2022/2023

Fifth Stage

Second Semester/ Pharm Biotechnology

Delivery of proteins
Lecture 9

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Approaches for rate-controlled delivery

- Rate control can be achieved by several different technologies similar to those used for conventional drugs.

Rate control through open loop type approach

- Continuous infusion with pumps: mechanically or osmotically driven input: constant/pulsatile/wave form
- Implants: biodegradable polymers, lipids
- Input: limited control

Rate control through closed loop approach/feed back system

- Biosensor-pump combination
- Self regulating system
- Encapsulated secretory cells

Table 9 Controlled release systems for parenteral delivery.

Osmotically driven systems:

- As example, osmotic mini-pump, which is S.C implant with continuous, constant infusion over a prolonged periods of time.
- The rate determining process is the influx of water through the rigid, semi-permeable external membrane.
- Then the release depend on **membrane properties and osmotic pressure differences** over this membrane (in presence of osmotic agent inside the pump).

For View

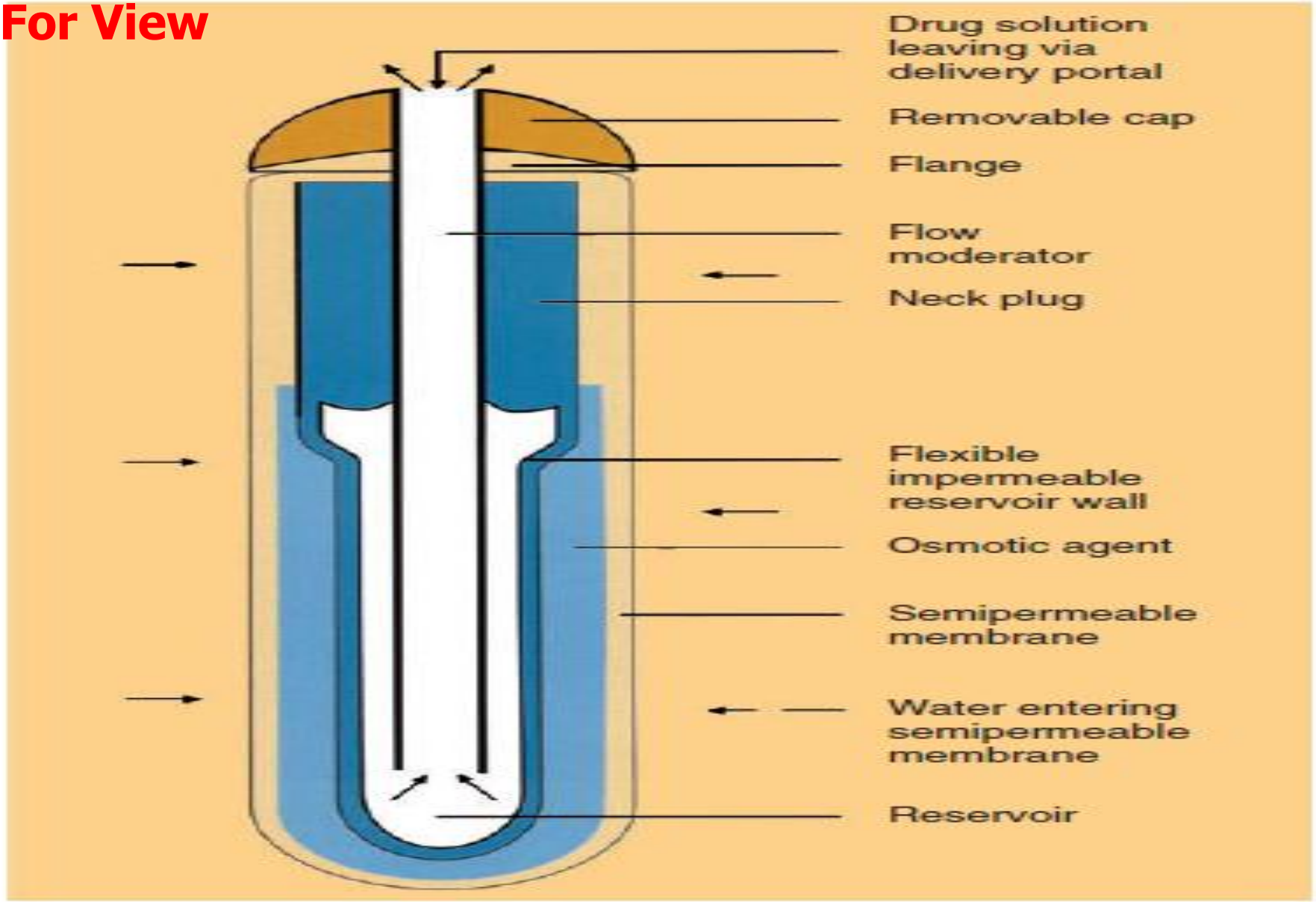


Figure 18 Cross section of functioning Alza Alzet osmotic minipump. *Source*: Adapted from Banerjee et al., 1991.

Bio-degradable microspheres:

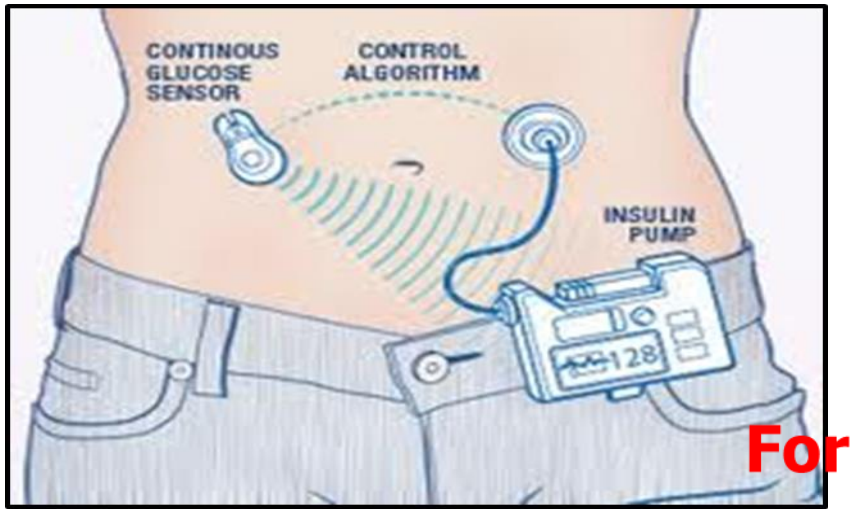
- Biodegradable polymers like PLGA (polylactic acid-polyglycolic acid can be used for enclosing certain types of proteins (like LHRH agonist =leuprolide) to be taken as implants with dose ranges 1-6 months.
- As requirement of this system, we need:

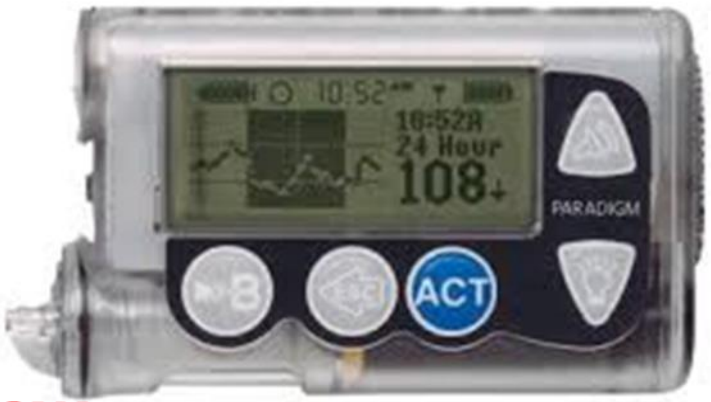
1) Highly potent drugs (low dose)
2) Sustained presence in the body
3) No adverse reaction at the administration site.

