ADMINISTRATION DE PETITS FRAGMENTS D'ACIDE NUCLEIQUES

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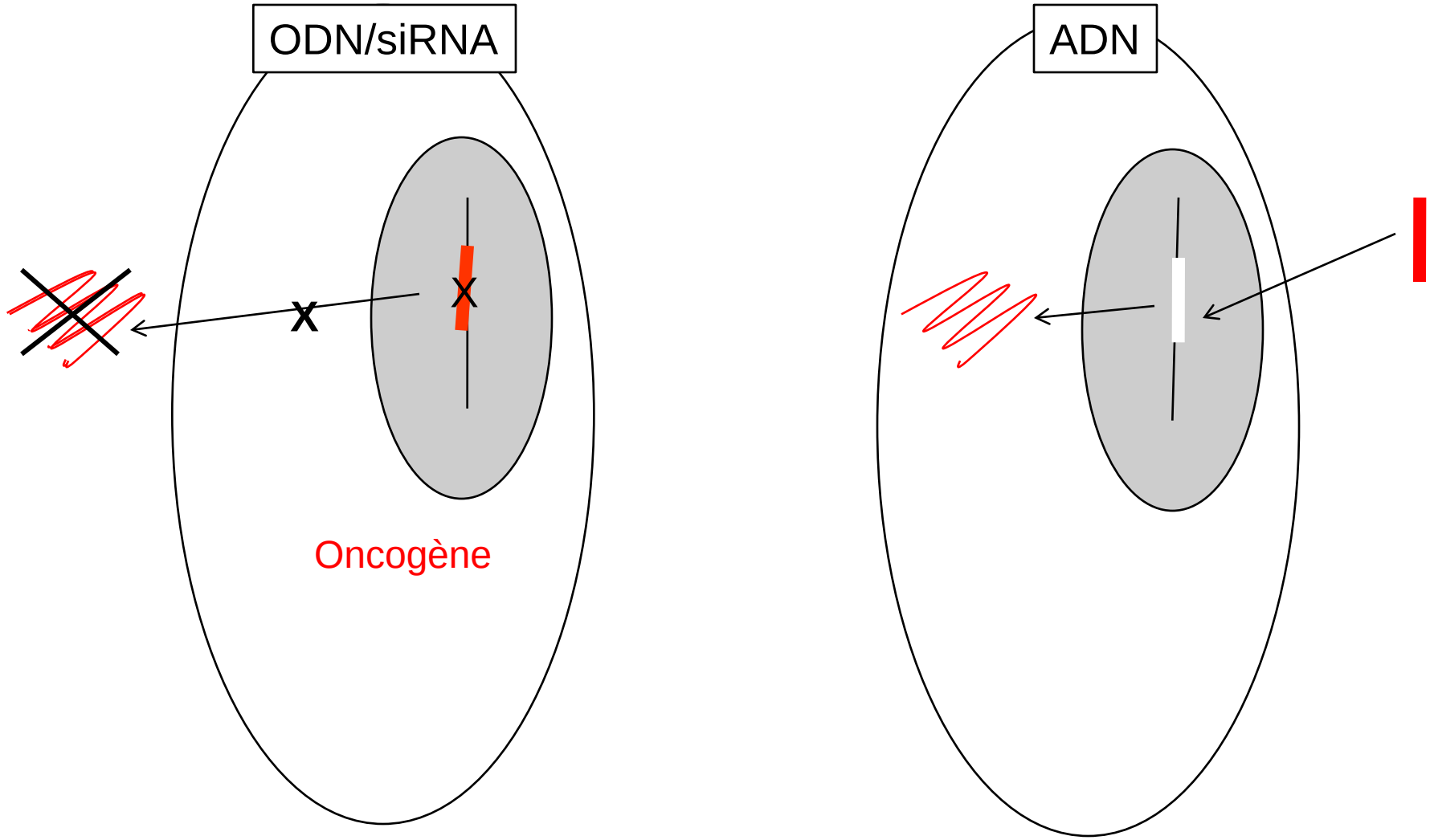
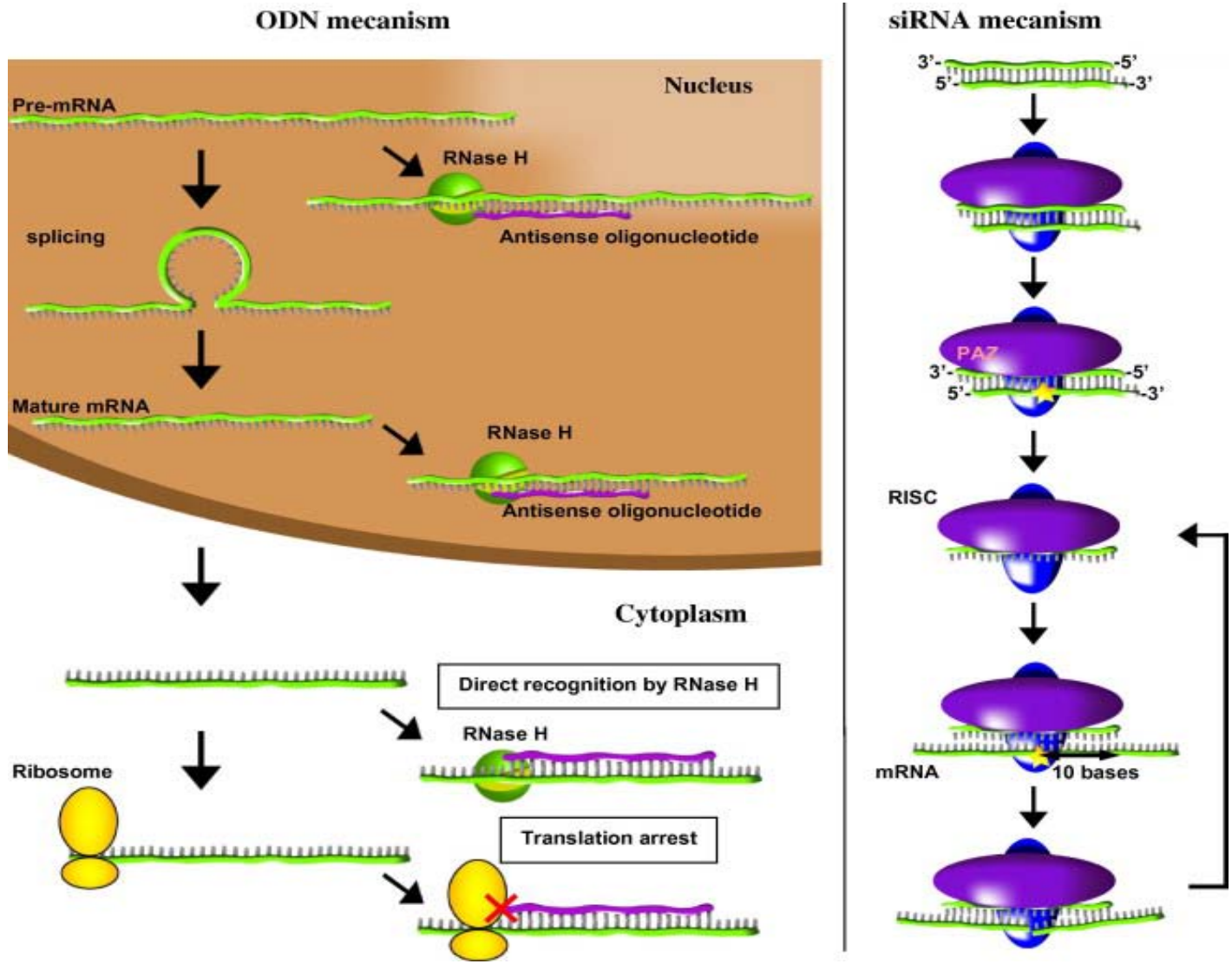
Professeur au Collège de France
Chaire d'Innovation Technologique 2009-2010

THERAPIE GENIQUE: SOIT ETEINDRE UN GENE (CANCEREUX/VIRAL) SOIT REMPLACER UN GENE MANQUANT

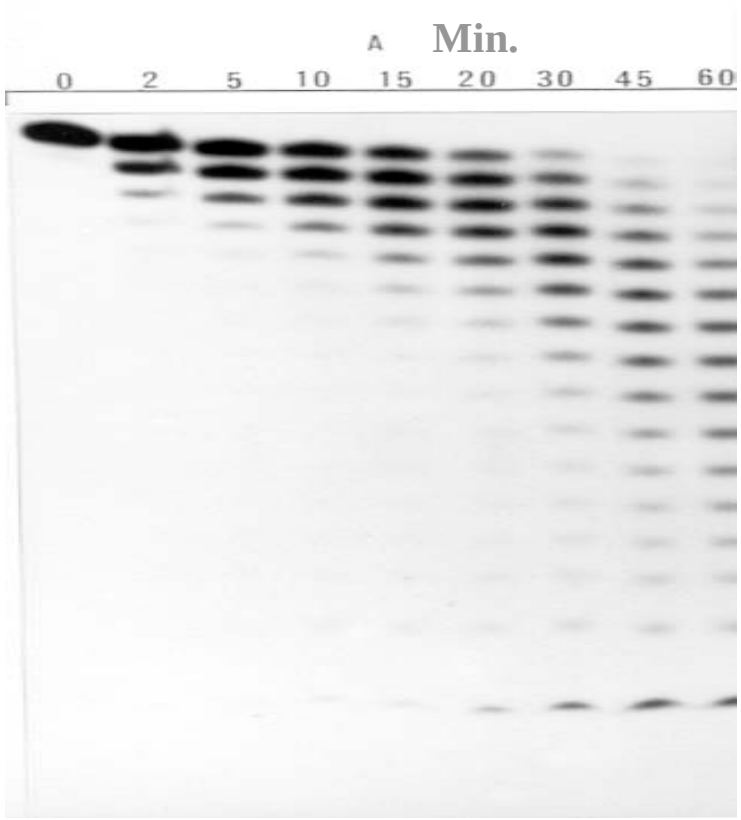


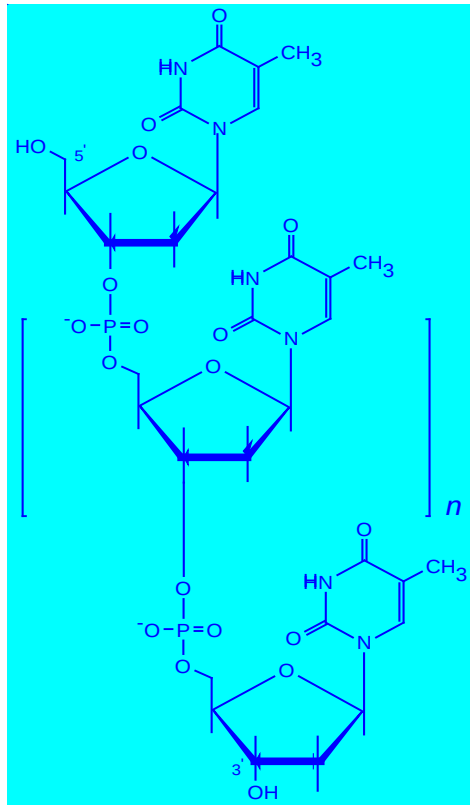
ODN AND siRNA MECHANISM

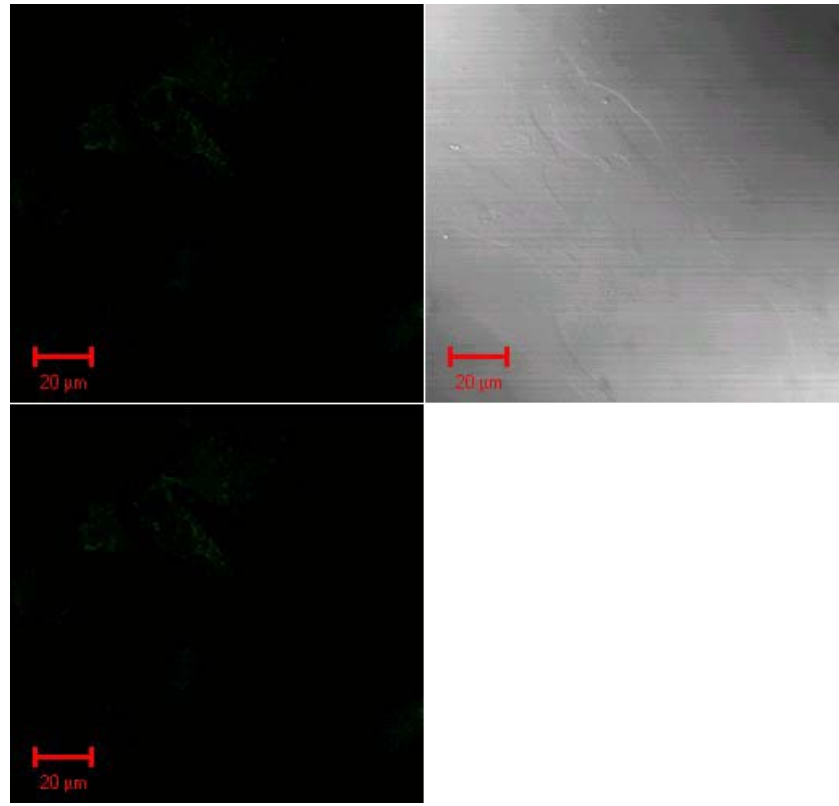
de Martymprey et al, European Journal of Pharmaceutics and Biopharmaceutics 71, 490-504 (2009)



COMMENT ADMINISTRER L'INDELIVRABLE ?







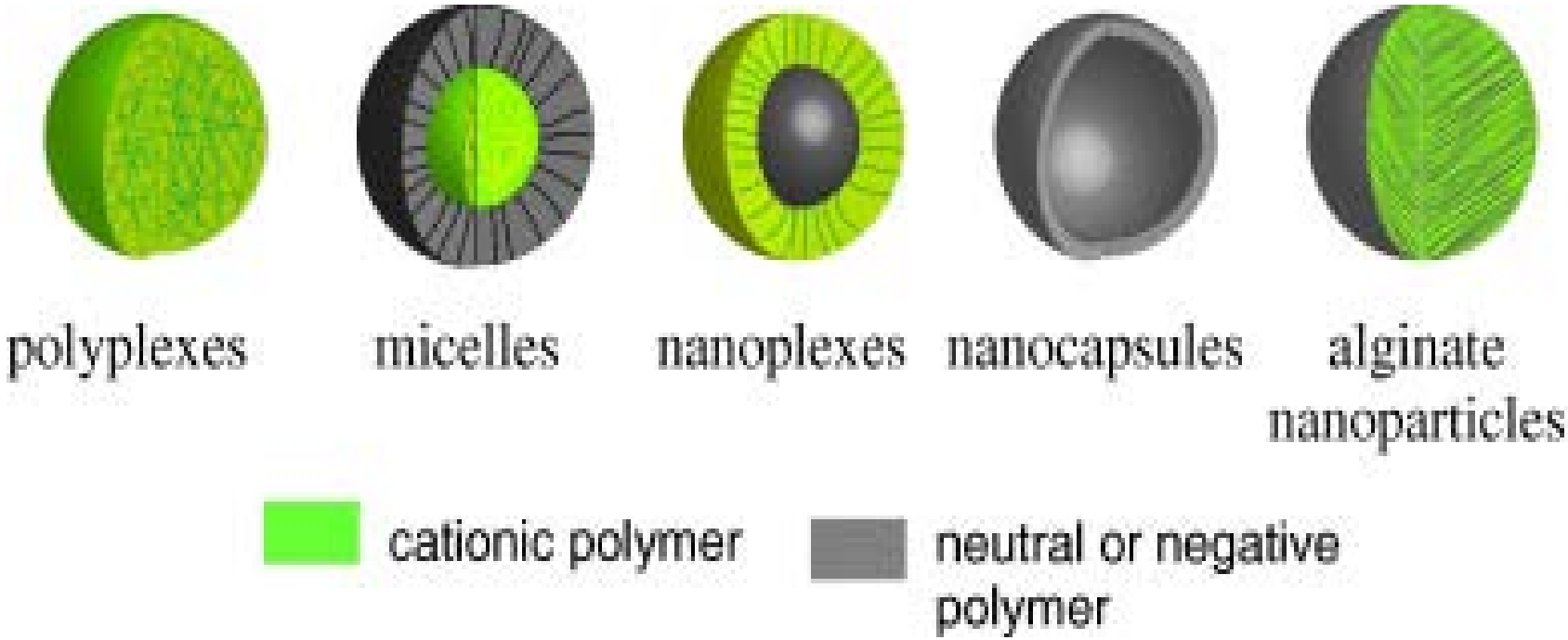
Degradation de ODN
Dans sérum (10%)

Pénétration intracellulaire

NANOVECTEURS POUR LA DELIVRANCE DES ODN ET siRNA

STRUCTURE OF THE DIFFERENT NANOCARRIERS

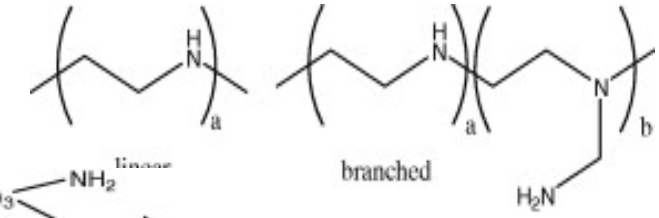
de Martymprey et al, European Journal of Pharmaceutics and Biopharmaceutics 71, 490-504 (2009)



