Immunoglobulins

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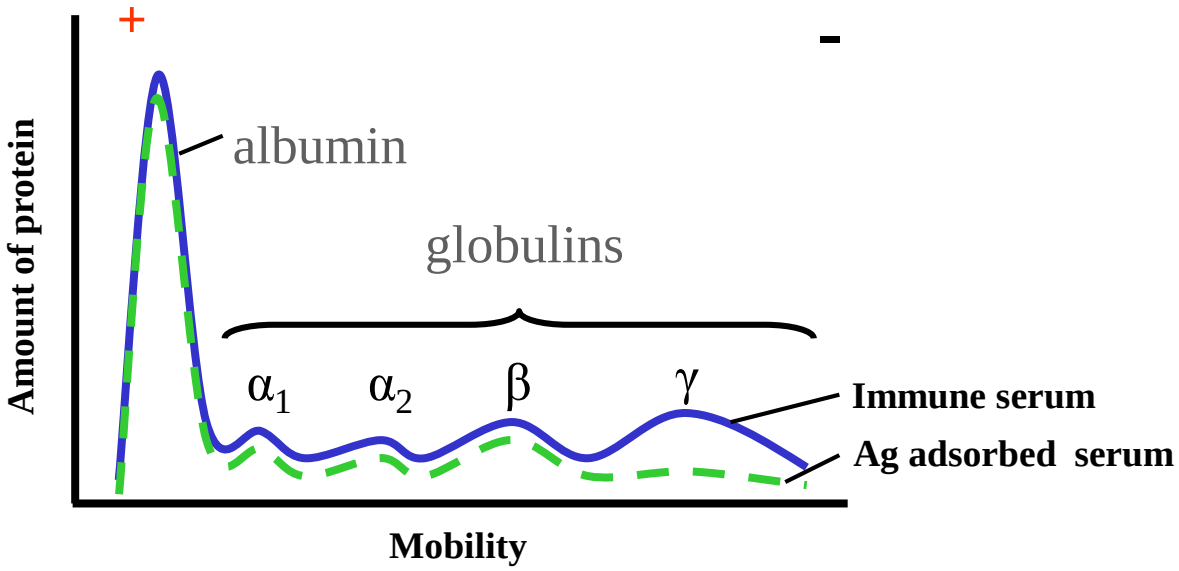
Structure and Functions

Definition

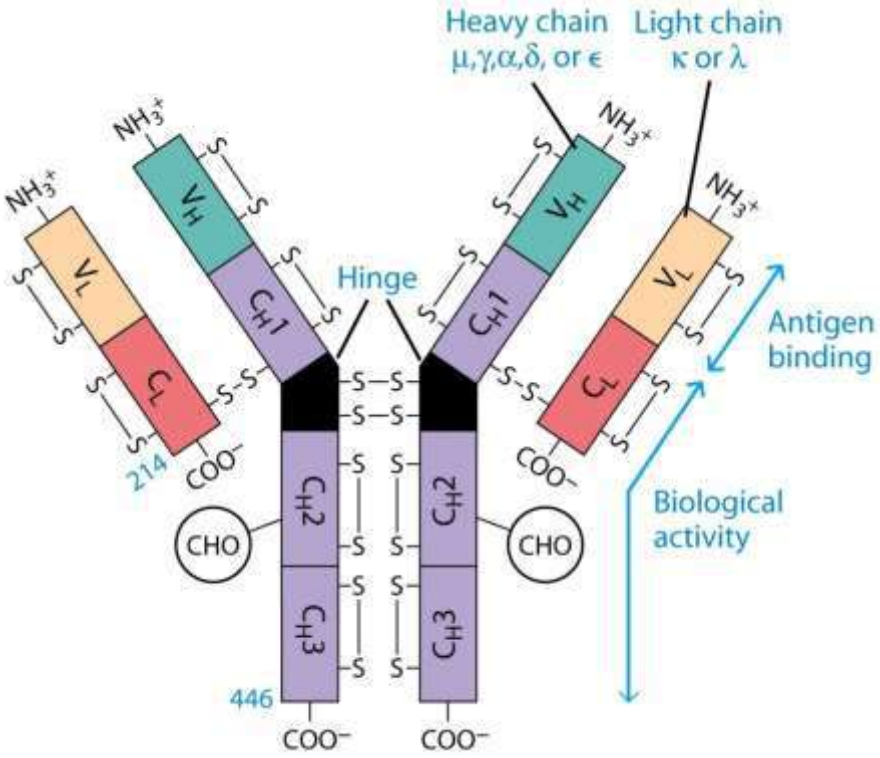
- Immunoglobulins are glycoprotein molecules belonging to γ-globulins class of plasma proteins produced in response to a non-self or an altered self immunogen and act as antibodies in humoral adaptive immune response.
- Immunoglobulins are produced in vertebrates by plasma cells, which are the terminally differentiated B lymphocytes

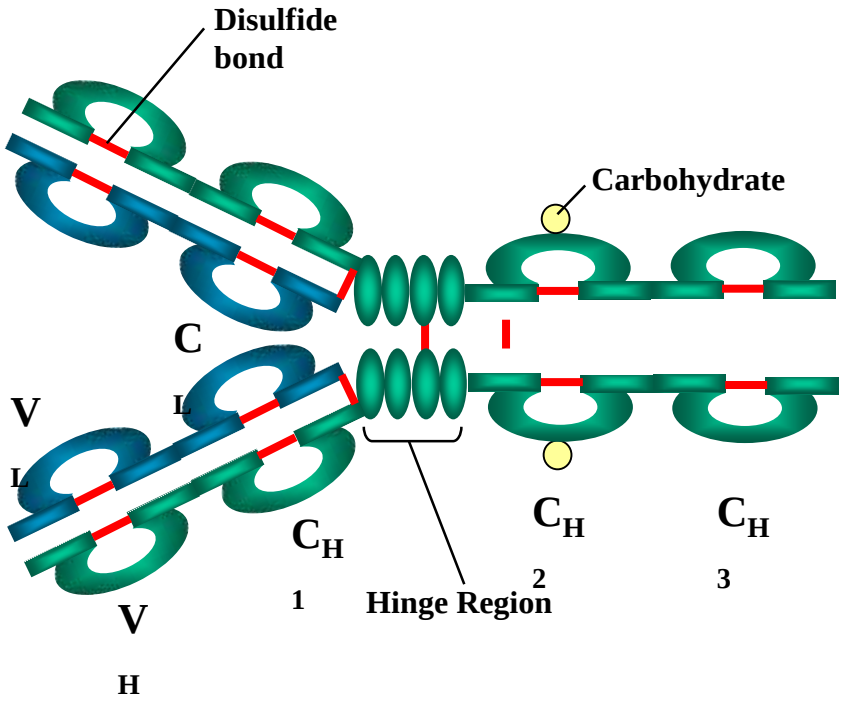


Basic Immunoglobulin Structure

- γ-globulin
- glycoprotein
- heterodimer
- ‘Y’ shaped molecule
- coded by immunoglobulin supergene family

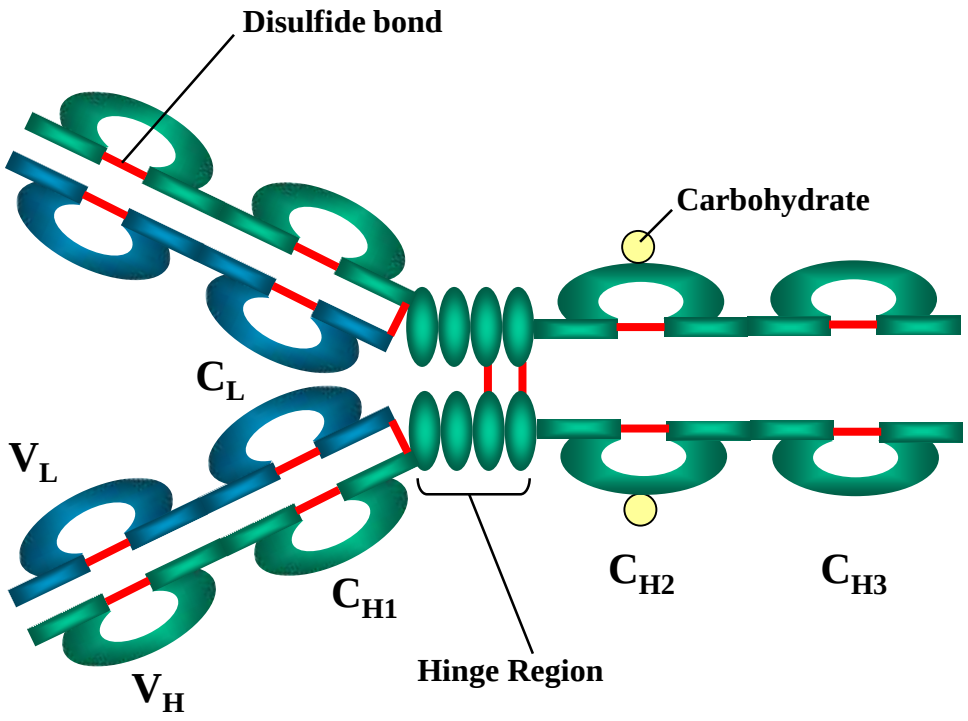
Basic Immunoglobulin Structure





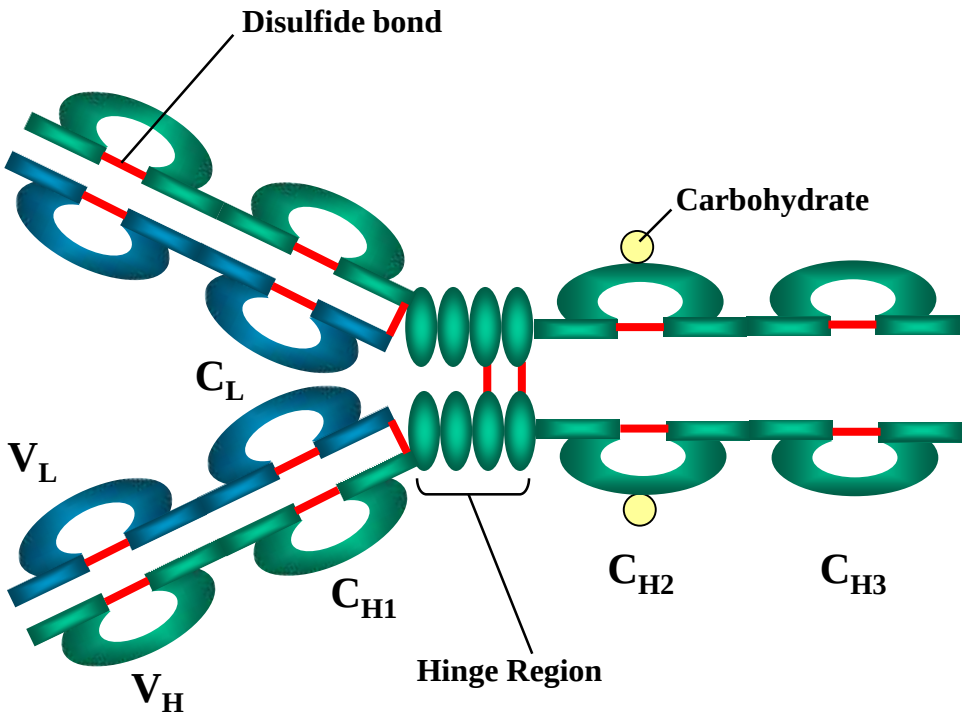
Immunoglobulin Structure – a monomer (H_2L_2)

