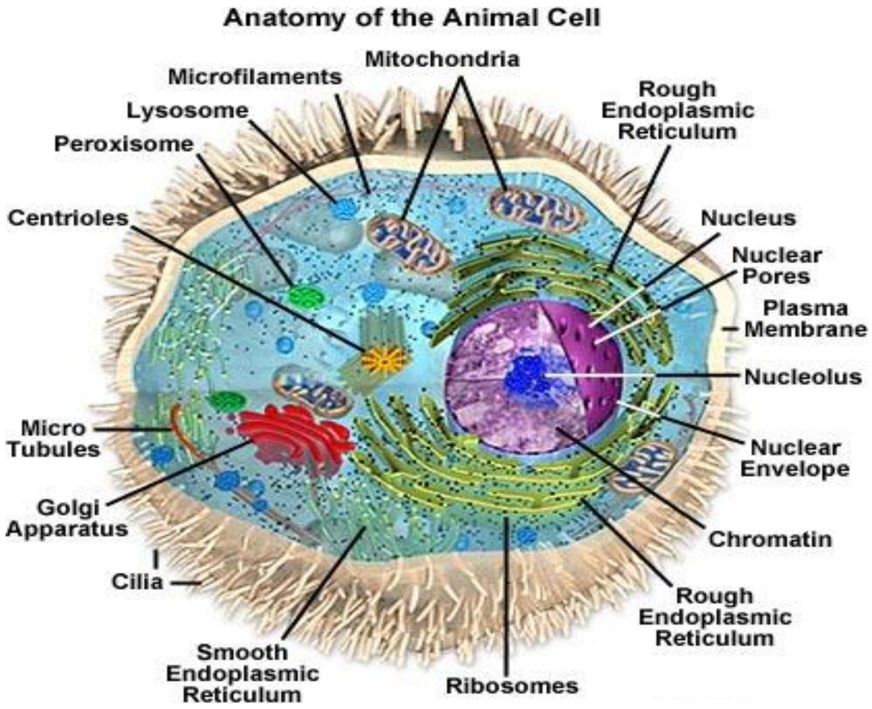
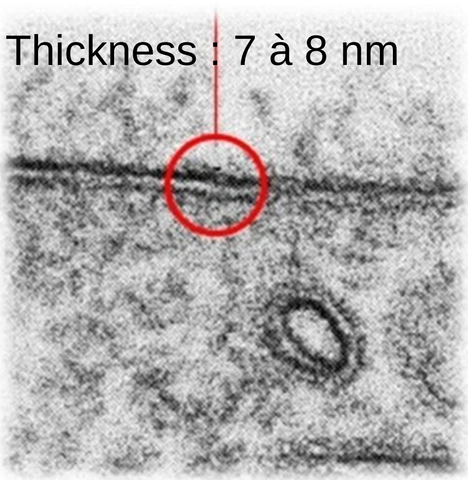
Transport across membranes
Insights from Molecular Dynamics Simulations

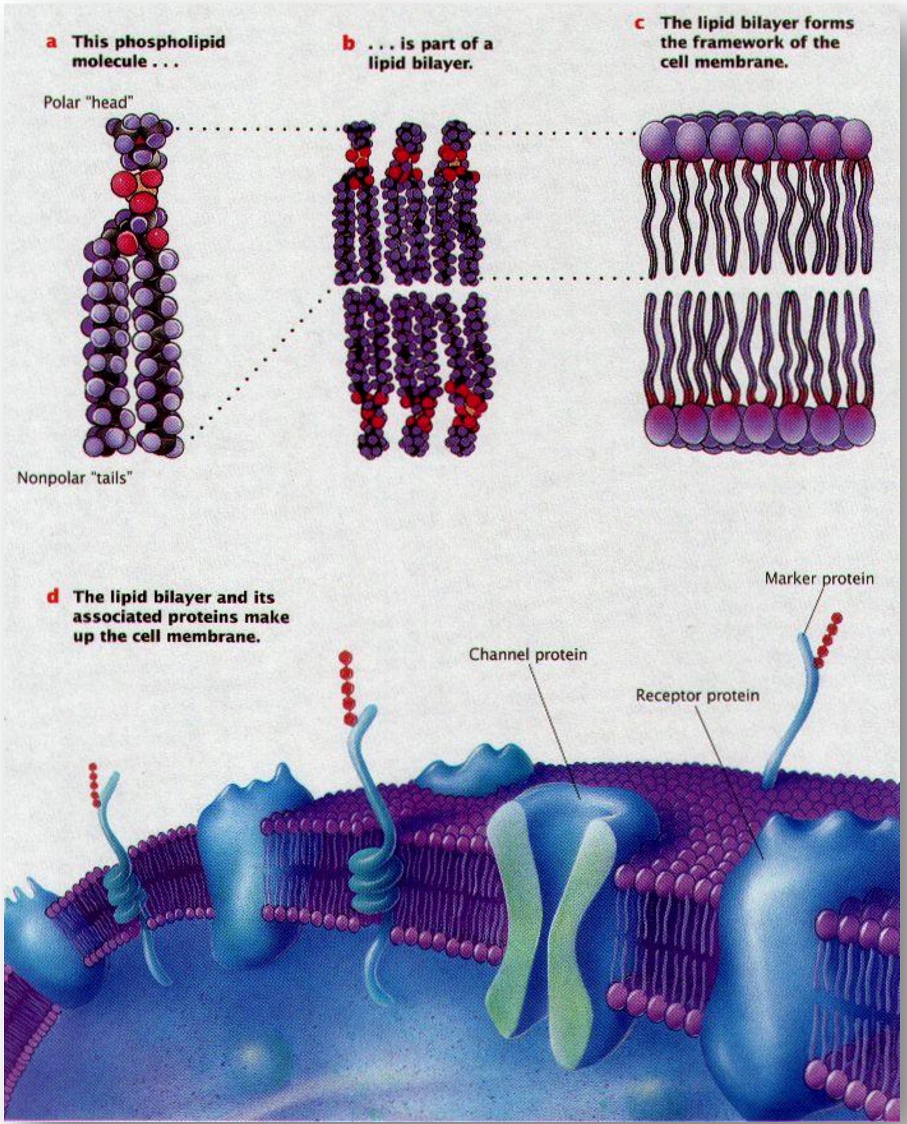
Mounir Tarek

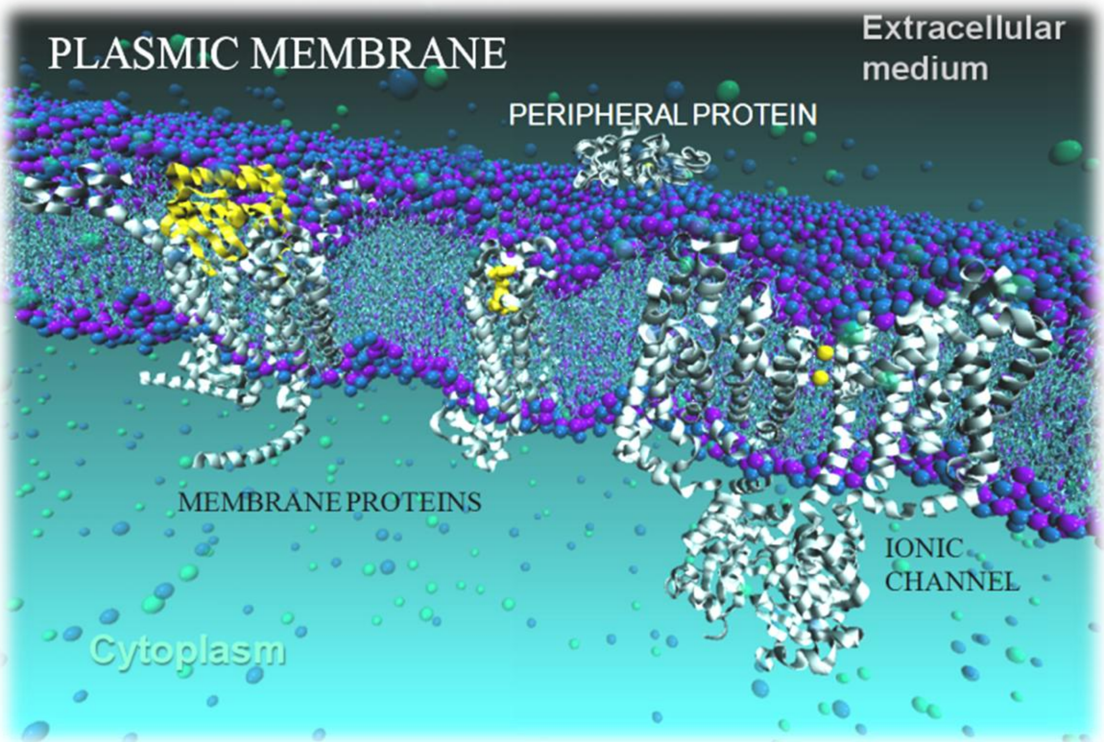
CNRS / Nancy Université





Two leaflets Electron-microscope





Investigate the structure and
dynamics of membranes
& associated membrane proteins

<u>Communication between</u>
<u>Intra and extra cellular media</u>

Molecular modeling

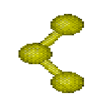
Molecular Mechanics (MM)

Empirical
force fields

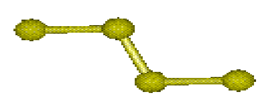
Classical
dynamics

$U_{elect}, U_{vdw}(q_i, \sigma, r_{min})$

$U_{bond}(F_{k1})$



$U_{bend}(F_{k2})$



$U_{dihed}(F_{k3})$

SYSTEM OF N PARTICLES

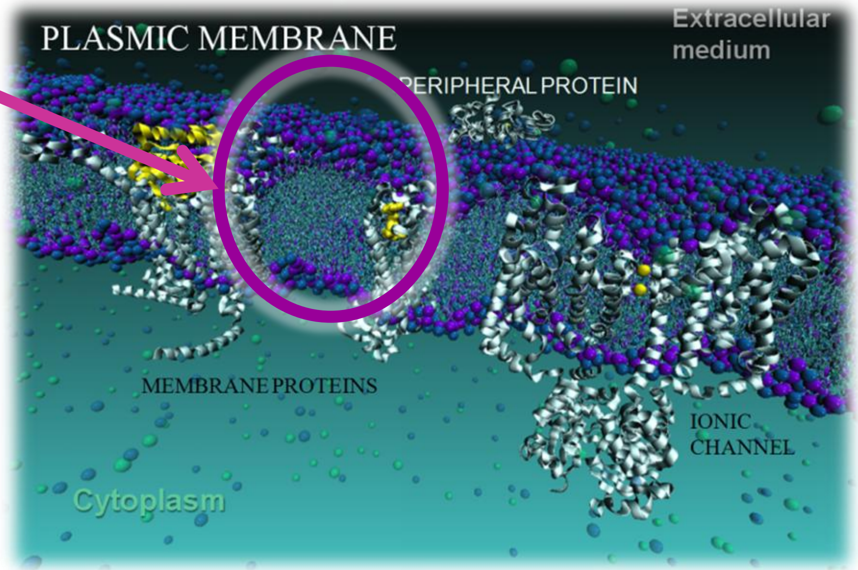
$$U(\mathbf{r}_1, \mathbf{r}_2, \ldots \mathbf{r}_N) \equiv U(\mathbf{r}^N)$$

FORCES ON PARTICLE i

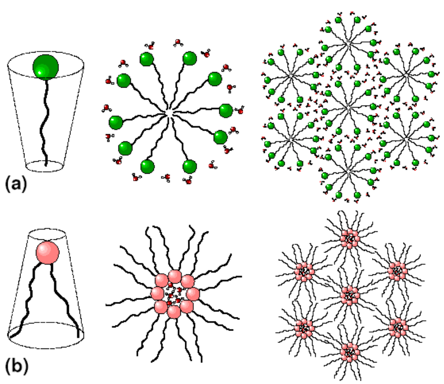
$$f_i(\mathbf{r}^N) = -\frac{\partial U(\mathbf{r}^N)}{\partial \mathbf{r}_i}$$

SOLVE THE EQUATIONS OF MOTION

$$m_i \ddot{\mathbf{r}}_i = f_i(\mathbf{r}^N)$$

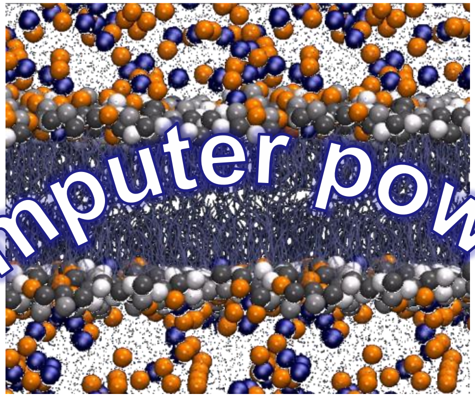


Surfactants

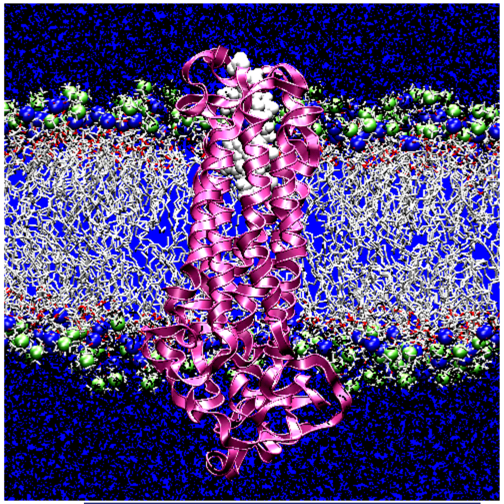


10^4 atoms
10^{-9} s

computer power



membranes

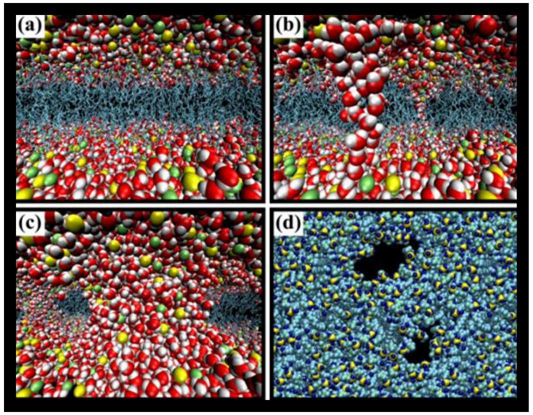


Membrane proteins

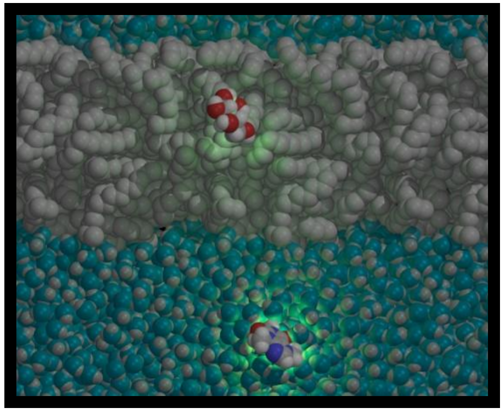
10^5 atoms
$10\text{-}100 \times 10^{-9}$ s