Biosensor-pump combinations:

- They consist of:

1) A biosensor.
2) An algorithm (calculate the required input rate).
3) A pump system (administer the drug at the required rate over prolonged periods of time).





For View

Self-regulating systems:

- The drug release is controlled by stimuli in the body.
- Two approaches for controlled drug release are being followed:

1) Competitive desorption
2) Enzyme-substrate reaction (depend on pH drop)

When glucose is converted to Gluconic acid in presence of Glucose oxidase, this induces Changes in acid-sensitive Delivery devices.

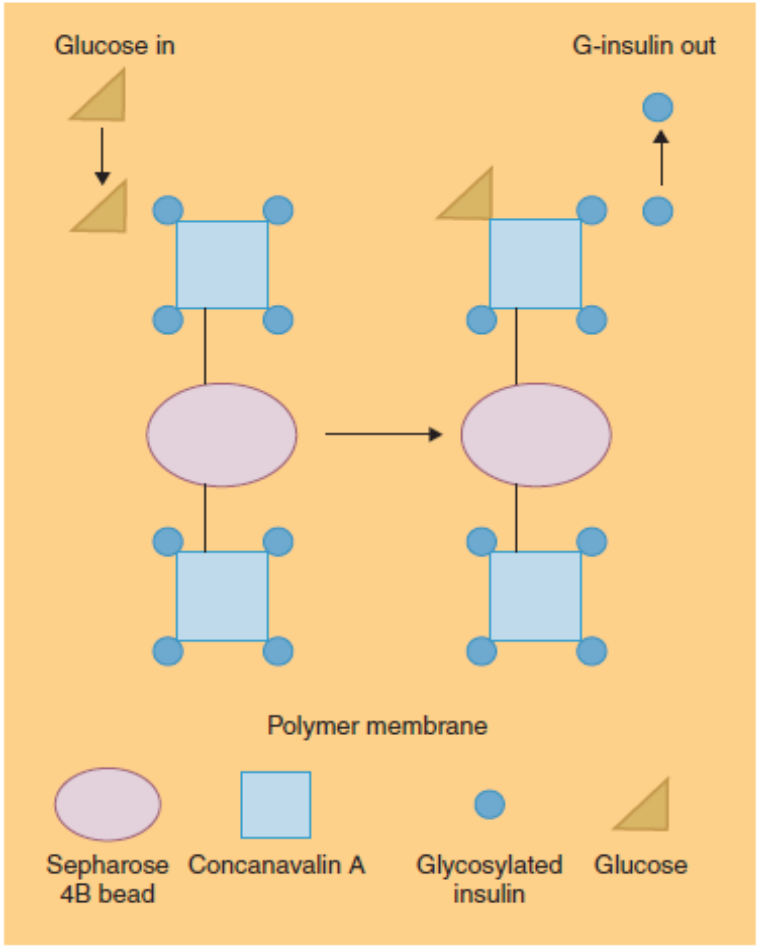
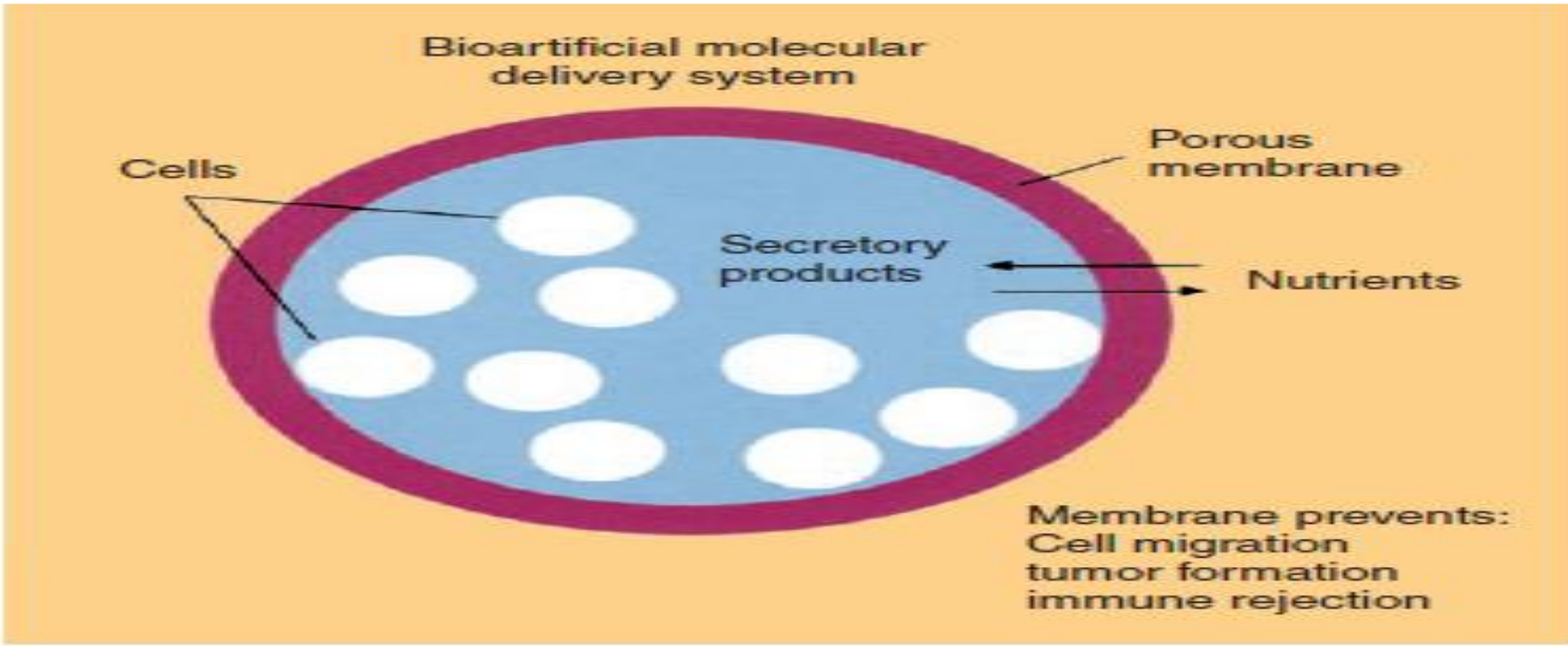


Figure 23 Schematic design of the Con A immobilized bead/G (*glycosylated*)-insulin/membrane self-regulating insulin deliv-

Microencapsulated secretory cells

- Like implantation of Langerhans cells in diabetics to restore their insulin production through biofeedback.
- They should be protected from the body environment (no rejection).



