

Antibodies

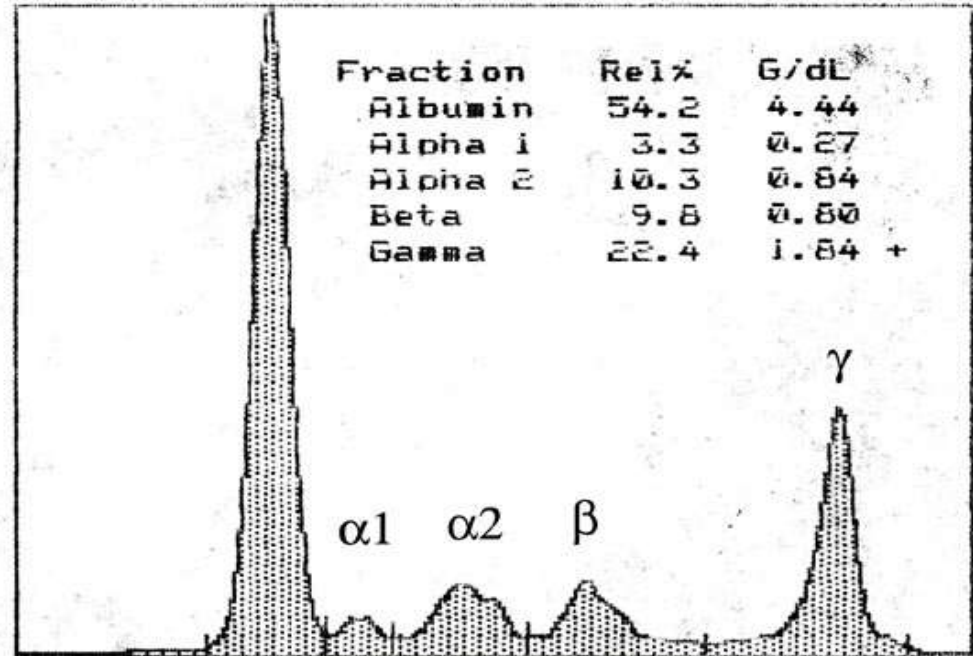
Dr. Pankaj Kumar
Department of Veterinary Microbiology

Humoral immunity is mediated by Antibodies

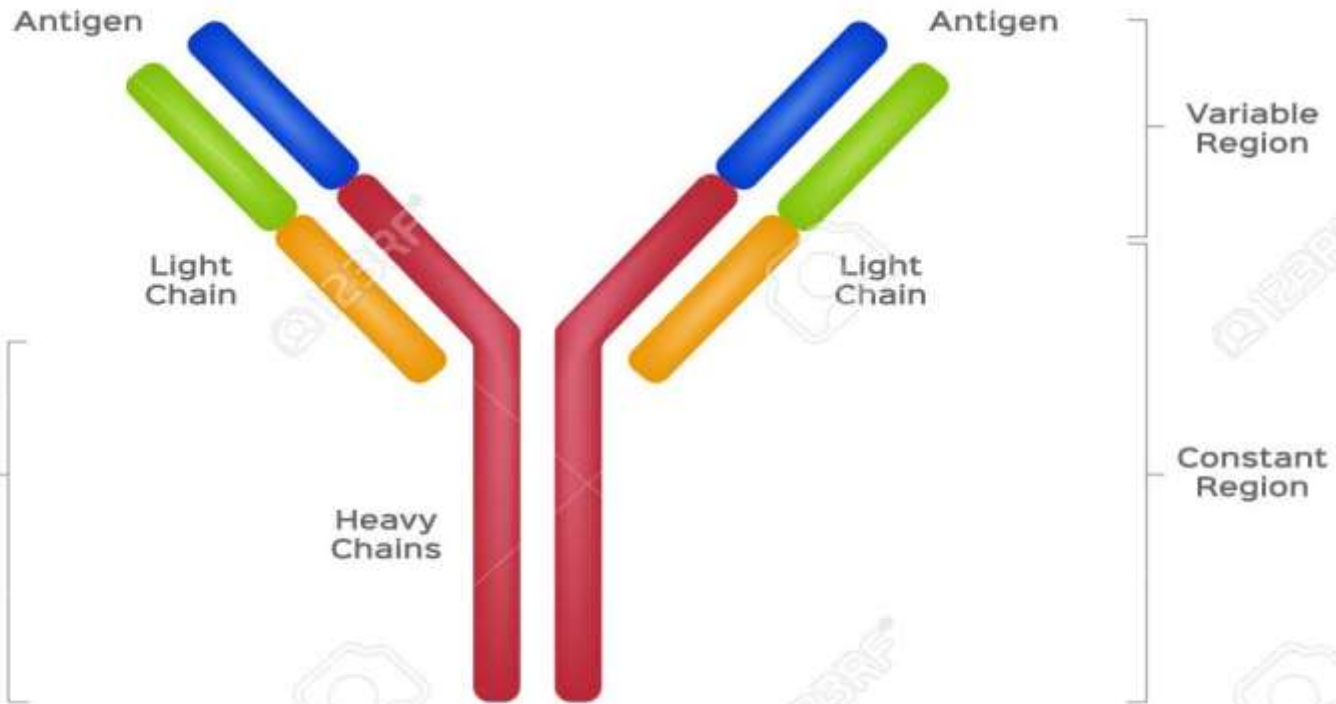
- Serum transferred from an immunized animal to non-immunized animals confers protection.
- Immunity is mediated by factors /components present in serum (*humoral theory of immunity*).
- Serum + Bacteria = Agglutination
- Serum + RBC = Haemagglutination
- Serum + Soluble antigen = Precipitation

Antibody activity is present in globulin fraction

- Tiselius carried out electrophoresis of the serum sample
- Four fractions (*Albumin, alpha-globulin, beta-globulin and gamma-globulin*).
- Most of antibody activity is found in Gamma fraction of the globulin
- (thus antibody molecule is also considered as “Gamma-globulins”).



