siRNA delivery systems: lipoplexes vs. polyplexes

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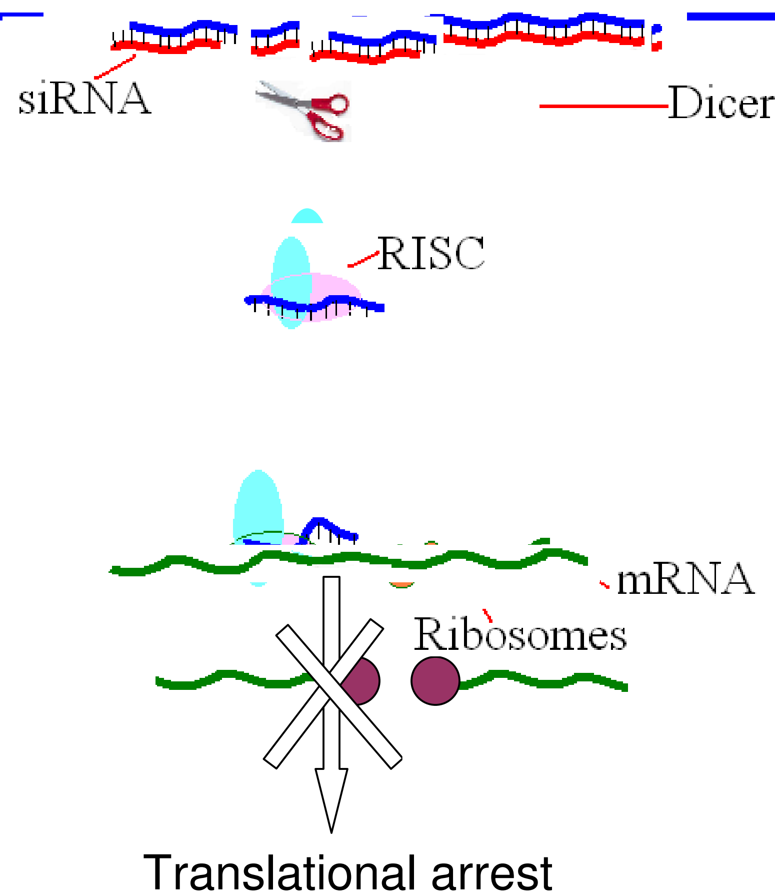
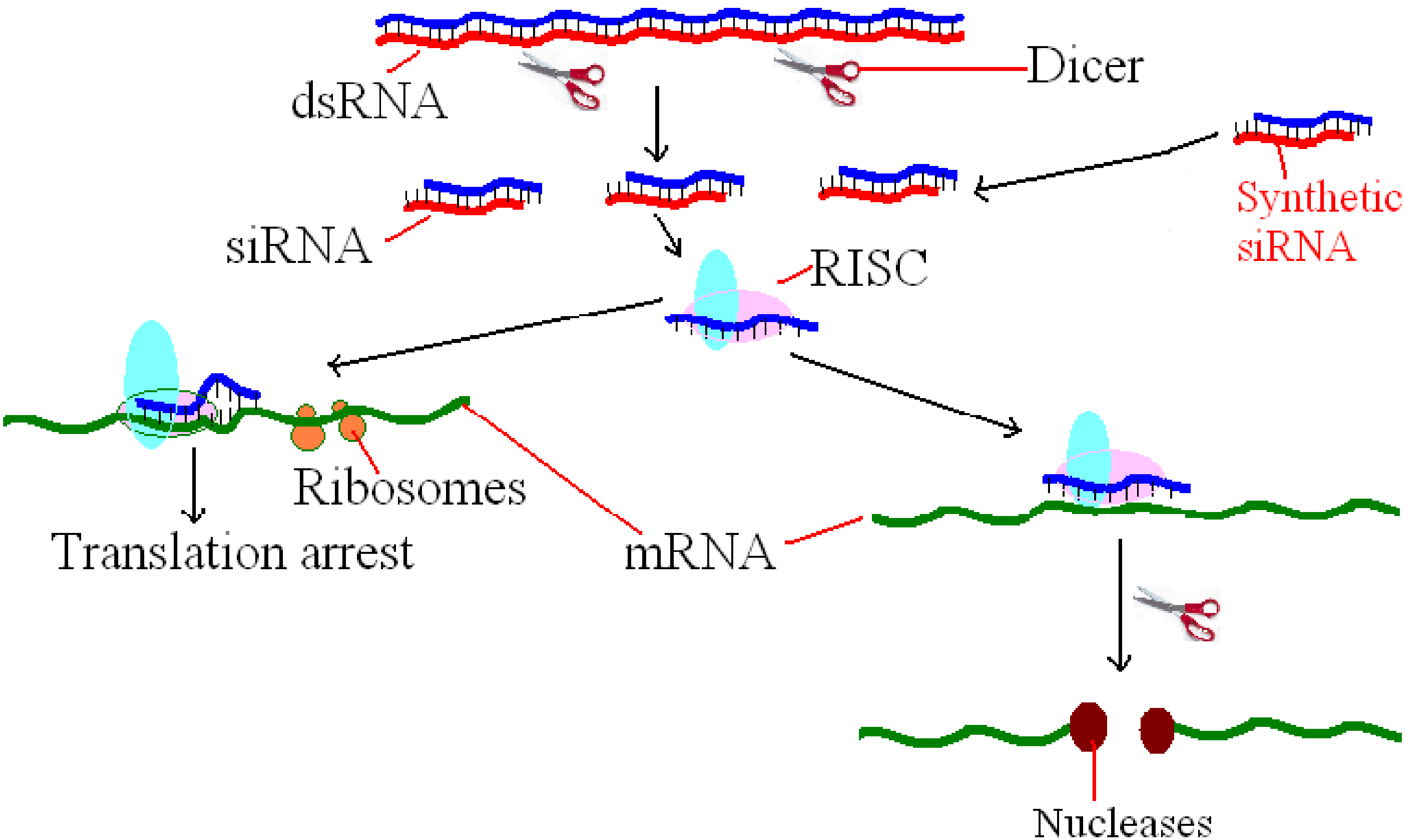
GPEN 2006

What is RNA interference?

RNAi is the natural process of sequence-specific, post-transcriptional gene silencing by double-stranded RNA (dsRNA) homologous in sequence to the target gene.


siRNA
Dicer
RISC
mRNA
Ribosomes
Translational arrest

What is RNA interference?



McManus et. al.; Hannon et. al.; Bernstein et. al.; Ketting et. al.; Hammond et. al.

Nobel Prize!!!!!!!

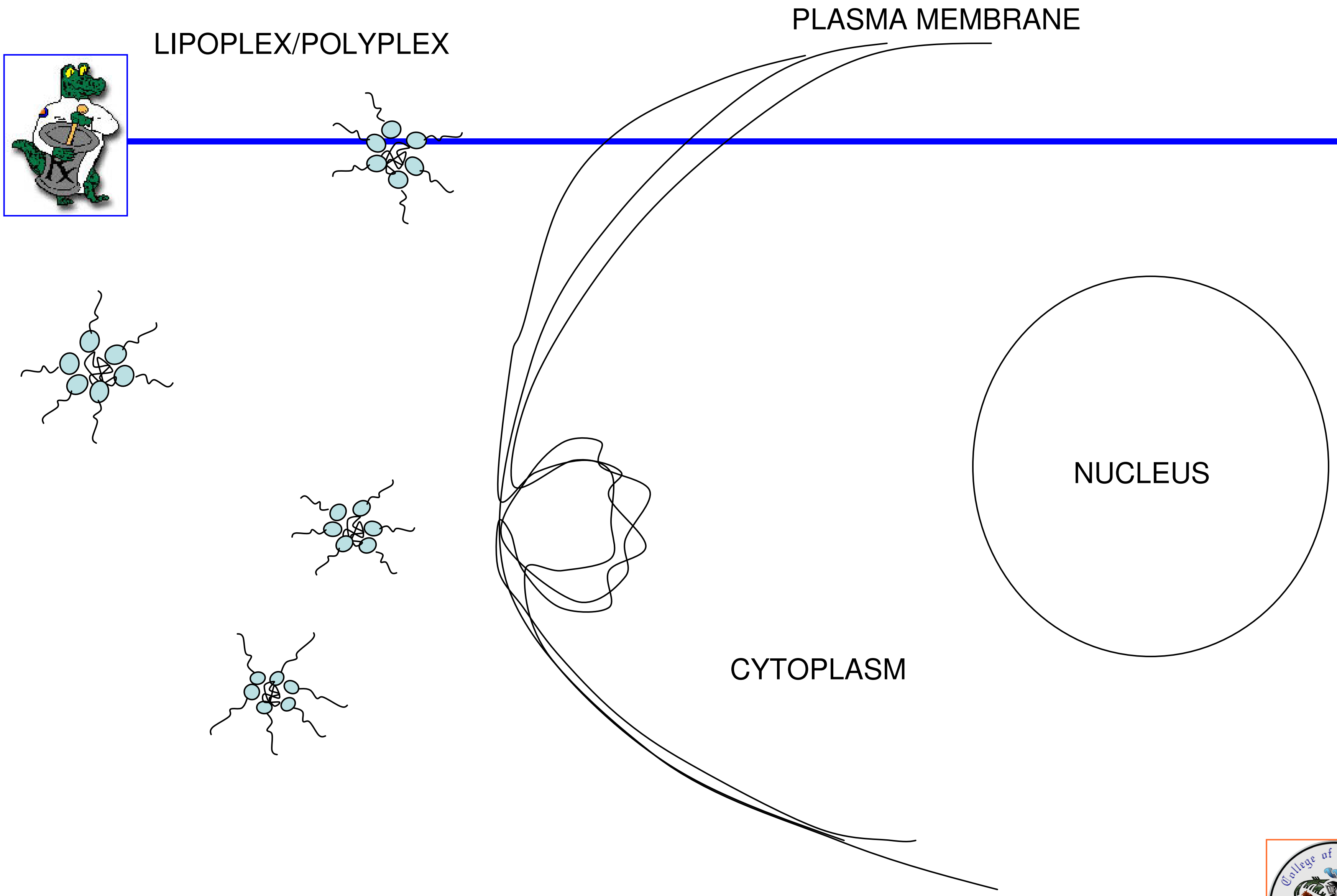
Andrew Fire (left)
Craig Mello (right)

