# Polymer Technology for Gene Delivery

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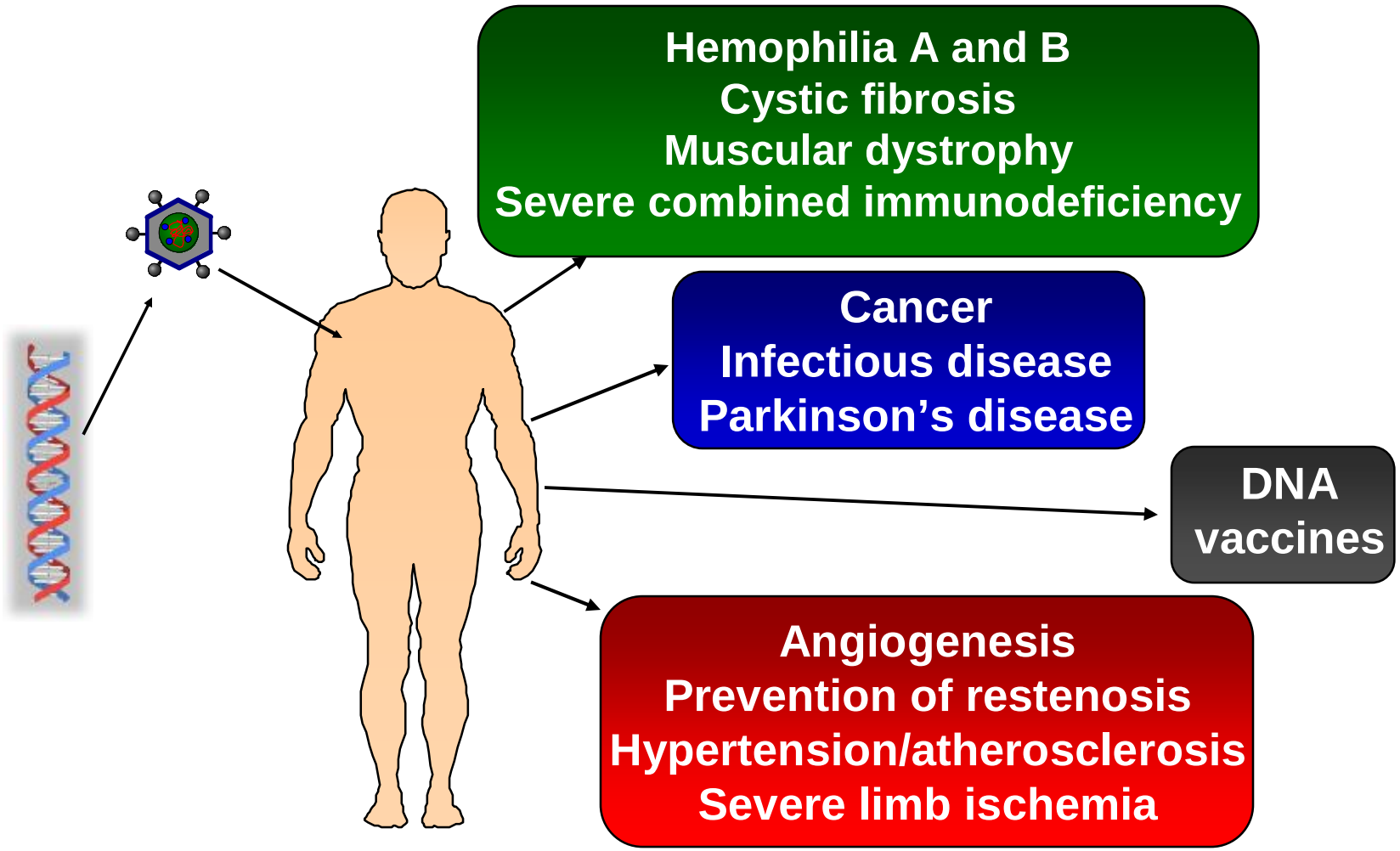
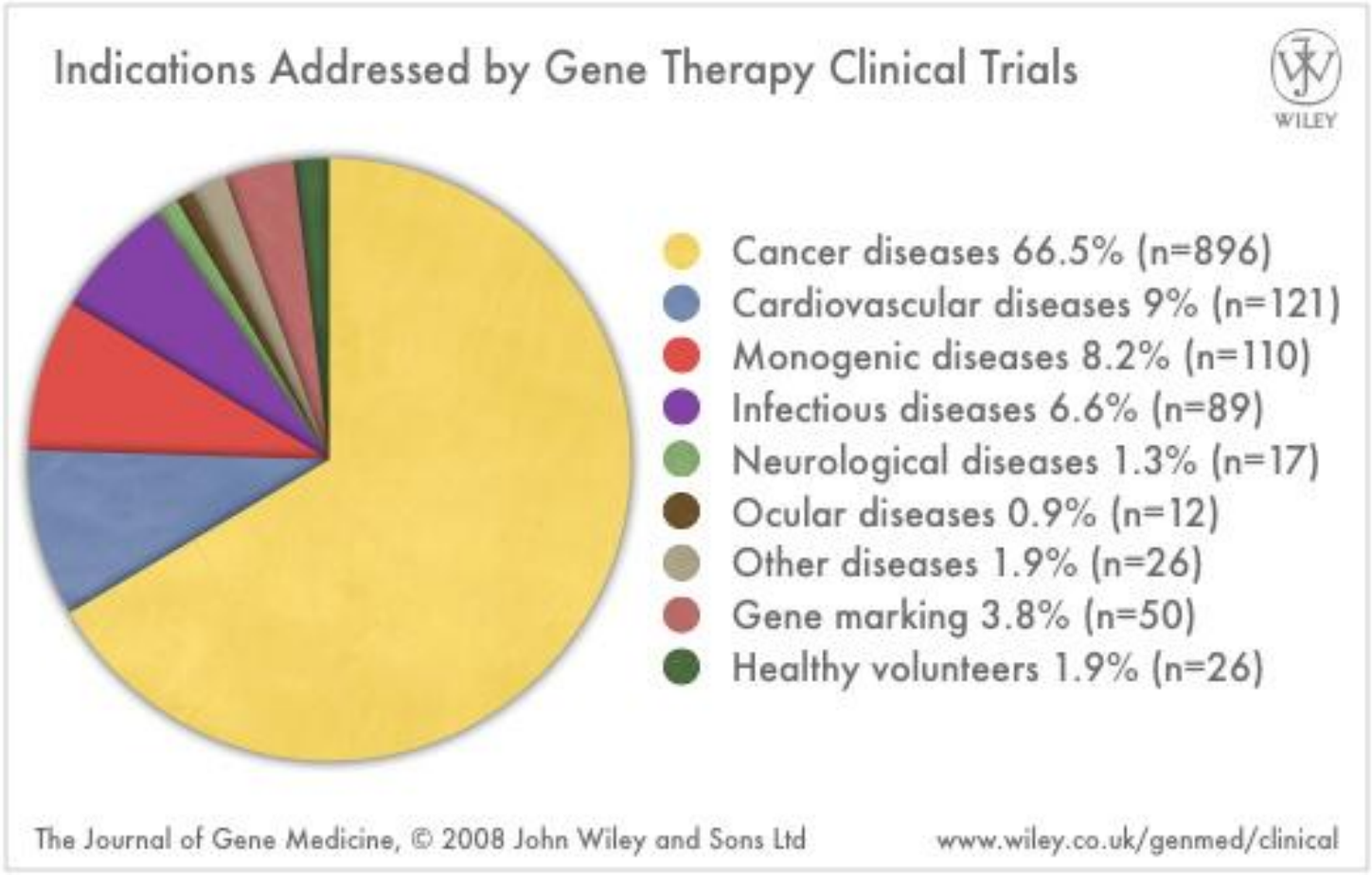

Hemophilia A and B
Cystic fibrosis
Muscular dystrophy
Severe combined immunodeficiency
Cancer
Infectious disease
Parkinson's disease
DNA vaccines
Angiogenesis
Prevention of restenosis
Hypertension/atherosclerosis
Severe limb ischemia


Indications Addressed by Gene Therapy Clinical Trials
WILEY
Cancer diseases 66.5% (n=896)
Cardiovascular diseases 9% (n=121)
Monogenic diseases 8.2% (n=110)
Infectious diseases 6.6% (n=89)
Neurological diseases 1.3% (n=17)
Ocular diseases 0.9% (n=12)
Other diseases 1.9% (n=26)
Gene marking 3.8% (n=50)
Healthy volunteers 1.9% (n=26)
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www.wiley.co.uk/genmed/clinical

# A brief history of gene therapy.

SCIENCE • VOL. 270 • 20 OCTOBER 1995

## T Lymphocyte–Directed Gene Therapy for ADA⁻ SCID: Initial Trial Results After 4 Years

R. Michael Blaese,* Kenneth W. Culver, A. Dusty Miller, Charles S. Carter, Thomas Fleisher, Mario Clerici,† Gene Shearer, Lauren Chang, Yawen Chiang, Paul Tolstoshev, Jay J. Greenblatt, Steven A. Rosenberg, Harvey Klein, Melvin Berger, Craig A. Mullen,‡ W. Jay Ramsey, Linda Muul, Richard A. Morgan, W. French Anderson§

In 1990, a clinical trial was started using retroviral-mediated transfer of the adenosine deaminase (ADA) gene into the T cells of two children with severe combined immunodeficiency (ADA⁻ SCID). The number of blood T cells normalized as did many cellular and humoral immune responses. Gene treatment ended after 2 years, but integrated vector and ADA gene expression in T cells persisted. Although many components remain to be perfected, it is concluded here that gene therapy can be a safe and effective addition to treatment for some patients with this severe immunodeficiency disease.

# A brief history of gene therapy.

The New York Times

## Patient Dies During a Trial Of Therapy Using Genes

By NICHOLAS WADE
Published: September 29, 1999

A patient has died while undergoing gene therapy in a trial study at the University of Pennsylvania in Philadelphia.

# A brief history of gene therapy.

www.sciencemag.org SCIENCE VOL 288 28 APRIL 2000

## Gene Therapy of Human Severe Combined Immunodeficiency (SCID)–X1 Disease

Marina Cavazzana-Calvo,*[1,2,3] Salima Hacein-Bey,*[1,2,3]
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# A brief history of gene therapy.

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www.sciencemag.org SCIENCE VOL 302 17 OCTOBER 2003

## *LMO2*-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1

S. Hacein-Bey-Abina,[1,2*] C. Von Kalle,[6,7,8] M. Schmidt,[6,7]
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I. Alexander,[16] U. Wintergerst,[17] T. Frebourg,[18] A. Aurias,[19]
D. Stoppa-Lyonnet,[20] S. Romana,[3] I. Radford-Weiss,[3] F. Gross,[2]
F. Valensi,[4] E. Delabesse,[4] E. Macintyre,[4] F. Sigaux,[20] J. Soulier,[21]
L. E. Leiva,[14] M. Wissler,[6,7] C. Prinz,[6,7] T. H. Rabbitts,[9]
F. Le Deist,[1] A. Fischer,[1,5†‡] M. Cavazzana-Calvo[1,2†]

# A brief history of gene therapy.

6 OCTOBER 2006 VOL 314 SCIENCE

## Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

Richard A. Morgan, Mark E. Dudley, John R. Wunderlich, Marybeth S. Hughes, James C. Yang, Richard M. Sherry, Richard E. Royal, Suzanne L. Topalian, Udai S. Kammula, Nicholas P. Restifo, Zhili Zheng, Azam Nahvi, Christiaan R. de Vries, Linda J. Rogers-Freezer, Sharon A. Mavroukakis, Steven A. Rosenberg*

Through the adoptive transfer of lymphocytes after host immunodepletion, it is possible to mediate objective cancer regression in human patients with metastatic melanoma. However, the generation of tumor-specific T cells in this mode of immunotherapy is often limiting. Here we report the ability to specifically confer tumor recognition by autologous lymphocytes from peripheral blood by using a retrovirus that encodes a T cell receptor. Adoptive transfer of these transduced cells in 15 patients resulted in durable engraftment at levels exceeding 10% of peripheral blood lymphocytes for at least 2 months after the infusion. We observed high sustained levels of circulating, engineered cells at 1 year after infusion in two patients who both demonstrated objective regression of metastatic melanoma lesions. This study suggests the therapeutic potential of genetically engineered cells for the biologic therapy of cancer.

