DRUG TARGETING

Getting Vaccines to Dendritic Cells

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:

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.

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

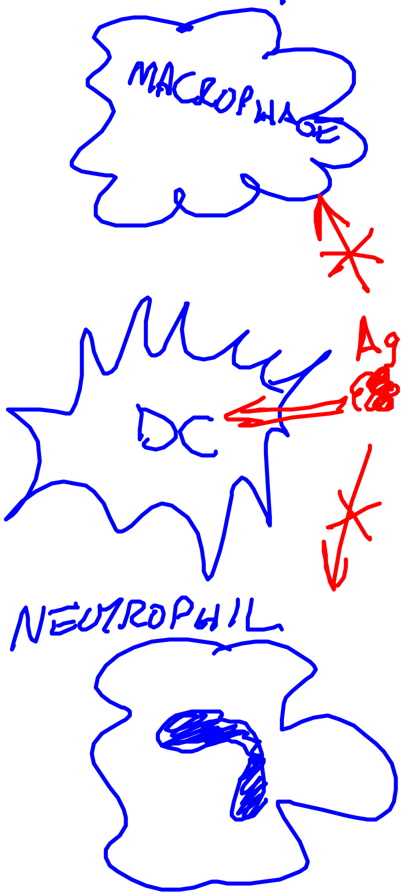
... TUMOR HAS TIME TO MUTATE

... 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!



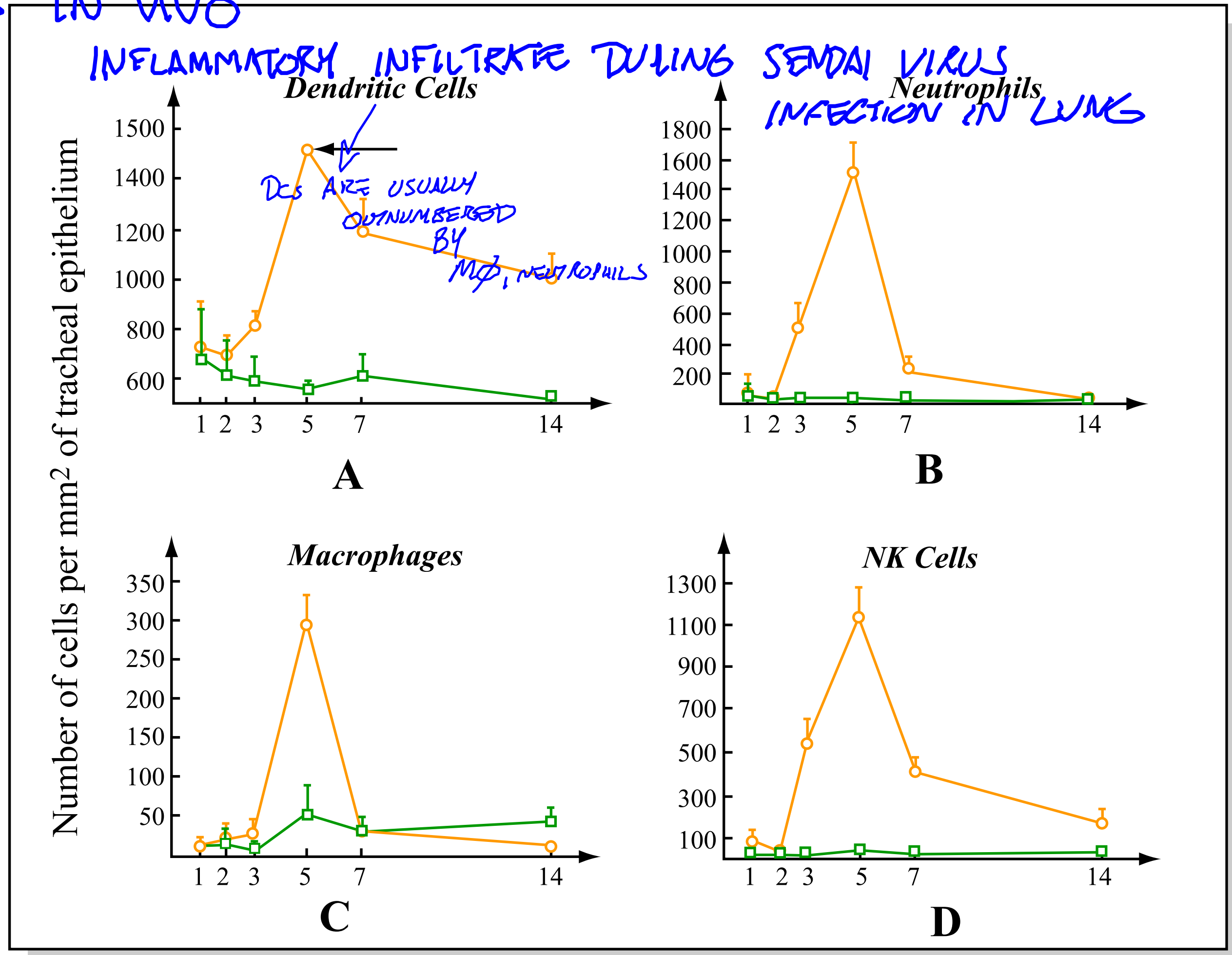
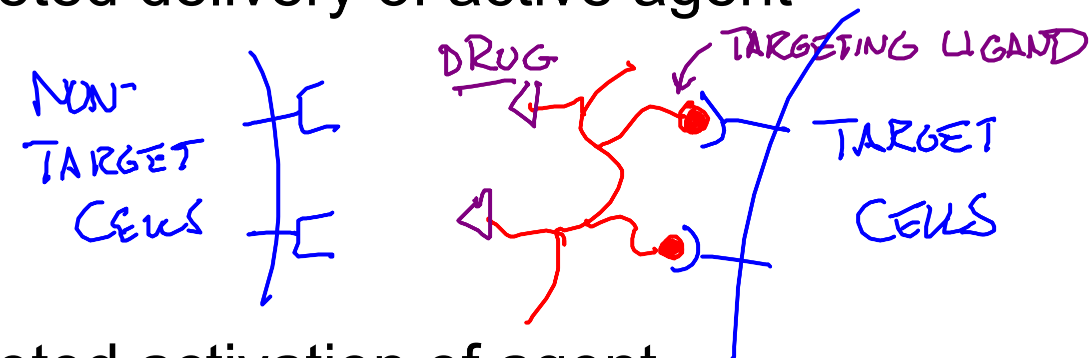


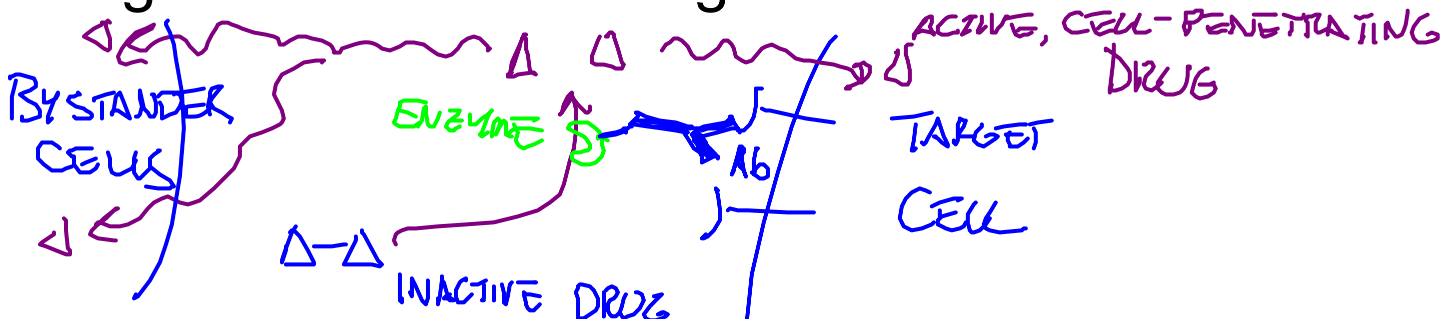
Figure by MIT OCW.

