Lecture 7 Introduction to Antibody Structure

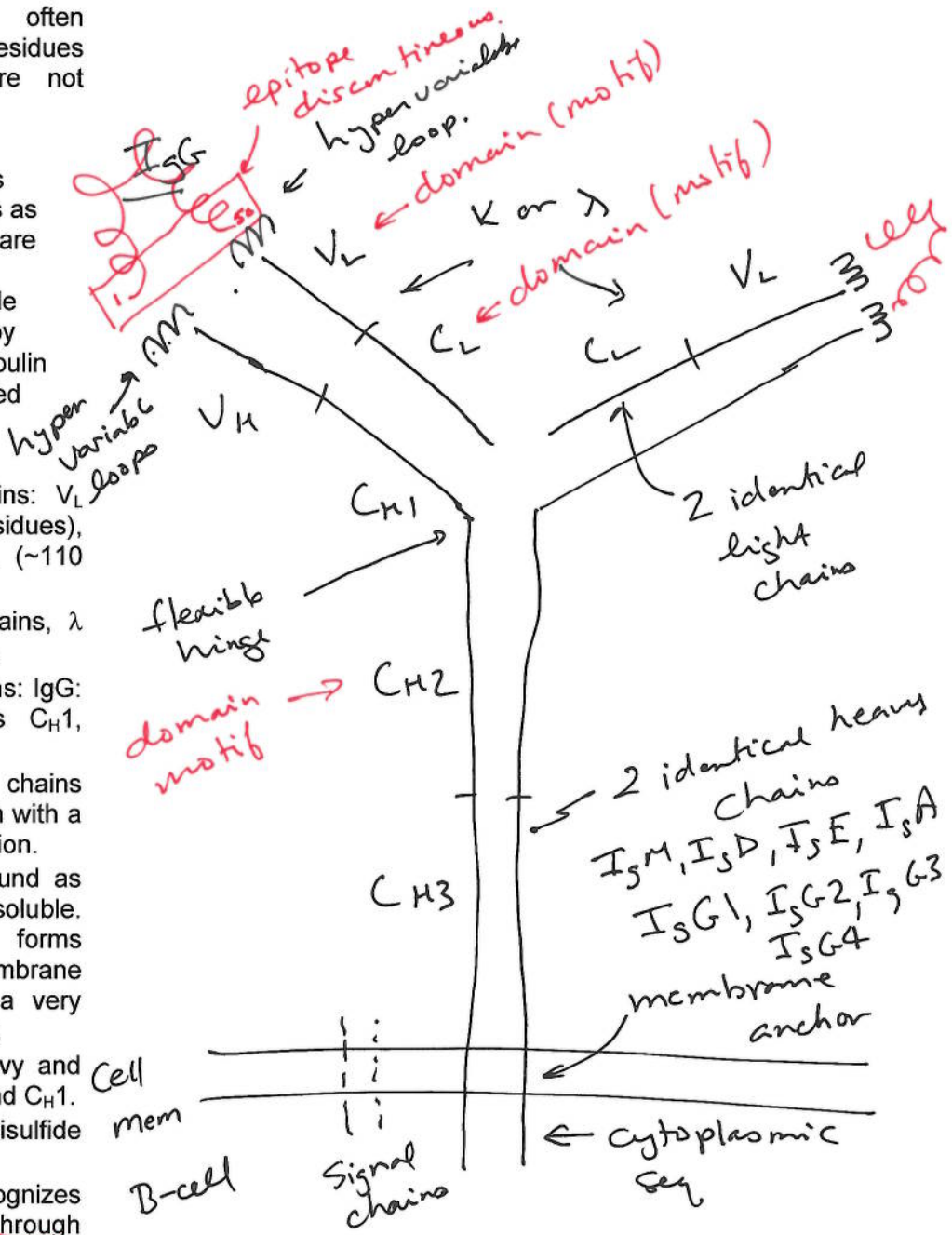
- **Epitope:** Region on antigen that binds to the antibody.
- **Antigen:** Molecule/structure that is recognized by antibody.
- **Immunogen:** Antigen that evokes an immune response.

