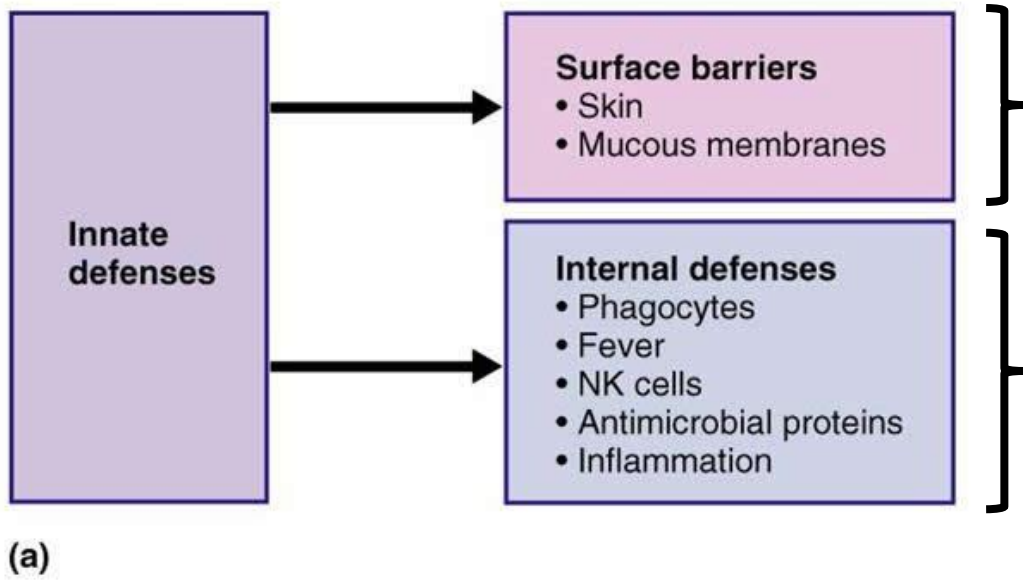
Globular proteins

Immunoglobulins

Professor Mamoun Ahram

Types of immunity



First-line defense (Physical; chemical)

Second-line defense (Non-specific, inflammatory; phagocytic)

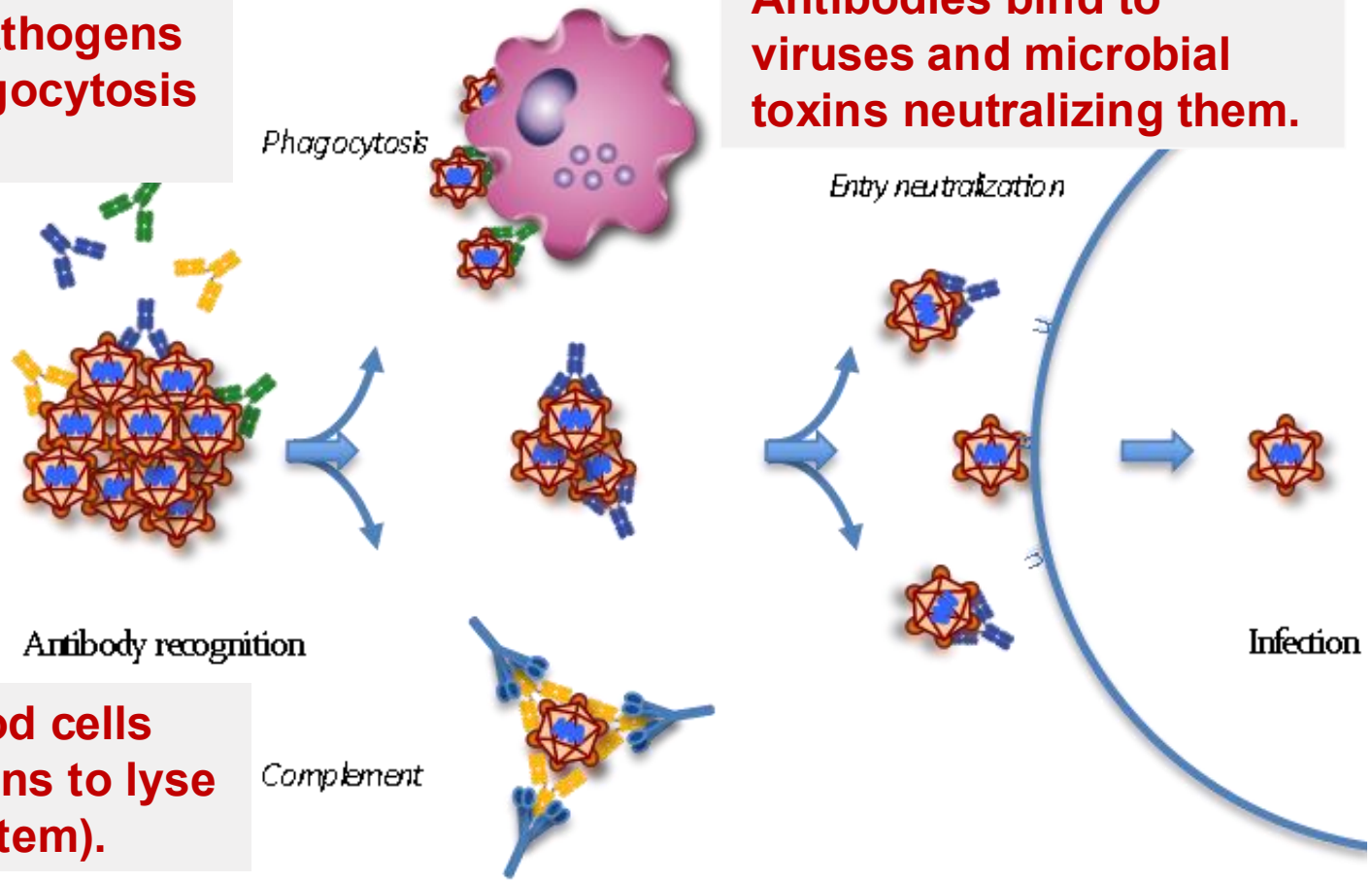
Third-line defense (Specific)

How do B cells work?

- B cells secrete immunoglobulins (also known as antibodies).
- Immunoglobulins have three roles:

Antibodies bind to pathogens and induce their phagocytosis into immune cells.

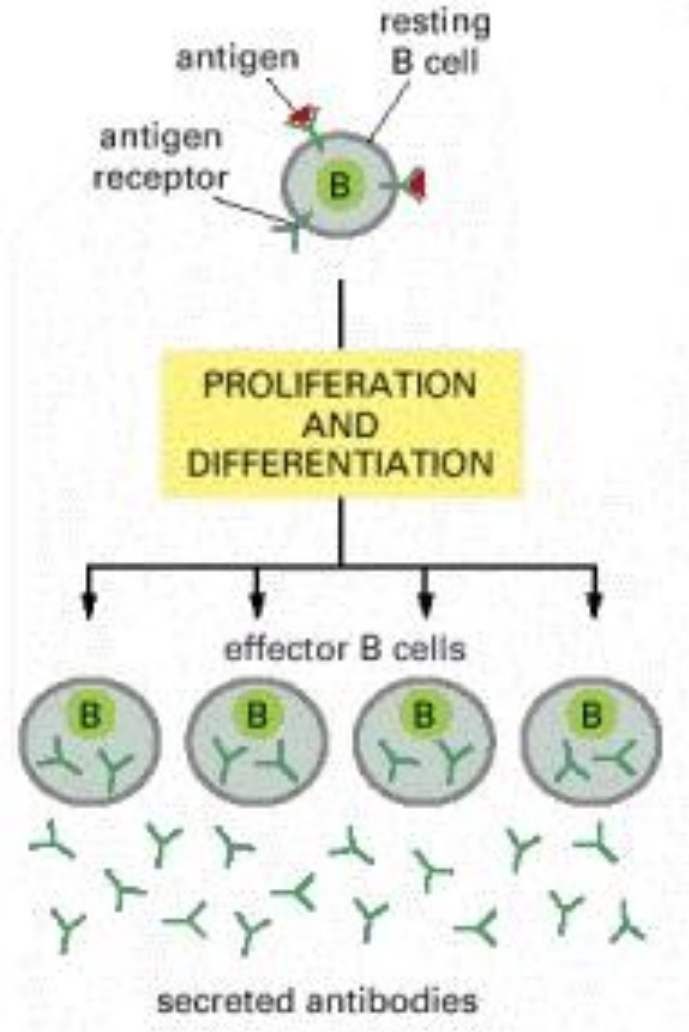
Antibodies bind to viruses and microbial toxins neutralizing them.



