Lecture 14: Nano- and micro-particle carriers

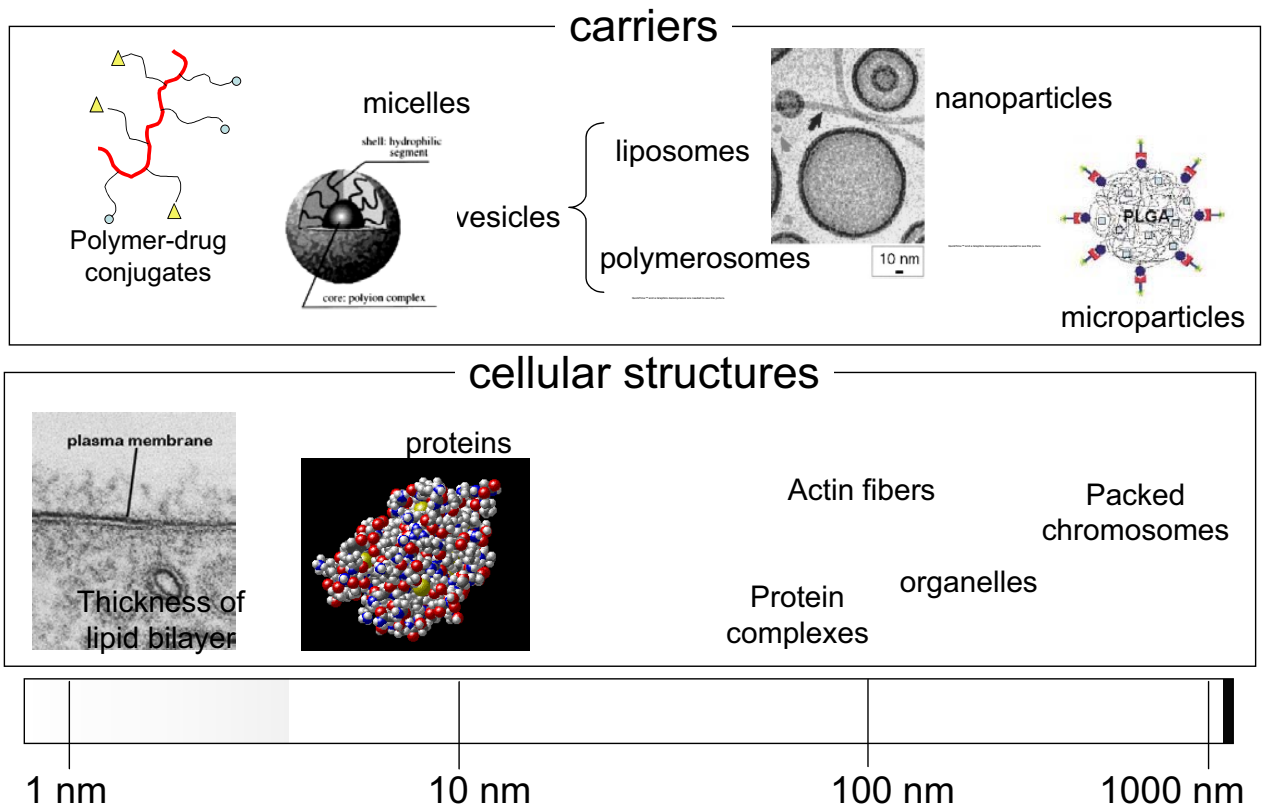
Last time: molecular switches
Proteins as motors in nanodevices

Today: nano- and micro-particle drug carriers

Reading: D.A. Hammer and D.E. Discher, 'Synthetic cells- Self-assembling polymer membranes and bioadhesive colloids,' *Annu. Rev. Mater. Res.*, **31**, 387-404 (2001)

Nano- and Micro-scale Drug Carriers and Detection Reagents

Nano- to Micro-particle polymer-protein conjugates



- Image sources:
  - Plama membrane EM: http://cellbio.utmb.edu/cellbio/membrane_intro.htm
  - Ribonuclease space-filling model: http://www.blc.arizona.edu/courses/181gh/rick/biomolecules/protein.html
  - Hepatitis B virus nucleocapsid: http://www.cryst.bbk.ac.uk/PPS2/course/section11/assembli.html

Applications of tiny drug carriers and cellular markers

Applications

- Delivery to tissues from circulation
  - Therapeutic drugs
    - Anti-cancer drugs
  - Markers for analysis/detection[1]
    - Detect tumors
    - Infected cells
    - Anti-pathogen lymphocytes

- Intracellular delivery
  - Vaccines
    - class I MHC loading – priming $CD8^+$ T cells
  - Gene delivery
    - Delivery of plasmid DNA
  - Anti-sense therapy
    - Shutting off production of certain proteins by delivery of anti-sense oligonucleotides to bind ribosomal mRNA
  - Intracellular toxins for cancer therapy
  - Ribozyme delivery
    - ?
  - drug delivery to organelles
    - drug delivery to mitochondria[2]

