Stealth particles (continued)
Biology of vaccination

Last Time: carriers continued; avoiding the RES

Today: polymer brush theory for protein resistant stealth particles
basic biology of primary immune responses and vaccination

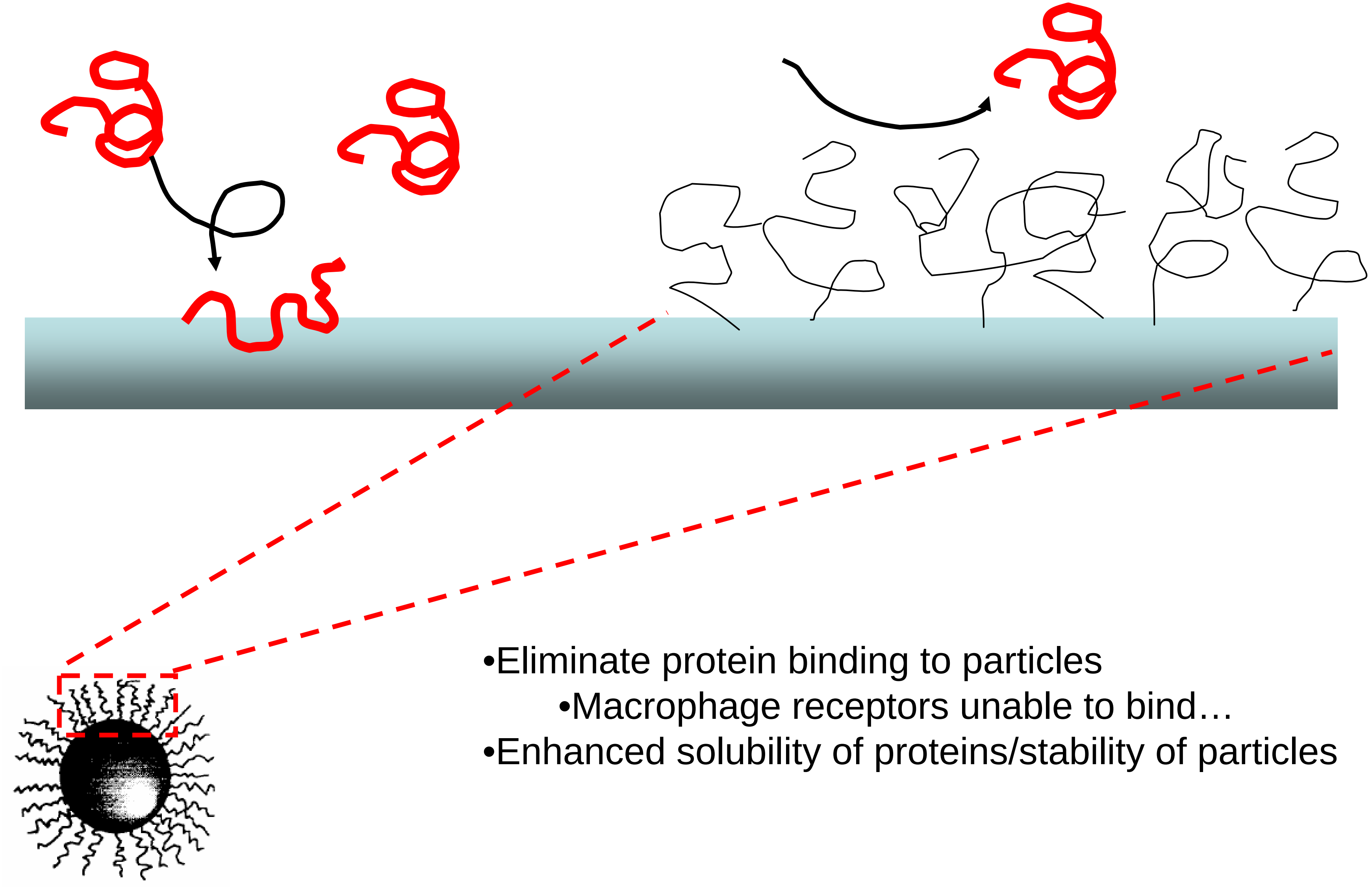
Reading: Plotkin and Orenstein, ‘The Immunology of Vaccination,’ from *Vaccines* 3rd ed., pp. 28-39
Abbas et al. ‘General properties of immune responses,’ from *Cellular and Molecular Immunology* 4th ed. Pp. 3-16

Supplementary Reading:

ANNOUNCEMENTS:

Take-home exam 2 out today– due last day of class

'stealth' particles: avoiding the reticulorendothelial system

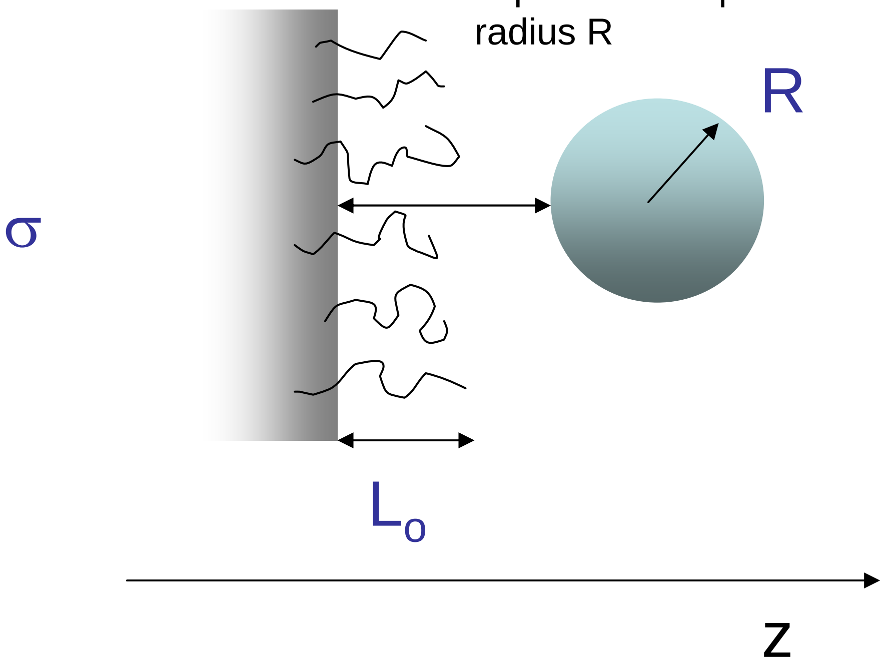


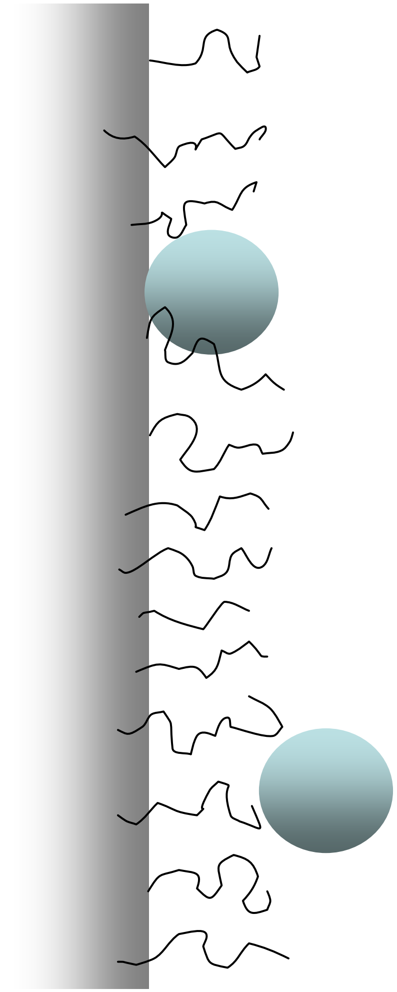
•Eliminate protein binding to particles
  •Macrophage receptors unable to bind…
•Enhanced solubility of proteins/stability of particles

