# DRUG TARGETING
## Getting Vaccines to Dendritic Cells

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**Last Time:** DNA vaccination

**Today:** Targeting particles/molecules to cells
Delivering activation signals to dendritic cells in vaccines

**Reading:** P. Carter, 'Improving the efficacy of antibody-based cancer therapies,' *Nat. Rev. Cancer* **1** 118 (2001)

**Supplementary Reading**:

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**ANNOUNCEMENTS:** REMINDER — TAKE HOME EXAMS DUE THURSDAY → 5 pm (8-425)

# What is drug targeting?

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Please see: Wickham. *Nature Medicine.* 9, no. 1 (2003): 135.

(Wickham, 2003)

# Motivation for drug targeting: General

- MANY DRUGS ARE TOXIC SYSTEMICALLY
  - → NONSPECIFIC RADIO / CHEMOTHERAPEUTIC DRUGS
    - TOP 6 CHEMOTHERAPEUTICS : NONSPECIFICALLY KILL PROLIFERATING CELLS
  - → PROTEIN DRUGS OFTEN PLEIOTROPIC EFFECTS
    - ↓
    - CAN ACT ON MANY CELL TYPES

IN THE SETTING OF CANCER THERAPY:

... THUS LOWER DOSES USED

... TUMOR HAS TIME TO MUTATE

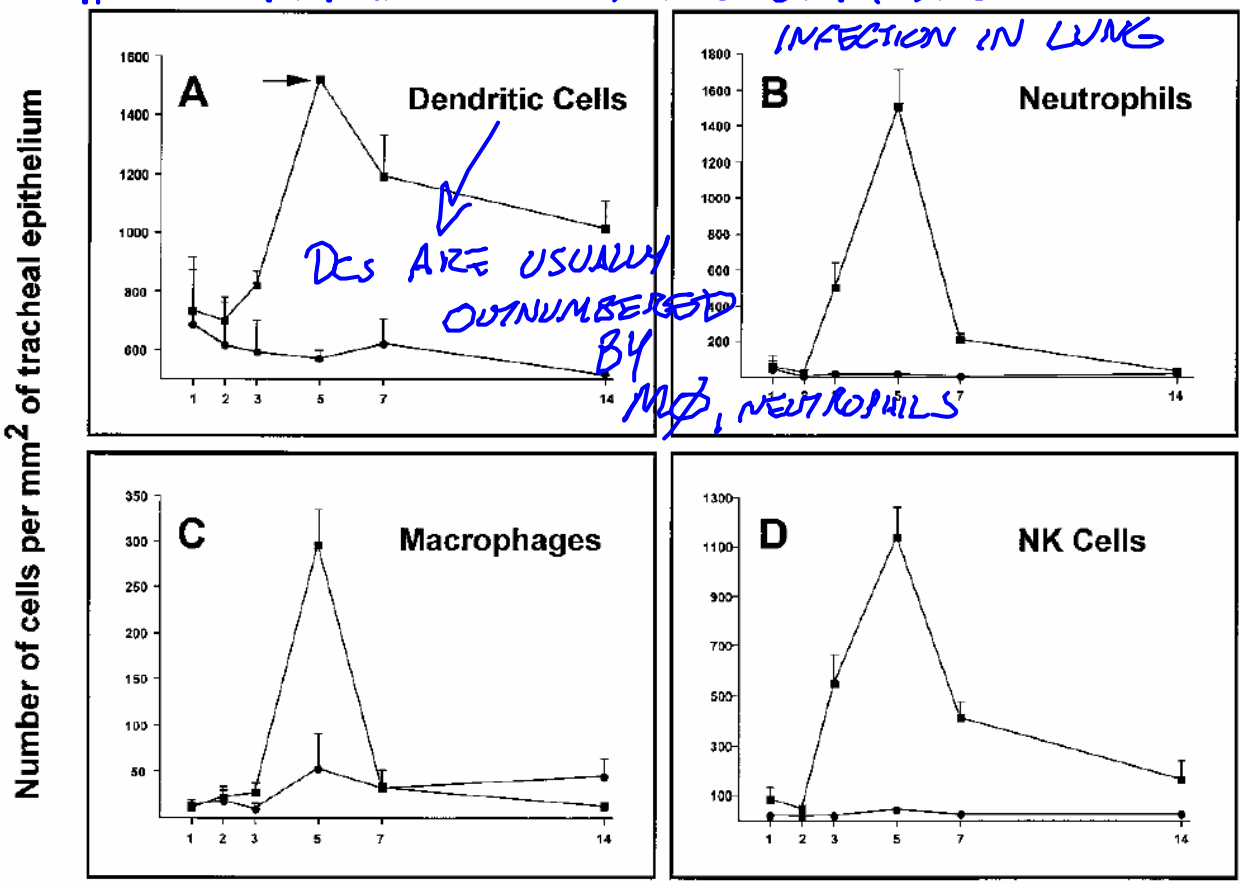
... DEVELOPMENT OF DRUG-RESISTANT TUMORS

# Motivation for drug targeting: Vaccines

DENDRITIC CELLS ARE ONLY CELL KNOWN TO ACTIVATE NAIVE T CELLS IN VIVO

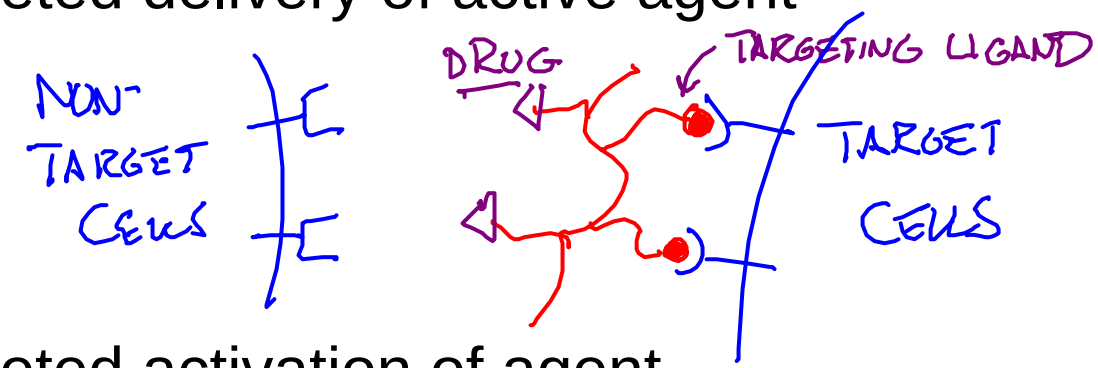
ALL PHAGOCYTES!


