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UNIVERSITÀ DI ROMA

NANODELIVERY LAB
Smart Nanocarriers for Drug and Gene Delivery

Intracellular Trafficking of Gene Delivery Systems Investigated by Single Particle Tracking

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Gene delivery

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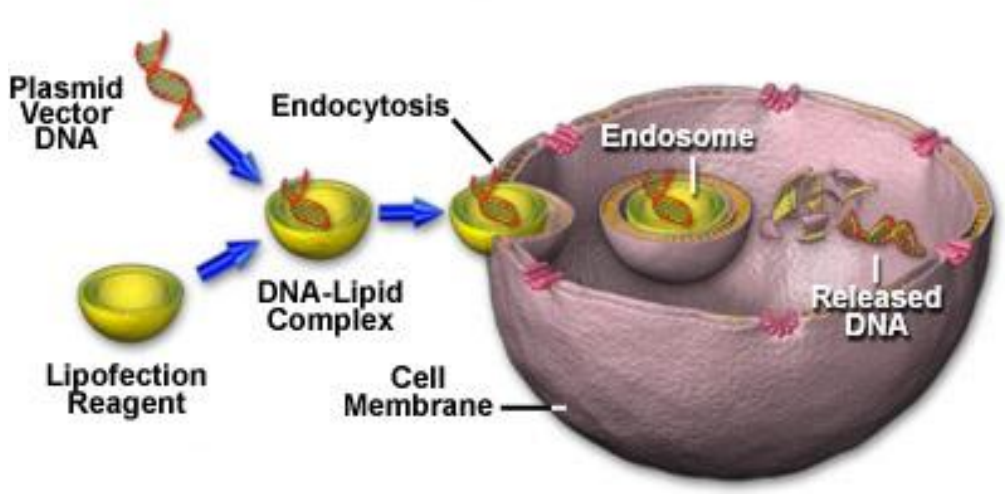
- Gene delivery: process of introducing foreign DNA into host cells to elicit a therapeutic benefit.
- It is generally accomplished by viral or non-viral vectors (e.g. lipid-based nanoparticles).



Intracellular trafficking

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1. Is the dynamic of nanocarriers inside the cytoplasm related to the transfection efficiency?
2. Which are the effects of the interactions with the cellular structure?
3. How can we give a satisfactory description of the gene delivery at the intracellular scale?

Intracellular trafficking

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1. Is the dynamic of nanocarriers inside the cytoplasm related to the transfection efficiency?
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3. How can we give a satisfactory description of the gene delivery at the intracellular scale?

Straightforward combination of fluorescence microscopy techniques

on different gene delivery systems.

Brownian Diffusion

mainly governs the intracellular trafficking of **highly efficicient** gene delivery vectors.

Flow Motion

along microtubules leads to **degradation** by specific cellular compartments, i.e. the lysosomes.

Lipid-based vectors

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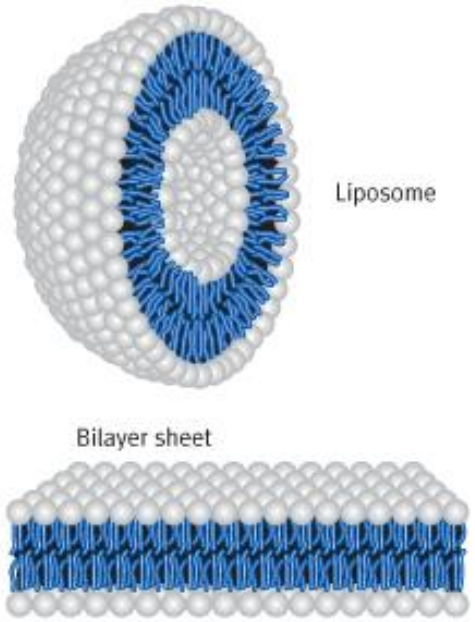

- Non-viral vectors;
- biocompatibility;
- self-assembling process with genetic material.