Current applications

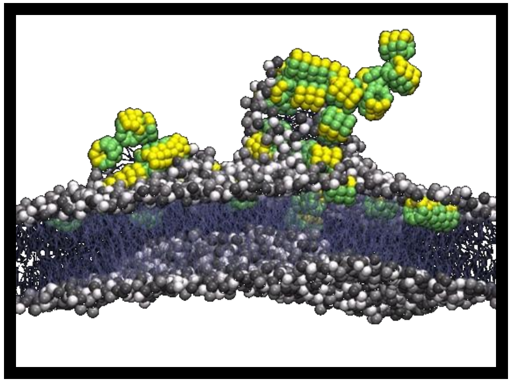
- Electroporation



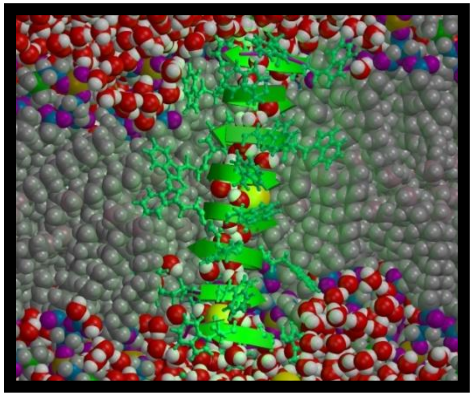
- Drug permeation



- Antimicrobial attack

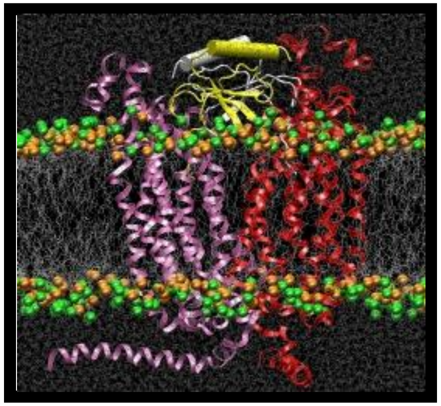


- Ionic transport



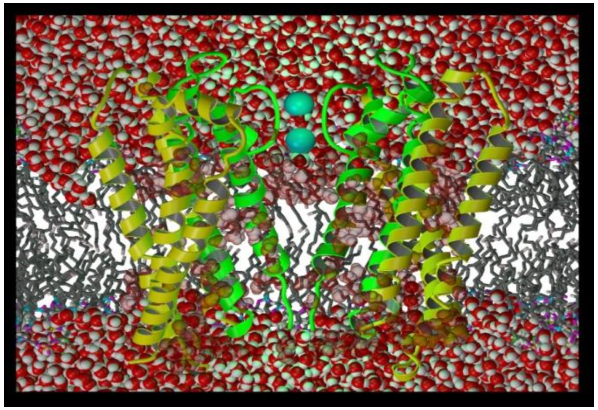
Peptide nanotubes

- Signal transduction



G Protein Coupled Receptors

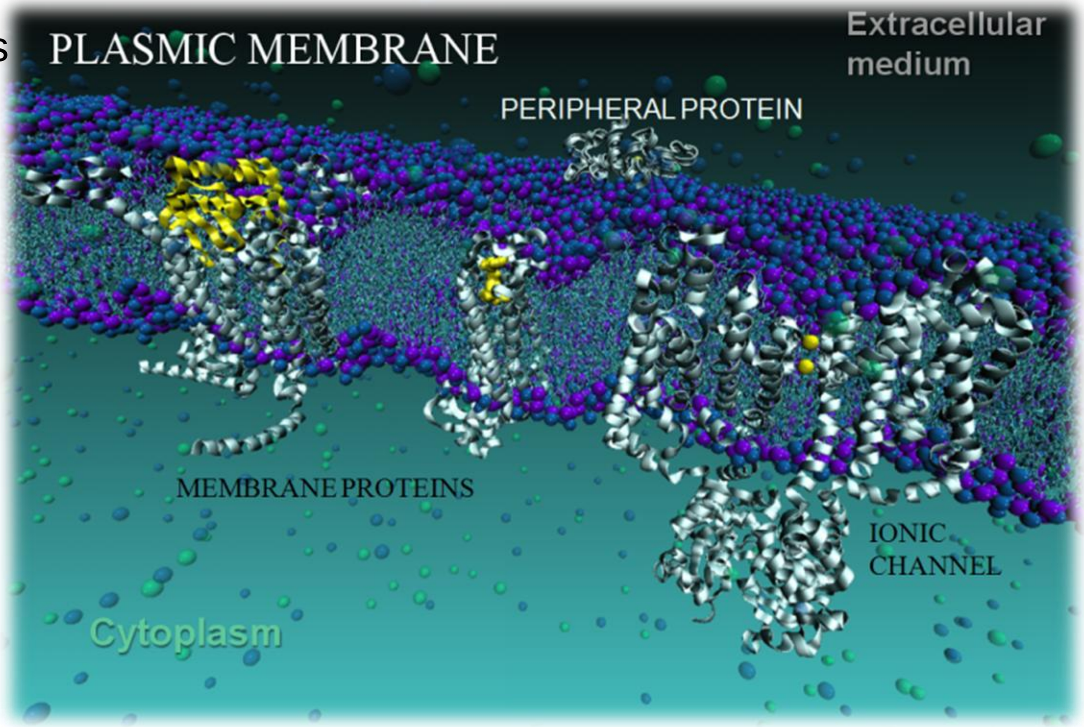
- Cellular excitability



Ionic channels

Molecular Insights into electroporation and siRNA electrotransfer through model cell membranes

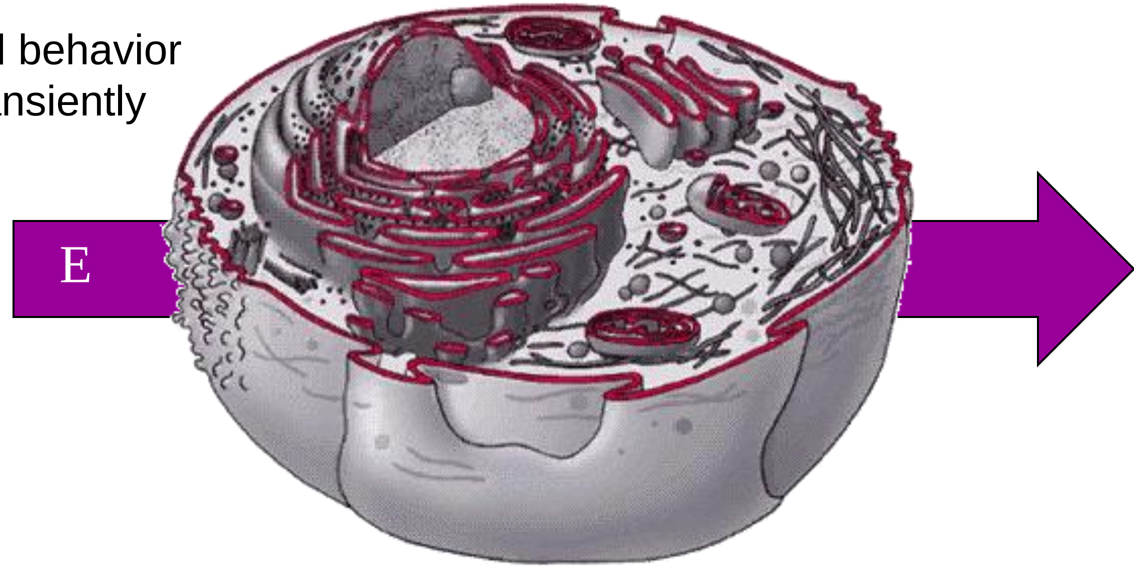
Lucie Delemotte
Equipe de Chimie et Biochimie Théoriques
UMR Structure et Réactivité des Systèmes Moléculaires Complexes, Nancy



Laboratoire Européen Associé (France - Slovenia)
"Pulsed Electric Fields Applications in Biology and Medicine" (LEA EBAM)

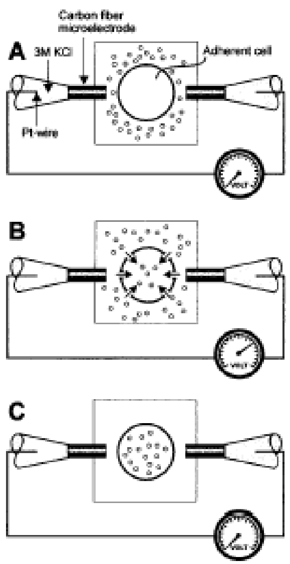
Electroporation

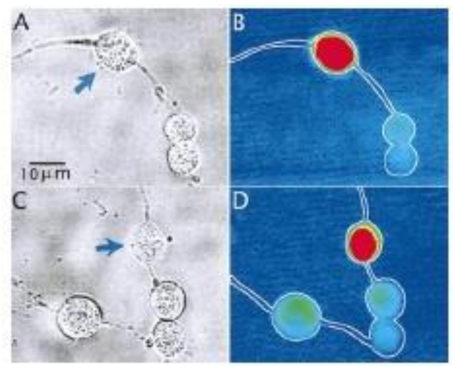
Electroporation affects the fundamental behavior of cells membranes since it disturbs transiently or permanently the integrity of their membranes



REVERSIBLE:

TRANSFER MOLECULES



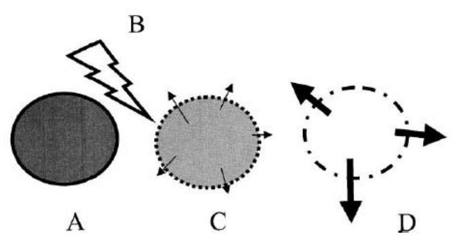


Lundqvist et al. PNAS 1998

Encycl. of Biomedical Engineering (John Wiley & Sons 2006)

IRREVERSIBLE:

KILL MICRO-ORGANISMS



Electroporation: historical background

~40 years-1st report of controlled <u>ELECTRO release</u> of intracellular compounds
Neumann, E. and Rosenheck, K., *J. Membr Biol.* 10: 279-290, **1972**

~ 30 years-1st report on gene <u>ELECTRO transfert</u>
Neumann, E., et al. *EMBO J. 1: 841-845,* ***1982***

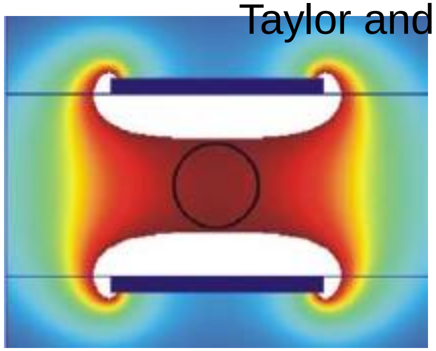
~ 20 years-1st s *in-vitro* et *in-vivo* experiments combining <u>ELECTROPORATION & bleomycin</u>
Okino M., et al. *Jpn J. Cancer Res 78: 1319-1321,* **1987.**
Orlowski S., et al. *Biochem. Pharma. 37: 4727-4733,* ***1988***

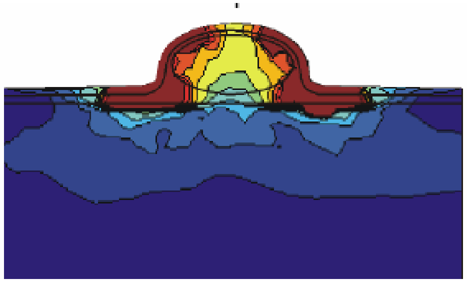
~15 years-1st use of <u>ELECTRO- chemotherapy</u> in veterinary medici
Mir L.M., et al. *British J. Cancer 76: 1617-1622,* ***1997***

Today: Electrochemotheray (melanoma, liver, ..) treatment in 80 Centers in Eu (IGR, …)

Advanced Electroporation Techniques in Biology and Medicine A. Pakhomov, D. Miklavcic and M. Markov, Editors, Taylor and Francis/CRC Press **(2010)**

Continuum models
To optimize the technique
Treatment Planning



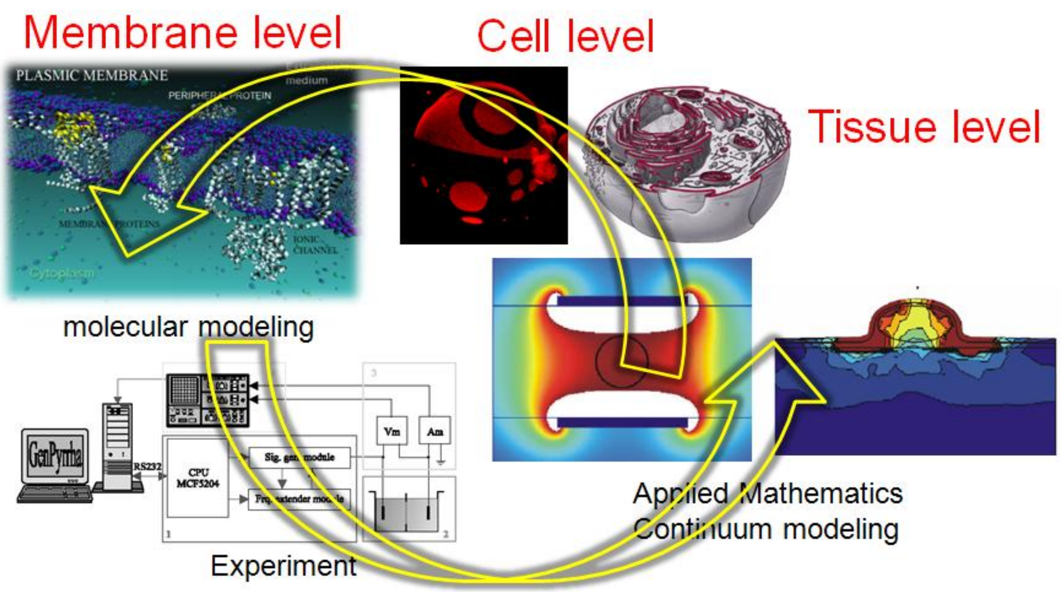


Remaining questions:

• Inderstand the process at the
Molecular Model

• Nature of the phenomena as a function of electric pulses intensity and duration

MODELING (MD simulations)



LEA EBAM
European Laboratory of Pulsed Electric Fields Applications in Biology and Medicine

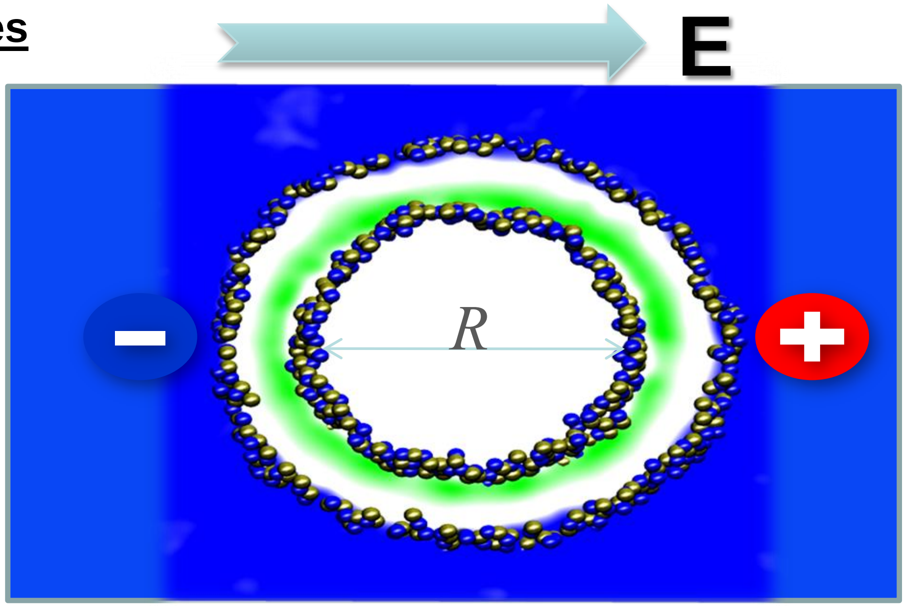
ANR

INTCELL (2011)

COST 2012- 2016 + **ITN** in prep

Subject to electric field

Pulses



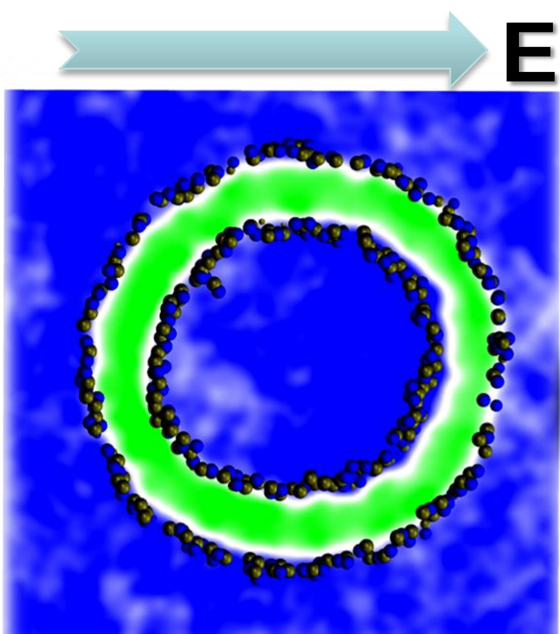
Classical electroporation

Low intensity E pulses (~KV/cm)

(µs to ms)

$$\Delta V = \frac{3}{2} E R \cos\varphi \left[1 - \exp(-t/\tau_m)\right],$$

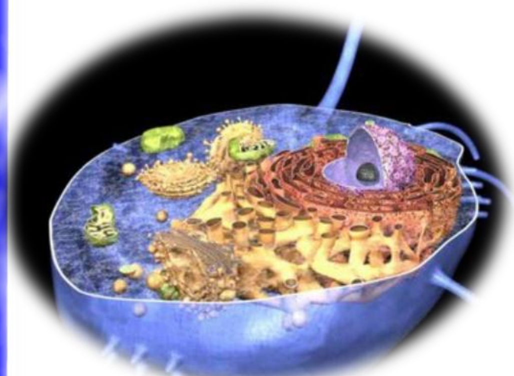
T effects ? ?

