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# A suitable delivery system is key in the concept of siRNA-mediated treatment


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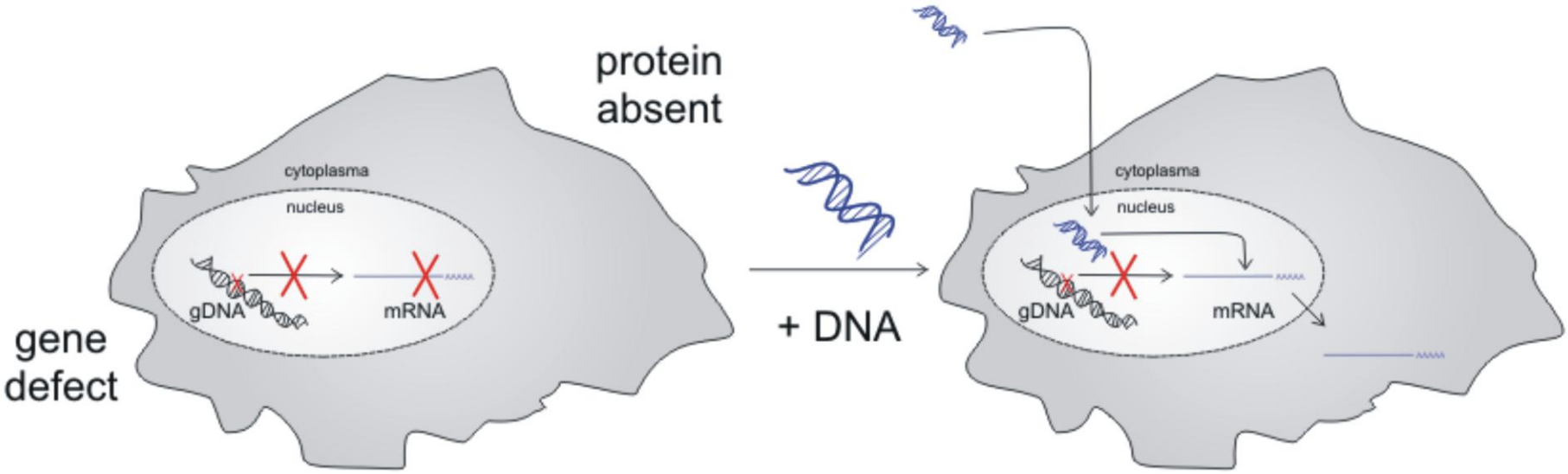
# What is gene therapy?



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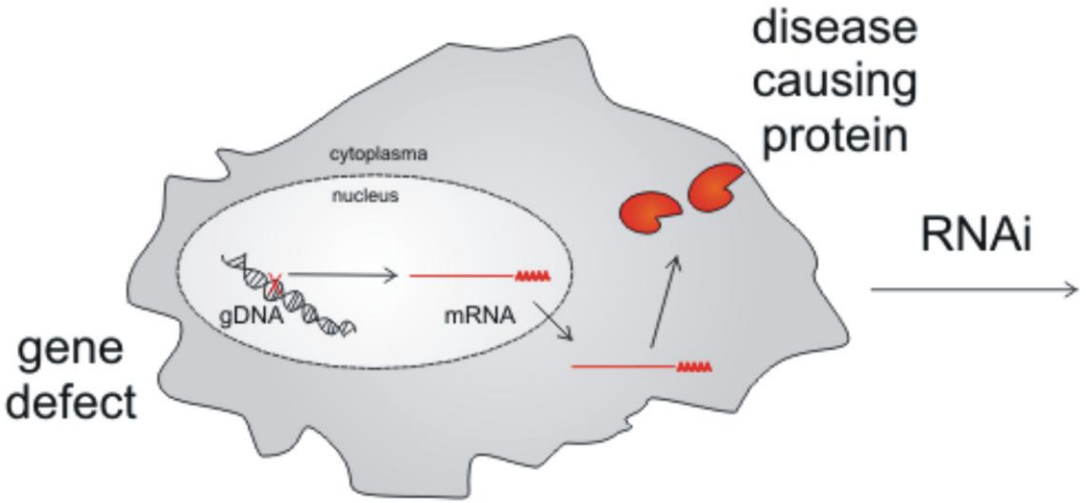
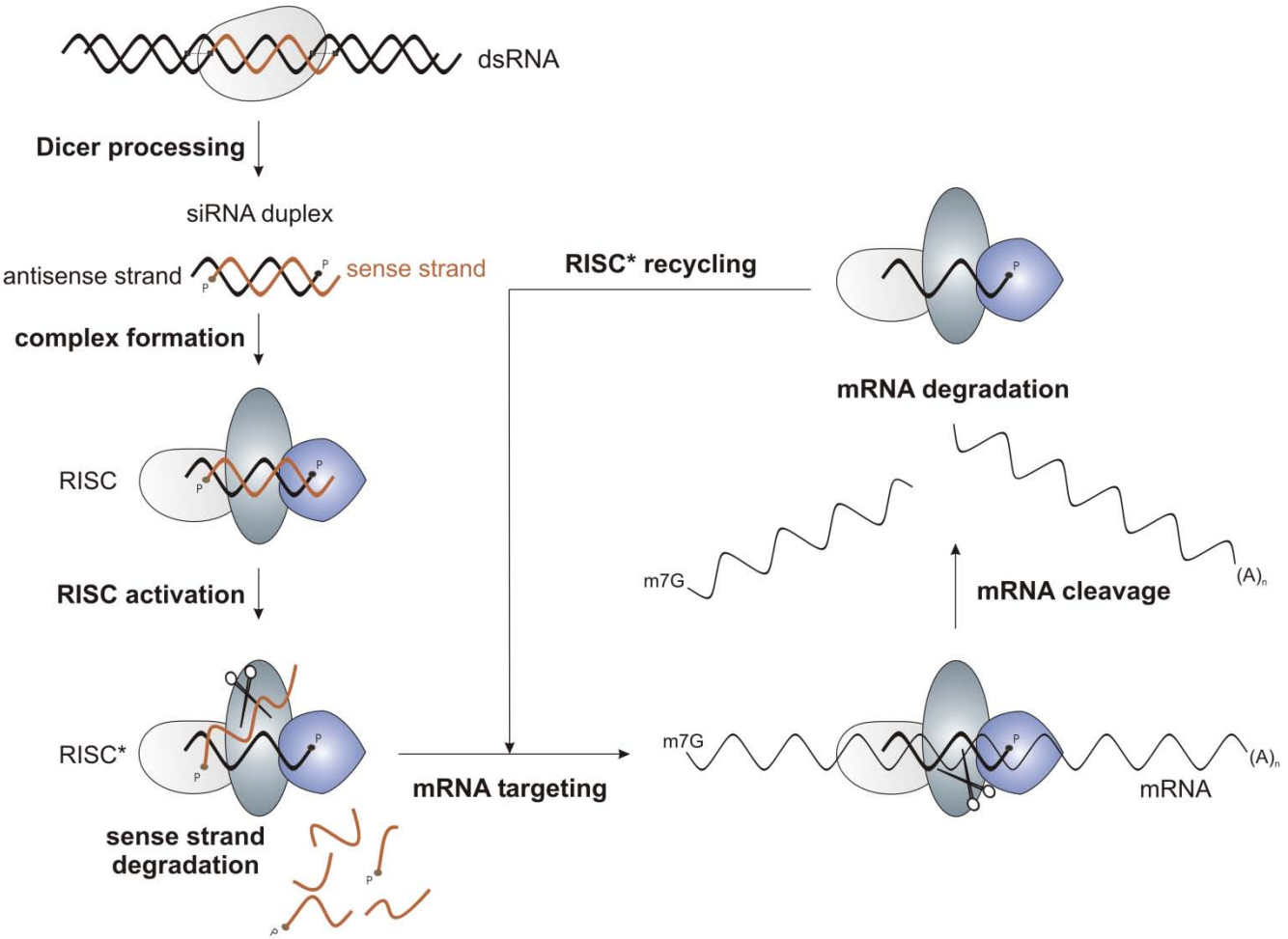
# What is gene therapy?



