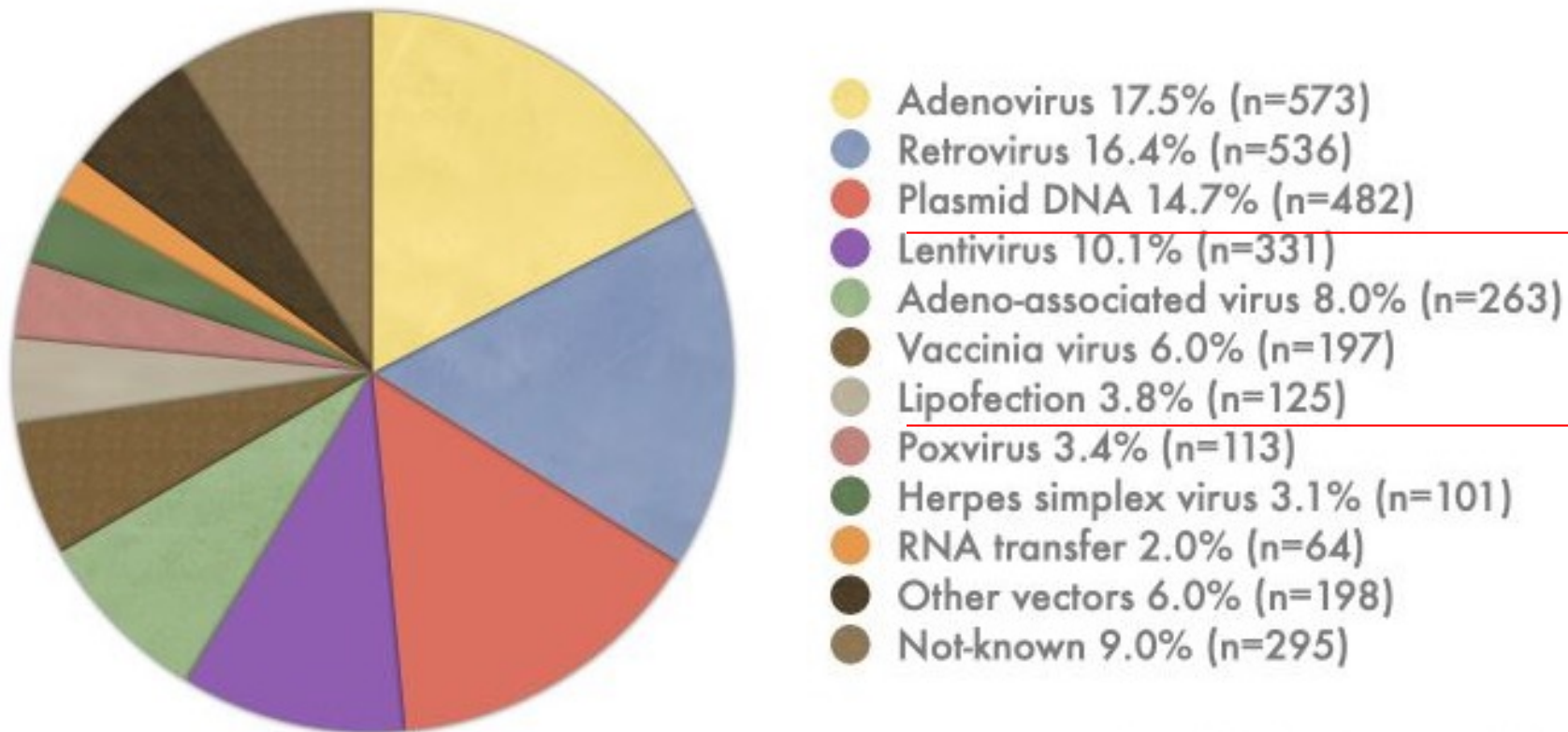# Which vectors for the genes

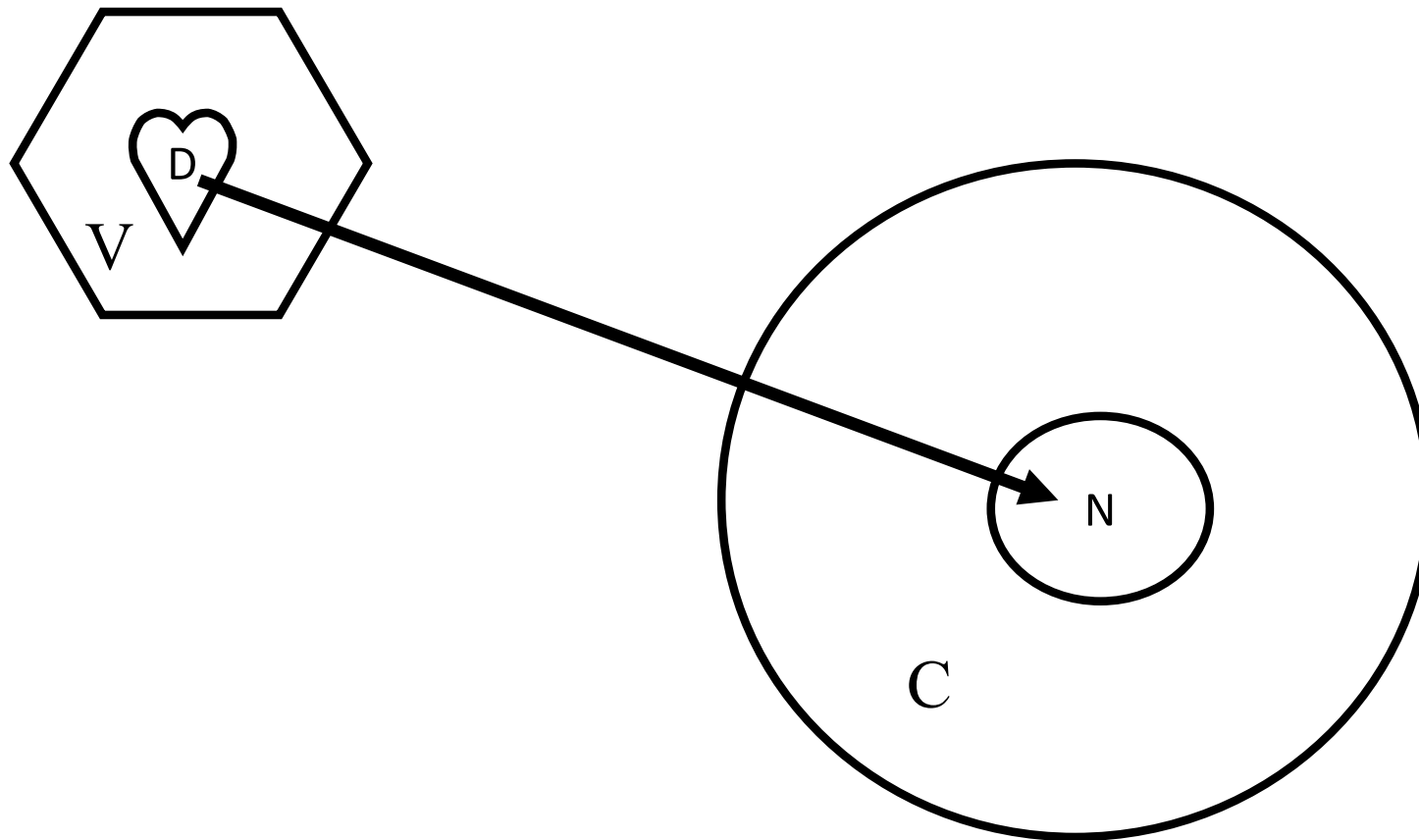

Vectors Used in Gene Therapy Clinical Trials

- Adenovirus 22.2% (n=506)
- Retrovirus 18.4% (n=420)
- Naked/Plasmid DNA 17.4% (n=397)
- Vaccinia virus 7.2% (n=165)
- Adeno-associated virus 6% (n=137)
- Lipofection 5% (n=115)
- Lentivirus 5% (n=114)
- Poxvirus 4.4% (n=101)
- Herpes simplex virus 3.2% (n=73)
- Other vectors 7.6% (n=174)
- Unknown 3.3% (n=76)