Approaches to targeted drug activity

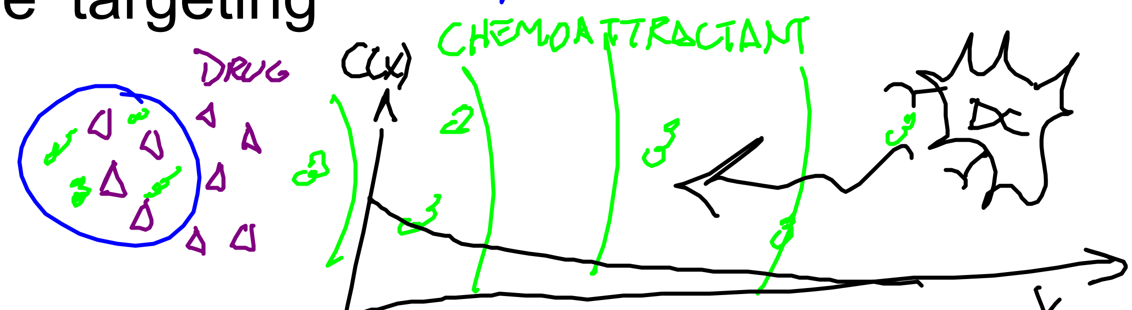
1) Targeted delivery of active agent



2) Targeted activation of agent

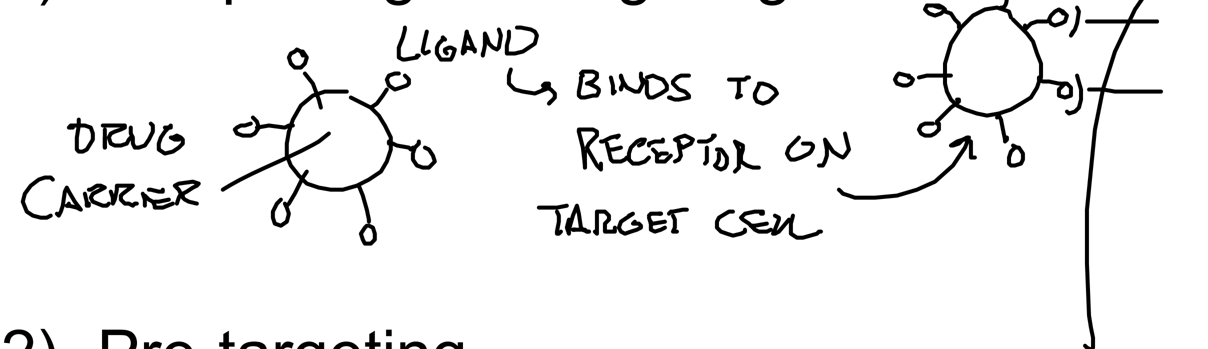


3) 'Reverse' targeting

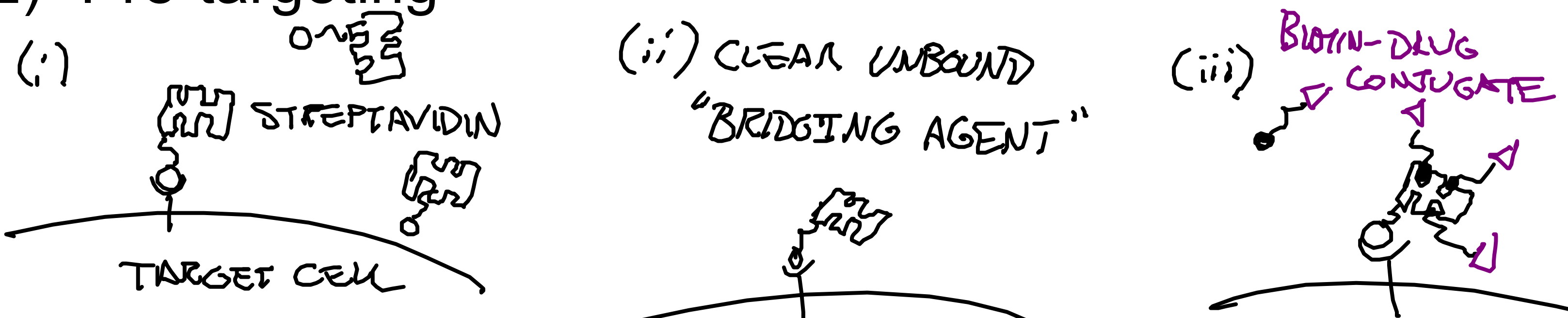


Major approaches for targeted delivery

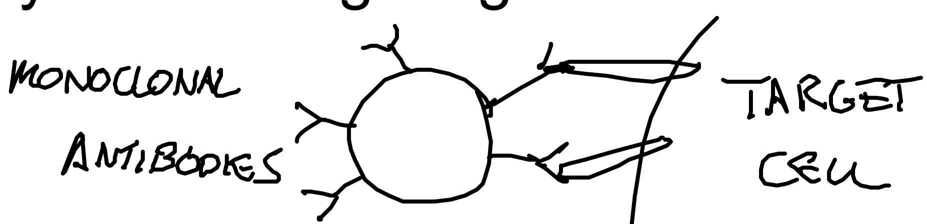
1) receptor-ligand targeting



2) Pre-targeting



3) Antibody-based targeting



Example approaches: receptor-ligand-mediated targeting to vasculature

Mimicking lymphocyte responses to inflammation:

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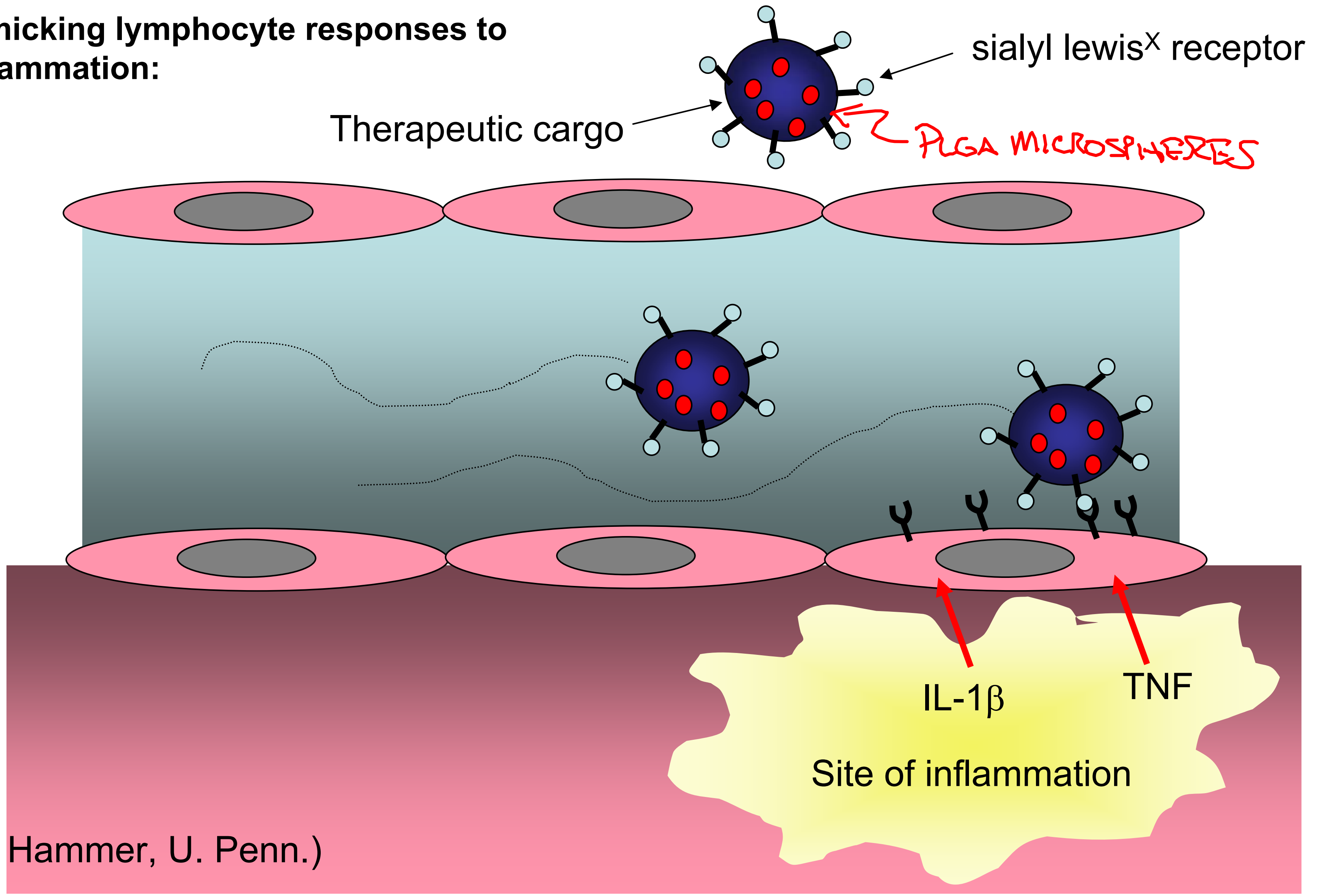
Please see: Figure 1 in Hogg, et al. *J Cell Sc* 116 (2003): 4695-4705.

