# NANOTECHNOLOGIES ET INTERACTION AVEC LES CELLULES

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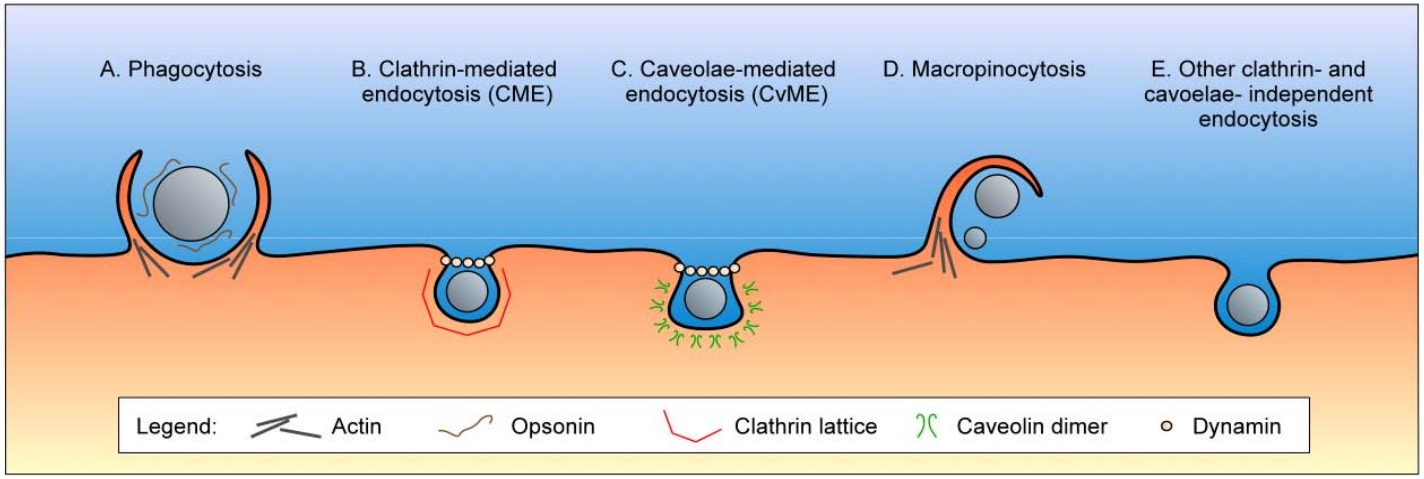
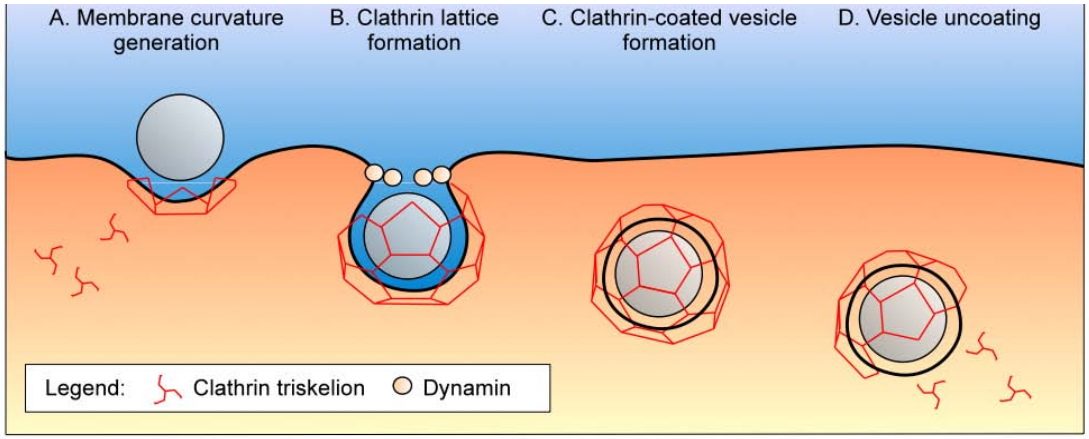
2009-2010