- 2 Heavy & 2 Light chains
- Disulfide bonds
  - Inter-chain
  - Intra-chain



Immunoglobulin Structure

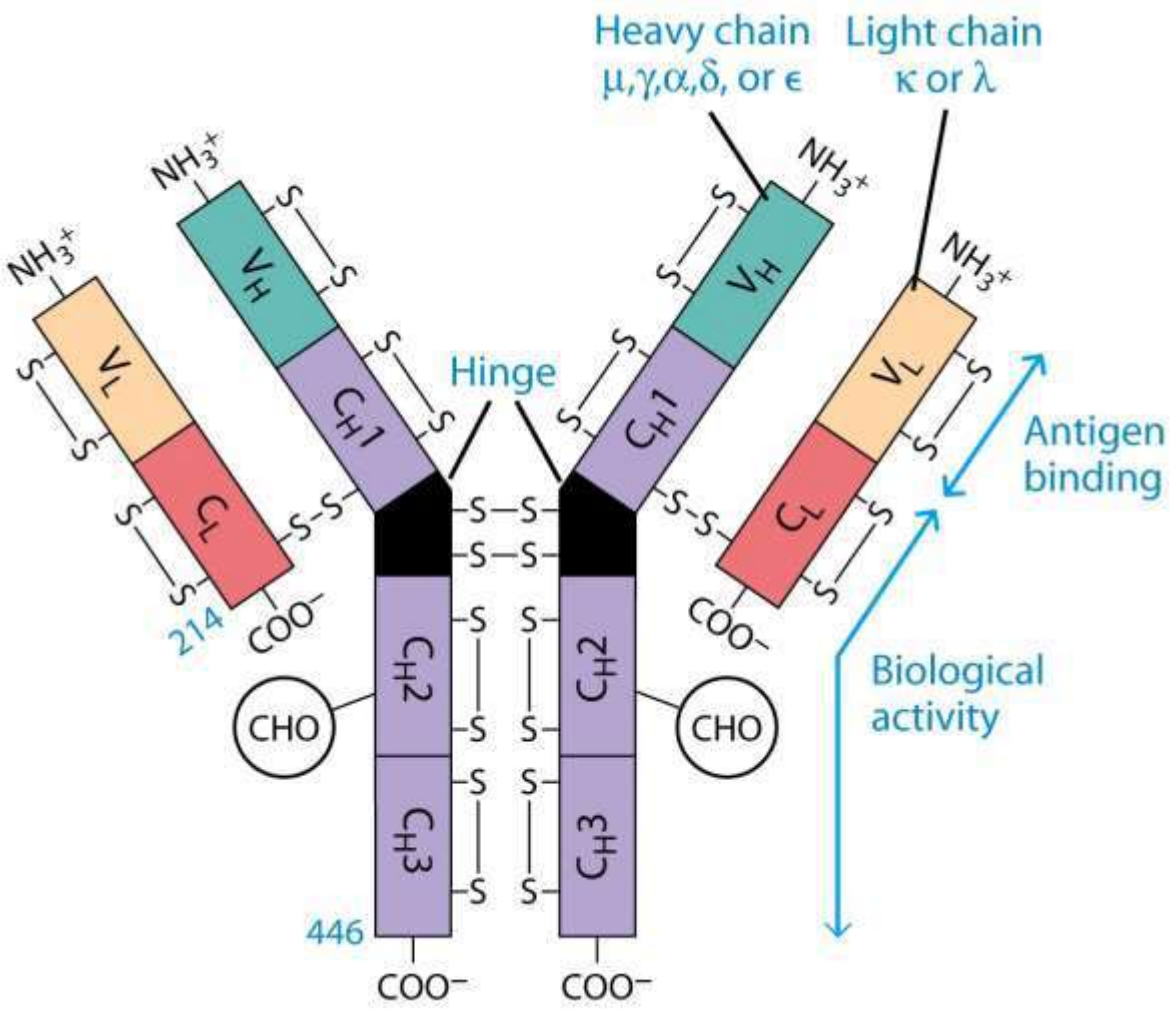
- Variable & Constant regions in each chain
  - V_L & C_L
  - V_H & C_H
- Forms globular loop like structure called as domains
- Hinge Region



Basic Immunoglobulin Structure

- A monomer (H_2L_2) of an immunoglobulin molecule is made up of:
  - 2 Light Chains (identical) ~25 KDa
  - 2 Heavy Chains (identical) ~50 KDa
- Each light chain bound to heavy chain by disulfide bonds (H-L)
- Each heavy chain bound to heavy chain by disulfide bonds (H-H)
- The ¼ portion of each H chain and ½ of each L chain towards amino terminal are more variable (110 aa each - V_H and V_L) in amino acid composition as compared to the remaining portion towards carboxyl terminal (C_H and C_L) in each monomer, which has nearly constant composition in each domain of a given isotype.
- CDR (Complementarity Determining Regions) are actual areas where antigen binds

Basic Immunoglobulin Structure

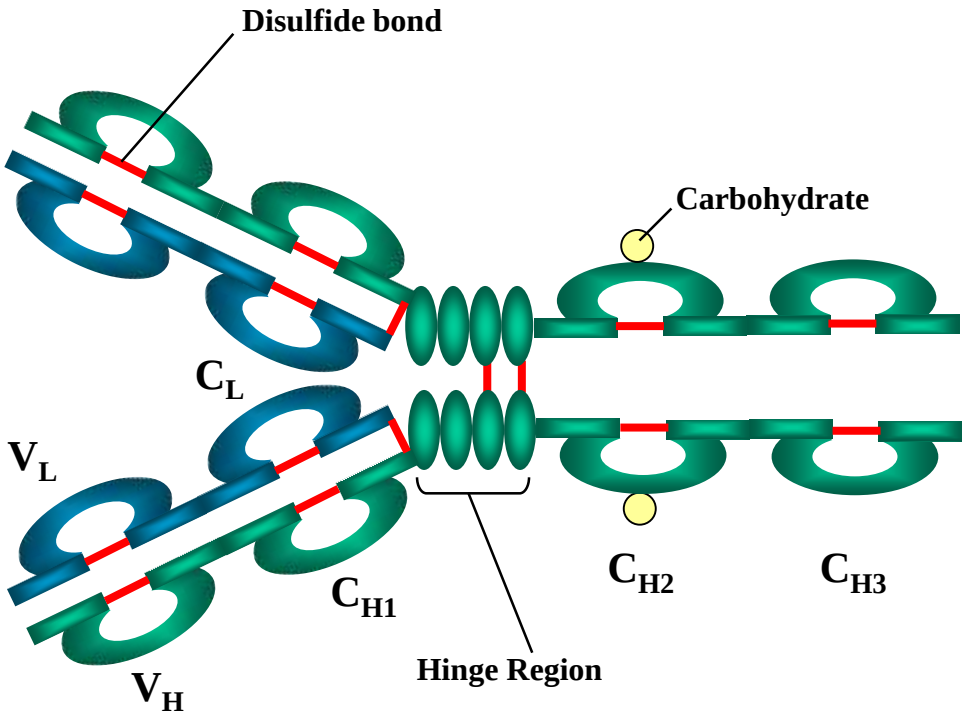


Basic Immunoglobulin Structure

- **Repeating Domains of ~110 a/a**
  - Intra-chain disulfide bonds within each domain
- **Heavy chains**
  - 1 V_H and either 3 or 4 C_H (C_H1, C_H2, C_H3, C_H4)
- **Light chains**
  - 1 V_L and 1 C_L
- **Hinge Region**
  - Rich in cysteine residues (disulfide bonds)
  - Rich in proline residues (flexible)
  - Proline residues are target for proteolytic digestion (papain and pepsin)
  - Hinge found in IgG, IgA and IgD
  - IgM and IgE lack hinge region
  - They instead have extra C_H4 Domain

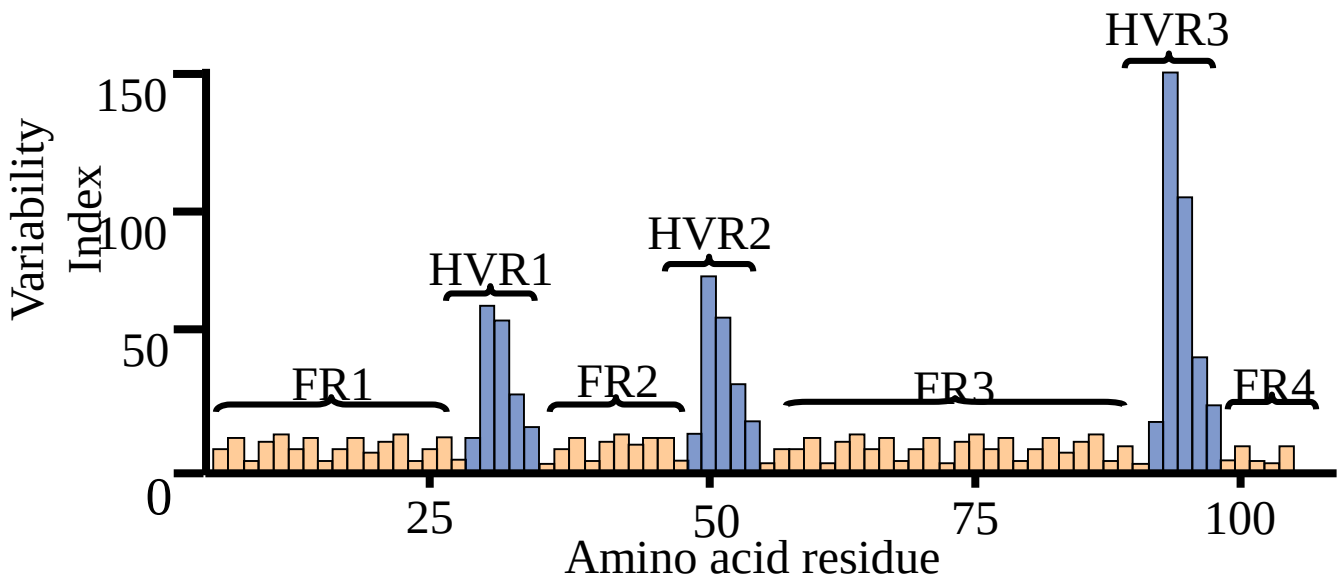
Immunoglobulin Structure

- Domains
  - V_L & C_L
  - V_H & C_{H1} - C_{H3} (or C_{H4})
  - each domain has approximately 100-110 aa
- Oligosaccharides