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Which vectors for the genes

Vectors Used for Gene Transfer in Gene Therapy Clinical Trials

- Adenovirus 17.5% (n=573)
- Retrovirus 16.4% (n=536)
- Plasmid DNA 14.7% (n=482)
- Lentivirus 10.1% (n=331)
- Adeno-associated virus 8.0% (n=263)
- Vaccinia virus 6.0% (n=197)
- Lipofection 3.8% (n=125)
- Poxvirus 3.4% (n=113)
- Herpes simplex virus 3.1% (n=101)
- RNA transfer 2.0% (n=64)
- Other vectors 6.0% (n=198)
- Not-known 9.0% (n=295)

First step:
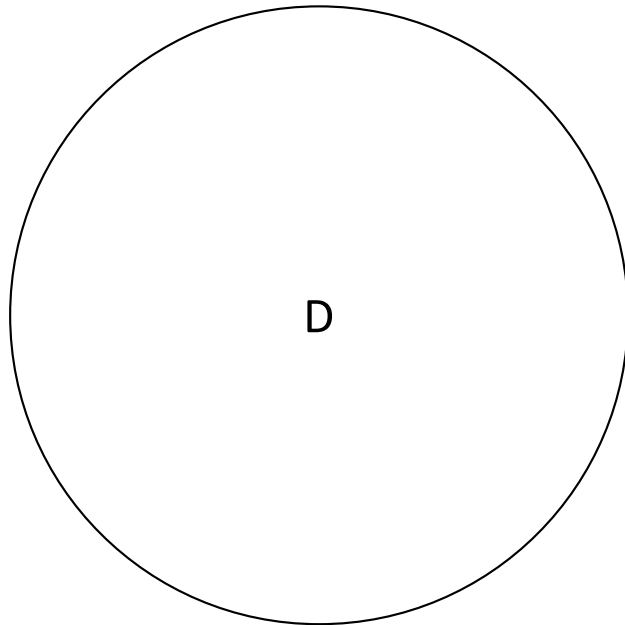# bring the transgene into the nucleus

2nd step:
the transgene is expressed

D
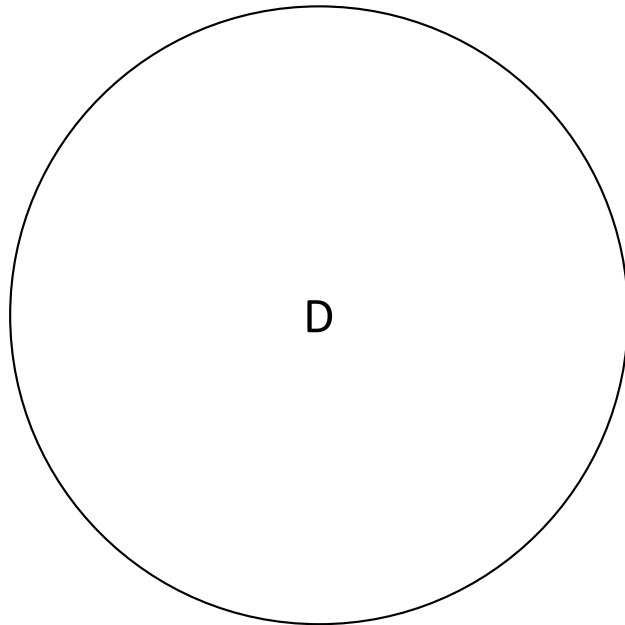
DNA must include:

2nd step:
the transgene is expressed

D

DNA must include:

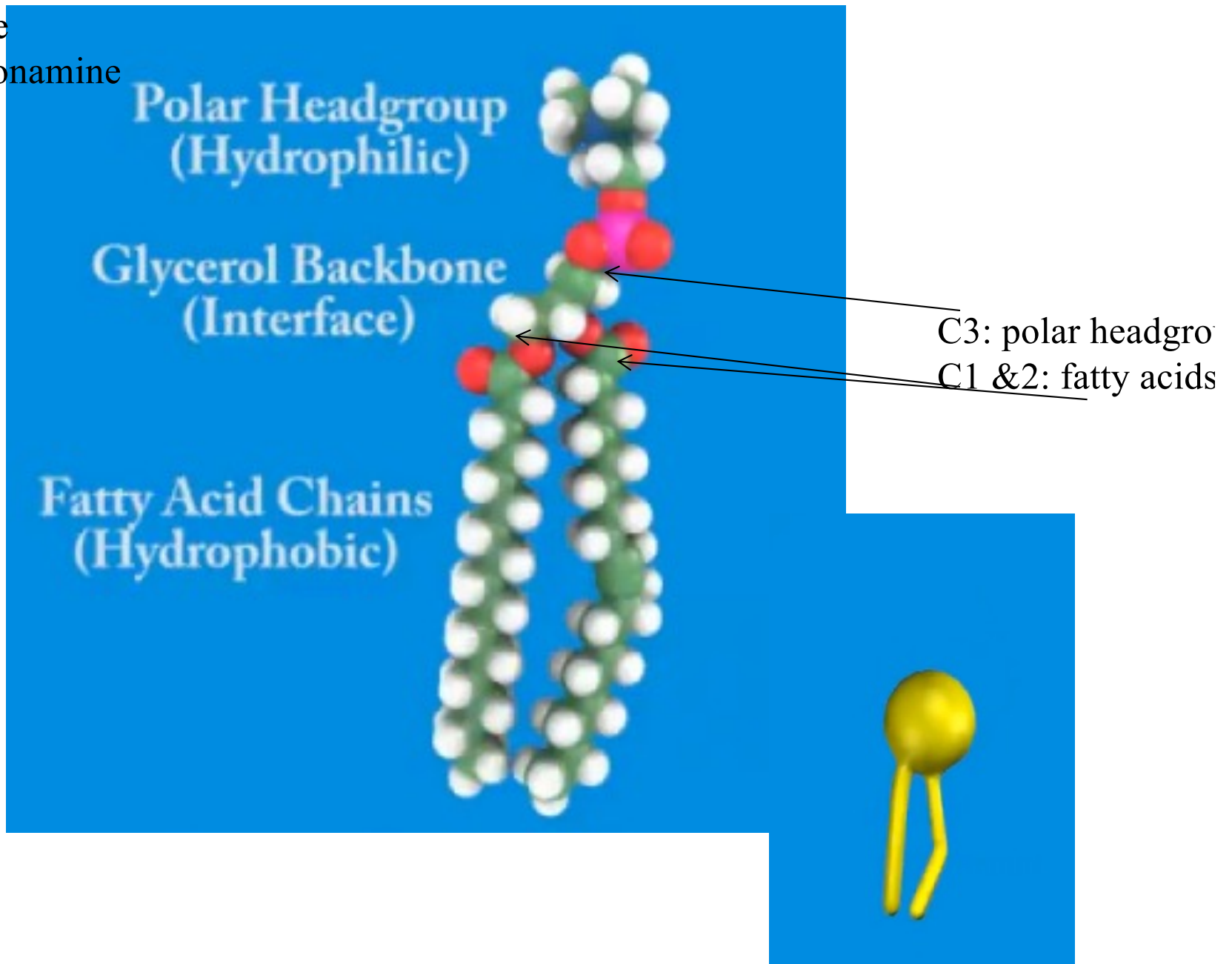- Antibiotic resistance
- *ORI*
- Eukaryotic expression casette

Liposome: microprecipates containing DNA

http://www.youtube.com/watch?v=04SP8Tw3htE&NR=1

video

Phospholipids (membrane component)
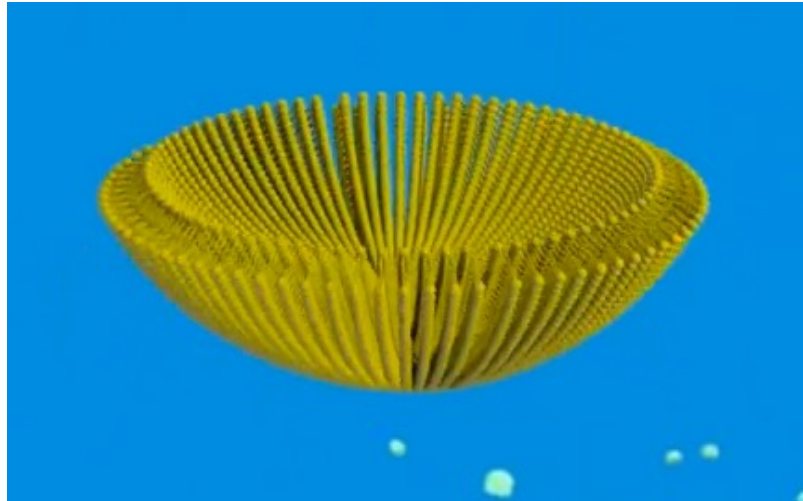
Phosphoryl-coline
Phosphoryl-ethalonamine
-Glycerol
-Inositol
-Serine
....