Theory of protein-resistant surfaces

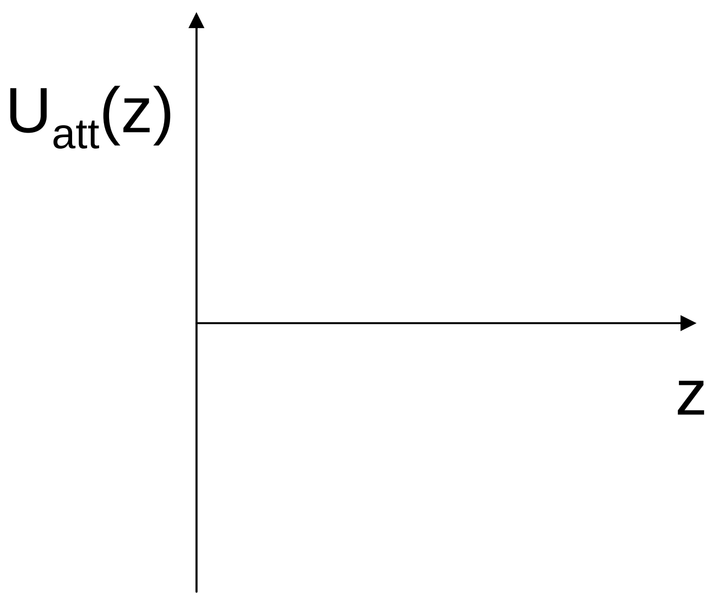
Model parameters

Protein modeled as an impenetrable sphere of radius R

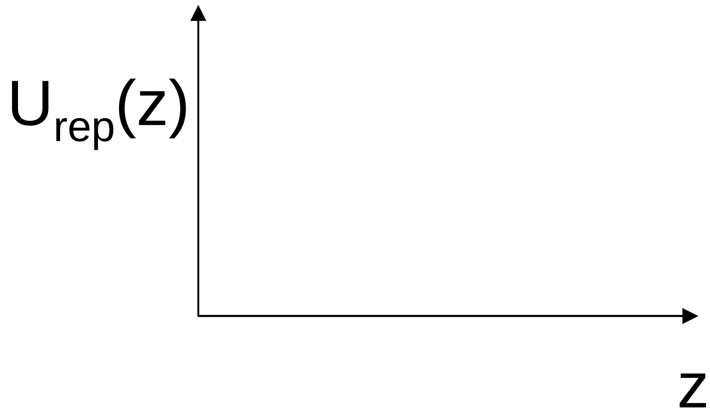


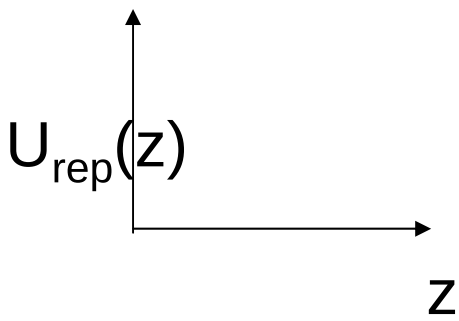


Attractive potential

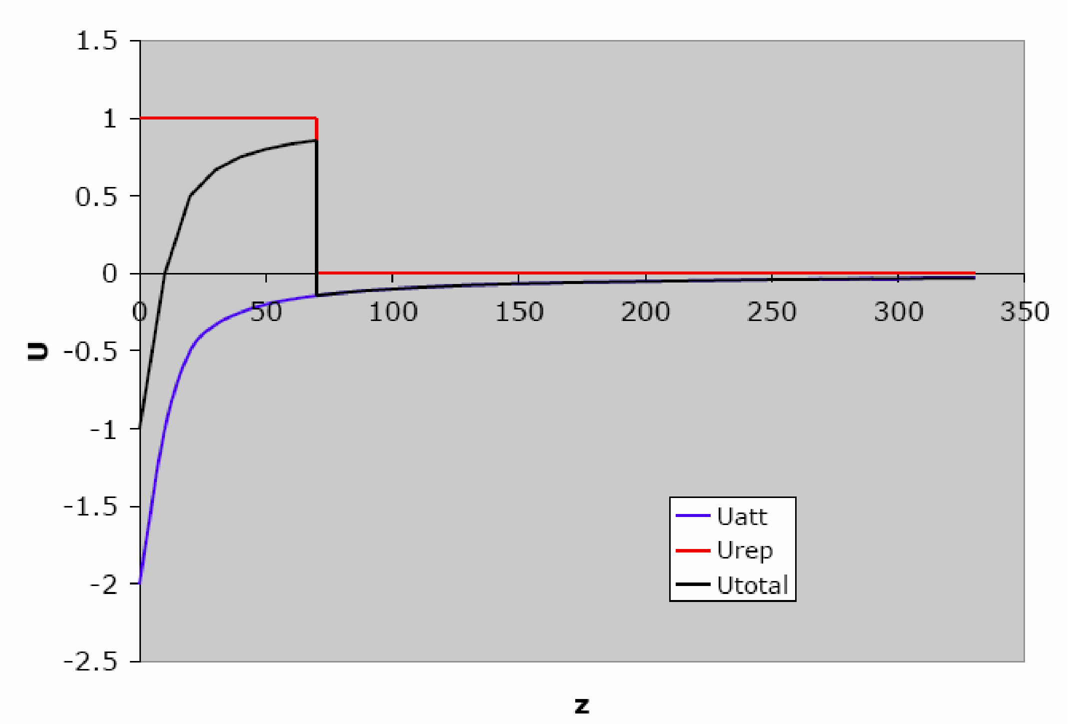


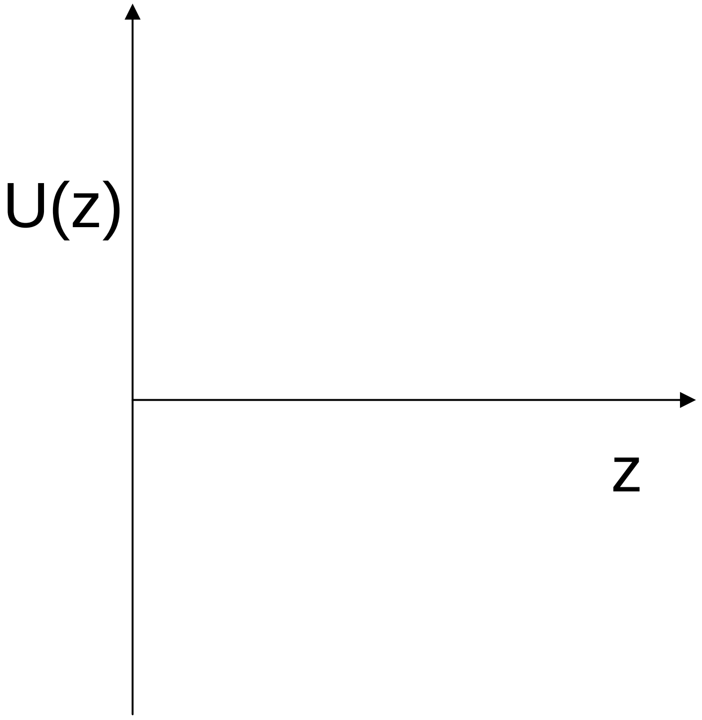
Repulsive potential



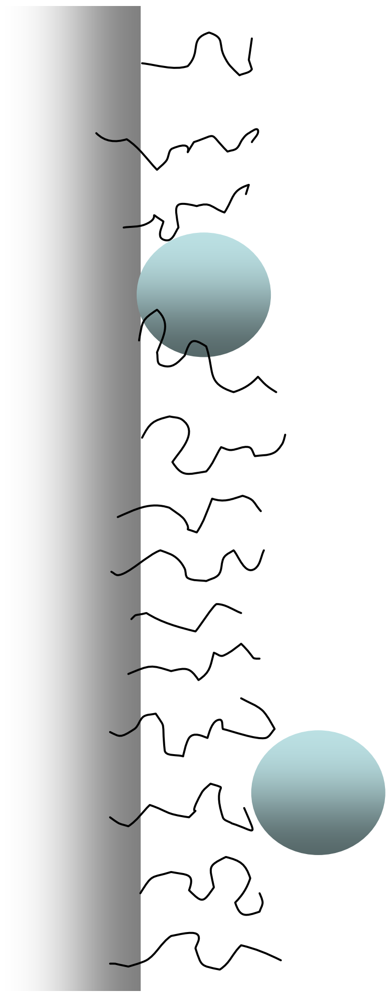


Total potential:





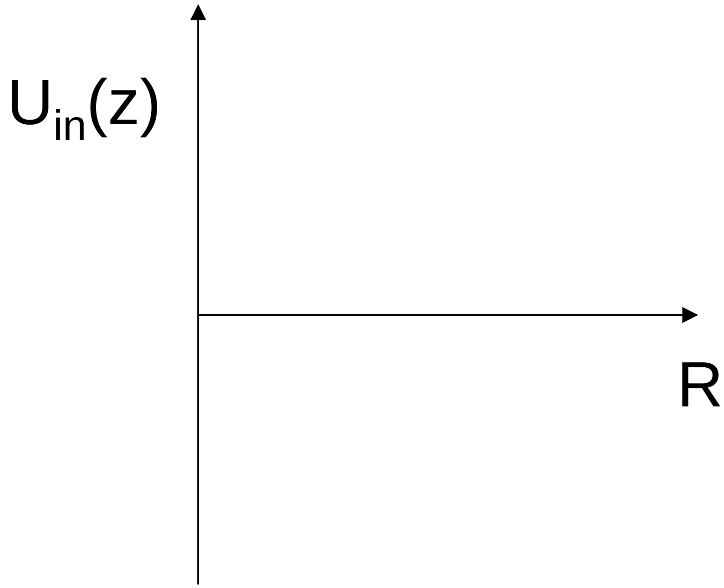
Adsorption of small proteins

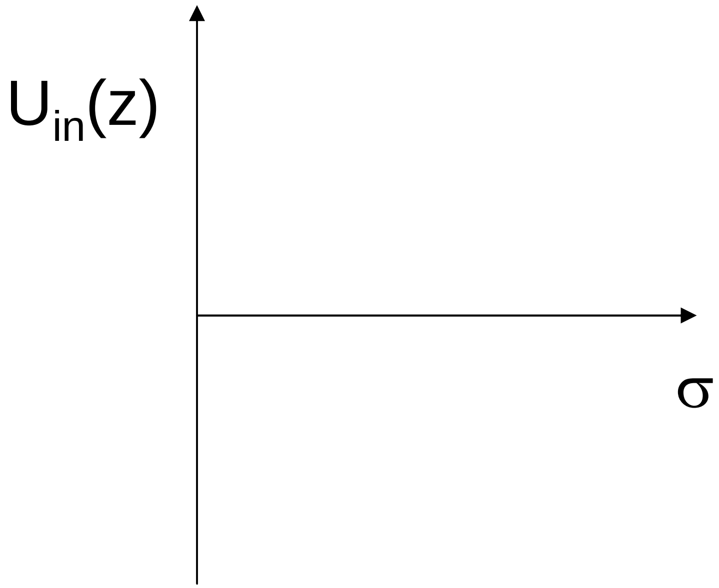


Langmuir binding model:

1) Proteins are dilute- do not interact with one another
2) Proteins bind to a finite number of unique surface sites

Achieving protein-resistant stealth particles





What condition for equilibrium primary protein adsorption resistance?

Adsorption of large vs. small proteins

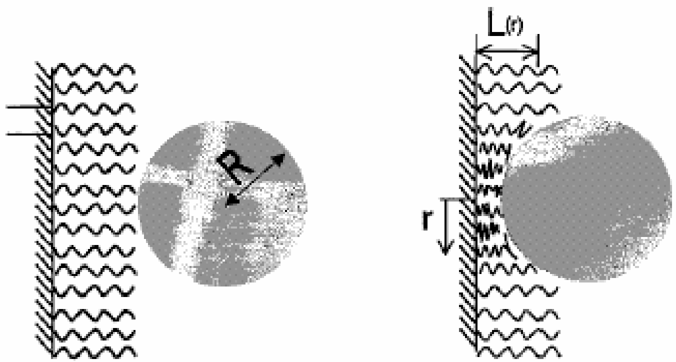


Figure 2. Large proteins can approach the surface only by compressing the brush. The free energy penalty associated with the compression mechanism favors secondary adsorption at the outer edge of the brush.

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Please see: Figure 3 in Halperin, A. "Polymer Brushes that Resist Absorption of Model Proteins: Design Parameters." *Langmuir* 15 (1999): 2525-2533.

Kinetic protein resistance: Depends on L_o and σ, but s,R dependence still dominates

Comparison of theory with experiment

Surface plasmon resonance measurements:

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Please see: Figure 7 in Efremova, et al. "Measurements of Interbilayer Forces and Protein Adsorption on Uncharged Lipid Bilayers Displaying Poly(ethylene glycol) Chains." *Biochemistry* 39 (2000): 3441-51.

Comparison of theory with experiment

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Please see: Figure 9 in Efremova, et al. "Measurements of Interbilayer Forces and Protein Adsorption on Uncharged Lipid Bilayers Displaying Poly(ethylene glycol) Chains." *Biochemistry* 39 (2000): 3441-51.