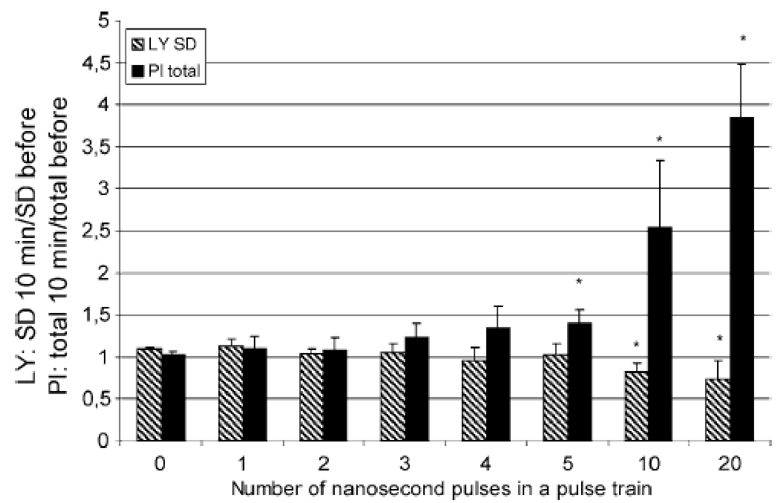


High intensity

(100s KV/cm)

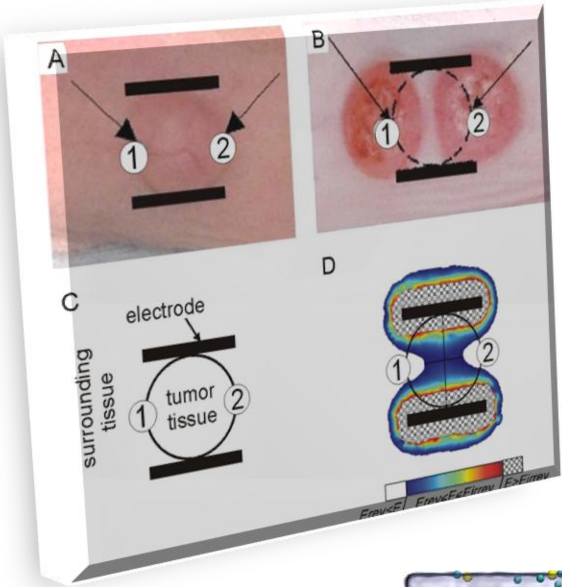
(ns **Nanopulses**)





T. Napotnik et al. Med Biol. Eng. Comput. (2010) 48:407–41

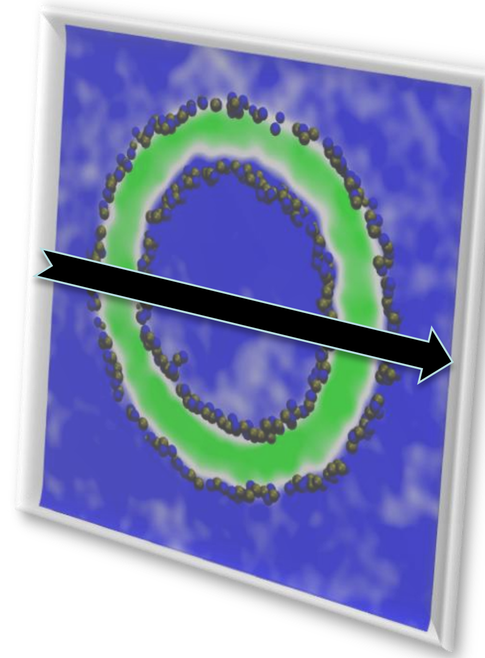
Modeling nano-pulses

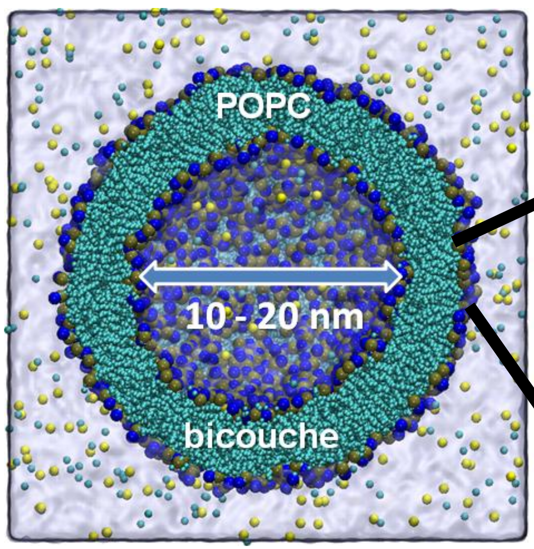


Pulses

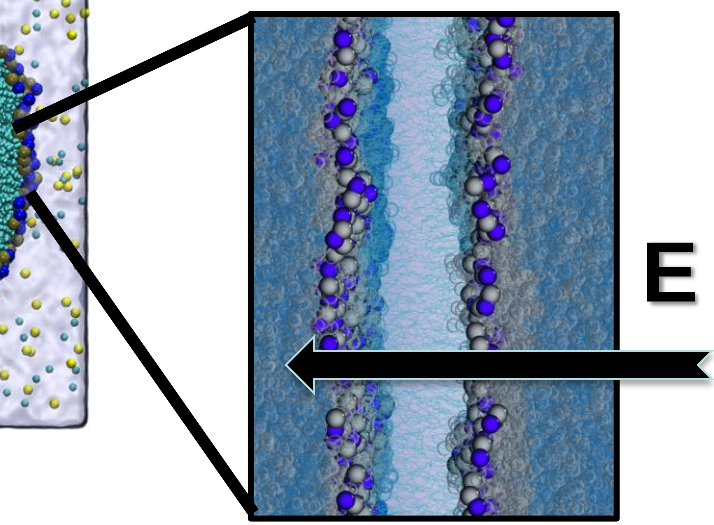
High intensity
(100s KV/cm)
short (ns)
(Nanopulses)

E

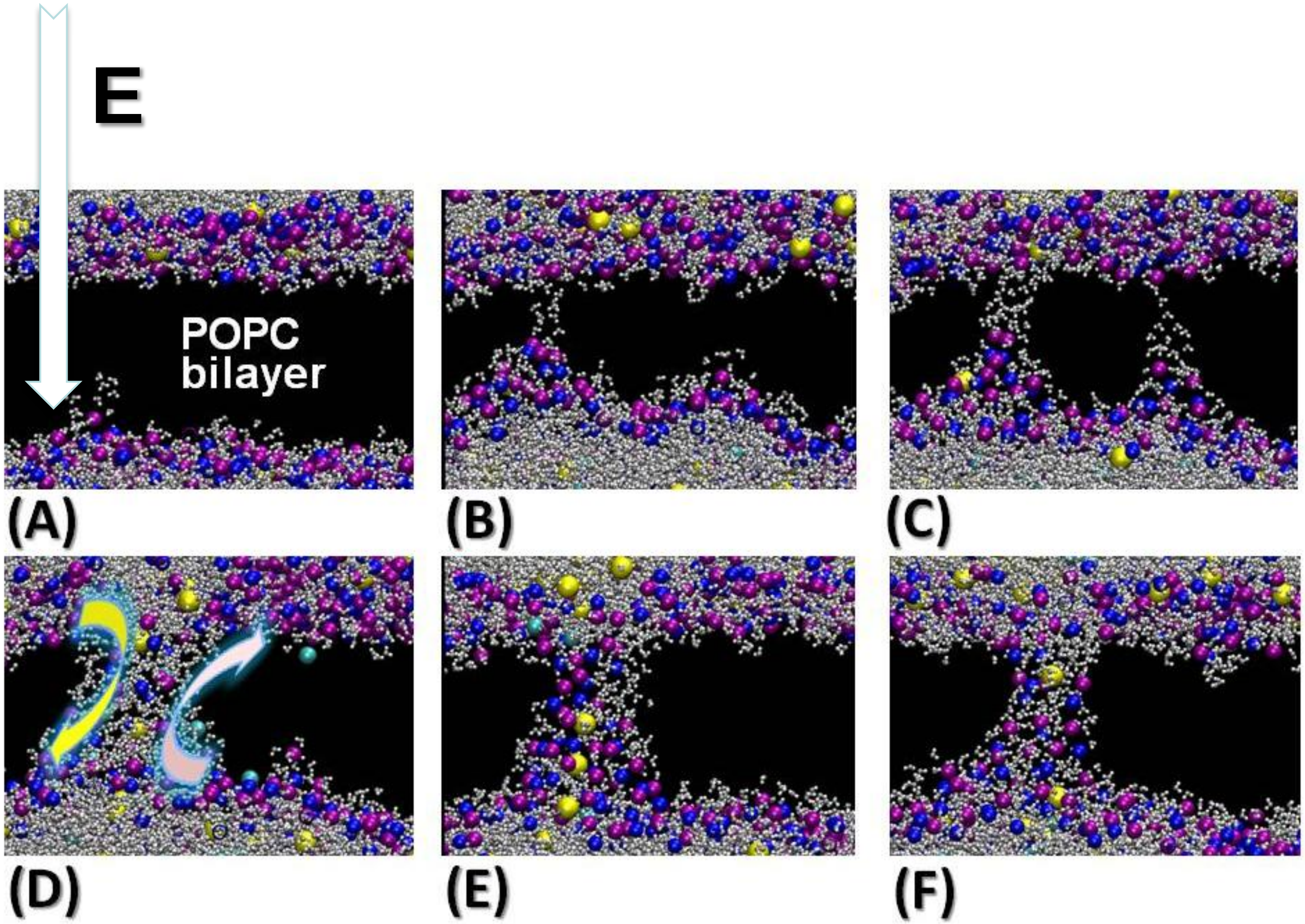


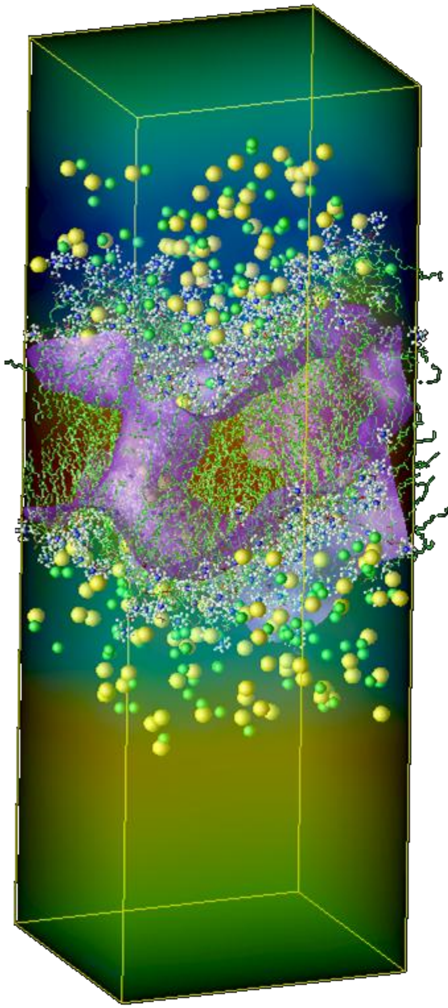


~ 1 - 2,5 M
atoms



Effect of nanopulses on lipid membranes



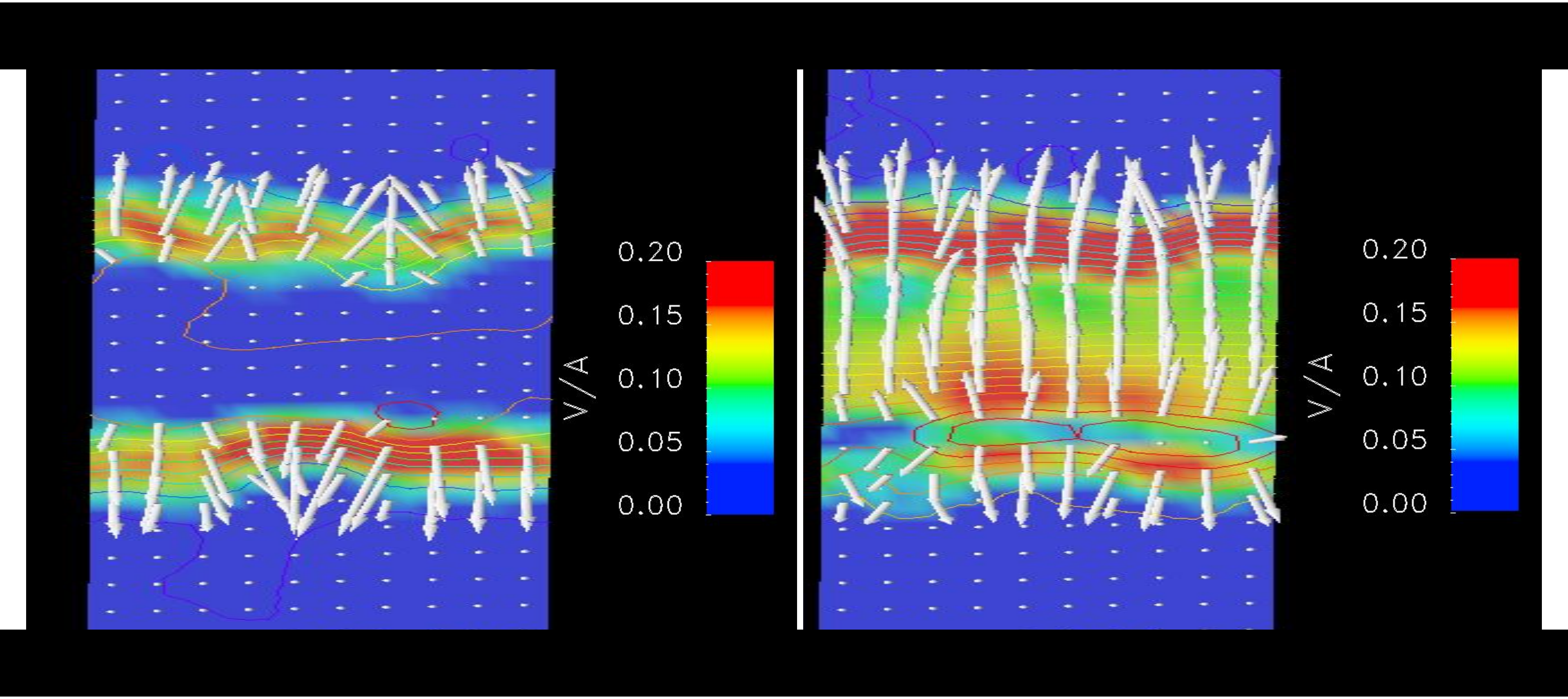


Tieleman P BMC Biochemistry **2004**
Tarek M. *Biophys. J.* **2005**

Electrostatic properties

No electric field

High electric field .