INFLAMMATORY INFILTRATE DURING SENDAI VIRUS INFECTION IN LUNG

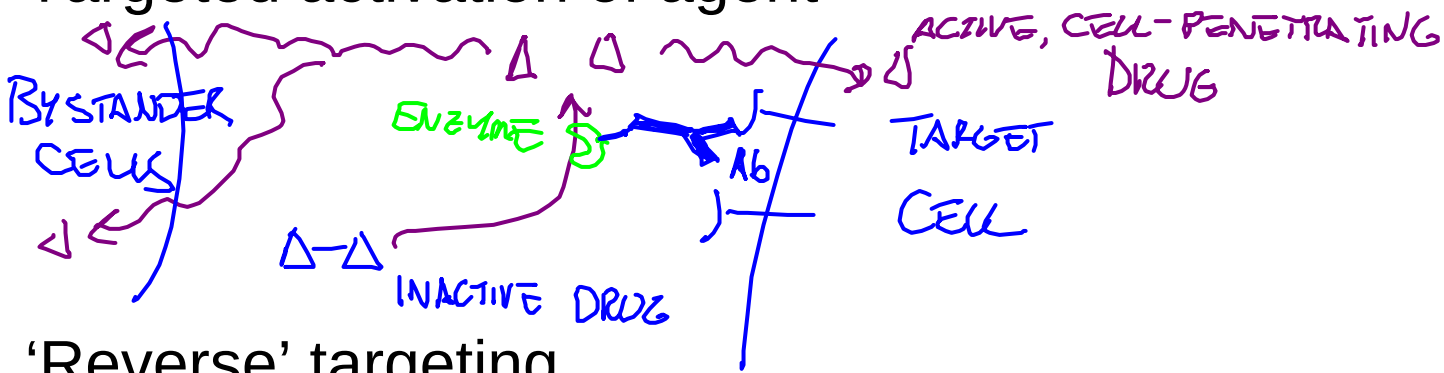


# Approaches to targeted drug activity

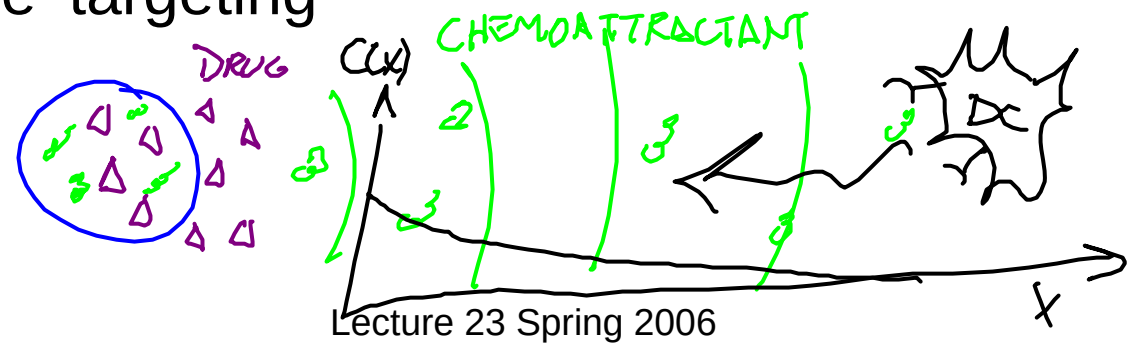
1) Targeted delivery of active agent




2) Targeted activation of agent

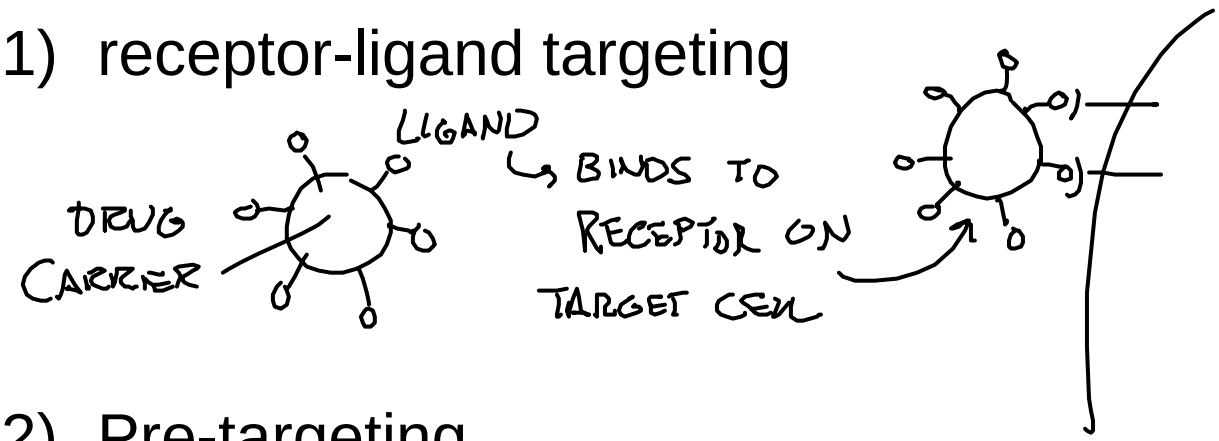



3) 'Reverse' targeting

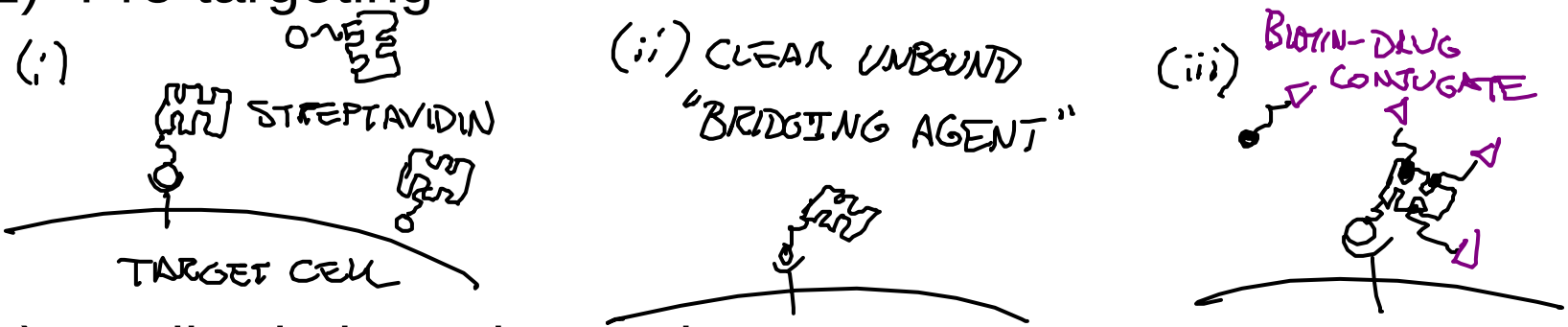


# Major approaches for targeted delivery

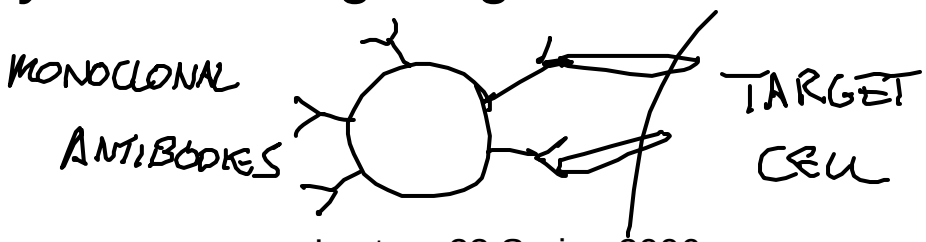
1) receptor-ligand targeting




2) Pre-targeting




3) Antibody-based targeting



# Example approaches: receptor-ligand-mediated targeting to vasculature

**Mimicking lymphocyte responses to inflammation:**

(Hogg et al. *J. Cell Sci.* **116** 4695-4705 (2003))

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Please see: Figure 1 in Hogg et al. *J. Cell Sci.* 116 (2003): 4695-4705.

# Example approaches: receptor-ligand-mediated targeting to vasculature

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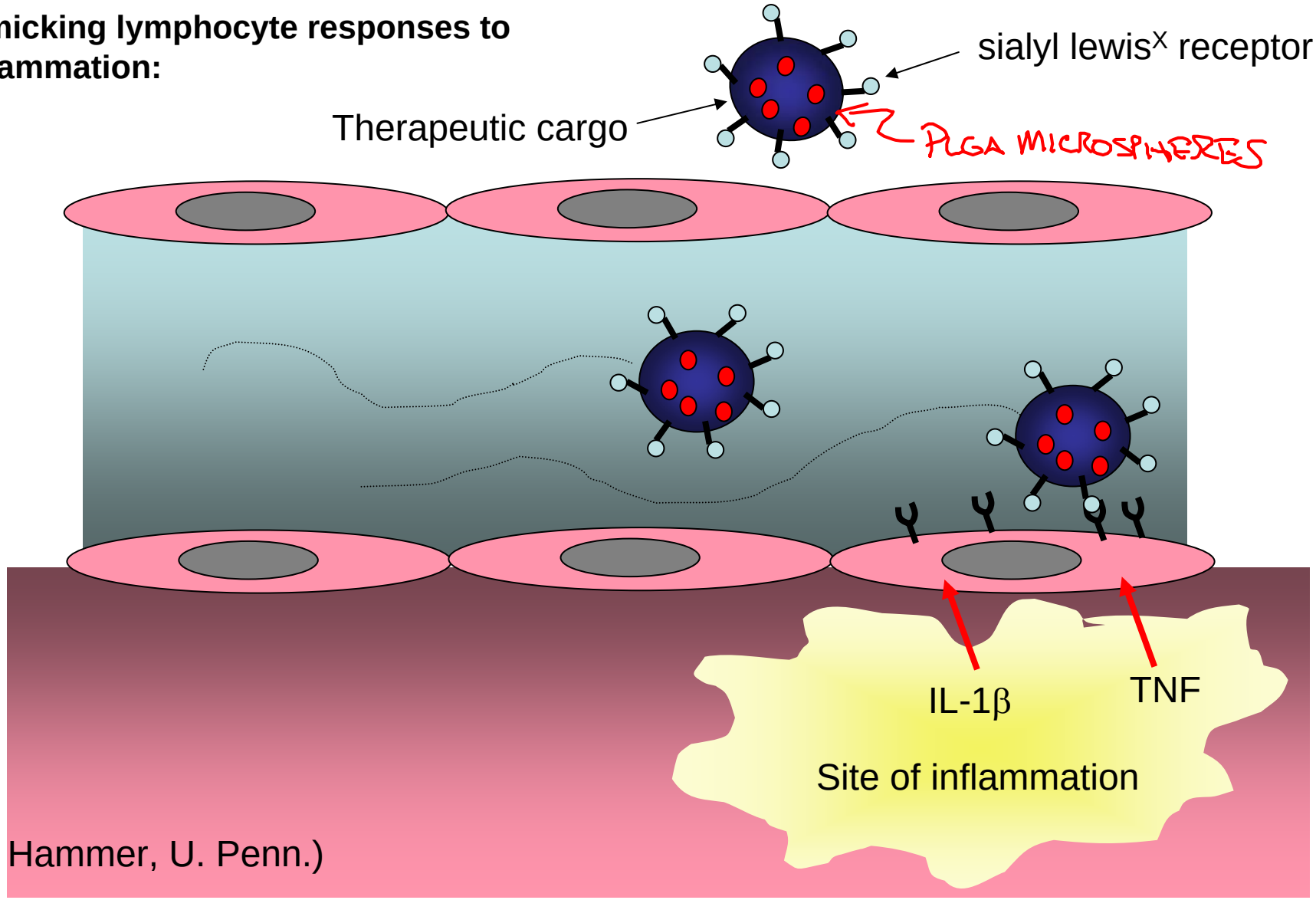
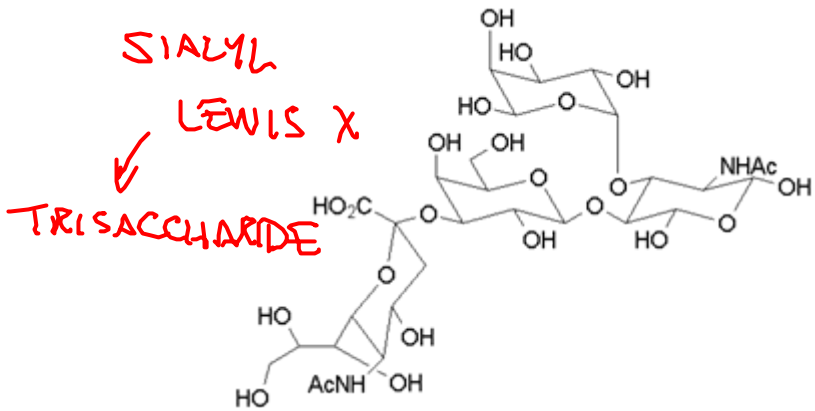



(D. Hammer, U. Penn.)