Site-specific delivery (Targeting) of protein drugs

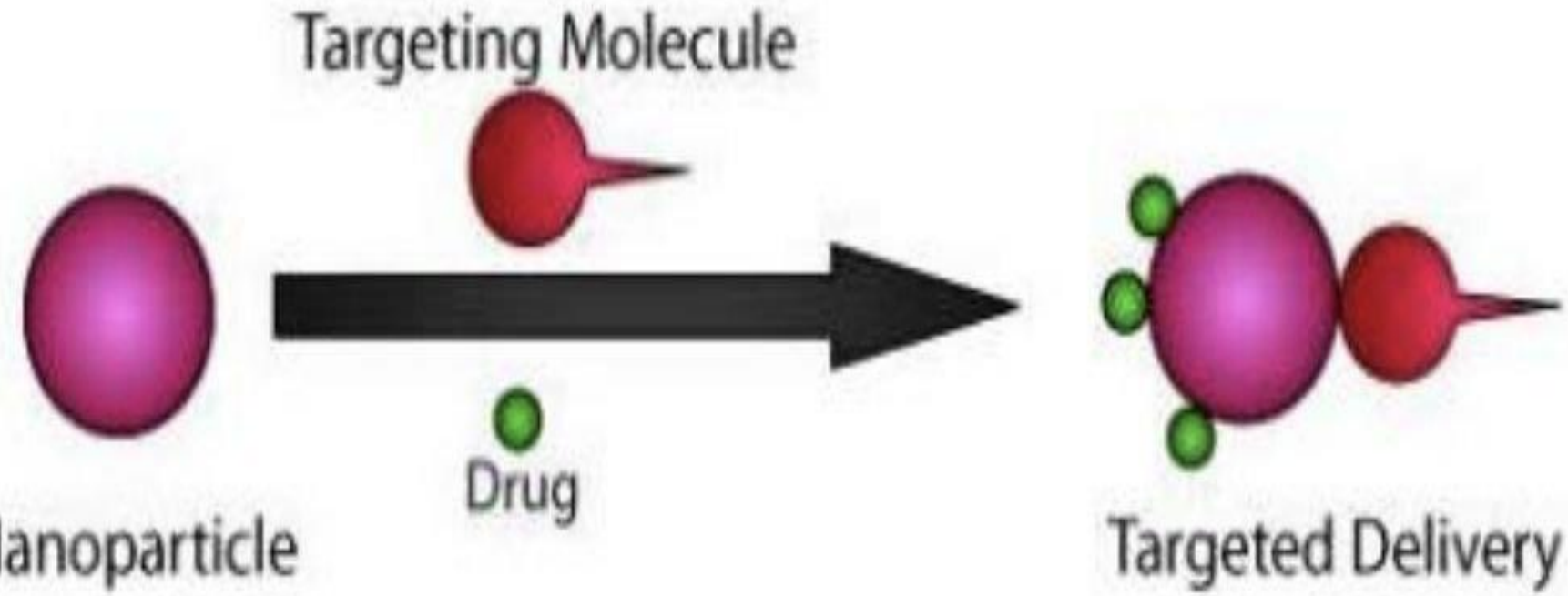
- It is used for:

1) Decrease the degradation in the body organs other than the site of action
2) More localization in the target organ and less non-target organs distribution

- Components of targeted drug delivery (carrier based) are:

1. An active moiety	For: therapeutic effect
2. A carrier	For: (metabolic) protection, changing the disposition of the drug
3. A homing device	For: specificity, selection of the assigned target site

Targeted Delivery



1. Drugs with high total clearance are good candidates for targeted delivery.
2. Response sites with a relatively small blood flow require carrier-mediated transport.
3. Increases in the rate of elimination of free drug from either central or response compartments tend to increase the need for targeted drug delivery; this also implies a higher input rate of the drug-carrier conjugate to maintain the therapeutic effect.
4. For maximizing the targeting effect, the release of drug from the carrier should be restricted to the response compartment.

Table 12 Pharmacokinetic considerations related to protein targeting.

Targeting can be classified into:

1) Passive: use of the natural disposition pattern of the carrier system as in macrophages action toward certain particulate carriers circulating in the blood and then accumulate in the liver and spleen.

2) Active: change the natural disposition of the carrier by some types of homing device to select one particular tissue or cell type.

Factors affecting protein targeting

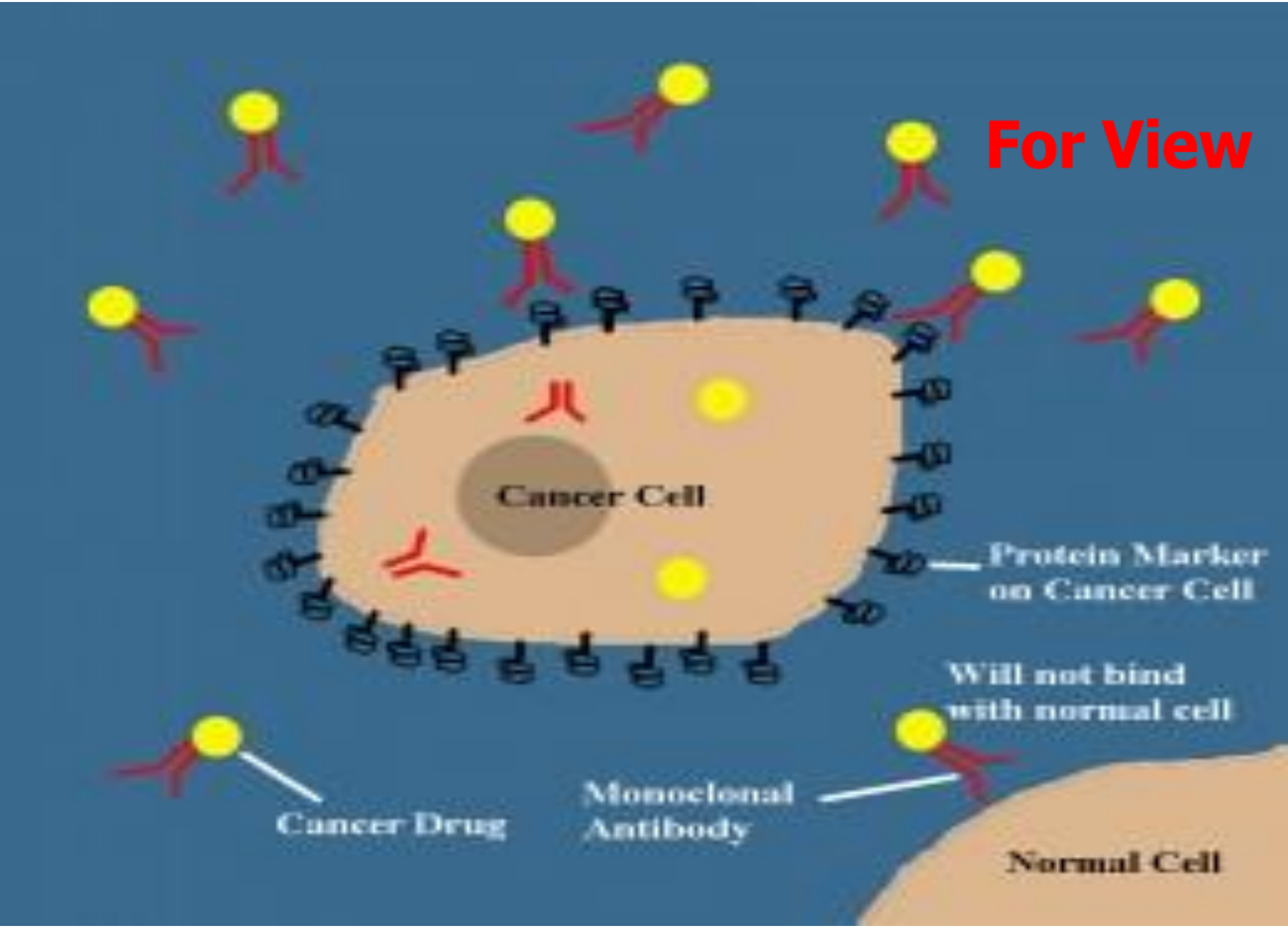
- The physicochemical properties of carrier (its charge, mol.wt., surface hydrophobicity and presence of ligands for interaction with surface receptors).
- The nature of endothelial barrier, healthy or non (inflamed, necrotic or tumorous)