# A brief history of gene therapy.

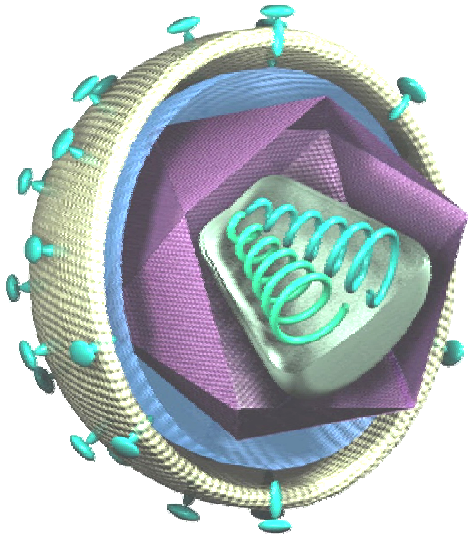
The New York Times

## Patient in Experimental Gene Therapy Study Dies, F.D.A. Says

By DENISE GRADY AND ANDREW POLLACK
Published: July 27, 2007

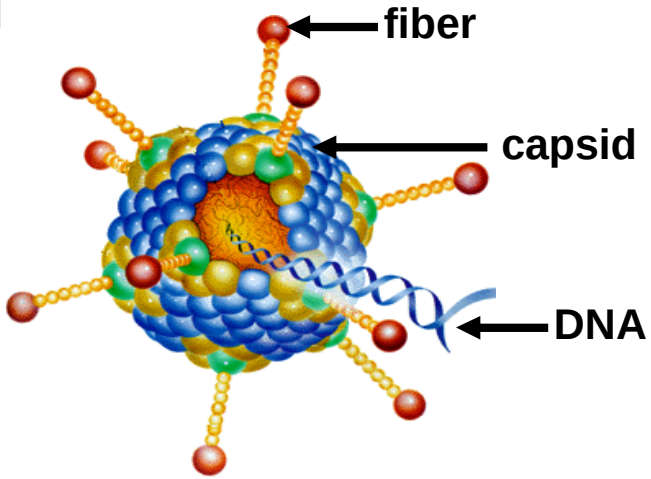
**A patient has died in a study of an experimental gene therapy, the Food and Drug Administration reported yesterday. The agency said it was investigating the death to determine whether the treatment was to blame.**

# Viruses are optimized for gene delivery...

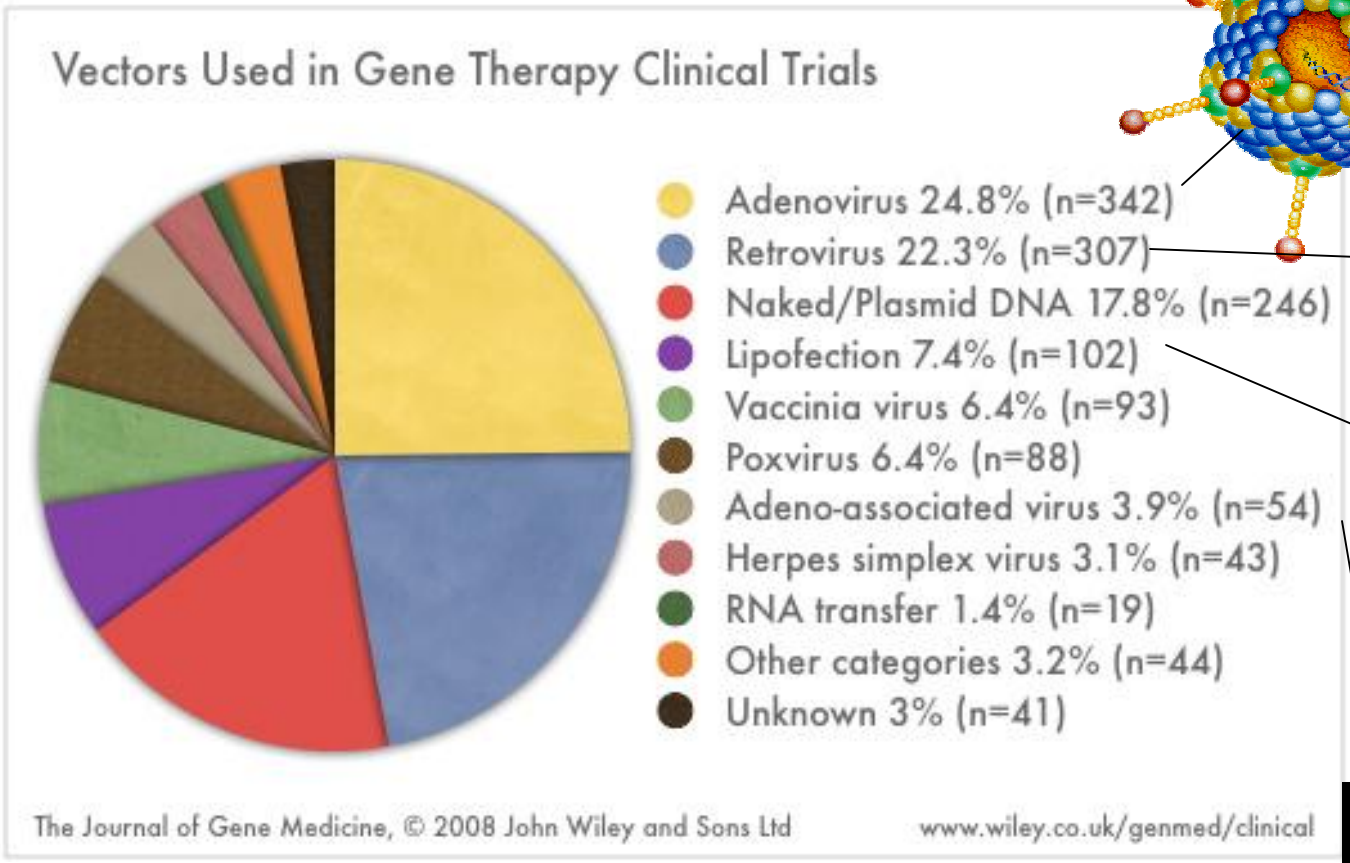


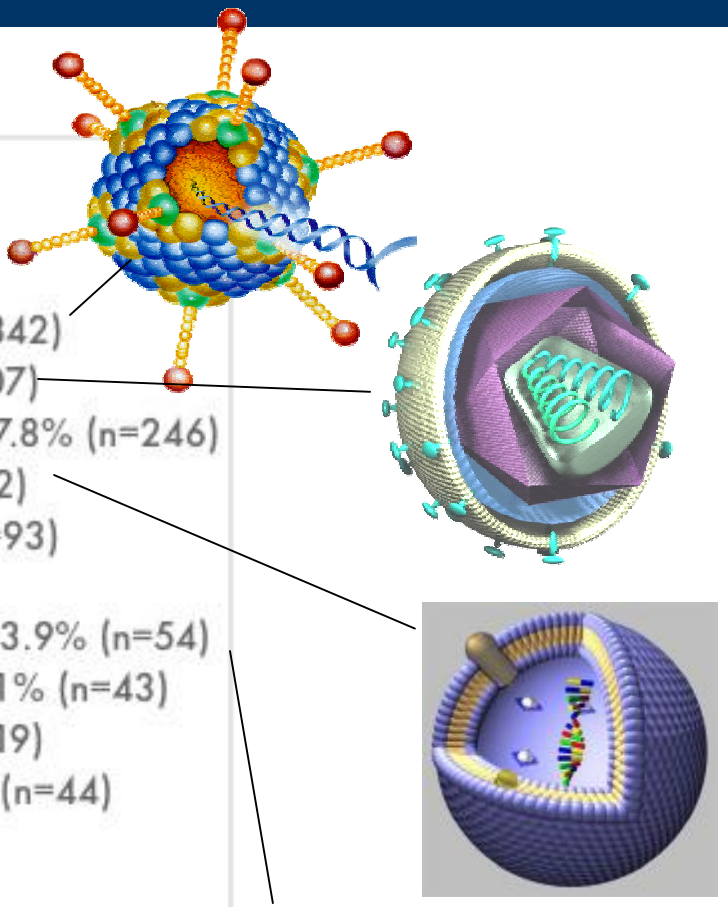
Viruses evolved to efficiently and specifically deliver genetic material!!

Recombinant viruses have been used in the majority of gene therapy clinical trials.



# Viral vectors are employed in two thirds of ongoing clinical trials.



# …but, viruses are NOT optimized for gene therapy.

Pre-determined **host-cell specificity**.

**Immunogenic** (immune system resists viruses).

**Pathogenic** (may cause diseases including cancer).

**Difficult** and expensive to **produce** in large quantities.

# Gene therapy "successes"

SCIENCE • VOL 270 • 20 OCTOBER 1995

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www.sciencemag.org SCIENCE VOL 288 28 APRIL 2000

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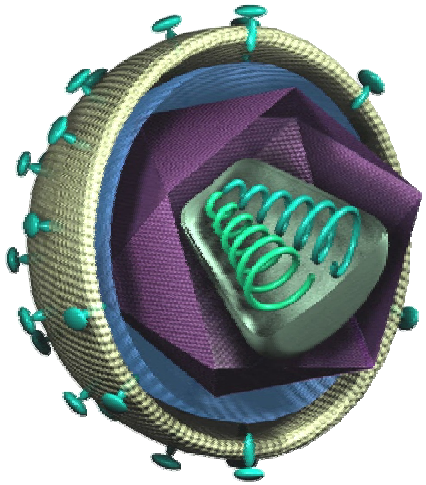
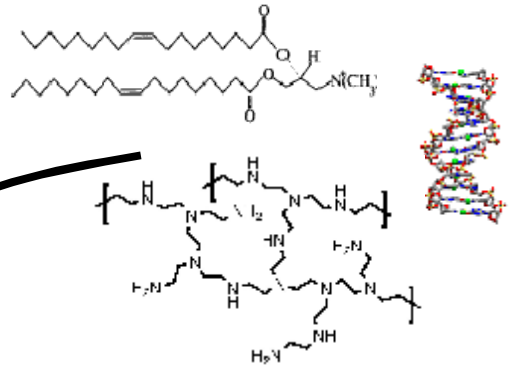
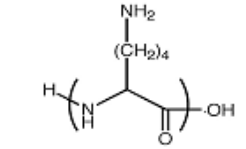
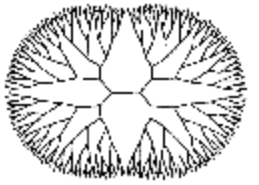
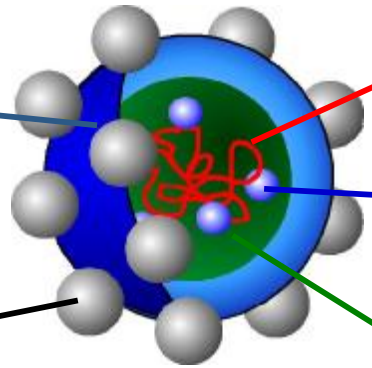
# Can one build an “artificial virus?”











Biodegradable, inert coat (pH sensitive?)

Condensed plasmid DNA

Nuclear localization signal

Targeting ligand (peptide, protein, sugar, etc.)

DNA-complexing “proton sponge”

