

Corso di Immunologia

A.A. 2011-12

Immunoglobuline

Struttura e Funzione

Definitions

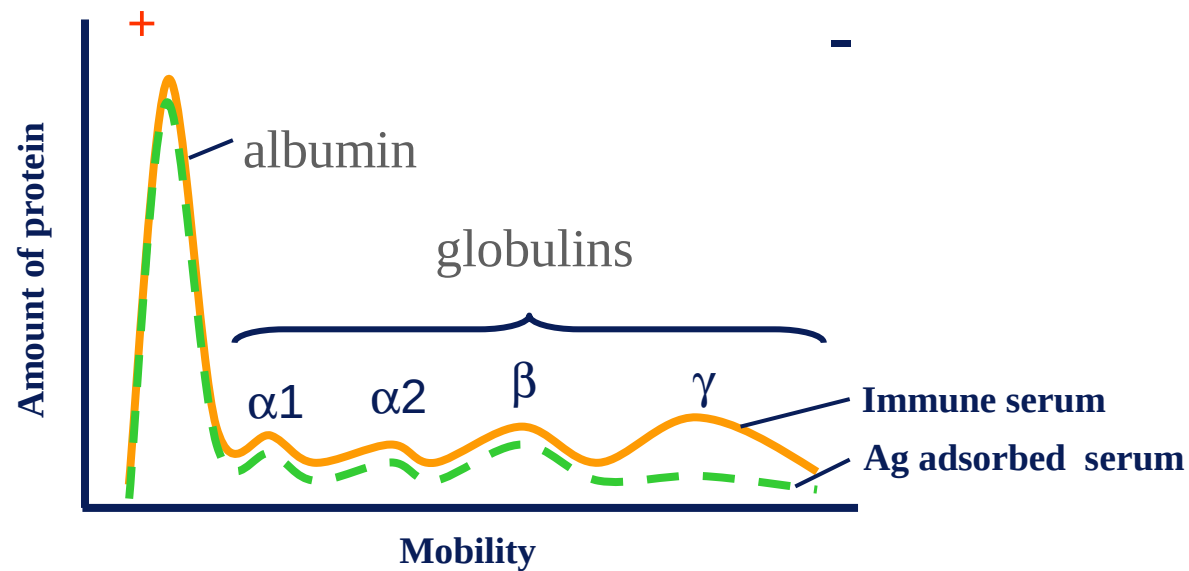
- **Immunoglobulins (Ig)** - Glycoprotein molecules which are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that when antibody-containing serum is placed in an electrical field the antibodies, migrated with the globular proteins.

Keywords

Immunoglobulin, Valence, Heavy chain, Light chain, Variable region, Constant region, Hinge region, Domain, Hypervariable region, Framework region, Groups & subgroups, Fab & Fc, F(ab')₂, Type & subtype, Class & subclass, Opsonin, J chain, Secretory component

Immunoglobulins: Structure and Function

- Definition: Glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies.



General Functions of Immunoglobulins

- **Ag binding** - Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host.
 - Can result in protection
 - **Valency** - The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.

General Functions of Immunoglobulins

- **Effector functions** - (Usually require Ag binding) - Often the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a consequence of secondary "effector functions" of antibodies. The immunoglobulins mediate a variety of these effector functions. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions.
 - **Fixation of complement** - lysis of cells, release of biologically active molecules.
 - **Binding to various cells** - phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins and the binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts. The binding results in transfer of the immunoglobulin across the placenta and the transferred maternal antibodies provide immunity to the fetus and newborn.

Alcune Funzioni Anticorpali

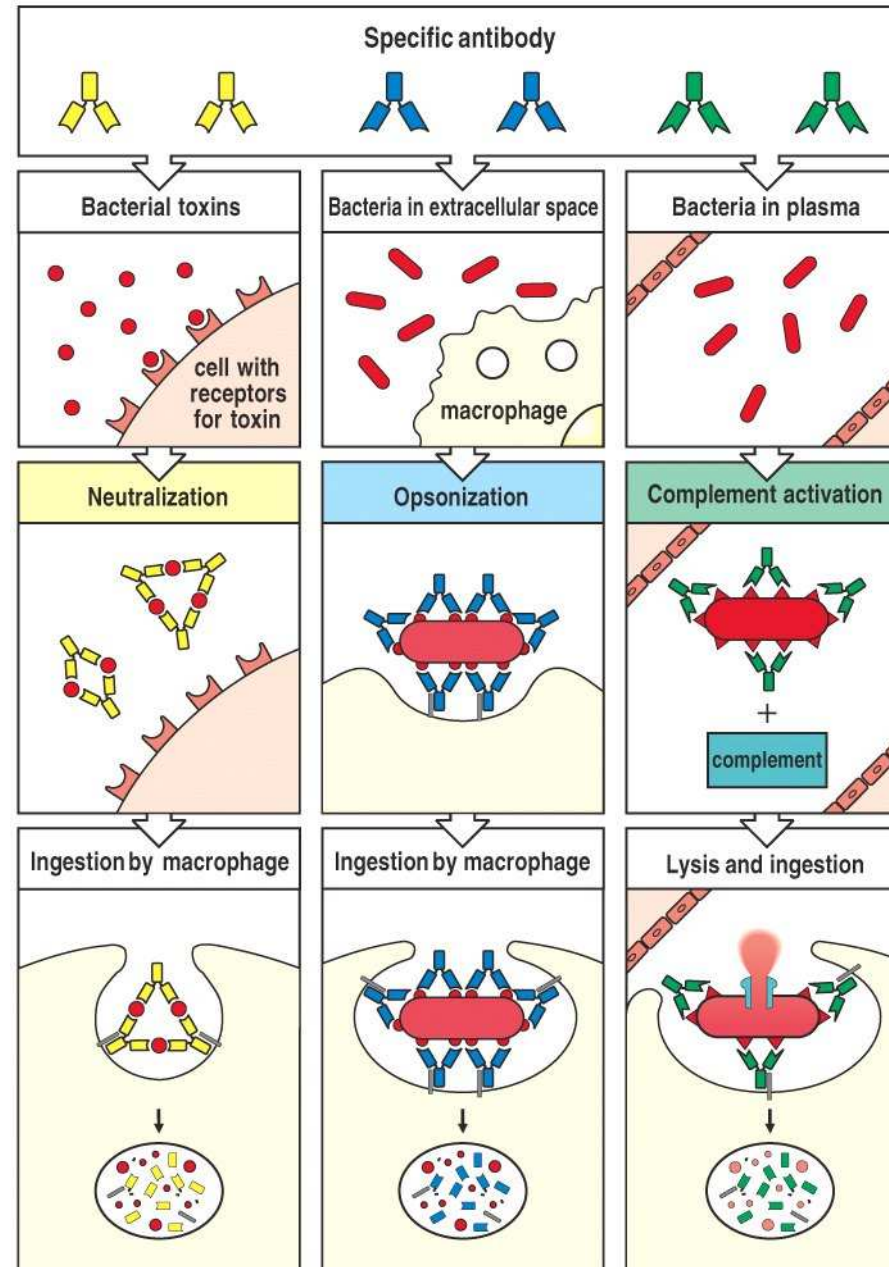


Figure 1-24 Immunobiology, 6/e. (© Garland Science 2005)

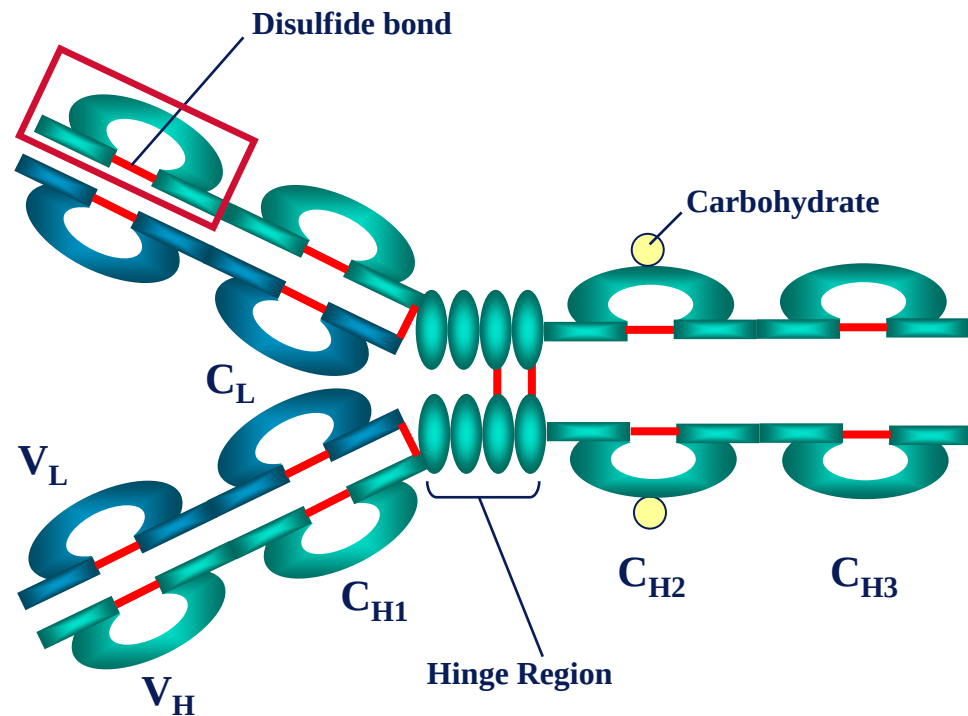
Basic Immunoglobulin Structure

Although different immunoglobulins can differ structurally they all are built from the same basic unit.

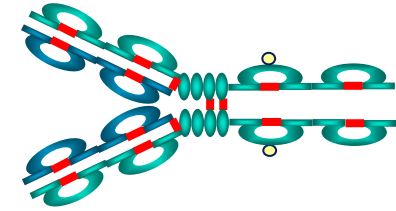
- Immunoglobulins - heterogeneous
- Myeloma proteins - homogeneous immunoglobulins

Dominio
Immunoglobulinico:

2 foglietti beta
stabilizzati da un
legame disolfuro
intracatena ripiegati.



Struttura delle immunoglobuline



Dominio Immunoglobulinico (110 aa): 2 foglietti beta stabilizzati da un legame disolfuro intracatena.

2 Catene pesanti (Heavy)

2 Catene leggere L (Light)

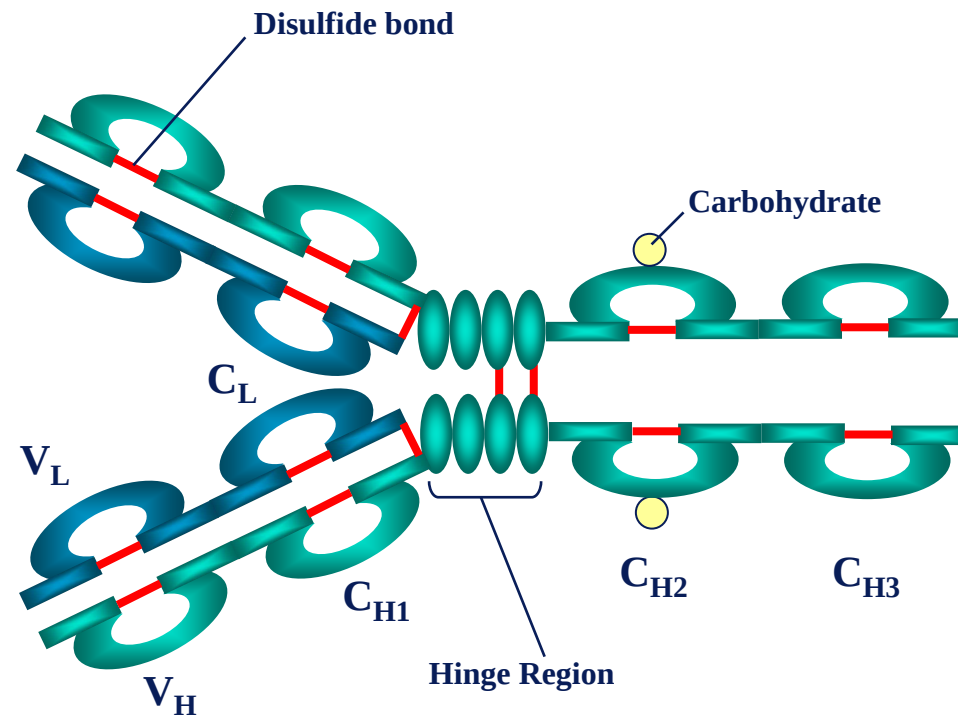
Ogni catena si compone di una **parte variabile (V)** e una **costante (C)**
9 classi o isotipi di catene H (mu, delta, gamma1-4, alfa1,2, epsilon)
e 2 classi o isotipi di L (kappa o lambda).

Nell' ambito delle catene pesanti alfa, gamma e delta, tra CH1 e CH2 e` presente una regione non globulare da circa 10 aminoacidi (in alfa1, 2, gamma1, 2 e 4) a circa 62 aminoacidi (in gamma3 e delta) chiamata regione **cerniera** nelle cui vicinanze sono localizzati i residui cisteinici che danno origine ai ponti disolfuro intercatenari. La regione cerniera fornisce la necessaria elasticita` ai frammenti Fab favorendone il movimento tra CH1 e CH2 e nelle molecole IgG le maggiori differenze tra le catene pesanti sono concentrate in questa regione.

Questa regione non e` presente nelle IgM ed IgE che sono dotate di un dominio aggiuntivo con funzioni di cerniera.

