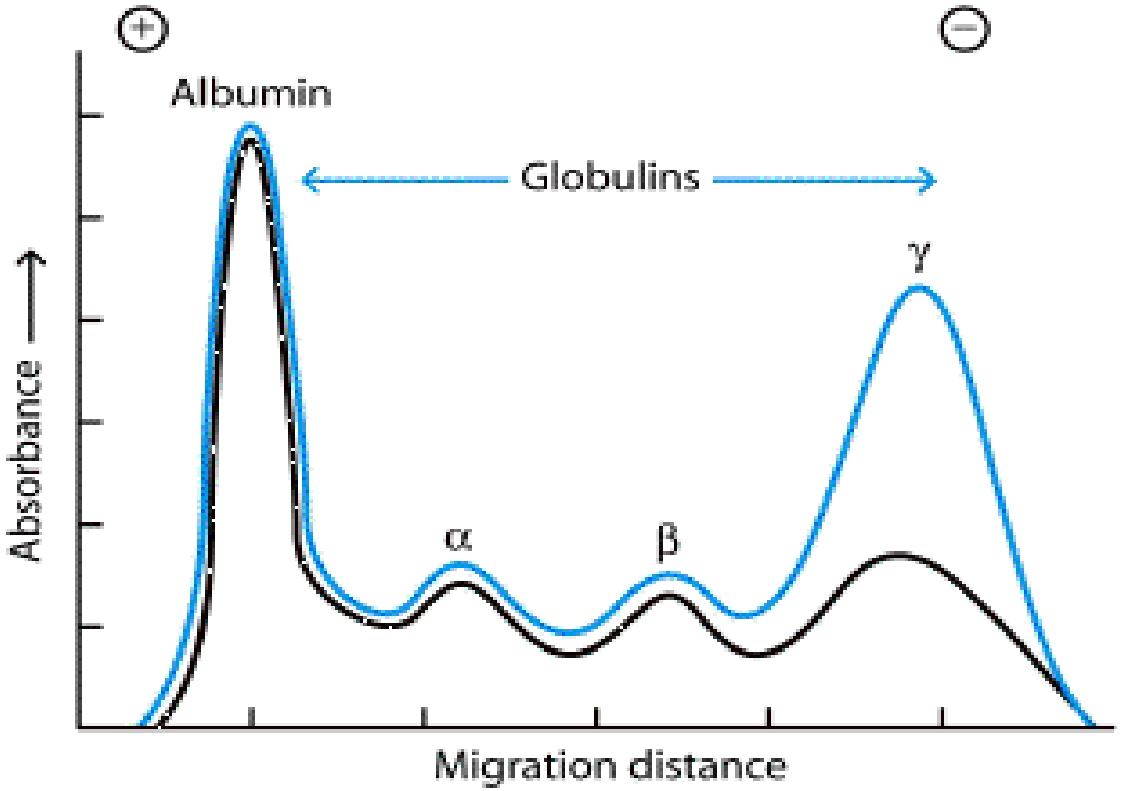