Lipid-based vectors

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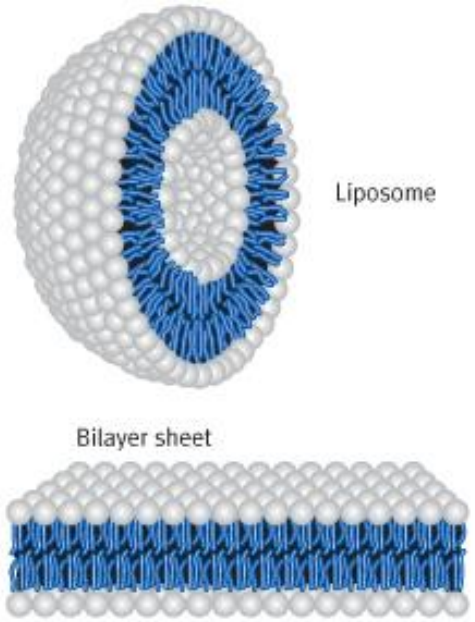

- Non-viral vectors;
- biocompatibility;
- self-assembling process with genetic material.

We adopted

1. **Lipofectamine**: a gold standard among the transfection reagents.
2. **Control nanoparticles**: cationic liposomes synthetized in lab.

- Cy3-labeled plasmidic DNA.
- Untreated and nocodazole*-treated Chinese Hamster Ovary (CHO) cells.

*antineoplastic agent which interferes with the polymerization of microtubules

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Single Particle Tracking

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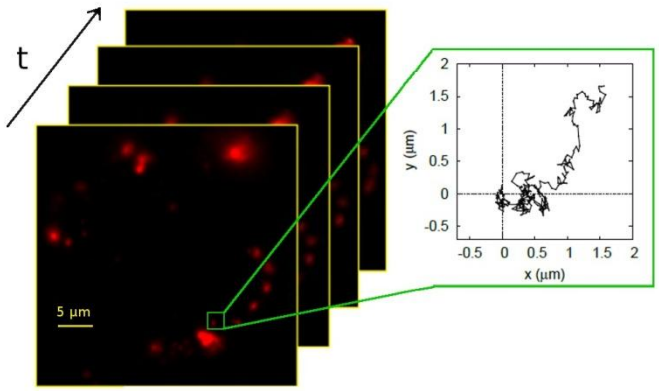
1. Incubation of nanoparticles with cells;
2. acquisition of image stacks;
3. particle identification and tracking process;
4. track analysis → **motion characterization**.



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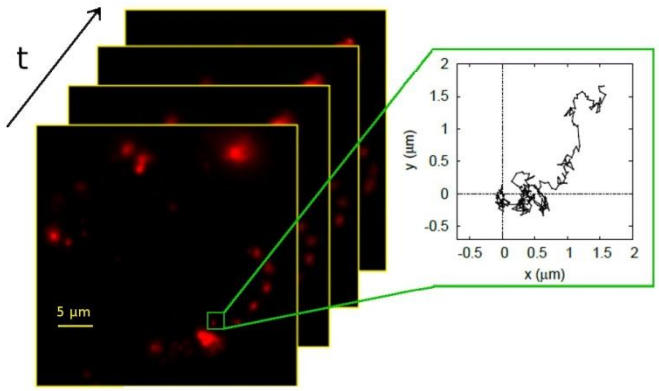

Is it a 2-dimensional **Brownian process** or is it significantly affected by **flow motion** along specific directions?

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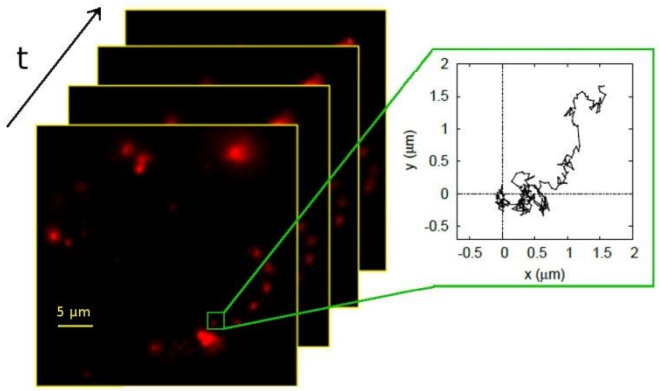

Is it a 2-dimensional **Brownian process** or is it significantly affected by **flow motion** along specific directions?

