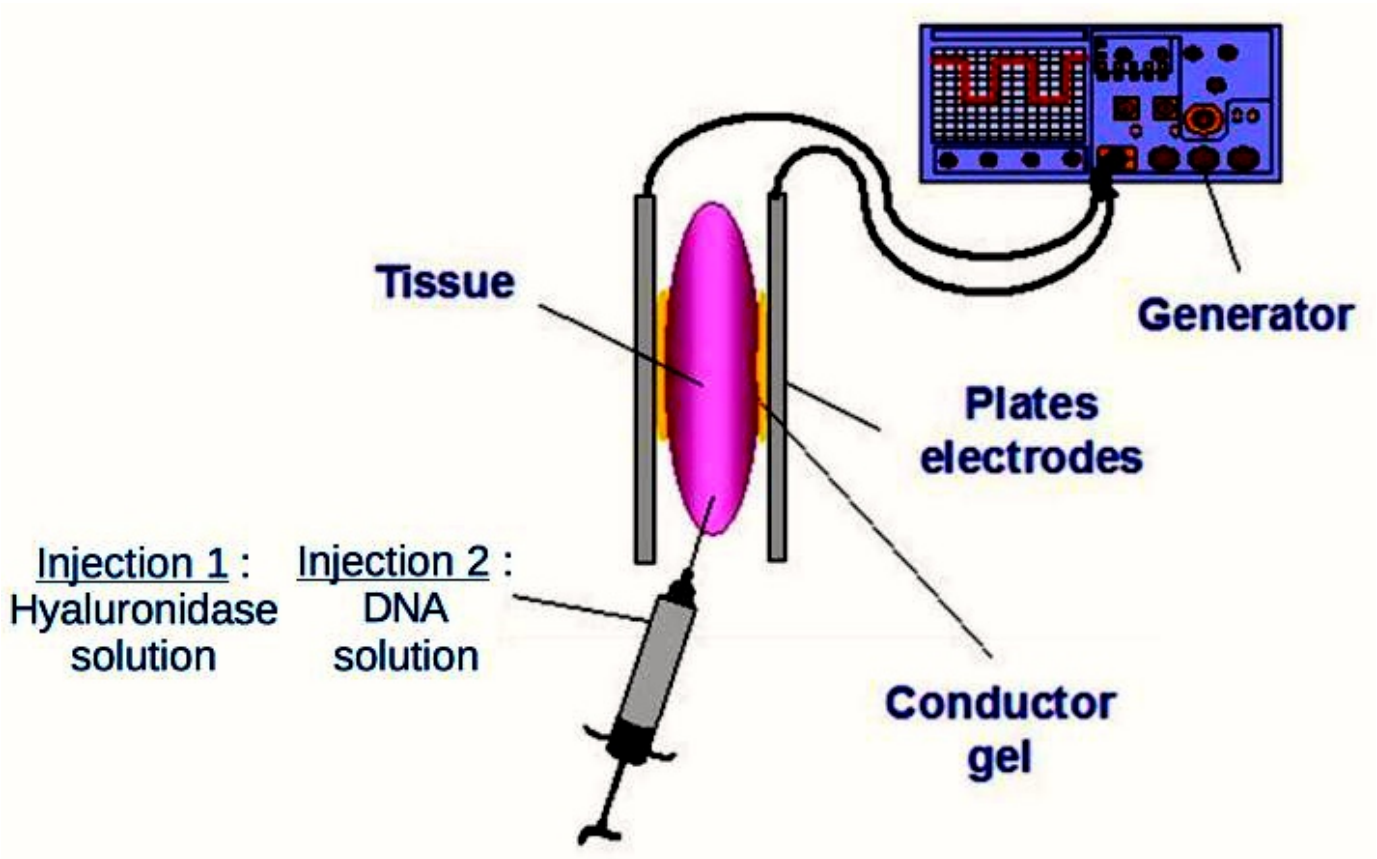
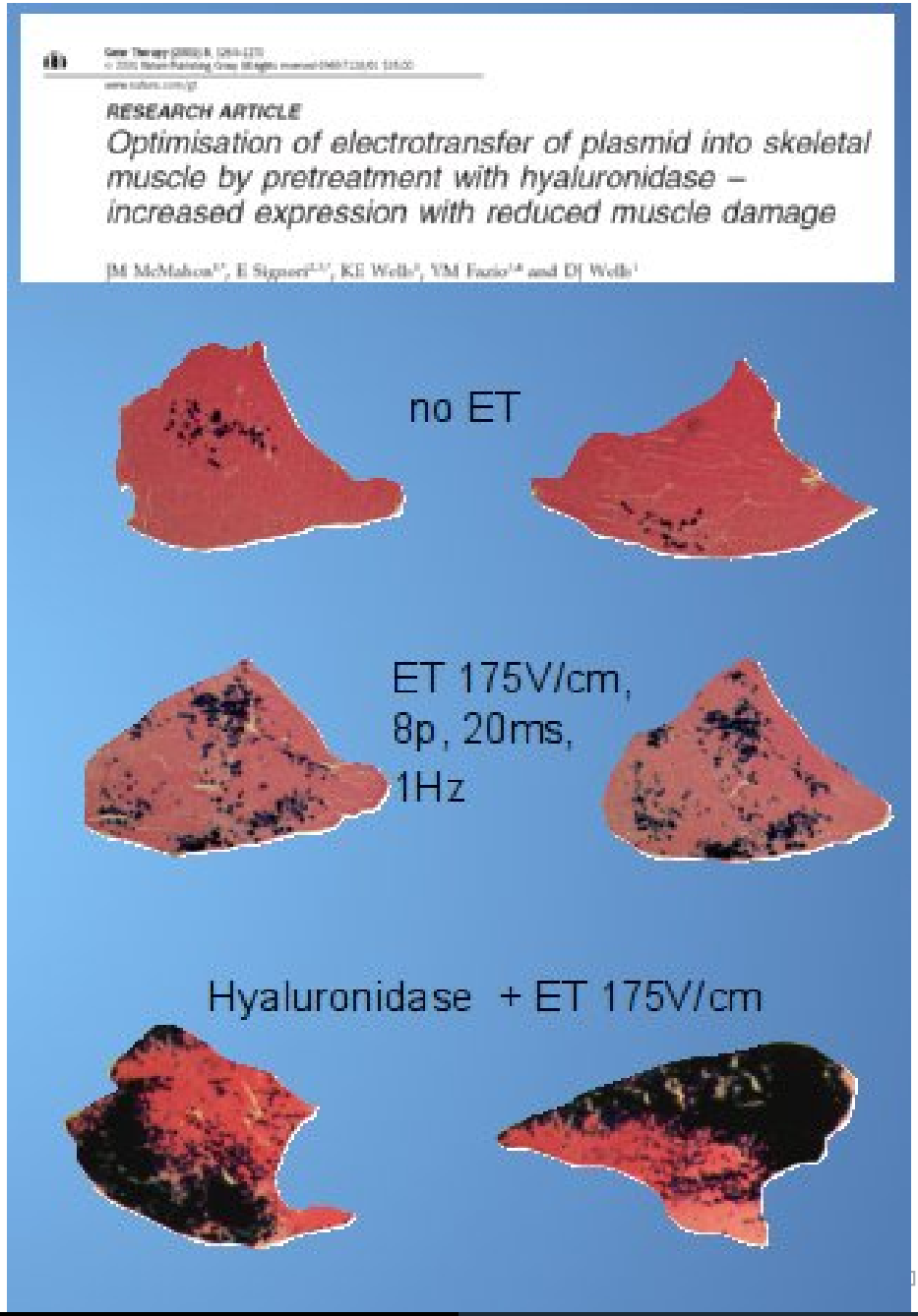


Model: Injection of hyaluronidase in the muscle
(work (in progress) in collaboration with Clair Poignard and Manon Deville)

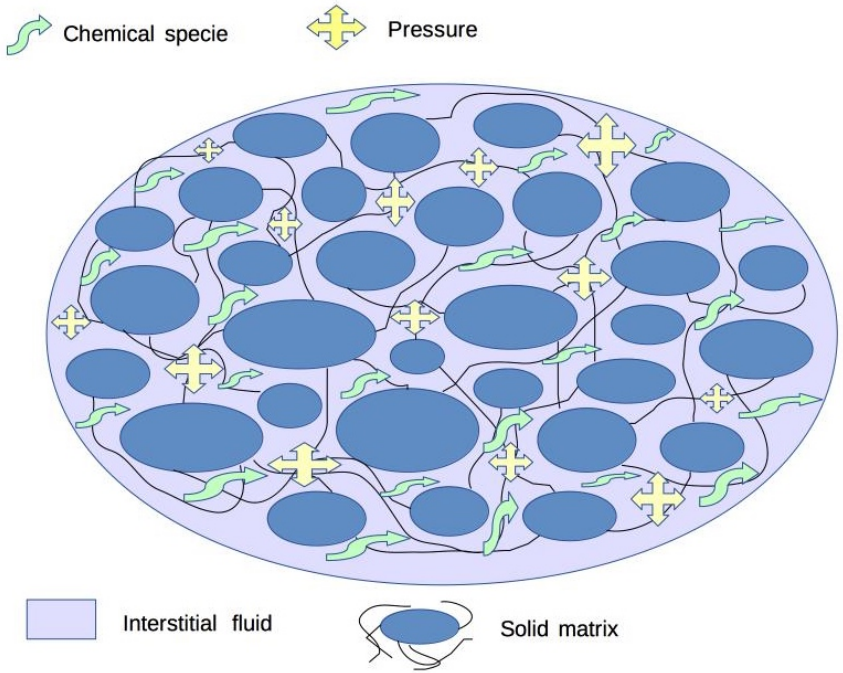
1. Injection of hyaluronidase
2. Injection of DNA plasmids
3. Electric pulses



Experimental results



Tissue = interstitial fluid + elastic fibers + cells



- The displacement of the solid matrix u,
- The interstitial velocity of the fluid V,
- The porosity of the medium f,
- The pressure P,
- The concentration of hyaluronidase in the tissue h.

Main ingredients of the model

The model is macroscopic and contains

- a constitutive law for the stress tensor
- Darcy's law on fluid flow in a porous medium
- equations of conservation of mass and momentum as applied to the porous space, the interstitial fluid and the chemical species

Displacement of the solid matrix

Linear constitutive law on the stress : $\sigma = 2G\varepsilon(u) + \lambda Tr(\varepsilon(u))I$,
where $\varepsilon(u) = \frac{\nabla u + \nabla u^T}{2}$.

