Intracellular drug delivery: aiding cross presentation of subunit vaccines

Last Time: basic vaccine concepts

Today: Using synthetic biomaterials to enhance cytosolic delivery of molecules

Reading: Wang et al. 'Molecularly engineered poly(ortho ester) microspheres for enhanced delivery of DNA vaccines,' *Nat. Mater*. **3** 190-196 (2004)

Supplementary Reading:

ANNOUNCEMENTS:

COURSE EVALUATION NEXT TUESDAY 5/16

Particle-stimulated cross presentation

ER-PHAGOSOME FUSION MODEL:

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Please see: Lehner, and Cresswell. *Curr Opin Immunol* 16, 82 (2004).

Graph removed due to copyright restrictions.
Please see: Kovacs-Bankowski et al. *PNAS* 90 (1993): 4942-4946.

INTRACELLULAR DRUG DELIVERY AND VACCINES:

(1) BOOST CROSS PRESENTATION OF PROTEIN ANTIGENS

(2) CYTOSOLIC DELIVERY OF DNA IS A STEP ON PATH TO NUCLEUS

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Please see: Vijayanathan, et al. 2002.

ENDOSOMAL ESCAPE:

Enhancing cross presentation
cytosolic delivery of large macromolecules

(1) ‘proton sponge’ effect

(2) pH-activated polymers
and peptides

INTRACELLULAR VS. EXTRACELLULAR ENVIRONMENT

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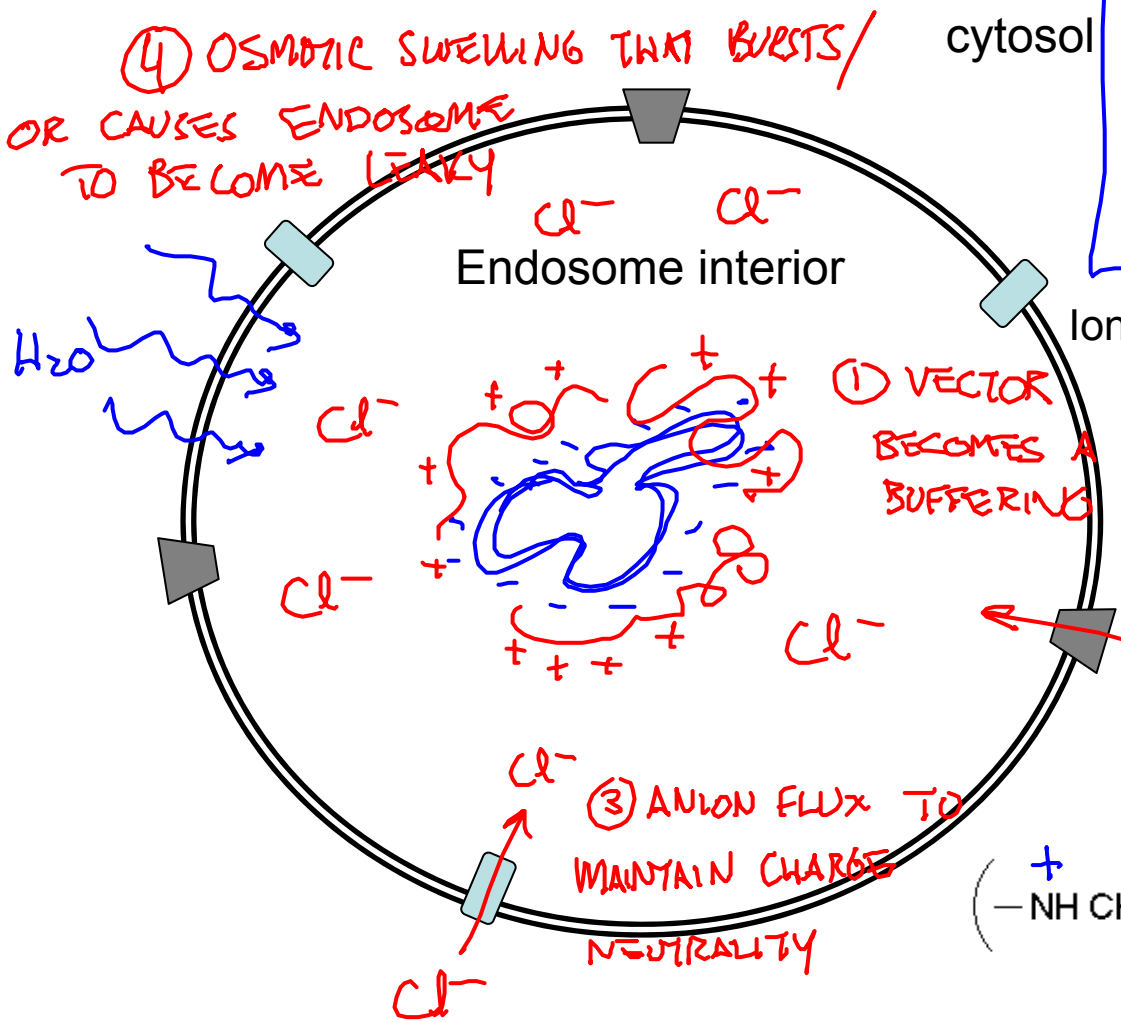
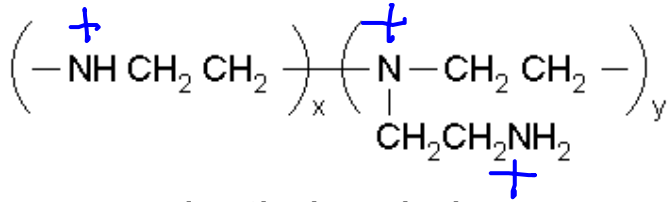
Please see: Alberts, Bruce, et al. *Molecular Biology of the Cell*. New York, NY: Garland, 2004.