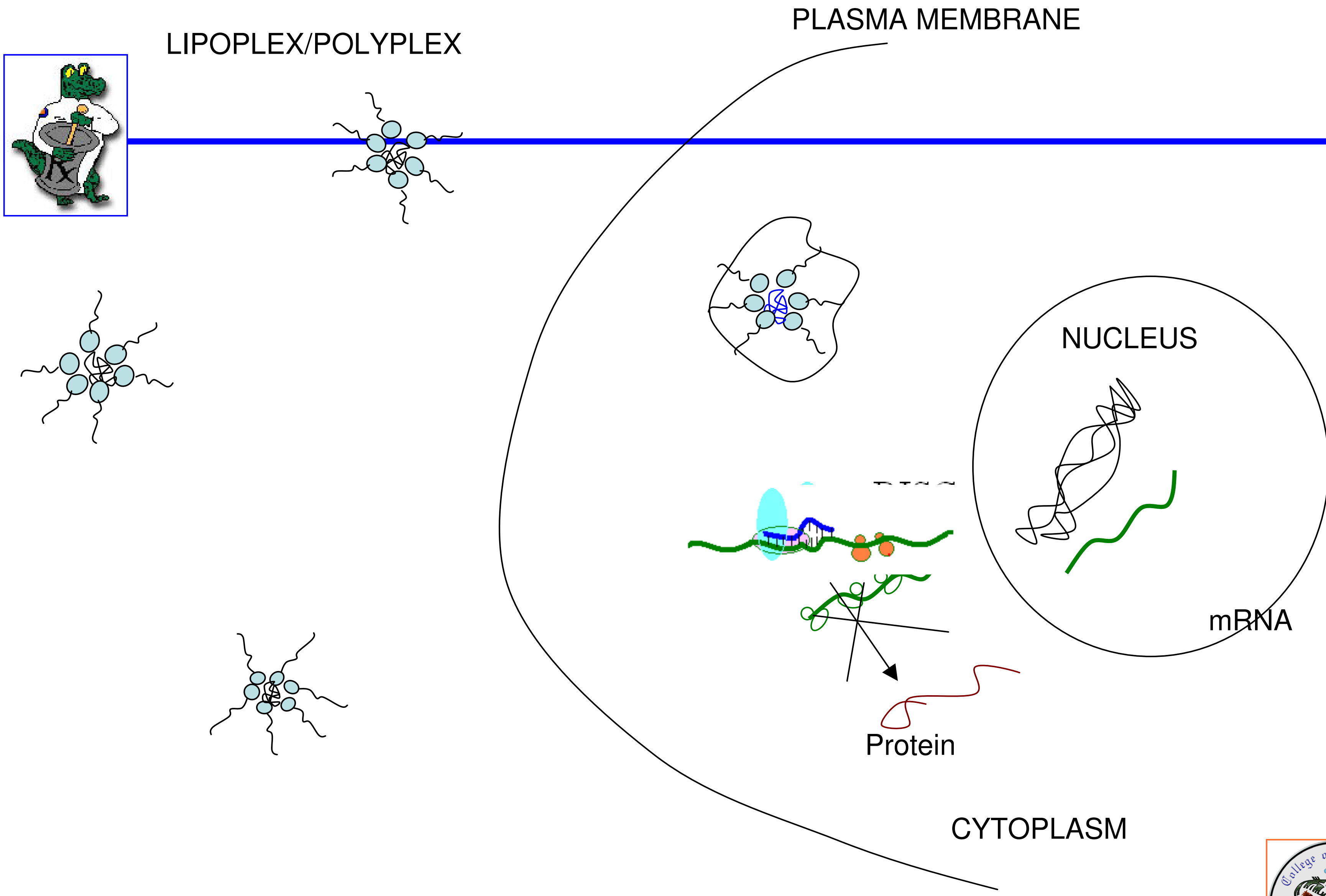
RNAi

Why siRNA?

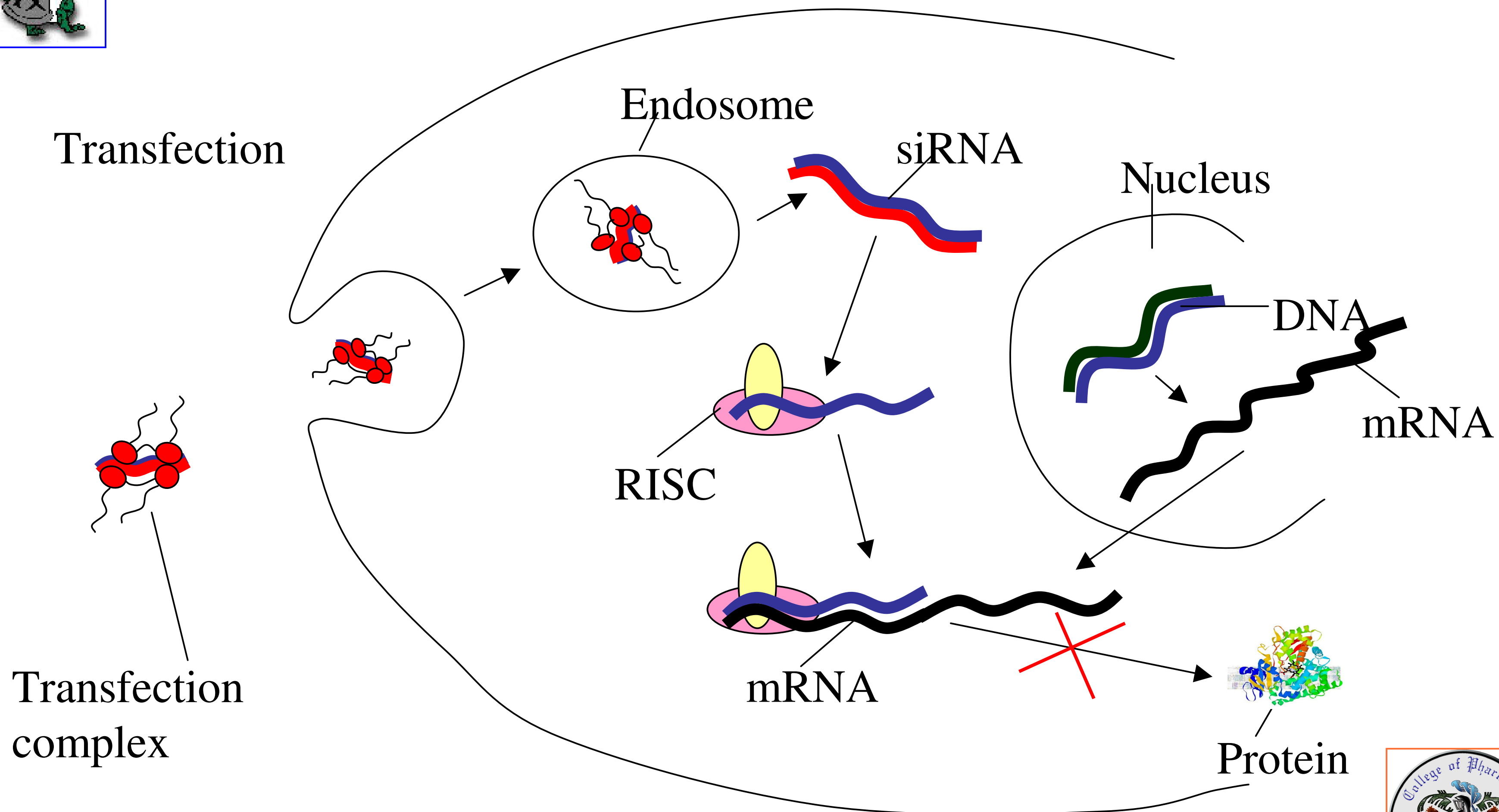
- Potent: exploits cellular machinery
- Specific
- Site of action: cytosol
- Long dsRNA: non-specific, immunogenic
- Concerns
  - Non-specific effects on gene expression
  - RNAi pathway can be saturated
  - Ribonuclease degradation

Kawasaki et. al.; Ding et. al.; Miyagishi et. al.; Khan et. al.; Scherer et. al; Xu et. al.