The equations of mechanical equilibrium are given by

$$\rho_s \frac{\partial^2 u}{\partial t} = \nabla.(\sigma) + F$$

where F represents the body force, that we assume to be proportional to the pressure gradient. We get the following (linear) elasticity equation

$$\rho_s \frac{\partial^2 u}{\partial t^2} = (\lambda + G)\nabla(\nabla.u) + G\Delta u + F(\nabla P)$$

We assume a gradient in pore pressure accompanies a relative velocity between the interstitial fluid and the network

$$f(V - \frac{\partial u}{\partial t}) = -\kappa \nabla P.$$

Mass balance law on the solid matrix :

$$\partial_t((1-f)\rho_s) + \nabla.(\rho_s(1-f)\partial_t u) = \underbrace{-K(1-f)h}_{effect\ \ of\ \ hyaluronidase} + \underbrace{a_r(f-f_0)}_{reconstruction}$$

Mass balance law on the fluid :

$$\partial_t(f\rho_f) + \nabla.(\rho_f f V) = K(1-f)h + \underbrace{\varphi_s(t,x)}_{source}$$

Mass balance law on the chemical specie :

$$\partial_t(fh) = \underbrace{\nabla.(fD_0\nabla h)}_{diffusion} - \underbrace{\nabla.(f(V-\partial_t u)h)}_{advection} + \underbrace{\varphi_s(t,x)c_{inj}}_{source} - \underbrace{k_r f h}_{degradation}$$

Collecting all pieces of the model described so far, we obtain the following PDE system :

$$f(V - \frac{\partial u}{\partial t}) = -\kappa \nabla P \tag{1a}$$

$$\rho_s \frac{\partial^2 u}{\partial t^2} = (\lambda + G)\nabla(\nabla . u) + G\Delta u - F(\nabla P) \tag{1b}$$

$$f\frac{\partial h}{\partial t} = fD_0\Delta h + \nabla h.(\kappa \nabla P + D_0 \nabla f) - (k_r f + \frac{\varphi_s}{\rho_f} - \nabla.(f\frac{\partial u}{\partial t}))h - \frac{K}{\rho_f}(1-f)h^2 + \varphi_s(x,t)c_{inj} \tag{1c}$$

$$\partial_t f - \nabla.((1-f)\frac{\partial u}{\partial t}) = \frac{K}{\rho_s}(1-f)h - \frac{a_r}{\rho_s}(f - f_0) \tag{1d}$$

$$-\nabla.(\kappa \nabla P) = K(\frac{1}{\rho_f} - \frac{1}{\rho_s})(1-f)h + \frac{\varphi_s(x,t)}{\rho_f} + \frac{a_r}{\rho_s}(f - f_0) - \nabla.(\frac{\partial u}{\partial t}) \tag{1e}$$

Numerical results : concentration in hyaluronidase

Numerical results : porosity

Model: Intracellular transport of antitumoral vaccine (work in collaboration with Maria Grazia Notarangelo and Emanuela Signori)

Intracellular Transport (microscopic level)

It is necessary to know

- the size of DNA plasmids used in a vaccine,
- how the DNA plasmids navigate through the cytoplasm until the nucleus,
- how the DNA plasmids pass the Nuclear Envelope (NE) and arrive and diffuse into the nucleus.

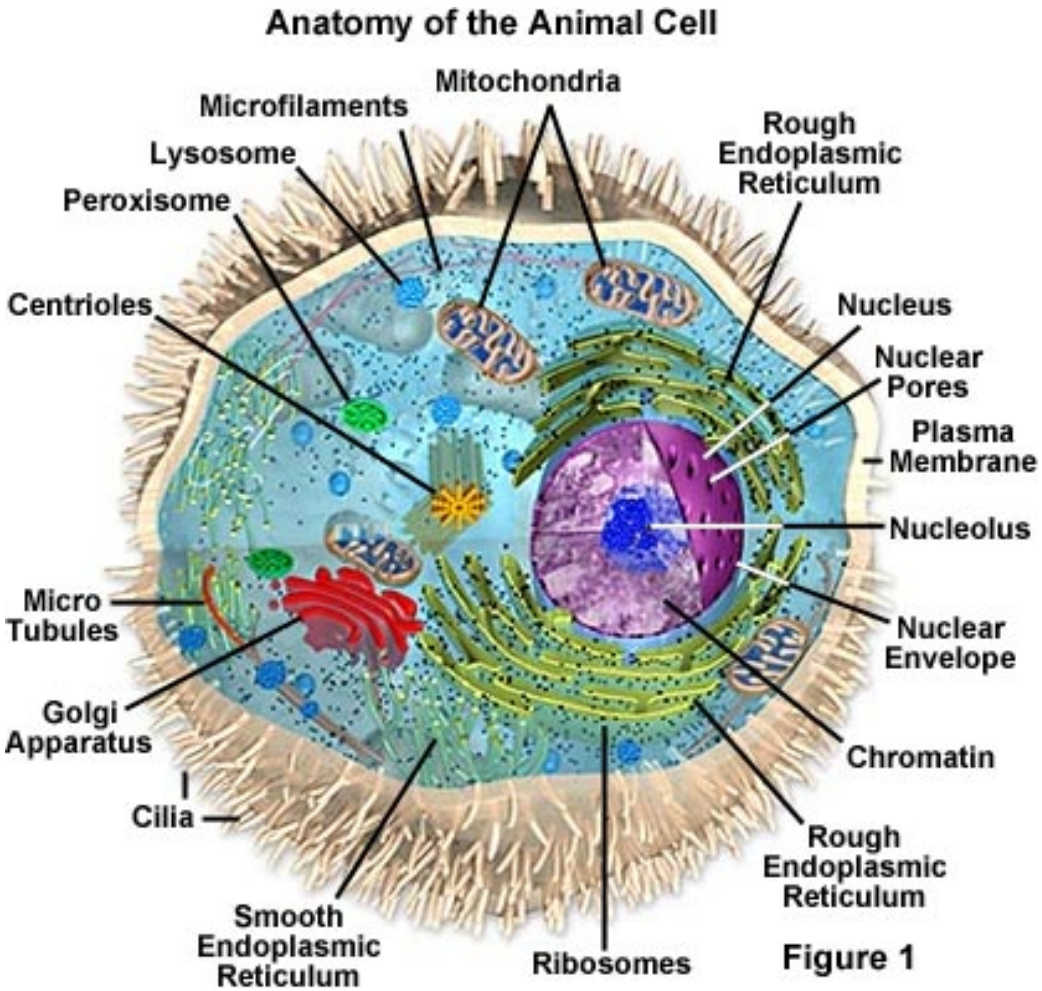


Figure 1

Main problem: the size of plasmids

The size of DNA plasmid vectors used as therapeutic molecules is between $\mathbf{5kb}$ and $\mathbf{20kb}$, which corresponds to molecular weight of around 3300 and 13200 kDa respectively.

Cytoplasm

Molecules of DNA larger than 2000 bp are unable to diffuse freely (*Dean et al. 2006*).

$\Downarrow$

Plasmids do NOT diffuse freely in the cytoplasm

Nuclear Envelope

Only those molecules of mass less than 40 kDa can freely diffuse through the Nuclear Pore Complexes (NPCs) (*Gorlich et al., 2003*).

$\Downarrow$

Plasmids do NOT diffuse freely through the NPCs

Low Gene Expression - Reasons

The large size of plasmids and the inner structure of skeletal muscle cells involve that

- DNA plasmids degrades in the cytoplasm-the dense latticework of citoskeleton impedes free diffusion.
- Nuclear Envelope is a barrier for reaching the nucleus.

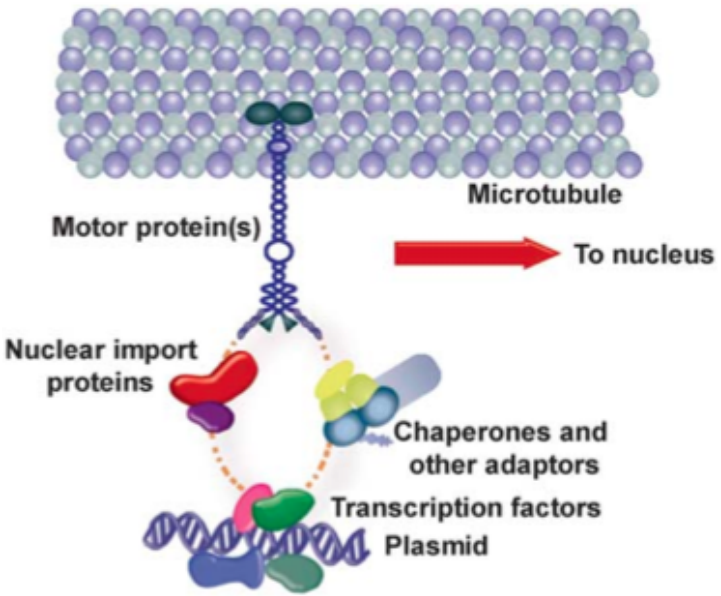
Critical Goal

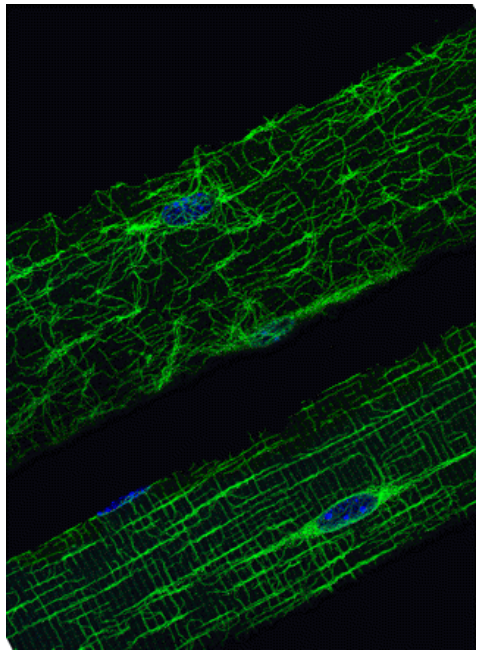
Know and enhance the principal processes that regulate the intracellular transport :

- plasmid-microtubule interaction
- plasmid-importin bound.