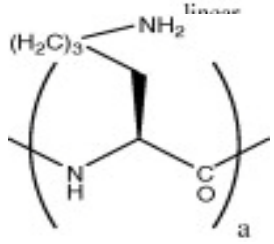
CHEMICAL STRUCTURE OF POLYMERS USED FOR ODN OR siRNA DELIVERY

Cationic **(POLYPLEXES)**

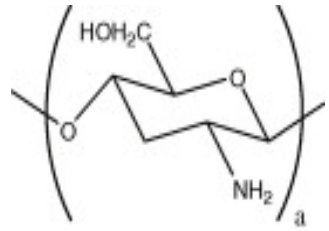
PEI



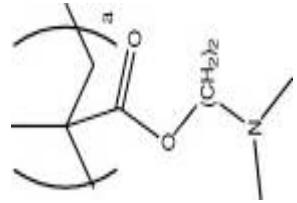
PLL



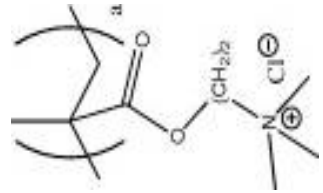
Chitosan



PDMAEMA

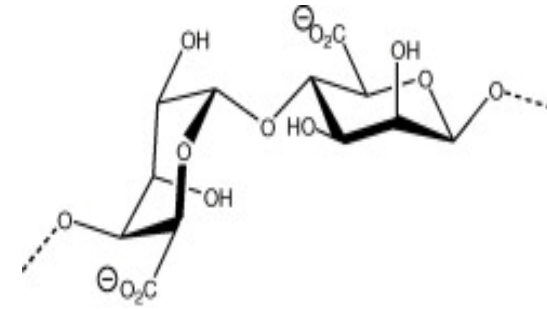


PTMAEMA



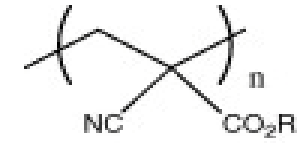
Negative

Alginate

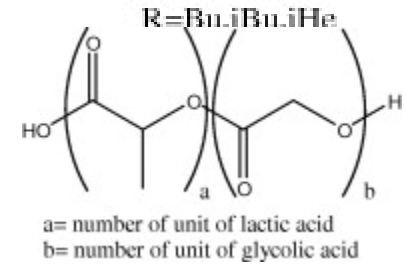


Neutral

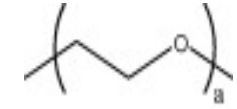
PACA



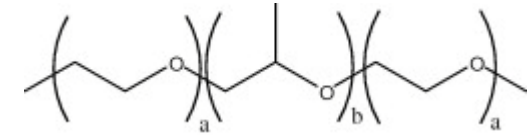
PLGA



PEG

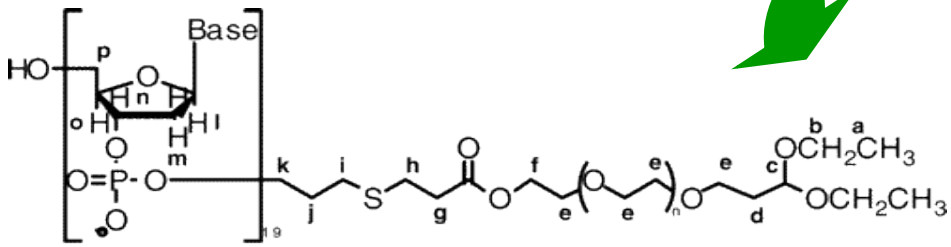


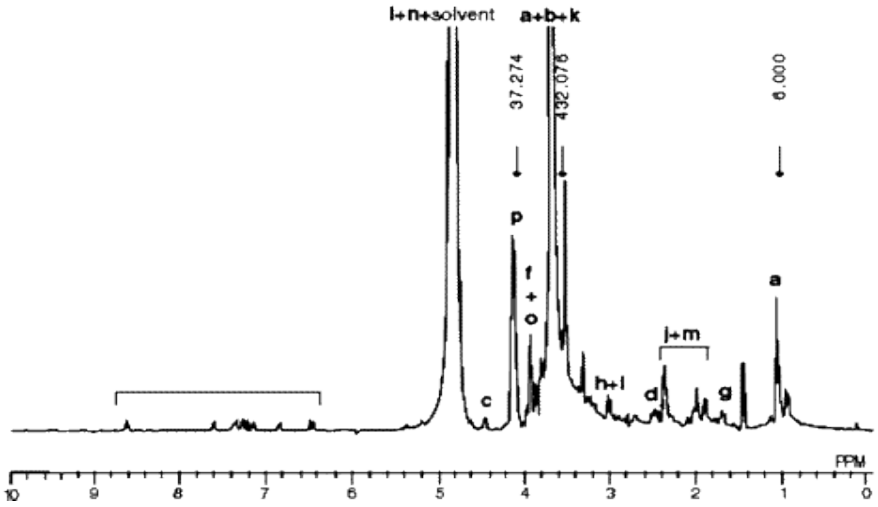
Pluronic

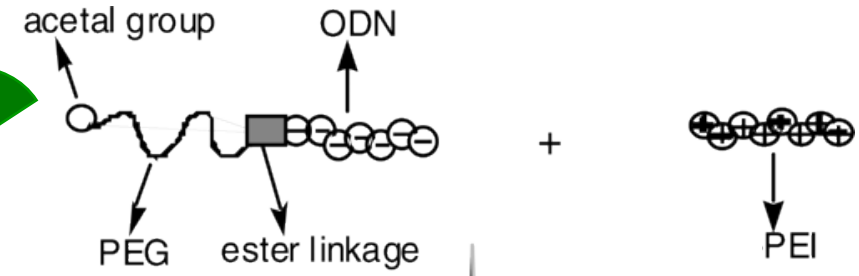


POLYION COMPLEX MICELLES

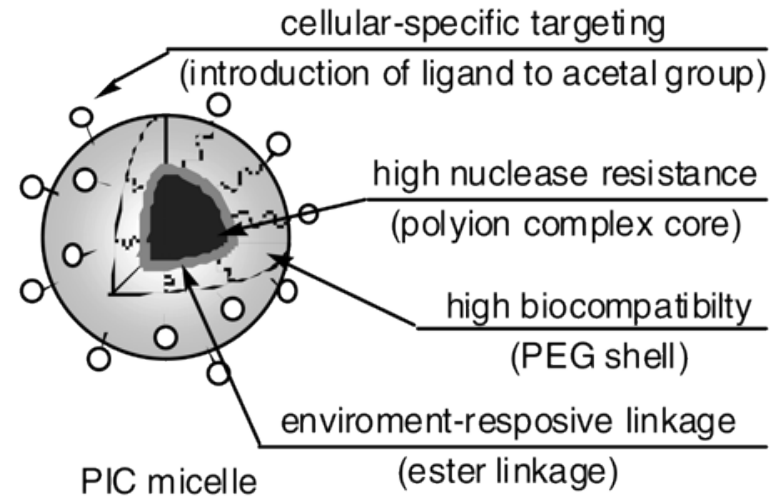
Motoi Oishi et al. Biomacromolecules 4, 1426-1432 (2003)





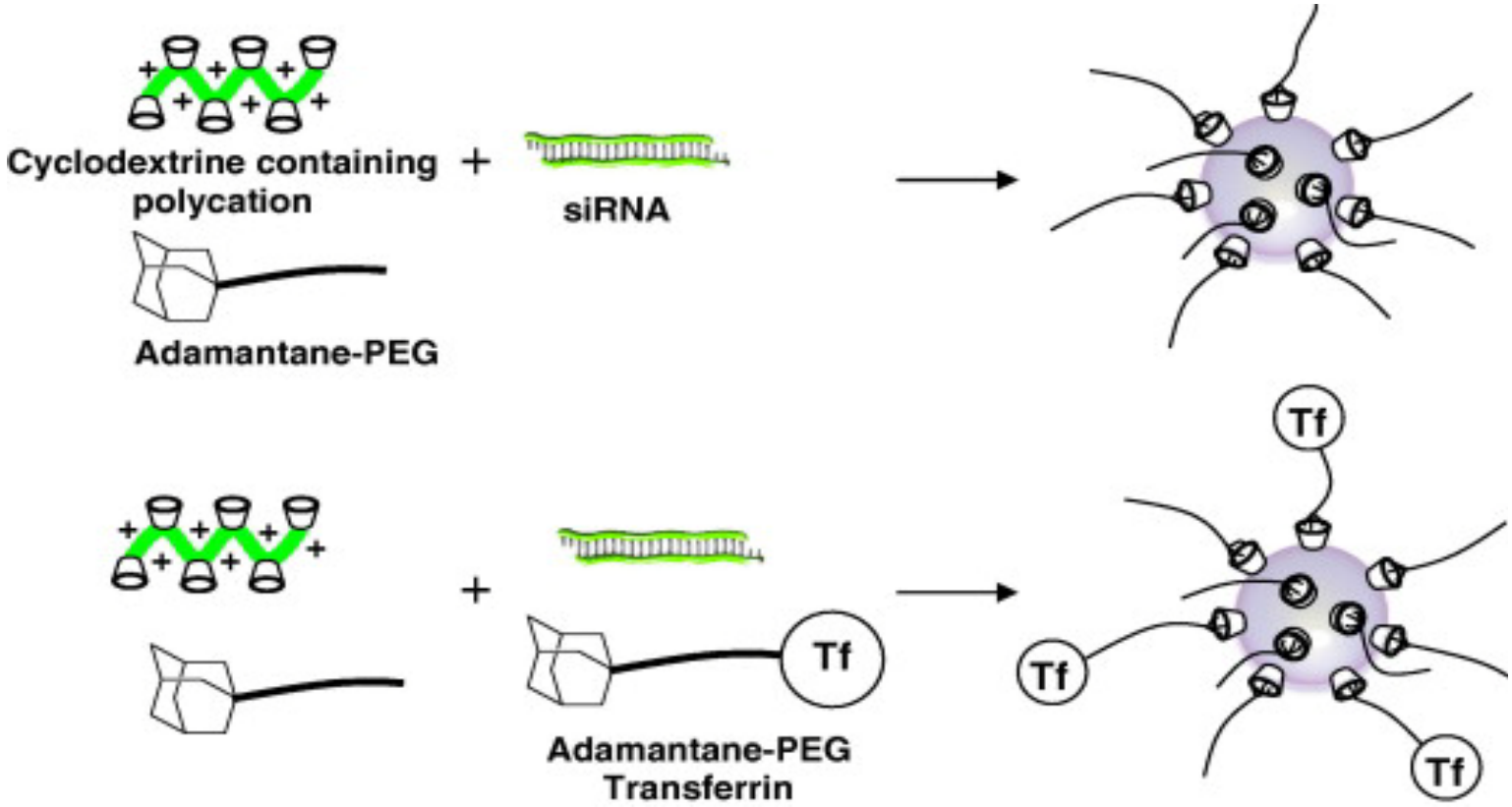


self-assembly



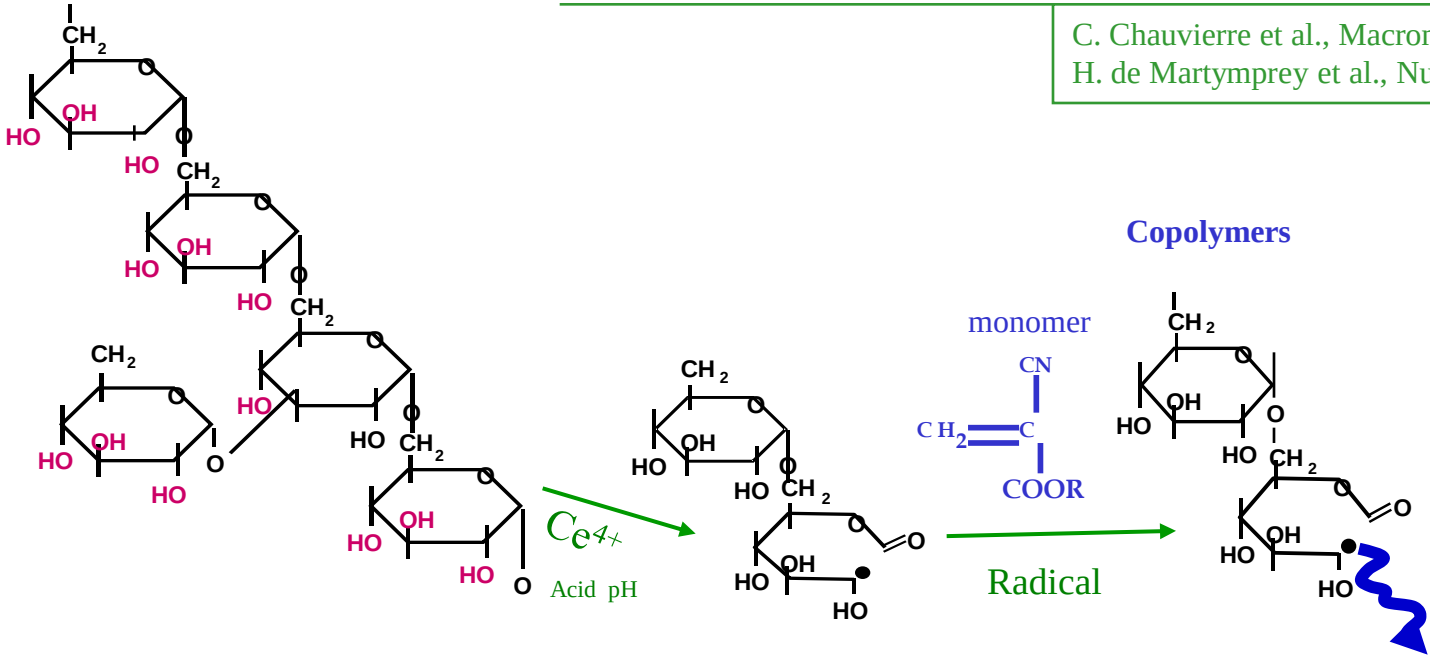
ASSOCIATION OF siRNA WITH POLYMER MICELLES

de Martymprey et al, European Journal of Pharmaceutics and Biopharmaceutics 71, 490-504 (2009)

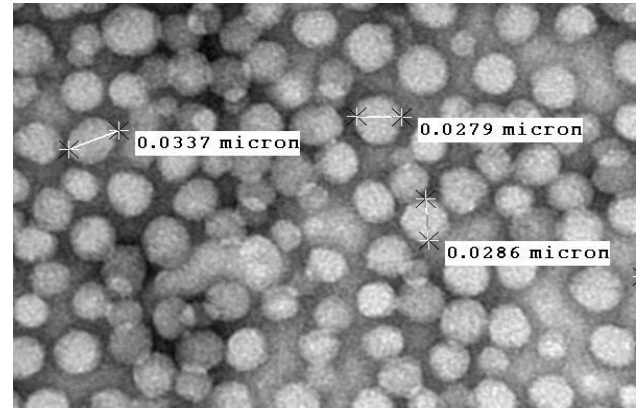


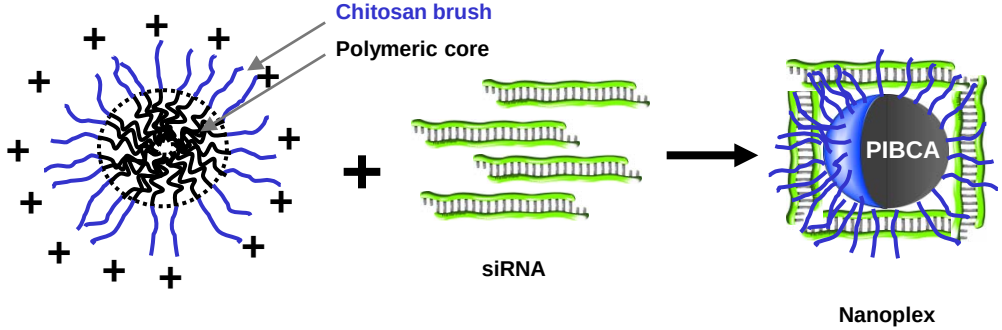
NANOPLEXES OF PACA/CHITOSAN AND siRNA LOADING

C. Chauvierre et al., Macromolecules, 36, 6018-6027 2003
H. de Martymprey et al., Nucl. Ac. Res., 36, 1-14, 2008

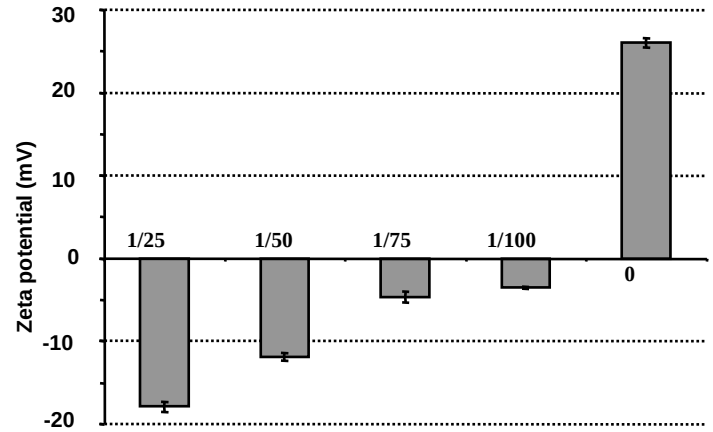


TEM



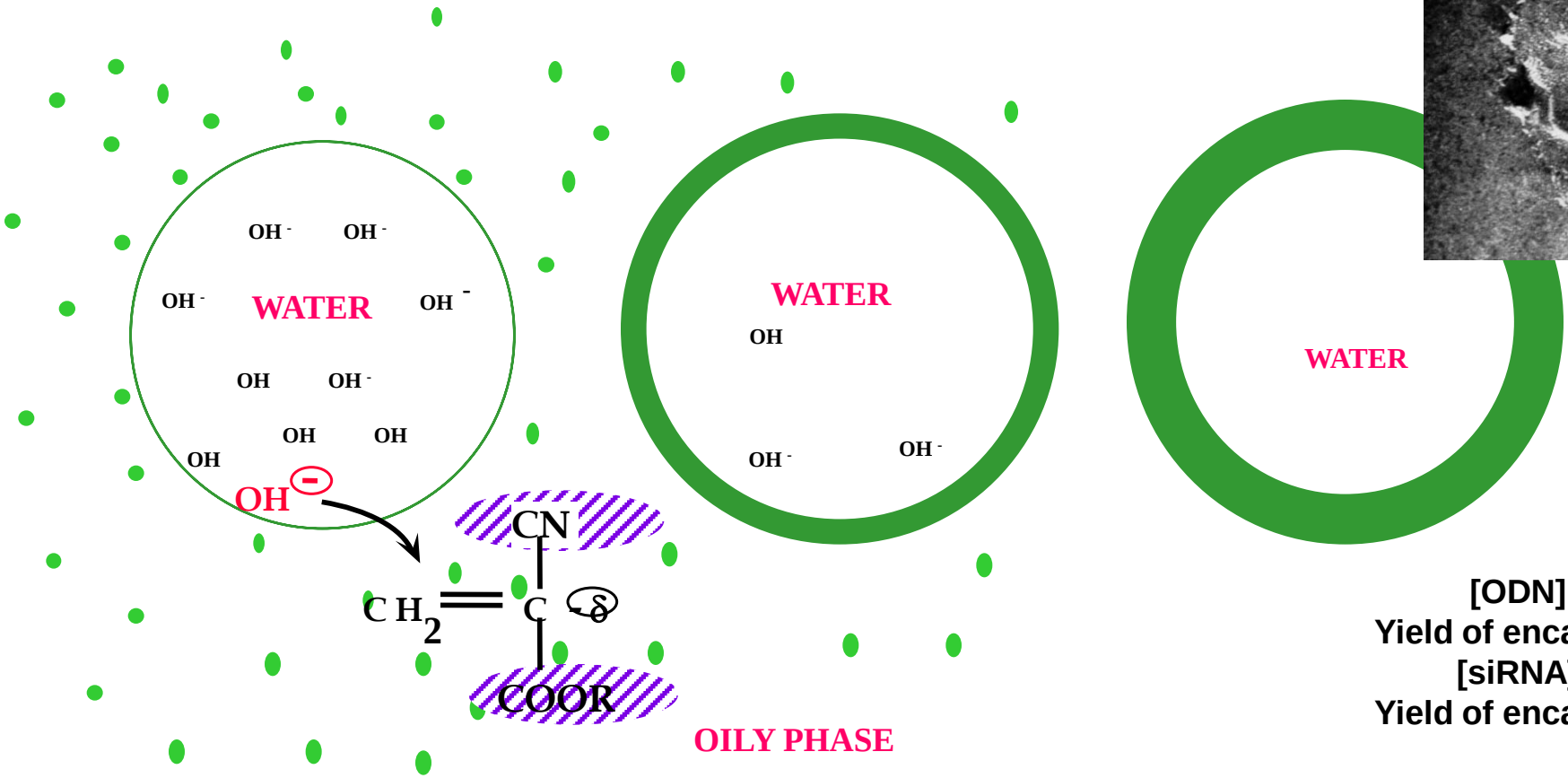


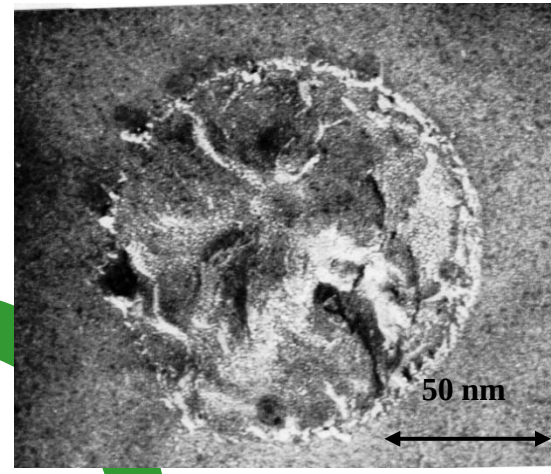
Zeta potential



PACA NANOCAPSULE FORMATION

G. Lambert et al., BBRC, 2001
N.Toub et al., Pharm. Res, 23, 892-900, 2006

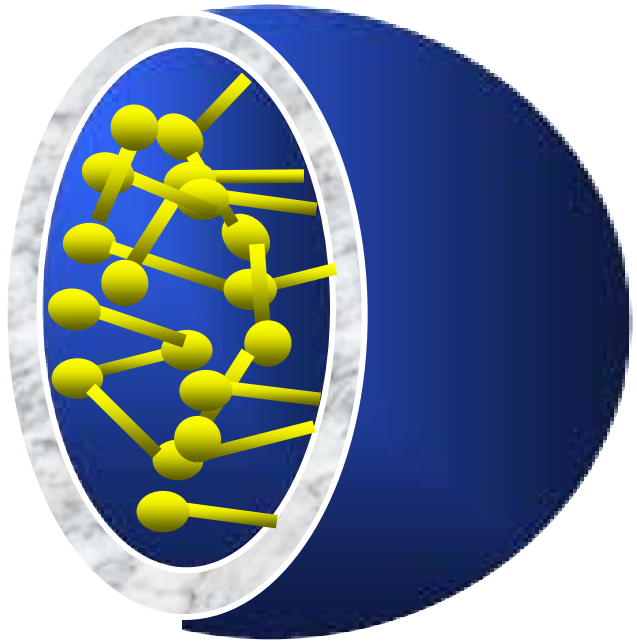


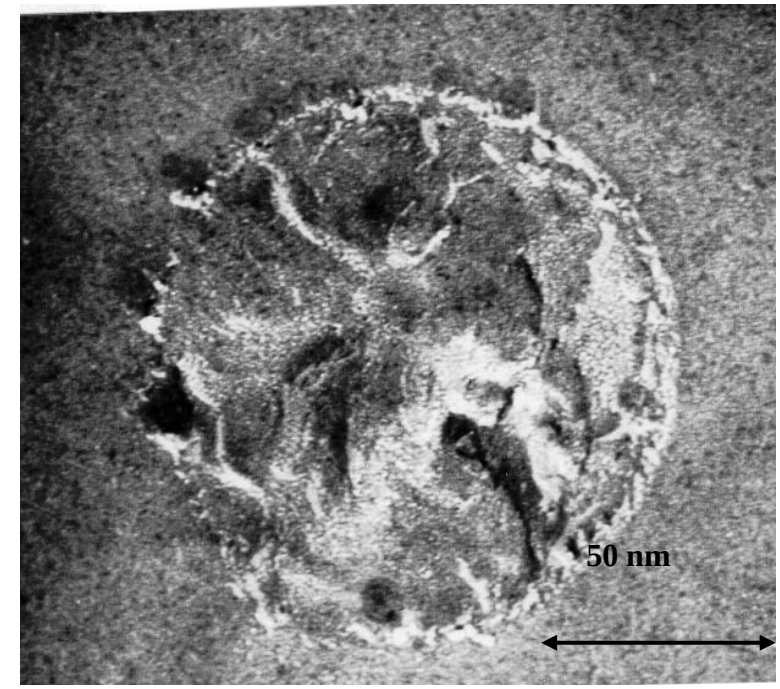


[ODN] = 2.5 mM
Yield of encapsulation: 81%
[siRNA] = 0.5 mM
Yield of encapsulation: 98%

