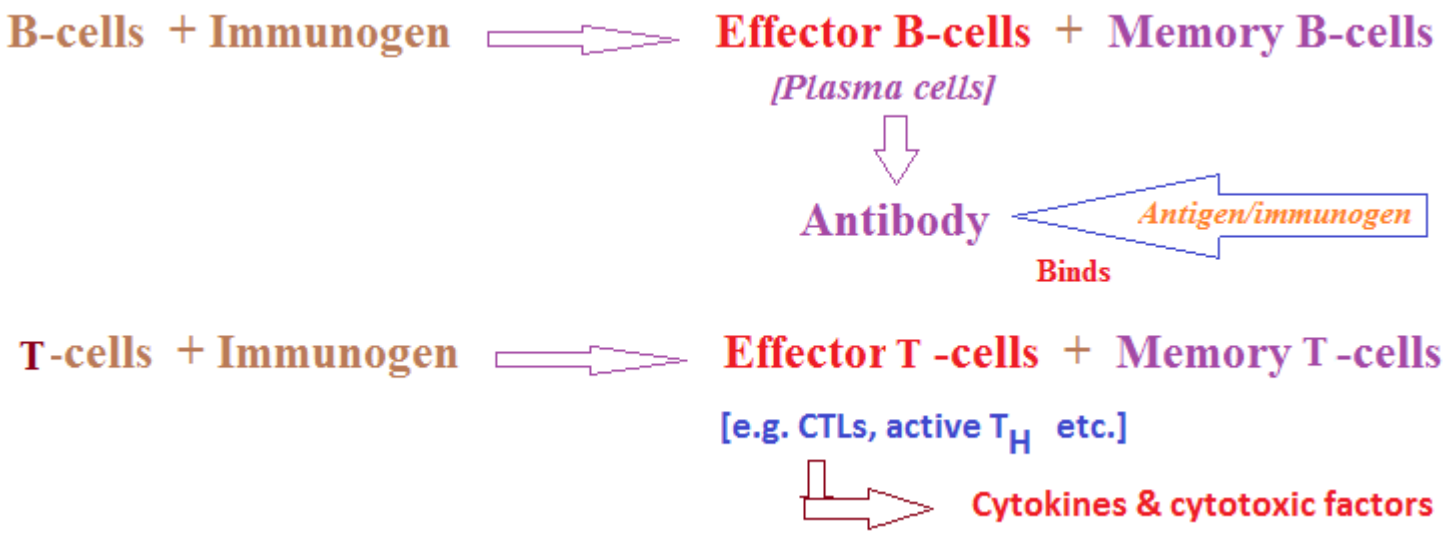
Antigens

To ‘B’ or not to ‘B’- that is the question

An immunogen refers to a molecule that is capable of eliciting an immune response (humoral and/or cell-mediated) by an organism’s immune system, whereas an antigen refers to a molecule that is capable of binding to the product of that immune response.

So, an immunogen is necessarily an antigen, but an antigen may not necessarily be an immunogen.

Hence , all immunogens are antigen but all antigens are not immunogen.



Haptens are low-molecular-weight compounds that may be bound by antibodies or T cell receptors, but cannot elicit an immune response by themselves.

Haptens themselves are nonimmunogenic and they cannot evoke an immune response until they are conjugated with a larger carrier immunogenic moleculesuch BSA. The hapten-carrier complex, unlike free hapten, can act as an immunogen and can induce an immune response.

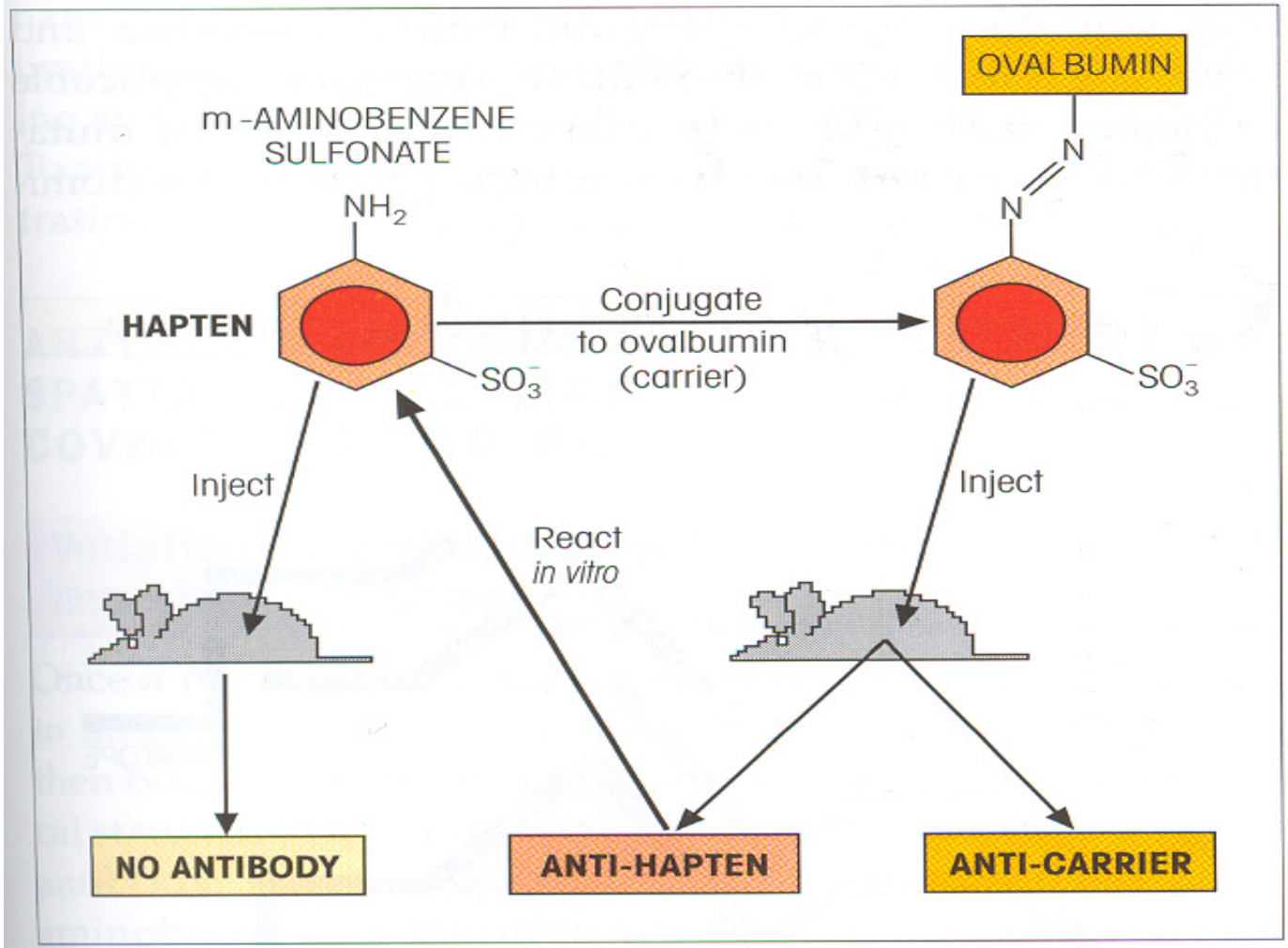
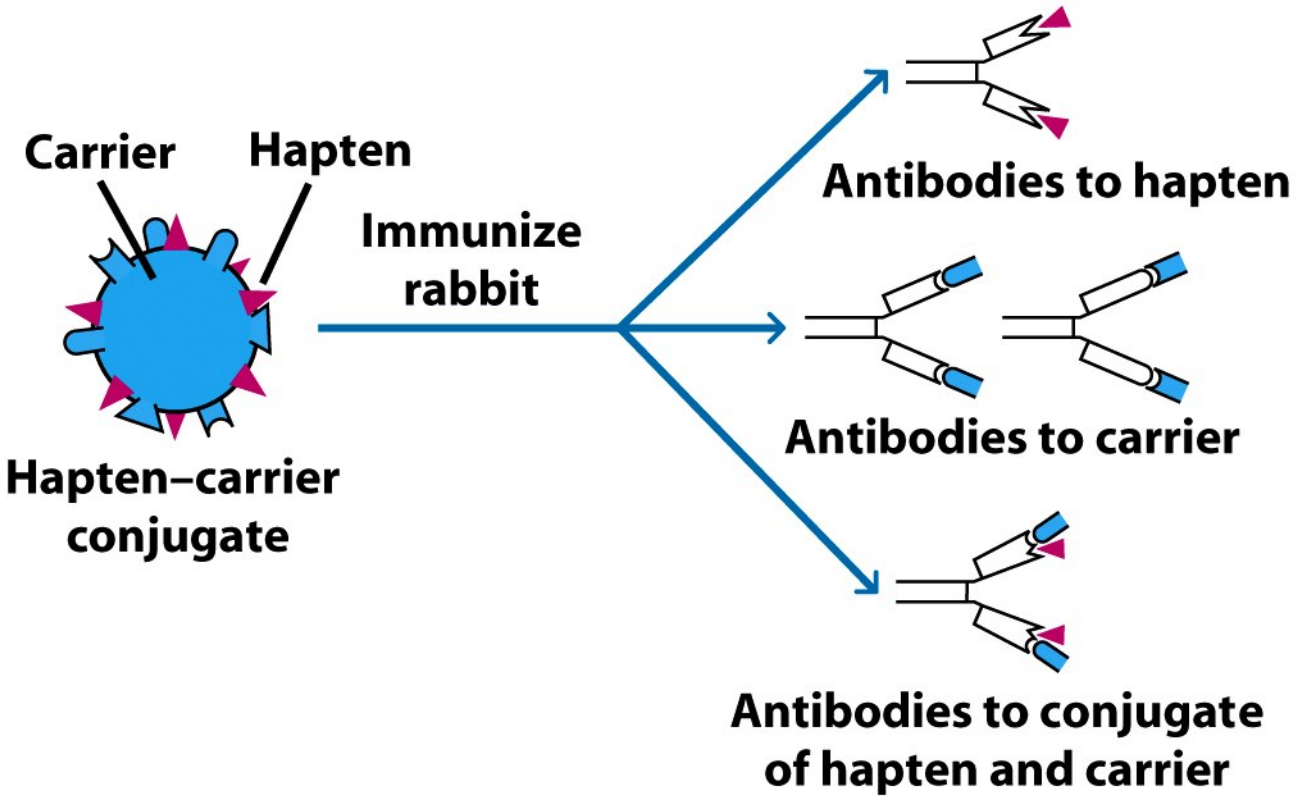