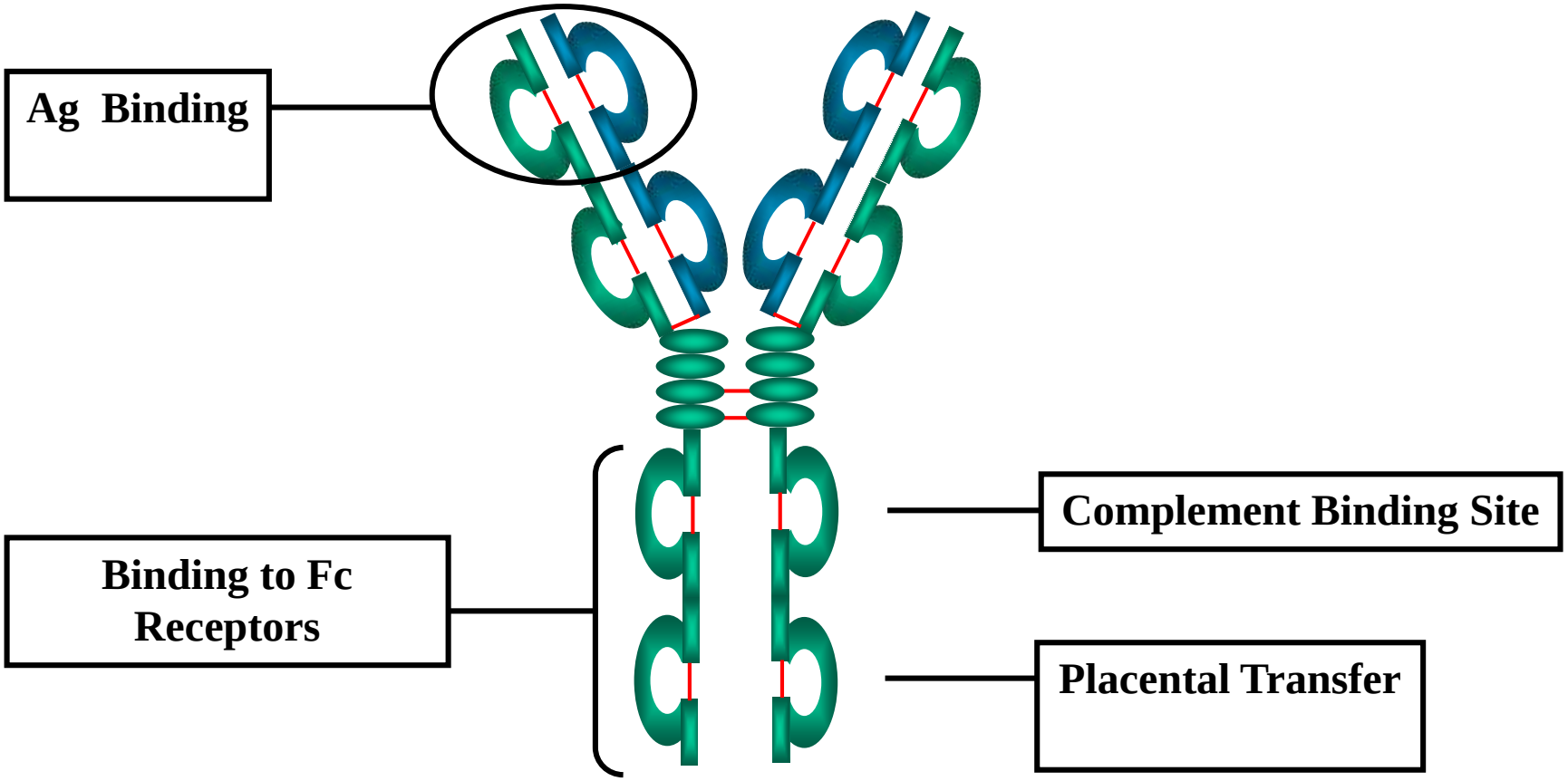
Structure of the Variable Region

- **Hypervariable (HVR) or complimentarity determining regions** (CDR) – hot spots within variable region of both H and L chains which exhibit more variation in aa composition than other regions
- HVRs form **paratope** – the epitope bindin region on antibody



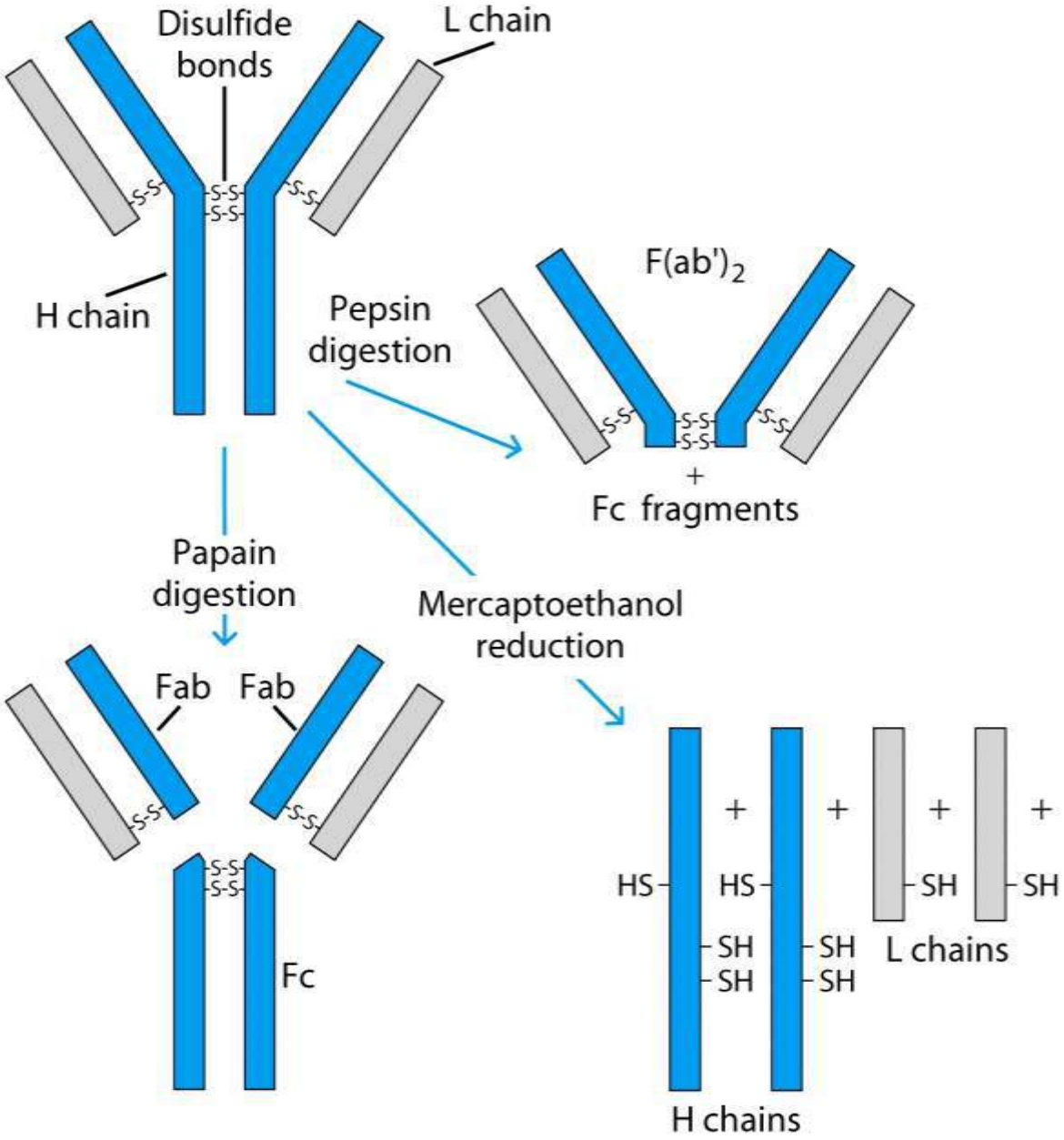
- **Framework regions (FR)**

Immunoglobulin Fragments: Structure/Function Relationships



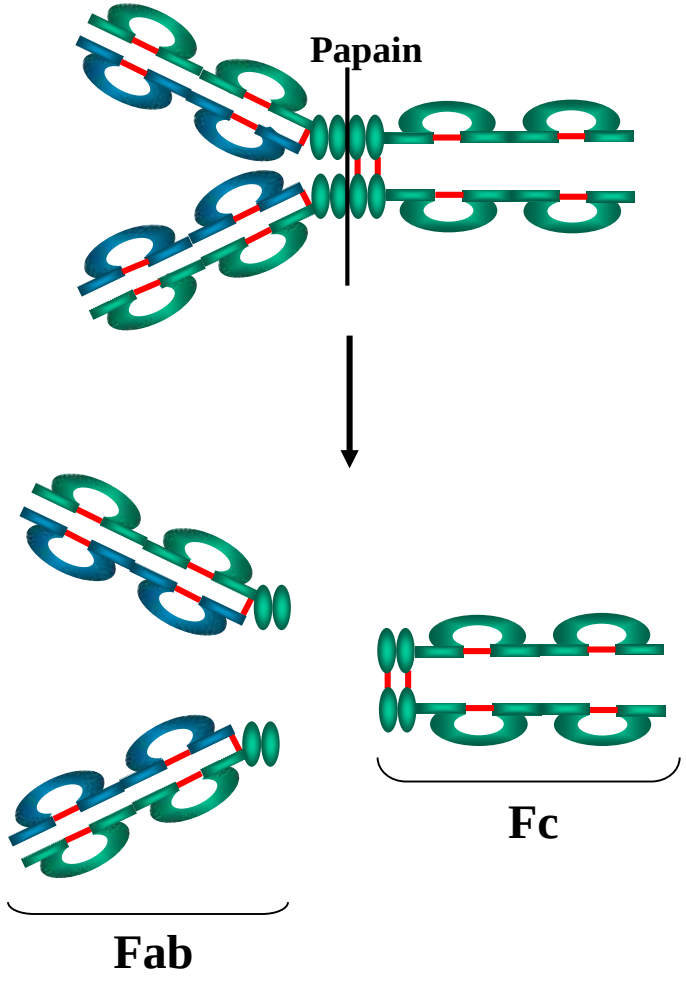
Enzymatic digestion of antibodies

- **Digestion with Papain yields**
  - 3 fragments
  - 2 identical Fab (each monovalent) and 1 Fc
  - Fab - fragment that is antigen binding
  - Fc - crystallize in cold storage
- **Digestion with Pepsin yields**
  - F(ab`)2 (divalent)
  - No Fc recovery; digested entirely
- Mercaptoethanol reduction eliminates disulfide bonds


Disulfide bonds
L chain
H chain
Pepsin digestion
F(ab')2
+
Fc fragments
Papain digestion
Mercaptoethanol reduction
Fab
Fab
Fc
HS
HS
SH
SH
SH
SH
SH
SH
L chains
H chains