Plasmid-Microtubule interaction

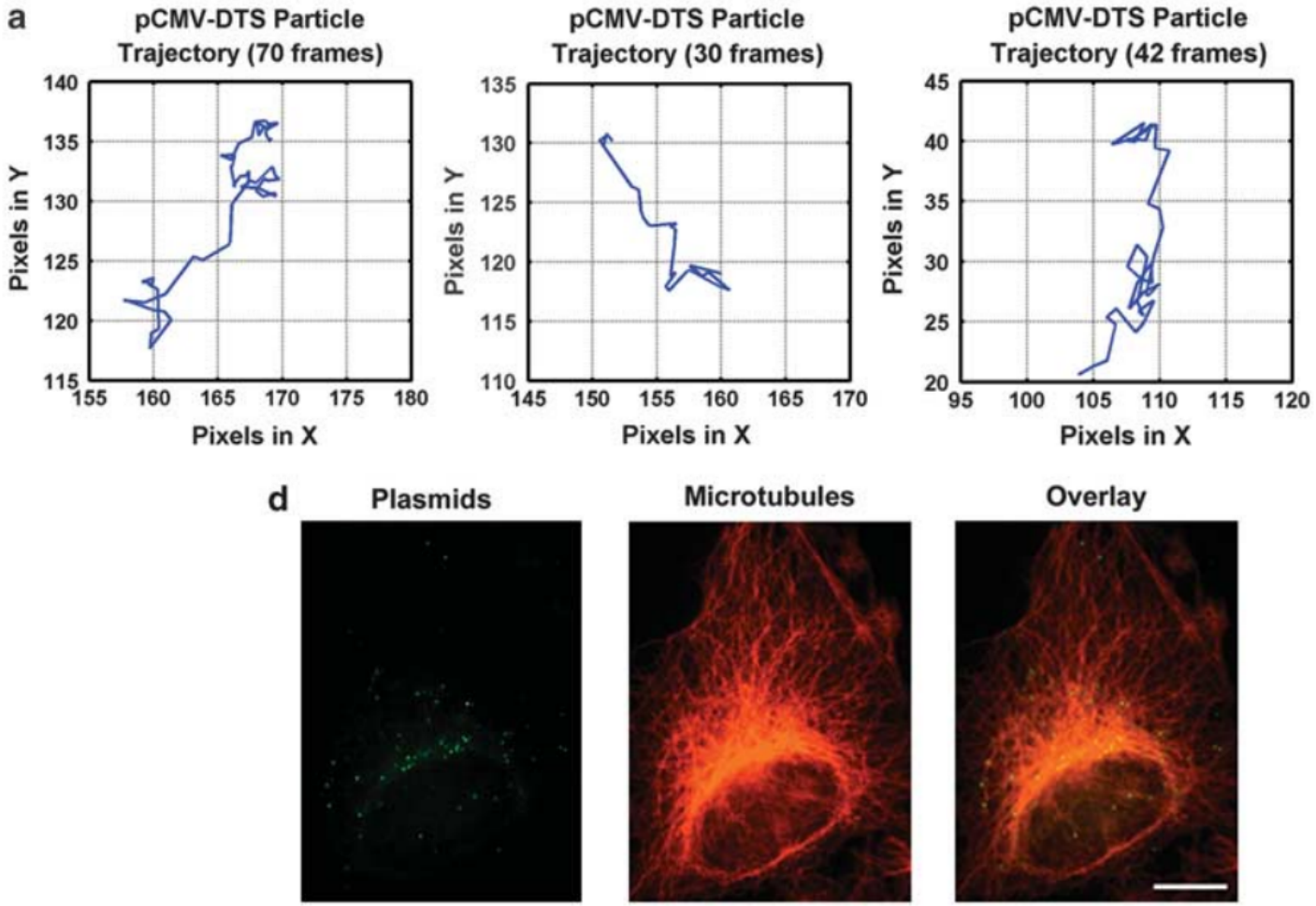
Microtubules are one component of the cytoskeleton.
Dynein (motor protein) moves along microtubule and it transports large molecules near the nucleus.





The plasmid-microtubule interaction require cytoplasmatic adapter proteins such as dynein, transcriptional factors (TFs) and importins (*Dean et al.2012*).
The plasmid-microtubule interaction have an important role in intracellular trafficking of plasmids (*Dean et al.2012*)

Plasmid-Microtubule interaction (Dean experiments)

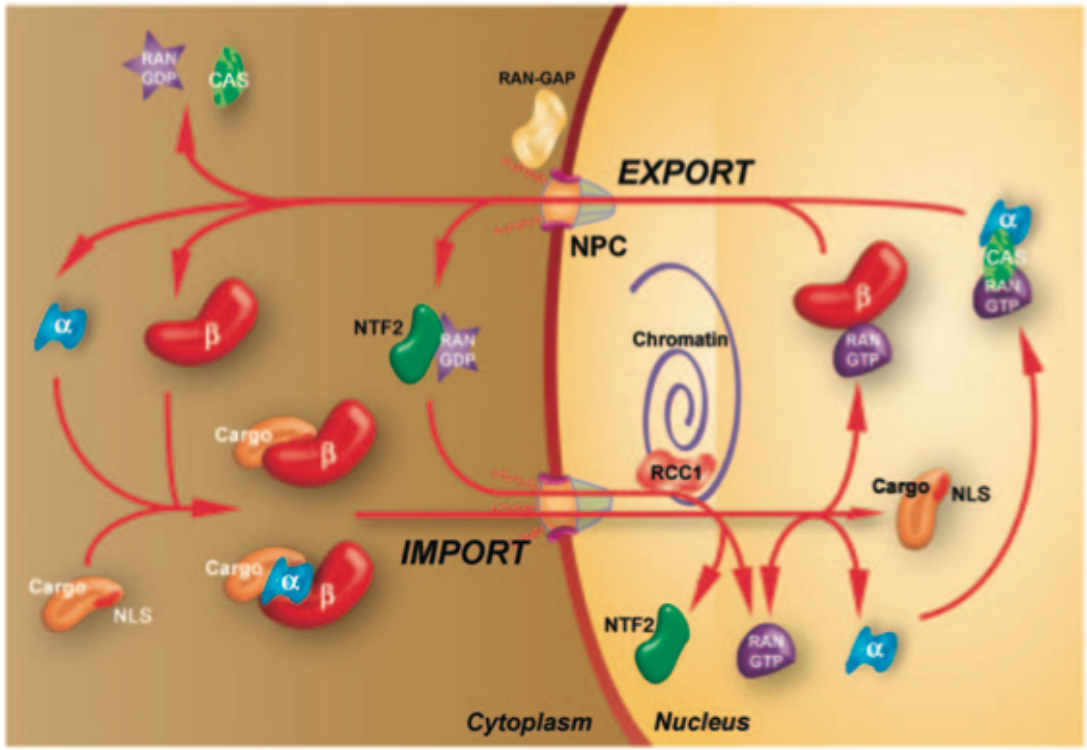


Importin and Ran Cycle

Importin is a protein that allows the translocation of plasmids across the nuclear envelope.

Plasmids bound to importin and enter in the nucleus.

The concentration of importin between cytoplasm and nucleus is monitored by Ran cycle (RanGDP and RanGTP) (Cangiani-Natalini, JTB 2010)



DNA target sequences (DTS) and CREB binding site allow that plasmids enter the nucleus (*Dean et al. 2011*)

Solutions to Low Gene Expression

Plasmids with sites for TF, NLS and dynein

- interact with microtubules and importin,
- enter into the nucleus,
- have a good gene expression

BUT

Plasmids are too big

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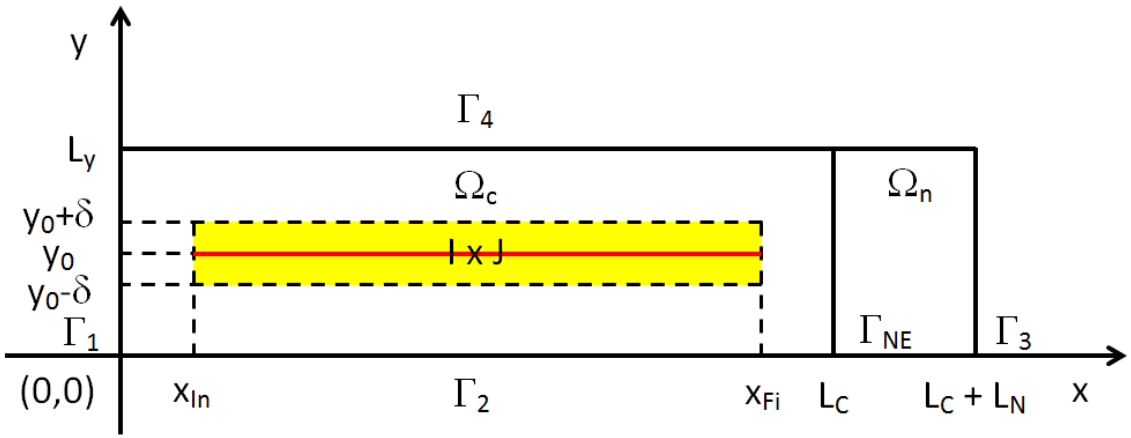
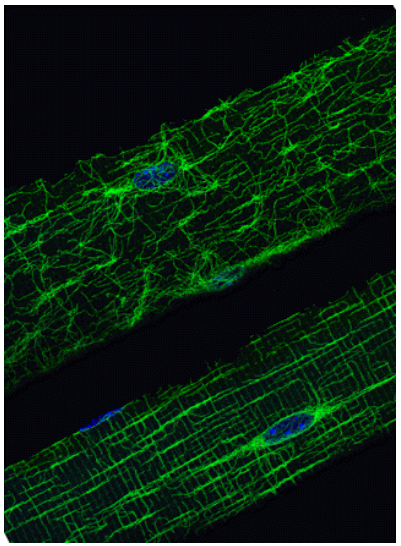
A simplified mathematical model





$I \times J$ attraction area of microtubule with $I = [x_{In}, x_{Fi}]$ and $J = [y_0 - \delta, y_0 + \delta]$.