Figure 5.1. **A hapten on its own will not induce antibodies.** However, it will react *in vitro* with antibodies formed to a conjugate with an immunogenic carrier.



Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti–BSA
Hapten–carrier conjugate (DNP-BSA)	Anti–DNP (major) Anti–BSA (minor) Anti–DNP/BSA (minor)

Figure 4-1
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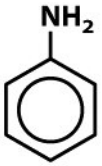
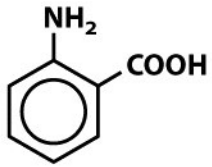
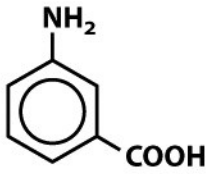
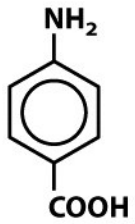
TABLE 4-1 Reactivity of antisera with various haptens

Antiserum against	REACTIVITY WITH: Aminobenzene (aniline) 	*o*-Aminobenzoic acid 	*m*-Aminobenzoic acid 	*p*-Aminobenzoic acid 
Aminobenzene	+	0	0	0
o-Aminobenzoic acid	0	+	0	0
m-Aminobenzoic acid	0	0	+	0
p-Aminobenzoic acid	0	0	0	+

KEY: 0 = no reactivity; + = strong reactivity

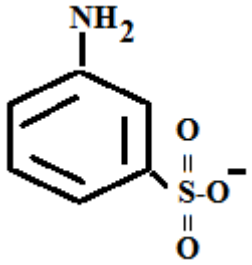
SOURCE: Based on K. Landsteiner, 1962, *The Specificity of Serologic Reactions,* Dover Press. Modified by J. Klein, 1982, *Immunology: The Science of Self-Nonself Discrimination,* Wiley.

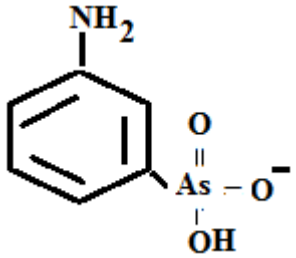
Table 4-1
Kuby IMMUNOLOGY, Sixth Edition

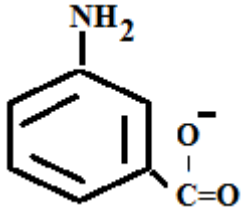
Cross reactivity between antisera against m-amino benzene sulfonate and *o*-, *m*- and *p*- amino benzene sulfonate/arsonate /carbonate.

	Ortho-	Meta-	Para-
Amino benzene sulfonate	+	+++	-
Amino benzene arsonate	-	+	-
Amino benzene carbonate	-	-	-

Explain these results.





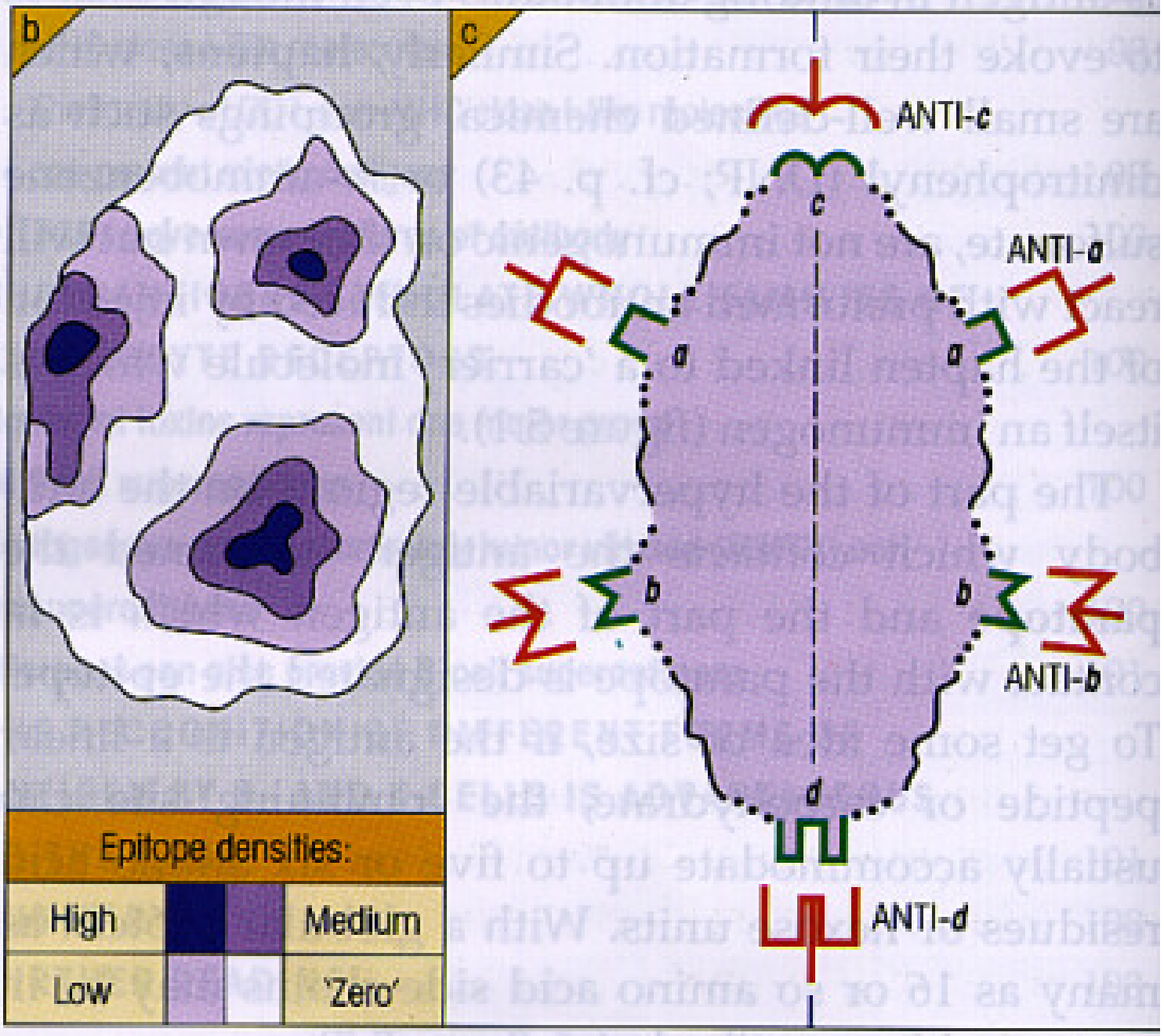


- **Epitope: the portion of an antigen that is recognized and bound by an Ab or TCR/MHC complex (antigenic determinant).**