LIPOPLEX/POLYPLEX
PLASMA MEMBRANE
NUCLEUS
CYTOPLASM

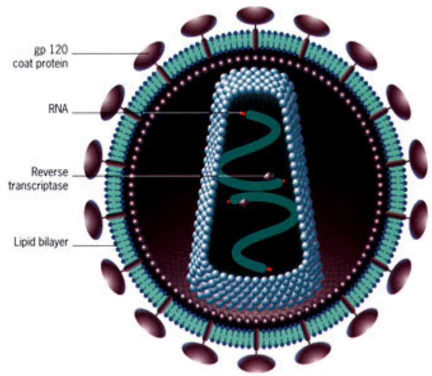

LIPOPLEX/POLYPLEX
PLASMA MEMBRANE
NUCLEUS
mRNA
Protein
CYTOPLASM

RNA/DNA Delivery

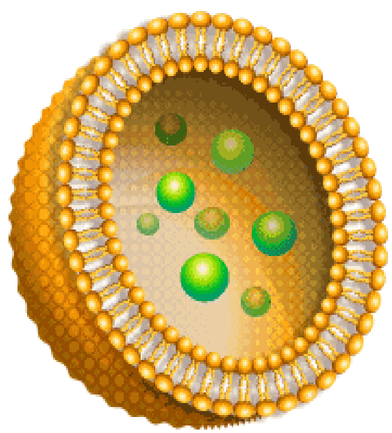


Delivery Systems

- Viral



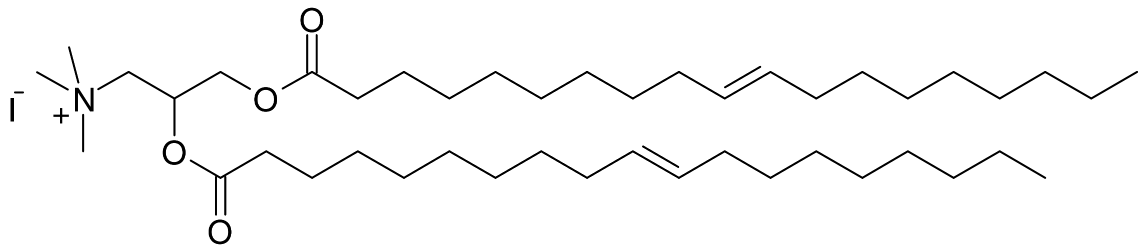
- Non-Viral
  - Polymers: polyplexes
  - Lipids:lipoplexes



Liposome

Delivery Systems

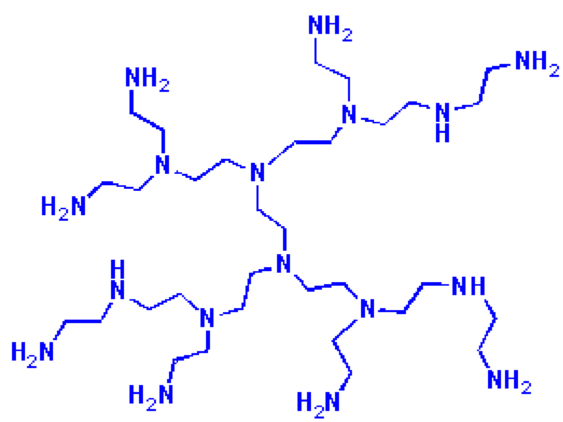
- Lipids: DOTAP-DOPE liposomes



DOTAP

- Polymers: PEI

Branched PEI



Liposomes

- Endosomal disruption:
  - fusogenic inverted hexagonal phase (Ellens et al,1989)
  - destabilization of lipid bilayers by cationic lipids (Hafez et al,2001;Rappolt et al,2003)
- Release their load in the cytosol (Remaut et al,2006)
- Stabilized by intra- and intermolecular interactions and hydration-repulsion forces. (Harvie et al., 1998)
- Local dehydration between DOPE -NH2 and DNA -PO_4^{2-} weakens the interaction between cationic lipids and DNA. (McIntosh,1996)

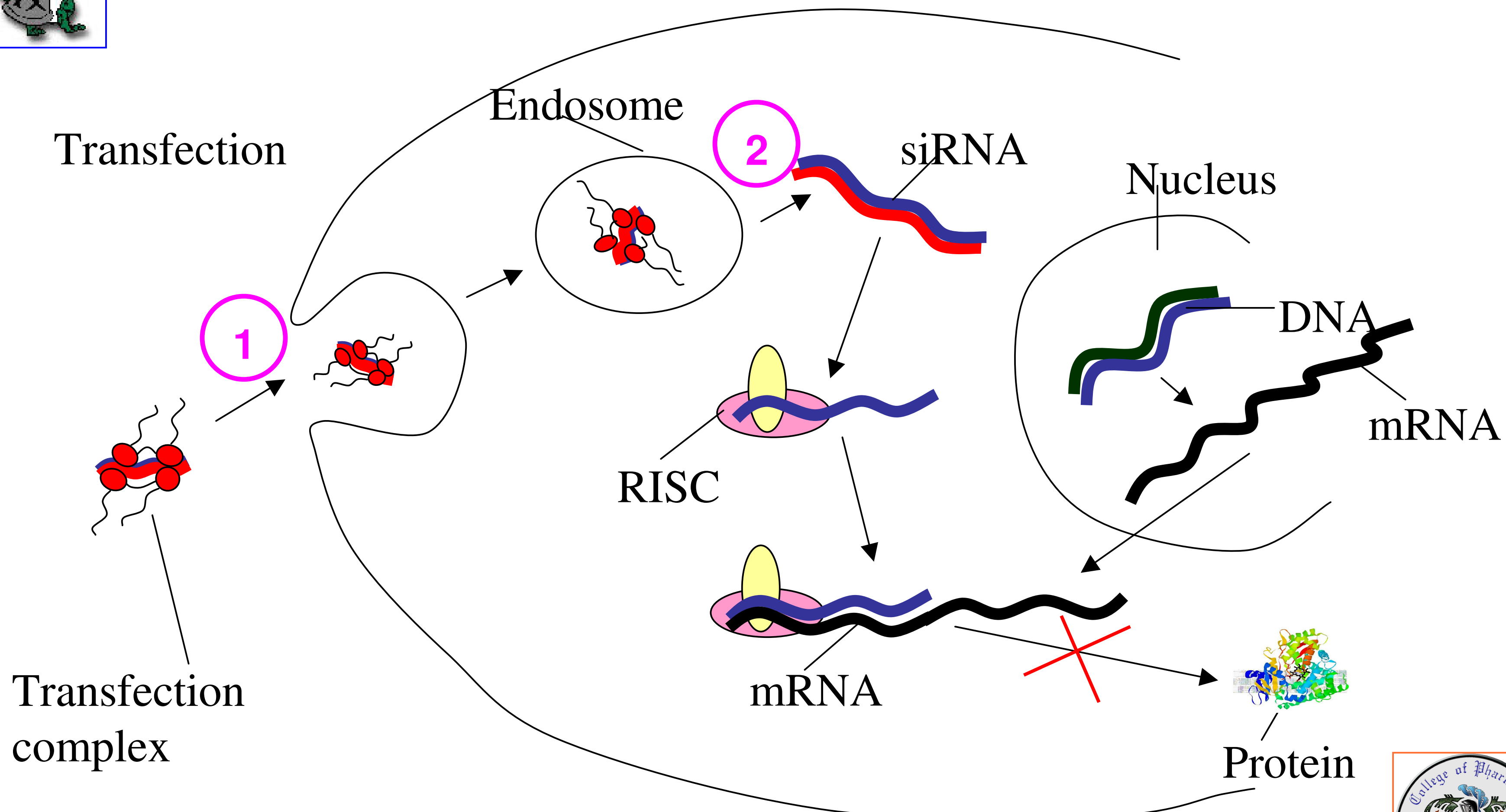
PEI

- Delivery: Large buffering capacity/ proton-sponge effect (Erbacher et al,1999; Kichler et al,2001;Gebhart et al, 2001;Boussif et al,1995)
- Endocytosed PEI undergoes nuclear localization (Godbey et al,1999; Oh et al,2002)
- PEI more efficient than DOTAP for delivering pRSV-α3-Luc plasmid into COS-1 and Calu-3 cells. (Florea et al.,2002)
- Polymers but not cationic lipids promote gene delivery from the cytoplasm to the nucleus (Pollard et al.,1998)
- Held together by covalent bonds

Hypothesis

The difference in efficiency between polyplexes and lipoplexes arises due to their delivery characteristics.

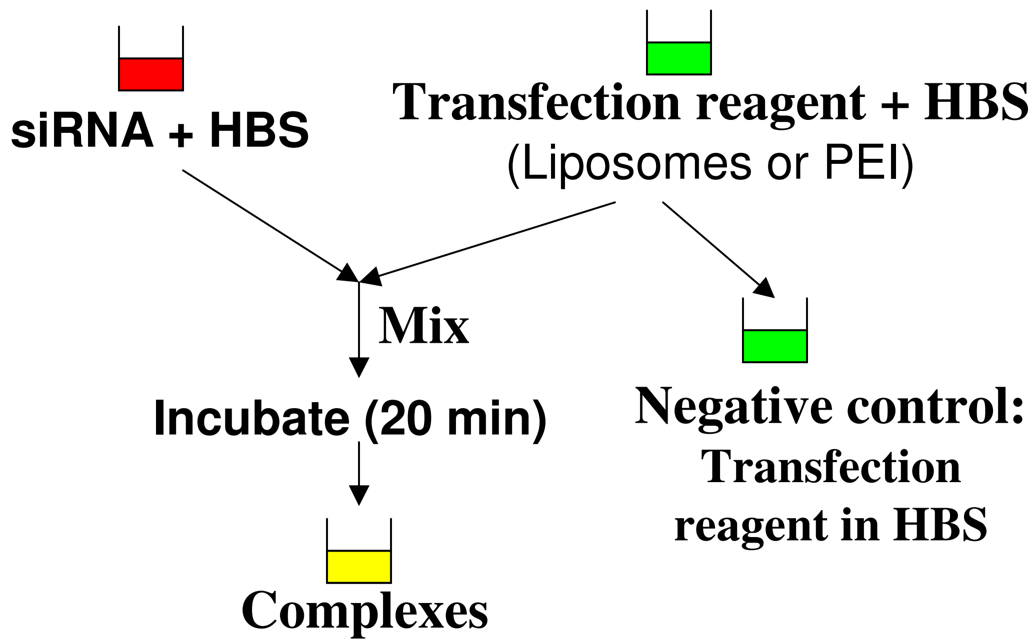
For the delivery of siRNA, the site of action for which is the cytoplasm, release in the cytosol is favorable.