B-cell Epitopes: Membrane bound (and soluble) antibodies produced by B-cells are capable of recognizing a broad array of antigens. In general, B-cell epitopes must be exposed on the surface of the pathogen, such that they can interact with the antibody component of the B-cell receptor. The list of possible antigens, in the order of frequency of occurrence, is:

1. Proteins 2. Carbohydrates 3. Haptens 4. Lipids 5. Nucleic acids

Protein epitopes are often discontinuous, involving residues on the epitope that are not adjacent to each other.

Structure of Antibodies:
Antibodies, when found as membrane bound proteins as part of the B-cell receptor are referred to as **immunoglobulins.** Soluble **antibodies** are secreted by plasma cells. Immunoglobulin and antibody are often used interchangeably.



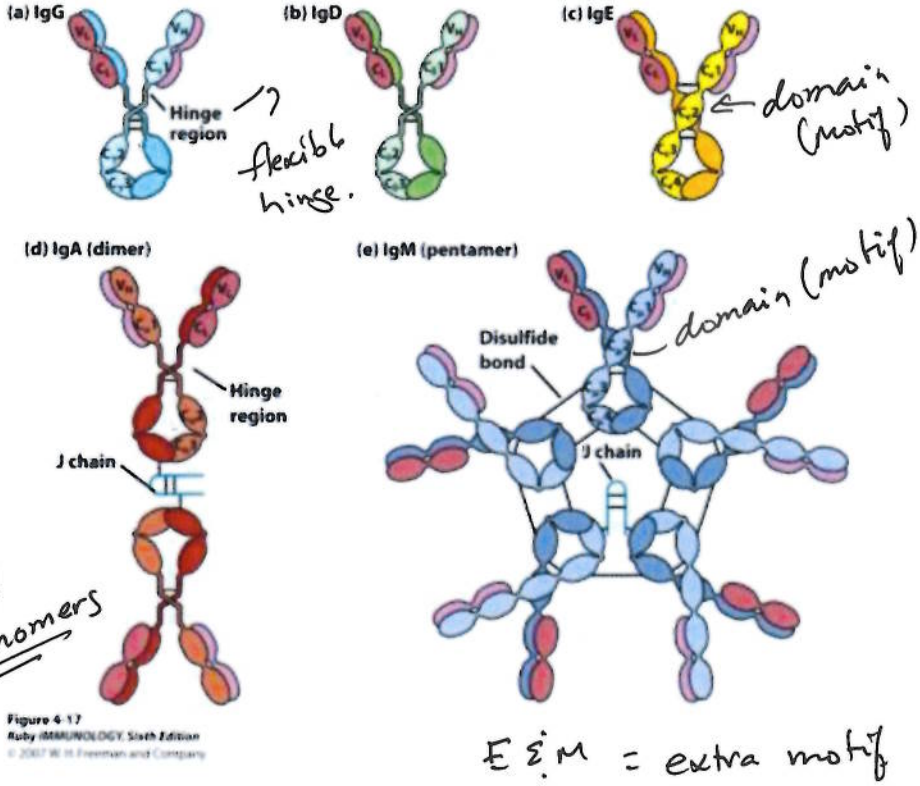
Quaternary Structure:

- 2 *Identical* Light Chains: V_L domain (~110 residues), Constant Domain, C_L (~110 residues).
  Two forms of light chains, λ (lambda) and κ (kappa).
- 2 *Identical* Heavy Chains: IgG: V_H, constant domains C_H1, C_H2, C_H3.
- 5 different heavy chains classes (isotypes), each with a different biological function.
- All isotypes can be found as membrane or soluble. **Membrane bound** forms contain a transmembrane segment followed by a very short cytosolic segment.
- V domains pair in heavy and light chains, as do C_L and C_H1.
- C_L and C_H1 linked by disulfide bond
- Variable region recognizes antigen, largely through hypervariable loops. (also called CDR, complementary determining regions). 3/chain.
- Constant region of heavy chain in Ab has **effector,** or biological activity functions. **Biological Effect depends on type of heavy chain.**

Isotypes of Antibodies:

- Five different heavy chain genes: γ, α, μ, δ, ε.
- Class of an immunoglobulin is defined by its type of heavy chain: IgG(γ), IgA(α), IgM(μ), IgD(δ), IgE(ε). These are termed **Isotypes**.
- IgM is the initial isotype produced by B-cells.
- IgA is secreted as a dimer, IgM is produced as a pentamer.
- These may be found as soluble or membrane bound (BCR)
- All forms found in the BCR will be monomeric.