Immunoglobulin Structure (1)

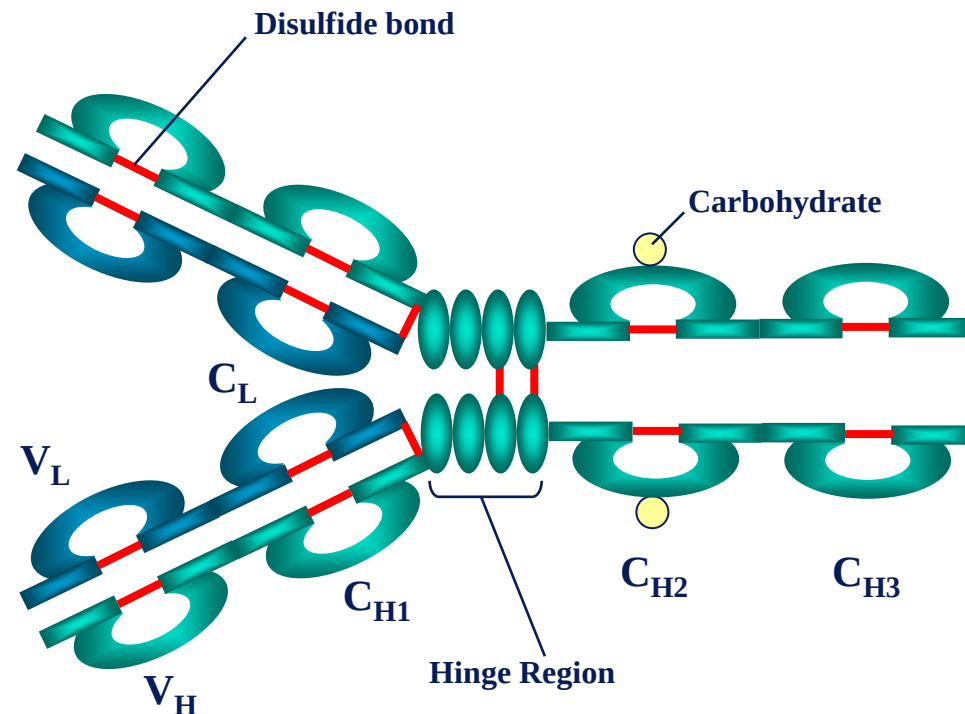
- **Heavy & Light Chains** - All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical **light chains** (23Kd) and two identical **heavy chains** (50-70Kd)



Immunoglobulin Structure (2)

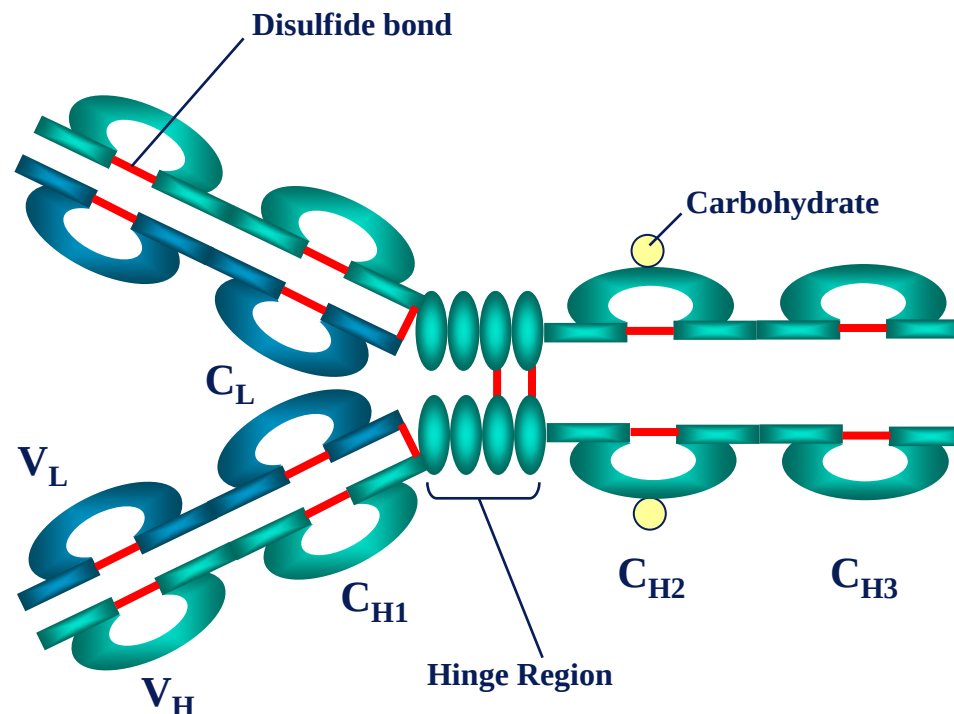
- **Disulfide bonds**

- **Inter-chain** - The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions. The number of interchain disulfide bonds varies among different immunoglobulin molecules.
- **Intra-chain** - Within each of the polypeptide chains there are also intra-chain disulfide bonds.



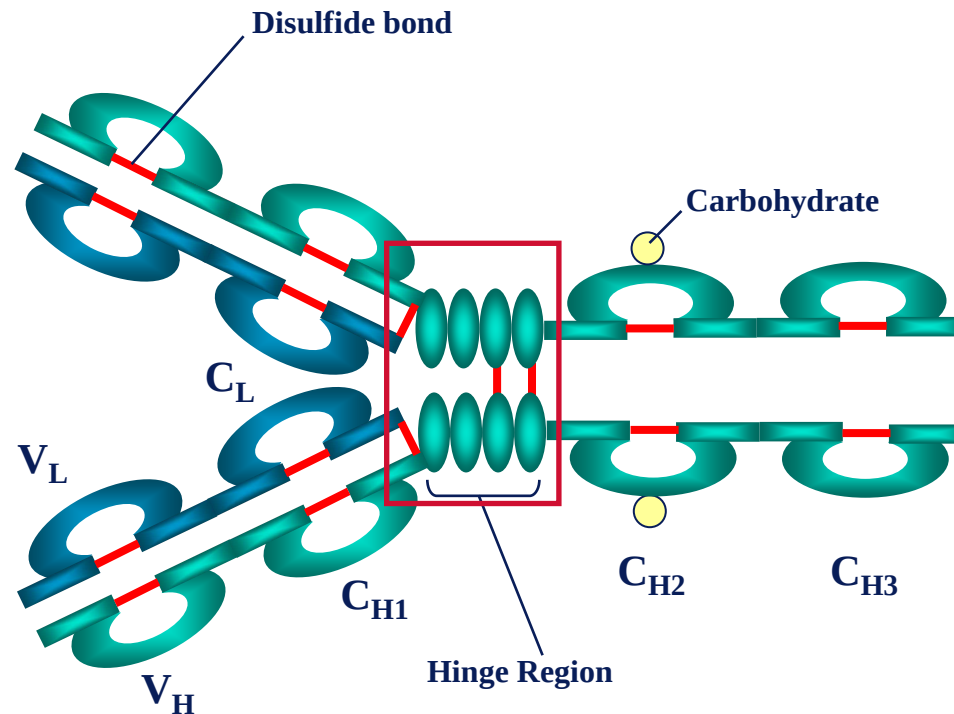
Immunoglobulin Structure

- **Variable & Constant Regions** - After the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the heavy and light chain could be divided into two regions based on variability in the amino acid sequences.
 - 1. Light Chain - V_L (110 aa) and C_L (110 aa)
 - 2. Heavy Chain - V_H (110 aa) and C_H (330-440 aa)



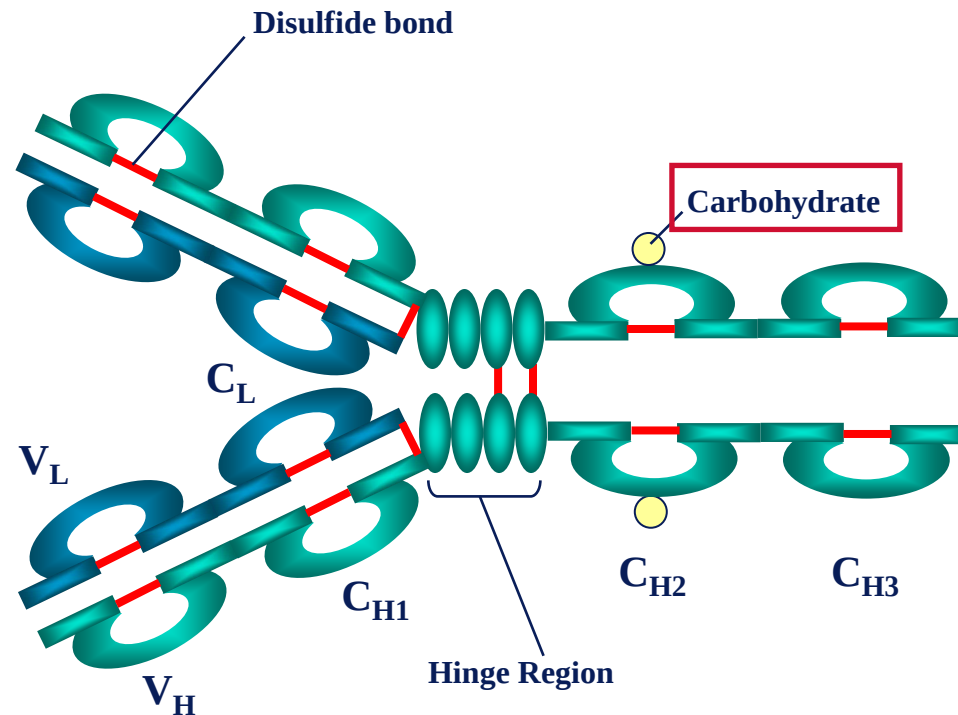
Immunoglobulin Structure

- **Hinge Region** - The region at which the arms of the antibody molecule forms a Y is called the hinge region because there is some flexibility in the molecule at this point.



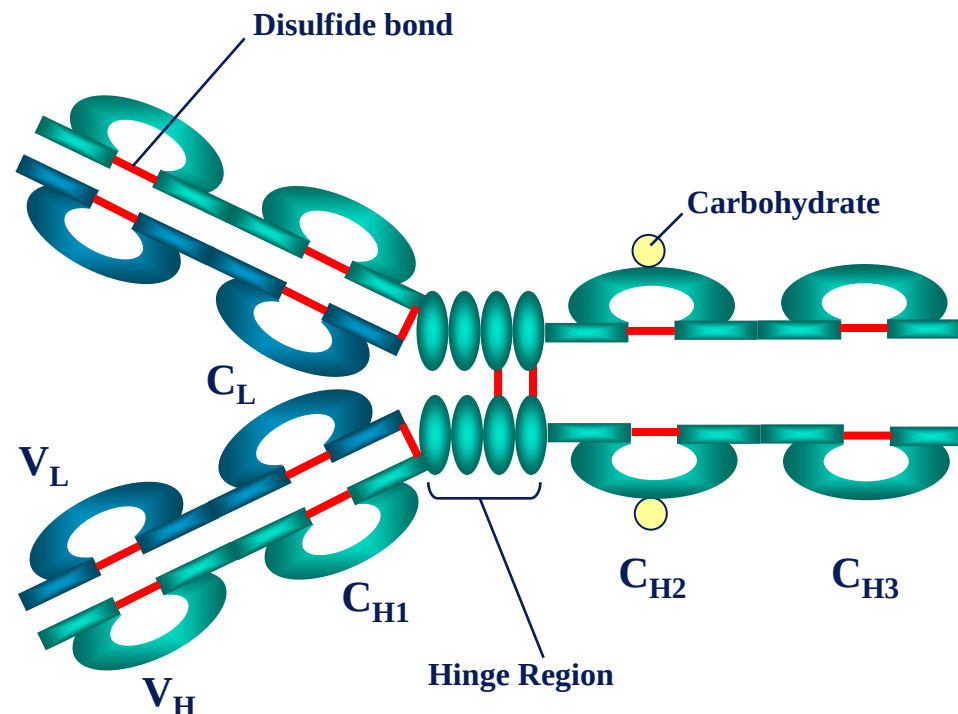
Immunoglobulin Structure

- **Oligosaccharides** - Carbohydrates are attached to the CH2 domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.



Immunoglobulin Structure

- **Domains** - 3D images of the immunoglobulin molecule shows that it is not straight as depicted in figure. Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond. These regions are called domains.
 - Light Chain Domains - V_L & C_L
 - Heavy Chain Domains - V_H & $C_{H1} - C_{H3}$ (or C_{H4})



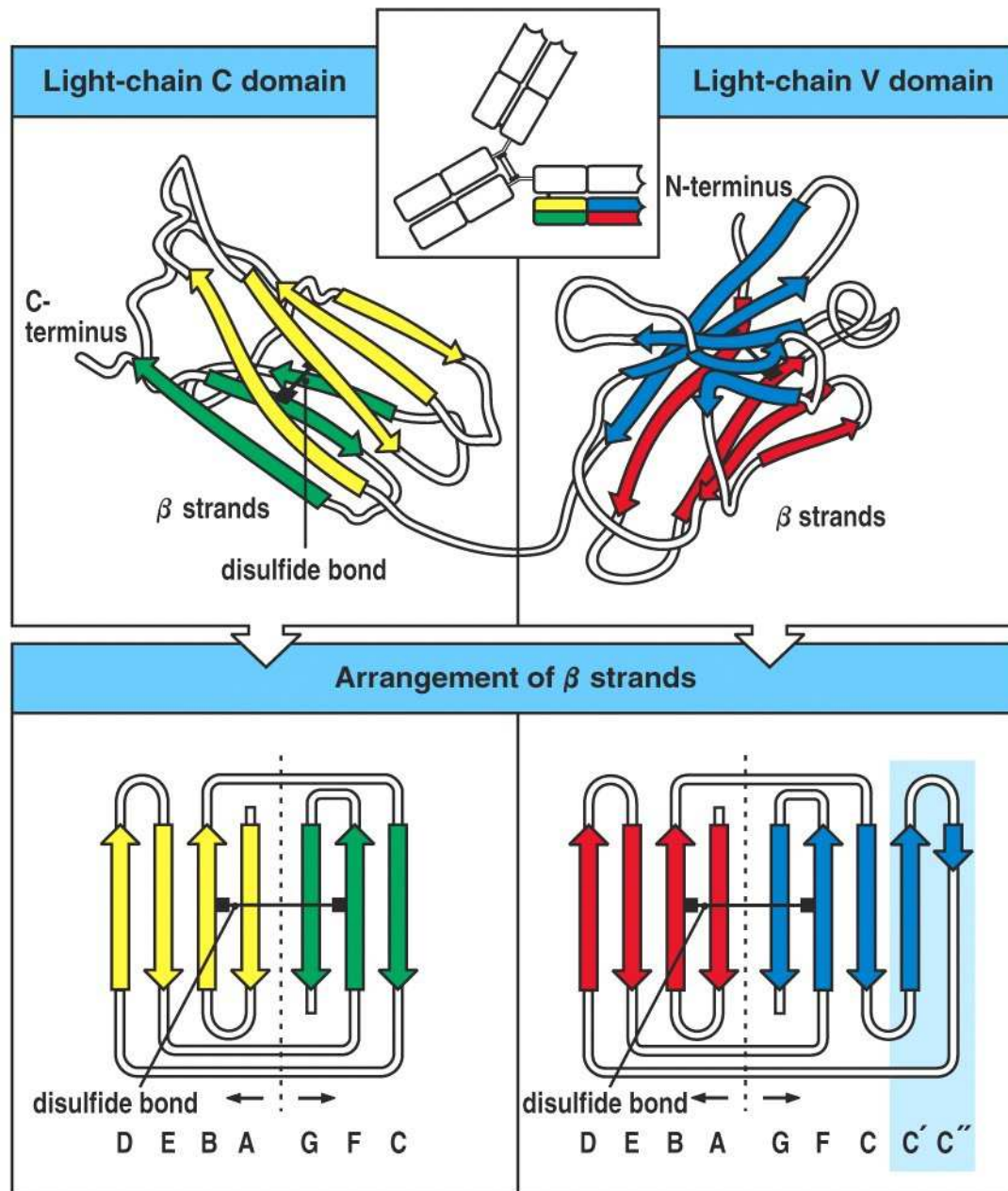


Figure 3-5 Immunobiology, 6/e. (© Garland Science 2005)

Dominio
Immunoglobulinico:

2 foglietti beta
stabilizzati da un
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intracatena ripiegati.