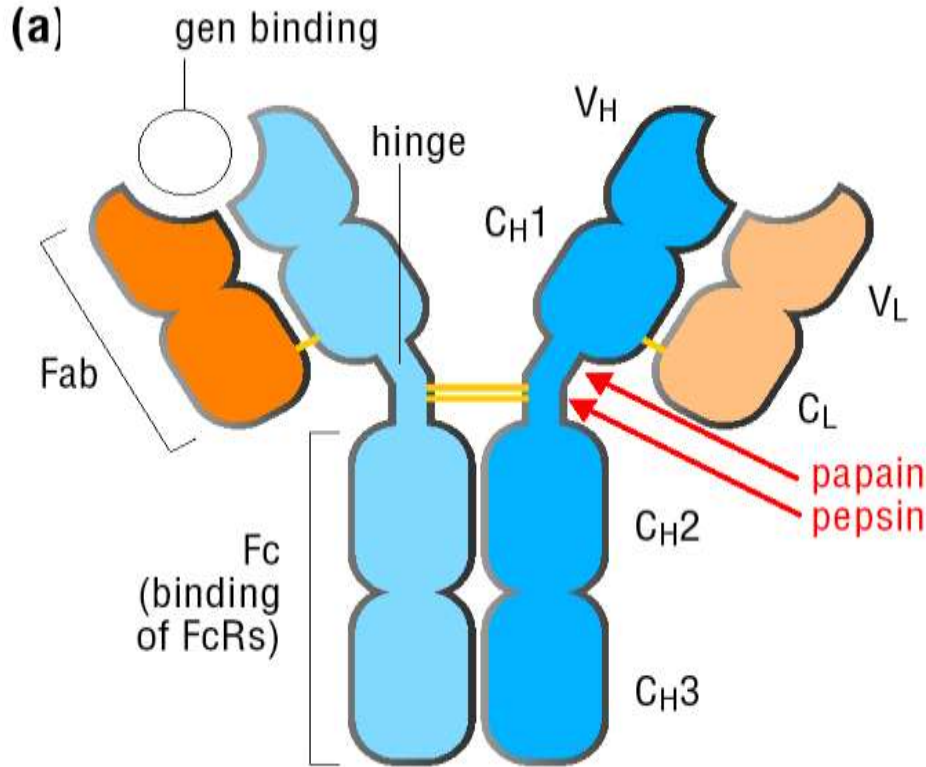
ANTIBODY



Structure of a typical antibody molecule:

- Antibodies are secretory form of B-Cell Receptors (BCRs).
- Antibodies are secreted by differentiated B cells called “PLASMA cells”.
- A typical antibody molecule is made up of four polypeptides i.e. two identical light and two identical heavy chains.
- Two types of light chains are known i.e., Kappa (κ) or lambda (λ) chain.
- Five types of heavy chains i.e., *Alpha*, *Gamma*, *Mu*, *Delta* and *Epsilon* are known.

Antibody Structure



Ig domain: 110 amino acids

Variable domains: Present on N terminal

Constant domains: Present on C terminal

Hinge Region: Provides flexibility to Fab arms of the antibody molecule

Fab: fragment antigen binding

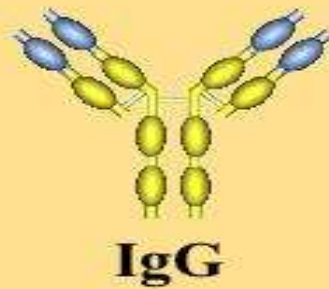
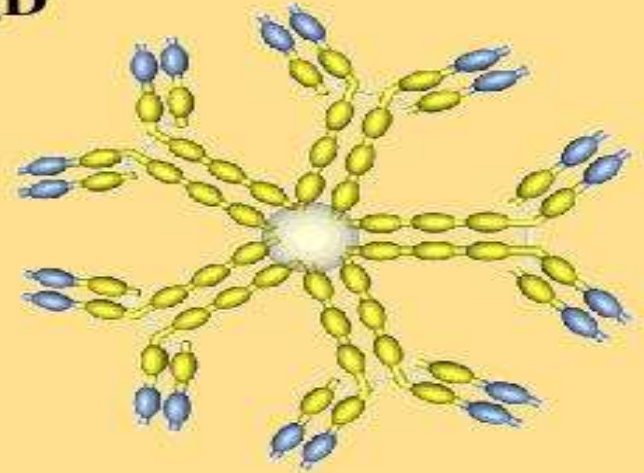
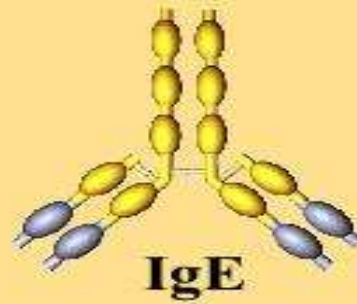
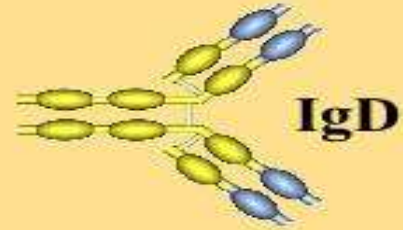
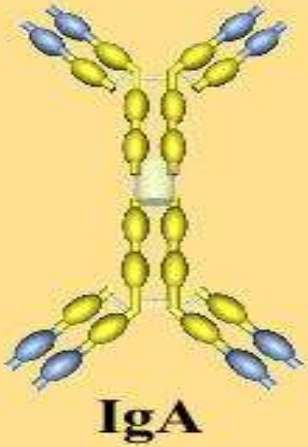
Fc: fragment crystallizable