NANOCAPSULE WITH AN AQUEOUS CORE AS A NANOCARRIER FOR NUCLEIC ACIDS

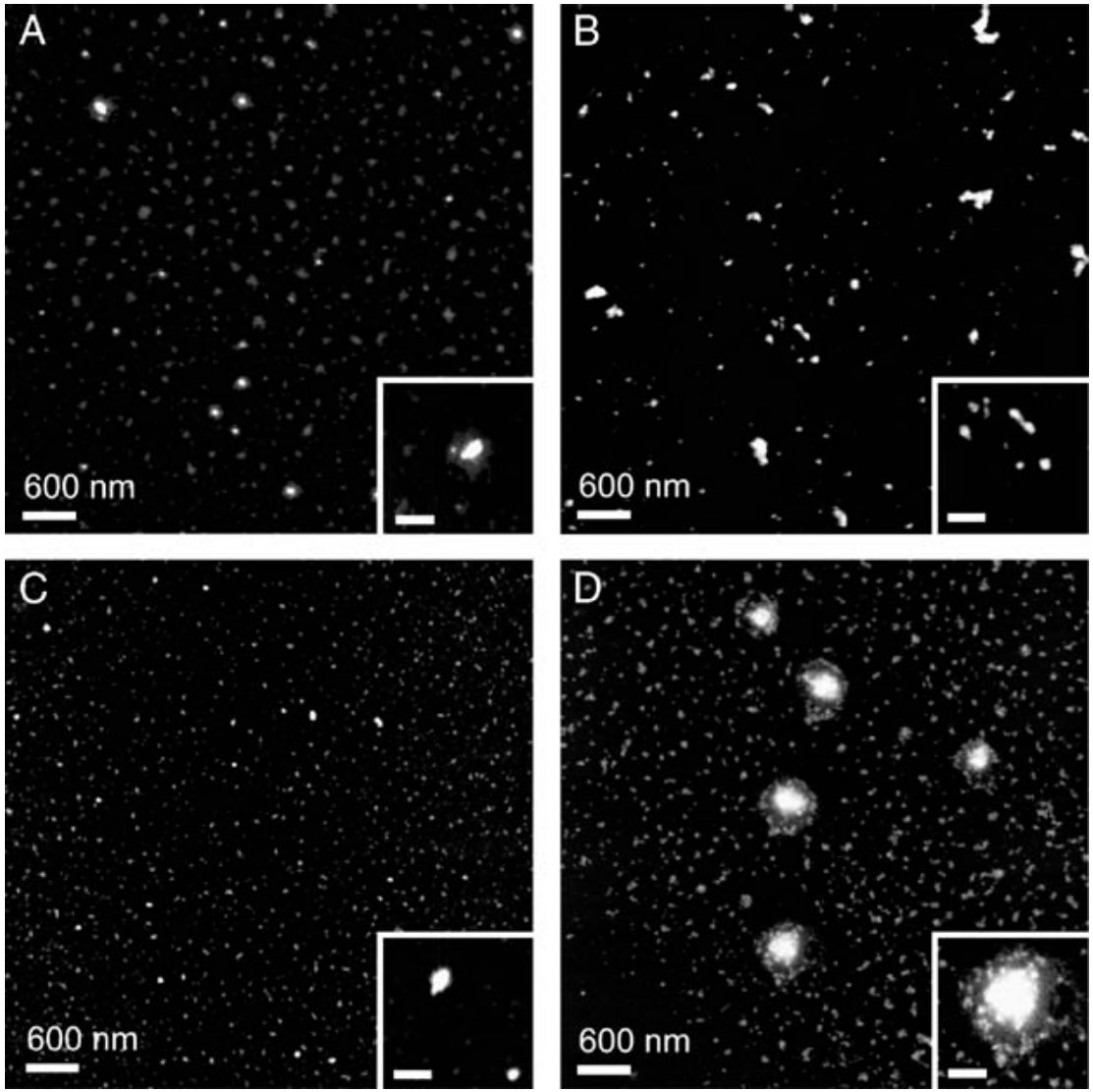
G. Lambert et al., Pharm. Research, 2000
G. Lambert et al., Int. J. Pharm., 2001
G. Lambert et al., Advanced Drug Delivery Reviews, 47, 99-112, 2001
G. Lambert et al., BBRC, 2001
A. Maksimenko et al., Pharm. Research, 2003





ATOMIC FORCE MICROSCOPY OF CHITOSAN/siRNA NANOPARTICLES AT DIFFERENT N/P RATIO

Kenneth A et al, Molecular Therapy, 14, 476-484 (2006)



SIZE INCREASES WITH N:P RATIO !

[A] N:P 71 and [B] N:P 6
(chitosan concentration: 250 μg/ml)

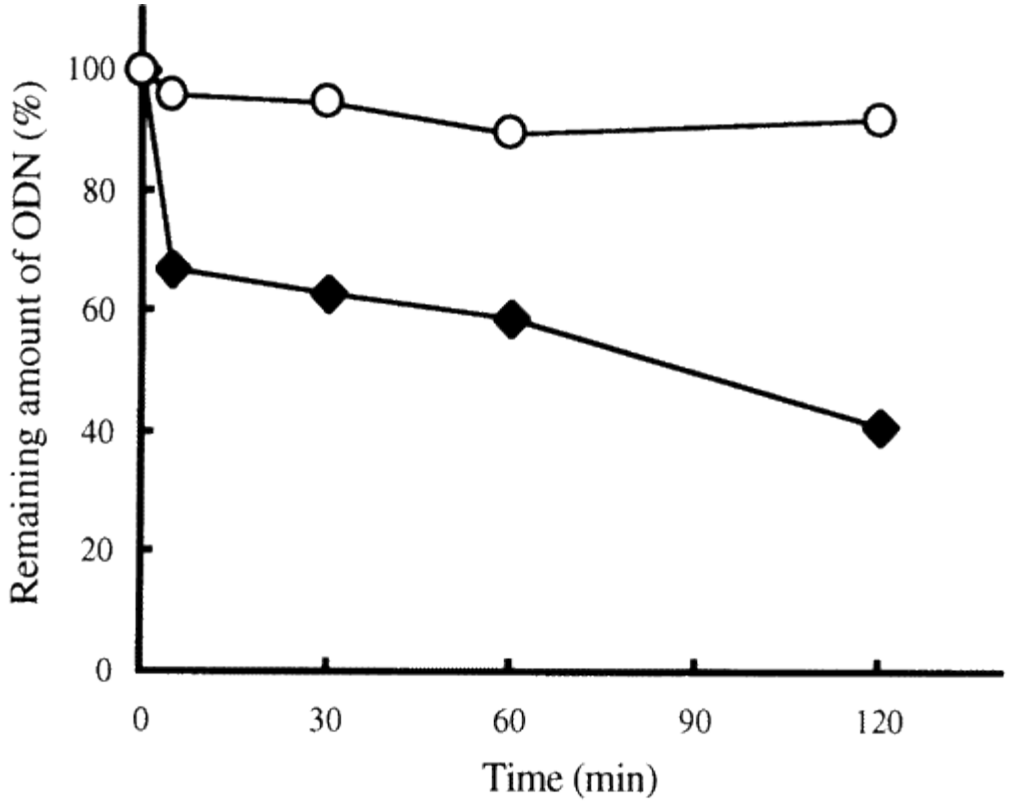
[C] N:P 285 and [D] N:P 23
(chitosan concentration 1 mg/ml)

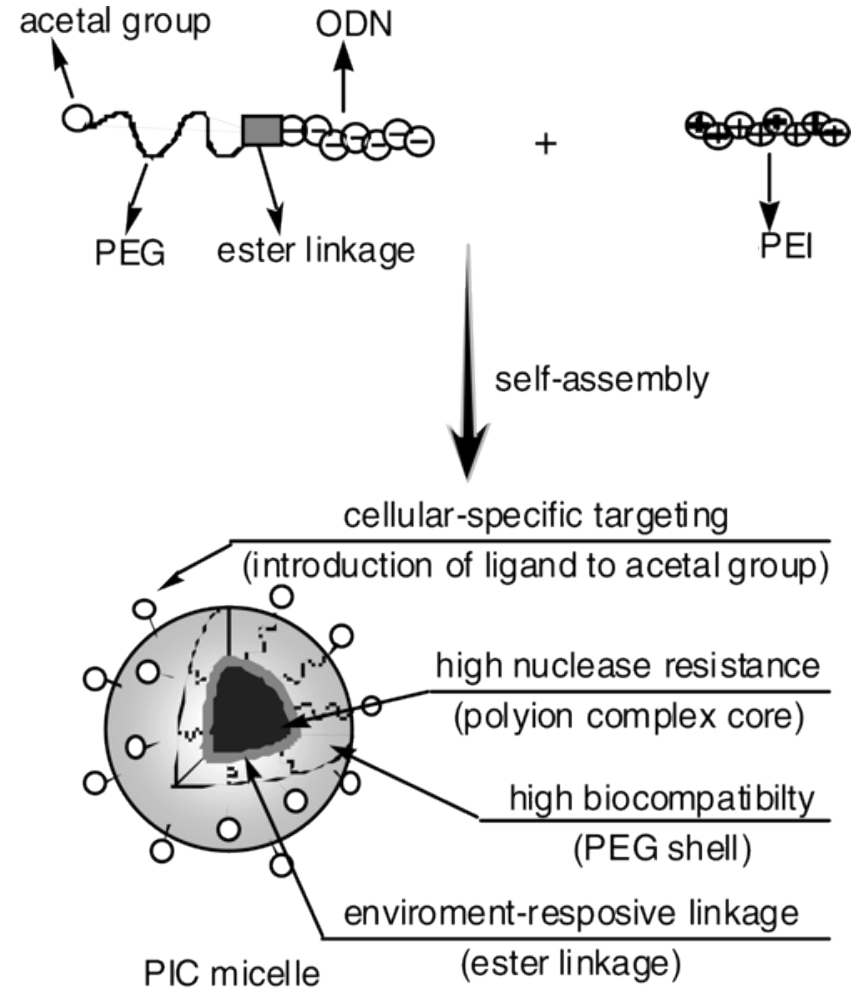
RESISTANCE TO DNA DIGESTION

Protection against 3’exo-nucleases

DNAse DIGESTION

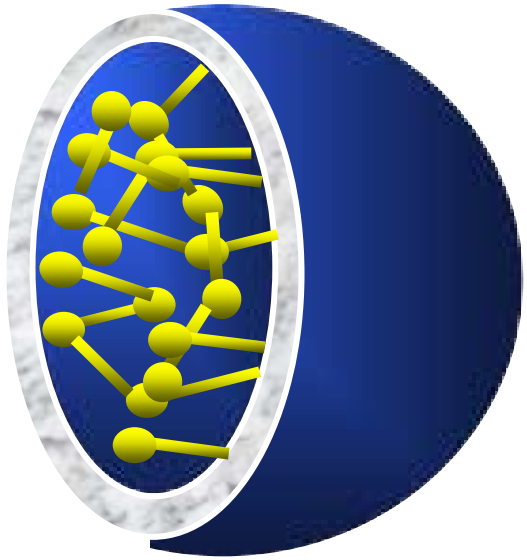
Motoi Oishi et al. Biomacromolecules 4, 1426-1432 (2003)