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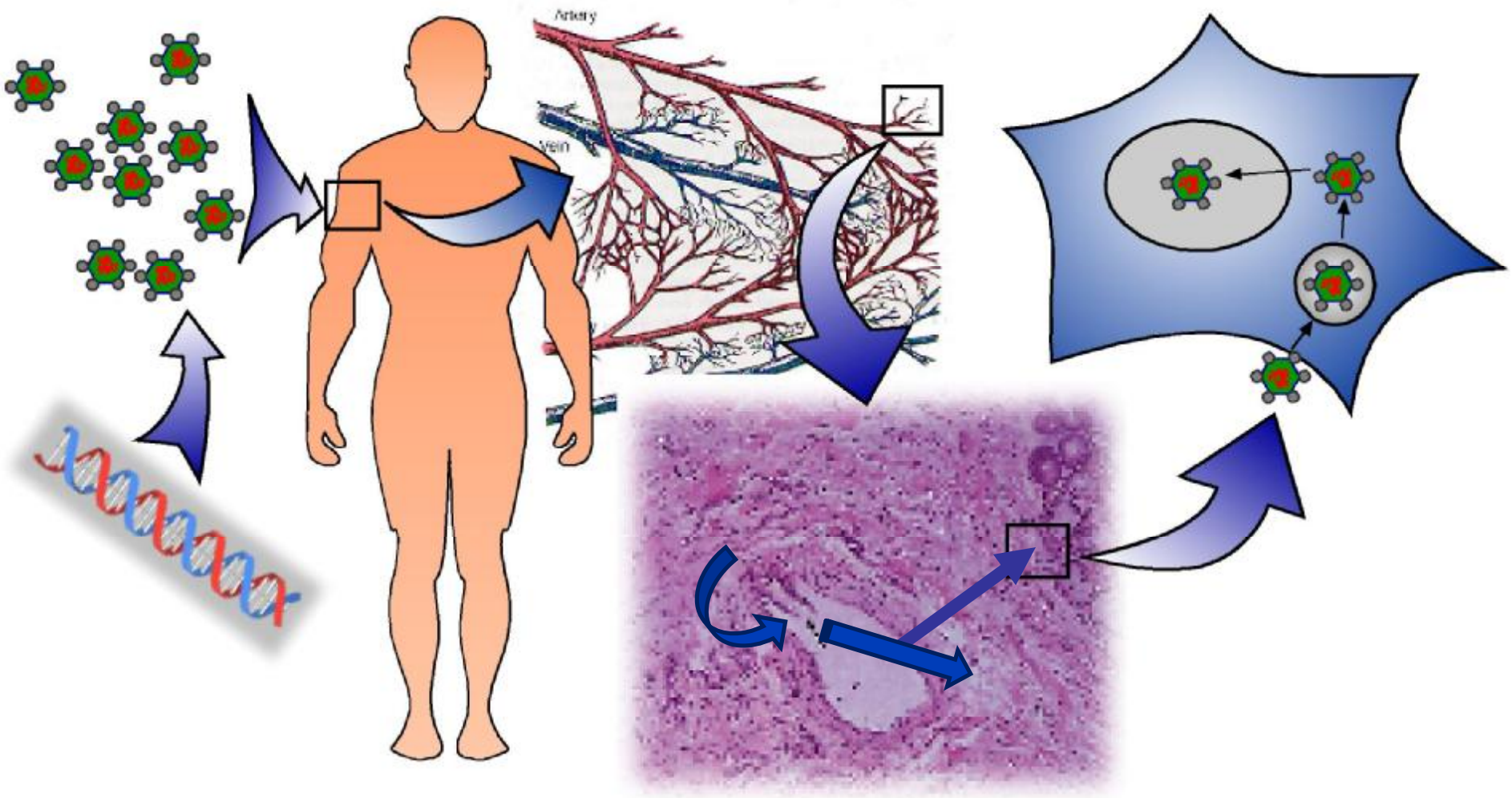
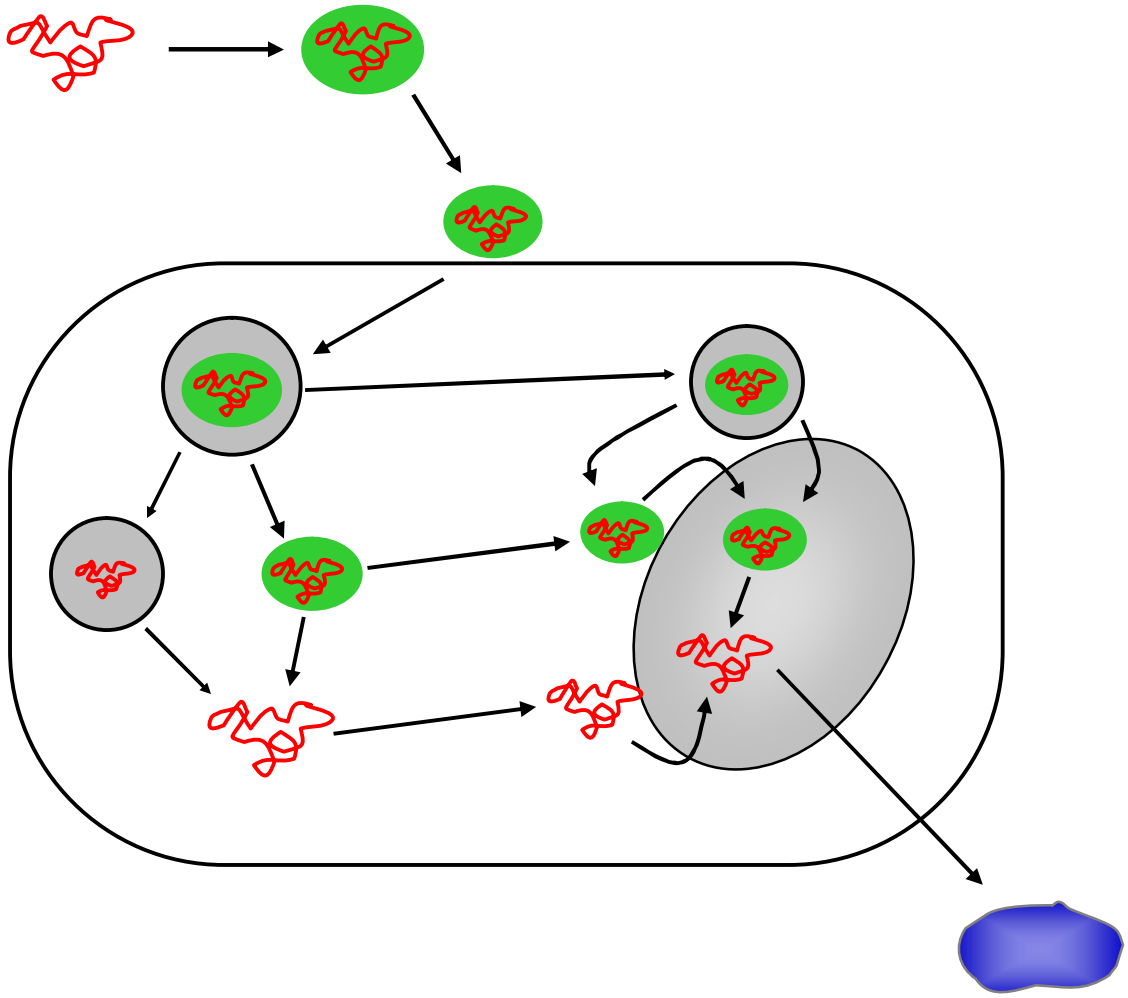
# The path from the test tube to the target cell poses many obstacles.



# Gene delivery vectors must navigate an intracellular obstacle course.



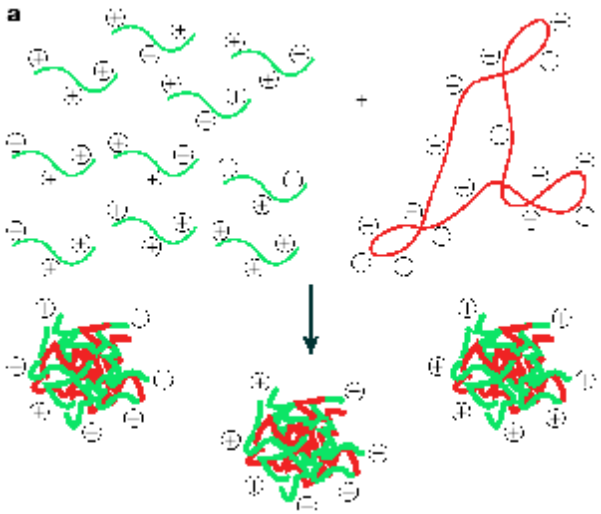
# Many design criteria = many functions.

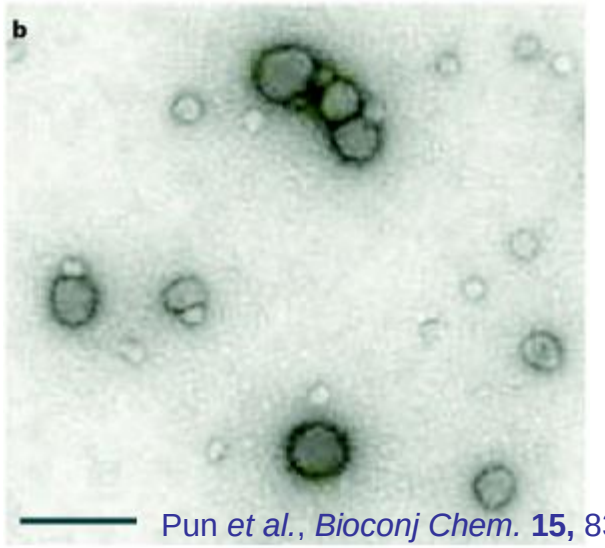
- Protect DNA
- Package large sequences
- Easy administration
  (inhalable, oral)
  injectable
  long circulation time
- Targeted to specific cell types
  by receptor usage
  by cell-specific promoters
- Facile fabrication
  inexpensive production
  easy to purify
  robust / stable
- High efficiency
  internalization
  endolysosomal escape
  nuclear transport
  efficient unpackaging
- Controlled expression
  stable
  drug-responsive
- Infection of non-dividing cells
- Safety
  non-toxic
  non-immunogenic
  non-pathogenic



Pack *et al.*, *Nature Rev. Drug Disc.* **4,** 581-593 (2005).

# Polymer-DNA complexes (polyplexes) self-assemble via electrostatic interactions.



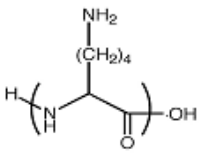



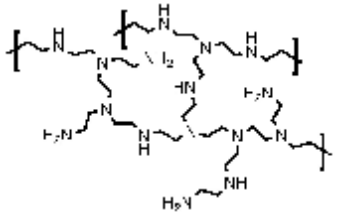

Pun *et al.*, *Bioconj Chem.* **15,** 831-840 (2004).

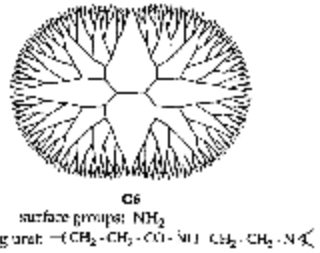
- Cationic polymers and DNA form simple electrostatic complexes (polyplexes).
- Polyplexes consist of several plasmids, with an excess of polymer, and are ~50-150 nm in diameter.

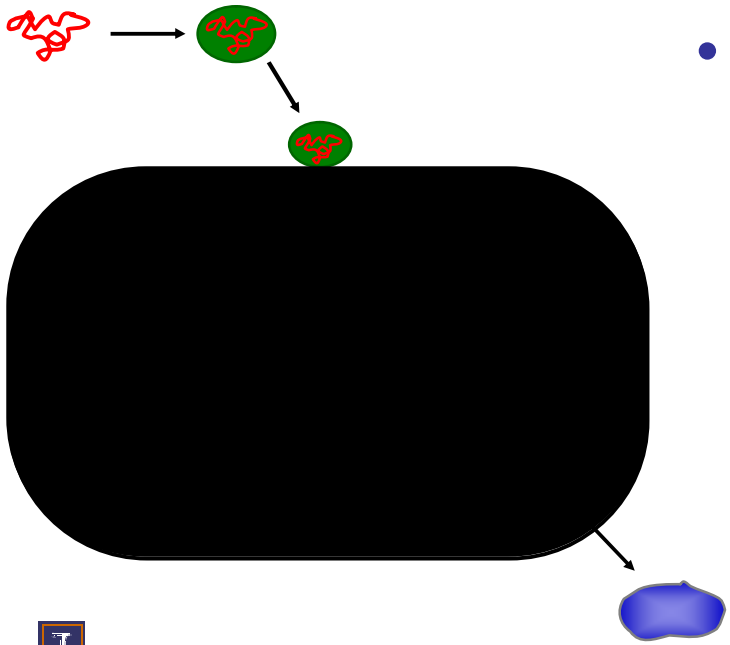
Pack *et al.*, *Nature Rev. Drug Disc.* **4,** 581-593 (2005).

# Limited understanding of intracellular obstacles hinders vector design.



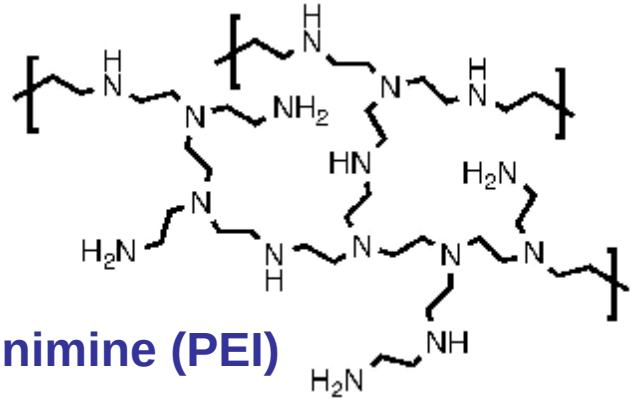





- Many of the early gene delivery agents were off-the-shelf materials.
- New materials are often designed with little understanding of intracellular trafficking mechanisms.

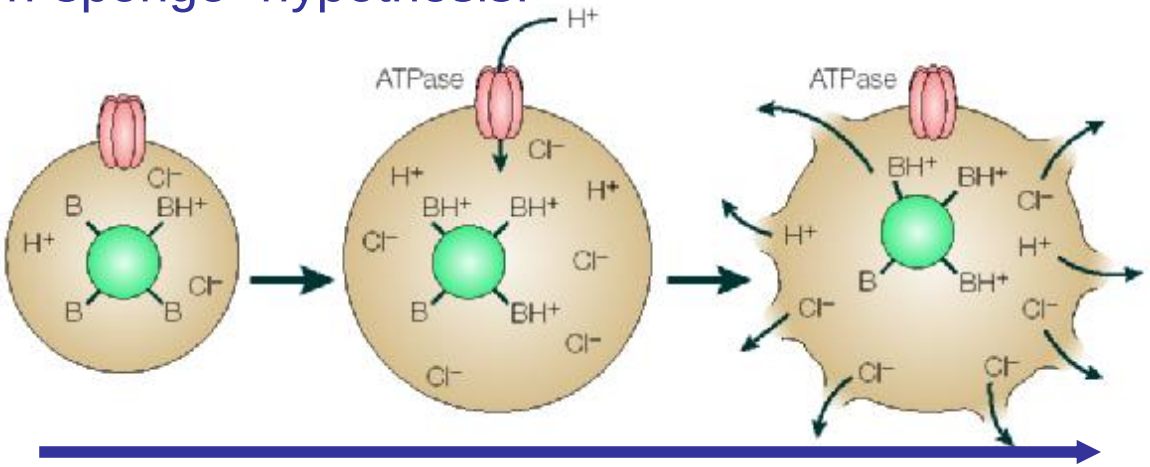
# Polyethylenimine is a relatively efficient gene delivery polymer.



**Polyethylenimine (PEI)**

PEI is hypothesized to take advantage of acidification of endocytic compartments for efficient escape into the cytoplasm.