Objectives

- protection of cargos from degradation
  - e.g. DNA protection from DNAses
  - protein protection from proteases, phosphatases

- avoid opsonization and production of antibodies against drug molecule
  - opsonization: coating foreign protein, small molecule, or particle with antibodies or complement proteins
    - leads to macrophage binding and destruction
    - source of opsonization animation: http://medtech.cls.msu.edu/ISL/immunology/opsonize.htm

Opsonization:

B cells binding to a foreign protein, drug, or particle can be triggered to produce antibodies against the drug or carrier:

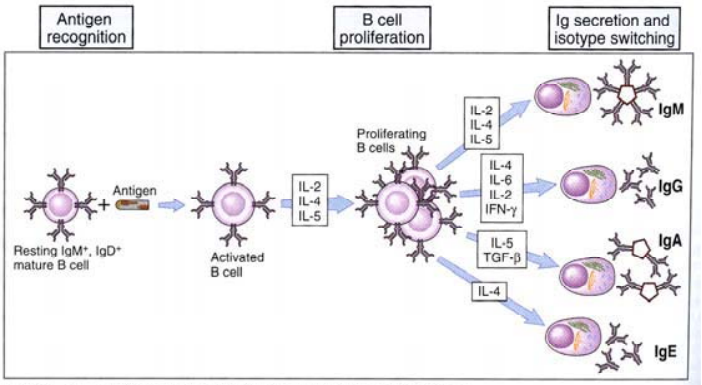


Figure 9–11 Functions of cytokines in B cell growth and differentiation.
Various cytokines stimulate different stages of B cell proliferation and differentiation in humans and mice. The same cytokines may have less striking effects at other stages that are not shown, and there may be differences among species. IFN, interferon; Ig, immunoglobulin; IL, interleukin; TGF, transforming growth factor.

- targeted delivery to select tissues, cells

Molecular Carriers

Role of polymeric carriers

1. Multivalency
   - High avidity binding to low-affinity receptors for detection/delivery
     - CD40-dextran conjugate example
   - Potent delivery on per-molecule basis
     - 1 carrier delivered = 10-50 drug molecules delivered
2. penetration of tissues
3. 'stealth' functions

Chemistry and physical chemistry of conjugation

- non-covalent linkages
  - Ni-histadine linkages
- Molecular size considerations

Micelle Carriers[3]

- Amphiphilic block copolymer structures form micelles in water
  - Monodisperse copolymers can form relatively monodisperse micelle spheres
  - Compartmentalization/association of cargo within the micelle
    - Electrostatic interactions[3]
    - Localization driven by hydrophilic/hydrophobic balance[4]
      - Hydrophobic core-hydrophobic drug
      - Hydrophilic drugs – only associate with corona
      - Amphiphilic drugs – localized at core-corona interface

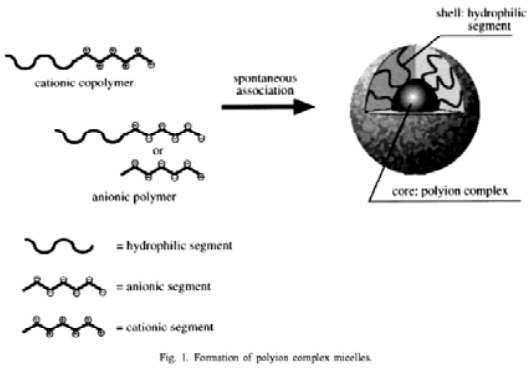


Fig. 1. Formation of polyion complex micelles.

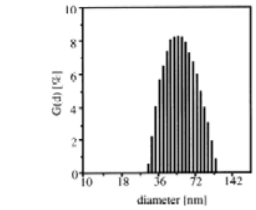
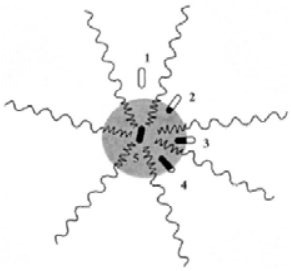


Fig. 5. Size distribution of PEG-*b*-PLL/oligonucleotide micelles determined by DLS (histogram analysis). (Reprinted with permission from Ref. [13]. Copyright 1996 American Chemical Society.)



(Kakizawa and Kataoka, 2002)

- Composition range for core-shell structure?