Lauric acid
Myristic acid
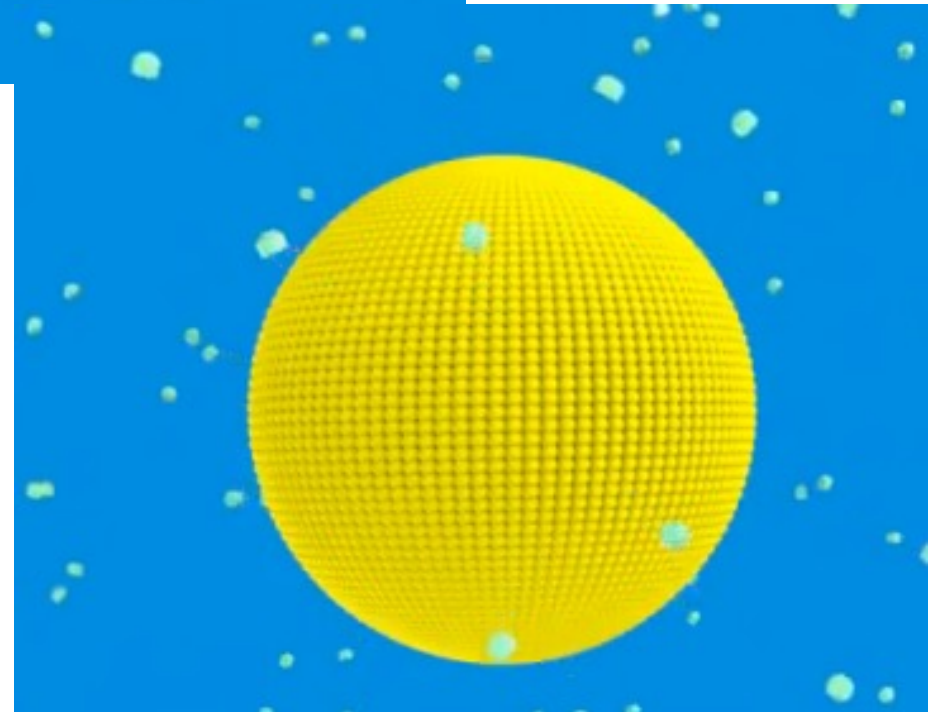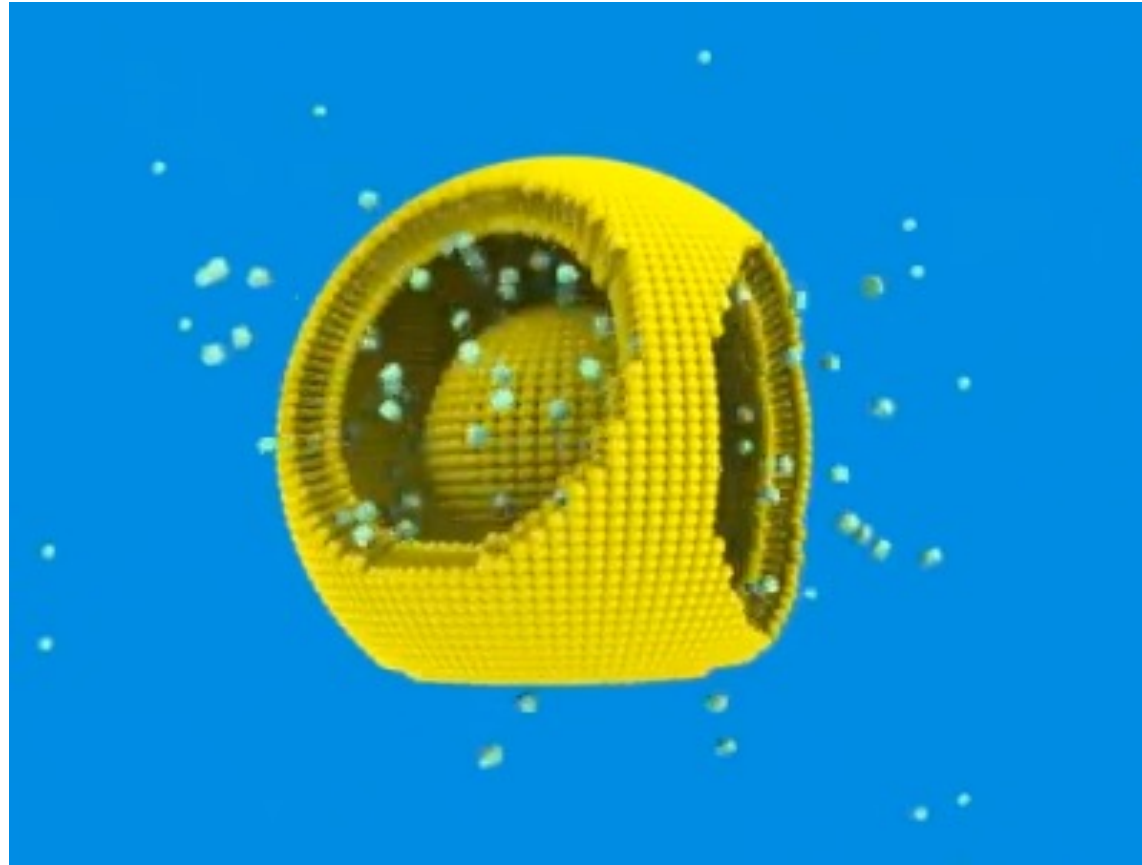Palmitic acid
Stearic acid
Oleic acid
...

Polar Headgroup
(Hydrophilic)

Glycerol Backbone
(Interface)

Fatty Acid Chains
(Hydrophobic)