# VOIES D'ENTREE DANS LA CELLULE

H. Hillaireau and P. Couvreur, CMLS, 2009

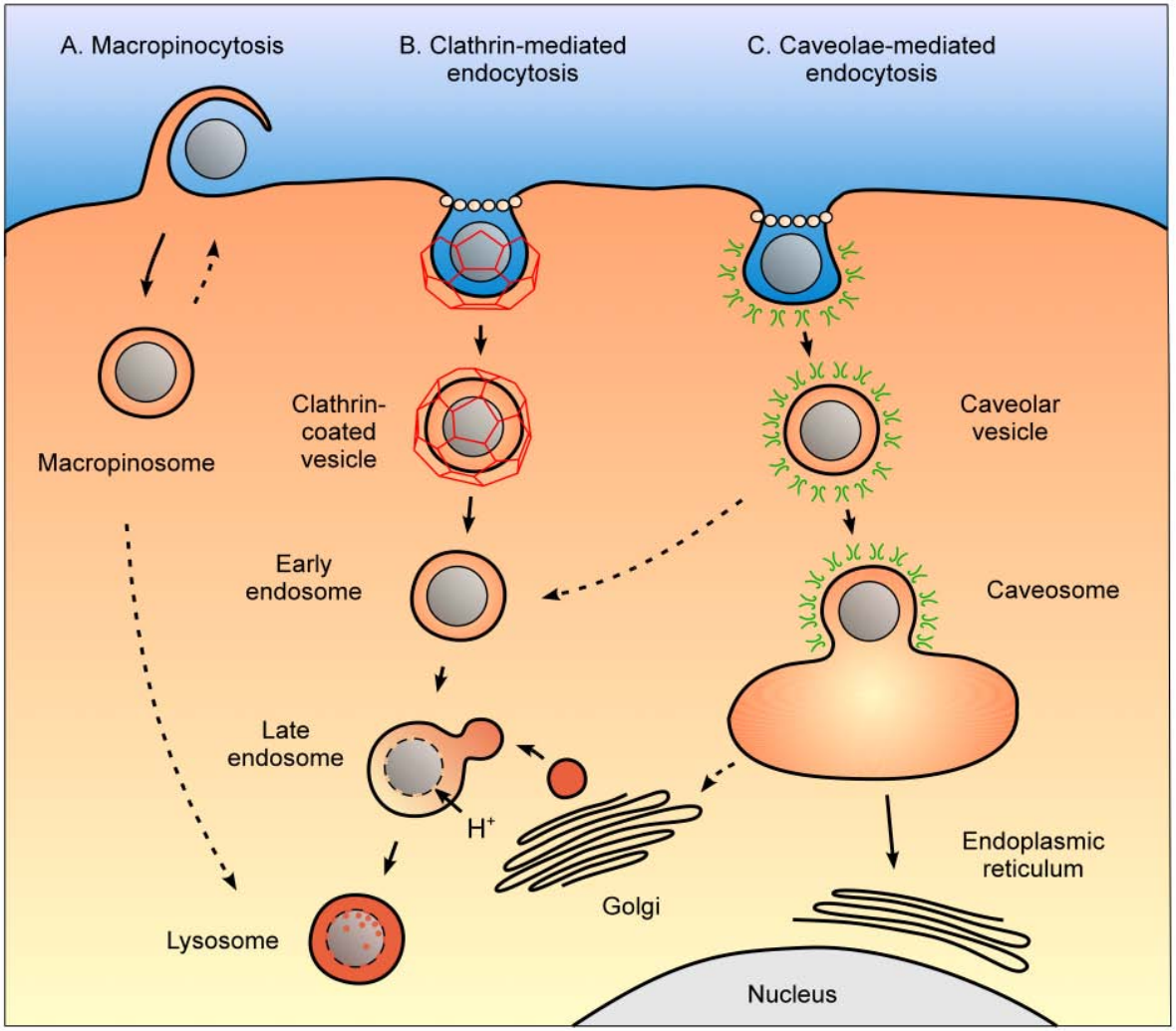



Clathrin mediated endocytosis



# TRAFIC INTRACELLULAIRE

H. Hillaireau and P. Couvreur, CMLS, 2009

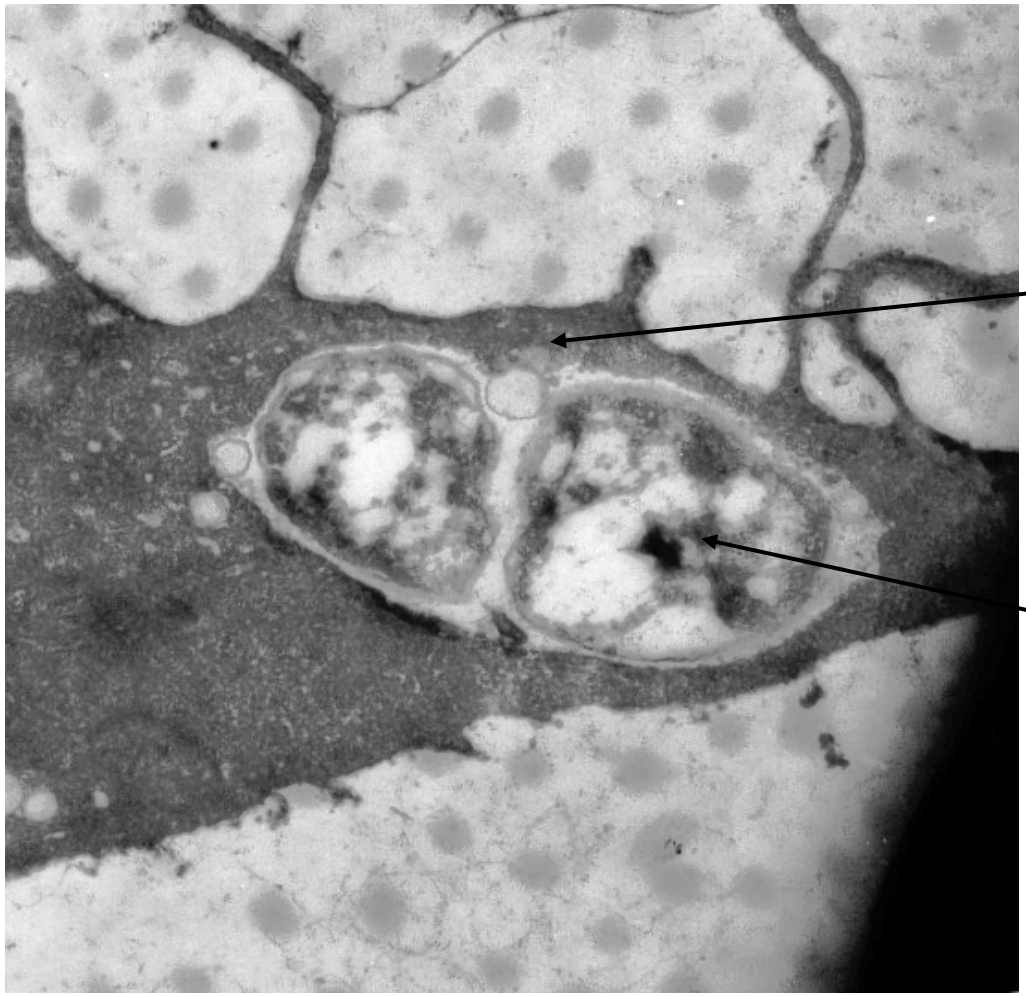


# INTERACTION DES NANOPARTICULES AVEC LES CELLULES

Macrophages

# NANOPARTICLES CAN DELIVER ANTIBIOTICS IN THE INTRACELLULAR COMPARTMENT WHERE THE BACTERIA ARE

O. Balland et al., J. Antimicr. Chemother., 1995

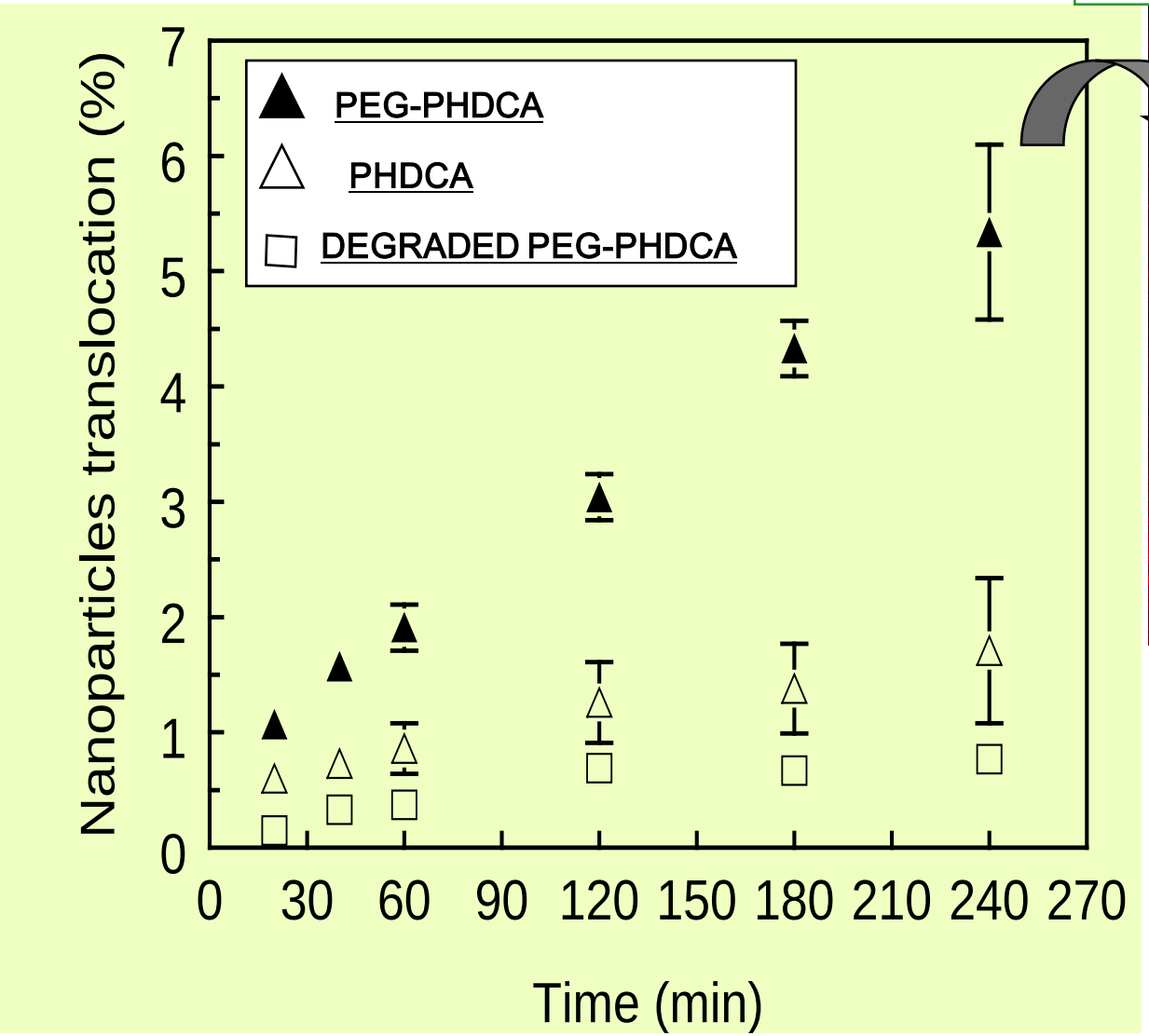


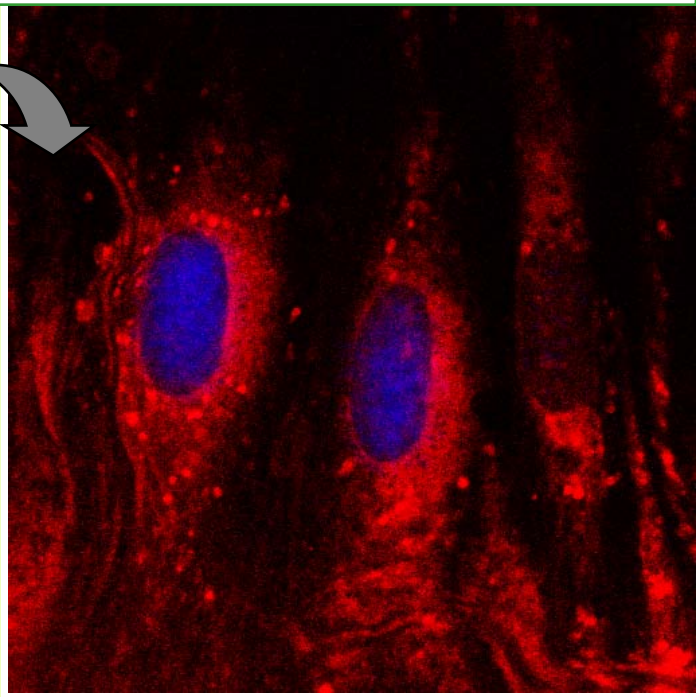
# INTERACTION DES NANOPARTICULES AVEC LES CELLULES

Cellules endothéliales

# NANOPARTICLES TRANSLOCATION THROUGH EXPERIMENTAL RAT BBB

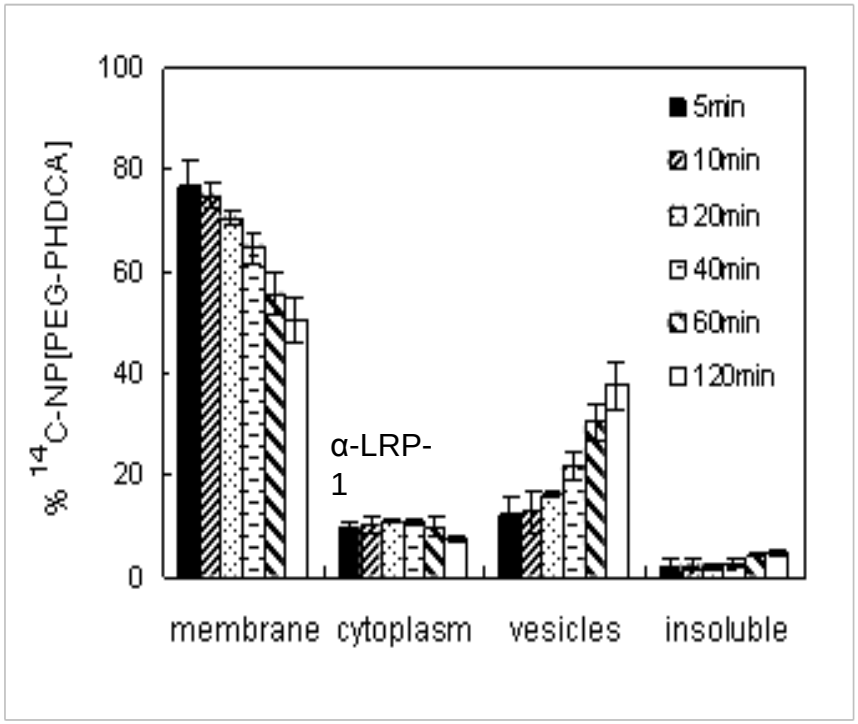
E. Garcia-Garcia et al., CMLS, 62, 1400-1408 2005

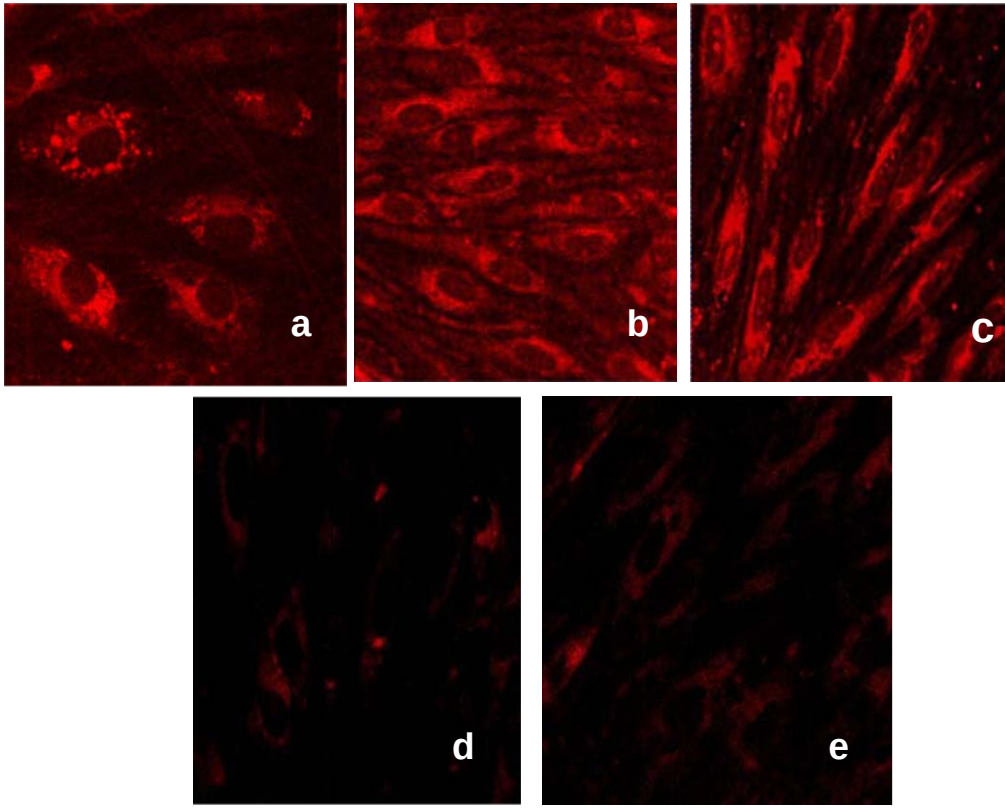


# PEGYLATED CYANOACRYLATE NANOPARTICLES ARE TAKEN UP BY RBEC THROUGH COATED PITS ENDOCYTOTIC PATHWAY

Kim HR et al., Cell Mol Life Sci., 64, 356-364 (2007)




α-LDLR

α-LRP-1

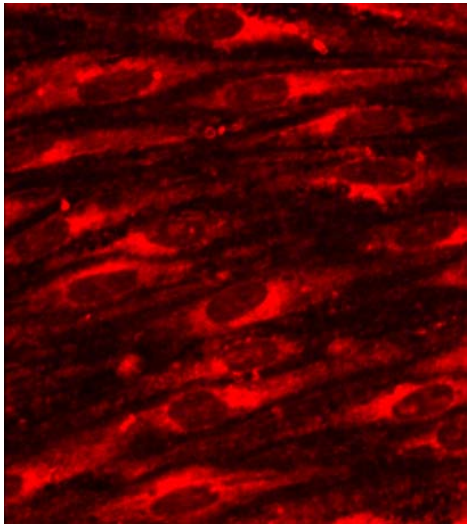
α-caveolin-1

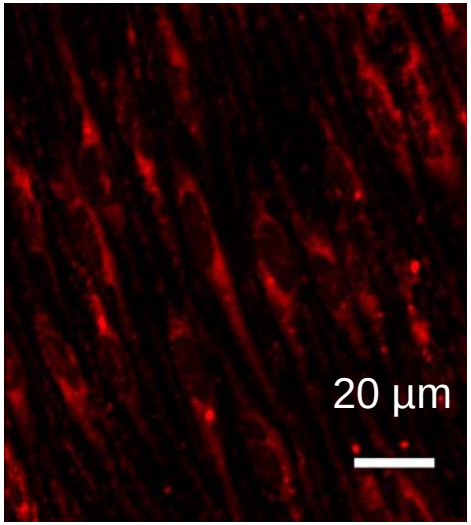
Control cells (a), 10 min pre-treatment with 3 µg/mL filipin (calveolae disrupter) (b), 3 µg/mL nystatin (calveolae disrupter) (c), 50 µM chlorpromazine (prevents coated pits formation) (d) and 0.1% sodium azide (inhibitor of energy-dependent receptor-mediated pathway) (e). Bar represents 20 µm.

