Molecular/particulate drug carriers (continued)
Stealth particles

Last Time: molecular, nano, and microcarriers for drug molecules

Today: carriers continued
'stealth' particles

Reading: S. Stolnik et al. 'Long circulating microparticulate drug carriers,' *Adv. Drug Deliv. Rev*. **16**, 195 (1995)

Supplementary Reading: Halperin – theory of protein-resistant brushes
Efremova et al. – experimental test of theory with model 'stealth' liposome surfaces

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ANNOUNCEMENTS:

ALSO A REVIEW ON INTERACTIONS OF COMPLEMENT SYSTEM W/ BIOMATERIALS (RELEVANT TO TODAY'S DISCUSSION)

Last Time: MOLECULAR/PARTICULATE DRUG CARRIERS

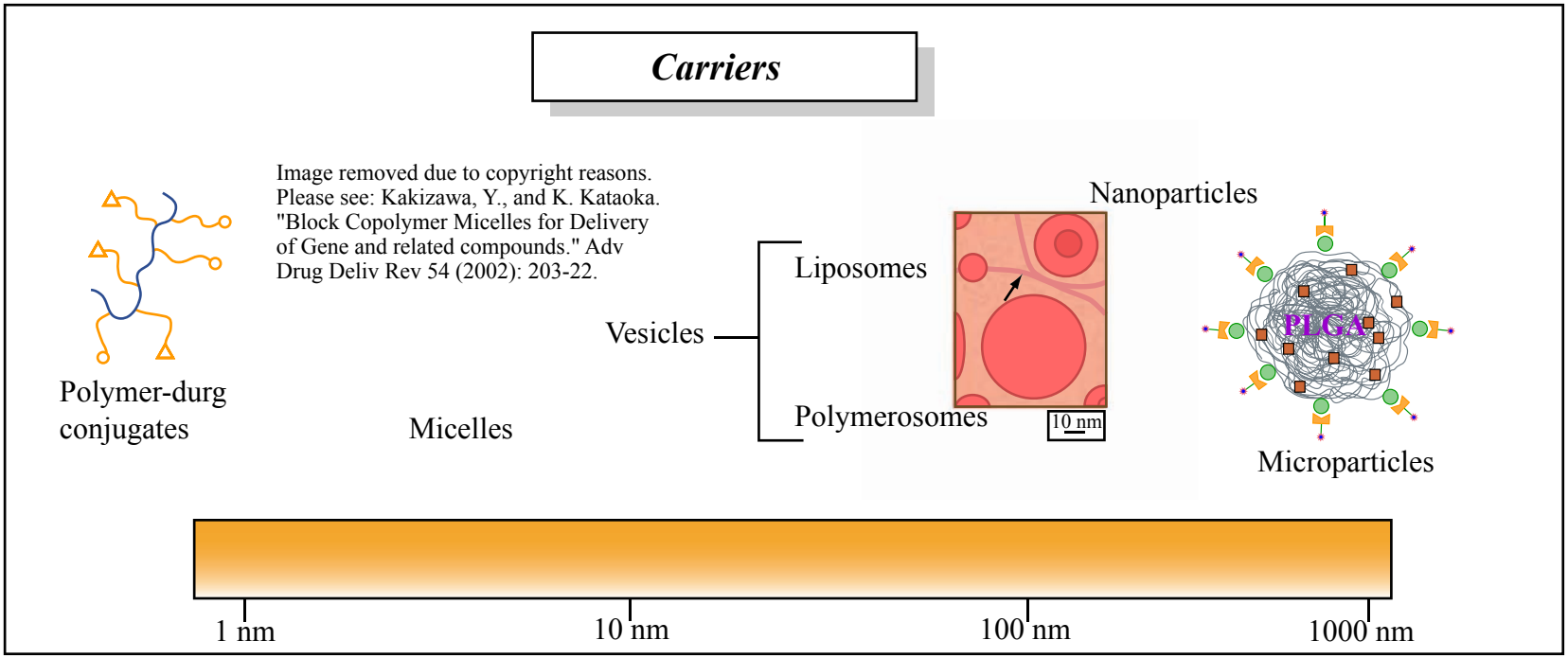


Figure by MIT OCW.

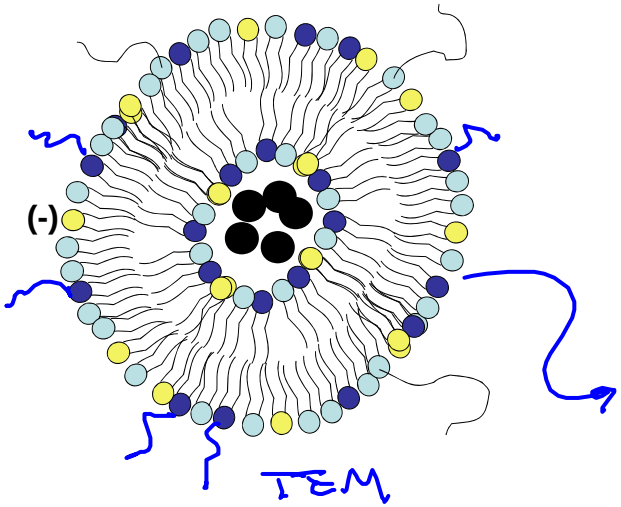
Vesicle carriers

Liposomes – lipid bilayer vesicles formed typically using phospholipids mimicking the plasma membrane of cells

Virosomes – hybrids formed by fusion of liposomes with viral particles

Polymerosomes – synthetic vesicles formed using block copolymers as analogs of small-molecule amphiphiles

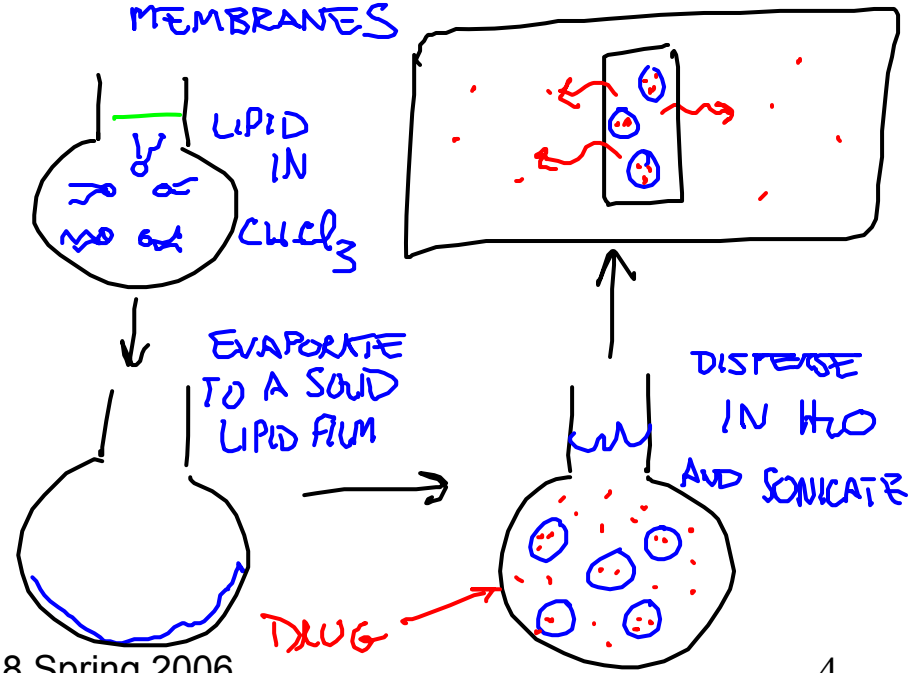
Liposome carriers



100 nm

Figure removed for copyright reasons.
Please see: Figure 2 in Bergstrand, and Edwards. *Langmuir* 17 (2001): 3245-3253.

1) PROTEIN-RESISTANT SURFACES
2) SPONTANEOUSLY FUSE WITH CELL MEMBRANES



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Please see: Bergstrand, and Edwards. *Langmuir* 17 (2001): 3245-3253.