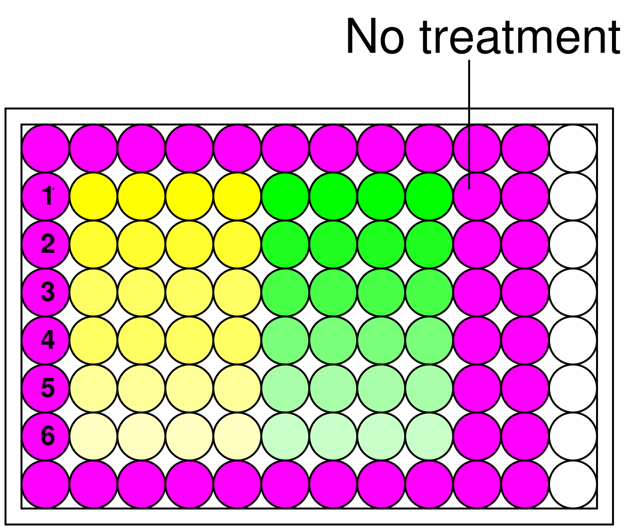


Transfection
Endosome
2
siRNA
Nucleus
1
DNA
mRNA
RISC
mRNA
Transfection
complex
Protein

Methods

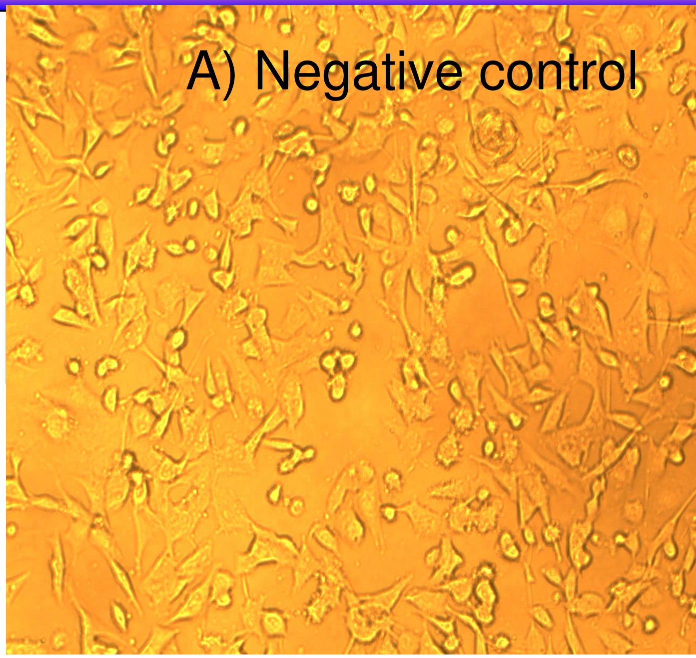
siTOX®: when successfully delivered into the cells causes cell death

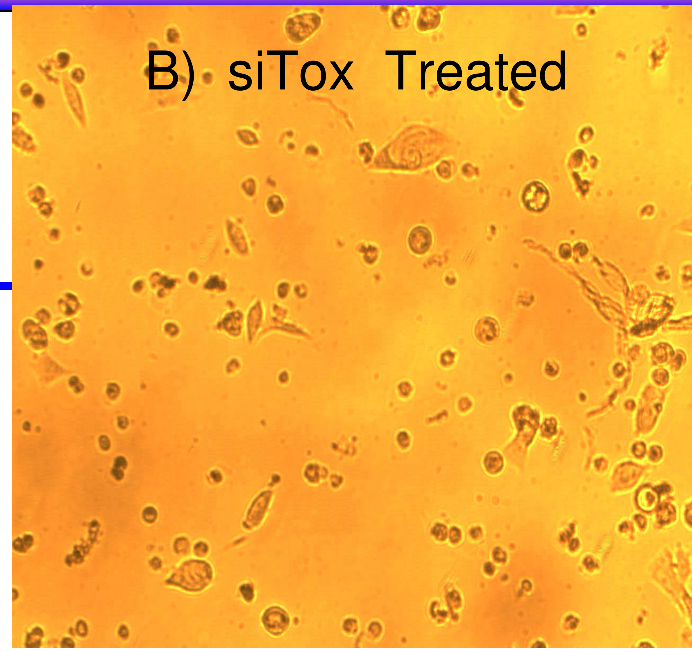




N/P = [Nitrogen (transfection reagent)] ÷[Phosphate (nucleic acid)]

Andric *et al.*; Chandra *et al.*; Garcia-Pedrero *et al.*; Oh *et al.*; http://www.dharmacon.com