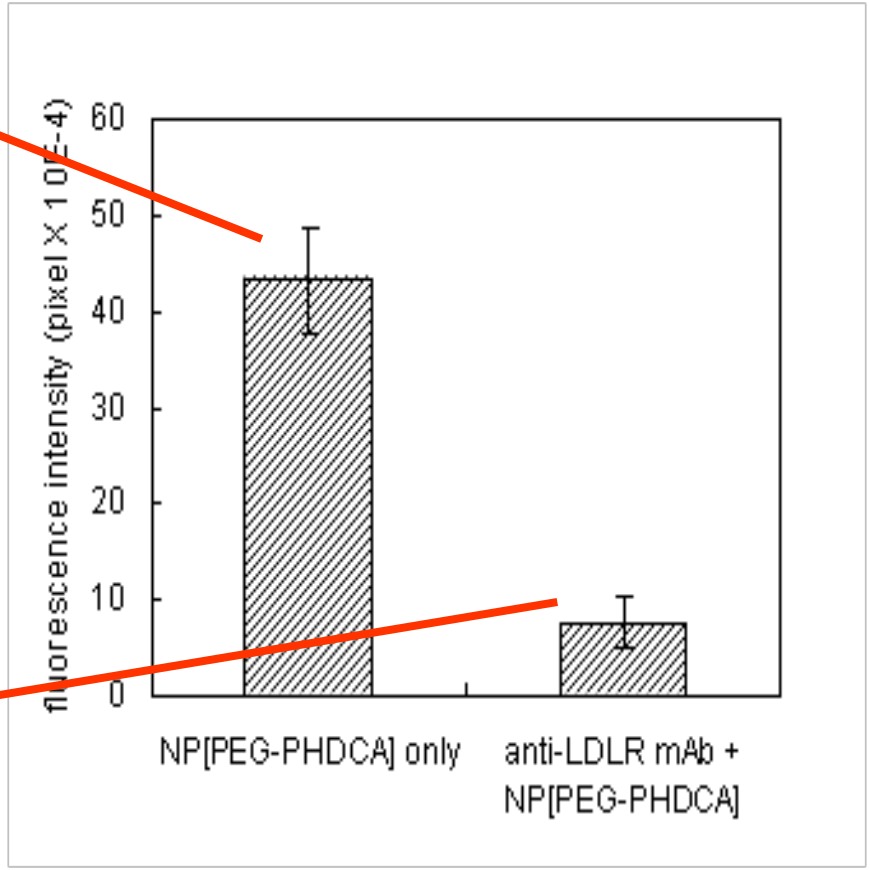
**Proteins involved in these vesicles-mediated endocytosis**

# … AND LDL RECEPTOR IS INVOLVED

Kim HR et al., Cell Mol Life Sci., 64, 356-364 (2007)





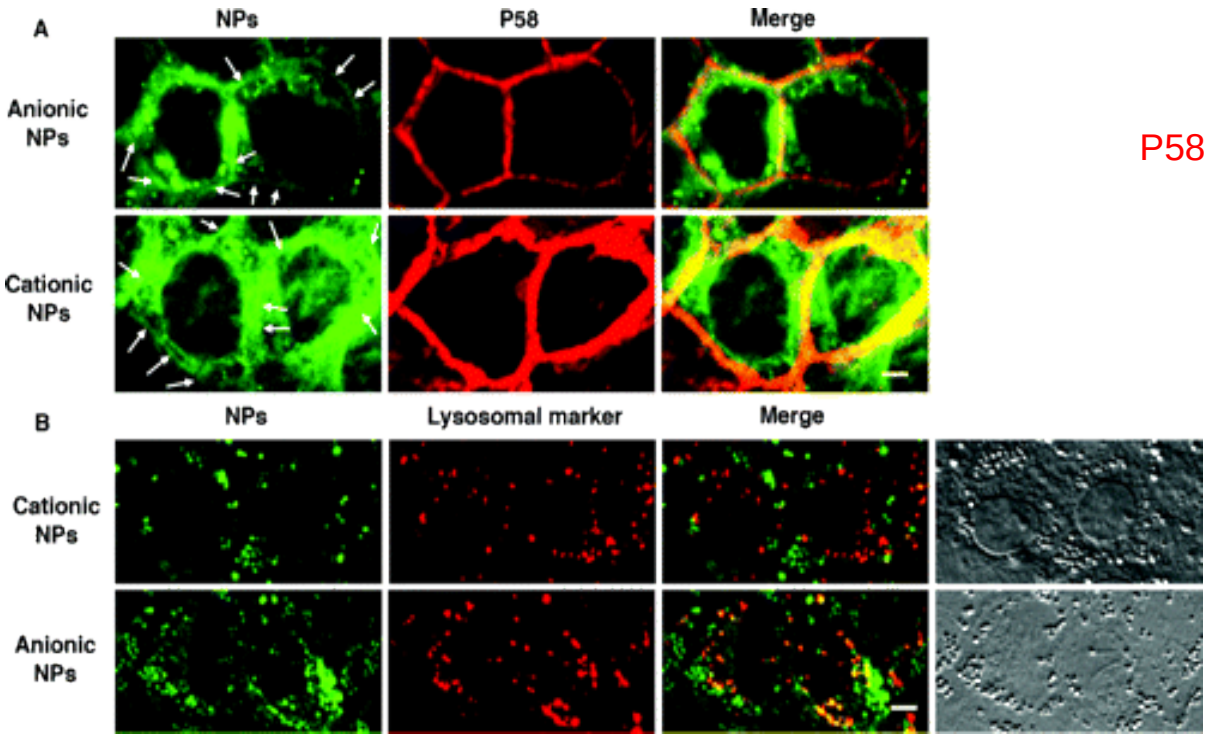



# INTERACTION DES NANOPARTICULES AVEC LES CELLULES

Cellules épithéliales

# INTRACELLULAR TRAFFICKING IN EPITHELIAL CELLS OF NEGATIVELY AND POSITIVELY CHARGED NANOPARTICLES

Harush-Frenkel O. et al, Biomacromolecules, 2008




P58 mouse antibody

Intracellular accumulation of NP formulations. (A) Polarized epithelial kidney MDCK cells incubated with cationic and anionic NPs (green), incubated mouse monoclonal antibody (red). Merged fluorescences (yellow) shows partial overlap of NPs and lateral plasma membrane in yellow staining. (B) MDCK cells incubated with cationic and anionic NPs green) and LysoTracker (red). Merged fluorescence shows overlap of anionic NPs and LysoTracker (yellow) but not cationic NPs and lysoTracker.

\+ NP escape from the lysosomes

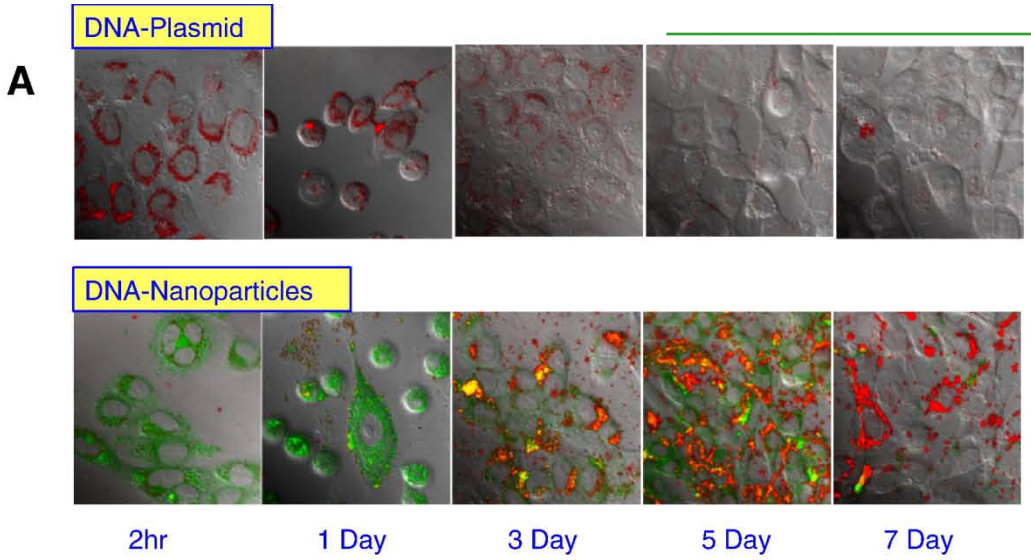
\- NP ended into lysosmes

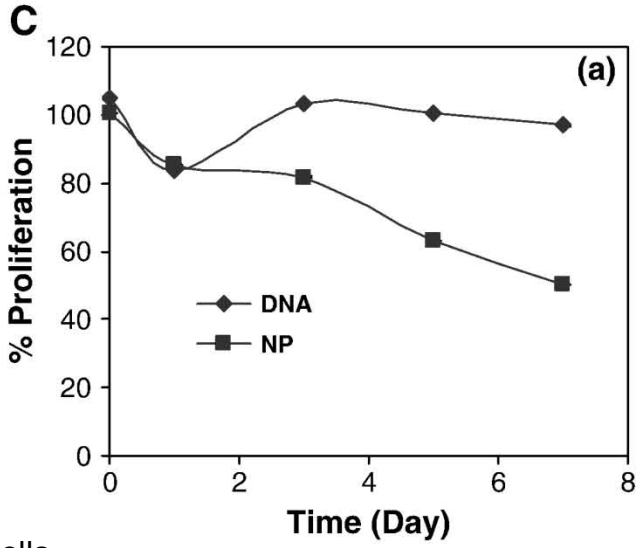
# INTERACTION DES NANOPARTICULES AVEC LES CELLULES

Cellules cancéreuses

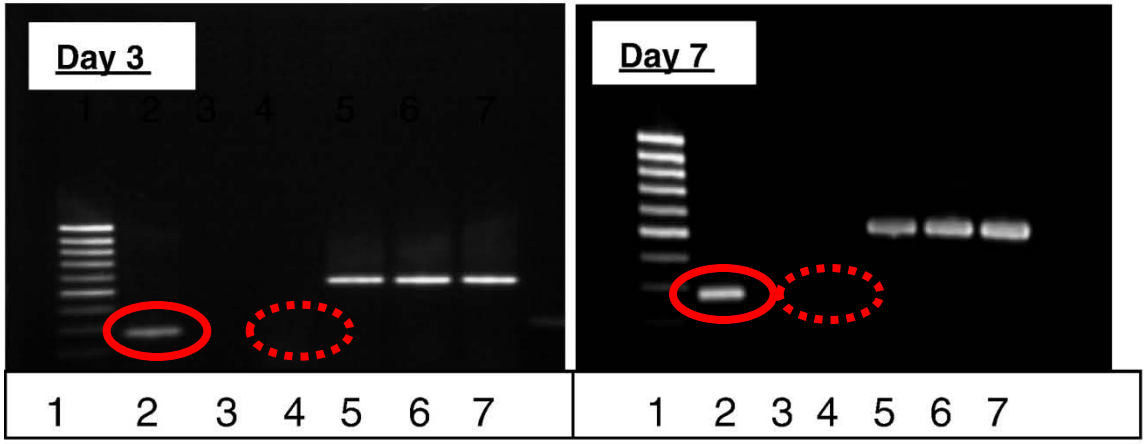
# PLGA NANOPARTICLES CONTAINING p53 DNA

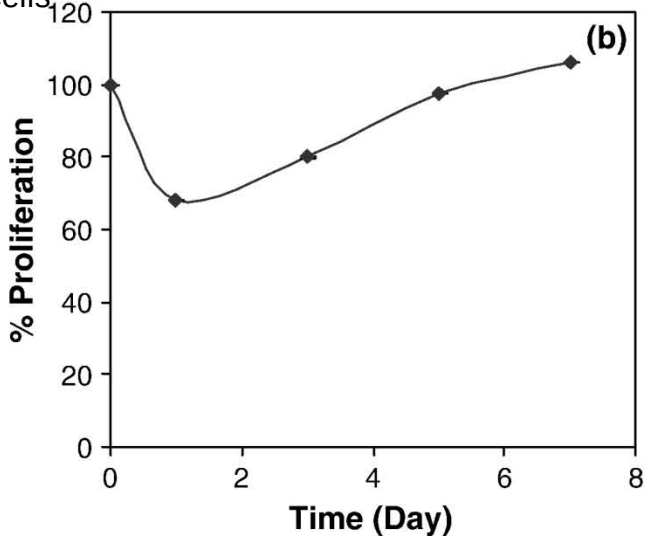
S. Prabha and V. Labhasetwar, Mol Pharmacol, 2004