Example approaches: receptor-ligand-mediated targeting to vasculature

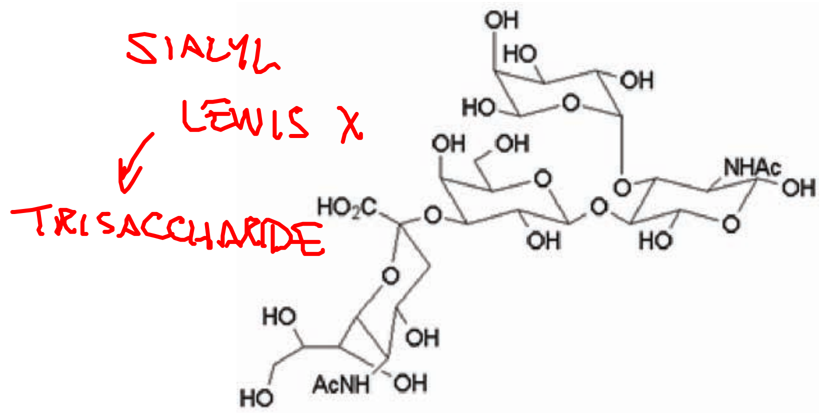
Mimicking lymphocyte responses to inflammation:

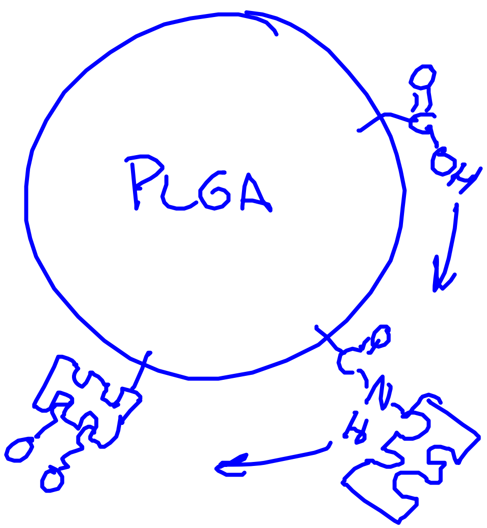


(D. Hammer, U. Penn.)

Example approaches: receptor-ligand-mediated targeting to vasculature

Mimicking lymphocyte responses to inflammation:





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Please see: Figure 2 in Cao, Y., and L. Lam. “Bispecific Anitbody Conjugates in Therapeutics.” *Adv Drug Delib Rev* 55 (2003): 171-97.

Pre-targeting drug delivery with bispecific antibodies

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

Antibody-based targeting

General structure of IgA, IgE, IgD, IgG:

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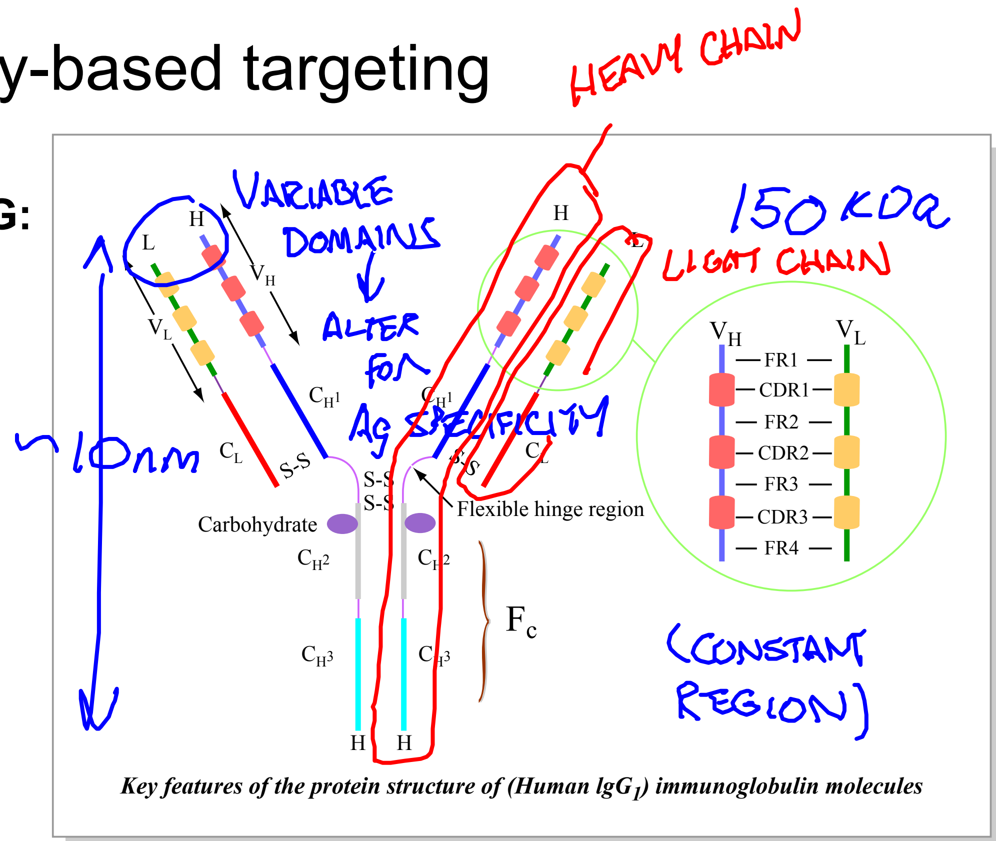
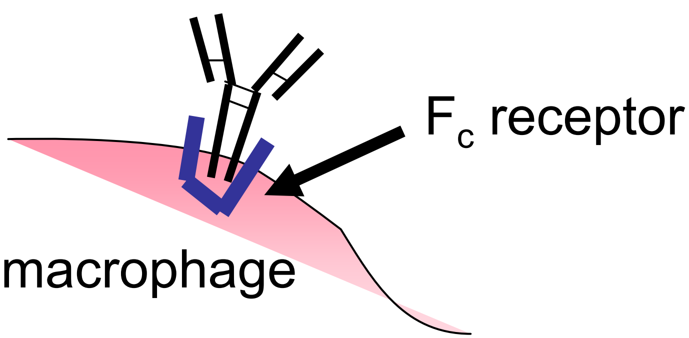


Key features of the protein structure of (Human IgG$_1$) immunoglobulin molecules

Figure by MIT OCW.



FAb/FAb'

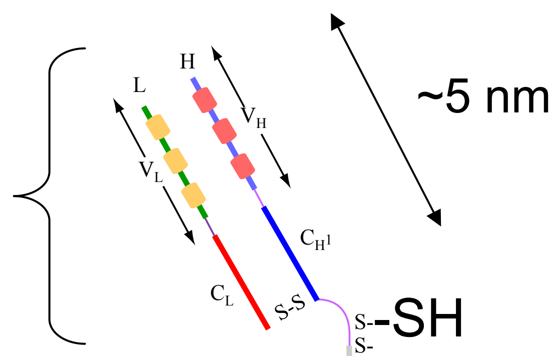


Figure by MIT OCW.

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, NY: Wiley-Liss, 1996.

Synthesizing antibodies which avoid recognition by the immune system

Figure removed due to copyright restrictions.
Please see: Figures 2 in Allen, T.M. "Ligand-targeted therapeutics in anticancer therapy."
Nat Rev Cancer 2 (2002): 750-63.