Variables: cytoplasm transport

- u, cargo (plasmid+NLS+DTS+TF)
- v_d, cargo + dynein complex
- v_i, cargo + importin complex (imp=impα+impβ)
- v_{di}, cargo + dynein+importin complex

transport along microtubule

- w_d, cargo + dynein complex on the microtubule
- w_{di} cargo + dynein + importin complex on the microtubule

nuclear transport

- I, importin
- R_d, RanGDP
- R_t, RanGTP
- I_R, Importin + RanGTP complex

We present the most significant reactions between the complexes that occurs in the cell

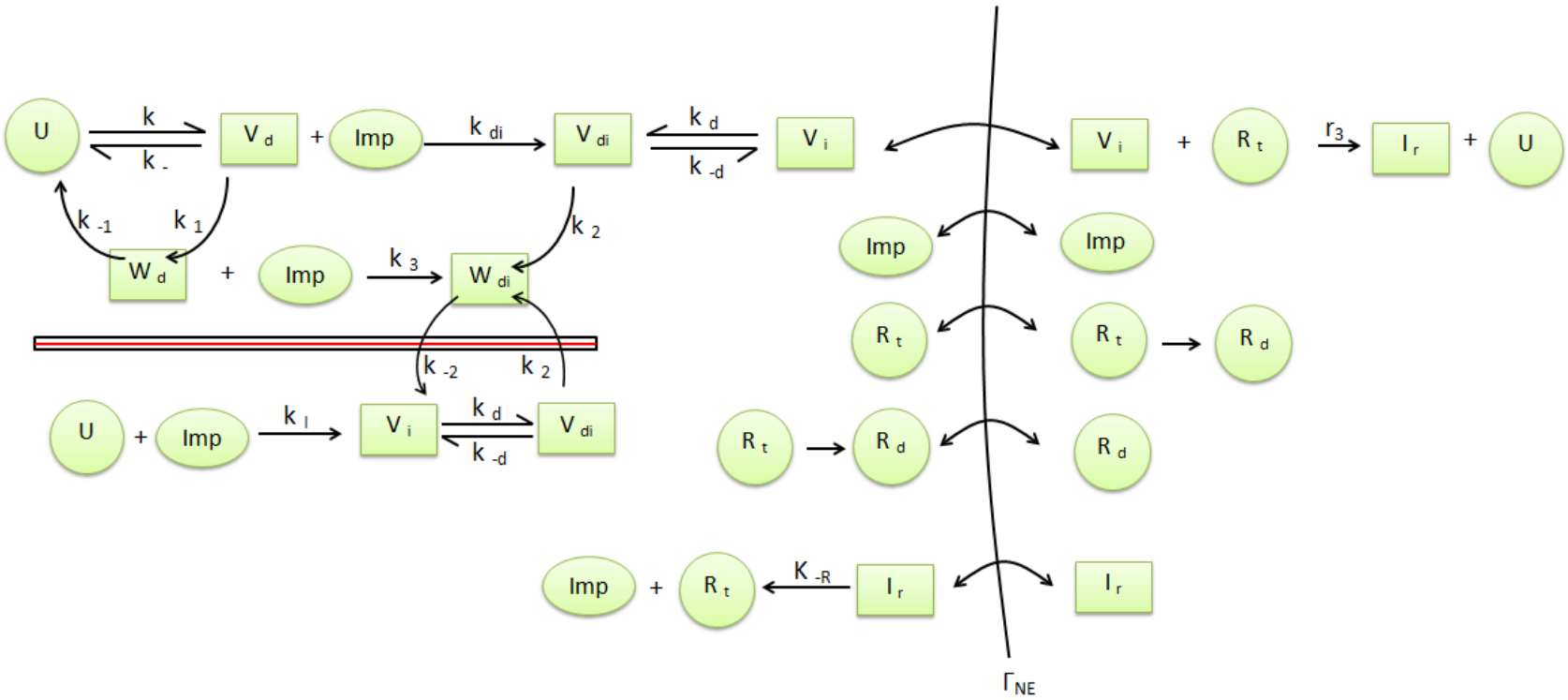


Figure : Reactions between constituents of the model.

Cargo and its complexes can be degradate.

In the cytoplasm Ω_C

Ω_C

$$u \underset{k_-}{\overset{k}{\longleftrightarrow}} v_d + I_{mp} \xrightarrow{k_{di}} v_{di},$$

$$u + I_{mp} \xrightarrow{k_i} v_i \underset{k_{-d}}{\overset{k_d}{\longleftrightarrow}} v_{di},$$

$$R_t \xrightarrow{m1} R_d,$$

$$I_r \xrightarrow{k_{-R}} R_t + I_{mp},$$

$I \times J$

$$v_d \overset{k_1}{\curvearrowright} w_d,$$

$$v_{di} \overset{k_2}{\curvearrowright} w_{di},$$

$y_0 \times [x_{in}, x_{Fi}]$

$$w_d \overset{k_{-1}}{\curvearrowright} u,$$

$$w_d + I \xrightarrow{k_3} w_{di} \overset{k_{-2}}{\curvearrowright} v_i,$$

Mathematical model (cytoplasm)

$$
\begin{cases}
\partial_t u = d_u \Delta u - k_{deg} u - k u + k_- v_d - k_i u I_{mp} + k_{-1} w_d \frac{\nVdash_{IxJ}}{|J|} + c_d \frac{w_d(x)}{|J|} \delta_0(x - x_{Fi}, y - \tilde{y}) \\
\partial_t I_{mp} = d_{I_{mp}} \Delta I_{mp} - k_i u I_{mp} - k_{di} v_d I_{mp} + r_{-1}(Ir) - k_3 I_{mp} w_d \frac{\nVdash_{IxJ}}{|J|}, \\
\partial_t R_d = d_{R_d} \Delta R_d + m1(Rt), \\
\partial_t R_t = d_{R_t} \Delta R_t - m_1(R_t) + r_{-1}(I_r), \\
\partial_t I_r = d_{I_r} \Delta I_r - r_{-1}(I_r), \\
\partial_t v_i = d_i \Delta v_i - k_{deg} v_i - k_d v_i + k_i u I_{mp} + k_{-d} v_{di} + k_{-2} w_{di} \frac{\nVdash_{IxJ}}{|J|} + c_{di} \frac{w_{di}(x)}{|J|} \delta_0(x - x_{Fi}, y - \tilde{y}) \\
\partial_t v_d = d_{vd} \Delta v_d - k_{deg} v_d - k_- v_d - k_{di} v_d I_{mp} + k u - k_1 v_d I_{IxJ}, \\
\partial_t v_{di} = d_{vdi} \Delta v_{di} - k_{deg} v_{di} - k_{-d} v_{di} + k_d v_i + k_{di} v_d I_{mp} - k_2 v_{di} I_{IxJ}, \\
\partial_t w_d = -c_d \frac{\partial w_d}{\partial x} - k_{-1} w_d - k_{deg} w_d + k_1 \int_J v_d(x,y) dy - k_3 w_d \int_J I_{mp}(x,y) dy \qquad \mathbf{y_0} \times (\mathbf{x_{In}}, \mathbf{x_{Fi}}) \\
\partial_t w_{di} = -c_{di} \frac{\partial w_{di}}{\partial x} - k_{-2} w_{di} - k_{deg} w_{di} + k_2 \int_J v_{di}(x,y) dy + k_3 w_d \int_J I_{mp}(x,y) dy
\end{cases}
\tag{2}
$$

- 1D in 2D:

$$W_d(x,y) = w_d(x)\frac{\mathbf{1}_{I\times J}}{|J|} = \begin{cases} \frac{w_d(x)}{|J|} & \text{if } (x,y)\in I\times J \\ 0 & \text{elsewhere} \end{cases}$$

- 1 point value in 2D:

$$c_d\frac{w_d(x)}{|J|}\delta_0(x-x_{Fi},y-\tilde{y}) \qquad c_{di}\frac{w_{di}(x)}{|J|}\delta_0(x-x_{Fi},y-\tilde{y}).$$

- 2D in 1D:

$$\int_J v_d(x,y)dy \qquad \int_J v_{di}(x,y)dy \qquad \int_J I_{mp}(x,y)dy,$$

In the nucleus, we have

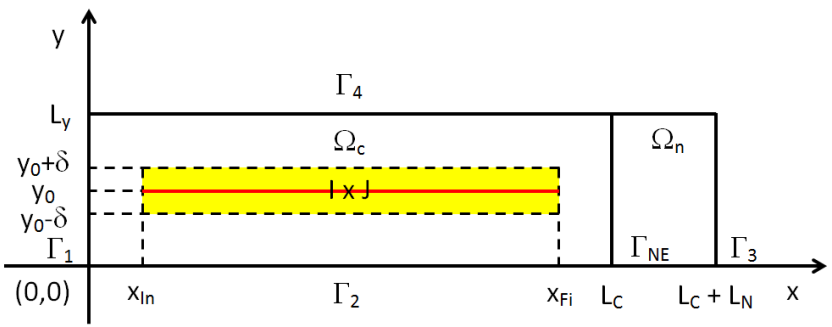
$$v_i + R_t \xrightarrow{r_3} I_r + U, \qquad\qquad R_d \xrightarrow{m_2} R_t.$$

$$\begin{cases} \partial_t v_i = d_i \Delta v_i - r_3(R_t, v_i), & in \quad \mathbf{\Omega_N} \\ \partial_t R_t = d_{R_t} \Delta R_t + m_2(R_d) - r_3(R_t, v_i), \\ \partial_t u = d_u \Delta u + r_3(R_t, v_i), \\ \partial_t I_r = d_{I_r} \Delta I_r + r_3(R_t, v_i), \\ \partial_t R_d = d_{R_d} \Delta R_d - m_2(Rd), \\ \partial_t I_{mp} = d_{I_{mp}} \Delta I_{mp}. \end{cases} \tag{3}$$

where $m_2(R_d) = K_C^D C_1 \frac{R_d}{K_m^d + R_d}$, and $r_3(R_t, v_i) = r_3 R_t v_i$.