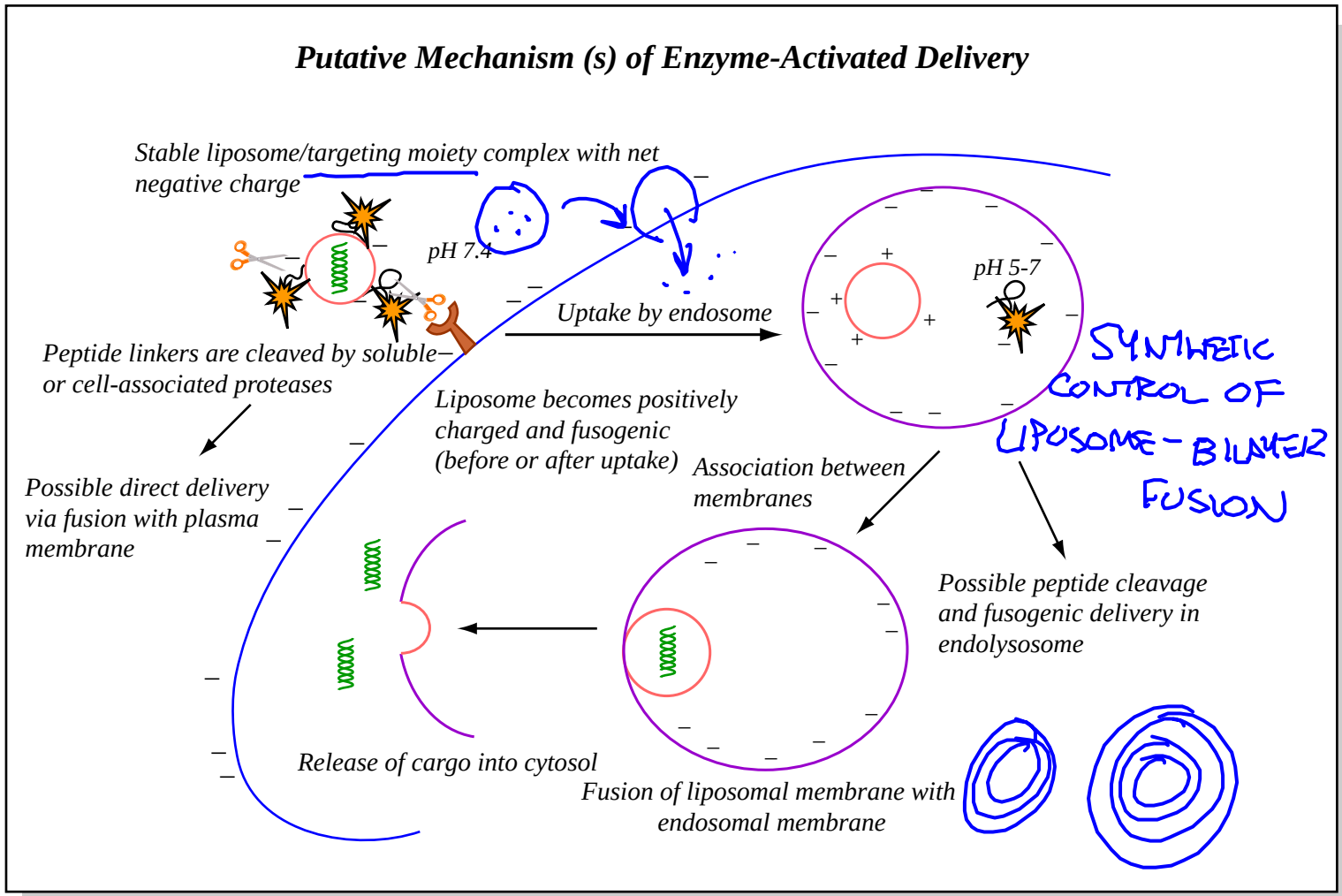
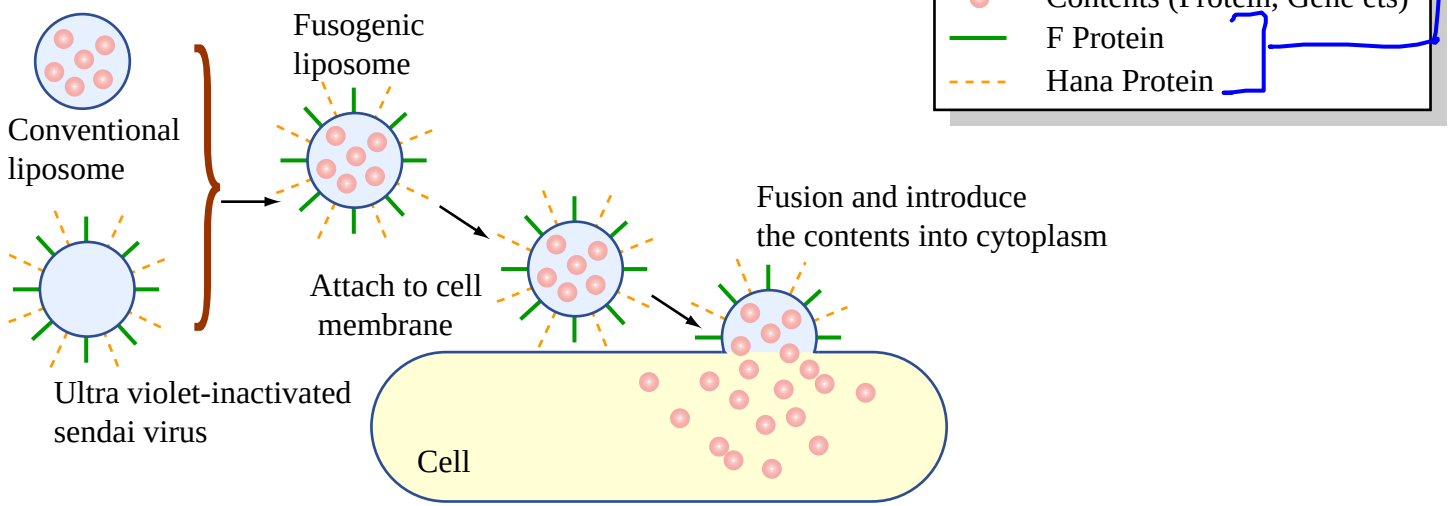


Figure by MIT OCW.

Virosomes: hybridizing synthetic liposomes with viral membranes

MANY VIRUSES HAVE VERY SMALL SIZES : 50-100 nM
↳ VERY SMALL CARGO SPACE
↳ DIFFICULT TO LOAD SYNTHETIC DRUGS INTO NATIVE VIRAL PARTICLES

CELL ENTRY PROTEINS



Features of fusogenic liposomes as efficient delivery vehicles into the cytoplasm. Fusogenic liposomes were prepared by fusing conventional liposomes with the Sendai virus at 37°C and purified by discontinuous sucrose centrifugation. Fusogenic liposomes bind to the cell surface via HANA proteins and fuse with the cell membrane with F proteins, then directly deliver encapsulated molecules into the cytoplasm.

Figure by MIT OCW.

Pros and cons of vesicular delivery

Advantages:

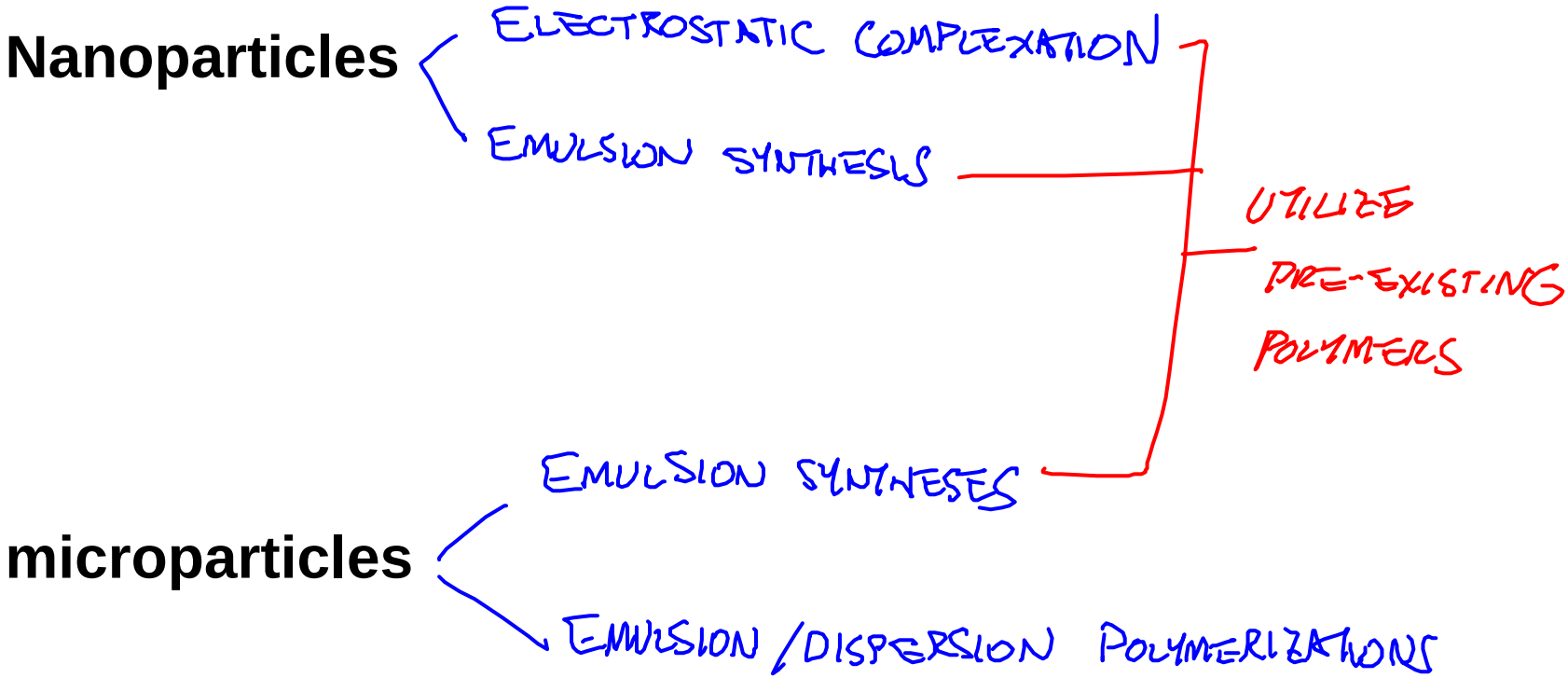
- LIPID BILAYER WHICH RESISTS OPSONIZATION
  - ANTIBODY BINDING
  - COMPLEMENT PROTEINS
- INSERT MOBILE COMPONENTS FOR CONTROLLING TARGETING/ FUSION W/ MEMBRANES

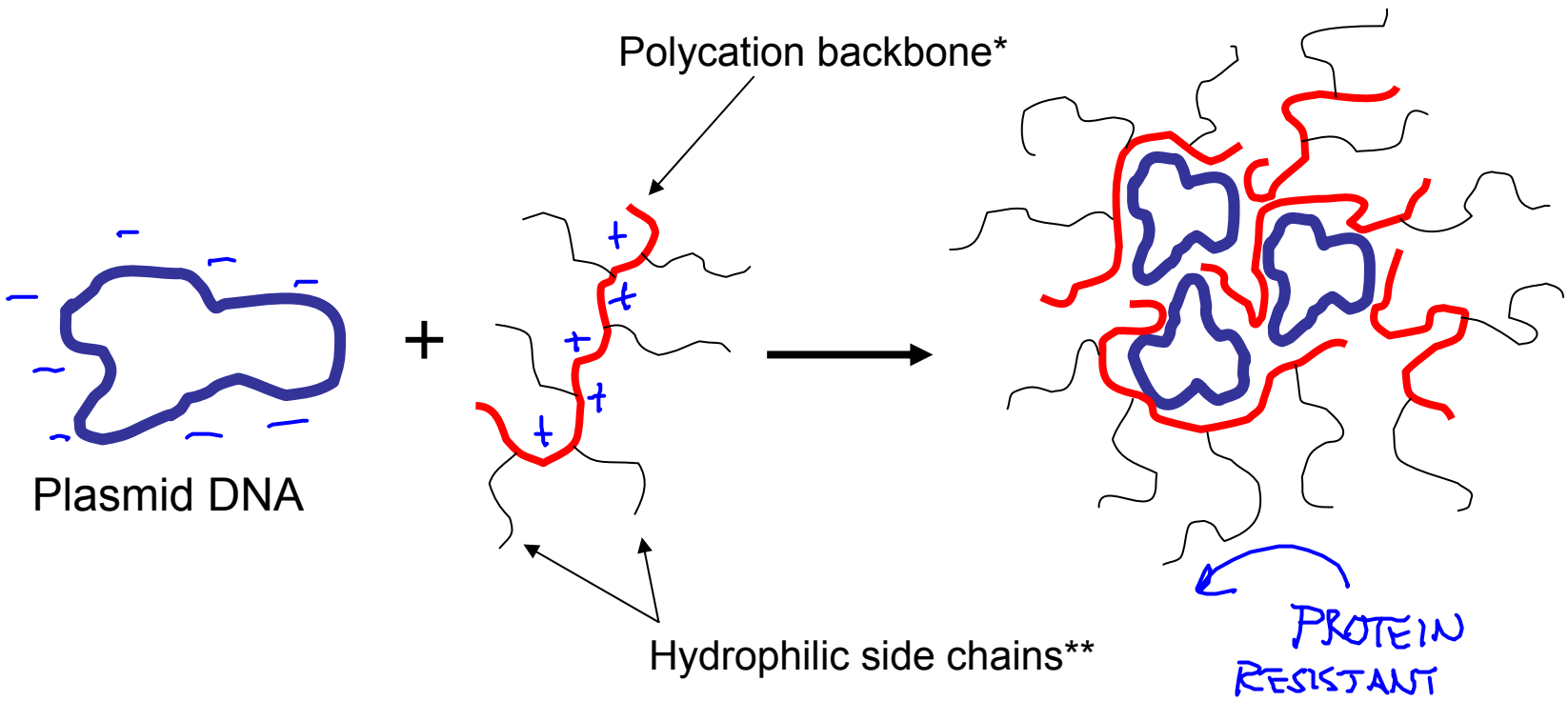
Disadvantages:

- LIPOSOMES NOTORIOUSLY 'LEAKY'
- CANNOT BE STORED / DIFFICULT TO PREPARE AS MONODISPERSE POPULATIONS

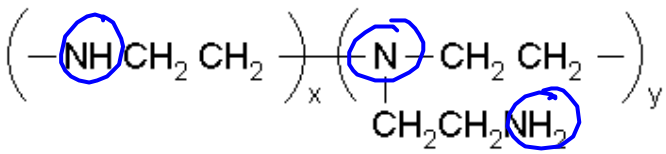
Synthetic polymer nano- and micro-particle carriers

Strategies for synthesis:



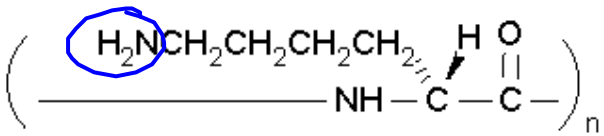


* Backbone components



PEI

pKa ≈ 9.8

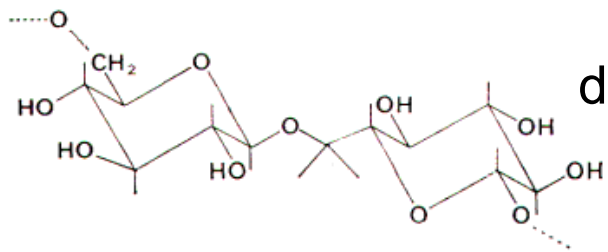


· HBr PLL

pKa ≈ 10.0-10.2

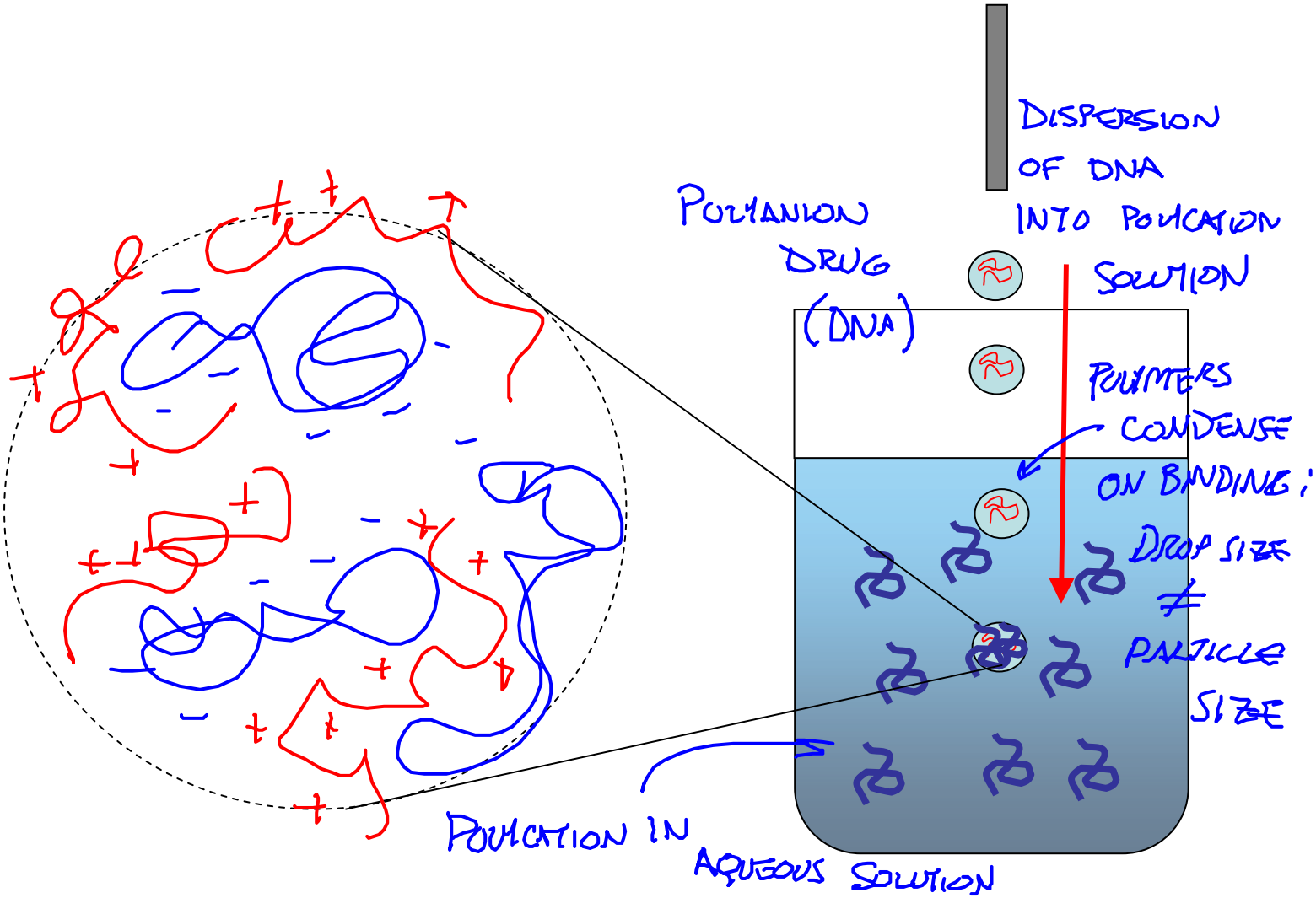
** side chain components

$-(CH_2-CH_2-O)_n-$ PEO



dextran

Synthetic polymer nano- and micro-particle carriers



Nanoparticle DNA packaging

Figure removed due to copyright reasons.
Please see: Figure 2 in Park, S., and K. E. Healy. “Nanopoarticulate DNA Packaging using Terpolymers of Poly(lysine-g-lactide-b-ethylene glycol).” *Bioconjugate Chemistry* 14 (2003): 311-319.

Protection from DNAses

Figure removed due to copyright reasons.
Please see: Figure 5 in Park, S., and K. E. Healy . “Nanopoarticulate DNA Packaging using Terpolymers of Poly(lysine-g-lactide-b-ethylene glycol).” *Bioconjugate Chemistry* 14 (2003): 311-319.

Figure removed due to copyright reasons.
Please see: Figure 6 in Park, S., and K. E. Healy. “Nanopoarticulate DNA Packaging using Terpolymers of Poly(lysine-g-lactide-b-ethylene glycol).” *Bioconjugate Chemistry* 14 (2003): 311-319.

Nanoparticle DNA packaging

Graph removed due to copyright reasons.

Please see: Wightman, et al. *J Gene Med* 3 (2001): 362-372.

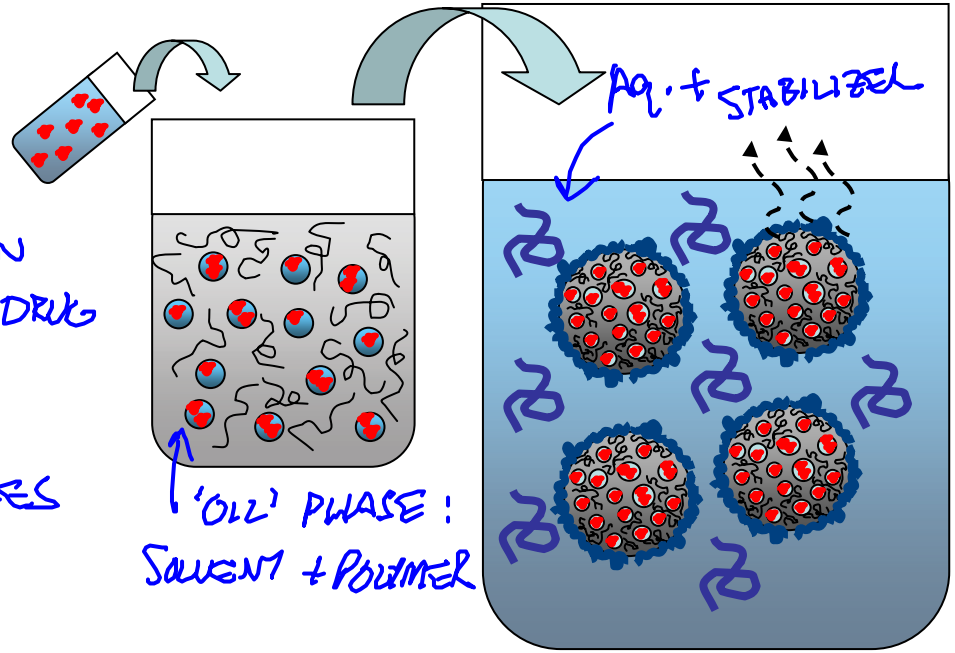
0.5X HBS (Hank's buffered saline) = 75 mM NaCl, 20 mM HEPES,2.5% glucose
0.5X HBG (HEPES-buffered glucose) = 20 mM HEPES, 5% glucose