B Green: 6-coumarin NPs; Red: TOTO-labeled DNA MDA breast cancer cells



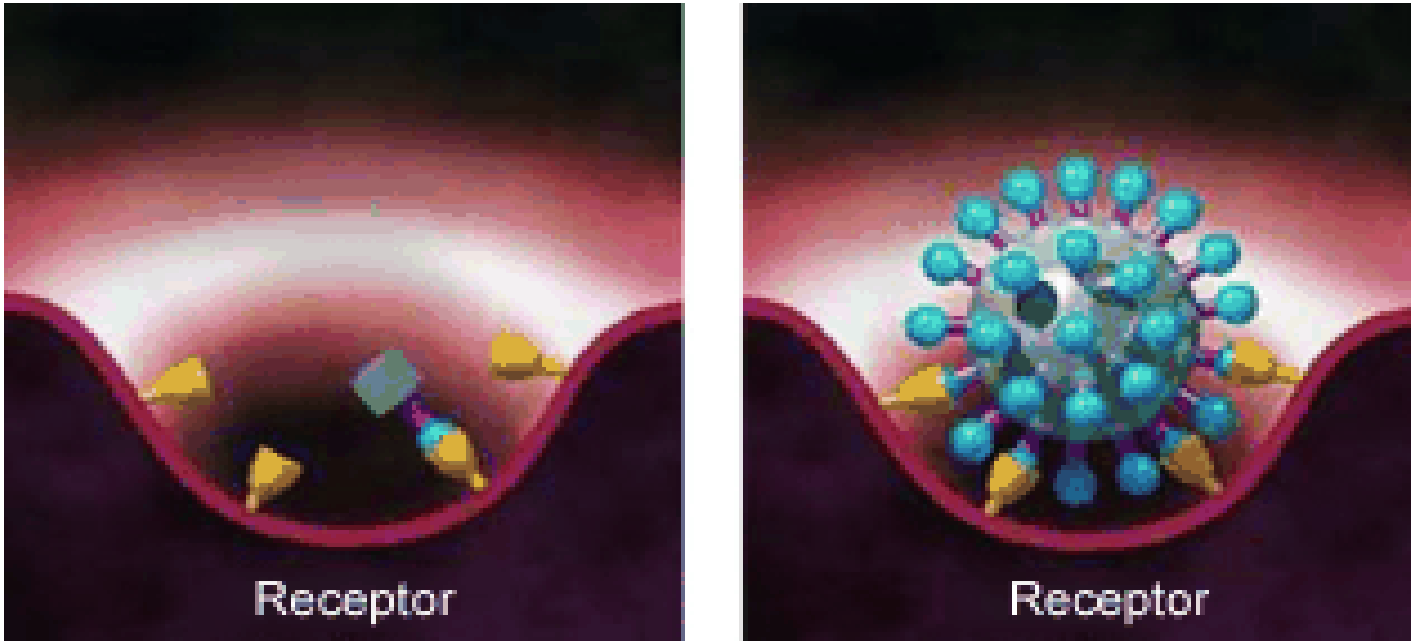




RT-PCR data of cells transfected with *wt*-p53 gene: Lane 1: Molecular weight marker, Lane 2: p53 DNA-loaded NPs, Lane 3: p53(− ve) DNA-loaded NPs, Lane 4: p53 DNA only; Lane 5: β-actin for p53 DNA-loaded nanoparticles, Lane 6: β-actin for p53(− ve) DNA-loaded nanoparticles, Lane 7: β-actin for p53 DNA. C) Antiproliferative activity of *wt*-p53 DNA: MDA-MB-435S cells were treated either with (a): *wt*-p53-plasmid DNA or *wt*-p53 DNA-loaded nanoparticles (NP) or (b): DNA-Lipofectamine™ complex.

# AMELIORATION DE LA PENETRATION INTRACELLULAIRE PAR FONCTIONNALISATION DES NANOVECTEURS

Fonction de reconnaissance

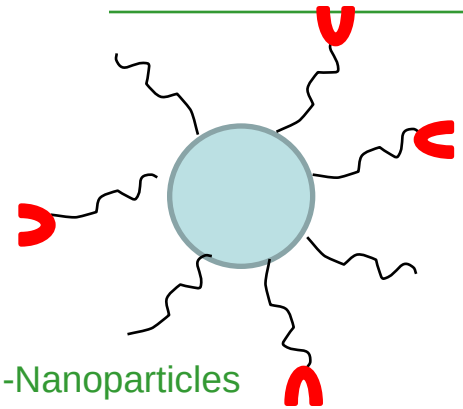
# MONOVALENT BINDING OF A DRUG VERSUS POLYVALENT BINDING OF A NANOCARRIER




(from Starpharma.)

# PLASMON RESONNANCE AND MULTIVALENT BINDING

B. Stella et al., J. Pharm.Sci., 2000
B. Stella et al., J Drug Target., **15,** 146-53 (2007)



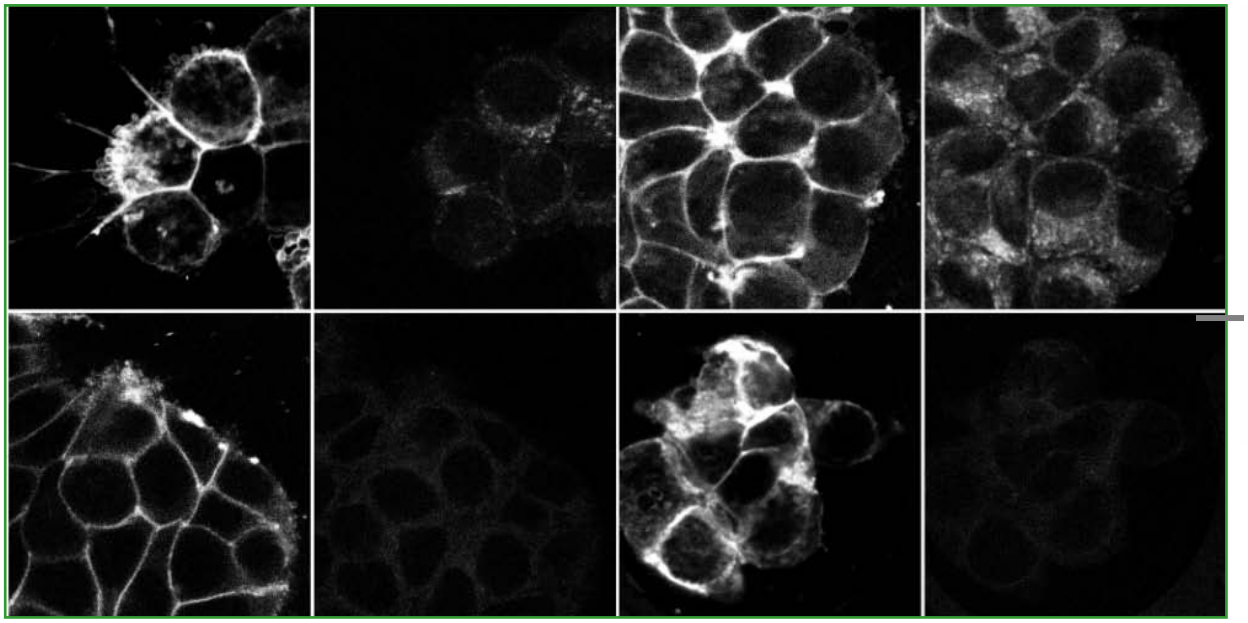
PEG-Nanoparticles

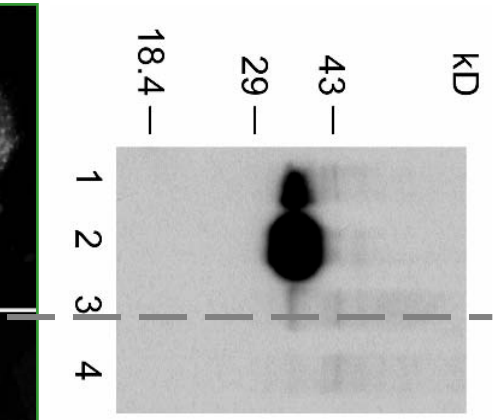
Folate-nanoparticles

**Acide folique**

**KB**

**MCF-7**






Membrane labelling

Nanoparticles fluorescence

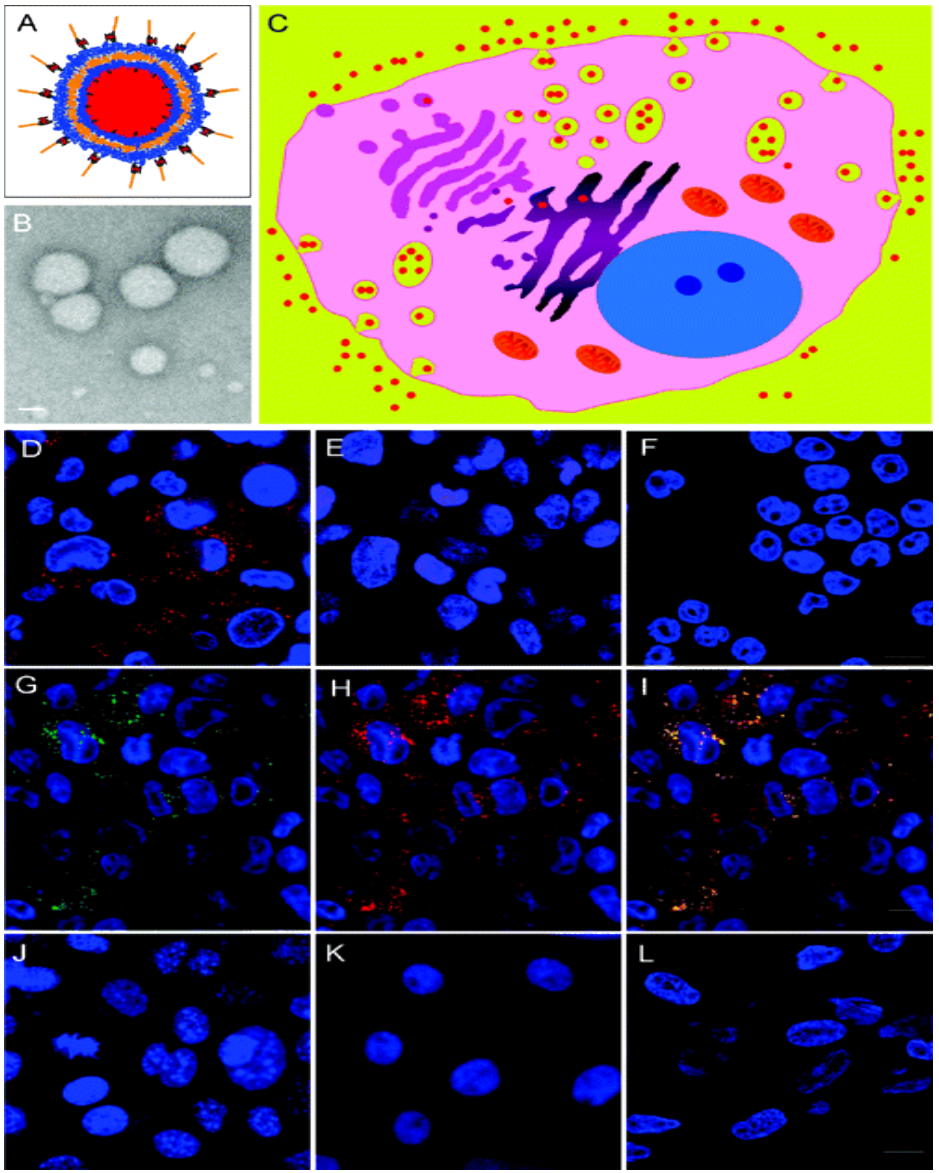
Membrane labelling

Nanoparticles fluorescence

# FUNCTIONALIZED POLYMER VESICLES BY POLY G

Ben-Haim N et al., nano Letters, 8, 1368-1373 (2008)

**ABA copolymer Poly(2-methyloxazoline)-b-poly(dimethylsiloxane)-b- poly(2-methyloxazoline) forming the core of the nanocarrier functionalized with polyguanilic acid** (PolyG recognizes macrophages scavenger receptors) **and filled with fluorescent BSA**