# Introduction



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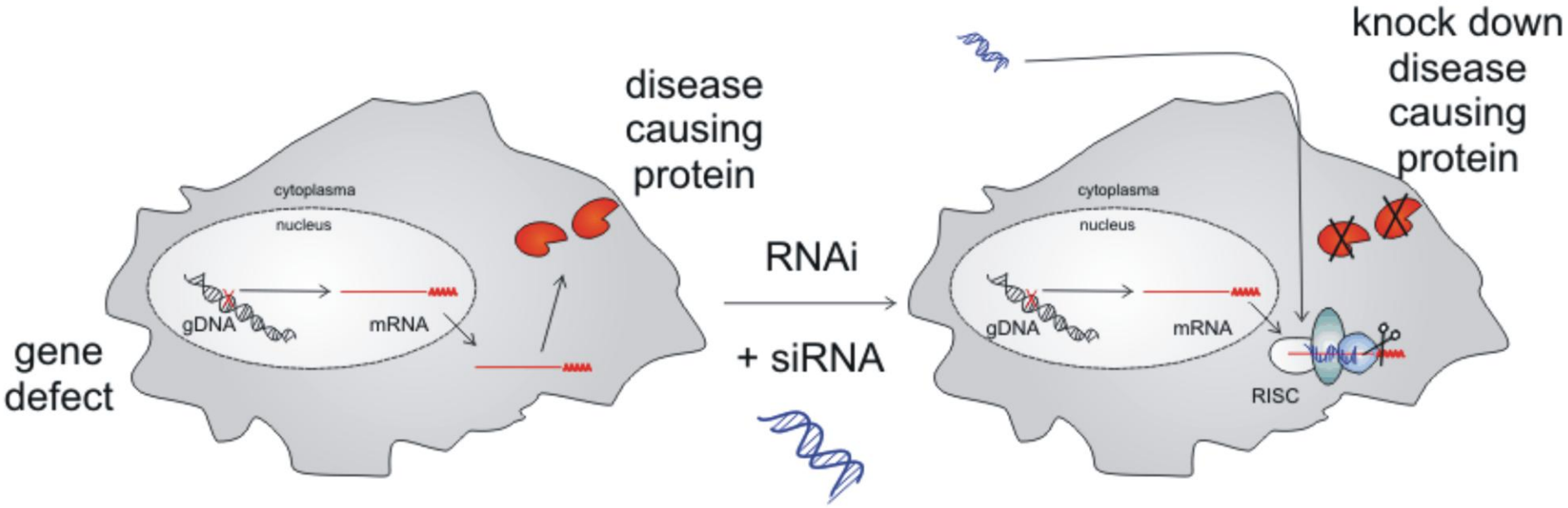
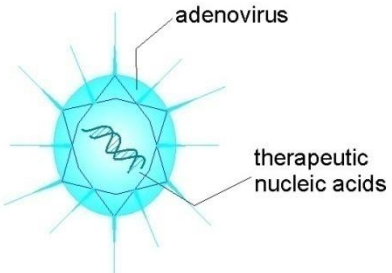
# What is gene therapy?



# Introduction

## viral




e.g. RV – LV – AV – AAV

\+ high transfection efficiency

transient or constitutive expression

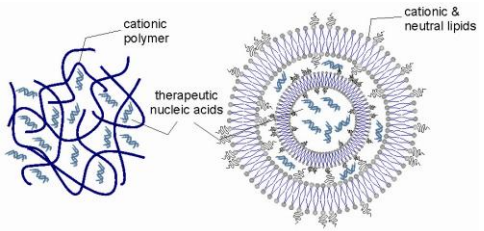
\- immunogenic respons

safety (recombination wt virus)

insertional oncogenesis

high production costs

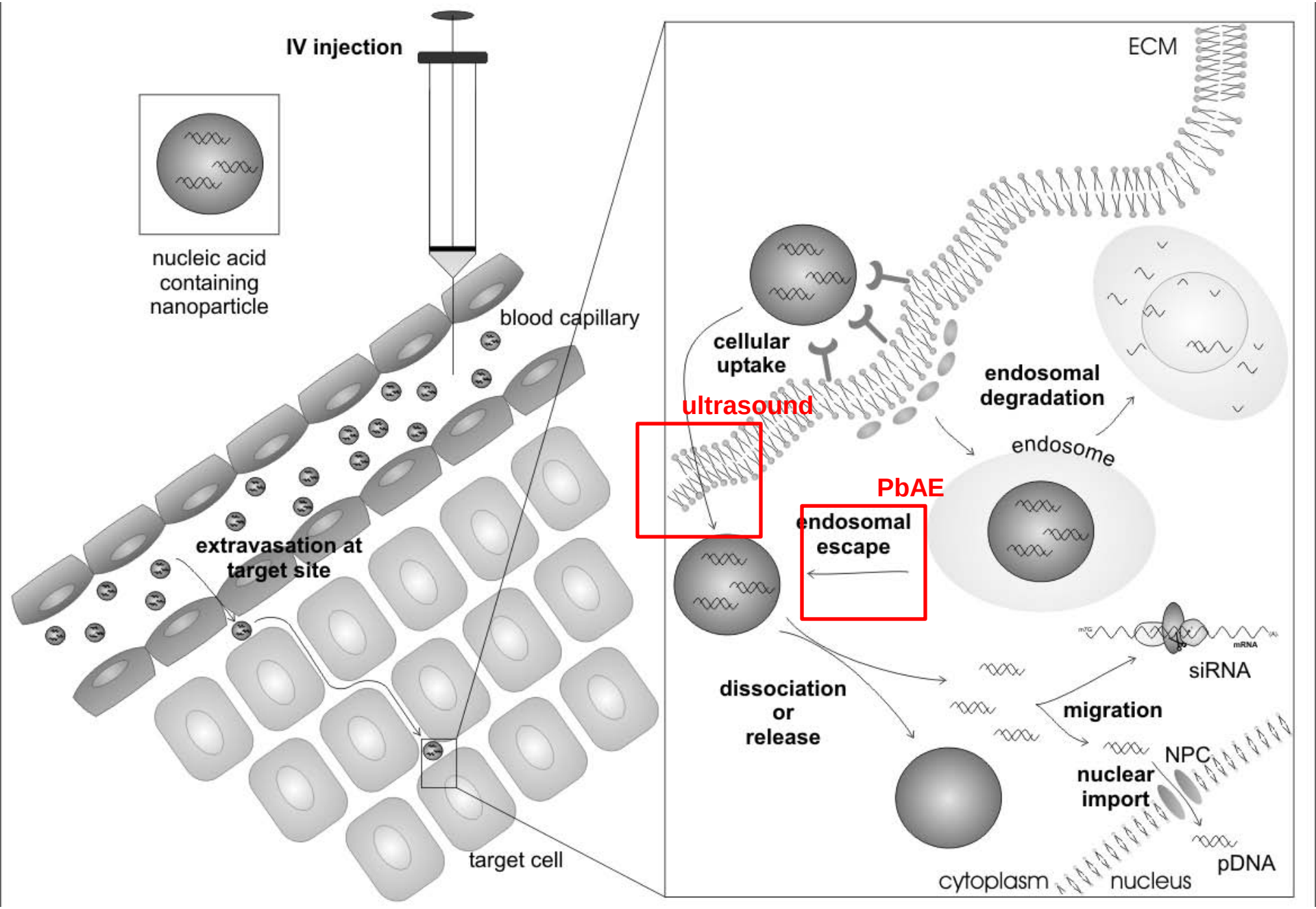
## non-viral




e.g. polymers – lipids - nanospheres

\+ synthetic
mass production
safe
non-immunogenic

\- low transfection efficiency
transient expression


IV injection
nucleic acid containing nanoparticle
blood capillary
extravasation at target site
target cell
ECM
cellular uptake
ultrasound
endosomal degradation
endosome
PbAE
endosomal escape
dissociation or release
siRNA
migration
NPC
nuclear import
pDNA
cytoplasm
nucleus

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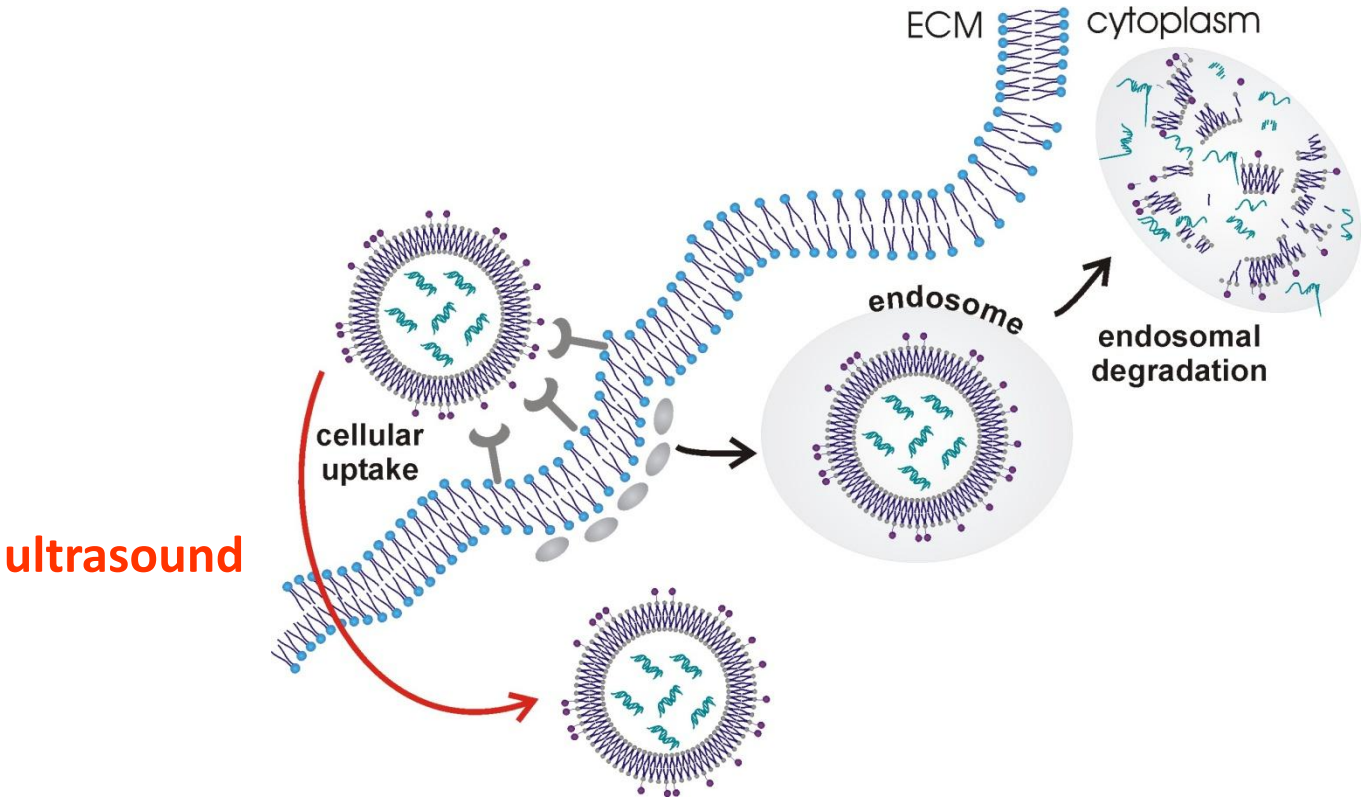
# The cell membrane: ultrasound & microbubbles