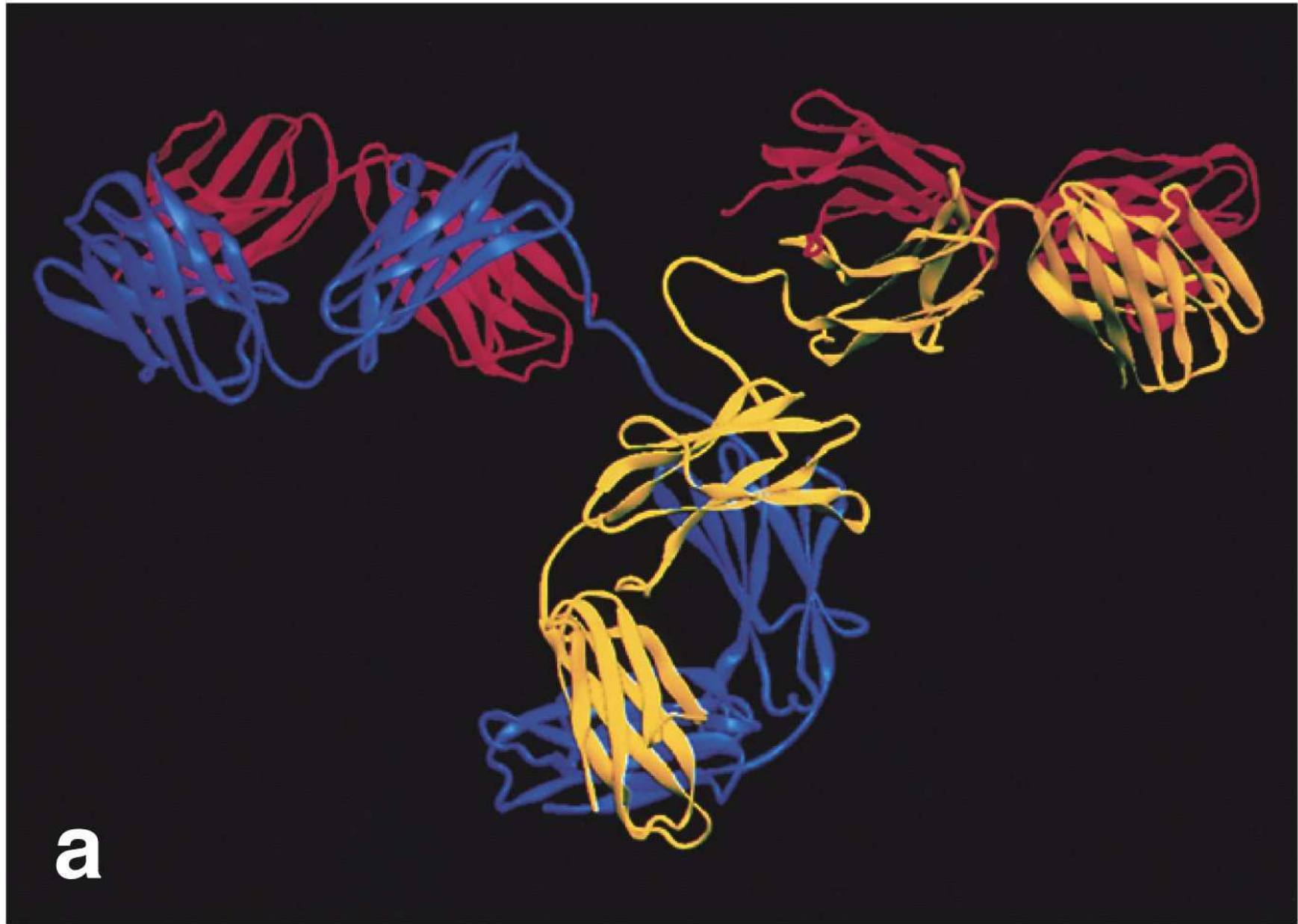


Figure 3-1 part 1 of 3 Immunobiology, 6/e. (© Garland Science 2005)

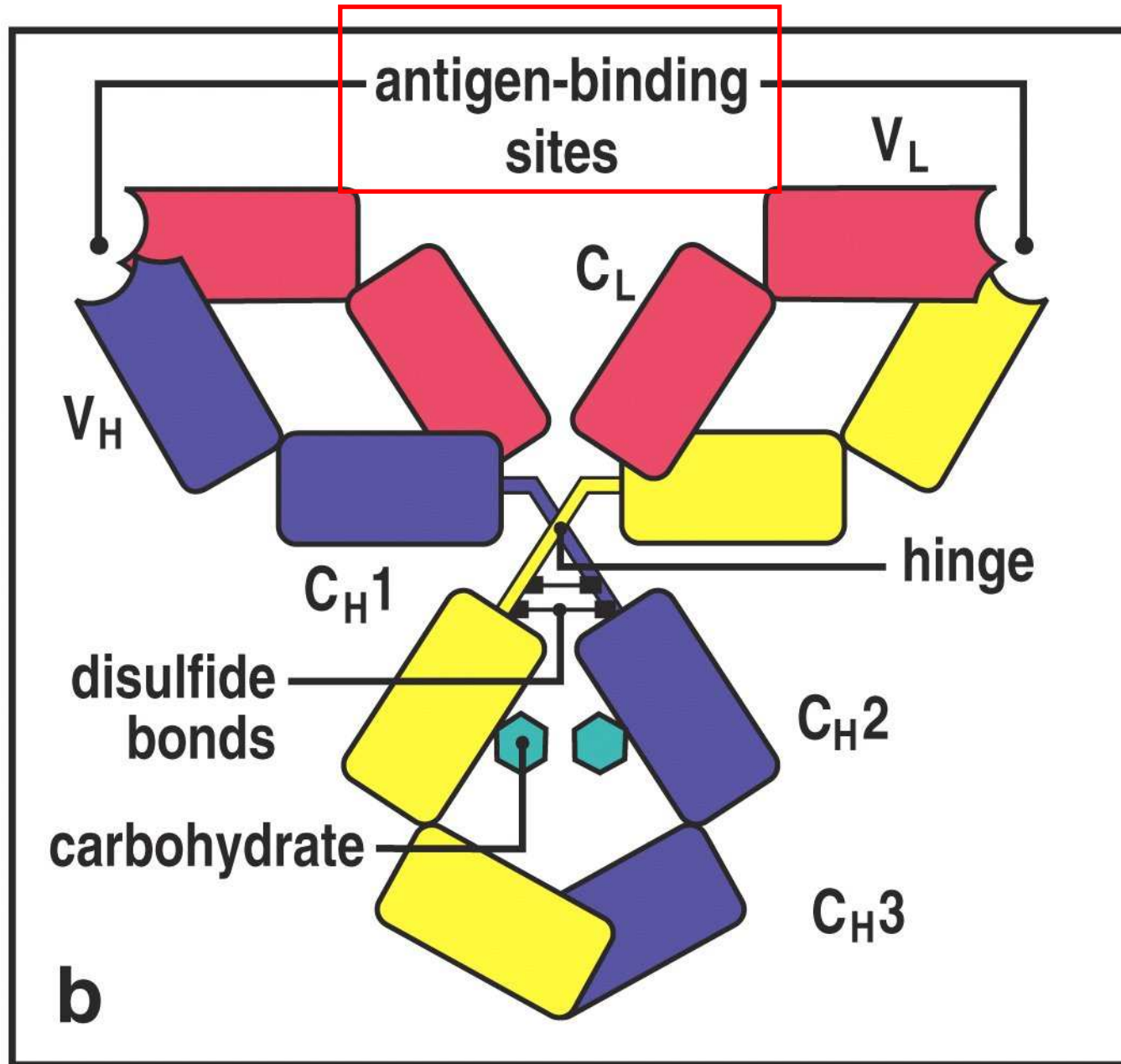


Figure 3-1 part 2 of 3 Immunobiology, 6/e. (© Garland Science 2005)

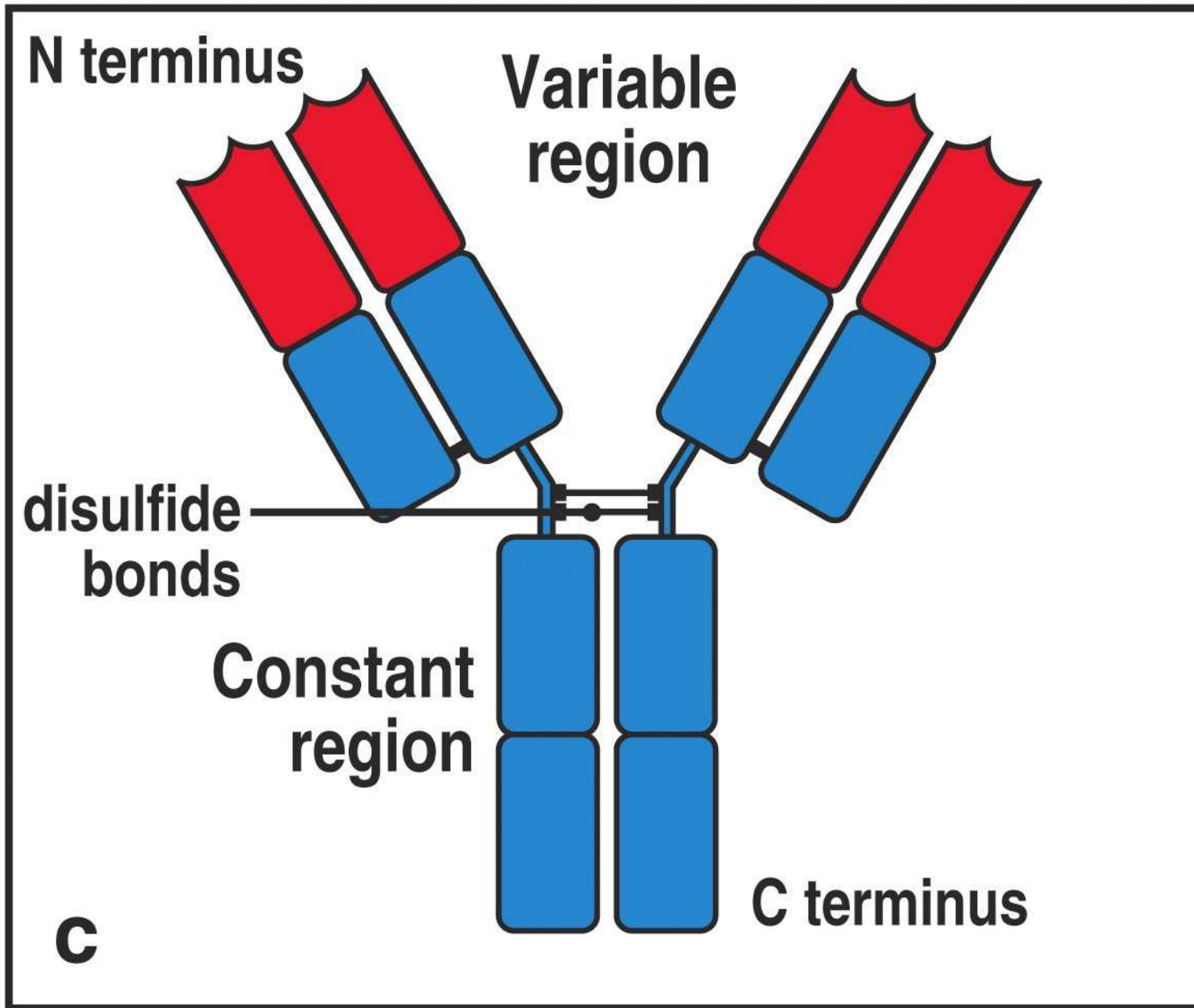


Figure 3-1 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)

