



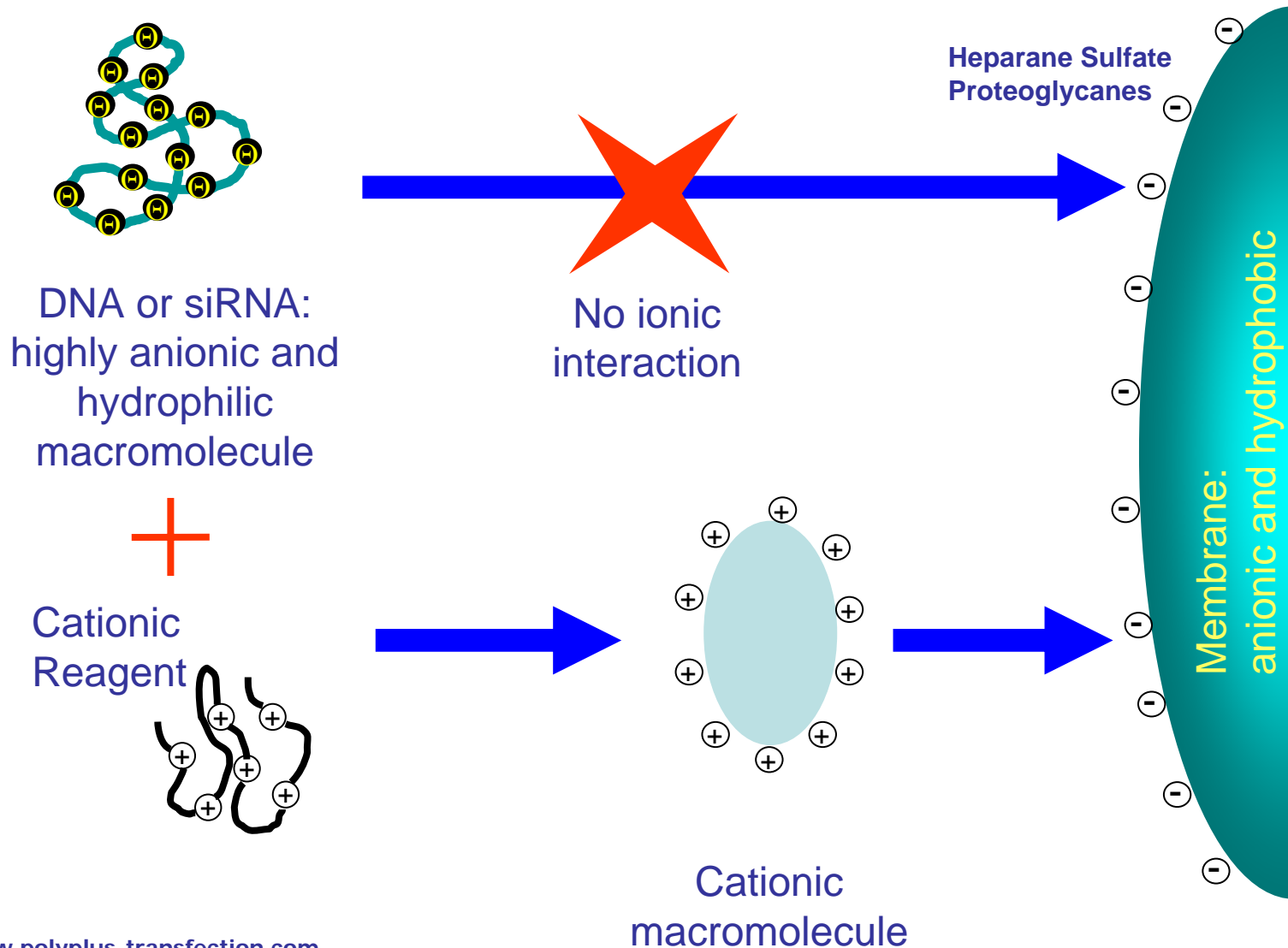
***Advance in DNA & siRNA delivery
in vitro & in vivo***

Alain Cuzange
2008

Seminar Content

- Introduction
 - How transfection is working?
- DNA delivery *in vitro*
 - Why polymers are better?
- siRNA delivery *in vitro*
 - How to reach a successful silencing?
- DNA & siRNA *in vivo* delivery
 - Polyplus transfection solutions

Why do we need transfection reagent?

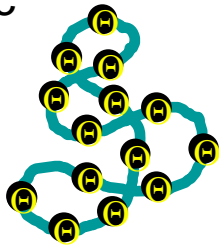


What is transfection ?

- plasmids
- oligonucleotides

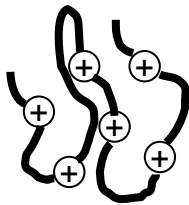
- siRNA oligo

nucleic acids

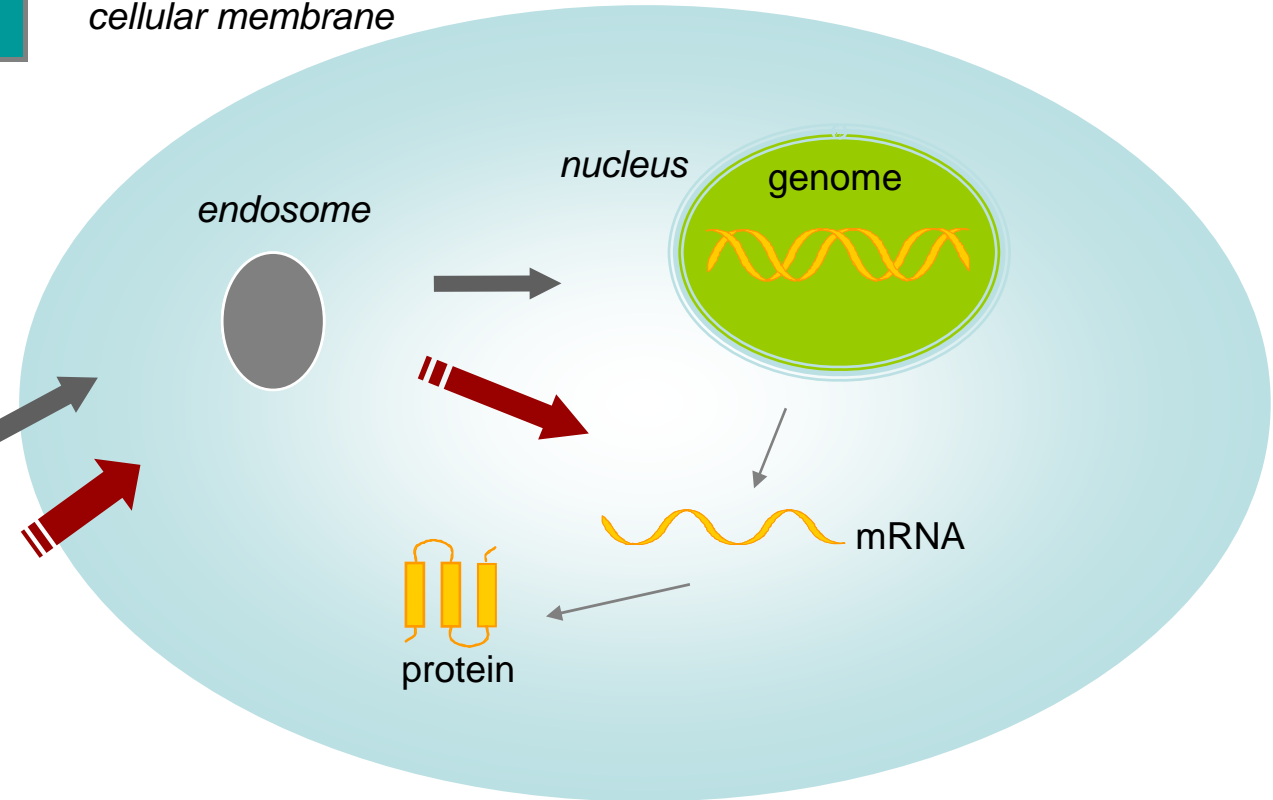


+

Cationic Reagent



cellular membrane



Gain or loss of function

3 Barriers to Transfection

Membrane	+	-
Endosome	+	+/-
Nucleus	+	+

Transfect.