Antibodies recruit white blood cells and a system of blood proteins to lyse pathogens (complement system).

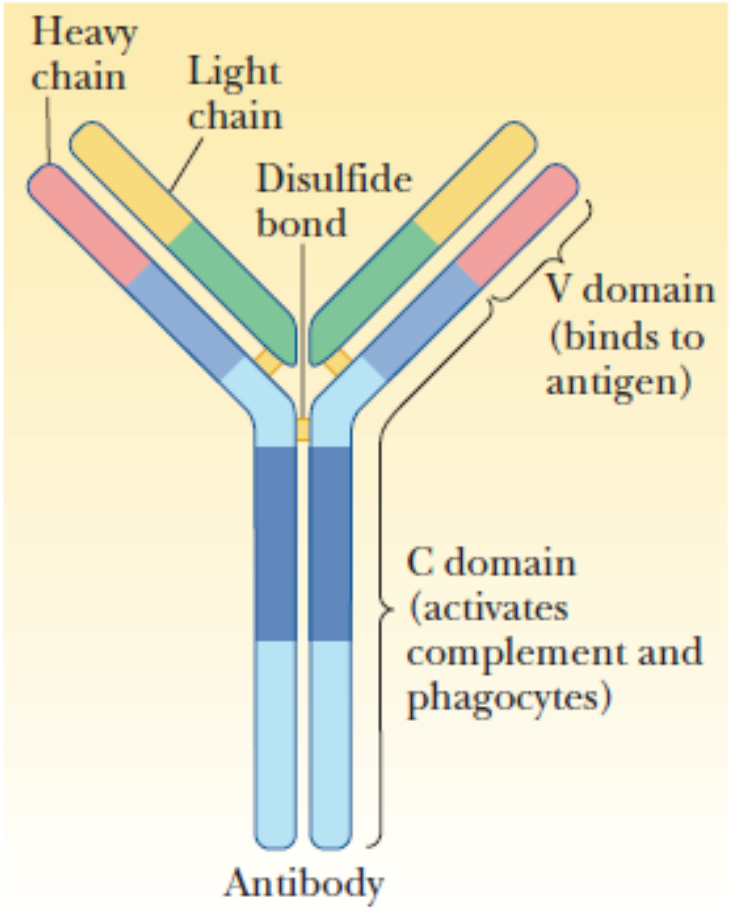
When B cells recognize an antigen…

- When a B cell is activated by antigen (a foreign body), it proliferates and differentiates into an antibody-secreting effector cell called plasma cell.
- Such cells make and secrete large amounts of soluble (rather than membrane-bound) antibodies at a rate of about 2000 molecules per second.
- B cells can produce more than 10^{11} different antibody molecules, but each cell produces one type of antibody.
- The antibody has high affinity and specificity to the antigen.



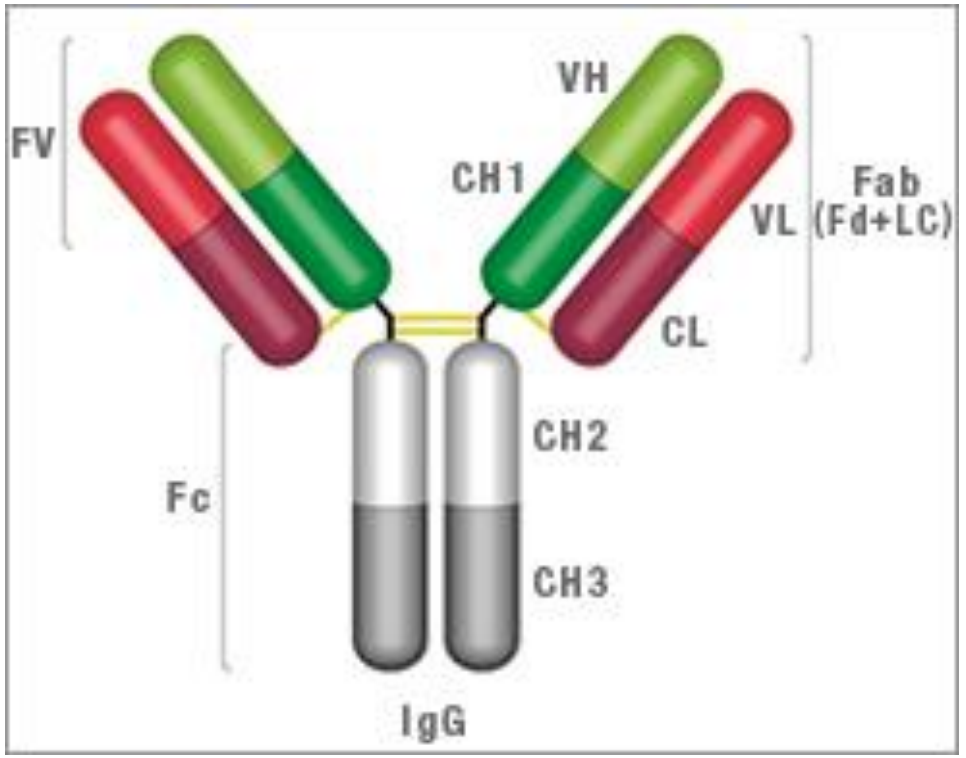
Structure of antibodies

- Antibodies are Y-shaped tetrameric glycoproteins consisting of two identical heavy chains (50 kDa each) and two identical light chains (25 kDa each).
- The four polypeptide chains are held together by covalent disulfide (-S-S-) bonds
- Within each of the polypeptide chains, there are also intra-chain disulfide bonds.
- The oligosaccharides are linked to the heavy chains.