## Incubation with activated THP-1 macrophages

D: BSA-polyG vesicles E:BSA free F: BSA vesicles

G: Ac LDL Alexa Fluor 488 H: BSA-polyG vesicles
I: Merge

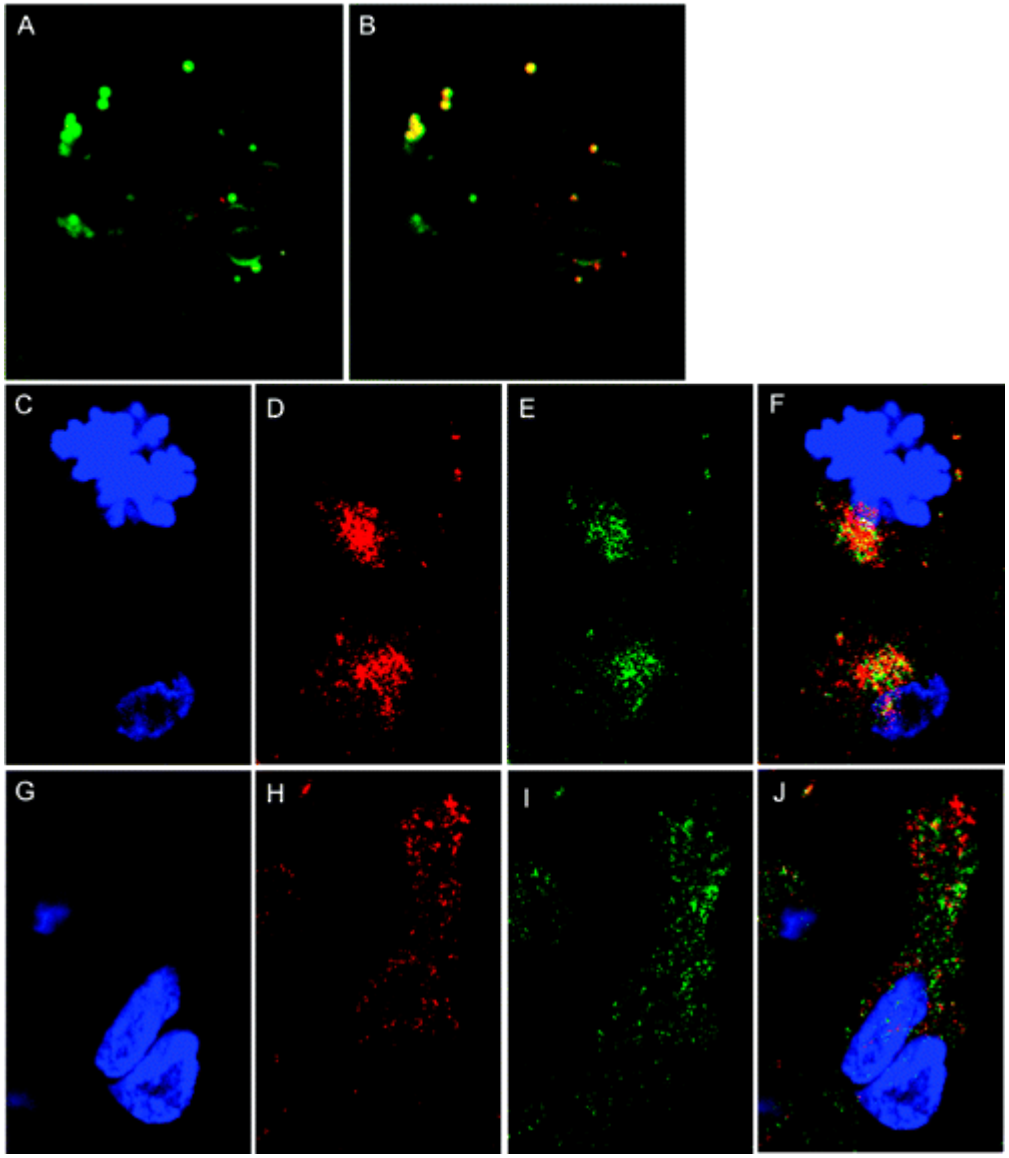
J: Incubation with mouse embr. Fibroblasts K: rat skeletal muscle cells
L: rat liver endothelial cells

# FUNCTIONALIZED POLYMER VESICLES ARE COLOCALIZED WITH LYSOSOMES AND GOLGI:RETICULUM ENDOPLASMIC BUT NOT WITH MITOCHONDRIA AND NUCLEUS

Ben-Haim N et al., Nano Letters, 8, 1368-1373 (2008)

PolyG-nanovesicles (red) + Lysosensor (green)
Merge (yellow)

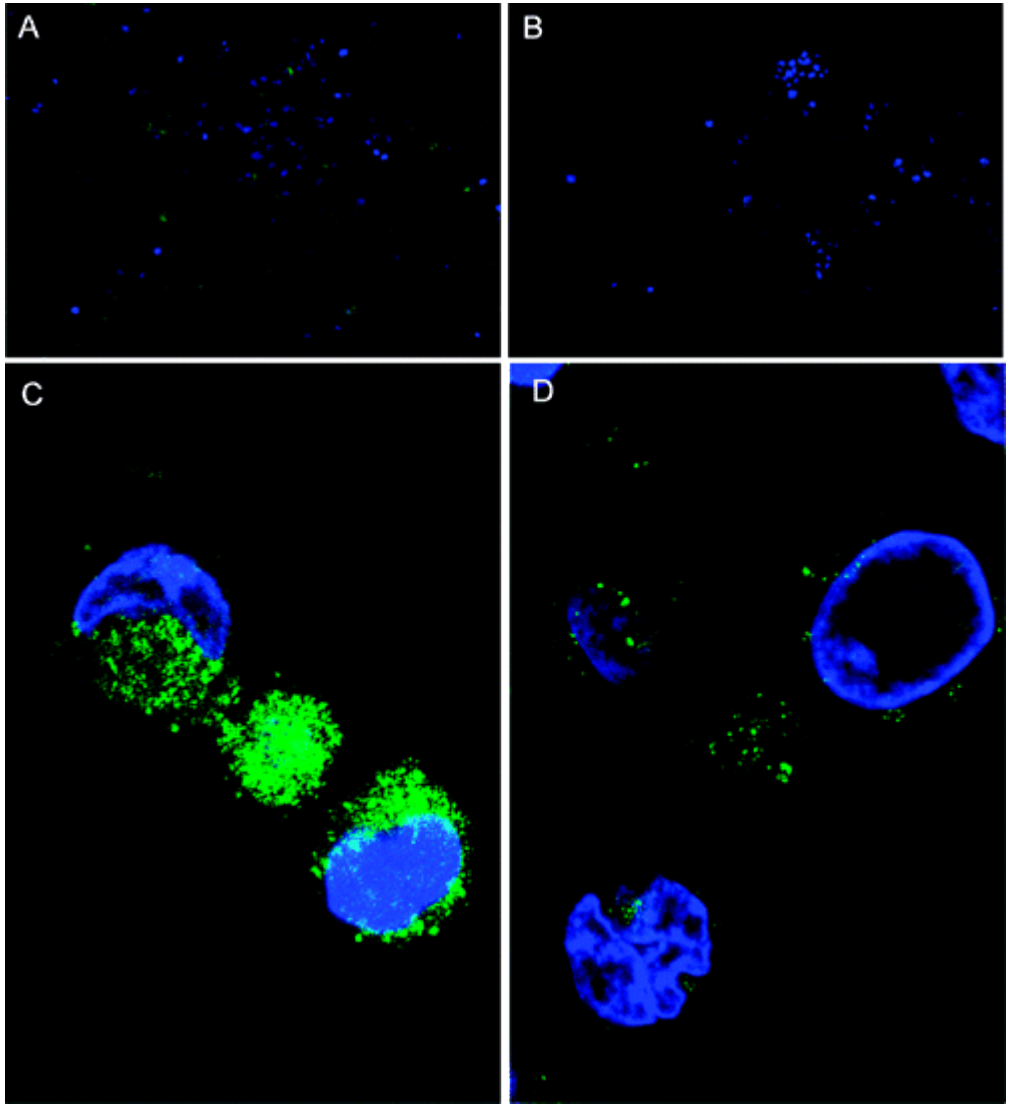
C and G: Nuclear stain (blue)
D and H: PolyG-nanovesicles (red)
E: Golgi and Reticulum Endoplasmic apparatus (green)
I: Mitochondria (green)
F and J: merge



# FUNCTIONALIZED POLYMER VESICLES CONTAINING TRYPSIN ARE FUNCTIONAL INTRACELLULARLY IN THP-1 MACROPHAGES

Ben-Haim N et al., nano Letters, 8, 1368-1373 (2008)

Trypsin polyG vesicles | Empty vesicles



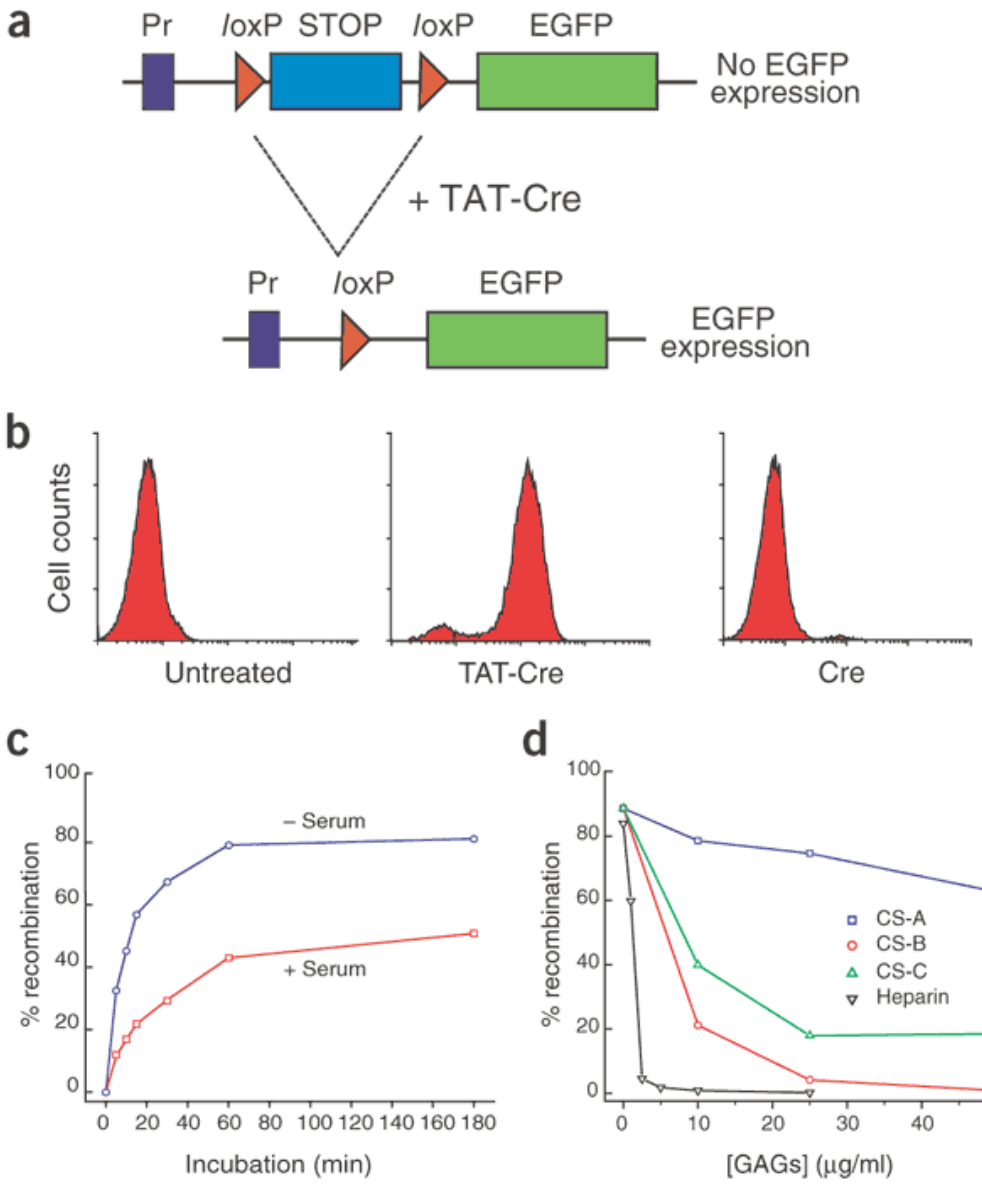

Hydrophobic substrate BZiPAR is converted into green fluorescent rhodamine when trypsin is released from the nanoreactor

# AMELIORATION DE LA PENETRATION INTRACELLULAIRE PAR FONCTIONNALISATION DES NANOVECTEURS

Fonction de pénétration intracellulaire

# TAT PEPTIDE

JS. Wadia et al., Nature Med, 10, 310-315 (2004)

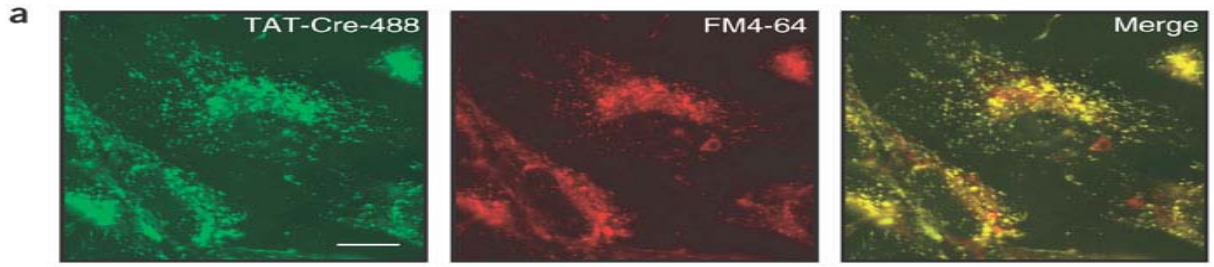



TAT-Cre protein enter the cell, translocate nucleus, excise STOP DNA segment, before scoring Green Fluorescent protein (EGFP) expression

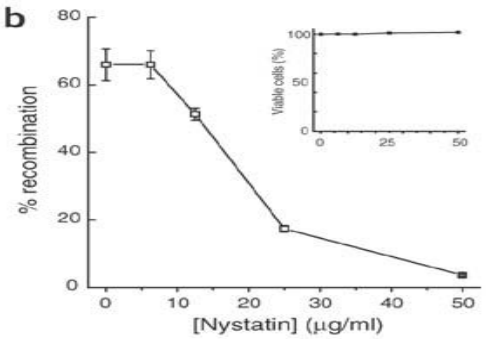
Chondroitin sulfate B and C and heparin prevent surface binding of TAT. Thus cell-surface binding of + charged TAT-Cre with – charged cell membrane is needed