ENDOSOMAL ESCAPE:
'PROTON SPONGES'

Proton sponge effect



CAVEATS:
POLYCATIONS/CATIONIC LIPIDS TEND TO BE TOXIC AT HI DOSES



polyethyleneimine

PEI

Role of additional structural features of PEI in efficient endosomal escape:

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Please see: Dubruel, et al. *Biomacromolecules* 5 (2004): 379-388.

ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS

Endosomal escape by direct membrane interactions

Both polycation and polyanion headgroups with pKas = 5-7 can promote endosomal escape:

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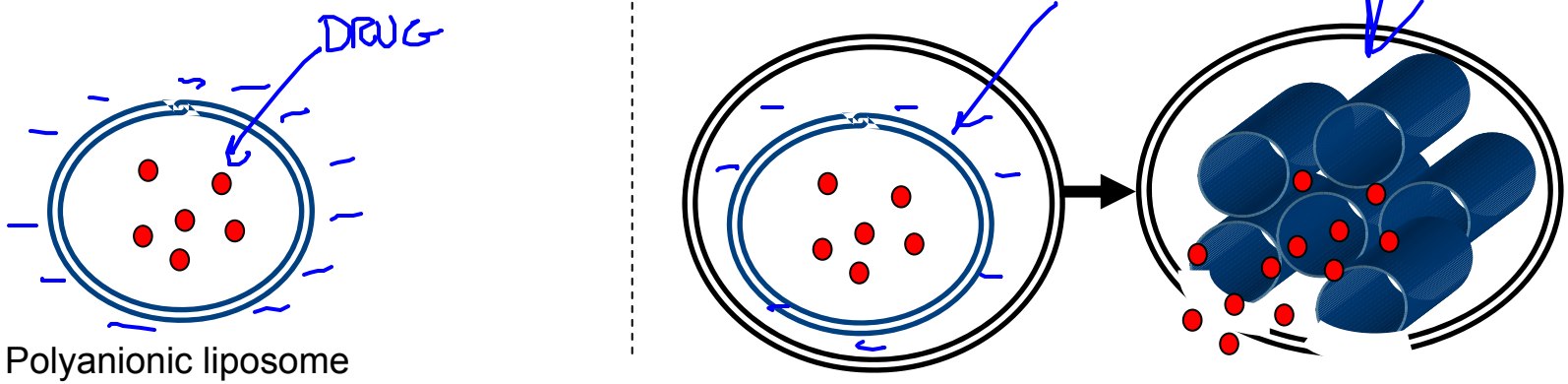
Please see: Mann, Stephen. Biomineralization: *Principles and Concepts in Bioinorganic Materials Chemistry*. New York, NY: Oxford University Press, 2001.