Experimental parameters

- Fit of the Mean Square Displacement (MSD);
- Gyration tensor → Asphericity (*shape* of trajectories)

Single Particle Tracking

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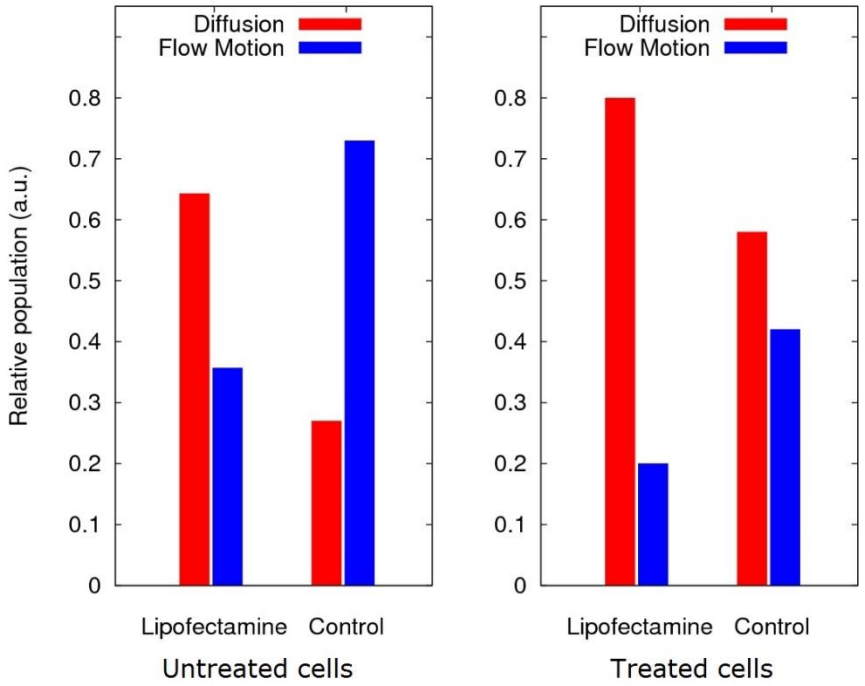

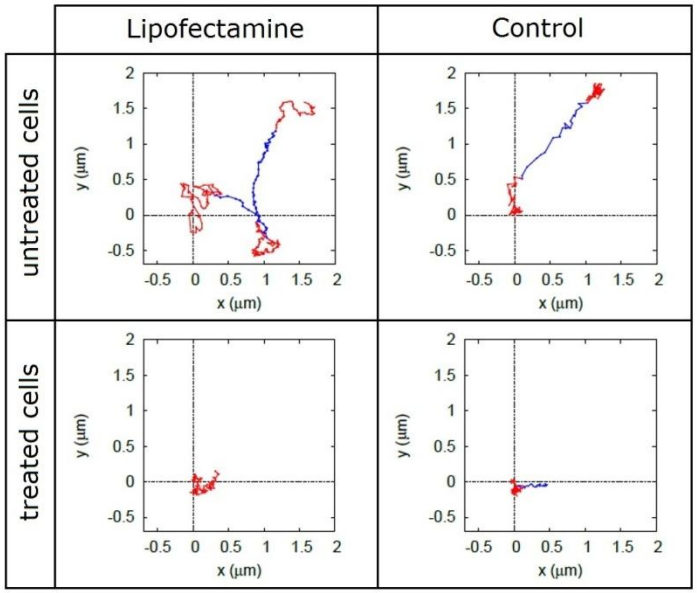

Representative tracks

	Not treated cells			Nocodazole-treated cells		
	Diffusion	**Flow motion**		**Diffusion**	**Flow motion**	
	D (nm^2/s)	v (nm/s)	D (nm^2/s)	D (nm^2/s)	v (nm/s)	D (nm^2/s)
Lipofectamine	984 ±179	7.5 ± 1.3	549 ± 155	226 ± 60	4.9 ± 0.5	356 ± 101
Control	370 ± 68	7.4 ± 1.1	255 ± 66	103 ± 13	6.6 ± 1.5	252 ± 103