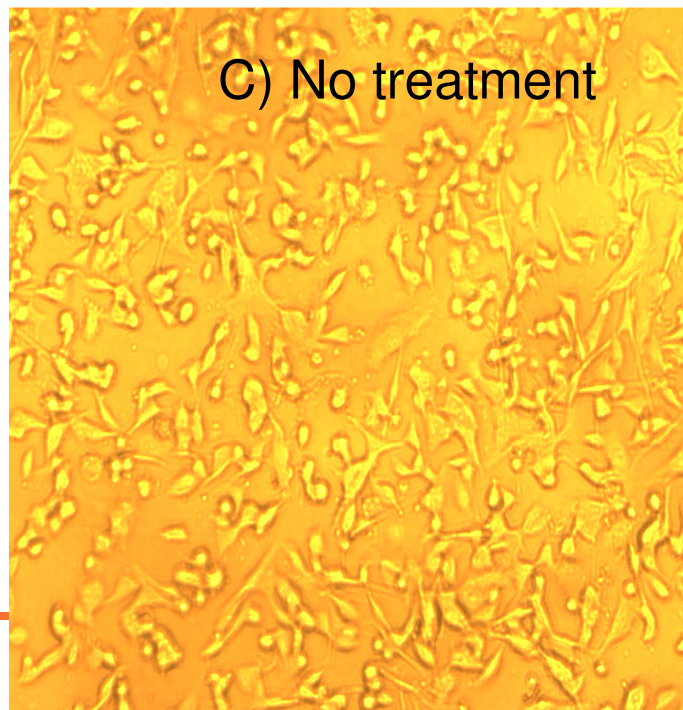


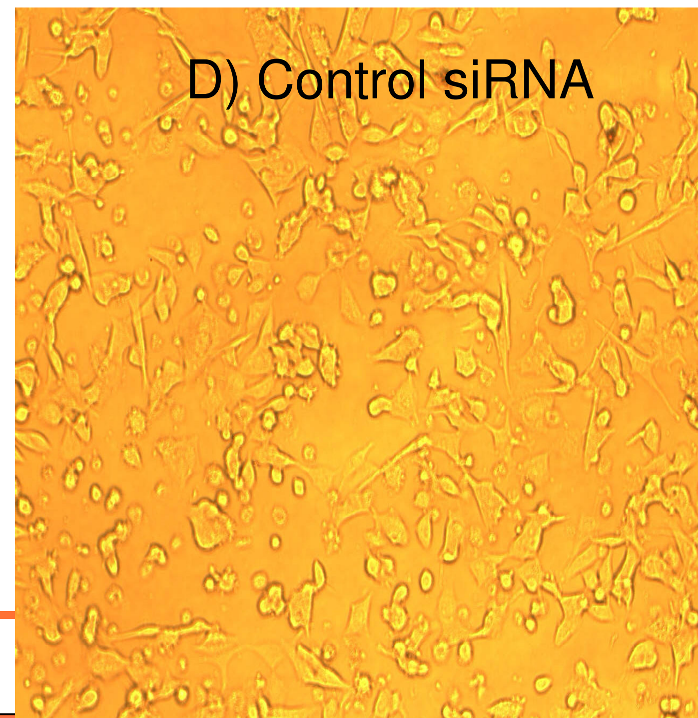


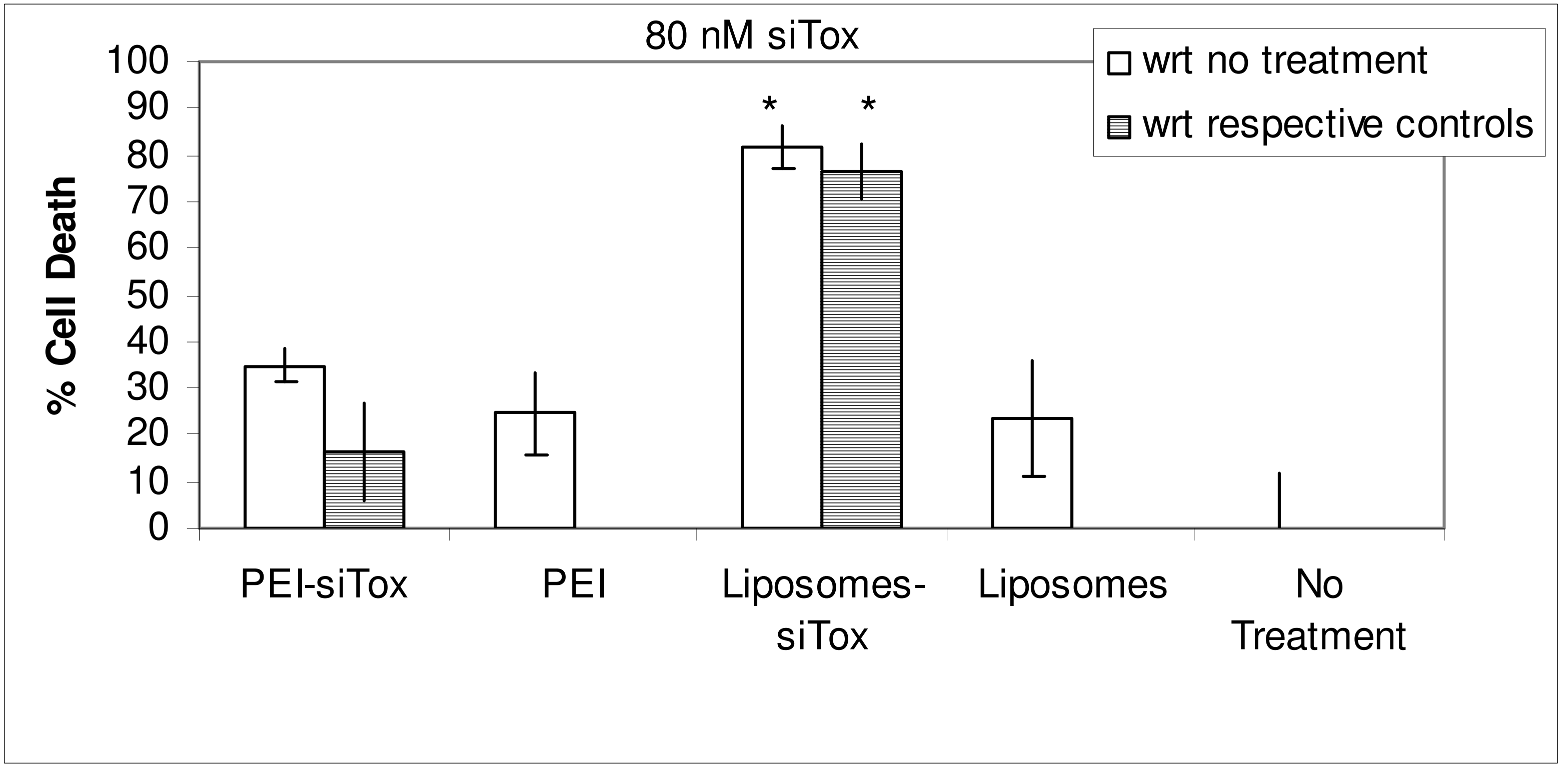
B16F10 cells

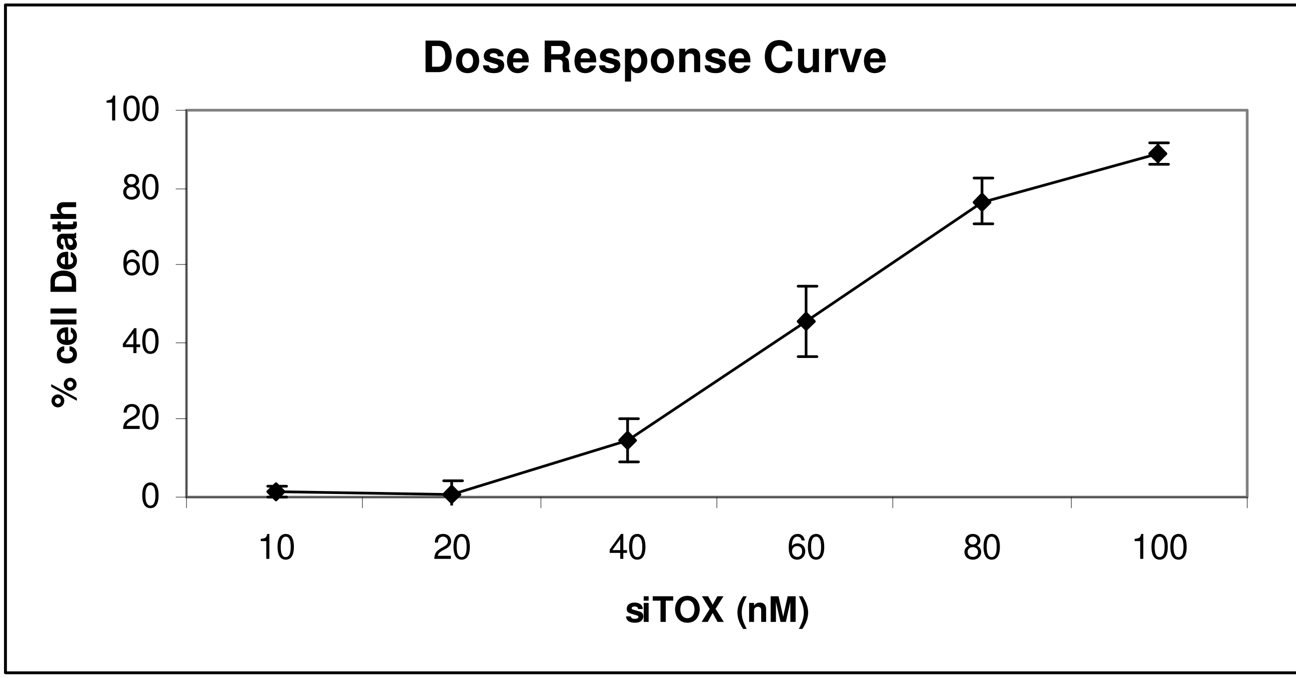
Other cell lines tested:

- A172
- RG2



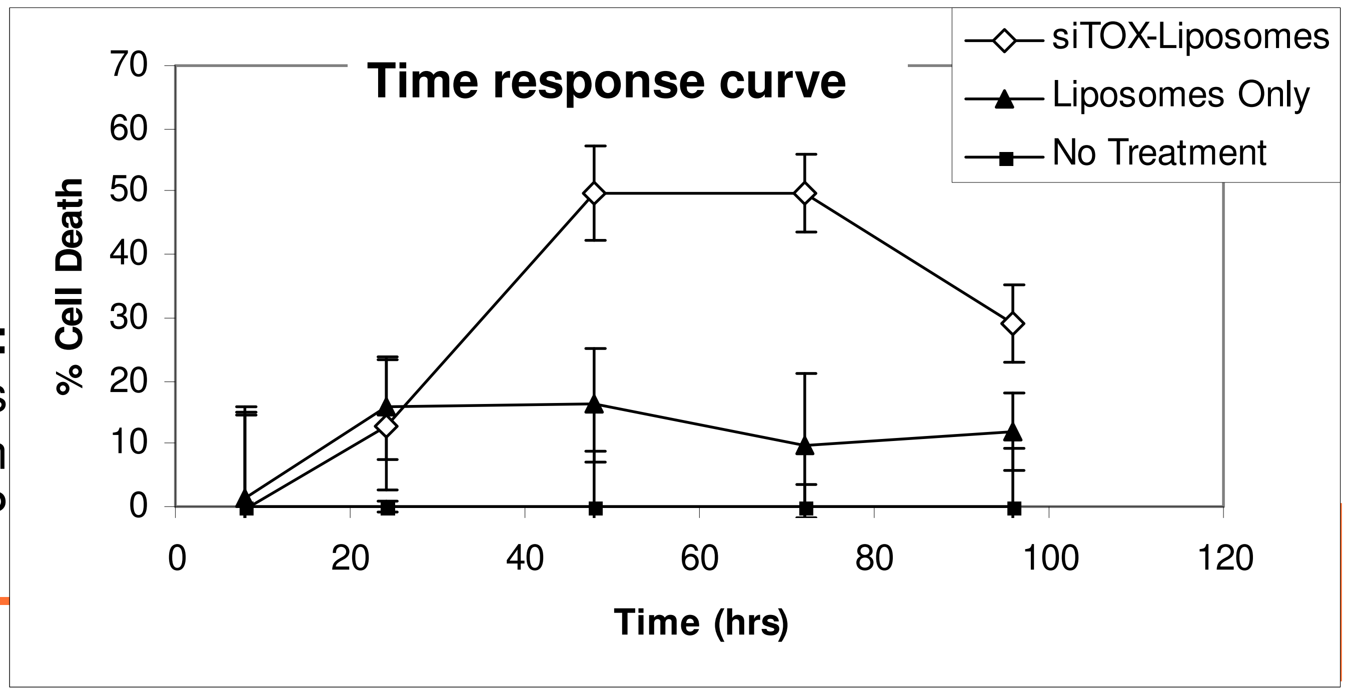



80 nM siTox
% Cell Death
100
90
80
70
60
50
40
30
20
10
0
*
*
wrt no treatment
wrt respective controls
PEI-siTox
PEI
Liposomes-siTox
Liposomes
No Treatment