Delemotte et al. J Membr. Biol. 2012

Effect of nanopulses on lipid membranes

Time scales

Nanosecondes to electroporate simples (lipid) membranes

1.5 to 2.5 Volts (Transmembrane voltage)

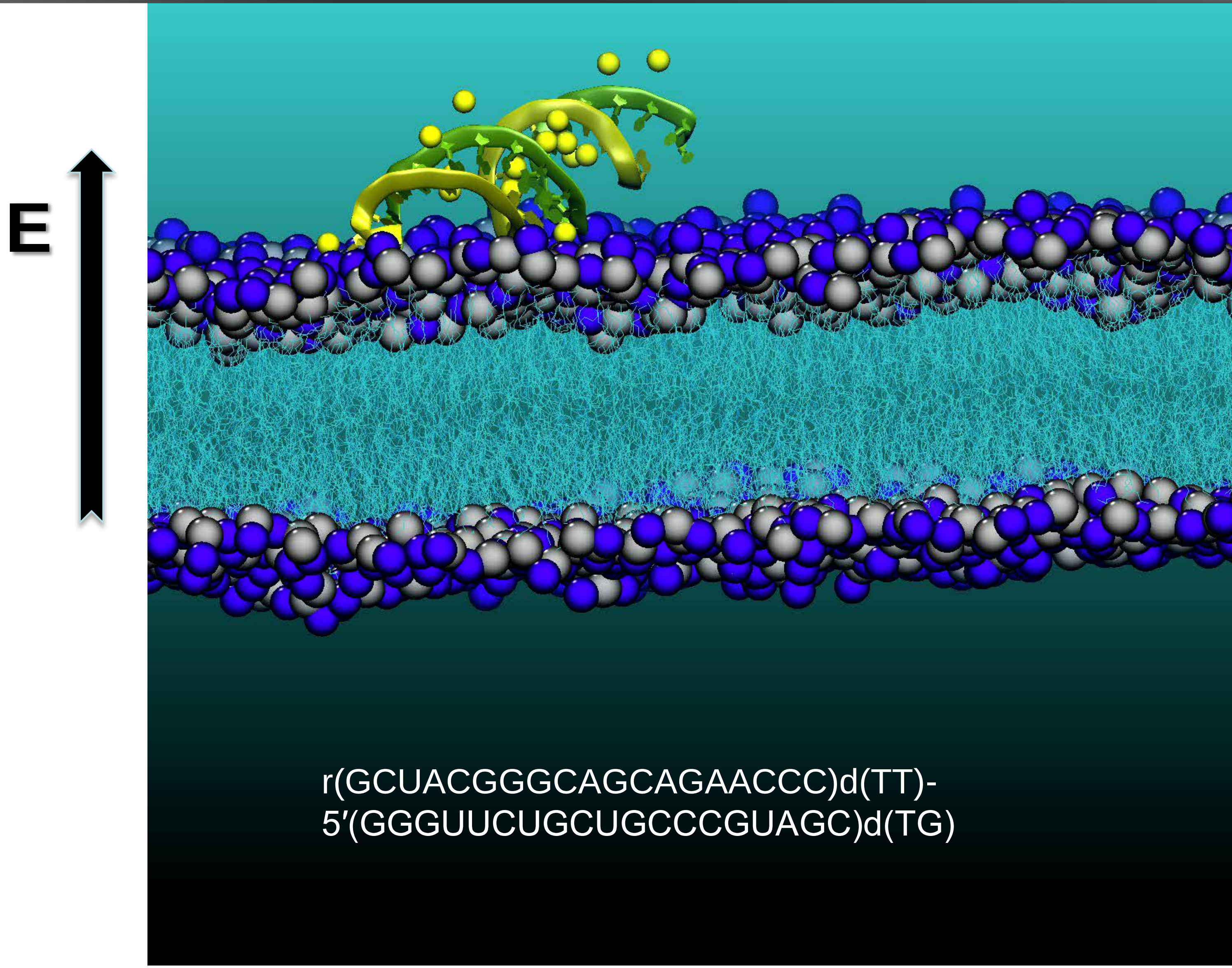
➢**10 ns pore resealing**
(Vernier and Ziegler, 2007; Bockmann et al., 2008)

Pore sizes

2 - 10 nm diameter

Tarek M. *Biophys. J.* **2005**
Tarek, M., & Delemotte, L. (**2010**). Electroporation of lipid membranes. In **Advanced Electroporation Techniques in Biology and Medicine** A.Pakhomov, D. Miklavcic and M. Markov, Editors. (Taylor and Francis/CRC Press).
Delemotte L &Tarek M. *J. Membr. Biol.* **2012**

Small interfering RNA (siARN)



SiRNA

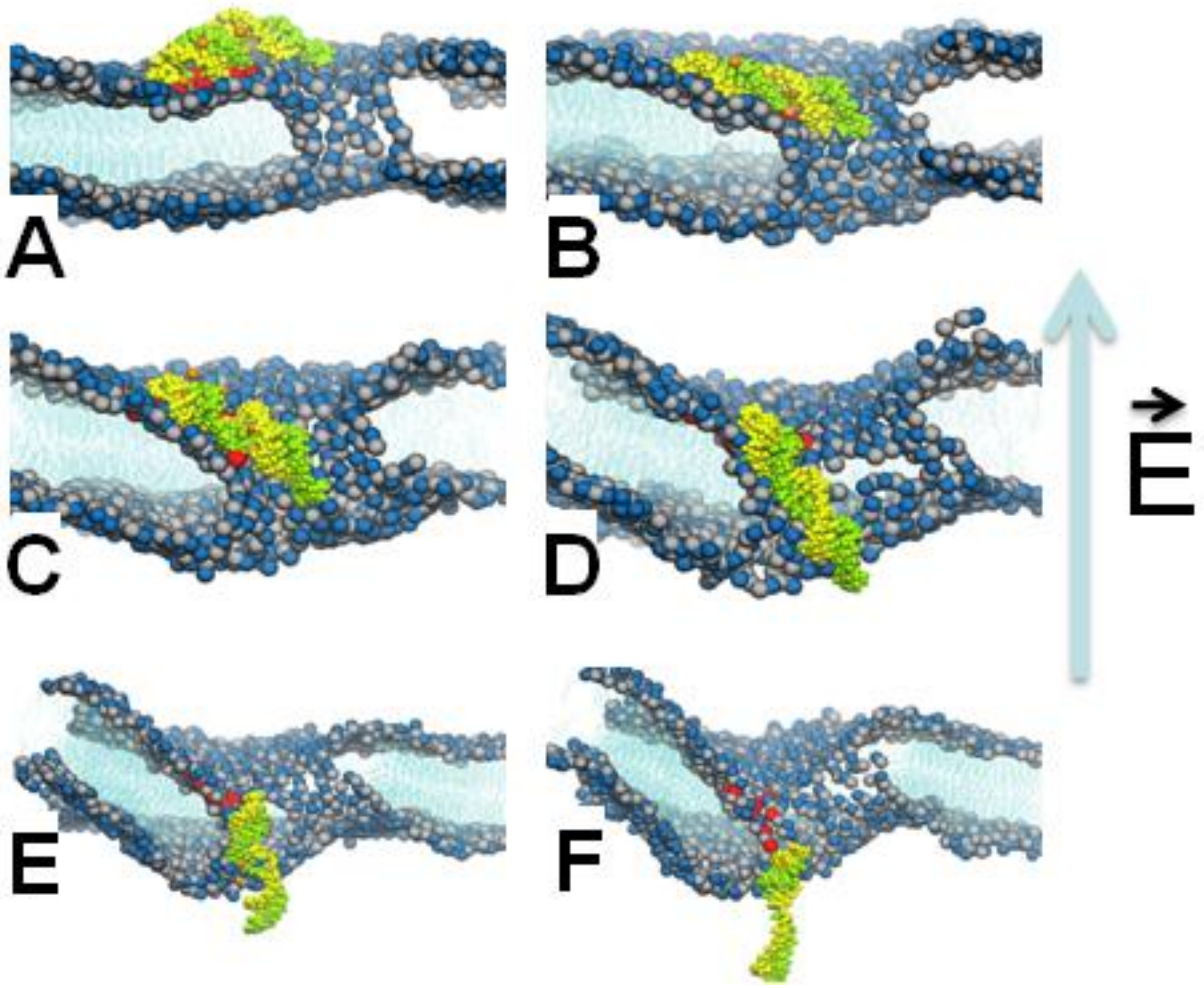
mediate sequence-specific gene expression

Paganin-G et al. *PNAS 2011*

Classical Electroporation Transfert :ms

Tarek M. *Biophys. J.* **2005**

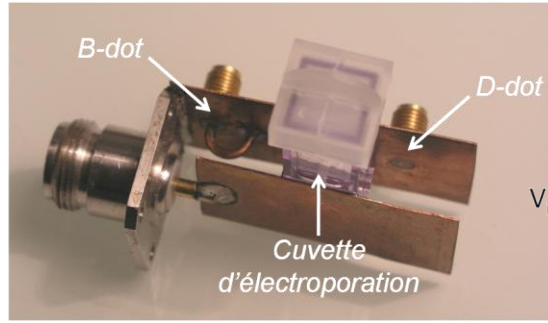
Small interfering RNA (siARN)

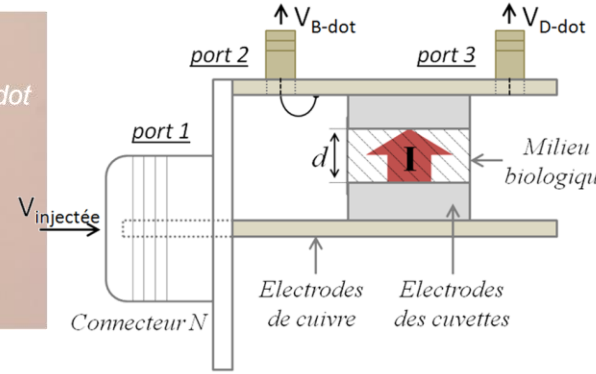


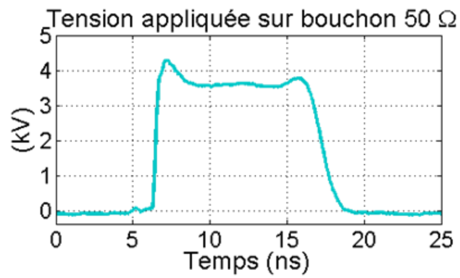
Time for translocation < 10 ns

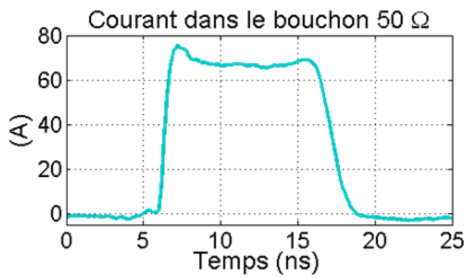
Experiment

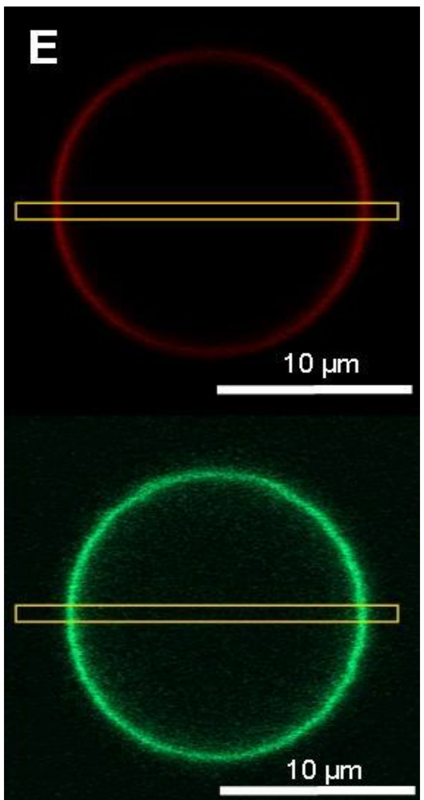
Marie Breton, Lluis M. Mir
Laboratoire de Vectorologie et Thérapeutiques Anticancéreuses, UMR8203,
Université Paris-Sud, CNRS,
Institut de cancérologie GUSTAVE ROUSSY
VILLEJUIF - www.igr.fr











Giant Unilamellar vesicles (GUV)
Diameter 10-100 µ (~ 20 µ)
(lipid DOPC)

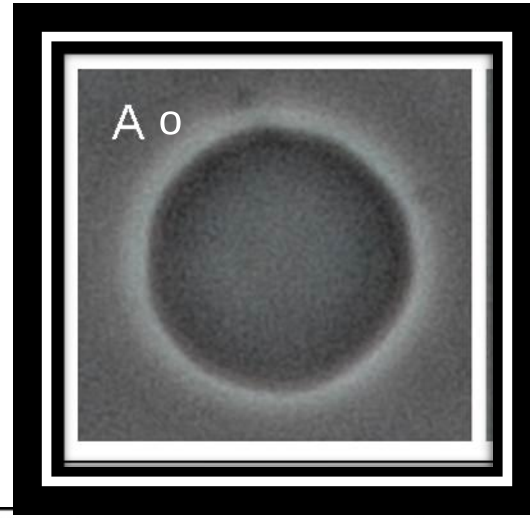
\+ IMAGING

Transmission microscopy

240 mM sucrose (intra)

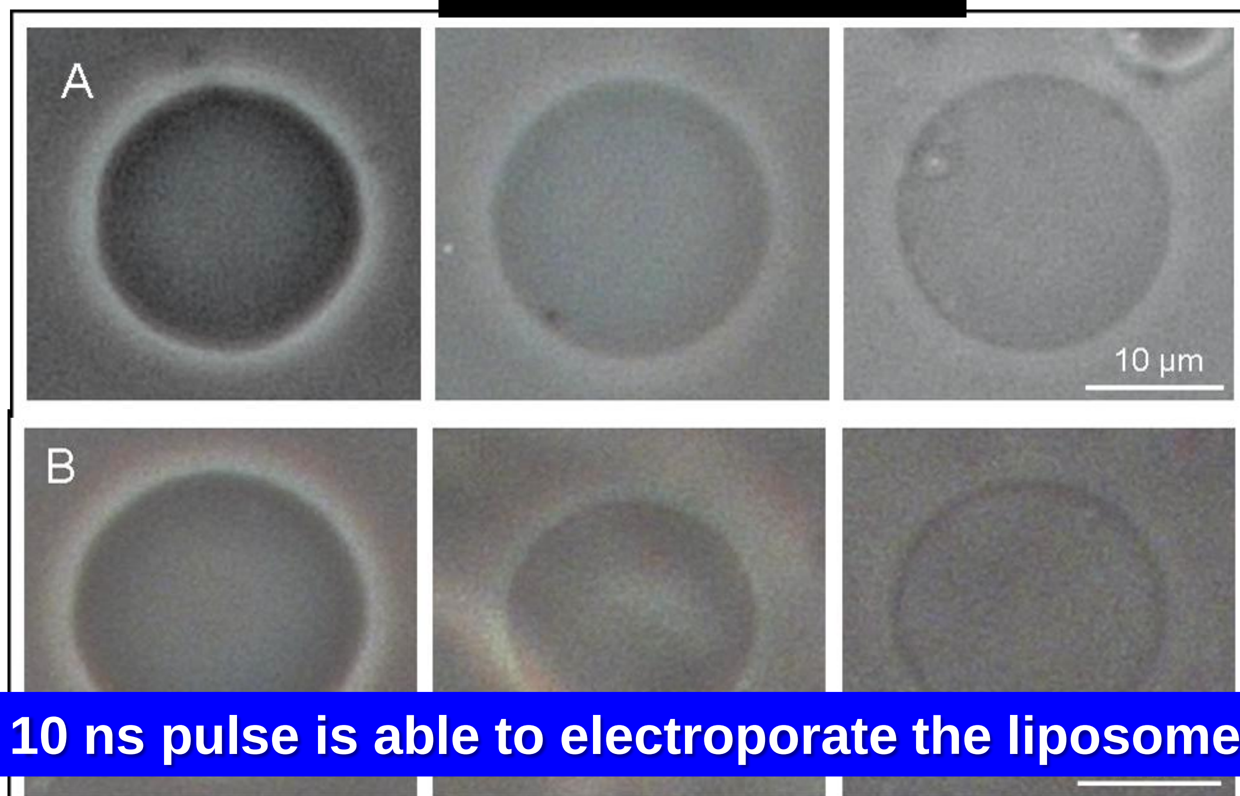
260 mM glucose (extra)