The “proton-sponge” hypothesis:

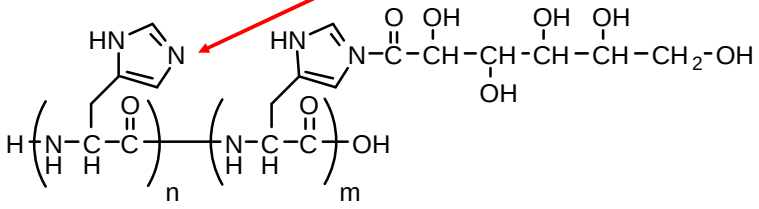




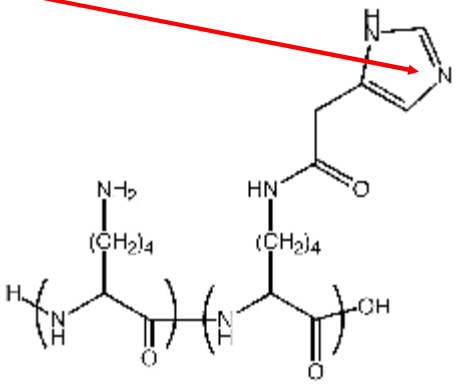

Behr, J.P., *Chimia* **51** 34-36 (1997).
Pack *et al.*, *Nature Rev. Drug Disc.* **4,** 581-593 (2005).

# Imidazole-containing polymers designed to be biocompatible proton sponges.

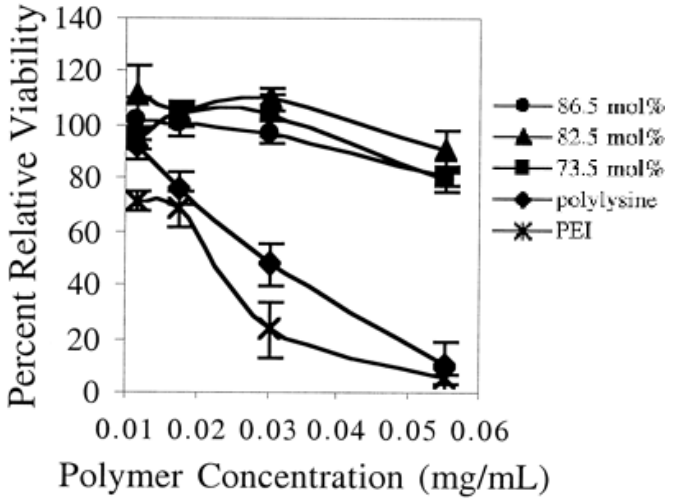
Imidazole $pK_a$ ~6.5

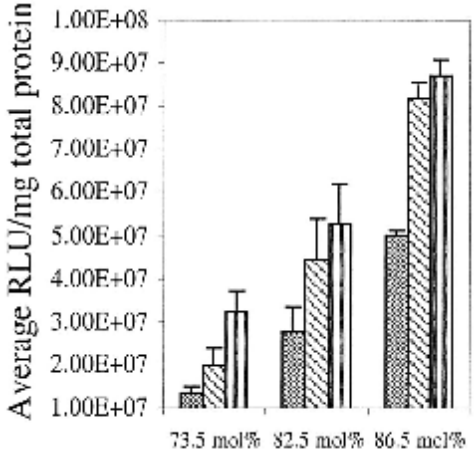


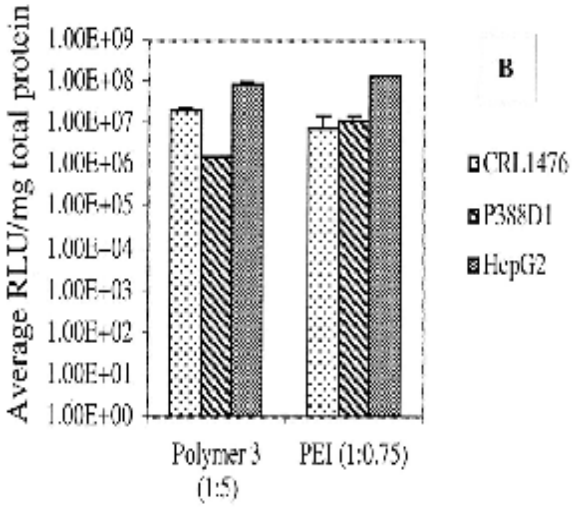
glycosylated poly(histidine)
Pack et al., *Biotechnol. Bioeng*. **67**, 217-223 (2000)



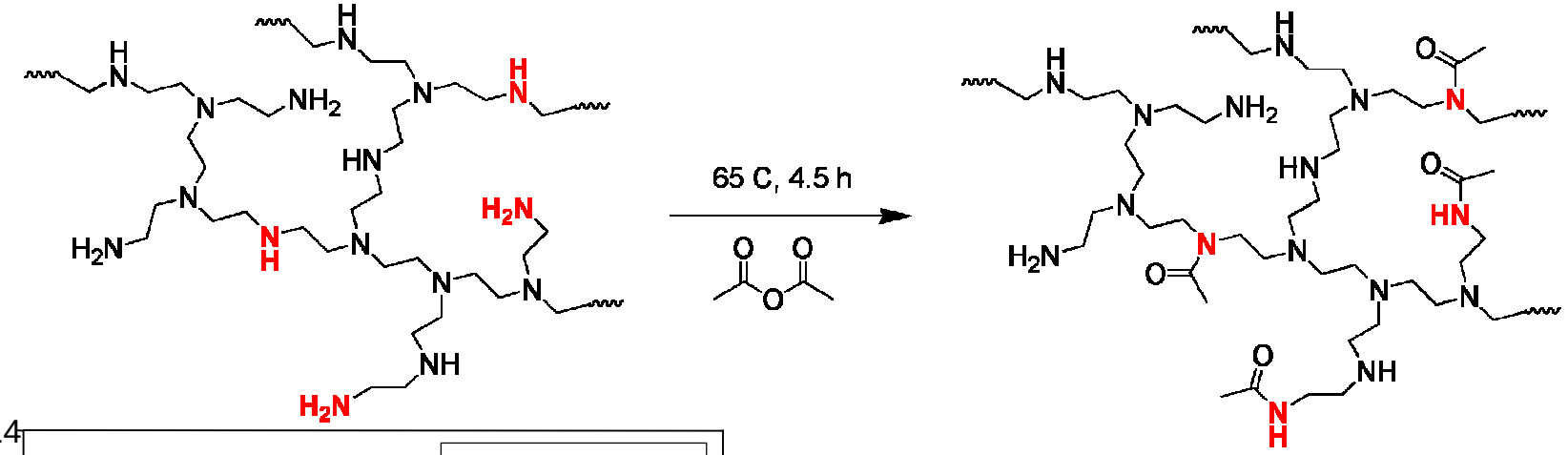
PLL-graft-imidazole
Putnam et al. *Proc. Natl. Acad. Sci.* **98**, 1200-1205 (2001)

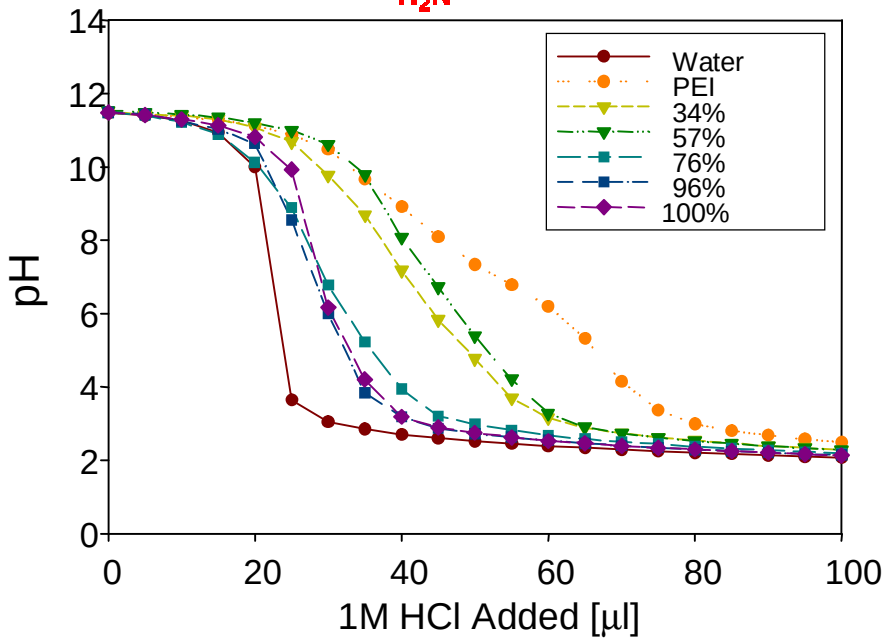


# “Capping” of PEI alters buffering capacity and DNA binding strength.




$$\text{Capping density} \propto \left(\text{Buffering capacity}\right)^{-1}$$

$$\text{Capping density} \propto \left(\text{DNA binding}\right)^{-1}$$

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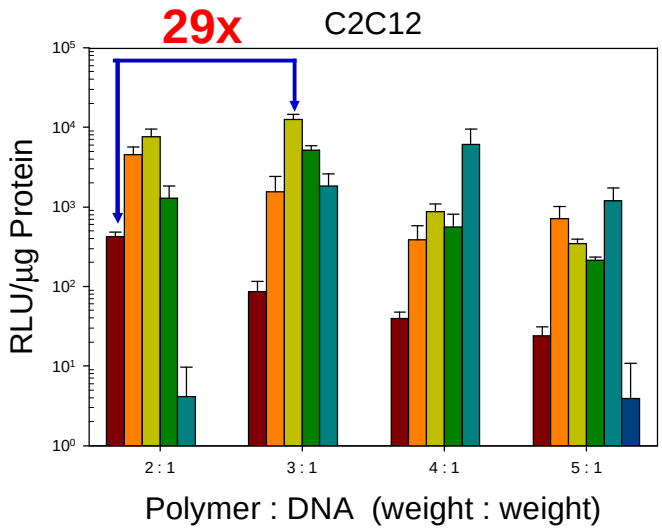
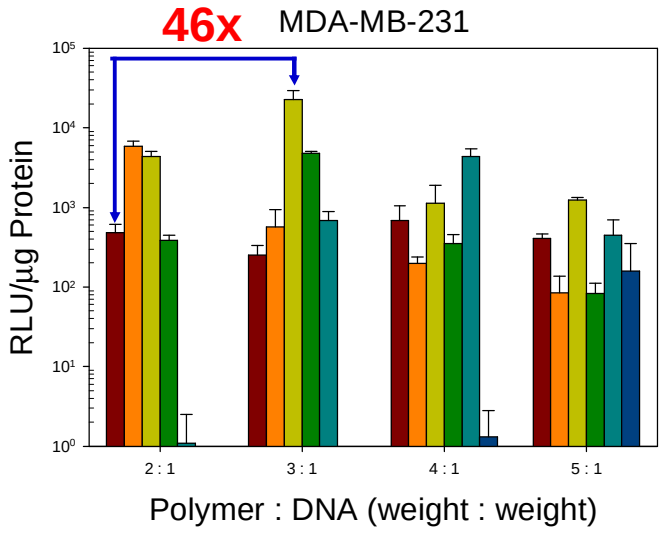
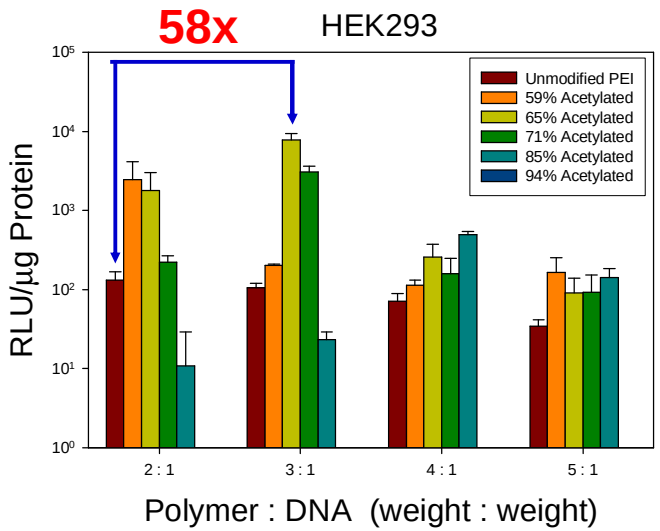
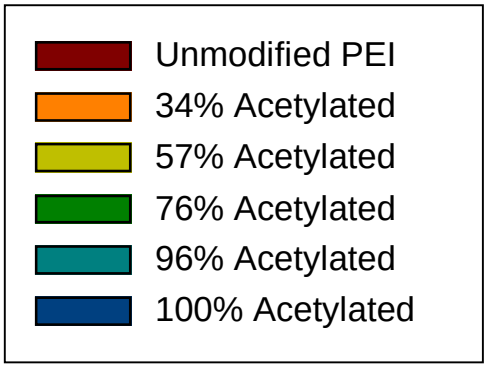
Forrest and Pack, *Pharm. Res.* **21**, 365 (2004).
Gabrielson and Pack, *Biomacromol.* **7**, 2427 (2006).

# Capped PEI is significantly more efficient than commercial PEI.




Gabrielson and Pack, *Biomacromol.* **7**, 2427 (2006).