We set

- homogeneous Neumann condition on Γ_1 and Γ_3 for all variables,
- periodic boundary conditions on Γ_2 and Γ_4 for all variables,
- Kedem-Katchalsky conditions on Γ_{NE} as in the system



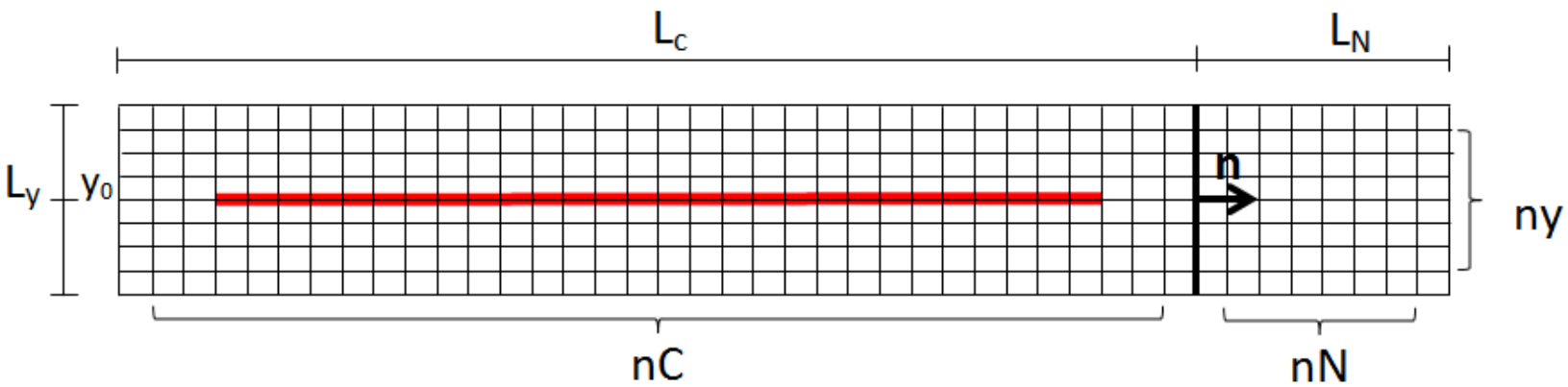
$$
\begin{cases}
d_{v_i} \dfrac{\partial v_i^{(c)}}{\partial \mathbf{n}} = d_{v_i} \dfrac{\partial v_i^{(n)}}{\partial \mathbf{n}} = p_{vi} \left(v_i^{(n)} - v_i^{(c)} \right), \\
d_{R_t} \dfrac{\partial R_t^{(c)}}{\partial \mathbf{n}} = d_{R_t} \dfrac{\partial R_t^{(n)}}{\partial \mathbf{n}} = p_{R_t} (R_t^{(n)} - R_t^{(c)}), \\
d_{R_d} \dfrac{\partial R_d^{(c)}}{\partial \mathbf{n}} = d_{R_d} \dfrac{\partial R_d^{(n)}}{\partial \mathbf{n}} = p_{R_d} (R_d^{(n)} - R_d^{(c)}), \\
d_{I_{mp}} \dfrac{\partial I_{mp}^{(c)}}{\partial \mathbf{n}} = d_{I_{mp}} \dfrac{\partial I_{mp}^{(n)}}{\partial \mathbf{n}} = p_{I_{mp}} (I_{mp}^{(n)} - I_{mp}^{(c)}), \\
d_{I_r} \dfrac{\partial I_r^{(c)}}{\partial \mathbf{n}} = d_{i_r} \dfrac{\partial I_r^{(n)}}{\partial \mathbf{n}} = p_{I_r} (I_r^{(n)} - I_r^{(c)}), \\
d_u \dfrac{\partial u^{(c),(n)}}{\partial \mathbf{n}} = 0, \\
d_{vd} \dfrac{\partial v_d^{(c)}}{\partial \mathbf{n}} = 0, \\
d_{vdi} \dfrac{\partial v_{di}^{(c)}}{\partial \mathbf{n}} = 0.
\end{cases}
$$

$$L_C = 10\mu m, \qquad L_N = 2\mu m, \qquad L_y = 0.02\mu m$$

Δ_x : space discretization step along $x-$ direction,
Δ_y : space discretization step along $y-$ direction

$$\Delta_x \neq \Delta_y$$

Δ_t : time discretization step.



$$(x_i, y_j) = (i\Delta_x, j\Delta_y), \qquad i = 0, \ldots n_C + n_N + 2,$$
$$j = 0, \ldots n_{y1}$$

$$u^n_{i,j} = u(x_i, y_j, \tilde{t}), \qquad \forall n : n\Delta_t = \tilde{t}$$

Schemes

- a third order time in Implicit-Explicit (IMEX) scheme for reaction-diffusion equations,
- a forward Euler scheme for advection equations.

CFL conditions

For reaction-diffusion equations we propose

$$\Delta_t^{IMEX} \leq \frac{\sqrt{4\gamma - 1}}{2\gamma} min\left\{\Delta_x, \Delta_y\right\}$$

for the stability of the scheme ($\gamma = \frac{3+\sqrt{3}}{6}$).
For advection equations, the stability of the scheme is satisfied for

$$\Delta_t^{Advection} \leq \frac{\Delta_x}{max(c_d, c_{di})}.$$

So we take

$$\Delta_t = min(\Delta_t^{IMEX}, \Delta_t^{Advection}).$$

Numerical Results

Experimental Data

From biological experiments we know

- data involved in the Ran Cycle,
- size of plasmids.

We have to deduce some parameter values as

- diffusion of plasmid and its complexes (?),
- rate of degradation
- rate of attach and detach by microtubule, (?)
- permeability coefficients of cargo + importin, p_{vi}, (?)
- rate of reactions between species, (?)
- velocity of movement along microtubule (?)
- how much DNA vaccine arrives in the cytoplasm, starting from 50 $\frac{\mu g}{30 \mu l}$ vaccine dose (?)

We will observe these behaviors

$T = 20sec$ $T = 40sec$ $T = 60sec$

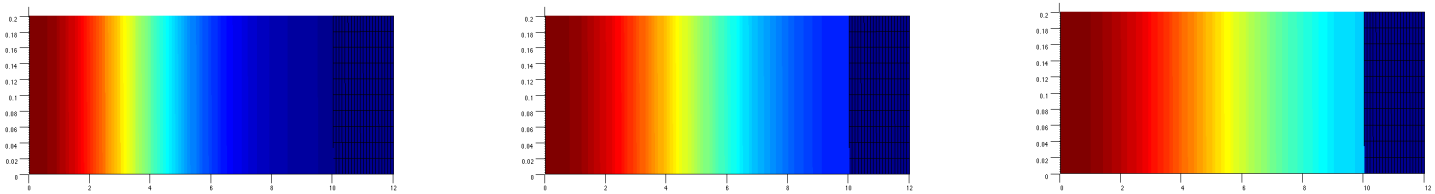


Figure : Spatial distribution of the cargo concentration and its complexes on the action of diffusion without MT activity and permeability on the nuclear envelope

$T = 20sec$ $T = 40sec$ $T = 60sec$

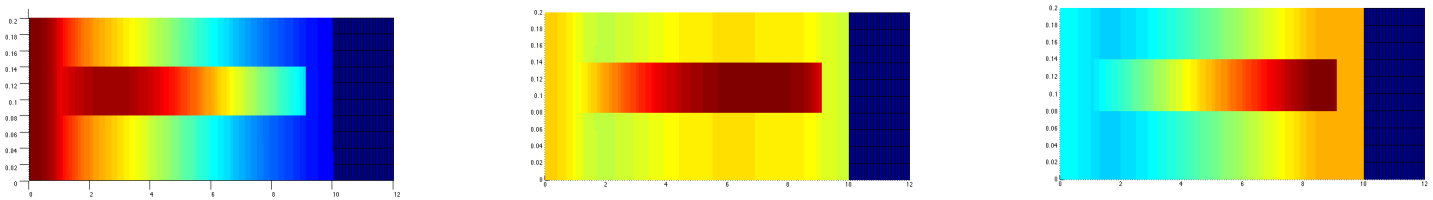


Figure : Spatial distribution of the cargo concentration and its complexes on the action of diffusion and MT. No permeability on the nuclear envelope

$T = 20sec$ $T = 40sec$ $T = 60sec$

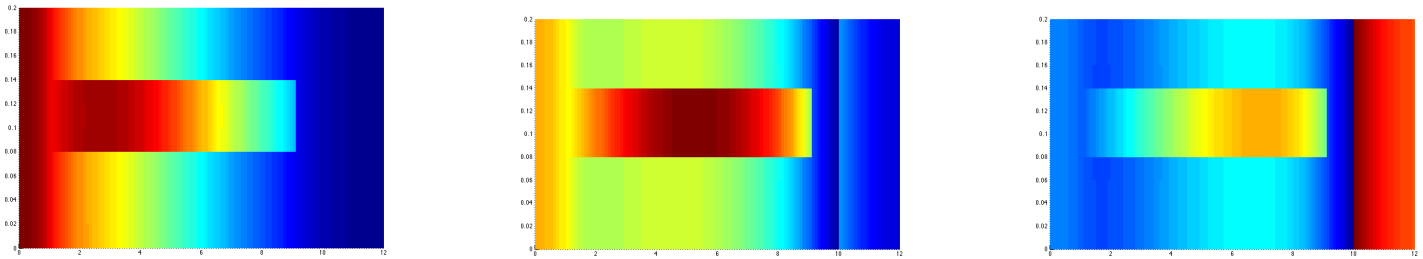


Figure : Spatial distribution of the cargo concentration and its complexes on the action of diffusion and MT. The permeability on the nuclear envelope is considered.