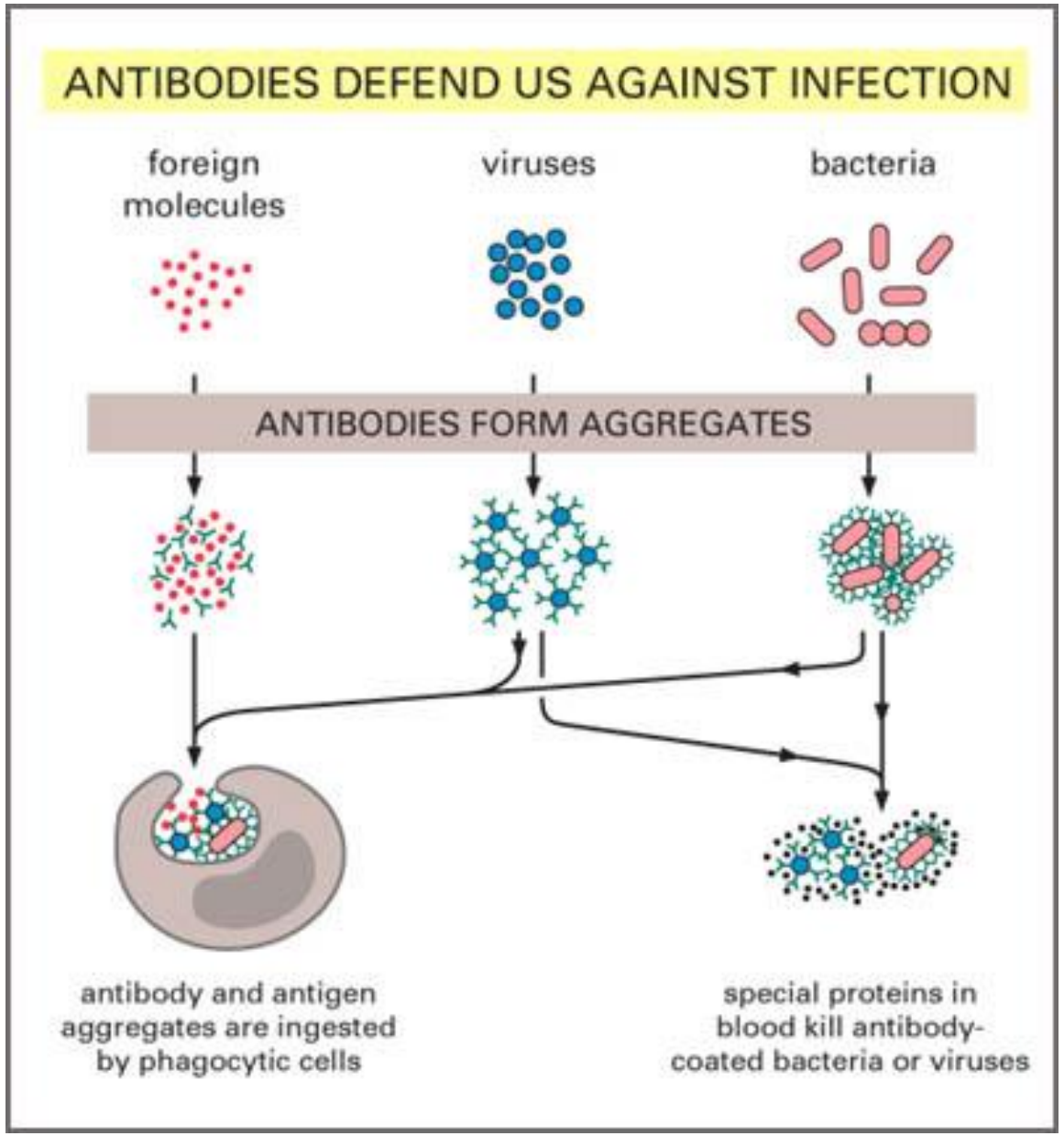
Antibodies