PHASE TRANSITIONS DRIVEN BY: CONCENTRATION, TEMP, pH

PHASE TRANSITION CAUSES FUSION W/VESICLE MEMBRANE

PROTONATION/ UNCHARGING

pH: **7.4** **5.0**



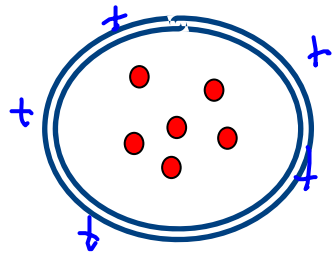
Polyanionic liposome

ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS

Endosomal escape by direct membrane interactions

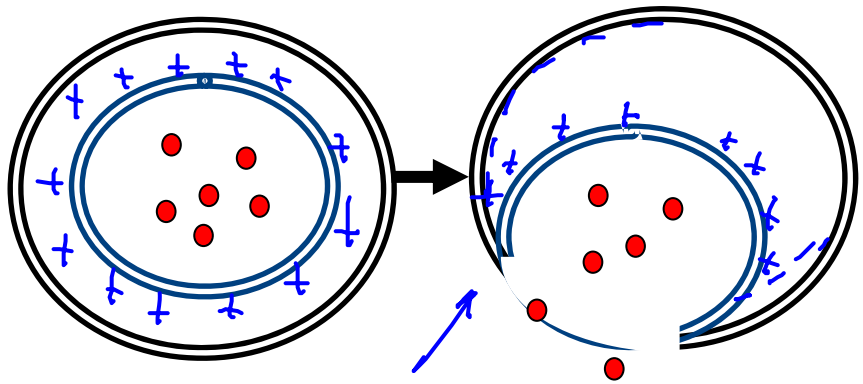
Both polycation and polyanion headgroups with pKas = 5-7 can promote endosomal escape:

pH: 7.4



polycationic liposome

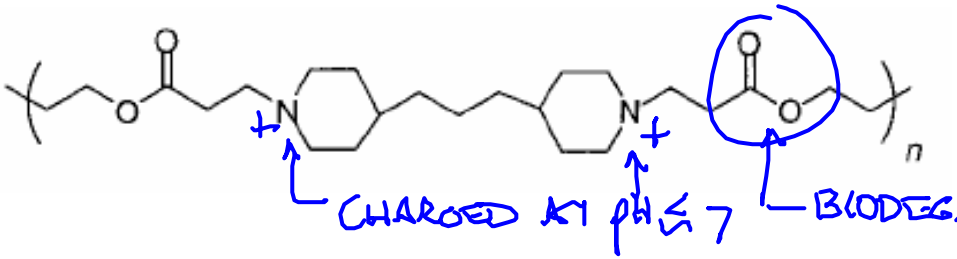
5.0



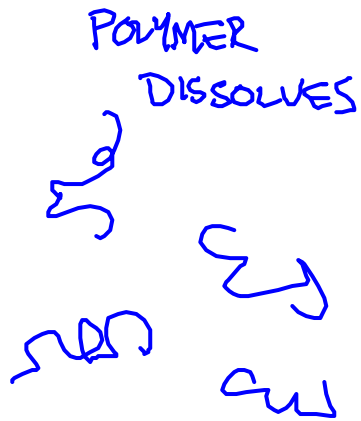
ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS

STRATEGIES FOR CUED 'BURST' RELEASE OF CARGO COINCIDENT WITH ENDOSOMAL ESCAPE

POLY (β-AMINO ESTERS)



PBAE MICROSPHERE LOADED W/DRUG



RAPID + TOTAL RELEASE OF DRUG

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Please see: Lynn, Langer, et al. *Angew Chem Int Ed* 40 (2001): 1707.

ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS

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Please see: Little, Langer, et al. *PNAS* 101 (2004): 9534-9539.

ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS

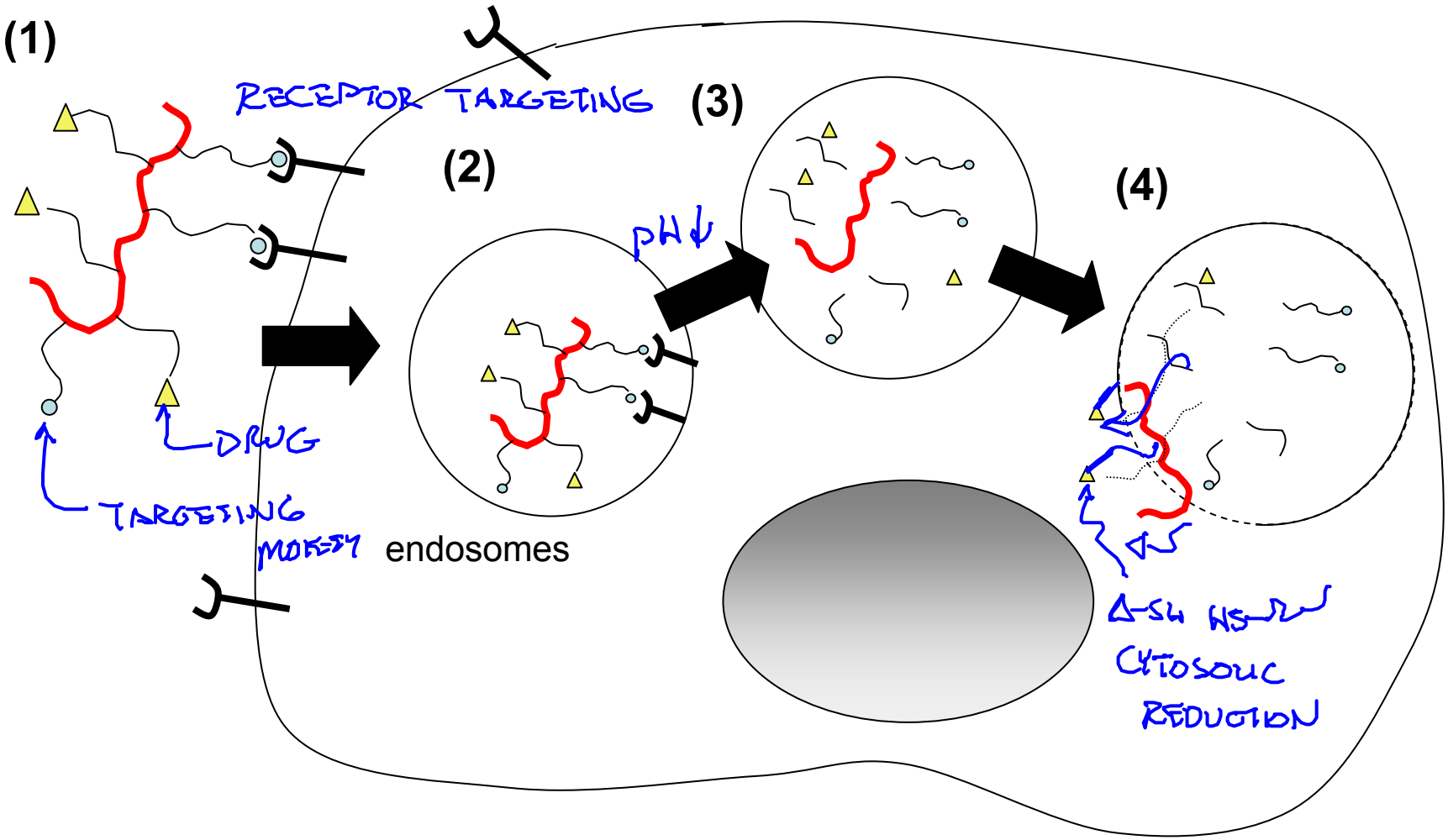
Approaches to endosome escape: 'encrypted' polymers