Colocalization analysis

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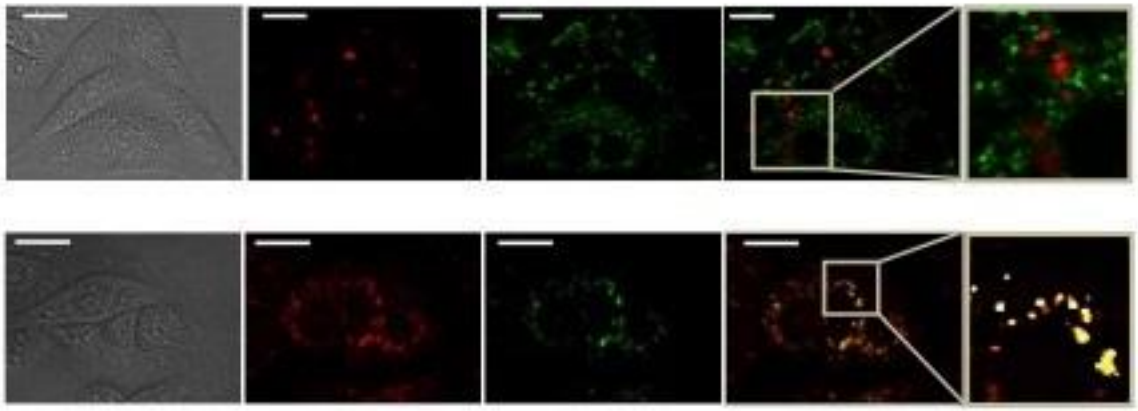
- Cy3-labeled plasmidic DNA
- Lysosensor-labeled cells.

→ Spatial overlap of the fluorescence signals.



Lipofectamine

Control

- Lipofectamine: low colocalization with lysosomes and high transfection efficiency.
- Control nanoparticles: high colocalization with lysosomes and low transfection efficiency.

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Conclusions

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- Brownian diffusion is an efficient route for complexes to avoid metabolic degradation, thus leading to optimal transfection.
- Active transport along microtubules results in DNA degradation and subsequent poor transfection.

Intracellular trafficking and lysosomal degradation can be viewed as strictly related phenomena, in such a way that they appear as a single barrier on the route for efficient transfection.

Thank you for your attention and thanks to

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Nanodelivery Lab Staff @ Sapienza, Roma

- Giulio Caracciolo
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NOVARTIS

NATIONAL INSTITUTES OF HEALTH

Asphericity

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N-length trajectory in a d-dimensional space.

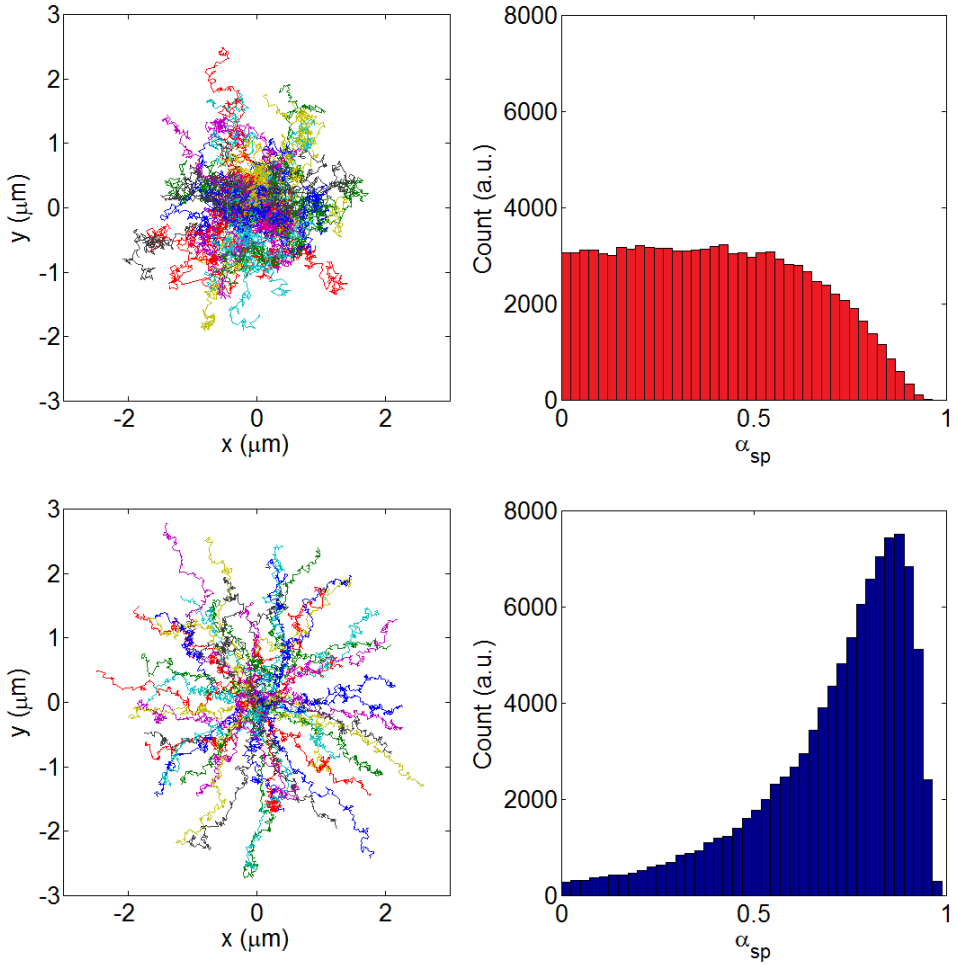
Gyration Tensor:

$$T_{ij} = \frac{1}{N}\sum_{k=1}^{N}\left(x_{ik} - \langle x_i \rangle\right)\left(x_{jk} - \langle x_j \rangle\right)$$

Ensemble of N-length trajectories in a d-dimensional space.

Asphericity parameter:

$$\alpha^{(d)} = \frac{d\left\langle T_r \mathrm{T}^2 \right\rangle - \left\langle \left(T_r \mathrm{T}\right)^2 \right\rangle}{(d-1)\left\langle \left(T_r \mathrm{T}\right)^2 \right\rangle}$$



Mean Square Displacement

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$$MSD(\tau)=\left\langle\left[r(t+\tau)-r(t)\right]^2\right\rangle=\iint P(\mathrm{r}')\left[r-r'\right]^2 P(\mathrm{r}\,|\,\mathrm{r}',\tau)\,dr\,dr'$$

Brownian Diffusion:

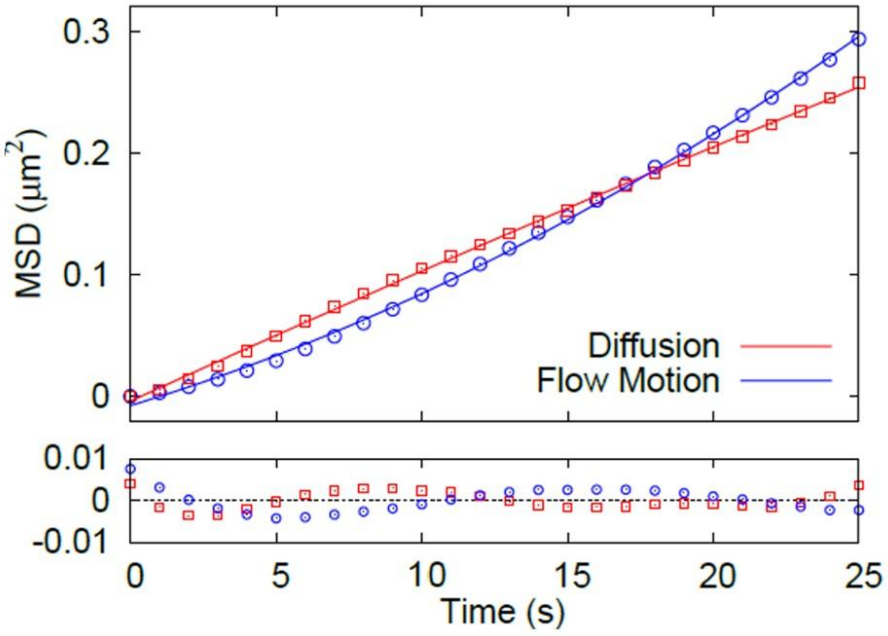
$$P(\mathrm{x}_i\,|\,\mathrm{x}'_i,\tau)=\frac{1}{\sqrt{4\pi D_i\tau}}\exp\left\{-\frac{(x_i-x'_i)^2}{4D_i\tau}\right\}\Rightarrow$$

$$\Rightarrow MSD(\tau)=2D_i\tau$$

Diffusion with flow motion:

$$P(\mathrm{x}_i\,|\,\mathrm{x}'_i,\tau)=\frac{1}{\sqrt{4\pi D_i\tau}}\exp\left\{-\frac{(x_i-x'_i-v_i\tau)^2}{4D_i\tau}\right\}\Rightarrow$$

$$\Rightarrow MSD(\tau)=2D_i\tau+v_i^2\tau^2$$



Track segmentation

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