C3: polar headgro
C1 &2: fatty acids

Liposomes

Nanometer size

In H_2O environment

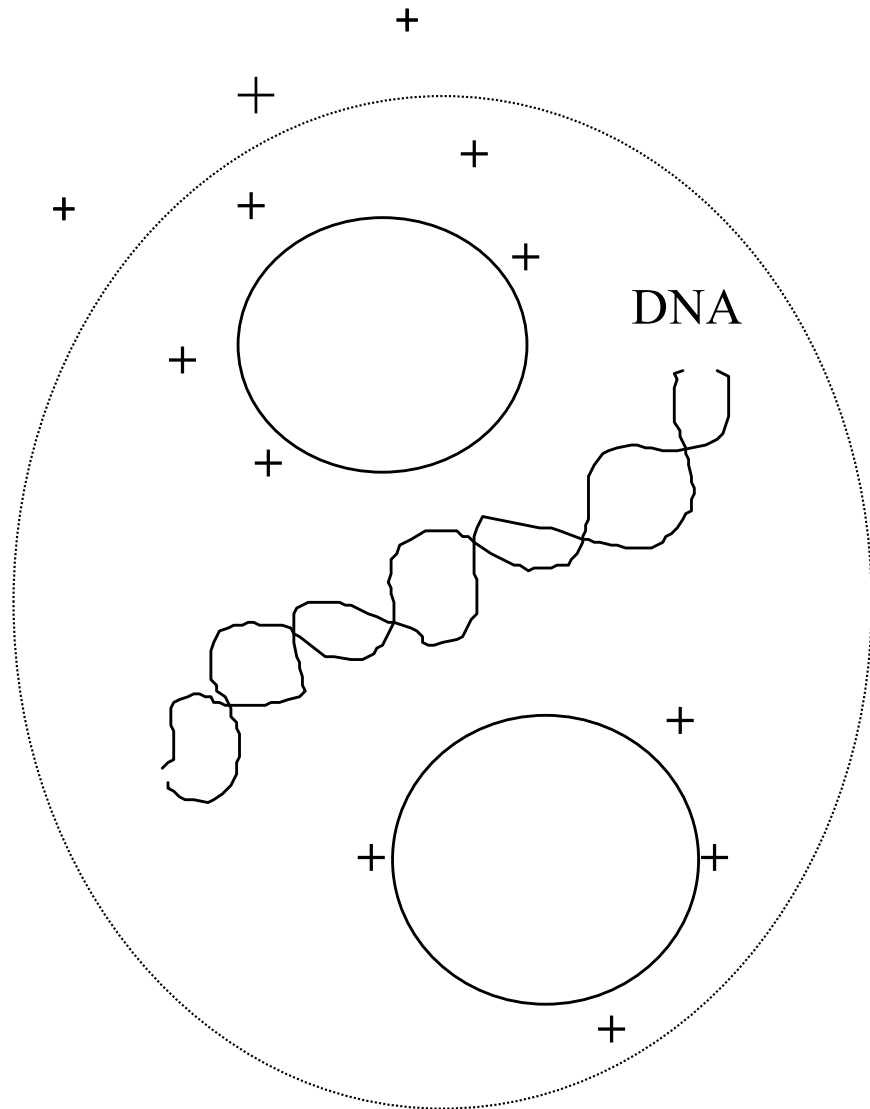# Enzymes and acids disrupt the liposome and let nucleic acids get out
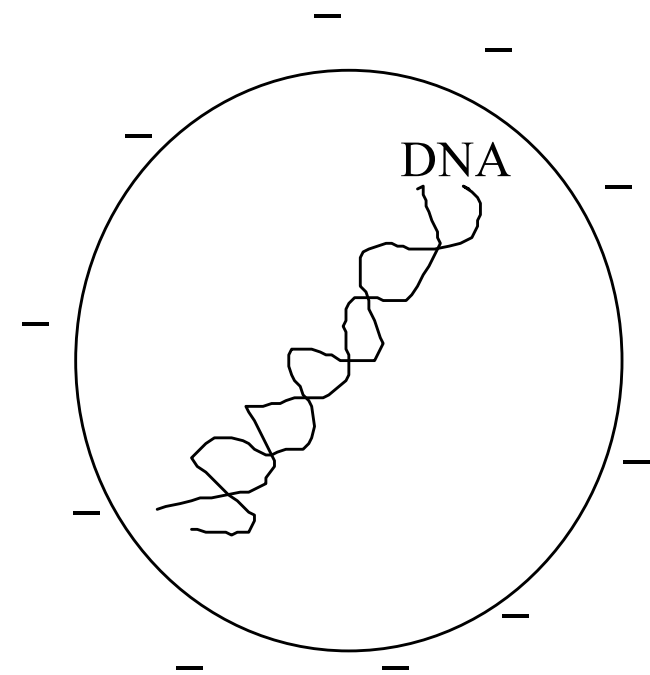
Liposomes/Lipoplexes
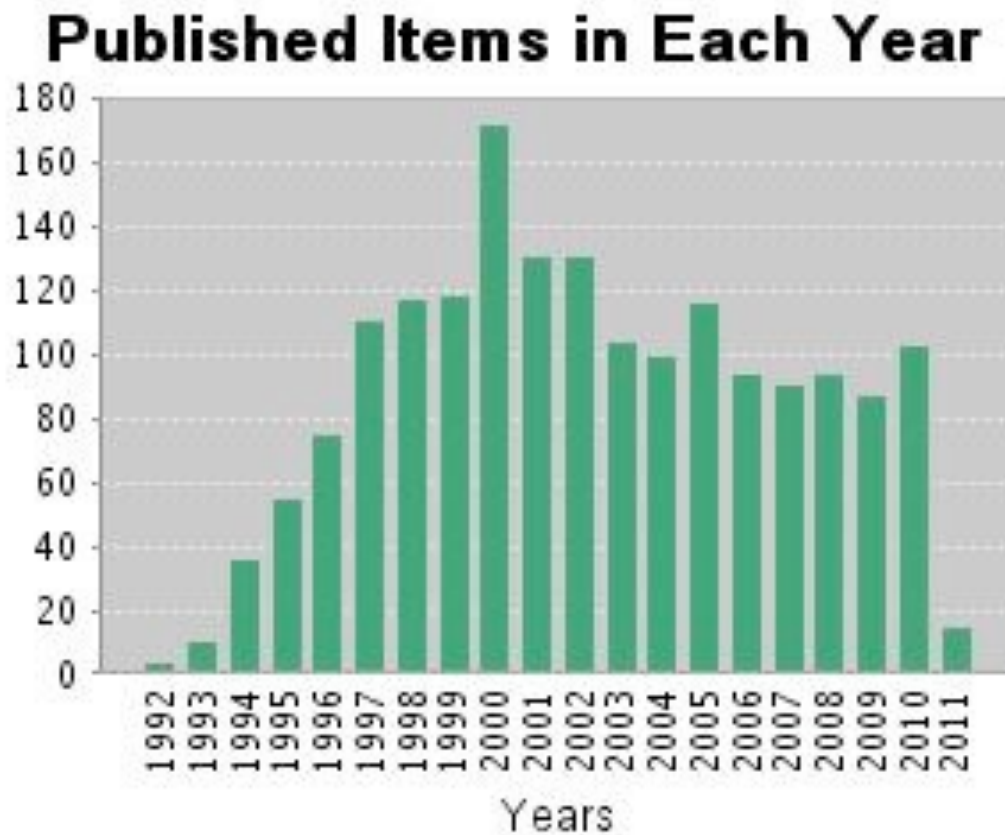## (12.1% clin tr.2003, 8.6% 2005,7.1% 2008; 6.5 2010)

DNA

DNA

Successful

Unsuccessful, pH sensitive, DOTAP

Liposome and gene therapy 1985 - 2011; ISI

Published Items in Each Year

h index 103

How does DNA-liposome enter the cell?

- Membrane fusion?

- Spontaneous endocytosis?

- mammalian cell surface is covered with negatively charged syalic acid

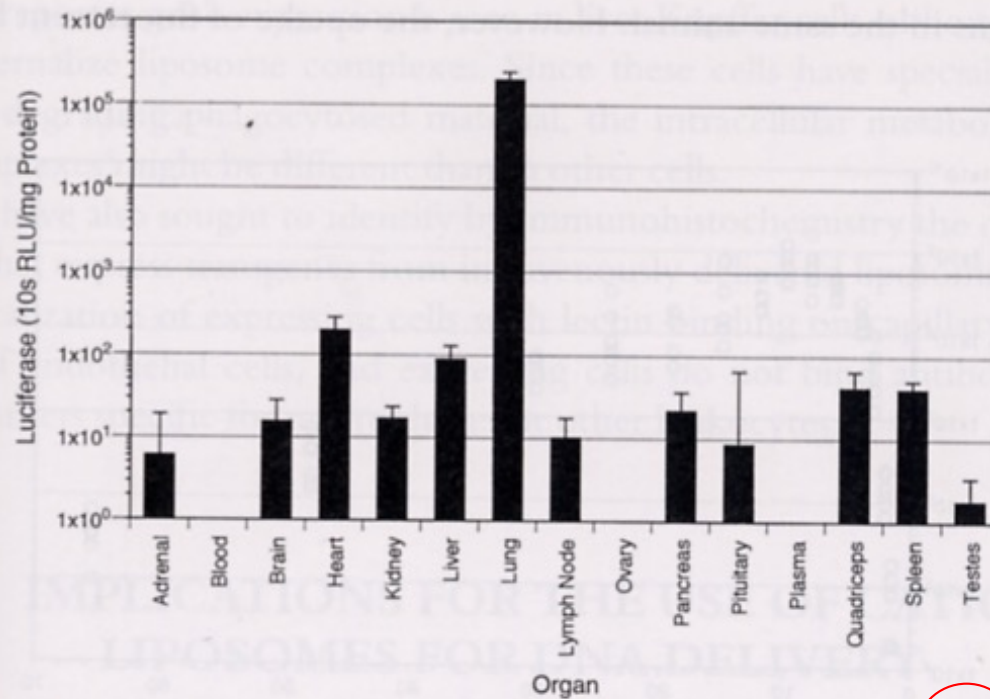# Liposome gene transfer in vivo (intraven.)
Organ distribution

Figure 7 Luciferase expression in organs at 24 h after the intravenous injection of 60 μg of luciferase plasmid complexed to 1440 nmoles of DOTAP/cholesterol (1/1) liposomes (mean ± SD, $n = 6$). No detectable expression above background was seen in blood, ovary, or plasma.