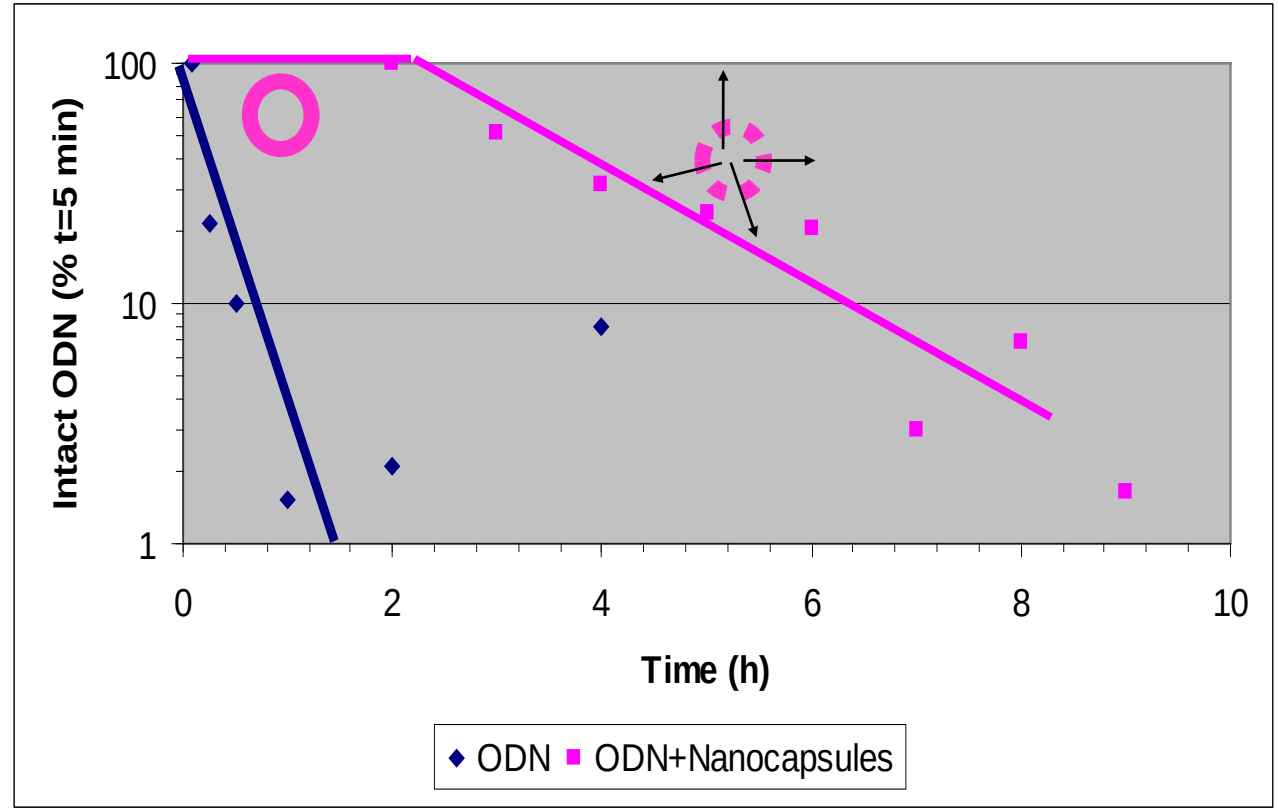




STABILITY OF ODN AND ODN NANOCAPSULES IN DMEM MEDIUM CONTAINING 10 % SERUM

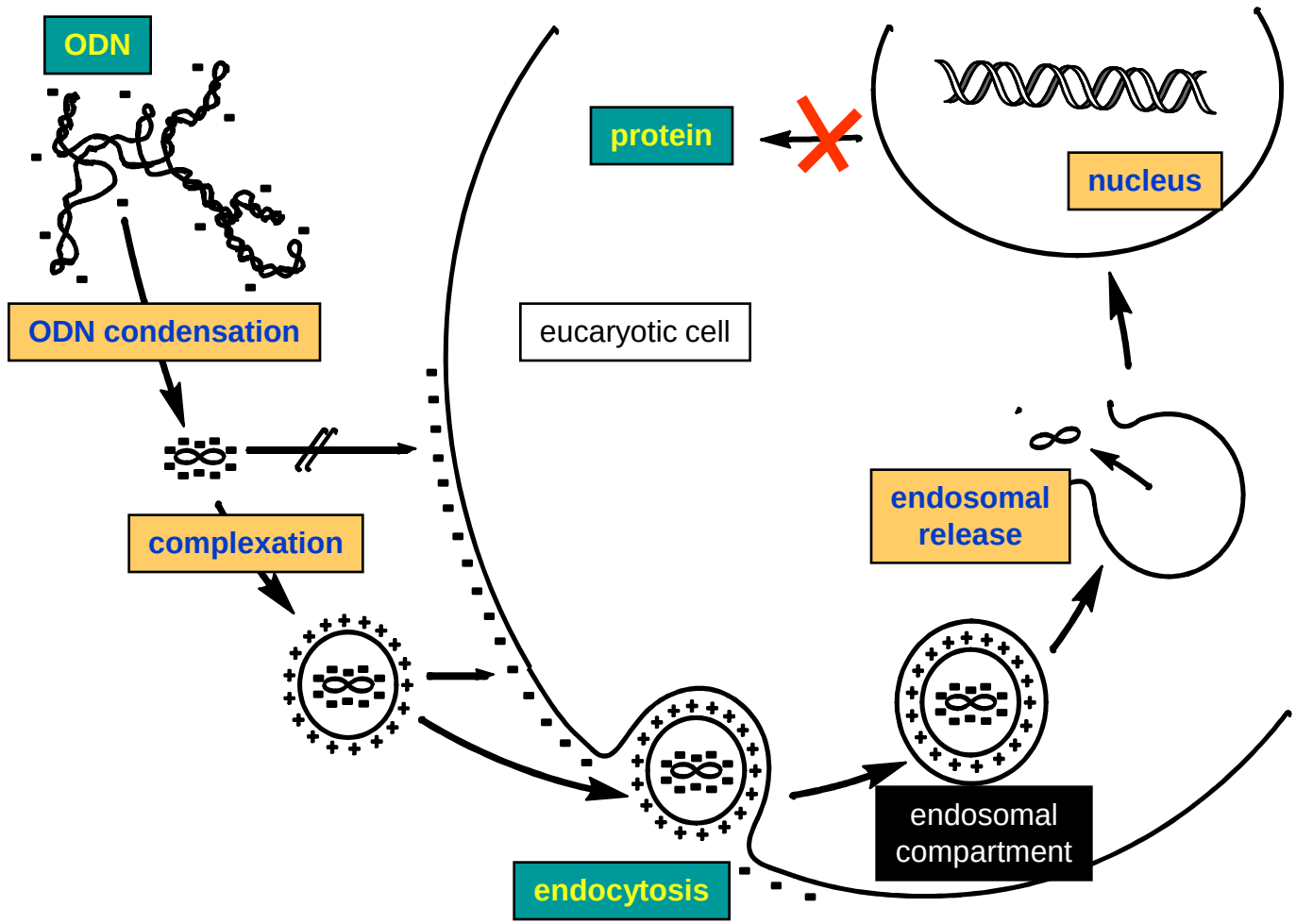
G. Lambert et al., BBRC, 2001





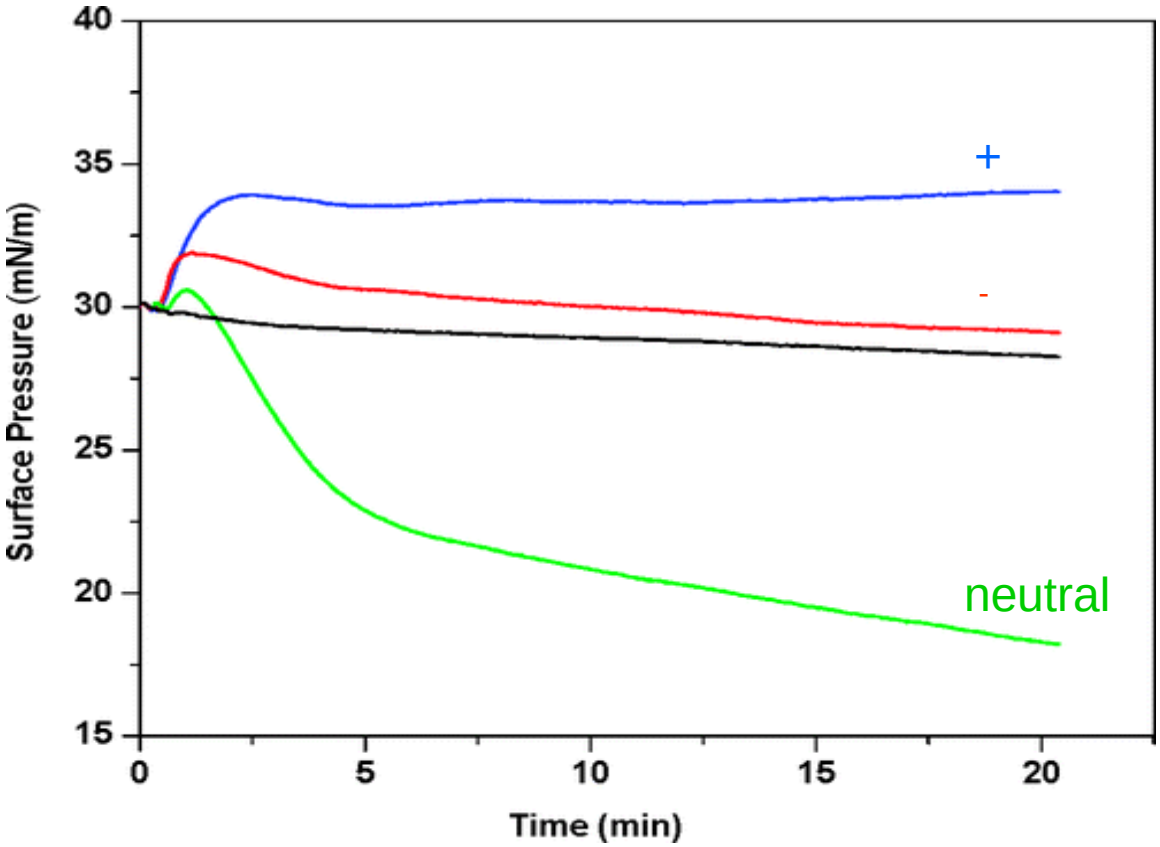
INTERACTION AVEC LA MEMBRANE CELLULAIRE

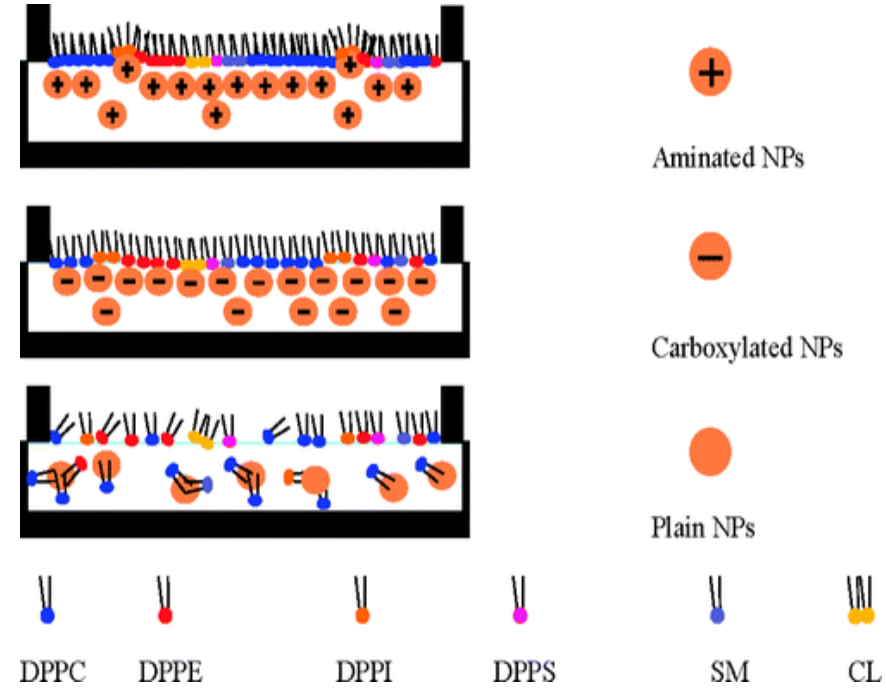
INTERACTION WITH THE CELL MEMBRANE



EFFECTS OF NANOPARTICLES SURFACE GROUPS ON ENDOTHELIAL CELL MODEL

C. Peetla, V. Labhasetwar, Molecular Pharmaceutics, 5, 418-429 (2007)





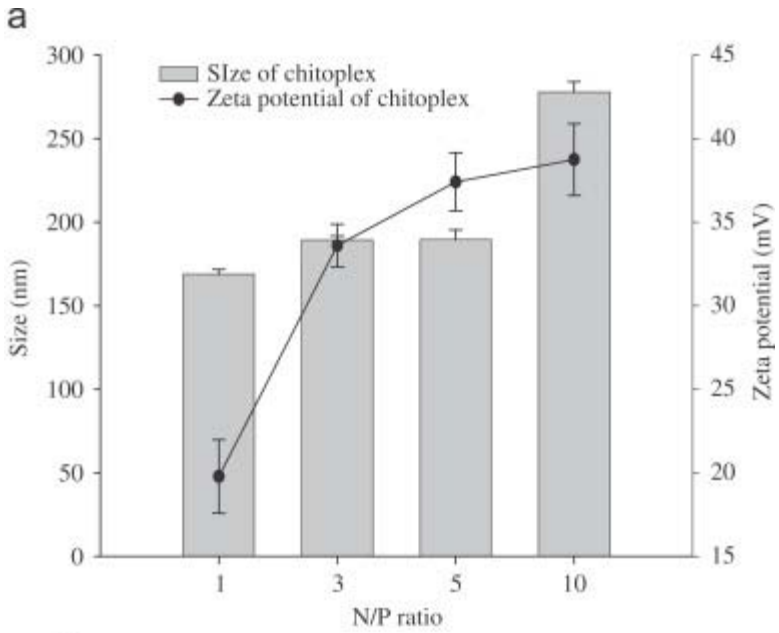
- Polystyrene nanoparticle size = 60 nm.
- blue, aminated; red, carboxylated; green, plain; black, water control without NPs.

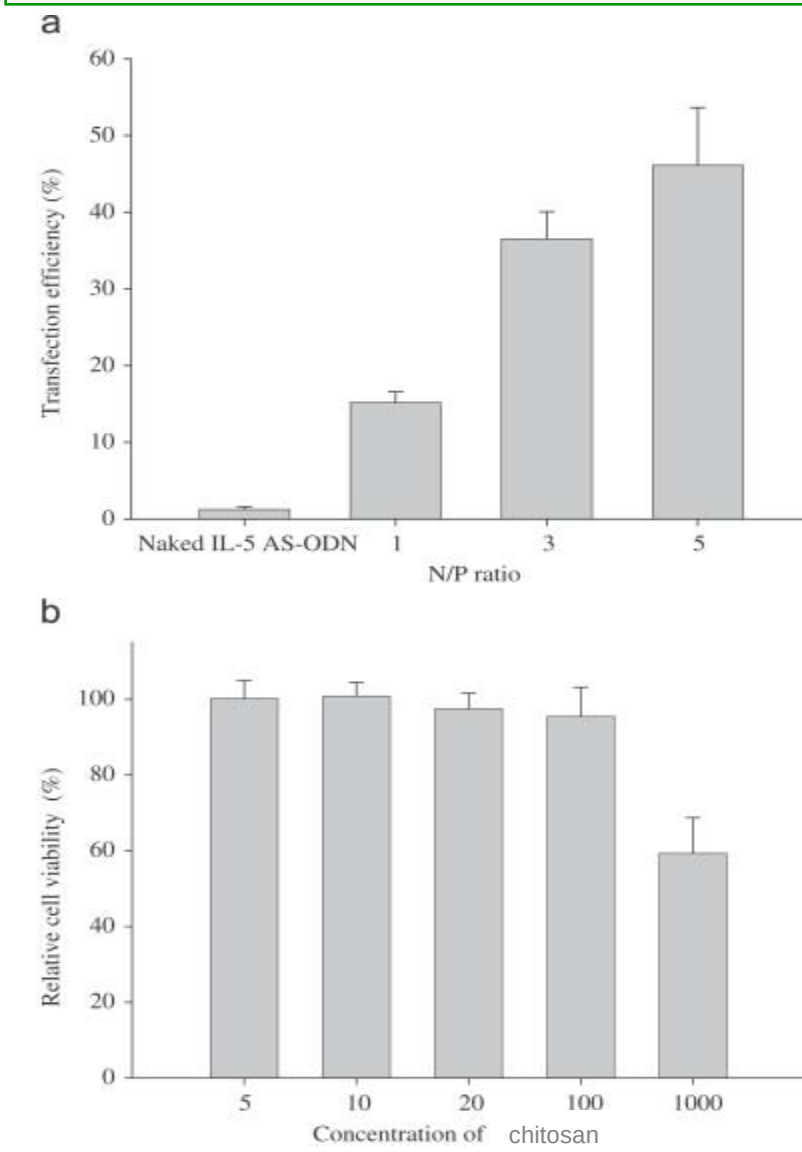
INTRACELLULAR DNA DELIVERY

N/P ratio influences intracellular penetration

CHITOSAN-IL5 ODN POLYPLEXES

S. Tae Kim, C. Kook Kim, Biomaterials 28, 3360-3368 (2007)

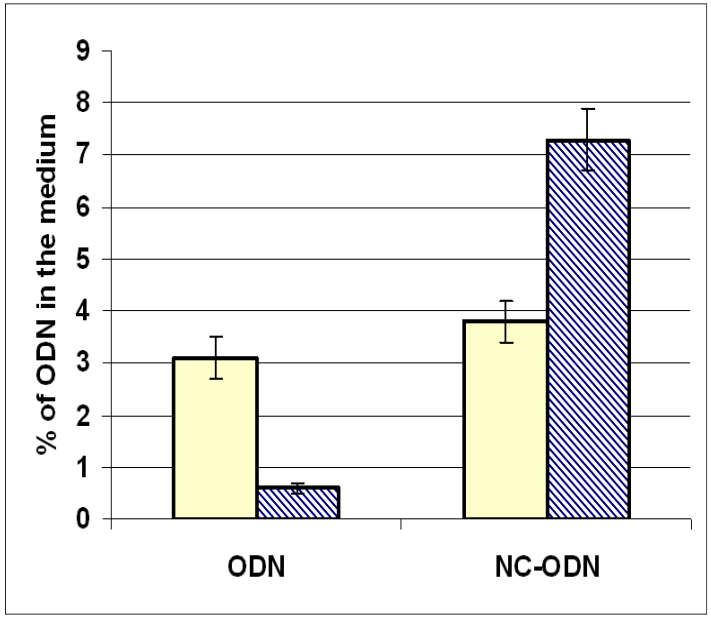


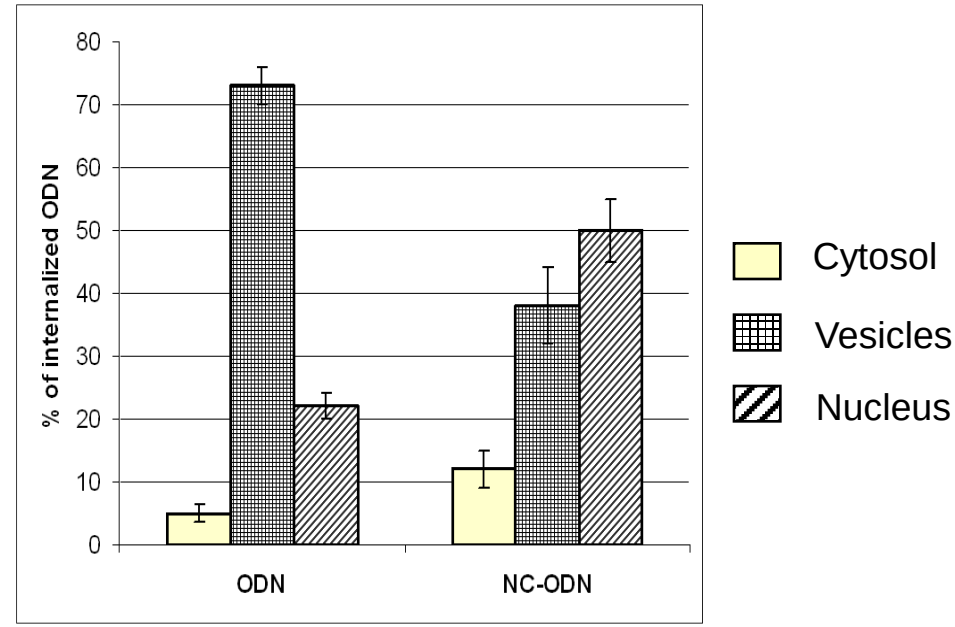


ODN IN NANOCAPSULES: INTRACELLULAR DISTRIBUTION

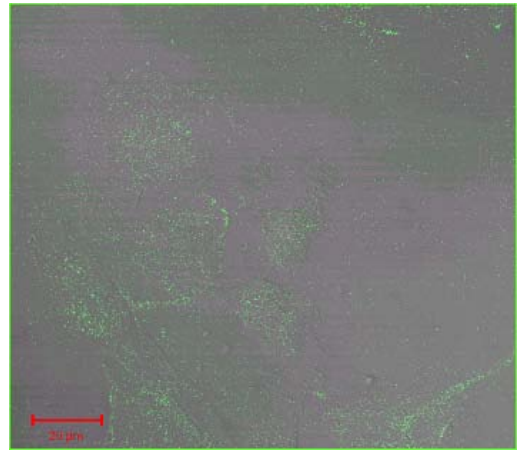
N. Toub et al., JCR, 106, 209-213, 2006

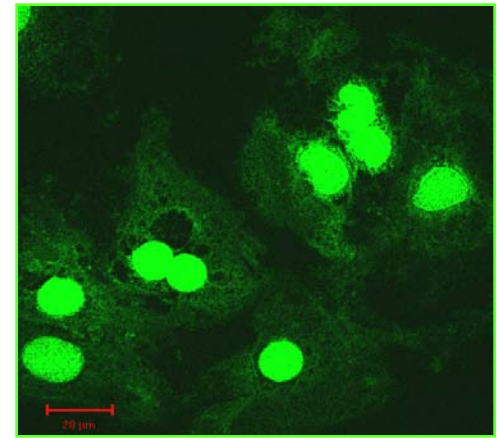
3T3 cells





FITC-ODN free

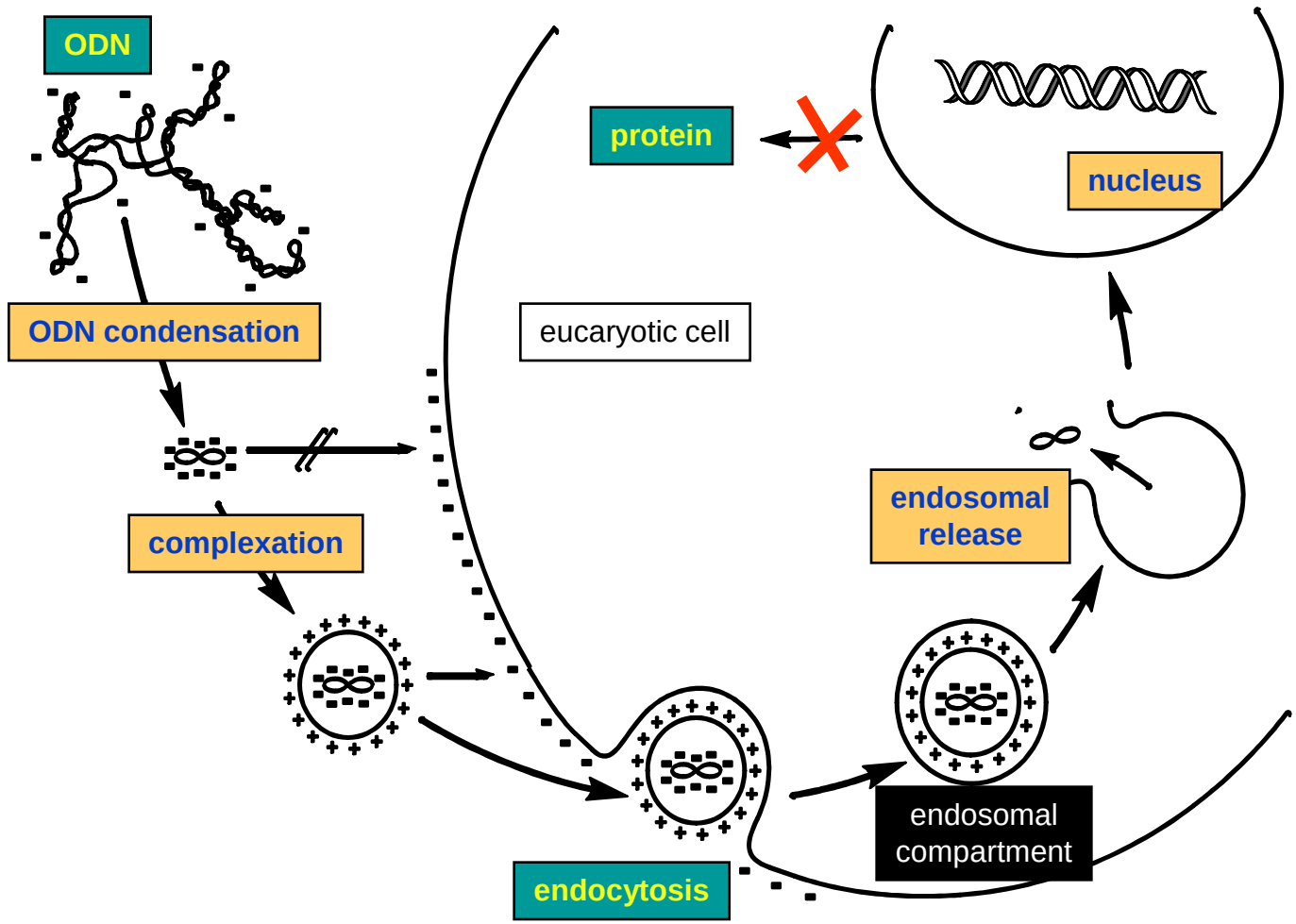




FITC-ODN in NC

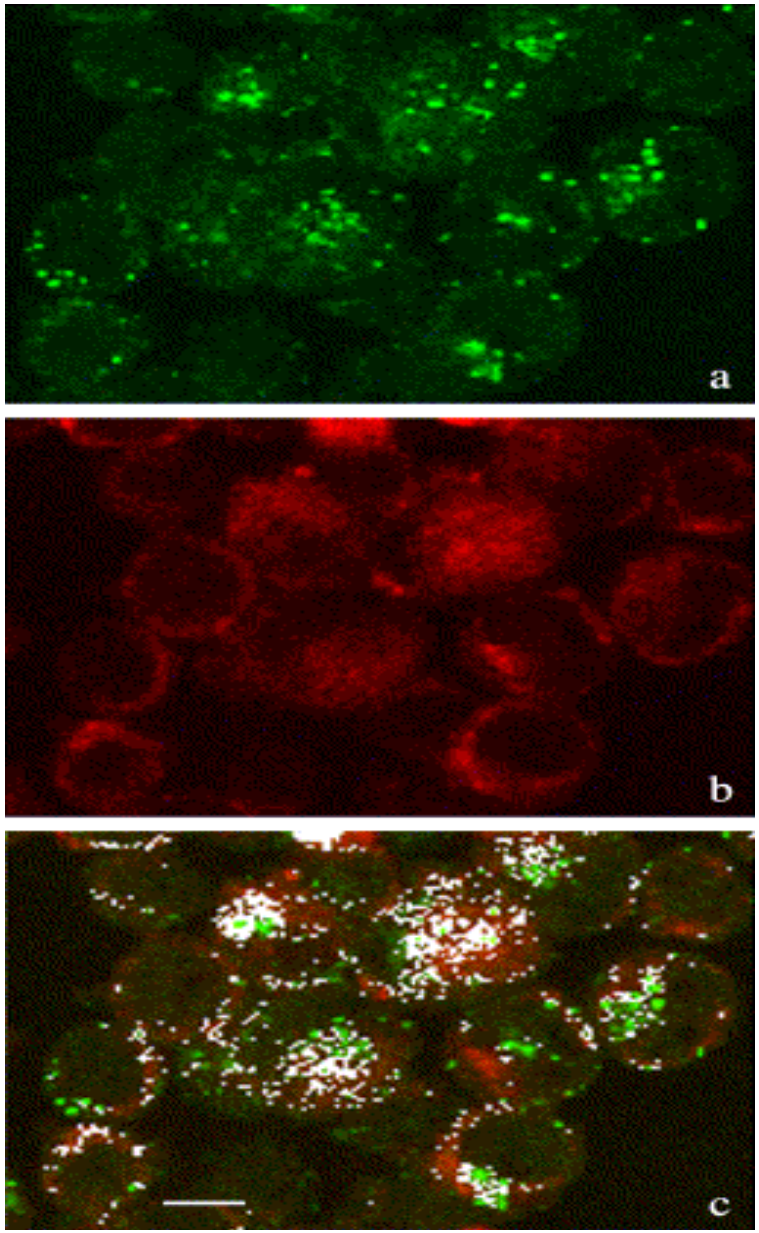
ENDO-LYSOSOMAL ESCAPE

INTERACTION WITH THE CELL MEMBRANE



CELL CAPTURE OF POLYDIMETHYLAMINOETHYLMETACRYLATE p(DMAEMA) OCCURS THROUGH EARLY ENDOSOMES

R A Jones et al, J Controlled Release, 96, 379-391 (2004)