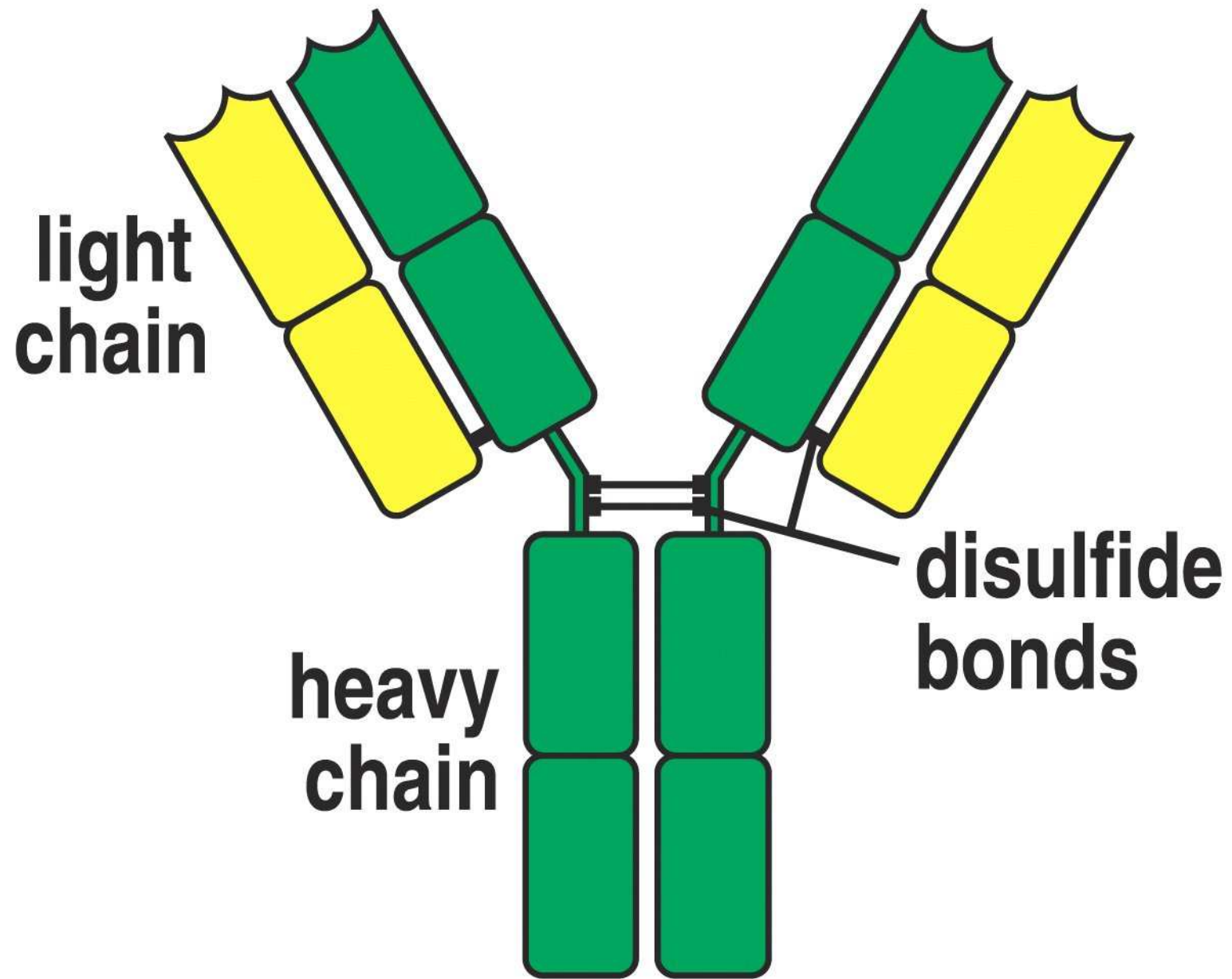


Figure 3-2 Immunobiology, 6/e. (© Garland Science 2005)

Complessi antigene-anticorpo

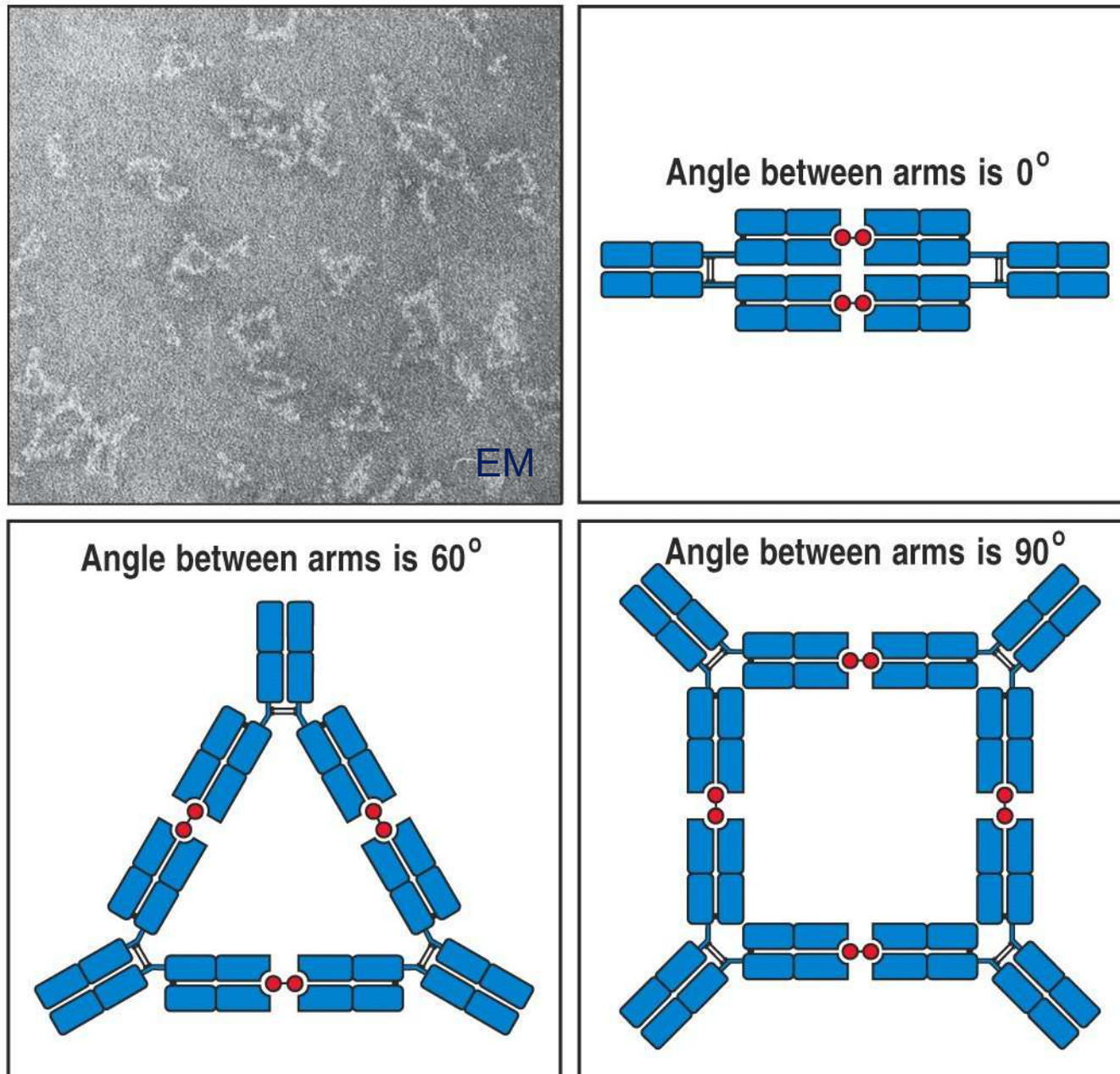
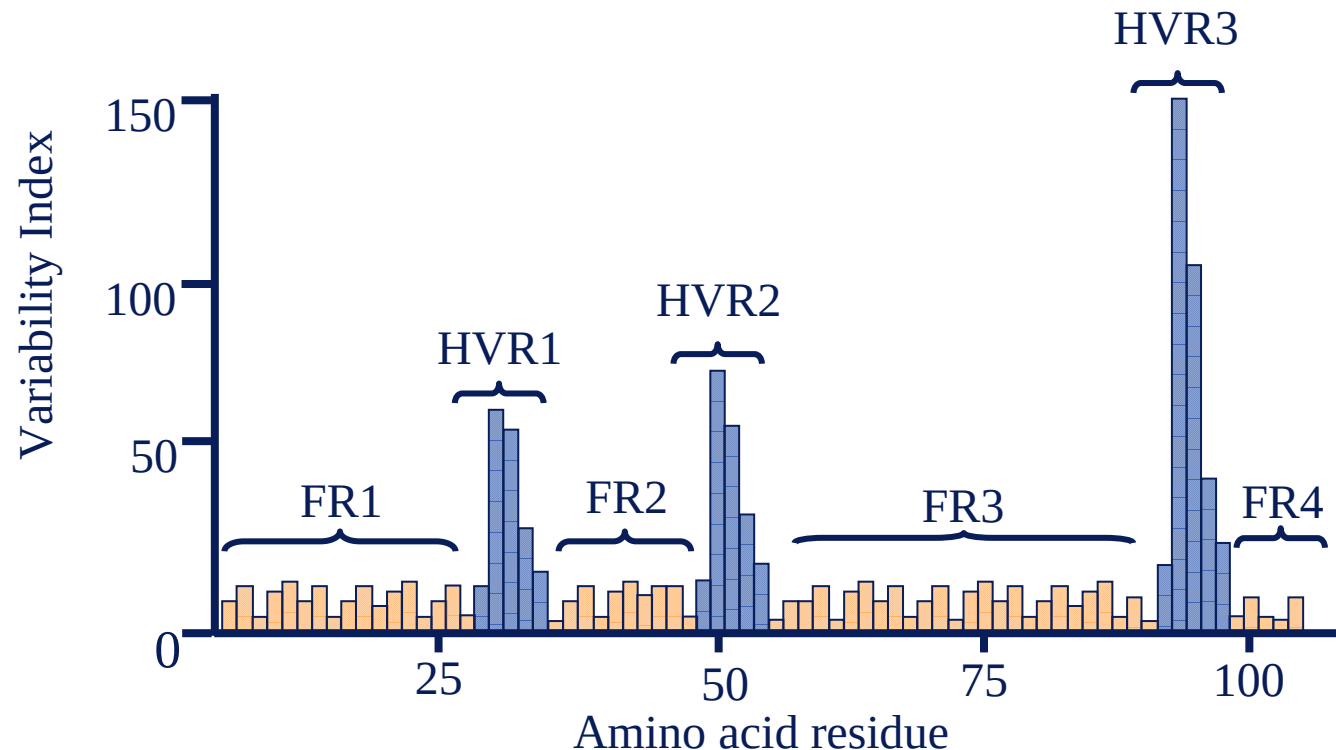


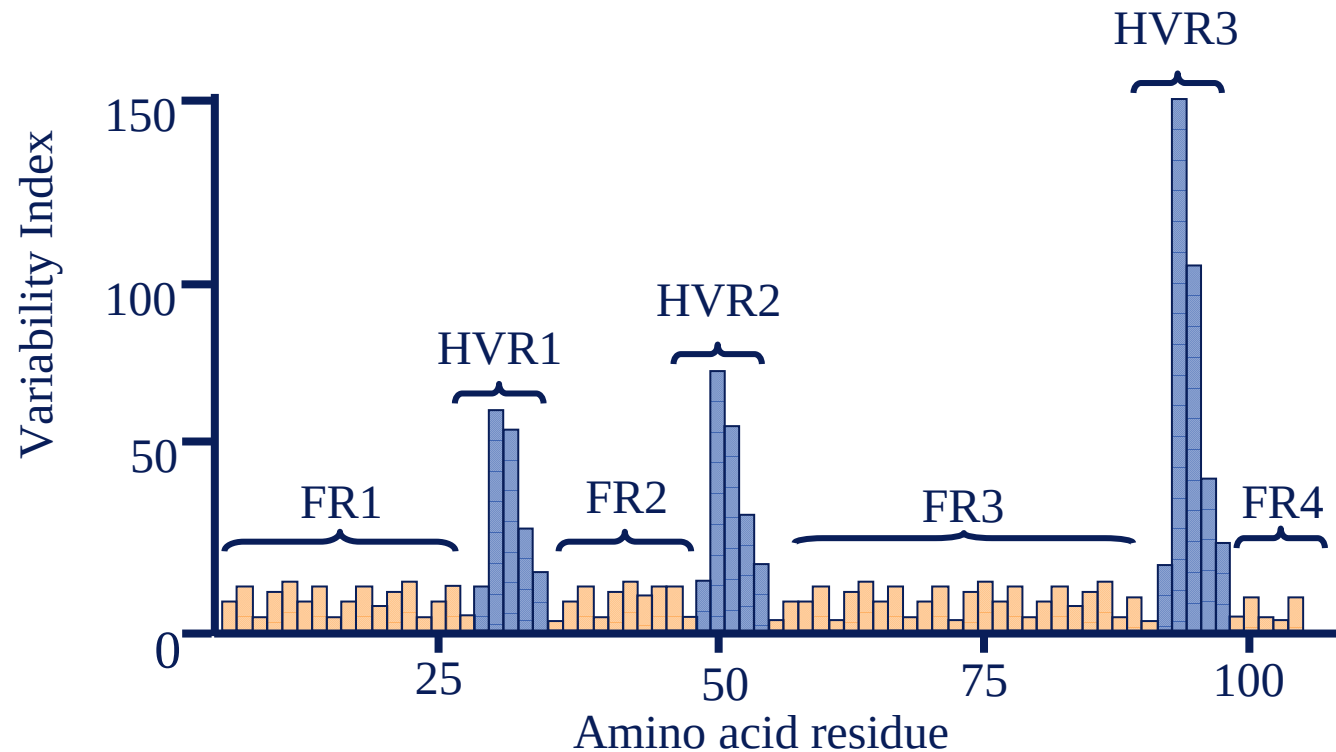
Figure 3-4 Immunobiology, 6/e. (© Garland Science 2005)

Structure of the Variable Region

- Hypervariable (**HVR**) or complementarity determining regions (**CDR**) - Comparisons of the amino acid sequences of the variable regions of Igs show that most of the variability resides in three regions are called the hypervariable regions or the complementarity determining regions as illustrated in figure. Antibodies with different specificities (i.e. different combining sites) have different CDR's while antibodies of the exact same specificity have identical CDR's (i.e. CDR --> Ab Combining site). CDR's are found in both the H and the L chains.



Structure of the Variable Region

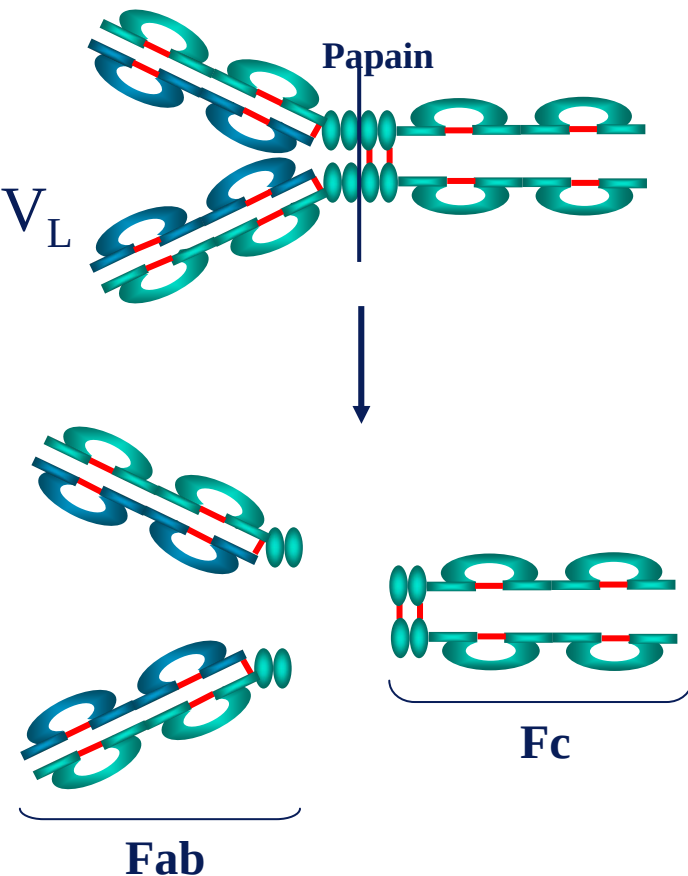


- Framework regions (**FR**) - The regions between the CDR's in the variable region are called the framework regions. Based on similarities and differences in the framework regions the immunoglobulin heavy and light chain variable regions can be divided into groups and subgroups. These represent the products of different variable region genes.