Multi-function molecular carriers:

Figure removed due to copyright restrictions.

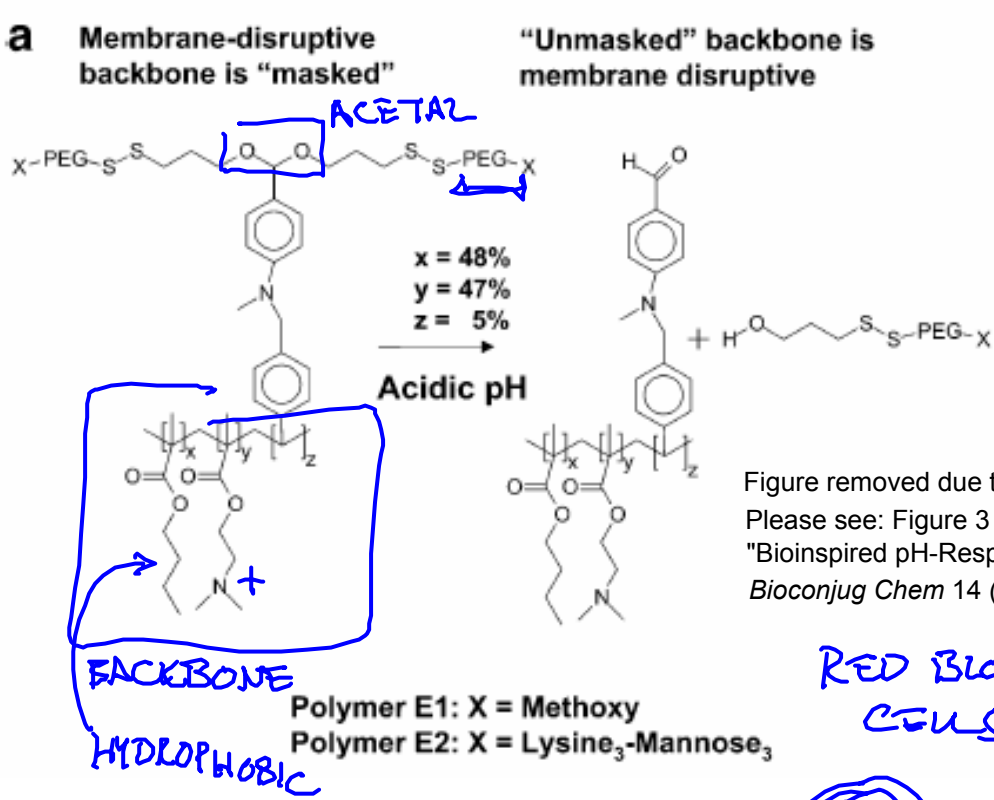
Please see: Figure 1 in Murthy, N. et al. "Bioinspired pH-Responsive Polymers for the Intracellular Delivery of Biomolecular Drugs." *Bioconjug Chem* 14 (2003): 412-9.

ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS



ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS

CLEAVAGE OF SIDE CHAINS:



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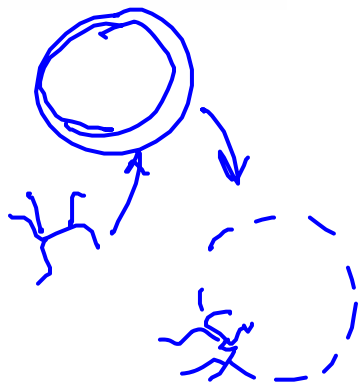
Please see: Figure 3 in Murthy, N. et al.
"Bioinspired pH-Responsive Polymers for the Intracellular Delivery of Biomolecular Drugs."
Bioconjug Chem 14 (2003): 412-9.

RED BLOOD CELLS



Results with peptide delivery by encrypted polymers

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ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS

Example vaccine results: pH-responsive gels as vaccines

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Please see: Murthy, Frechet, et al. *PNAS* 100 (2003): 4995-5000.

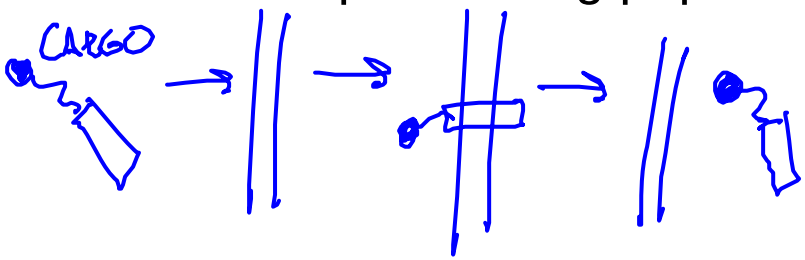
ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS

Example vaccine results: pH-responsive gels as vaccines

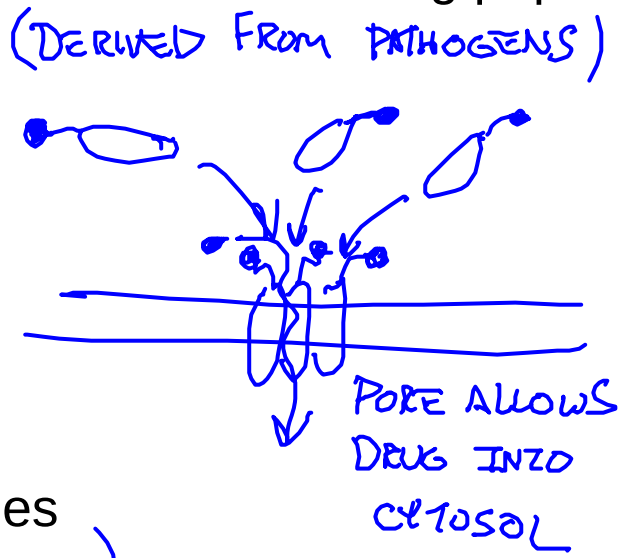
Figure removed due to copyright restrictions.
Please see: Murthy, Frechet, et al. *Bioconj Chem* 15 (2004): 1281-1288.