Secreated forms



flexible hinge.

domain (motif)

BCR

IsM IsA IsG all monomers

EεM = extra motif

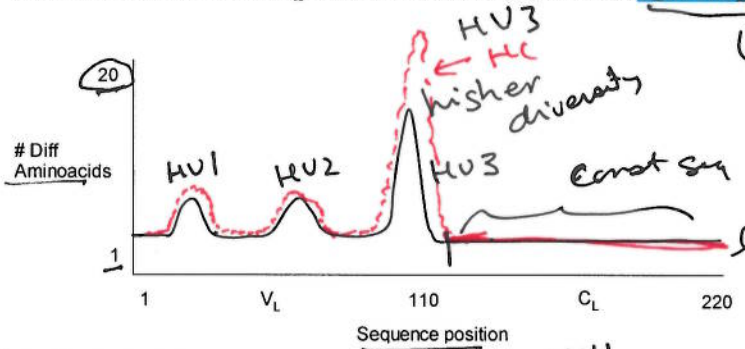
Primary Structure of Antibodies:

Lambda Light Chain Sequences:

HV1 HV2 – highly variable – contact antigen

```
Antibody A   QSVLTQPPSVSGAPRQRVTISCSGGNSNIGN-NAVNWYQQLPGQAPKLLIHYDDLLPSGV  61
             QS LTQP SVSG+P Q +T+SC+G +S++GN N V+WYQQ PG+ PKL+I+  + PSGV
Antibody B   QSALTQPASVSGSPGQSITVSCTGTSSDVGNYNYVSWYQQHPGKVPKLMIYDVNNRPSGV  62

62           SDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNAQVFGGGTKLTVLGQPKAAPSV  119
             S+RFSGSKSG +ASL ISGLQ+EDEA YYC+++  S +  VFG GTKLTVLGQPKAAPSV
63           SNRFSGSKSGNTASLTISGLQAEDEAHYYCSSYTTS-DTWVFGEGTKLTVLGQPKAAPSV  119
```

Same DNA is used to code CL



Heavy Chain Sequences:

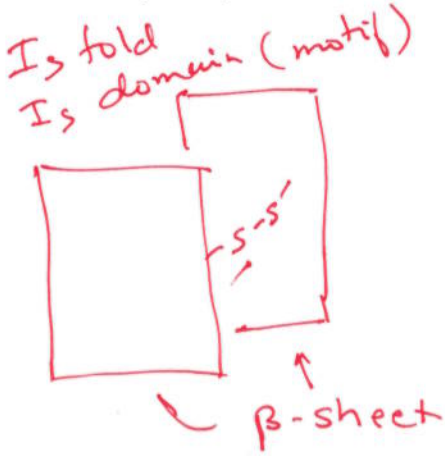
HV1 HV2 HV3

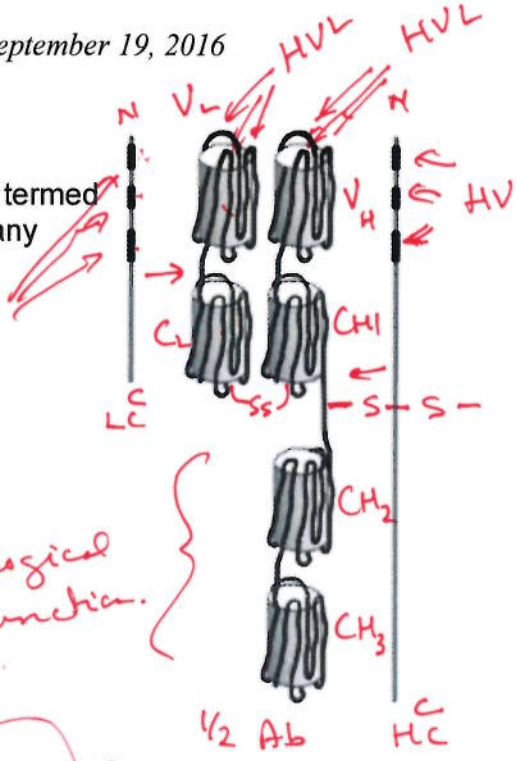
```
Antibody A   LVALLRGVQCQV-LVQSGGGVVQPGRSLRLSCVTSGFTFSNFGIDWVRQAPGKGPEWVAY  59
             LVALL+GVQC++ LV+SGG  VQPG SLRLSC  SGF FS+  I WVRQA GK  EWV +
Antibody B   LVALLKGVQCEMQLVESGGAFVQPGGSLRLSCAASGFNFSDSTIHWVRQASGKSLEWVGH  59

60           ISNDGTNIN--YADSVKGRFTVSRDTSKNTLSLAMNSLRLEDTAVYYCARQPRYFDSGGY  117
             I      +  SVKGRF +SRD SKNT  L M+SLR +DTA+YYC   P
60           IEEKSKKYATIFRASVKGRFIISRDDSKNTAFLQMDSLRPDDTALYYCTPPPEV-----E  114

118          YIDYWGQGTLV  128
               + WG+GTLV
115          SLRSWGRGTLV  125
```

Same DNA used to code CH

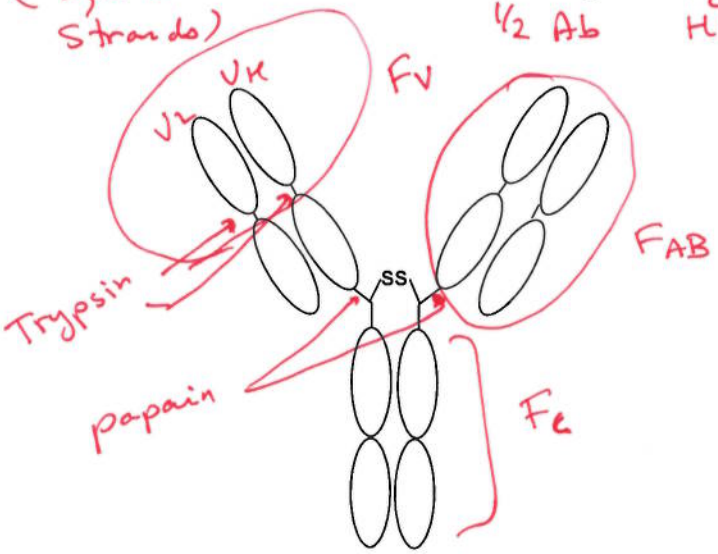
Domains & Tertiary Structure:

Each domain (e.g. V_L, C_H3) consists of 7 stranded (4+3) β-sandwich, crosslinked by a disulfide bond. This structure is termed the **Immunoglobulin fold/domain/motif** and is found in many proteins that participate in the immune response.

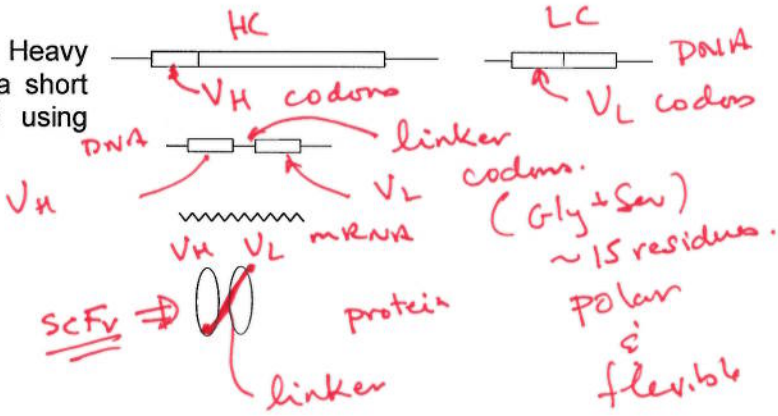




Proteolytic Fragments of Antibodies: The protease papain (protease) cuts γ-globulin into two identical F_{ab} fragments (fragment-antigen binding) and F_c (fragment that crystallized). F_v fragments, containing only the heavy and light V-regions can be produced by trypsin digestion or by recombinant DNA methods.