Strategies for conjugation of antibodies to biomaterials

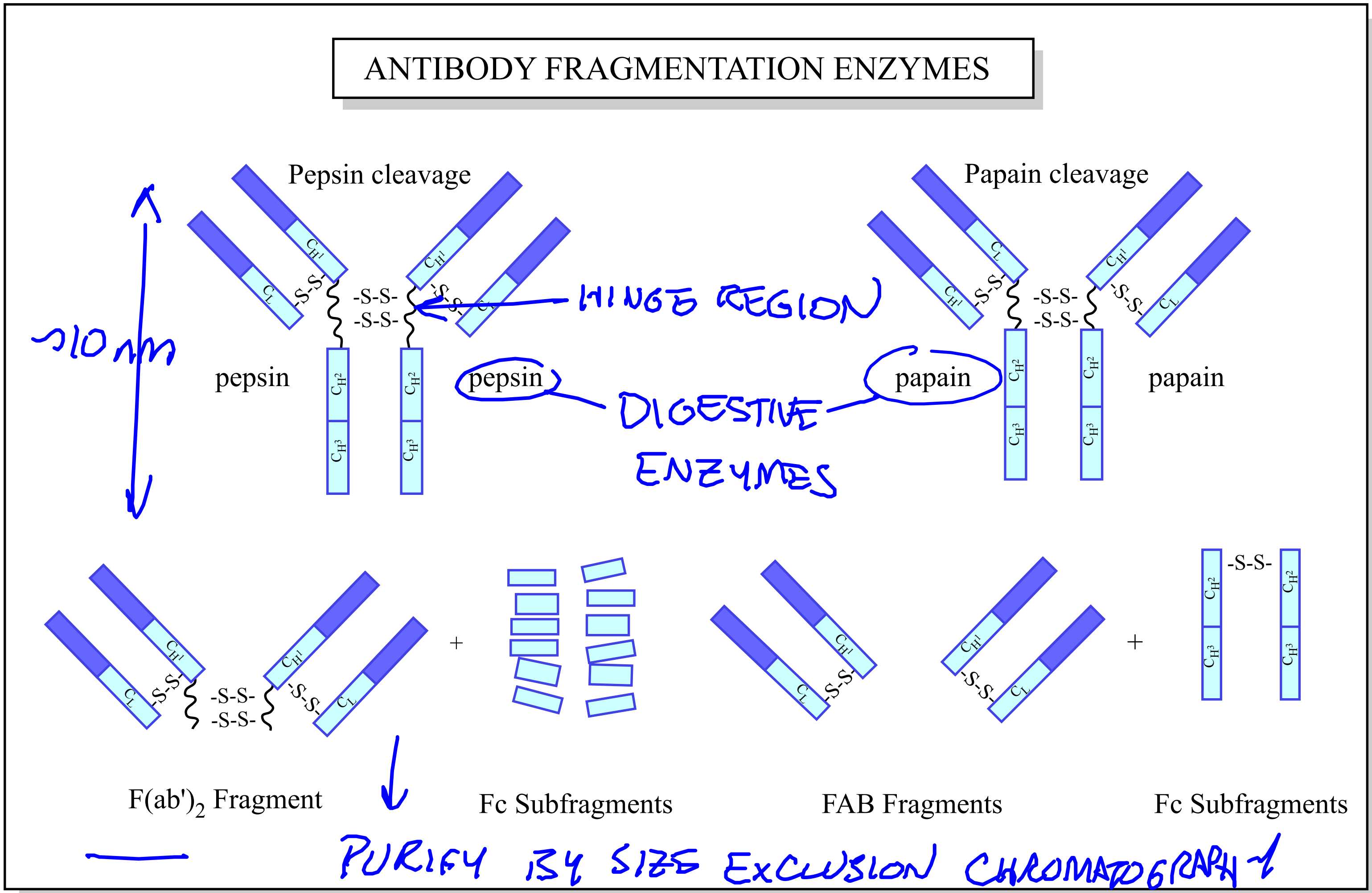
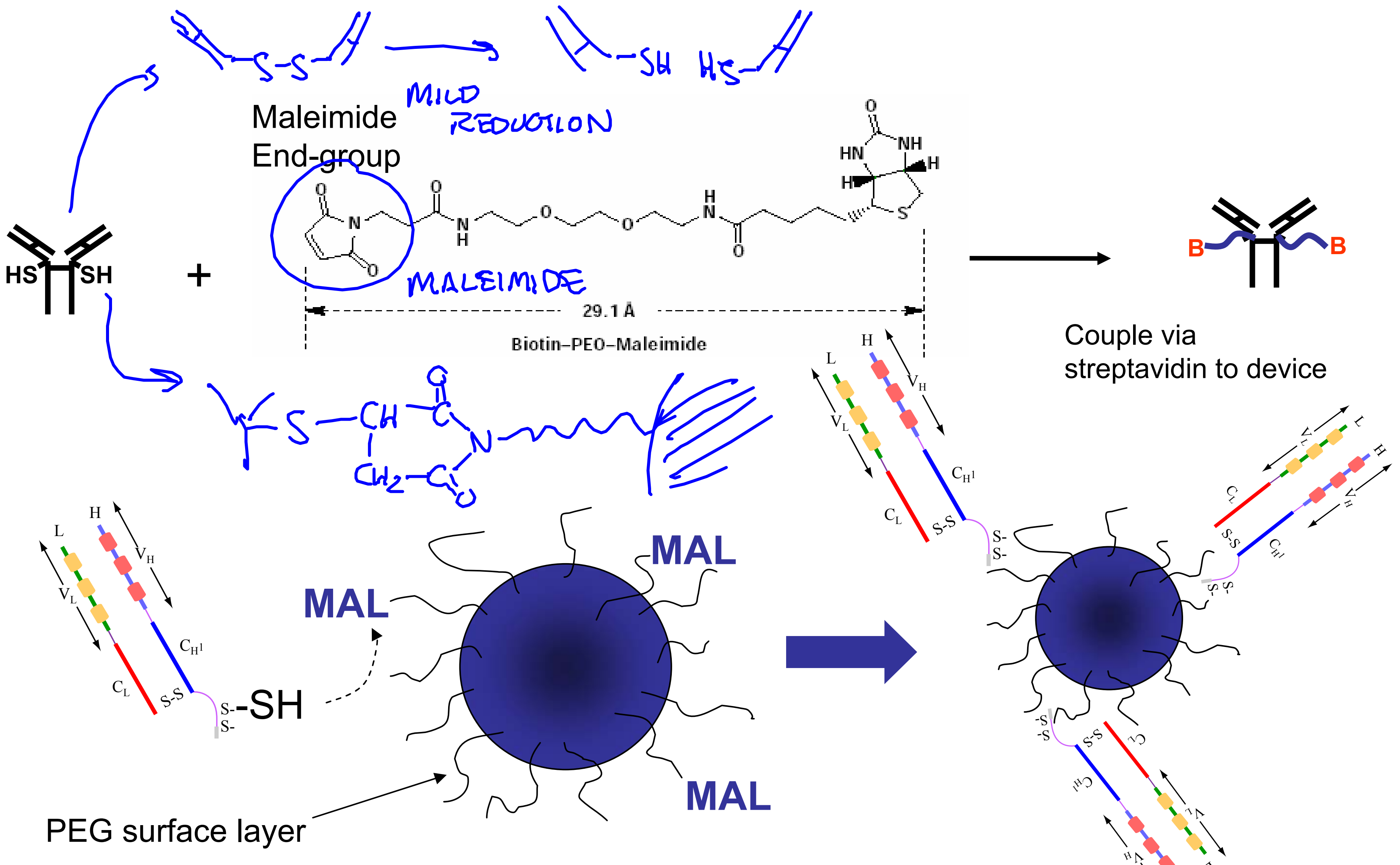


Figure by MIT OCW.

Strategies for conjugation of antibodies to biomaterials



Figures by MIT OCW.

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.