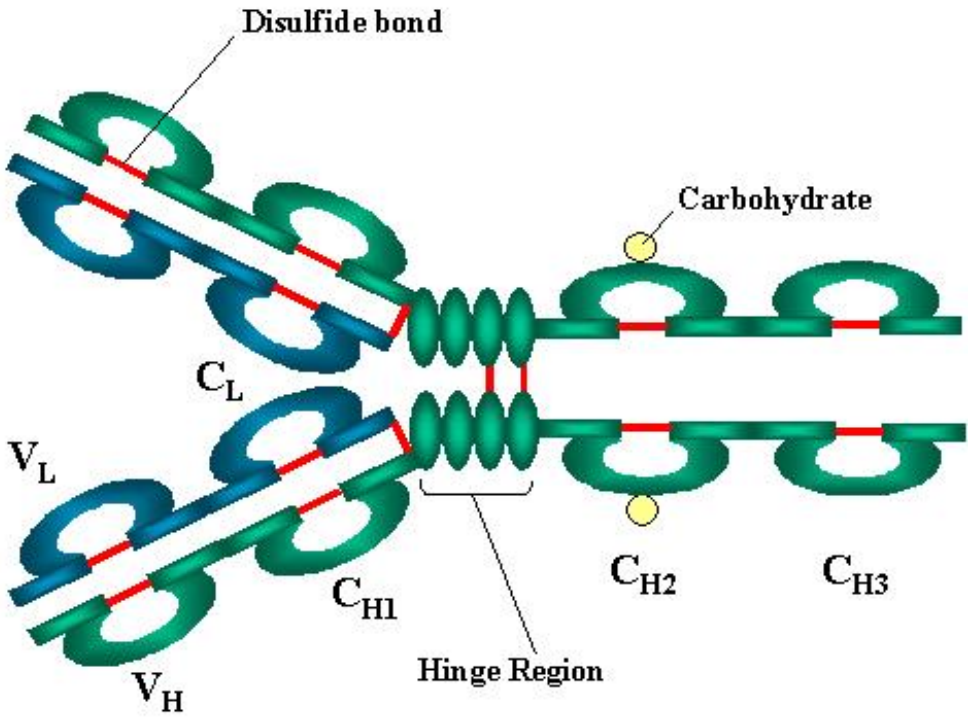
Antibody regions

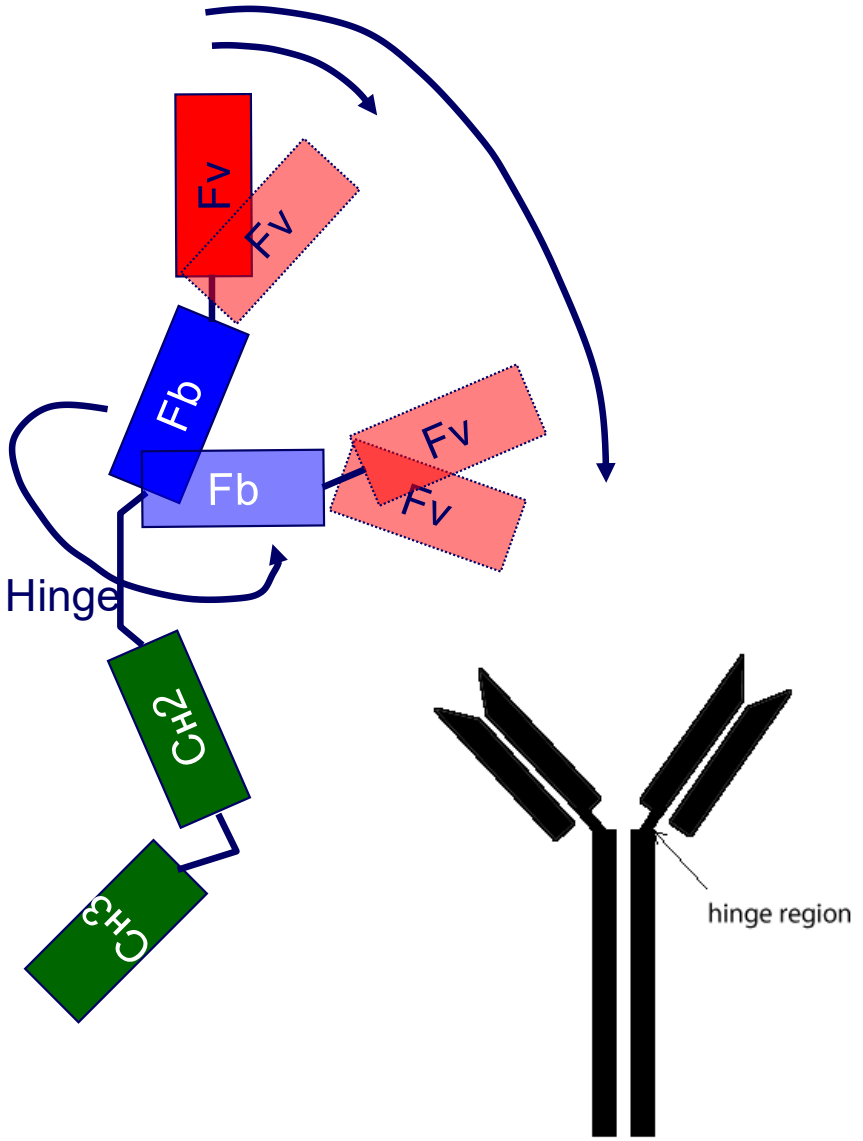
- A light chain consists of one variable (V_L) and one constant (C_L) domain.
- The heavy chain consists of one variable region or domains (V_H) and three constant regions/domains (C_{H1}, C_{H2}, and C_{H3}).
  - V_L and C_L pair with V_H and C_{H1}, respectively.
- Constant regions are uniform from one antibody to another within the same isotype.
- The Fc domain of antibodies is important for binding to phagocytic cells, allowing for antigen clearance.



Hinge region

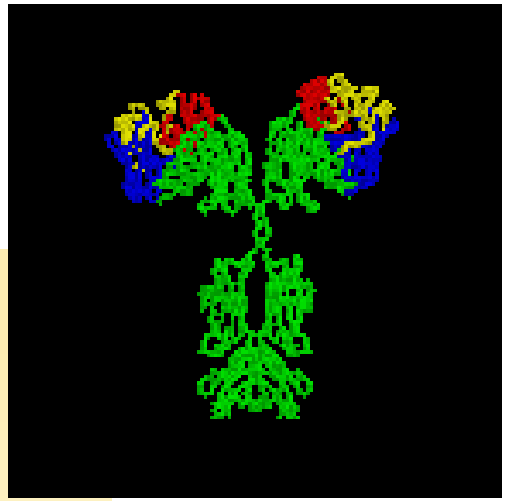
- A hinge region exists where the arms of the antibody molecule forms a Y structure.
- It adds some flexibility to the molecule.