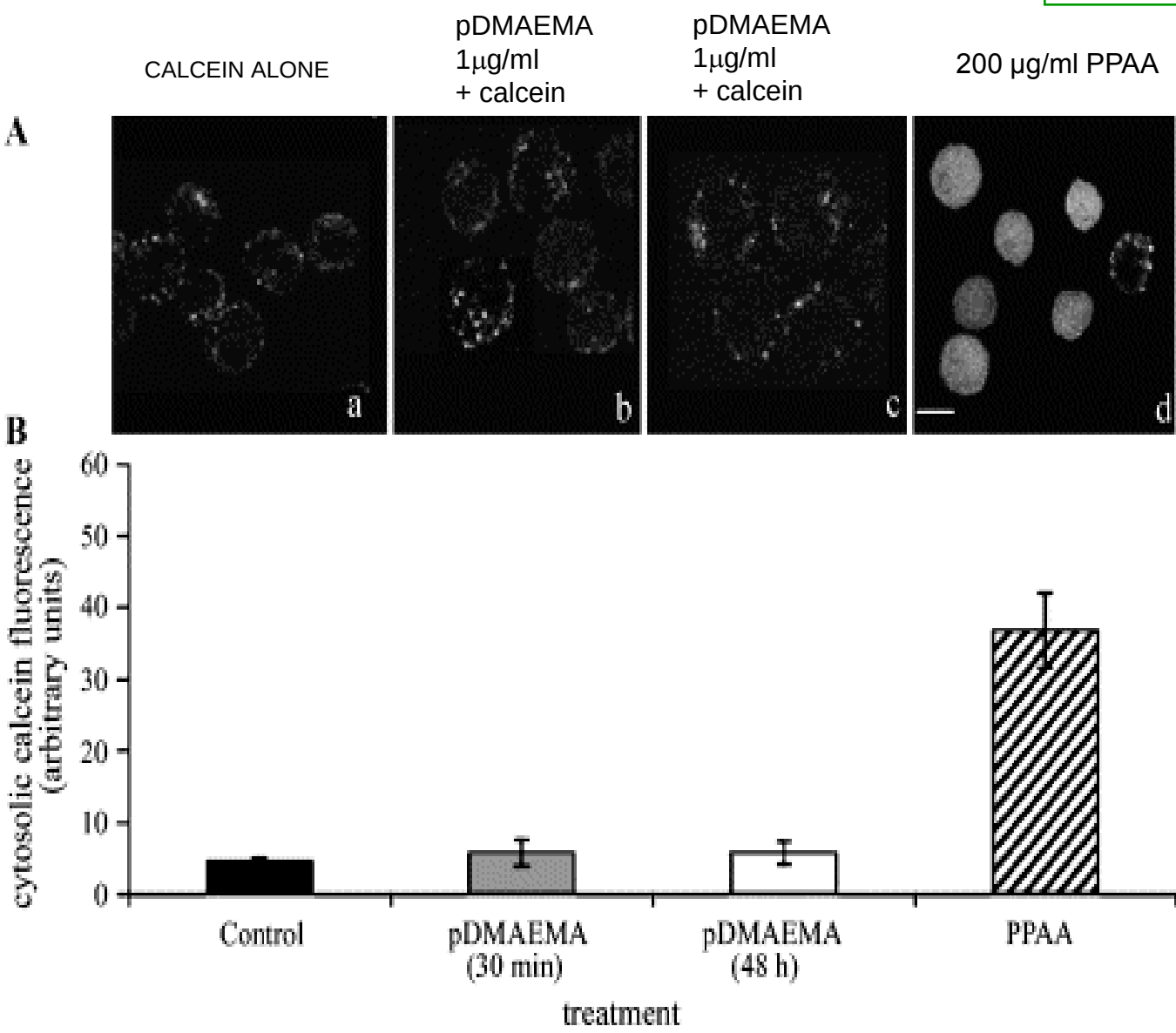
Green EEA1 immunofluorescence:
a marker of early endosomes

Red fluorescence associated with p(DMAEMA-*co*-AEMA)-biotine
Biotinylated p(DMAEMA-co-AEMA) reacts with streptavidin-Alexa 633

overlay image of (a) and (b)
showing pixels with both green and red fluorescence in white

DETERMINATION OF LYSOSOMAL MEMBRANE INTEGRITY BY CALCEIN ASSAY

R A Jones et al, J Controlled Release, 96, 379-391 (2004)

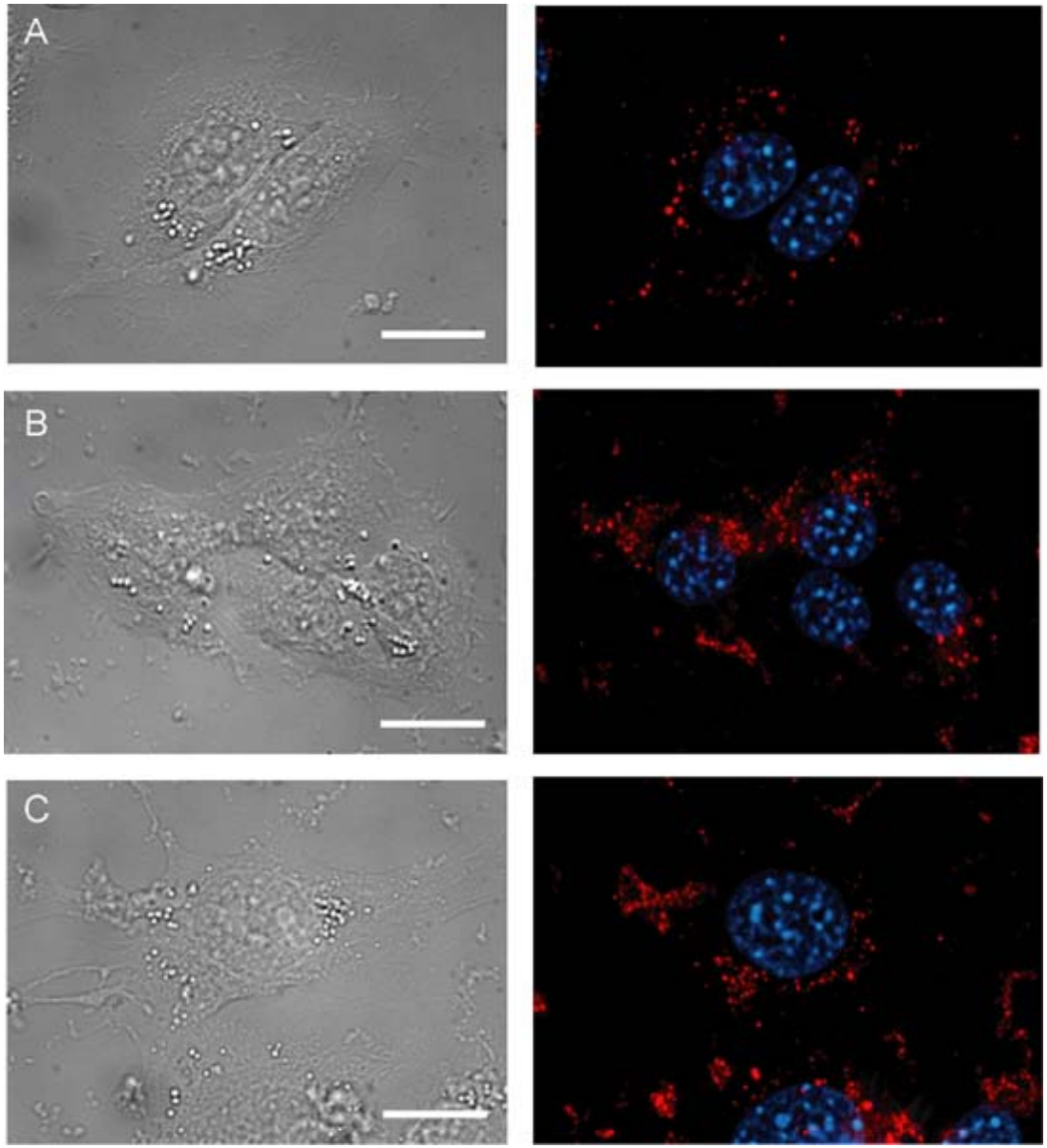


In the absence of lysosomal disruption, calcein doesn't diffuse into the cell cytoplasm PPAA is a compound known to disrupt endo-lysosomal membranes

pDMAEMA doesn't show endosomal disruptive properties !

CELL UPTAKE OF CHITOSAN/siRNA NANOPARTICLES

Kenneth A et al, Molecular Therapy, 14, 476-484 (2006)



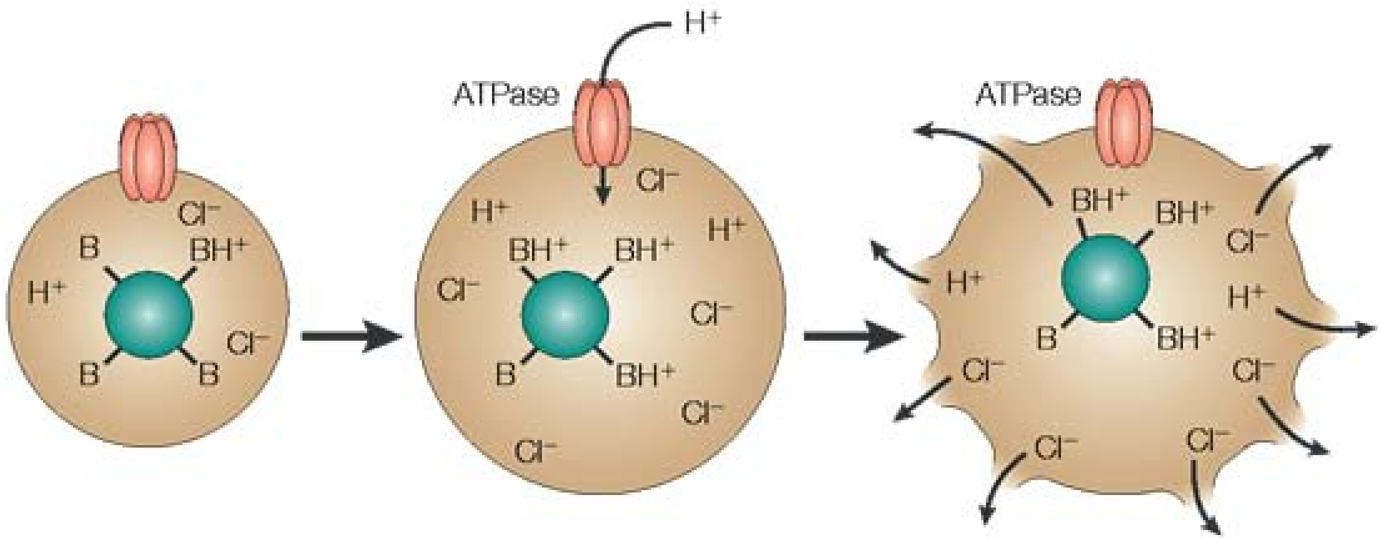
NIH 3T3 cells. Fluorescence microscopy was used to visualize cellular uptake and translocation of Cy5-labeled siRNA (red)

1h: punctuated fluorescence

4h: more diffuse fluorescence

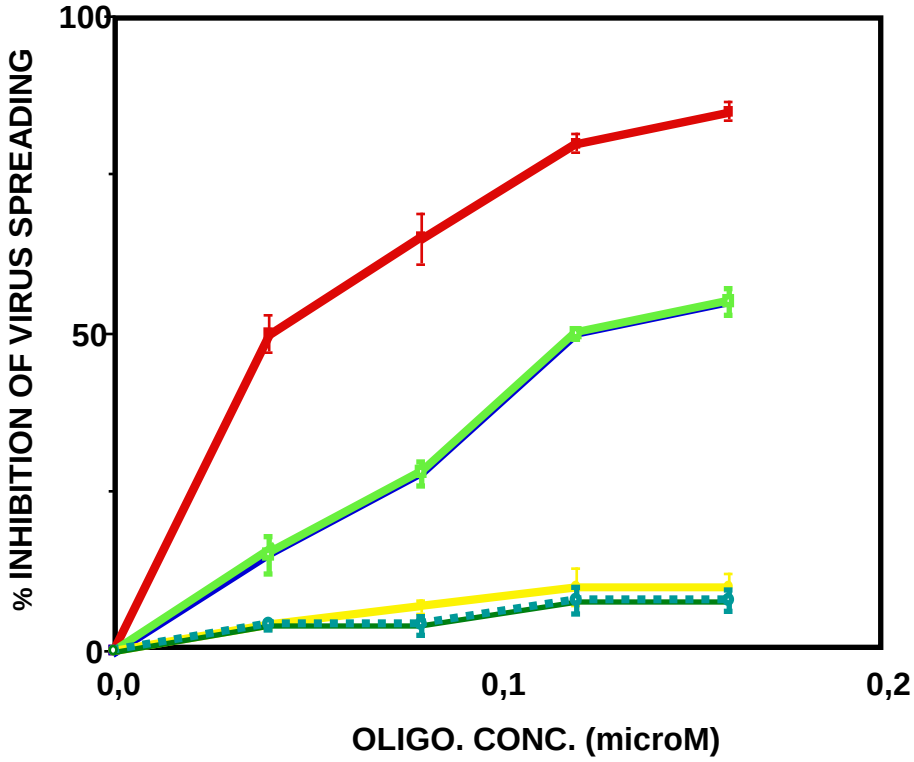
24h: fluorescence througout the whole cytoplasm

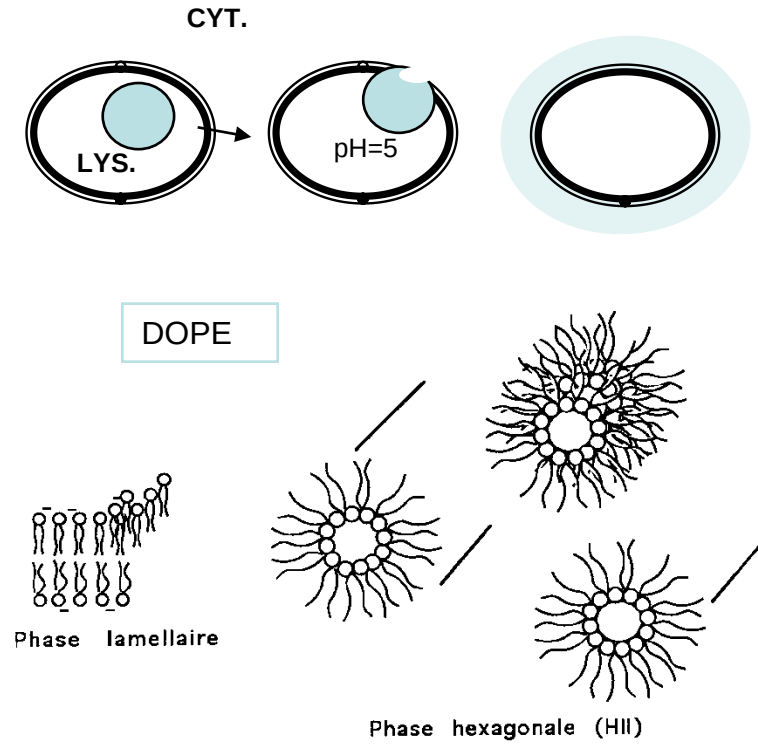
ENDO-LYSOSOMAL DISRUPTION THROUGH « PROTON SPONGE » EFFECT



INHIBITION DE LA MULTIPLICATION VIRALE PAR LIPOSOMES pH-SENSIBLES

C. Ropert, Z. Mishal, JM. Rodriguez, C. Malvy, P. Couvreur, BBA, 1996





PHARMACOLOGICAL ACTIVITY

Anticancer activity

IN VIVO STUDIES OF NANODEVICES CONTAINING ANTICANCER siRNA

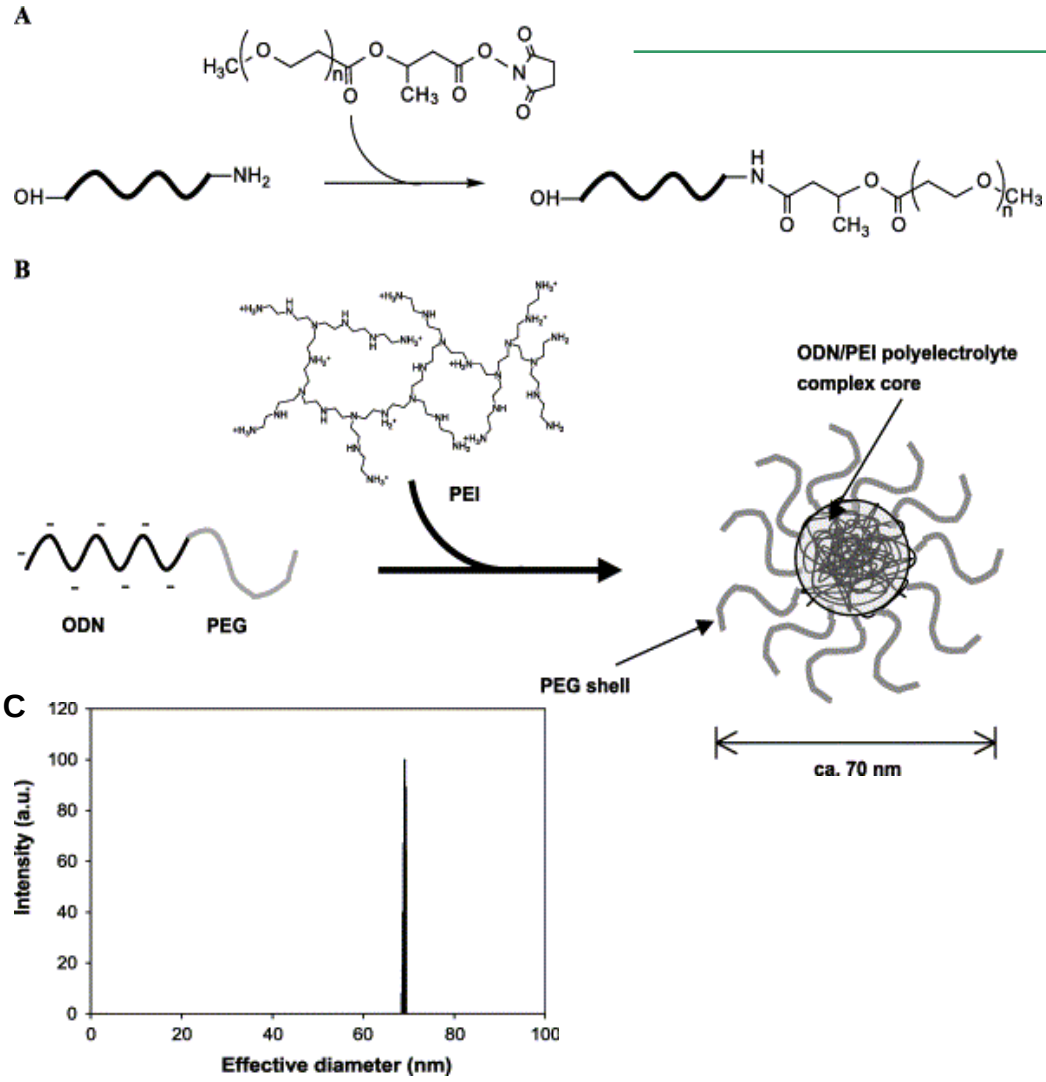
de Martymprey et al, European Journal of Pharmaceutics and Biopharmaceutics 71, 490-504 (2009)

Table 3
In vivo studies of RNAi based treatment vectorized with nanocarriers.