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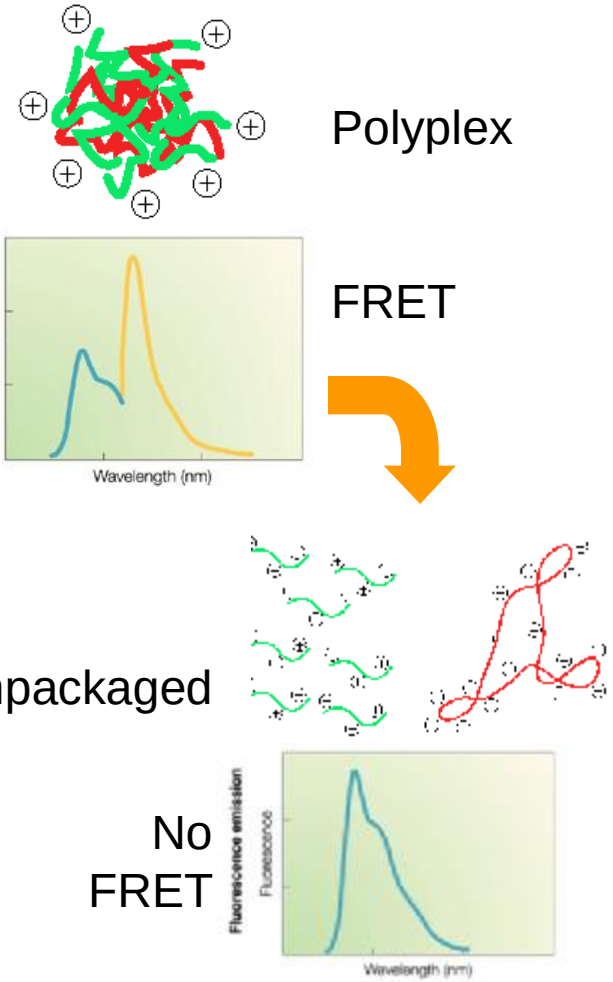
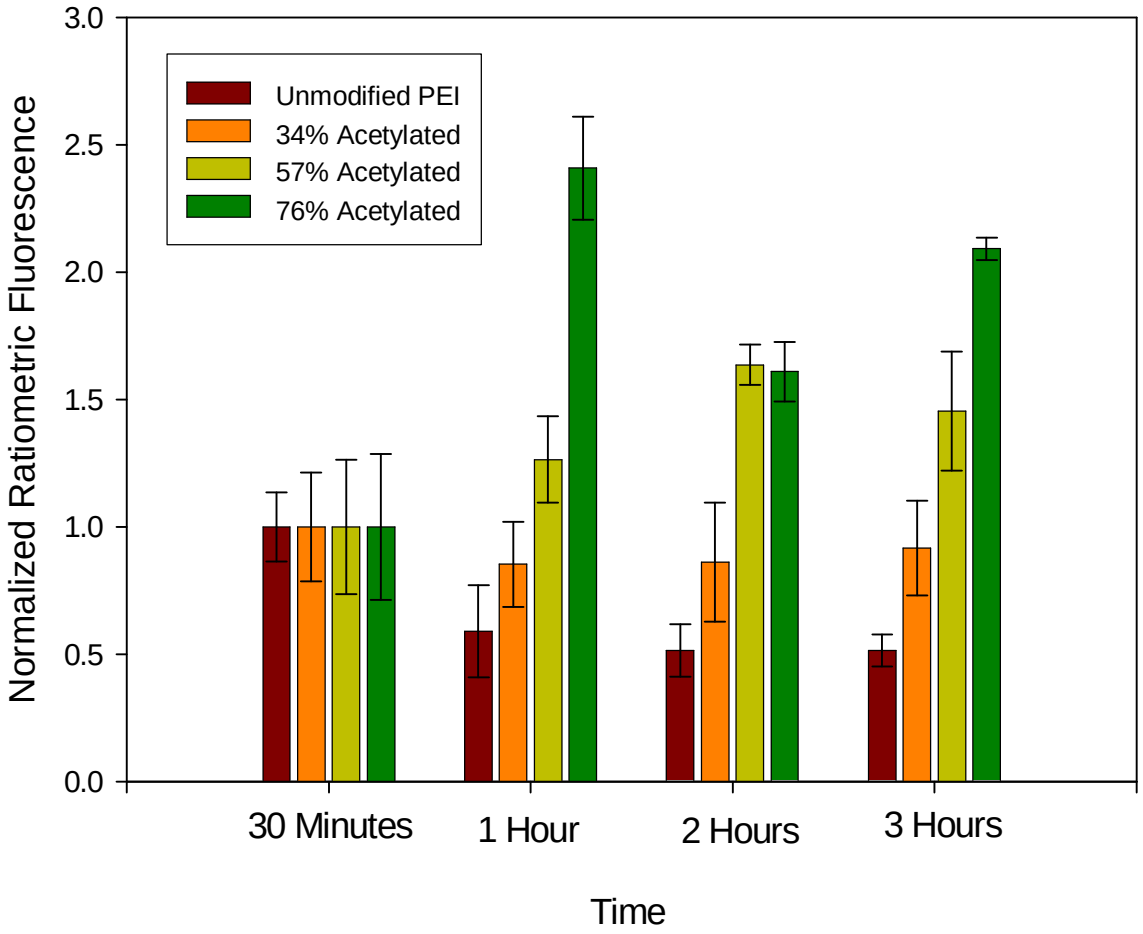
# Unpackaging is enhanced by capping of PEI.

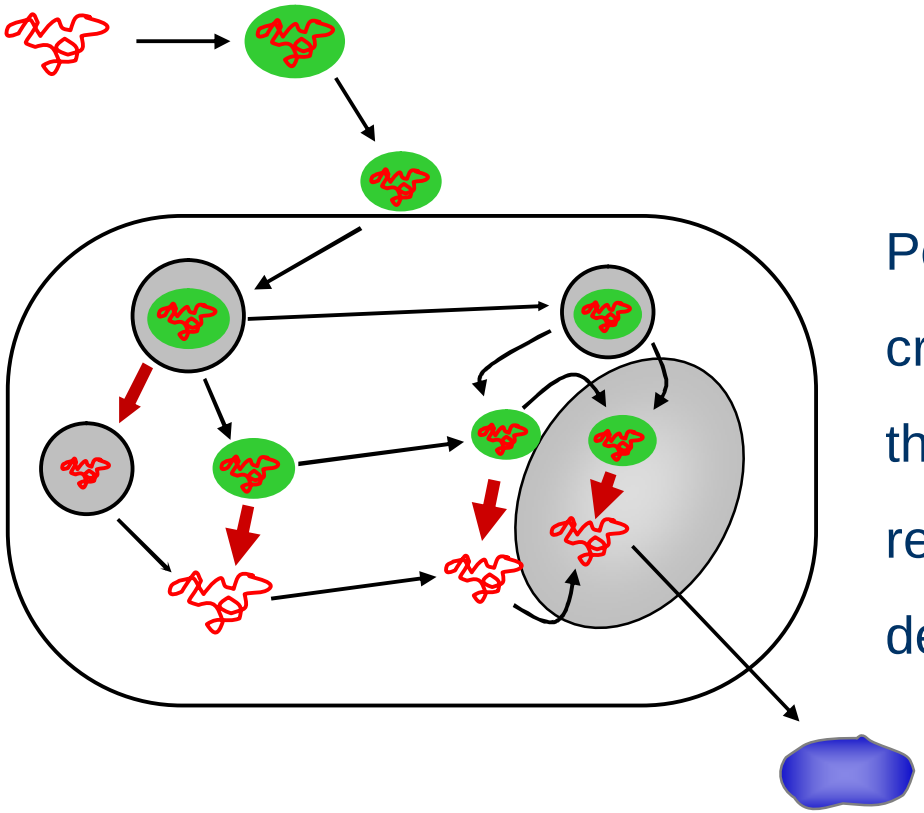




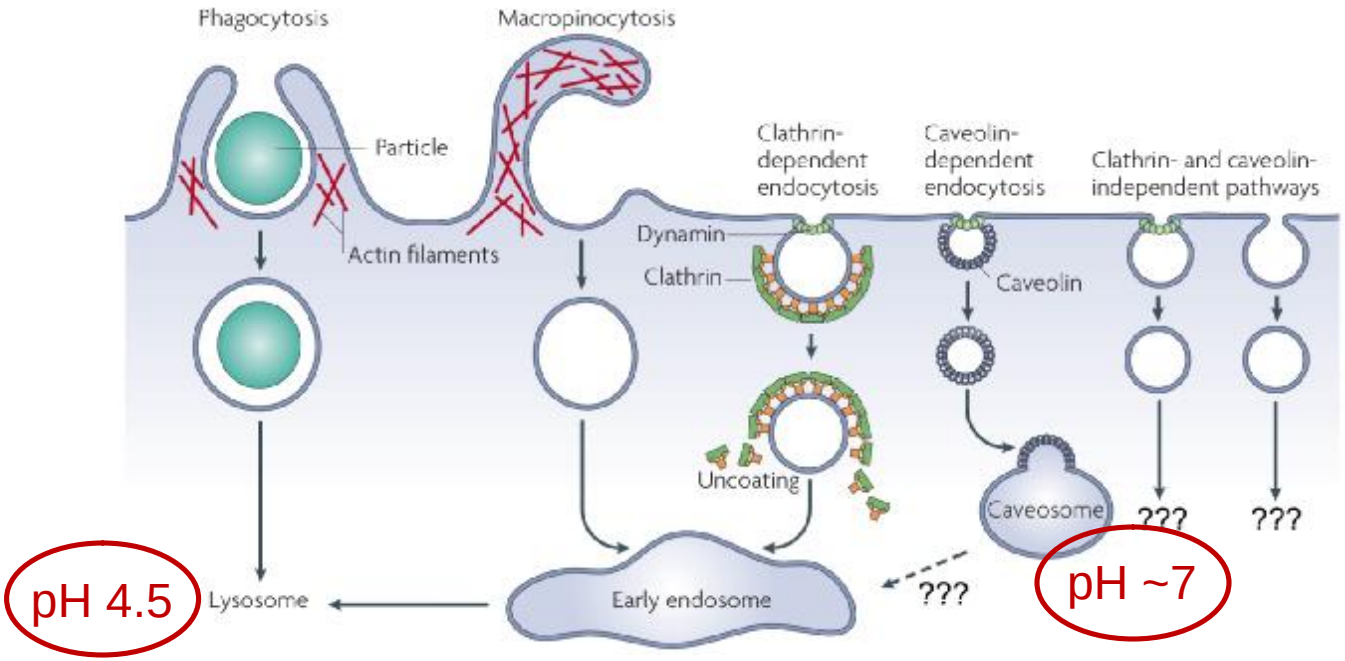

Gabrielson and Pack, *Biomacromol.* **7**, 2427 (2006).

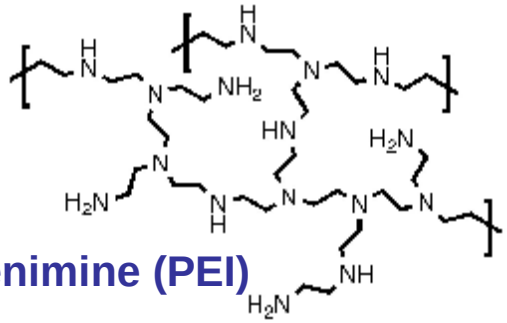
# “Unpackaging” of polyplexes is an often-overlooked intracellular barrier.



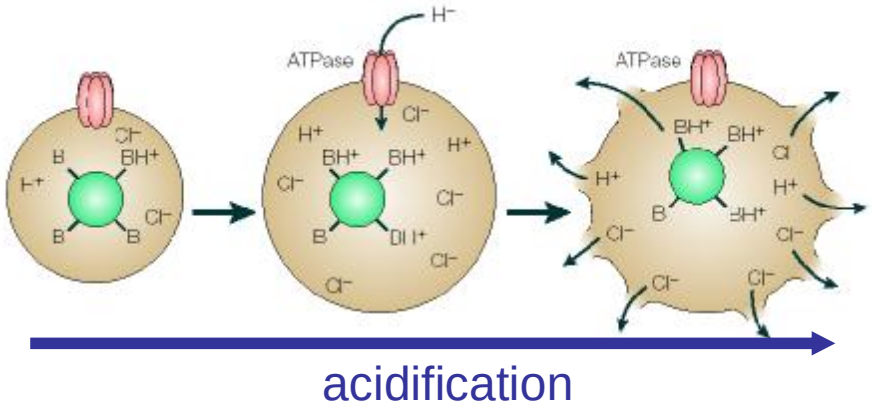
Polymer-DNA unpackaging is a critical barrier to efficient delivery that was not previously recognized as an important design criterion.

# “Endocytosis” is a broad term representative of several uptake mechanisms.



Polyethylenimine (PEI)

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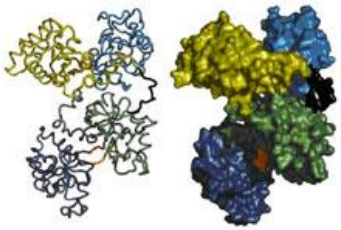
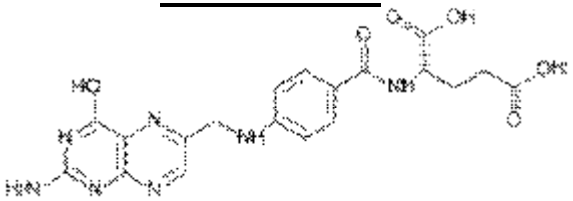
# Does clathrin- vs. caveolin-dependent uptake affect gene delivery?

