Determining the Mode of DNA Delivery by Non-viral Gene Delivery Vectors

Supervisor: Cyrus Safinya

Mentor: Nathan Bouxsein

Chris McCalister (Bio)

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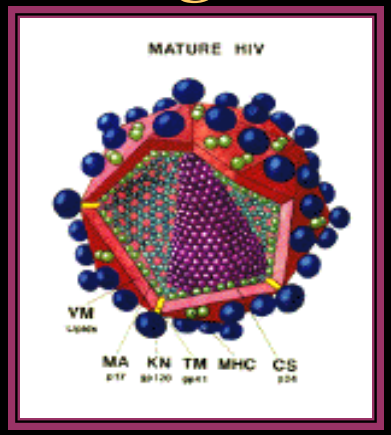
RET I Summer Research Presentation by Lisa Boyer 07-27-06

Gene Therapy
Gene Delivery Systems

Gene Delivery Systems

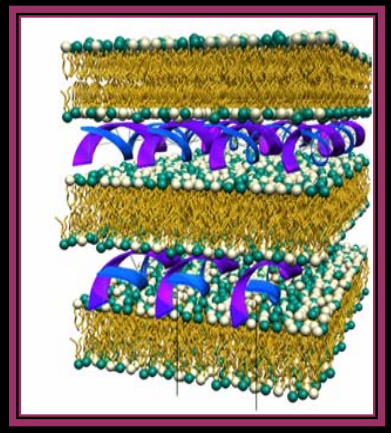
Biological :



•<u>Retrovirus</u>: integrates transgene into chromosome

•<u>Adenovirus:</u> no integration

Chemical :



•Polymers

•Peptides

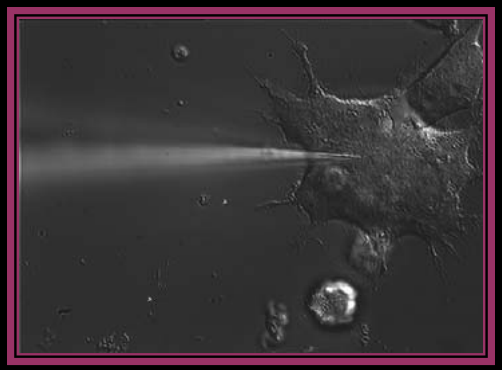
•Cationic Liposomes

Low immune response

No size limitation

Ease of Production

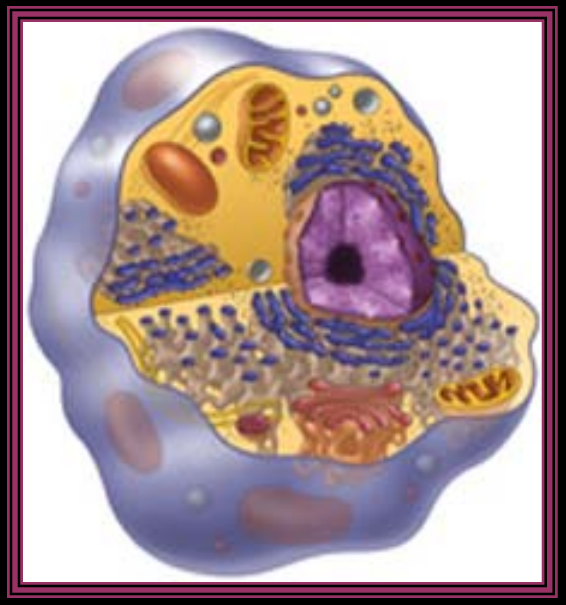
Physical :



•Electroporation

•Microinjection

Research Goals



Successful Vector:
1. Package DNA
2. Deliver DNA to Cell
3. Release DNA into nucleus

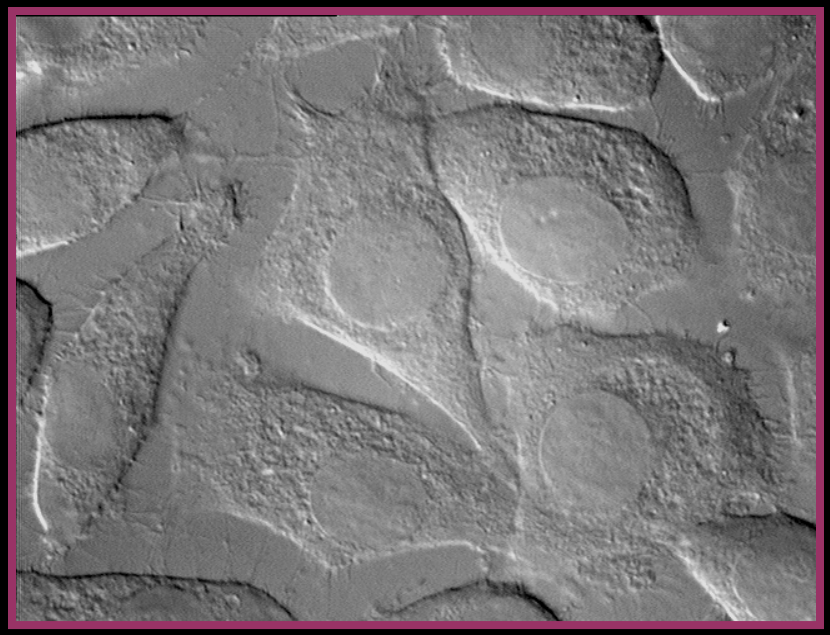
- **KNOWN**: When DOTAP/PE and DOTAP/PC are at ratios of high neutral lipid, DOTAP/PE has a higher transfection efficiency than DOTAP/PC

- **GOAL**: To figure out why this difference occurs
  - Is it due to method of delivery?