Easy

Hard

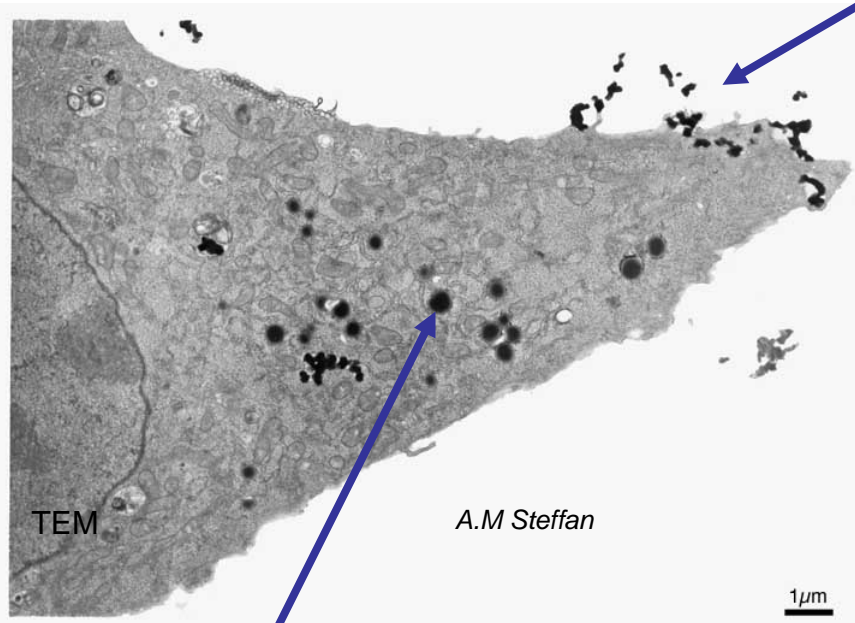


HeLa

Lymph T

Electron microscopy pictures

Transfection of NIH 3T3 cells

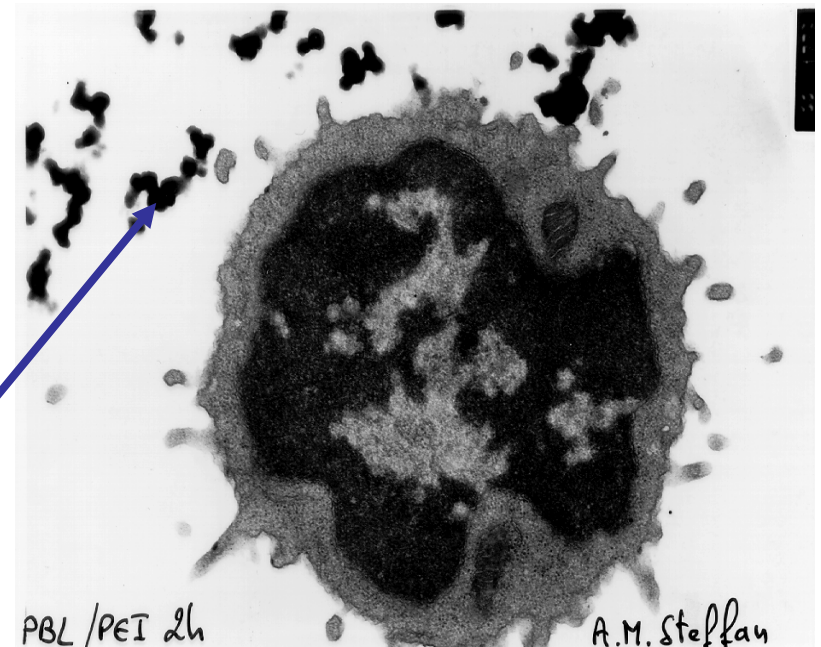


Transfection complexes in interaction with cell membrane

Complexes in endosomes

Complexes do not interact with cell membrane

Transfection of lymphocytes



3 Barriers to Transfection

Membrane	+	-	+
----------	---	---	---

Endosome	+	+/-	+
----------	---	-----	---

Nucleus	+	+	-
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Transfect.	Easy	Hard	Hard
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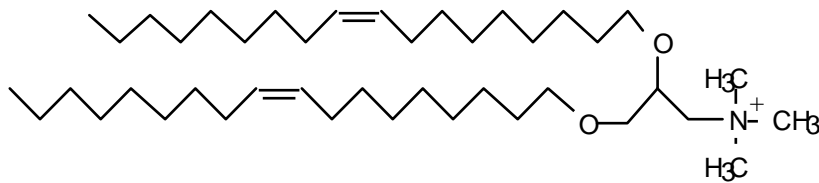
HeLa

Lymph T

Neurons

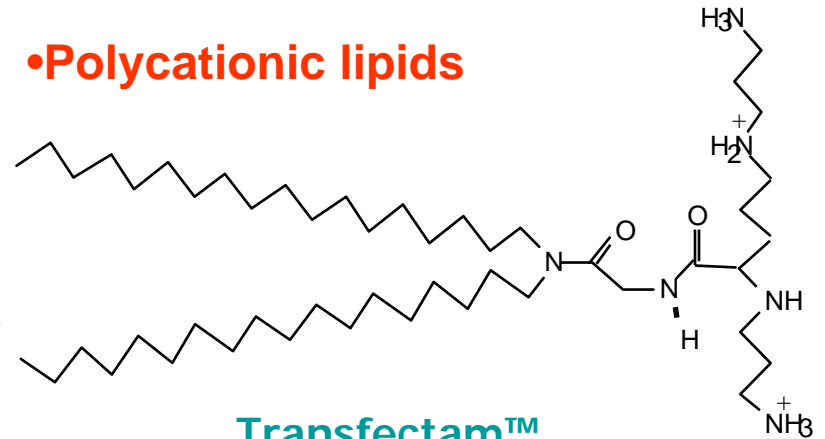
Transfection reagents

•Monocationic lipids



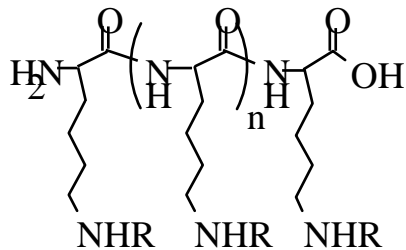
DOTMA (Lipofectin®)

•Polycationic lipids

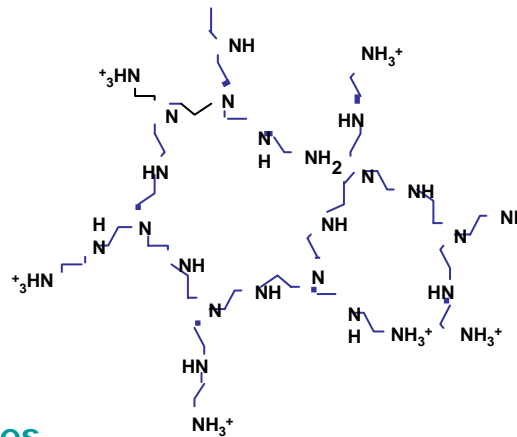


Transfectam™

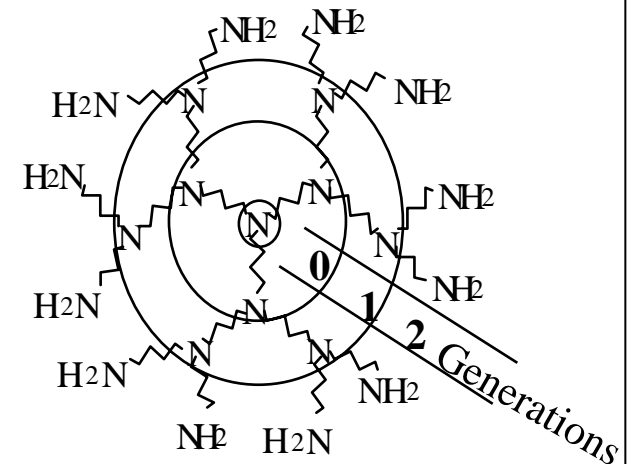
•Cationic polymers



Polylysine and derivatives



Polyethylenimine



PAMAM Dendrimer



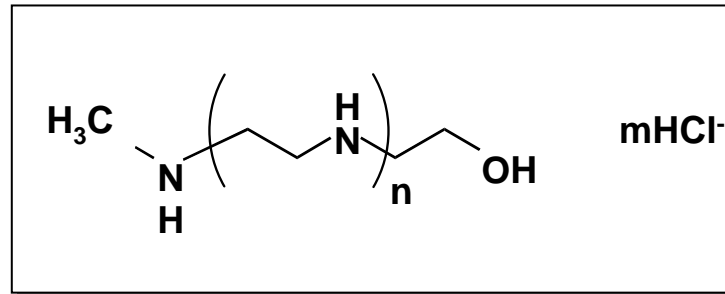
DNA transfection *in vitro*

DNA delivery *in vitro*: jetPEI™

What is jetPEI?

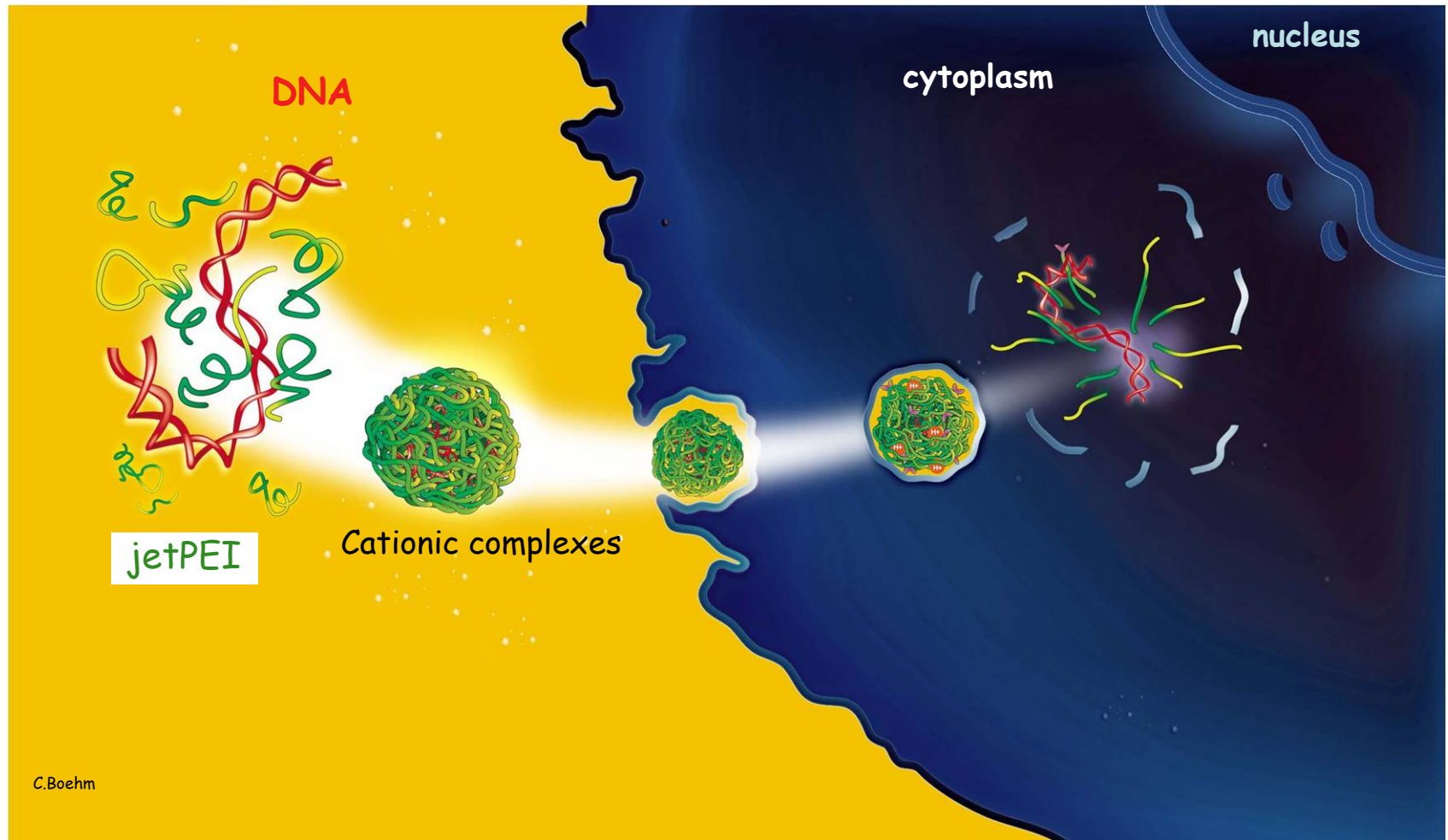
Linear Polyethylenimine

→ it is not a lipid – it is a **polymer**



- Contains only amine functions
- Interact with DNA and lead to complexation
- High transfection efficiencies (Proton sponge)
- Exclusively licensed for transfection