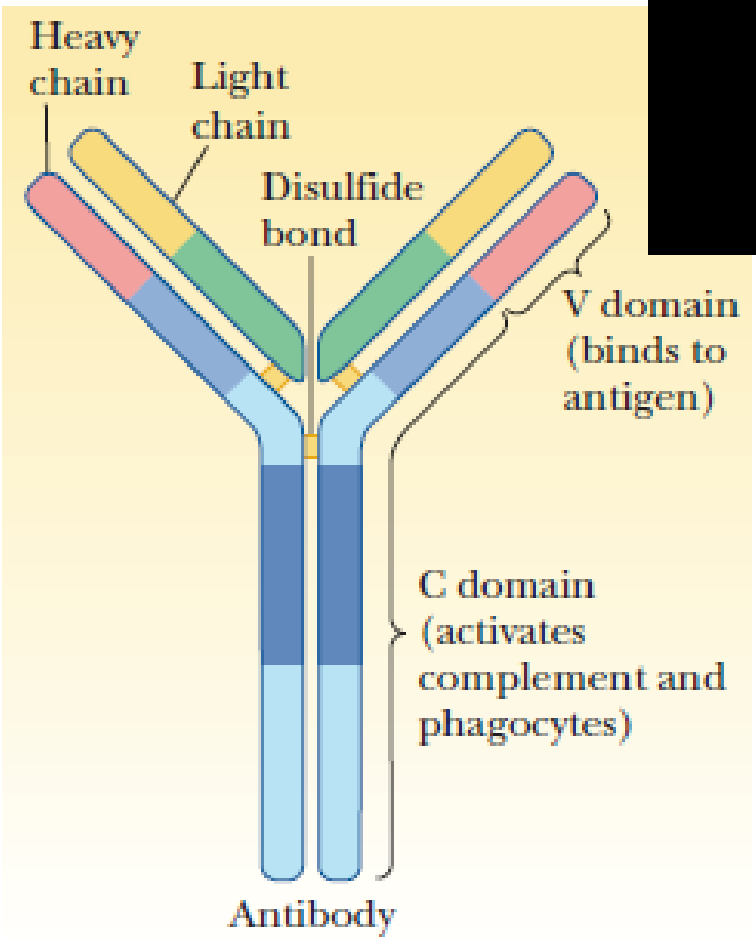




Variable regions

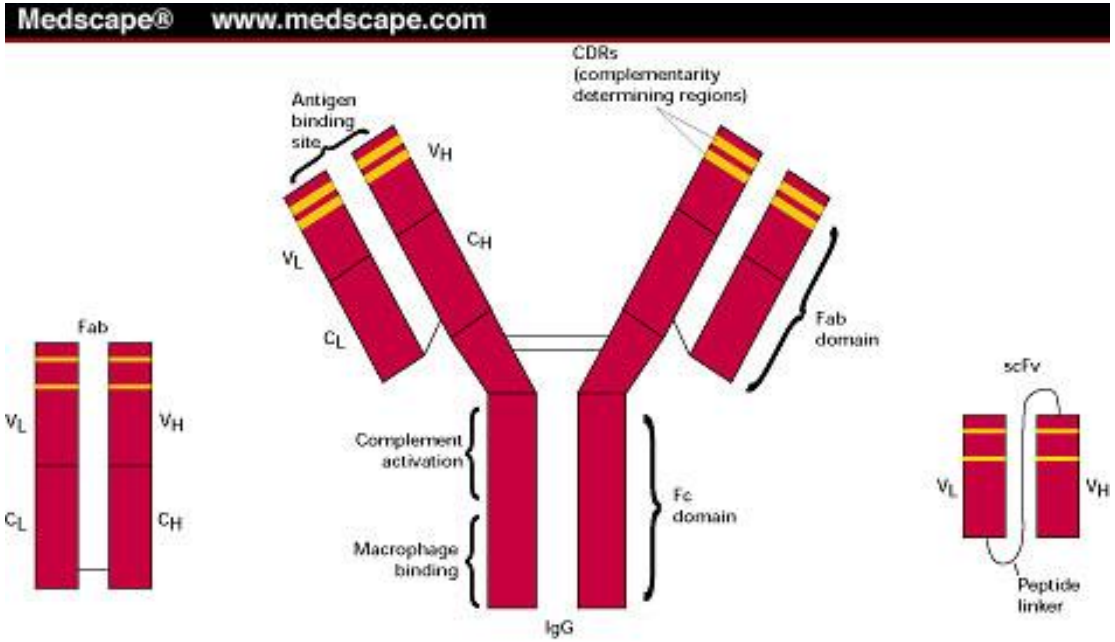
- The variable region is found at the tips of the Y and is the part of the antibody that binds to part of the antigen (called epitope).
- Each antibody can bind to two antigens.
- The primary sequences of the variable regions among different antibodies are quite distinct.
- Each B cell produces only one kind of antibody.
- No two variable regions in different humans are identical.
- Relatively invariable regions and other hypervariable regions.
- L chains have 3 hypervariable regions (in V_L) and H chains have four (in V_H).





Hypervariable" regions

- Hypervariable" regions, or "Complementarity Determining Regions" (CDRs) are found within the variable regions of both the heavy and light chains.
- These regions serve to recognize and bind specifically to the antigen with high affinity (dissociation constant (K_D) 10^{-12}-10^{-7}).

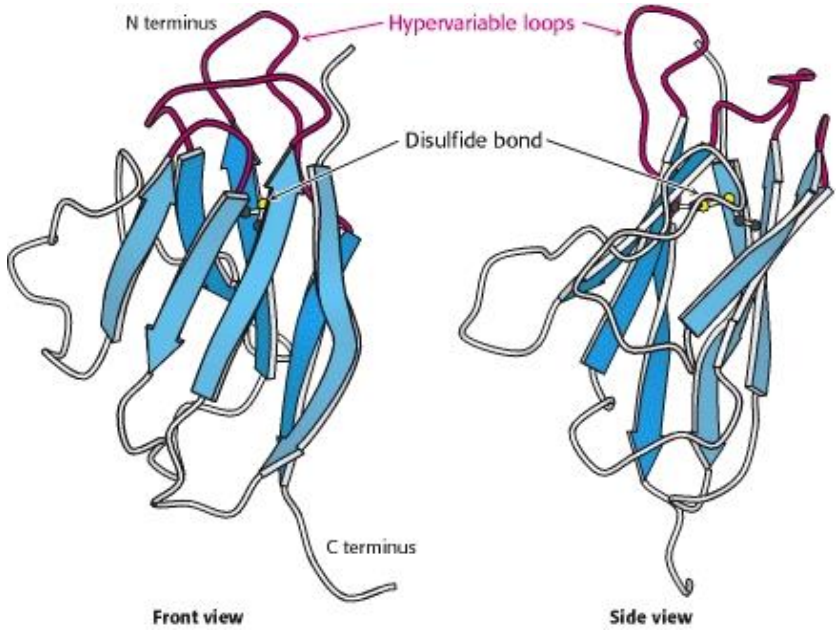


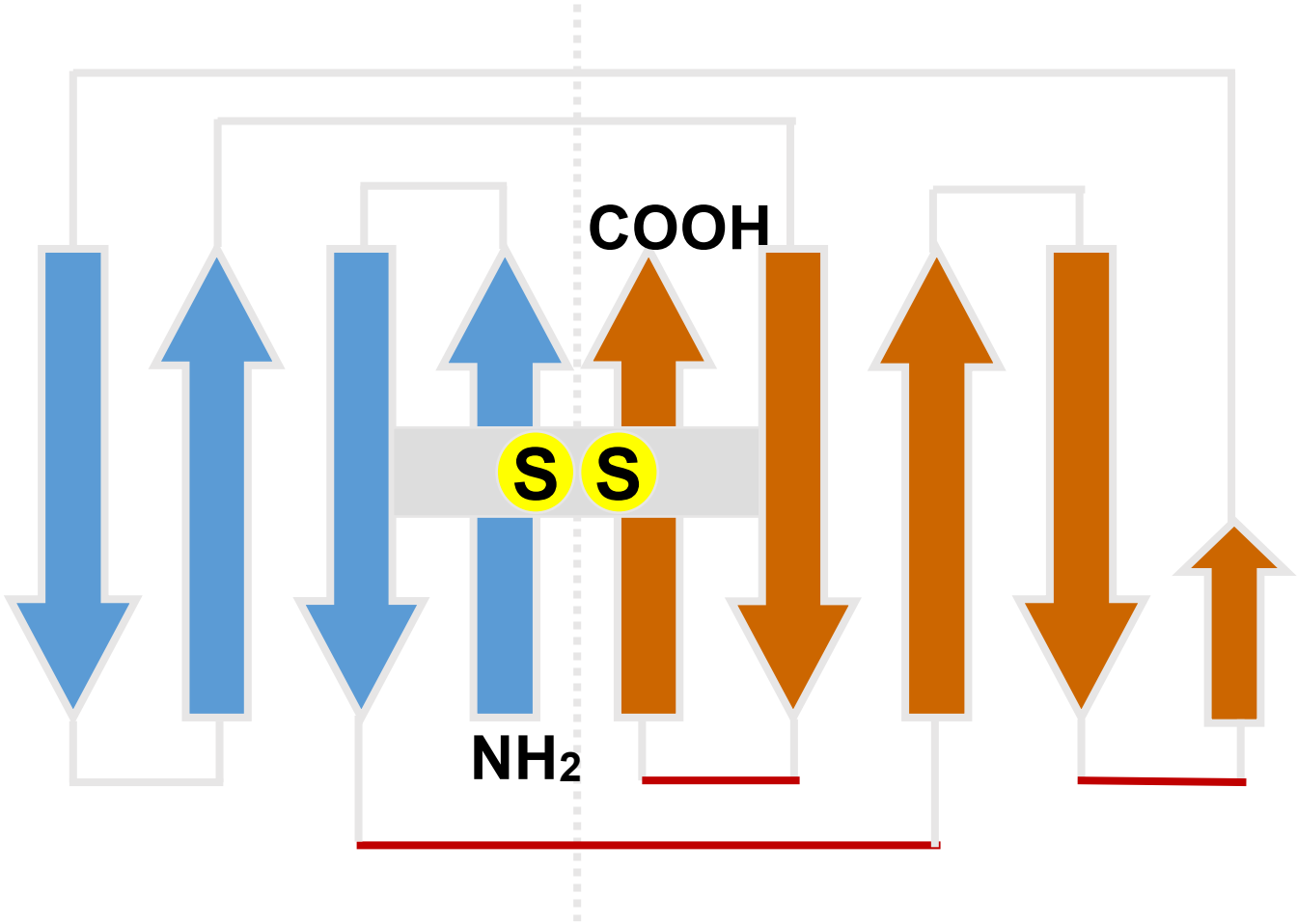
The dissociation constant (K_D) is used to measure the rate at which the antibody dissociates from its target. K_D is inversely proportional to affinity, so the lower the K_D value (the lower the concentration), the higher the affinity of the antibody.