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Antibodies

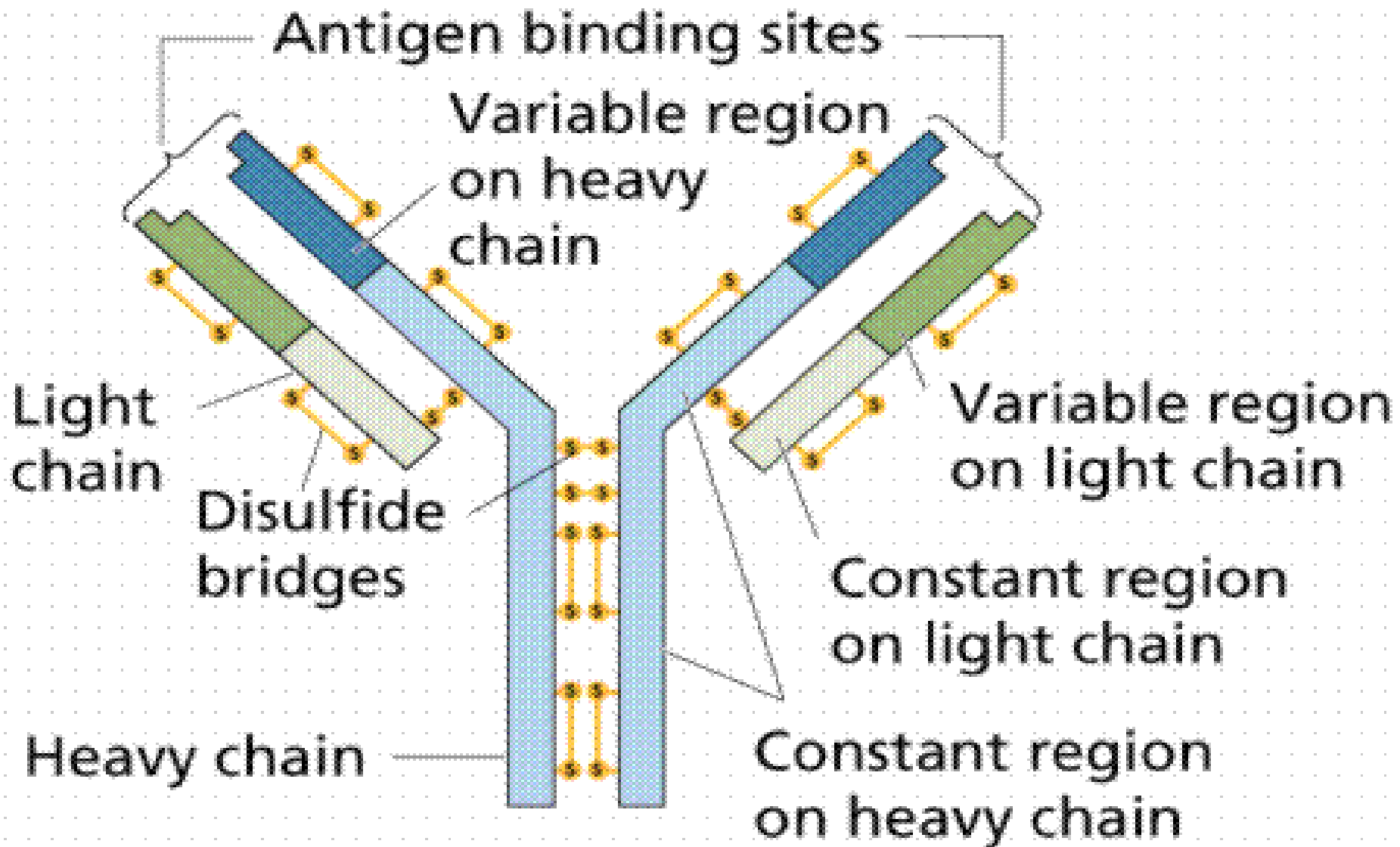
- Of a typical antibody molecule, each light chain is of 25kDa while heavy chain is of 50kDa.
- Thus, a antibody molecule is of 150kDa ($2 \times 25 + 2 \times 50 = 150$ kDa).
- Depending upon the type of heavy chains antibody molecules are of 5 different isotypes viz.,
 - **Ig G, - Ig M, - Ig A, - Ig E and - Ig D types**
- These isotypes differ significantly in their biological activities.

ISOTYPES



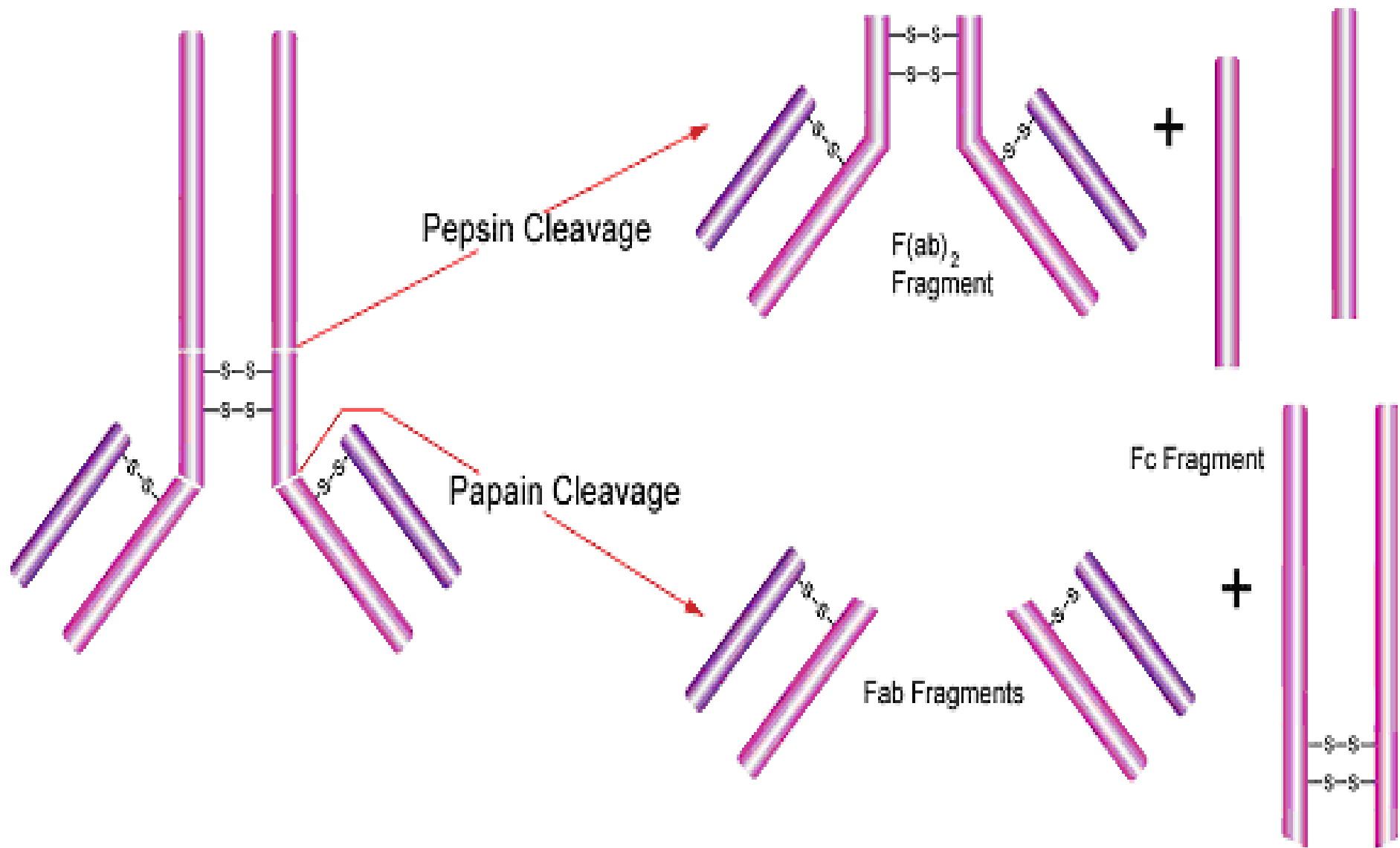
Antibody: Structure

- Light chain has two domains. The amino terminus domain is “variable” whereas carboxy terminal domain is “constant”.
- Variable and Constant domains of light chains are designated as “V_L” and “C_L”, respectively.
- Heavy chain typically has four domains. One domain situated at the amino terminus is “variable” while rest three domains are “constant”.
- Starting from amino terminus, these variable and constant domains are designated as “V_H”, “C_{H 1}”, “C_{H 2}” and “C_{H 3}” domains, respectively.