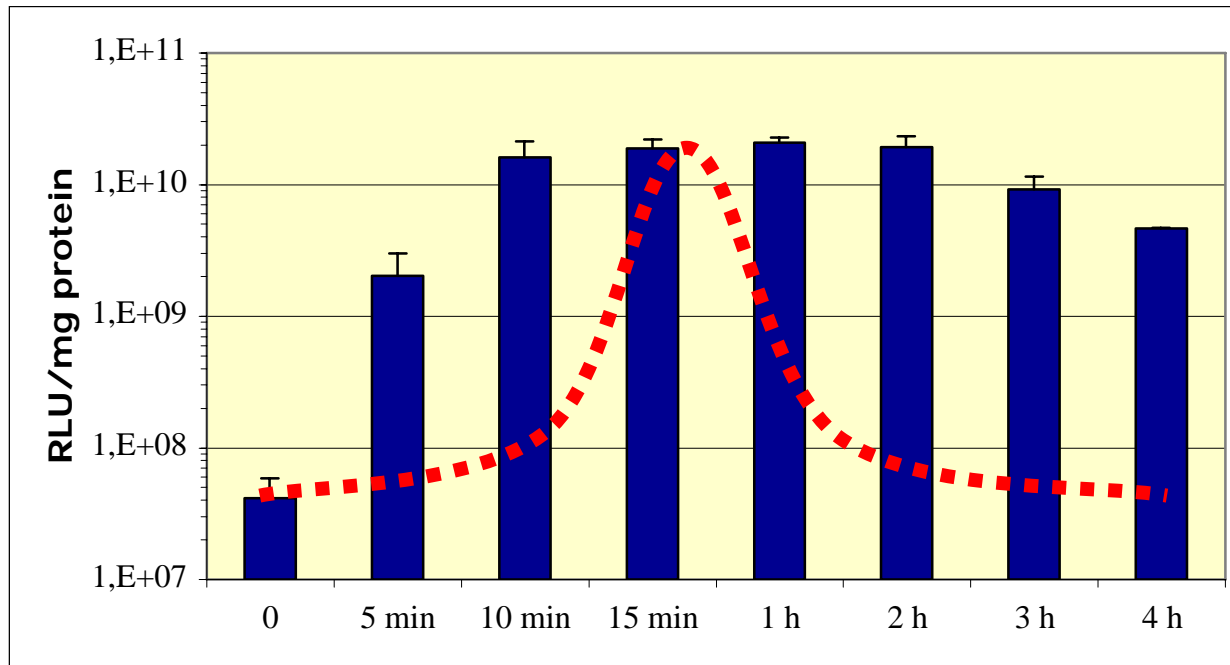
DNA delivery *in vitro*: jetPEI



jetPEI: Complexes Formation

HeLa cells

Effect of incubation time

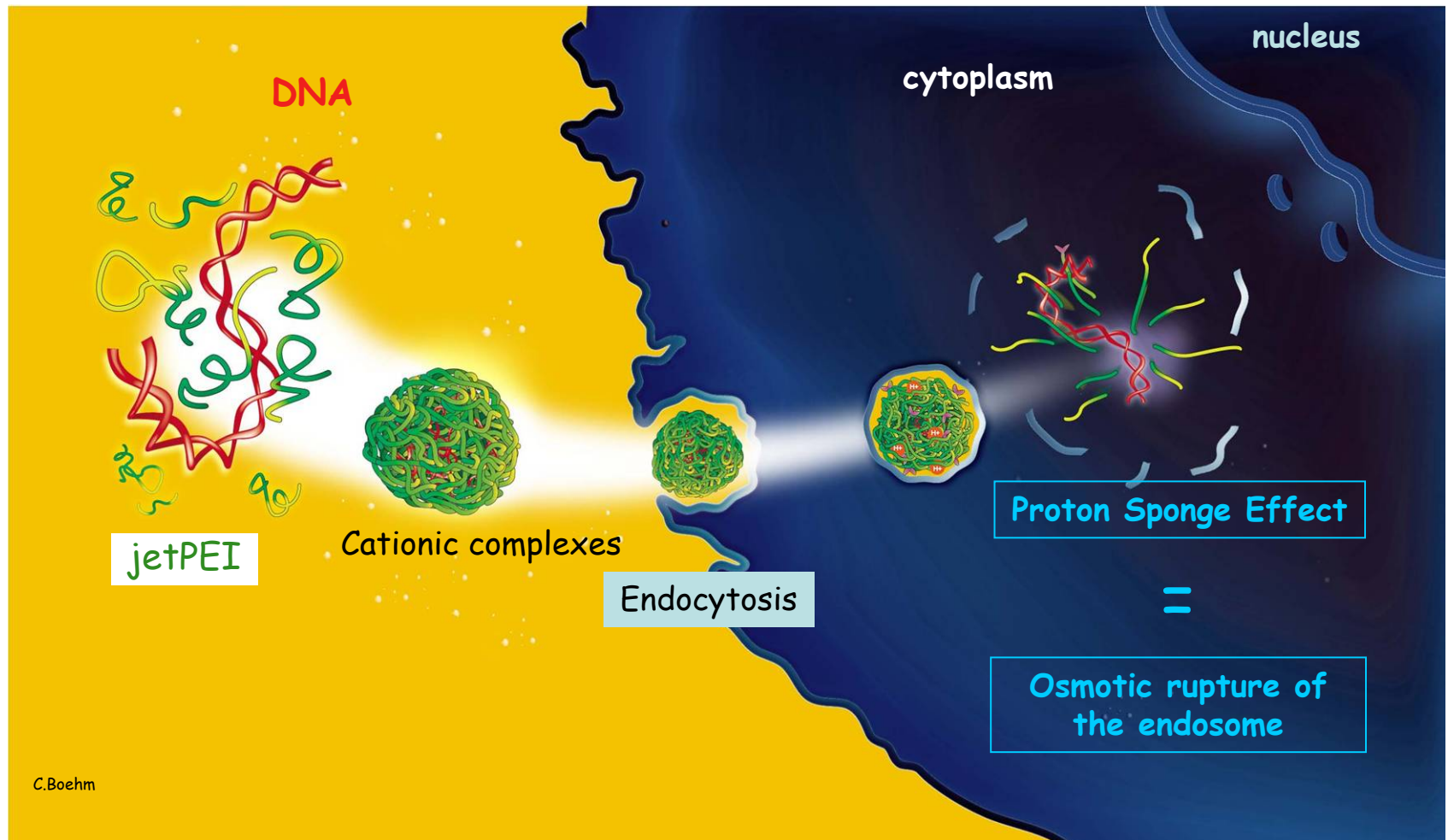


1 μ g of pCMVLuc, N/P 5



jetPEI complexes more stable than Lipid based complexes

DNA delivery *in vitro*: jetPEI

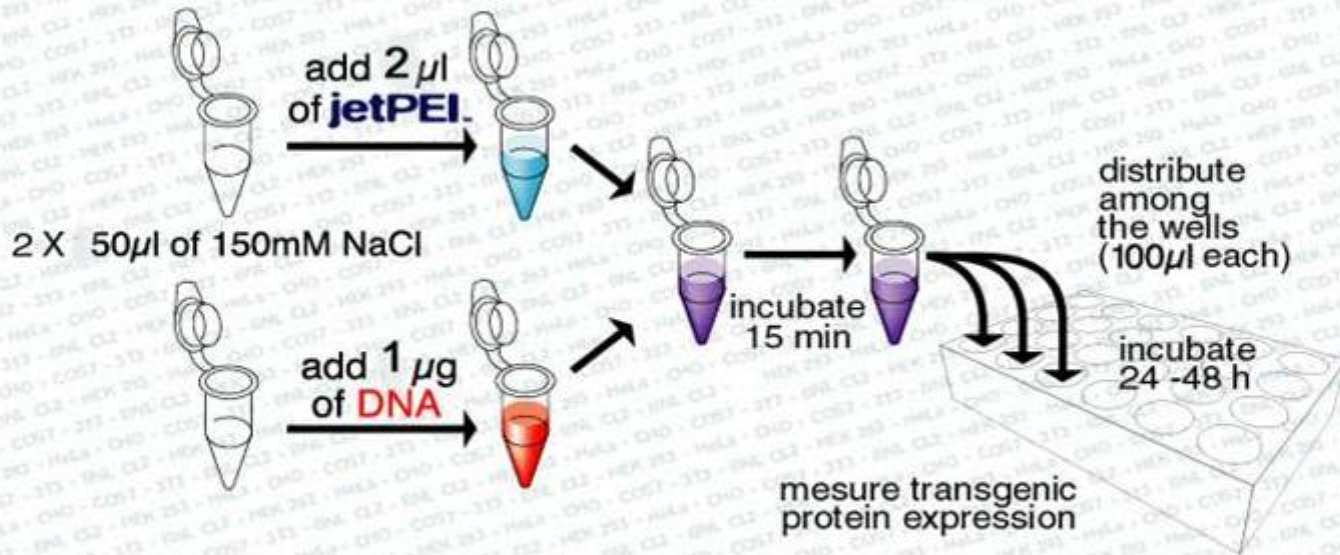


Fast and efficient release of DNA

jetPEI™: Transfection protocol

2 μ l of jetPEI for 1 μ g of DNA

+ serum

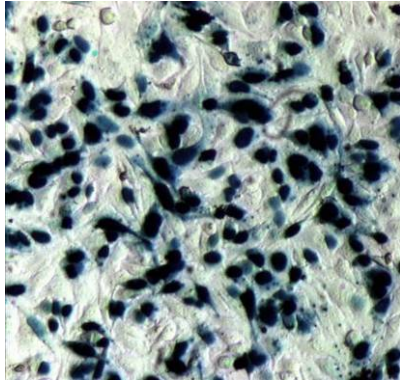


1 ml of jetPEI = 500 transfections in 24-well plates
or = 2500 transfections in 96-well plates

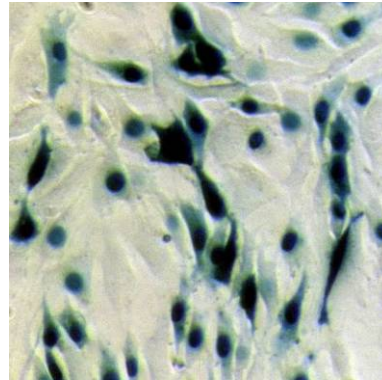
jetPEI™ transfection efficiency

β-galactosidase

HeLa



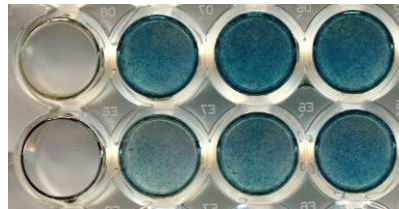
NIH-3T3



BHK

control

pCMVLacZ

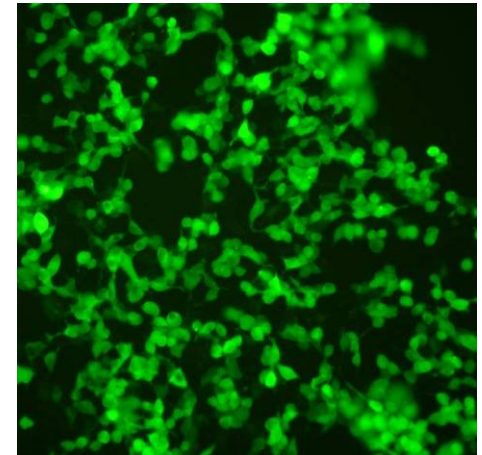


N/P = 5

N/P = 3

GFP

HEK-293



Cells transfected in 24-well plates with jetPEI (N/P = 5) with a β-galactosidase-expressing plasmid (pCMVLacZ) or pEGFP plasmid. Expression was visualized by X-gal staining after 24 h or fluorescence microscopy

jetPEI derivatives

jetPEI-Macrophage

jetPEI-Mannose / Mannose receptor

Applications : Macrophages

jetPEI-Hepatocyte

jetPEI-Galactose / Galactose receptor

Applications : Hepatocytes

jetPEI-HUVEC

jetPEI-Glucose

Applications : HUVEC cells

jetPEI-Fluo & Biotin

Applications : Transfection positive control

jetPEI™ transfection: database

- **Cell database**

- More than 150 cell lines efficiently transfected

- Epithelial-like cells, Fibroblast-like cells, Endothelial-like cells, Hepatocyte-like cells, Neuroblastoma, Leukemia cells / lymphoblasts, Melanoma, Monocytes / macrophages, Myotubes / myoblasts / muscle cells, Chondrocytes, Glioblastoma