# Example approaches: receptor-ligand-mediated targeting to vasculature

**Mimicking lymphocyte responses to inflammation:**



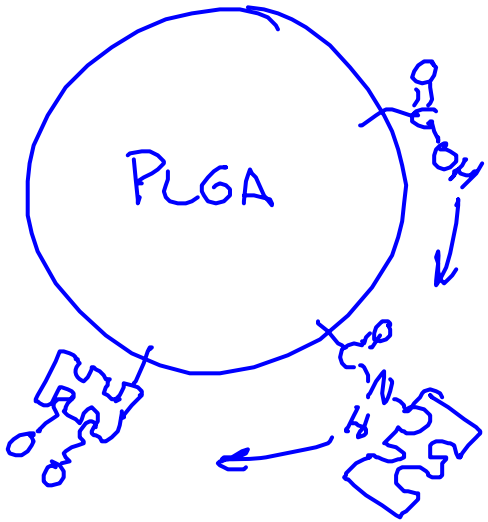




Figure removed due to copyright restrictions. Please see: Figure 2 in Cao, Y and L. Lam. "Bispecific Anitbody Conjugates in Therapeutics." *Adv Drug Delib Rev.* 55 (2003): 171-97.

(D. Hammer, U. Penn.)

# Pre-targeting drug delivery with bispecific antibodies

Figure removed due to copyright restrictions.
Please see: Figure 2 in Eniola, A. O. and Hammer D. A. *Biomaterials.* 26 (2005): 661.

(Cao and Lam, 2003)

# Antibody-based targeting

**General structure of IgA, IgE, IgD, IgG:**

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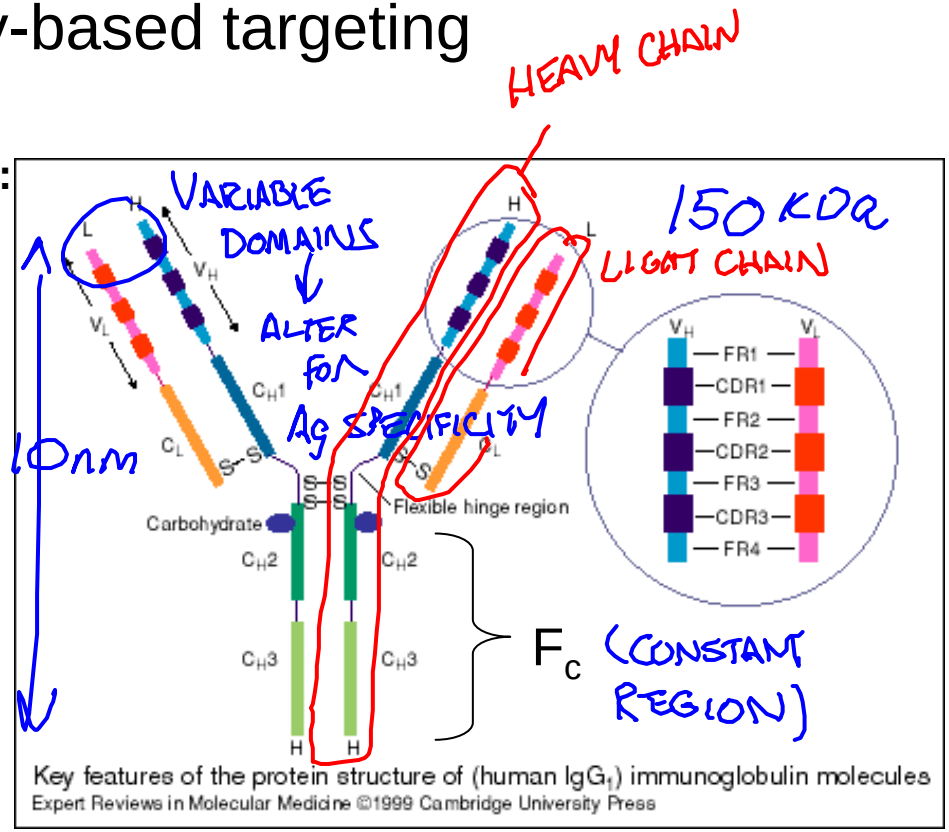
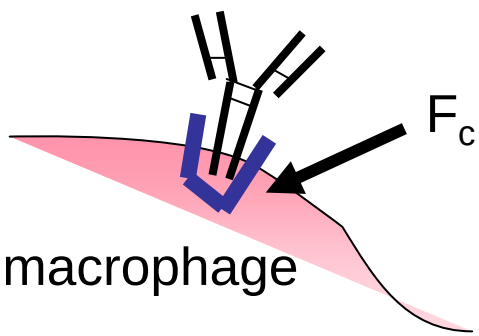
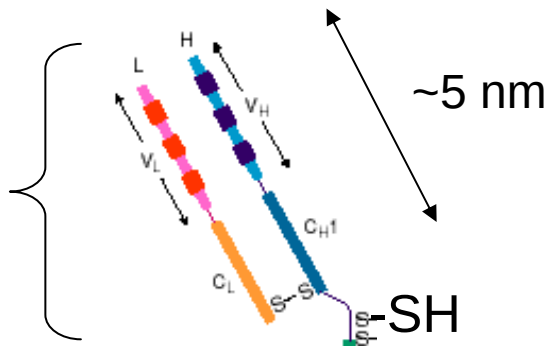



FAb/FAb'



# Generation of monoclonal antibodies against selected molecular targets

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Please see: Figures 4-12 in Elgert, K. D. *Immunology: Understanding the Immune System*. New York: Wiley-Liss, 1996.

(Elgert 1996)

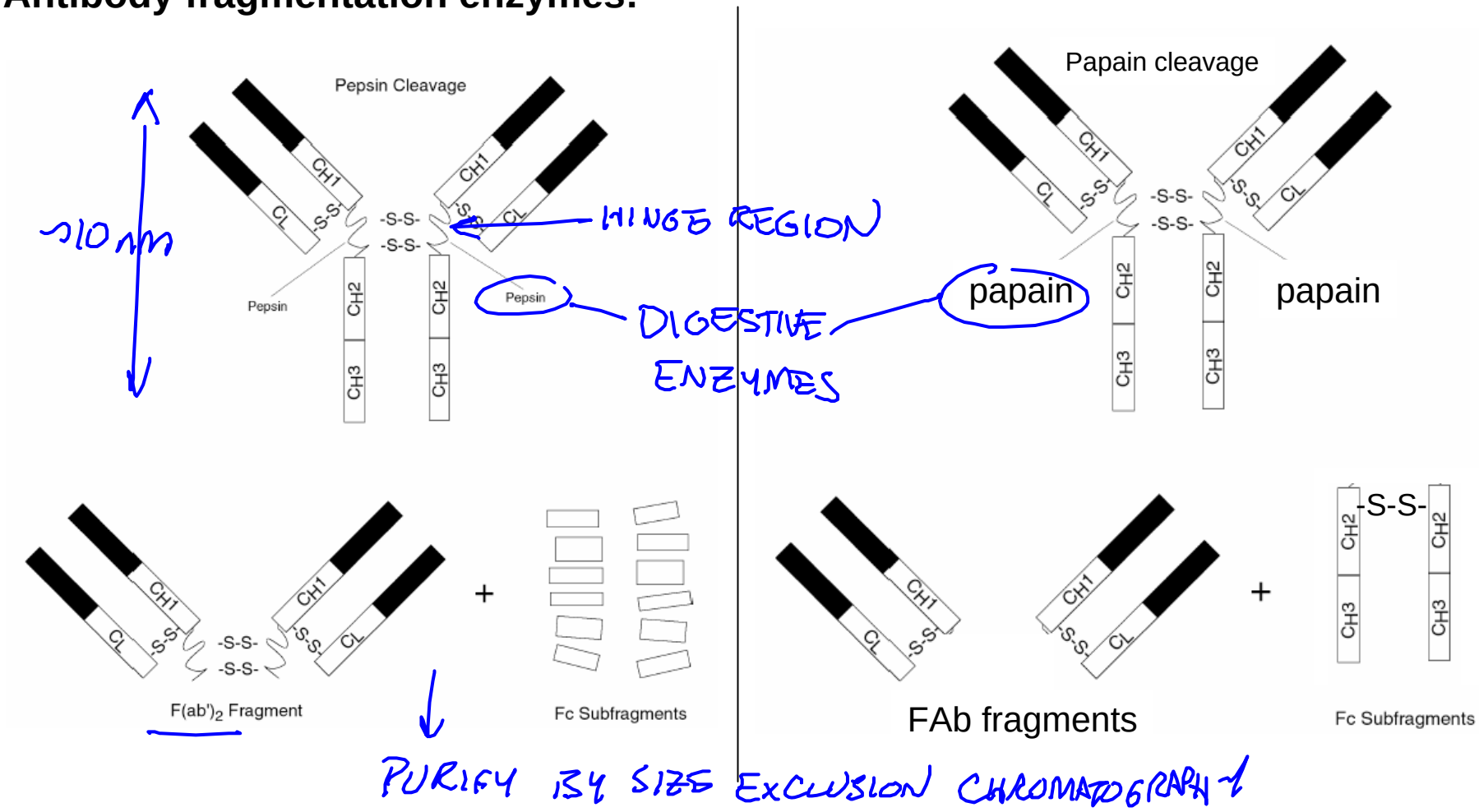
# Synthesizing antibodies which avoid recognition by the immune system