Immunoglobulin Fragments: Structure/Function Relationships

- Fab (*Fragment Antigen Binding*) - Immunoglobulin fragments produced by proteolytic digestion have proven very useful in elucidating structure/function relationships in immunoglobulins.

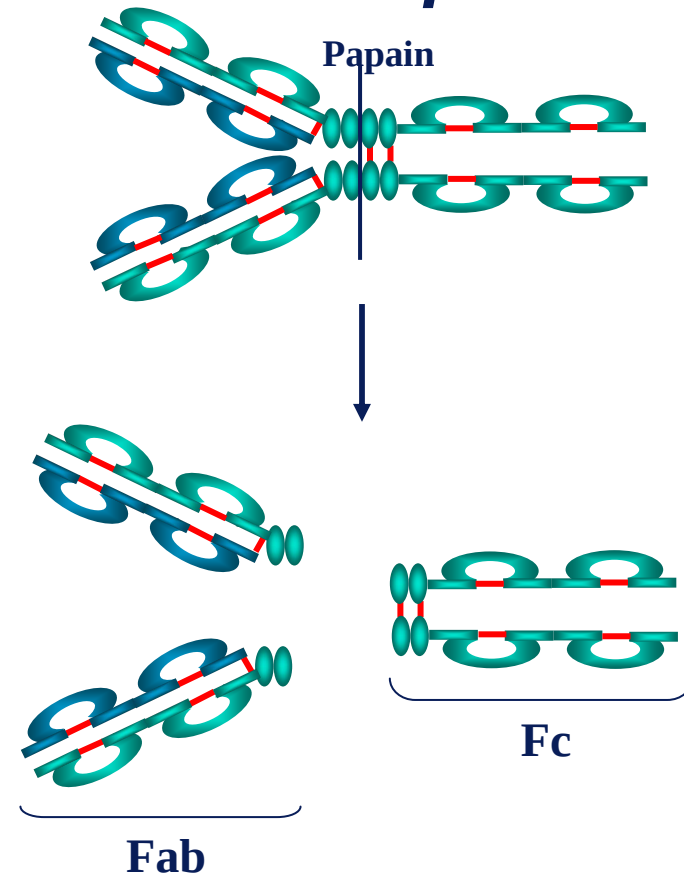
- Ag binding
- Valence = 1
- Specificity determined by V_H and V_L



- Fc
 - Effector functions

Immunoglobulin Fragments: Structure/Function Relationships

- Fab -
 - Ag binding
 - Valence = 1
 - Specificity determined by V_H and V_L



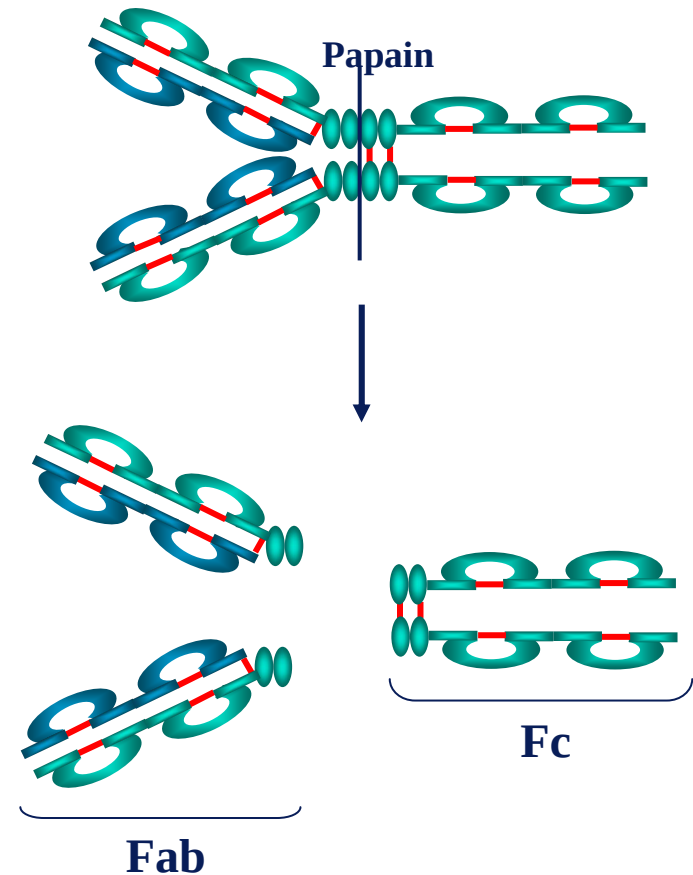
- **FC** (*Crystallizing Fragment*)- Digestion with papain also produces a fragment that contains the remainder of the two heavy chains each containing a CH2 and CH3 domain. This fragment was called Fc because it was easily crystallized.
 - Effector functions

Immunoglobulin Fragments: Structure/Function Relationships

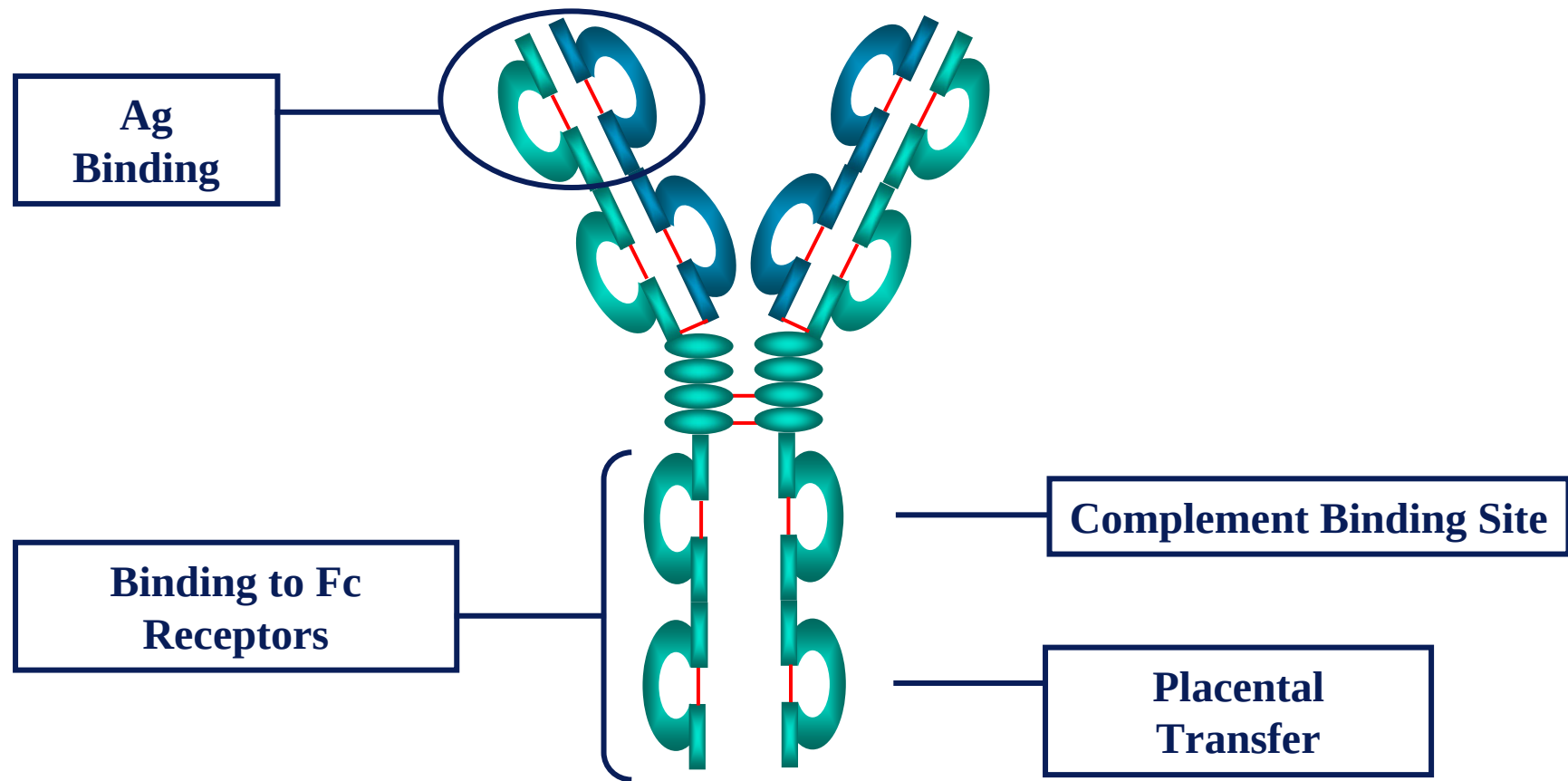
- Fab
 - Ag binding - These fragments were called the Fab fragments because they contained the antigen binding sites of the antibody. Each Fab fragment is monovalent whereas the original molecule was divalent. The combining site of the antibody is created by both V_H and V_L . An antibody is able to bind a particular antigenic determinant because it has a particular combination of V_H and V_L . Different combinations of a V_H and V_L result in antibodies that can bind a different antigenic determinants.

Immunoglobulin Fragments: Structure/Function Relationships

- Fc
 - Effector functions - The effector functions of immunoglobulins are mediated by this part of the molecule. Different functions are mediated by the different domains in this fragment. Normally the ability of an antibody to carry out an effector function requires the prior binding of an antigen. However, there are exceptions to this rule.

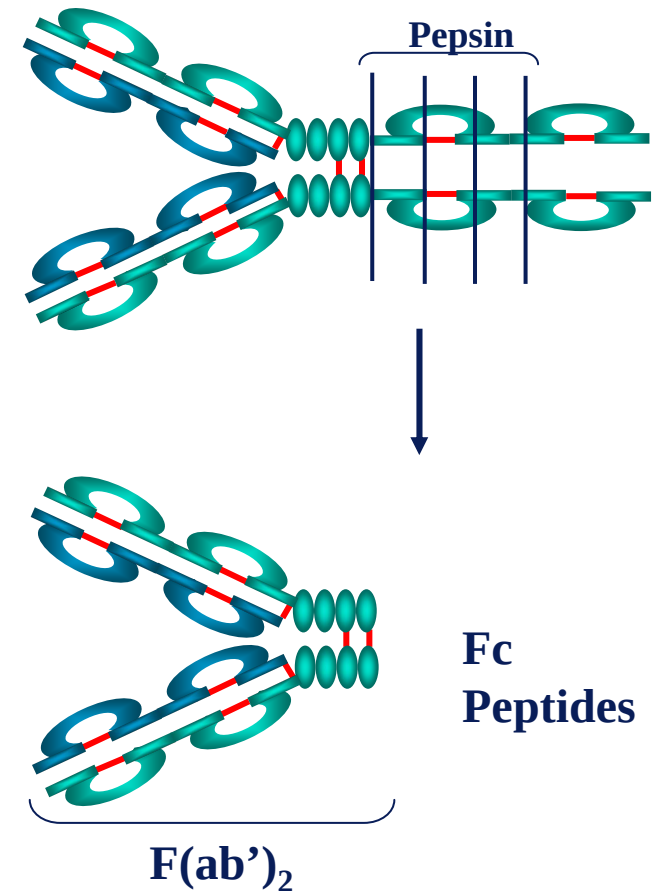


Immunoglobulin Fragments: Structure/Function Relationships



Immunoglobulin Fragments: Structure/Function Relationships

- Fab
 - Ag binding
- Fc
 - Effector functions
- $F(ab')_2$ - Treatment of immunoglobulins with pepsin results in cleavage of the heavy chain after the H-H inter-chain disulfide bonds resulting in a fragment that contains both antigen binding sites. This fragment was called $F(ab')_2$ because it was divalent. The Fc region of the molecule is digested into small peptides by pepsin. The $F(ab')_2$ binds antigen but it does not mediate the effector functions of antibodies.