Methods
Care of Cells

- Mouse L-cells are used for transfecting DNA, so the cell line must be maintained

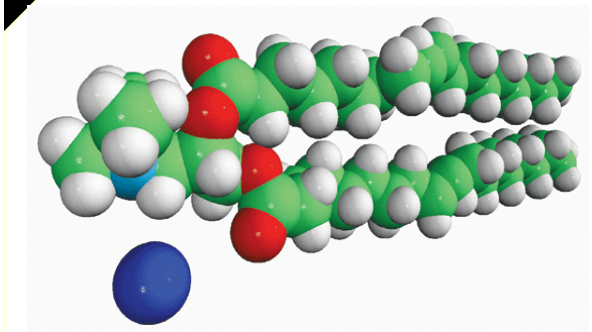
- Splitting Cells
  - Cells are cleaned and diluted into new media every 3 days

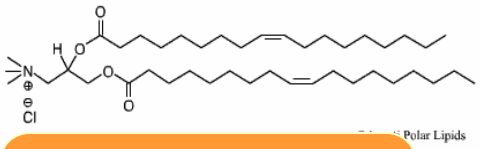


Methods

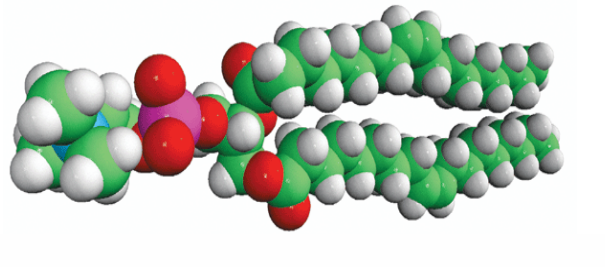
Lipid Stock TAP/PC & TAP/PE

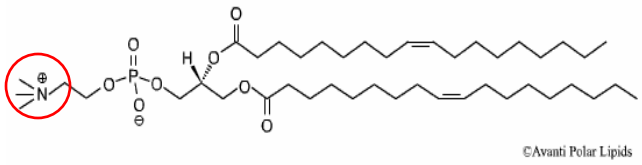
- Make DOTAP, DOPE,
  & DOPC @ 5mM concentrations
- Create molar ratios of DOTAP to PC or PE



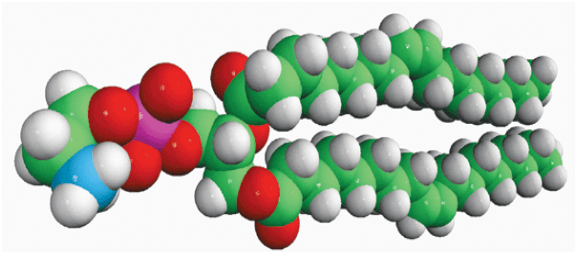


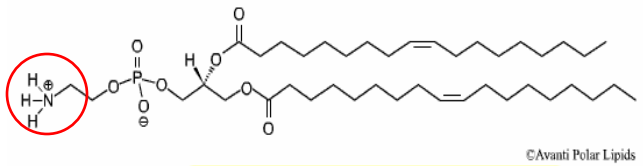
DOTAP
Cationic Lipid





DOPC
Neutral Lipid





DOPE
Neutral Lipid

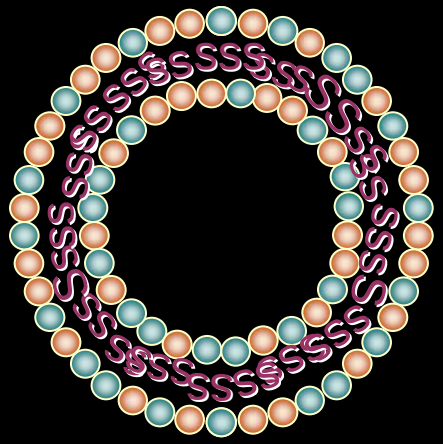
Complex Formation

Lipids:

Self-Assembly

Driving Force:
HYDROPHOBIC EFFECT

Liposomes:

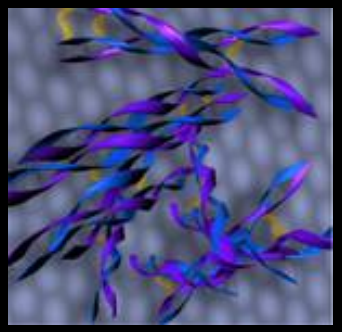


Mixture of Cationic And Neutral Lipids

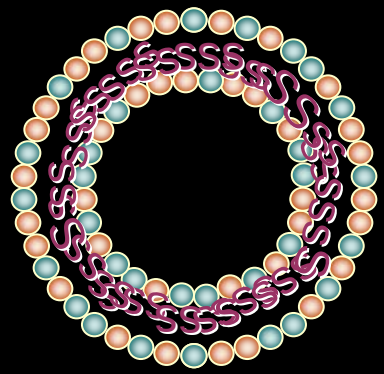
Methods
Forming Complexes of DNA and Lipids

- Determine volume of mixture so there is a charge ratio of 2.8:1 (CL:DNA)
  - Firefly Luciferase DNA is used for transfection

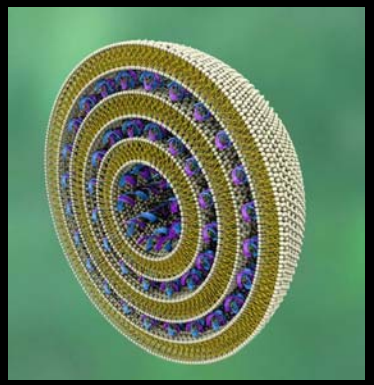
Complex Formation:



DNA



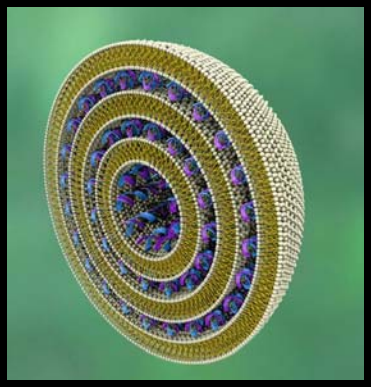
Liposome



Complex

Methods
Transfecting Cells

- Seed a 24 well plate by placing L-cells and media in each well & allowing to incubate
- Place complexes into wells & allow to incubate