Dr. Imran Riaz Malik
Immunology

Antibodies constitute a major component of serum



(Tiselius and Kabat in 1939)



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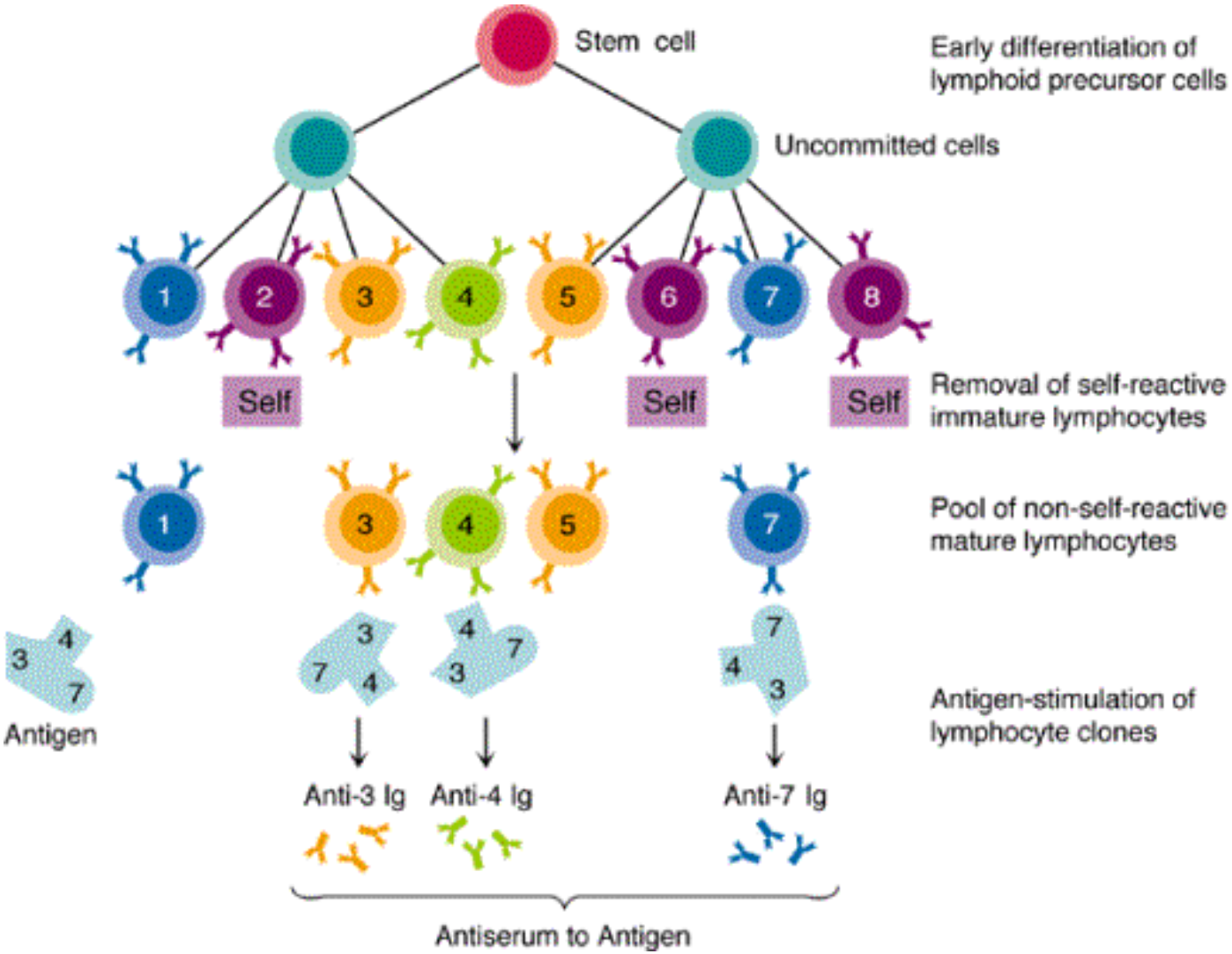
B cell clones are selectively expanded in response to specific antigen

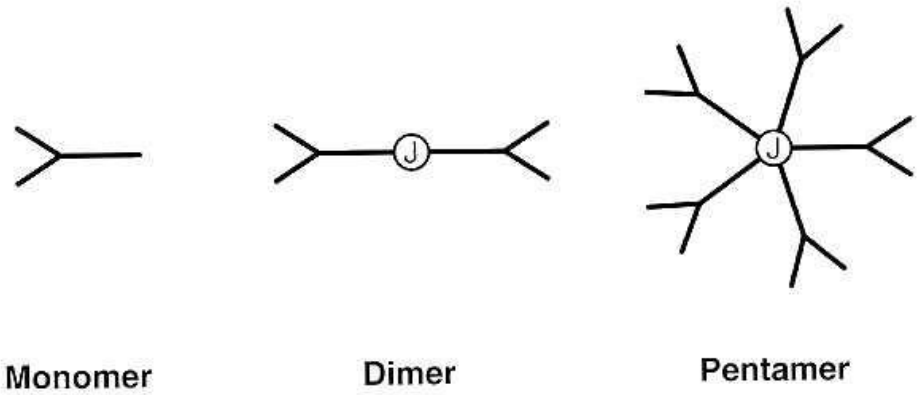


Antibodies bind antigen. This interaction is non-covalent and generally highly specific.