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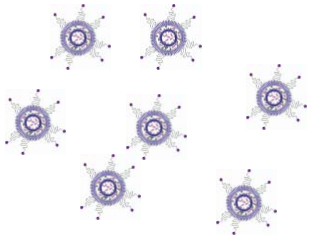
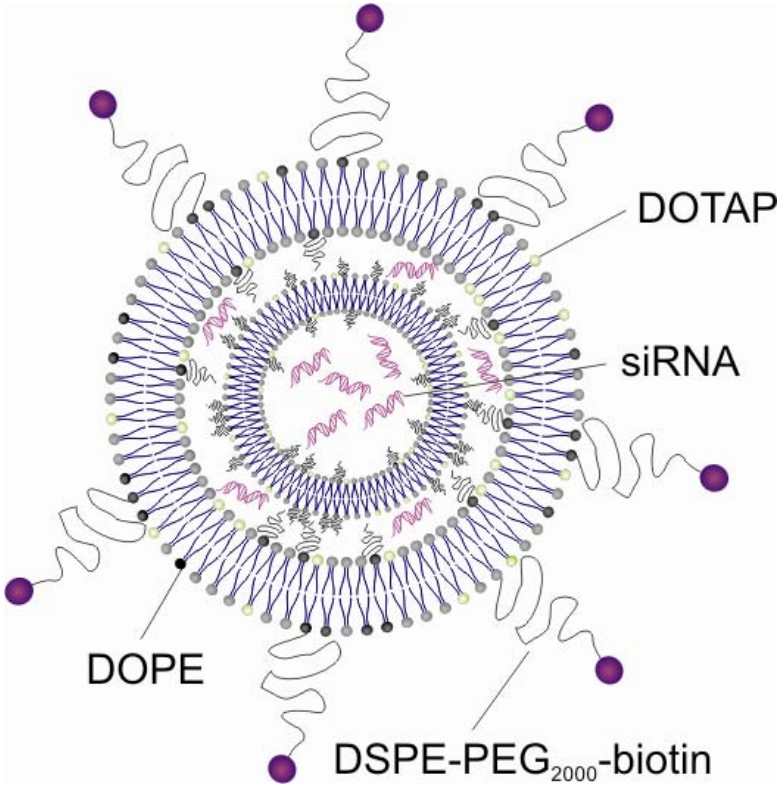
# Aim



**siRNA containing DOTAP:DOPE LPXs**

- Equal amounts of DOTAP and DOPE
- 0, 2, 5 or 15 mol% DSPE-$PEG_{2000}$-biotin

# siRNA containing LPXs




## cationic LPXs

*In vivo*:

- Interaction with negatively charged serum proteins
- Rapid clearance from the bloodstream

⇒ **PEGylation is necessary!**

**BUT**

**Low transfection efficiency:**

- Reduced uptake
- Reduced endosomal release

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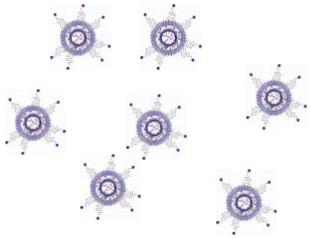
# Aim



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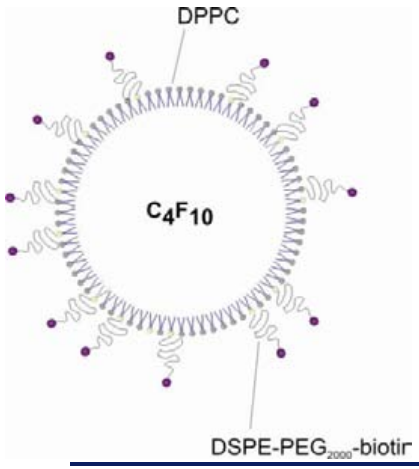


## lipid coated microbubbles

- 95 mol% DPPC
- 5 mol% DSPE-$PEG_{2000}$-biotin

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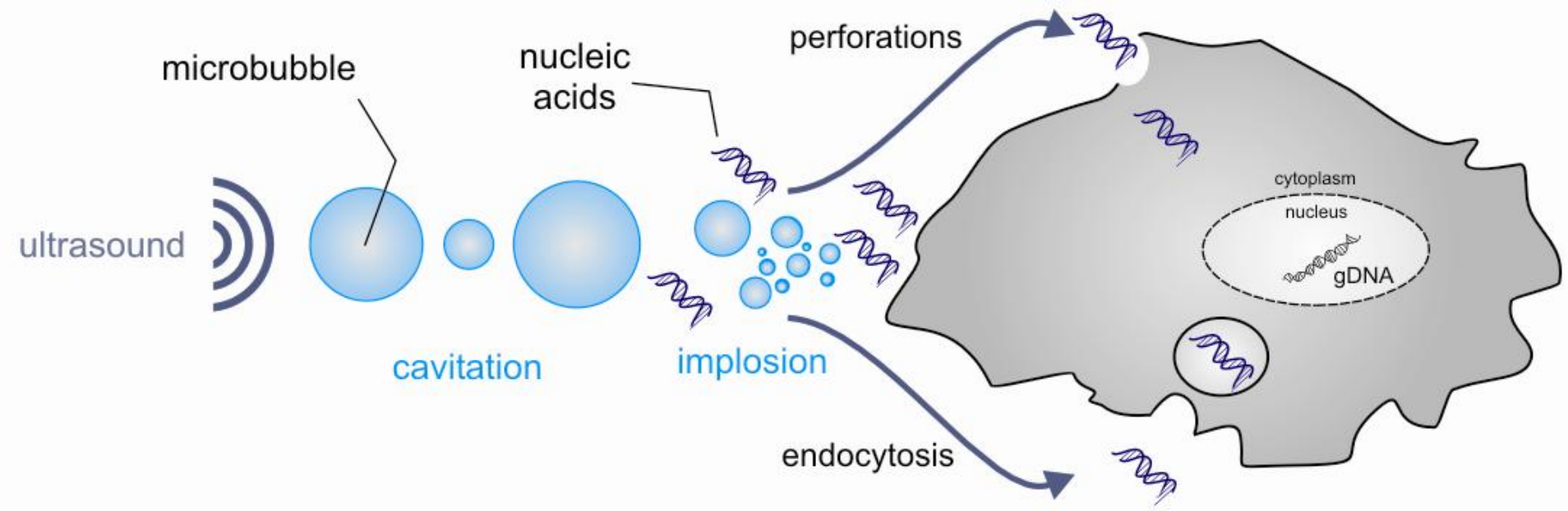
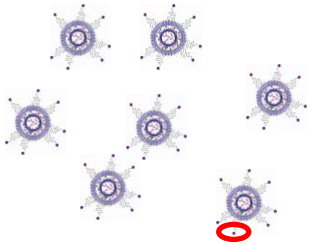
# Microbubbles