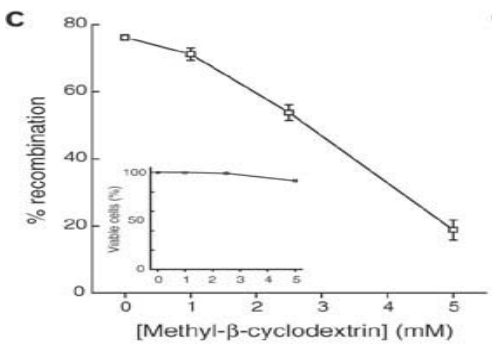
# TAT MEDIATED TRANSDUCTION IS LIPID RAFT DEPENDANT

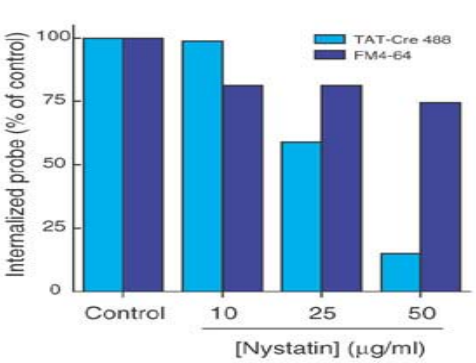
JS. Wadia et al., Nature Med, 10, 310-315 (2004)




TAT-Cre colocalize in Endosomic vesicles with FM4-64, a endocytic vesicle marker



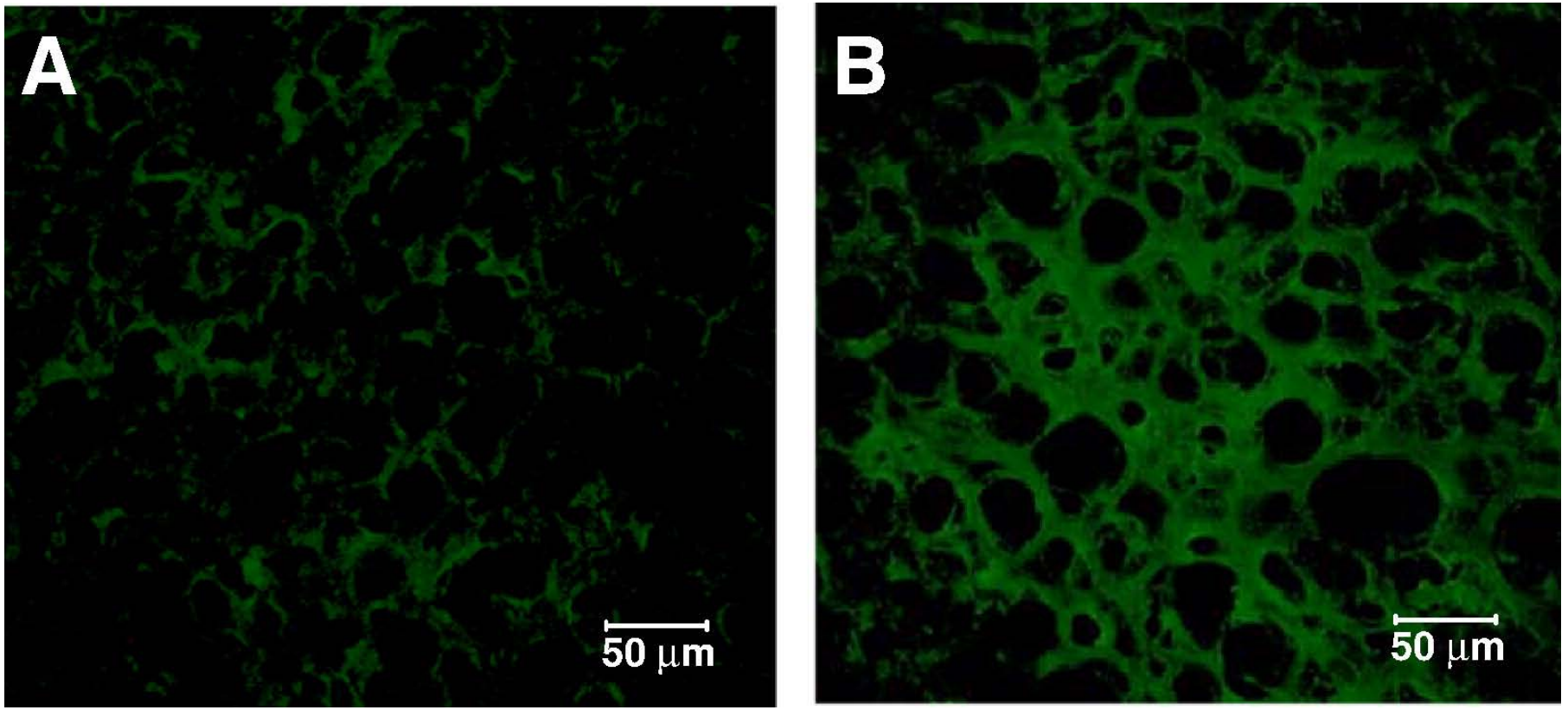




Nystatine or β-cyclodextrin which deplete or sequester cholesterol, results in dose-dependent inhibition of EGFP

# BRAIN TUMOR TRANSFECTION BY TAT-LIPOSOMES

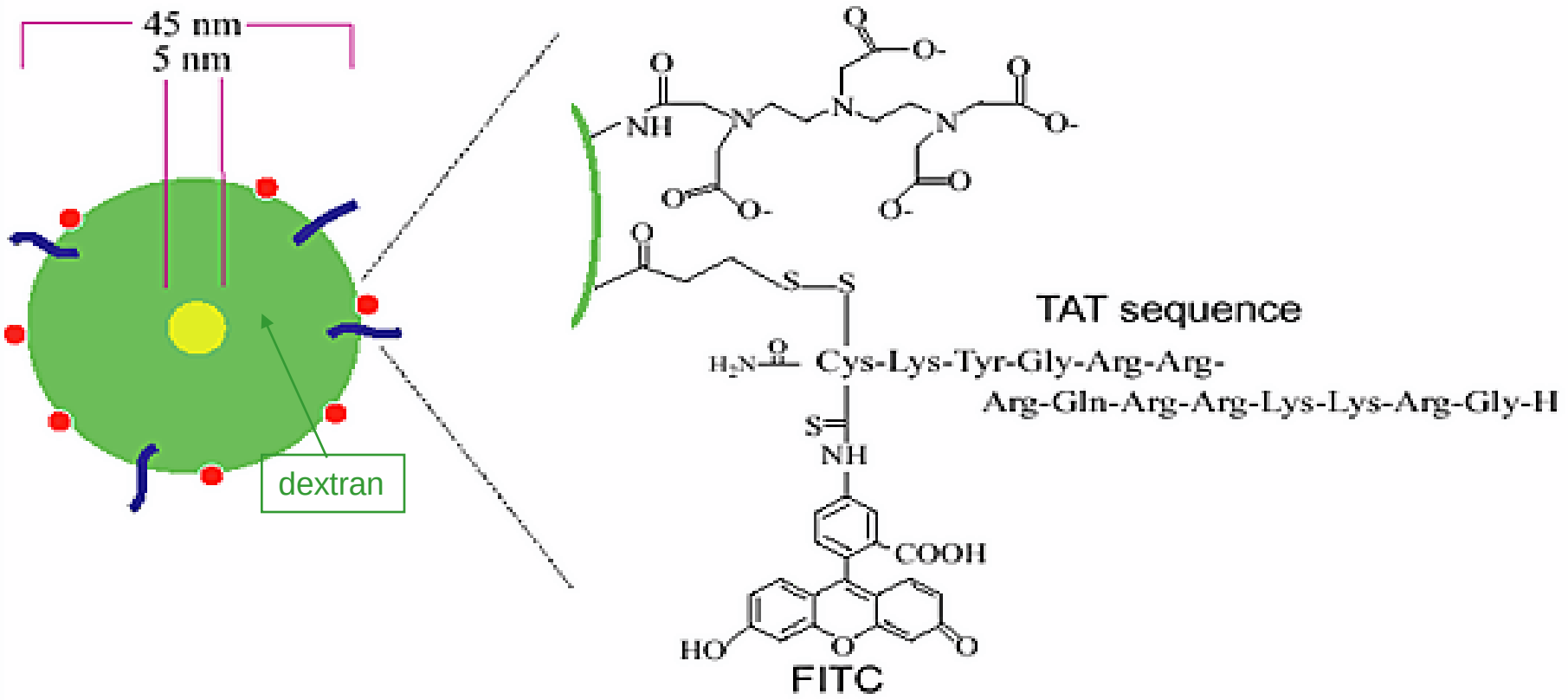
V. Torchilin et al., ADDR 2007




(A) Mouse injected with TATp-free and (B) mouse injected with TATp-modified DNA–liposome complexes as seen following the **GFP** fluorescence on brain tumor coronal sections (200×)

# TAT-DECORATED IRON OXIDE NANOPARTICLES

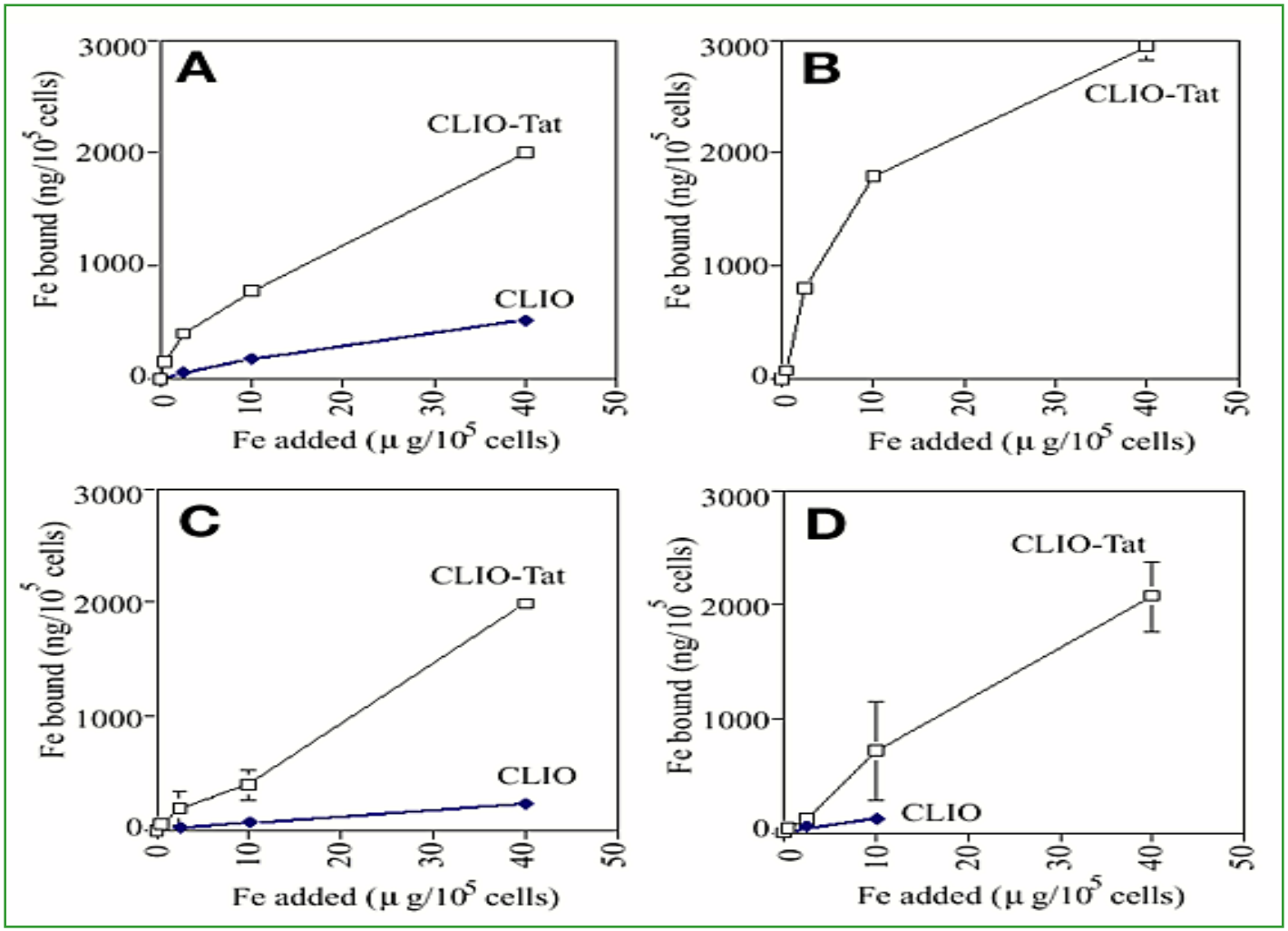
Lewin M et al., Nature Biotechnol, 18, 410-414, 2000




The developed magnetic particle consist of a central superparamagnetic iron oxide core (yellow), sterically shielded by crosslinked dextran (green). The particle core measures 5 nm and the overall particle size is 45 nm. The FITC-derivatized Tat peptide (blue) was attached to the aminated dextran, yielding an average four peptides per particle. The dextran surface was also modified with the chelator DTPA (red) for isotope labeling.