Antibody: Structure

- One light chain is attached to one heavy chain by means of inter-chain disulphide bonds.
- Both the heavy + light chain complexes are further stabilized by means of inter-chain disulphide bonds between two heavy chains in the “Hinge region”.
- The *antigen binding site* is made up of variable domain of both the light and heavy chains.
- Thus, one antibody molecule has two antigen binding sites.



Papain digestion of the antibody molecule:

- Papain cleaves antibody molecule just above the interchain disulphide bond in the hinge region. Thus, Papain yields three fragments: 2 Fab + 1 Fc

Fab portion:

- Of these three fragments, two are capable of binding with antigen.
- They are called “*fragment antigen binding*” (*Fab*).
- The specificity of the antibody molecule for epitope (antigen) is determined by this “Fab portion”.

Fc portion:

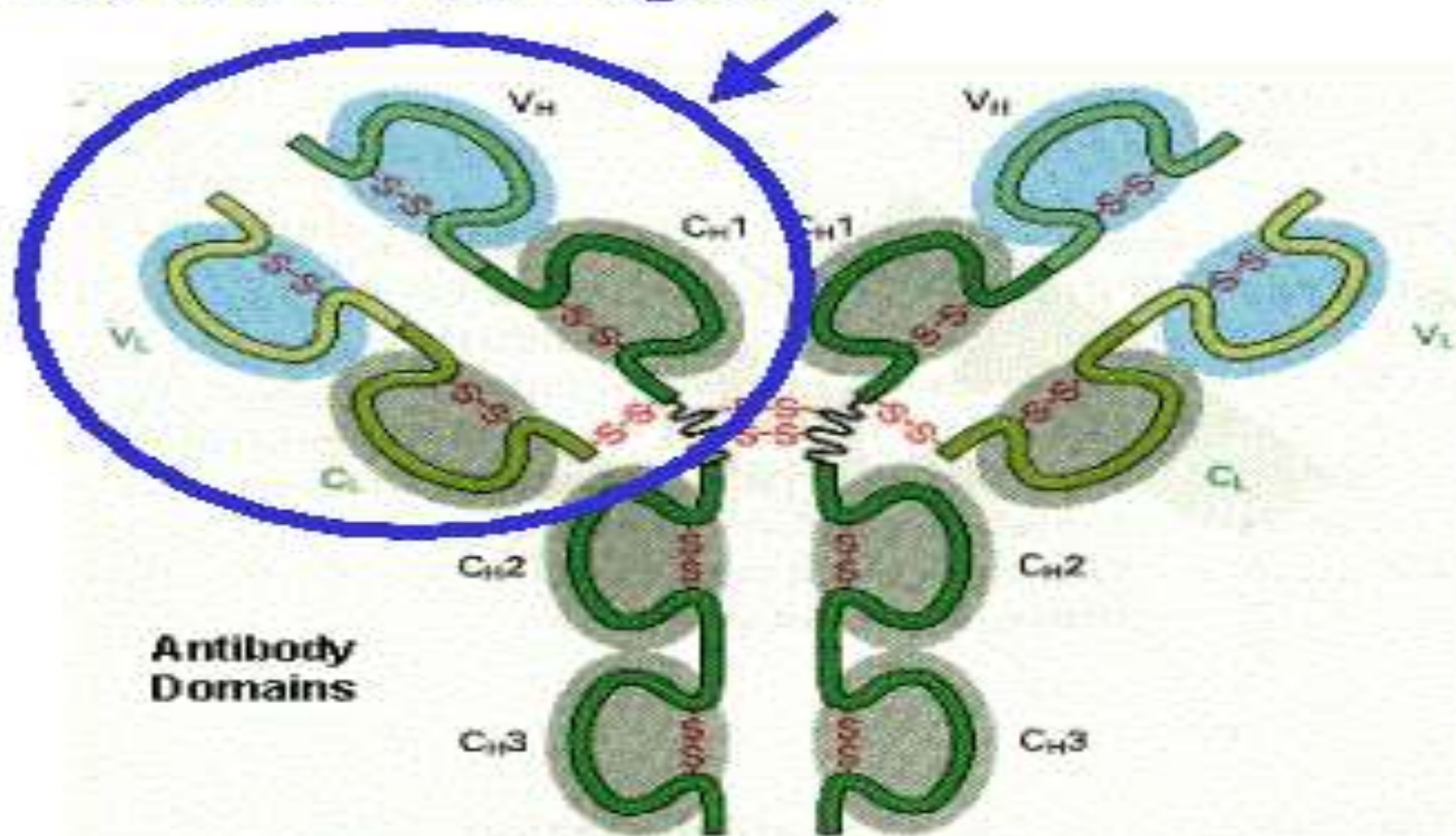
- Other fragment is crystallizable, thus called “*fragment crystallizable*” (Fc). This fragment does not bind to antigen.
- Most of the biological activities of the antibody molecule is determined by this “Fc portion”.

Pepsin digestion of immunoglobulin molecule

- Pepsin cleaves antibody molecules just below the interchain disulphide bond in the hinge region.
- Digestion of antibody molecule yields one (Fab)₂ fragment where as the other fragment is not recognized

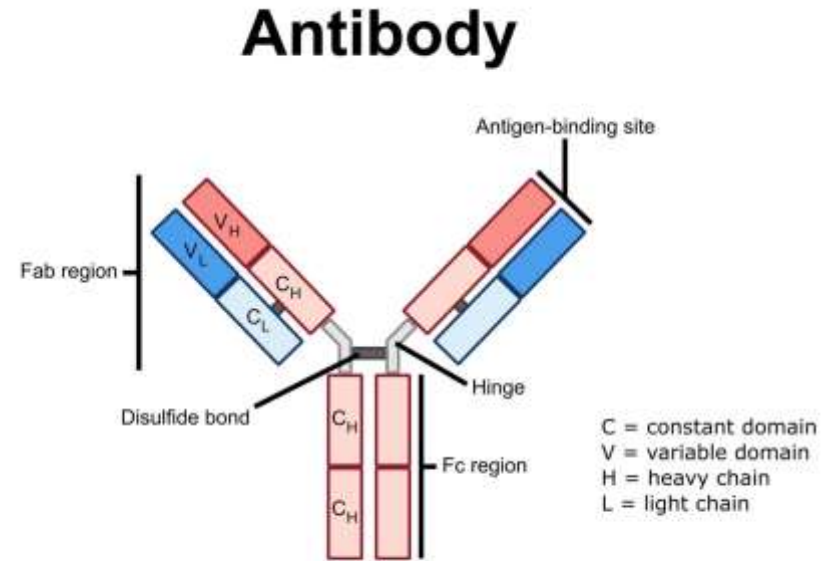
(It is completely digested)

This is the Fab fragment



Hinge region:

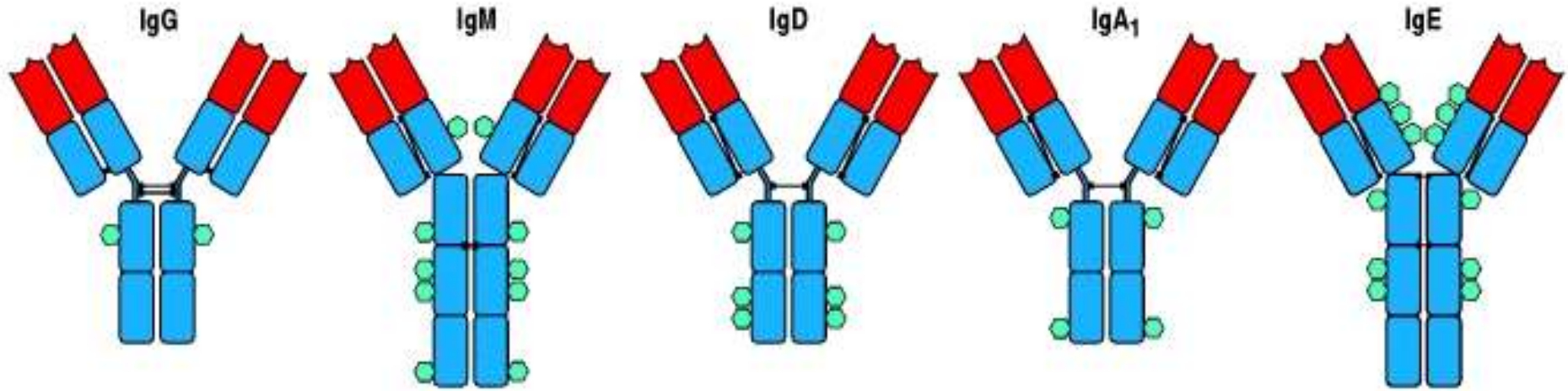
- Present between C_{H1} and C_{H2} domains of IgG, IgA and IgD molecules.
- Heavy chains are attached in the hinge region by inter-chain disulphide bonds.
- Hinge region gives flexibility to the Fab arms of the antibody molecule.
- This region is rich in **Proline and Cystein**.
- Hinge region is absent in IgM and IgE antibodies.