- Advantages: - non-invasive & locally applicable
  - safe

# Aim



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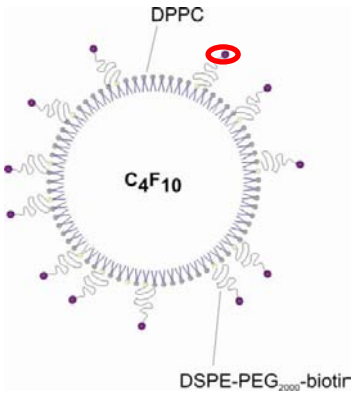
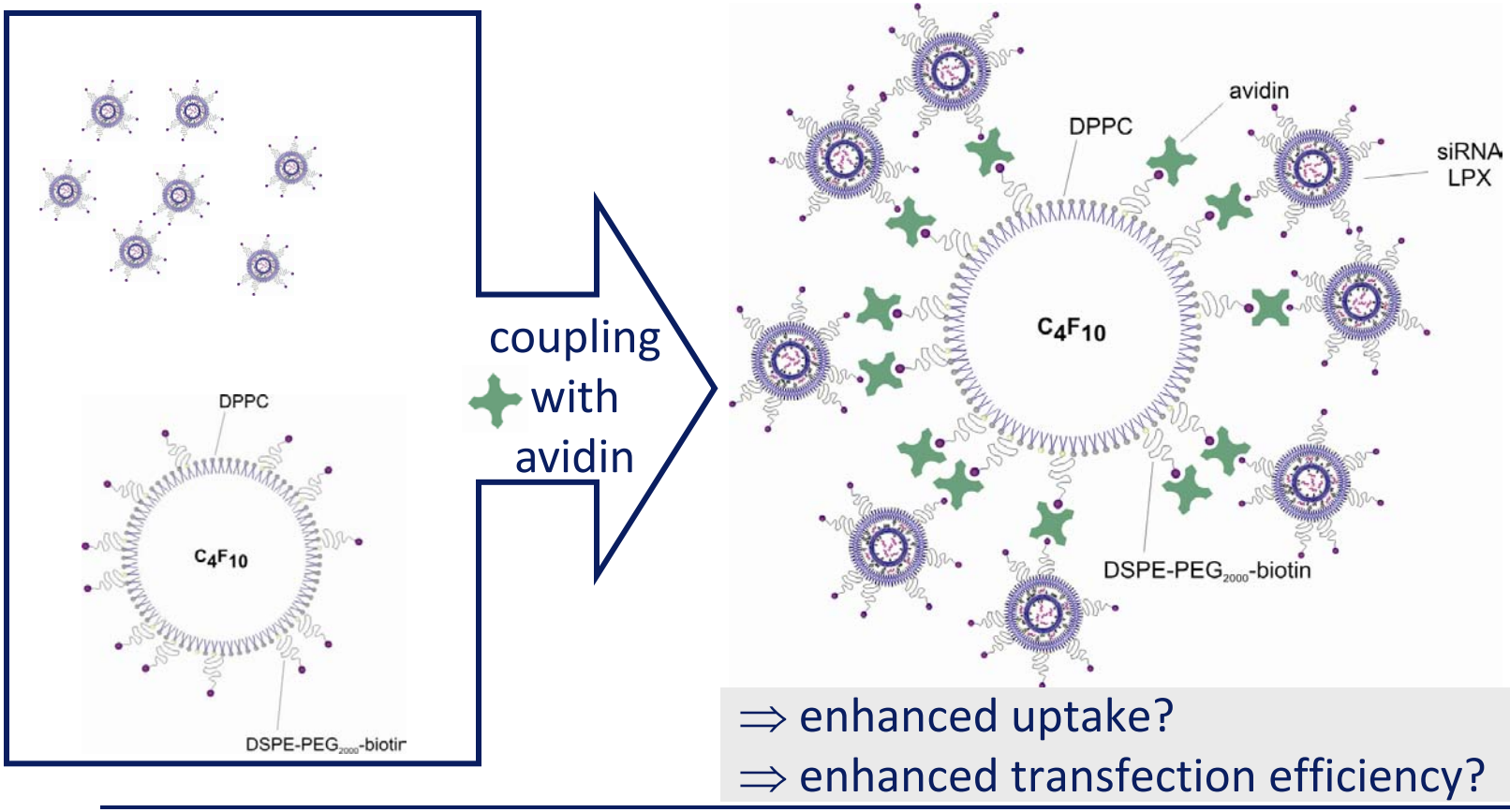



## lipid coated microbubbles

- 95 mol% DPPC
- 5 mol% DSPE-$PEG_{2000}$-biotin

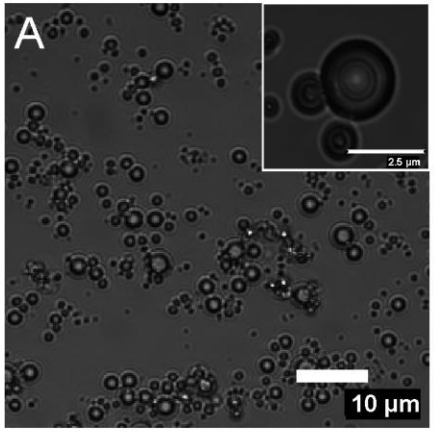
# Part I

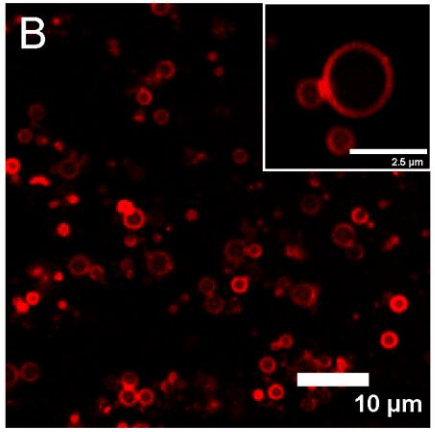
## Aim



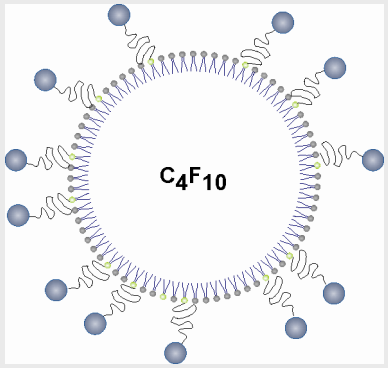

⇒ enhanced uptake?
⇒ enhanced transfection efficiency?

# Characterization of the microbubbles




Lipid coated microbubbles incubated with Cy5 labelled streptavidin




**Biotin molecules are present at the outside of the microbubble coat.**

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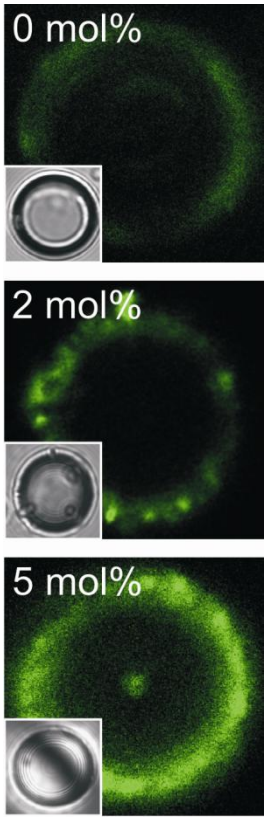
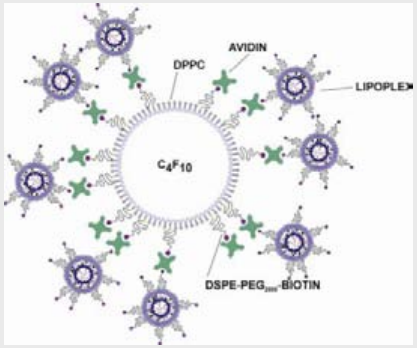
# Binding of LPXs onto the microbubble surface