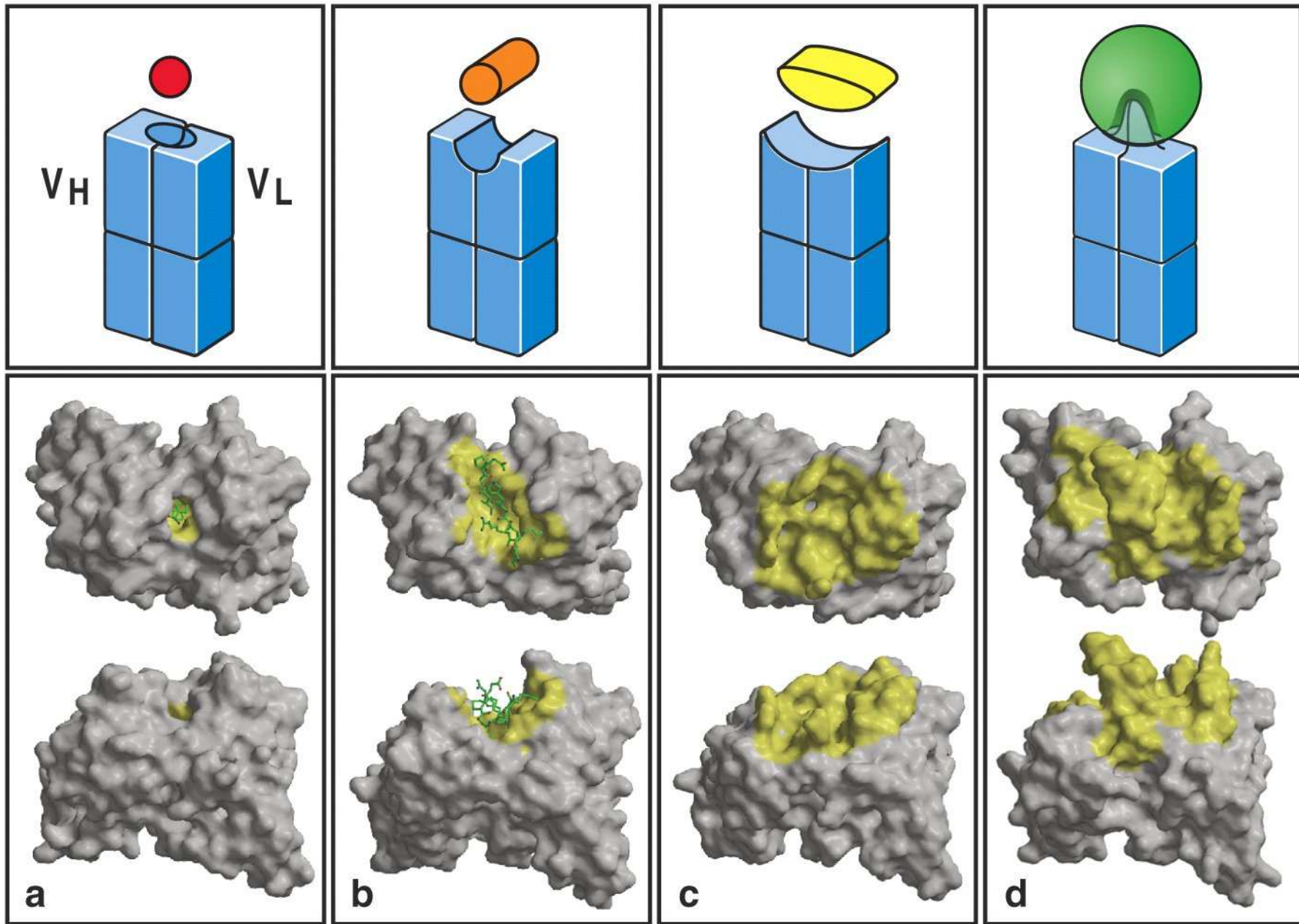


Figure 3-8 Immunobiology, 6/e. (© Garland Science 2005)

Legame antigene-anticorpo

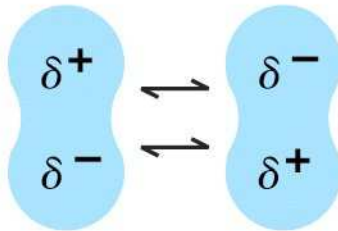
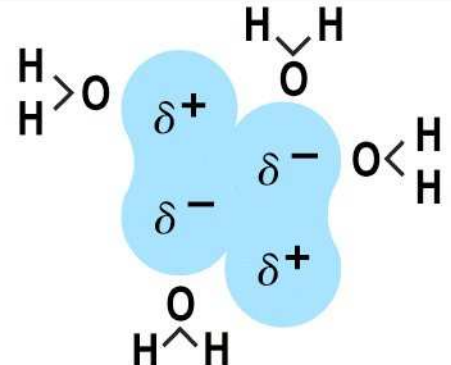
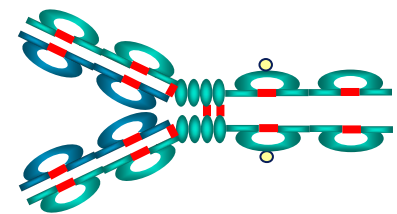
Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	$\text{—NH}_3^+ \quad ^-\text{OOC—}$
Hydrogen bonds	Hydrogen shared between electronegative atoms (N,O)	$\begin{array}{c} \diagup \text{N} \text{—} \text{H} \text{—} \text{O}=\text{C} \diagdown \\ \delta^- \quad \delta^+ \quad \delta^- \end{array}$
Van der Waals forces	Fluctuations in electron clouds around molecules oppositely polarize neighboring atoms	
Hydrophobic forces	Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces	

Figure 3-9 Immunobiology, 6/e. (© Garland Science 2005)

Human Immunoglobulin Classes

The immunoglobulins can be divided into 5 different **classes** based on differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a given class will have very similar heavy chain

- IgG - Gamma heavy chains
- IgM - Mu heavy chains
- IgA - Alpha heavy chains
- IgD - Delta heavy chains
- IgE - Epsilon heavy chains



Human Immunoglobulin Subclasses

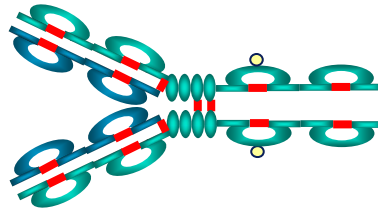
The classes of immunoglobulins can be divided into **subclasses** based on small differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a subclass will have very similar heavy chain constant region amino acid sequences. Again these differences are most commonly detected by serological means.

- IgG Subclasses
 - IgG1 - Gamma 1 heavy chains
 - IgG2 - Gamma 2 heavy chains
 - IgG3 - Gamma 3 heavy chains
 - IgG4 - Gamma 4 heavy chains
- IgA subclasses
 - IgA1 - Alpha 1 heavy chains
 - IgA2 - Alpha 2 heavy chains

Human Immunoglobulin Light Chain Types

Immunoglobulins can also be classified by the **type** of light chain that they have. Light chain types are based on differences in the amino acid sequence in the constant region of the light chain. These differences are detected by serological means.

- Kappa
- Lambda



Human Immunoglobulin Light Chain Subtypes

The light chains can also be divided into **subtypes** based on differences in the amino acid sequences in the constant region of the light chain.

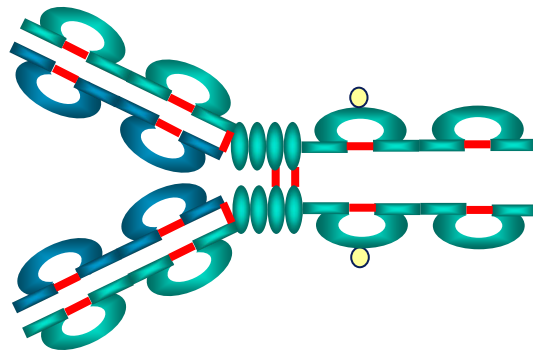
- Lambda light chains
 - Lambda 1
 - Lambda 2
 - Lambda 3
 - Lambda 4

Immunoglobulins

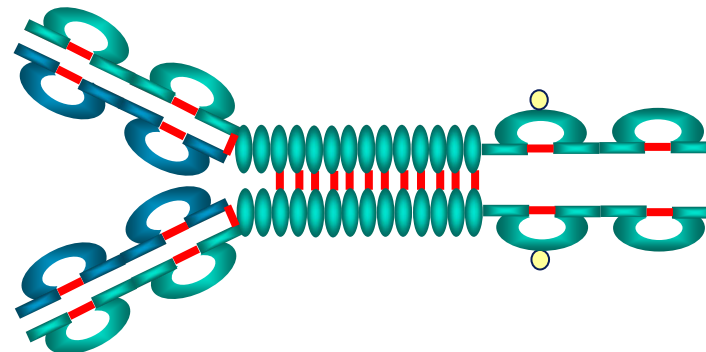
- **Nomenclature** - Immunoglobulins are named based on the class, or subclass of the heavy chain and type or subtype of light chain. Unless it is stated precisely you are to assume that all subclass, types and subtypes are present. IgG means that all subclasses and types are present.
 - IgM (kappa)
 - IgA1(lambda 2)
 - IgG
- **Heterogeneity** - Immunoglobulins considered as a population of molecules are normally very heterogeneous because they are composed of different classes and subclasses each of which has different types and subtypes of light chains. In addition, different immunoglobulin molecules can have different antigen binding properties because of different V_H and V_L regions.

IgG

- **Structure** - The structures of the IgG subclasses are presented in figure. All IgG's are monomers (7S immunoglobulin). The subclasses differ in the number of disulfide bonds and length of the hinge region.
 - Monomer (7S)



IgG1, IgG2 and IgG4



IgG3

IgG

- Structure
- **Properties** - Most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules.
 - Major serum Ig
 - Major Ig in extravascular spaces
 - Placental transfer – Does not require Ag binding (IgG2 not well)
 - Fixes complement (except IgG4)
 - Binds to Fc receptors (IgG2, IgG4 do not bind)
 - Phagocytes - opsonization
 - K cells - ADCC

IgG

- Structure
- Properties
 - Major serum Ig - 75% of serum Ig is IgG
 - Major Ig in extravascular spaces
 - Placental transfer – Does not require Ag binding (IgG2)
 - Fixes complement (IgG4)
 - Binds to Fc receptors (IgG2, IgG4)
 - Phagocytes - opsonization
 - K cells - ADCC

IgG

- Structure
- Properties -
 - Major serum Ig
 - Major Ig in extravascular spaces
 - Placental transfer – Does not require Ag binding (IgG2)
 - Fixes complement (IgG4)
 - Binds to Fc receptors (IgG2, IgG4)
 - Phagocytes - opsonization
 - K cells - ADCC

IgG

- Structure
- Properties -
 - Major serum Ig
 - Major Ig in extravascular spaces
 - Placental transfer – Does not require Ag binding - IgG is the only class of Ig that crosses the placenta. Transfer is mediated by receptor on placental cells for the Fc region of IgG. Not all subclasses cross equally; IgG2 does not cross well.
 - Fixes complement (IgG4)
 - Binds to Fc receptors (IgG2, IgG4)
 - Phagocytes - opsonization
 - K cells - ADCC

IgG

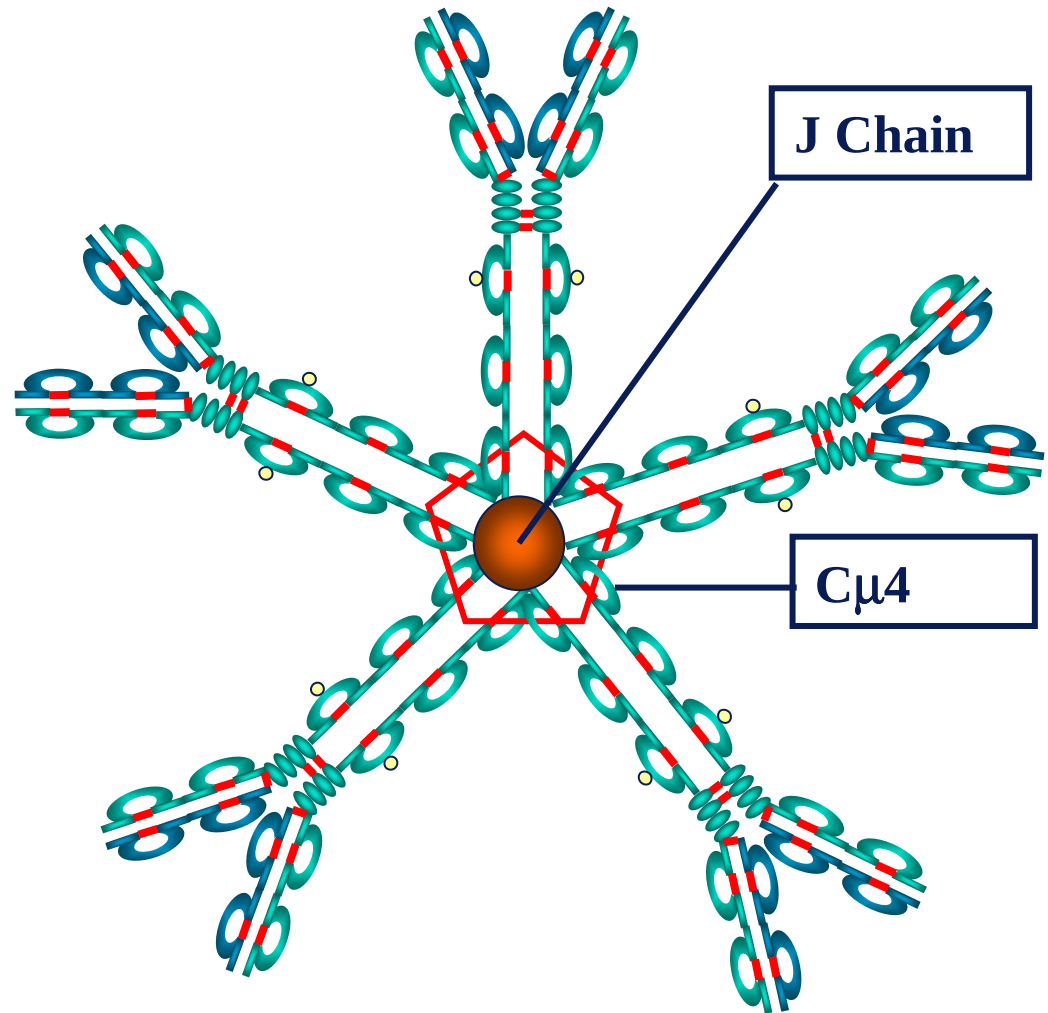
- Structure
- **Properties**
 - Major serum Ig
 - Major Ig in extravascular spaces
 - Placental transfer – Does not require Ag binding (IgG2)
 - **Fixes complement - Not all subclasses fix equally well; IgG4 does not fix complement**
 - Binds to Fc receptors (IgG2, IgG4)
 - Phagocytes - opsonization
 - K cells - ADCC