Complex Formation

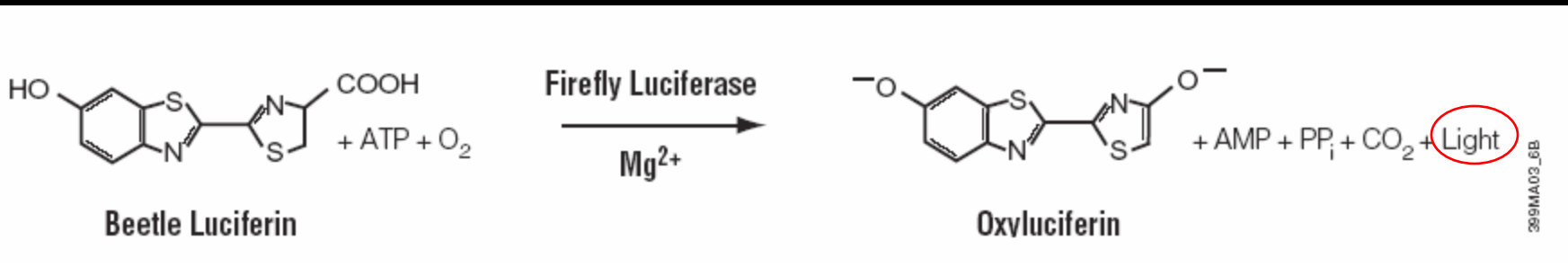
Cells: Mouse Fibroblast L-cells

Methods
Readings for Transfection

- Add lysis buffer to well to lyse cells
- Transfer extract to 96 well plate
- Use PerkinElmer plate reader to measure luminescence
  - Firefly Luciferase assay added during readings

Measure Luciferase Protein Expressed

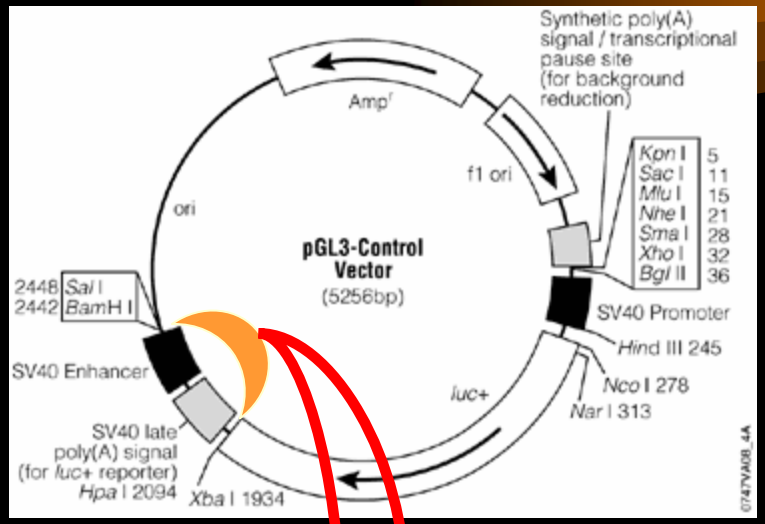
Emission of light @ 560nm

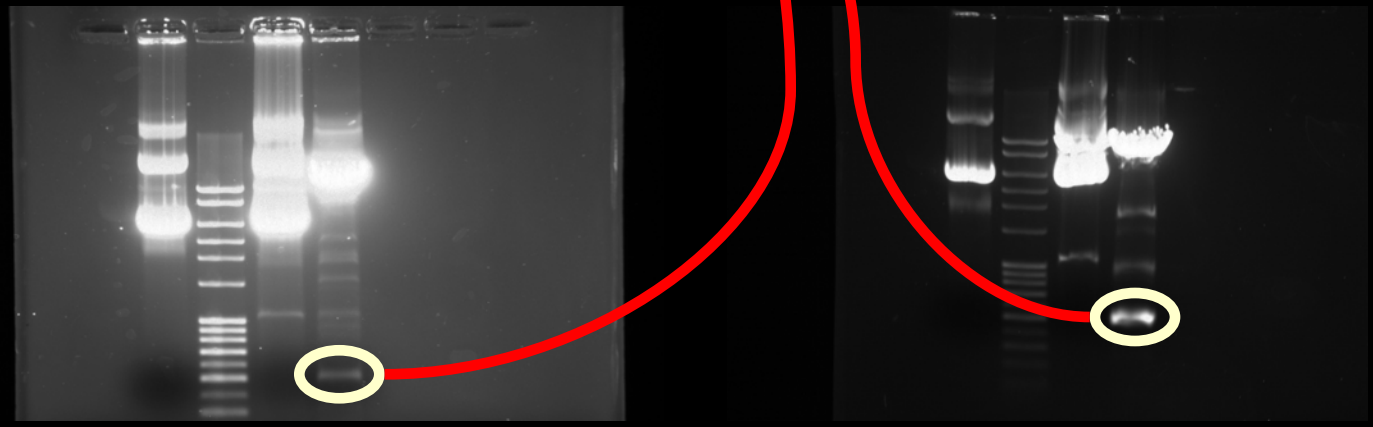


Methods

Dot Blot ~ Making the Probe

1. Cut plasmid with Xba1 and BamH1 restriction enzymes.
2. Separated DNA on agrose gel
3. Purify selected bands to recover DNA
4. Add α^{32}P to DNA