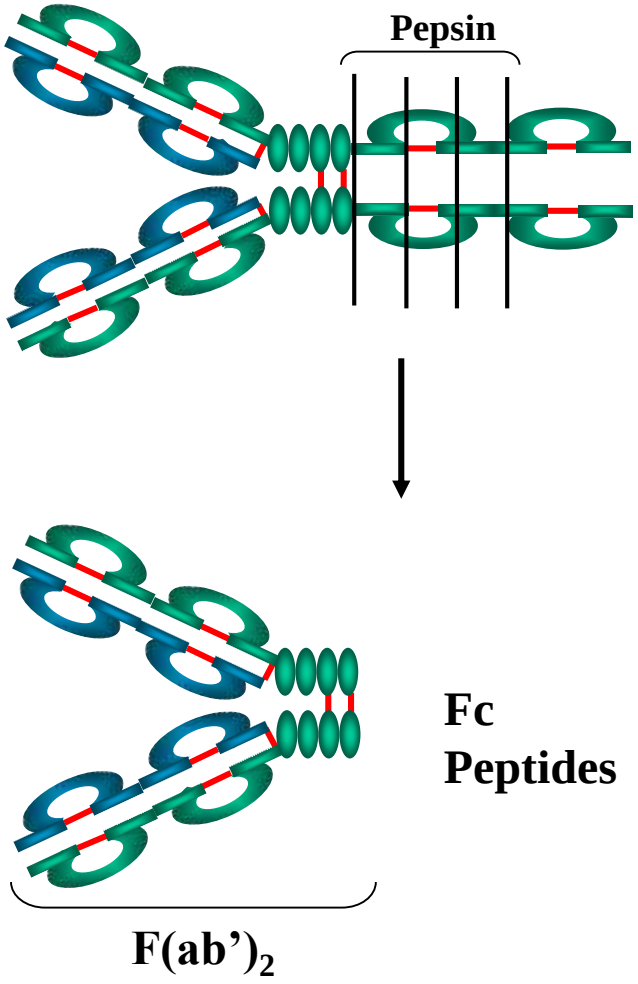
Immunoglobulin Fragments: Structure/Function Relationships

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



Immunoglobulin Fragments: Structure/Function Relationships

- $F(ab')_2$
  - Ag binding
  - Valence = 2
  - Specificity determined by V_H and V_L
- Fc
  - digested



Immunoglobulin Classes

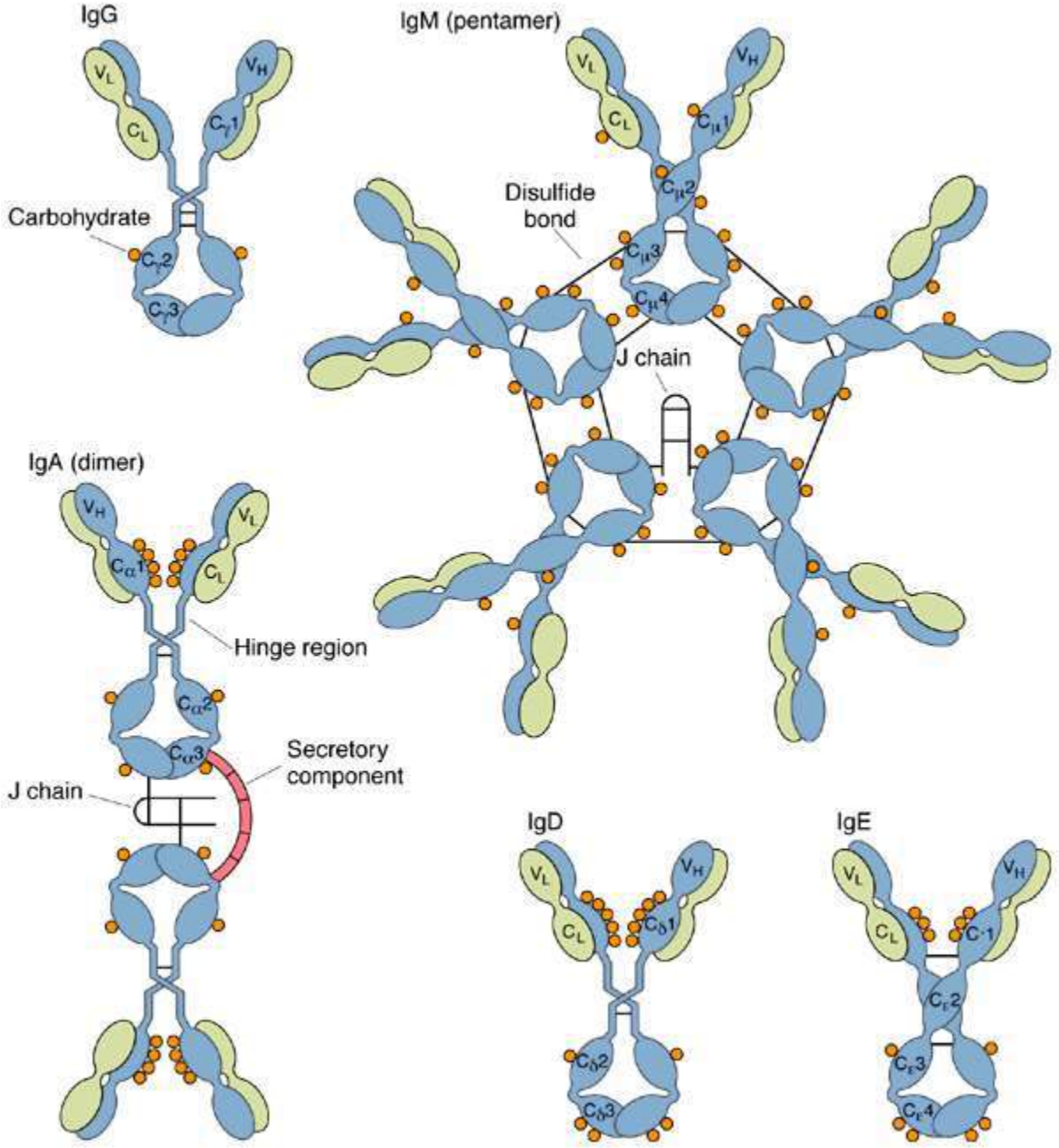
- **Sequencing of heavy chains of several immunoglobulins in human beings and mice revealed:**
  - A highly variable (V) region of 100-110 amino acids at amino terminus of each H chain
  - Five basic amino acid sequence patterns in remaining constant (C) region of H chains which differ between H chains of each pattern, but not in all H chains of a given pattern
  - α,γ, δ, ε, μ types of heavy chains
  - IgA, IgG, IgD, IgE and IgM classes of immunoglobulins
  - The above classes are called isotype named on basis of type of heavy chain
  - κ or λ light chains; each class can have either of these
  - Minor differences led to sub-classes

Human Immunoglobulin Heavy chain Subclasses

- **IgG Subclasses**
  - IgG1 - Gamma 1 (γ1) heavy chains
  - IgG2 - Gamma 2 (γ2) heavy chains
  - IgG3 - Gamma 3 (γ3) heavy chains
  - IgG4 - Gamma 4 (γ4) heavy chains
- **IgA subclasses**
  - IgA1 - Alpha 1 (α1) heavy chains
  - IgA2 - Alpha 2 (α2) heavy chains

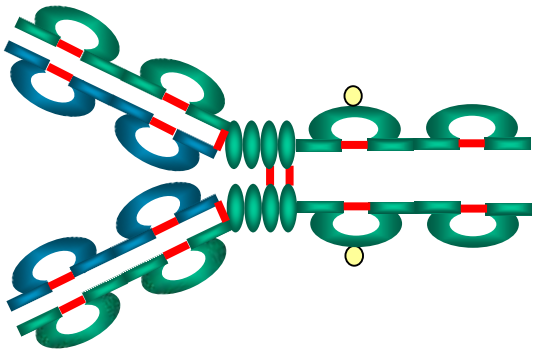
Human Immunoglobulin Light chain Subclasses

- **Kappa (κ)**
  - No subclass
- **Lambda (λ)**
  - Lambda 1 (λ1)
  - Lambda 2 (λ2)
  - Lambda 3 (λ3)
  - Lambda 4 (λ4)

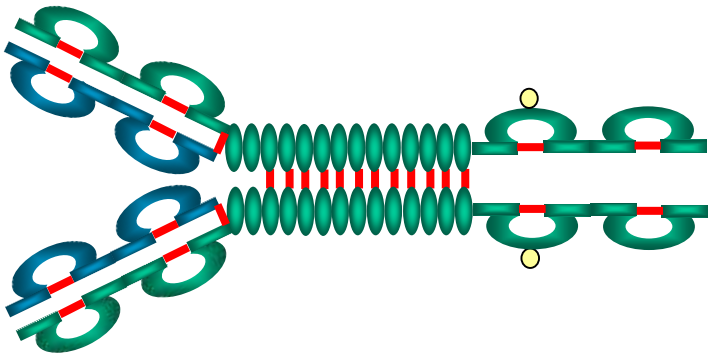

IgG
V_L
V_H
C_L
$C_\gamma 1$
Carbohydrate
$C_\gamma 2$
$C_\gamma 3$
IgM (pentamer)
V_L
V_H
C_L
$C_\mu 1$
$C_\mu 2$
Disulfide bond
$C_\mu 3$
$C_\mu 4$
J chain
IgA (dimer)
V_H
V_L
$C_\alpha 1$
C_L
Hinge region
$C_\alpha 2$
$C_\alpha 3$
Secretory component
J chain
IgD
V_L
V_H
C_L
$C_\delta 1$
$C_\delta 2$
$C_\delta 3$
IgE
V_L
V_H
C_L
$C_\varepsilon 1$
$C_\varepsilon 2$
$C_\varepsilon 3$
$C_\varepsilon 4$

IgG - structure

- Y-shaped
- monomer (H_2L_2)
- 7S
- γ isotype of H chain
- one variable (V_H) and 3 constant (C_{H1}, C_{H2}, C_{H3}) regions in H chain
- hinge region present