- **Paratope: "The site in the variable (V) domain of an antibody or T-cell receptor that binds to an epitope on an antigen**

Epitopes

- Antigenic determinants recognized by B cells and T cells
  - B cell epitopes tend to be on the outside of the antigen
    - For example, the hydrophilic amino acids on a protein's surface
  - T cell epitopes from proteins derived from enzymatic digestion of peptide and then association with MHC


b
c
ANTI-c
ANTI-a
ANTI-b
ANTI-d
c
a
a
b
b
d
Epitope densities:
High
Medium
Low
'Zero'

Receptor-Ligand Interactions

- Antigen receptors of the adaptive immune system are transmembrane proteins
  - B cells – the B cell receptor
  - T cells – the T cell receptor
- Multiple noncovalent bonds
  - Hydrogen bonds
  - Ionic bonds
  - Van der Waals
  - Hydrophobic interactions

Immunoglobulin Superfamily

- All have similar structures
- Examples:
  - Antibodies
  - T-cell receptors
  - Class I and II MHC molecules
  - Part of B cell receptor
  - Most members of immunoglobulin superfamily cannot bind antigen

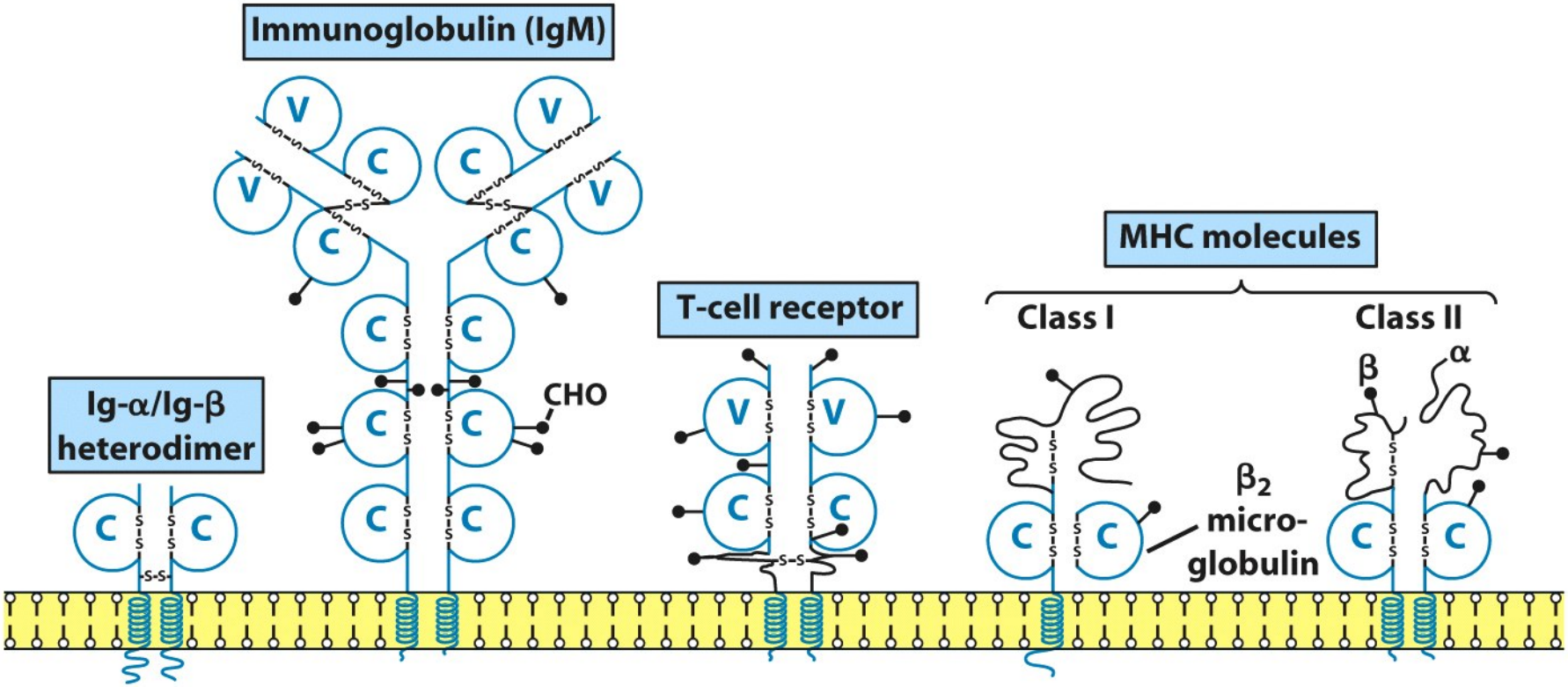


Figure 4-24 part 1
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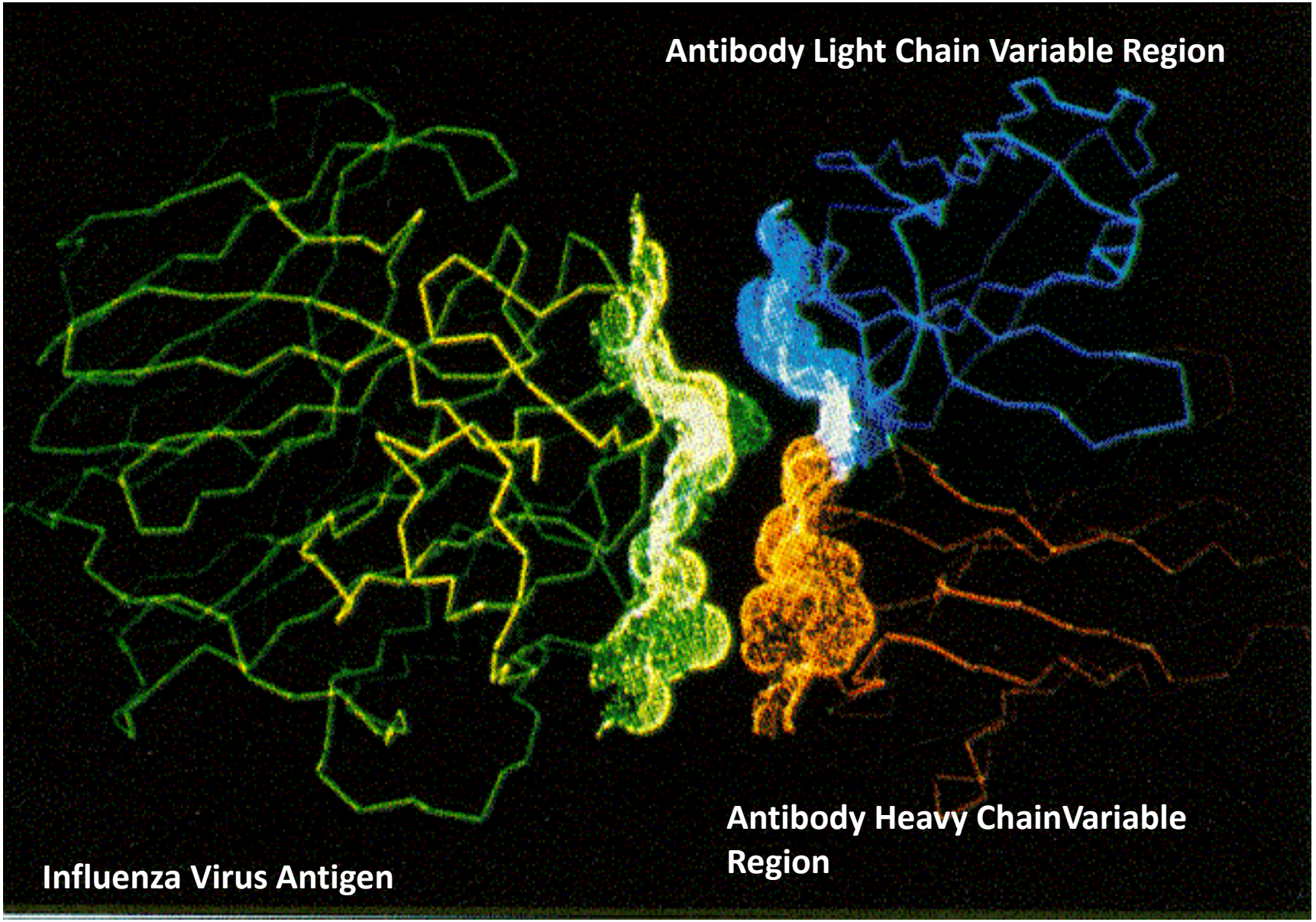
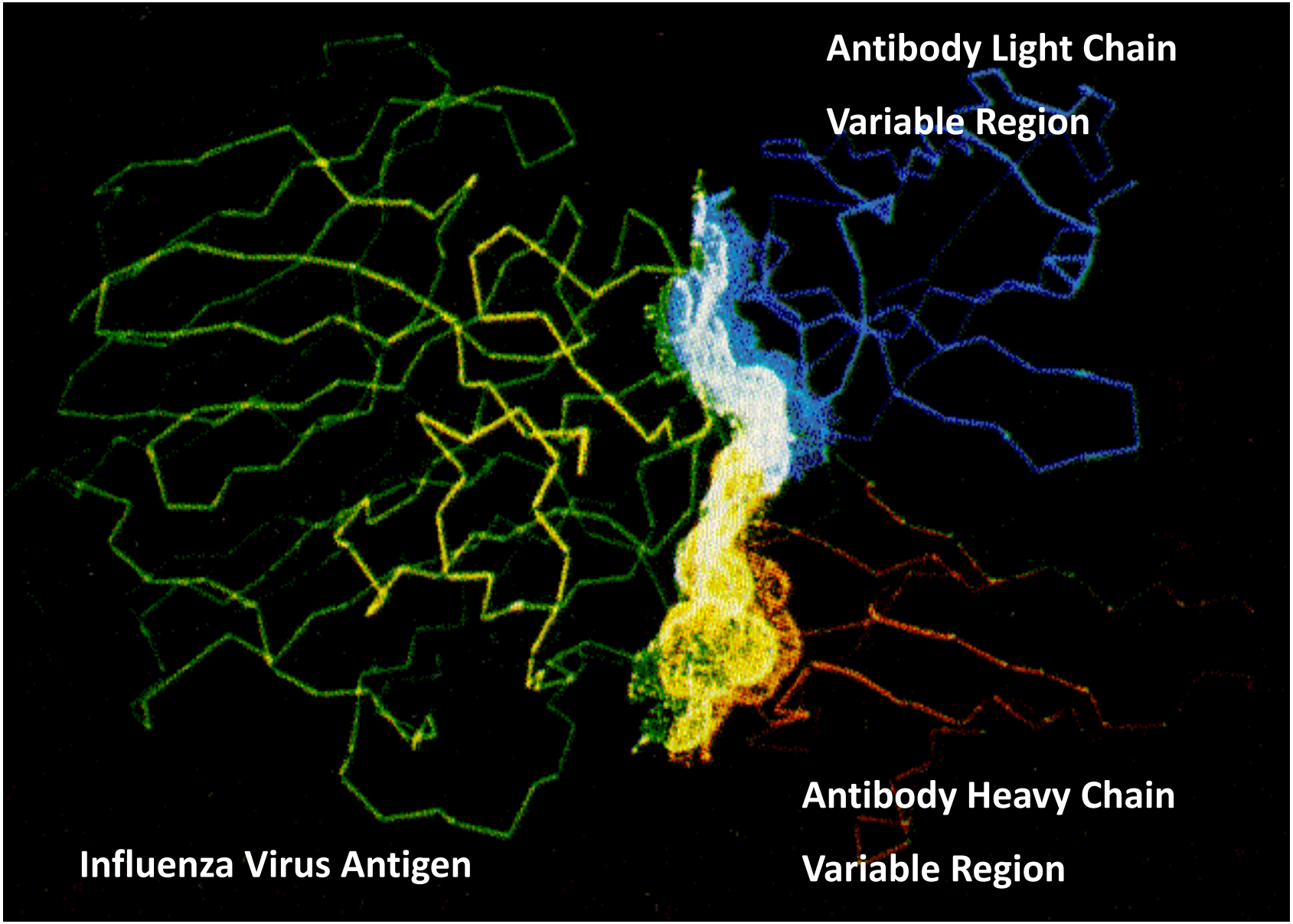
Characteristics of B-cell epitopes

- **B cell epitopes on native proteins generally are composed of hydrophilic amino acids on the protein surface that are topographically accessible to membrane bound or free antibody.**
  - **When talking about proteins, the epitopes can be sequential or nonsequential (referring to amino acid sequence) depending on protein folding**