Types of carriers for targeting

- Soluble (Molecular) carrier systems: ex. **MAb** (monoclonal antibodies) and Antibody-Drug conjugates (ADC)
- Colloidal particulate carrier systems

MAb

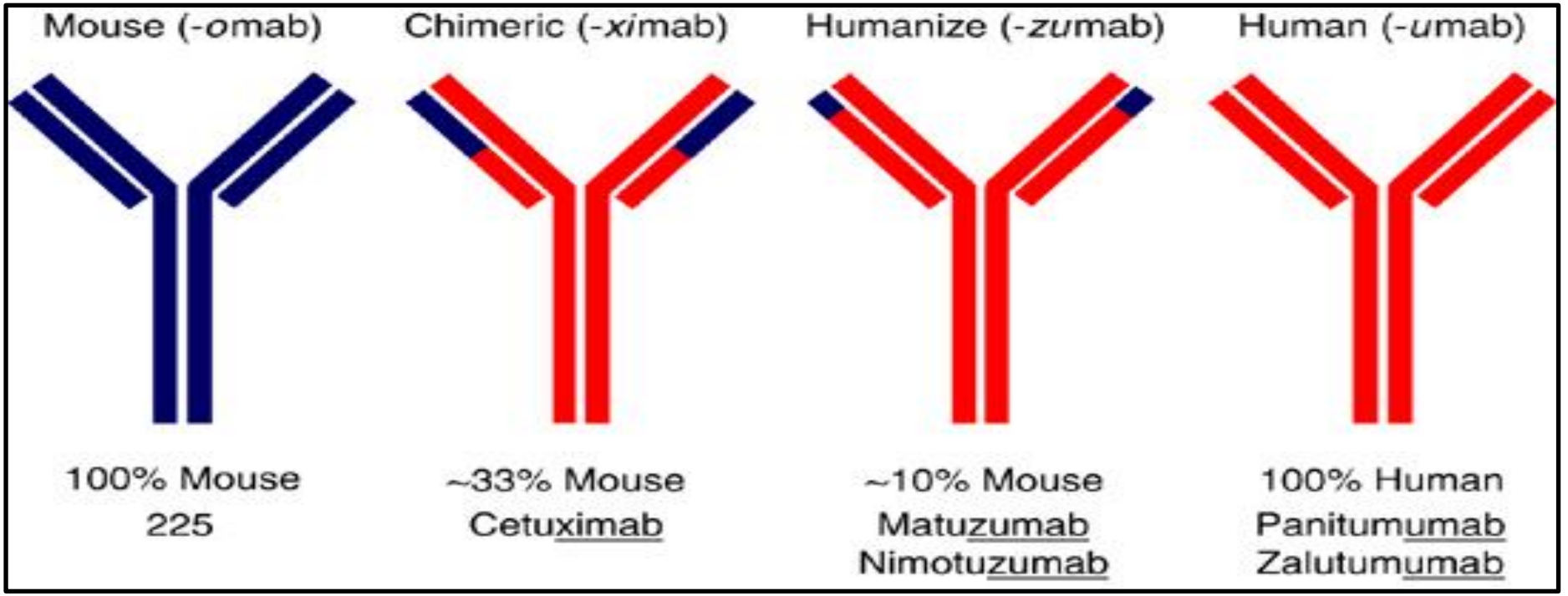
- **Antibodies produced by immortalized cell lines (hybridoma) derived from single B cells.**
- **They are Specific to only one Antigen.**
- **Used as a diagnostic agent as well as therapeutic.**
- **Mab can be coupled with other agents e.g., anticancer drugs (ADC), liposomes and others**