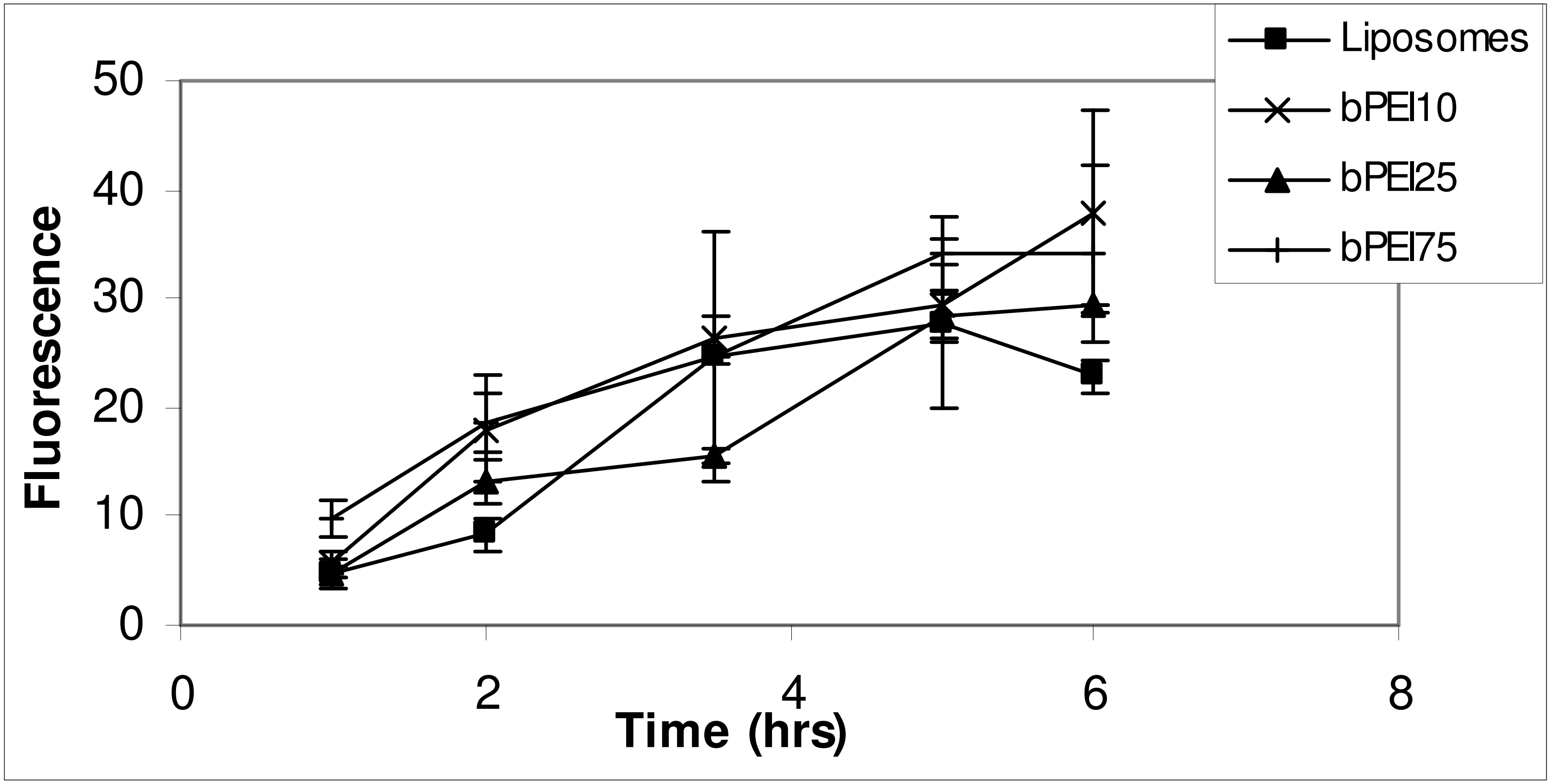
B16F10 cells

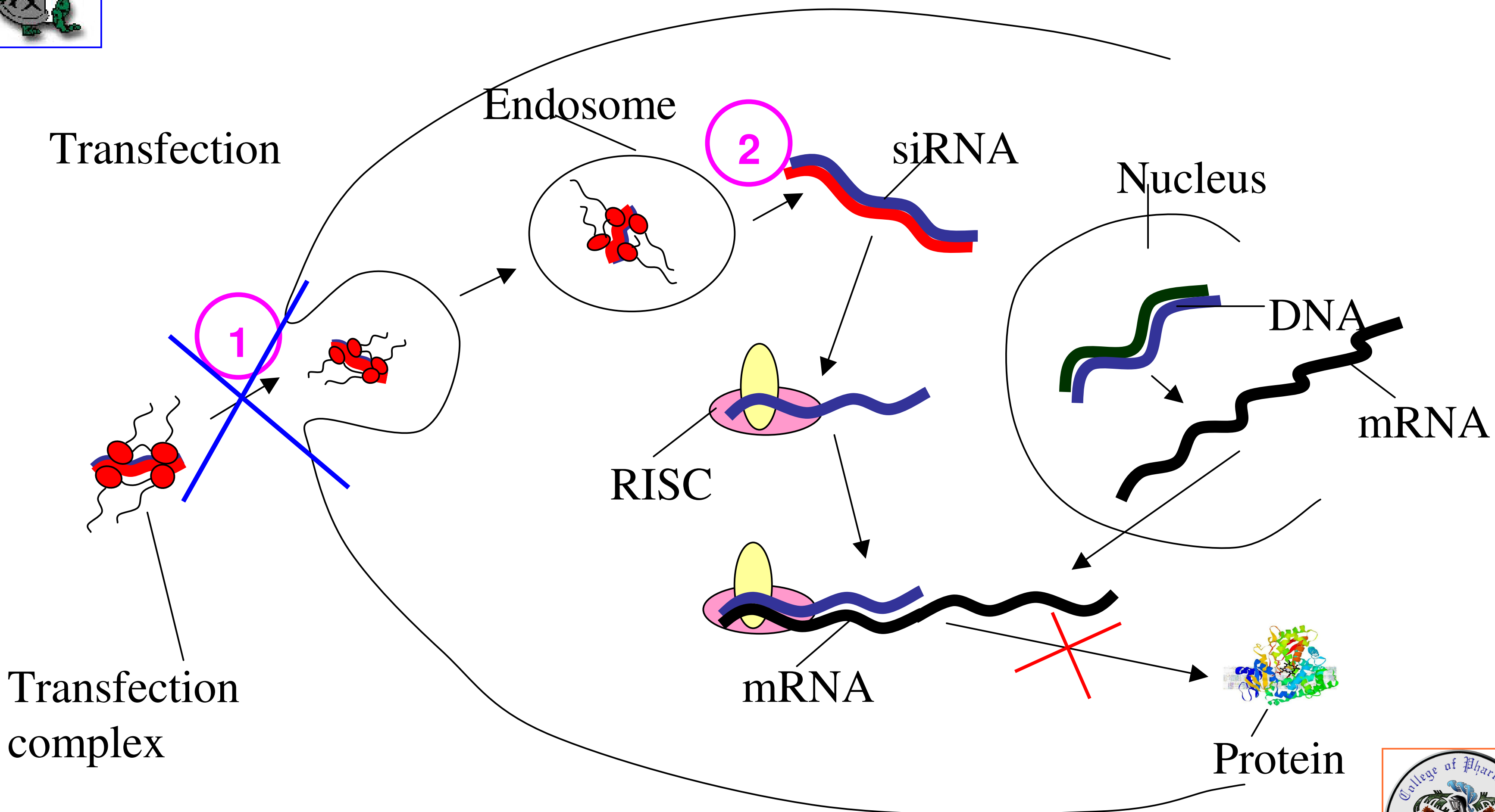
Dose response curve: siTox were complexed with liposomes at an N/P ratio of 4



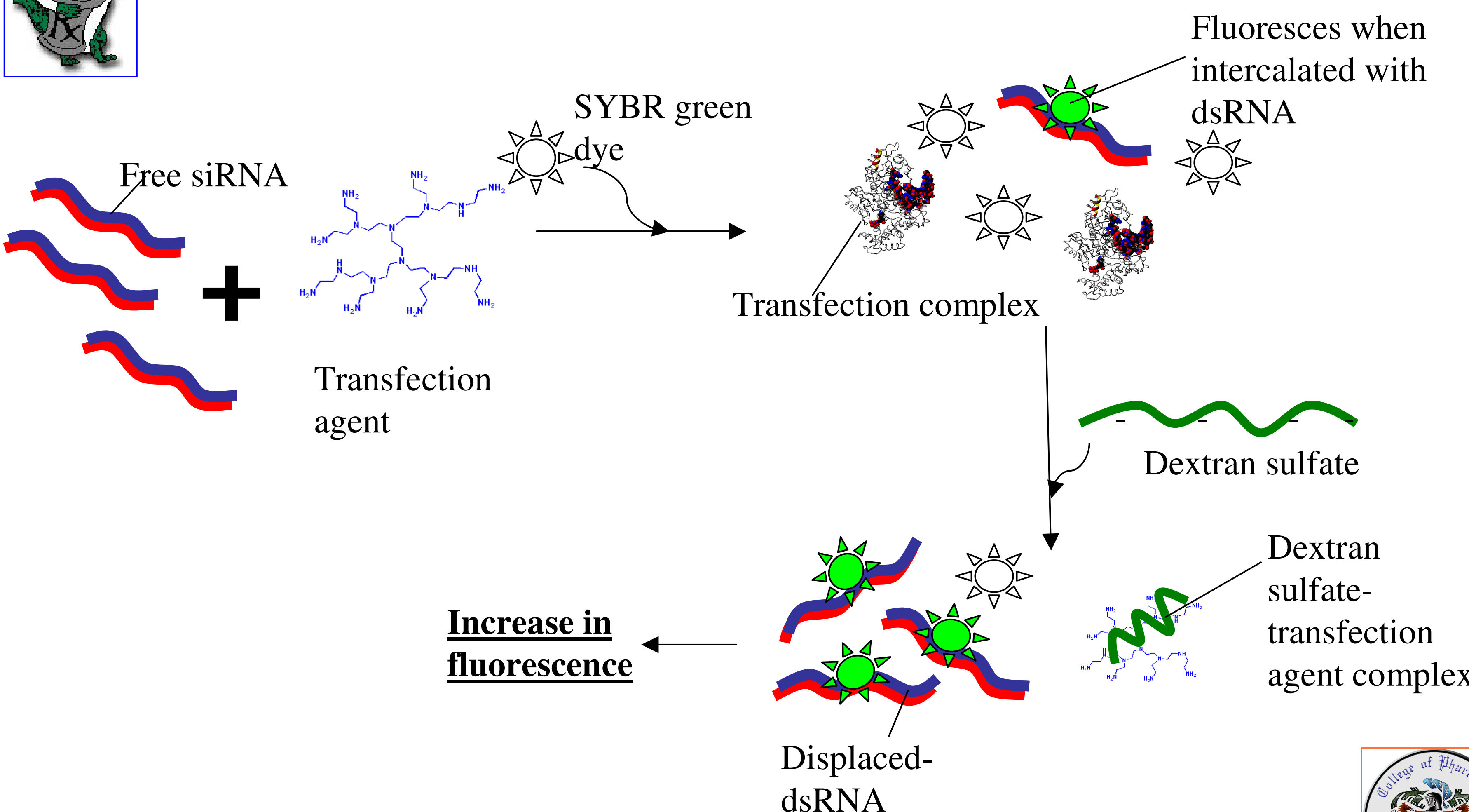
Time response curve: 60 nM siTox was complexed with liposomes at an N/P ratio of 4