Figure removed due to copyright restrictions.
Please see: Figures 4-12 in Elgert, K. D. *Immunology: Understanding the Immune System*. New York: Wiley-Liss, 1996.

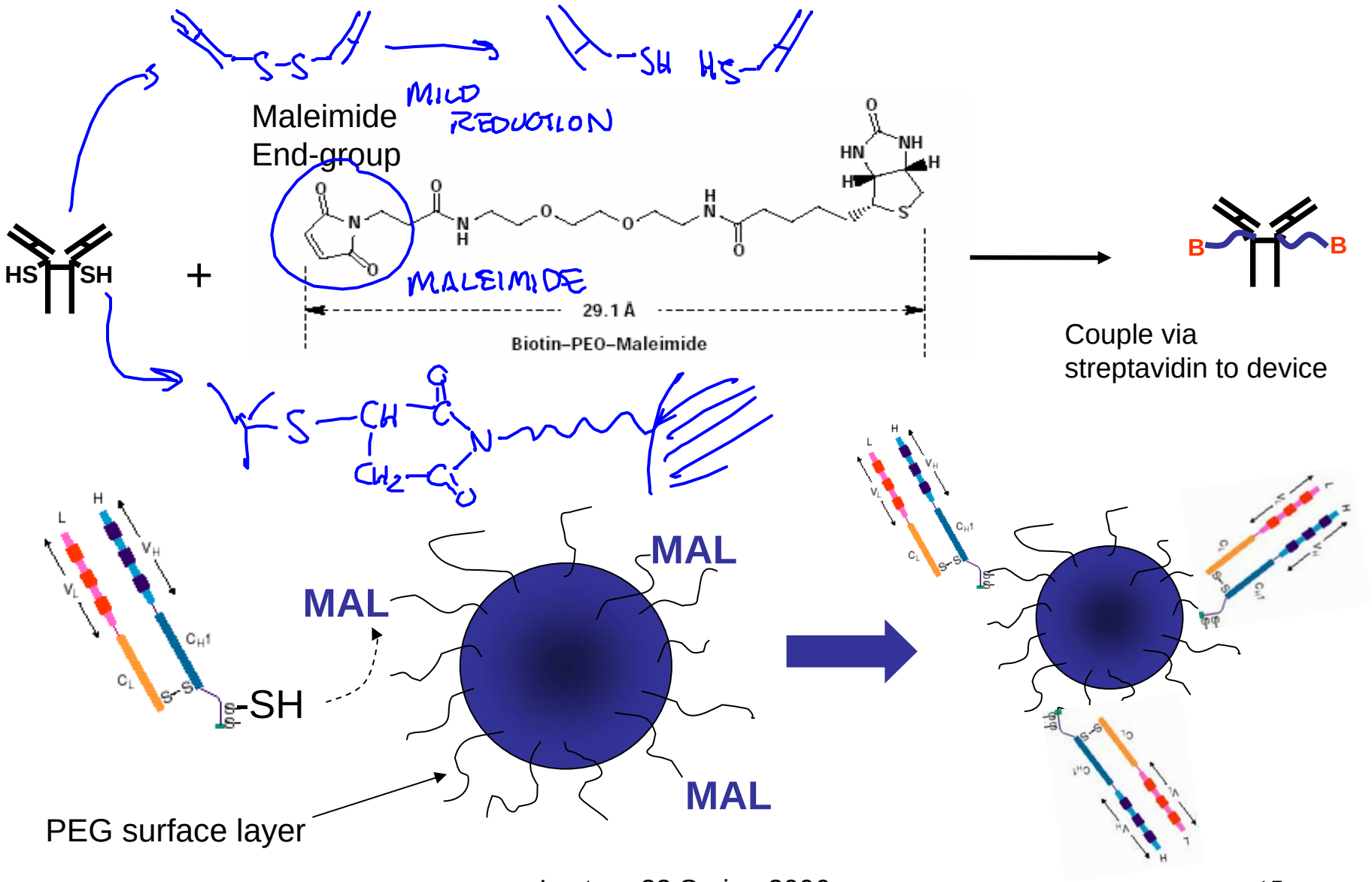
(Allen 2002)

# Strategies for conjugation of antibodies to biomaterials

**Antibody fragmentation enzymes:**



# Strategies for conjugation of antibodies to biomaterials



# Results from mAb-targeting

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Please see: Figure 4 in Daan, J. A. et al. "N Anotechnological Approaches for the Delivery of Macromolecules." *J. Controlled Release.* 87, 81 (2003).

Graph removed due to copyright restrictions.
Please see: Park, J. W. et al. "Anti-HER2 Immunoliposomes: Enhanced Efficacy Attributable to Targeted Delivery." *Clin Cancer Res.* 8 (2002): 1172-81.

(Park et al. 2002)

(Crommelin et al. 2003)

Fig. 4. Immunoliposomes binding to the surface of an ovarian carcinoma cell. This electron micrograph depicts a human OVCAR-3 cell taken from the peritoneal cavity of nu/nu mice after injecting the animals intraperitoneally with OV-TL3-Fab'-immunoliposomes. A more detailed analysis of the cell-immunoliposome interaction showed very little endocytic uptake. A search was started to identify endocytosis inducing antibodies. mAB with human ovarian cancer cell specificity were identified (e.g., mAB 425). These 425 immunoliposomes loaded with DTA and a pH-dependent fusogen (diINF-7) were tested in vitro [15,17].

| Application | Cellular target | Molecular target | Targeting ligand | Ligand type |
|---|---|---|---|---|
| Anti-cancer therapy | Various tumor cells | Folate receptor<br>EGF receptor | Folate<br>EGF | Protein ligand for target receptor preferentially expressed on target cells |
| | Neovascular tissue | B-FN (fibronectin isoform) | anti-B-FN antibody | antibody against fibronectin isoform only expressed during embryonic development and in aggressive tumors |
| Anti-cancer therapy, pulmonary, cardiovascular, and inflammatory diseases | Endothelial cells | E-selectin<br>P-selectin | sialyl Lewis$^X$ receptor | receptor expressed at sites of inflammation |
| Anti-cancer therapy (leukemias and B cell lymphomas) | Transformed B lymphocytes | CD20 | Anti-CD20 antibody | Antibody against target cell-surface protein unique to target class of cells (e.g. B cells) |
| Anti-cancer therapy (T cell lymphomas) | Transformed T lymphocytes | IL-2Rα (Interleukin-2 receptor a chain | Anti-IL-2Rα antibody | Antibody against target cell-surface protein not expressed on normal resting cells |