EMULSION SYNTHESIS

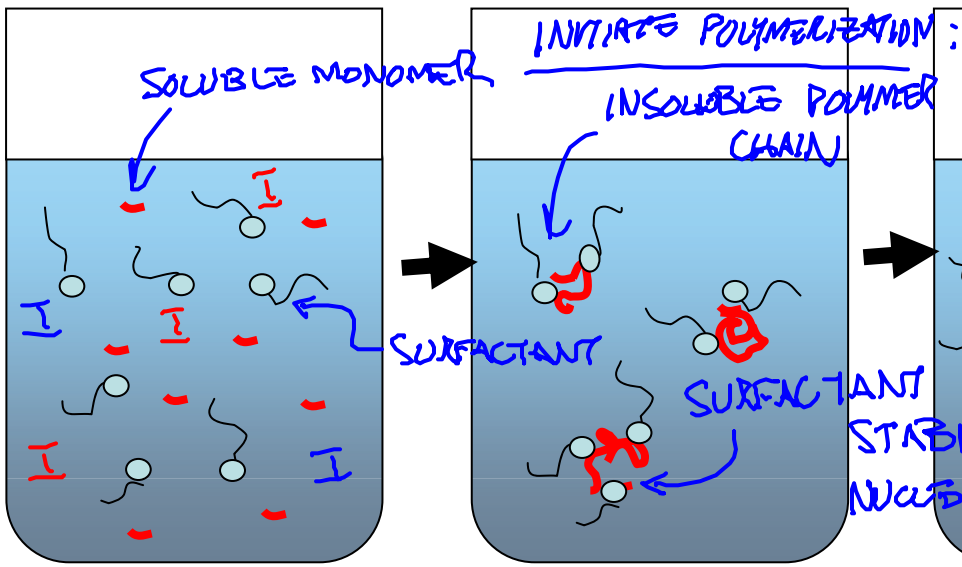
AS SMALL AS 50-100 nm

✓ POLYDISPERSE PARTICLES

✓ EASILY BIODEGRADABLE

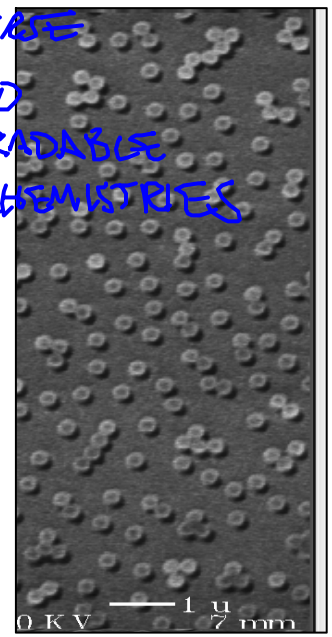


EMULSION/DISPERSION POLYMERIZATION

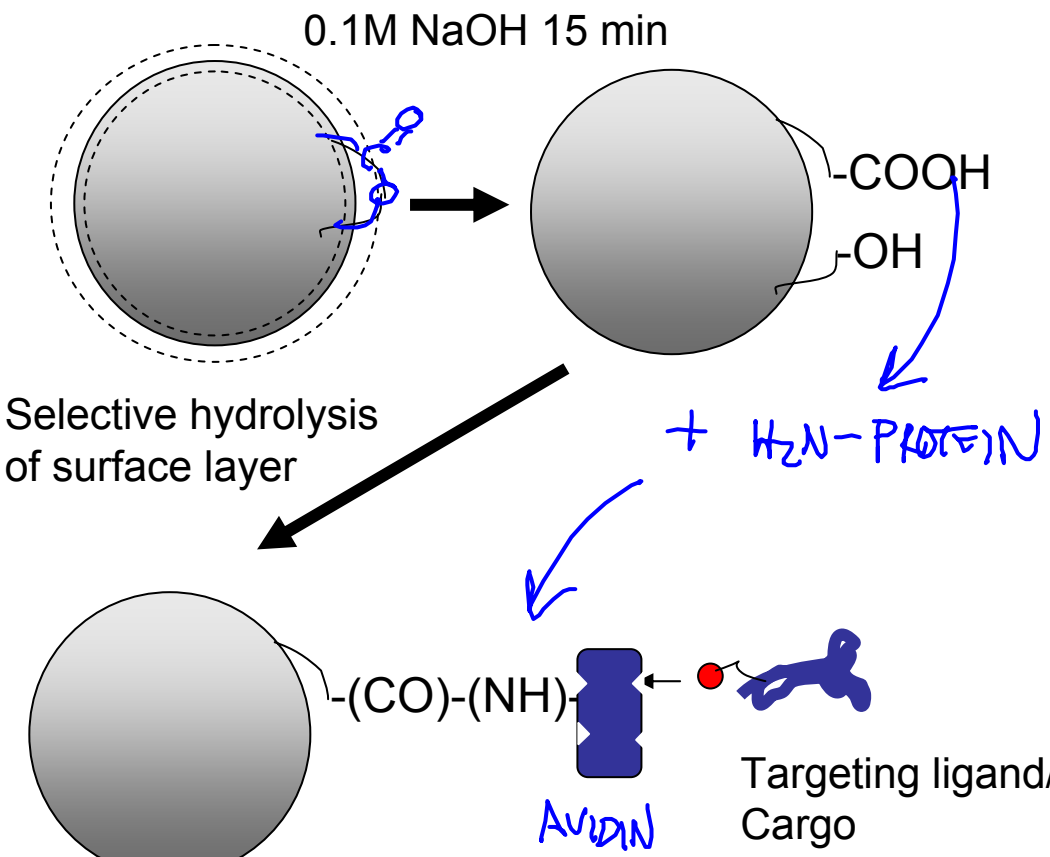


✓ MONODISPERSE

✓ DIFFICULT TO FIND BIODEGRADABLE CHEMISTRIES



Surface modification of biodegradable micro/nanoparticle carriers



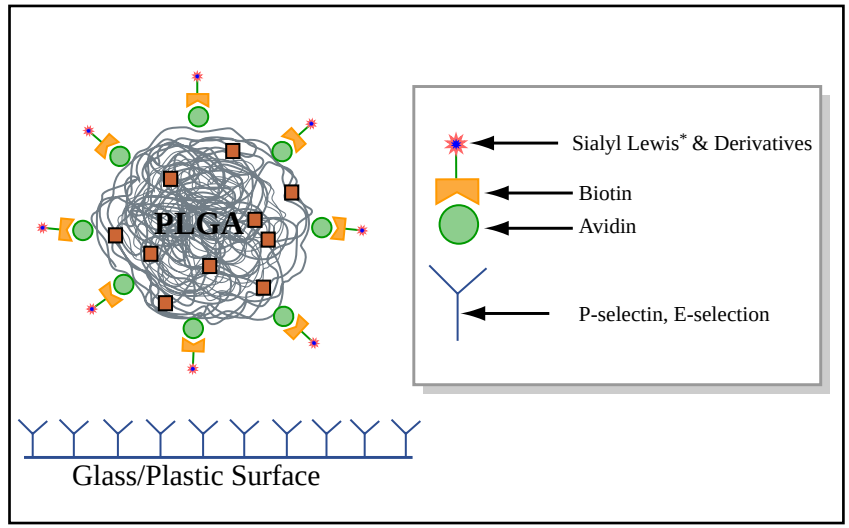
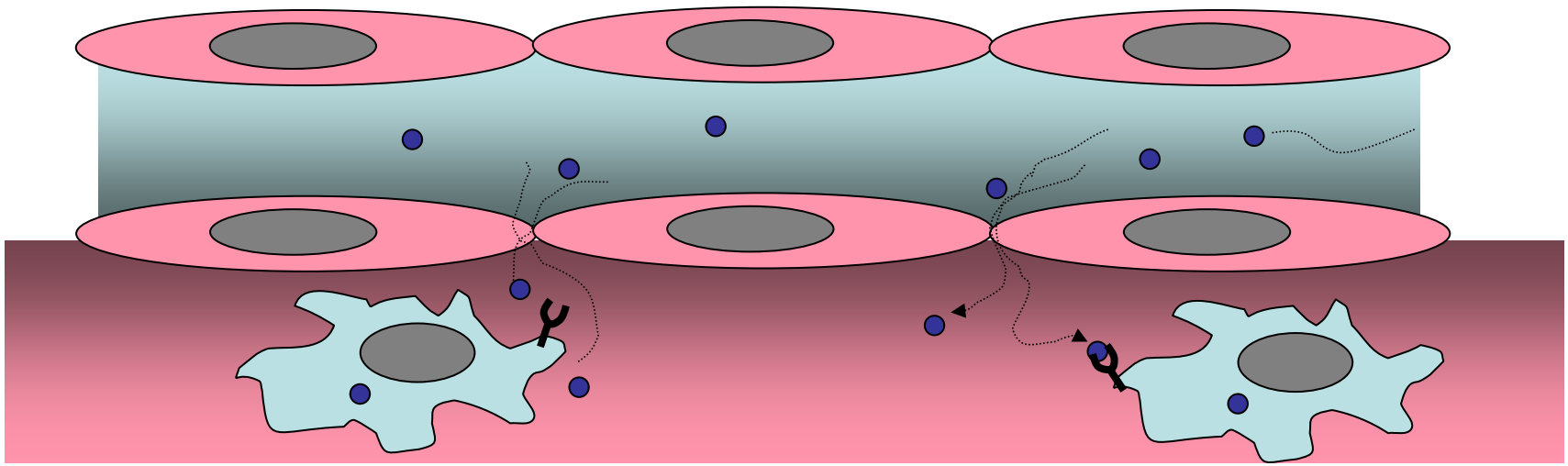


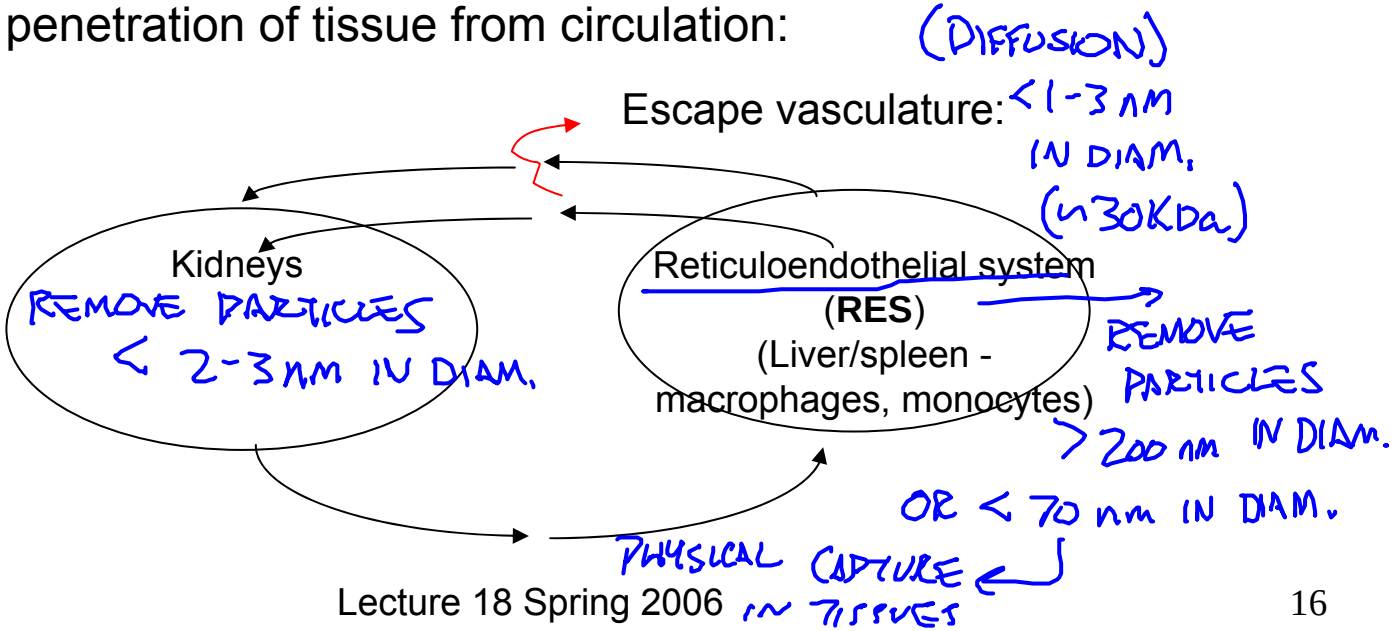
Figure by MIT OCW.