Application	Cellular target	Molecular target	Targeting ligand	Ligand type
Anti-cancer therapy	Various tumor cells	Folate receptor EGF receptor	Folate 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 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

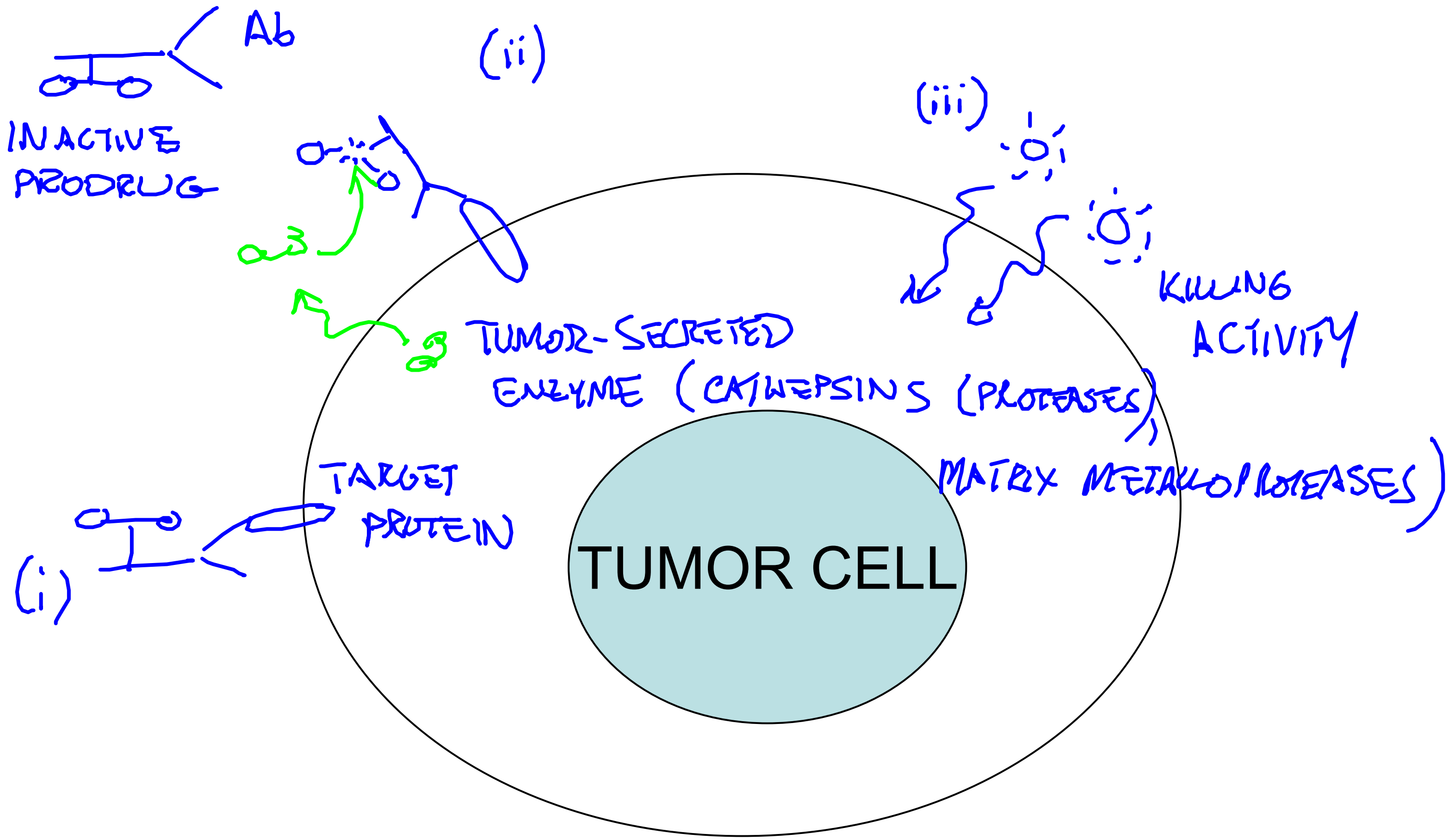
ONLY KILL ACTIVATED T CELLS ... ACCEPTABLE SIDE EFFECT

"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 (2002): 750-63.

Example approaches: targeted activation of active agent

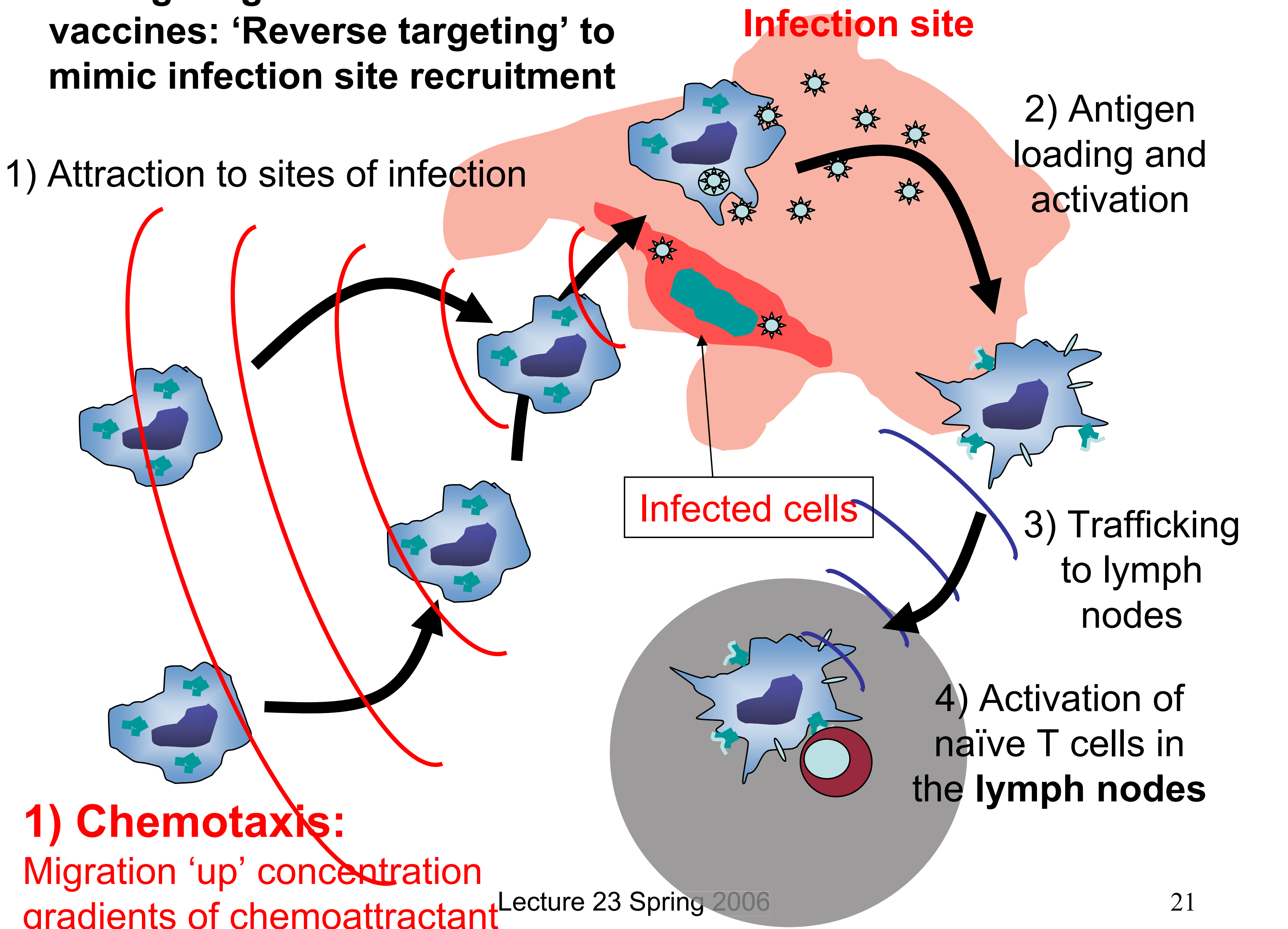
Antibody-directed enzyme prodrug therapy (ADEPT):



‘Reverse targeting’

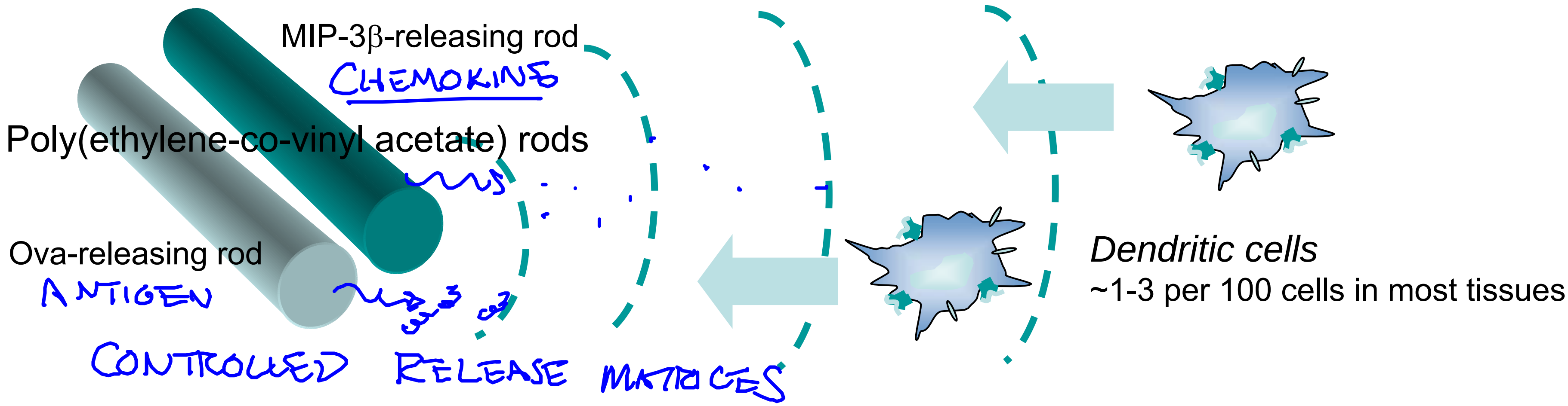
Bringing cells to the drug

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



Targeting dendritic cells to vaccines

Attraction of target cells to device via chemotaxis:



Advantages relative to bolus chemoattractant injection:

1. CHEMOATTRACTANTS CLEAR IN LESS < 24 HRS IN VIVO (IN TISSUE)
2. ENGINEER CONCENTRATION GRADIENT TO OPTIMIZE ATTRACTION

 (Kumamoto et al. 2002)

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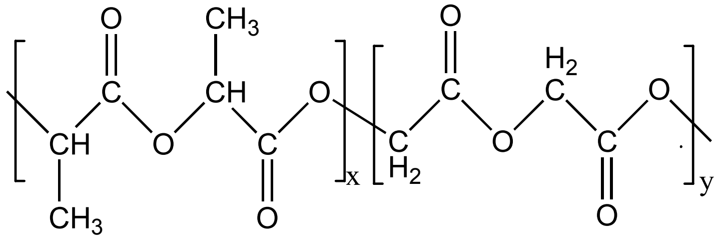
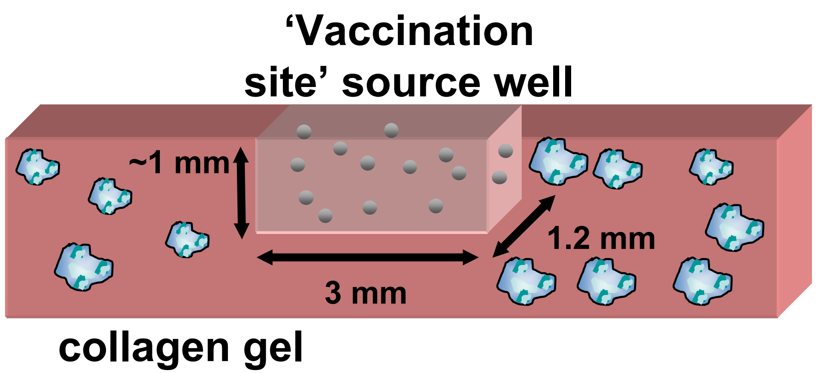
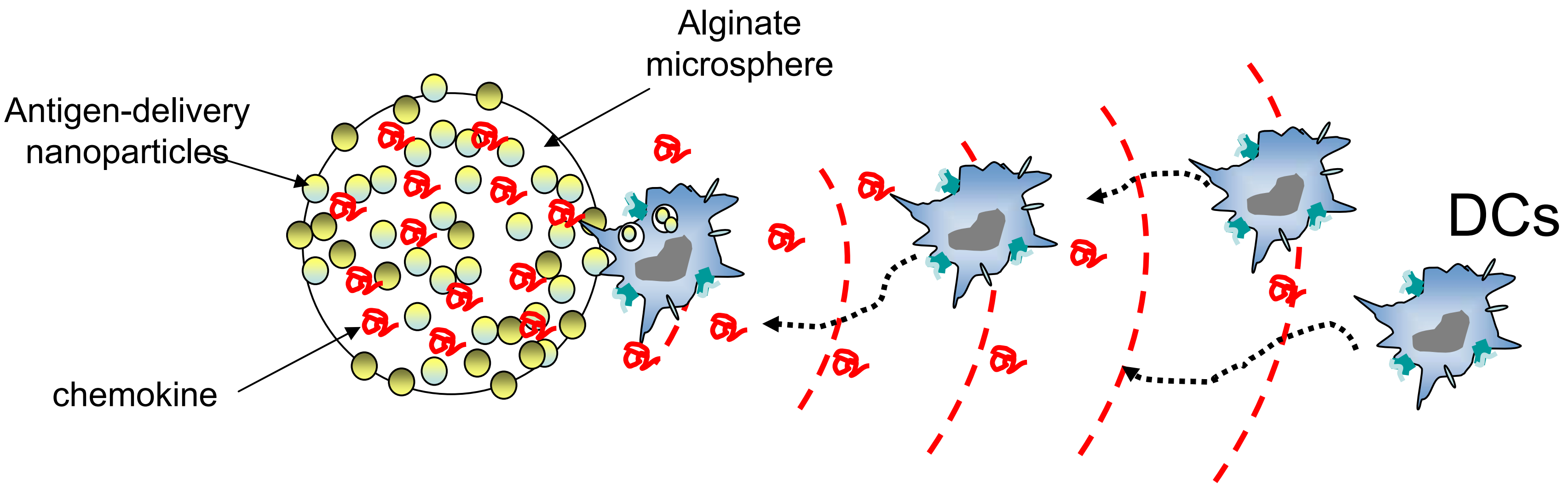
Please see: Kumamotos, T., et al. ”Induction of Tumor-specific Protective Immunity by in Situ Langerhans Cell Vaccine." *Nat Biotechnol* 20 (2002): 64-9.

PLGA





Dendritic cell attraction, antigen loading, and activation



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

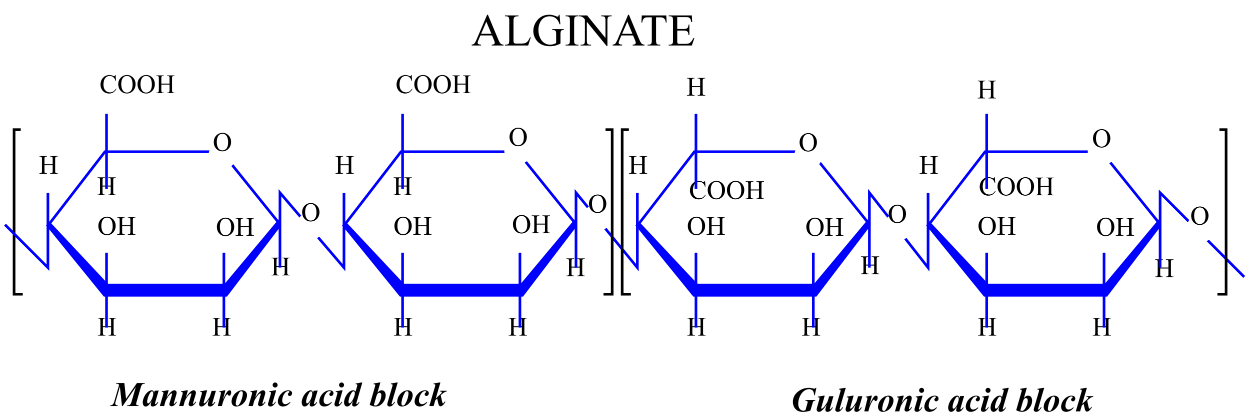


Figure by MIT OCW.