IgG1, IgG2 and IgG4



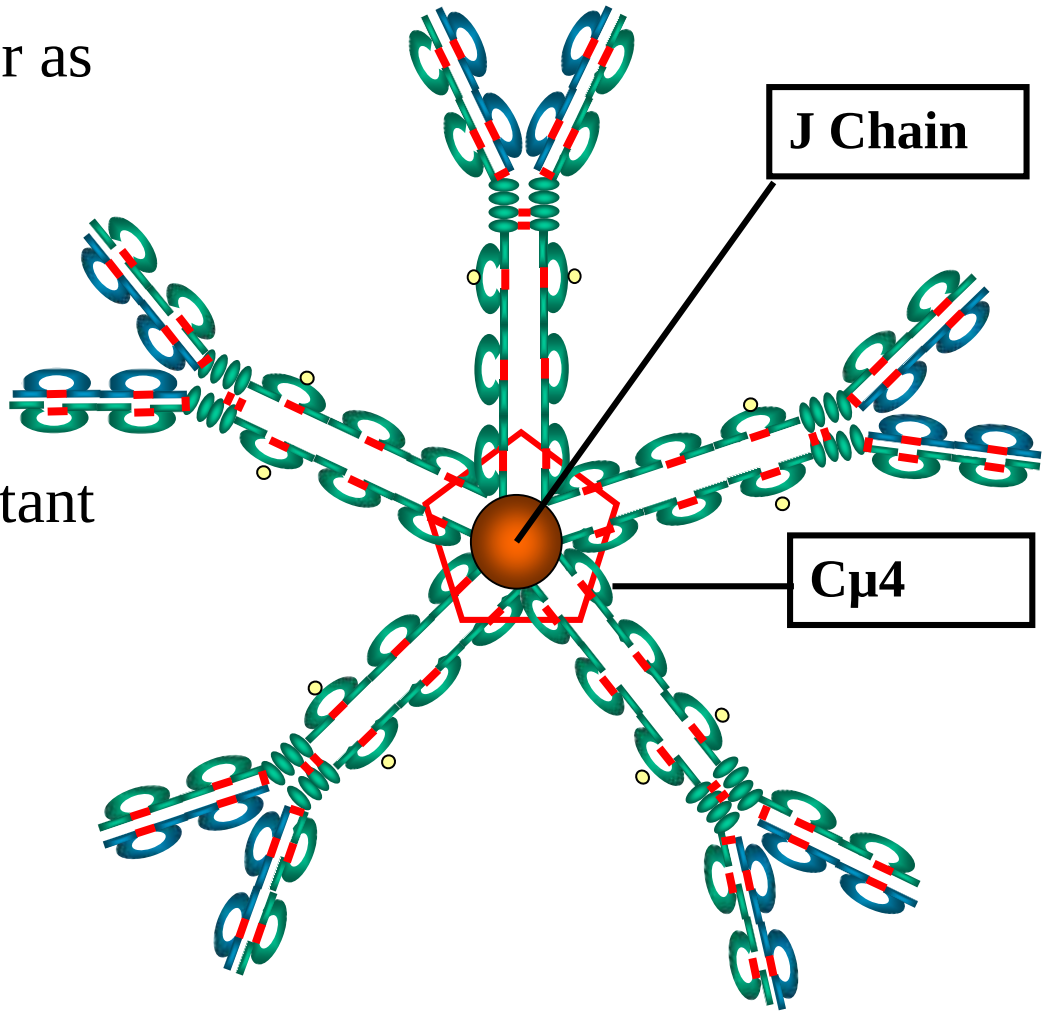
IgG3

IgG - properties

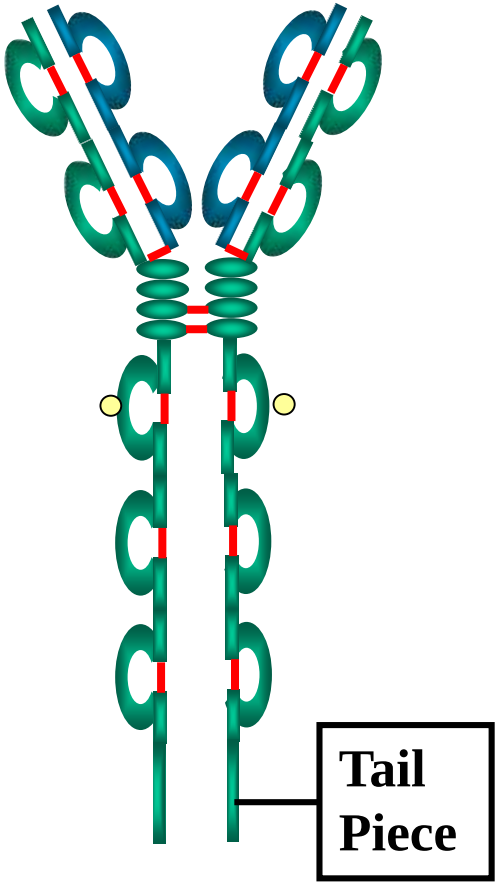
- major serum Ig (systemic immunity – 80% of serum Ig)
- ~10mg/mL
- IgG1,2,3,4 (decreasing serum concentration in this order)
- antigen binding valency: 2
- major Ig in extravascular spaces
- IgG1, IgG3 and IgG4 cross placenta (± IgG2)
- complement activation (IgG3 most effective)
- neutralization (most effective)
- agglutination
- binds to Fc receptors (IgG1 and IgG3 have high affinity) (± IgG2, IgG4)
  - Phagocytes - opsonization
  - K cells – ADCC

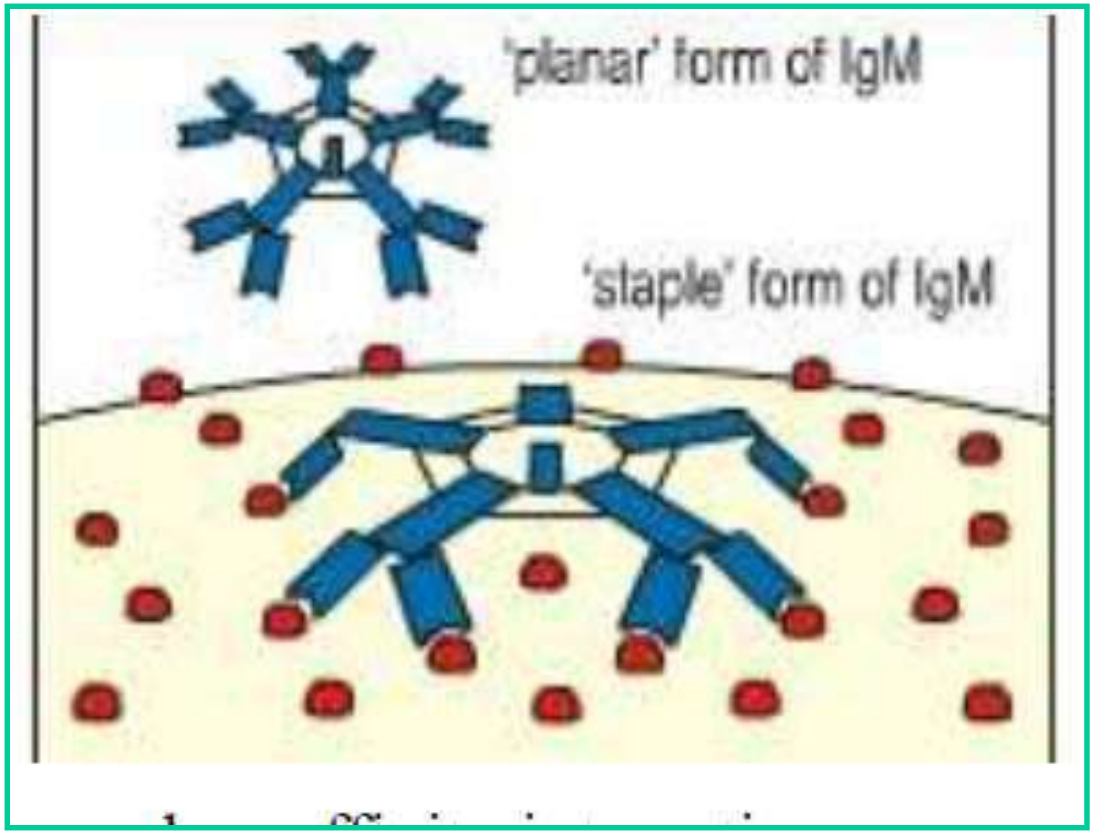
IgM - structure

- pentamer (H_5L_5); monomer as BCR (mIgM)
- star or crab shaped
- 19S
- μ isotype of H chain
- one variable and four constant regions in H chain
- extra domain (C_{H4})
- J chain
- no hinge region
- mIgM have a short intra-cytoplasmic tail



IgM – structure (mIgM)



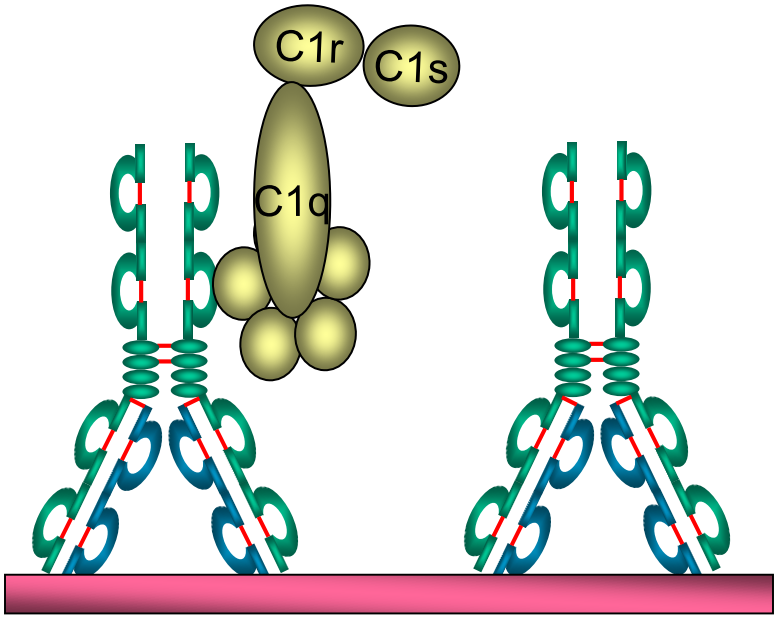


Unliganded, the IgM molecules appear “planar” or “star-shaped”, while bound to the surface of antigens they form “staple-like” or “crab-shaped” structures with the Fab arms bent down and away from the Fc region

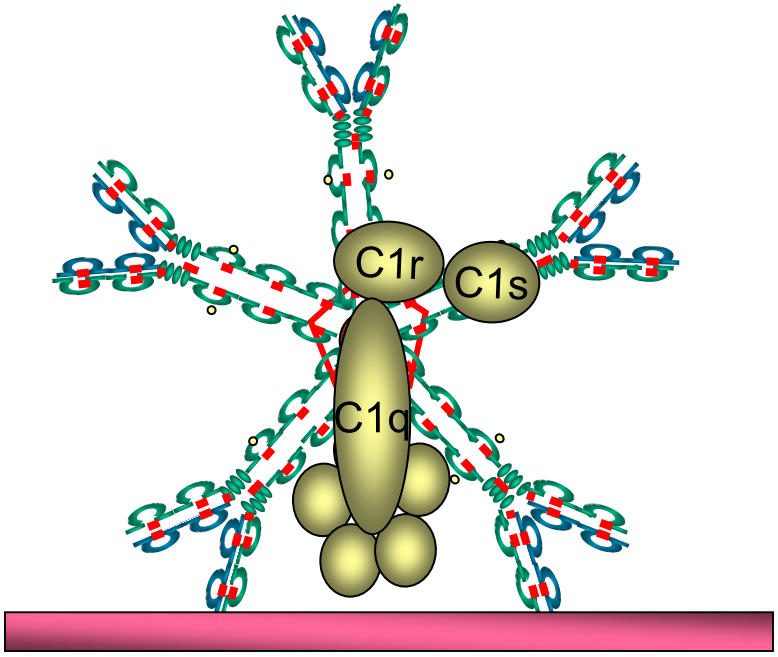
IgM - properties

- 3rd highest serum Ig
- 5-10% of serum immunoglobulin
- 1.5mg/mL
- first Ig made by fetus and B cells
- first Ig of primary immune response
- monomeric version (mIgM) is membrane bound
- pentameric version is secreted
- high valence Ig (10 theoretical), 5 empirical (stearic hinderance)
- fixes complement (most efficient in complement activation)
- agglutination