## Transferrin

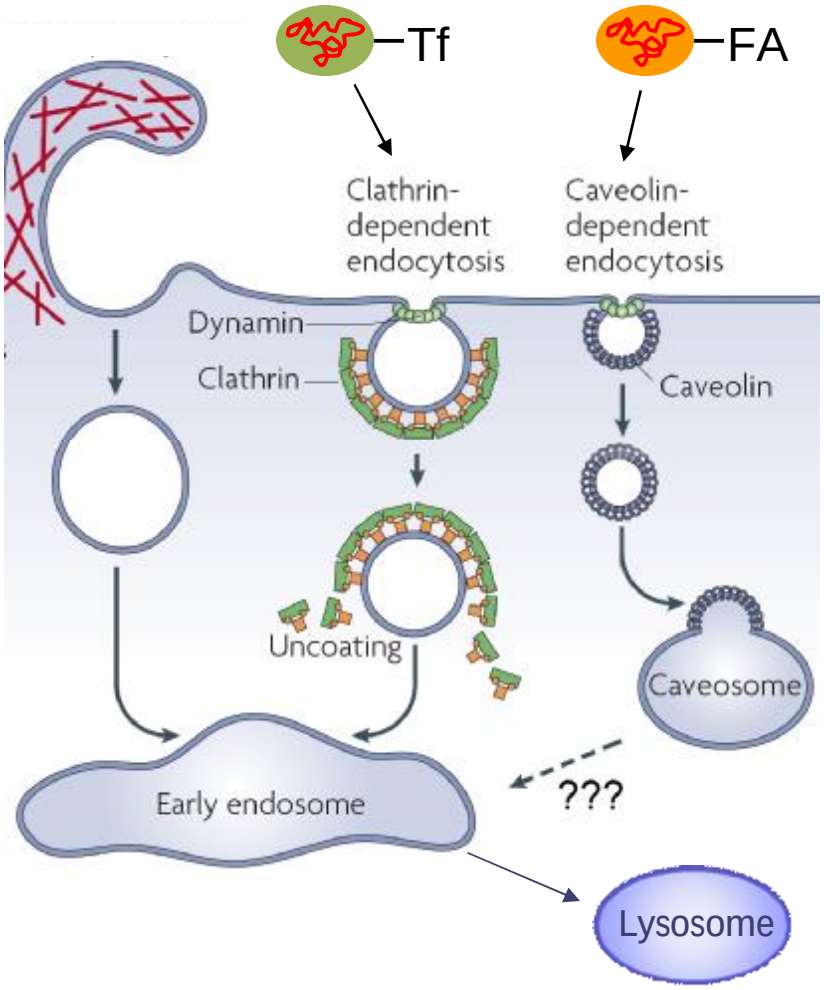


- 77-kDa serum iron transport protein
- Internalized by **clathrin pathway**

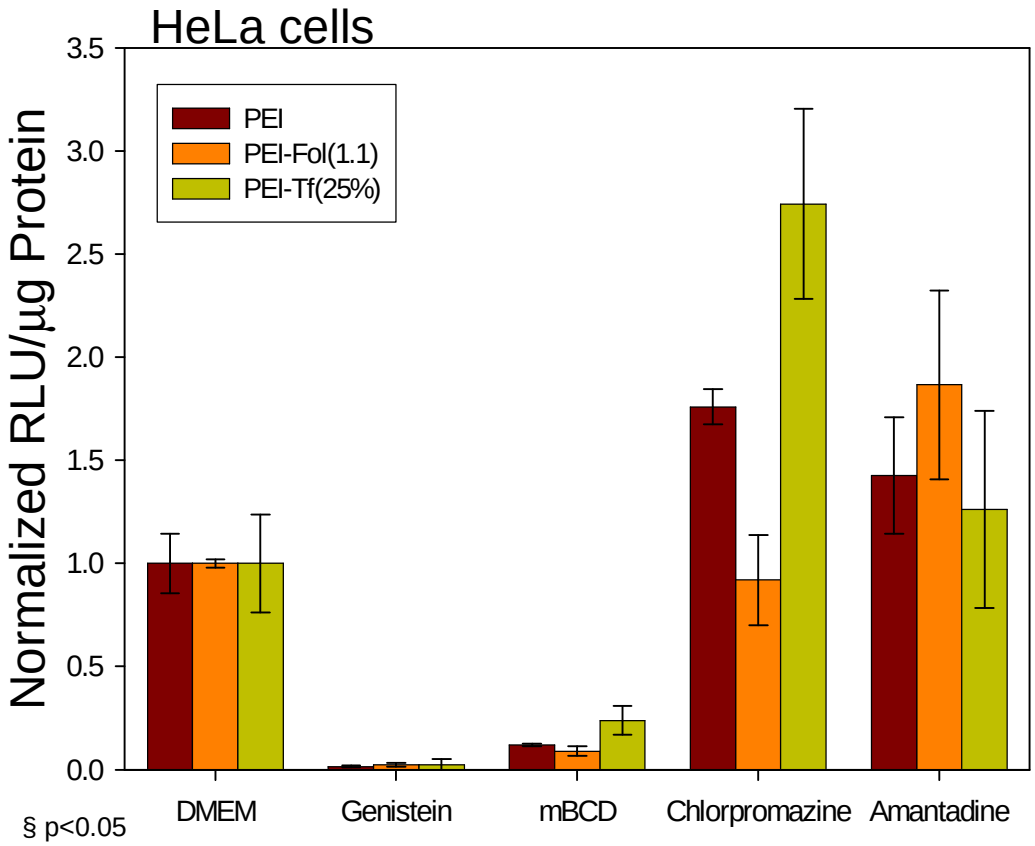
## Folic Acid

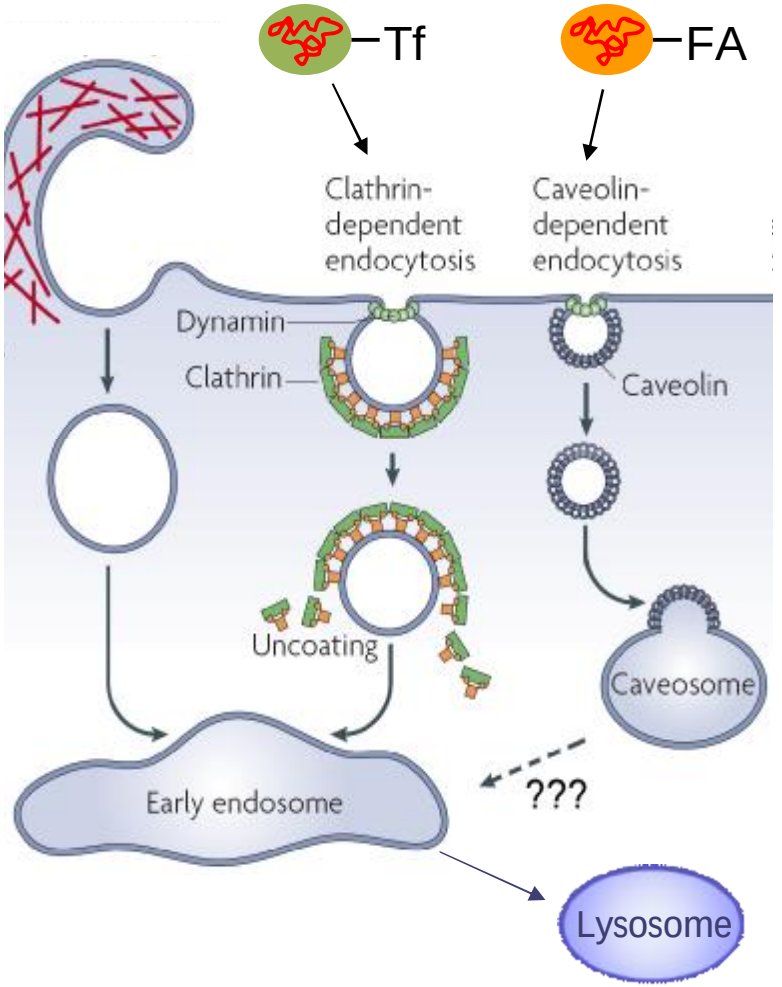


- Vitamin B9
- Internalized by **caveolae pathway**



# Only caveolar uptake is effective for PEI-mediated gene delivery in HeLa cells.




Gabrielson and Pack, submitted.

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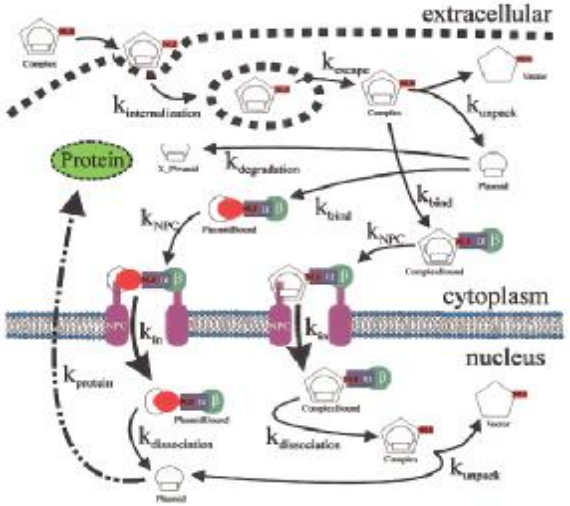
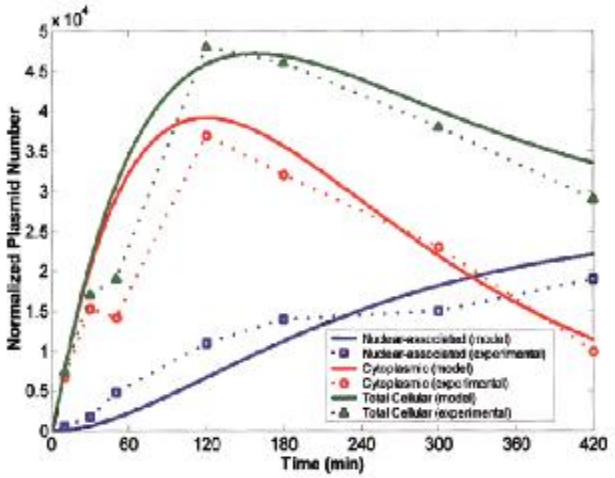
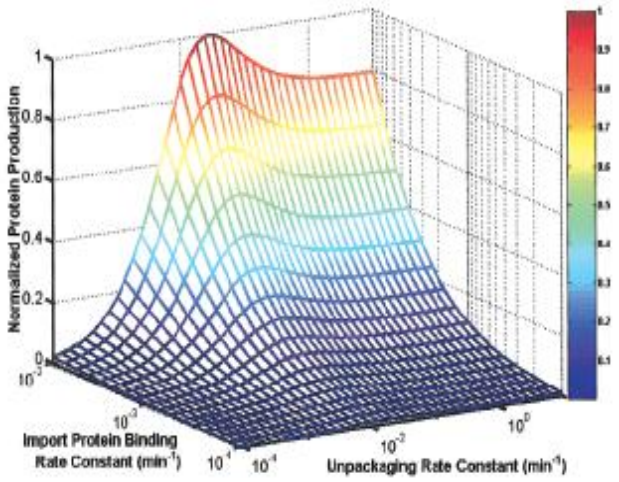
# Modeling can provide key insights to critical gene delivery barriers and vector design.