IgG

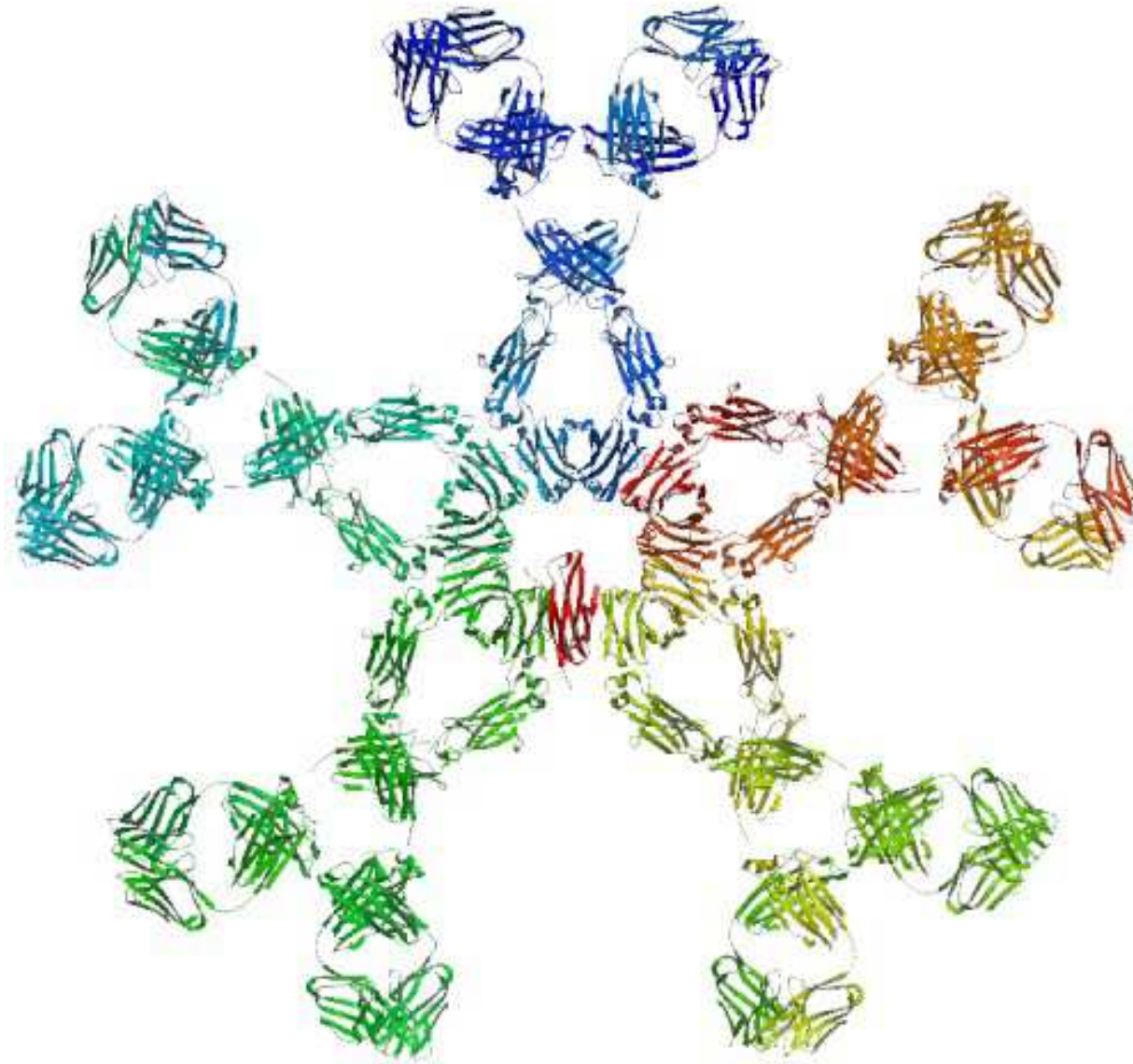
- Structure
- Properties
 - Major serum Ig
 - Major Ig in extravascular spaces
 - Placental transfer – Does not require Ag binding (IgG2)
 - Fixes complement - (IgG4)
 - **Binds to Fc receptors** - Macrophages, monocytes, PMN's and some lymphocytes have Fc receptors for the Fc region of IgG. Not all subclasses bind equally well; IgG2 and IgG4 do not bind to Fc receptors. A consequence of binding to the Fc receptors on PMN's, monocytes and macrophages is that the cell can now internalize the antigen better. The antibody has prepared the antigen for eating by the phagocytic cells. The term **opsonin** is used to describe substances that enhance phagocytosis. IgG is a good opsonin. Binding of IgG to Fc receptors on other types of cells results in the activation of other functions.
 - Phagocytes - opsonization
 - K cells - ADCC

IgM

- **Structure** - IgM normally exists as a pentamer (19S immunoglobulin) but it can also exist as a monomer. In the pentameric form all heavy chains are identical and all light chains are identical. Thus, the valence is theoretically 10. IgM has an extra domain on the μ chain (C_{H4}) and it has another protein covalently bound via a S-S bond called the **J chain**. This chain functions in polymerization of the molecule into a pentamer.
 - Pentamer (19S)
 - Extra domain (C_{H4})
 - J chain



IgM



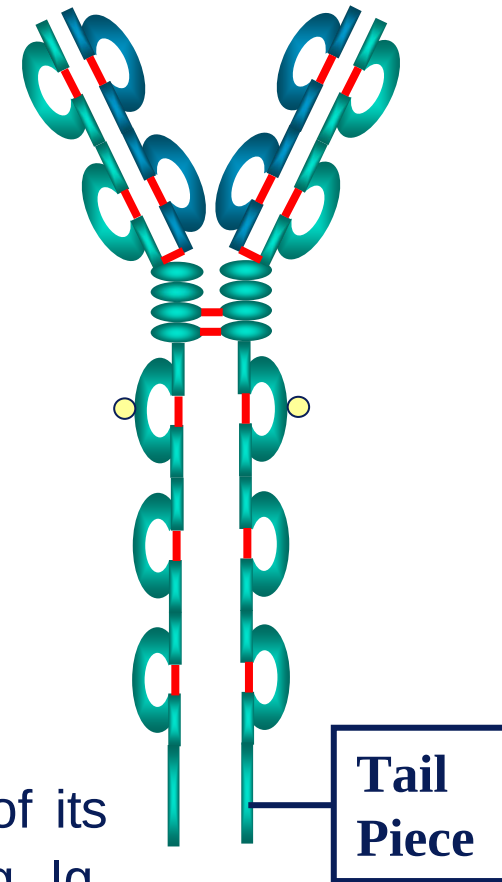
<http://www.rcsb.org/pdb/explore.do?structureId=2RCJ>

IgM

- Structure
- Properties
 - 3rd highest serum Ig - IgM is the 3rd most common serum Ig.
 - First Ig made by fetus and B cells - IgM is the first Ig to be made by the fetus and the first Ig to be made by a virgin B cells when it is stimulated by antigen.
 - Fixes complement - As a consequence of its pentameric structure, IgM is a good complement fixing Ig. Thus, IgM antibodies are very efficient in leading to the lysis of microorganisms.

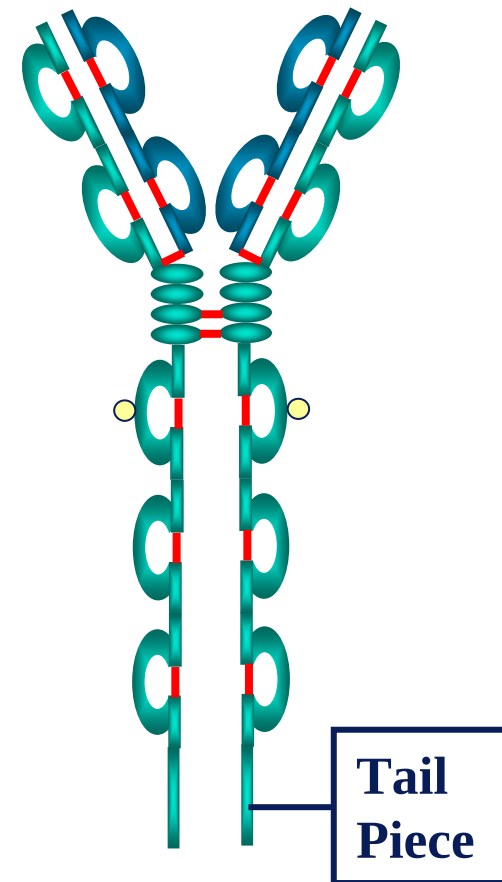
IgM

- Structure
- Properties
 - 3rd highest serum Ig
 - First Ig made by fetus and B cells
 - Fixes complement
 - Agglutinating Ig - As a consequence of its structure, IgM is also a good agglutinating Ig. Thus, IgM antibodies are very good in clumping microorganisms for eventual elimination from the body.
 - Binds to Fc receptors - IgM binds to some cells via Fc receptors.
 - B cell surface Ig



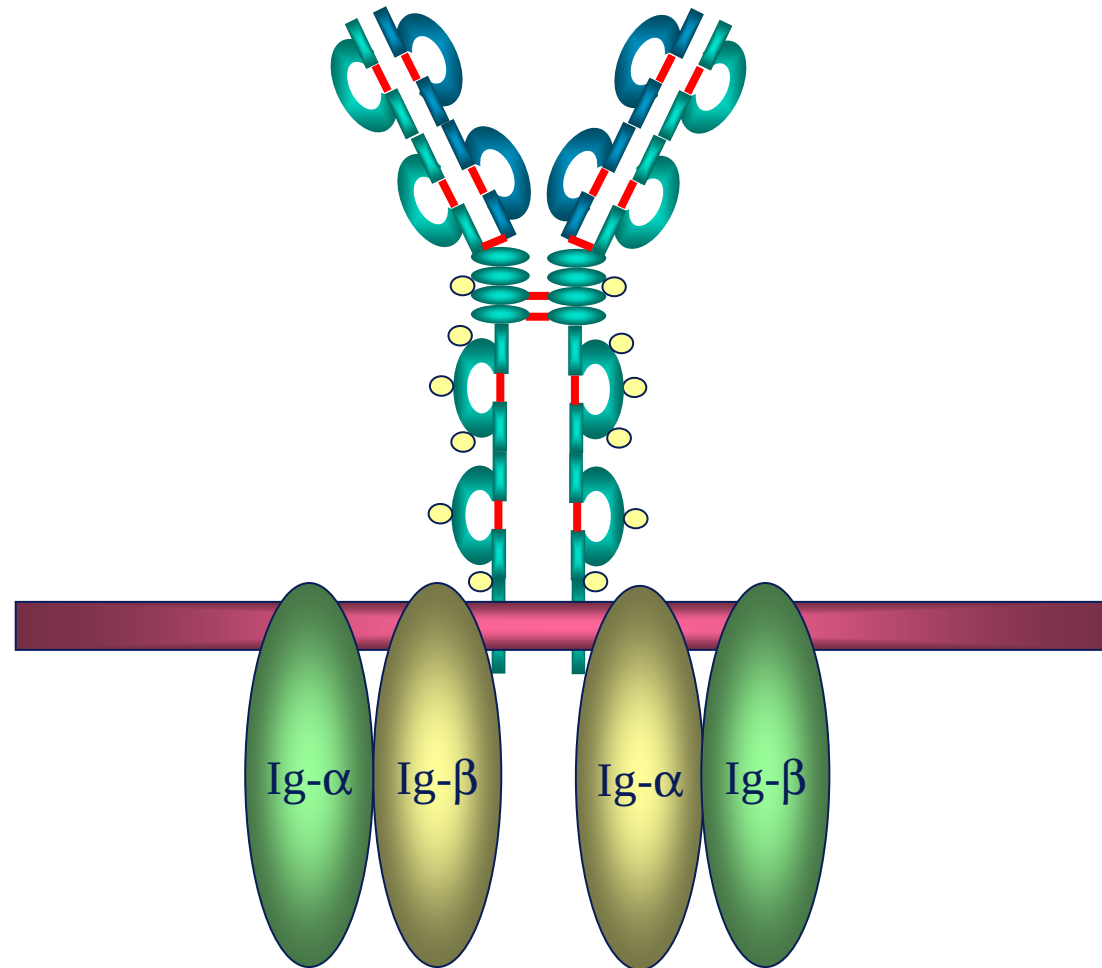
IgM

- Structure
- Properties
 - 3rd highest serum Ig
 - First Ig made by fetus and B cells
 - Fixes complement
 - Agglutinating Ig
 - Binds to Fc receptors



- B cell surface Ig - Surface IgM exists as a monomer and lacks J chain but it has an extra 20 amino acids at the C-terminal end to anchor it into the membrane (see figure). Cell surface IgM functions as a receptor for antigen on B cells. Surface IgM is noncovalently associated with two additional proteins in the membrane of the B cell called **Ig- α** and **Ig- β** as indicated in the next figure. These additional proteins act as signal transducing molecules since the cytoplasmic tail of the Ig molecule itself is too short to transduce a signal. Contact between surface immunoglobulin and an antigen is required before a signal can be transduced by the Ig- α and Ig- β chains. In the case of T-independent antigens (LPS batterico, capsule polisaccaridiche, flagelli batterici), contact between the antigen and surface immunoglobulin is sufficient to activate B cells to differentiate into antibody secreting plasma cells. However, for T-dependent antigens, a second signal provided by helper T cells is required before B cells are activated.

B Cell Antigen Receptor (BcR)

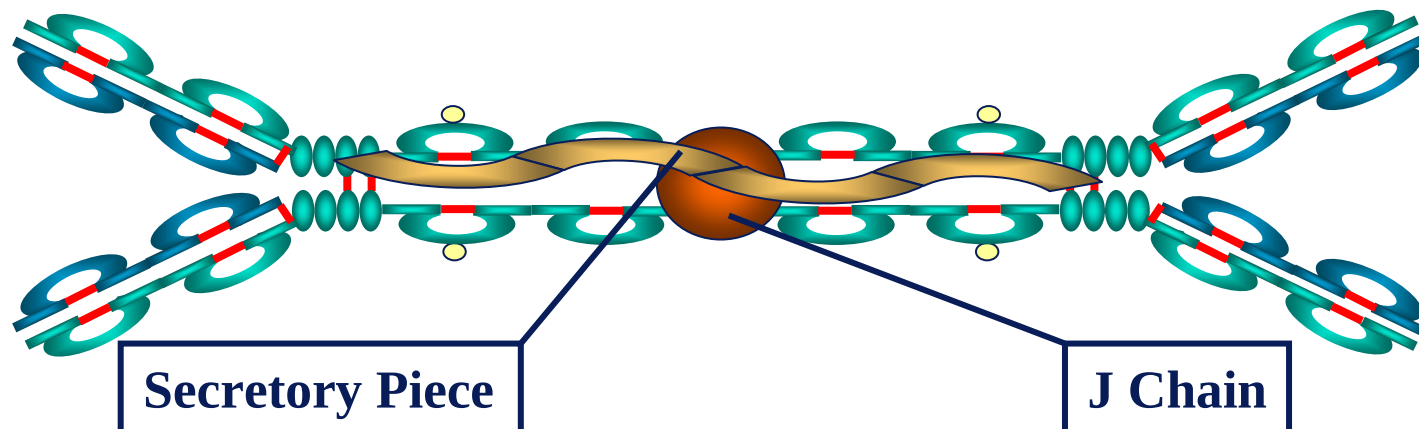


Vi sono due forme di molecole immunoglobuliniche: di membrana (recettori Ig) e secretorie (immunoglobuline circolanti). Queste due forme si differenziano per l'estremità' COOH terminale: la forma secretoria possiede un peptide terminale altamente idrofilo chiamato **Ts (secretory tail)**, mentre la forma di membrana termina con la sequenza idrofoba **Tm (membrane tail)** che ne permette l'ancoraggio alla membrana