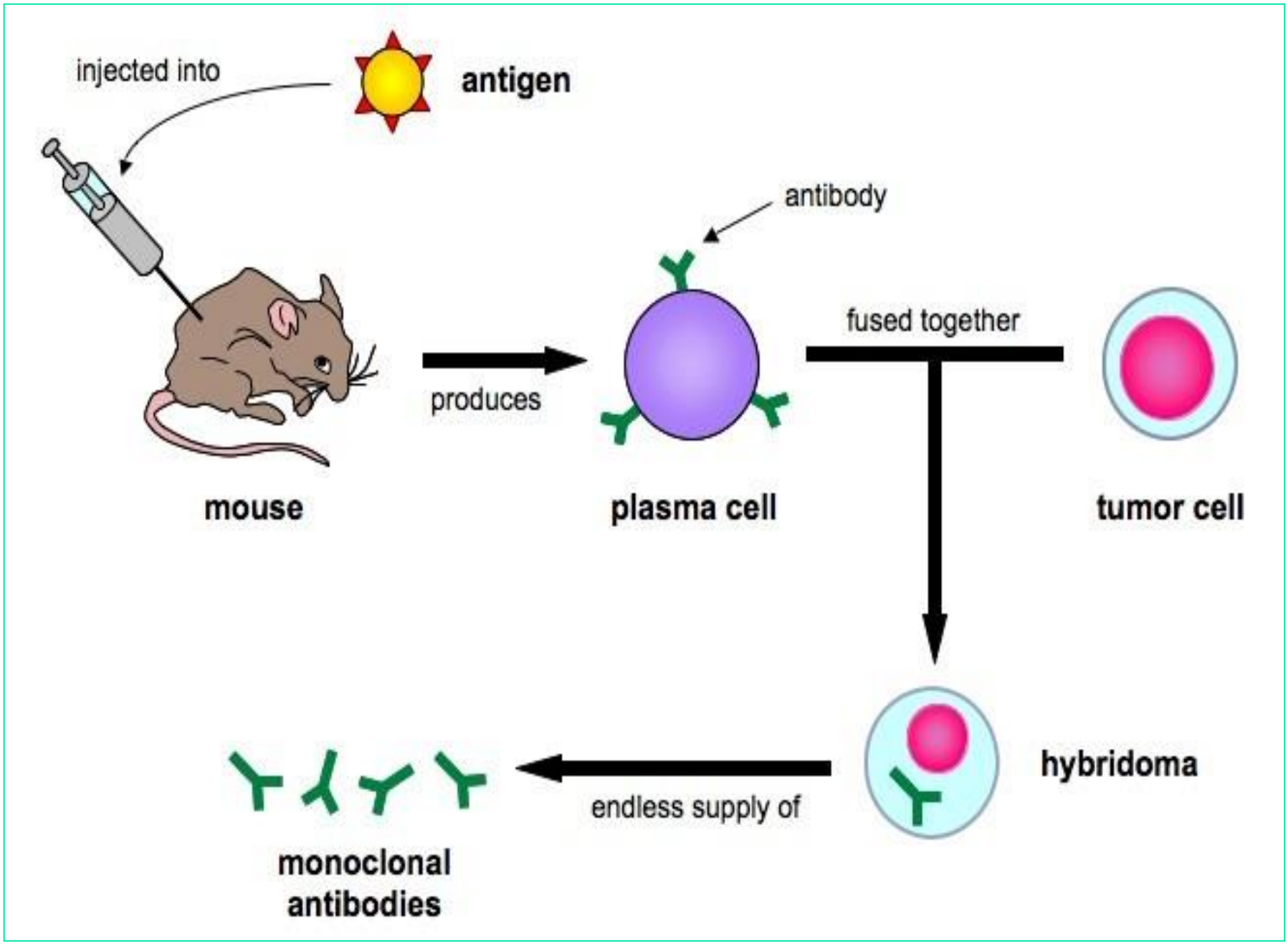

For View
Cancer Cell
Protein Marker
on Cancer Cell
Will not bind
with normal cell
Cancer Drug
Monoclonal
Antibody
Normal Cell

Monoclonal antibodies can be produced in two major ways:

Murine (mouse) origin

Human origin

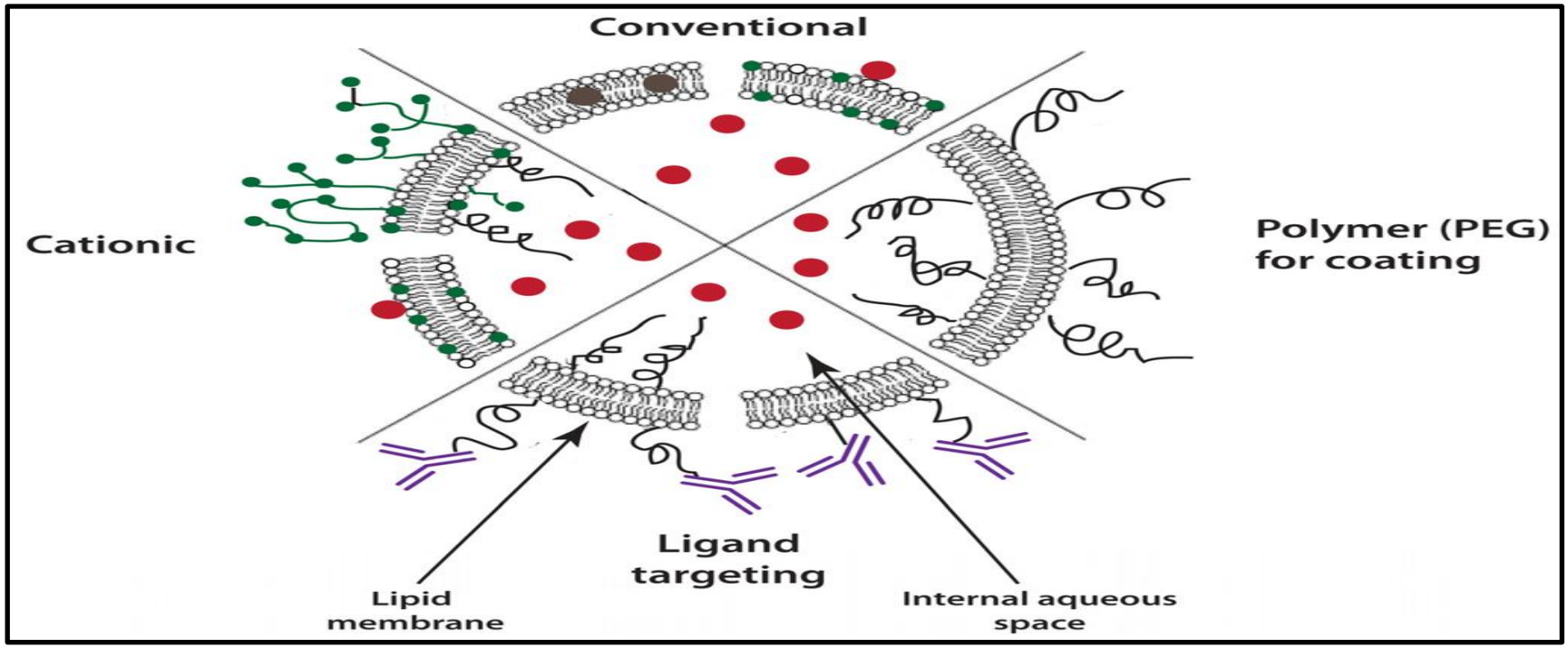


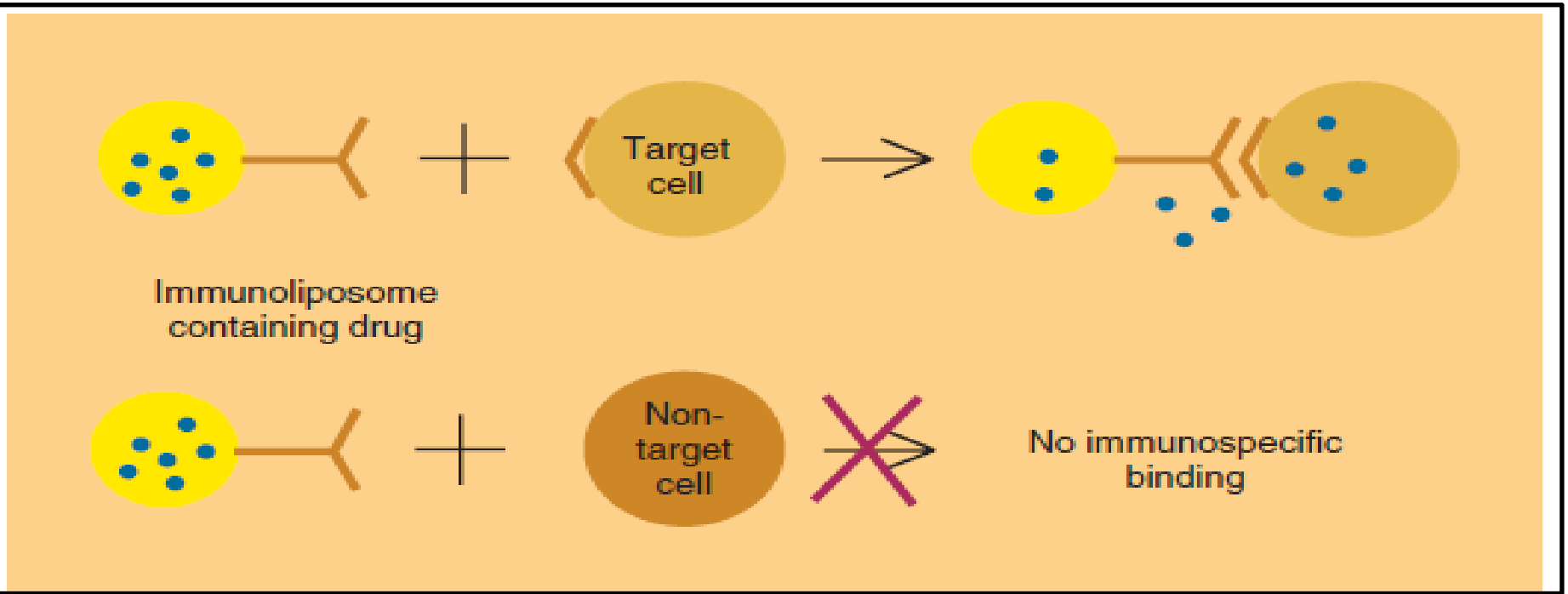

injected into
antigen
antibody
mouse
produces
plasma cell
fused together
tumor cell
hybridoma
endless supply of
monoclonal antibodies


Antigen

Colloidal particulate carrier systems

- Liposomes (Nanosized vesicles)
- Immuno-liposomes
- Nanoparticles
- Microspheres

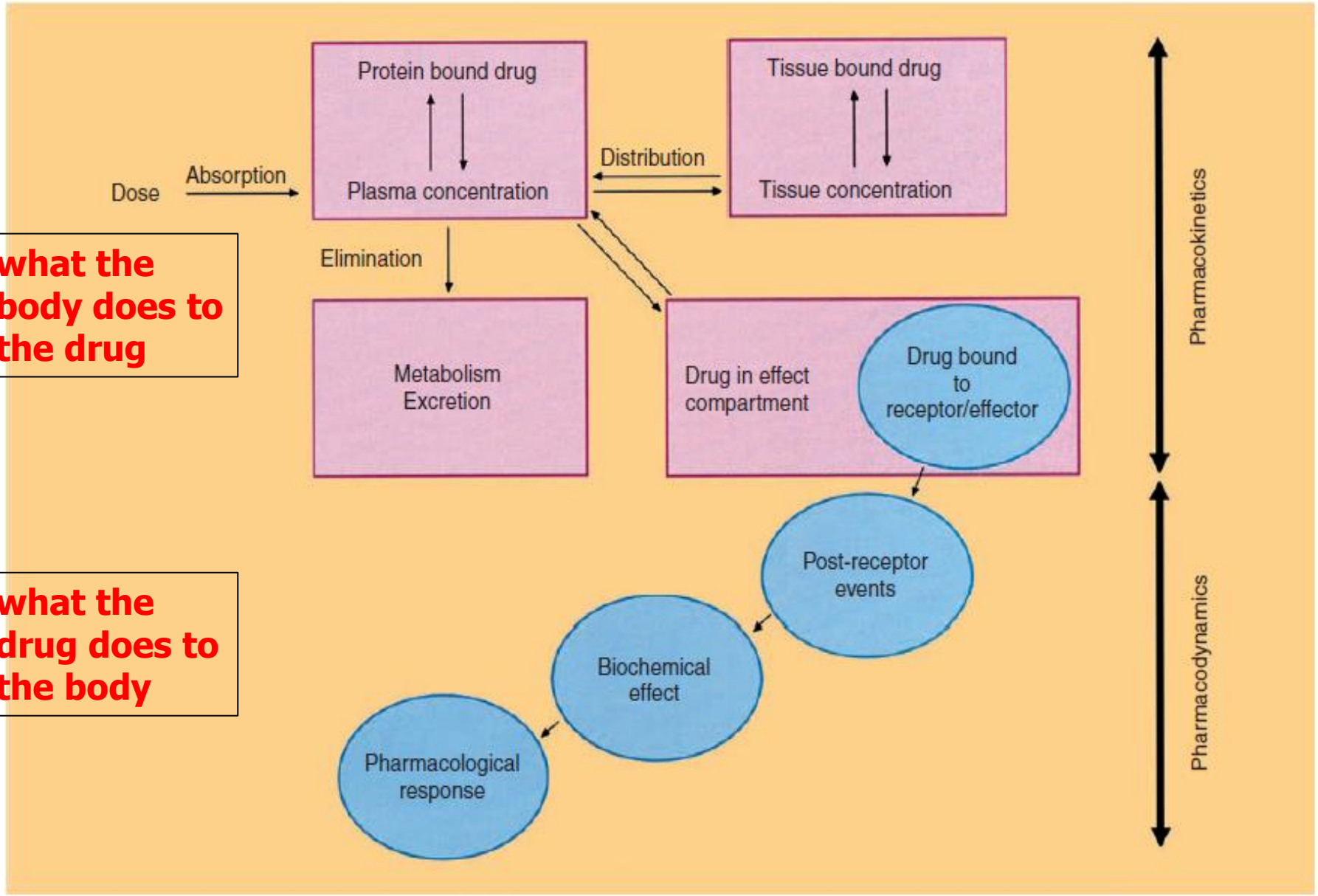

Conventional
Cationic
Polymer (PEG)
for coating
Ligand
targeting
Lipid
membrane
Internal aqueous
space


Target
cell
Immunoliposome
containing drug
Non-
target
cell
No immunospecific
binding

- Nanoparticles are smaller than microspheres.
- Formulation of drugs into the nanoparticles can occur at the surface of the particles and in nucleus, depending on the physicochemical properties of the drug.

- The site of drug incorporation significantly affects its release rate from the particle.
- After systemic administration or transportation, they quickly distribute to the target site and subsequently become internalized by the cells of the phagocytic system.

Pharmacokinetics of peptides and proteins



what the body does to the drug

what the drug does to the body

Figure 2 Physiological scheme of pharmacokinetic and pharmacodynamic processes.

- Pharmacokinetics for proteins may be different from that for conventional drugs.(Why?)

This is related to:

1) The structural similarity to some endogenous compounds.
2) With regulatory feedback mechanisms.
3) Difficulties in analysis (interferences).
4) Their large molecular weights.

Absorption of protein therapeutics

- Poorly absorbed orally. (as taken before).
- Mainly administered parenterally (I.V, I.M, and S.C.) depending on type of protein.
- The potential limitations of SC and IM administrations are the pre-systemic degradation processes, local blood flow, injection trauma and the capillaries sizes.

then **Ka = F . K app**

Ka= the true abs. rate constant

F= The bioavailability compared to IV adm.

Kapp= apparent abs. rate constant for IM,SC adm

Distribution of protein therapeutics

- The rate and extent of protein distribution are largely determined by their size and Mol.wt, physicochemical properties (like charge, lipophilicity), protein binding, and their dependency on active transport.
- The lymphatic system play important role in distribution of proteins depending on size.
- Protein charge is important for electrostatic attraction of +vely charged proteins with –vely charged cell membranes (containing glycoaminoglycans).
- I.V. administered proteins may follow one or two-compartmental model or may be non-compartmental (with rapid elimination rates) in distribution.

- Then for distribution characterization :

1) Biopsy or necropsy for determination of actual proteins concentrations in the tissue.