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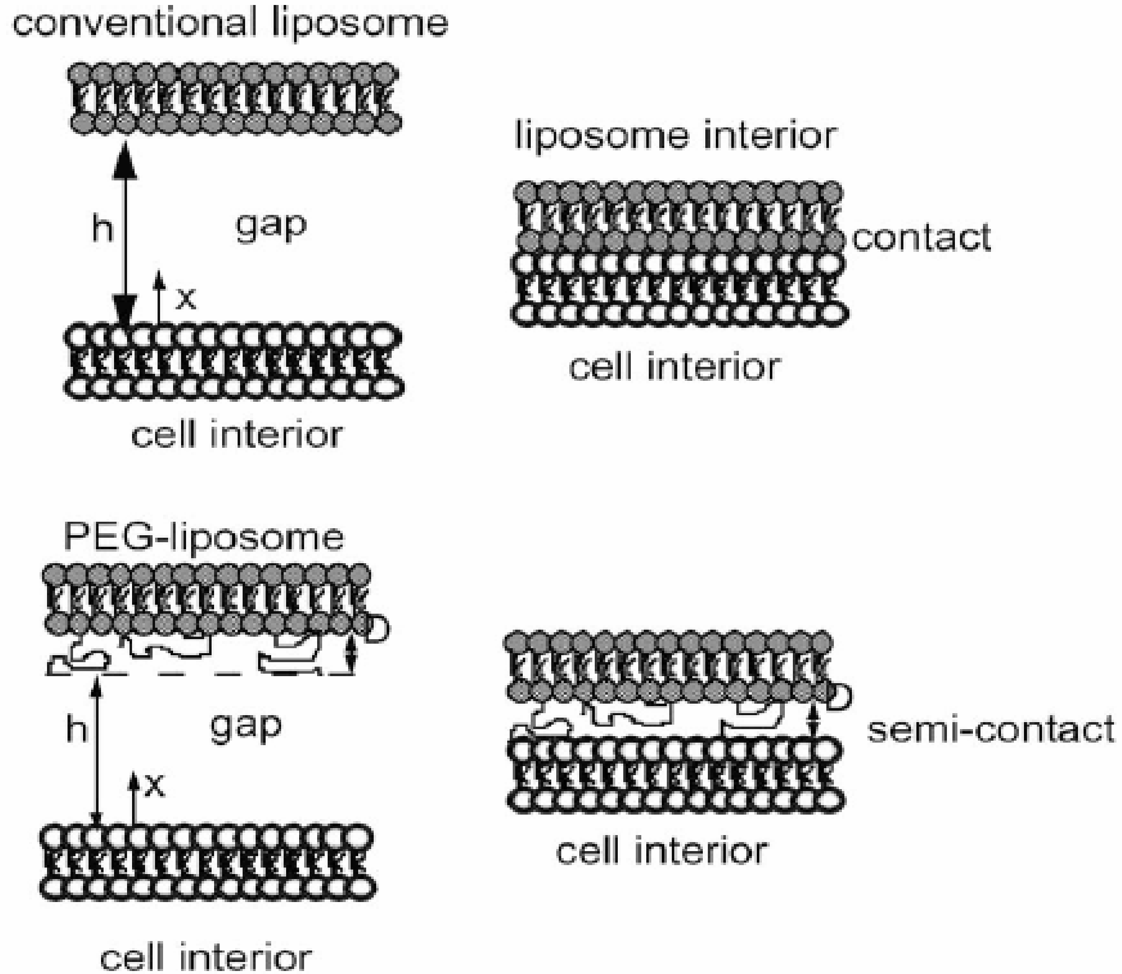
Please see: Figure 9 in Efremova, et al. "Measurements of Interbilayer Forces and Protein Adsorption on Uncharged Lipid bilayers Displaying Poly(ethylene glycol) Chains." *Biochemistry* 39 (2000): 3441-51.

Additional benefits of PEGylated carriers: improved carrier stability

Liposomes:



Synthesis of ‘stealth’ particles

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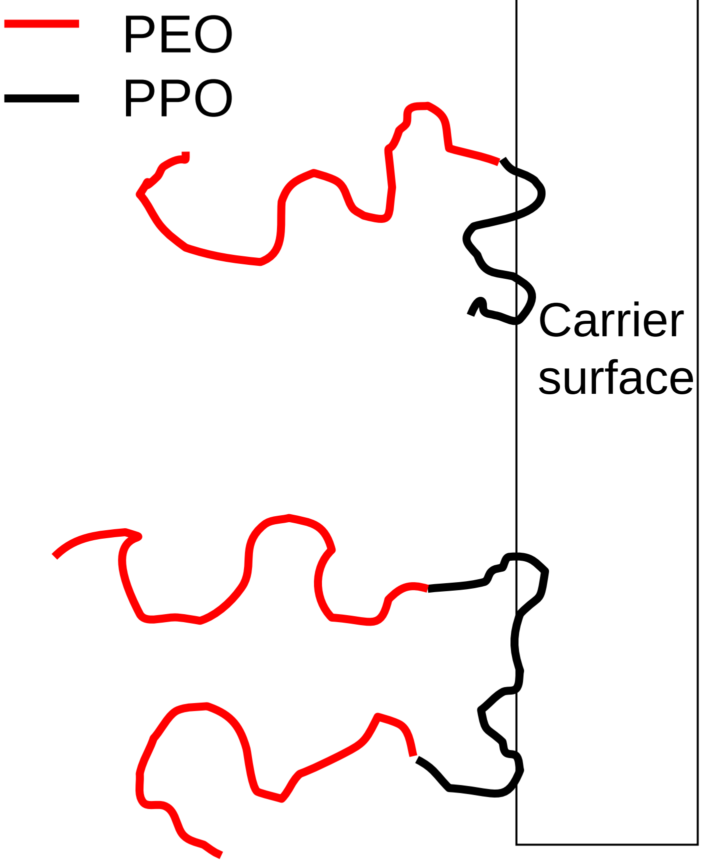
Please see: Stolnik, et al. "Long Circulating Microparticulate Drug Carriers." *Advanced Drug Delivery Reviews* 16 (1995): 195-214.

e.g. Pluronics:



Example stealth particle results: PEGylated PLGA nanoparticles

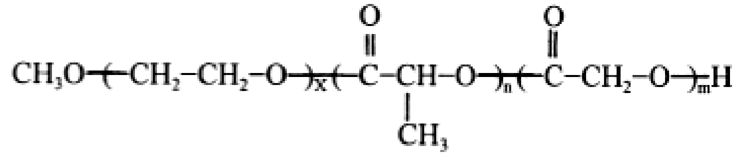
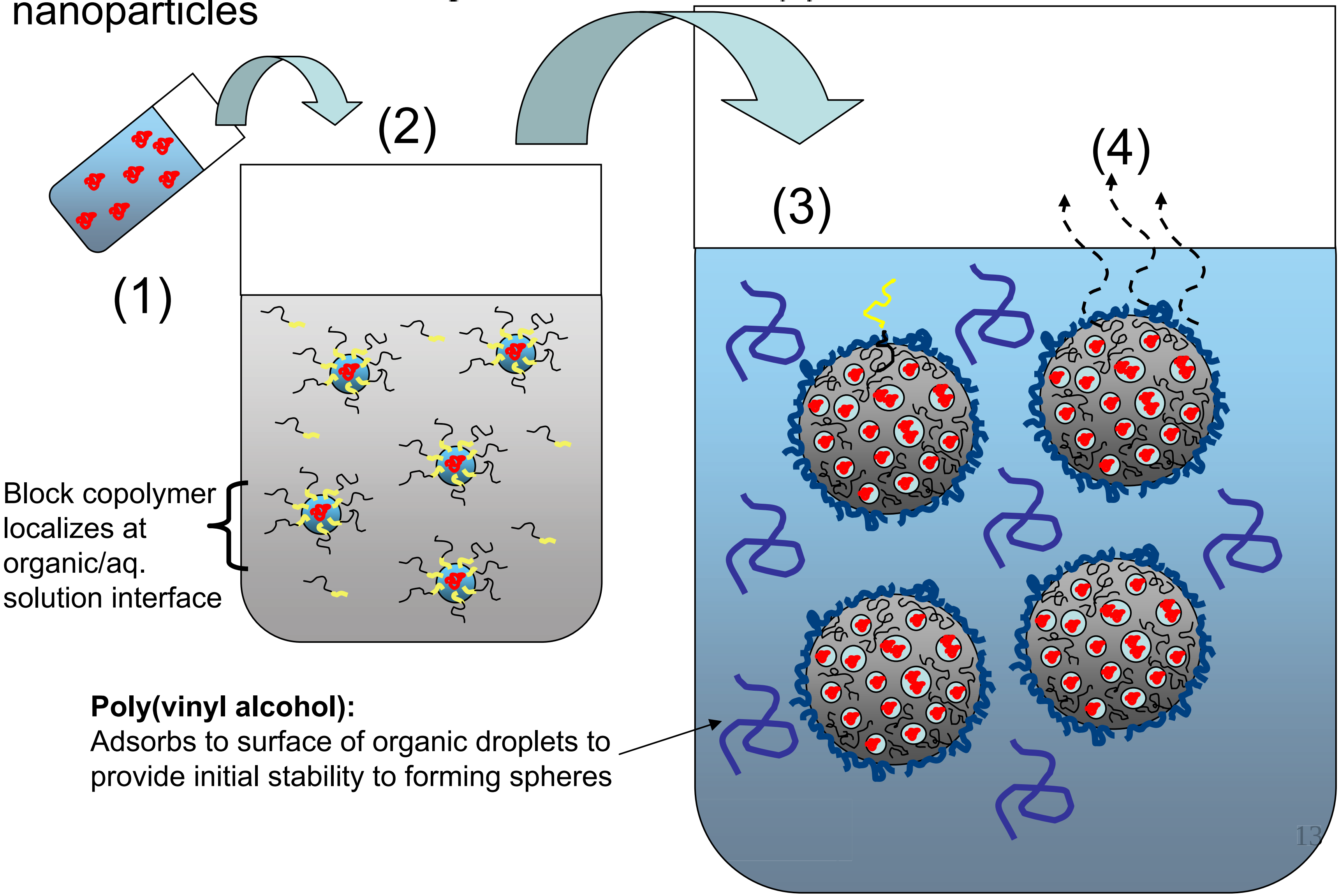
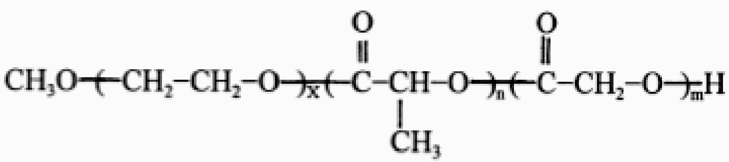