DELIVERY USING CARRIERS THROUGH SYSTEMIC/ORAL ROUTES

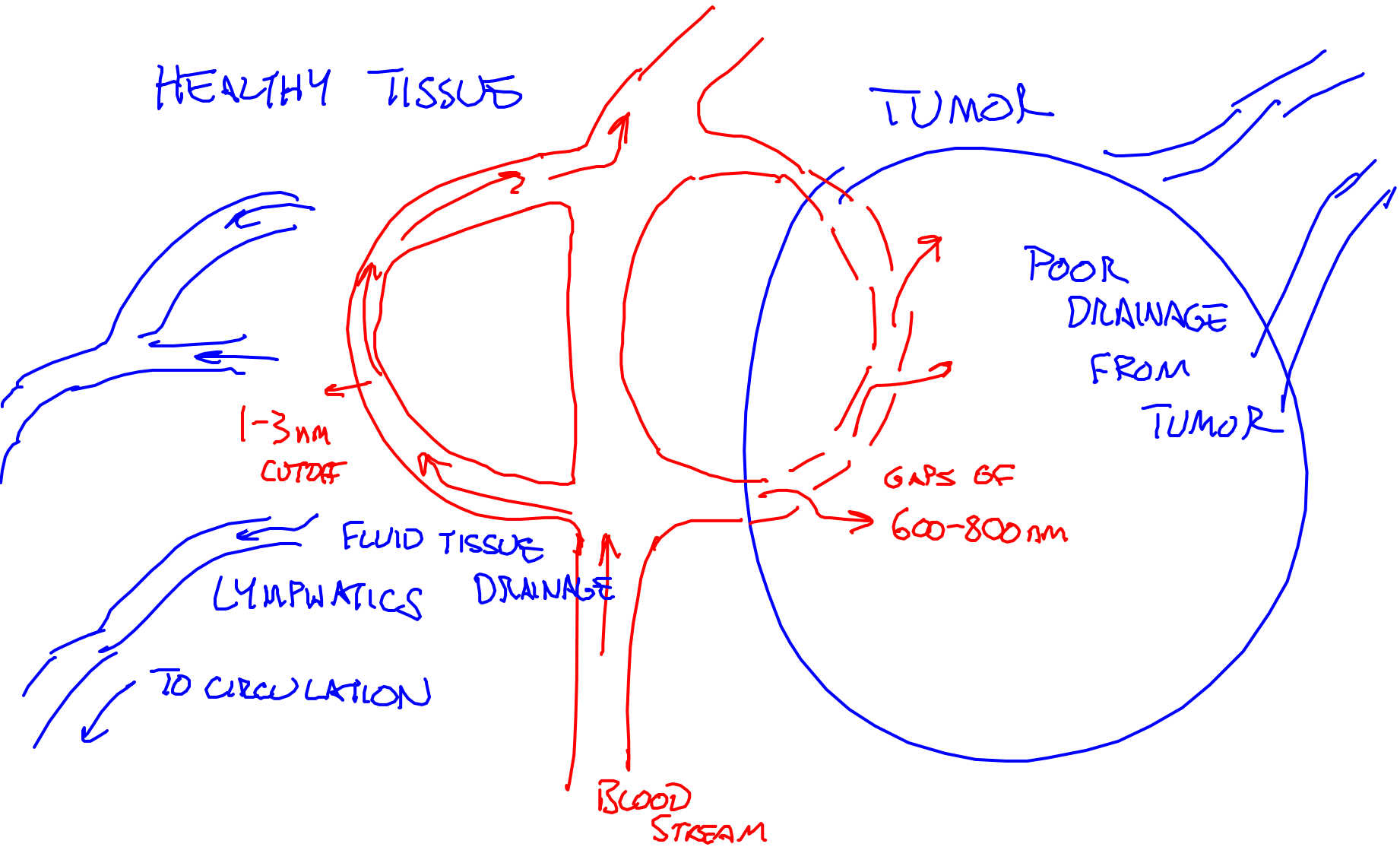
Systemic delivery from bloodstream



Size limits for penetration of tissue from circulation:



Enhanced permeation and retention (EPR) effect in tumors:



How to avoid the RES?

'STEALTH PARTICLES'

C. Van Oss (1978): showed that many bacteria which remain in circulation have a highly hydrophilic, hydrated surface layer of protein, polysaccharide, and glycoprotein

Image removed due to copyright reasons.
Please see: *Annu Rev Microbiol* 32, 19 (1978).

T. Paustian,
http://www.bact.wisc.edu/MicrotextBook/BacterialStructure/CellWall.html

F.F. Davis (1977): showed showed that poly(ethylene glycol) conjugated to a protein is non-immunogenic and greatly increased protein half-lives *in vivo*

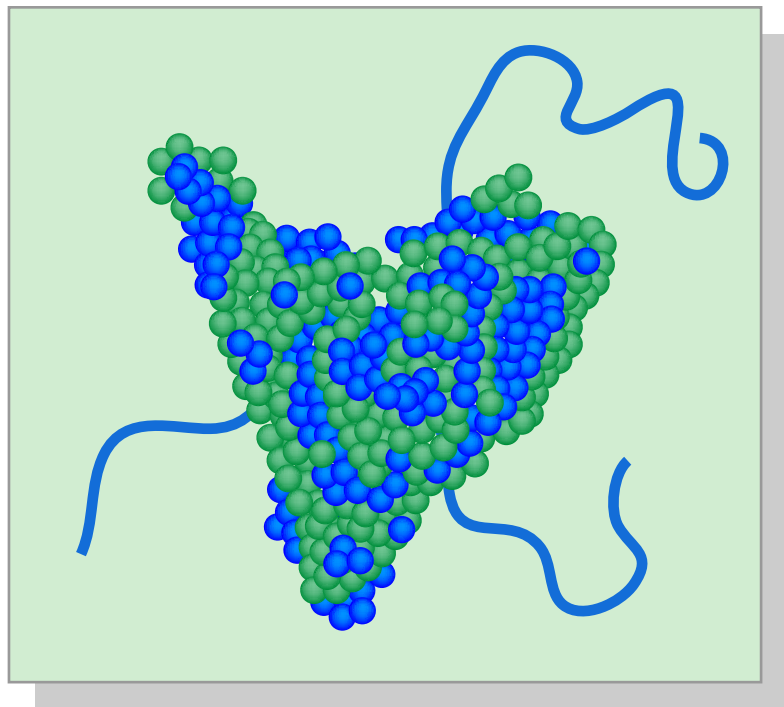
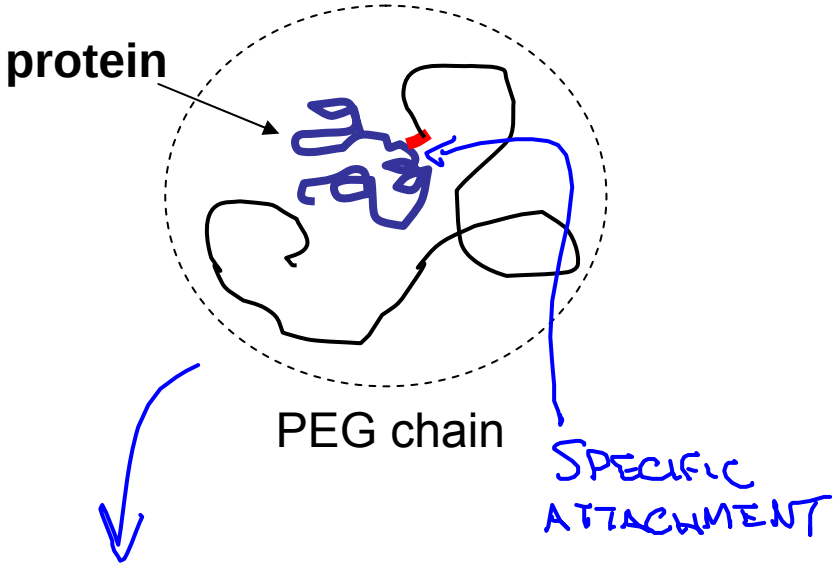


Figure by MIT OCW.
Image by MIT OCW after Davis, F.F. *Journal of Biol Chem* 252, 3578 (1977).

PEGylated molecules:



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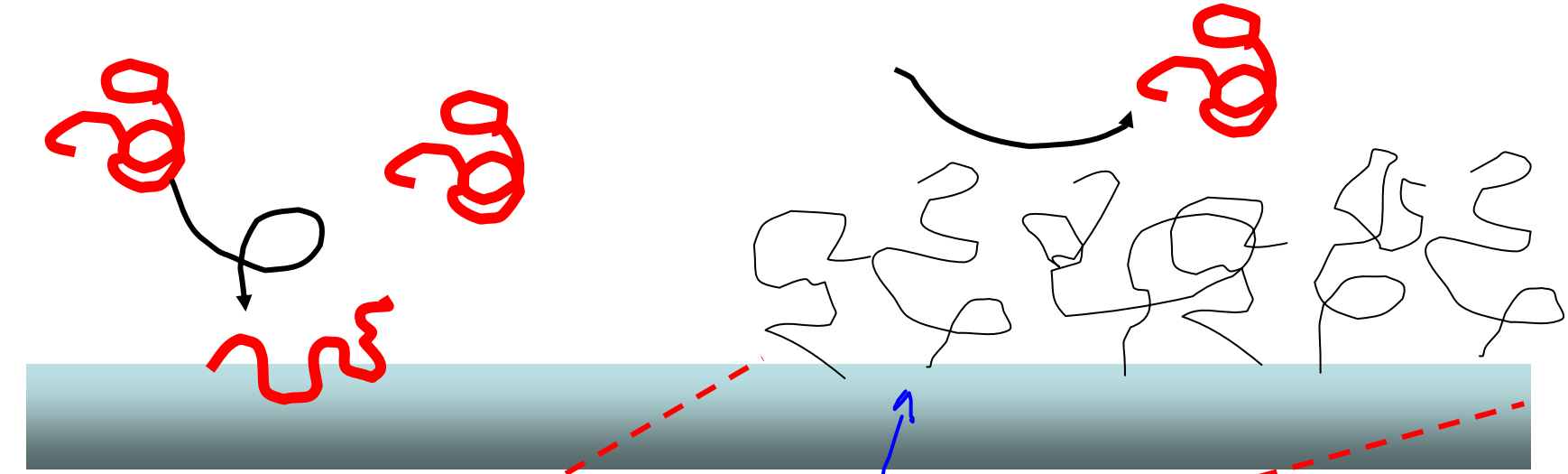
Please see: Table 1 in Harris, J. M., and R. B. Chess. "Effect of Pegylation on Pharmaceuticals." *Nat Rev Drug Discov* 2 (2003): 214-21.

Figure removed due to copyright reasons.
Please see: Figure 4 in Harris, J.M., and R.B. Chess. "Effect of Pegylation on Pharmaceuticals." *Nat Rev Drug Discov* 2 (2003): 214-21.