Cytotoxic drugs
- AraC
- Doxorubicin

OVEREXPRESSED BY 95% OF OVARIAN CARCINOMAS

Anti-tumor cytokines
- Interleukin-2
- Interleukin-12

LOSS OF HEALTHY B CELLS OK: BONE MARROW TRANSPLANT

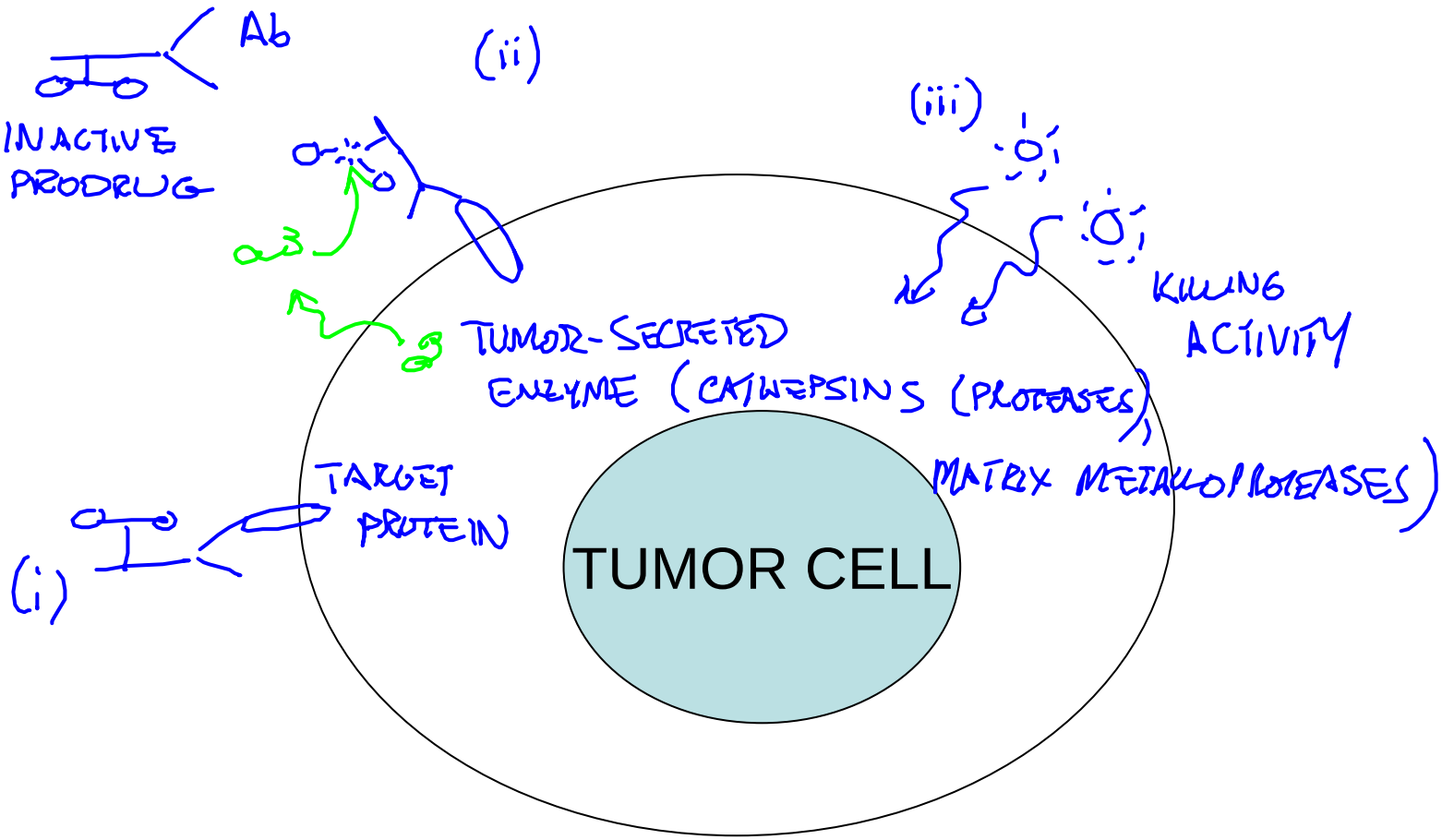
ONLY KILL ACTIVATED T CELLS ... ACCEPTABLE SIDE EFFECT

"LOOK" LIKE ACTIVATED T CELLS

Table removed due to copyright restrictions.
Please see: Table 1 in Allen, T. M. “Ligand Targeted Therapeutics in Anticancer Therapy.” *Nat Rev Cancer* 2 750-63 (2002).

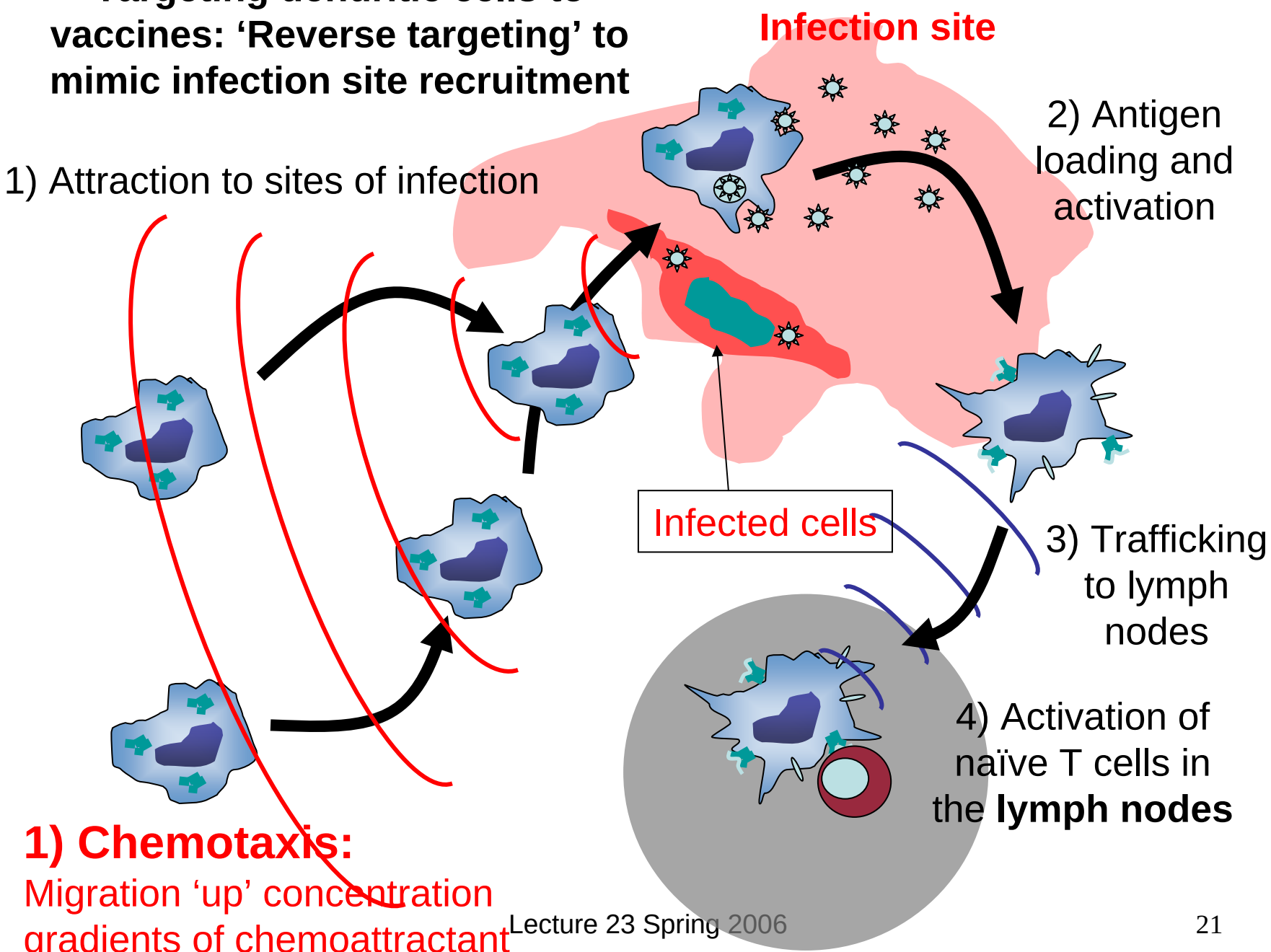
# Example approaches: targeted activation of active agent

**Antibody-directed enzyme prodrug therapy (ADEPT):**



**‘Reverse targeting’**
Bringing cells to the drug

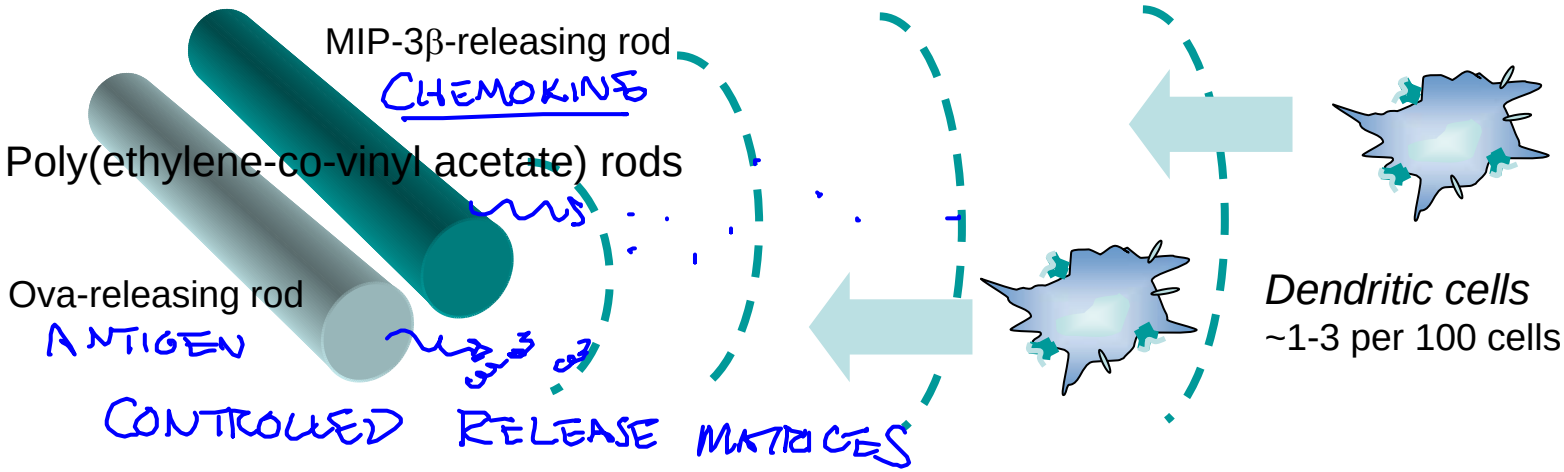
# Targeting dendritic cells to vaccines: 'Reverse targeting' to mimic infection site recruitment