- Primary Cells

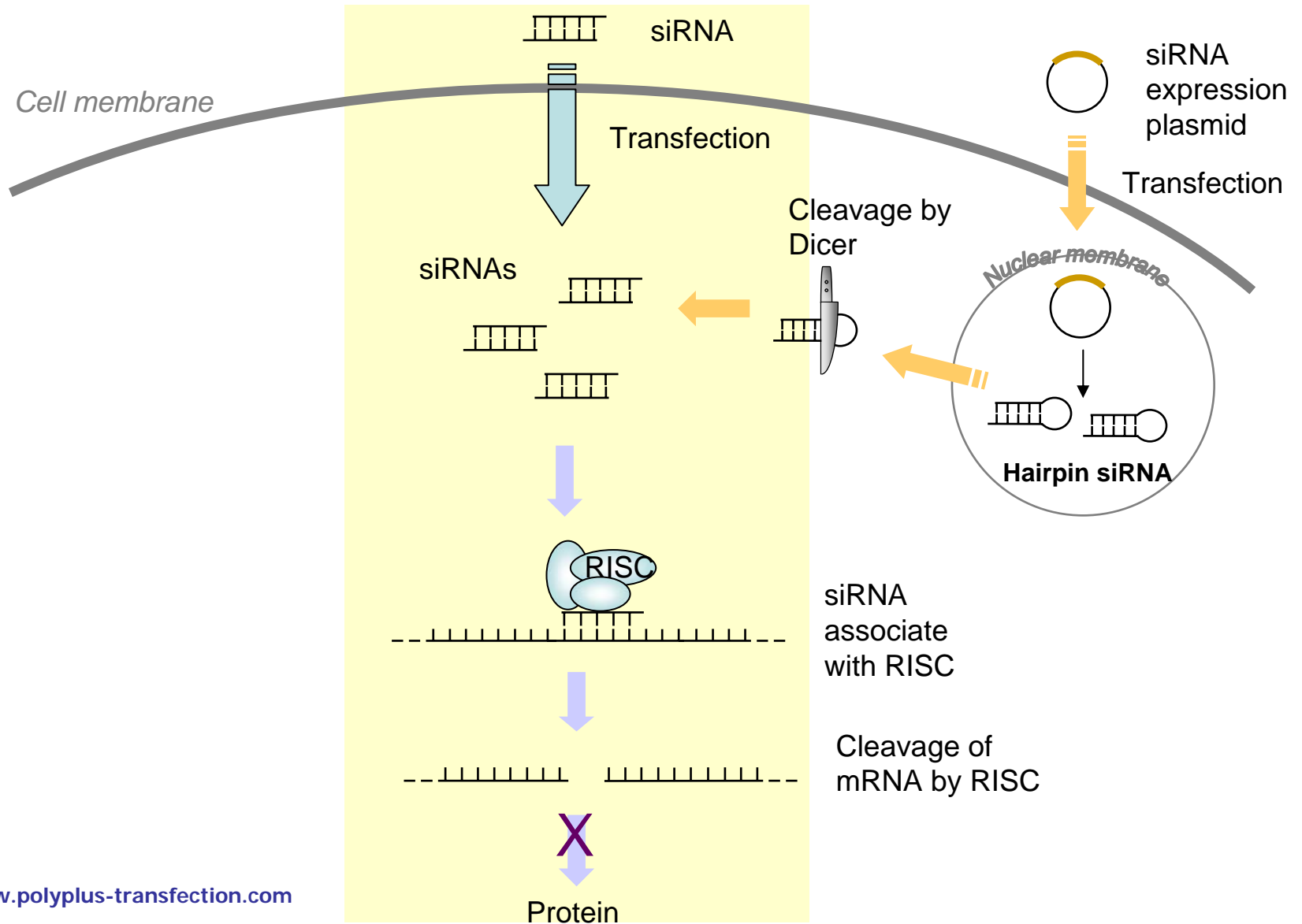
- **Publication data base**

More than 500 publications → see new search web site



siRNA transfection *in vitro*

Mechanism of RNA interference



2 Barriers to siRNA Transfection

Membrane	+	-	+
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Endosome	+	+/-	+
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Nucleus	+	+	-
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Transfect.	Easy	Hard	Easier
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HeLa

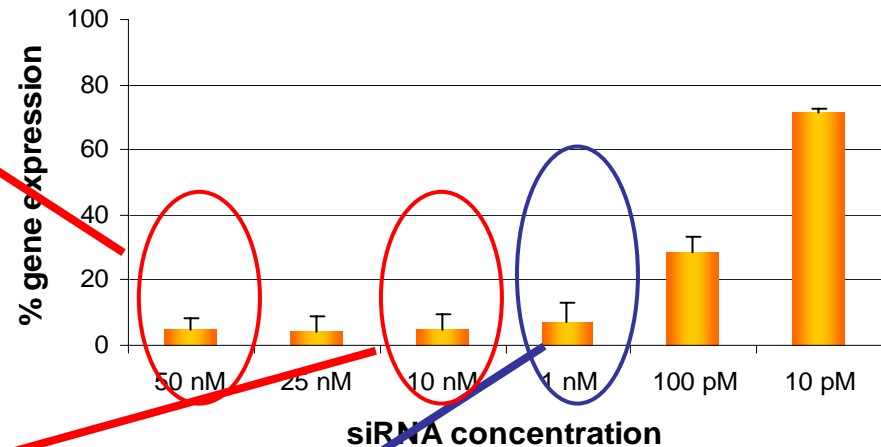
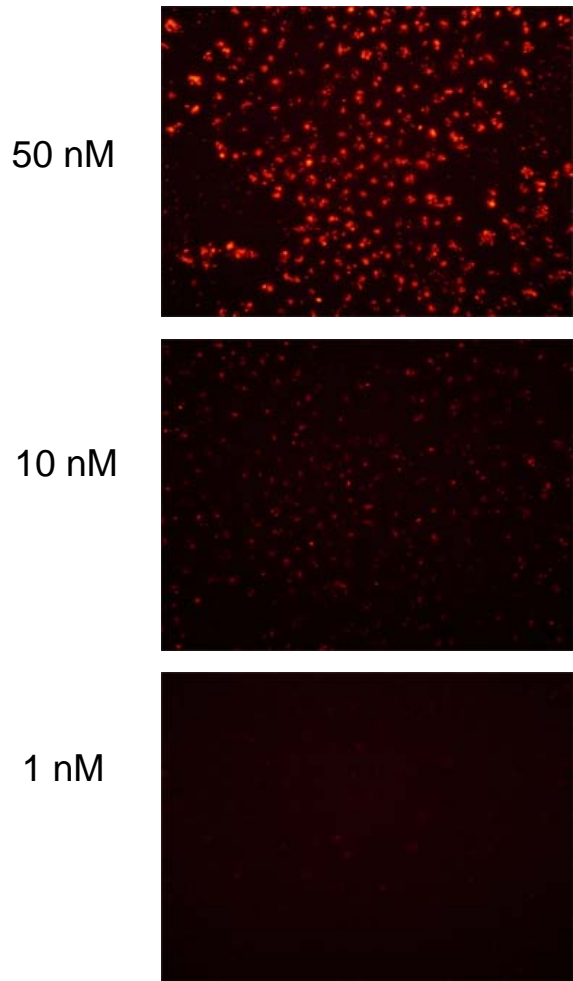
Lymph T