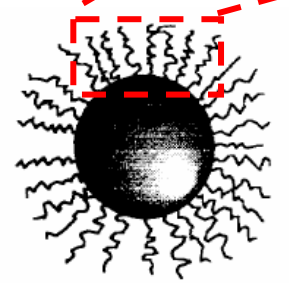
Translation to submicron carriers: 'stealth' particles



PEG OR POLYSACCHARIDE

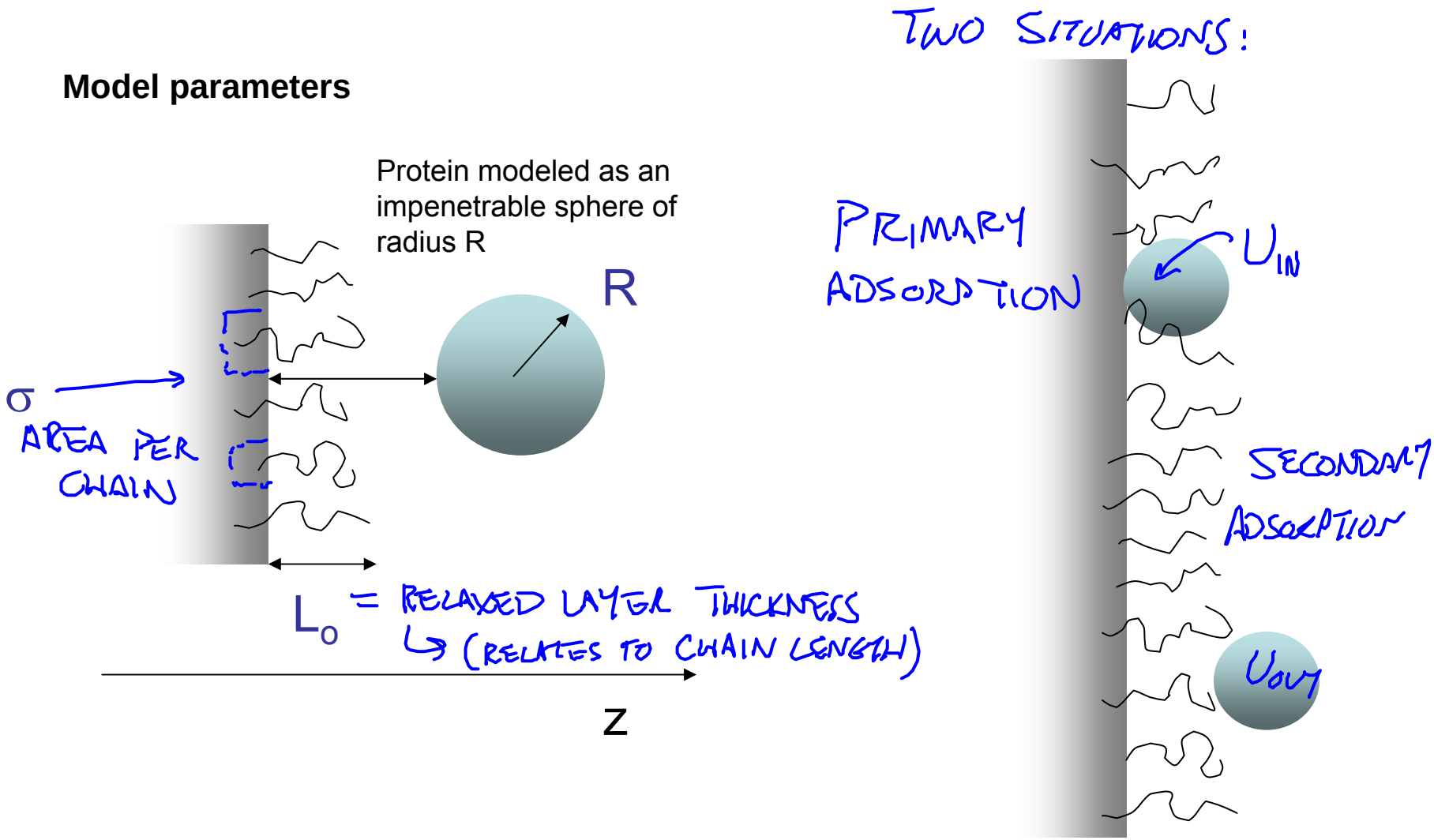
— ELIMINATES PROTEIN BINDING (→ OPSONIZATION → PHAGOCYTOSIS)

— INCREASED STABILITY OF PARTICLE IN SUSPENSION



Theory of protein-resistant surfaces

Model parameters



Attractive potential

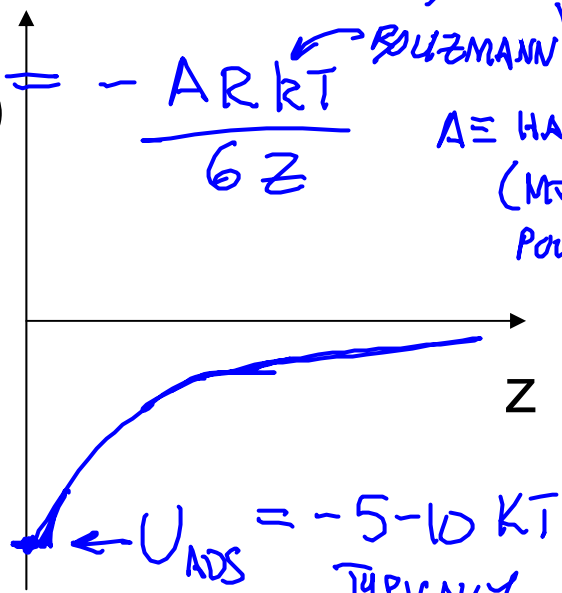
MODEL ATTRACTION AS PURELY VAN DER WAALS INTERACTIONS

↓

OFTEN SEEN TO DOMINATE IN EXPERIMENTS (SCREENING LENGTH IN BLOOD < 1nm ; IONIC STRENGTH ≈ 0.15 M) → ELECTROSTATICS VERY COMPLICATED

$U_{att}(z) = -\frac{A R k T}{6 z}$ ← BOLTZMANN CONSTANT

A ≡ HAMAKER CONSTANT (MEASURE OF PROTEIN'S POLARIZABILITY)



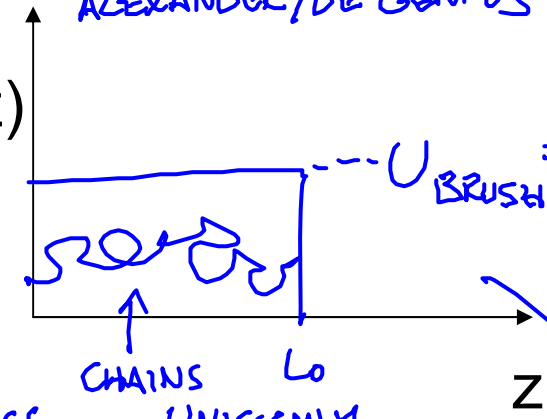
← $U_{ADS} = -5\text{-}10\ kT$ TYPICALLY

Repulsive potential

ALEXANDER/DE GENNES

OSMOTIC PRESSURE

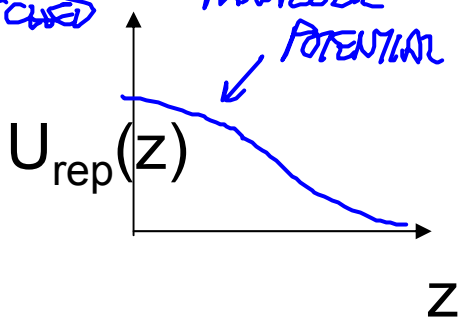
$U_{rep}(z)$



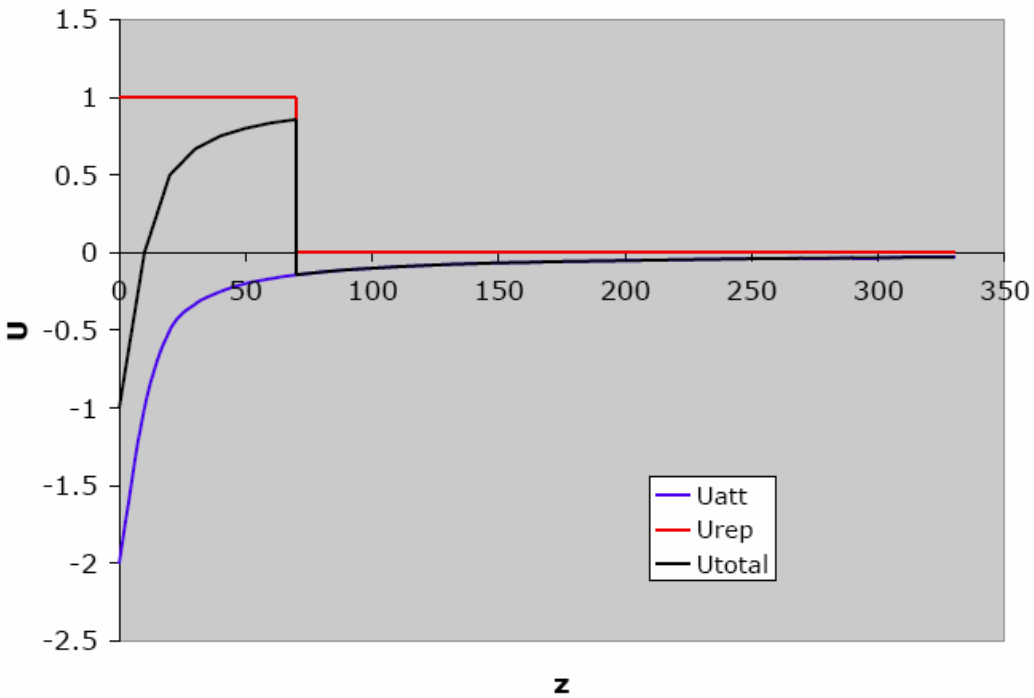
MORE REALISTIC

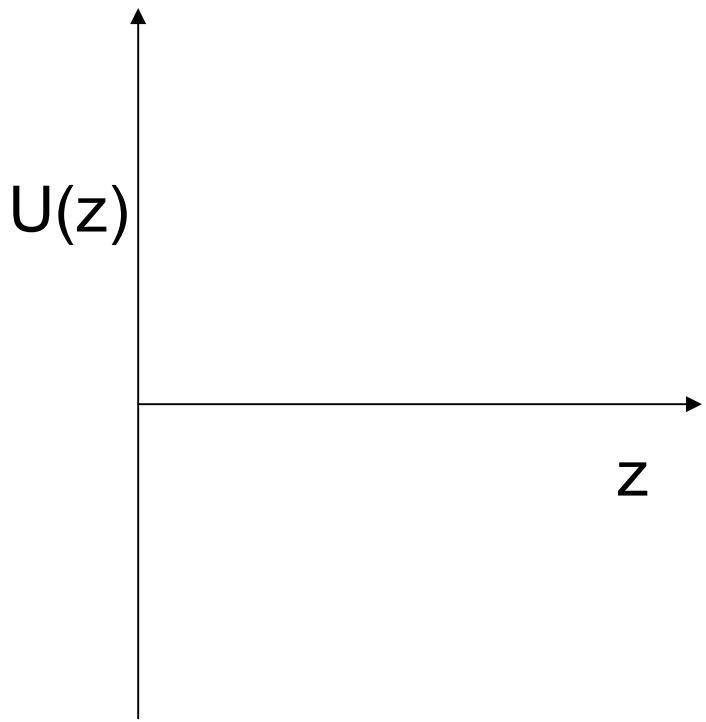
PARABOLIC POTENTIAL

$U_{rep}(z)$

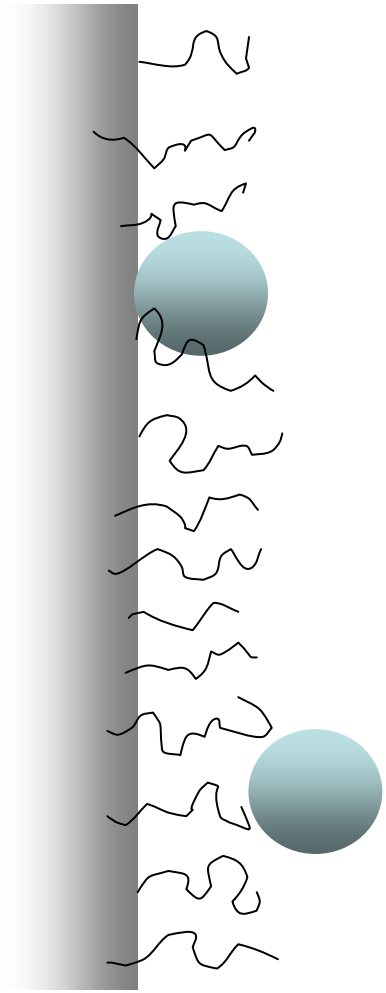


Total potential:





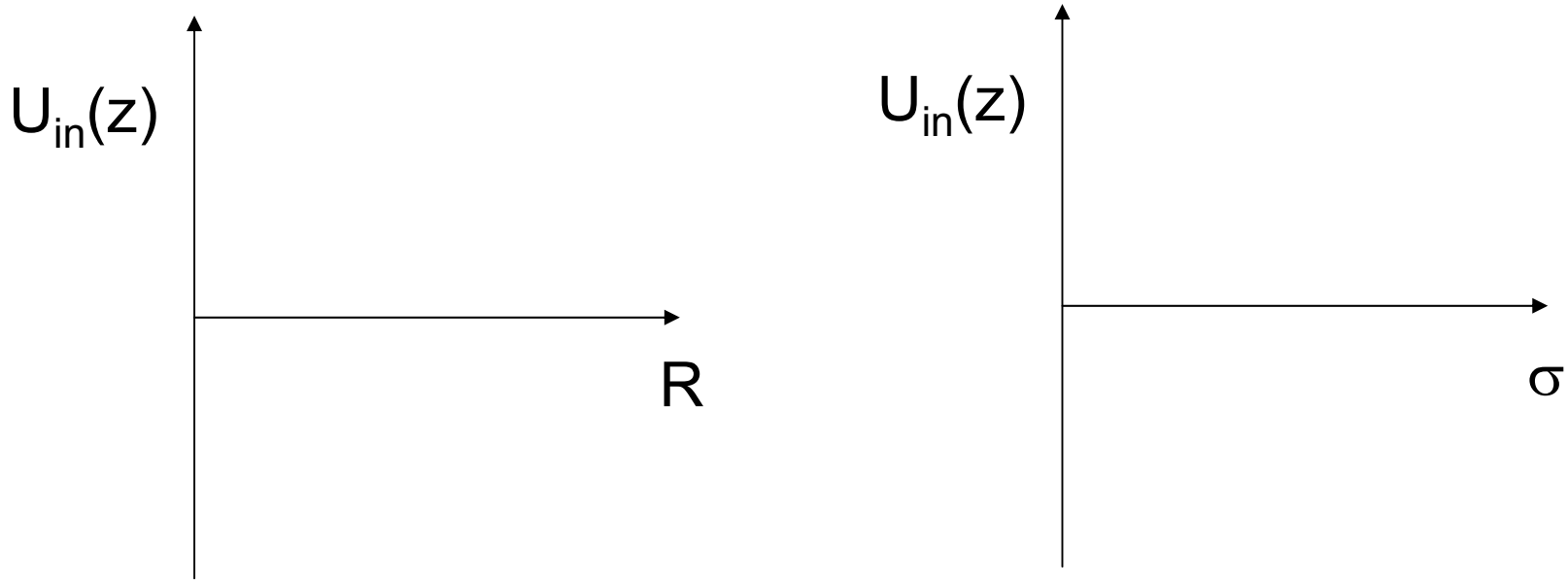
Adsorption of small proteins



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

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Please see: Figure 2 in Halperin, A. "Polymer Brushes that Resist Absorption of Model Proteins: Design Parameters." *Langmuir* 15 (1999): 2525-2533.

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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:

Figure removed for copyright reasons.
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

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.

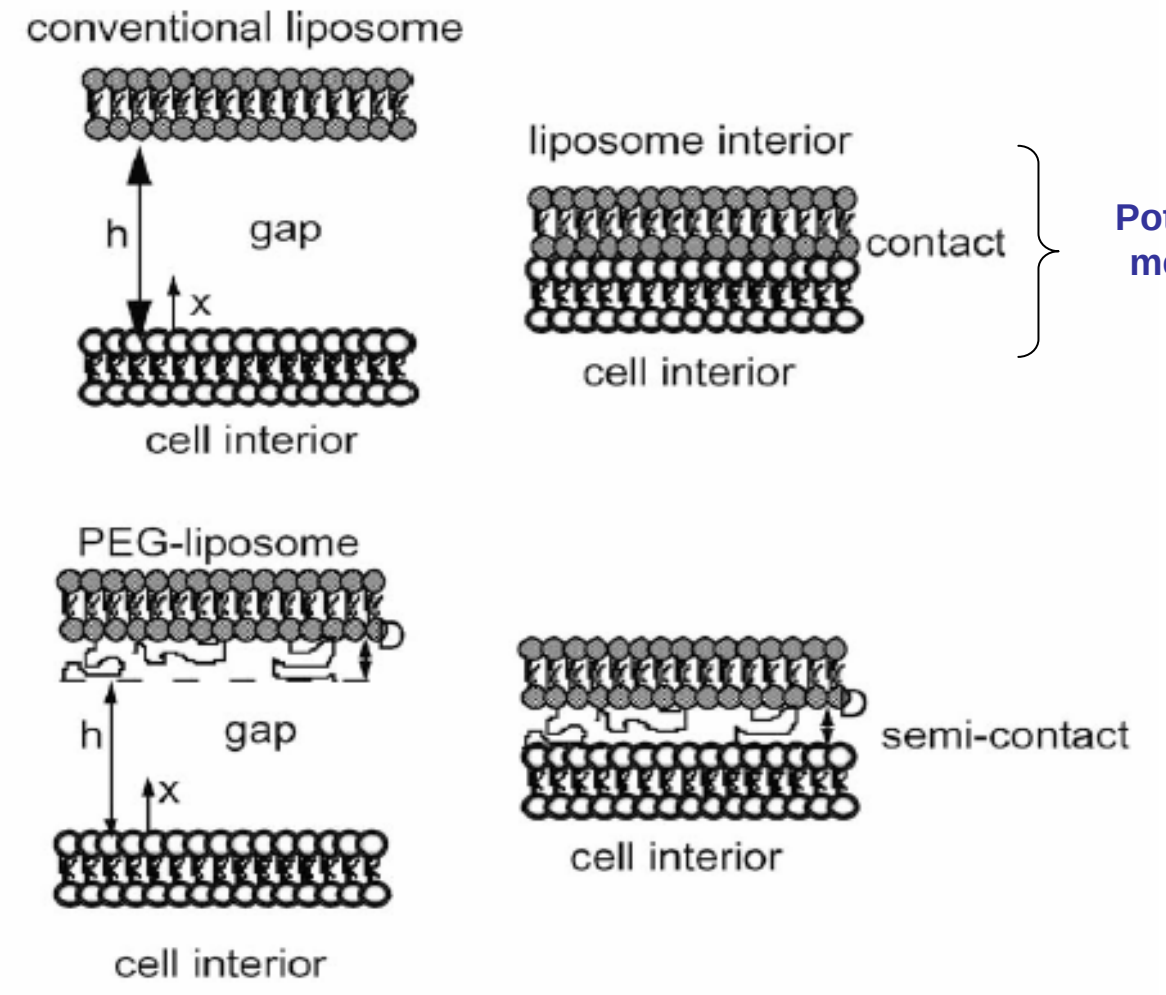
Figure removed for copyright reasons.
Please see: Figure 10 in Efremova, et al. "Measurements of Interbilayer Forces and Protein Adsorption on Uncharged Lipid Bilayers Displaying Poly(ethylene glycol) Chains." *Biochemistry* 39 (2000): 3441-51.

BPTI = bovine pancreatic trypsin inhibitor (enzyme), 6 KDa, 21x21x30 Å

HSA = human serum albumin, 66 KDa, 38x38x150 Å
FBN = fibrinogen, 340 KDa, 55x55x460 Å

Additional benefits of PEGylated carriers: improved carrier stability

Liposomes:



Synthesis of ‘stealth’ particles

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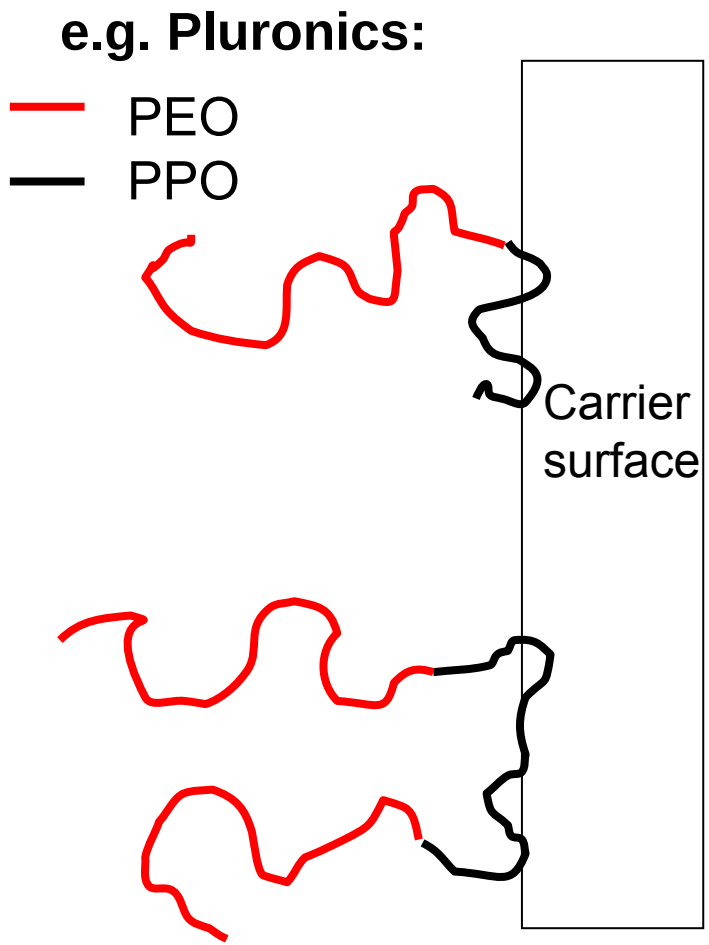
Please see: Figure 1 in Stolnik, et al. "Long Circulating Microparticulate Drug Carriers." *Advanced Drug Delivery Reviews* 16 (1995): 195-214.