DIRECT ENTRY TO THE CYTOSOL

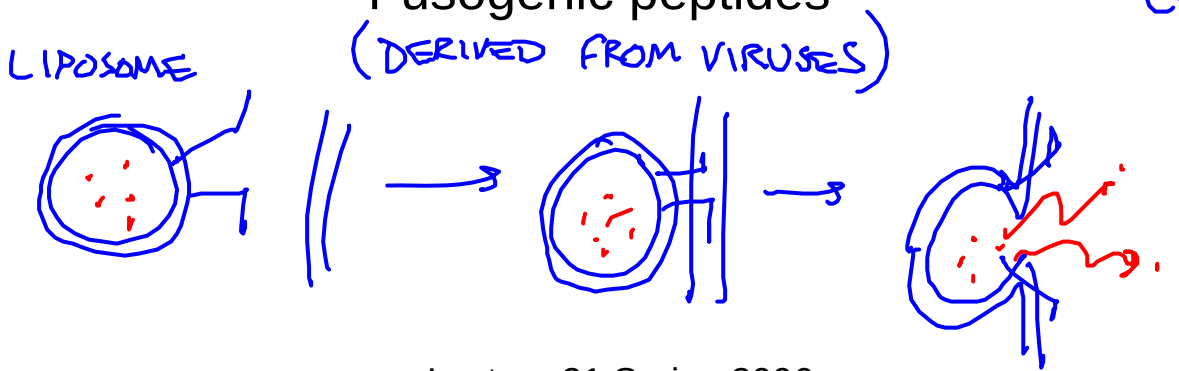
Membrane-penetrating peptides



Pore-forming peptides



Fusogenic peptides



DIRECT ENTRY TO CYTOSOL:
MEMBRANE-PENETRATING PEPTIDES

Cell-penetrating peptides (CPPs)
[aka Protein Transduction Domains (PTDs)]

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Please see: Joliot, A., and A. Prochiantz. "Transduction Peptides: from Technology to Physiology." *Nat Cell Biol* 6 (2004): 189-96.

DIRECT ENTRY TO CYTOSOL:
MEMBRANE-PENETRATING PEPTIDES

Sources and sequences

CPPS TEND TO HAVE :
- HYDROPHOBIC SEQUENCE
- CATIONIC SEQUENCE

CATIONIC: HIS (H) LYS (K) ARGININE (R)
HYDROPHOBIC: ALA (A) VAL (V) TRP (W) ...

Table removed due to copyright restrictions.
Please see: Table 1 in Joliot, A., and A. Prochiantz. "Transduction Peptides: from Technology to Physiology." *Nat Cell Biol* 6 (2004): 189-96.

DIRECT ENTRY TO CYTOSOL:
MEMBRANE-PENETRATING PEPTIDES

Models of membrane-penetrating peptide function

Penetratin:
Short peptide sequence from drosophila transcription factor protein Antennapedia

RQIKIWFQNRRMKWKK

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Please see: Figure 7 in Derossi, D., et al.
"Cell Internalization of the Third Helix of the Antennapeadia Homeodomain is Receptor-Independent." *J Biol Chem* 271 (1996): 18188-93.

DIRECT ENTRY TO CYTOSOL: MEMBRANE-PENETRATING PEPTIDES

Uptake of penetratin by primary neuronal cells:

Images removed due to copyright restrictions.
Please see: Derossi, D., et al.
"Cell Internalization of the Third Helix of the Antennapeadia Homeodomain is Receptor-Independent." *J Biol Chem* 271 (1996): 18188-93.

CPP function in vitro

CAVEAT 1:
TYPICALLY CAN DELIVER PEPTIDES UP TO 100 aa IN LENGTH → ~60 KDa
REPORTED FOR PENETRATIN

CAVEAT 2:
SOME QUESTION ABOUT "UNIVERSALITY" – WORK IN ALL CELLS?

Protein delivery using HIV tat peptide:

Images and graph removed due to copyright restrictions.
Please see: Schwarze, S. R., et al. "Vivo Protein Transduction: Delivery of a Biologically Active Protein into the Mouse." In *Science* 285 (1999): 1569-72.

DIRECT ENTRY TO CYTOSOL:
MEMBRANE-PENETRATING PEPTIDES

CPP function in vivo

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Please see: Schwarze, S. R., et al. "In Vivo Protein Transduction: Delivery of a Biologically Active Protein into the Mouse." *Science* 285 (1999): 1569-72.

DIRECT ENTRY TO CYTOSOL:
MEMBRANE-PENETRATING PEPTIDES

ACTIVATION ON ENTRY TO THE CYTOSOL
Selective bond dissociation using reversible disulfide linkages

Figure removed due to copyright restrictions.
Please see: Falnes, P. O., and K. Sandvig. "Penetration of Protein Toxins into Cells." *Curr Opin Cell Biol* 12 (2000): 407-13.

DIRECT ENTRY TO CYTOSOL:
PORE-FORMING PEPTIDES

Pore-forming proteins/peptides as a tool for membrane-penetrating drug carriers

Figure removed due to copyright restrictions.
Please see: Figure 1 in Bhakdi, S., et al.
"Staphylococcal Alpha-Toxin, Streptolysin-O and Escherichia Coli Hemolysin: Prototypes of Pore-Forming Bacterial Cytlysins."
Arch Microbiol 165: 73-9.