Neurons

Visualisation and gene knockdown

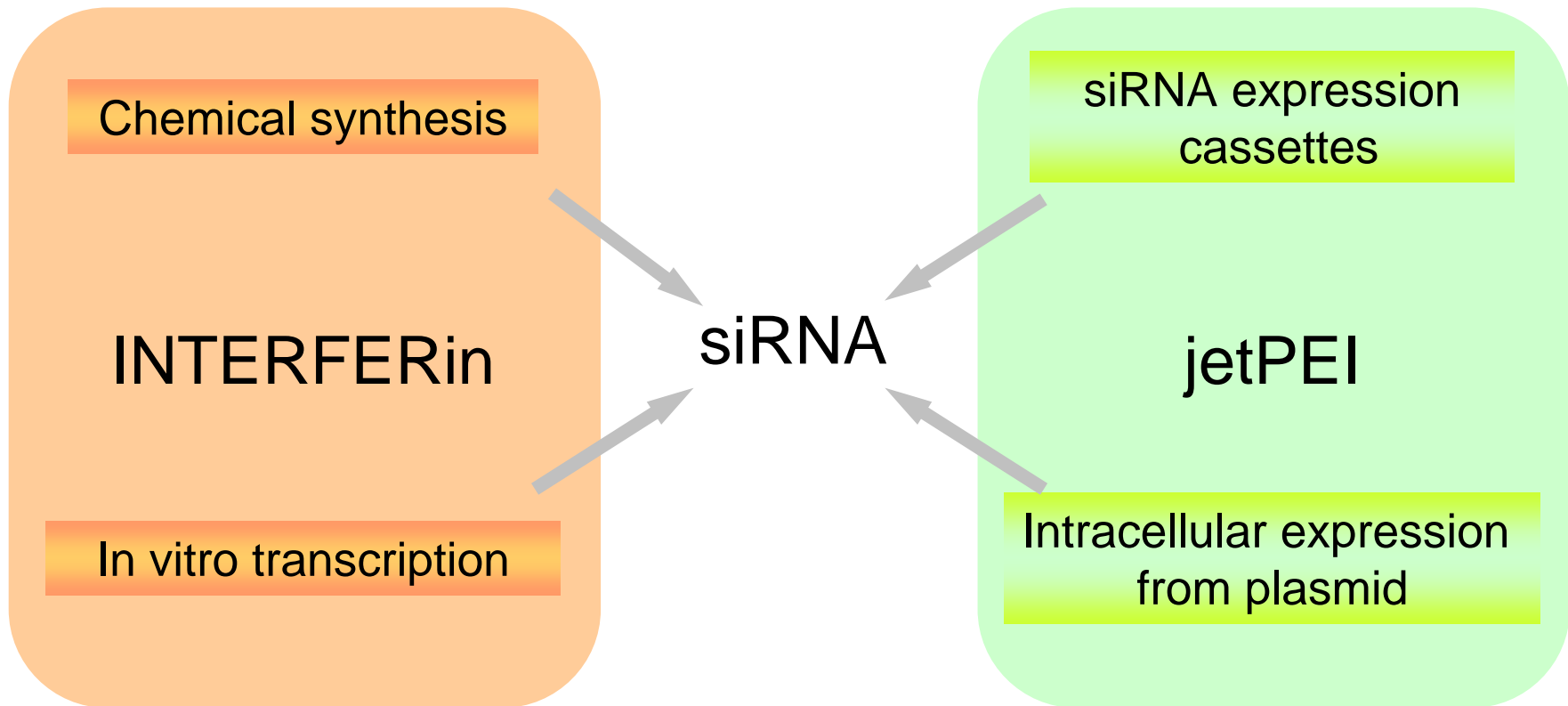
Rhodamine siRNA

Gene silencing efficiency



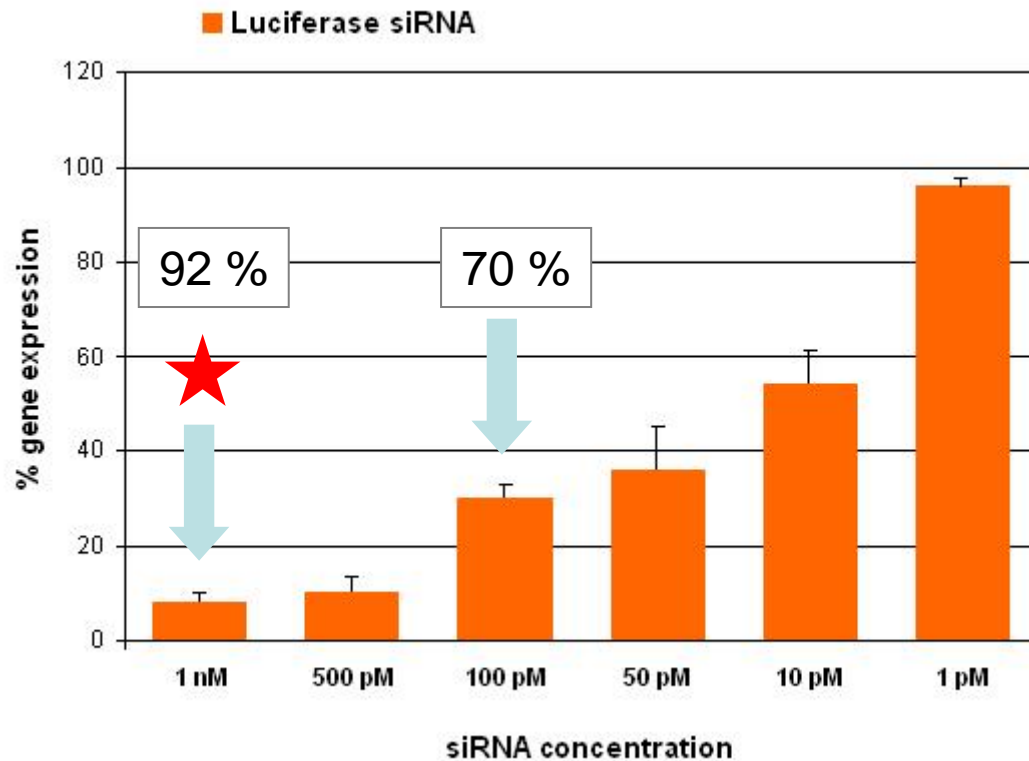
- **Detected transfection efficiency does not correlate with gene silencing**
- **Use a functional siRNA rather than fluorescent siRNA for low siRNA concentration experiment**

siRNA: Different origins



Efficient at low siRNA concentration

Great silencing down to **picomolar** siRNA concentration



- A549-GL3Luc cells (24-well plate)
- Luciferase

INTERFERin™: Silencing efficiency

Adherent (1 nM siRNA)		
A549-Luc	Luciferase	> 90%
Caski	GAPDH	
HeLa	GAPDH	
MCF7	GAPDH	
NIH-3T3	Vimentin	
RAW 264.7	Eg5	
SiHa	GAPDH	
Suspension (5 nM siRNA)		
K562	GAPDH	> 80%
THP-1	GAPDH	

INTERFERin™: Other positive feedbacks

- **Neurons**

- Embryonic neurons
- Adult hippocampal neurons
- Neuroblastoma cell line (SK-N-SH)
- Chick brain organotypic culture

- **Hepatocytes**

- Rat hepatocarcinoma (DU145)
- Primary hepatocytes

- **Muscle cell types**

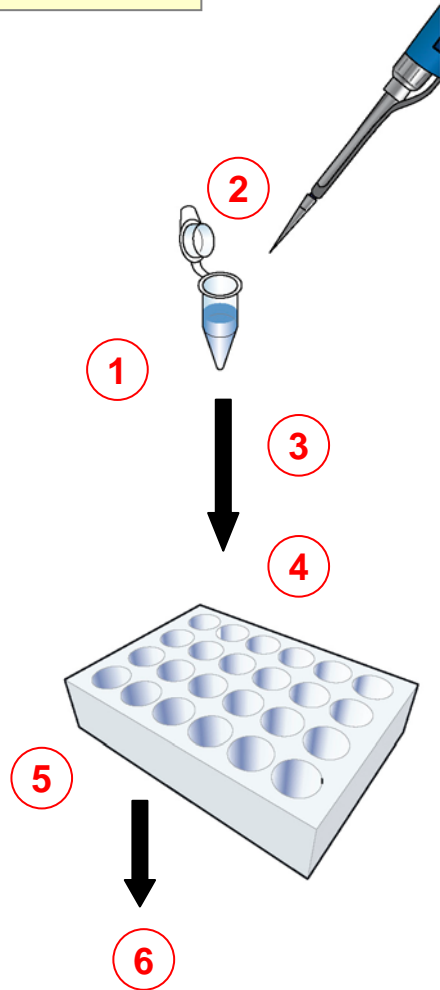
- cardiomyocytes,
- differentiated C2C12
- smooth muscle cells types

- **Endothelial cells**

- Human saphenous vein endothelial cells
- Rat aortic endothelial cells

Extremely simple protocol

INTERFERin™



Protocol for cells in 24-well plate

- ① **siRNA (1 nM) in serum-free medium**
- ② **Add INTERFERin™**
- ③ **Vortex and incubate 10 min at r.t.**
- ④ **Add to well containing cells in complete medium**
- ⑤ **Incubate for 48 hours at 37° C**
- ⑥ **Gene silencing assay**



DNA & siRNA *in vivo* delivery



Polyplus-transfection solutions

DNA and shRNA in vivo

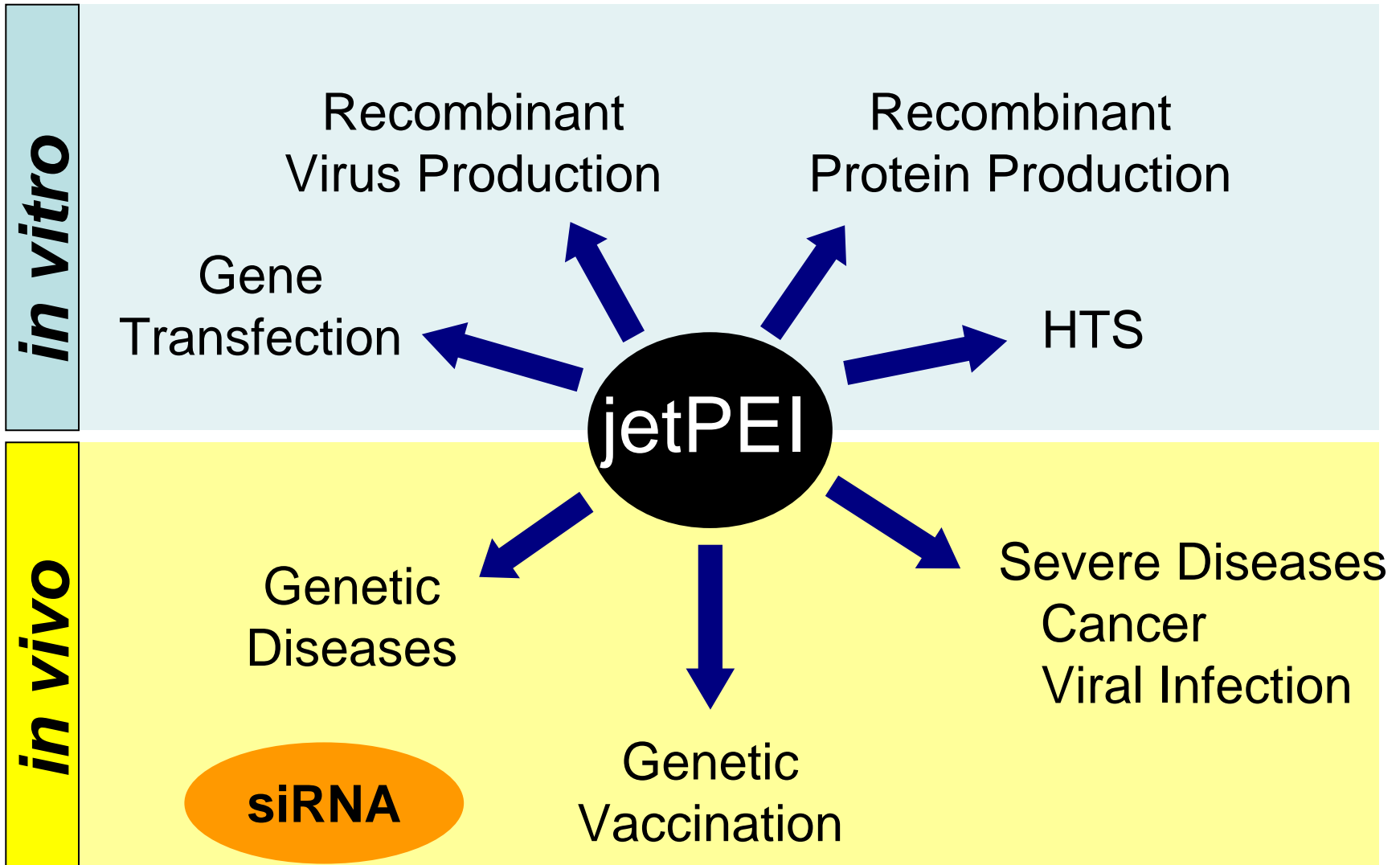
in vivo jetPEI

siRNA in vivo

in vivo jetPEI (except brain)

jetSI 10mM (brain)

jetPEI: Applications





in vivo DNA delivery

in vivo DNA delivery: jetPEI vs. *in vivo*!!!