Antigen-Antibody Complementarity



Antigen - Antibody Binding



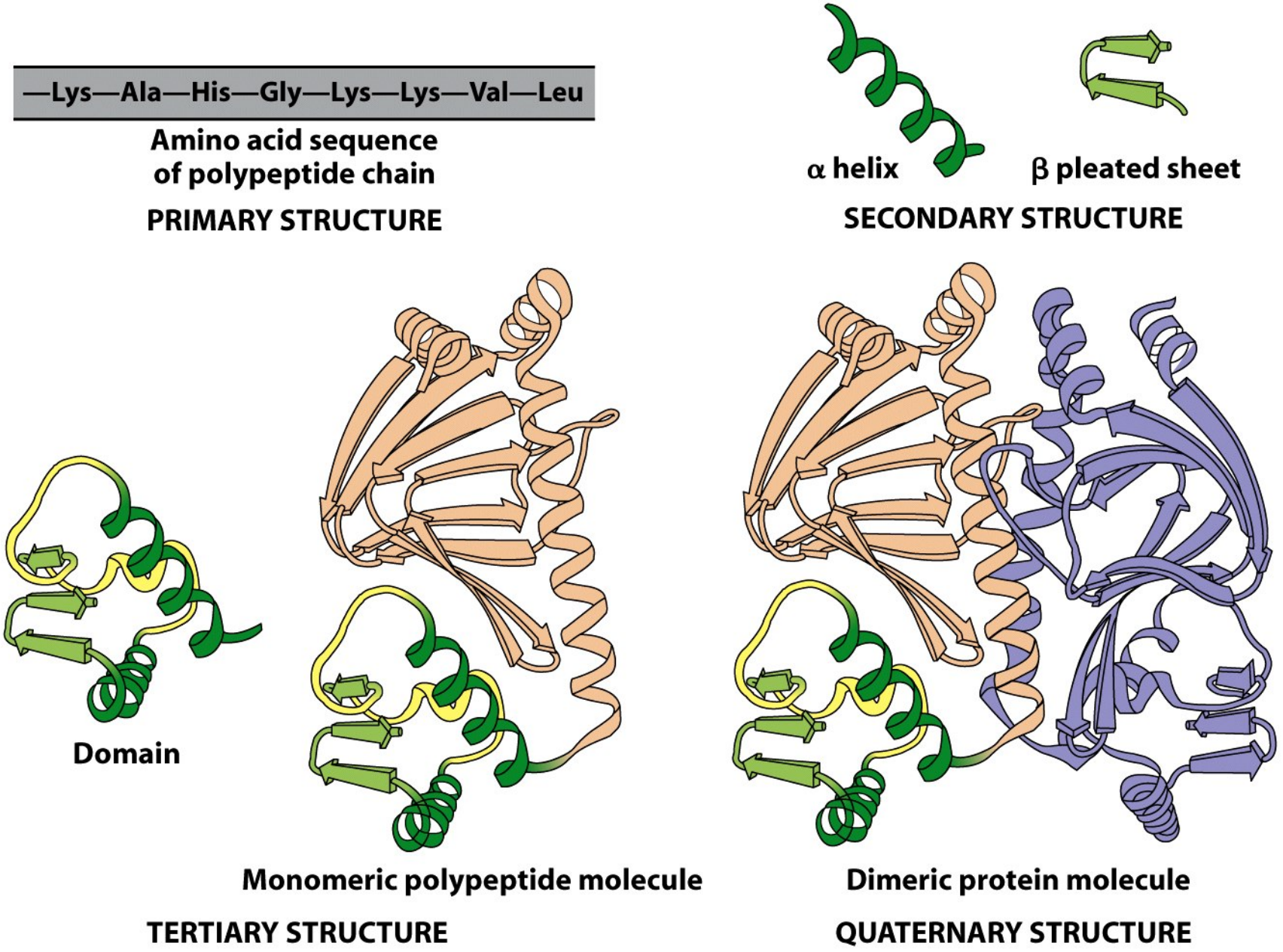


Figure 4-2
Kuby IMMUNOLOGY, Sixth Edition

TABLE 4-2 Comparison of antigen recognition by T cells and B cells

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules

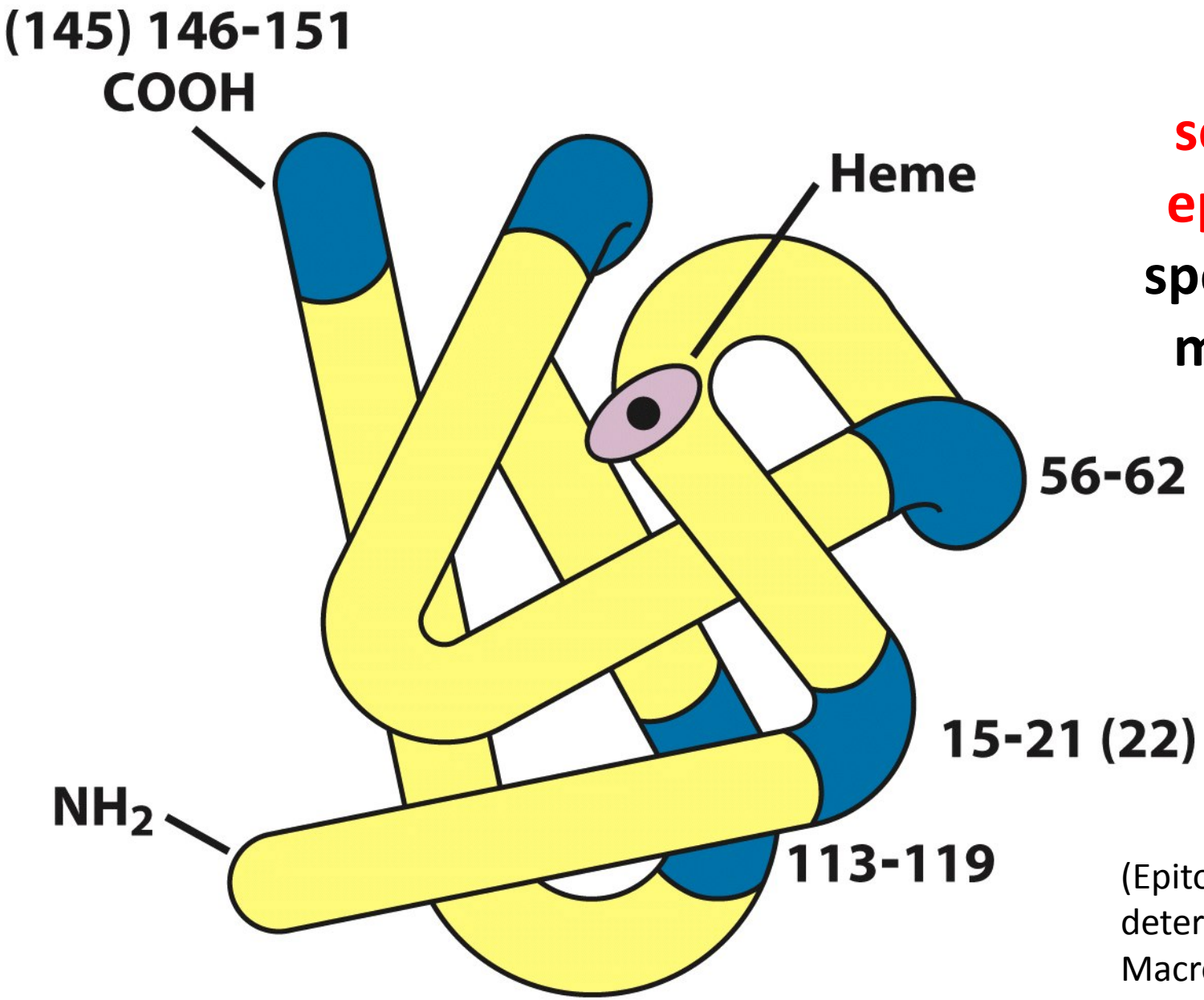
Table 4-2
Kuby IMMUNOLOGY, Sixth Edition

Naïve T cells: cells never meet antigens before.
They can only be activated by dendritic cells.

- **Effector cells: short-lived cells with special** functions such as cytokine secretion and B-cell help and cytotoxic killing activity. Effector cells are derived from naïve or memory cells after antigen activation. TH1 and TH2 subsets.

- **Memory cells: long-lived resting cells that are** derived from naïve and effector cells. They respond faster and stronger to a subsequent challenge with the same antigen.

Primary sequential epitopes in sperm whale myoglobin



(Epitopes are antigenic determinants in Macromolecules)