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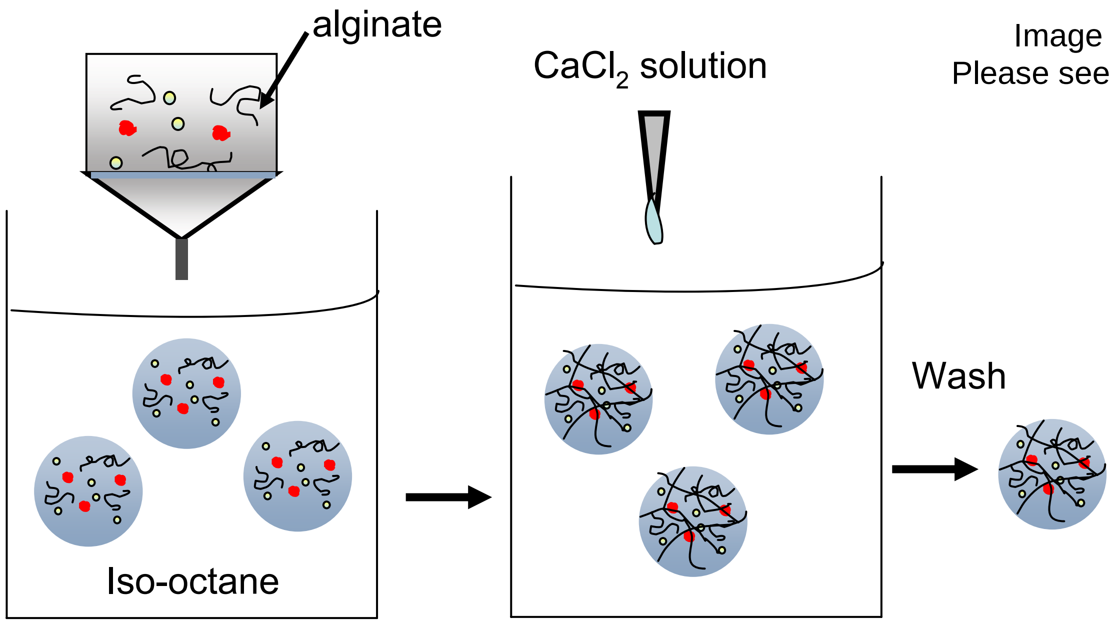
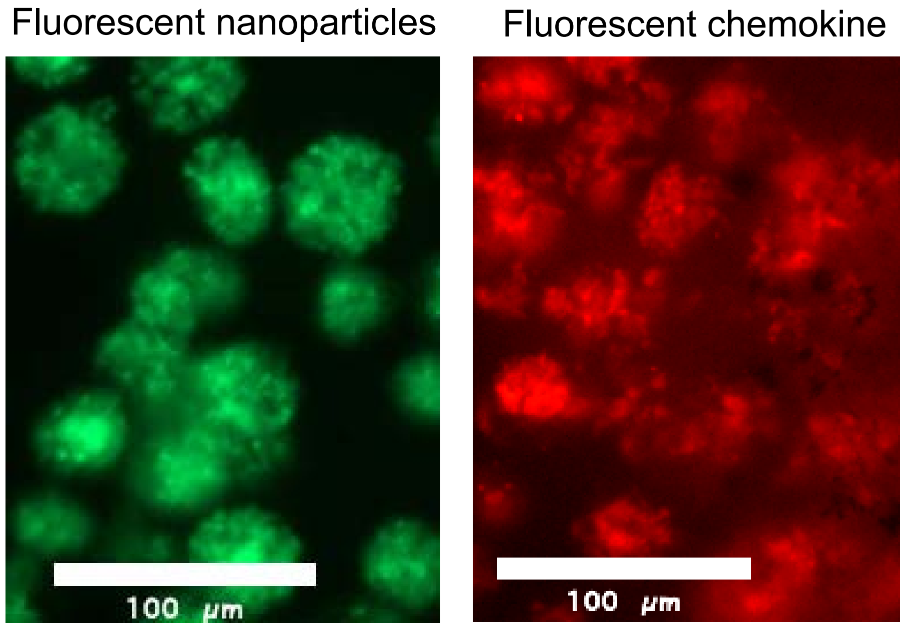
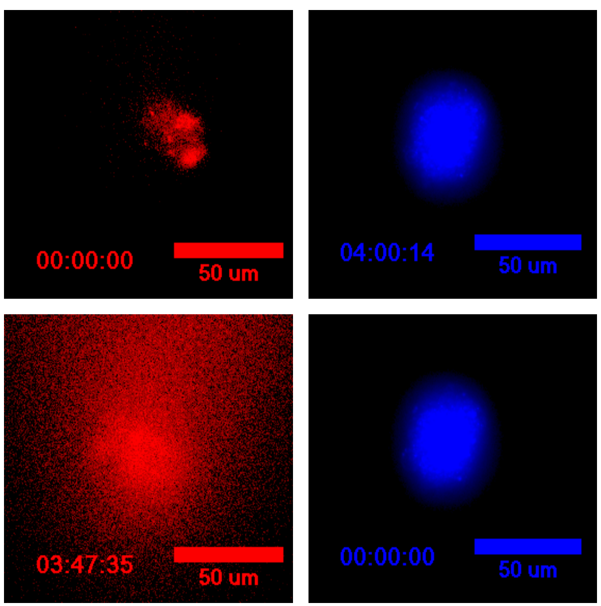
Please see: http://www.lsbu.ac.uk/water/hyalg.html





Fluorescent nanoparticles
Fluorescent chemokine
100 μm
100 μm



00:00:00
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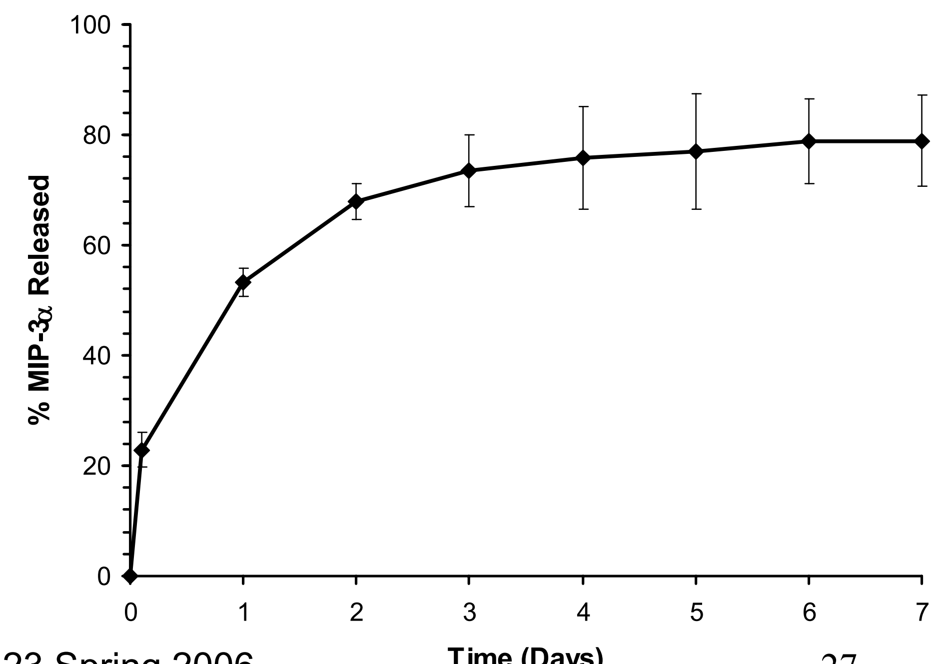
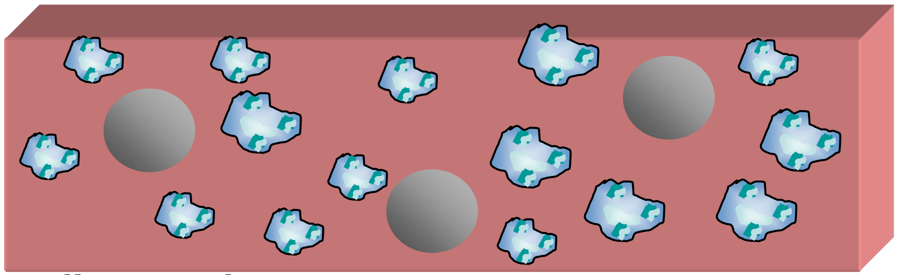
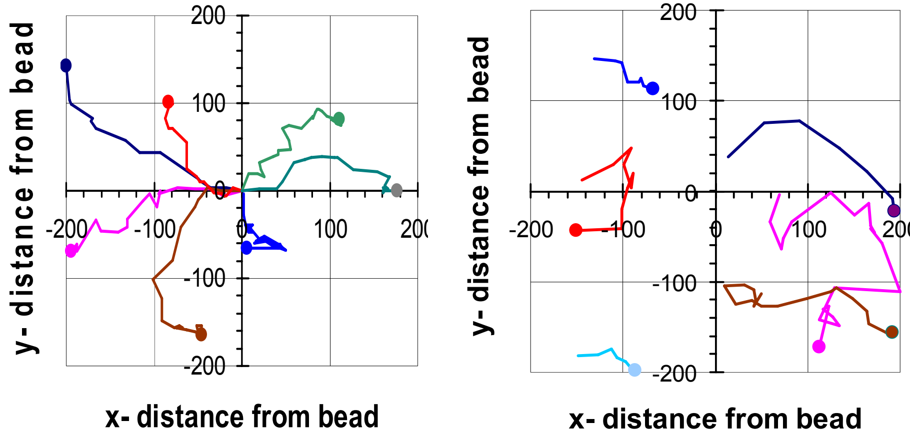
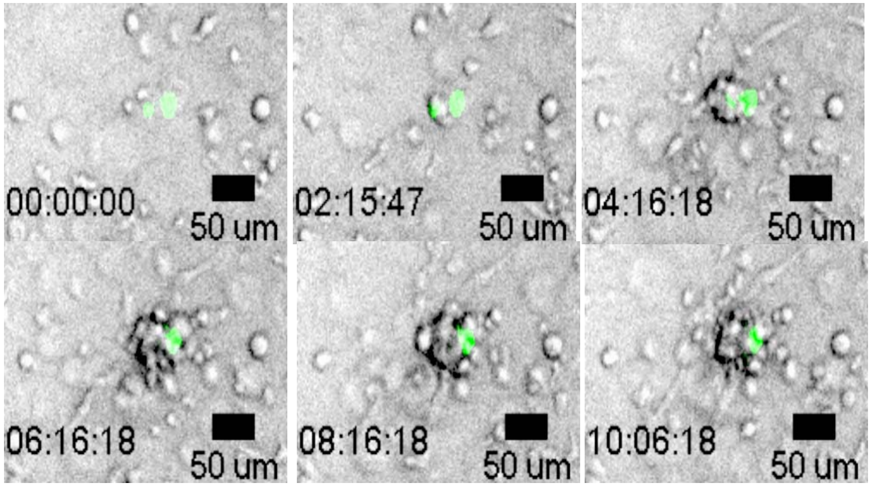
04:00:14
50 um
03:47:35
50 um
00:00:00
50 um