Fig. 1. Structure of the PEG–PLGA copolymer.

PEG = 5KDa, PLGA = 40 KDa



Block copolymer localization at aqueous/polymer interfaces



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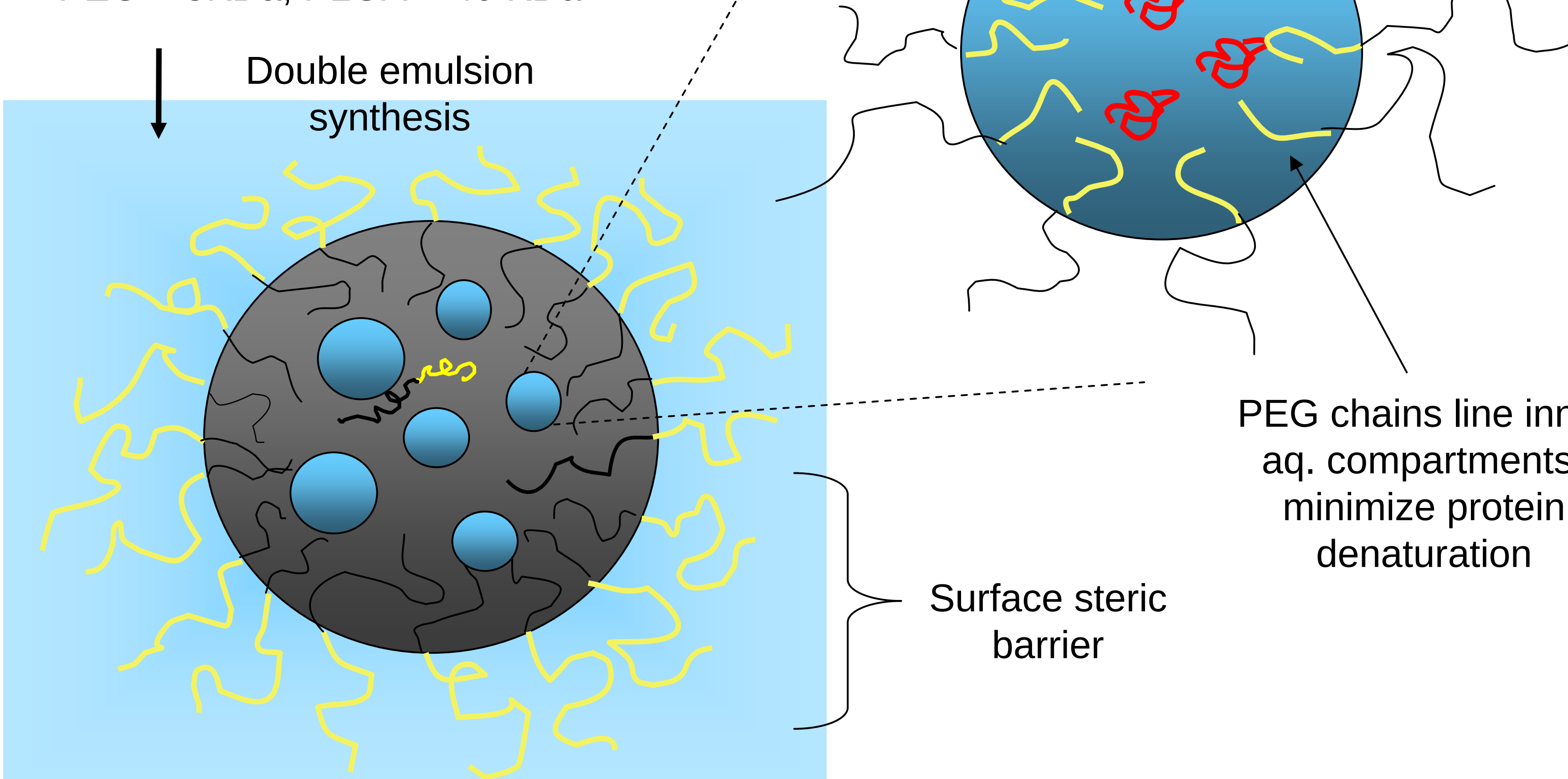

TEM of nanoparticles

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Please see: Li, Y., et al. "PEGylated PLGA Nanoparticles as Protein Carriers: Synthesis, Preparation and Biodistribution in Rats." *Journal of Control Release* 71 (2001): 203-11.

Release properties of diblock particles

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Please see: Figure 6 in Li, Y., et al. "PEGylated PLGA Nanoparticles as Protein Carriers: Synthesis, Preparation and Biodistribution in Rats." *Journal of Control Release* 71 (2001): 203-11.

Increased $t_{1/2}$ in blood:

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Please see: Figure 7 in Li, Y., et al. "PEGylated PLGA Nanoparticles as Protein Carriers: Synthesis, Preparation and Biodistribution in Rats." *Journal of Control Release* 71 (2001): 203-11.

Altered biodistribution:

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Please see: Li, Y., et al. "PEGylated PLGA Nanoparticles as Protein Carriers: Synthesis, Preparation and Biodistribution in Rats." *Journal of Control Release* 71 (2001): 203-11.

Clinically-approved stealth carriers

- PEG-GCSF (granulocyte colony stimulating factor, Amgen) 2002
    - Pegylated GCSF (cytokine)
    - Reduction of febrile neutropenia associated with chemotherapy
- Pegademase (Adagen) 1990
    - Pegylated adenosine deaminase (enzyme)
    - Treatment of severe combined immunodeficiency (SCID)- hereditary lack of adenosine deaminase
- Pegaspargase (Oncaspar)
    - Pegylated asparaginase (enzyme)
    - Treatment of leukemia
        - Leukaemic cells cannot synthesize asparagines; asparaginase kills cells by depleting extracellular sources of this amino acid
- Pegylated IFN-α2a (Pegasys) 2001
    - Treamtent of hepatitis C
- Doxil (Alza) 1995-2003
    - Pegylated liposomes carrying anti-cancer drug doxorubicin
    - Improves treatment from daily 30min injections for 5 days every 3 weeks to once-a-month single injections
    - Approved for treatment of Karposi's sarcoma, ovarian cancer, and breast cancer[8]

Delivery into cells once the target tissue is reached: Cell type-dependent endocytosis limits

Internalization of 200nm-diam particles by carcinoma cell line:

Image removed for copyright reasons.
Please see: Zuner, et al. *J Contr Rel* 71, 39 (2001).