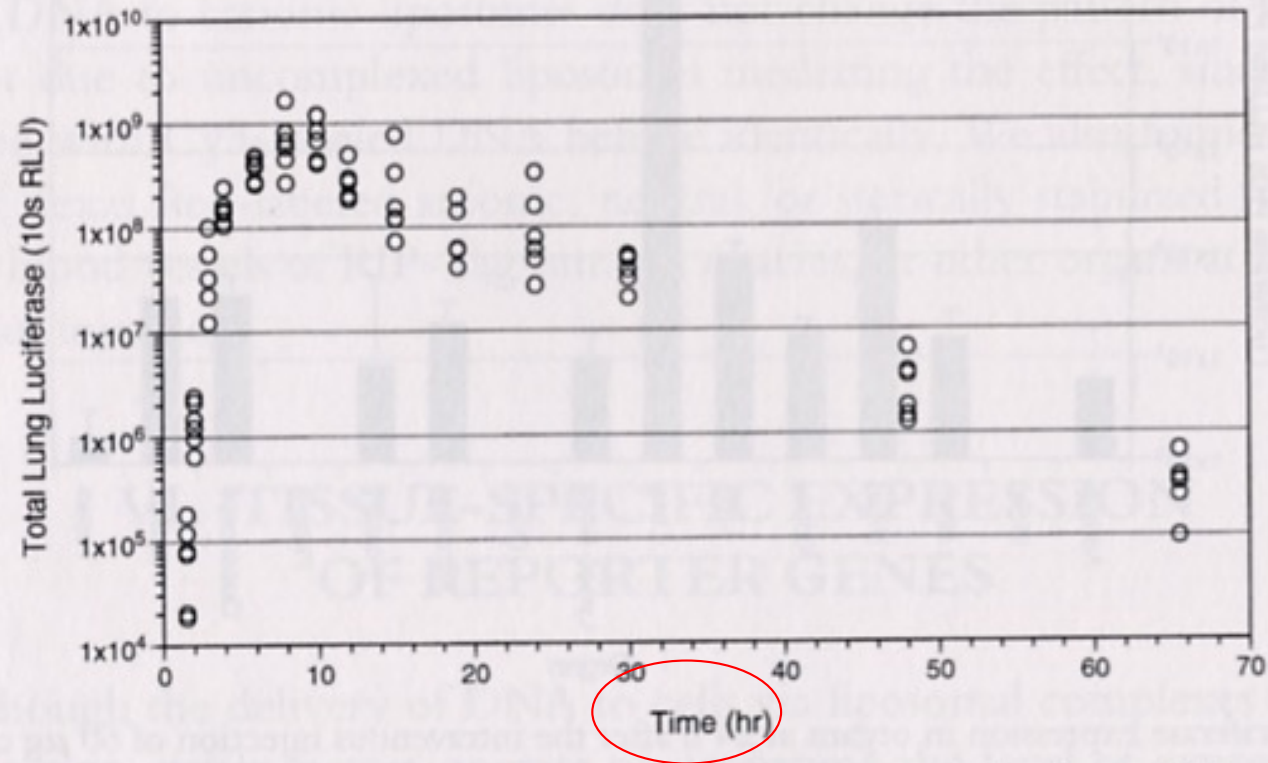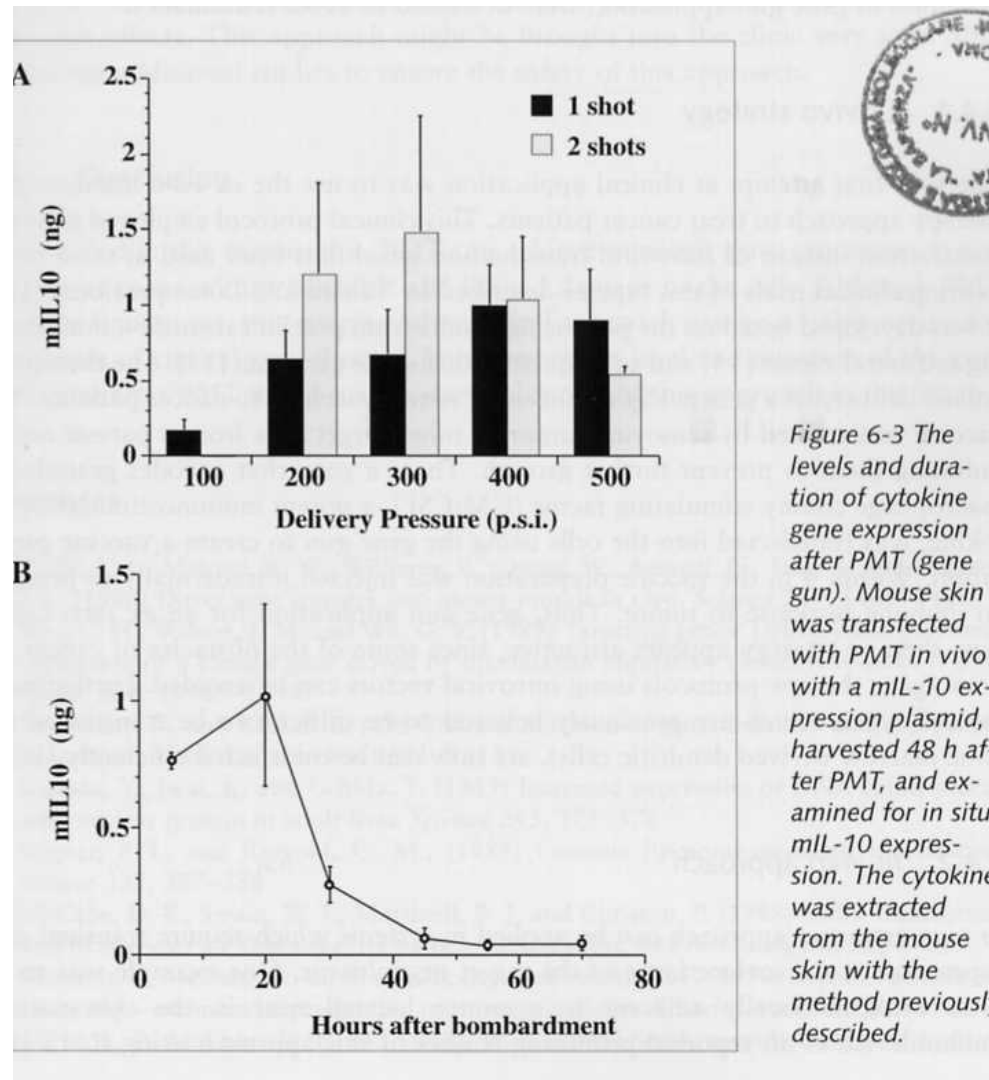# Liposome gene transfer in vivo (intraven.) Time course

Figure 8 Time course of expression in lungs after the intravenous injection of 30 μg of luciferase plasmid complexed to 540 nmoles of DDAB/cholesterol (1/1) liposomes ($n = 6$ per time point).

Gene gun transfection in vivo, injection in mouse skin

Figure 6-3 The levels and duration of cytokine gene expression after PMT (gene gun). Mouse skin was transfected with PMT in vivo with a mIL-10 expression plasmid, harvested 48 h after PMT, and examined for in situ mIL-10 expression. The cytokine was extracted from the mouse skin with the method previously described.