System type	Composition	Application	Nucleic acid dose*	Reference
Polyplexes	jetPEI, anti-HER-2 siRNA	Human ovarian cancer Intra-peritoneal	2.4 mg/kg	[111]
	jetPEI, anti-PTN siRNA	Human glioblastoma Intra-peritoneal Intracranial	3.6 mg/kg 10 μg/kg	[35]
	Chitosan 114 kDa, DD = 84% Anti-GFP siRNA	Pulmonary delivery in GFP transgenic mice	7.5 mg/kg	[45]
Nanoplexes	Core-shell PIHCA/chitosan Anti-RhoA and anti-RhoC siRNA	Human breast cancer Intravenous	1.5 μg/kg	[81]
	Core-shell PIHCA/chitosan Anti-EWS/Fli1 ODN	Ewing sarcoma model Intratumoral injection	1 mg/kg	[79]
	Core-shell PIBCA/chitosan Anti-ret/PTC1 siRNA	Thyroid carcinoma model Intratumoral	1 mg/kg	[82]
Nanocapsules	PIBCA siRNA	Ewing sarcoma model	1 mg/kg	[92]
	PLGA Anti-PDGFβR	Restenosis treatment Local administration	0.67 mg/kg (1 nmol)	[96]
	PIBCA Anti-EWS/Fli1 ODN	Ewing sarcoma model Intratumoral administration	9.6 mg/kg (14.4 nmol)	[90]
Micelles	PEG-ODN/PEI (2 kDa, 25 kDa) Anti-c-raf ODN	Human long carcinoma Tail vein injection	2.5 mg/kg	[103]
	PEG-siRNA/PEI (25 kDa) Anti-VEGF siRNA	Murine neuroblastoma Intravenous	2 mg/kg	[36,115]
	PEG/cyclodextrin containing polycation/siRNA	Human Ewing sarcoma Intravenous	2.5 mg/kg	[116]

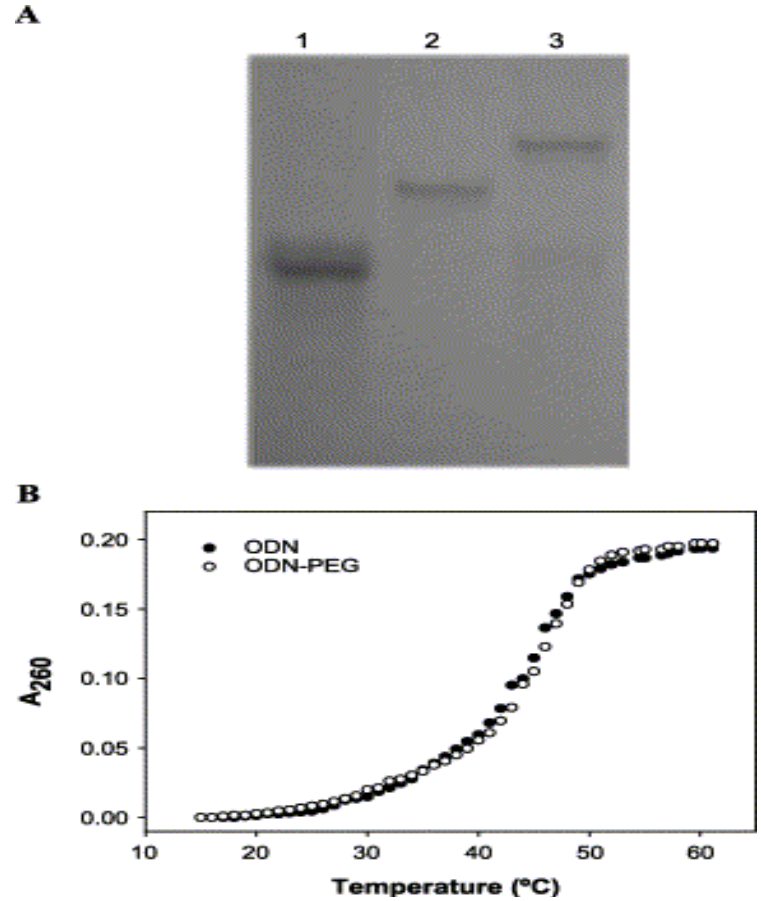
* For the comparison, a typical mouse weight of 20 g and a molar mass of 13,400 g mol $^{-1}$ where used in the calculation.

SELF-ASSEMBLED ODN-PEG HYBRID MICELLES



(A) Synthesis of the ODN–PEG conjugate
(B) Formation of micelles between ODN–PEG and PEI
(C) Diameter

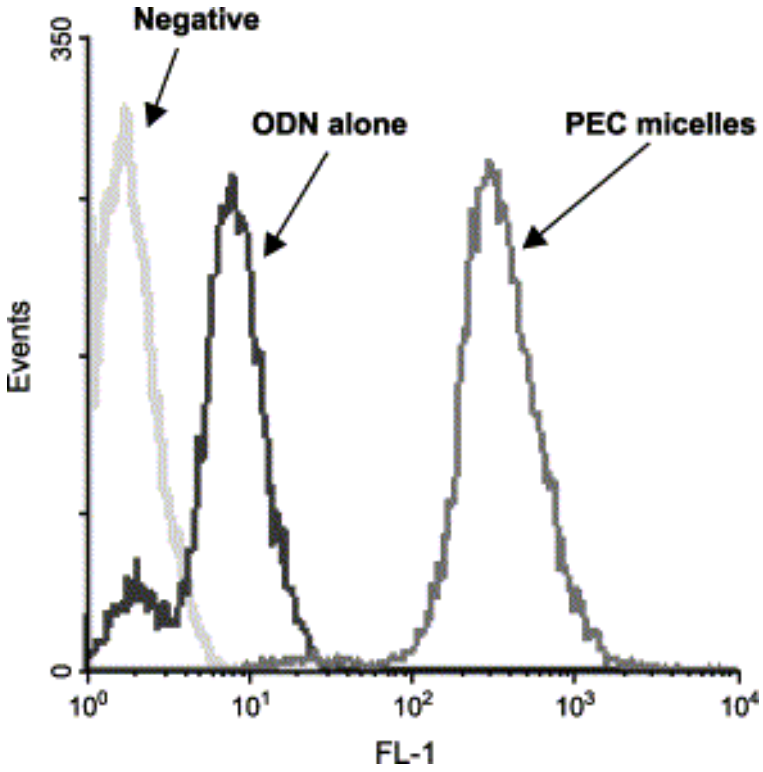
Ji Hoon Jeong and al, J of Controlled Release, 93, 183-191, 2003



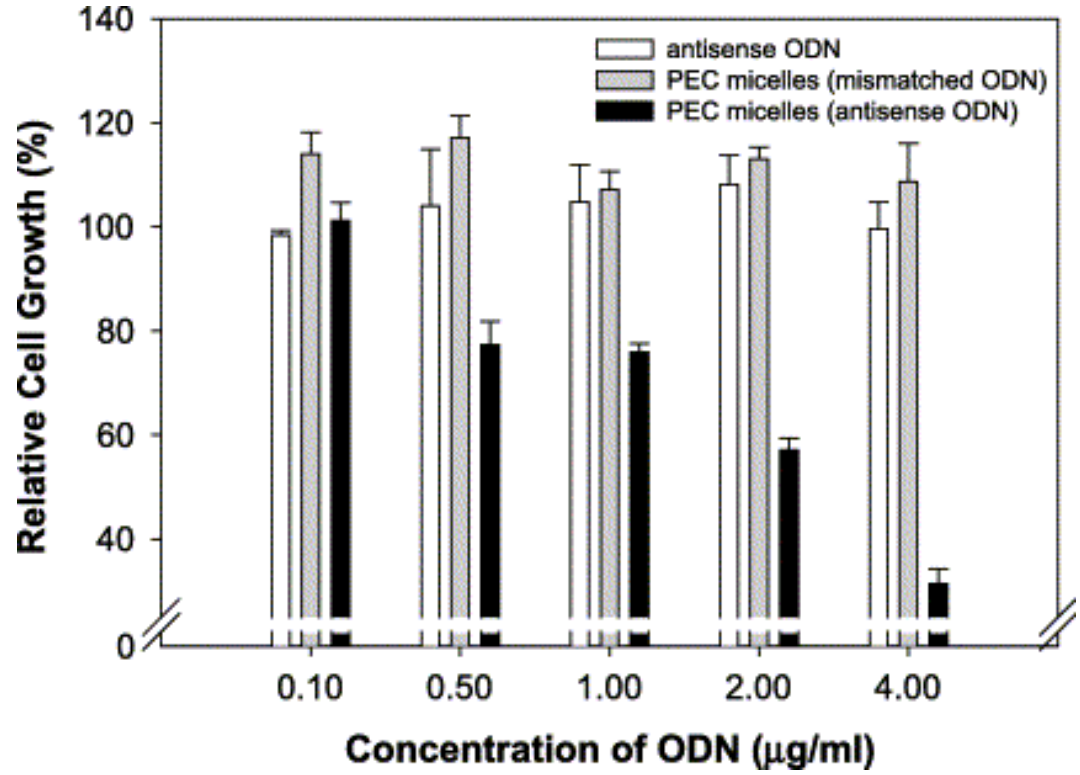
(A) Migration profiles of unmodified ODN (lane 1), ODN–PEG conjugate (lane 2), ODN–PEG conjugate hybridized with its complementary ODN (lane 3)
(B) Thermal melting transitions of ODN and antisense ODN–PEG when hybridized with sense ODN

ANTICANCER ACTIVITY ON A2780 OVARIAN CANCER CELLS

Ji Hoon Jeong and al, J of Controlled Release, 93, 183-191, 2003



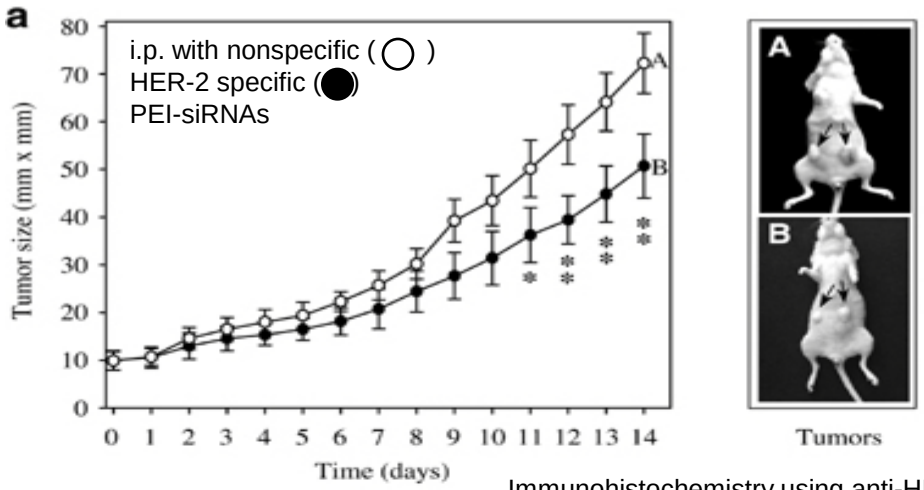
Ovarian carcinoma cell uptake (flow cytometric analysis)



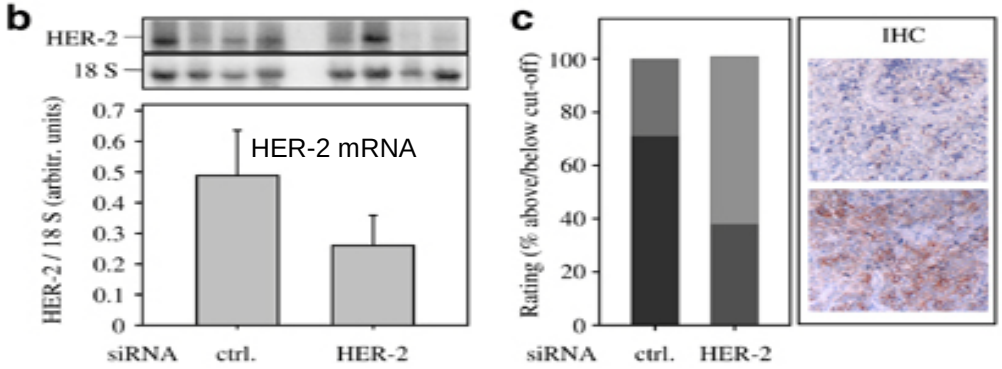
Anticancer activity

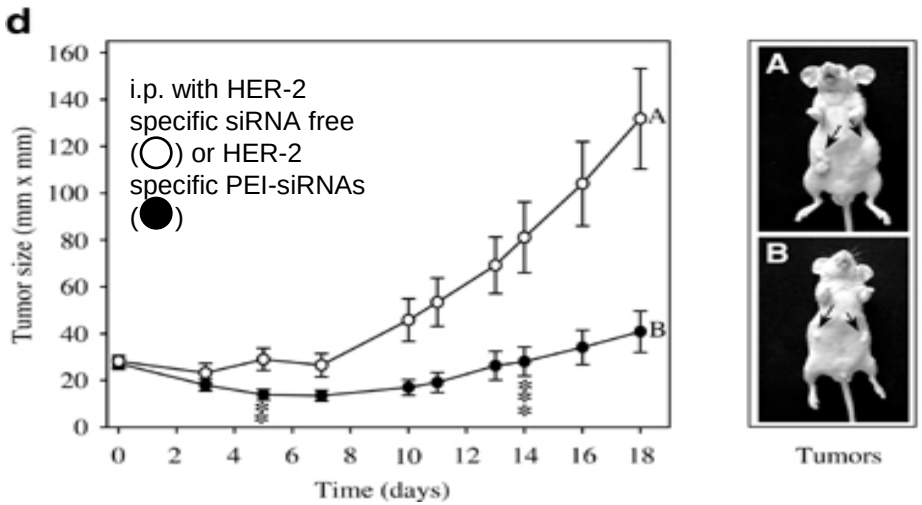
IN VIVO ANTICANCER ACTIVITY OF HER-2 siRNA COMPLEXED WITH PEI

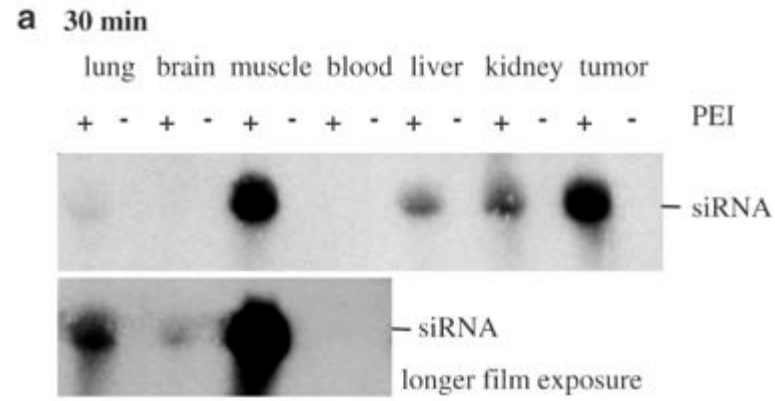
B Urban-Klein and al, Gene Therapy, 12, 461-466 (2005)