Table removed for copyright reasons.
Please see: Table 1 in Zuner, et al. *J Contr Rel* 71, 39 (2001).

Endpoint for most particles: endosomal compartments

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Please see: Figure 2 in Chithranl, et al. *Nano Lett* 6 (2006): 662-668.

FOCUS TOPIC: INTEGRATING BIOLOGICAL KNOWLEDGE INTO BIOMATERIALS DESIGN FOR VACCINES

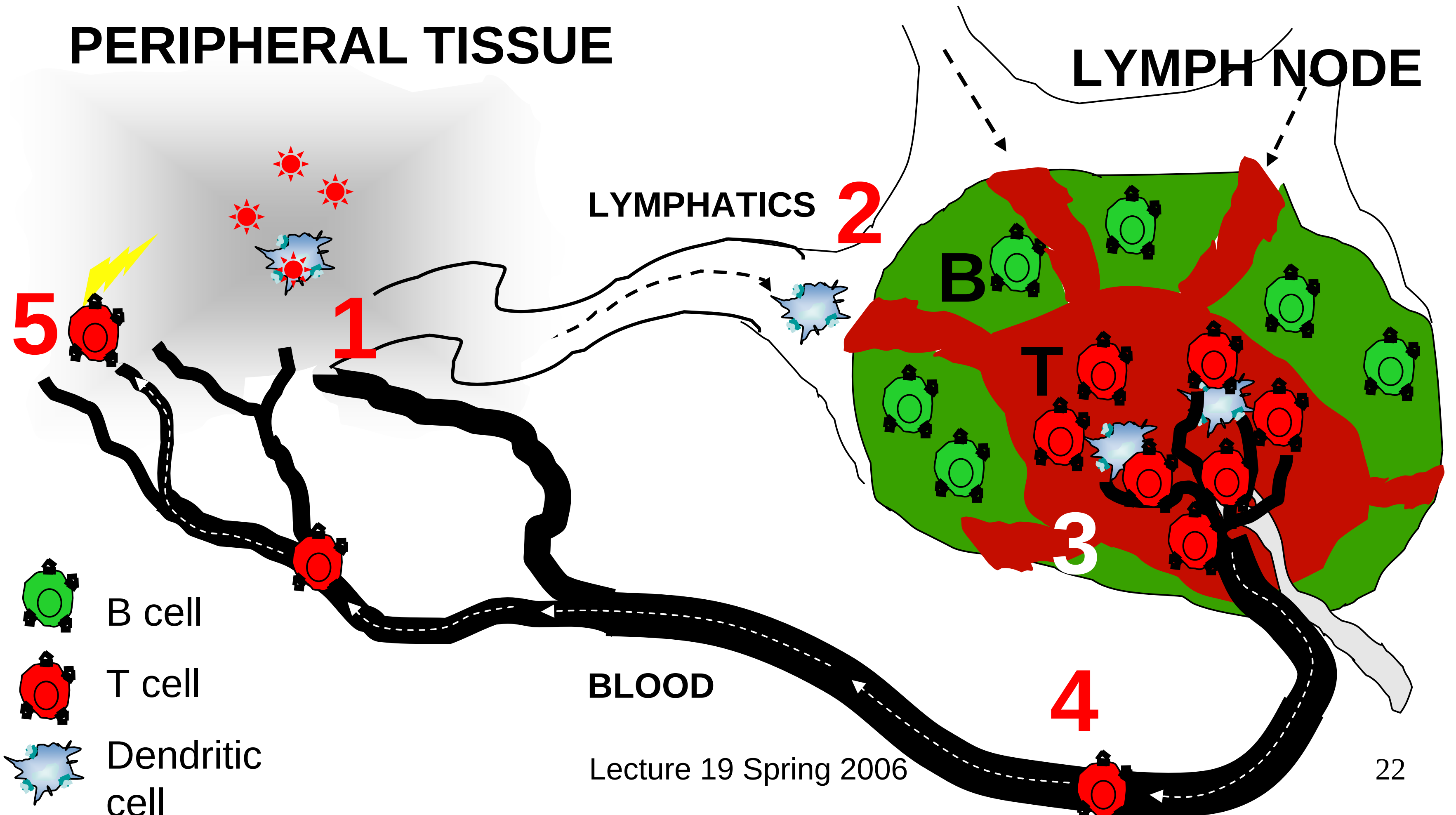
Basic Biology of Vaccination

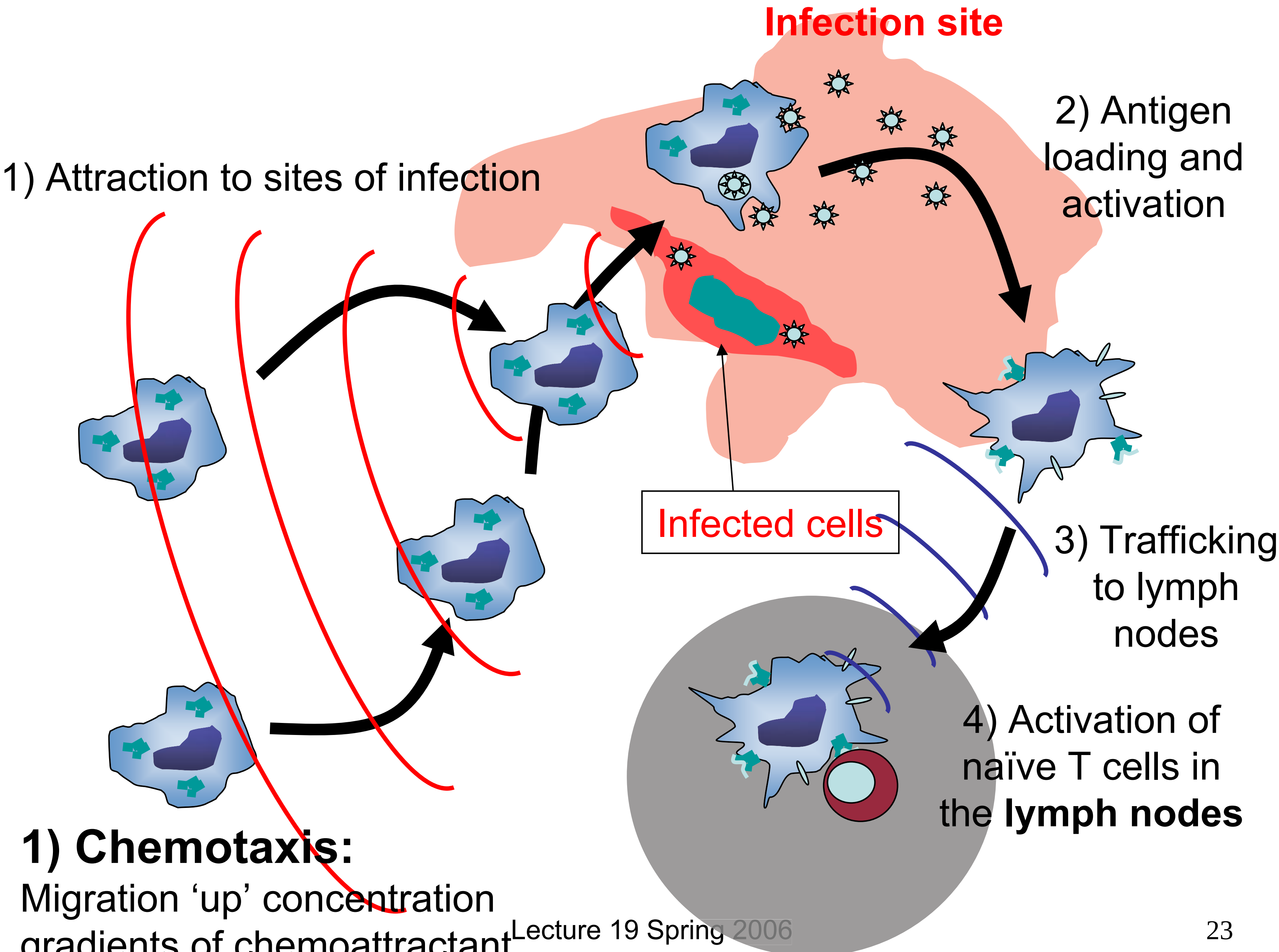
KEY EFFECTORS OF ADAPTIVE IMMUNITY

THE CLONAL IMMUNE SYSTEM

Arstila et al. *Science* **286**, 958 (1999)
Blattman et al. *J. Exp. Med*. **195**, 657 (2002)

