Stealth particles (continued)
Biology of vaccination

<table>
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<tr>
<th>Last Time:</th>
<th>carriers continued; avoiding the RES</th>
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<td>Today:</td>
<td>polymer brush theory for protein resistant stealth particles basic biology of primary immune responses and vaccination</td>
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Supplementary Reading:  

ANNOUNCEMENTS:  

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

Accessory journal articles will be posted this afternoon
‘stealth’ particles: avoiding the reticuloendothelial 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

\[ \sigma \quad \text{Area per chain} \]

\[ L_0 = \text{Relaxed layer thickness} \]

Protein modeled as an impenetrable sphere of radius \( R \)

End-grafted polymer layer

Two situations:

Primary adsorption \( (U_{in}) \)

Secondary adsorption \( (U_{out}) \)
Attractive potential

MODEL ATTRACTIONS AS VAN DER WAALS FORCES (IONIC INTERACTIONS, SCREENED, DEBYE LENGTH < 1 nm; ALSO THEORETICALLY EXTREMELY COMPLEX)

\[ U_{\text{att}}(z) = -\frac{A_R kT}{6z} \]

HAMAKER CONSTANT

Repulsive potential

AXELANDER / DE GENNES

\[ U_{\text{rep}}(z) \]

ASSUMES CHAINS ALL UNIFORMLY STRETCHED

OSMOTIC PRESSURE:

\[ RT = \frac{kT}{\sigma^{3/2}} \]

3 REPULSIVE ENERGY OF

CONCENTRATING POLYMER

\[ U_{\text{brush}} = \left( \frac{kT}{\sigma^{3/2}} \right)^3 \]

NO DEPENDENCE ON \( L_0 \)!
Total potential:

\[ F = -\frac{\partial U}{\partial z} \]

RELATES TO FORCE PROTEIN FEELS!

FOR REAL SYSTEMS:

CONTROLS KINETIC PROTEIN ADSORPTION RESISTANCE

KINETIC BARRIER TO ADSORPTION

DETERMINE THERMODYNAMIC RESISTANCE

U(z)

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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

\[ \theta = \frac{K_{IN} C}{1 + K_{IN} C} \]

\[ K_{IN} = K_0 e^{-U_{IN}/kT} \]

\[ U_{IN} = U_{AOS} + \frac{kTR^3}{\sigma^{3/2}} \]

\[ R < L_0 \]

\[ \text{EQUILIBRIUM} \]

\[ \text{PROTEIN BINDING:} \]

\[ \text{MEASURE} \ \theta, \ \text{GET} \ K_{IN} \]
Achieving protein-resistant stealth particles

What condition for equilibrium primary protein adsorption resistance?

\[ U_{\text{in}} \geq kT \quad \text{for stable adsorption} \]

\[ U_{\text{in}} \geq 1 \]

\[ \frac{U_{\text{ads}}}{kT} + \frac{R^3}{\sigma^*} \geq 1 \]

\[ \sigma^* = \frac{R^2}{\left(1 - \frac{U_{\text{ads}}}{kT}\right)^{2/3}} = \frac{R^2}{\left(1 - \frac{U_{\text{ads}}}{kT}\right)^{2/3}} \]

\[ \sigma^*/kT \gg 1 \]
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.

Kinetic protein resistance:
Depends on $L_0$ and $\sigma$, but $\kappa R$ dependence still dominates

Figure removed for copyright reasons.
Comparison of theory with experiment

Surface plasmon resonance measurements:

Figure removed for copyright reasons.

Comparison of theory with experiment

Figure removed for copyright reasons. 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

e.g. Pluronics:
- PEO
- PPO

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Example stealth particle results:
PEGylated PLGA nanoparticles

1. Poly(vinyl alcohol): Adsorbs to surface of organic droplets to provide initial stability to forming spheres.
2. Block copolymer localizes at organic/aq. solution interface.
3. PEG = 5KDa, PLGA = 40 KDa

Fig. 1. Structure of the PEG–PLGA copolymer.
Double emulsion synthesis

PEG = 5KDa, PLGA = 40 KDa

Surface steric barrier

PEG chains line inner aq. compartments-minimize protein denaturation

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

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TEM of nanoparticles

Release properties of diblock particles

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

Altered biodistribution:

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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.

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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

- **Pegasparagase** (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
  - Treatment 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

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Delivery into cells once the target tissue is reached:
Cell type-dependent endocytosis limits
Internalization of 200nm-diam particles by carcinoma cell line:

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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

Figure removed due to copyright restrictions.
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

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THE CLONAL IMMUNE SYSTEM

\[ \Rightarrow 10^{12} \text{ TOTAL T CELLS IN ADULT HUMAN} \]

\[ \Rightarrow 25-100 \times 10^6 \text{ DISTINCT CLONES} \]

\[ \Rightarrow \text{ONLY SEVERAL 1000 T CELLS AT MOST RESPOND TO ANY INDIVIDUAL ANTIGEN} \]

**Precursor frequency of antigen-specific cells:**
- **CD8\(^+\)** T CELLS: 1 in 200,000
- **CD8\(^+\)** T CELLS MAY EXPAND \( \approx 100,000 \) FOLD DURING RESPONSE

Physiology of the primary immune response

1. IMMUNIZATION OR INFECTION
2. APC \rightarrow DENDRITIC CELL
3. ANTIGEN DRAINS THRU LYMPHATICS DIRECTLY TO B CELLS
4. T CELL ACTIVATION ALL T CELLS SCAN LN WITHIN 48 HOURS
5. KILL INFECTED CELLS ETC.
6. GENERATE MEMORY CELLS

PERIPHERAL TISSUE

LYMPH NODE

BLOOD

B cell
T cell
Dendritic cell

4) Activation of naïve T cells in the lymph nodes

3) Trafficking to lymph nodes

2) Antigen loading and activation

1) Attraction to sites of infection

- Pathogen-associated molecular patterns (PAMPs)
- ‘Danger’ signal

Chemokines

DC

1% of cells in tissue

Infected cells

Infection site

1) Chemotaxis:
Migration ‘up’ concentration gradients of chemoattractant
Biology of dendritic cells in T cell activation

Classical pathways of antigen processing and presentation:

- **Classical Class I antigen loading pathway**
- **Exogenous ANTIGEN**
- **Class II antigen loading pathway**

CD8+ T cells

CD4+ T cells
Antigen is one of (at least) two signals that must be delivered by a vaccine.

- Signal 1 - antigen
- Signal 2 - costimulation

+ANTIGEN
+DC ACTIVATION

• MAXIMAL T CELL PROLIFERATION
• GENERATION OF FULL EFFECTOR FUNCTIONS
• GENERATION OF MEMORY T CELLS

+DC ACTIVATION
+ANTIGEN

• T CELLS TOLERIZED

• NO T CELL ACTIVATION
B cell activation

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Induction of immunological memory (the basis of vaccination)

Number of Pathogen-specific T cells

Antibody titer

Mean antibody affinities

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OBJECTIVES OF VACCINATION

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Prophylactic vs. therapeutic immunization

Two situations where vaccination is of interest:

(1) Therapeutic vaccine:

(2) Prophylactic vaccine:
ROUTES OF IMMUNIZATION

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Further Reading

Further Reading