Similarities

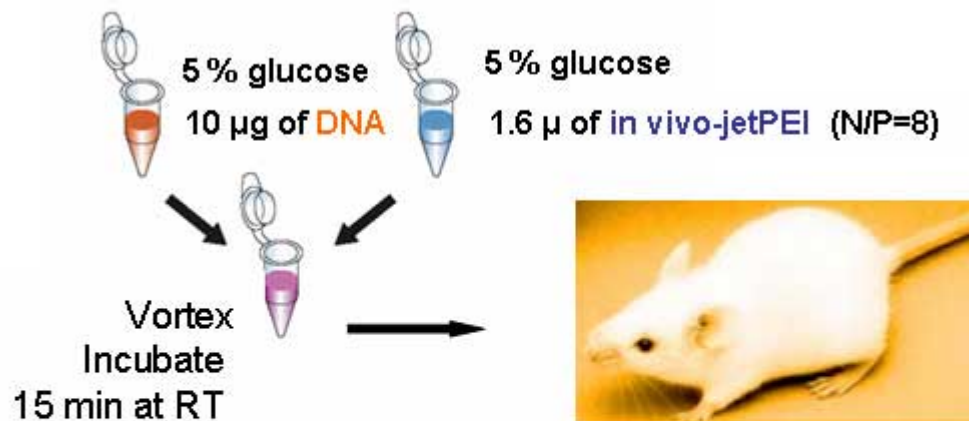
- Same active linear PEI (same size/mass)
- Acts through proton sponge effect
- Fully synthetic
- Animal-free origin

Differences between *in vivo* jetPEI and jetPEI:

- *In vivo* jetPEI is more concentrated
- *In vivo* jetPEI is controlled for endotoxin
- *In vivo* jetPEI/DNA complexes are stable at least 24h in glucose (in vitro 2h only)
- Can be produced on cGMP quality
- ...

In vivo-jetPEI™ protocol

- Very simple
- Prepare the complexes in 5% glucose final concentration

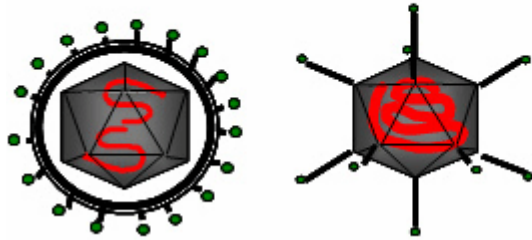


Analysis

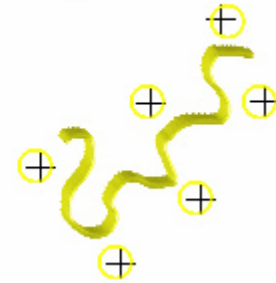
- Bioluminescence
- Luciferase expression per organ

Virus vs. *in vivo* jetPEI

Virus



in vivo-jetPEI™



+++

Efficiency/
specificity

+

3 - 30

gene size
(kbp)

> 400

Yes

Immune response

none

Yes (L2 – L3)

Lab requirement

none

Less

Safety

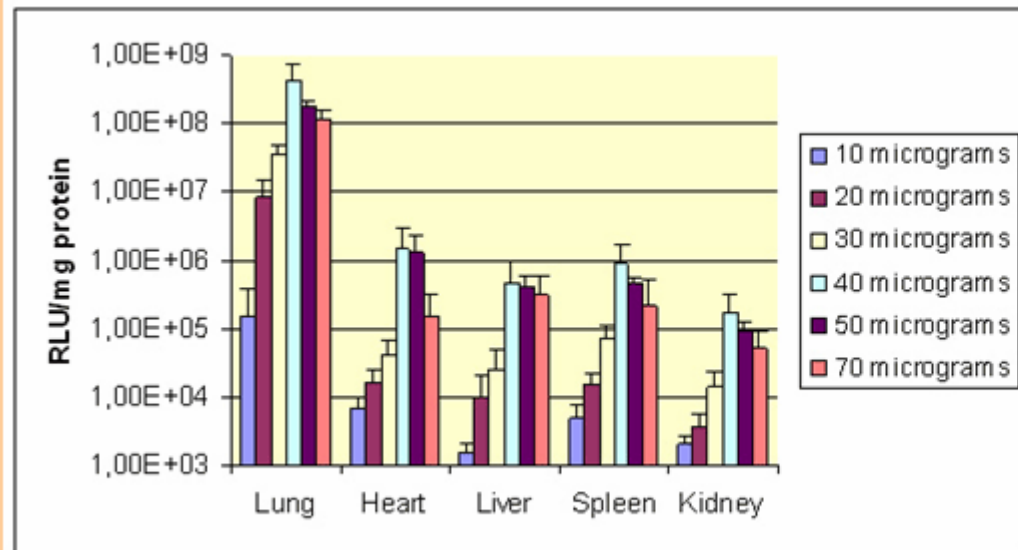
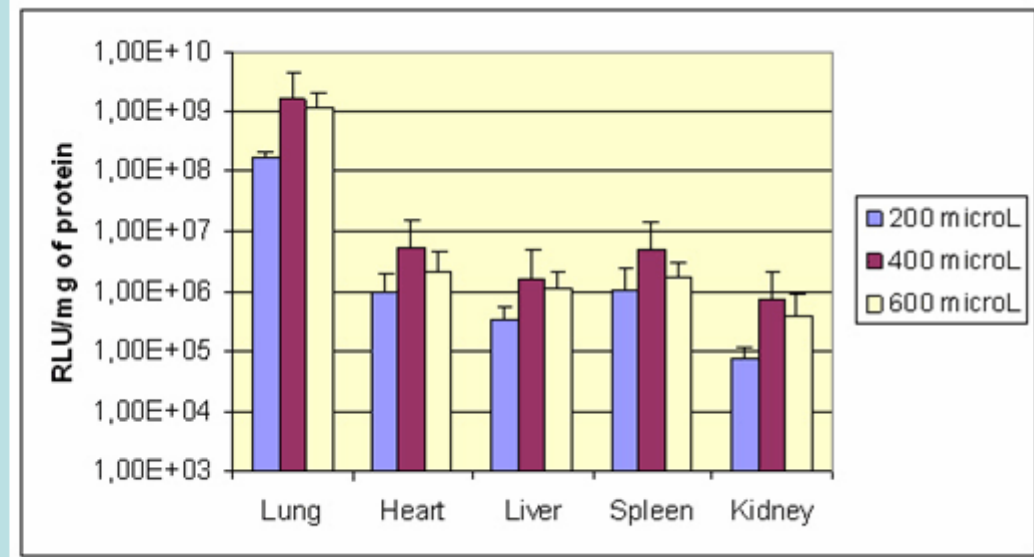
more

In vivo DNA transfection: Retro-Orbital

Volume effect

pCMVLuc/in vivo jetPEI™
NP8 50 µg Gluc5%
24h n=4

Optimum = 400 µl



Dose effect

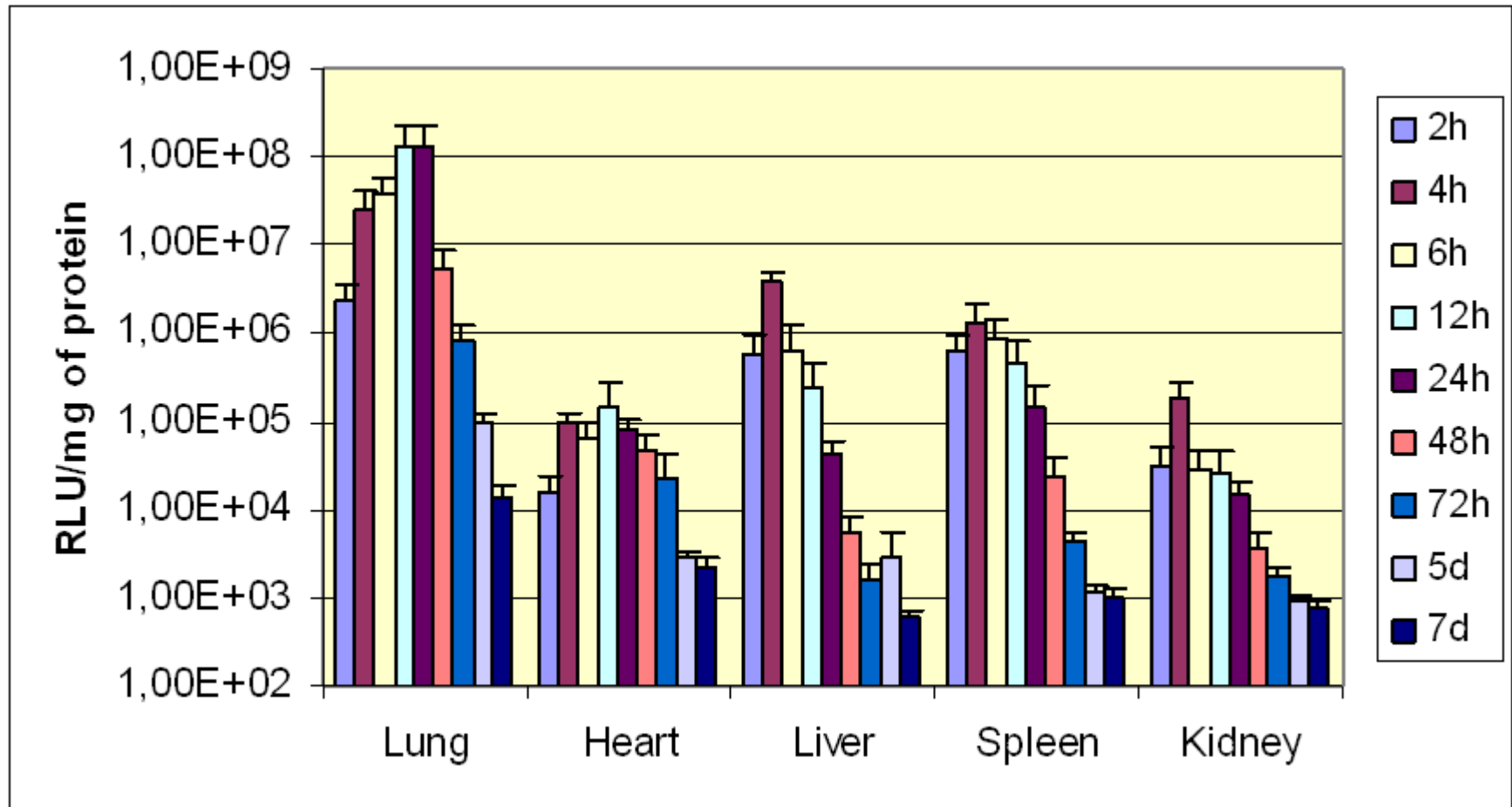
pCMVLuc/in vivo jetPEI™
NP8 400 µl Gluc5%
24h n=4