DIRECT ENTRY TO CYTOSOL:
FUSOGENIC PEPTIDES

fusogenic peptides: using viral entry strategies for drug delivery

Images removed due to copyright restrictions.
Please see: Hawiger, J. "Noninvasive Intracellular Delivery of Functional Peptides and Proteins." *Curr Opin Chem Biol* 3 (1999): 89-94.

Further Reading

1. Moghimi, S. M., Hunter, A. C. & Murray, J. C. Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol Rev* **53**, 283-318 (2001).
2. Hawiger, J. Noninvasive intracellular delivery of functional peptides and proteins. *Curr Opin Chem Biol* **3**, 89-94 (1999).
3. Derossi, D. et al. Cell internalization of the third helix of the Antennapedia homeodomain is receptor-independent. *J Biol Chem* **271**, 18188-93 (1996).
4. Falnes, P. O. & Sandvig, K. Penetration of protein toxins into cells. *Curr Opin Cell Biol* **12**, 407-13 (2000).
5. Joliot, A. & Prochiantz, A. Transduction peptides: from technology to physiology. *Nat Cell Biol* **6**, 189-96 (2004).
6. Schwarze, S. R., Ho, A., Vocero-Akbani, A. & Dowdy, S. F. In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science* **285**, 1569-72 (1999).
7. Snyder, E. L. & Dowdy, S. F. Cell penetrating peptides in drug delivery. *Pharm Res* **21**, 389-93 (2004).
8. Thoren, P. E. et al. Membrane binding and translocation of cell-penetrating peptides. *Biochemistry* **43**, 3471-89 (2004).
9. Asokan, A. & Cho, M. J. Exploitation of intracellular pH gradients in the cellular delivery of macromolecules. *J Pharm Sci* **91**, 903-13 (2002).
10. Sandgren, S., Cheng, F. & Belting, M. Nuclear targeting of macromolecular polyanions by an HIV-Tat derived peptide. Role for cell-surface proteoglycans. *J Biol Chem* **277**, 38877-83 (2002).
11. Yatvin, M. B., Kreutz, W., Horwitz, B. A. & Shinitzky, M. Ph-Sensitive Liposomes - Possible Clinical Implications. *Science* **210**, 1253-1254 (1980).
12. Lee, K. D., Oh, Y. K., Portnoy, D. A. & Swanson, J. A. Delivery of macromolecules into cytosol using liposomes containing hemolysin from Listeria monocytogenes. *J Biol Chem* **271**, 7249-52 (1996).
13. Bhakdi, S. et al. Staphylococcal alpha-toxin, streptolysin-O, and Escherichia coli hemolysin: prototypes of pore-forming bacterial cytolysins. *Arch Microbiol* **165**, 73-9 (1996).
14. Raychaudhuri, S. & Rock, K. L. Fully mobilizing host defense: building better vaccines. *Nat Biotechnol* **16**, 1025-31 (1998).
15. Falo, L. D., Jr., Kovacsovics-Bankowski, M., Thompson, K. & Rock, K. L. Targeting antigen into the phagocytic pathway in vivo induces protective tumour immunity. *Nat Med* **1**, 649-53 (1995).
16. Murthy, N., Campbell, J., Fausto, N., Hoffman, A. S. & Stayton, P. S. Bioinspired pH-Responsive Polymers for the Intracellular Delivery of Biomolecular Drugs. *Bioconjug Chem* **14**, 412-9 (2003).
17. Shi, G., Guo, W., Stephenson, S. M. & Lee, R. J. Efficient intracellular drug and gene delivery using folate receptor-targeted pH-sensitive liposomes composed of cationic/anionic lipid combinations. *J Control Release* **80**, 309-19 (2002).