© Lineage

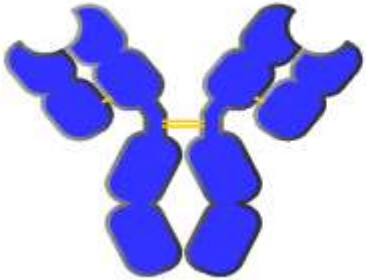
Moises Dominguez

Antibody Classes: Structure

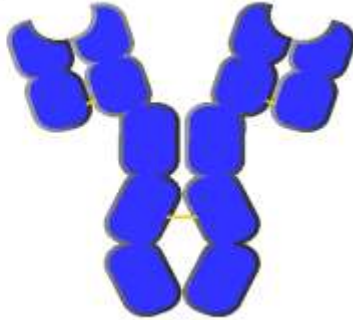


Antibody Classes: Structure

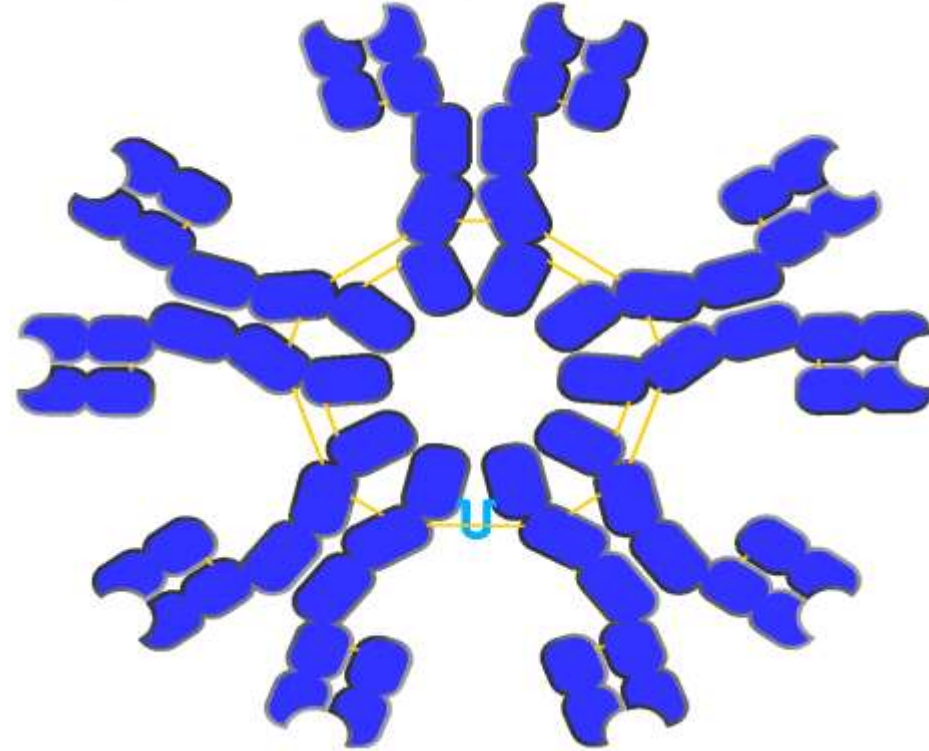
(a) IgG, IgD
monomeric IgA



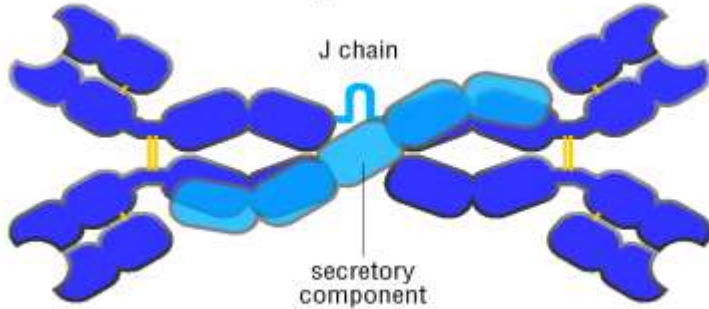
(b) IgE and IgM



(d) IgM pentamer



(c) IgA dimer





Monomer
IgD, IgE, IgG



Monomer
IgD, IgE, IgG



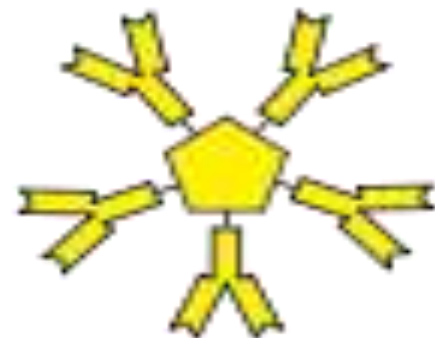
Dimer
IgA



Dimer
IgA



Pentamer
IgM



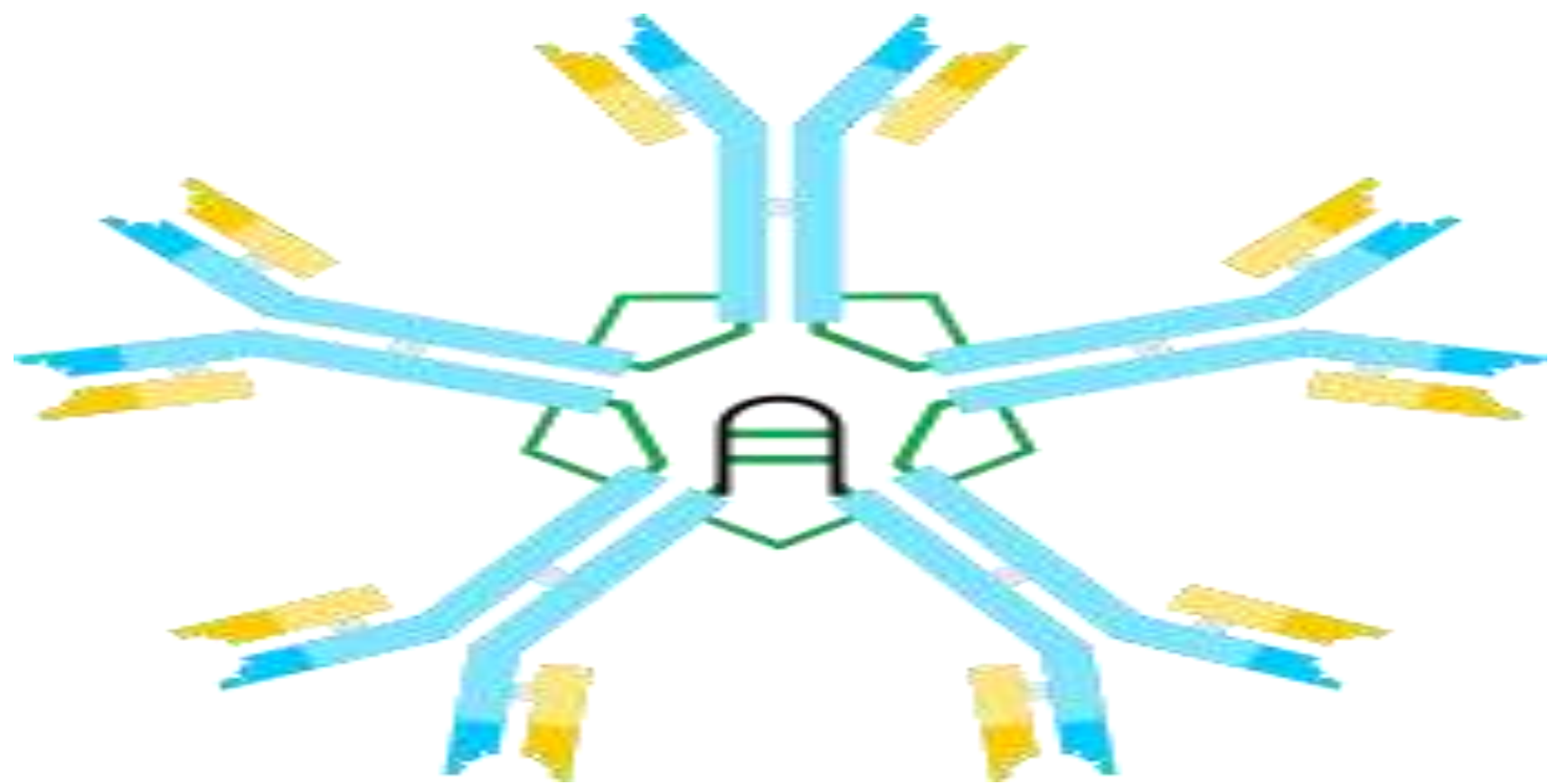
Pentamer
IgM

Immunoglobulin G (IgG)

- Present in highest concentration in Serum.
- Has molecular weight of 180 kDa.
- Smallest in size.
- Major class of antibody induced during secondary immune response.
- Only class of antibody that can cross placenta.

Immunoglobulin M (IgM)

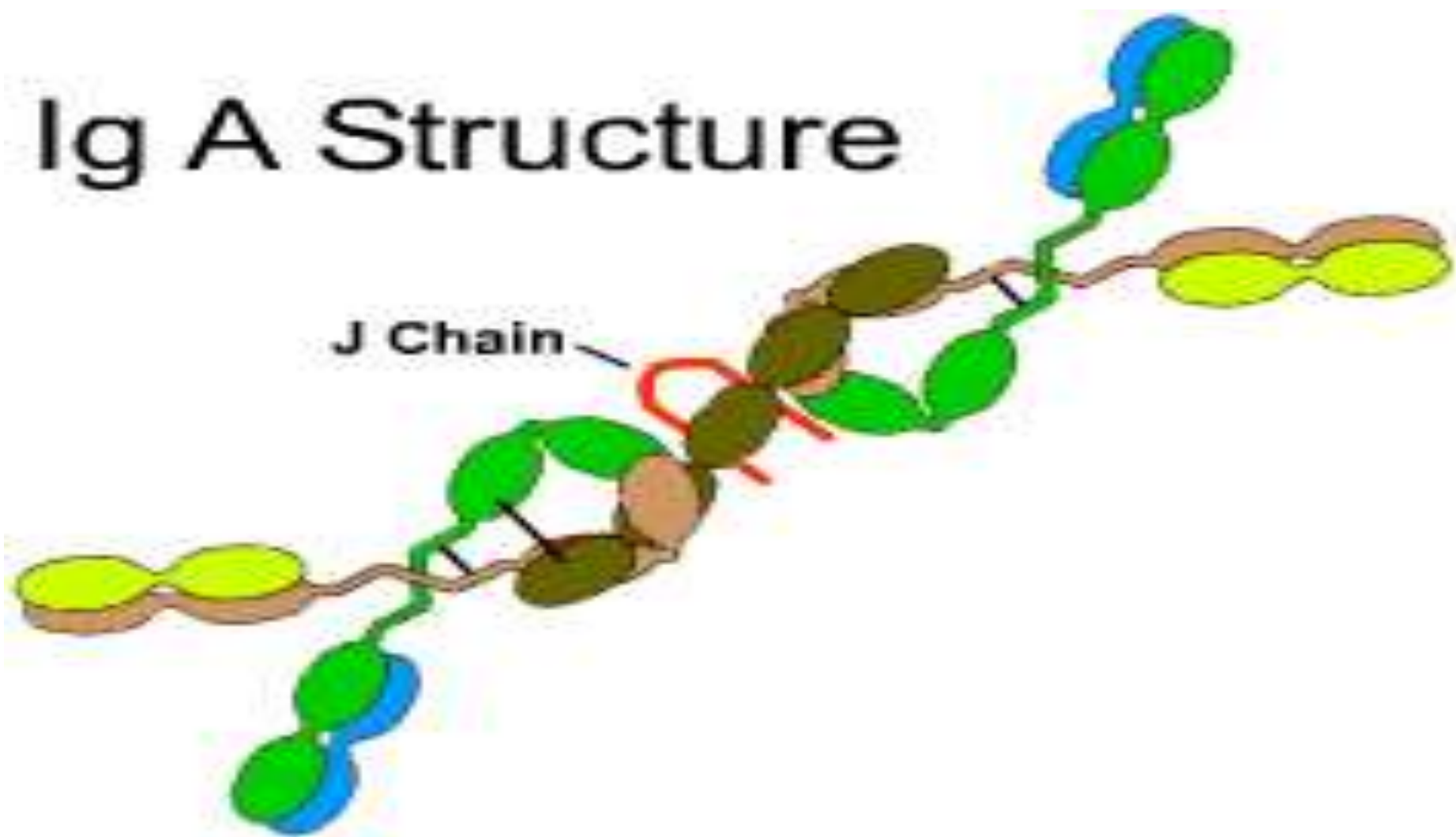
- Largest in size (molecular weight of pentameric form 900 kDa).
- Major class of antibody produced during primary immune response (also first to appear in life).
- IgM monomer is present on the surface of B cells as a component of BCRs.
- Secreted in the Pentameric form (consists of 5 monomers).
- Have an additional constant domain (C_H4) in the basic structure.
- Do not contain hinge region