Figure 4-3a
Kuby IMMUNOLOGY, Sixth Edition

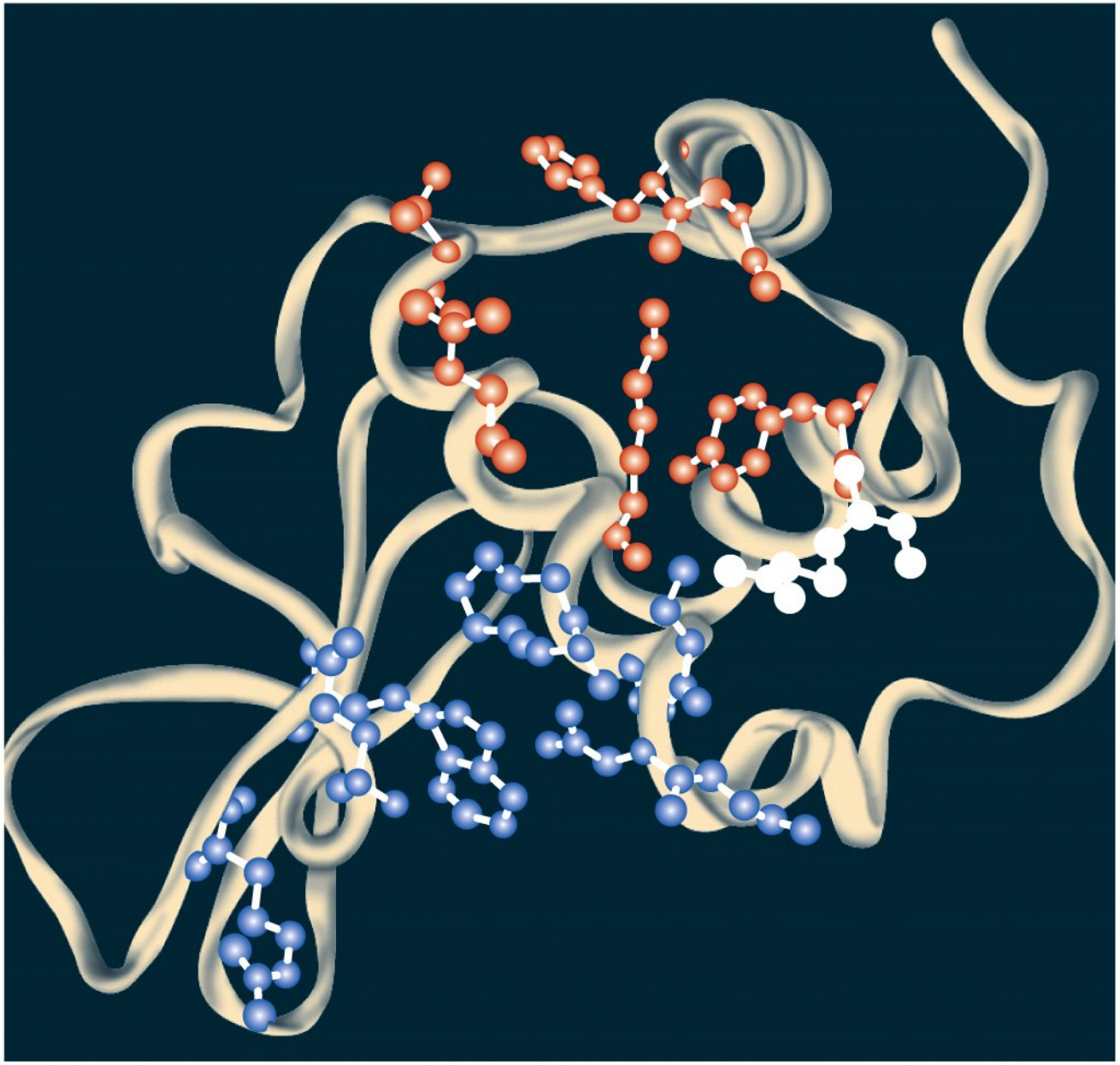


Figure 4-3b
Kuby IMMUNOLOGY, Sixth Edition

Conformational (non-sequential) Epitope in Hen Egg White Lysozyme.

(Colors show amino acid side chains contacting the two different chains of the antibody, or contacting both chains. Antibody structure to be covered later)

Properties of Immunogen contribute to Immunogenicity

- Properties
  - **Foreignness**
  - **Molecular size**
  - **Chemical composition and complexity**
  - **Ability to be processed and presented on MHC**

- Foreignness
  - Lymphocytes that do not bind to self antigens are allowed to further develop
    - Therefore they will later only recognized nonself antigens
  - For example:
    - Bovine serum albumin (BSA) is not immunogenic when injected into cow but is when injected into chicken
    - Some macromolecules are highly conserved throughout evolution and display little immunogenicity
      - » Cytochrome c, collagen

- Molecular Size
  - Active (good) immunogens
    - » > 100,000 Daltons
  - Poor immunogens
    - » < 5,000-10,000 Daltons

- Chemical Composition
  - Polymers composed of multiple copies of same amino acid or sugar tend to be poor immunogens
  - Lipids are haptens and need to be congugated with carrier to produce antibodies
    - Important for assays for detection of some steroids, vitamins

- Susceptibility to antigen processing
  - Large, insoluble macromolecules are more likely to be phagocytized for processing

The biological system contributes to immunogenicity

- Host Genetic make-up
- Manner in which material is presented
- Use of agents (adjuvants) to enhance immunogenicity

- Genotype of recipient animal
  - Genes of MHC
  - Genes in coding for specific antibodies

FACTORS GOVERNING IMMUNOGENICITY

Properties of the Host

Genetics of the responding host

1. **Major Histocompatibility Complex (MHC)**

Immune response genes

IA and IE: Class II in Mouse

ImgenFx2.pcx

Called “H2” in Mice; “HLA” in Human

HLA = Human Leucocyte-associated Antigens

TABLE 4.2

Effect of MHC haplotype on the immune response to the in mice

Two different antigens

Ag&MHCd.pcx
Ruby 2nd Ed (4th Build)

MHC Haplotype	Representative mouse strains	Antibody response to Ag 1	Antibody response to Ag 2
H-2 b	C57	Low	High
H-2 b	C57BL/6	Low	High
H-2 b	C3H.SW	Low	High
H-2 b	I29/J	Low	High
H-2 d	BALB/c	Intermediate	Intermediate
H-2 d	B10.D2	Intermediate	Intermediate
H-2 d	DBA/2	Intermediate	Intermediate
H-2 d	NZB	Intermediate	Intermediate
H-2 k	CBA	High	Low
H-2 k	C3H/HeJ	High	Low
H-2 k	C58J	High	Low
H-2 k	B10.BR	High	Low
H-2	B10.S	Low	Low
H-2	SJL	Low	Low

FACTORS GOVERNING IMMUNOGENICITY

Properties of the Host

Genetics of the responding host

2. Variable region (V-region) genes available for Antibody and T-cell Receptor generation

3. Genes controlling factors for Immune regulation

ImGenFx3.pcx

Immunogen dosage and route of Administration

- Too low or high of dosage can induce tolerance (WHY?)
- Single dose is often not enough – booster is needed
- Route
  - Intravenous (iv)
  - Intradermal (id)
  - Subcutaneous (sc)
  - Intramuscular (im)
  - Intraperitoneal (ip)
    - » Antigen administered iv would travel to spleen; administered sc would travel to lymph nodes