Fixation of C1 by IgG and IgM Abs



No activation

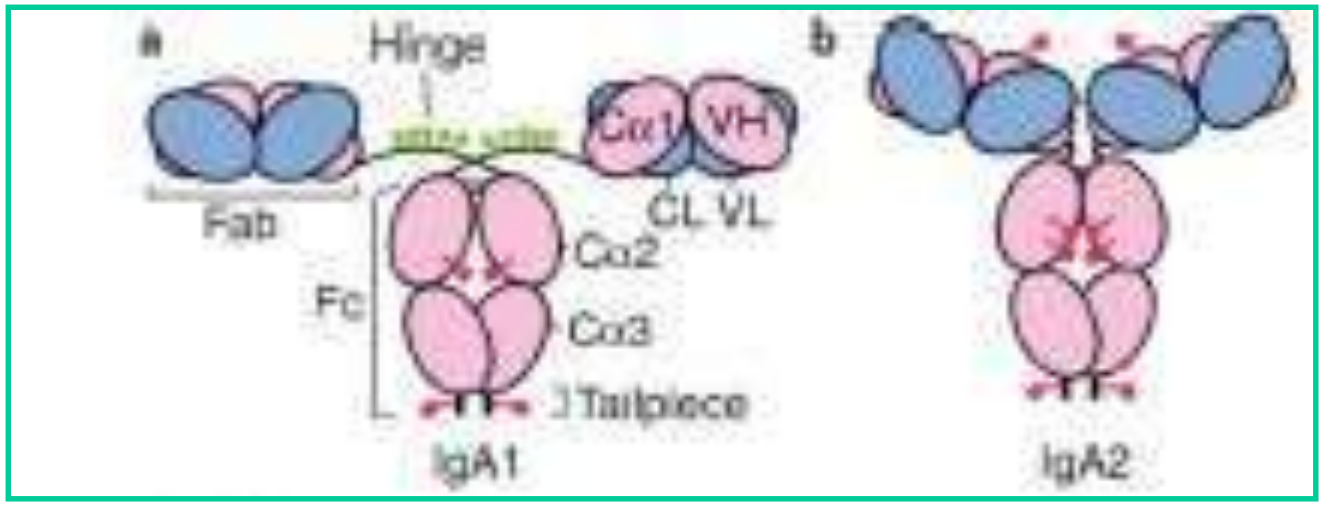


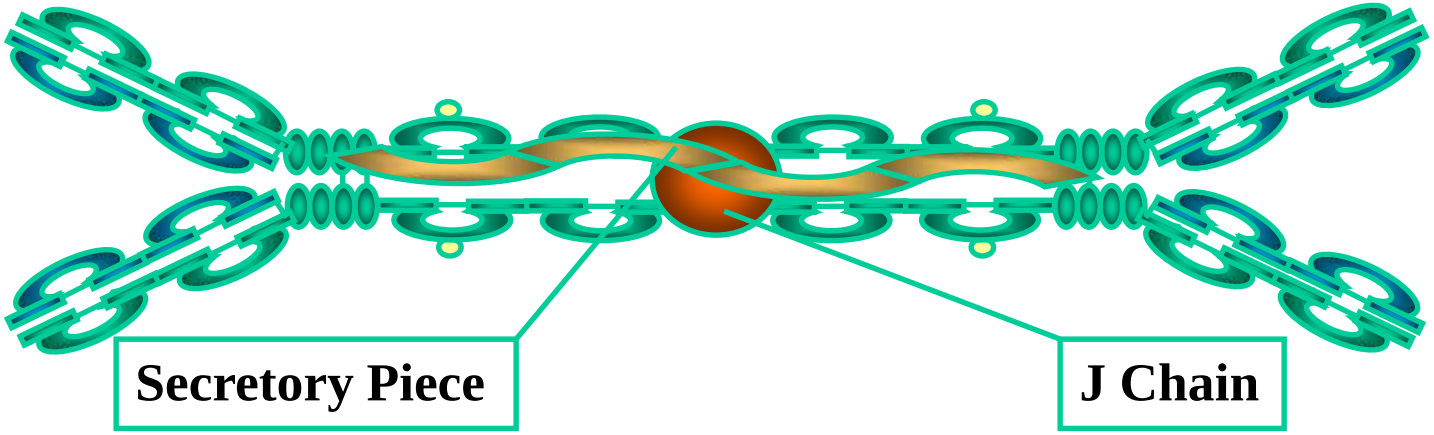
Activation

IgA- structure

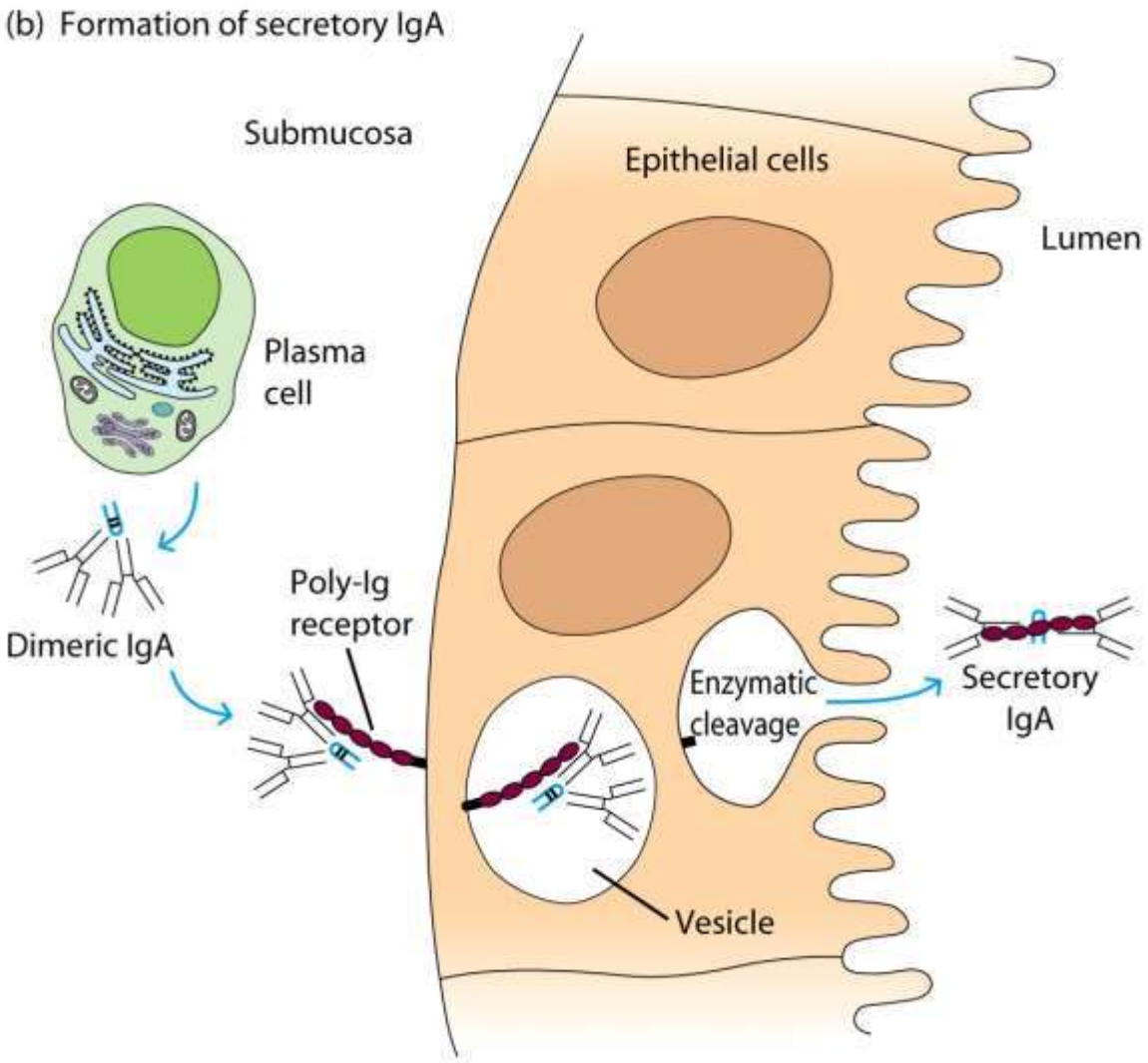
- α isotype of H chain
- one variable and three constant regions in H chain
- hinge region present; elongated in IgA1
- a short tail is present
- Two forms: monomer and dimer, polymers possible not common though
- **Serum:**
    - monomer (H_2L_2)
    - 7S
    - J chain absent
    - SP absent
- **Secretions (sIgA)**
    - dimer (H_4L_4) or even as trimer
    - 11S
    - J chain present
    - Secretory component or piece (SP) present

IgA - structure





sIgA Transport Mechanism (Transcytosis)



IgA - properties

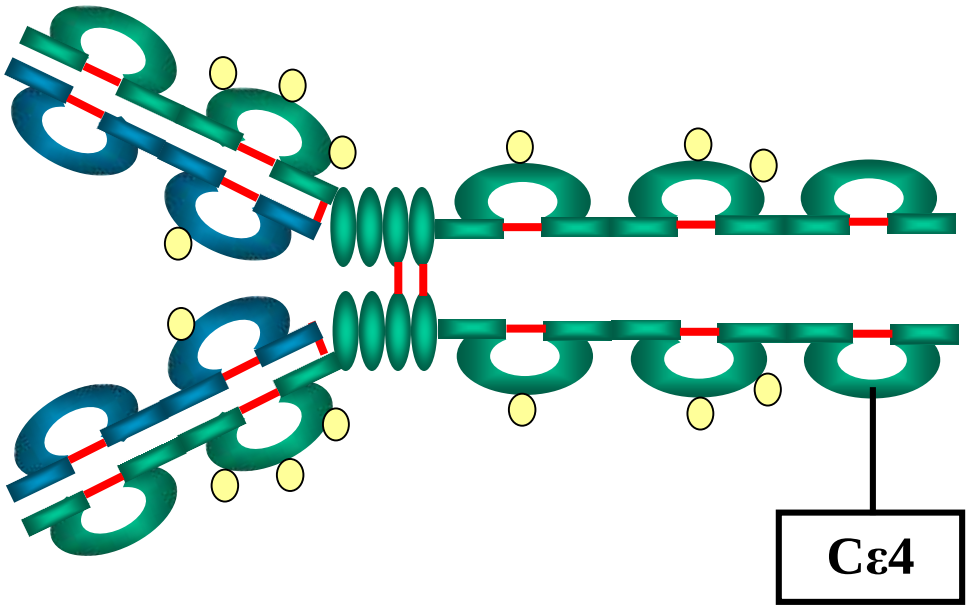
- 2nd highest serum Ig (10-15% of serum IgG)
- in humans, the IgA in serum is comprising ~ 90 % IgA1 and 10 % IgA2
- major secretory Ig (mucosal or local Immunity)
  - milk, tears, saliva, mucus, gastric and pulmonary secretions
  - subclass proportions vary with mucosal site
- 5-15 g of IgA released in secretions!!!!
- antigen valency: 2/4
- does not fix complement (unless aggregated?)
- binds to Fc receptors on some cells
  - Phagocytes - opsonization
  - K cells – ADCC
- neutralization

J chain

- The J chain itself is an extremely highly conserved polypeptide.
- In polymeric Igs such as dIgA, larger IgA polymers, or pentameric IgM, a single J-chain molecule is incorporated.
- It has eight Cys residues, six of which form intrachain disulfide bridges, while the remaining two form covalent links to the tailpiece.
- The J chain has an N-linked oligosaccharide which along with the oligosaccharide attached to the IgA tailpiece contributes to correct dimer formation.