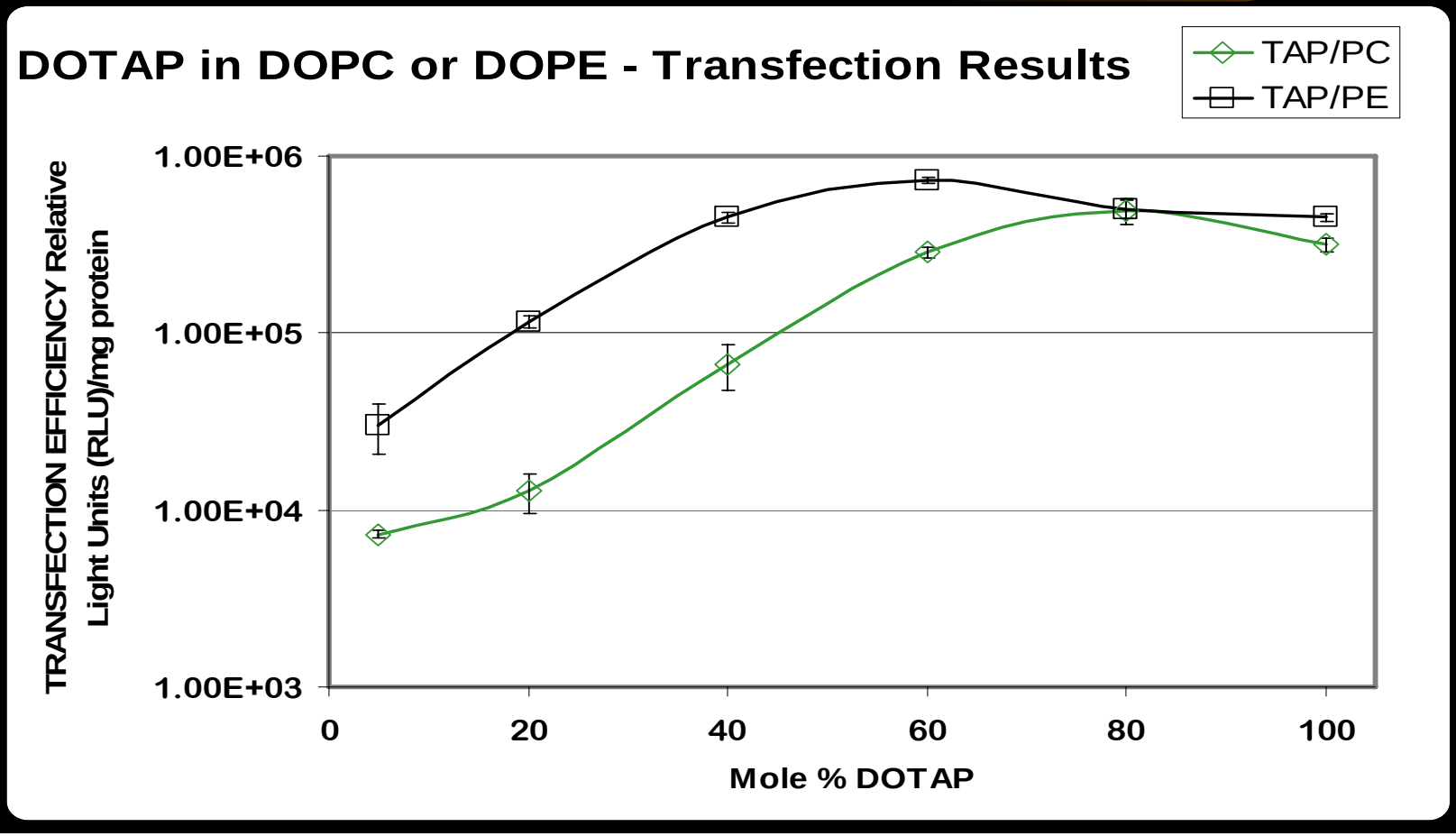


Methods
Dot Blot

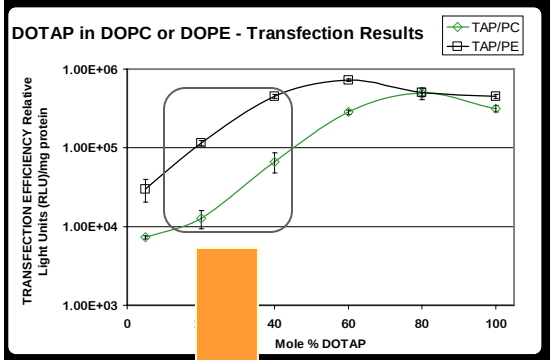
- Create 11 2x dilutions of DNA, SSC, & formaldehyde mixture in 96 well plate
- Set up apparatus with nylon membrane inside placing samples on membrane
- Cross link DNA to membrane using UV Stratalinker
- Add probe to membrane
- Use X-ray Film to capture image of radioactivity on Dot Blot membrane