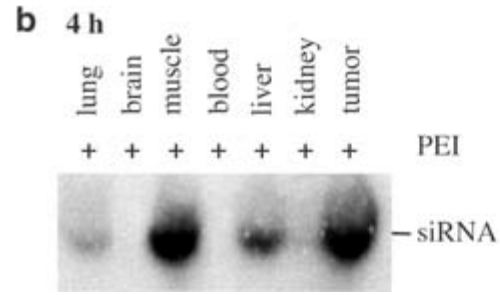


Immunohistochemistry using anti-HER2 antibodies





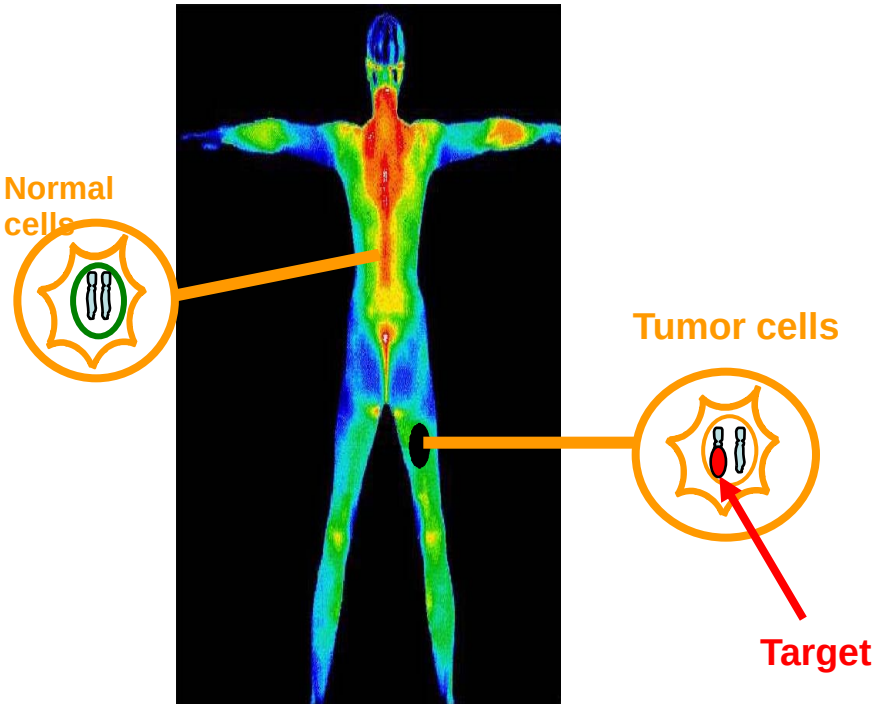


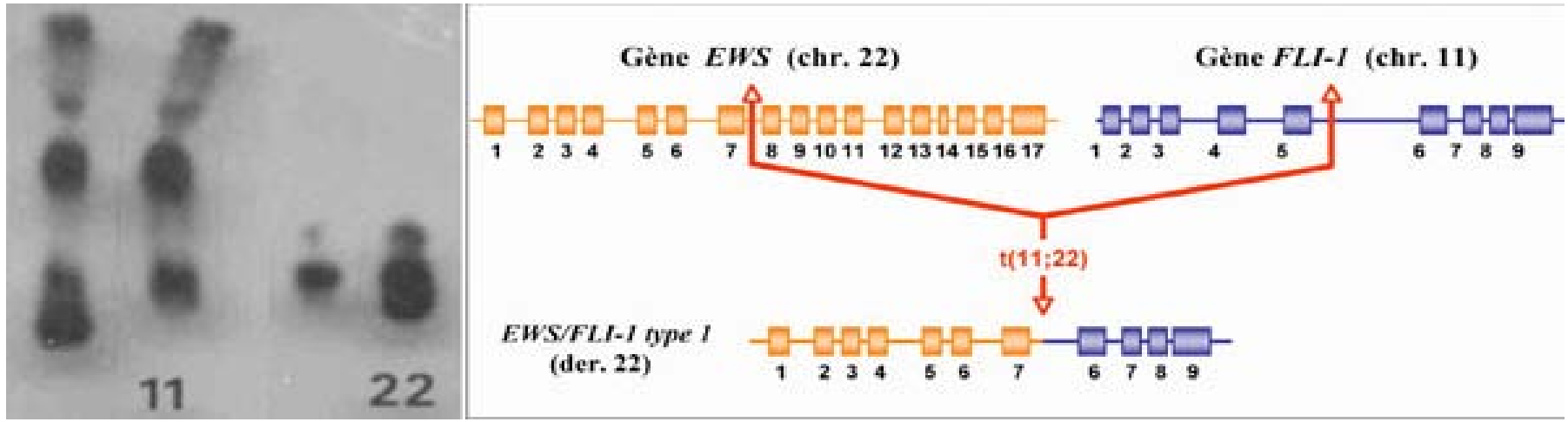


PEI-32P-labeled siRNA in organs

D’ après O. Delattre

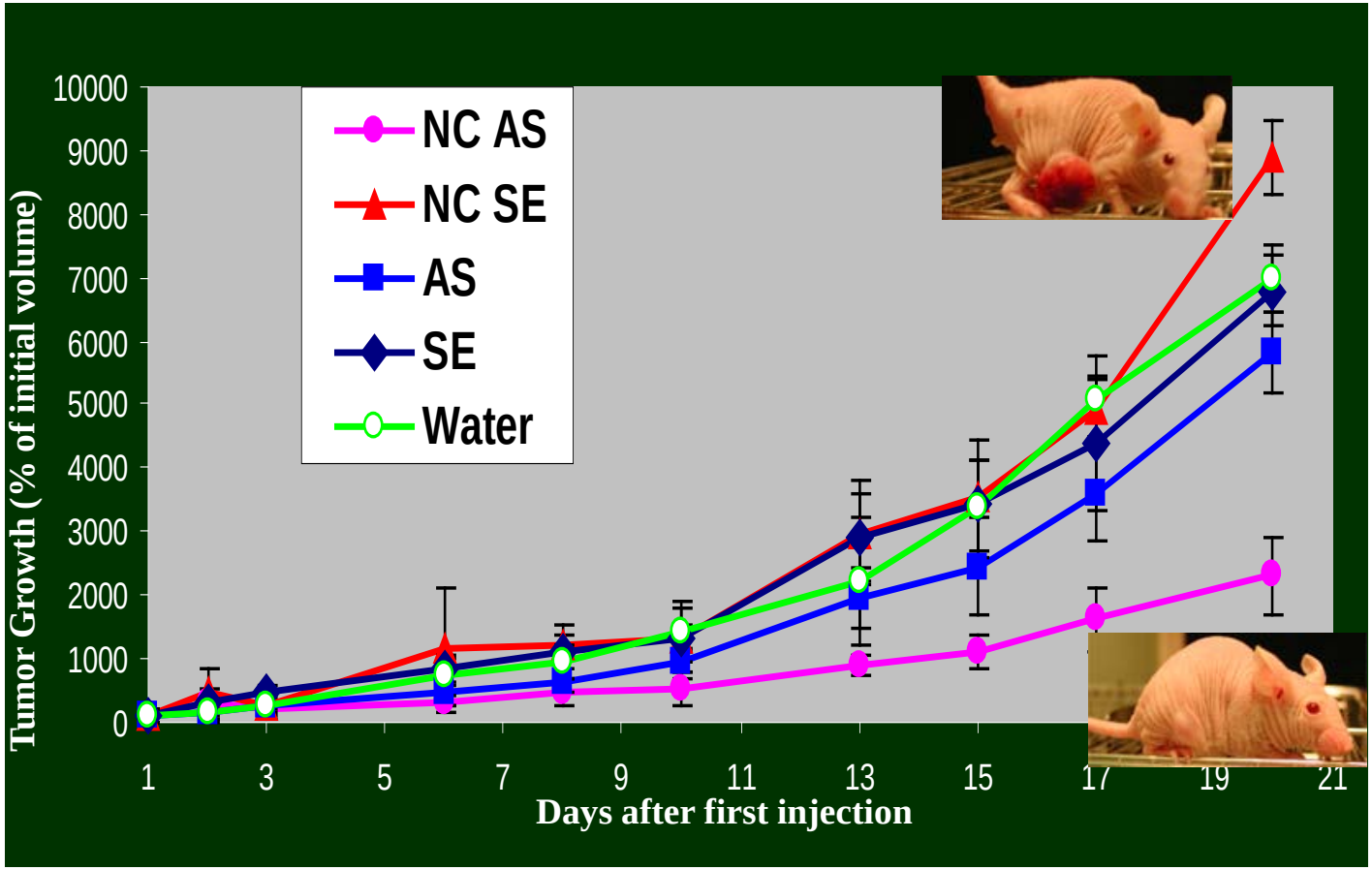
LE SARCOME D’EWING

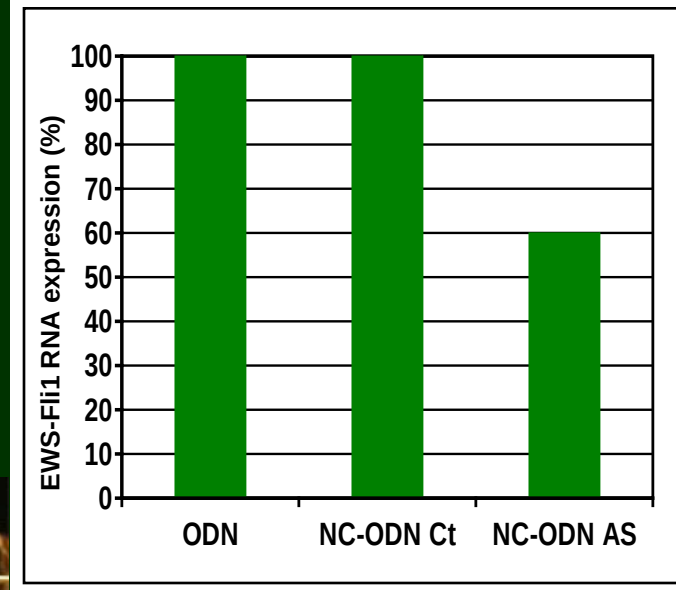




EFFECT OF ODN AND siRNA NANOCAPSULES ON SUBCUTANEOUSLY GRAFTED EWING SARCOMA

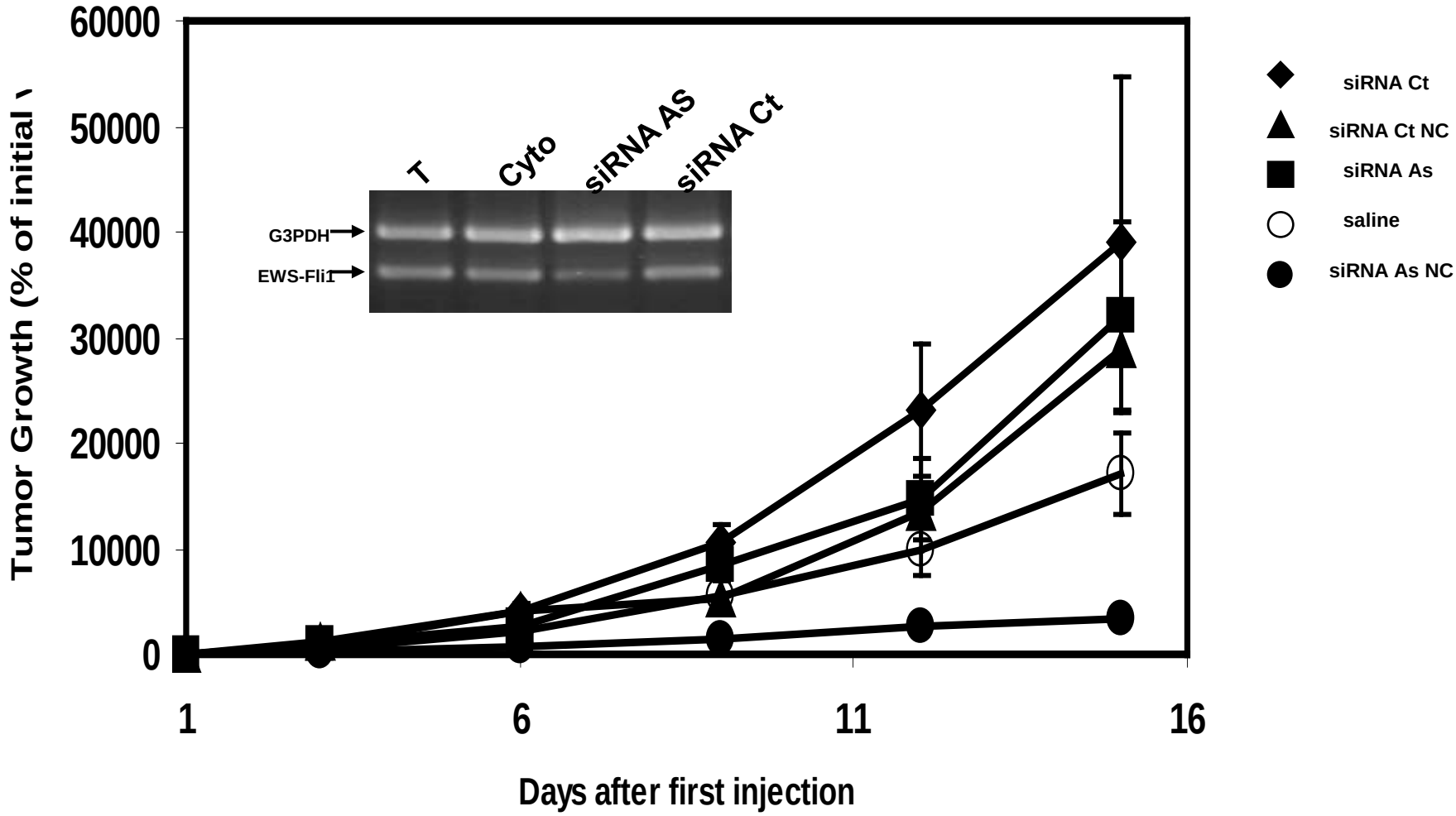
Lambert, et al., **279**, 401-406, BBRC (2001)
Maksimenko et al., Pharm. Res, **20**, 1565-1567 (2003)
Toub et al., Pharm Res, **23**, 892-900 (2006)





EFFECT OF siRNA NANOCAPSULES ON SUBCUTANEOUSLY GRAFTED EWING SARCOMA

N.Toub et al., Pharm. Res, 23, 892-900, 2006



EWS-Fli1ODN AND siRNA NANOCAPSULES: *IN VIVO* COMPARISON

Lambert et al, BBRC, 2000.
Toub et al, J. Pharm Res, 2006.

NC TYPE	DOSE	FREQUENCY	CUMULATIVE DOSE	EFFICACY
NC-ODN (1)	**0.55 mg**	**9 inj (1 inj/2dys)**	**5 mg/Kg**	**60 %**
NC-siRNA (2)	**0.22 mg**	**5 inj (1 inj/3days)**	**1.1 mg/Kg**	**90 %**

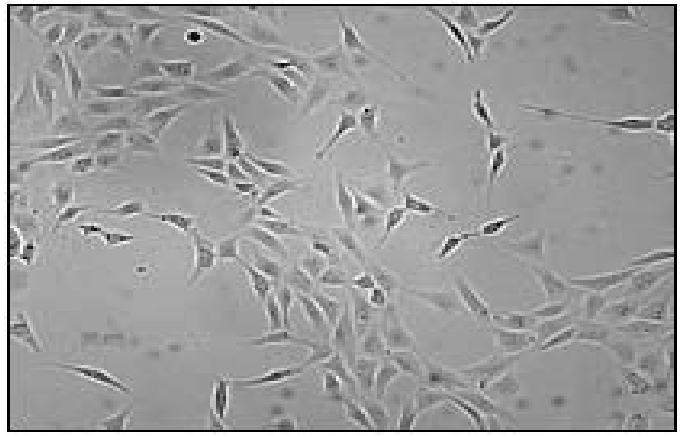
CARCINOME PAPILLAIRE DE LA THYROIDE

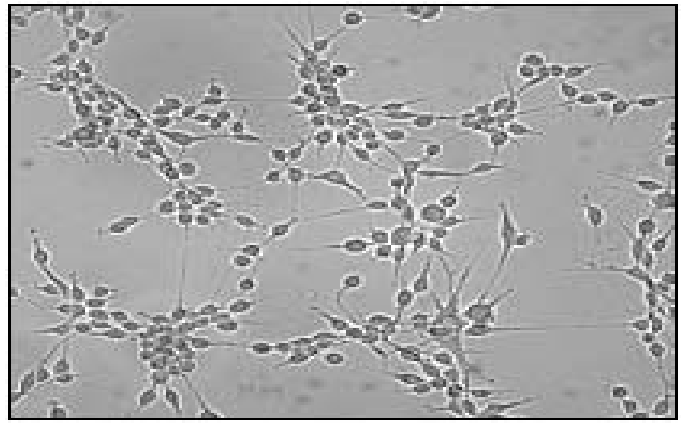
- Fusion entre le gène *ret* (récepteur de thyrosine kinase) avec le gène *H4* (gène ubiquitaire) → oncogène de jonction
- Induit la synthèse de la protéine chimère ret/PTC1→ la cellule devient tumorale

NIH/3T3

RP1

A





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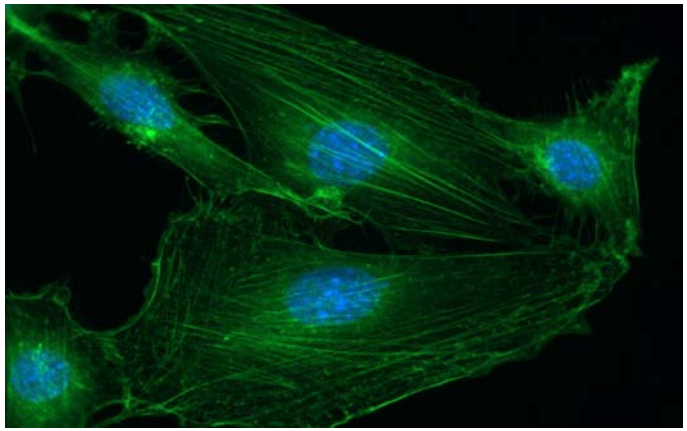

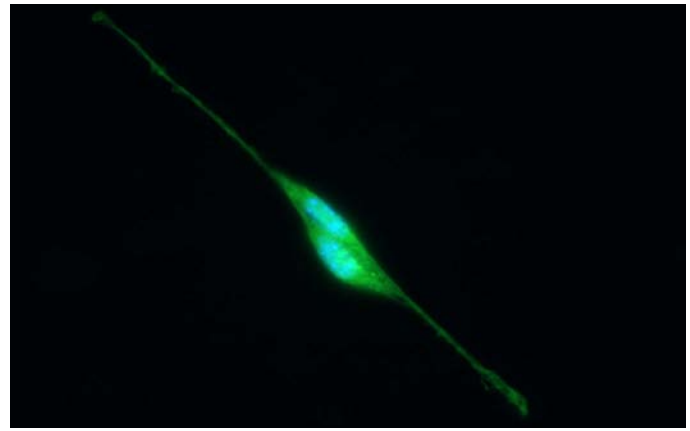

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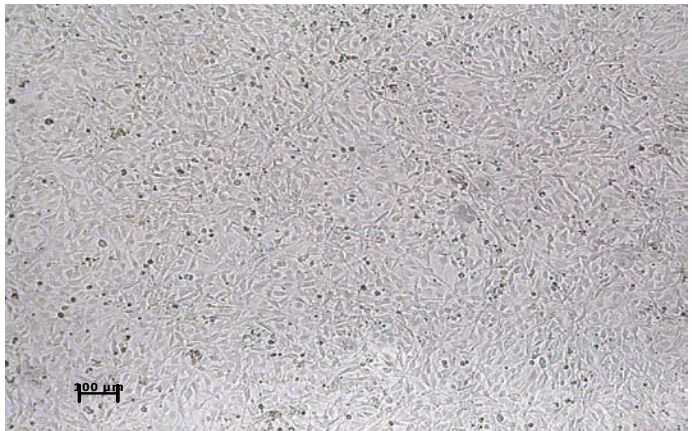
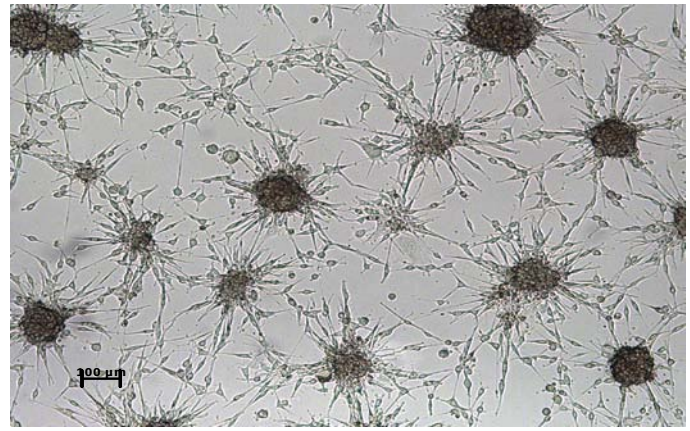




PHENOTYPICAL FEATURES OF NIH/3T3 CELLS AND ret/PTC1 TRANSFORMED NIH/3T3 CELLS (RP1)

H. de Martymprey et al.,
Nucl. Ac. Res., 36, 1-14, 2008

DESIGN OF ret/PTC1 siRNA AND shRNA

A

siRNA #1 CGUUACCAUCGAGGAUCCAdAdA
siRNA #2 UUACCAUCGAGGAUCCAAAdGdT
siRNA #3 UACCAUCGAGGAUCCAAAGdTdG
siRNA #4 GUUACCAUCGAGGAUCCAAdAdG
siRNA #5 GCCAGCGUUACCAUCGAGGdAdT

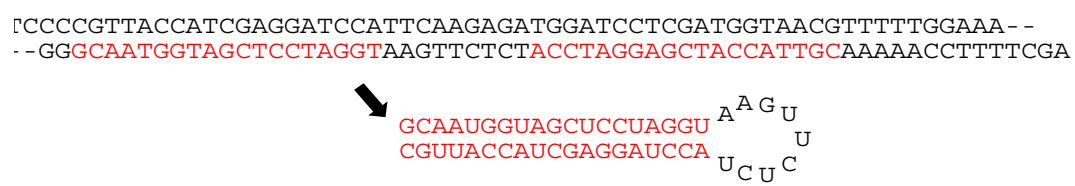
B

siRNA #5 5' – GCCAGCGUUACCAUCGAGGdAdT – 3'
3' – dTdTCGGUCGCAAUGGUAGCUCC – 5'

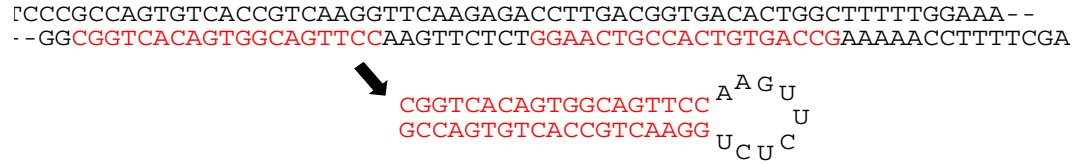
siRNA Ct 5' – GCCAGUGUCACCGUCAAGGdAdG – 3'
3' – dTdTCGGUCACAGUGGCAGUUCC – 5'

C

Structure of shRNA1 :



Structure of negative control shRNA :



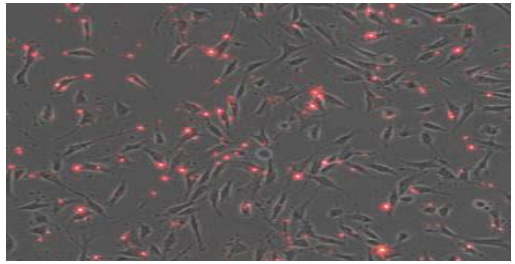
H. de Martymprey et al., Nucl. Ac. Res., 36, 1-14, 2008

Figure 1.

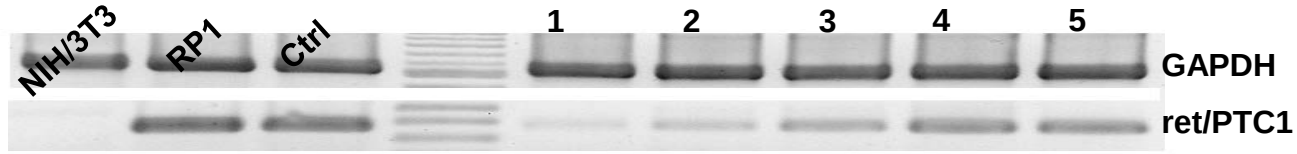
IN VITRO siRNA INHIBITION

H. de Martymprey et al., Nucl. Ac. Res., 36, 1-14 2008

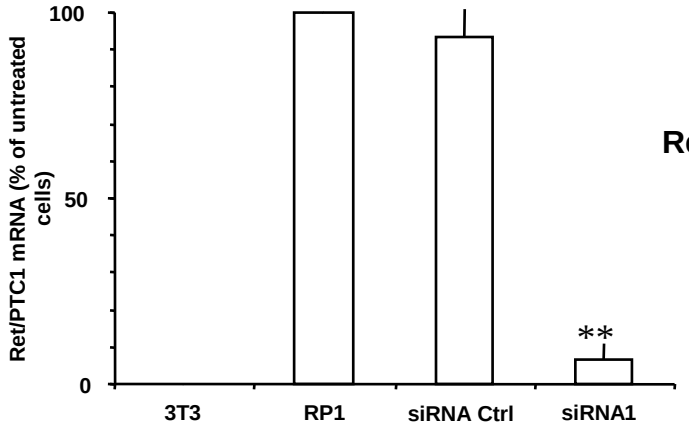
A Penetration of Rhodamine-labelled siRNA (cytofectine)



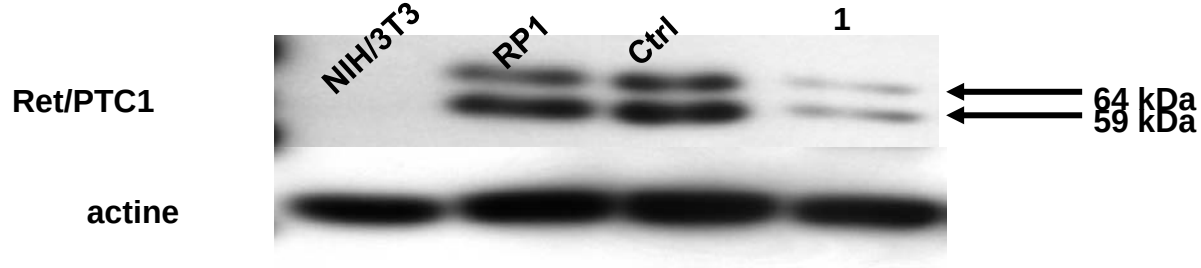
B Effect of siRNA sequence on ret/PTC expression