Immunoglobulin A (IgA)

- Antibody class predominantly present in secretions.
- Present in monomeric form in serum but secretory form is dimeric.
- Have **J** and **S** chains in addition to the basic structure.
- Provide protection on Respiratory, Gastrointestinal, Urogenital surfaces.

Ig A Structure



Immunoglobulin E (IgE)

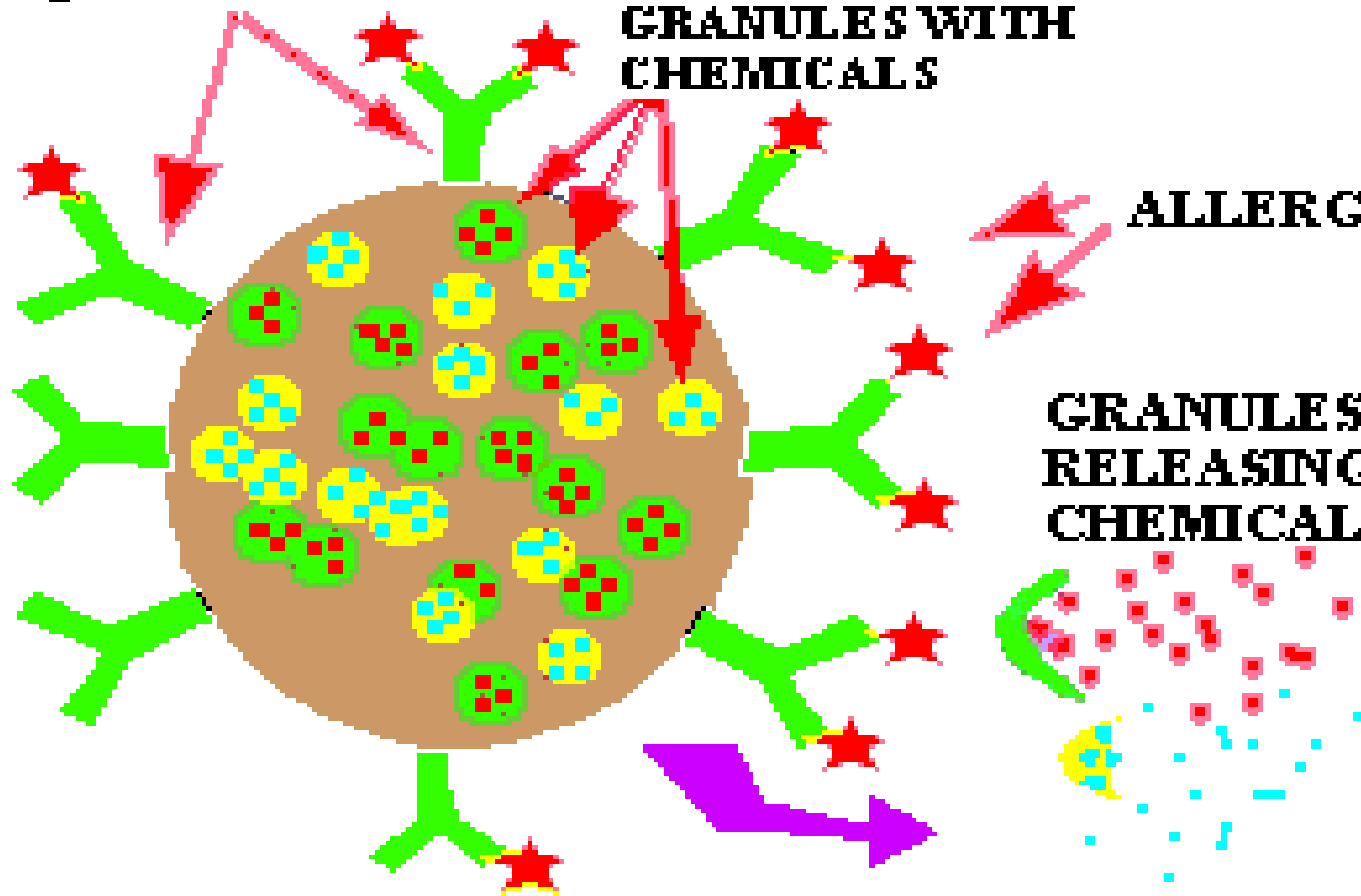
- Molecular weight of **190 kDa**.
- Has an additional constant domain C_H4 (Similar to IgM).
- Provides immunity against parasites.
- Also responsible for allergic reactions.
- Present in extremely low concentration.
- Also known as “Reaginic antibody”.
- Destroy by mild heat treatment

IgE ANTIBODIES

**GRANULES WITH
CHEMICALS**

ALLERGENS

**GRANULES
RELEASING
CHEMICALS**



Immunoglobulin D (IgD)

- Mainly present on B cells as BCRs (along with IgM).
- Very susceptible to digestion with proteases.
- Destroyed by mild heat treatment (Similar to IgE)