Unlabelled microbubbles incubated with green labelled siRNA LPXs

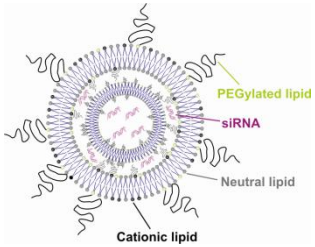



**Higher biotinylation degree**
**=**
**Higher loading degree of the LPXs on the microbubble surface.**

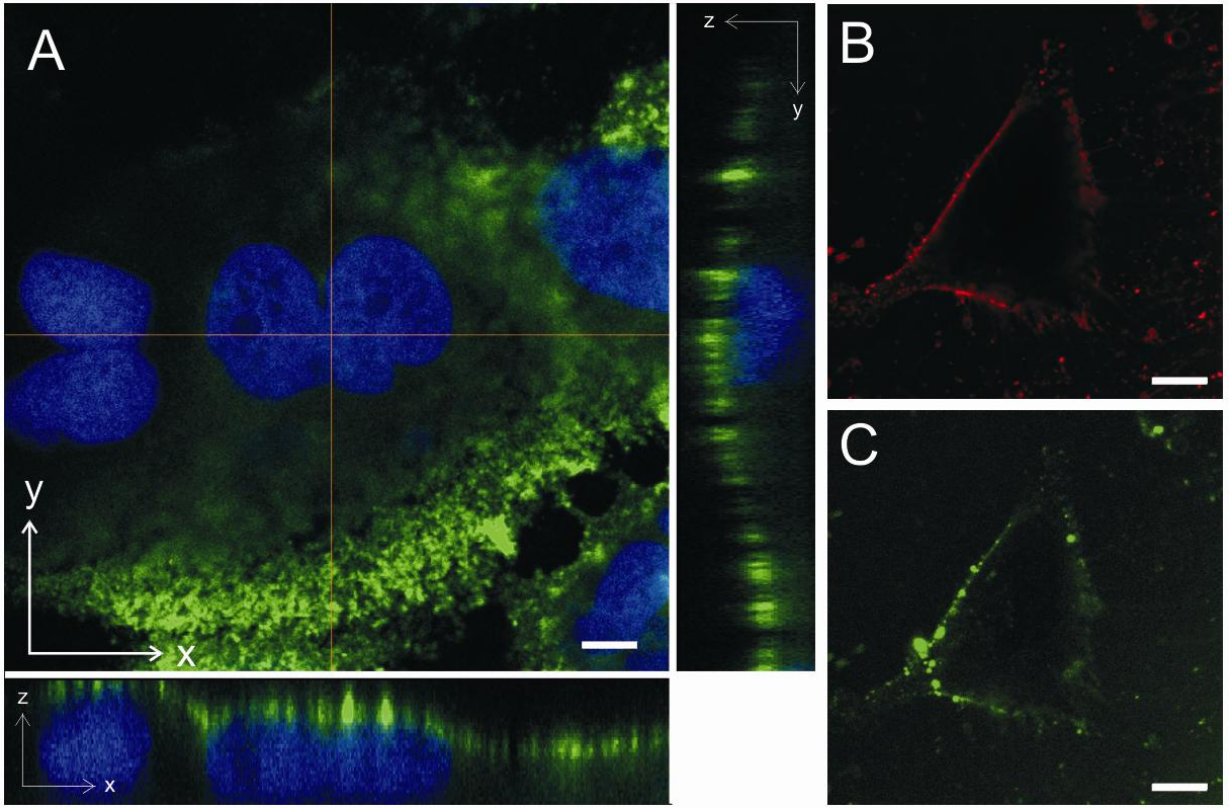
# Part I

## Cellular distribution w/o microbubbles and ultrasound

**5 mol% LPXs**
**cell membrane**
**nucleus**



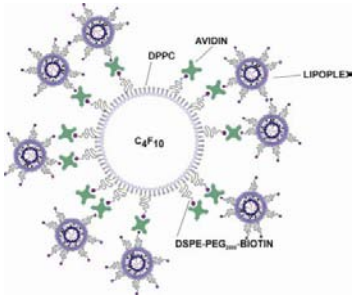

**LPXs are bound to the cellular membrane**



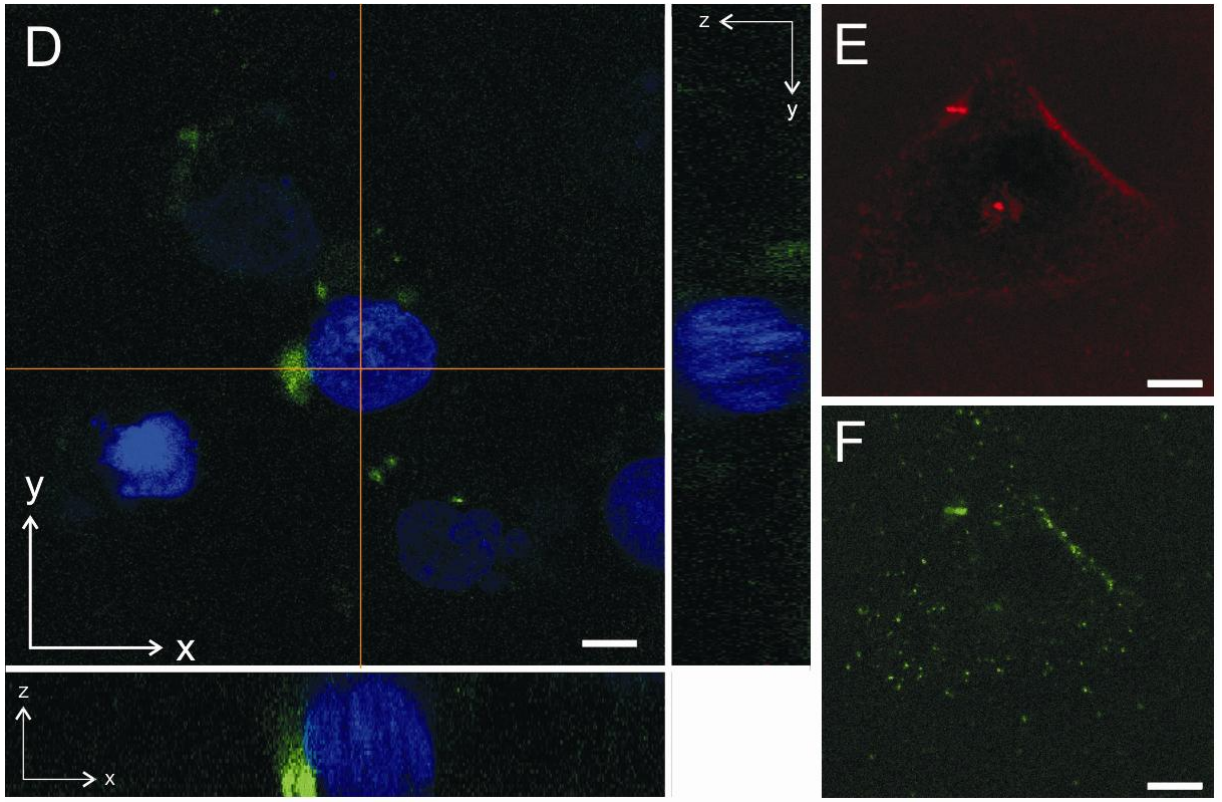
Part I

# Cellular distribution w/ microbubbles and ultrasound

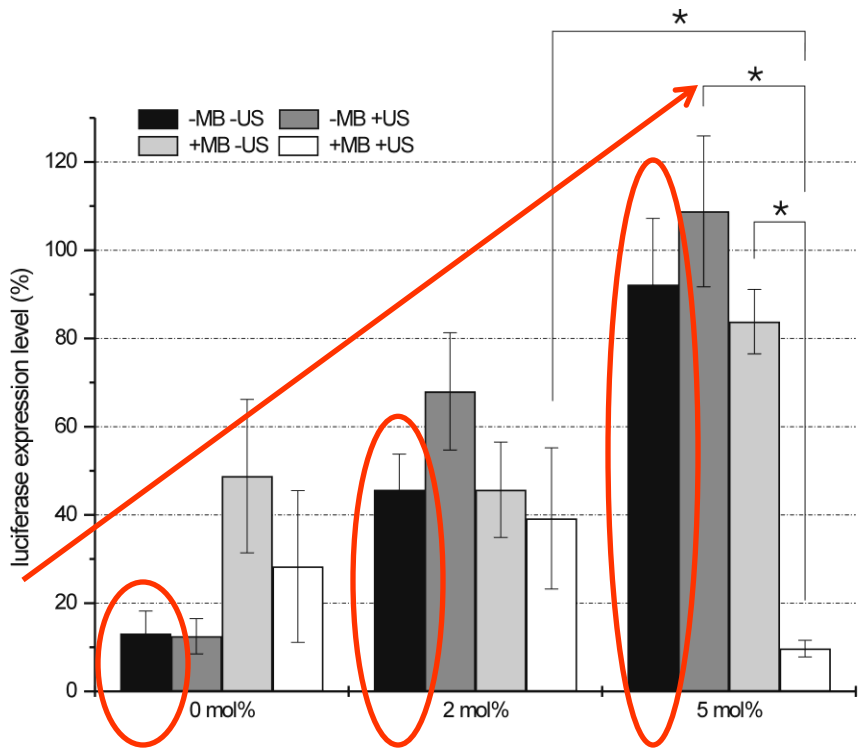
5 mol% LPXs
cell membrane
nucleus




LPXs are translocated to the cp



# Transfection efficiency analysis




**Black bars**

Gene silencing of siRNA LPXs **without** the use of microbubbles and ultrasound