Conformational Properties of Epitopes for Antibodies

Hen egg–white lysosome

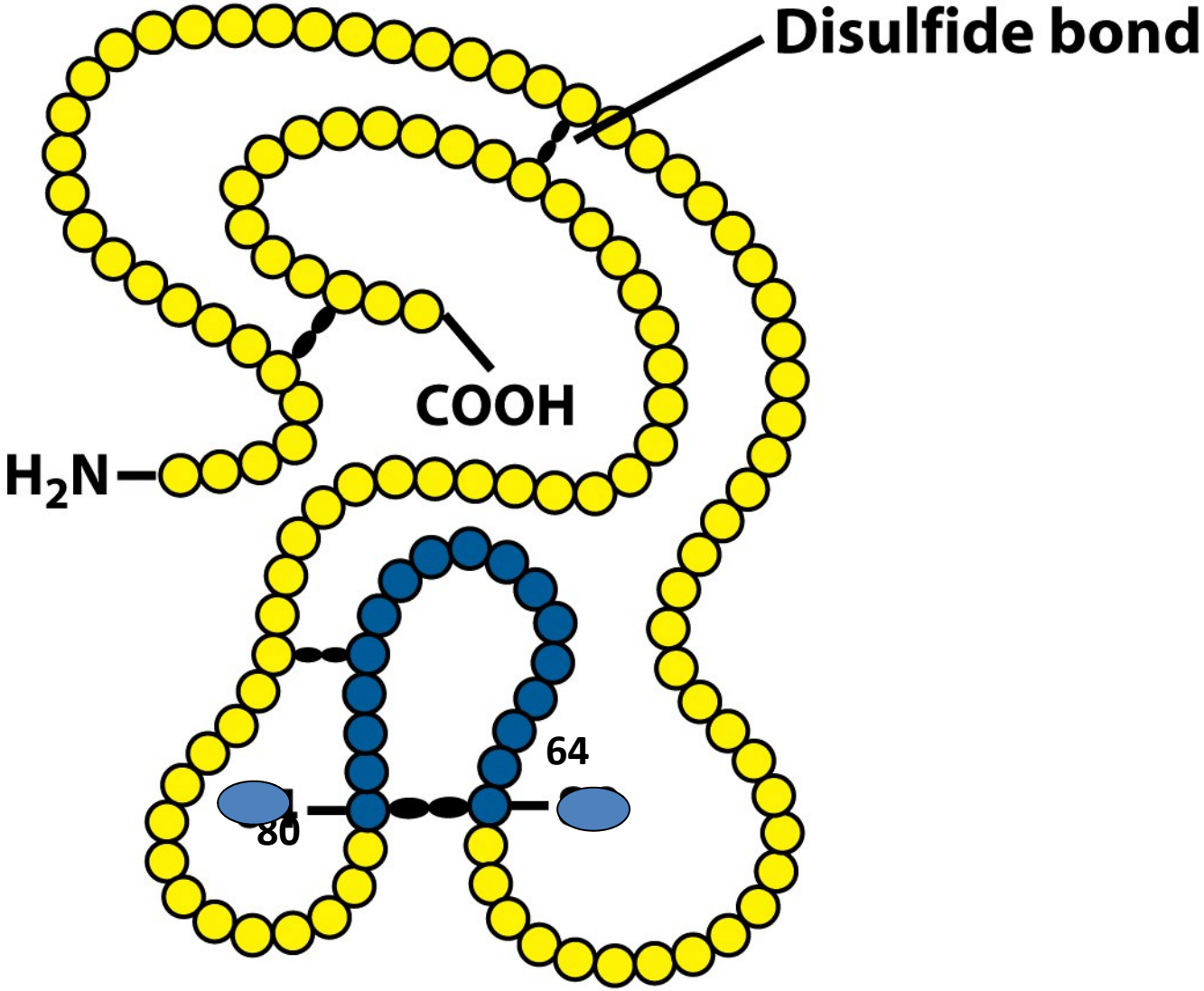


Figure 4-4a
Kuby IMMUNOLOGY, Sixth Edition

Hen Egg-White Lysozyme: Conformations of Sequence 62 to 80

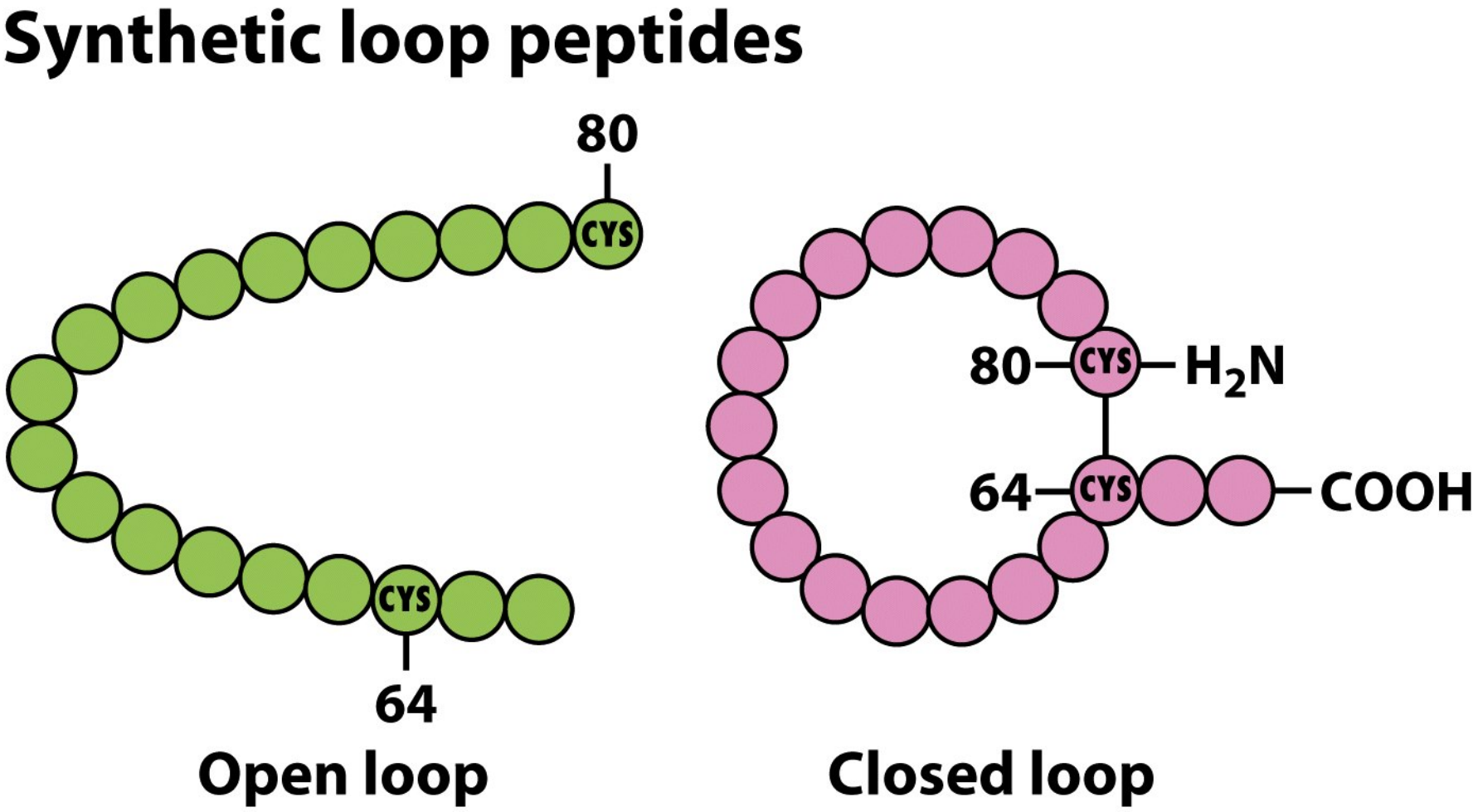


Figure 4-4b
Kuby IMMUNOLOGY, Sixth Edition

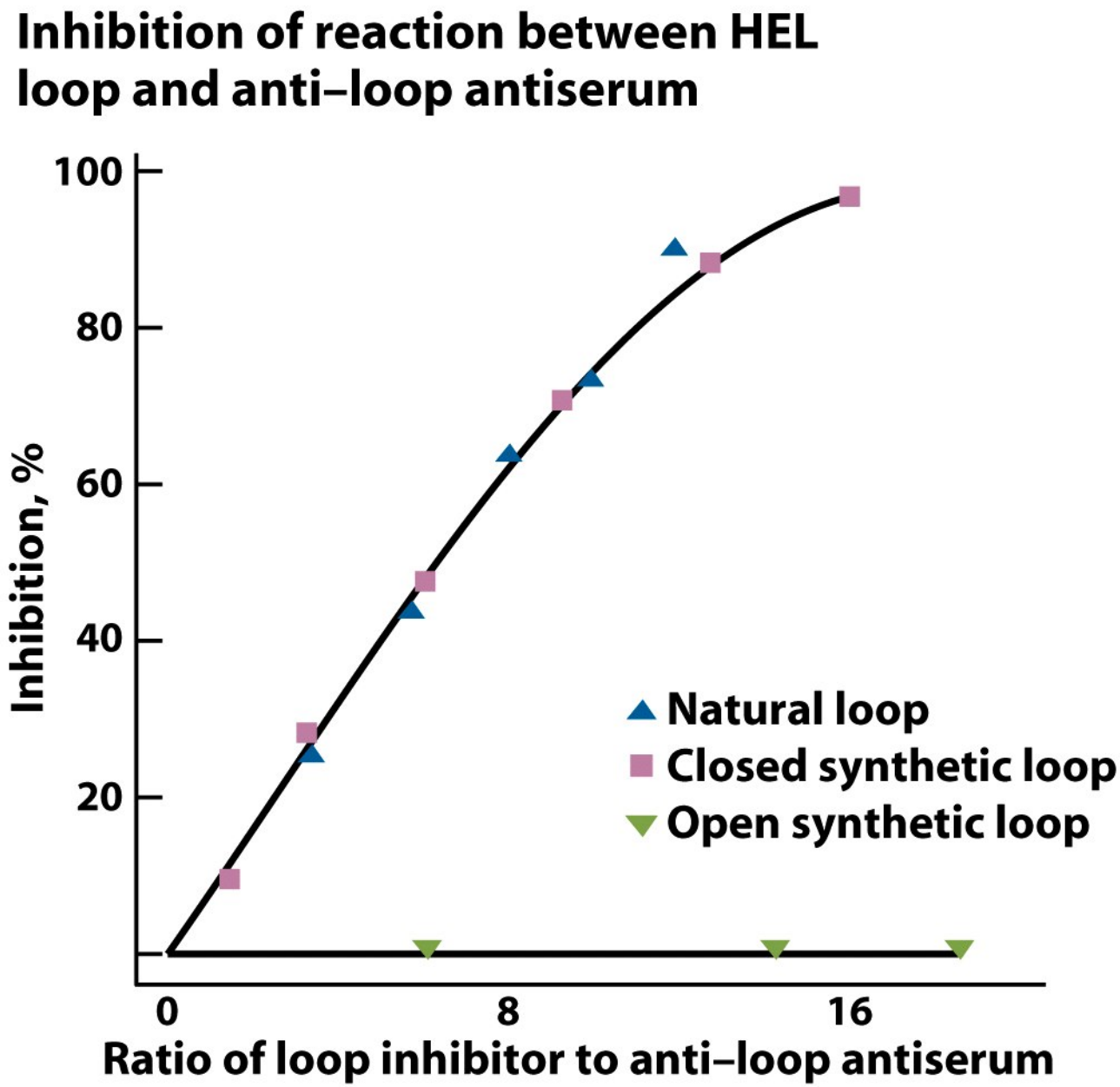


Figure 4-4c
Kuby IMMUNOLOGY, Sixth Edition

ADJUVANTS

An adjuvant (from Latin adjuvare – to help) is any substance, distinct from antigen, which enhances immune response by various mechanisms: by recruiting of professional Antigen-Presenting Cells (APCs) to the site of antigen exposure, by increasing the delivery of antigens by its delayed/slow release (depot generation), immunomudulation by cytokine production (selection of Th1 or Th2 response), by induction of T-cell response (prolonged exposure of peptide-MHC complexes (signal 1) and stimulation of expression of T-cell-activating co-stimulators (signal 2) on the APC's surface).

Freund's adjuvant (toxic for human use, used only for mice , rabbit etc.)
Freund's complete adjuvant – antigen in aqueous solution, mineral oil, emulsifying agent (mannide monooleate), heat killed cells of Mycobacteria (muramyl di-peptide activates dendritic cells and macrophages)

Freund's incomplete adjuvant – antigen in aqueous solution, mineral oil, and emulsifying agent.

Aluminum potassium sulfate (Alum) → Approved for general human use

- Adjuvants

  Enhance immunogenicity by:

  – Prolong antigen persistence (Antigen is released very slowly from injection site)
  – Enhance co-stimulatory signals (B7 etc.)
  – Induce granuloma formation (Chronic inflammatory response attract both phagocytes and lymphocytes formation of dense, macrophage-rich mass)