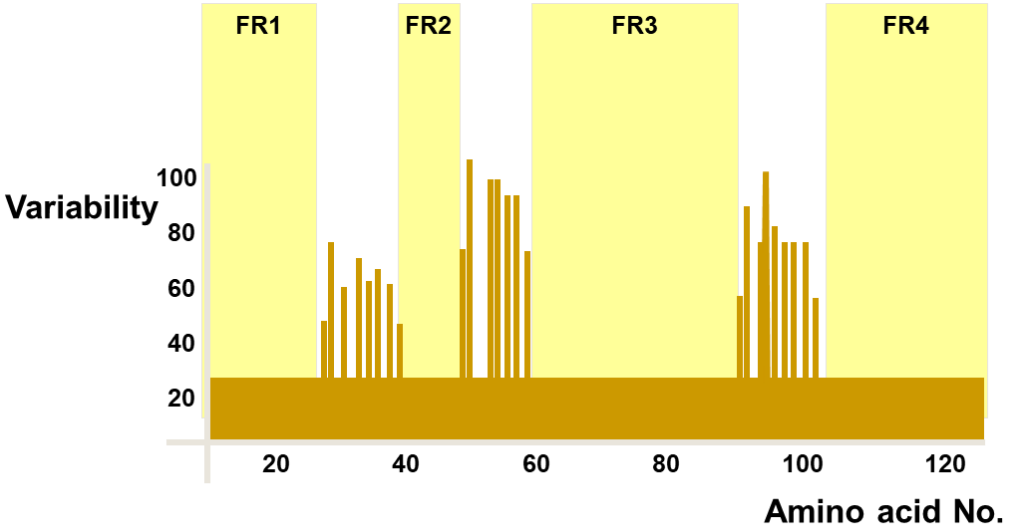
Immunoglobulin fold

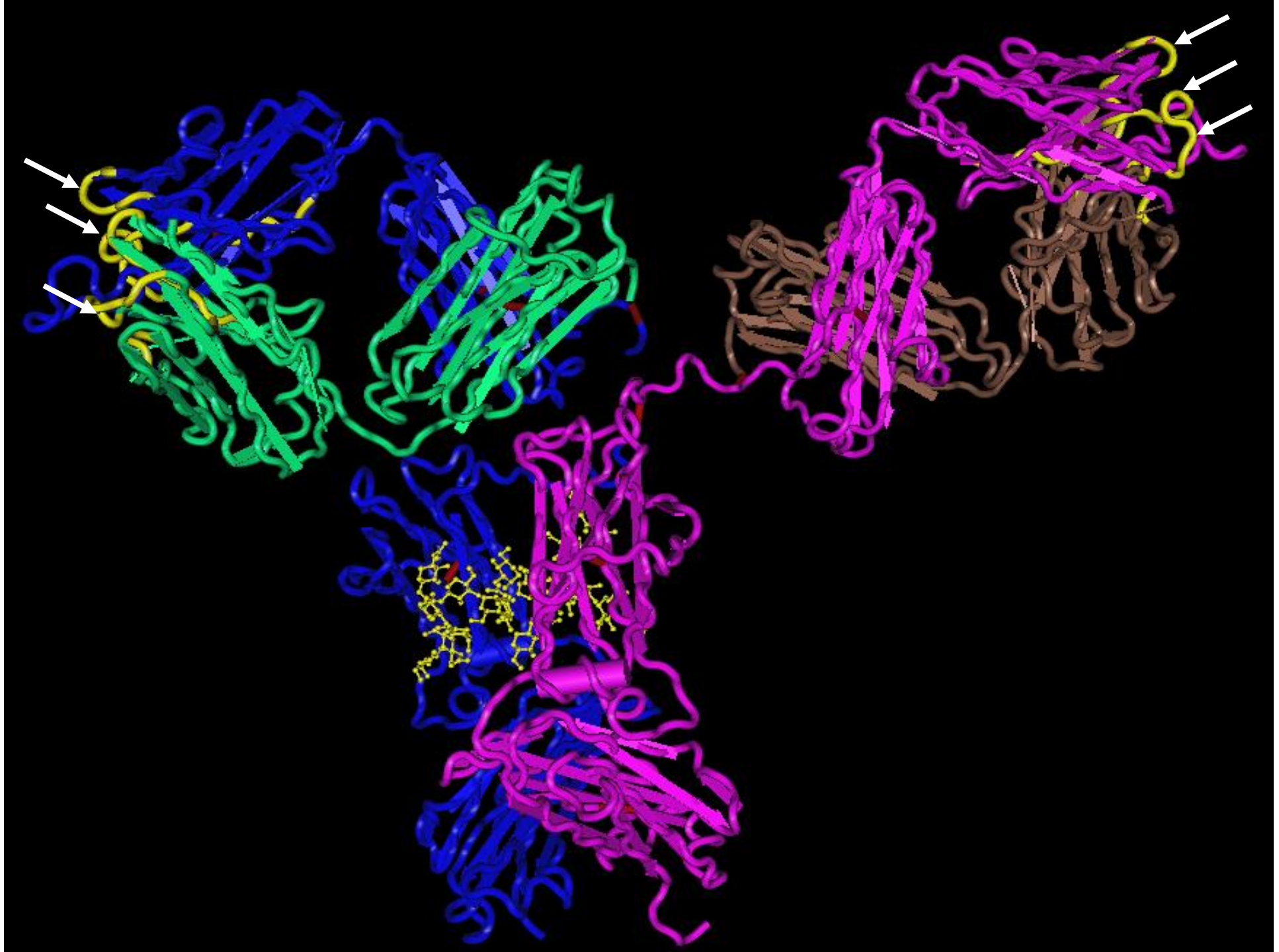
- The hypervariable regions exist in a specialized domain called “Immunoglobulin fold”, which is a domain that is present in every immunoglobulin.
- The hypervariable regions are specifically in three loops connecting the antiparallel β sheets to each other.
- About 7-12 amino acids in each one that contribute to the antigen-binding site.

It consists of a sandwich of two anti-parallel β sheets of 4 β strands each held together by a disulfide bond making a shape of a barrel, hence known as “beta barrel”.




COOH
S S
NH2


FR1
FR2
FR3
FR4
Variability
100
80
60
40
20
20
40
60
80
100
120
Amino acid No.