Antibodies are only produced by B lymphocytes and are exported through the usual constitutive exocytosis pathway in both integral plasma membrane and secretory forms.

Antibodies are found (i) in the plasma and (ii) bound to specific receptors for the invariant (Fc) region of immunoglobulin.

They are also found in secretory fluids such as mucus, milk and sweat.



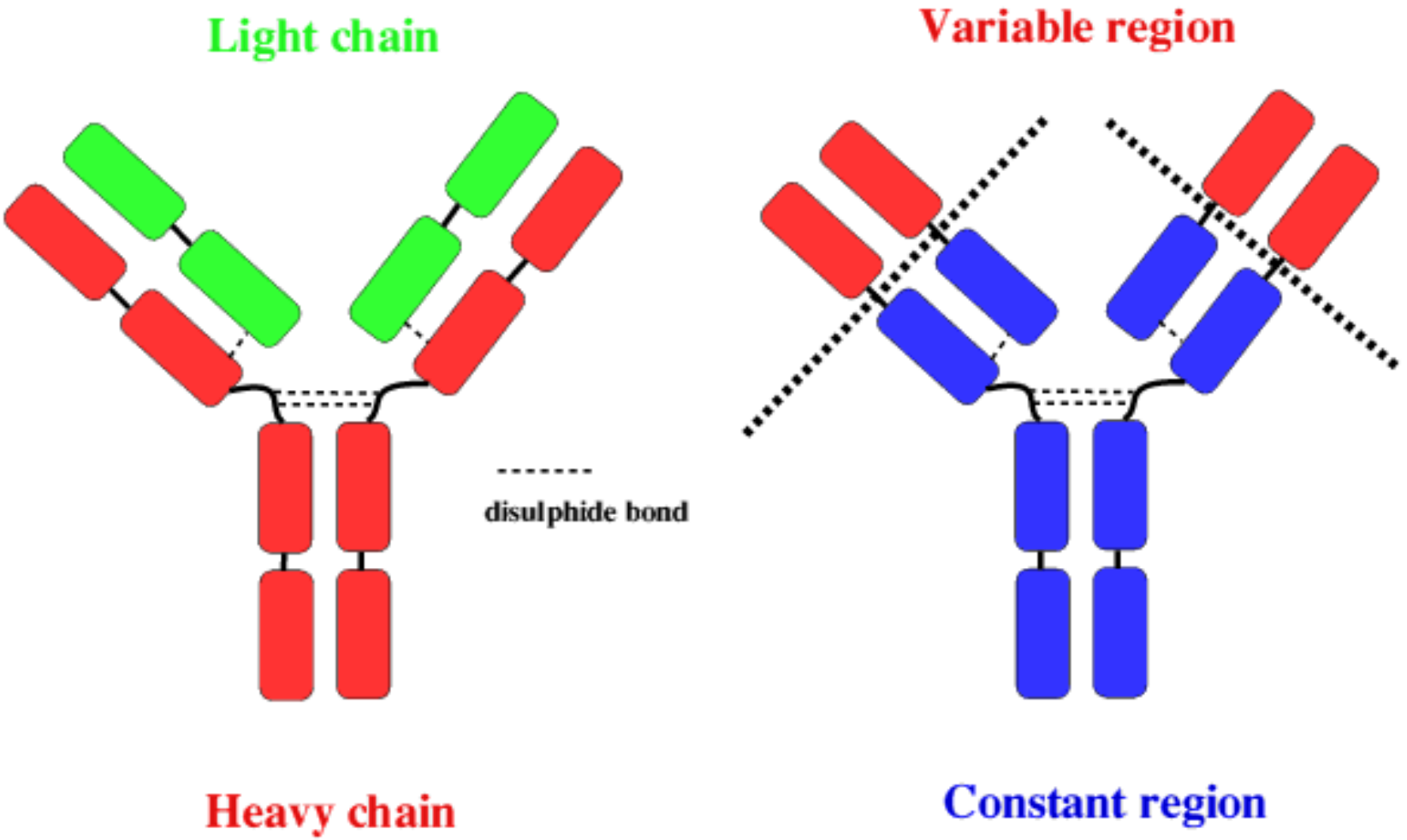
All antibodies have a similar overall structure with two light and two heavy chains. These are linked by both covalent (disulphide bridges) and non-covalent forces.