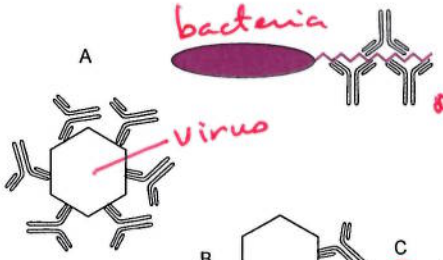


scF_V – Single chain Fragment Variable: Heavy and light variable fragments are joined by a short polypeptide linker. These are generated using recombinant DNA technology.



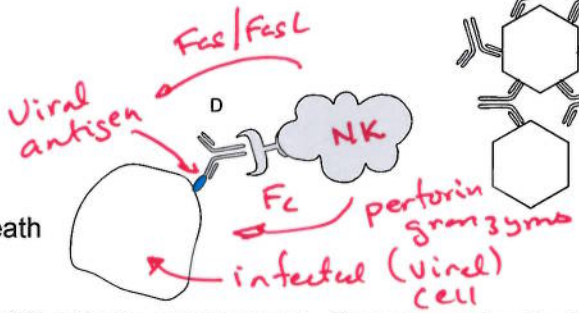
Property - Isotype	IgG1	IgG2	IgG3	IgG4	IgA	IgM	IgE	IgD
Polymeric state (soluble form)	M	M	M	M	Monomer/ Dimer	Penta-meric	M	M
a Physical Blocking (neutralization)	+++	+++	+++	+++	++	-	-	-
b Agglutination	-	-	-	-	-	+++	+/-	-
c Binds to F_c receptors on macrophages	++	-	++	-	-	+	-	-
d Binds to F_c receptors on NK cells (ADCC)	++	-	++	-	-	+	-	-
e Activates Complement	+	-	++	-	-	+++	-	-
f Histamine release from Mast Cells via IgE - Fc recep.	-	-	-	-	-	-	+	-
g Secretion outside body.	-	-	-	-	+	-	-	-
h Neonatel Immun.	+++	+++	+++	+++	+++			

a) **Physical Blocking** of viruses and bacteria, attachment to flagella:



b) **Agglutination** – formation of antigen-antibody crosslinks, destroyed by macrophages.

c) Enhanced clearance of immune complexes due to F_C receptors on macrophages. Phagocytosis is greatly enhanced if multiple F_C receptors are occupied.

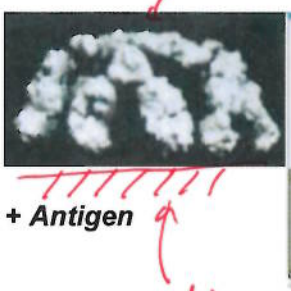


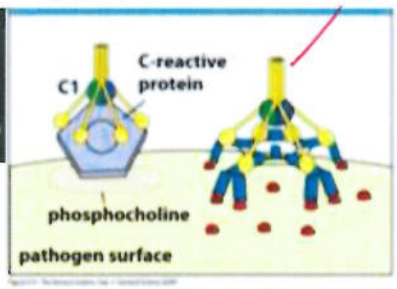
d) Antibody Dependent Cell-mediated Cytotoxicity (ADCC).
F_c receptors on NK cells induces cell death of antibody coated cells by apoptosis.

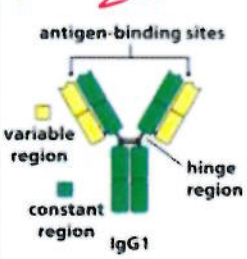
e) Activation of Complement: IgM and IgG3 activate complement. Complement activation requires a high density of F_C on the antigen. This situation is more readily achieved with pentameric IgM, however IgG3 has an extra long hinge that facilitates the interaction of two F_C regions with C1.