IgE - Structure

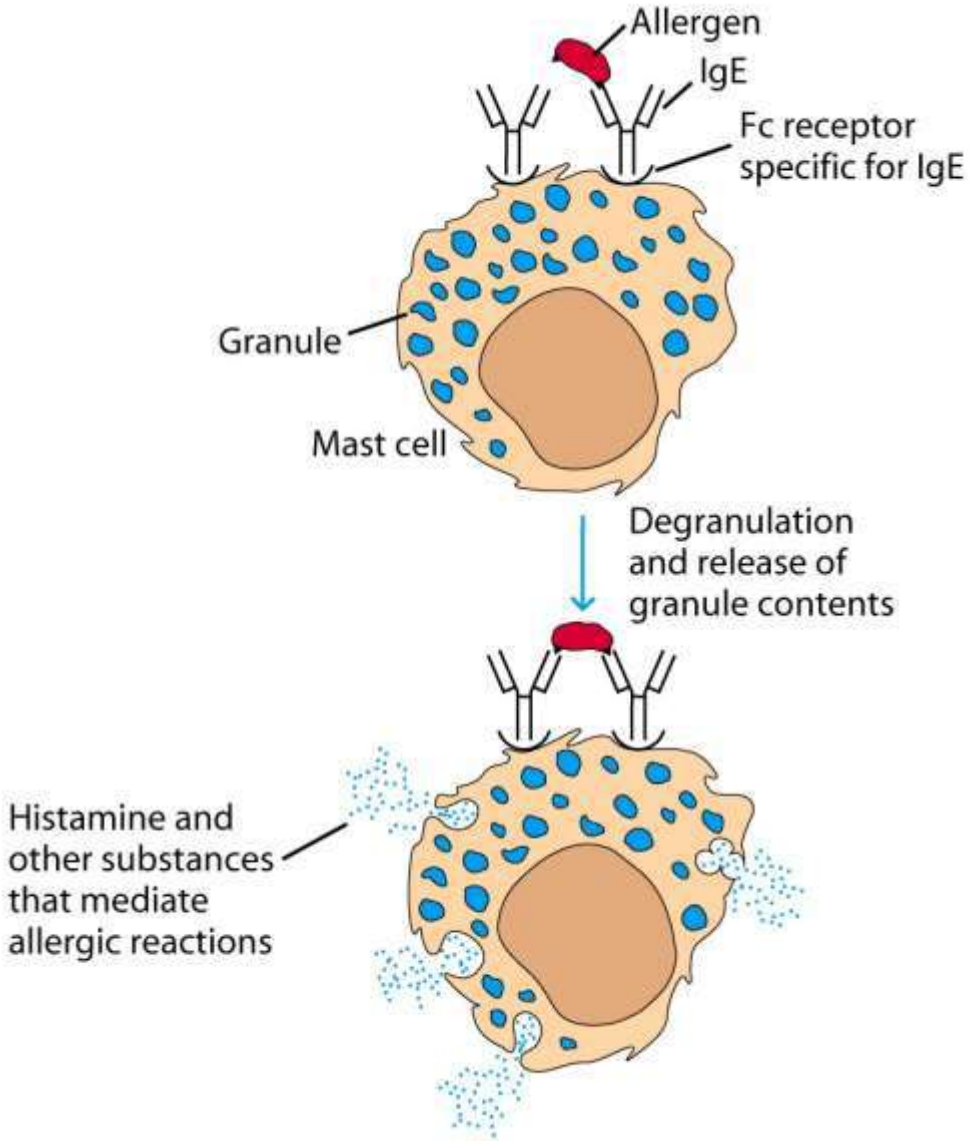
- monomer (H_2L_2)
- 7S
- ε isotype of H chain
- extra domain (C_{H4})
- one variable (V_H) and 4 constant (C_{H1}, C_{H2}, C_{H3}) regions in H chain
- no hinge region



IgE - Properties

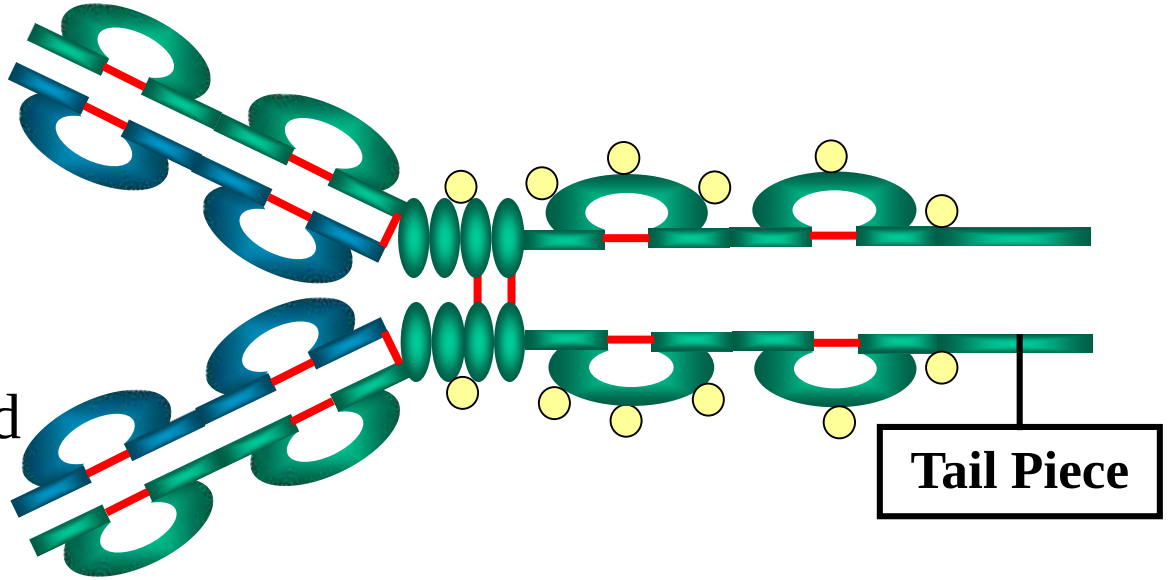
- least common serum Ig ; 0.3μg/mL
- most IgE is cell bound: binds to basophils, eosinophls and mast cells, which have high affinity FcR for IgE (FcεRI)
- anti-parasitic (helminths) –
  - binds to Fc receptor on eosinophils
  - cross links
  - promotes peace meal degranulation and digestion
- Type-I hypersentivity (anaphylaxis and allergic reactions)
  - binds to Fc receptor on mast cells and basophils
  - cross links
  - promotes peace meal degranulation releasing in massive release of vasoactive amines, such as histamine

Cross-Linkage of Bound IgE Antibody With Allergen Causes Type-I hypersensitivity



IgD- structure

- Y-shaped
- monomer (H_2L_2)
- 7S
- δ isotype of H chain
- one variable (V_H) and 3 constant (C_{H1}, C_{H2}, C_{H3}) regions in H chain
- hinge region present
- tail piece present



IgD- properties

- 4th highest serum Ig
- ~30μg/mL
- B cell surface Ig
- No other known function
- Does not bind complement
- Not found in all animals

Antigenic determinants on immunoglobulins

- Immunoglobulins, being protein in nature, are themselves immunogenic for other individuals of same or different species, i.e. Igs also have epitopes,

- Antigenic Determinants on Abs are of three types:

  – Isotypic

  – Allotypic

  – Idiotypic

Isotypic determinants

- prefix ‘Iso’ means same in all members of the same species
- Antigenic determinants that characterize the classes and subclasses of heavy chains and types and subtypes of light chains in a species are called as isotypic determinants
- the isotypic determinants are present in the constant region of heavy and light chains
- the isotypic determinants between different species are not the same
- if you inject an Ab from one species in a different species then the injected antibodies are recognized as foreign, resulting in the induction of antibodies (anti-antibodies) - a**nti-isotype is generated**
- **if within same species, no anti-isotype produced**

Allotypic determinants

- the prefix ‘Allo’ means that different in individuals of the same species.
- even though same isotype, within one species small differences (1-4 a/a) arise in different individuals (due to polymorphism).
- antigenic determinants specified by allelic forms of the Ig genes are called as allotypic determinants.
- if an animal of one species is injected with such Ab from another animal of same species, the former will generate anti-allotype Abs, provided that the two animals differ in their allotypic determinants, e.g. A2m (1), A2m (2)
    - during pregnancy
    - blood transfusion

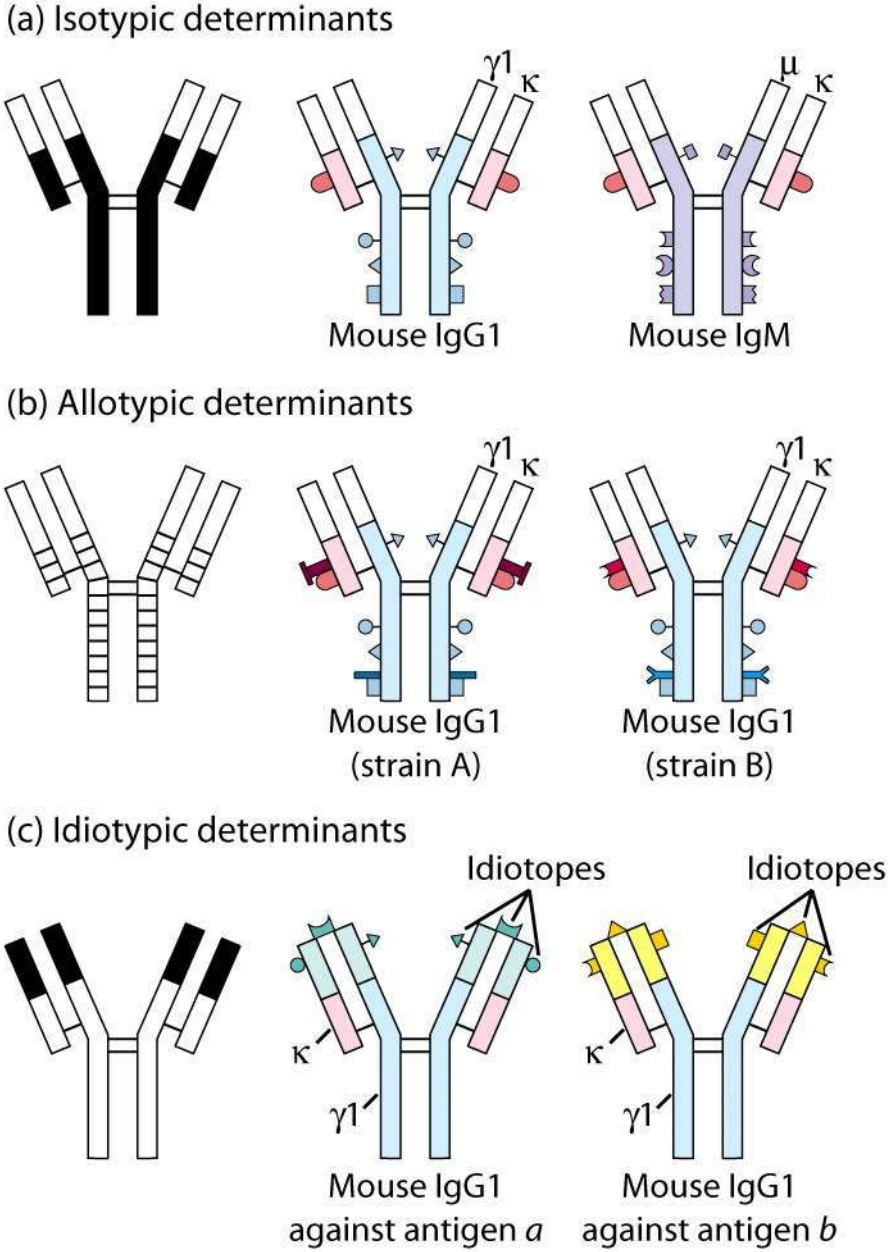
Human Immunoglobulin Allotypes

- **Nomenclature**
  - **G1m(3)**
  - **Km(1)**

Chain	Domain	Allotype	Amino Acid	Position
IgG1	C_{H1}	G1m(f) = (3)	Arg	214
	C_{H1}	G1m(z) = (17)	Lys	214
	C_{H3}	G1m(a) = (1)	Arg, Asp, Glu, Leu	355-358
κ light chain	C_L	Km(1)	Val, Leu	153, 191
	C_L	Km(3)	Ala, Val	153, 191