**The higher the PEGylation degree of the LPXs, the lower their silencing efficiency**

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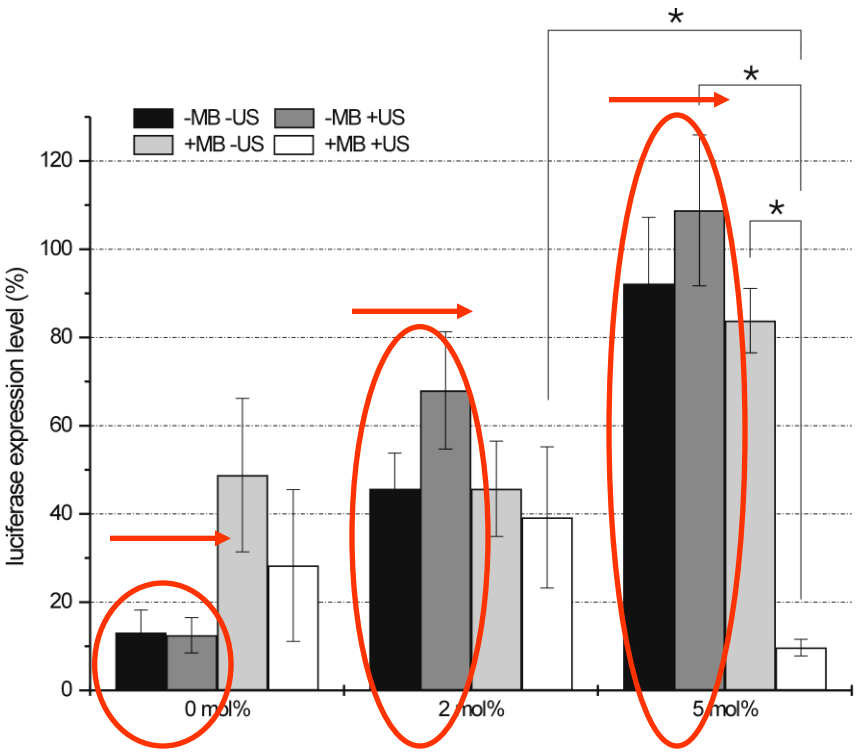
# Transfection efficiency analysis




Dark grey bars

Gene silencing of **free** lipoplexes **with** the use of **ultrasound**

**No significant influence of ultrasound on the silencing efficiency of siRNA LPXs**

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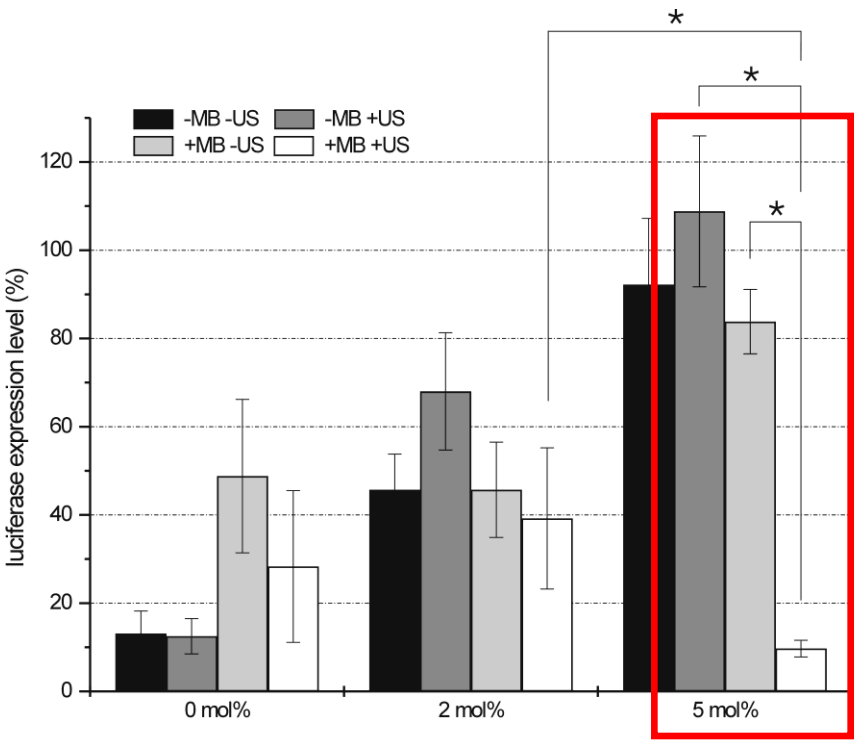
# Transfection efficiency analysis




White bars

Gene silencing of siRNA lipoplexes **with** the use of **microbubbles** and **ultrasound**

**Drastic increase in silencing efficiency with highly PEGylated siRNA lipolexes**

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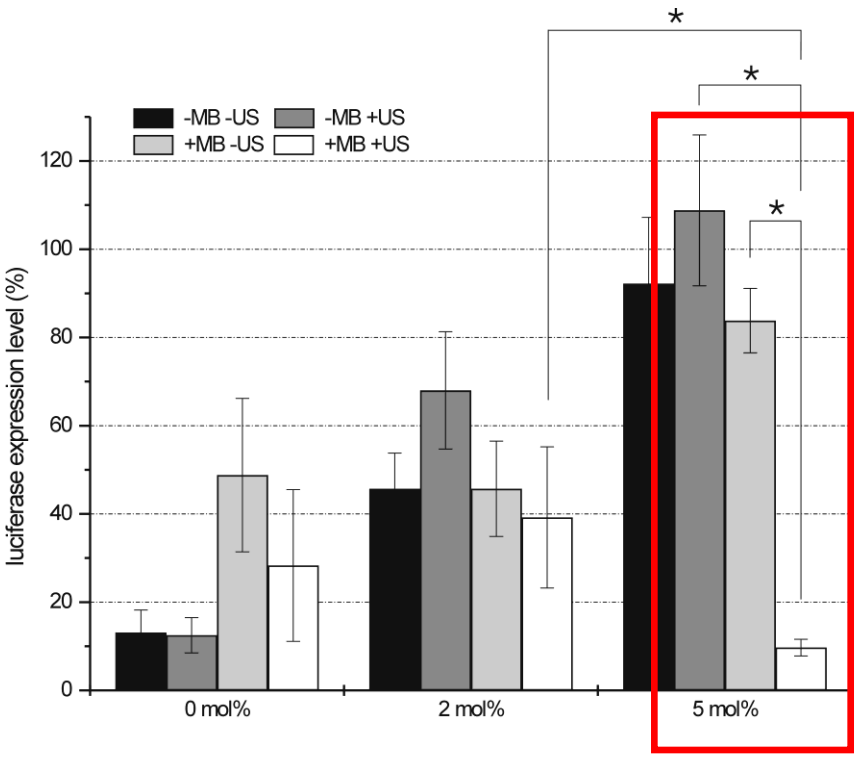
# Transfection efficiency analysis




Light grey bars

Gene silencing of siRNA lipoplexes **with** the use of **microbubbles** but **without ultrasound**