**1) Chemotaxis:**
Migration 'up' concentration gradients of chemoattractant

# Targeting dendritic cells to vaccines

**Attraction of target cells to device via chemotaxis:**




Advantages relative to bolus chemoattractant injection:

(1) CHEMOKINES CLEAR IN LESS < 24 HRS IN VIVO (IN TISSUE)

(2) ENGINEER CONCENTRATION GRADIENT TO OPTIMIZE ATTRACTION

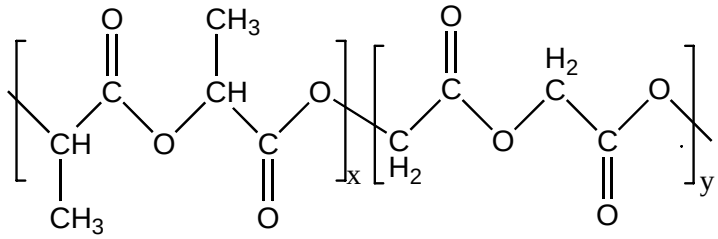
 (Kumamoto et al. 2002) 

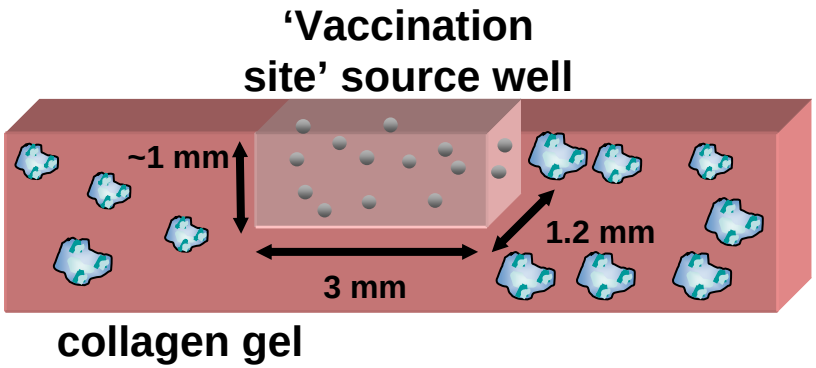
Images removed due to copyright restrictions.
Please see: Kumamotos, T. et al. ”Induction of Tumor-specific Protective Immunity by in Situ Langerhans Cell Vaccine. *Nat Biotechnol* 20 (2002): 64-9.

Tissue sections stained for MHC class II (expressed by antigen-presenting cells)

(Kumamoto et al. 2002)

**PLGA**

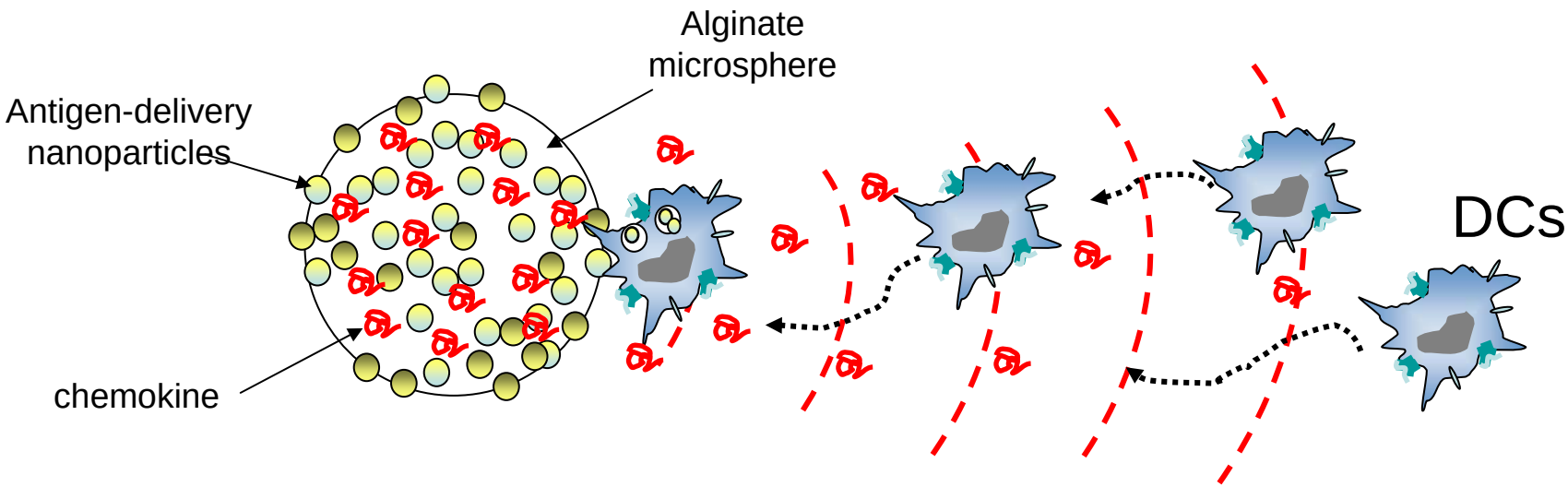



Images removed due to copyright restrictions.
Please see: X. Zhao et al. *Biomaterials* 26 (2005): 5048.

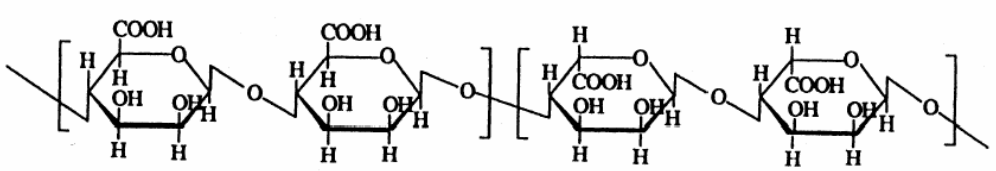
(X. Zhao et al., *Biomaterials* 2005)

# Dendritic cell attraction, antigen loading, and activation



How to encapsulate multiple factors under mild conditions for ‘reverse targeting’?

**alginate**



mannuronic acid block  guluronic acid block

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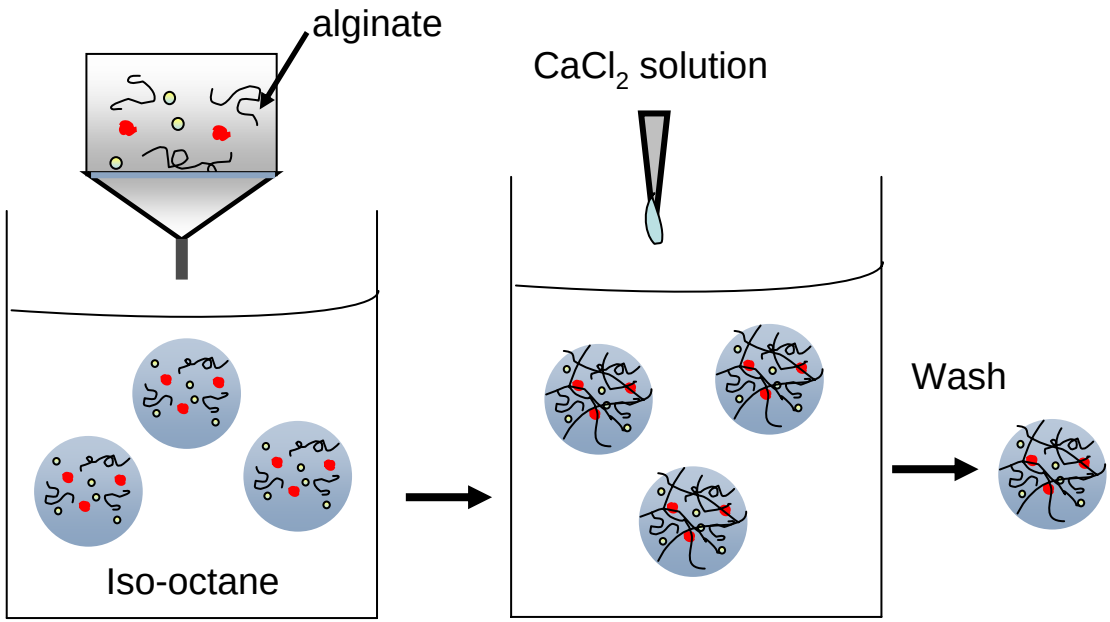
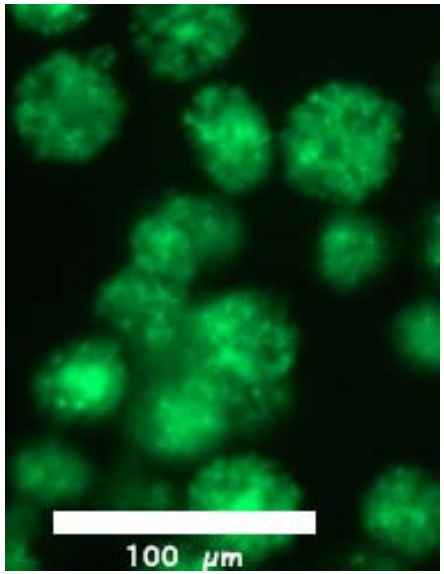
Please see: http://www.lsbu.ac.uk/water/hyalg.html