CONTARST



1 pulse

10 ns



3.2 kV/mm

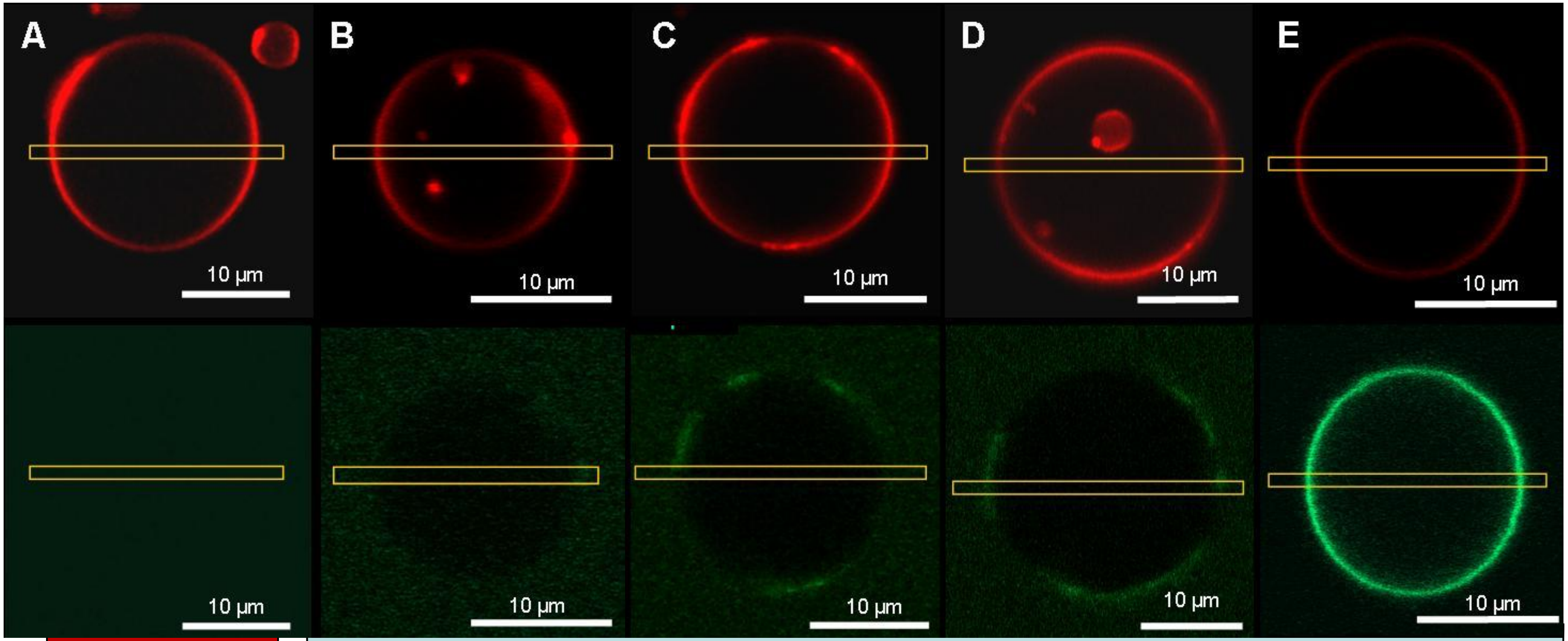
5.8 kV/mm

1 single 10 ns pulse is able to electroporate the liposome

Confocal Microscopy

GUV lipid: DOPC - Rhodamine- (Red Fluorescence)

siRNA -FITC fluorecine isothiocyanate) (Green Fluorescence)



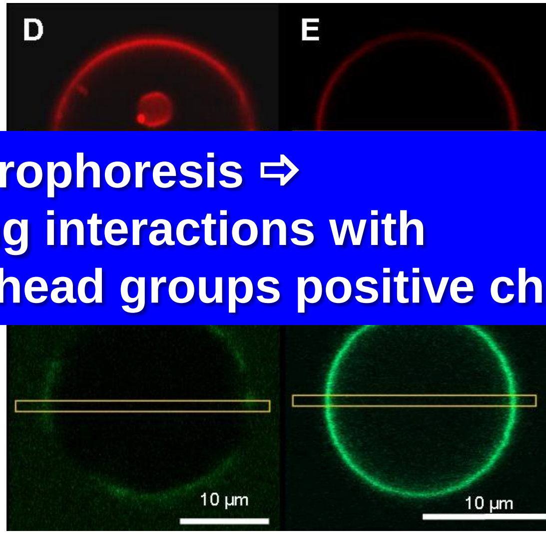
2 kV/mm

3.2 kV/mm

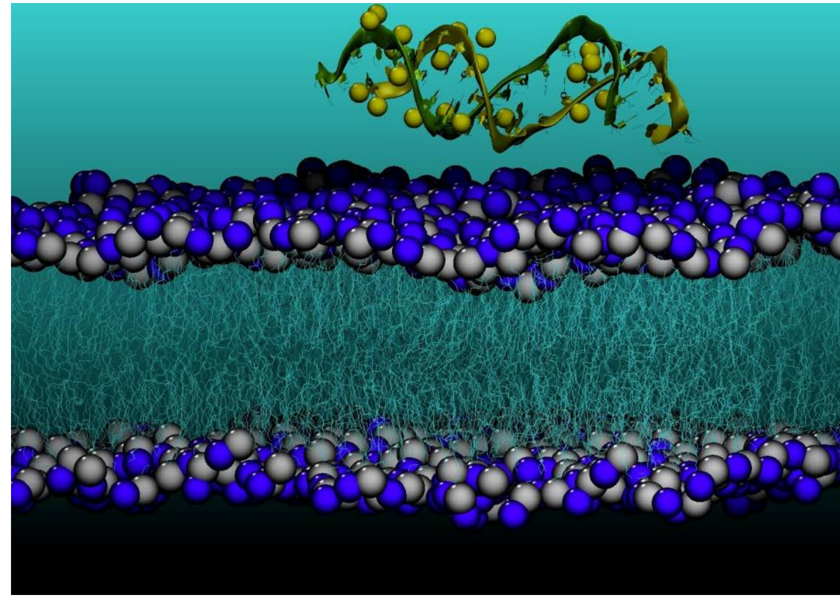
5.8 kV/mm

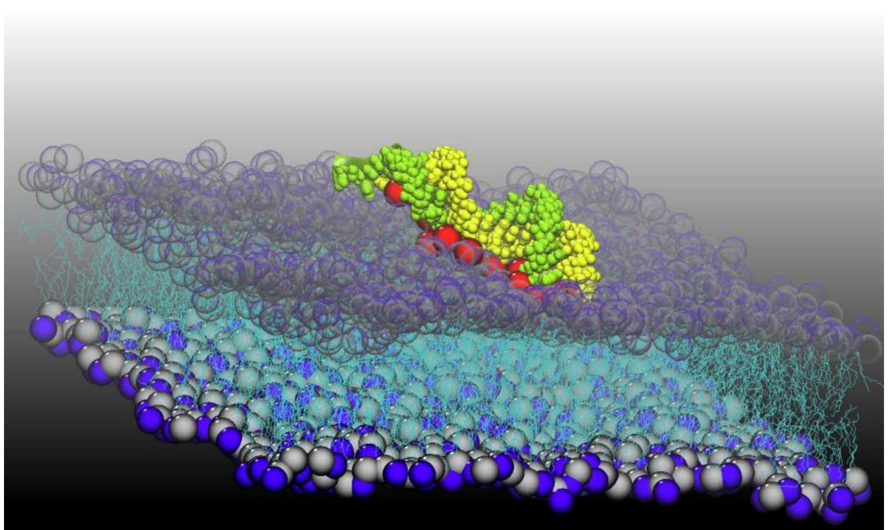
1 single 10 ns pulse is able to translocate siARN

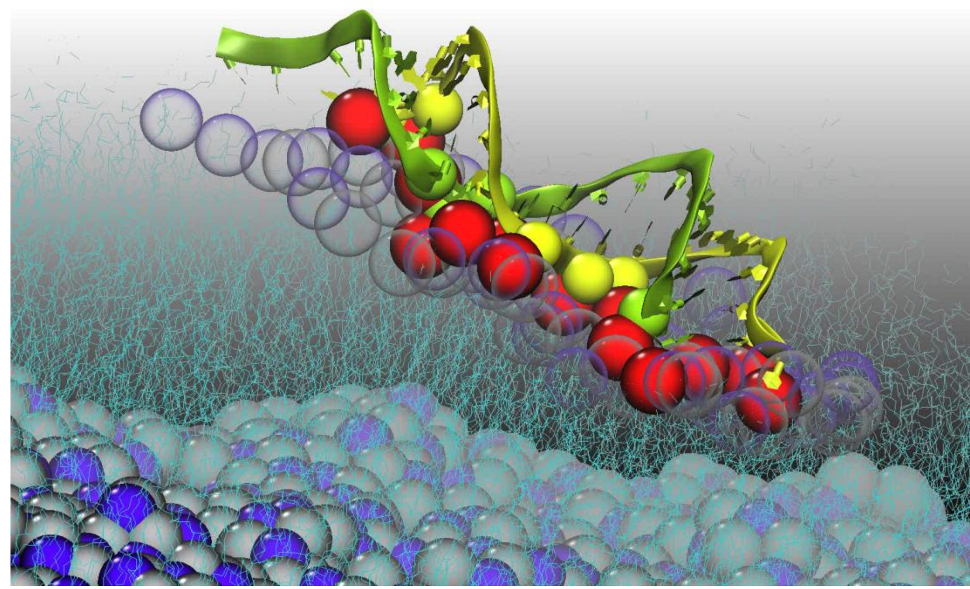
RNA adsorbs at the VUG



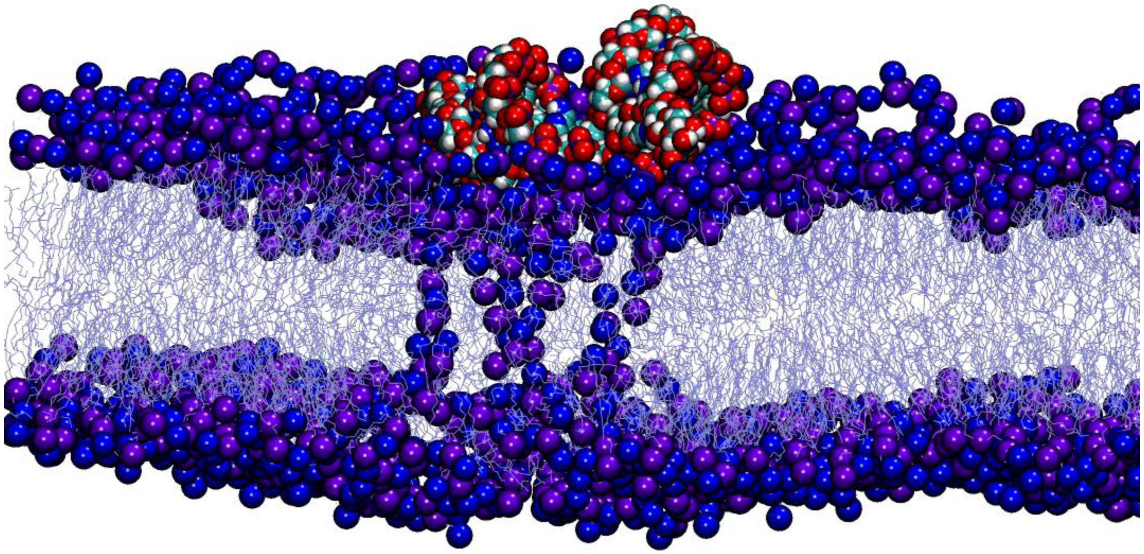
Electrophoresis ⇨
strong interactions with
lipid head groups positive charges

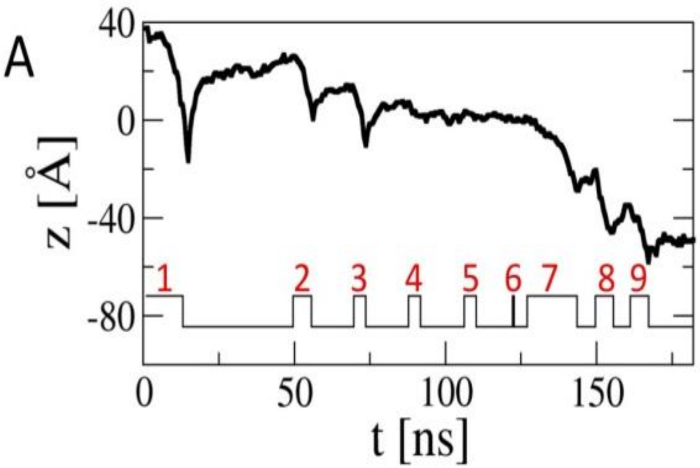






Optimise electro-transfer?





Several consecutive pulses of 2 to 4 ns delayed by > 100 ns

Under experimental investigation

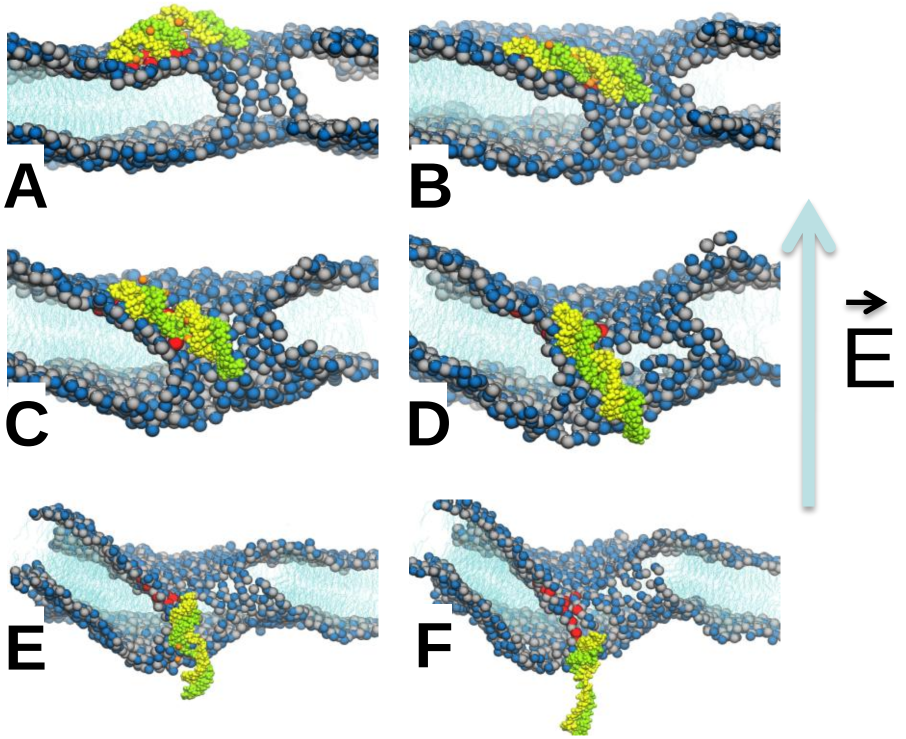
Conclusions

Combining Modeling and Experiments
Characterize the molecular mechanisms involved in plasmids Electro transfer

Open new venues for application of Nanosecond electric pulses

Next: From GUVs to cells, complex lipids, side effects, …..