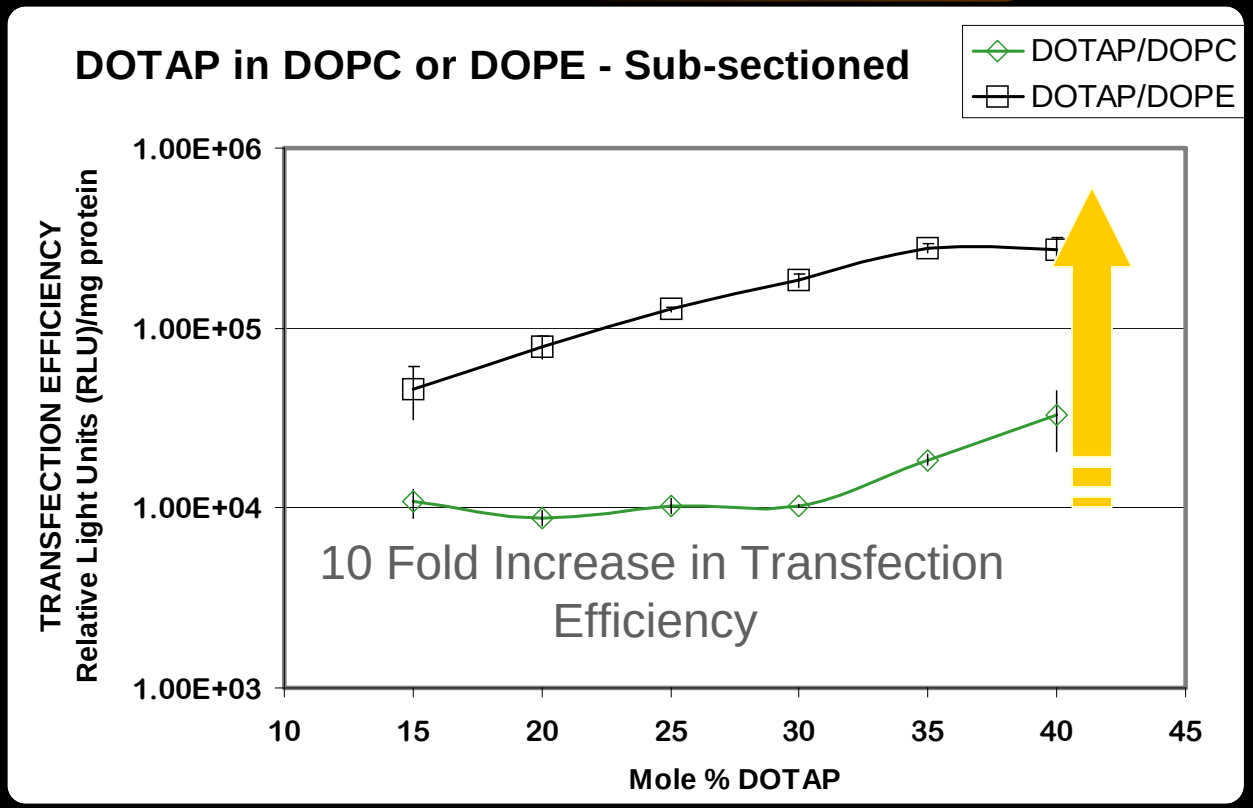
Results Transfection

- pGL3 Luciferase plasmid DNA (0.4mg/sample)

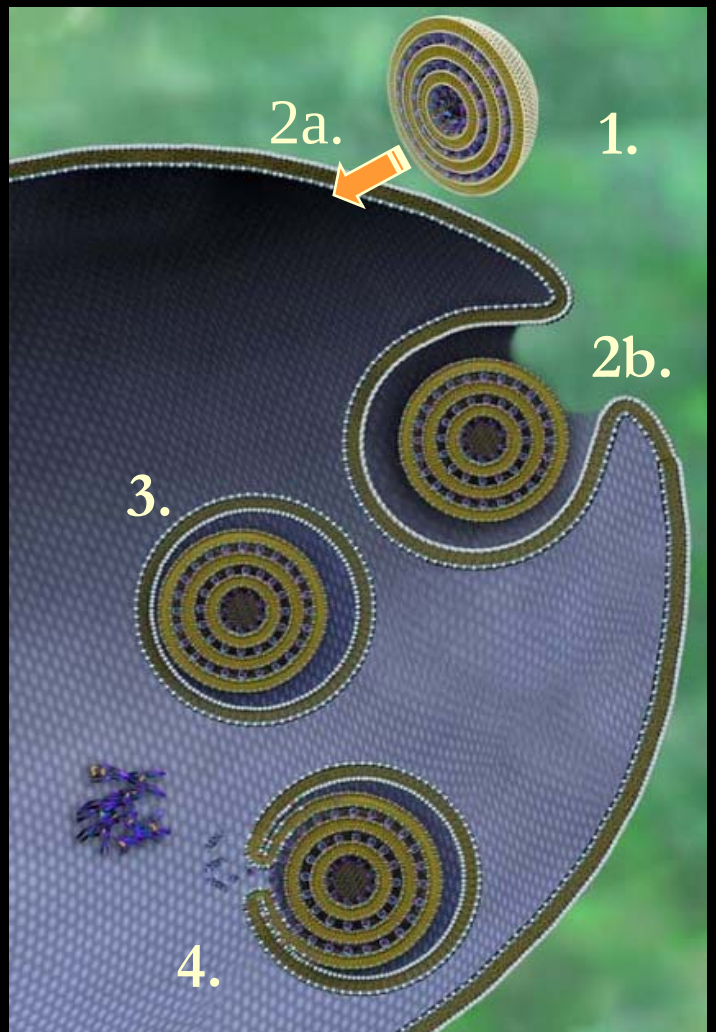


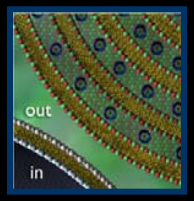
Results Transfection





Pathways of DNA Delivery

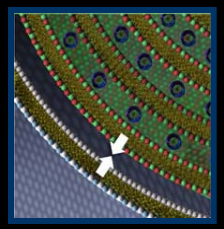




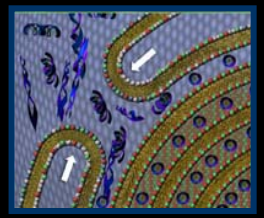
1. Initial Adhesion: Predominantly Electrostatics

2a. Direct Fusion – TAP/PE only

2b. Endocytosis: Uptake of foreign material
Engulfs material
Forms an intracellular vesicle