Optimum = 40 µg

In vivo DNA transfection: Retro-Orbital

Kinetic

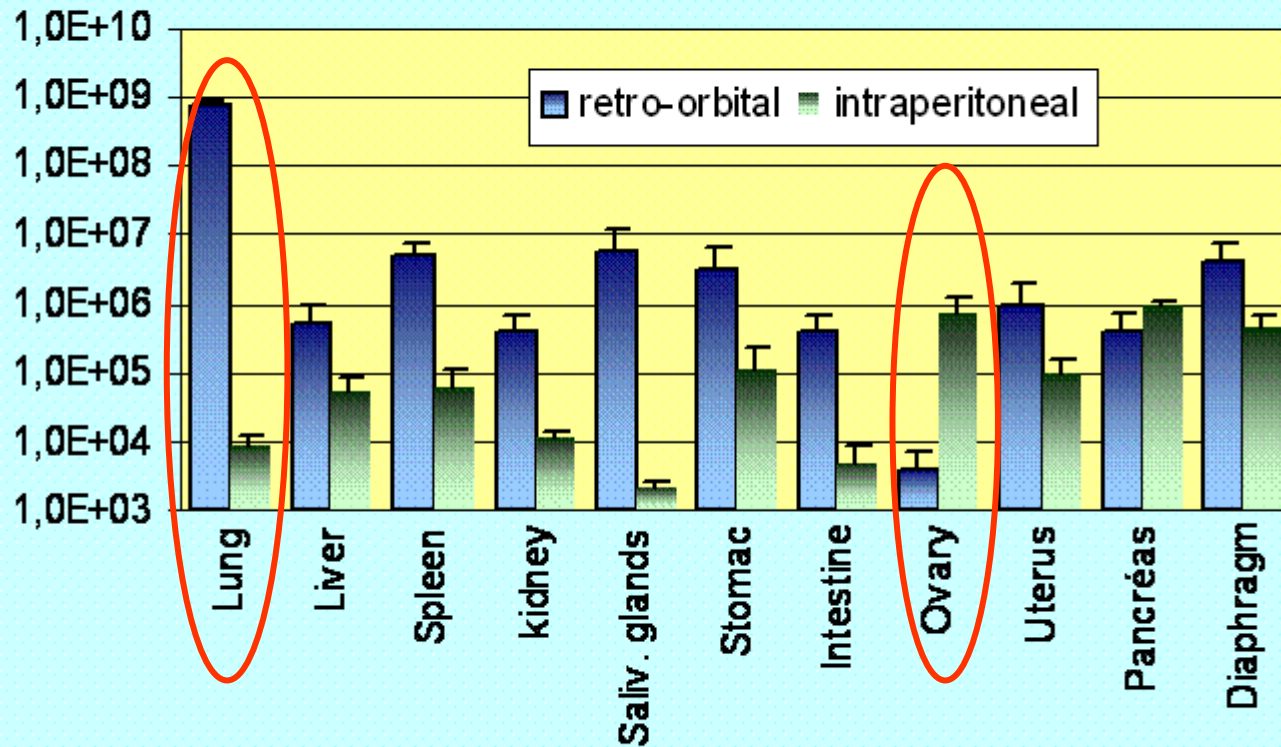
pCMVLuc/in vivo jetPEI™
NP8 400µl 40µg
Gluc5% n=6



In vivo DNA transfection: Bio-distribution

OF1 mice (n = 6)
pCMVLuc/*in vivo* jetPEI™ (N/P of 8)
5% glucose

Retro Orbital (50µg DNA / 200 µl)
Intraperitoneal (100 µg DNA/ 1ml)



depends on the route of administration

Bladder Cancer Strategy

Strategy

Use a plasmid with diphtheria toxin A gene expression being driven by the H19 regulatory sequences (pH19-DTA)

This gene should be expressed in and kill only cancer cells

Background

H19 gene product (RNA not protein) was highly expressed in rat and human bladder carcinoma tissues while nearly undetectable in surrounding tissues

Procedure

pH19-DTA was complexed with *in vivo-jetPEI*[™]

Rat: Intravesical administration of complexes on an orthotopic bladder carcinoma

Human: Introduce into the bladder of 2 human patients with recurrent superficial transitional cell carcinoma that was refractory to all commonly used treatments

Bladder Cancer Results

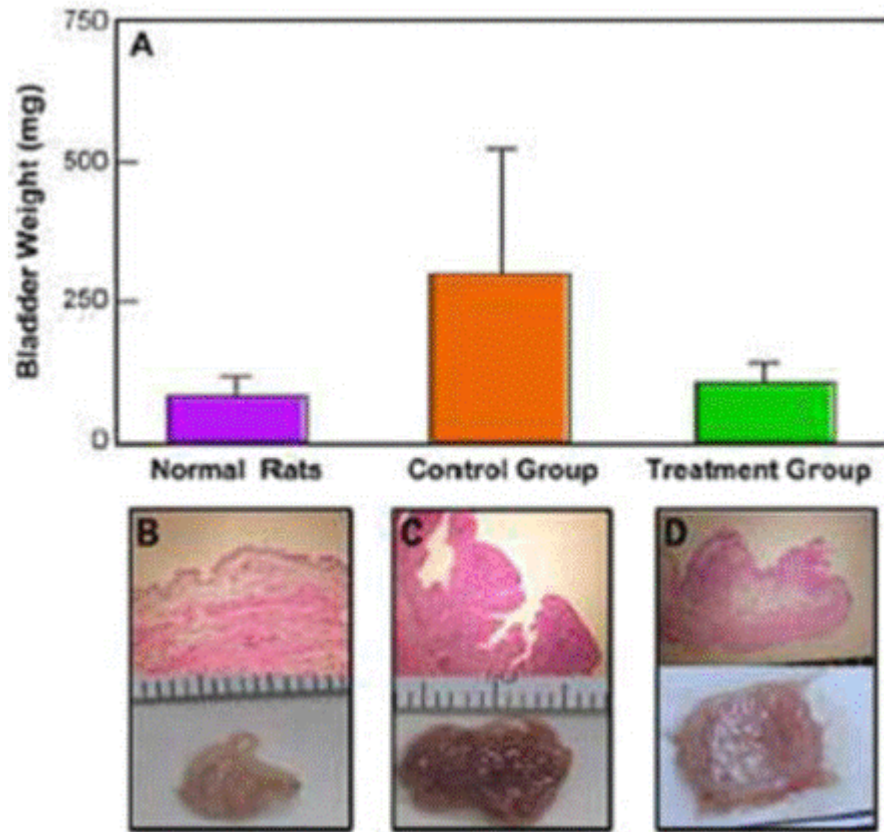
In Rats

Intravesical administration of H19-DTA plasmid on the orthotopic bladder carcinoma in rats

NBT-II cells

50µg DNA at D+4 & D+8

D+11 rats were sacrificed

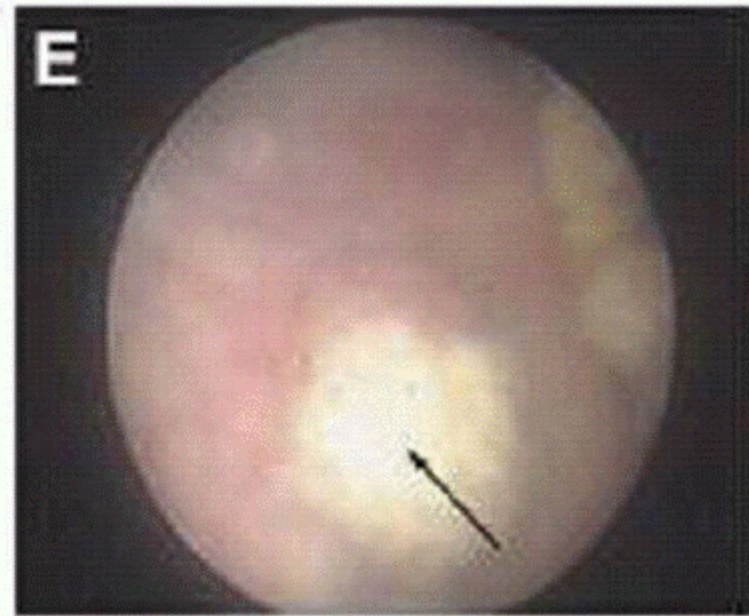
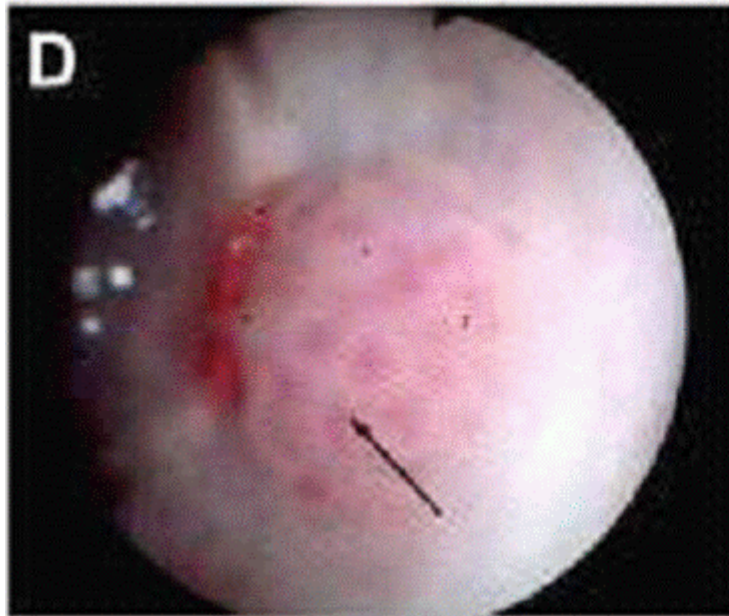


- Decrease in bladder weight
- Inhibition of tumor growth

Bladder Cancer Results

In Human

Treatment of **human** patient with the therapeutic H19-DTA plasmid following instillation with in vivo-jetPEI™



(D) Video-cystoscopy performed before the treatment and three weeks after the completion of the 6th treatment, showing a large necrotic area replacing the tumor region (E).