**Ultrasound targeted gene silencing possible**

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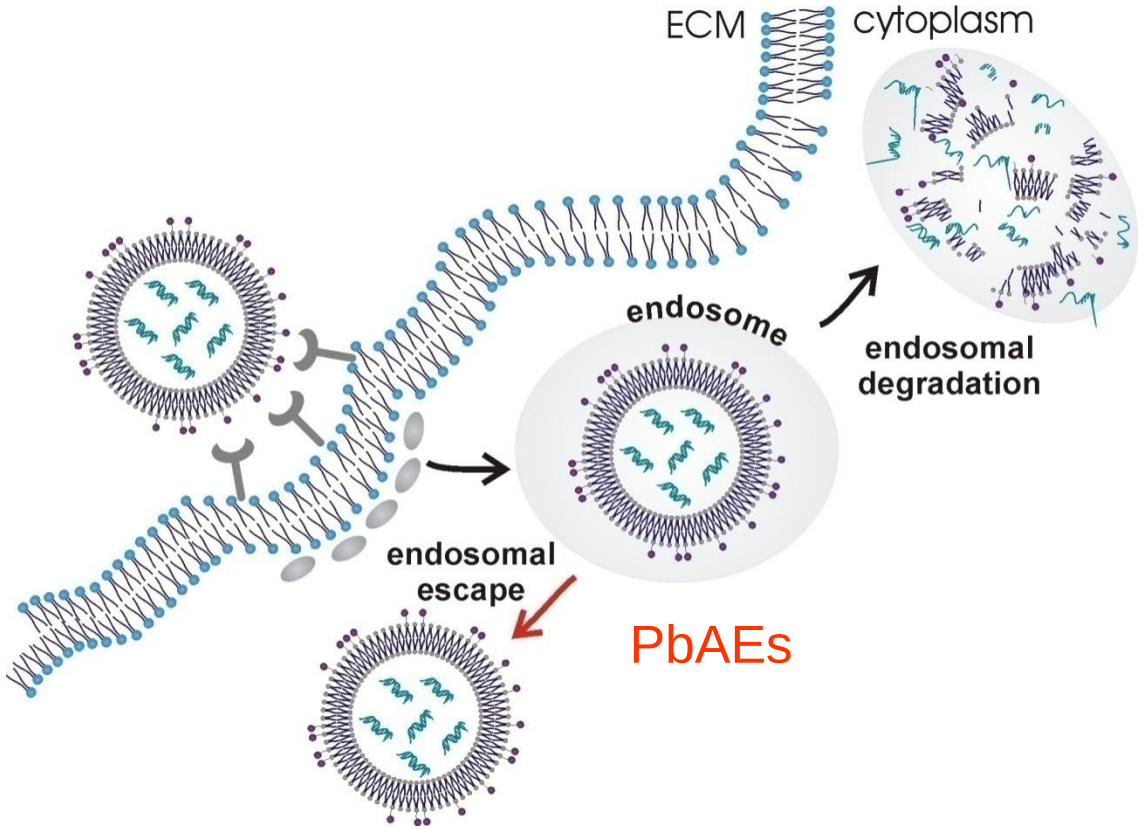
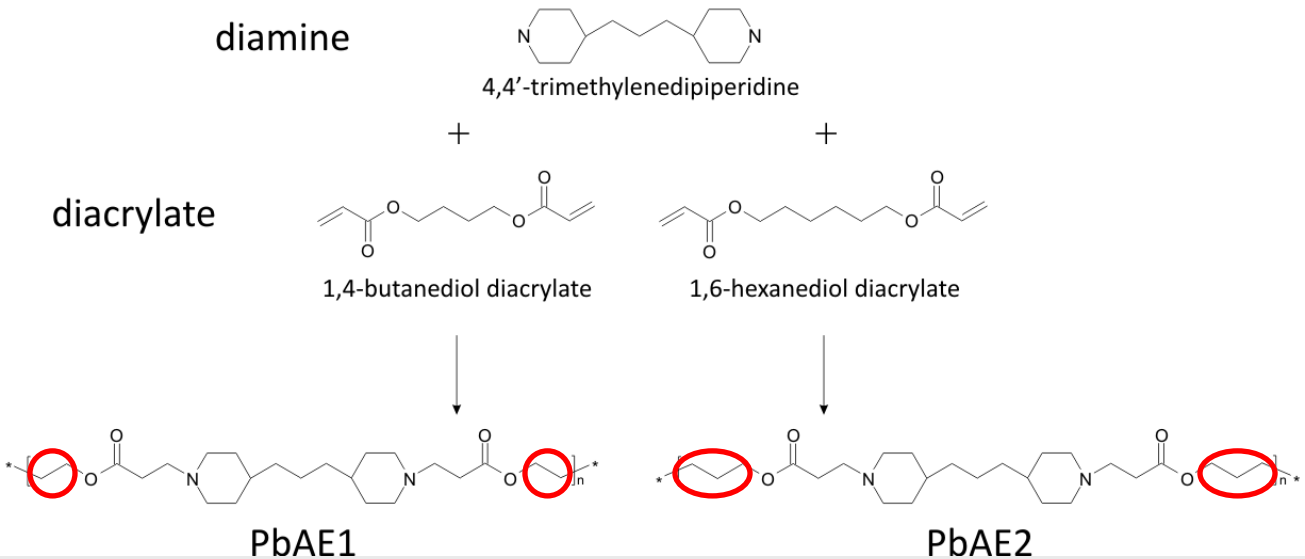
# The endosomal barrier - PbAEs for siRNA delivery



## Aim

synthesize different poly(β-amino ester)s (PbAEs):




⇒ **cytotoxicity - siRNA complexation - gene silencing**

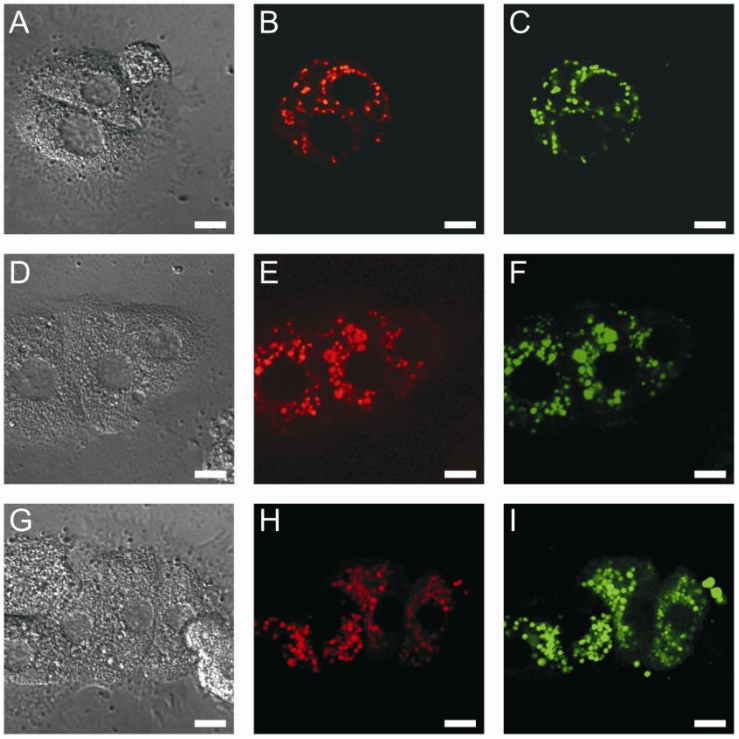
# Uptake, cytotoxicity and silencing efficiency

**cellular uptake**

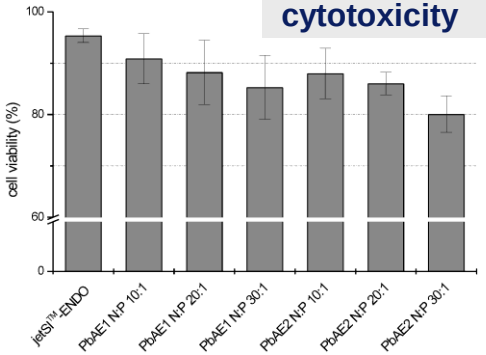
**JetSI™-ENDO**

**PbAE1 (N:P 30:1)**

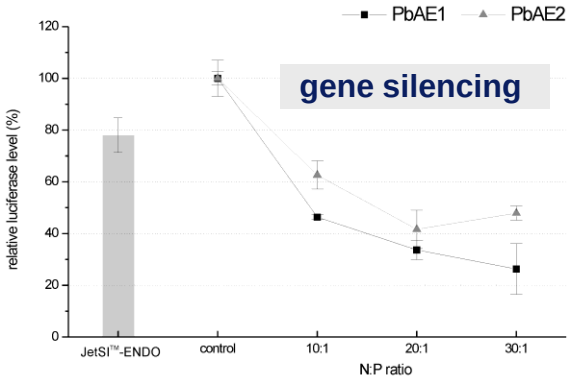
**PbAE2 (N:P 30:1)**




**cytotoxicity**




**gene silencing**



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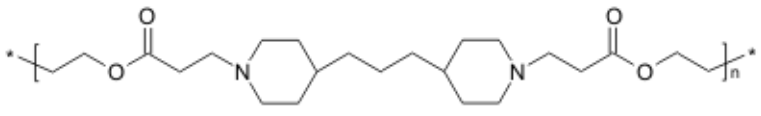
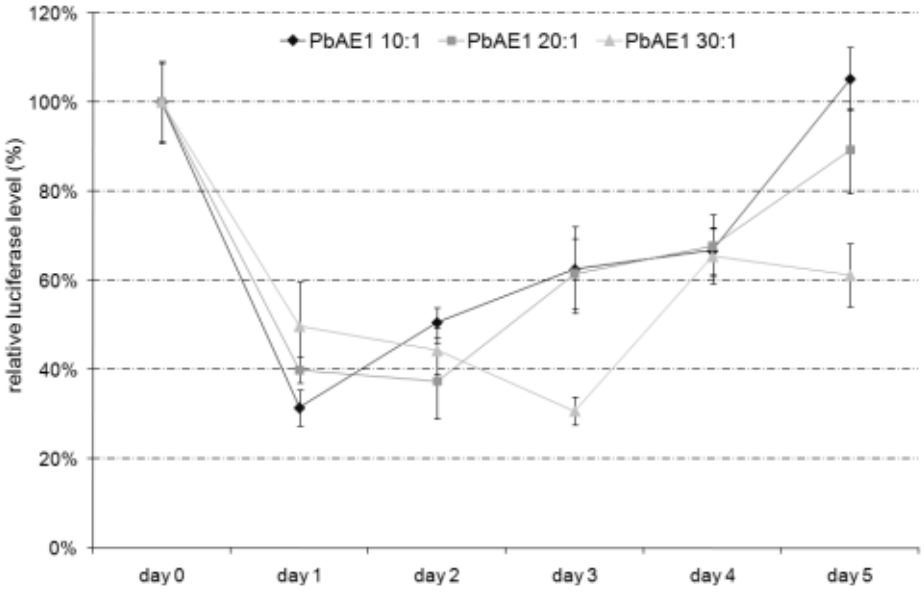
# Results