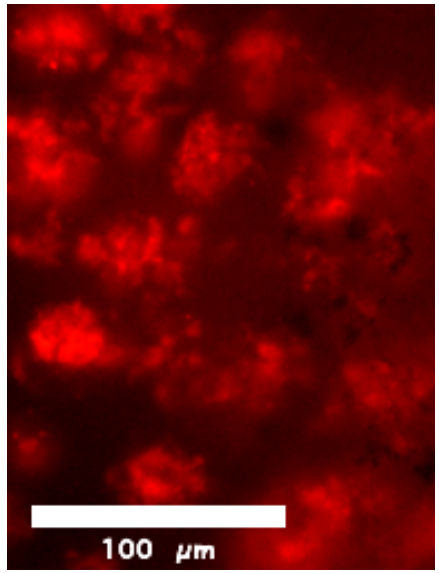
*

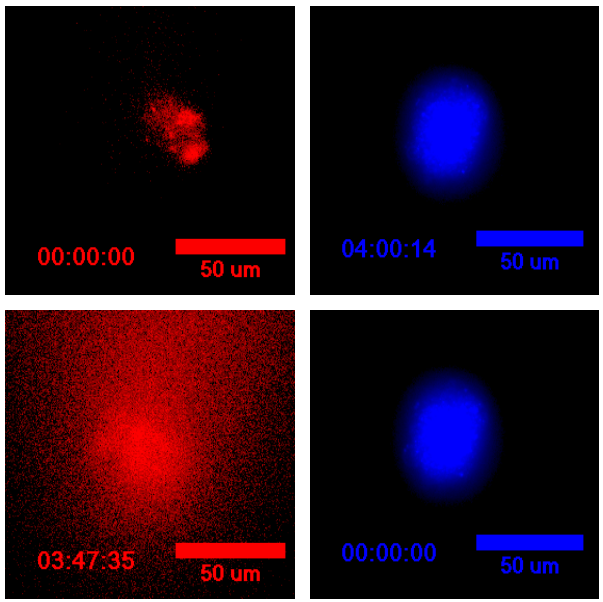


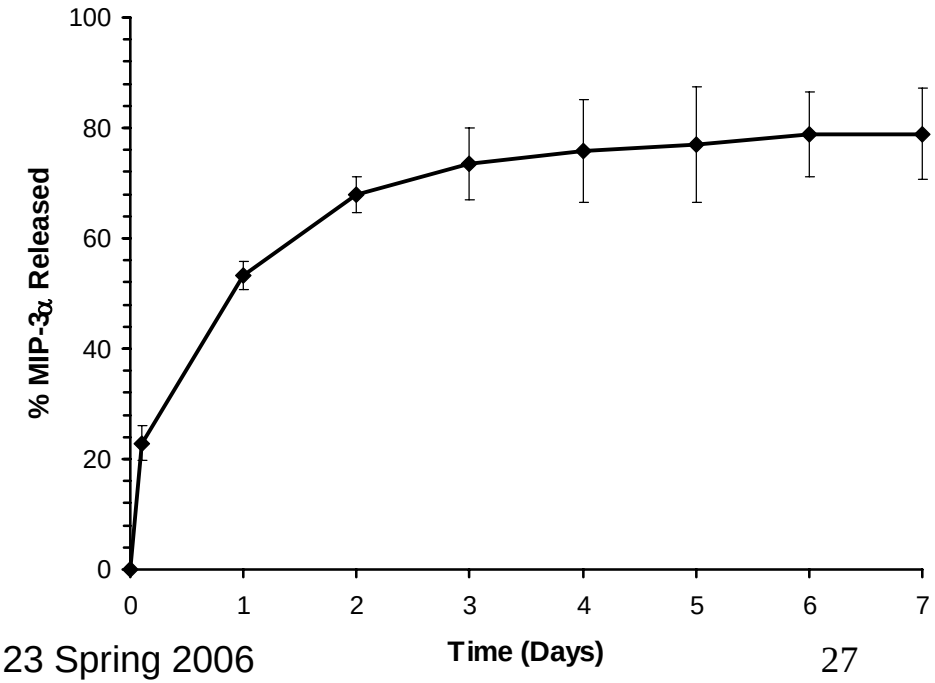
Fluorescent nanoparticles

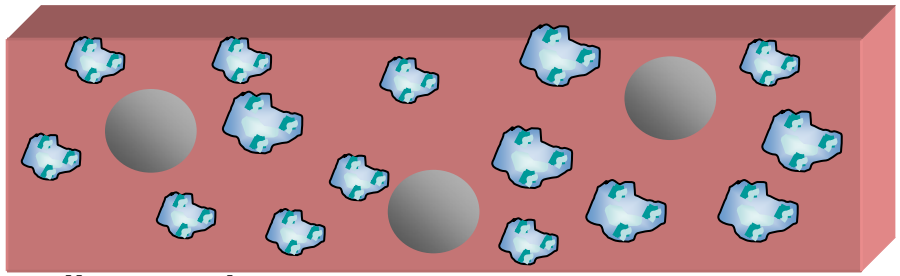


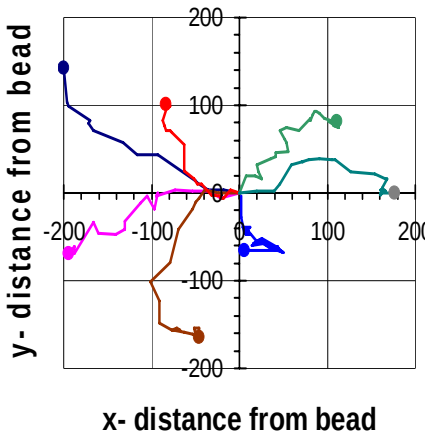

Fluorescent chemokine




collagen gel


y- distance from bead
x- distance from bead
200
100
0
-100
-200
-200
-100
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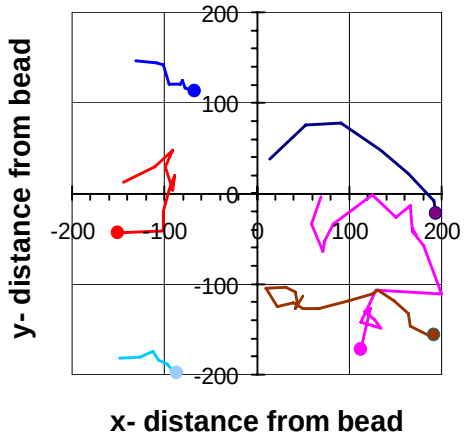
100
200