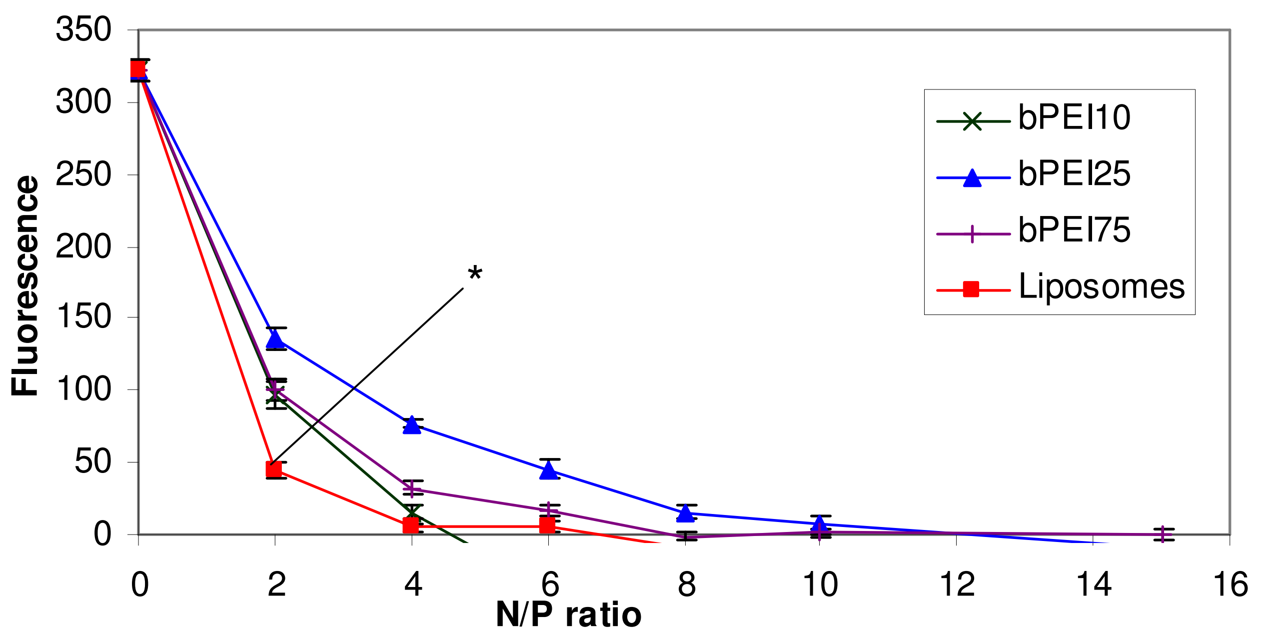
Cellular Uptake: FITC labeled siRNA (FACS)



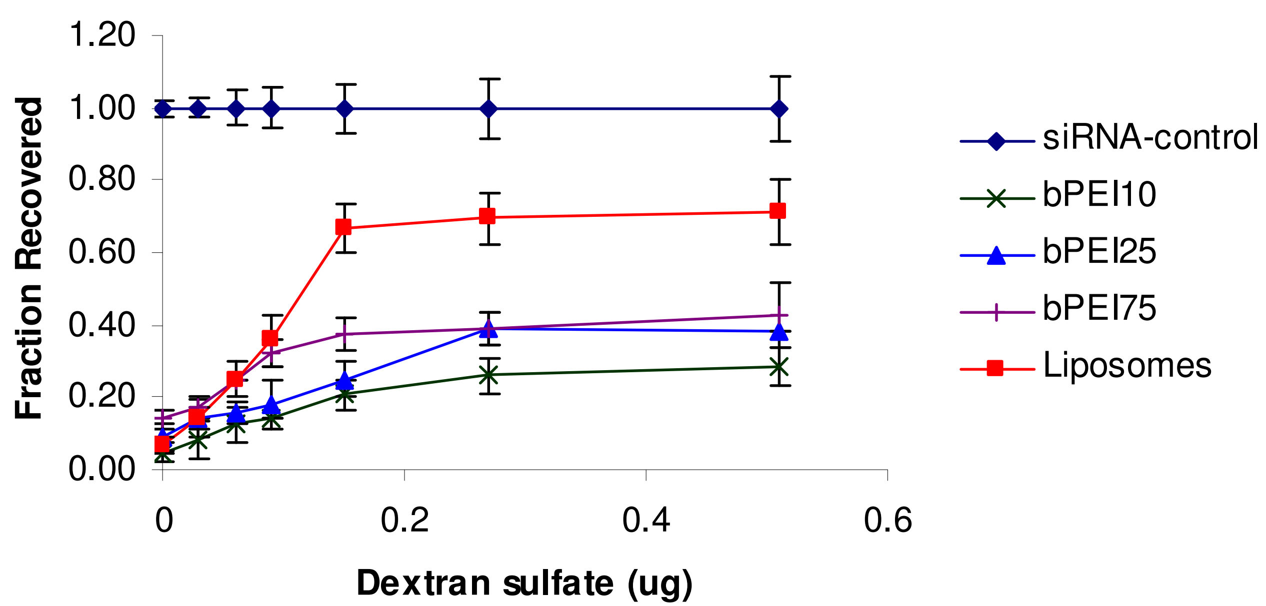

Transfection
Endosome
2
siRNA
Nucleus
1
DNA
mRNA
RISC
mRNA
Transfection complex
Protein

Complexation-decomplexation





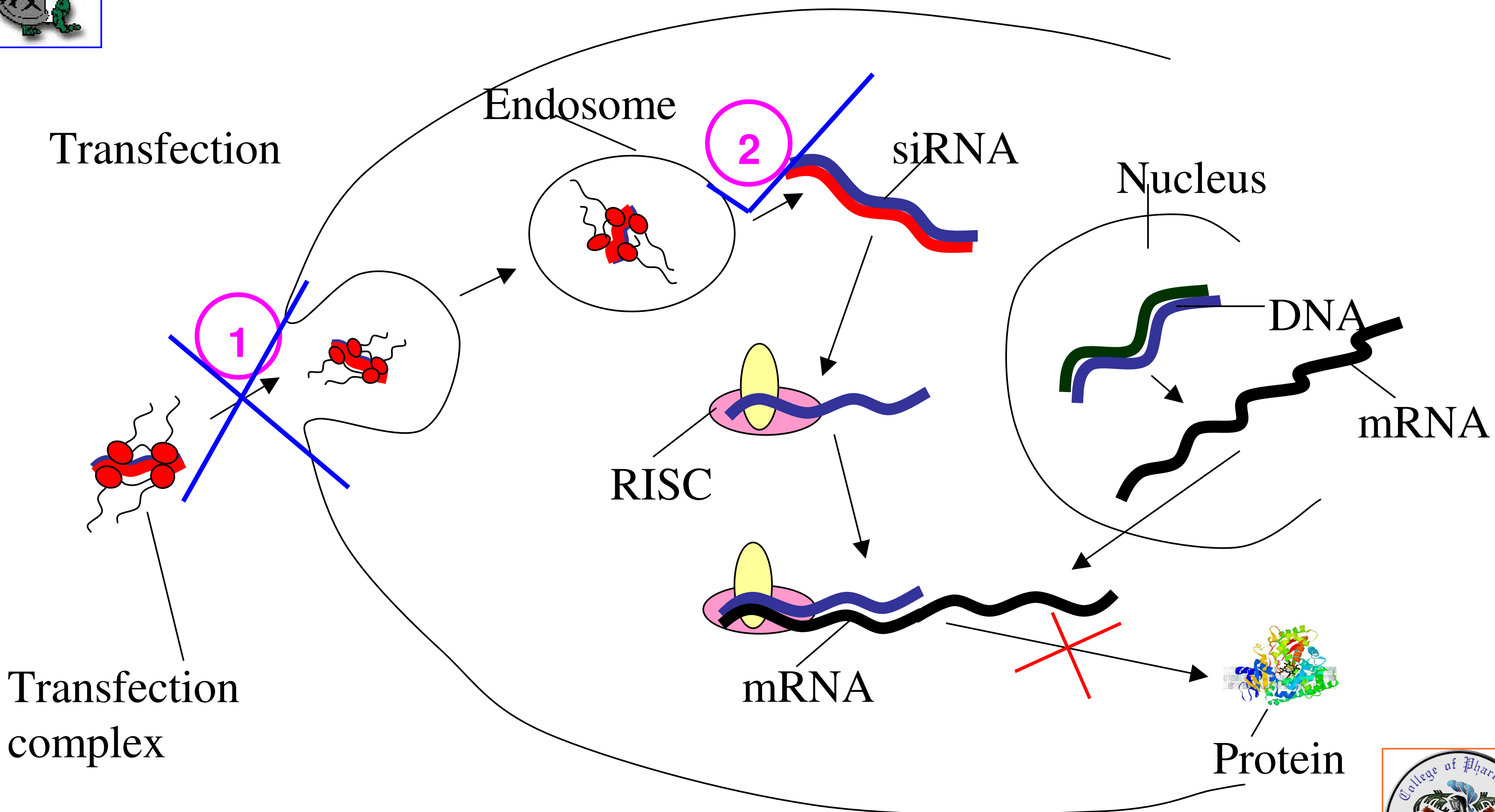
350
300
250
200
150
100
50
0
Fluorescence
*
0
2
4
6
8
10
12
14
16
N/P ratio
bPEI10
bPEI25
bPEI75
Liposomes



1.20
1.00
0.80
0.60
0.40
0.20
0.00
Fraction Recovered
0
0.2
0.4
0.6
Dextran sulfate (ug)
siRNA-control
bPEI10
bPEI25
bPEI75
Liposomes