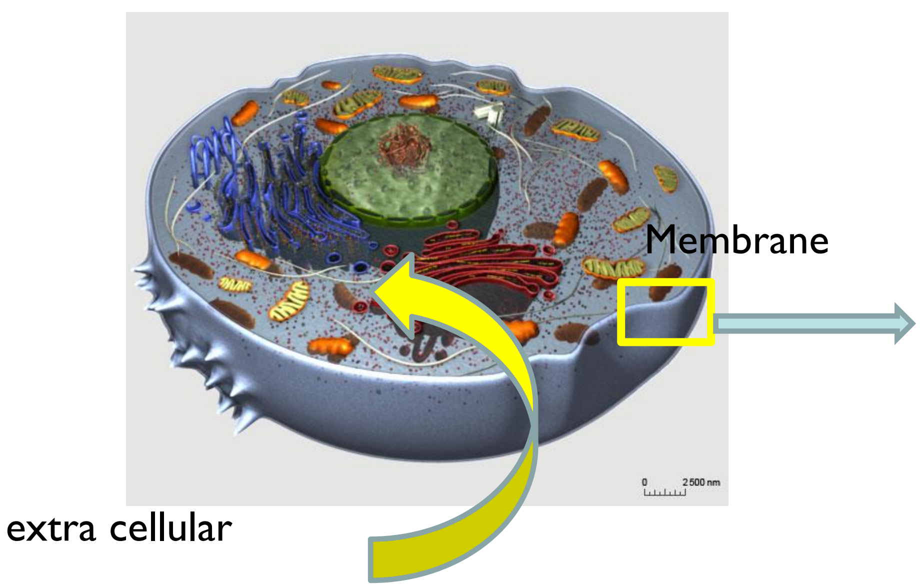
European Laboratory of Pulsed Electric Fields Applications in Biology and Medicine

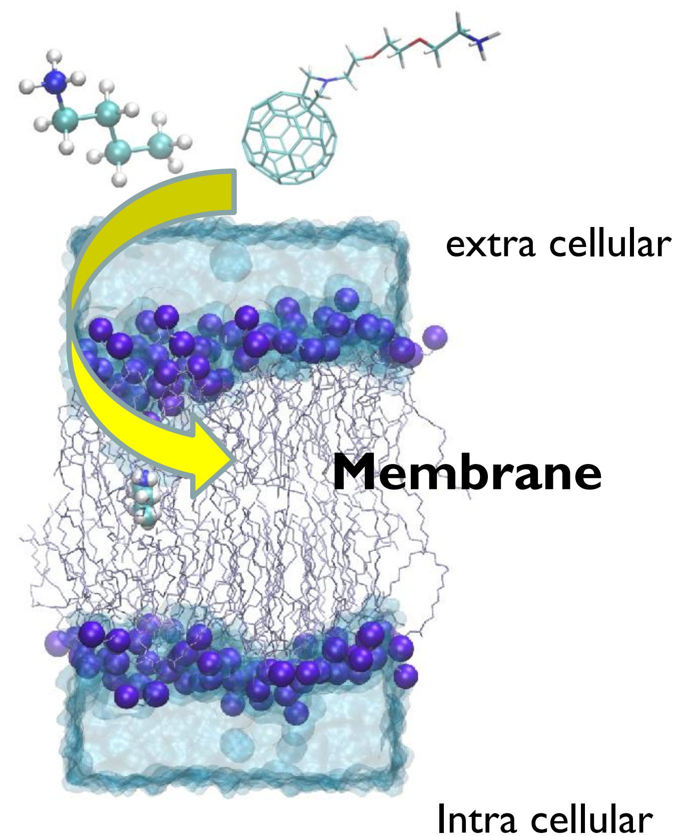


Unpublished

Transport of functionalized nanostructures through model cell membranes:

S. Kraszewski **Université Besonçon**





Lacerda al. (Review)
Nanotoday 2, (2007)

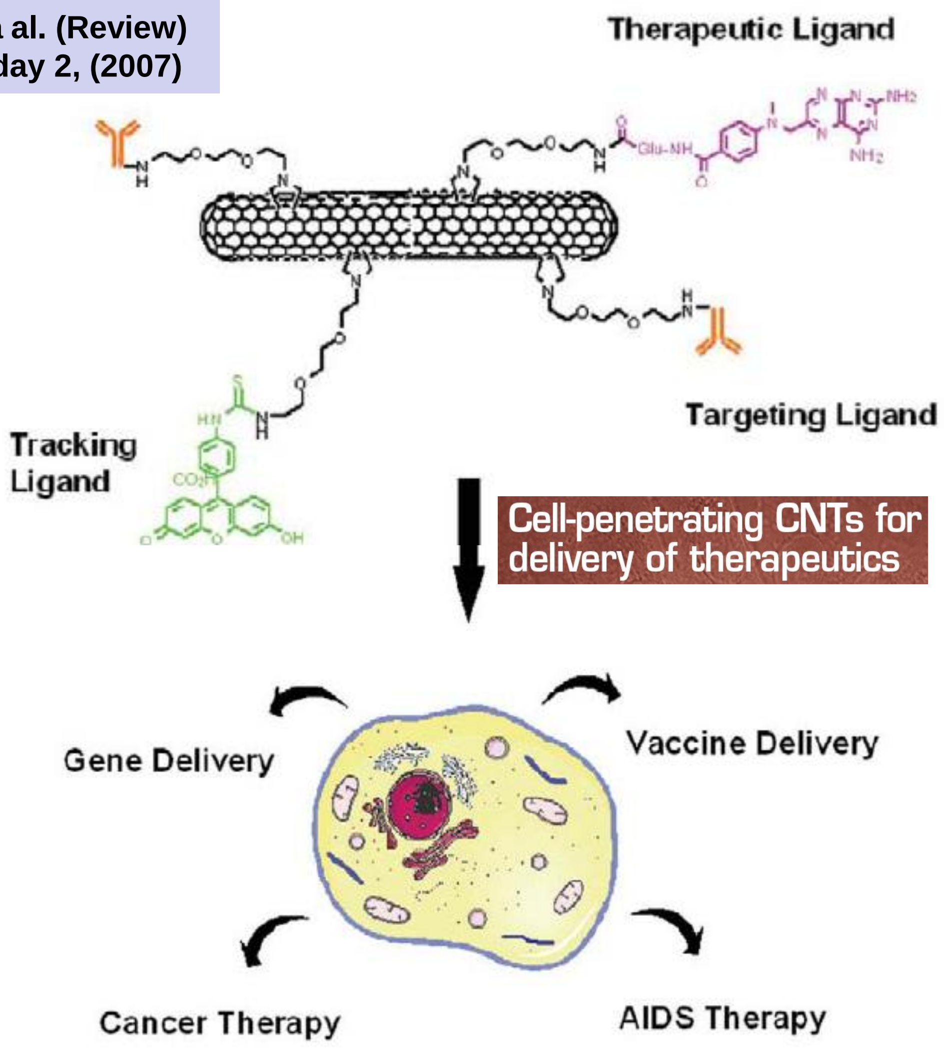
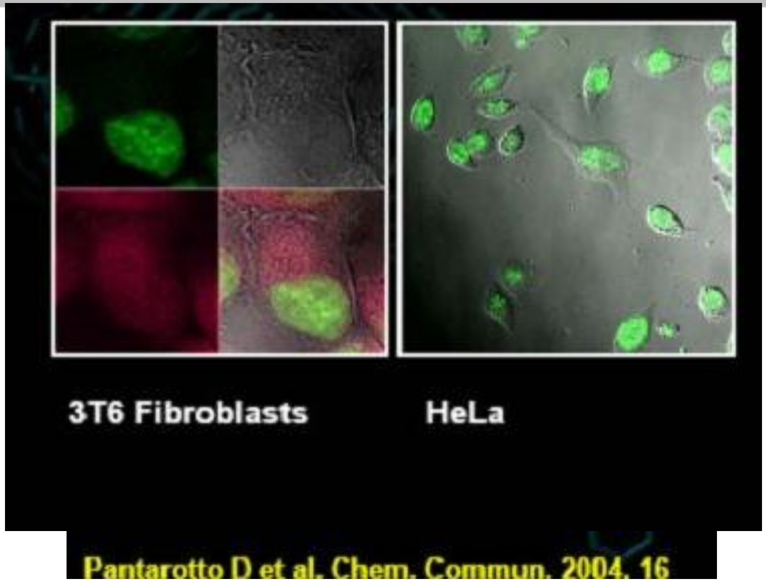


Fig. 4 Some specific biomedical applications of CNTs being explored by various groups as novel delivery systems.



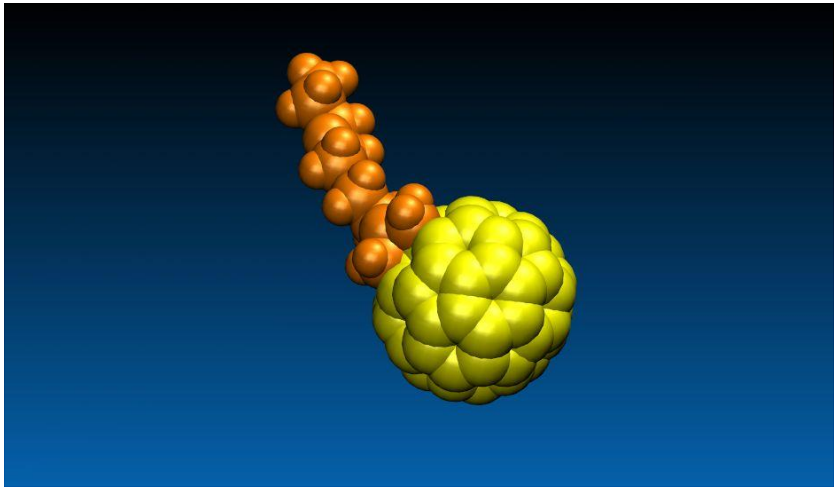
Bianco, A., Kostarelos, K. & Prato, M. (2008) Opportunities and challenges of carbon-based nanomaterials for cancer therapy. *Expt. Opin. Drug Deliv.* 5, 331-342.

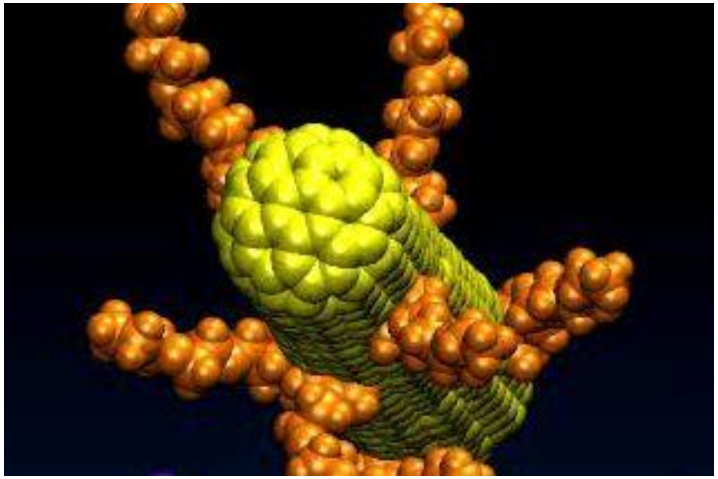
Characterize the cell penetration

Molecular modeling

↓

Support / Validation / Improvements





Ammonium based cationic peptide
CCNCCOCCOCCONH3$^+$,
Pantarotto et al. 2004
Angew. Chem. Int. Ed. 43, 5242.

Table 3. Characteristics of functionalised carbon nanotubes,

	Nanotubes
Shape	Tubular/cylindrical
Dimensions	Diameter: 1 – 100 nm Length: 0.01 – several microns/mm
Hybridisation	*sp*^{2}
Non-covalent functionalisation	Yes
Covalent functionalisation	Yes
Biocompatibility	Yes
Biodegradability	None
Cell uptake	Good
Cytotoxicity	Very low*
In vivo organ accumulation	Yes
Rapid elimination	Yes

*Assessed *in vitro* and *in vivo*.

Functionalized Carbon Nanotubes Are Non-Cytotoxic and Preserve the Functionality of Primary Immune Cells

Hélène Dumortier,*,† Stéphanie Lacotte,† Giorgia Pastorin,† Riccardo Marega,‡ Wei Wu,† Davide Bonifazi,‡ Jean-Paul Briand,† Maurizio Prato,*,‡ Sylviane Muller,† and Alberto Bianco*,†

NANO LETTERS
2006
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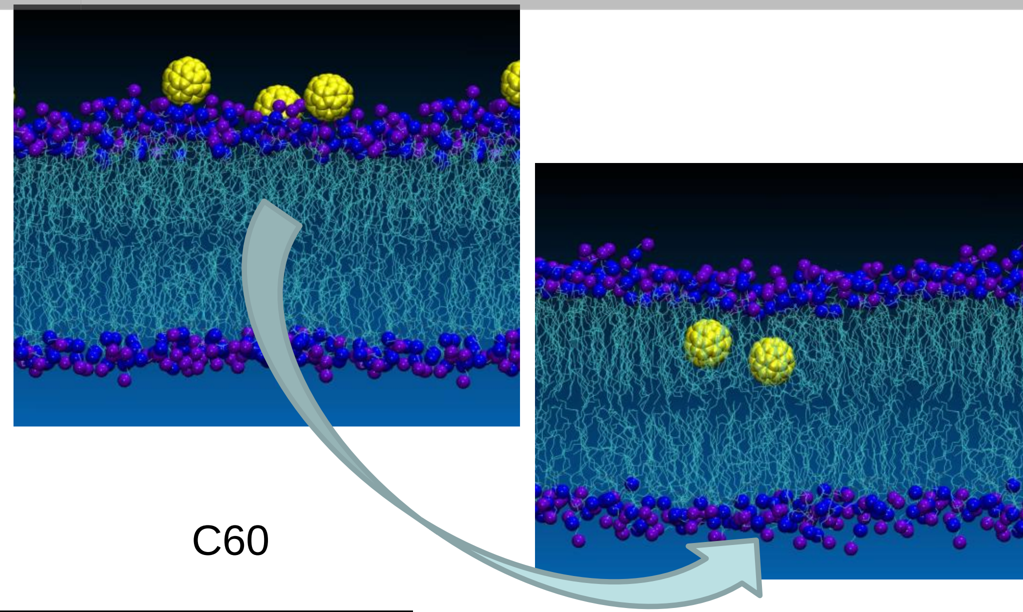
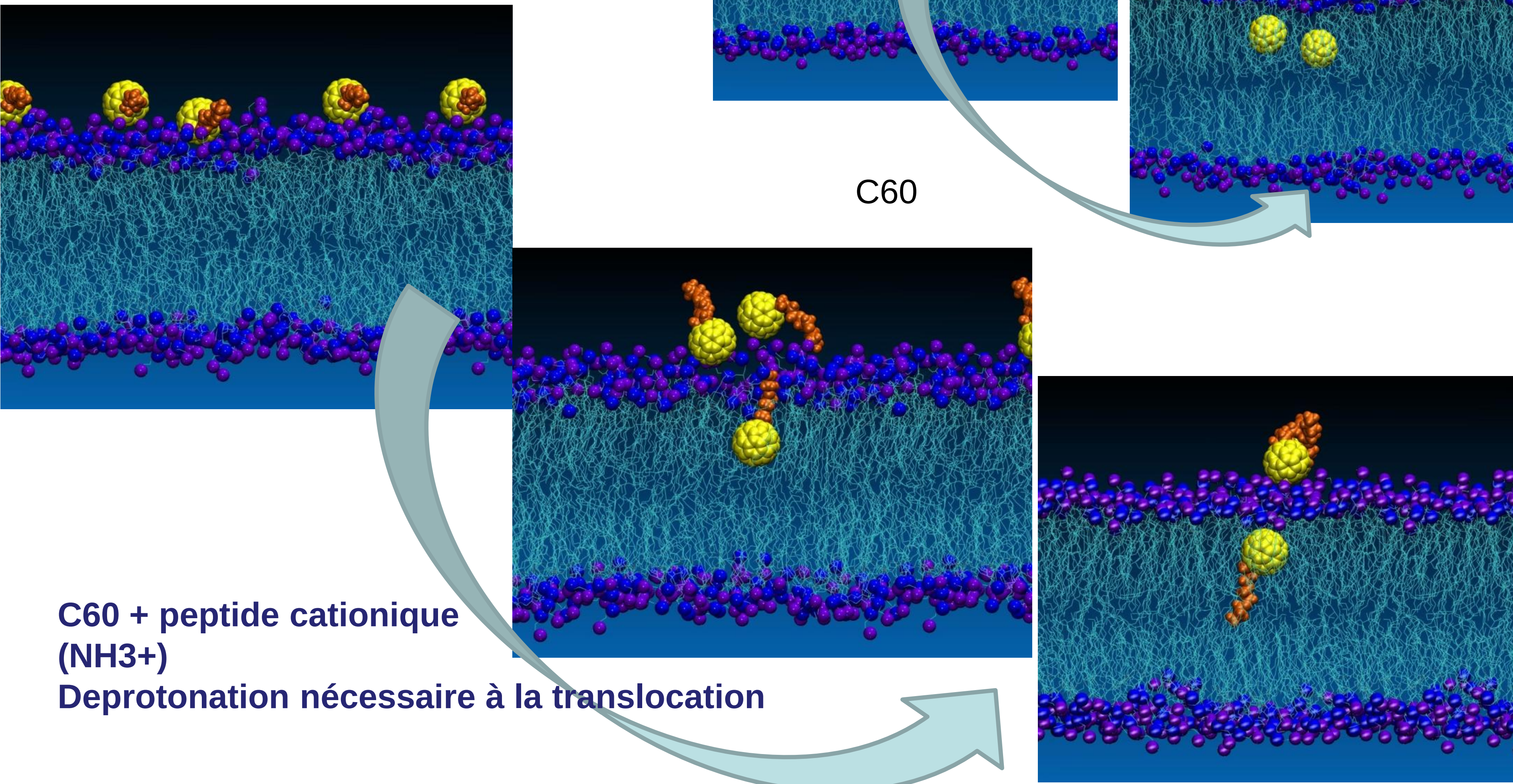
1522–1528