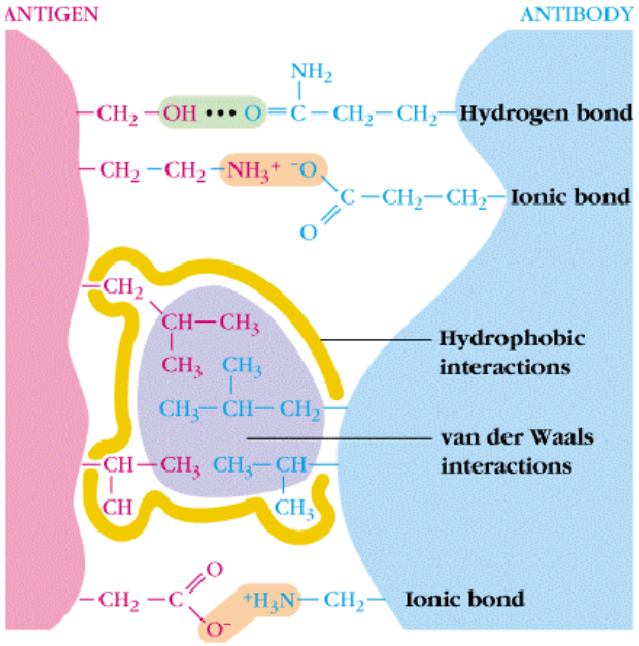
Antigen-antibody interaction

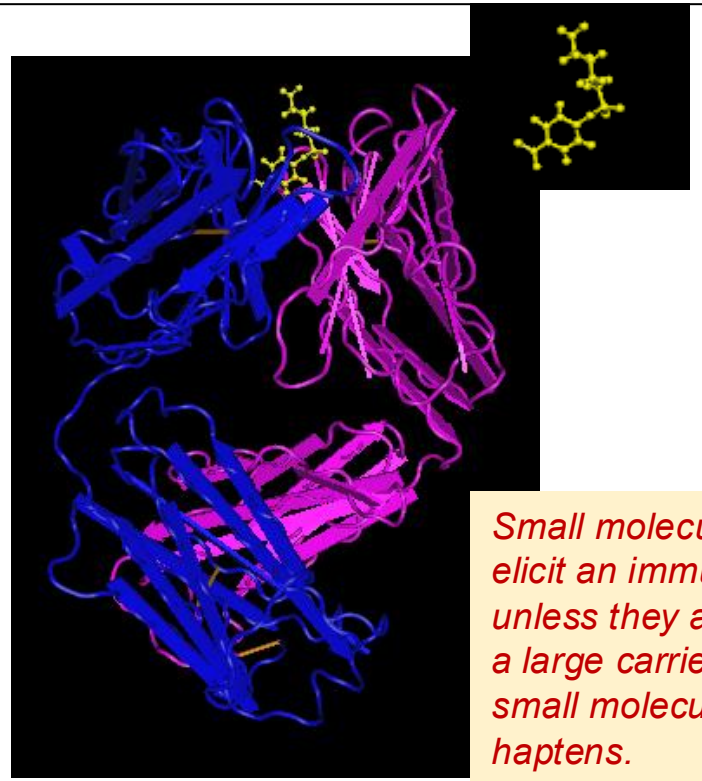
Antigen-antibody binding is mediated by noncovalent interactions between the complementary surfaces.

Large antigens interact with all of the CDRs of an antibody.

Small antigens interact with only one or a few CDRs that form a pocket or groove in the antibody molecule.

Human antibody complexed with influenza hemagglutinin





Small molecules cannot elicit an immune response unless they are attached to a large carrier. These small molecules are called haptens.

Hapten: 5-(para-nitrophenyl phosphonate)-pentanoic acid

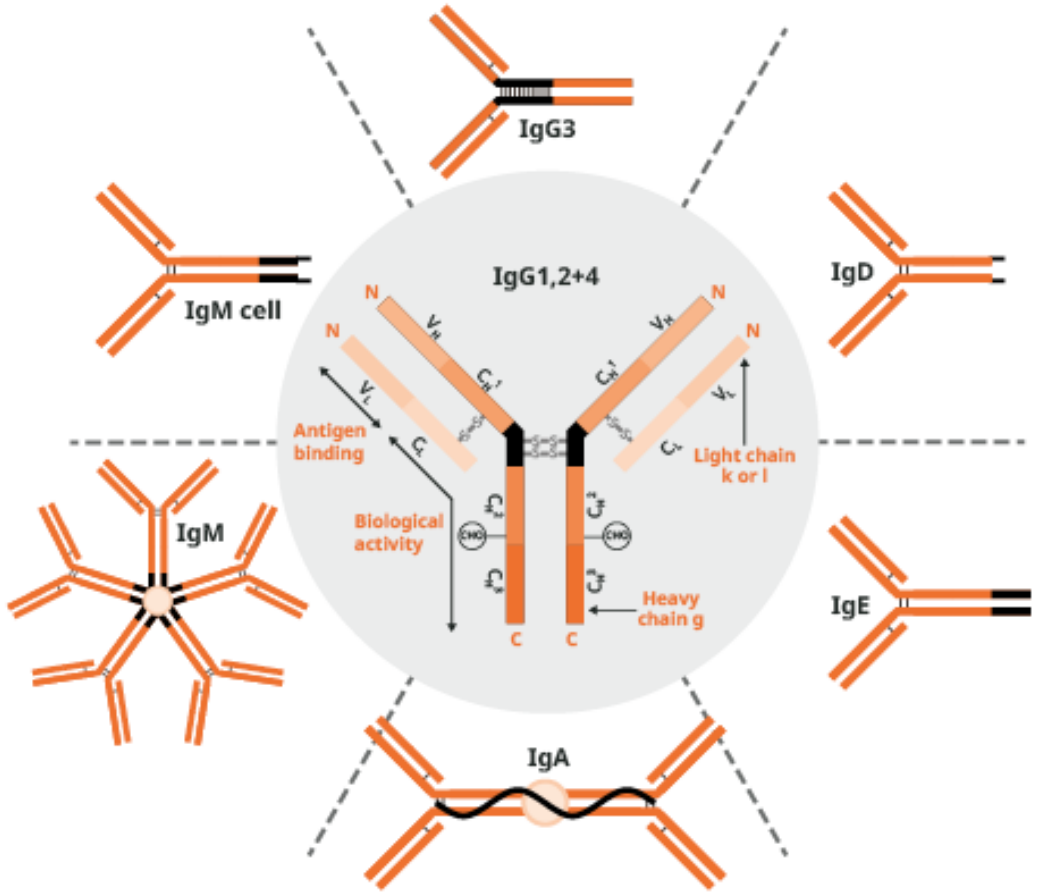
How is diversity of antibodies created?

- The enormous diversity of the amino acid sequence of the antigen-binding sites that are generated by
  - Erroneous combinations (genetic recombination) of the many components of the genes.
  - The introduction of genetic mutations during B cell differentiation resulting in changes of the lengths and amino acid sequences of the hypervariable loops.
- The combinations of different heavy and light chains.
- The overall three-dimensional structure of the antigen-binding sites.

https://www.youtube.com/watch?v=Na-Zc-xWCLE

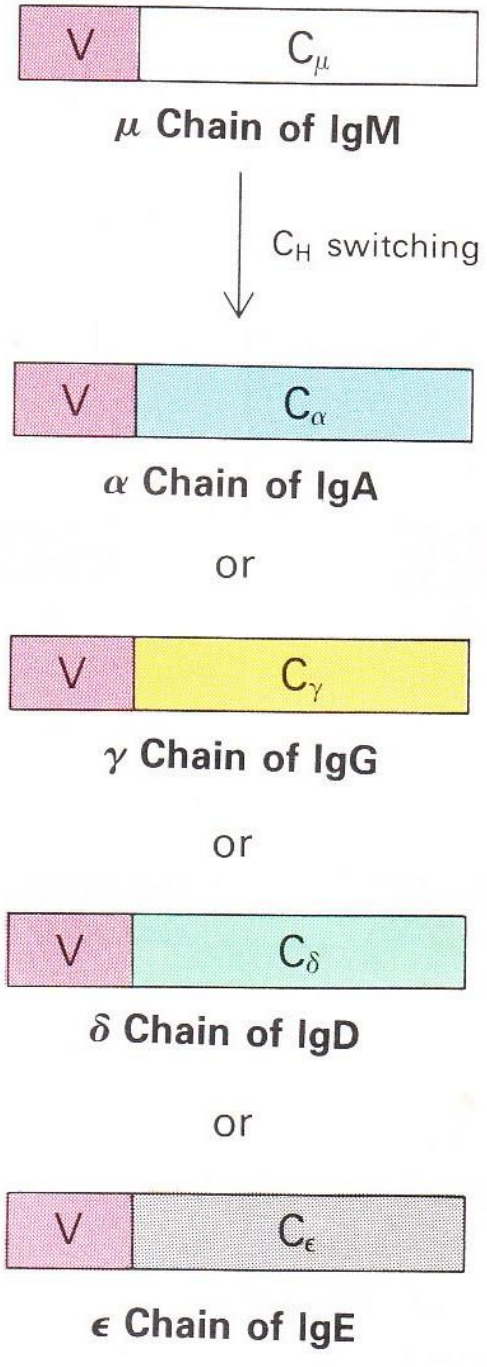
More diversity: immunoglobulin classes

- There are two constant regions of the "light" chains (lambda or kappa).
- There are five constant "heavy" chains (alpha, delta, gamma, epsilon or mu) that make five types of immunoglobulins known as immunoglobulin isotypes (IgA, IgD, IgG, IgE, IgM).