Values of parameters

Domain	Parameter	Value	Reference(s)
Ω_C	k_{deg}		proposed
	k	0.2	Dimitrio et al.(2012)
	k_-	0.2	Dimitrio et al.(2012)
	k_d	0.2	proposed
	k_{-d}	0.2	proposed
	k_i	0.2	proposed
	k_{di}	0.2	proposed
	k_R	0.2	proposed
	K_c^t	10.6	Gorlich et al.(2003)
	k_m^t	0.7	Makara et a (2005)
	R_g	0.5	Riddick et al. (2005)
$\mathbf{I} \times \mathbf{J}$	k_1	0.2	proposed
	k_{-1}	0.05	proposed
	k_2	0.2	proposed
	k_{-2}	0.2	proposed
	k_3	0.2	proposed
Ω_N	r_3	0.1	proposed
	K_c^d	8	Gorlich et al (2003)
	k_m^t	1.1	Gorlich et al(2003)
	C_1	0.7	Gorlich et al (2003)

Domain	Parameter	Value	Reference(s)
Ω	$d_{I_{mp}}$	12	estimated
	d_{R_t}	17	Gorlich et al. (2003)
	d_{R_d}	20	estimated
	d_{I_r}	10	Gorlich et al. (2003)
	d_u	2.52	calcultated
	d_{v_i}	2.47	calcultated
Ω_C	d_{v_d}	2. 12	calcultated
	$d_{v_{di}}$	2. 09	calcultated
$\mathbf{y_0} \times (\mathbf{x_{In}}, \mathbf{x_{Fi}})$	c_d	0.38	Dean et al.
	c_d	0.38	Dean et al.
Γ_{NE}	p_{R_t}	3.73	Smith et al. 2002
	p_{R_d}	3.73	Smith et al. 2002
	p_{Imp}	1.87	Smith et al. 2002
	p_{I_r}	1.87	Smith et al. 2002
	p_{v_i}	1	proposed
	$p_u, p_{v_d}, p_{v_{di}}$	0	proposed

Initial Data

Type	Concentration μM	Cell
I_{mp}	4	Cytoplasm
R_d	3	Cytoplasm
R_t	3	Nucleus
I_r	0	Cell
v_d	0	Cell
v_i	0	Cell
v_{di}	0	Cell
w_d	0	Cell
w_{di}	0	Cell

$$u(x,y,0) = \begin{cases} 10 & \text{if} \quad 0 \le x \le x_0 \\ 10e^{-(x-x_0)^2} & \text{if} \quad x_0 \le x \le L_C \end{cases}$$

Fixing x_0, the initial mass of the cargo $M_u = 2.77$

Degradation Coefficient

Experiment 1

During a typical 24 hour transfection experiment, unless the DNA reaches the nucleus quickly, less than 0.4% of the input DNA would remain in the cytoplasm by 24 hours (Dean et al 2006).

$$\Downarrow$$

k_{deg}

$$\begin{cases} \frac{du}{dt} = -k_{deg} u \\ \\ u(0) = u_0 \end{cases}$$

$$u(t) = u(0) e^{-k_{deg} t}$$

For T=24 h, and $u(T) = 0.04 \times u_0$, we obtain

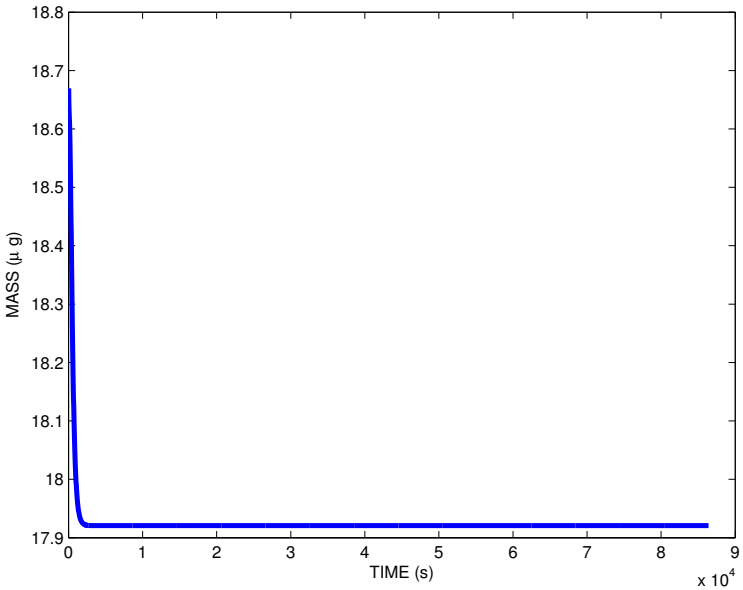
$$k_{deg} = 0.6 * 10^{-4} (1/s)$$

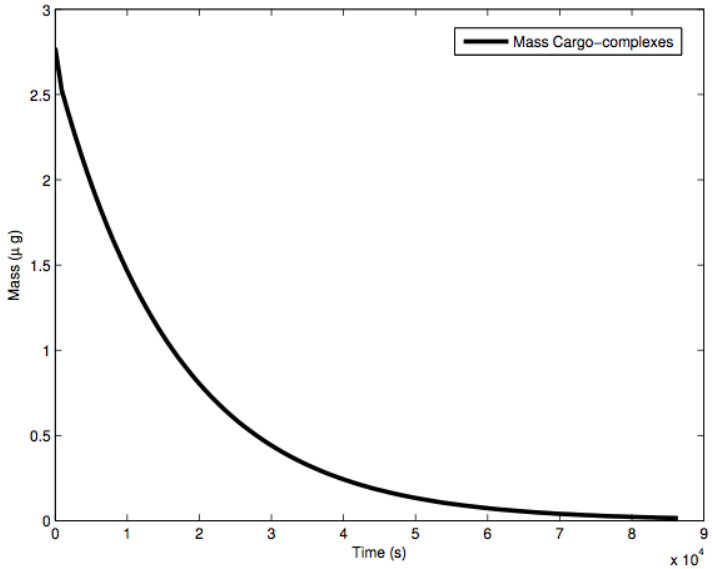
Degradation Coefficients

We set

$$M_T = M_u + M_{vi} + M_{vd} + M_{vdi} + M_{wd} + M_{wdi} + M_{Imp} + M_{Rt} + M_{Rd} + M_{Ir}$$

$$M_{complexes} = M_u^{(c)} + M_{vd} + M_{vdi} + M_{vi}^{(c)} + M_{wd} + M_{wdi}$$





Time evolution of the total mass M_T in the cell without the degradation coefficient (on the left) and time evolution of mass cargo complexes effected by degradation with $k_{deg} = 0.6 * 10^{-4}$ (on the right).

Experiment 2

Thanks to plasmid-microtubule interaction, a strong nuclear localization of plasmids occurs in a cell by 3 h (Dean et al 2006). The plasmids with CREB binding site start to reach the nucleus after 60 min (Dean et al 2012).

$$\Downarrow$$

Plasmids pRC110: 7752 bp, 5.1 MDa.
We estimate the diffusion coefficients according to

- Prazeres relations

$$D = \frac{A_1 T}{\eta} M^{-2/3}$$

Complex	**M** (Da)	**Diffusion Coefficient** $(\mu m^2 s^{-1})$
Dynein	1500000	
Cargo	5116320	2.52
Cargo + Dynein	6616320	2.12
Cargo + Importin	5266320	2.47
Cargo +Importin + Dynein	6766320	2.09

Diffusion Coefficients

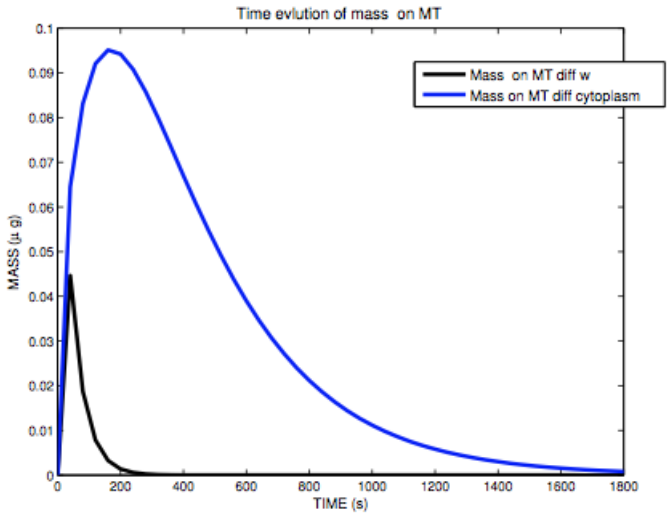
- Luckas relations

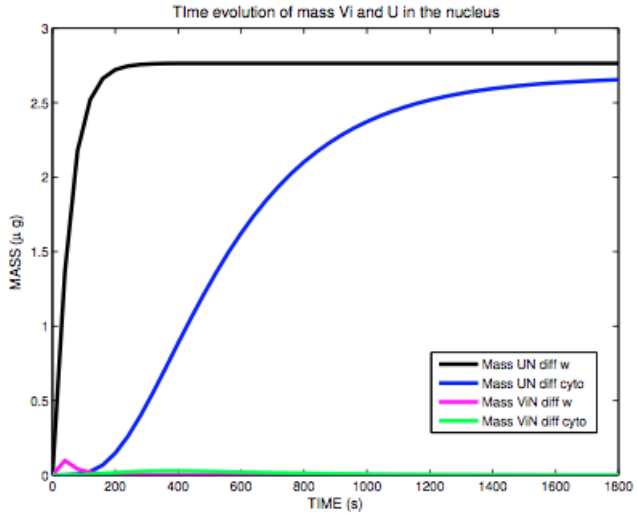
$$D_{cyto}/D_w < 0.01 \qquad \text{for bp } > 2000$$

Diffusion Coefficients	**Species**
$d_u = 0.0252$	Cargo
$d_{v_d} = 0.0212$	Cargo-Dynein complex
$d_{v_i} = 0.0247$	Cargo-Importin complex
$d_{v_{di}} = 0.0209$	Cargo-Dynein-Importin complex

We compare the simulations for

- D_w according to Prazeras,
- D_{cyto} according to Luckas.

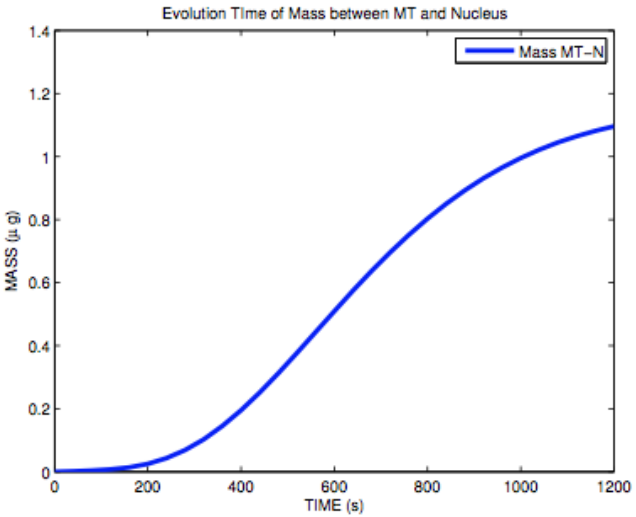


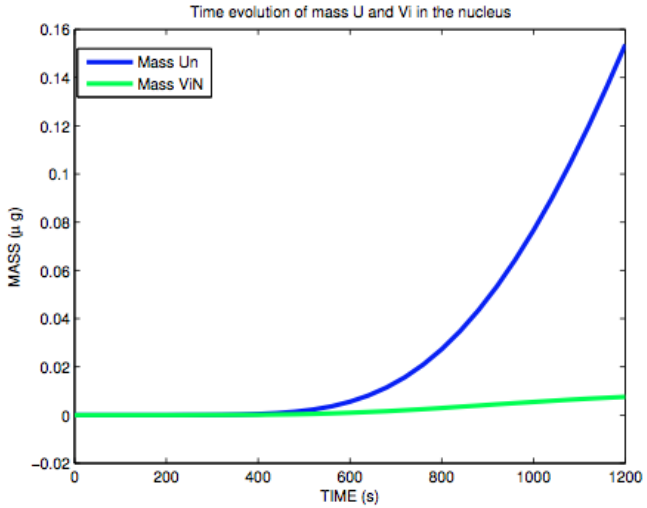


Time evolution of mass complexes M_{onMT} according to D_w (black) and D_{cyto} (blue) (on the left) and time evolution of $M_u^{(N)}$ and $M_{vi}^{(N)}$ with D_w (black and magenta, respectively) and with D_{cyto} (blue and green, respectively) (on the right).