  – Stimulate lymphocyte proliferation non-specifically

Activation of Systemic Inflammatory Responses by Potent Inflammatory Signals from Infectious Organisms and Plants

Endotoxins
Exotoxins
Super-antigens
Mitogens and Lectins

Immunopathology of Bacterial Septic Shock

Caused by Gram Negative Bacterial Endotoxins:
Examples -
E. coli
Pseudomonas aeruginosa
Neisseria meningitidis
Meningococcus

70,000 Deaths per year
Diarrhea, Fever, Blood Clotting, Blood Pressure Drop

Macrophage Activation by Endotoxin:
Generates systemic cytokine production -
IL1 and TNF-alpha (Tumor necrosis factor)

Immunopathology of Bacterial Toxic Shock

Exotoxins Secreted or Presented Membrane Bound:

Act as "Superantigens"

Examples -

Staphylococcus aureus:

TSST1 (Toxic Shock Syndrome Toxin)

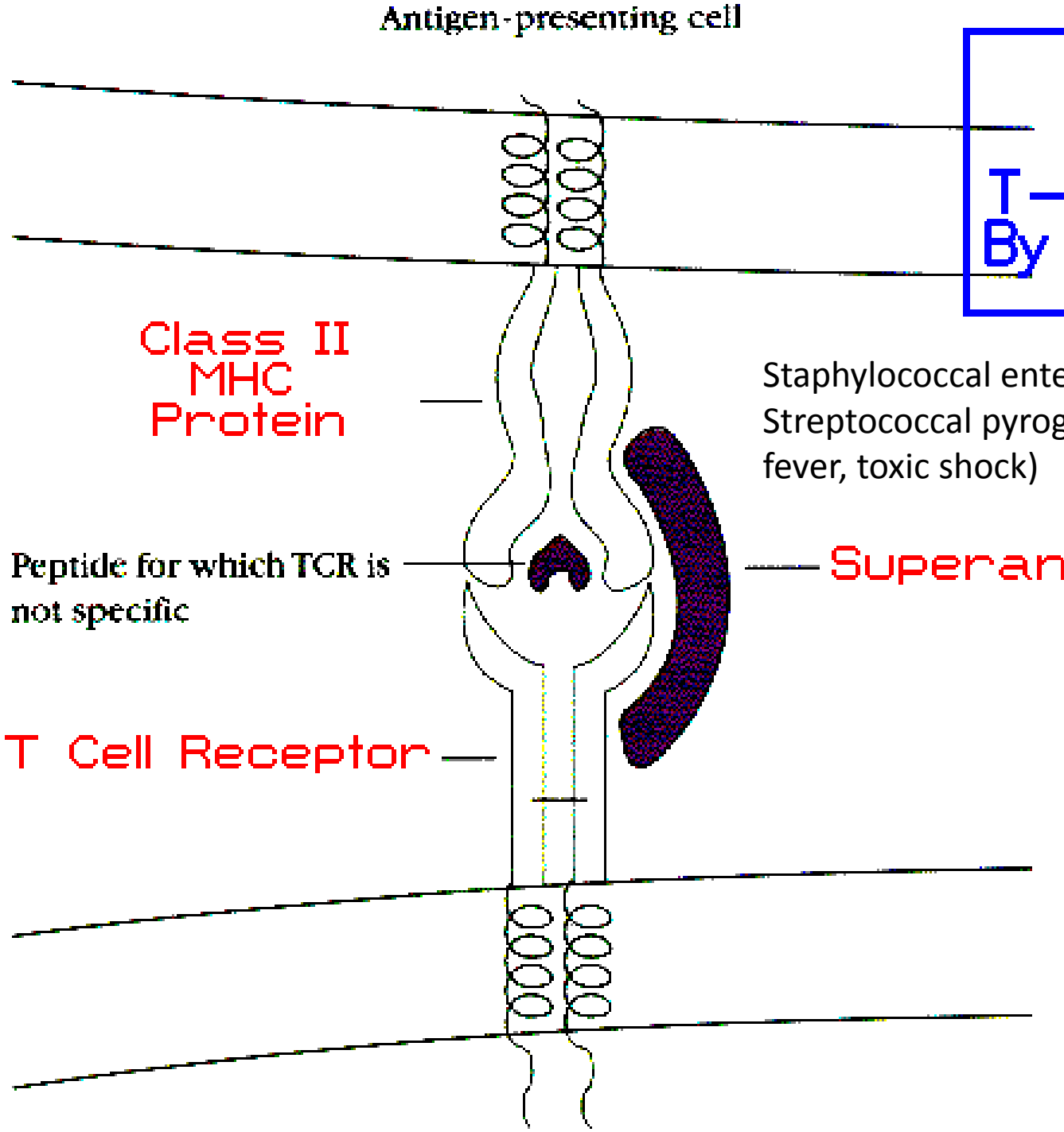
Streptococcus pyrogenes - Rheumatic fever and shock

Staphylococcus enterotoxins: Food poisoning

Superantigens cross-link TCR with Antigen-presenting Cell MHC Class II Proteins

Generates systemic cytokine production - IL1 and TNF-alpha (Tumor necrosis factor)

Generalized Pathological T-Cell Activation By Superantigens



Staphylococcal enterotoxins (food poisoning)
Streptococcal pyrogenes exotoxins (rheumatic fever, toxic shock)

SuperAg.pcx
Figure 4-15
Kuby 2nd Ed

See Figure 11-6
7th Edition p. 367

Mitogens and Lectins

Mitogens: Stimulate mitosis and cell division non-specifically
(non-specific therefore stimulate polyclonally)

Lectins: Proteins that bind to carbohydrate in glycoproteins
(Can be potent mitogens)

Glycoproteins: Conjugated protein with covalently attached carbohydrate residues