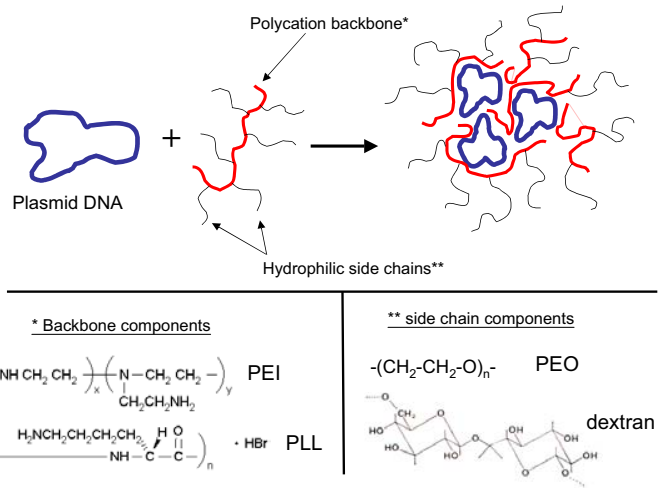
Vesicle carriers

- Liposomes
  - Mechanisms of cargo delivery
    - Membrane fusion
    - Receptor-mediated endocytosis (internalization by cell)
  - Functionalizing liposomes
  - 'stealth' functions
  - limitations
    - difficulty in storage/stability
    - rapid drug leakage (T.M. Allen, *Drugs* **54 suppl. 4**, 8-14 (1997))
      - unstable drug entrapment
      - hydrophobic drugs interact with bilayer and destabilize structure
    - drug instability within liposomes
      - proteins interact with bilayer and become denatured
    - unmodified liposomes activate complement
      - causes pseudo-allergic reactions that damate heart and liver cells[5]
- polymerosomes
  - larger amphiphilic molecules than lipids
    - larger hydrophobic blocks increase membrane stability and mechanical strength
    - can be polymerized to make membrane associations covalent

Nano- and Microparticle carriers

Approaches

- ***Electrostatic Complexation of cargo with carrier***
  - E.g. DNA delivery by polycationic polymers
    - Plasmid DNA + comb with cationic backbone hydrophilic side chains -> nano- to micro-particles[6]
      - Reduce adsorption of proteins to particle surface that could trigger phagocytosis
      - Hydrophilic, steric barrier to block uptake by RES



Nanoparticle DNA packaging

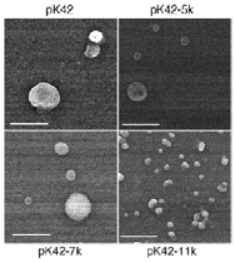


Figure 2. Cold field emission scanning electron microscopy on complexes coated with ~10 nm carbon. Complexes were made at an amine to phosphate charge ratio (N:P) of 1.0. The scale bars equal 500 nm in all images.

(Park and Healy, 2003)

Protection from DNAses

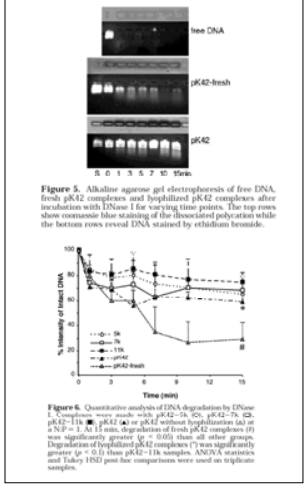
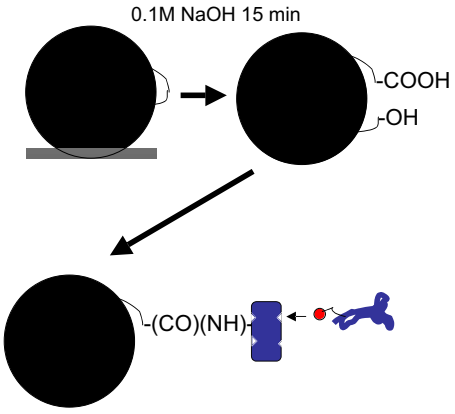
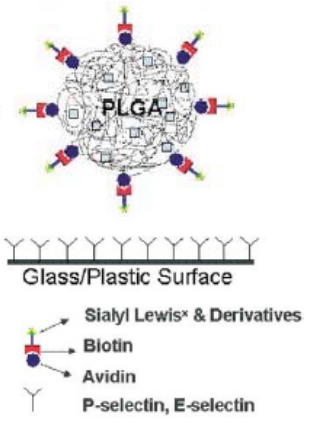


Figure 5. Alkaline agarose gel electrophoresis of free DNA, fresh pK42 complexes and lyophilized pK42 complexes after incubation with DNase I for varying time points. The top rows show coomassie blue staining of the dissociated polycation while the bottom rows reveal DNA stained by ethidium bromide.

Figure 6. Quantitative analysis of DNA degradation by DNase I. Complexes were made with pK42–5k (○), pK42–7k (□), pK42–11k (■), pK42 (▲) or pK42 without lyophilization (Δ) at a N:P = 1. At 15 min, degradation of fresh pK42 complexes (#) was significantly greater ($p < 0.05$) than all other groups. Degradation of lyophilized pK42 complexes (*) was significantly greater ($p < 0.1$) than pK42–11k samples. ANOVA statistics and Tukey HSD post-hoc comparisons were used on triplicate samples.

- Encapsulation
- Surface immobilization
  - Conjugation of cargos/targeting agents to surface of microparticles





References

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