Treatment shows > 75% tumor regression



in vivo siRNA delivery

In vivo siRNA: HER-2 silencing

Strategy

Silence HER-2 in ovarian carcinoma xenografts with siRNA

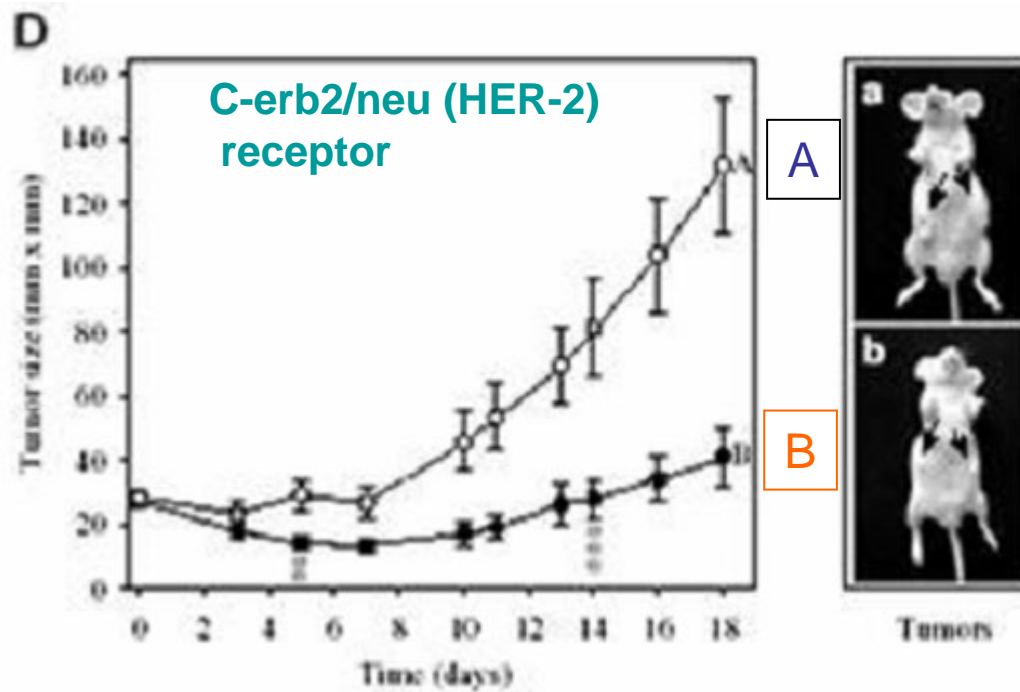
Background

HER-2 (proto-oncogene) belongs to EGF receptor family
Overexpression of HER 2 is observed in a wide variety of human cancers and cancer cell lines

Procedure

Complexation of jetPEI™ with siRNA targeting HER-2
Intra-peritoneal injection of the complexes into mice with ovarian carcinoma xenografts

In vivo sRNA: HER-2 silencing



i.p. in athymic nude mice

Subcutaneous tumor xenografts
SKOV-3 (ovarian carcinoma cell)

A) Naked HER-2 specific
siRNA (0.6 nmole)

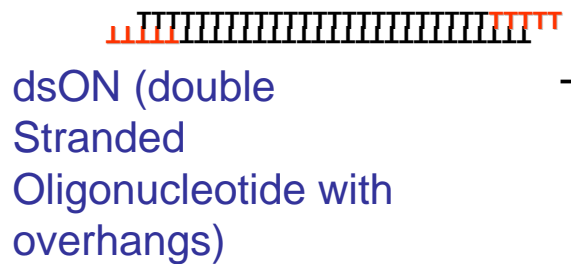
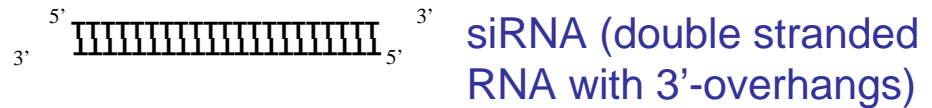
B) HER-2 siRNA/PEI
polyplexes

HER-2 down regulation

Tumor growth inhibition during 18 days

Sticky siRNA: Novel approach to in vivo RNAi

Goal: To make siRNA “look like a gene” to restore the original delivery power of jetPEI



+

+



Oligomerization by the overhangs

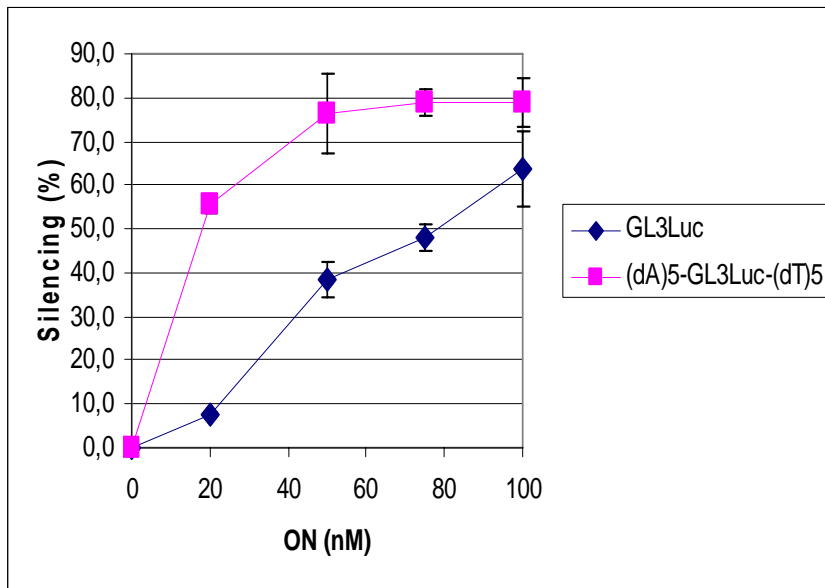


jetPEI™ promotes the siRNA oligomerization when the complex is formed.

Long double stranded RNA

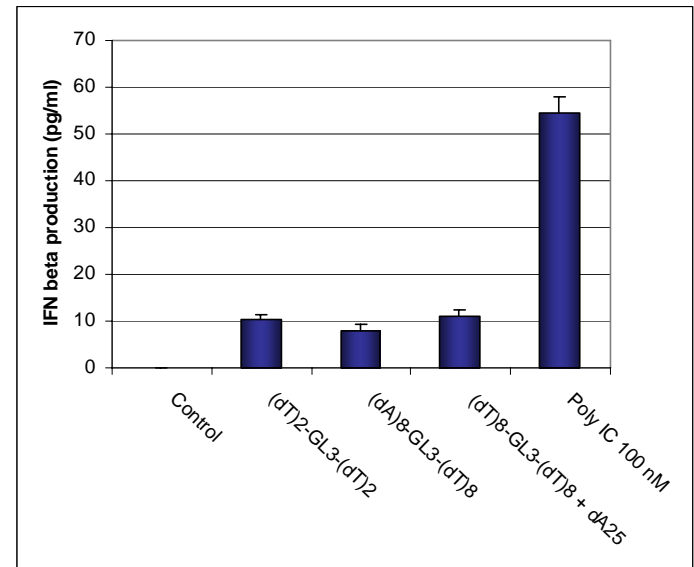
Sticky siRNA: in vitro

Gene silencing with ssiRNA



Sticky overhangs enhance siRNA-mediated gene silencing

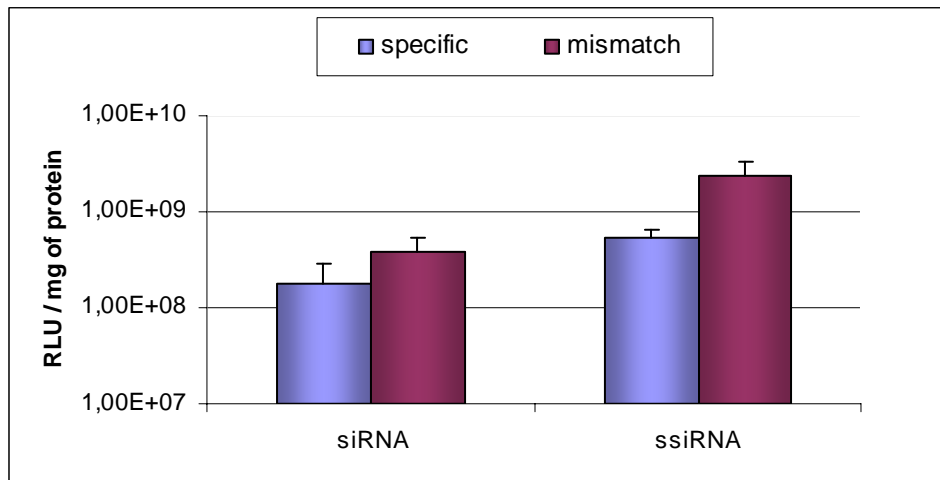
Interferon beta production of transfected MCF-7 with 100 nM siRNA



Similar low level of Interferon beta production were observed 24h after transfection of siRNA and sticky siRNA.

Sticky siRNA: Enhanced silencing in vivo

sticky siRNAs and *in vivo*-jetPEI™



Retro-orbital injection

- 30 µg of pGL2Luc
- 20 µg of siRNA or ssiRNA

in 50 µl 5% glucose mixed with *in vivo*-jetPEI™ at N/P=8.

The DNA and siRNA complexes were prepared independently and then mixed prior to injection.

Luciferase activity was measured in the lung, 24 h after injection of the complexes.

ssiRNAs delivery with *in vivo*-jetPEI™ leads to significantly higher gene silencing compared to that of classical siRNAs in mouse lung.

In vivo jetPEI™: Routes & Animals

Administration routes



- IV (tail vein, jugular)
- Intraperitoneal
- Intratumoral
- Intravesicular, intracortical
- Instillation
- Intravitreal
- Intracerebral
- ...

Animal models



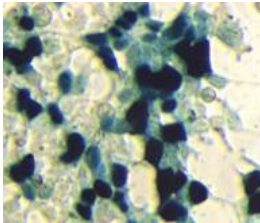
- Mouse, Rat
- Guinea pig, Hamster, Rabbit
- Duck, Chicken, Quail
- Tadpole, Shrimp, Fish, Jellyfish
- Cow, Sheep
- Monkey, **Human**

towards therapeutics and clinical applications

Polyplus-transfection products

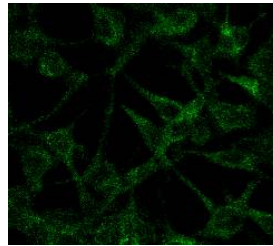
DNA delivery

jetPEI™ range



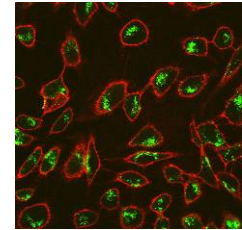
siRNA delivery

INTERFERin™



Protein and antibody delivery

Pulsin™

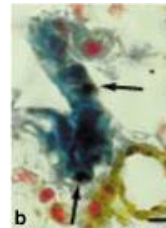


Protein production in synthetic medium

Fecturin™

In vivo delivery of DNA and siRNA

In vivo-jetPEI™ range





Thank you
for your kind attendance

Dakewe Biotech

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