The values of D_w and D_{cyto} are not in agreement with biological results.

We propose that the diffusion coefficient for these large plasmids is around 10^{-4}.
hp: D $\sim 10^{-4}$





Time evolution of mass M_{MT-N} (on the left) and $M_u^{(N)}$ (blue) and $M_{vi}^{(N)}$ (green) (on the right).

Experiment 3

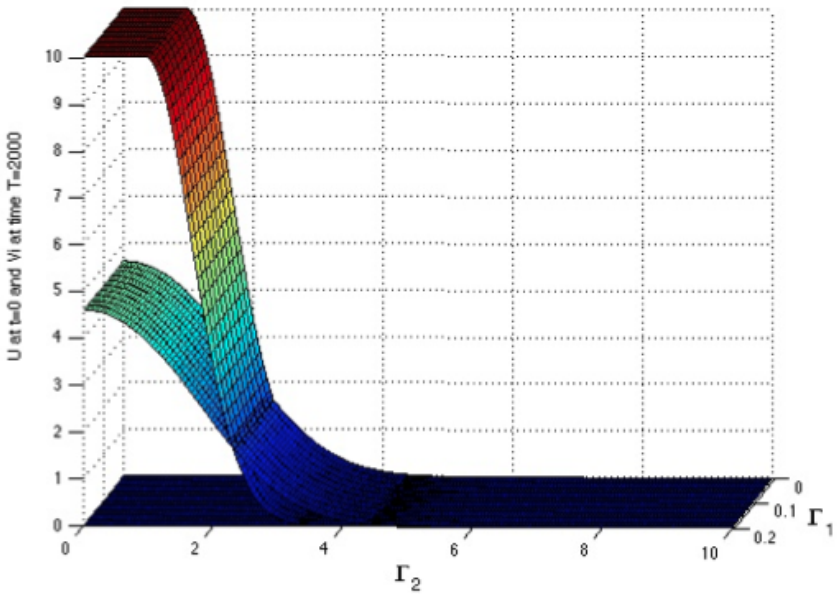
20 min later the microinjection, the cells show that 78% of the cargo is on the microtubule (Dean et al 2012).
No microtubule interaction, no nuclear localization after 5 h.

Tests:

- $k_1 = k_2 = 0$,
- $k_1 = k_2 = 0.2$,
- $k_1 = k_2 = 100$.

hp:

- T=40 min
- $D \sim 10^{-4}$,
- $p_{vi} \sim 10^{-4}$.

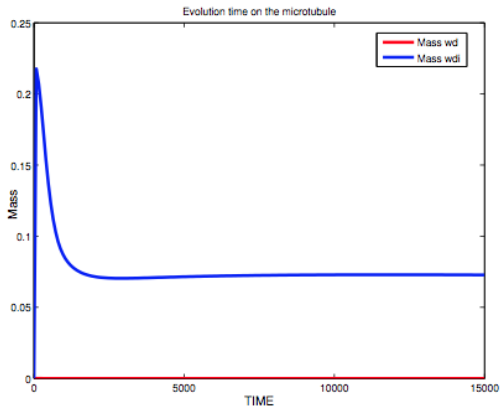


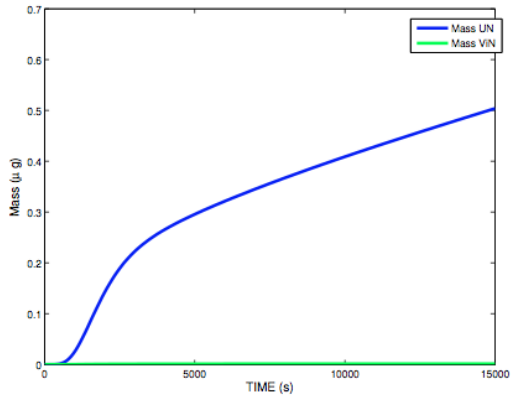
Initial condition of cargo at T=0 and state of cargo with importin then T=1200 s.

Start Movie k0

hp:

- $D \sim 10^{-4}$,
- $p_{vi} \sim 10^{-4}$.



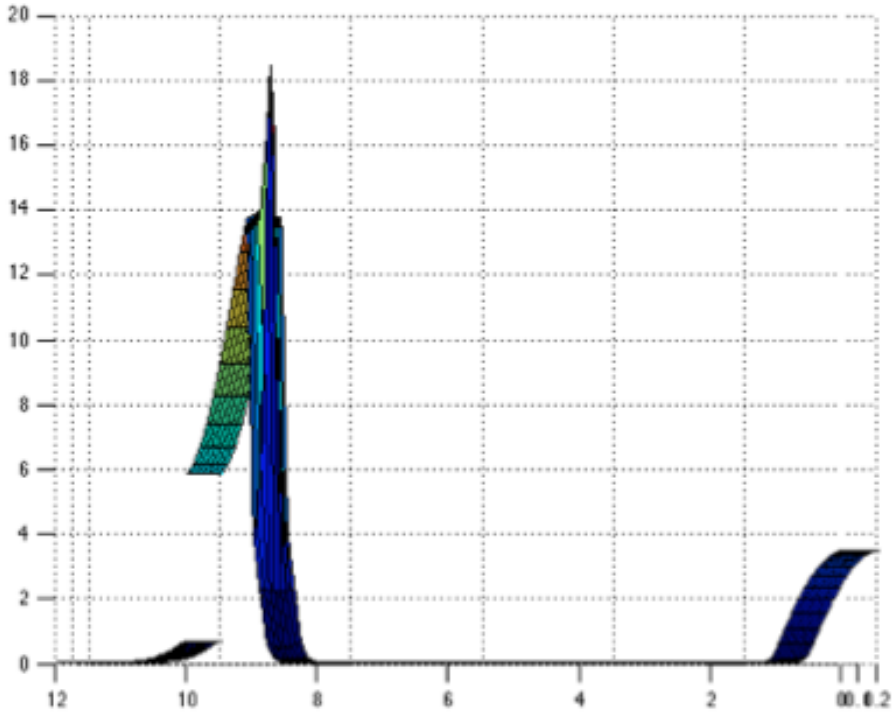


Time evolution of the mass M_{onMT} on the microtubule (on the left) and time evolution of mass $M_u^{(N)}$ and $M_{vi}^{(N)}$ in the nucleus (on the right).

Start Movie k02 front
Start Movie k02 top

hp:

- $D \sim 10^{-4}$,
- $p_{vi} \sim 10^{-4}$.



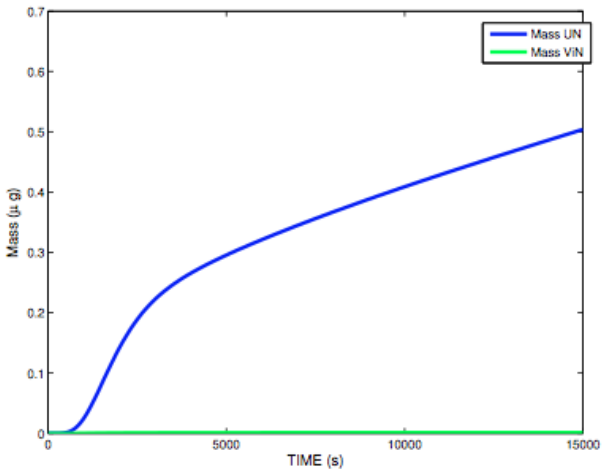
Cargo with importin in whole cell at time T=2000s.

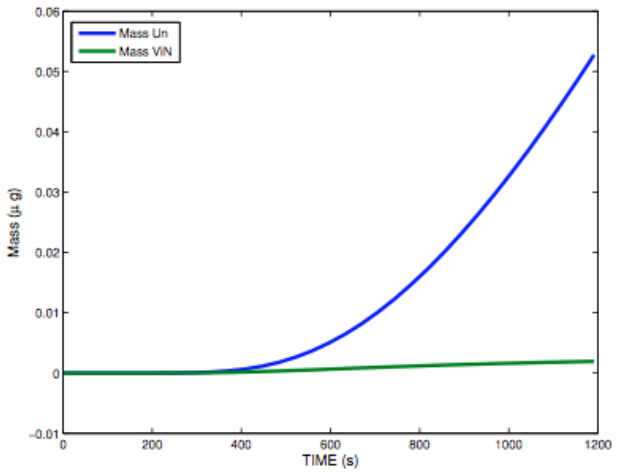
Start Movie k100 front
Start Movie k100 top

Comparing the evolution time of mass of cargo when $k_1 = 0.2, k_2 = 0.2$ (on the left) and $k_1 = 100, k_2 = 100$ (on the right), with

- $D \sim 10^{-4}$,
- $p_{vi} \sim 10^{-4}$.

give us these behaviors





k1=100, k2=100, high permeability

hp:

- $D \sim 10^{-2}$,
- $p_{vi} = 1$.

In this movie we note how the amount of cargo in the nucleus changes when the permeability changes.