Idiotypic determinants

- Antigen-binding site in antibody molecule is formed by the hypervariable regions of the V_H and V_L chains. These HVRs also act as immunogen.
- The antigenic determinants of the V_H and V_L region, unique to an antibody molecule of a given specificity, are called idiotypic determinants or idiotopes.
- One antibody molecule has many idiotopes in the antigen-binding site or adjacent to it. The sum of the individual idiotopes in an antibody molecule is called the idiotype of the antibody (antigenic determinants created by the HVR = Idiotypes)
- The idiotopes are further designated alpha, beta, and gamma idiotopes.
  - Alpha idiotope lie outside the antigen-binding site of hyper-variable region.
  - Beta idiotope lie close to the antigen binding site of hyper- variable region.
  - Gamma idiotope is formed by the amino acids of the antigen binding site.
- If a monoclonal antibody against an idiotype is injected into a genetically identical recipient then anti-idiotypic antibodies are generated; no anti-isotypic and no anti-allotypic Abs will be generated


(a) Isotypic determinants
γ1
κ
μ
κ
Mouse IgG1
Mouse IgM
(b) Allotypic determinants
γ1
κ
γ1
κ
Mouse IgG1
(strain A)
Mouse IgG1
(strain B)
(c) Idiotypic determinants
Idiotopes
Idiotopes
κ
γ1
κ
γ1
Mouse IgG1
against antigen a
Mouse IgG1
against antigen b

Immunoglobulin Genetics
(Antibody diversity)

Antibody diversity - Introduction

- **Antibody diversity** is defined as the phenomenon of immense variability characteristic of **antibodies**, which enables the immune system to react specifically against the essentially unlimited kinds of antigens it encounters.
- Antibody diversity in humans comes from several stages of immunoglobulin development, including both pre-immune repertoire and the post-immune repertoire
- In the pre-immune repertoire, there are six major sources of antibody diversity in human beings, generating a potential pre-immune diversity of $>10^{16}$ different antibodies.
- In the post-immune repertoire following exposure to antigen there occurs somatic hypermutation which results in affinity maturation of antibodies with higher affinity to the targeted antigen epitope, resulting in more effective binding and elimination of the antigen from circulation during the secondary immune response.
- It has been demonstrated that during the affinity maturation process, the average number of mutations in VH and VL are eight and five, respectively.

Antibody diversity - mechanisms

To date, seven means of antibody diversification have been identified in mice and humans:

- Multiple germ-line gene segments
- Combinatorial V-(D)-J joining
- Junctional flexibility
- P-region nucleotide addition (P-addition)
- N-region nucleotide addition (N-addition)
- Somatic hypermutation
- Combinatorial association of light and heavy chains

Generation of Diversity

	B cell receptor (Immunoglobulin)	
	Heavy	Light
V gene segments	1000	300
D gene segments	15	-
J gene segments	4	4
N region insertion	++	-
Junctional diversity	+++	+
Somatic mutation	+	+
	V x D x J 1000 x 15 x 4	V x J 300 x 4
Total	6×10^4	1.2×10^3
Combinatorial association	7.2×10^7	

Immunoglobulins of animals

Salient differential features

- There are five major immunoglobulin isotypes in mammals: IgM, IgD, IgG, IgA, and IgE.
- IgM is widely conserved throughout vertebrates, with the exception of the coelacanth.
- IgM, IgD, and IgA, or its analog, IgX, have been described in non-mammalian tetrapods.
- Birds, reptiles, and amphibians express IgY, a likely evolutionary precursor to IgG and IgE.
- A unique immunoglobulin isotypes, such as IgX in amphibians or IgW in sharks have been reported.
- Variability also exists in the type and usage frequencies of light chains. In certain species, the κ and λ light chains are utilized equally, while in others one or the other is preferred.

Table 4.1 Different immunoglobulin isotypes found in each species.

	IgM	IgD	IgG	IgA	IgE	IgD2	IgNAR	IgT	IgW	IgX	IgY	HCAbs
Rat/mouse	X	X	X	X	X							
Cat/dog	X	X	X	X	X							
Pig	X	X	X	X	X							
Cow	X	X	X	X	X							
Camel	X	X	X	X	X							X
Chicken	X			X							X	
Sauropsida	X	X		X		X					X	
Xenopus	X									X	X	
Teleost	X	X						X				
Shark	X						X					

Adapted from Vadanis *et al,* 2016

Table 4.2 Presence and use of light chains in various species.

	λ	κ	σ	σ-cart
Rat/mouse	X	X*		
Cat/dog	X*	X		
Pig	X	X		
Cow	X*	X		
Camel	X	X		
Chicken	X*			
Sauropsida	X	X		
Xenopus	X	X		X
Teleost	X	X		
Shark	X	X	X	X

Asterisks indicate known preferential use.

Adapted from Vadanis *et al*, 2016

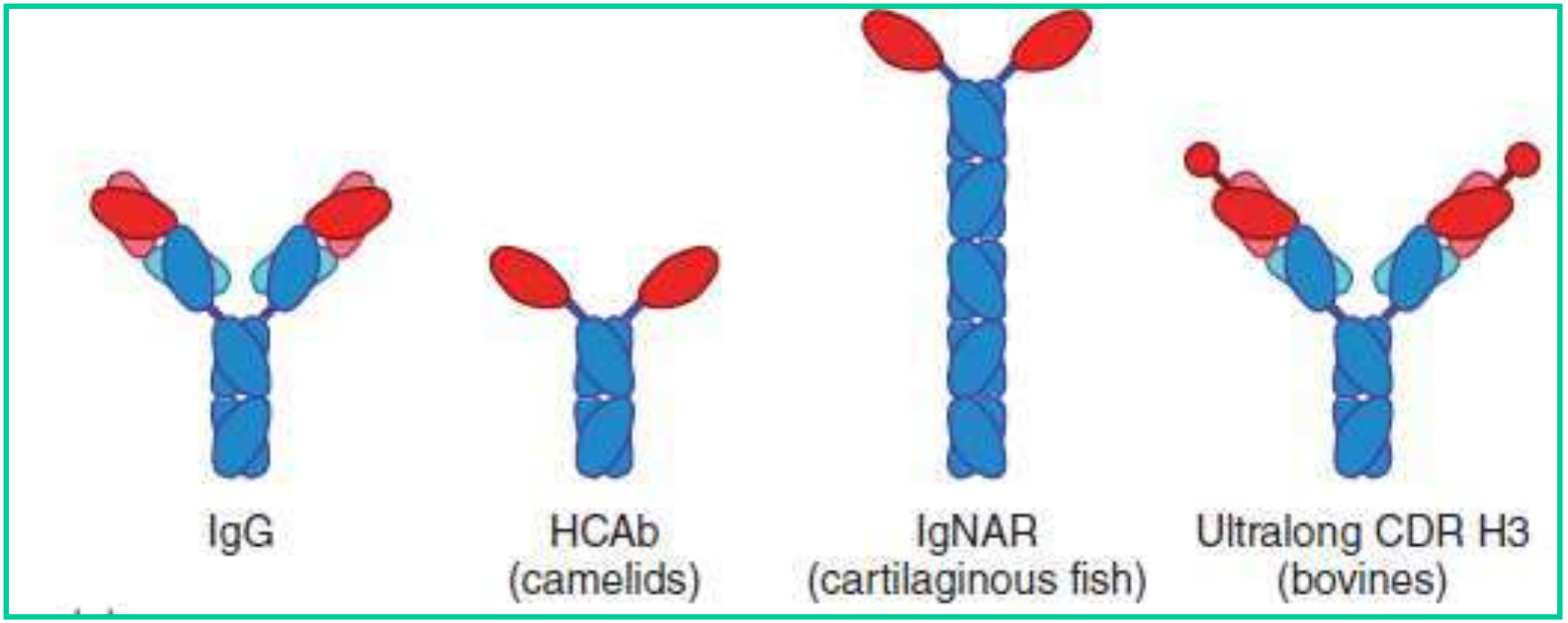
Salient differential features (contd.)

- The majority of maternal antibody transport in animals occurs through the consumption of colostrum and milk after birth
- In the cats, IgA is present as a dimer both in serum and mucosa, while in most species IgA is present as a dimer only in its secreted form.
- Class-switch recombination (CSR) to IgD is possible in cattle and perhaps in porcine due to their unique IgD switch region, which is absent in humans and rodents.
- Cows can have an unusually long complementarity-determining region 3 of the heavy chain (CDR H3), the biological function of which remains unknown (recombinant monoclonal humanized cow antibodies with ultra long CDR H3s are a potential candidate for immunotherapy of COVID-19)

Salient differential features (contd.)

- Camels have IgG type of antibodies (also called as "nanobodies") which lack light chains and C_{H1} domain; only posess two H chains, each with one V_H (referrd as VHH domain) and two C_H domains (HCAbs).
- Alpacas and llamas also have HCAbs that are very similar to those found in camels
- Llamas Abs are the a potential candidate for immunotherapy of COVID-19.
- Chickens have serum IgM, IgA, and IgY, the first two being homologs of their mammalian counterparts; however, they do not have IgE or IgD.
- IgY appears to be related to both mammalian IgG and IgE and may be an evolutionary common ancestor to both

Novel antibodies in animals



Colostral antibodies in bovines

- In bovines colostrum is rich in IgG1type of antibody (human colostrum has IgA), which is secreted into udder from dam's blood.
- Provides natural passive immunity
- Colostrum is rich in antibodies against pathogens to which dam has been exposed either naturally or through vaccination
- Colostrum of dam or from foster mother of same form should be used
- Clostrum feeding may cause loose faeces in calves, do not give antibiotics.
- Anti-rotaviral antibodies appear after 3 days, hence colostrum feeding should be continued for 5-6 days
- Vaccination of dams in third trimester of gestation against pathogens which cause disease in neo natal claves is recommended