y- distance from bead
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200
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0
100
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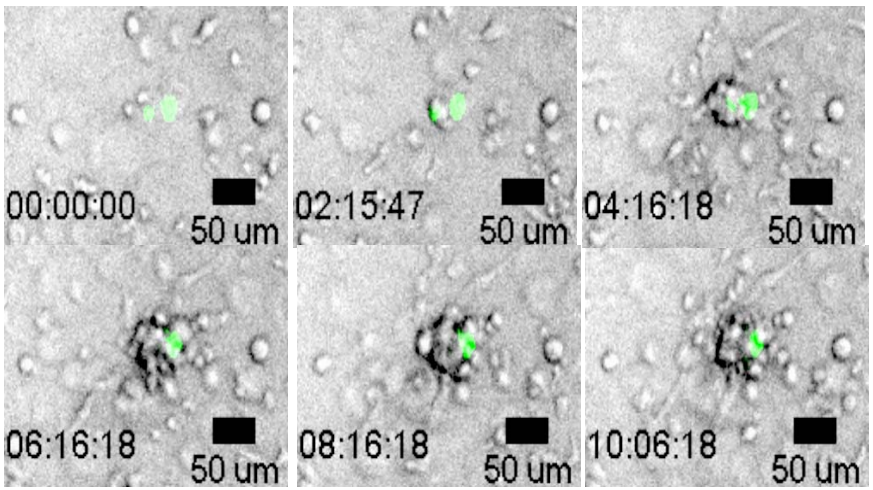
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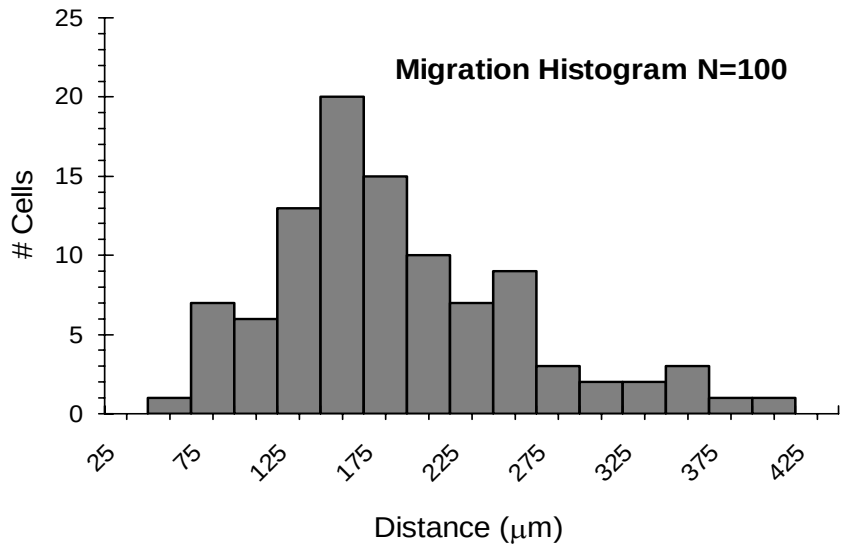
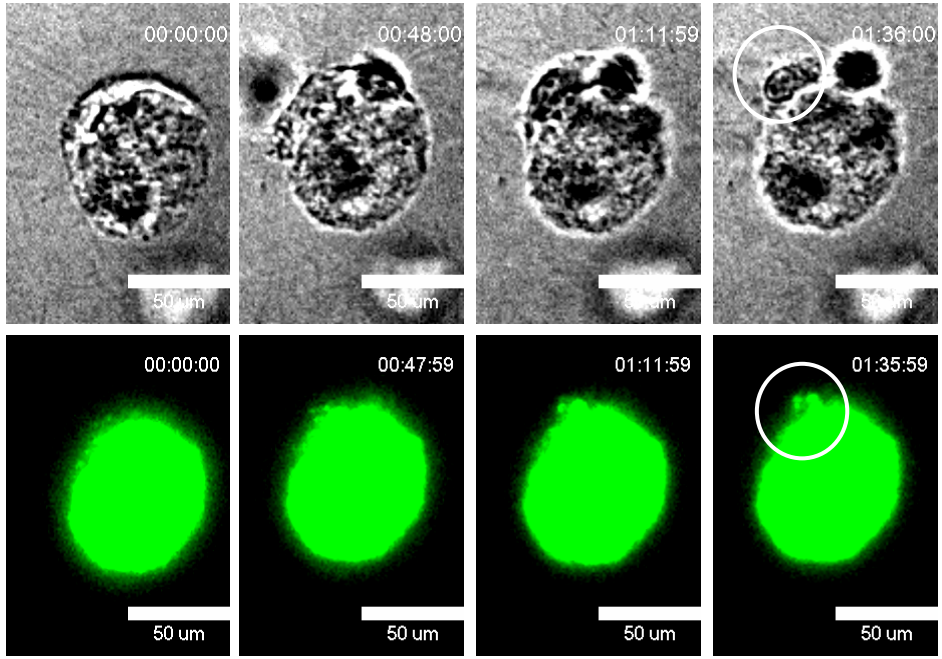
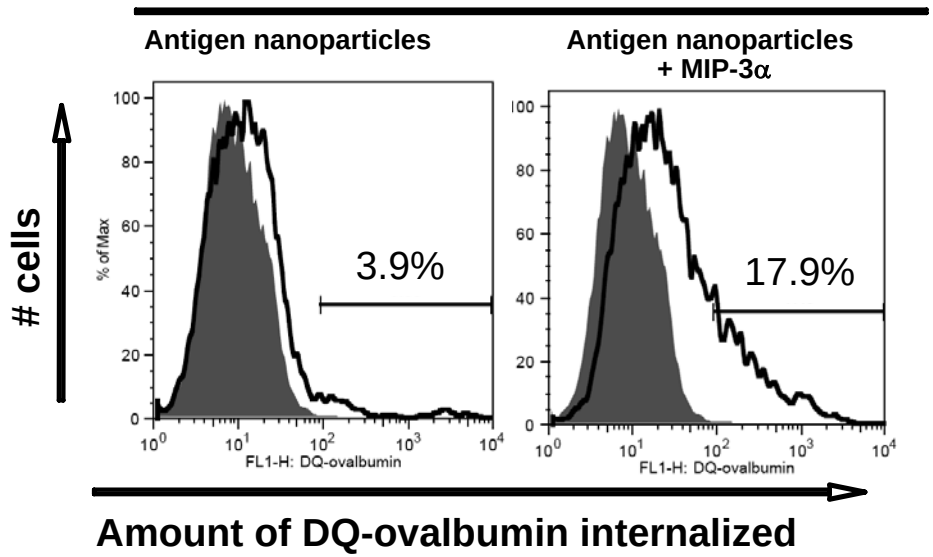

Migration Histogram N=100
# Cells
Distance (μm)
25
20
15
10
5
0
25
75
125
175
225
275
325
375
425




Alginate microspheres loaded with:



# Issues in targeted delivery

# Further Reading


1. Stayton, P. S. et al. Molecular engineering of proteins and polymers for targeting and intracellular delivery of therapeutics. *J Control Release* **65**, 203-20 (2000).
2. Eniola, A. O. & Hammer, D. A. Artificial polymeric cells for targeted drug delivery. *J Control Release* **87**, 15-22 (2003).
3. Halin, C. et al. Enhancement of the antitumor activity of interleukin-12 by targeted delivery to neovasculature. *Nat Biotechnol* **20**, 264-9 (2002).
4. Pardridge, W. M. Drug and gene targeting to the brain with molecular Trojan horses. *Nat Rev Drug Discov* **1**, 131-9 (2002).
5. Wickham, T. J. Ligand-directed targeting of genes to the site of disease. *Nat Med* **9**, 135-9 (2003).
6. 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).
7. Sakhalkar, H. S. et al. Leukocyte-inspired biodegradable particles that selectively and avidly adhere to inflamed endothelium in vitro and in vivo. *Proc Natl Acad Sci U S A* **100**, 15895-900 (2003).
8. Allen, T. M. Ligand-targeted therapeutics in anticancer therapy. *Nat Rev Cancer* **2**, 750-63 (2002).
9. Vingerhoeds, M. H. et al. Immunoliposome-mediated targeting of doxorubicin to human ovarian carcinoma in vitro and in vivo. *Br J Cancer* **74**, 1023-9 (1996).
10. Nassander, U. K. et al. In vivo targeting of OV-TL 3 immunoliposomes to ascitic ovarian carcinoma cells (OVCAR-3) in athymic nude mice. *Cancer Res* **52**, 646-53 (1992).
11. Crommelin, D. J. et al. Nanotechnological approaches for the delivery of macromolecules. *J Control Release* **87**, 81-8 (2003).
12. Elgert, K. D. *Immunology: Understanding the Immune System* (Wiley-Liss, New York, 1996).
13. Wittrup, K. D. Protein engineering by cell-surface display. *Curr Opin Biotechnol* **12**, 395-9 (2001).
14. Cao, Y. & Lam, L. Bispecific antibody conjugates in therapeutics. *Adv Drug Deliv Rev* **55**, 171-97 (2003).
15. Park, J. W. et al. Anti-HER2 immunoliposomes: enhanced efficacy attributable to targeted delivery. *Clin Cancer Res* **8**, 1172-81 (2002).
16. Hong, K. et al. Anti-HER2 immunoliposomes for targeted drug delivery. *Ann N Y Acad Sci* **886**, 293-6 (1999).
17. Kumamoto, T. et al. Induction of tumor-specific protective immunity by in situ Langerhans cell vaccine. *Nat Biotechnol* **20**, 64-9 (2002).

# Further Reading


1. Varga, C. M., Hong, K. & Lauffenburger, D. A. Quantitative analysis of synthetic gene delivery vector design properties. *Mol Ther* **4**, 438-46 (2001).
2. Varga, C. M., Wickham, T. J. & Lauffenburger, D. A. Receptor-mediated targeting of gene delivery vectors: insights from molecular mechanisms for improved vehicle design. *Biotechnol Bioeng* **70**, 593-605 (2000).
3. Segura, T. & Shea, L. D. Materials for non-viral gene delivery. *Annual Review of Materials Research* **31**, 25-46 (2001).
4. Segura, T. & Shea, L. D. Surface-tethered DNA complexes for enhanced gene delivery. *Bioconjugate Chemistry* **13**, 621-629 (2002).
5. Vijayanathan, V., Thomas, T. & Thomas, T. J. DNA nanoparticles and development of DNA delivery vehicles for gene therapy. *Biochemistry* **41**, 14085-94 (2002).
6. Demeneix, B. et al. Gene transfer with lipospermines and polyethylenimines. *Adv Drug Deliv Rev* **30**, 85-95 (1998).
7. Boussif, O. et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proc Natl Acad Sci U S A* **92**, 7297-301 (1995).
8. Zanta, M. A., Boussif, O., Adib, A. & Behr, J. P. In vitro gene delivery to hepatocytes with galactosylated polyethylenimine. *Bioconjug Chem* **8**, 839-44 (1997).
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