They are made up of a series of domains of related amino acid sequence which possess a common secondary and tertiary structure.

This conserved structure is found frequently in proteins involved in cell-cell interactions and is especially important in immunology.

The proteins utilising this structure are members of the immunoglobulin supergene family.

Basic structure of an Antibody



Antibodies are made up of V (for variable) and C (for constant) regions

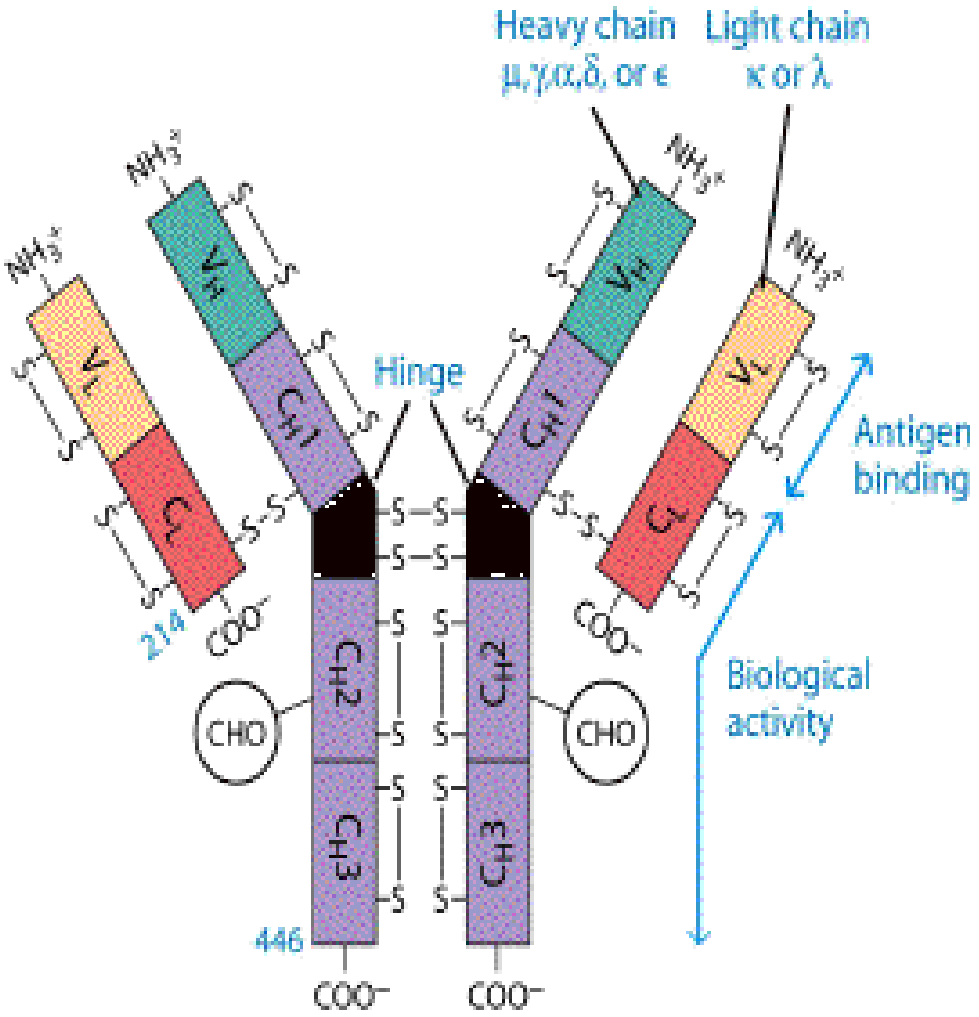
The antigen binding activity is found in the V region whereas the complement fixing and Ig receptor binding activity is found in the C region.