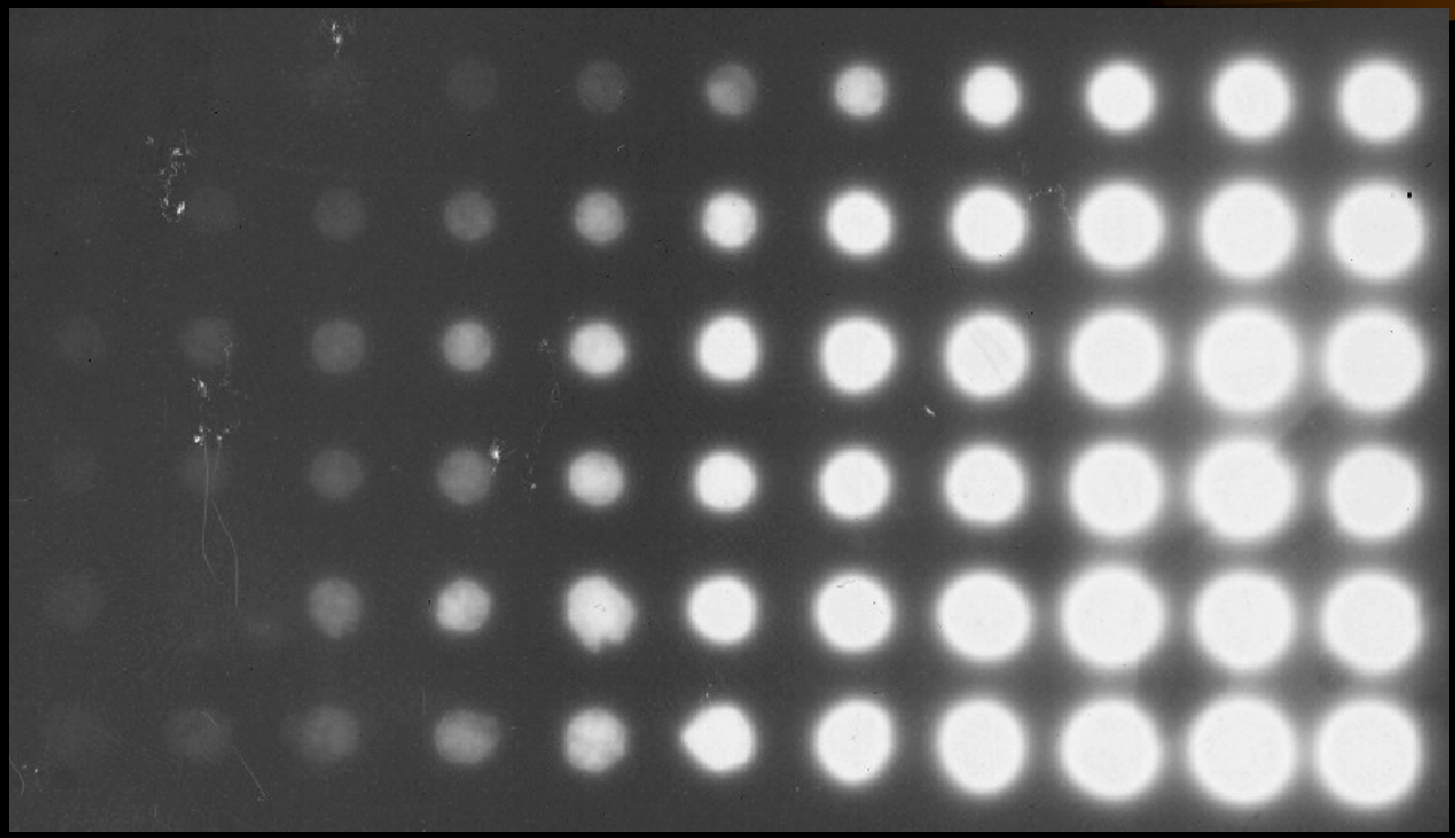


3. Endosomal Interaction



4. Lipid Fusion and DNA release

Results
Dot Blot



DNA ONLY

L2000

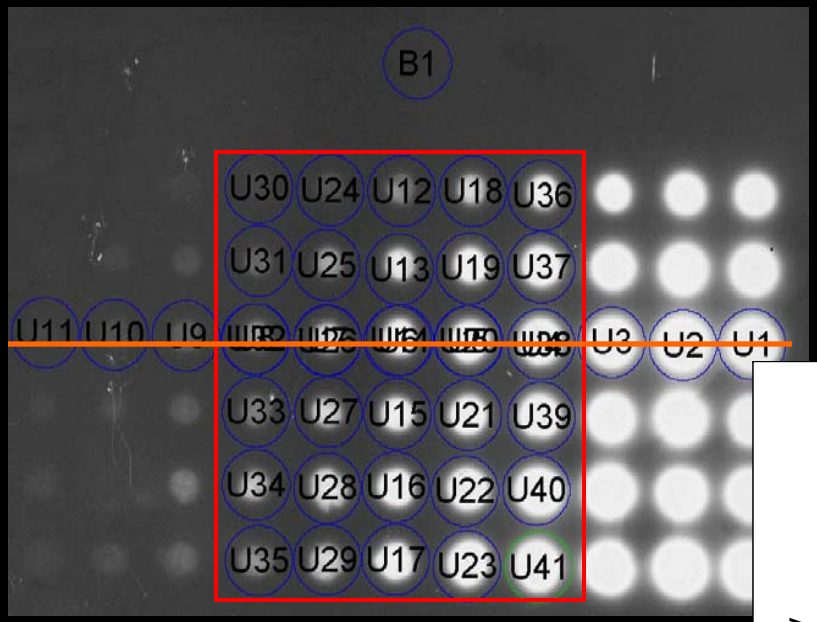
DOTAP 40% PE 60%

DOTAP 40% PC 60%

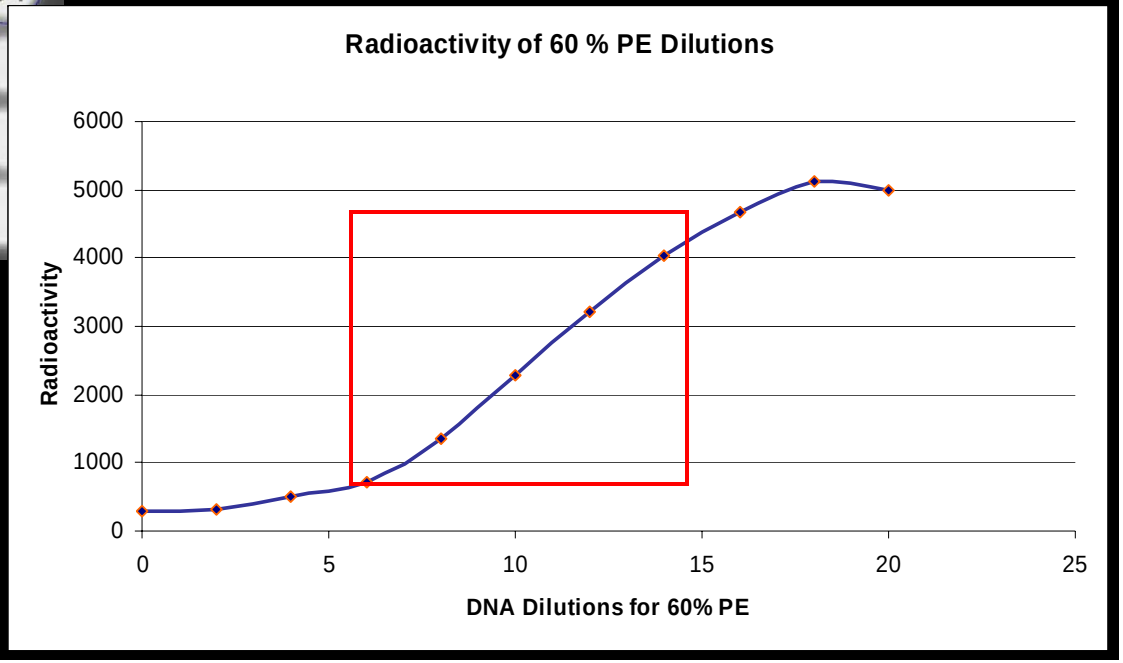
DOTAP 80% PE 20%

DOTAP 80% PC 20%

Results
Dot Blot

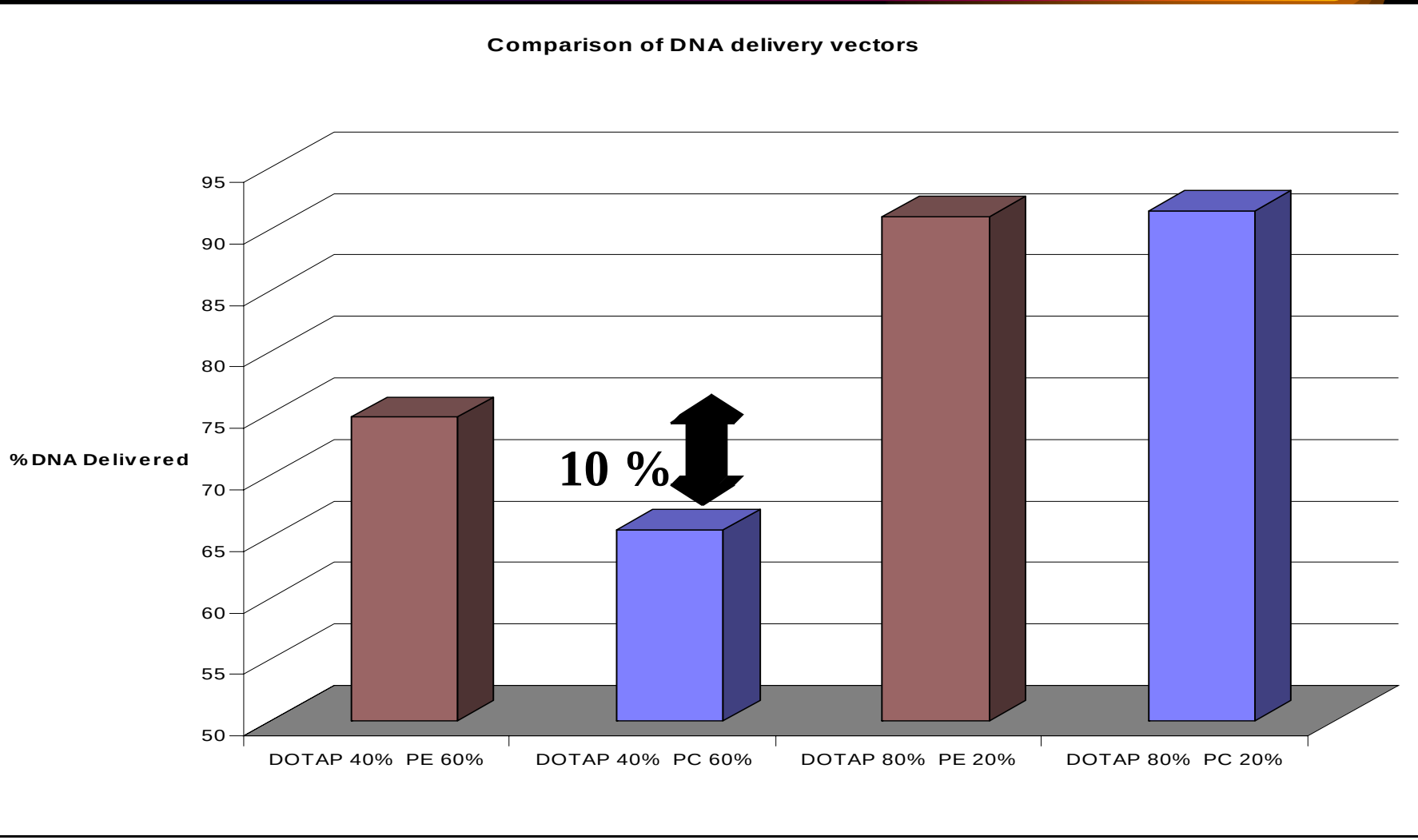


- Use data to find a linear range

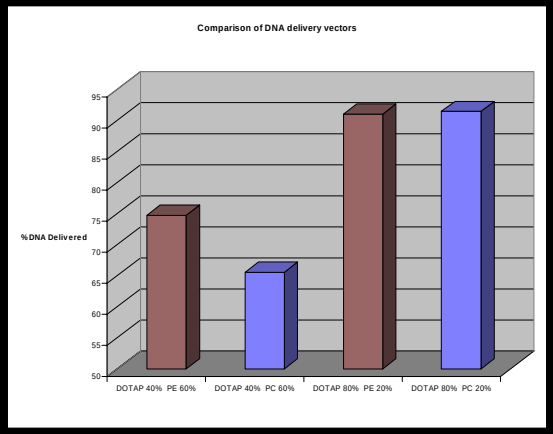


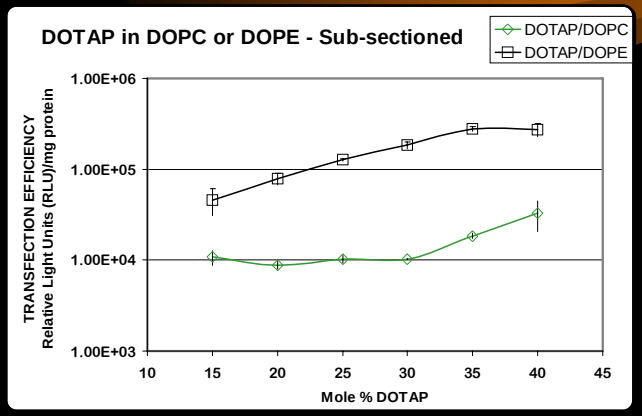
Results

Dot Blot ~ % DNA Delivery



Analysis ~ Discussion