EC50 (µM dextran sulfate)

	Transfection reagent	siRNA (EC_{50}) (µM dextran sulfate)	Std. dev.	DNA (EC_{50}) (µM dextran sulfate)	Std. dev.
1	Liposomes	0.08**	0.03	0.03*	0.02
2	Branched PEI 10,000	0.67	0.16	0.17	0.02
3	Branched PEI 25,000	0.29	0.08	0.10	0.02
4	Branched PEI 75,000	0.40	0.12	0.24	0.04


Transfection
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mRNA
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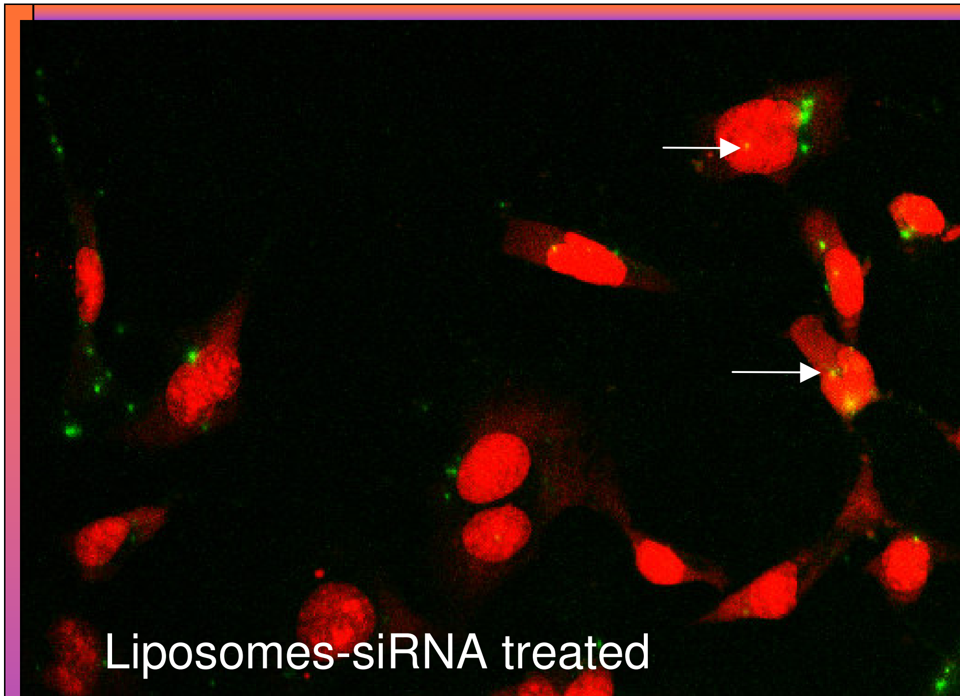
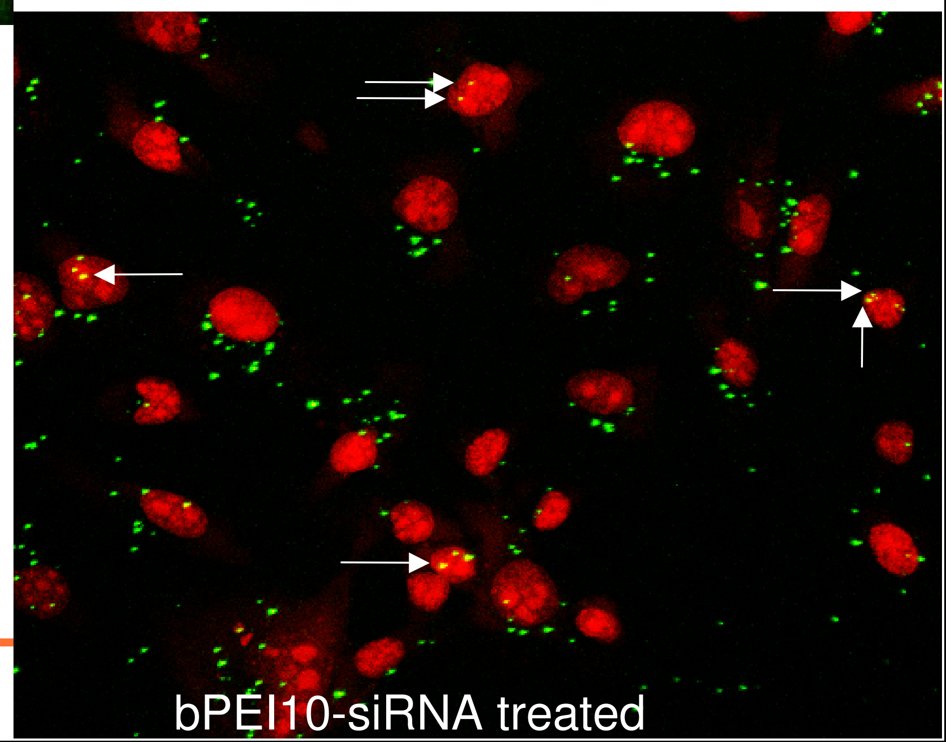
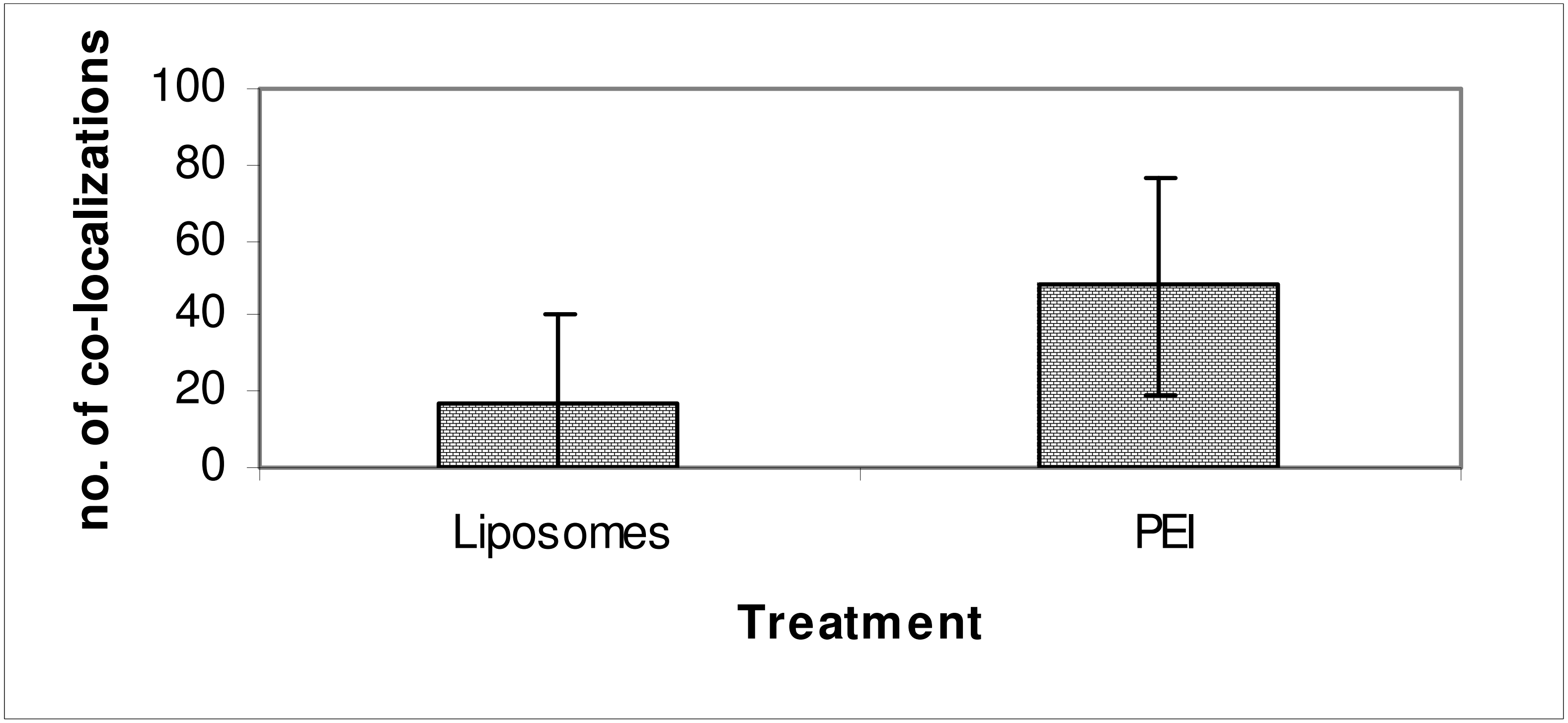


Confocal Analysis

Green: FITC labeled siRNA, Red: cell nucleus stained with Propidium iodide, Yellow: co-localization



Confocal analysis



Conclusions

- DOTAP:DOPE (2:1) liposomes lead to efficient siRNA activity with low amounts of siTox (80nmol), 76.4 ± 5.9% cell death due to apoptosis induced by siTox 48 hours post-transfection. This activity was dose-dependent.
- Complete complexation of free siRNA occurs at N/P =4 with liposomes and N/P = 8 for PEI.
- No significant difference in uptake

Conclusions

- EC50: Liposomes < Branched PEIs
- In vitro liposomes are more efficient siRNA delivery systems as compared to PEI based polyplexes.
- This difference in transfection efficiency of lipoplexes and polyplexes may be explained on the basis of release characteristics of liposomes.

Future Studies

- Targeting of liposomes-siTox complexes using FGF
- Further improve uptake by use of peptides eg TAT
- Test the system to deliver siRNA against a known mRNA target
- In- vivo studies

Acknowledgement

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- Dr. Sean Sullivan
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- Dr. Aaron Hirko
- Daniel Roura
- Members of Hughes lab
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Questions?

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