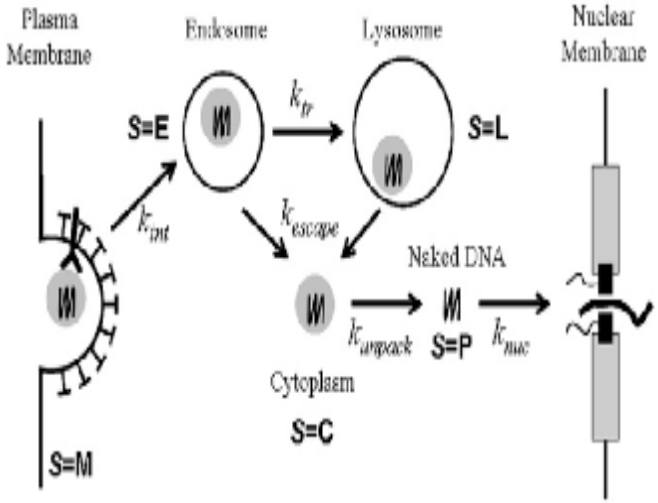
Varga et al. *Molecular Therapy* 2001

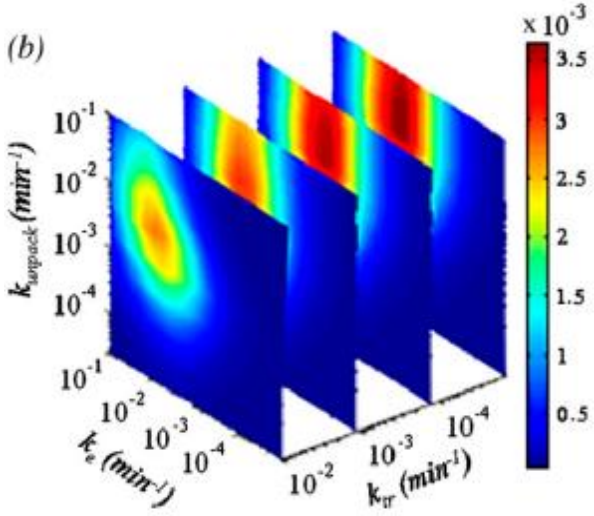


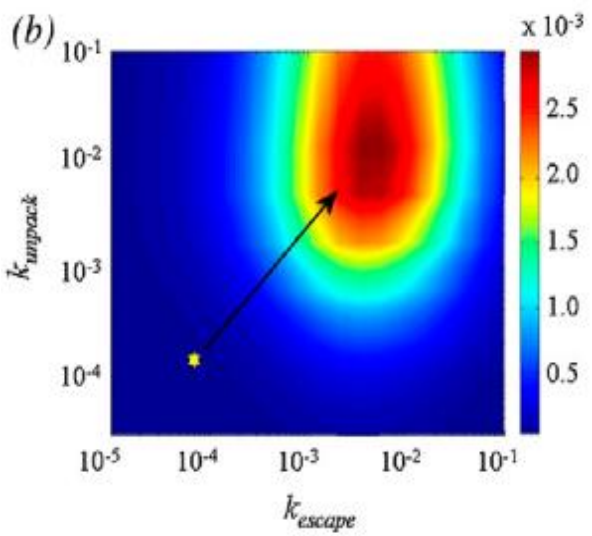



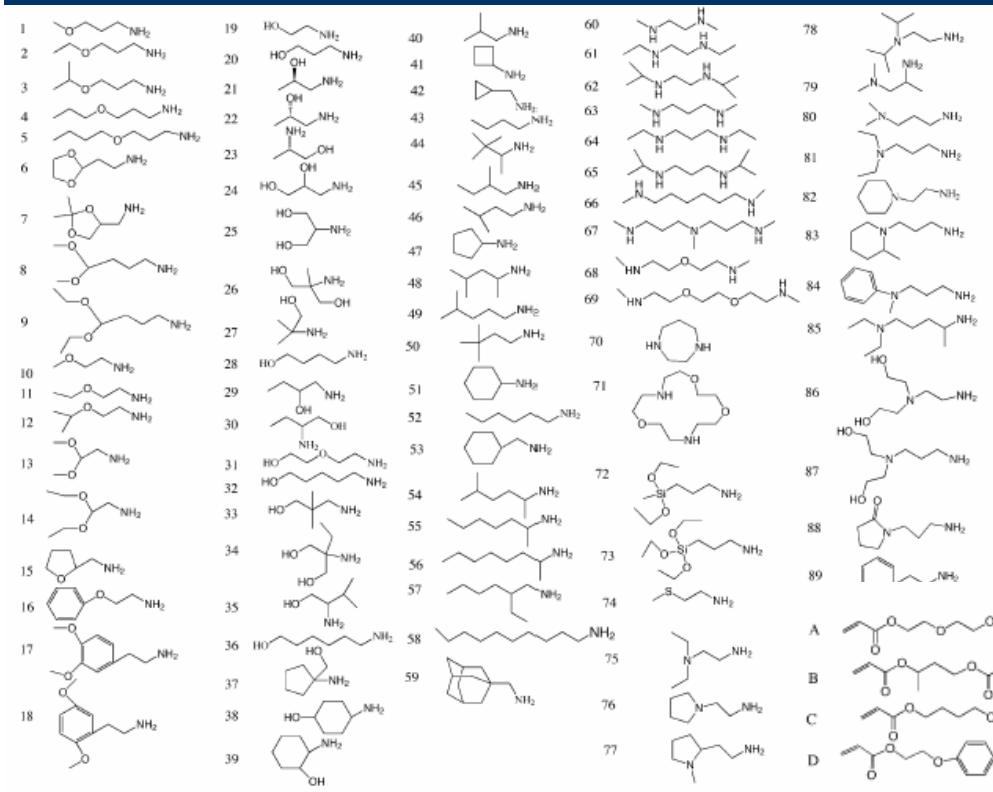

Dinh et al. *Biophysical Journal* 2007



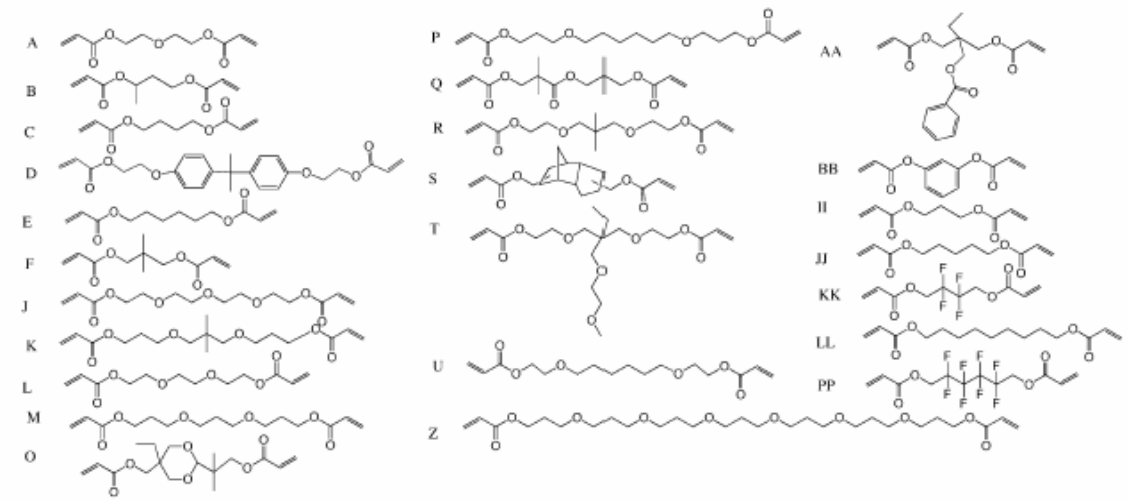



# Combinatorial polymer synthesis can circumvent lack of design criteria.

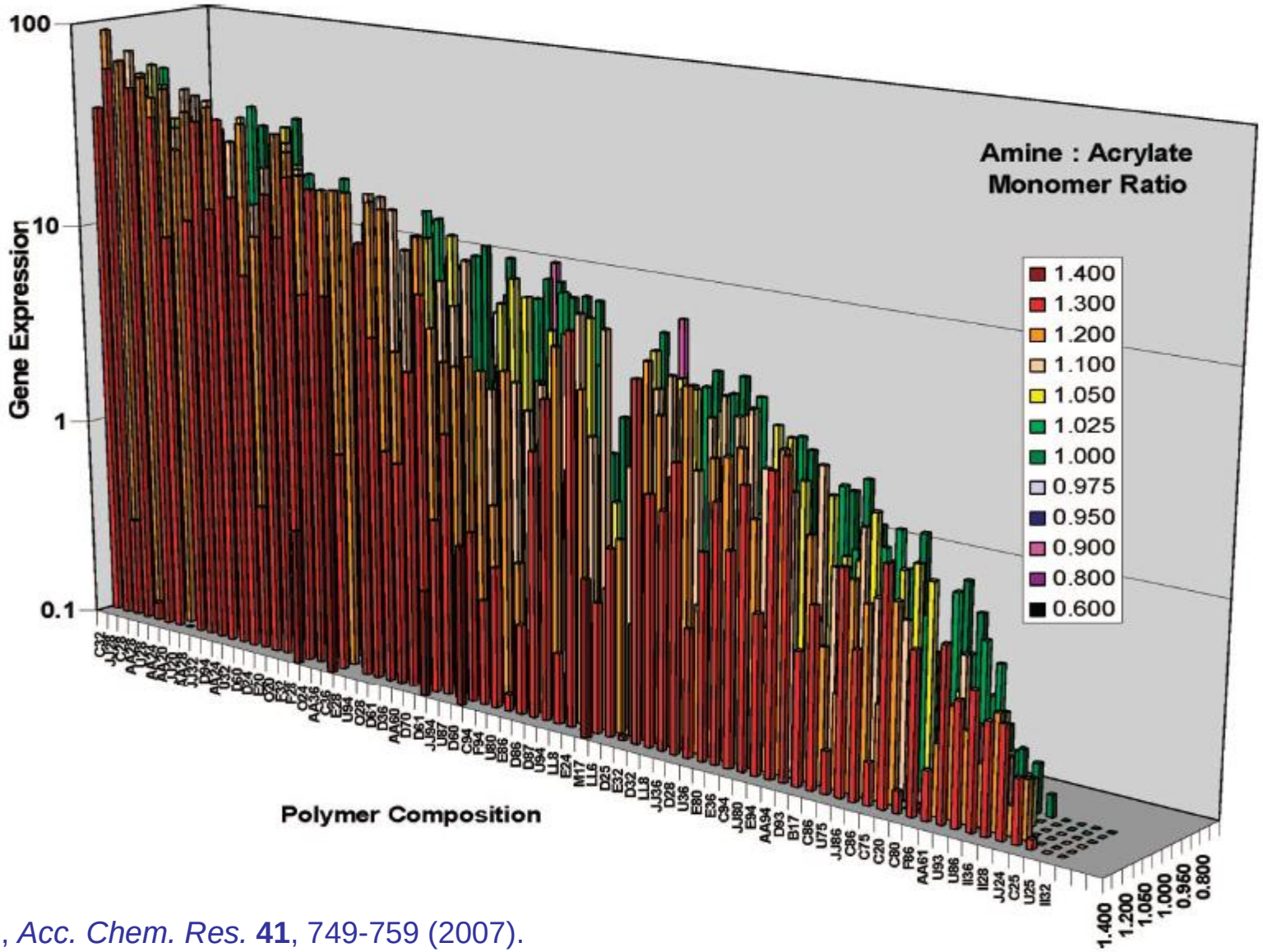


2350 unique polymers



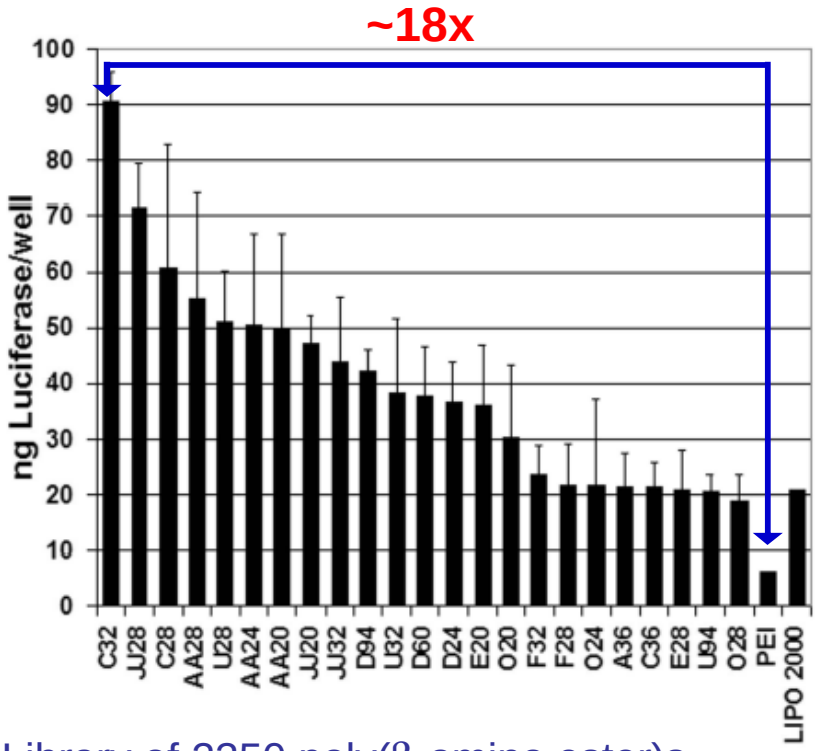
Anderson *et al.*, *Angew. Chem. IEE* **42**, 3153-3158 (2003).
Anderson et al., Molec. Ther. 11, 426-434 (2005).

# Library screening identified highly efficient gene delivery polymers.



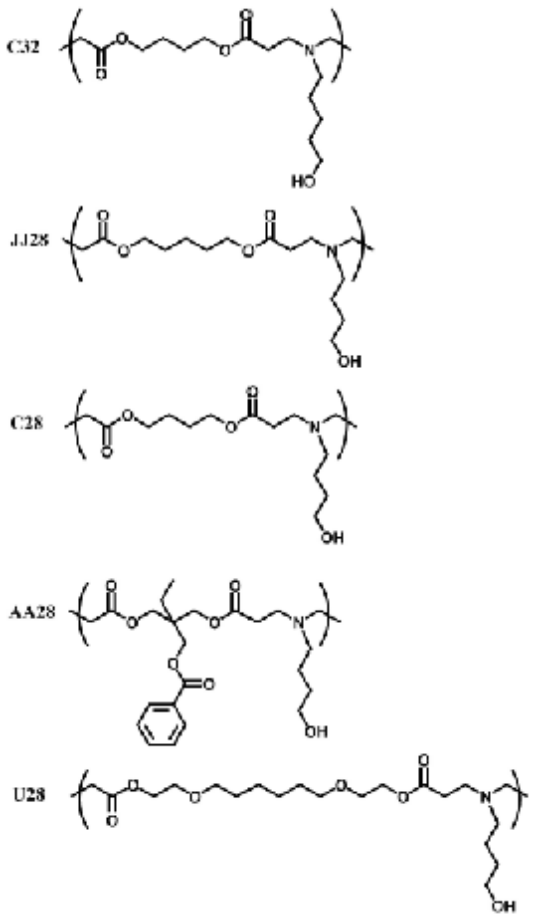

Green *et al*., *Acc. Chem. Res.* **41**, 749-759 (2007).