Features	Ig G	Ig A	Ig M	Ig D	Ig E
1. Molecular weight (Da)	1.8 Lakh	1.6 to 3.0 Lakh	9.0 Lakh	1.8 Lakh	1.9 Lakh
2. Sedimentation coefficient	7S	9-11S	19S	7S	8S
3. Heavy chain	Gamma	Alpha	Mu	Delta	Epsilon
4. Light chain	Kappa or lambda	Kappa or lambda	Kappa or lambda	Kappa or lambda	Kappa or lambda
5. Occurrence	Monomer	Monomer (serum) Dimer (Secretions)	Pentamer	Monomer	Monomer
6. Presence of J chain and S piece	-----	Both J and S	Only J	-----	-----
7. Carbohydrate content (%)	3	8	12	13	12
8. Half life (in days)	21	6	5	3	2
9. Presence in secretions	No	Yes	No	No	No
10. Placental transfer	Yes	No	No	No	No
11. Involvement in allergic response	No	No	No	No	Yes
12. Relative agglutinating activity	+	++	+++	---	----
13. Precipitation	+++	+	---	----	----
14. Neutralization	+	+++	----	-----	-----

Major functional properties of antibodies

Antibody class	Major Functional properties
IgM	complement activation; antigen trapping; antigen receptor of naïve B cells
IgG	complement activation, phagocytosis, ADCC, transfer of adaptive immunity to offspring, regulation of antibody production
IgA	mucosal immunity, phagocytosis
IgE	activation of mast cells, basophils, eosinophils
IgD	antigen receptor on naïve B cells

Role of antibody molecule in immune response:

- **Neutralization:** Antibodies play an important role in the neutralization of viruses and toxins.
- **Precipitation:** Antibodies cause the precipitation of soluble antigens.
- **Agglutination:** Antibodies cause the agglutination of particulate antigens.
- **Complement activation:** Antibodies have sites for complement binding. When an antibody molecule binds to an antigen, the complement binding sites are exposed, allowing complement binding and its activation.

Role of antibody molecule in immune response:

- **Opsonization:** binding of antibody molecule to antigen facilitates phagocytosis of antigen.
- **ADCC:** Binding of antibody to the antigen present on the cell surface allows lysis of cells by cytotoxic cells having receptors for Fc region of antibody molecule.
- **Allergy:** Allergic responses are mediated by IgE type of antibodies which remain bound on the surface of Mast cells. Binding of IgE antibody with antigen induces degranulation of mast cells.

ANTIBODIES

POLYCLONAL.

Derived from different B Lymphocytes cell lines

Batch to Batch variation affecting Ab reactivity & titre

NOT Powerful tools for clinical diagnostic tests

MONOCLONAL.

Derived from a single B cell clone

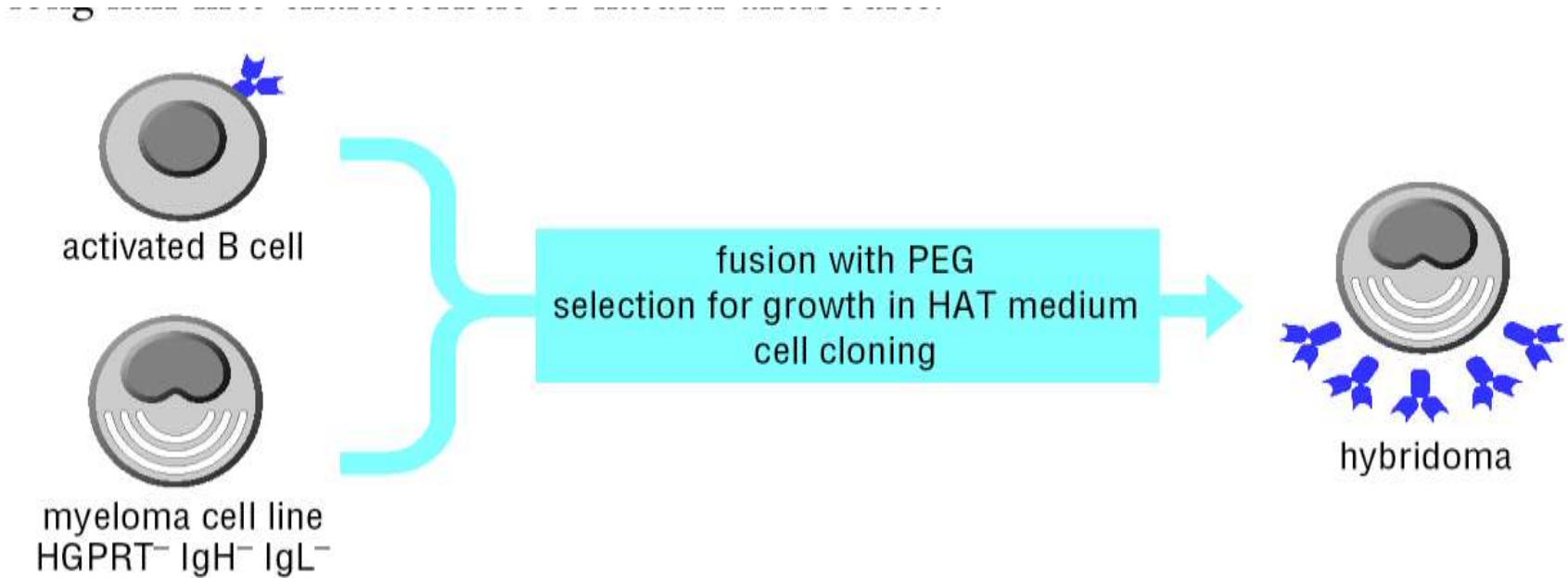
mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity

Enable the development of secure immunoassay systems.

Monoclonal antibodies (Mab):

- Antibodies produced by a clone of single plasma cells and having specificity against single epitope are called “Monoclonal antibodies”.
- Monoclonal antibodies are produced by the technique called “Hybridoma technology”.
- In brief, Plasma cell is allowed to fuse with Myeloma cell (cancerous Plasma cells) using chemical “poly ethylene glycol”.
- The hybrid cell produced is called “Hybridoma cells” secreting antibodies of single specificity.
- These immortal “Hybridoma cells” are sharing attributes from both the partner cells i.e., antibody specificity from plasma cells and immortality from Myeloma cell.

Generation of Monoclonal Antibodies



Bence-jones protein:

- A protein occurring in the serum and urine of patients with certain diseases, especially *multiple myeloma*.
- Myeloma cells are “Neoplastic Plasma Cells”.
- A Bence Jones protein is a monoclonal globulin protein found in the blood or urine, with a molecular weight of 22-24 kDa.
- The proteins are immunoglobulin light chains and can be kappa (most of the time) or lambda.

THE END

The images for slides are taken from resources available on internet and used for the purpose of teaching students