Physiology of the primary immune response

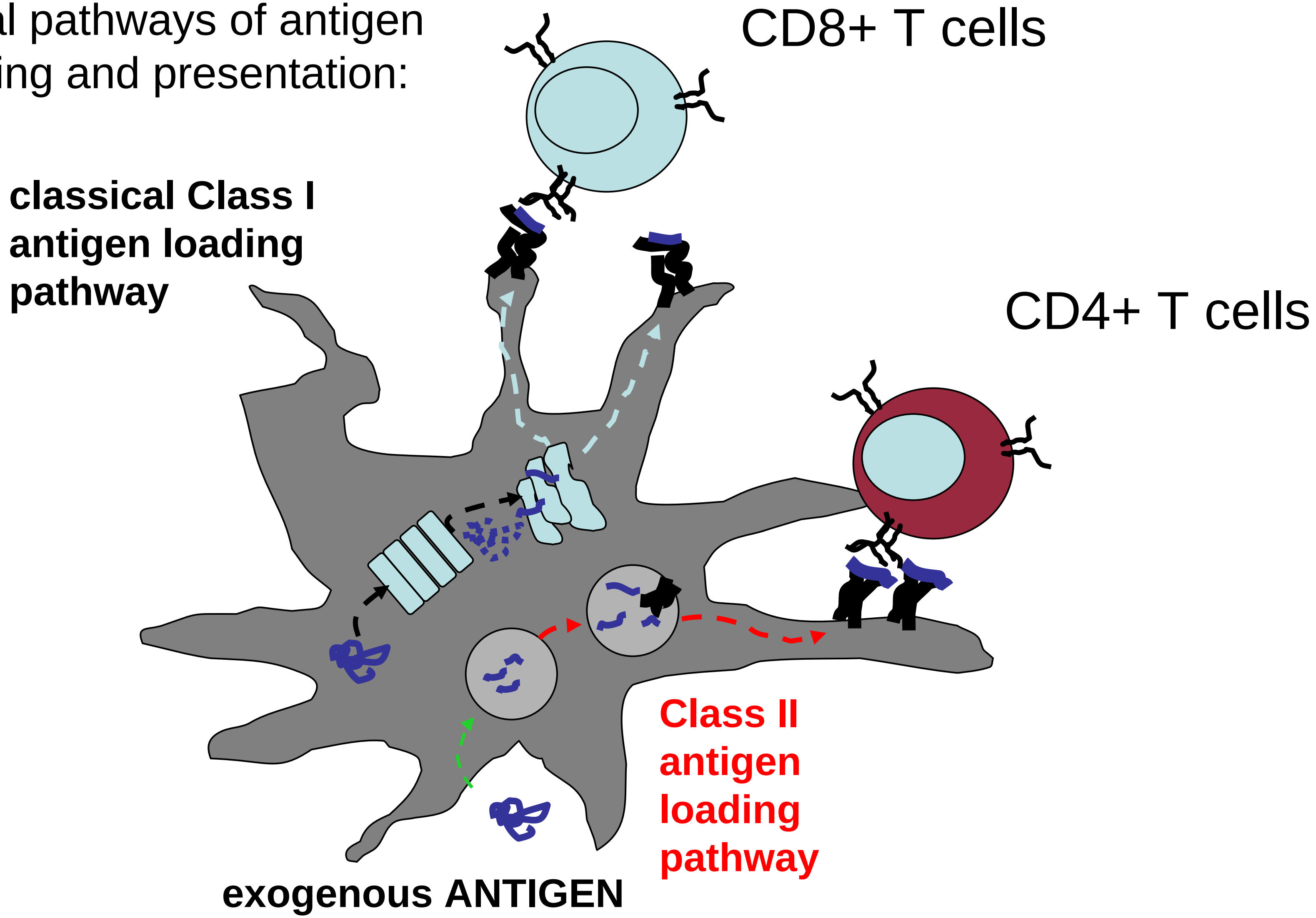




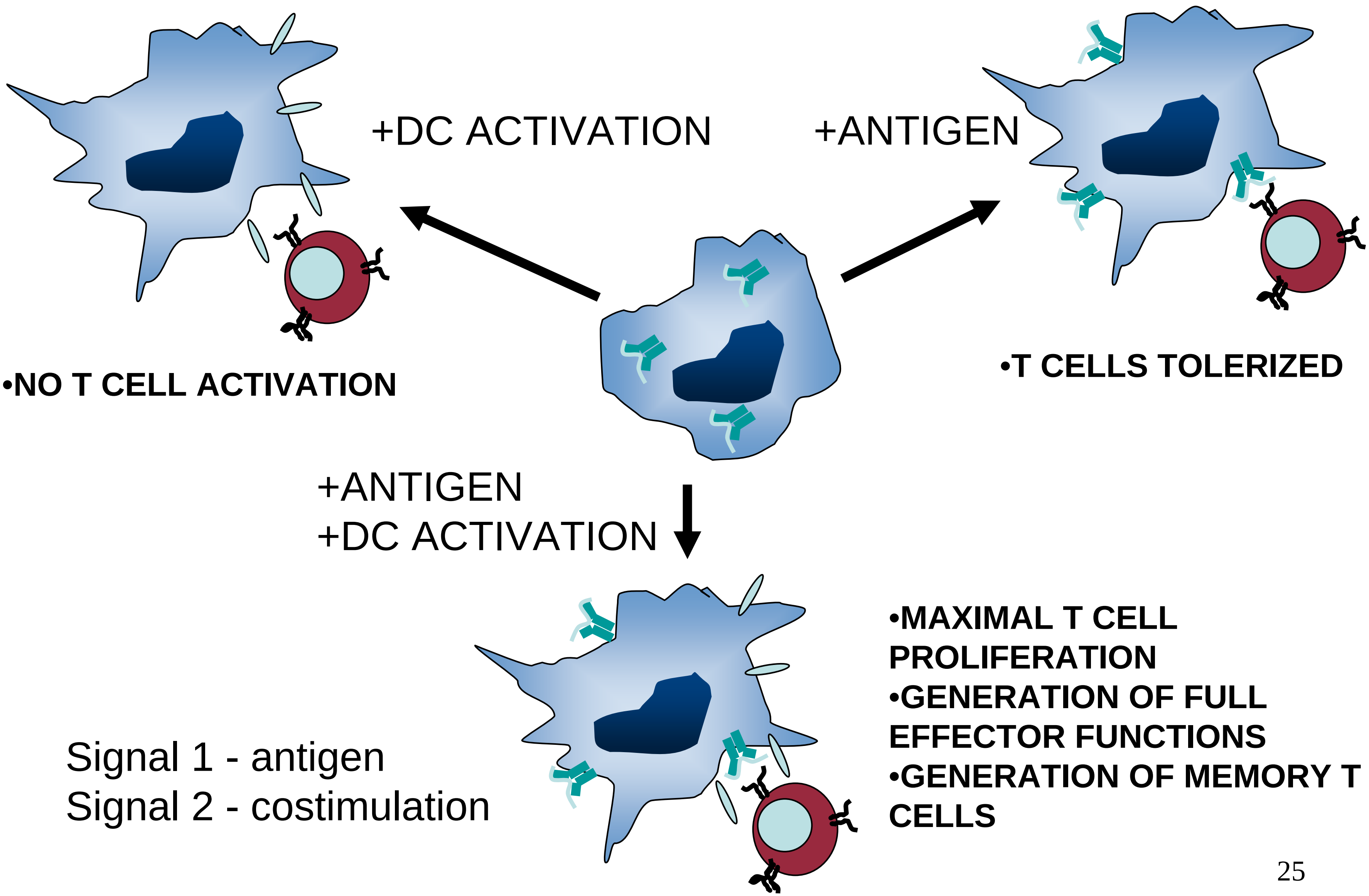
1) Chemotaxis:
Migration ‘up’ concentration gradients of chemoattractant