W/O antigen

cannot activate complement



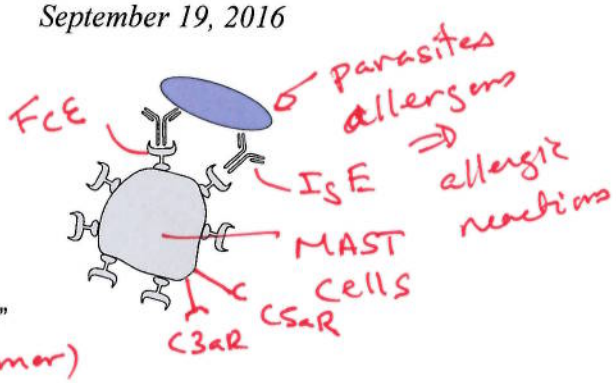




less flexible ∴ higher surface density

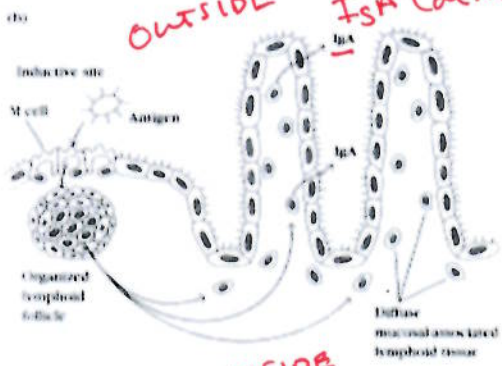
more flexible ∴ lower surface density

f) Hypersensitivity I: IgE bound to the surface of Mast Cells – response to parasites and allergic reactions – release of compounds that activate smooth muscle cells, increase vascular permeability (e.g. histamine).

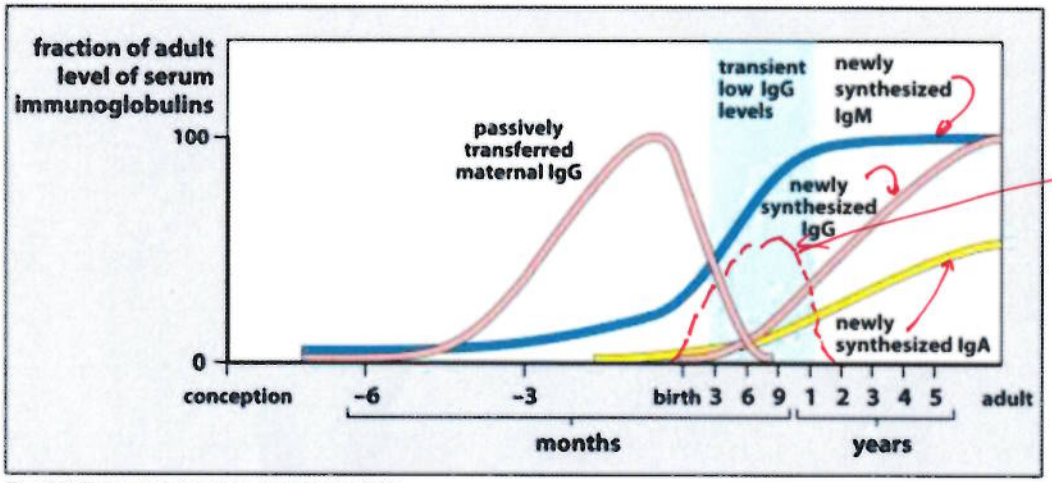


g) Secretion of IgA into intestines (and most other "mucosy" places):

MALT
mucosal assoc lymphatic tissue



h) Immunology of the neonate and passive immunization from the mother.



IgA from mother via breast milk.

Figure 9.24 The Immune System, 3ed. © Garland Science 2009)

i) $t_{1/2}$ of 6 months for beginning IgM production, 2.5 years for IgG and IgA
ii) Parental IgG reaches maximum at birth, decays with a $t_{1/2}$ of 3 months
iii) Plasma cells from MALT tissue in mother migrate to mammary glands, secreting pathogen specific IgA in milk.
iv) WHO recommends breastfeeding for ~6 months:
http://www.who.int/mediacentre/news/statements/2011/breastfeeding_20110115/en/