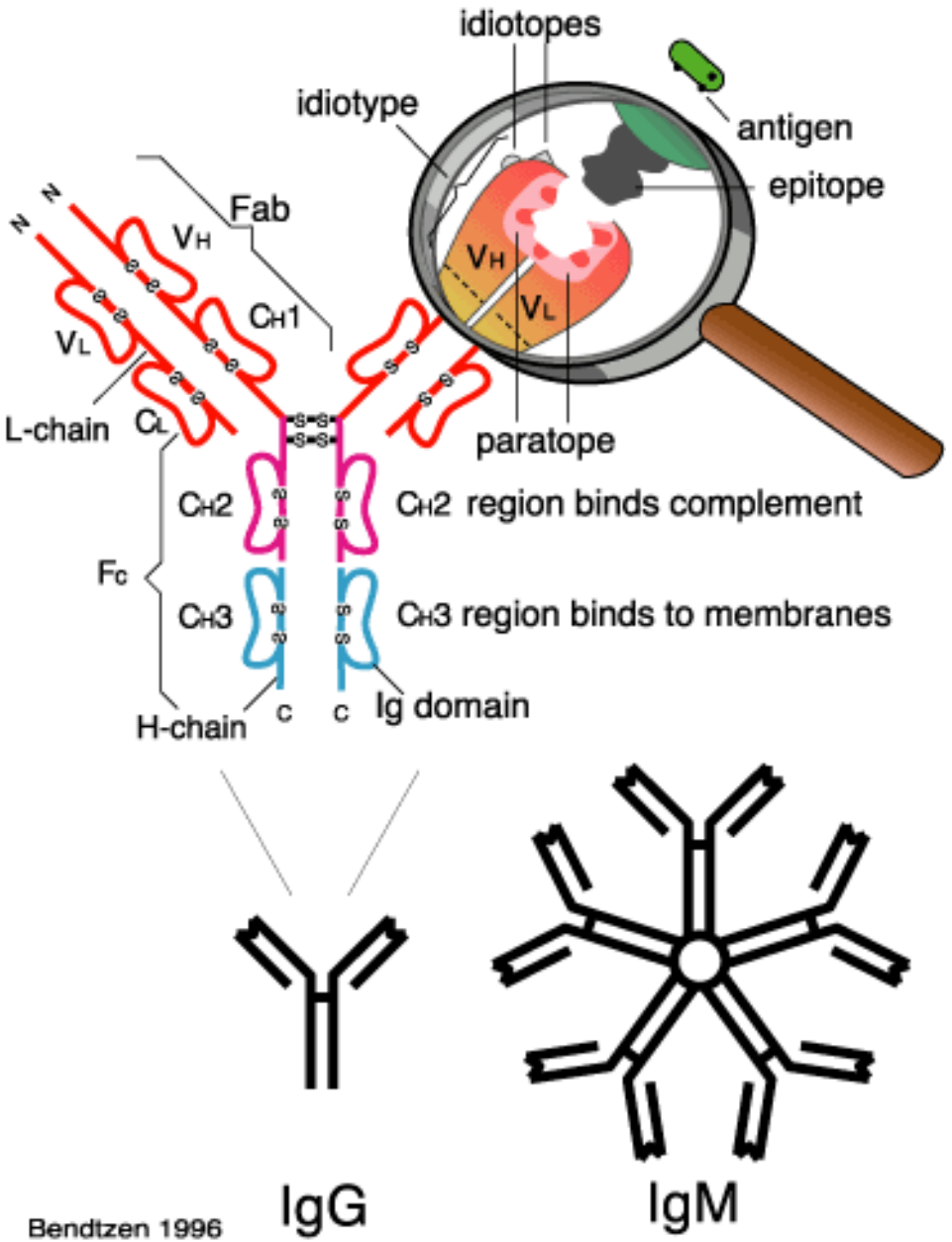
The variation is mostly restricted to three regions within the N-terminal domain of both the heavy (H) and light (L) chains.

In the 3-dimensional structure these regions form loops at the surface of the antibody molecule and these provide the binding surface between antibody and antigen.

Because these regions determine the 'fit' between antibody and antigen they are referred to as the complementarity determining regions or CDRs.

CDR3 shows more variation that either CDR1 or 2.