Start Movie k100 high permeability and diffusion

Conclusion

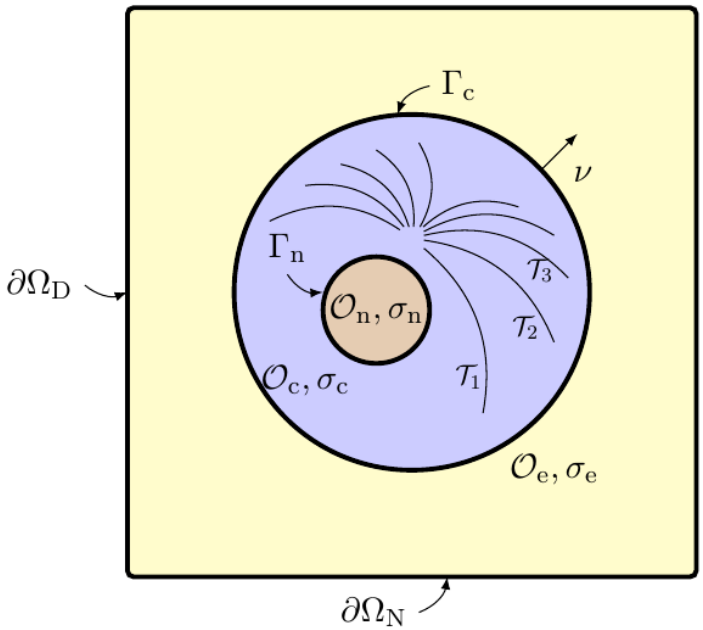
- The vaccine in the cell is effected by degradation.
- The diffusion coefficient of the cargo and its complexes is of order of $\leq 10^{-4}$.
- The permeability of v_i is very low, of the order of 10^{-4}.
- The attachment rate on the microtubule is not easy to estimate and depends by others factors.

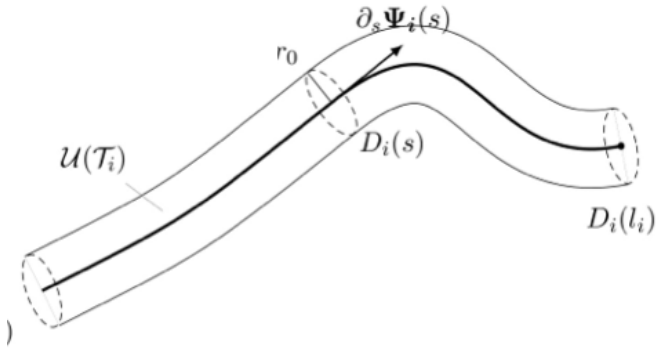
Combining models: MATHEMATICAL MODEL FOR TRANSPORT OF DNA PLASMIDS FROM THE EXTERNAL MEDIUM UP TO THE NUCLEUS BY ELECTROPORATION

work in collaboration with M.G. Notarangelo, C.Poignard, M. Leguèbe , M. Twarogowska

A 3D cell model with microtubules and electroporation

Electroporation is a molecular biology technique in which an electric field is applied to cells or tissue in order to increase the permeability of the cell membrane. It allows chemicals, drug or DNA to be introduce into the cells.





$$
\begin{cases}
\partial_t M - d_{\mathrm{e}}\Delta M + \mu_{\mathrm{e}}\nabla.(M\nabla U) = 0 & \text{in} \quad \Omega_e, \\
\partial_t M - d_{\mathrm{c}}\Delta M = -k^* M & \text{in} \quad \Omega_c, \\
\partial_t M^* - d_{\mathrm{c}}^*\Delta M^* = k^* M - k_M^* 1_{\mathcal{U}(\mathcal{T})} + \dfrac{v}{|D|}\displaystyle\sum_{i=1}^{N_{\mathcal{T}}} W_i(\mathcal{E}_i)\delta_{D(\mathcal{E}_i)}(x) & \text{in} \quad \Omega_c \\
\partial_t W_i(t,s) - v_{\partial s} W_i(t,s) = k\displaystyle\int_{D(s)} M^*(x)\,\mathrm{d}x & \text{on} \quad \mathcal{U}(\mathcal{T})
\end{cases}
$$

$\mathsf{d}_{\mathrm{e}}\partial_\nu M|_{\Gamma_{\mathrm{c}}^+} - \mu_{\mathrm{e}} M\partial_\nu U|_{\Gamma_{\mathrm{c}}^+} = d_{\mathrm{c}}\partial_\nu M|_{\Gamma_{\mathrm{c}}^-}$
$\mathsf{P}_{\mathrm{c}}([U]_{\Gamma_{\mathrm{c}}})\,[M]_{\Gamma_{\mathrm{c}}} = d_{\mathrm{c}}\partial_\nu M|_{\Gamma_{\mathrm{c}}^-}$
$\partial_\nu M|_{\Gamma_{\mathrm{n}}^+} = 0$
with U electrical potential, i.e

$$
\begin{aligned}
\Delta U &= 0 && \text{in } \mathcal{O}_{\mathrm{e}} \cup \mathcal{O}_{\mathrm{c}} \cup \mathcal{O}_{\mathrm{n}} \\
\sigma_{\mathrm{e}}\partial_{\boldsymbol{\nu}} U|_{\Gamma_{\mathrm{c}}^+} &= \sigma_{\mathrm{c}}\partial_{\boldsymbol{\nu}} U|_{\Gamma_{\mathrm{c}}^-}, \\
\sigma_{\mathrm{c}}\partial_{\boldsymbol{\nu}} U|_{\Gamma_{\mathrm{n}}^+} &= \sigma_{\mathrm{n}}\partial_{\boldsymbol{\nu}} U|_{\Gamma_{\mathrm{n}}^-}, \\
C_{\mathrm{c}}\partial_t [U]_{\Gamma_{\mathrm{c}}} + S_{\mathrm{c}}([U]_{\Gamma_{\mathrm{c}}})\,[U]_{\Gamma_{\mathrm{c}}} &= \sigma_{\mathrm{c}}\partial_{\boldsymbol{\nu}} U|_{\Gamma_{\mathrm{c}}^-}, \\
C_{\mathrm{n}}\partial_t [U]_{\Gamma_{\mathrm{n}}} + S_{\mathrm{n}}\,[U]_{\Gamma_{\mathrm{n}}} &= \sigma_{\mathrm{n}}\partial_{\boldsymbol{\nu}} U|_{\Gamma_{\mathrm{n}}^-}, \\
U &= g \text{ on } \partial\Omega_{\mathrm{D}}, \\
\partial_{\boldsymbol{\nu}} U &= 0 \text{ on } \partial\Omega_{\mathrm{N}}, \\
U(t=0) &= 0.
\end{aligned}
$$

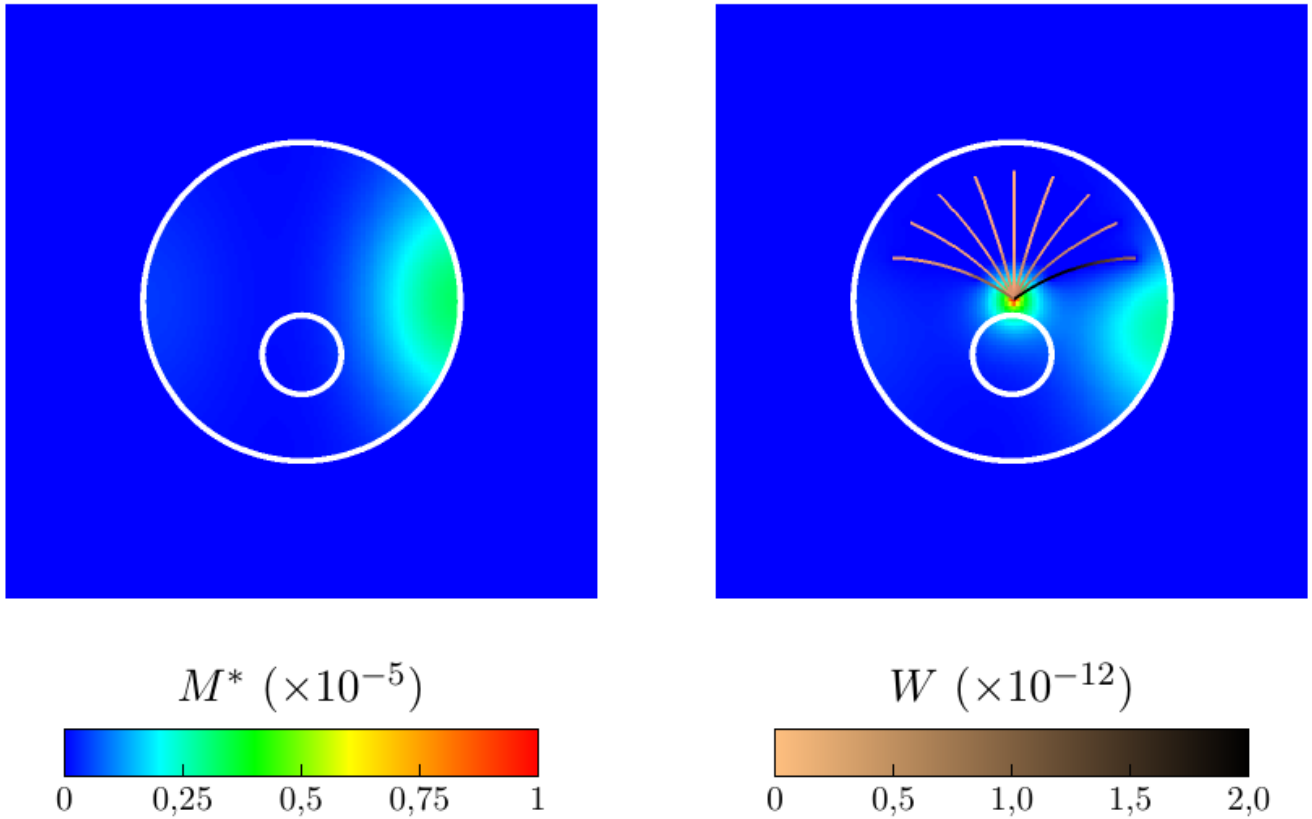


Figure : Spatial repartition of plasmids concentration at t = 10 s in simulations with and without microtubules

Work in progress and Perspectives

- mathematical model with more microtubules in a multi nucleate cell,
- complete the model with hyalunoridase
- more data to have a more reliable calibration of the model
- optimization of the procedure at each step (injection, electropration, transport)

List of pubblications

1 Notarangelo MG, PhD Thesis, Sapienza Univ. Rome 2014.

2 Notarangelo MG, Natalini R, Signori, E.
Gene Therapy: the Role of Cytoskeleton in Gene Transfer Studies Based on Biology and Mathematics. Current Gene Therapy 2014, 14, 2, 121-127.

3 Notarangelo MG, Natalini R.
A Mathematical model of intracellular transport: role of microtubules and Ran cycle in the nucleocytoplasmic plasmids transport in gene therapy anti-tumor in preparation.

4 Natalini R, Deville M, Poignard C. *A poro-elastic model of hylunoridase and vaccine transport in muscular tissue* in preparation

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