Dose dependent inflammation following intranasal instillation of lipoplexes into lungs of BALB/c mice. 48hrs after DNA inj.
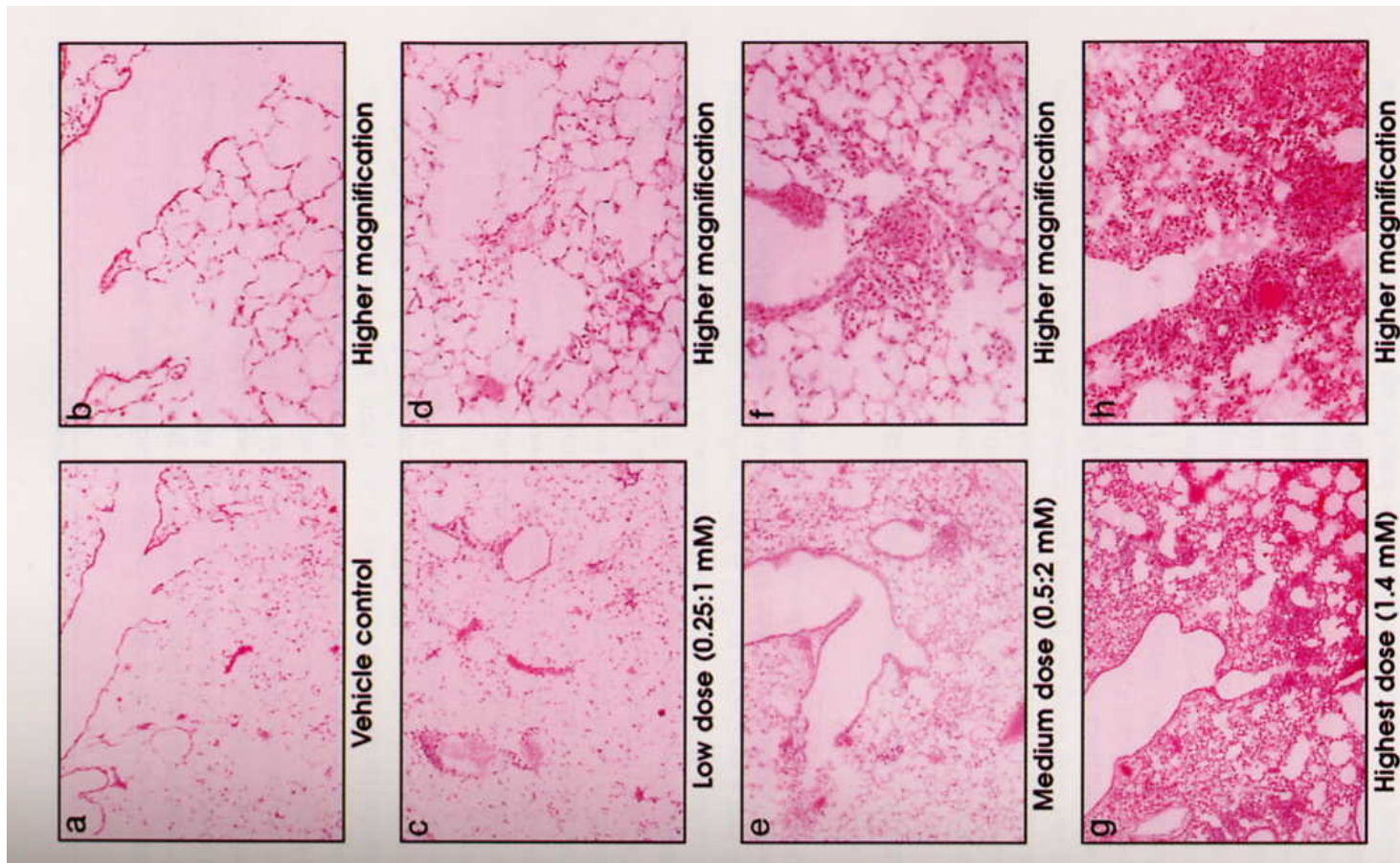
NB toxicity reduced with aerosol

Intracellular transport and plasmid biodistribution after cationic lipid mediated transfection

- 1.000.000 plasmids/cell transfected
- 300.000 plasmids/cell in pellet
- 50.000 plasmids/cell intracellular
- 1.000 plasmids/cell intranuclear (1/1000)

Delivery barriers that a vector must overcome

- ## <u>Extracellular barriers</u>
  Opsonins (act as binding enhancers for the process of phagocytosis)
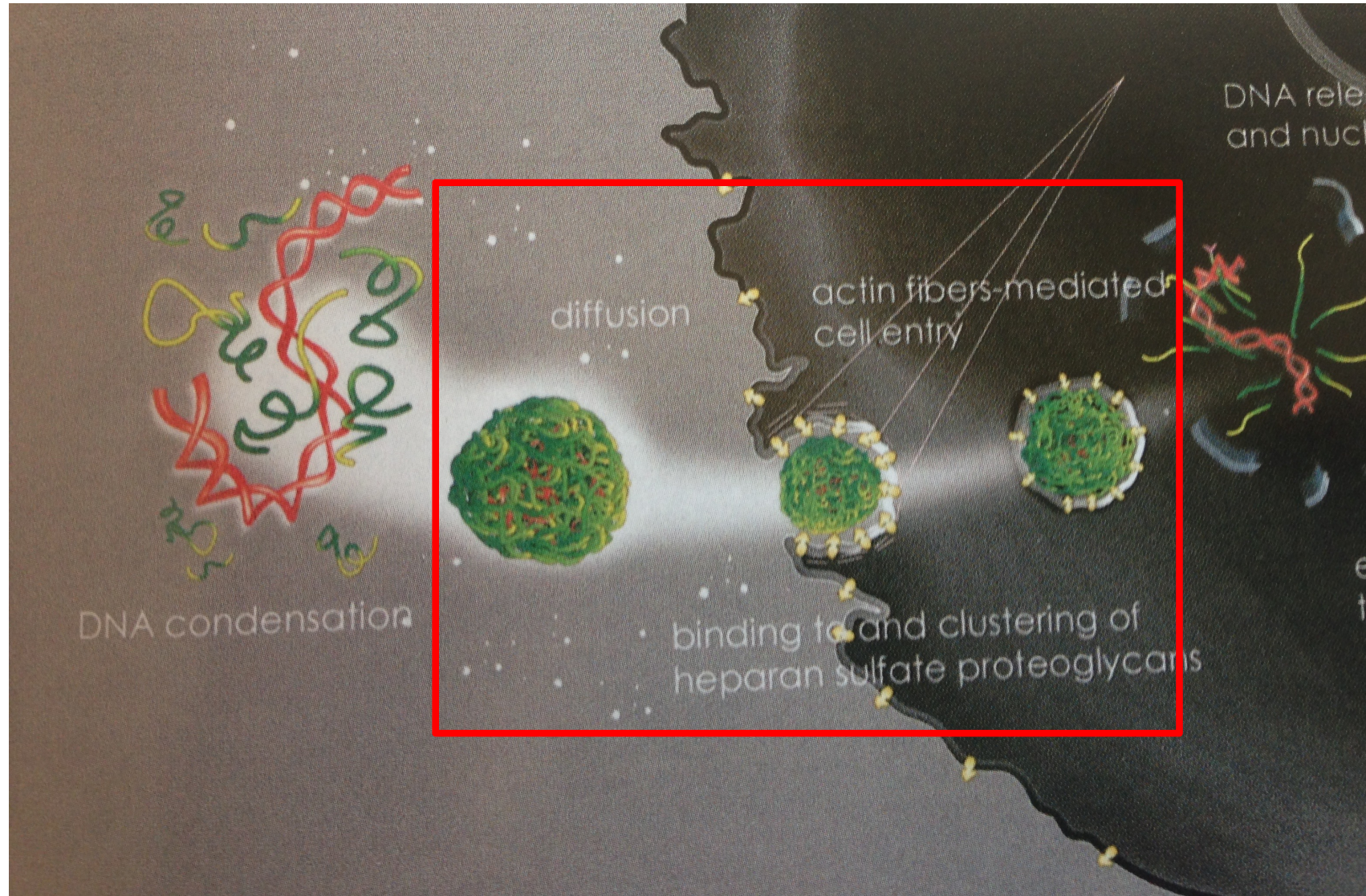 Phagocytic cells
  Extra-cellular matrix
  Digestive enzymes