% MIP-3α Released
Time (Days)
0
20
40
60
80
100
0
1
2
3
4
5
6
7


collagen gel


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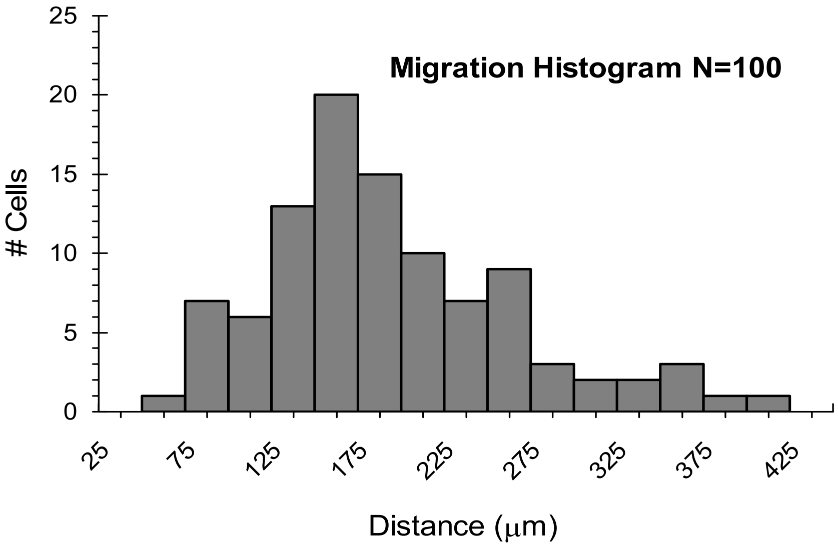
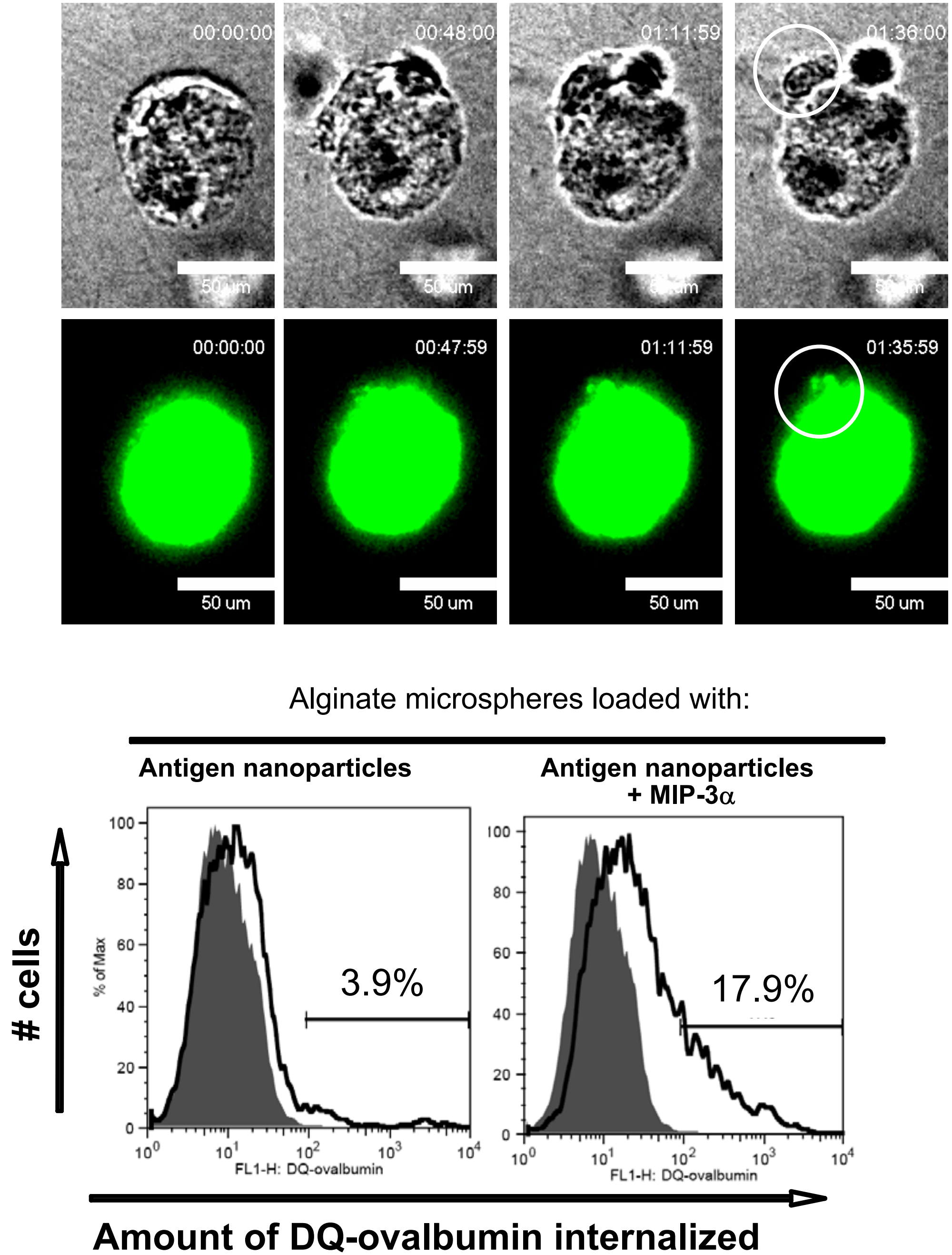

Migration Histogram N=100
Cells
Distance (μm)
0
5
10
15
20
25
75
125
175
225
275
325
375
425


00:00:00
00:48:00
01:11:59
01:36:00
00:00:00
00:47:59
01:11:59
01:35:59
50 um
Alginate microspheres loaded with:
Antigen nanoparticles
Antigen nanoparticles
+ MIP-3α
# cells
% of Max
3.9%
17.9%
FL1-H: DQ-ovalbumin
Amount of DQ-ovalbumin internalized

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).
9. Rungsardthong, U. et al. Effect of polymer ionization on the interaction with DNA in nonviral gene delivery systems. *Biomacromolecules* **4**, 683-90 (2003).
10. Rungsardthong, U. et al. Copolymers of amine methacrylate with poly(ethylene glycol) as vectors for gene therapy. *J Control Release* **73**, 359-80 (2001).
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14. Bonifaz, L. C. et al. In Vivo Targeting of Antigens to Maturing Dendritic Cells via the DEC-205 Receptor Improves T Cell Vaccination. *J Exp Med* **199**, 815-24 (2004).
15. Kircheis, R., Wightman, L. & Wagner, E. Design and gene delivery activity of modified polyethylenimines. *Advanced Drug Delivery Reviews* **53**, 341-358 (2001).