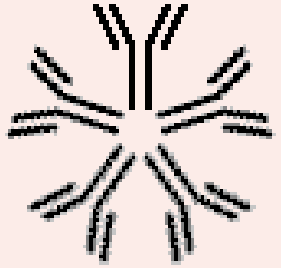
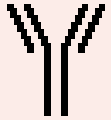
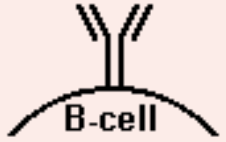
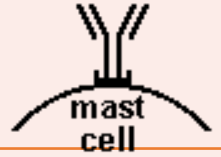
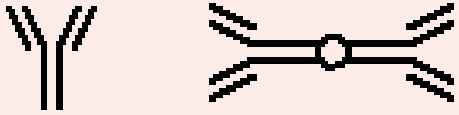


Class switching

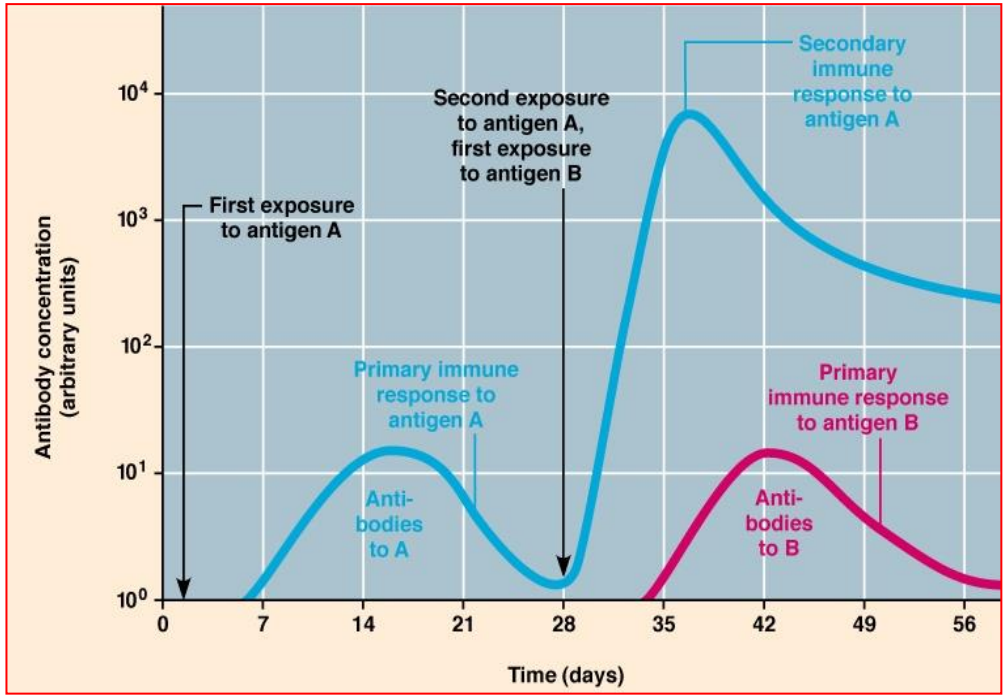
- Before binding an antigen, B cells contain IgM molecules only.
- Following antigen binding, class switching occurs.
- Class switching refers to a DNA rearrangement changing the heavy chain constant gene.
- The variable regions stays the same.
- That causes production of IgG, IgA, and IgE.

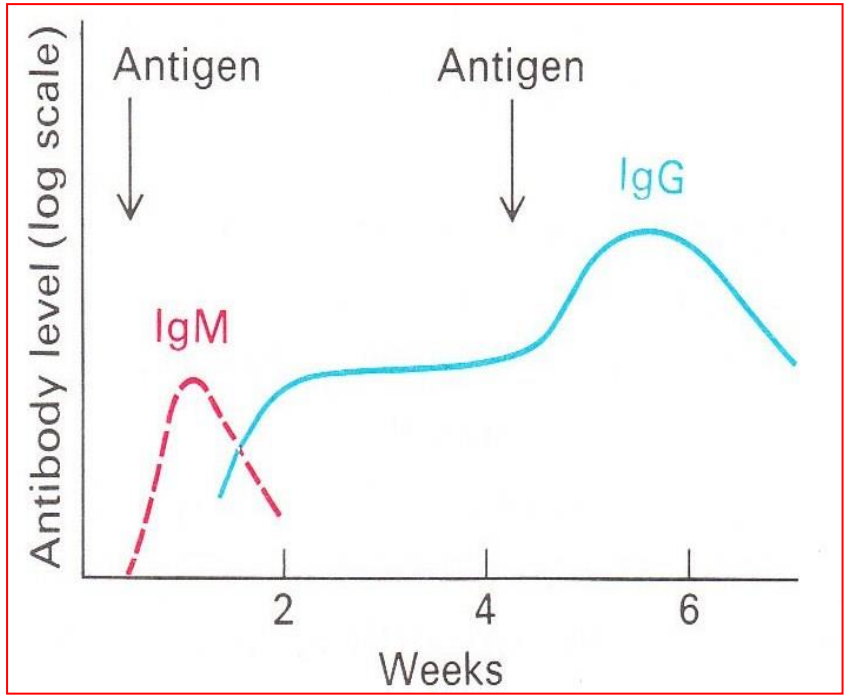


Types of antibodies

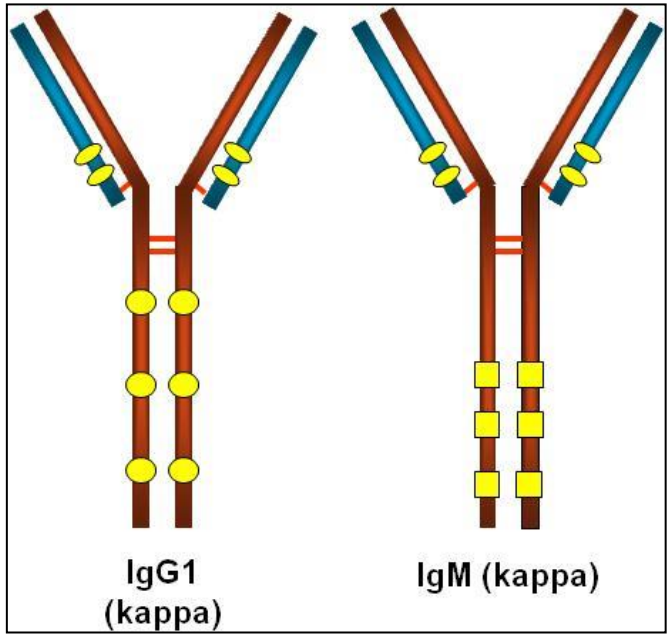
Isotype	Structure	Notes
IgM		Contains Mu heavy chains Expressed on the surface of B-cells (as a monomer only) The first antibodies produced in significant quantities against an antigen Appears usually as a pentamer when released, but can exist as a monomer and hexamer
IgG		Contains Gamma chains Monomers Most abundant immunoglobulins in sera Produced as a second immune response Only kind of antibodies that can cross the placenta
IgD		Contains Delta heavy chains Presents on surface of B-cell and can initiate immune responses
IgE		Heavy chains type Epsilon A monomer Plays an important role in allergic reactions
IgA		Contains Alpha chains Found mainly in mucosal secretion The initial defense in mucous against pathogen agents Appears usually as dimers, but can appear as monomer and trimer