Functionalied C_{60} placed near a lipid bilayer

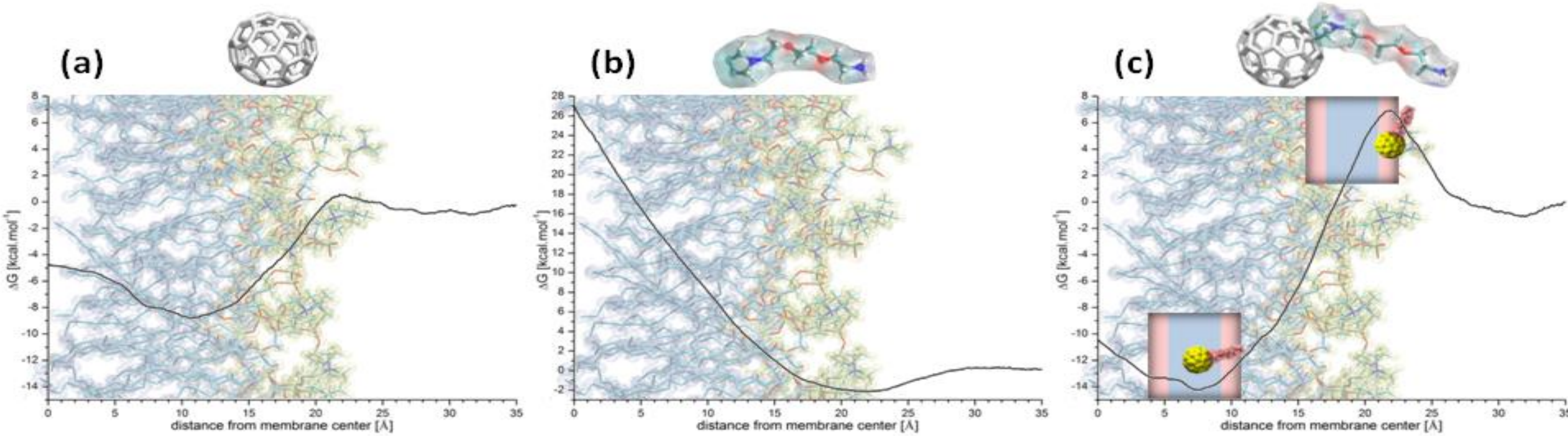




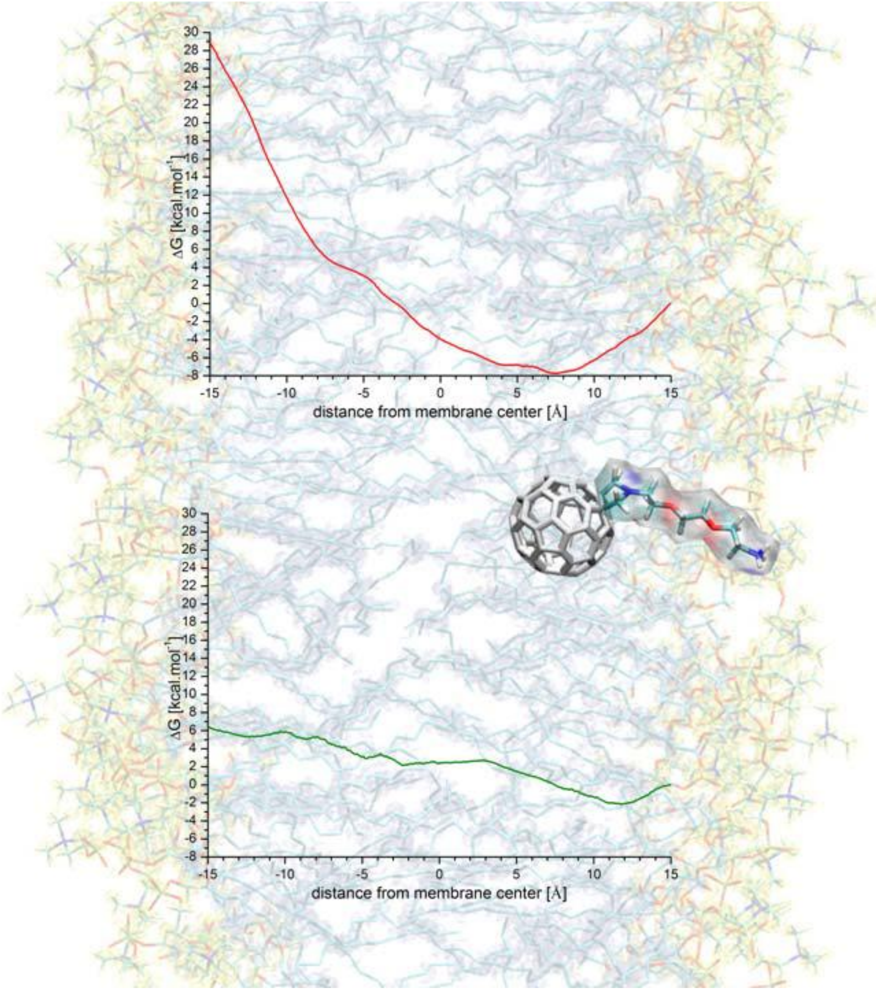
C60 + peptide cationique (NH3+)

Deprotonation nécessaire à la translocation

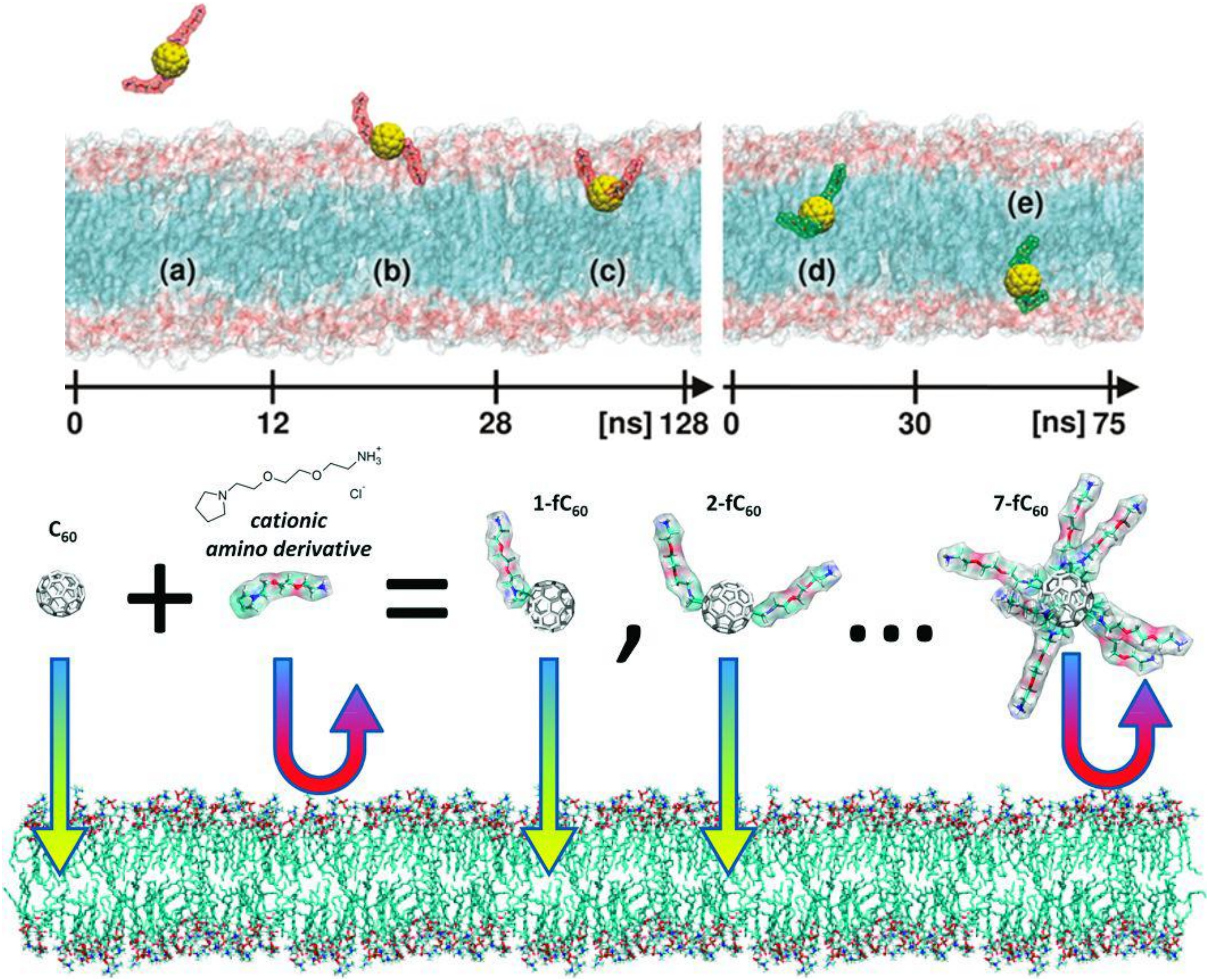
Free energy calculations to estimate the barrier to translocation



Free energy Calculations to estimate the barrier to translocation

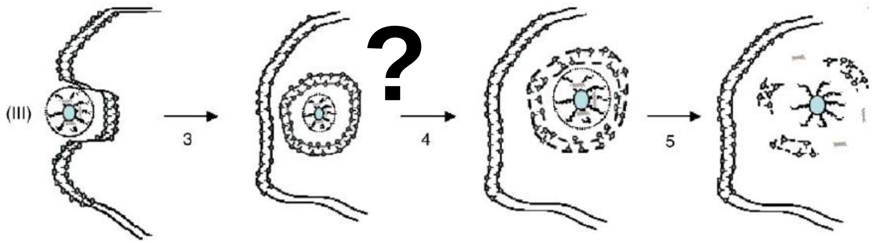


Functionalied C_{60} placed near a lipid bilayer

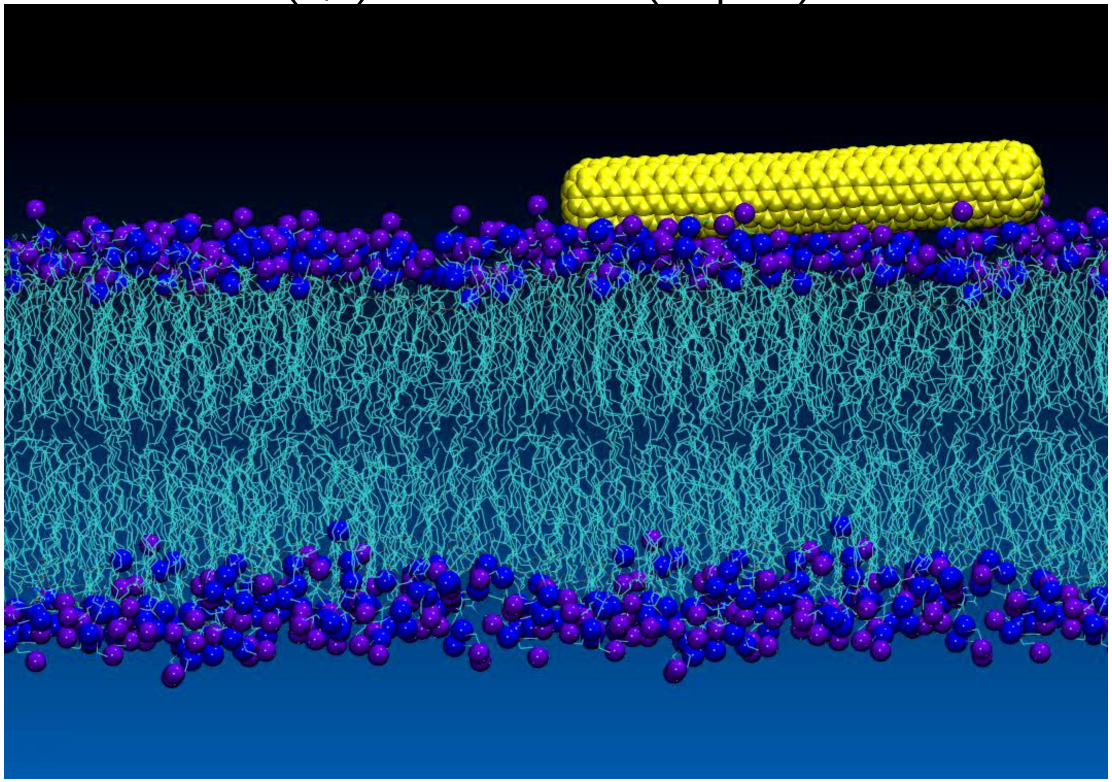


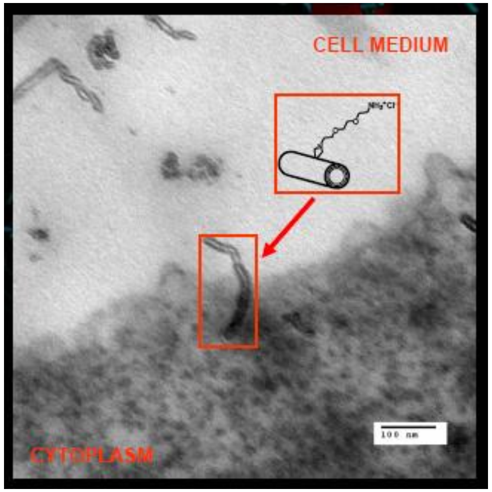
Kraszewski, Tarek et al. ACS Nano 2011

~~Cellular uptake?~~



CNT(6,6) Diameter 8 Å (caped)