IgA

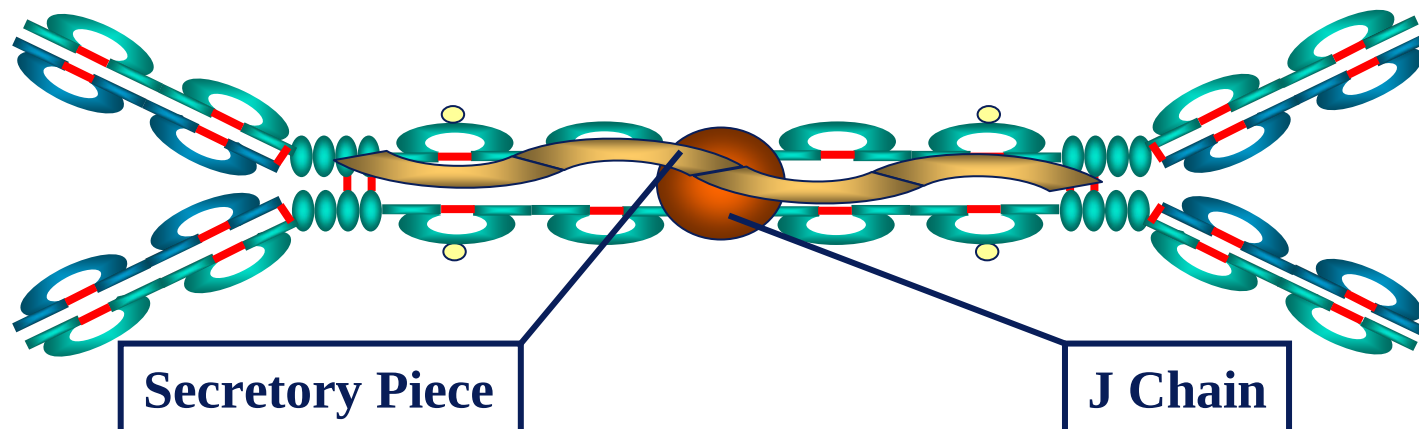
- Structure

- Serum - monomer - Serum IgA is a monomer but IgA found in secretions is a dimer as presented in figure. When IgA exits as a dimer, a **J chain** is associated with it.
- Secretions (sIgA)
 - Dimer (11S)
 - J chain
 - Secretory component



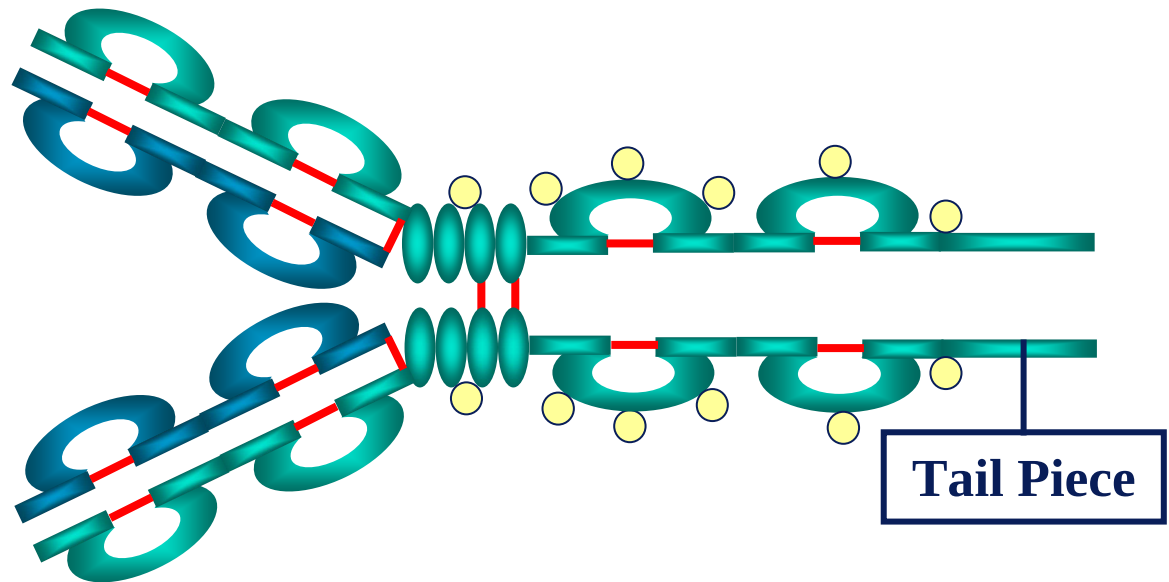
IgA

- Properties
- IgA is the 2nd most common serum Ig.
- IgA is the major class of Ig in secretions - tears, saliva, colostrum, mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity.
- Normally IgA does not fix complement, unless aggregated.
- IgA can binding to some cells - PMN's and some lymphocytes.



IgD

- Structure
 - Monomer
 - Tail piece

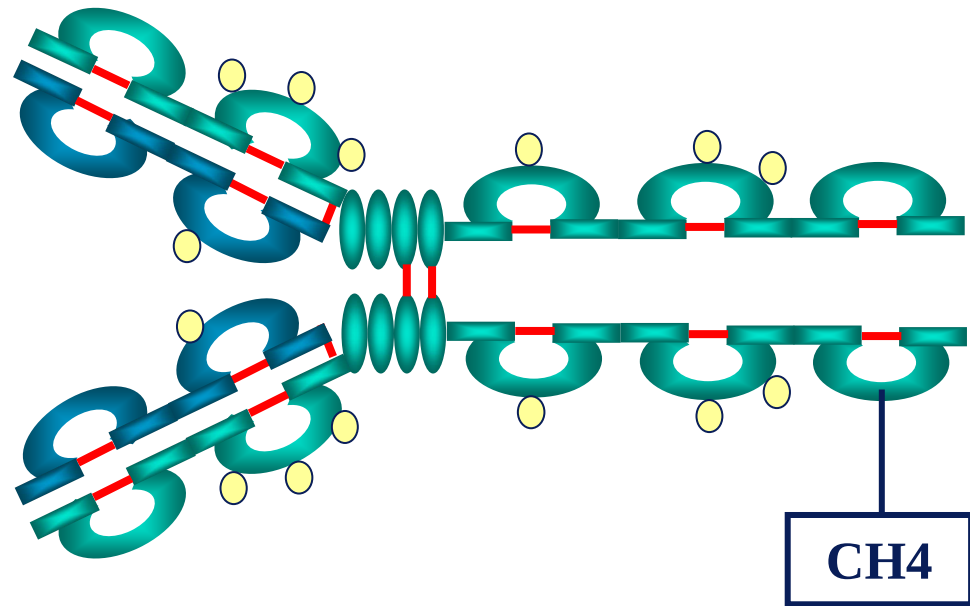


IgD

- Structure
- Properties
 - 4th highest serum Ig - IgD is found in low levels in serum; its role in serum uncertain.
 - B cell surface Ig - IgD is primarily found on B cell surfaces where it functions as a receptor for antigen. IgD on the surface of B cells has extra amino acids at C-terminal end for anchoring to the membrane. It also associates with the **Ig- α** and **Ig- β** chains.
 - Does not bind complement

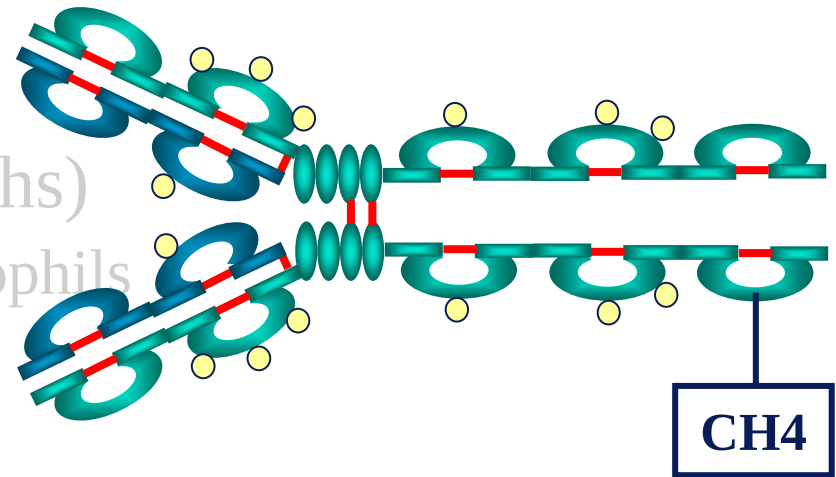
IgE

- **Structure** - IgE exists as a monomer and has an extra domain in the constant region.
 - Monomer
 - Extra domain (C_{H4})



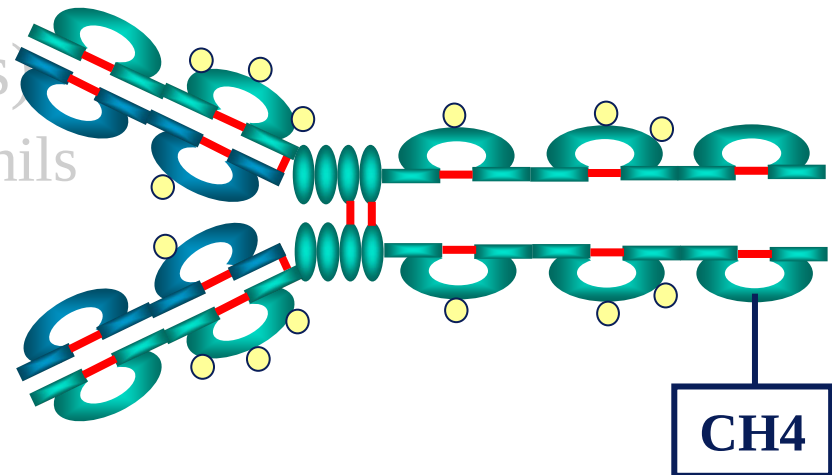
IgE

- Structure
- Properties
 - IgE is the least common serum Ig since it binds very tightly to Fc receptors on basophils and mast cells even before interacting with antigen.
 - Least common serum Ig -
 - Binds to basophils and mast cells (Does not require Ag binding)
 - Allergic reactions
 - Parasitic infections (Helminths)
 - Binds to Fc receptor on eosinophils
 - Does not fix complement

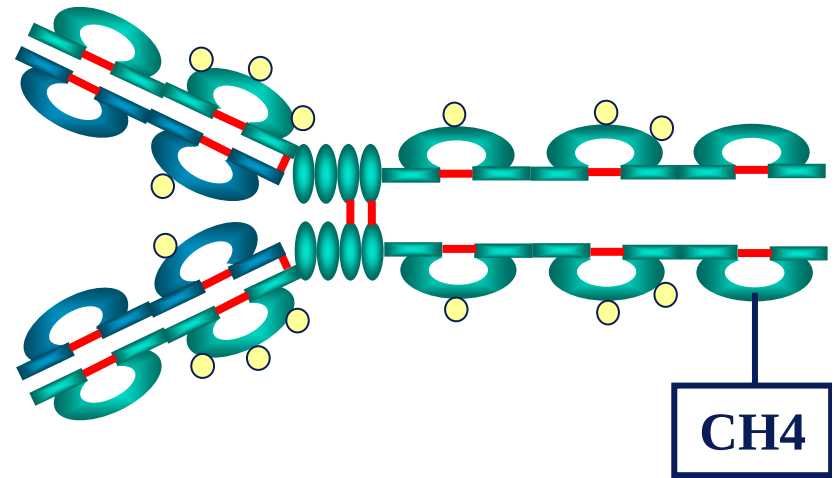


IgE

- Structure
- Properties
 - Least common serum Ig
 - Binds to basophils and mast cells (Does not require Ag binding)
 - Allergic reactions - Involved in allergic reactions - As a consequence of its binding to basophils and mast cells, IgE is involved in allergic reactions. Binding of the allergen to the IgE on the cells results in the release of various pharmacological mediators that result in allergic symptoms.
 - Parasitic infections (Helminths)
 - Binds to Fc receptor on eosinophils
 - Does not fix complement

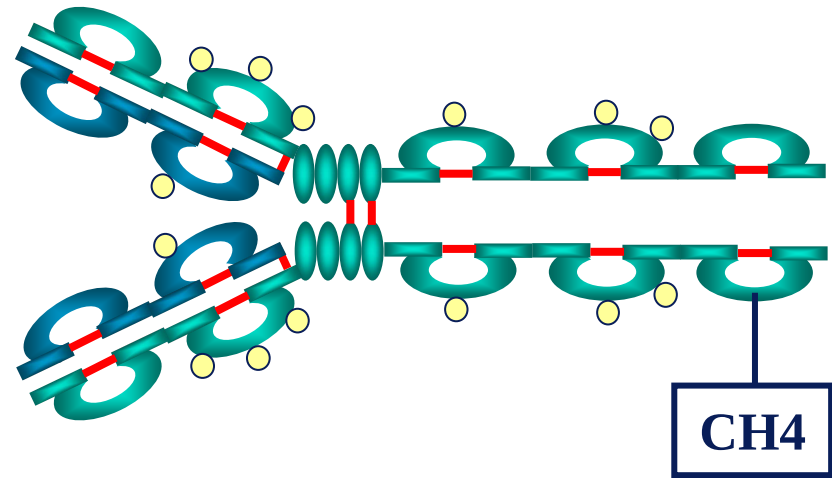


IgE



- Structure
- Properties
 - Least common serum Ig
 - Binds to basophils and mast cells (Does not require Ag binding)
 - Allergic reactions
 - Parasitic infections (Helminths) - IgE also plays a role in parasitic helminth diseases. Since serum IgE levels rise in parasitic diseases, measuring IgE levels is helpful in diagnosing parasitic infections. Eosinophils have Fc receptors for IgE and binding of eosinophils to IgE-coated helminths results in killing of the parasite.
 - Binds to Fc receptor on eosinophils
 - Does not fix complement

IgE



- Structure
- Properties
 - Least common serum Ig
 - Binds to basophils and mast cells (Does not require Ag binding)
 - Allergic reactions
 - Parasitic infections (Helminths)
 - Binds to Fc receptor on eosinophils
 - Does not fix complement