Heavy chain
μ, γ, α, δ, or ε
Light chain
κ or λ
Hinge
Antigen binding
Biological activity
CHO
CHO
214
446
COO⁻


idiotopes
idiotype
antigen
epitope
Fab
VH
VL
CH1
L-chain
CL
VH
VL
paratope
CH2
CH2 region binds complement
Fc
CH3
CH3 region binds to membranes
H-chain
C
C
Ig domain
IgG
IgM
Bendtzen 1996

2 heavy (H) and 2 light (L) chains

- **H: ~50 kD, five classes (m,d,g,a,e)**
- **L: ~25 kD, two classes (k, l)**

Two activities:

- **Antigen binding (H + L)**
- **Biologic (effector) function (H only)**

Each chain has one Variable (V) domain and one or more Constant (C) domains.

Flexibility provided by hinge region.

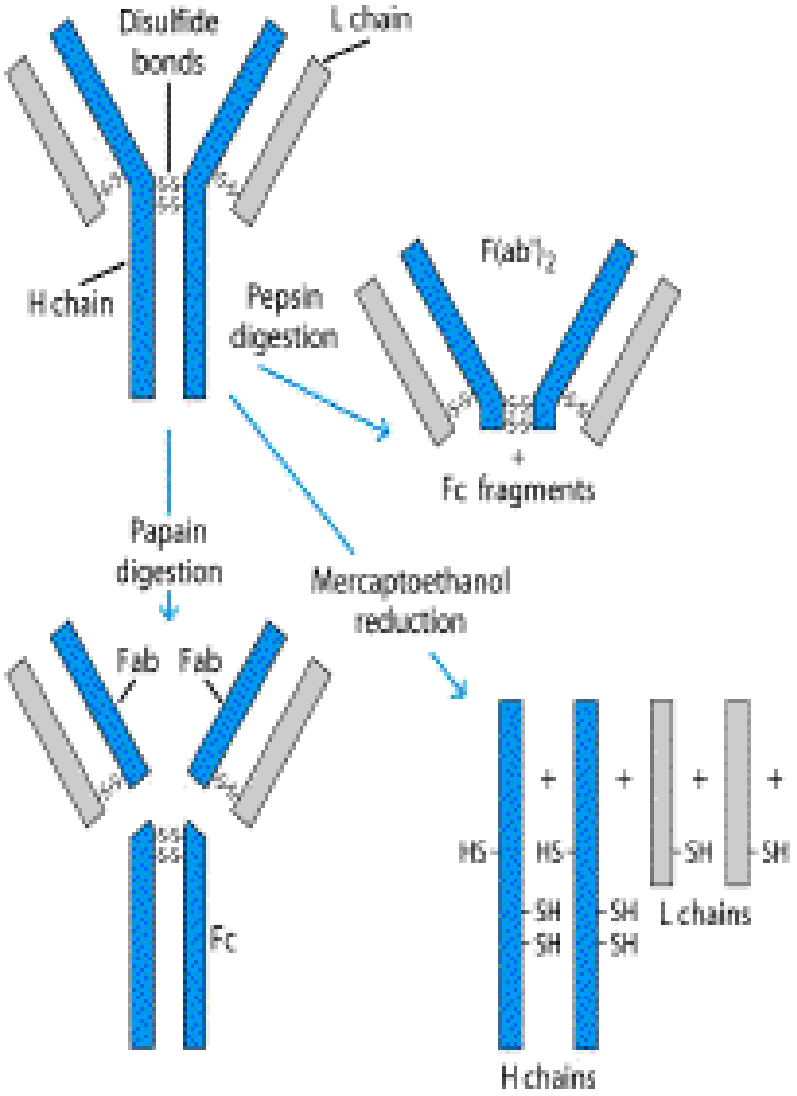
Enzymatic characterization of antibodies

Papain yields F(ab) and Fc fragments:

- **F(ab)** = **“fragment, antigen binding”**
- **Fc** = **“fragment, crystallizable”**

Pepsin yields F(ab’)2 + other small fragments

β-mercaptoethanol reduces disulfides and yields two polypeptide chains (H and L)


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
+
+
+
+
L chains
H chains