Biology of dendritic cells in T cell activation

Classical pathways of antigen processing and presentation:



Antigen is *one* of (at least) *two* signals that must be delivered by a vaccine



Signal 1 - antigen
Signal 2 - costimulation

B cell activation

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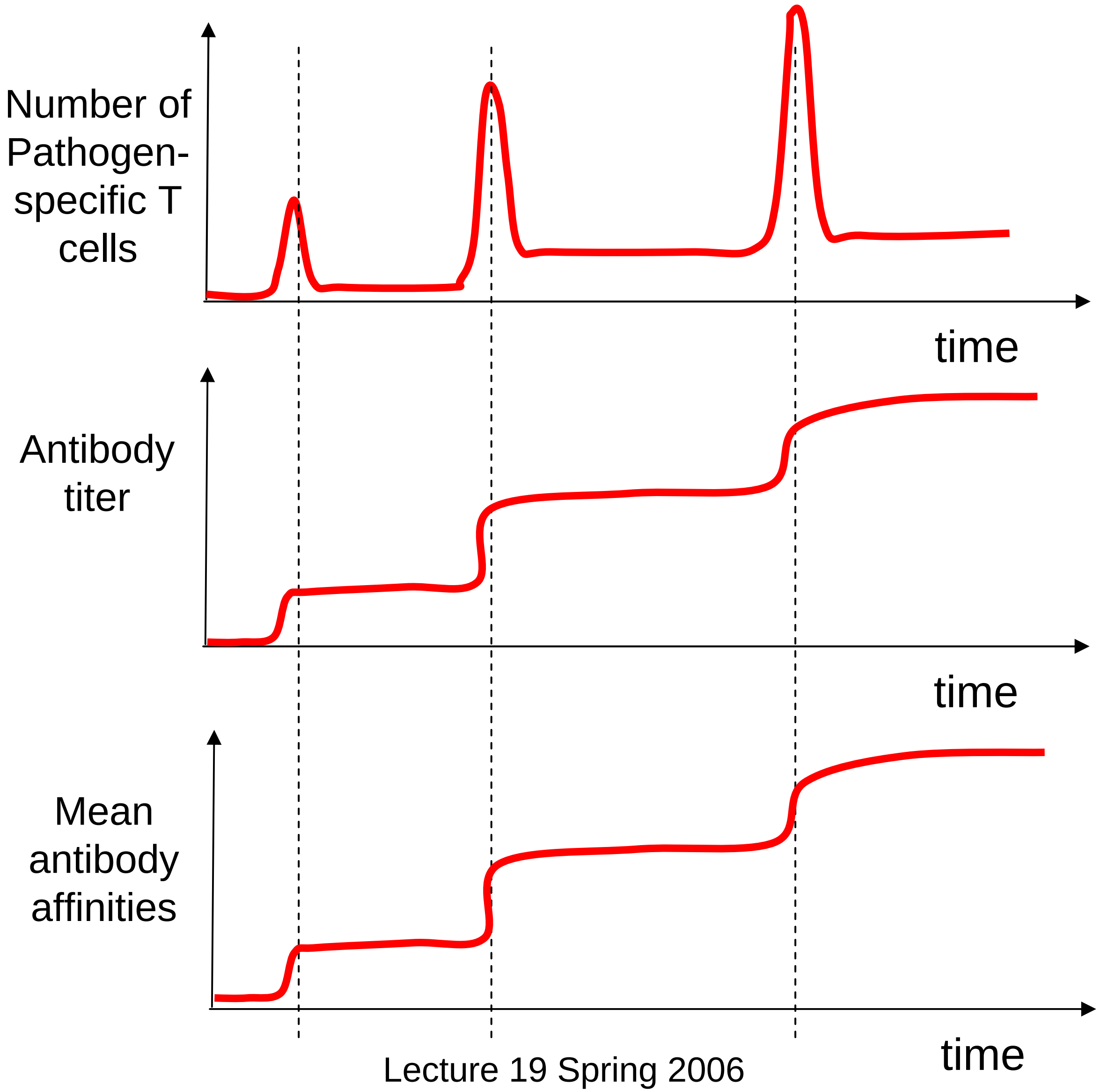
Please see: Abbas, A. K., and A. H. Lichtman. *Cellular and Molecular Immunology*. San Diego, CA: Elsevier, 2005. ISBN: 1416023895.

Induction of immunological memory (the basis of vaccination)



OBJECTIVES OF VACCINATION

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Please see: Neutra, and Kozlowski. *Nat Rev Immunol* 6 (2006): 148-158.

Prophylactic vs. therapeutic immunization

Two situations where vaccination is of interest:

(1) Therapeutic vaccine:

(2) Prophylactic vaccine:

ROUTES OF IMMUNIZATION

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Please see: "Mitragotri." *Nat Rev Immunol* 5 (2005): 905-916.

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).
11. Oupicky, D., Parker, A. L. & Seymour, L. W. Laterally stabilized complexes of DNA with linear reducible polycations: strategy for triggered intracellular activation of DNA delivery vectors. *J Am Chem Soc* **124**, 8-9 (2002).
12. Ewert, K. et al. Cationic lipid-DNA complexes for gene therapy: understanding the relationship between complex structure and gene delivery pathways at the molecular level. *Curr Med Chem* **11**, 133-49 (2004).
13. Martin-Herranz, A. et al. Surface functionalized cationic lipid-DNA complexes for gene delivery: PEGylated lamellar complexes exhibit distinct DNA-DNA interaction regimes. *Biophys J* **86**, 1160-8 (2004).
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).

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. Li, Y. et al. PEGylated PLGA nanoparticles as protein carriers: synthesis, preparation and biodistribution in rats. *J Control Release* **71**, 203-11 (2001).
3. Stolnik, S., Illum, L. & Davis, S. S. Long Circulating Microparticulate Drug Carriers. *Advanced Drug Delivery Reviews* **16**, 195-214 (1995).
4. Kozlowski, A. & Harris, J. M. Improvements in protein PEGylation: pegylated interferons for treatment of hepatitis C. *J Control Release* **72**, 217-24 (2001).
5. Harris, J. M. & Chess, R. B. Effect of pegylation on pharmaceuticals. *Nat Rev Drug Discov* **2**, 214-21 (2003).
6. Efremova, N. V., Bondurant, B., O'Brien, D. F. & Leckband, D. E. Measurements of interbilayer forces and protein adsorption on uncharged lipid bilayers displaying poly(ethylene glycol) chains. *Biochemistry* **39**, 3441-51 (2000).
7. Halperin, A. Polymer brushes that resist adsorption of model proteins: Design parameters. *Langmuir* **15**, 2525-2533 (1999).
8. Allen, T. M. & Cullis, P. R. Drug delivery systems: entering the mainstream. *Science* **303**, 1818-22 (2004).