# CELL CAPTURE OF TAT-DECORATED IRON OXIDE NANOPARTICLES

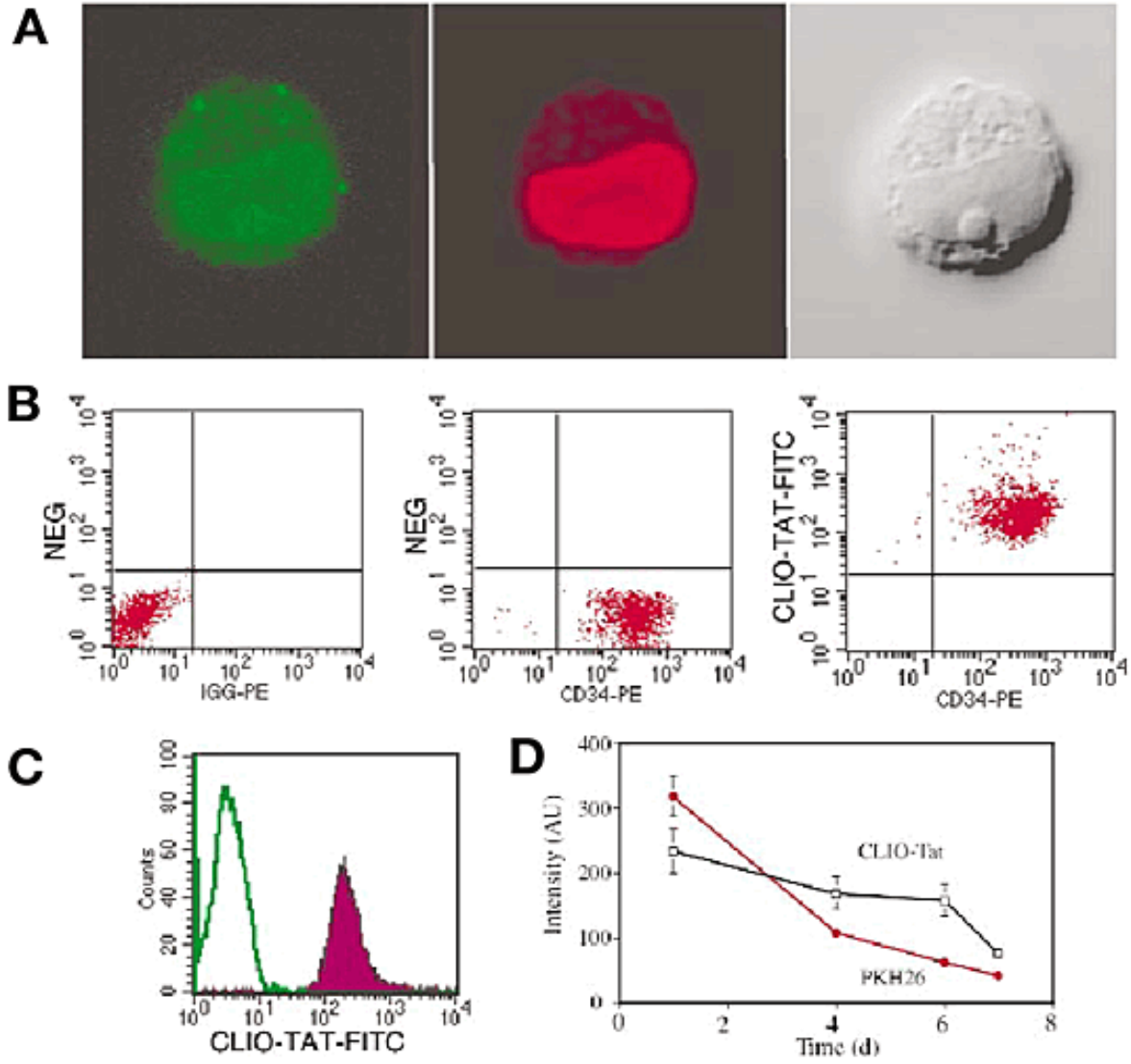
Lewin M et al., Nature Biotechnol, 18, 410-414, 2000




**(A) CD34+ cells (B) mouse neural progenitor cells (C17.2) (C) human CD4+ lymphocytes, and (D) mouse splenocytes.**

# INTRACELLULAR LOCALIZATION AND CELL UPTAKE RETENTION OF TAT-DECORATED IRON OXIDE NANOPARTICLES

Lewin M et al., Nature Biotechnol, 18, 410-414, 2000




(A) Confocal microscopy of CLIO-Tat (green) into CD34+ cell, propidium iodide-labeled nucleus (middle), and using Nomarski optics (right).

(B) Dual-color flow cytometry analysis of CLIO-Tat-labeled CD34 + cells. The y-axis represents the mean intensity of CLIO-Tat-FITC fluorescence, whereas the x-axis represents CD34-PE. NEG on the y-axis (left dot blot) refers to PE-conjugated immunoglobulins control.

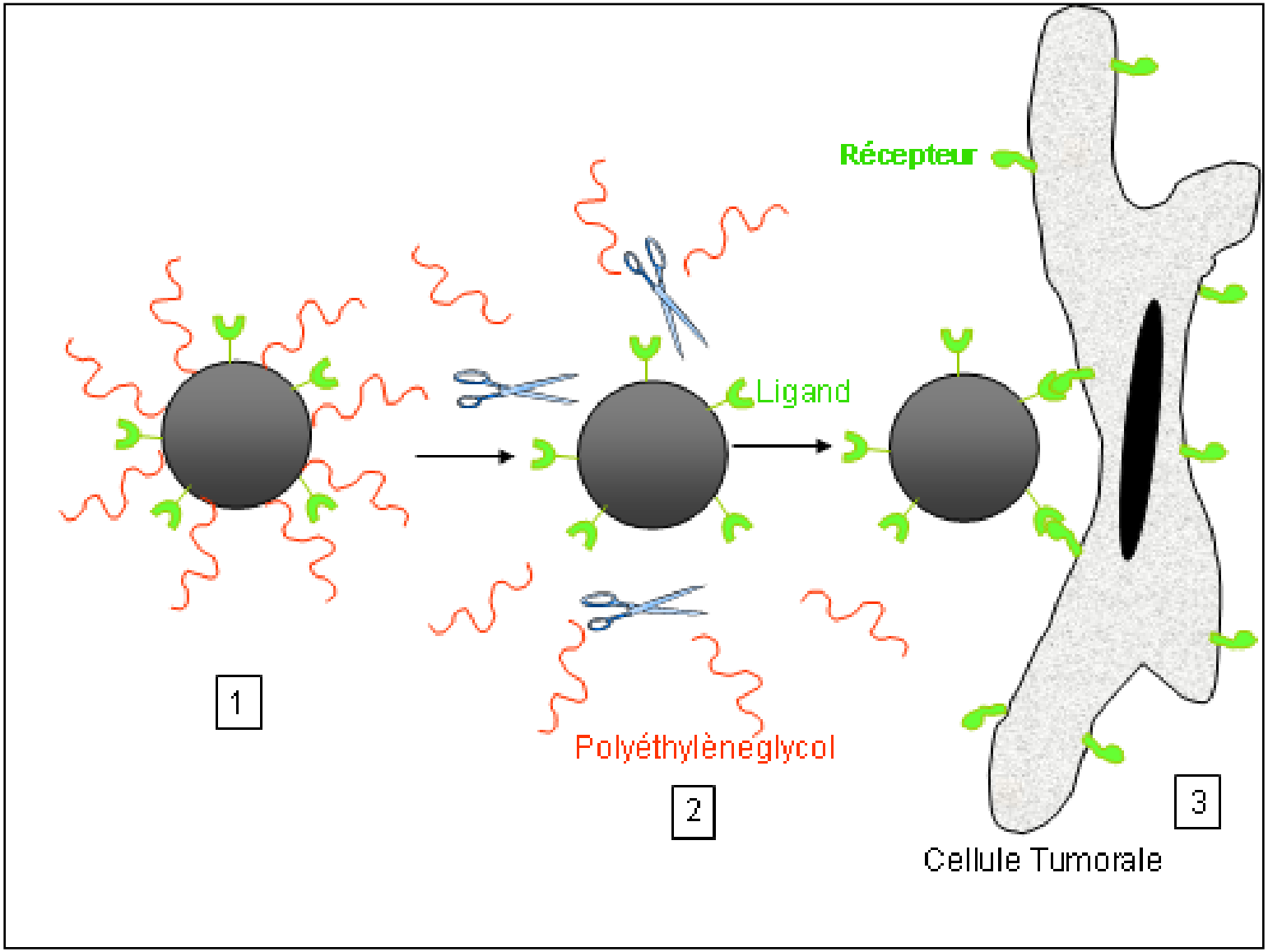
(C) Mean intensity diagram of CLIO-Tat-FITC-labeled cells (right; filled curve) and unlabeled CD34+ cells (left, solid line).

(D) Kinetics of CLIO-Tat retention in CD34+ cells over time compared to the cell membrane marker PKH26.

# CONCEPTION DE NANOVECTEURS « INTELLIGENTS »

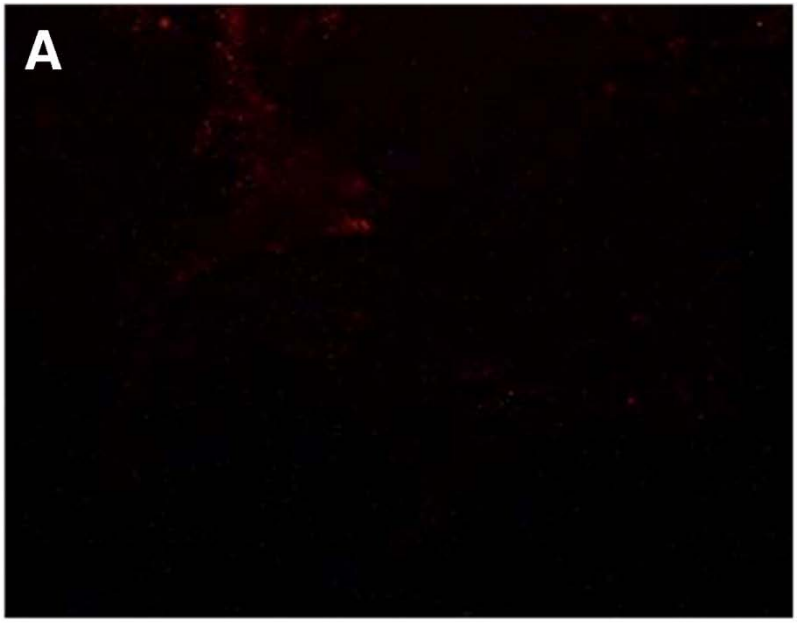
# LE CONCEPT DU LIGAND DEMASQUE

Torchilin V, Adv. Drug Del Rev, 58, 1532-1555 (2006)

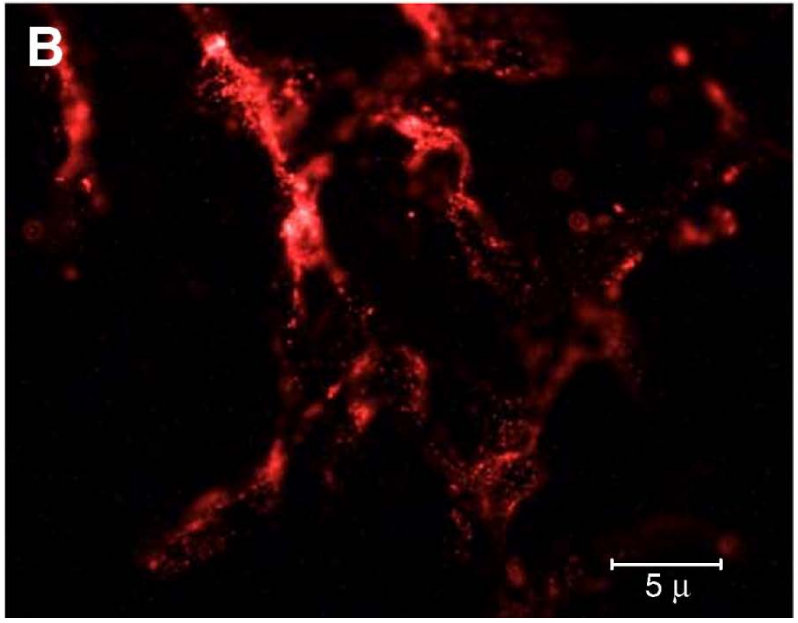


# RHODAMINE-LABELLED TAT DECORATED LIPOSOMES INCUBATED WITH U-87 ASTROCYTOMA CELLS

V. Torchilin et al., ADDR, 2007




Liposomes contain 18% mol pH-non-cleavable PEG–PE (PEG completely prevents TATp-mediated liposome internalization by shielding TATp groups).