Example stealth particle results: PEGylated PLGA nanoparticles

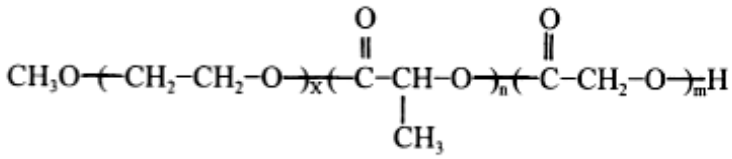
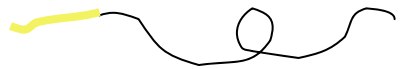
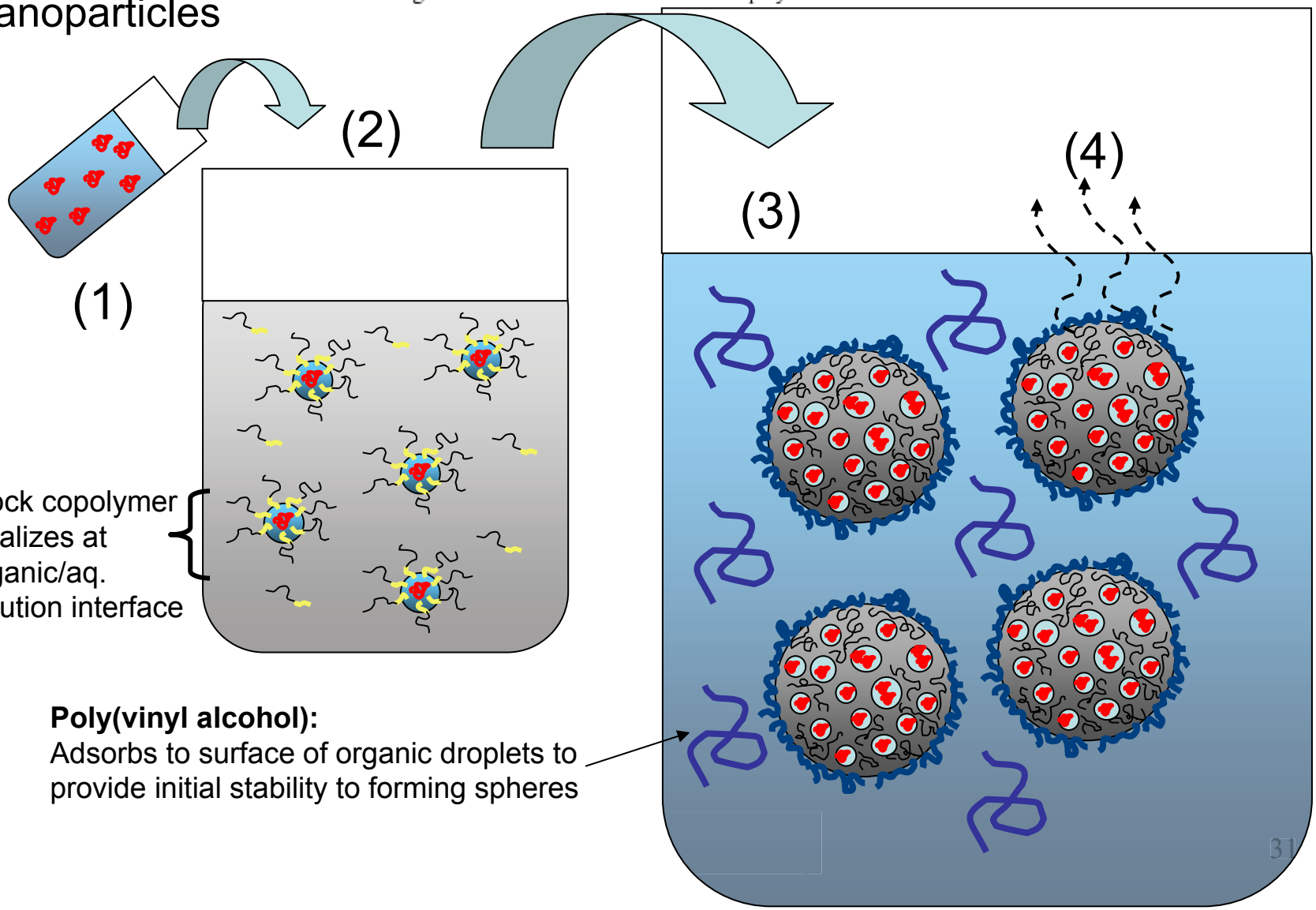


Fig. 1. Structure of the PEG–PLGA copolymer.

PEG = 5KDa, PLGA = 40 KDa





Block copolymer localization at aqueous/polymer interfaces

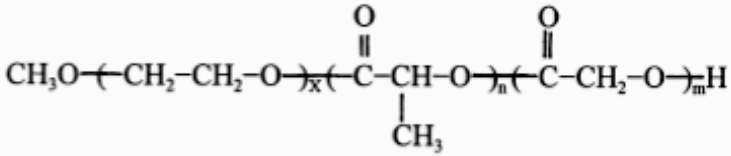
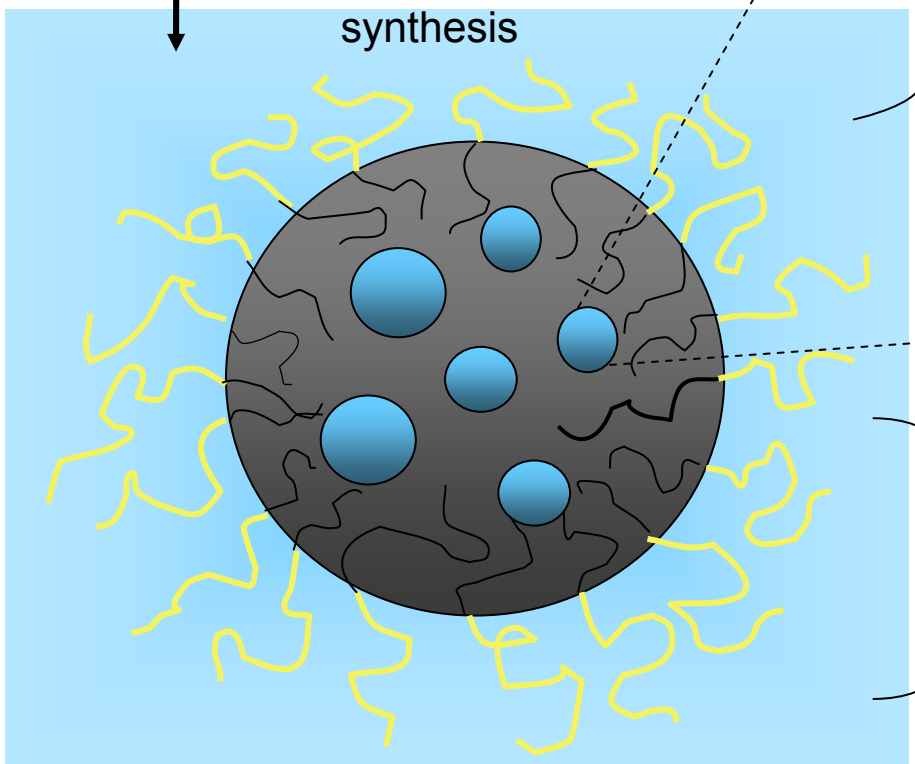
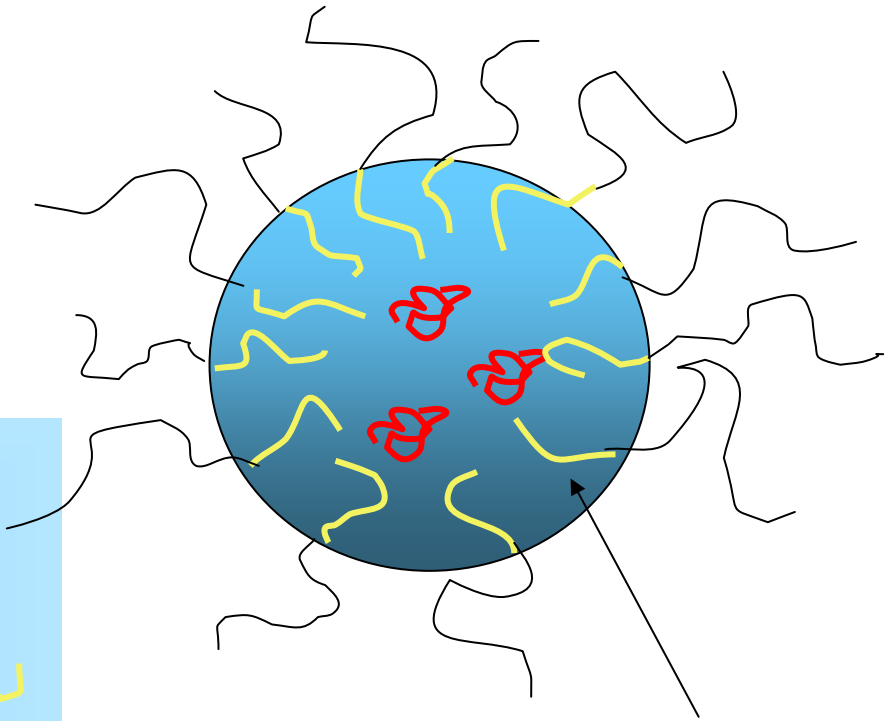


Fig. 1. Structure of the PEG–PLGA copolymer.

PEG = 5KDa, PLGA = 40 KDa

Double emulsion synthesis





PEG chains line inner aq. compartments- minimize protein denaturation

Surface steric barrier

TEM of nanoparticles

Image removed for copyright reason.
Please see: Li, et al. PEGylated PLGA Nanoparticles as Protein Carriers: Synthesis, Preparation and Biodistribution in Rats." *J Control Release* 71 (2001): 203-11.

Release properties of diblock particles

Figure removed for copyright reasons.
Please see: Figure 6 in Li, et al. "PEGylated PLGA nanoparticles as Protein Carriers: Synthesis, Preparation and Biodistribution in Rats." *J Control Release* 71 (2001): 203-11.

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

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

Altered biodistribution:

Chart removed for copyright reason.
Please see: Li, et al. "PEGylated PLGA Nanoparticles as Protein Carriers: Synthesis, Preparation and Biodistribution in Rats." *J 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]

Cell type-dependent endocytosis limits

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

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

Oral delivery barriers

Transcytosis in gut:

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Please see: Lodish, et al. *Molecular Cell Biology*.
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