- ## <u>Intracellular barriers</u>
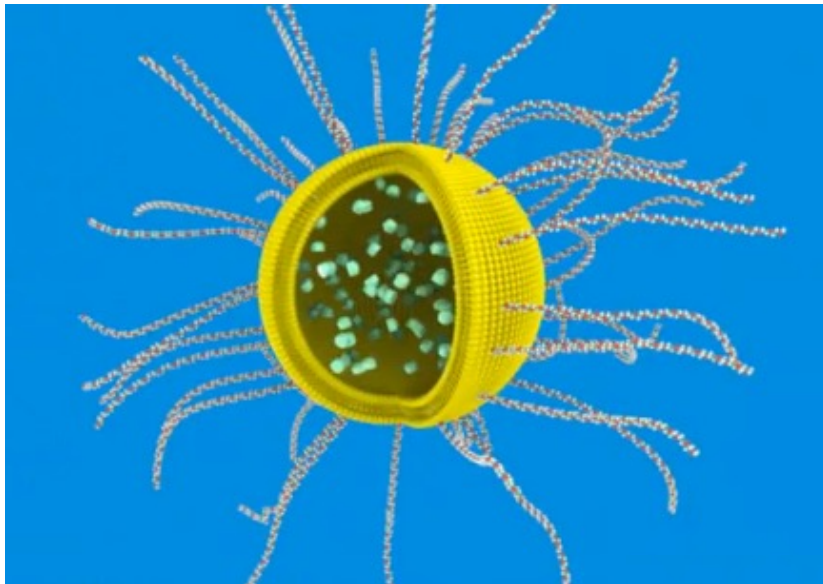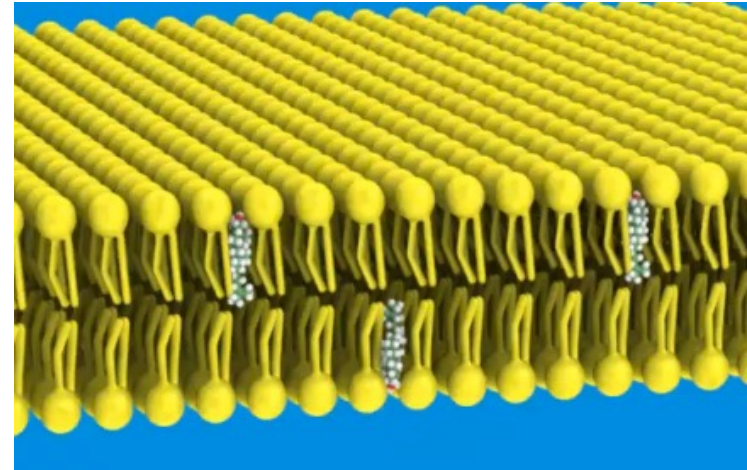 Plasma membrane
  Endosome/lysosome
  Nuclear membrane

Non viral vectors: the objectives of nanotech studies applied to gene transfer
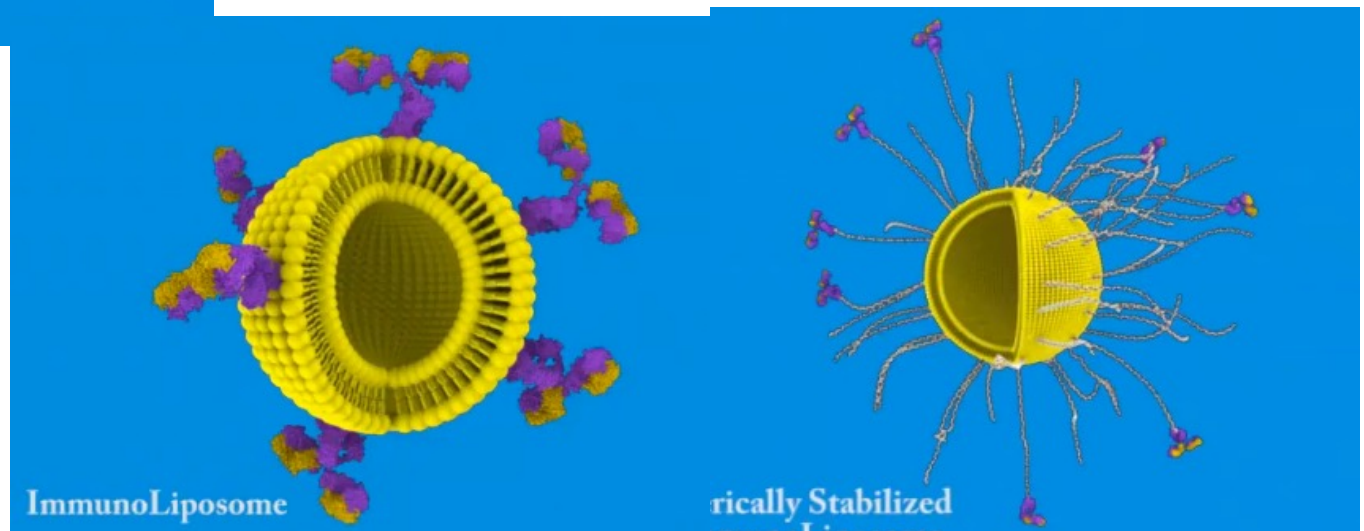
Liposomes/improvements

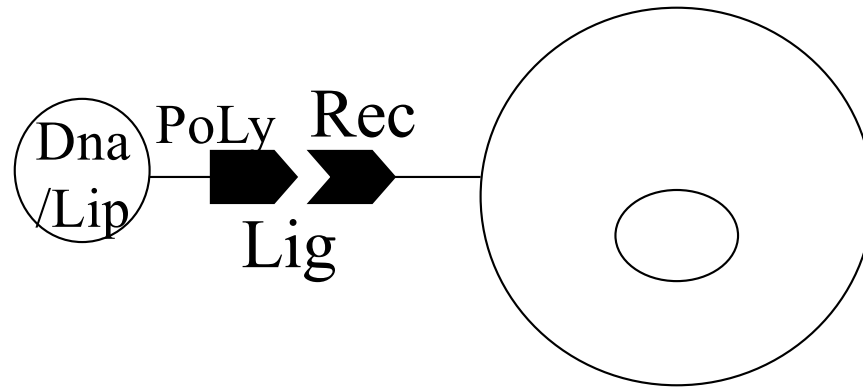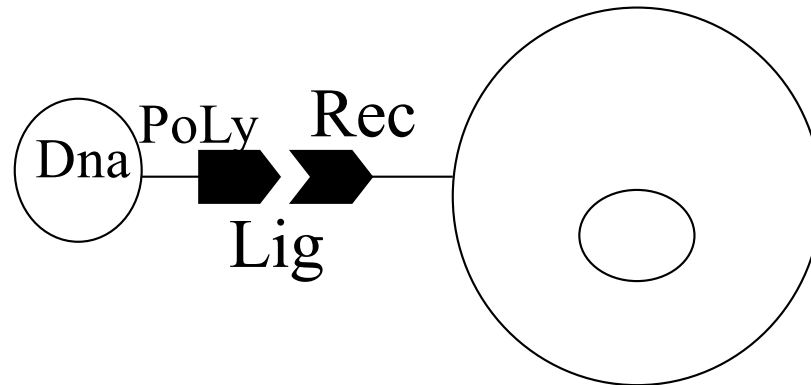+cholesterol-increase stability in vivo

+PEG, more stable

+Ab, targeting

ImmunoLiposome

rically Stabilized

Use of receptor ligands:
to facilitate binding to cell membrane

or

Which receptor ligand?