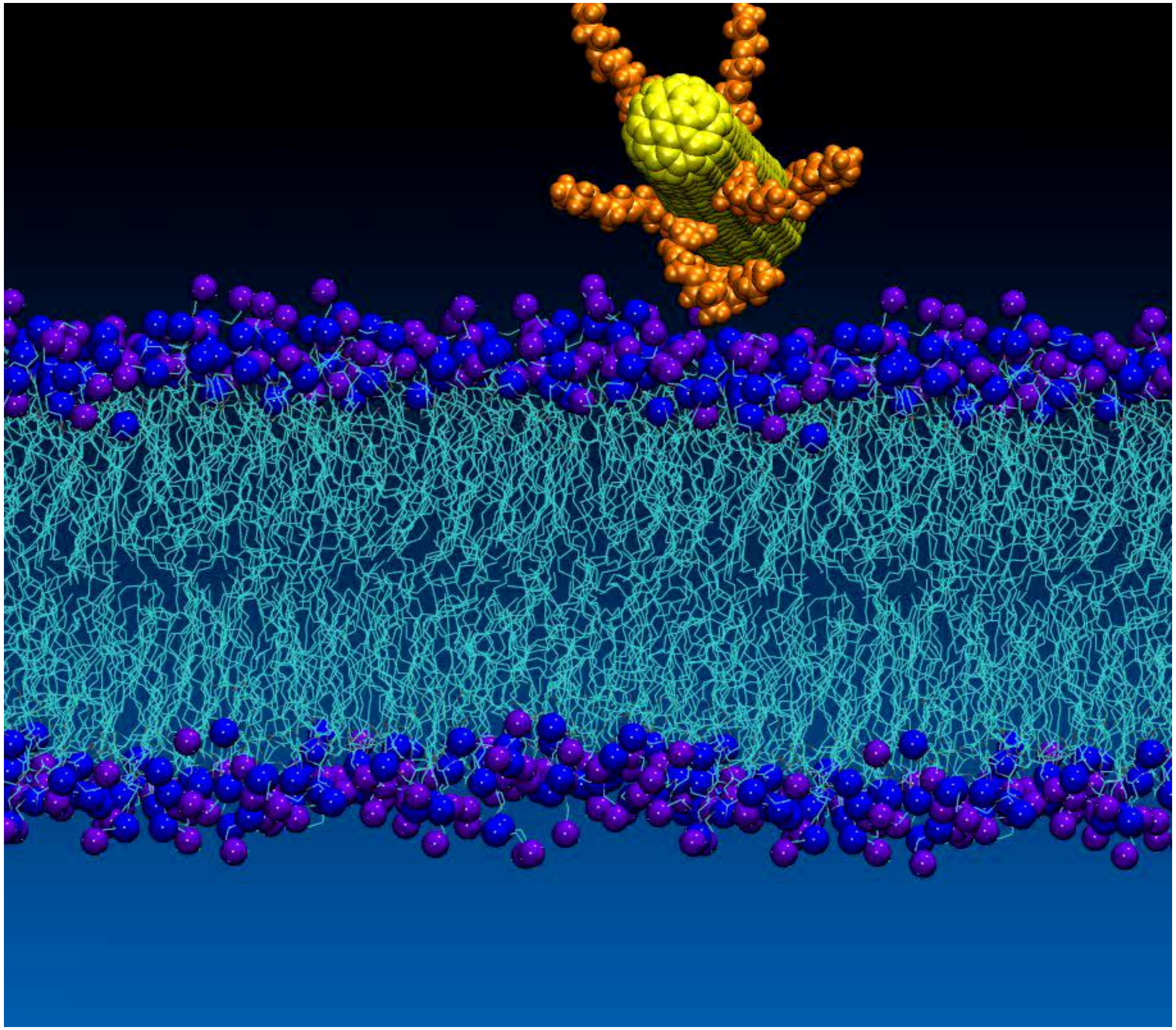
Nanoneedle Cell Penetration of Carbon Nanotubes

Pantarotto D et al. Angew. Chem. Int. Ed. 2004, 43, 5242

MD simulation
100 ns time scale

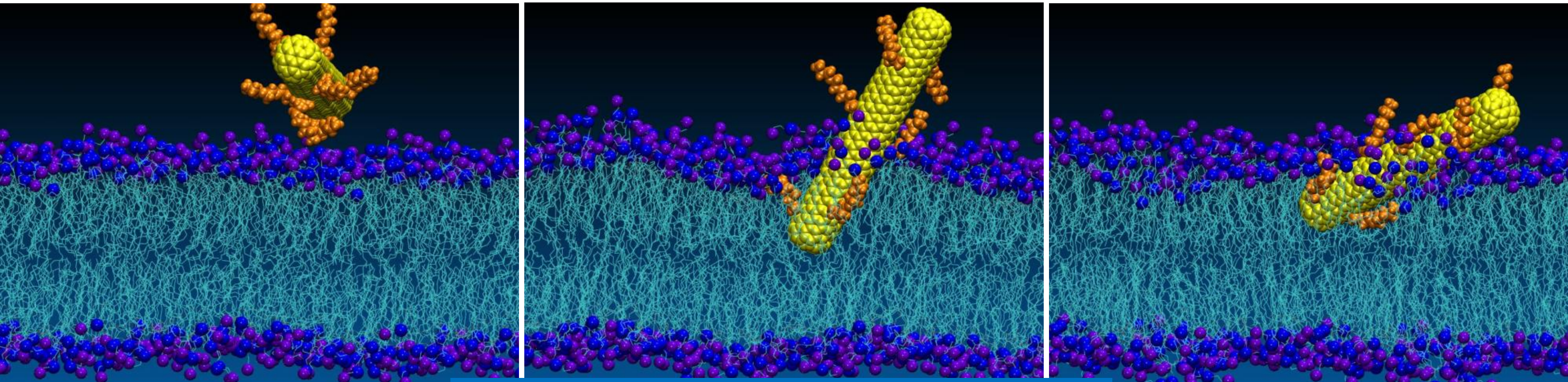
CNT(6,6) Diameter 8 Å (caped) + **CCNCCOCCOCCONH3^{+},**

MD simulation
100 ns time scale

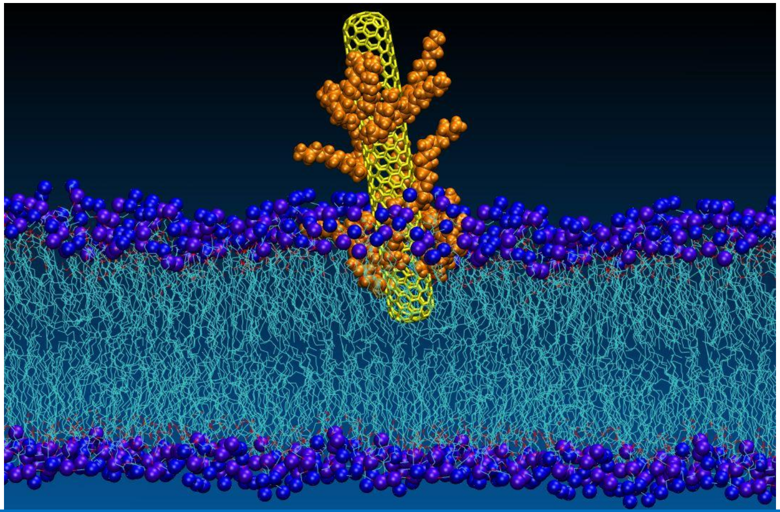


Fonctionalized nanotubes placed near a lipid bilayer

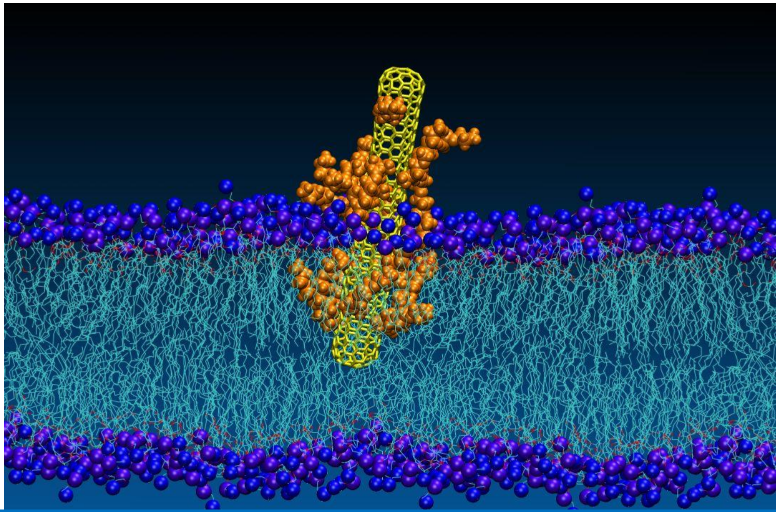
1F / 150 C



Barrier too high to cross



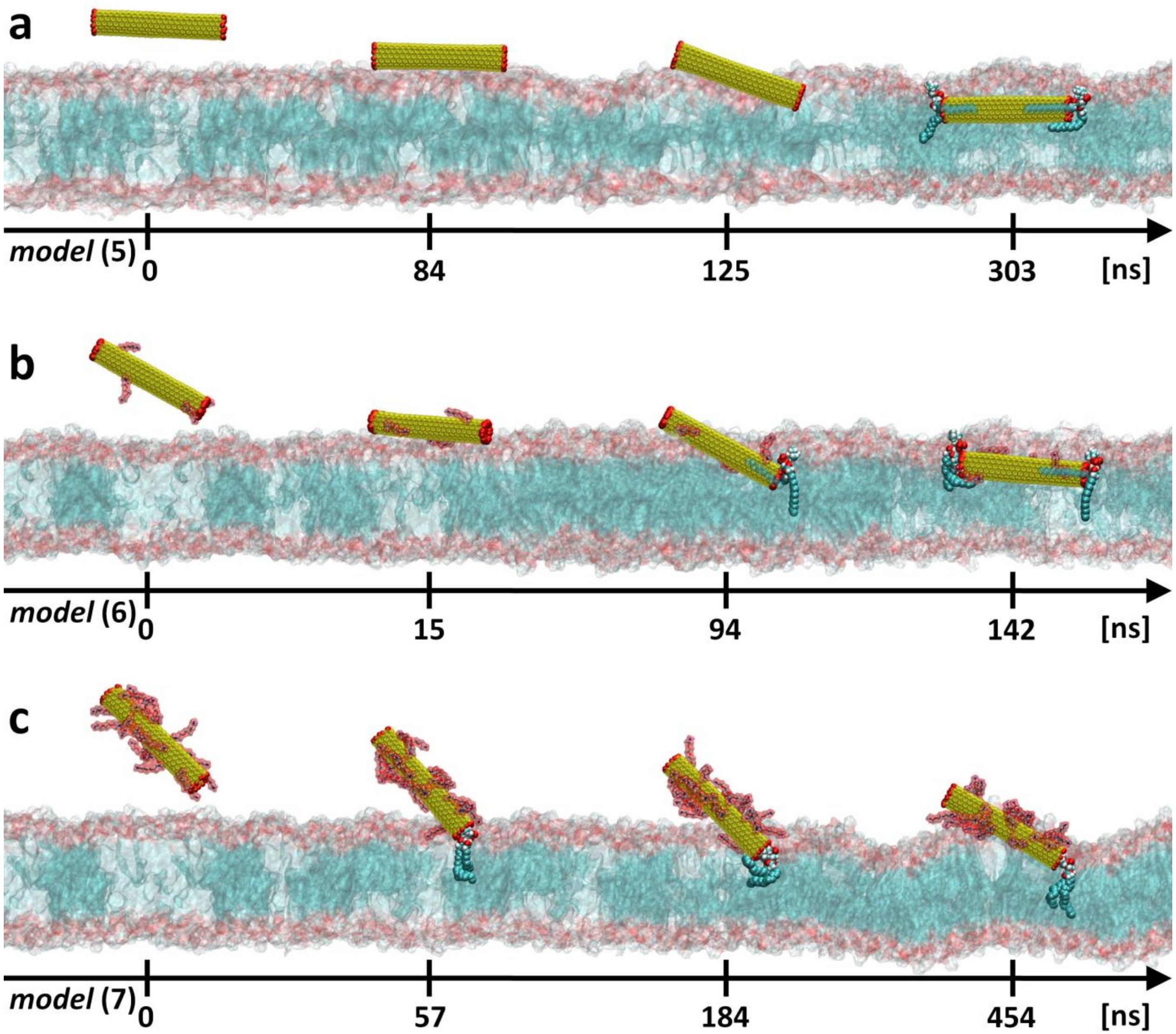
1F / 50 C



Deprotonation of the peptides as they reach the interface

molecular system	system description	total simulation time [ns]
(1)	non functionalized closed CNT	509
(2)	low degree side functionalized closed CNT	423
(3)	low degree side and edges functionalized closed CNT	395
(4)	highly side functionalized closed CNT	779
(5)	explicitly H-ended open CNT	443
(5)'	open CNT non explicitly H-ended	200
(6)	low degree functionalized, open CNT non explicitly H-ended	142
(7)	explicitly H-ended, highly functionalized open CNT	466

Kraszewski, Tarek et al. PLOS One 2012

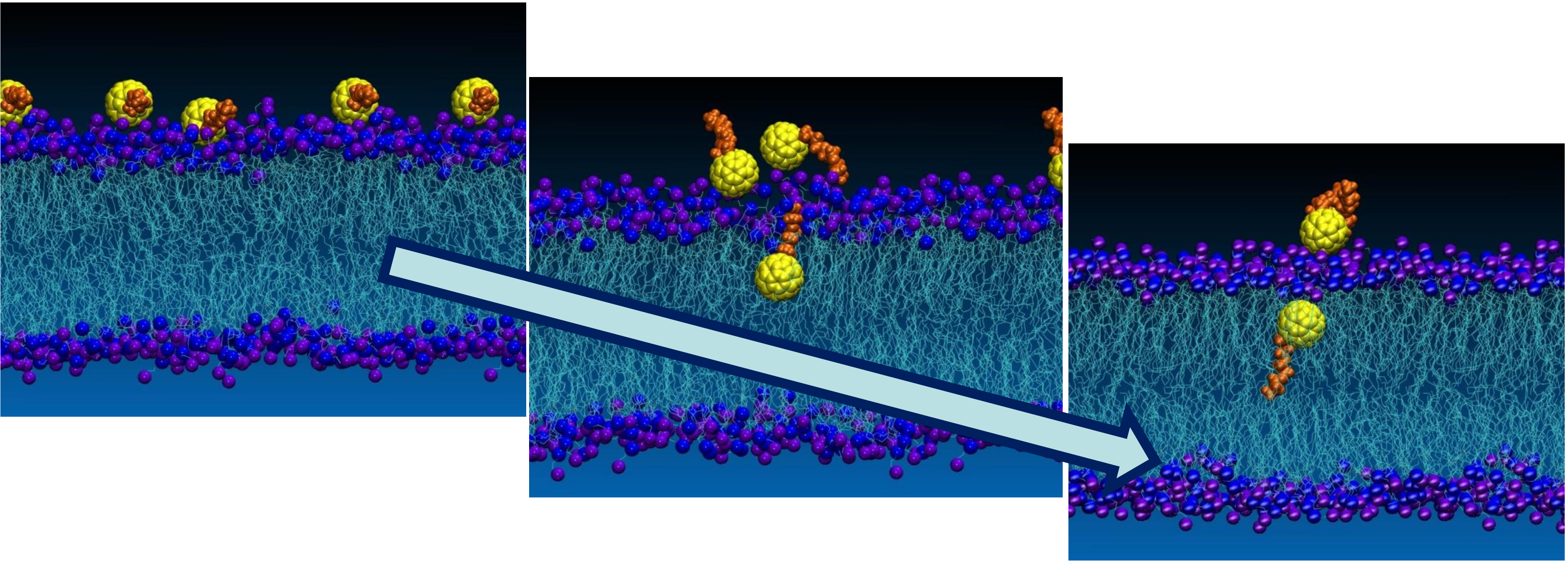

a
model (5)
0
84
125
303
[ns]
b
model (6)
0
15
94
142
[ns]
c
model (7)
0
57
184
454
[ns]

- Functionalized carbon nanostructures penetrate cell membranes through a passive process

- Barrier for translocation depends on the nature of the ligand

- Protonation state of the $CCNCCOCCOCCONH3^{+}$ ligand near the lipid head group yet to be fully characterized.

- Under study: transport of non covalently bound peptides / DNA…



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Université Besonçon

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