# Results from high-throughput screening can yield design insights.



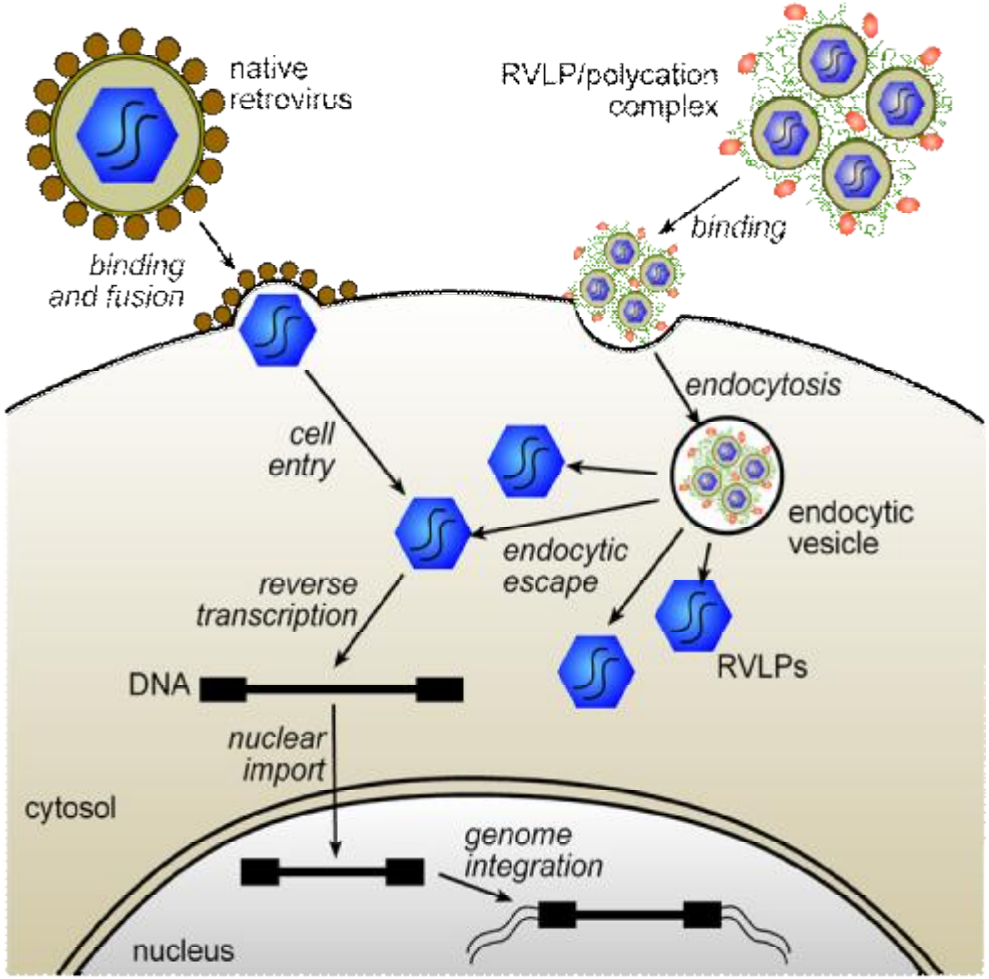

- Library of 2350 poly(β-amino ester)s.
- 46 polymers better than PEI; 26 better than Lipofectamine 2000.
- Hydrophobic diacrylates; alcohol side chains; >10,000 Da; small (<150 nm) polyplexes were most effective.

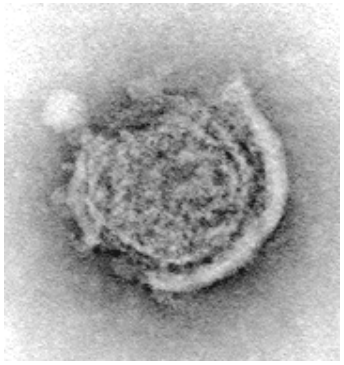
Five best polymers:

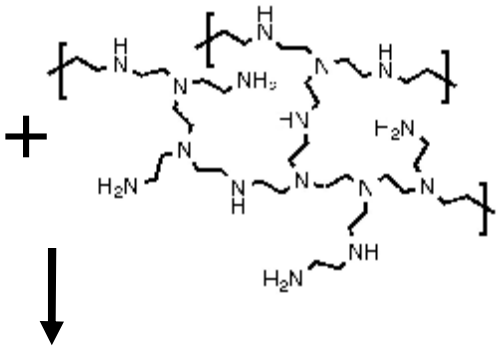


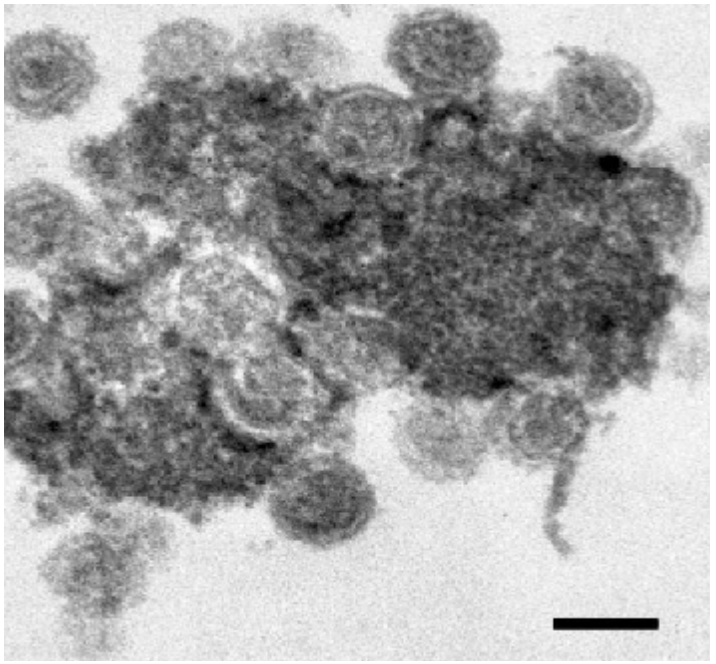

Anderson *et al.*, *Angew. Chem. IEE* **42**, 3153-3158 (2003).
Anderson et al., Molec. Ther. 11, 426-434 (2005).

# Polymer/virus hybrids may provide advantages compared to traditional vectors.



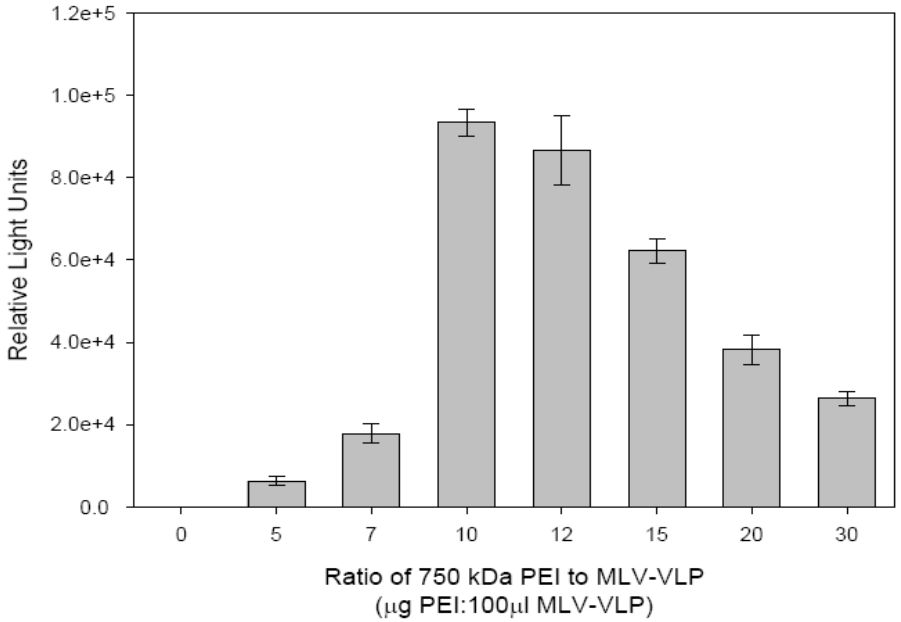



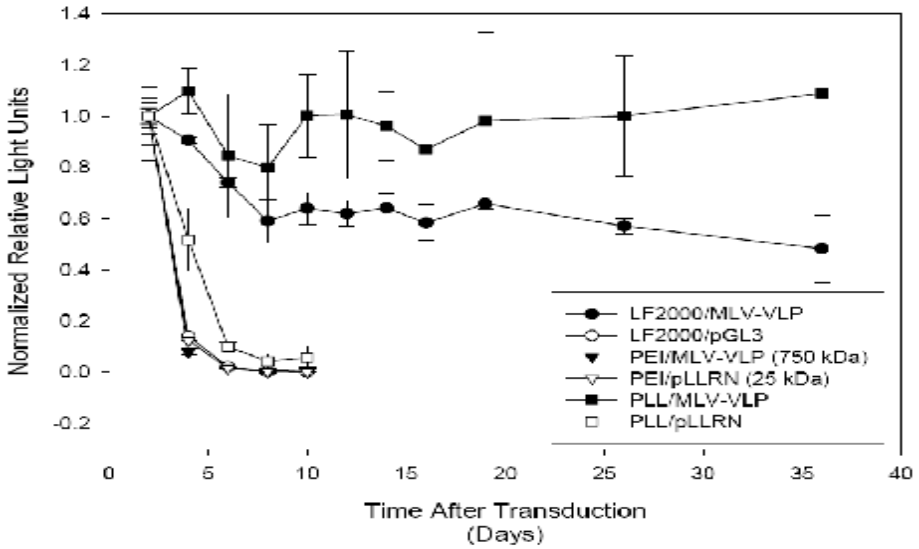


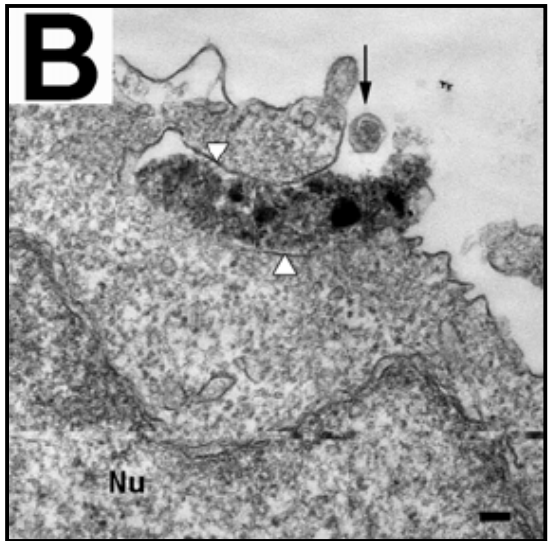


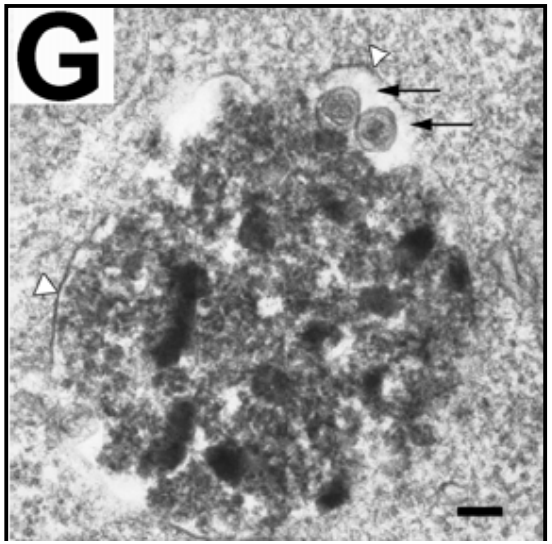
Ramsey, Vu and Pack, submitted.

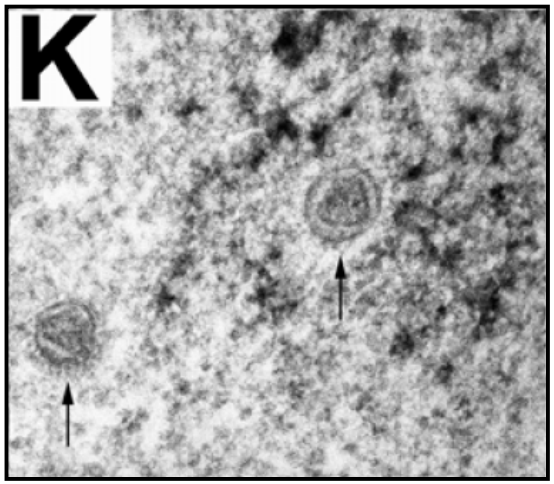
# Hybrid vectors show early promise.




Ramsey, Vu and Pack, submitted.

# Summary and conclusions.

- Routine clinical implementation of human gene therapy awaits development of safe, efficient, and practical gene delivery methods.
- An engineering design approach, based on **quantitative** understanding of **structure-activity relationships** and **quantitative models of intracellular processing**, will facilitate the design of more efficient gene delivery materials.
- New approaches including combinatorial synthesis and viral/synthetic hybrids hold promise for design of next-generation gene delivery methods.