C Ret/PTC1 mRNA expression



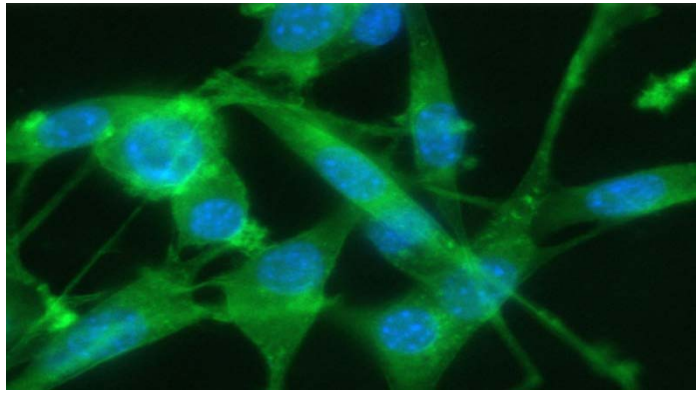
D Western blotting



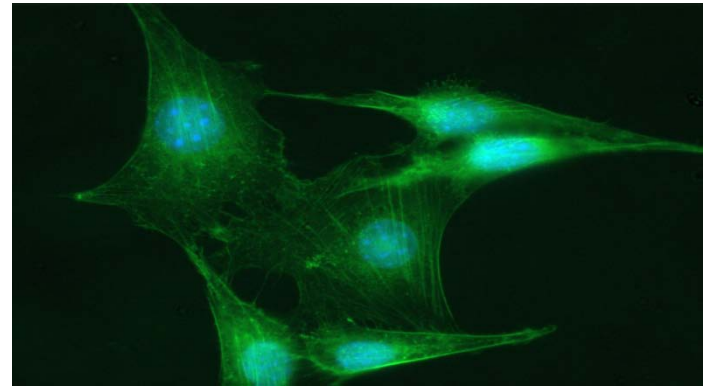
PHENOTYPICAL REVERSION

E

siRNA Ctrl



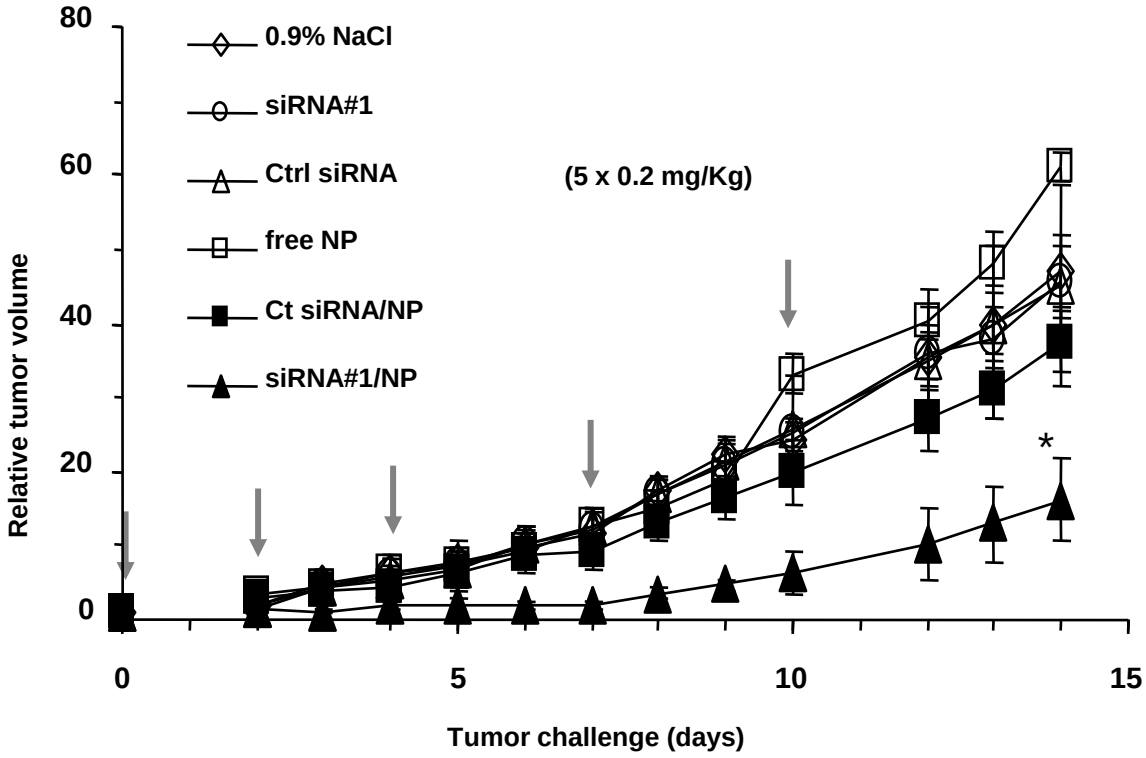
siRNA #1



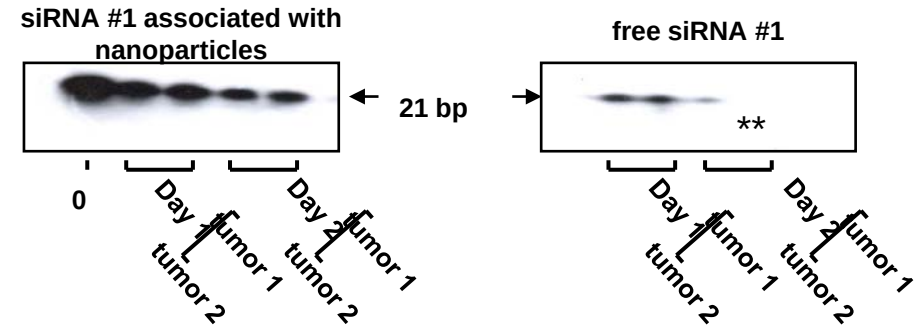
IN VIVO STUDIES

H. de Martymprey et al., Nucl. Ac. Res., 36, 1-14, 2008

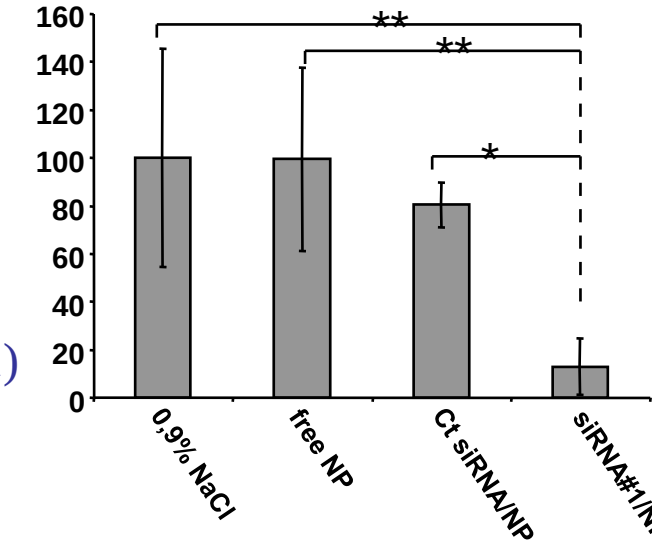
A Antitumor effect of siRNA formulations



B Stability of siRNA after intratumoral injection



C QPCR of ret/PCT1 mRNA expression in tumors (day 11)

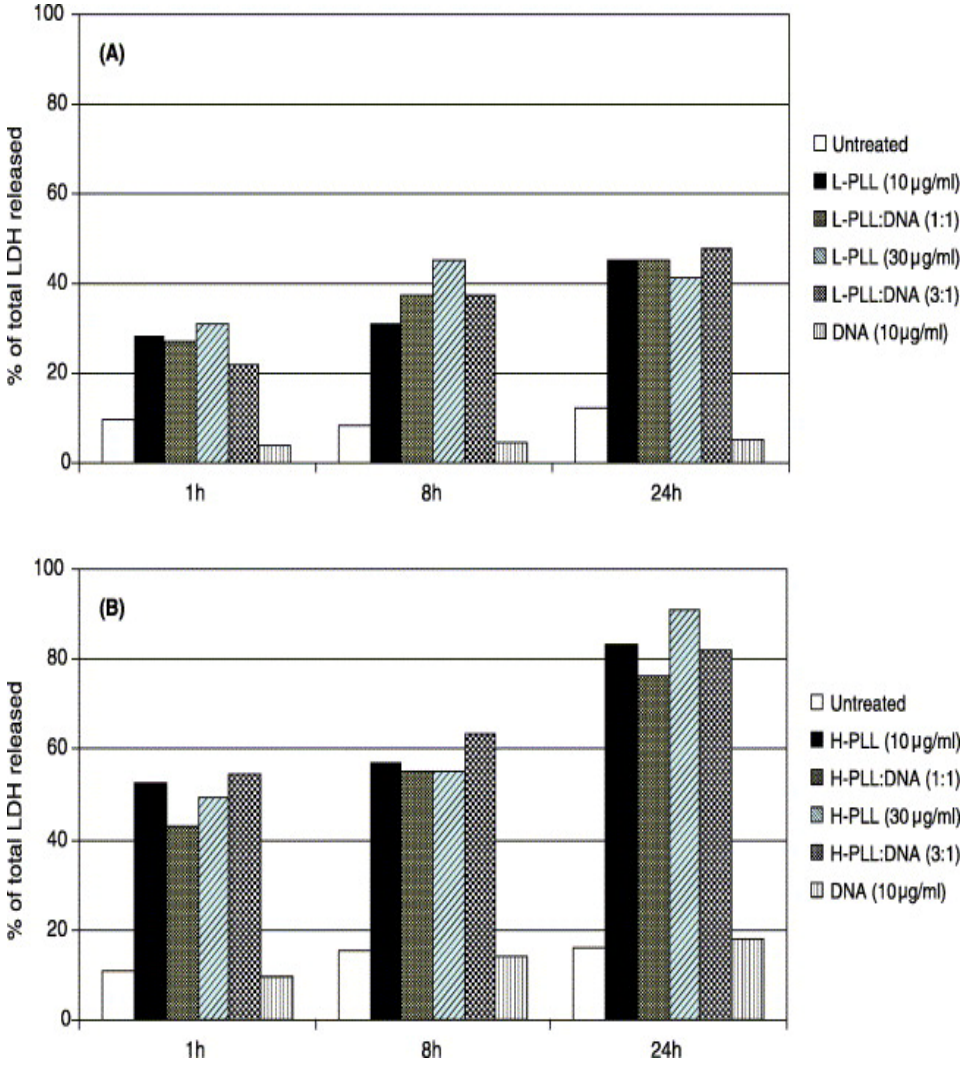


TOXICOLOGICAL ASPECTS OF POLYCATIONS

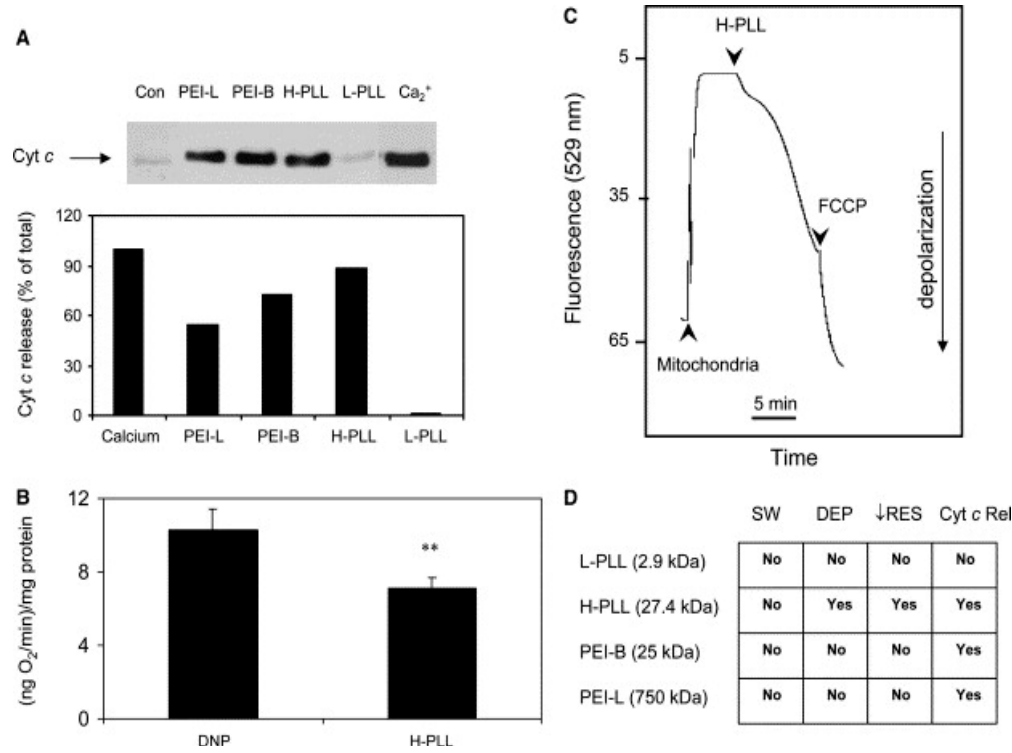
Polylysine and polyethyleneimine

TOXICITY OF POLYLYSINE

P. Symonds et al., FEBS Letters, 579, 6191-6198 (2005)



LDL release from Jurkat cells following incubation with low Mw or High Mw polylysine condensed or not with DNA

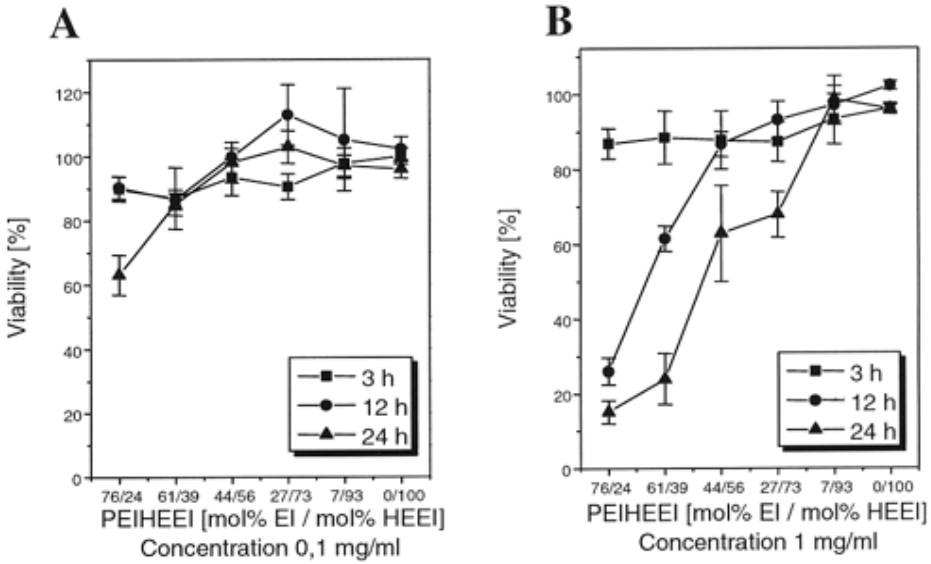


[A] Effect of H-PLL and L-PLL on cyt c release from mitochondria (Ca++ = positive control)
[B] Ability of H-PLL in suppressing mitochondrial respiration rate
[C] H-PLL is capable of affecting mitochondrial transmembrane potential
[D] Comparison of H-PLL and L-PLL with PEI on mitochondrial functions

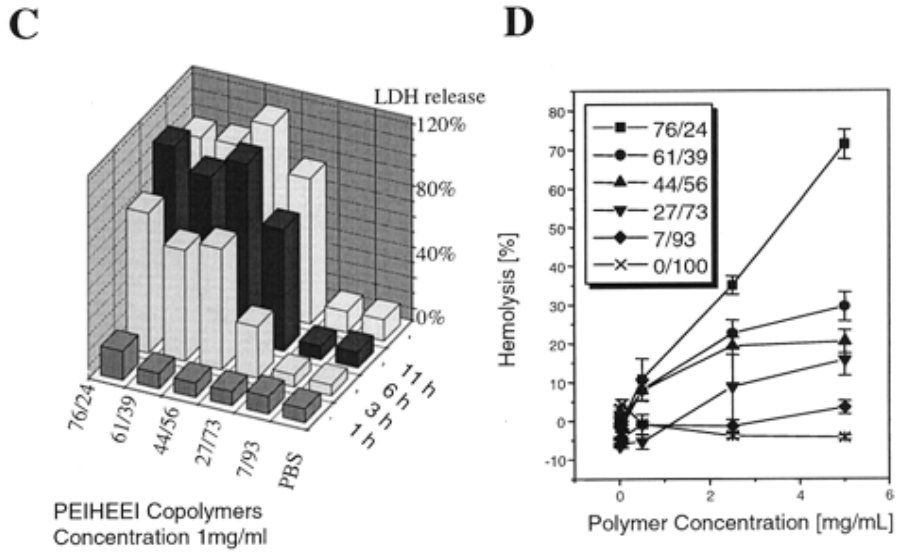
CYTOTOXICITY OF PEI POLYMERS WITH SIMILAR Mw BUT DIFFERENT DEGREES OF BRANCHING

Branching

D. Fischer et al., Bioconjugate Chem, 13, 1124-1133, 2002



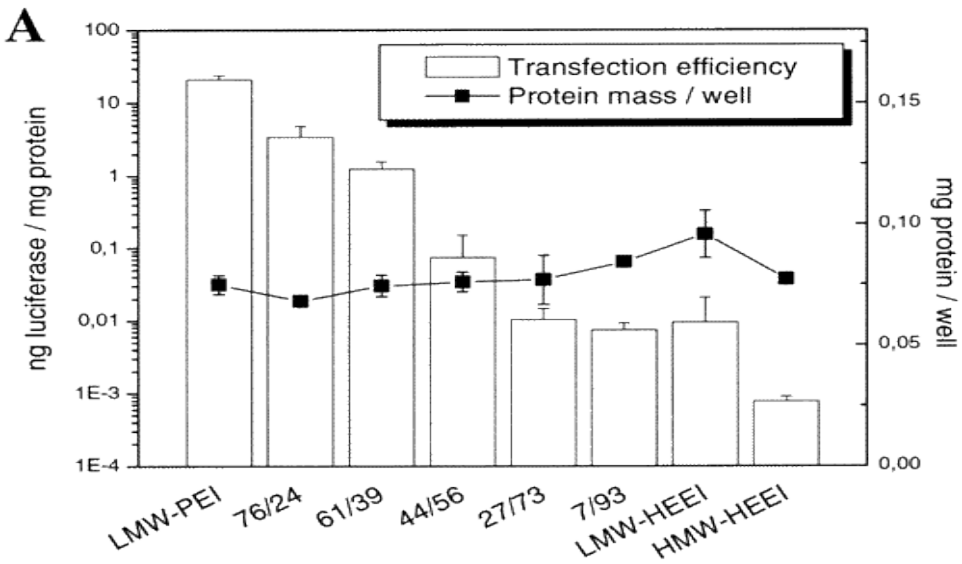
HEEI content decreases the degree of branching [A] and [B] **cytotoxicity (MTT)** as a function of the decreased degree of branching [A] 0.1 mg/ml [B] 1 mg/ml

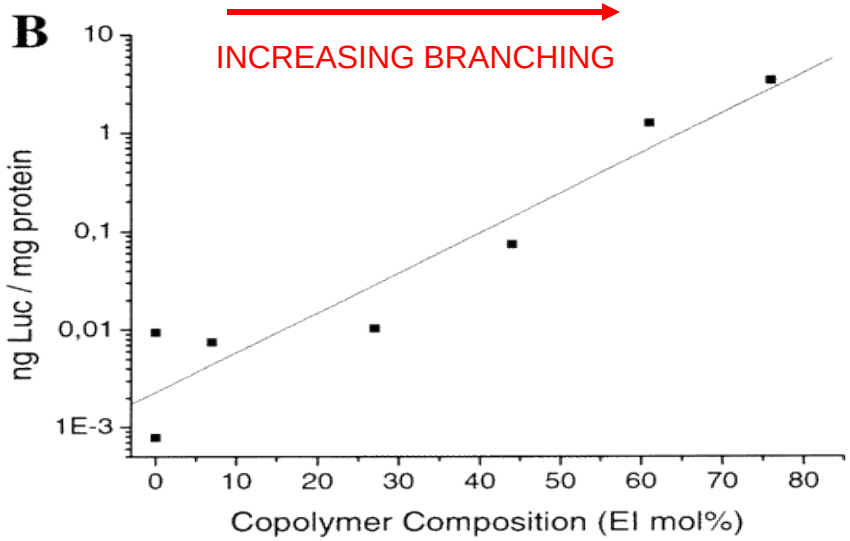


(C) The membrane damaging effects were characterized by **LDH-release i**ncubating L929 cells with 1 mg/mL polymer solution for 1, 3, 6, and 11 h (*n* = 3). (D) After 1 h incubation, the **hemolytic activity** of different concentrations of the polymers

TRANSFECTION EFFICIENCY OF PEI WITH DIFFERENT BRANCHING

D. Fischer et al., Bioconjugate Chem, 13, 1124-1133, 2002





Increasing branching increases transfection efficacy as well as cytotoxicity

VIVE LA GALENIQUE !

« La manière de donner est au moins aussi important que ce que l'on donne »

(Molière, L'Avare)