2) Bio-distribution studies with radiolabeled compound and/or imaging techniques.

- The binding to endogenous protein structures (specific) can affect the distribution, pharmacodynamics (PD) and disposition properties of proteins.
- The binding may be non specific to plasma proteins (albumin and lipoproteins).
- Site-specific receptor mediated uptake can also substantially influence and contribute to the distribution, elimination and PD of proteins.

Elimination of protein therapeutics

- The exogenous proteins are subjected to the same catabolic pathways as endogenous ones.
- The end products of protein metabolism are thus amino acids that are reutilized in the endogenous amino acids pool for synthesis of endogenous proteins.
- The elimination pathways includes:

1) **Proteolysis**
2) **GIT protein metabolism**
3) **Renal protein metabolism and excretion**
4) **Hepatic protein metabolism**
5) **Receptor-mediated protein metabolism.**

Molecular weight	Elimination site	Predominant elimination mechanisms	Major determinant
<500	Blood, liver	Extracellular hydrolysis Passive lipoid diffusion	Structure, lipophilicity
500–1,000	Liver	Carrier-mediated uptake Passive lipoid diffusion	Structure, lipophilicity
1,000–50,000	Kidney	Glomerular filtration and subsequent degradation processes (see Fig. 4)	Molecular weight
50,000–200,000	Kidney, liver	Receptor-mediated endocytosis	Sugar, charge
200,000–400,000		Opsonization	α_2-macroglobulin, IgG
>400,000		Phagocytosis	Particle aggregation

Note: Other determining factors are size, charge, lipophilicity, functional groups, sugar recognition, vulnerability for proteases, aggregation to particles, formation of complexes with opsonization factors, etc. Mechanisms may overlap and endocytosis may occur at any molecular weight range.
Source: After Meijer and Ziegler, 1993.

Table 1 Molecular weight as major determinant of the elimination mechanisms of peptides and proteins.

- The kidneys are a major site of protein metabolism for smaller sized proteins that undergo glomerular filtration (GF).
- The size- selective cut-off for GF is approx. **60kD**

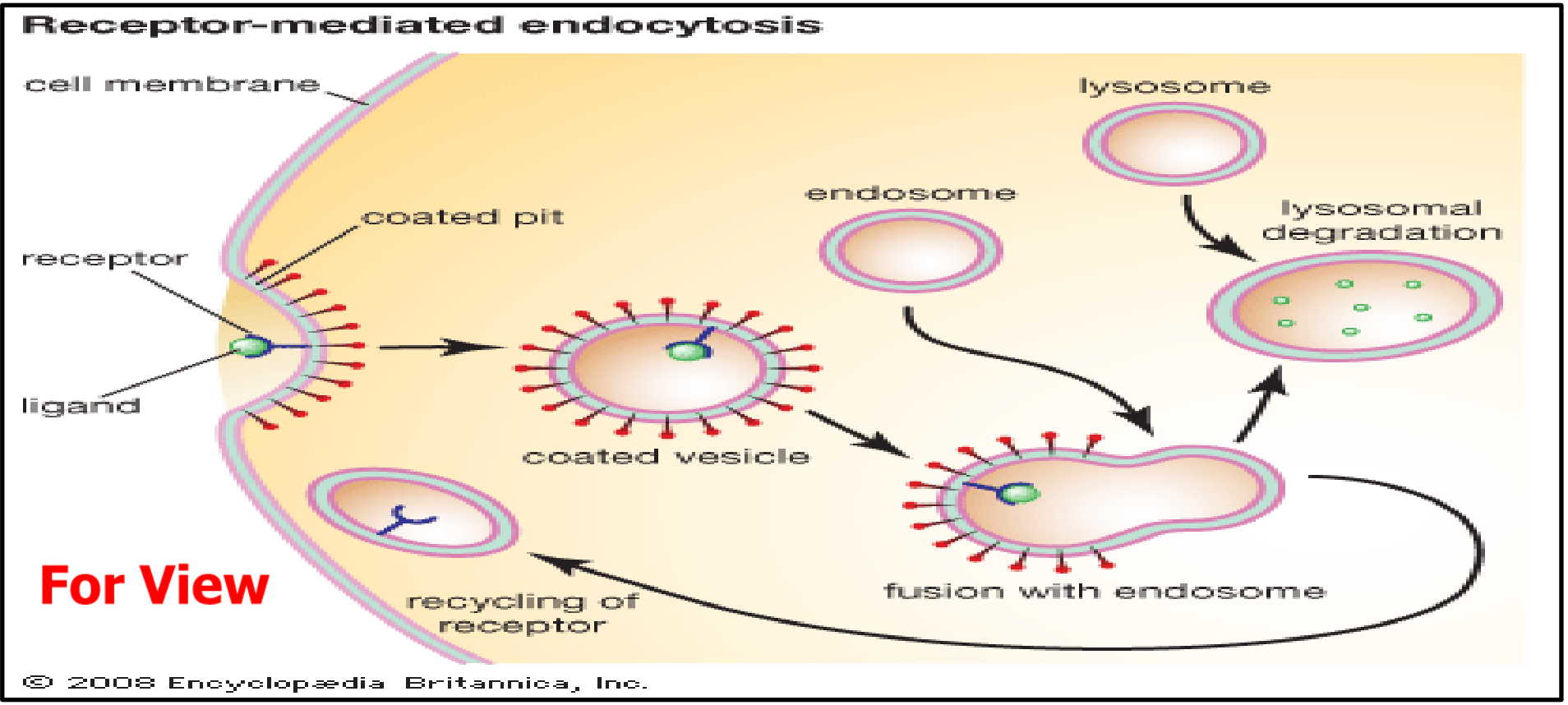
Q/ What about Albumin? Pass or not? And why?

- **Charge selectivity** is important here, where anionic compounds (Ex. TNF- alpha) pass less readily than neutral ones which in turn pass through less readily than cationic ones, due to the –ve charge of GF (presence of abundance of glycosaminoglycan).

Hepatic protein metabolism:

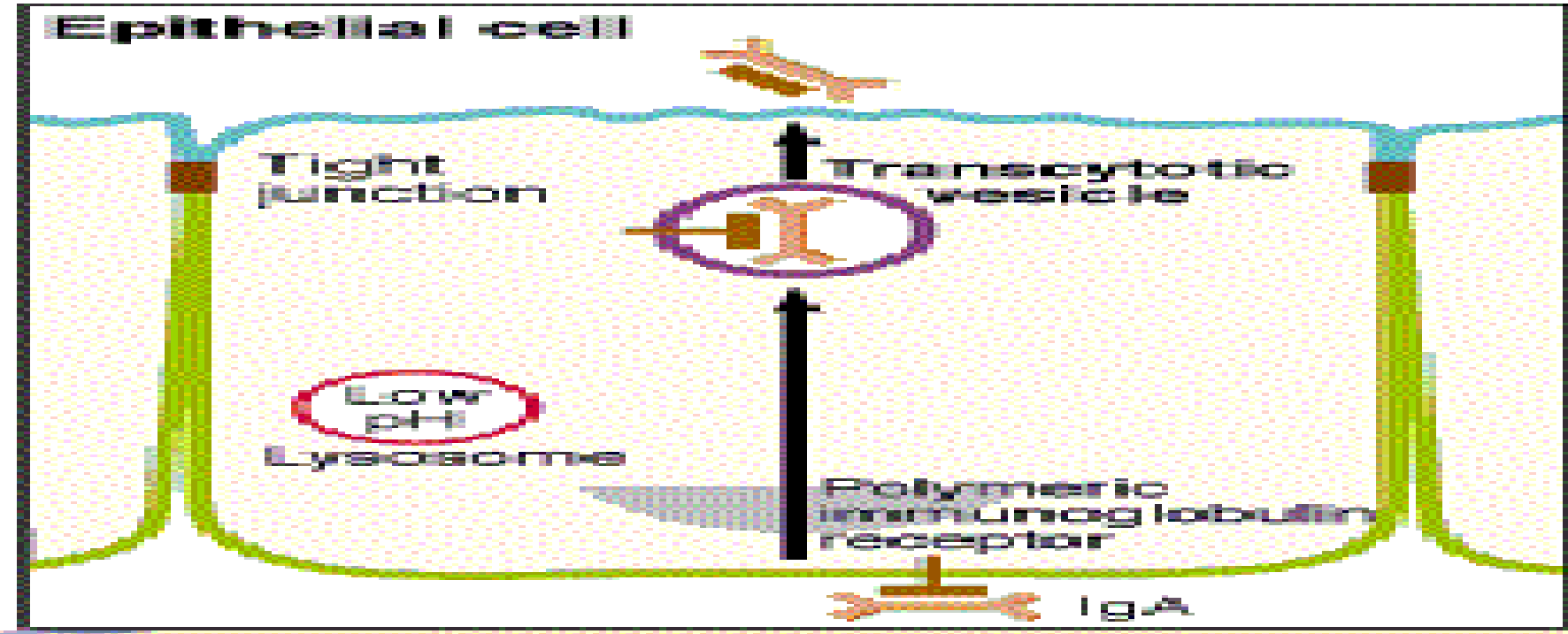
- The rate of hepatic metabolism is largely dependent on the specific a.a. sequence of the protein (endopeptidases or exopeptidases action).
- Mechanisms of hepatic uptake for proteins depend on the size and hydrophobicity.
- There are different hepatic cells (Hepatocytes, Kupffer, endothelial and fat storing cells) for this uptake mechanisms (simple passive diffusion, carrier mediated transport, **RME** and **trans-cytosis**)

- Here, recycling of receptors occurred, so depending on type of protein receptor, sometimes degradation may be occurred leading to a decrease in the concentration of receptors on the cell surfaces (**Receptor down-regulation**) as for interferon and insulin.



In trans-cytosis, the endocytotic vesicle formed at the cell surface traverses the cell, degradation, and exocytosis into bile, as for polymeric immunoglobulin A.

For View



Receptor-mediated protein metabolism

- Occurred for proteins that bind with high affinity to membrane-associated receptors on the cell surface.
- Includes endocytosis and subsequent intracellular lysosomal metabolism.
- It is not constant (dose-dependent), decreases with increasing the dose.
- It is not limited for a specific organ or tissue type, but depend on number of protein drug receptors.
- As in metabolism of some proteins by linking to a receptor- mediated uptake into macrophages.

Note:

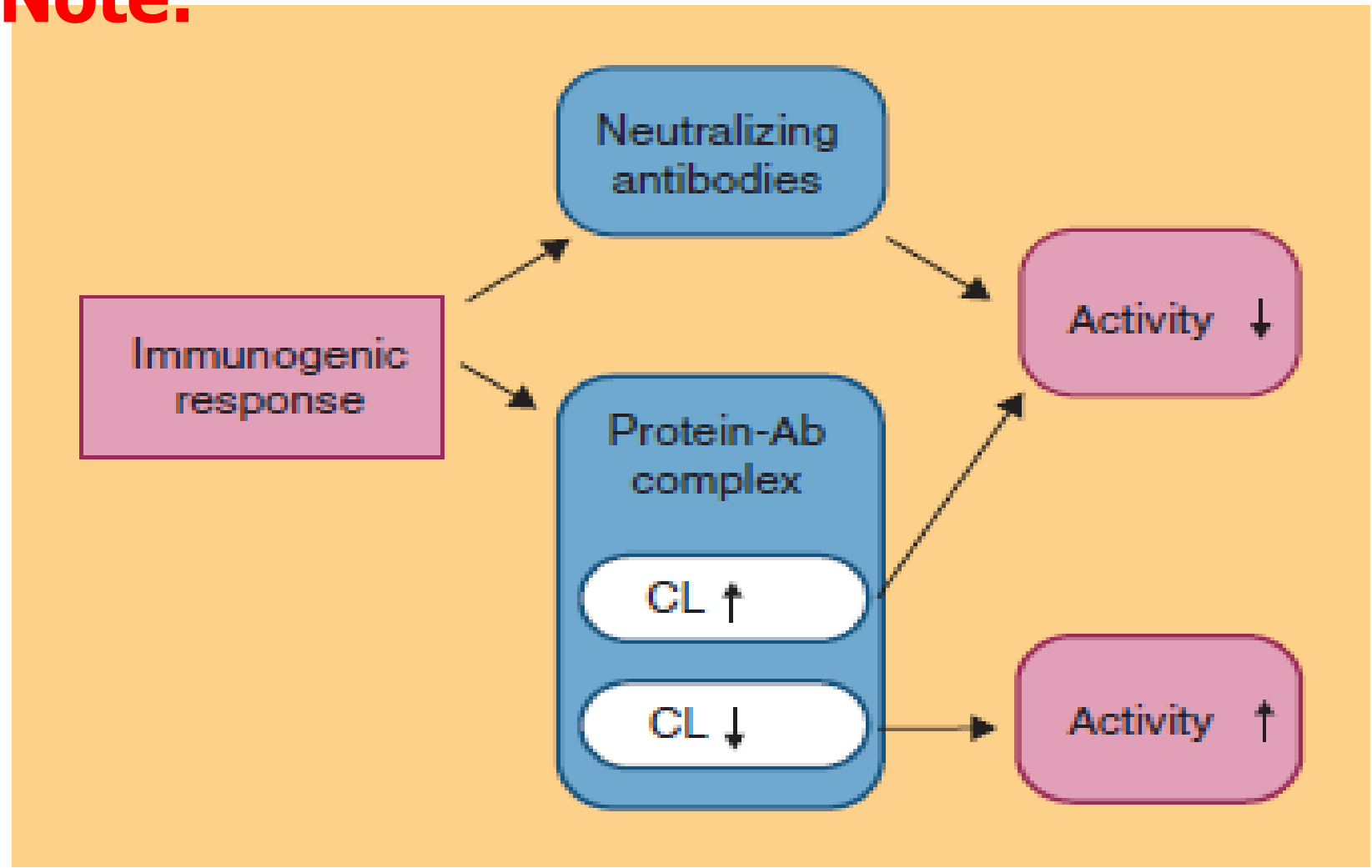


Figure 6 Effect of antibody formation on pharmacokinetics and pharmacodynamics of protein drugs.

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