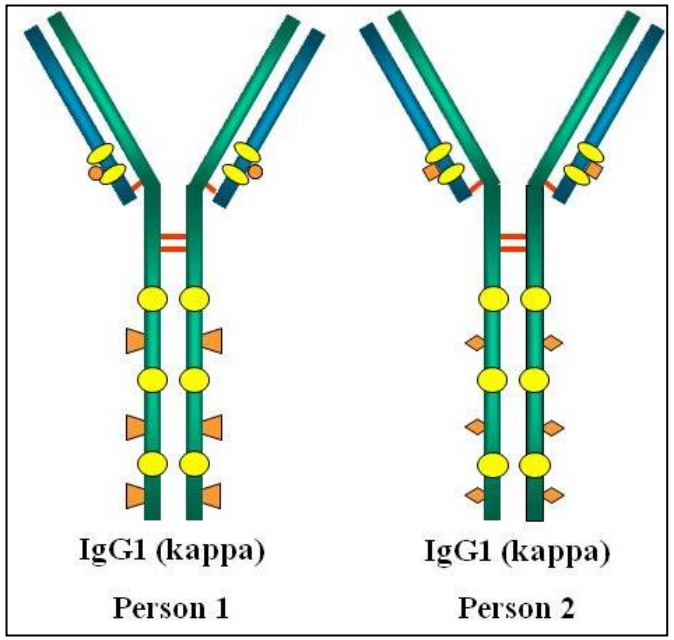
Immunological Memory

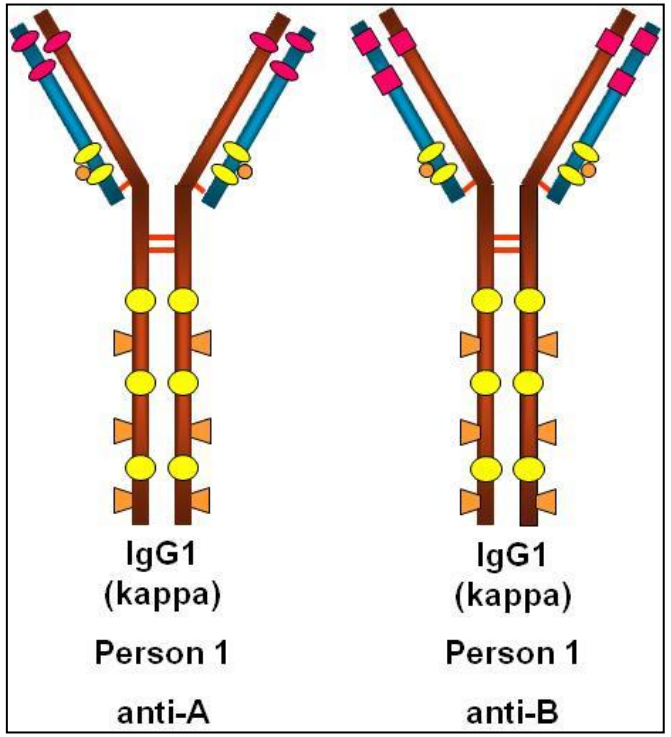




Isotypes: Immunoglobulins of different classes based on their different constant regions.





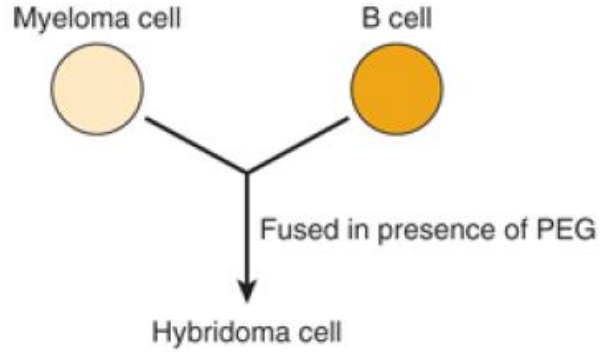


Allotypes: Immunoglobulins of **the same class but different among individuals** of the same species due to different genetics are called allotypes.

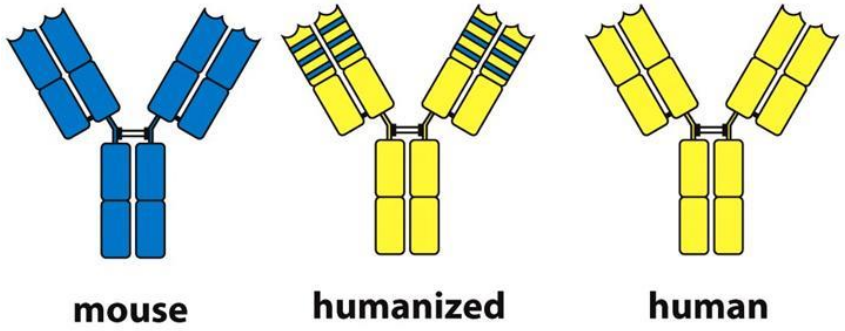
Idiotype: immunoglobulin molecules that have the same constant regions, but **different variable domains** within their light (VL) chains and heavy (VH) chains

Hybridoma and monoclonal antibodies

- When an antigen is injected into an animal, the resulting antibodies are polyclonal, meaning they are directed against a number of different epitopes on the antigen.
- In order to “create” an immortal B cell that produces a single antibody (monoclonal), a B cell hybridizes with a B cancer cell (myeloma).



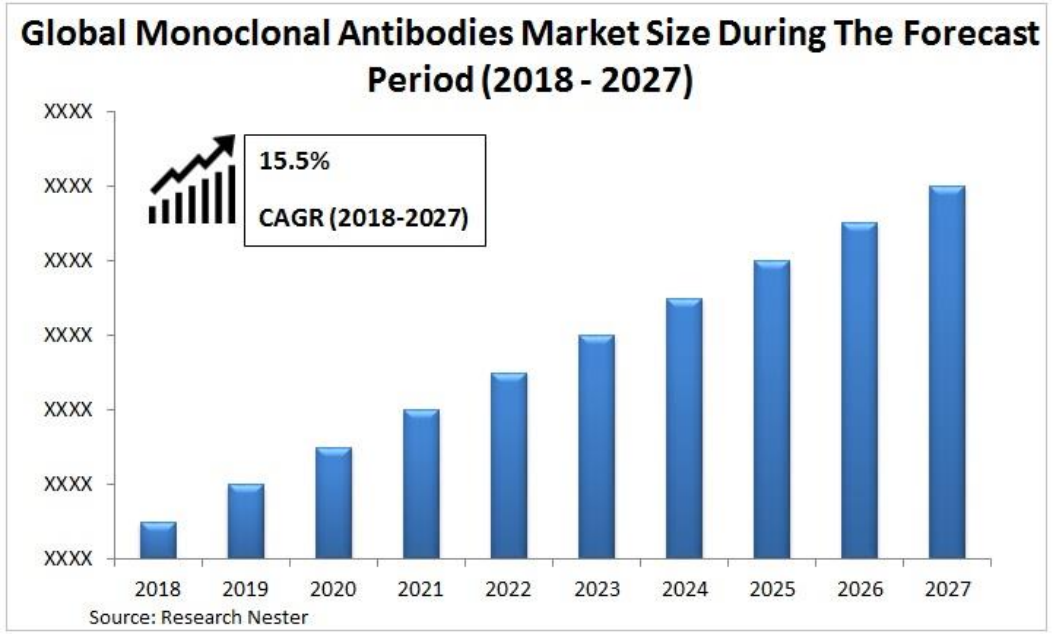
Monoclonal antibodies made in mice can be humanized by attaching the CDRs onto appropriate sites in a human immunoglobulin molecule.



https://www.youtube.com/watch?v=CNPwxbeP7B8
https://www.youtube.com/watch?v=U76LI3OuBsU

Benefits of monoclonal antibodies

- Measure the amounts of many individual proteins and molecules (e.g. plasma proteins, steroid hormones).
- Determine the nature of infectious agents (e.g. types of bacteria).
- Used to direct therapeutic agents to tumor cells.
- Used to accelerate the removal of drugs from circulation when they reach toxic levels.



Diseases

- Multiple myeloma: a neoplastic condition where plasma cells grow out of control in the bone marrow and overproduce abnormal monoclonal proteins (M proteins) instead of useful antibodies.
- Decreased production may be restricted to a single class or may involve underproduction of all classes (ex. agammaglobulinemia)