**prolonged effect?**

**PbAE1**

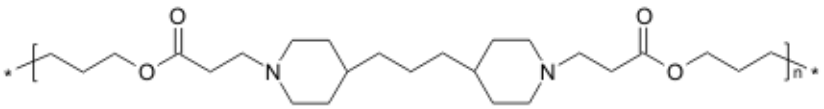


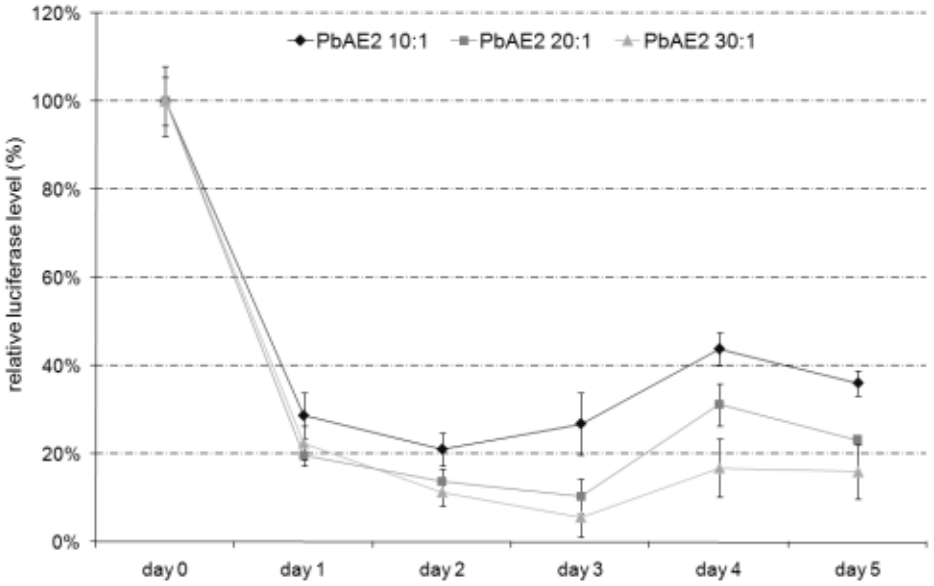



# Results

**prolonged effect?**

**PbAE2**



# Results

**prolonged effect?**

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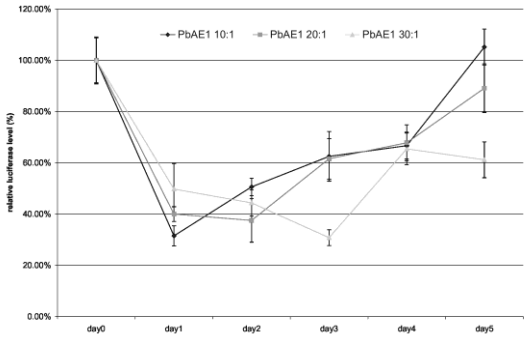
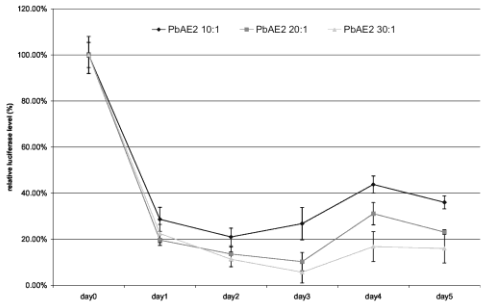
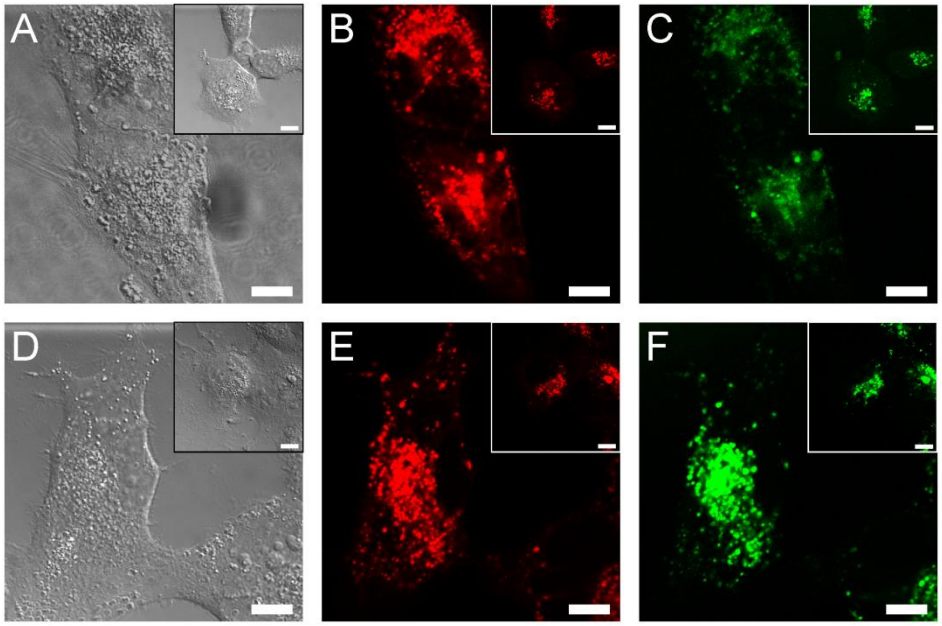
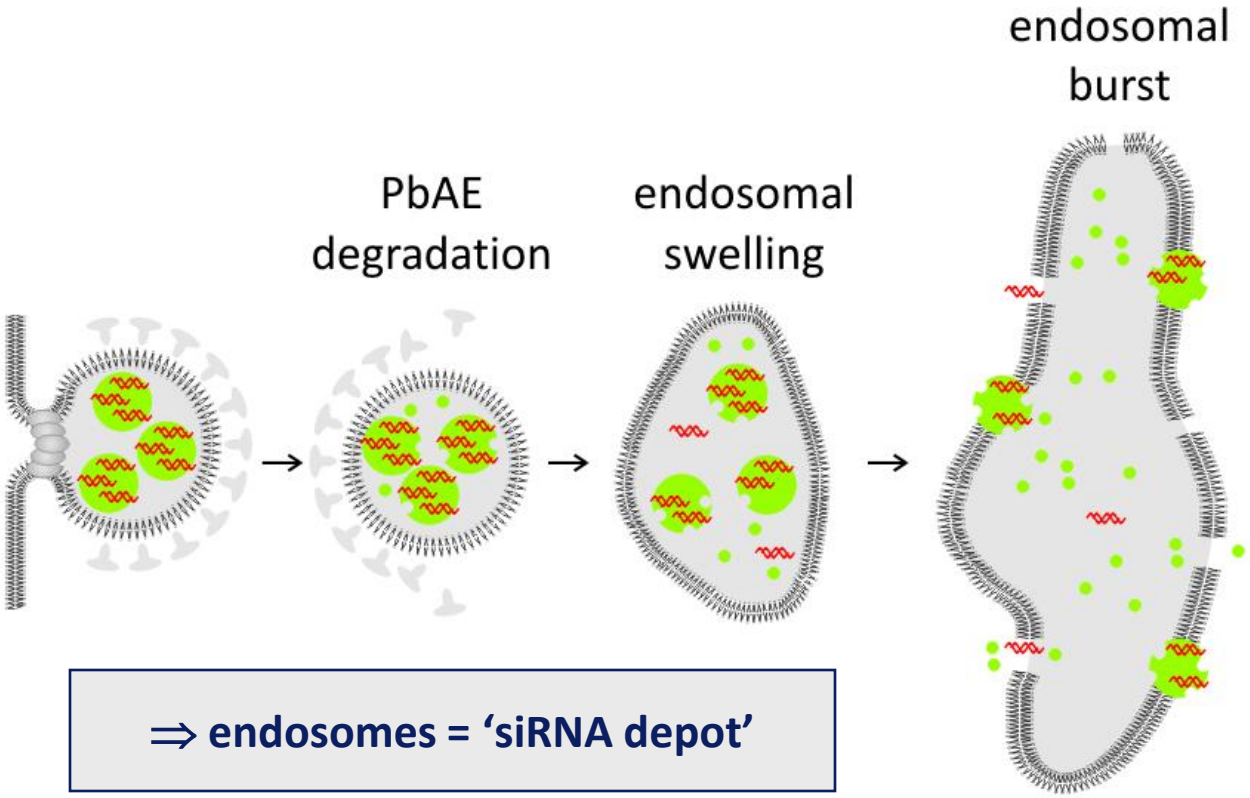



**PbAE2**



# Proposed mechanism




⇒ **endosomes = 'siRNA depot'**

# Conclusions

**Cellular** barrier

→ **ultrasound & microbubbles**: targeted, enhanced siRNA delivery

**Endosomal** barrier

→ **PbAEs**: non-toxic, efficient siRNA delivery systems acting by creating an 'siRNA depot' inside the endo/lysosomes

# Thanks to…

**Laboratory of General Biochemistry and Physical Pharmacy, Ghent University, Ghent**

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Prof. Stefaan De Smedt

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Prof. Harry Heimberg
Stefan Bonné

**Dept. of Toxicology, VUB, Brussels**
Prof. Vera Rogiers
Tamara Vanhaecke
Mathieu Vinken

**Dept. of Pharmacy, Munich, Germany**
Prof. Ernst Wagner