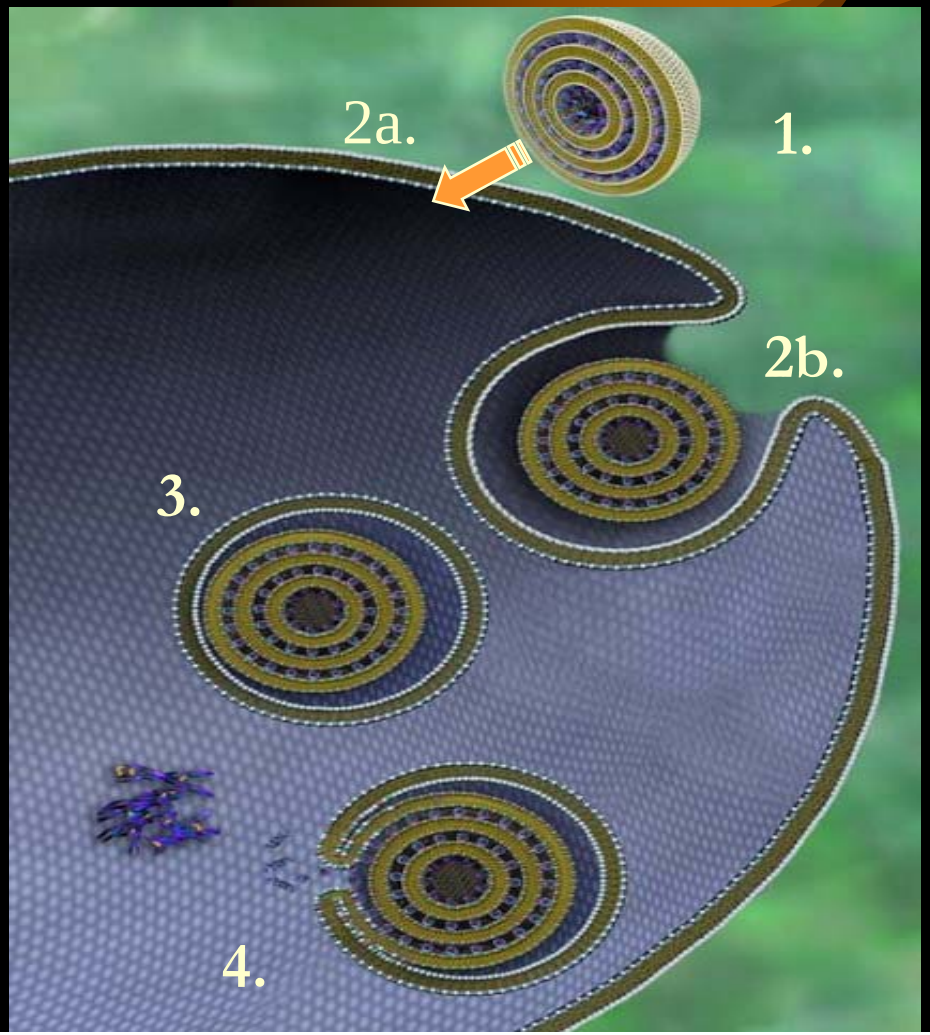
- The 10% increase in DNA released by PE is likely due to direct fusion with the cell membrane
- Is the increase in delivered DNA by PE enough to account for the 10 fold difference in gene expression?
  - A single DNA molecule produces many protein

Conclusion

- Possible Pathways for releasing DNA

A. PE is able to release DNA from endosome (step 4), while PC is trapped in endosome (step 3)

B. PE directly fuses with cell membrane



Further Research

- Measure the amount of nuclear DNA & cytoplasmic DNA for both PC & PE using techniques similar to dot blot.
- If we see that PE has less cytoplasmic DNA than PC, but has more nuclear DNA than PC we can infer that PE facilitates endosomal release.
- If we see that PE & PC have equal amounts of cytoplasmic DNA, but PE had more nuclear DNA than the 10% increase in DNA delivery can possibly account for the 10 fold increase in gene expression.

Acknowledgements

- MRL and RET Program
- Safinya's and Samuel's Labs
- Martina
- My Dumb & Dumber Mentors

  aka: Nate & Chris