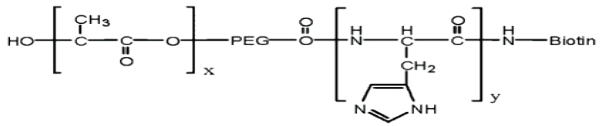



Liposomes contain 18% mol pH-cleavable PEG–Hz–PE and were pre-incubated at pH 5.0 for 20 min (the removal of PEG coat allows for the effective TATp-mediated liposome uptake by the cells).

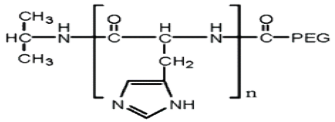
# pH-SENSITIVE MULTIFUNCTIONAL POLYMERIC MICELLES

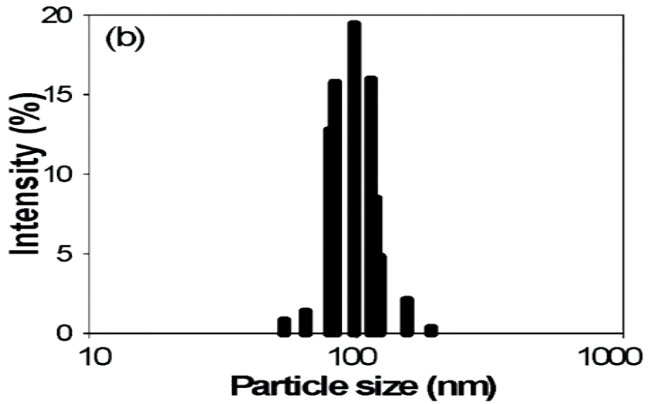
ES. Lee et al., Nano Letters, 5, 325-329 (2005)

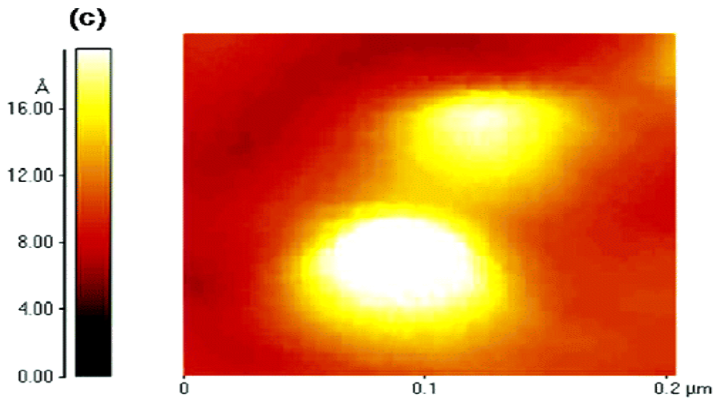
(a) pLLA-*b*-PEG-*b*-polyHis-biotin




polyHis-*b*-PEG

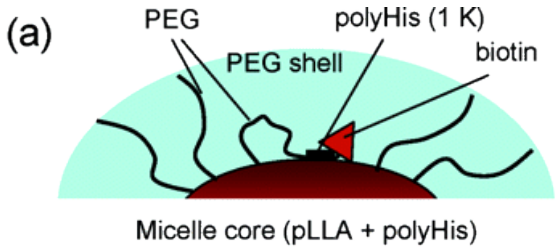


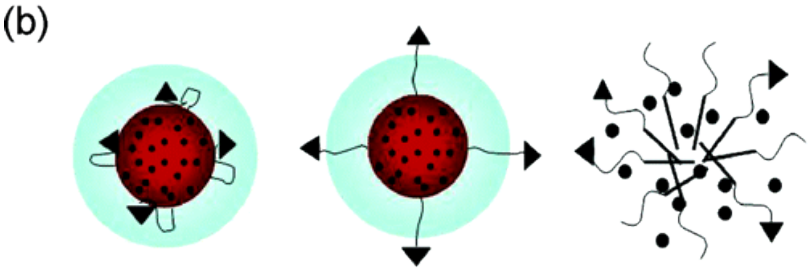




Polylactic acid-PEG-polyhistidine
Polyhistidine is responsible for micelle destabilization at acid pH (pKb histidine = 6.5)

pH-sensitive drug carrier



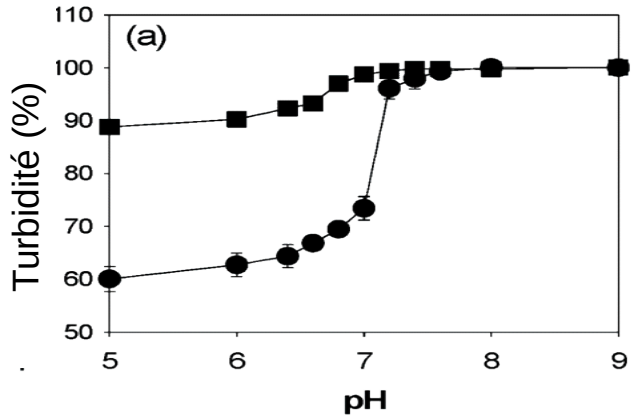




Biotine is exposed and tigger biotine receptor mediated endocytosis

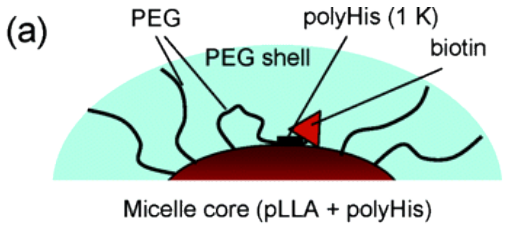
Micelle destabilization

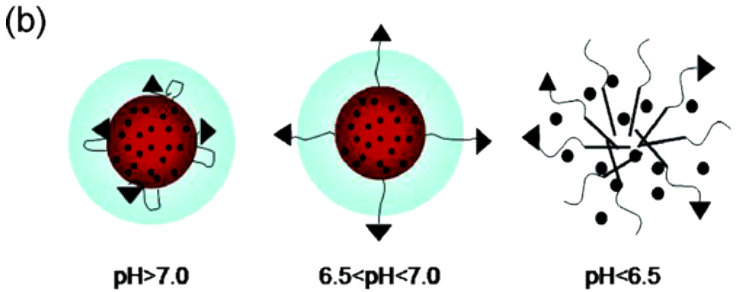
# EVIDENCE FOR BIOTINE EXPOSURE AT pH 6.5-7

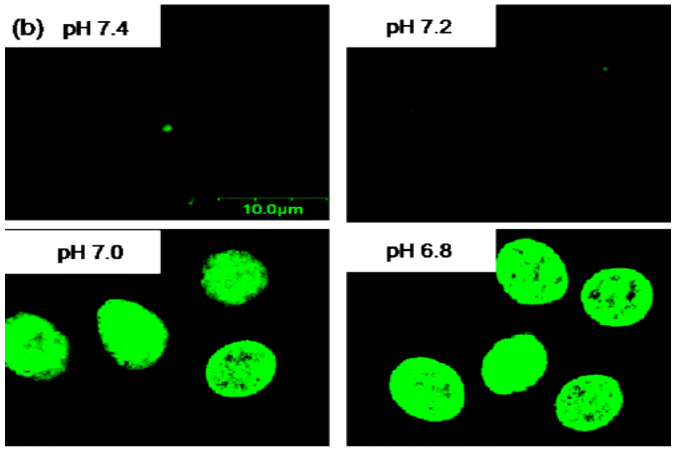
ES. Lee et al., Nanoletters, 5, 325-329 (2005)

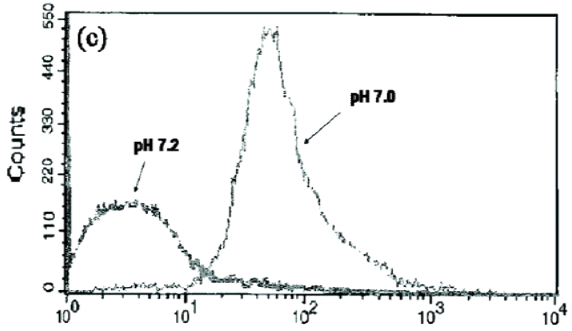



- Biotine pH-sensitive test micelle + avidin
- Control micelle without biotine



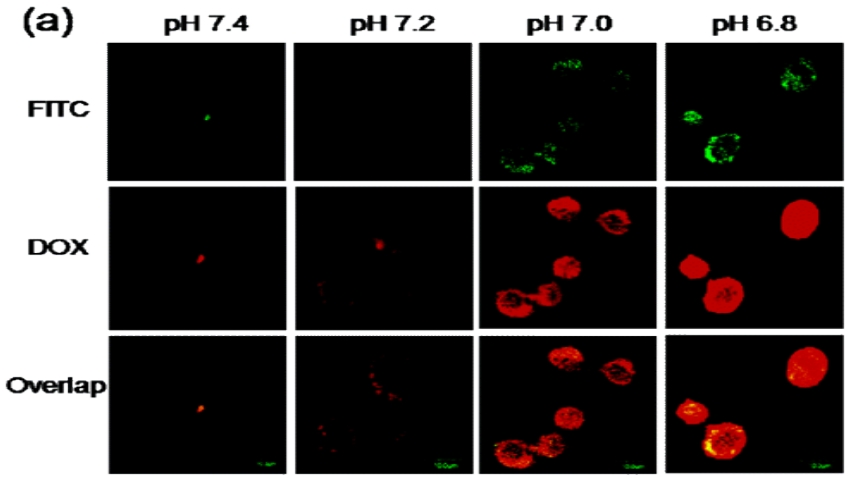



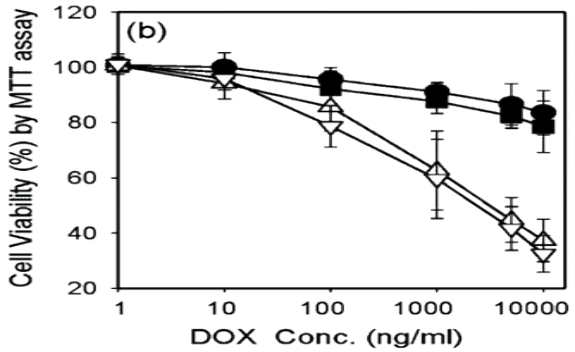

Cell capture of biotine-micelles by MCF-7 cells expressing the Biotine-receptor at different pH. The spread of the dye to the nucleus was attributed to the « proton sponge » effect

# CYTOTOXICITY OF DOXORUBICIN LOADED pH SENSITIVE MICELLES ON MCF-7 CELLS

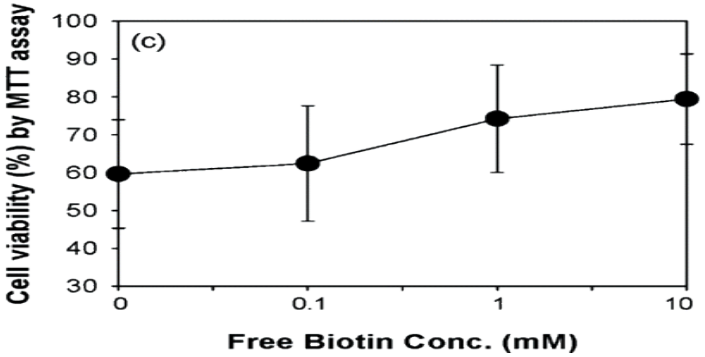
ES. Lee et al., Nanoletters, 5, 325-329 (2005)




Distribution of doxorubicin (red) loaded onto FITC micelles (green). Merge (yellow)




Cytotoxicity of doxorubicin-loaded biotin micelles at pH 7.4 (black), 7.2 (black), 7.0 (white) and 6.8 (white)




Competition between dox biotin micelles and biotin (cytotoxicity at pH 7)

# CONCLUSIONS

- L’interaction des nanovecteurs (nanoparticules, liposomes, micelles…) avec les cellules dépend de la nature du vecteur et de celle de la cellule
-Mais les mécanismes d’internalisation les plus fréquents sont l’endocytose et la phagocytose
- La fonctionnalisation à l’aide de ligands permet d’accroitre la pénétration intracellulaire des vecteurs
- Les nanotechnologies permettent donc de favoriser la pénétration intracellulaire de molécules qui ne diffusent pas spontanément à l’intérieur de la cellule