Table 1 Ligands Used in Receptor-mediated Gene Transfer

Ligands	Refs.
Alpha2 macroglobulin	24,25
Anti-CD3	26,27
Anti-CD5	28
Anti-CD117	29
Anti-EGF	30
Anti-HER2	31
Anti-IgG	32,33
Antisecretory component Fab	34–36
Anti-Tn	37
Antithrombomodulin	38
Antibody ChCE7	39
Asialoglycoproteins	40–49
EGF	50–52
Fibroblast growth factor 2(FGF2)	9,53
Folate	54–56
Glycosylated synthetic ligands	57–69
IgG (FcR ligand)	32,70
Insulin	10,71
Invasin	72
Lectins	73–75
Malarial circumsporozoite protein	76
RGD-motif (integrin binding)	77
Steel factor (CD117 ligand)	78
Surfactant proteins A and B	79,80
Transferrin	13,81–87

Liposomes-evolution

liposome drug-loaded liposome targeted liposome "stealth" liposome

mRNA-carrying lipid nanoparticle solid lipid nanoparticle nanostructured lipid carrier cubosome
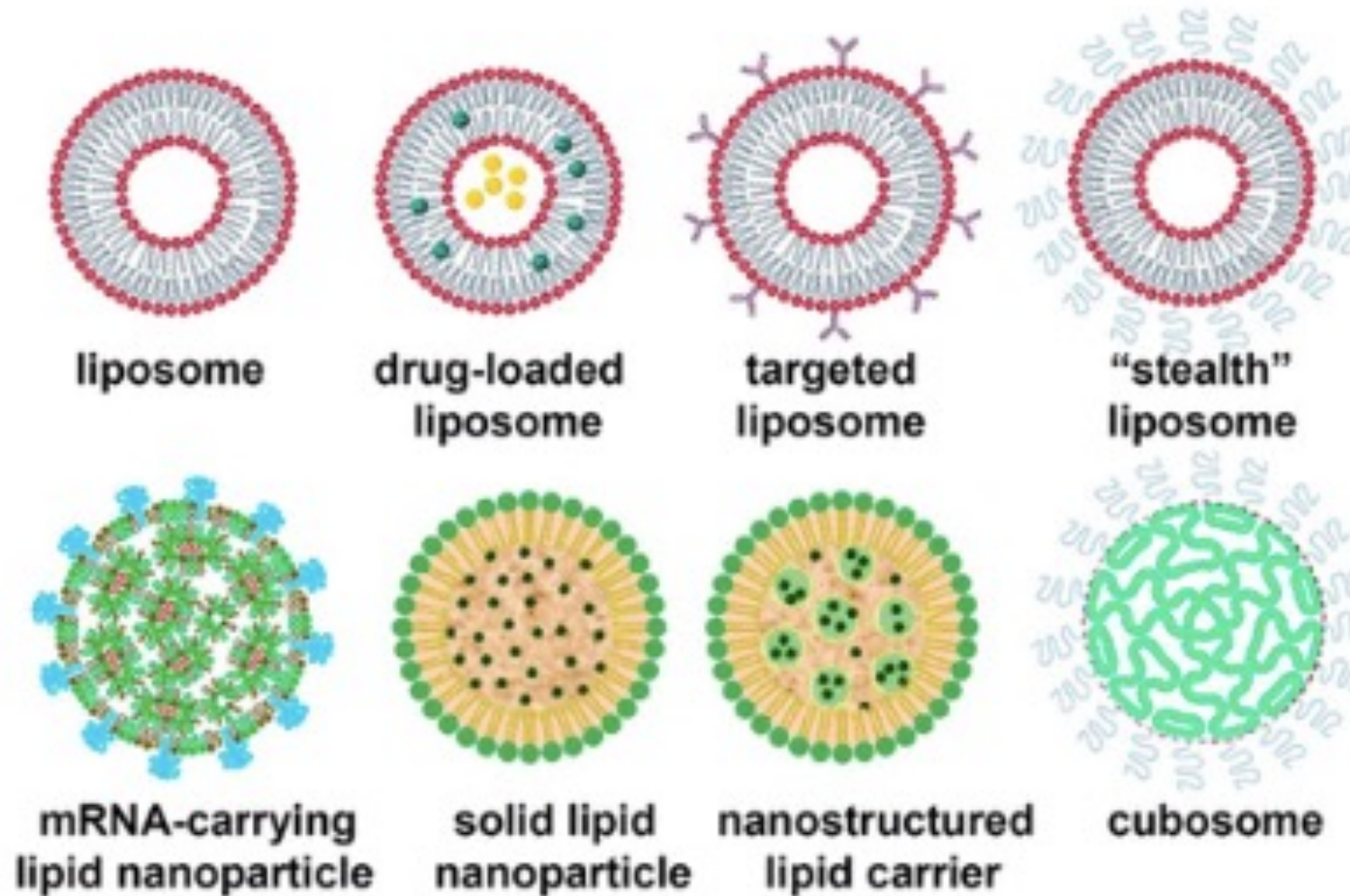
Liposomes- time line evolution

Tenchov et al, ACS Nano 2021
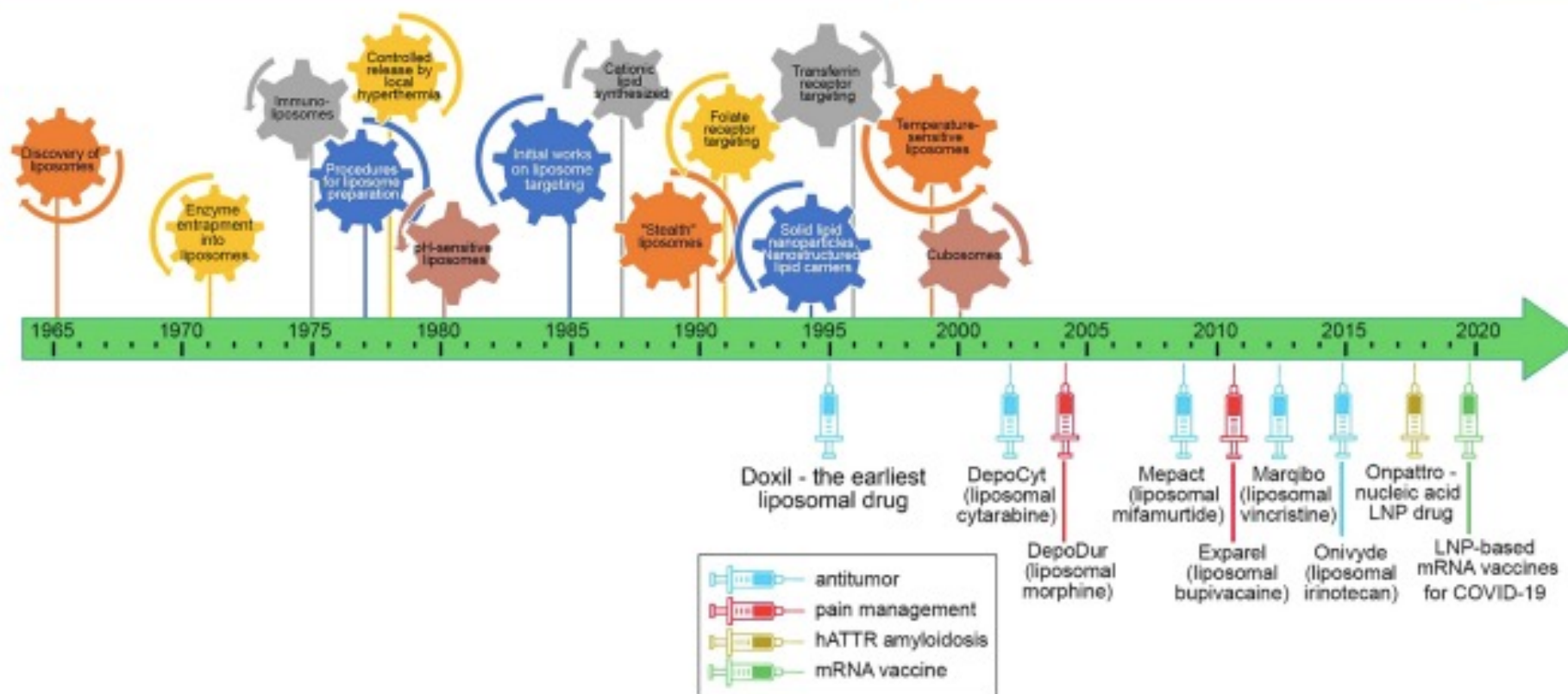
Gene therapy project

Theme I: Aging
Group A: Bernardi, Ilie, Colonnelli, Bastianelli
Charcot marie tooth – x linked – gjb1
Group B: Hazrati, Bartolini, Glaudo, Montrone
Sarcopenia - CD38

Theme II: Cancer
Group C: Belvedere, Jeong, Majaliwa, Virgilio (new entry)
Retinoblastoma – rb1
Group D: Santacroce, Pace, Serra, Fanelli, Duarte
tbd

Gene therapy project

30/10/23

1 slide/group

Title
General Idea