Administrative issues:

Recommended text: Goldsby/Kuby Immunology, 6th edition
(Note that Innate Immunity is not adequately covered in the 5th edition.)

Text book reading assignments are to supplement the lecture. Exam questions will be drawn primarily from lecture material.

Discussion sections start next week. The journal article Akira et al, and the relevant problem set questions will be covered. Both are available on the website.

Office Hours: Questions about the lecture material are best addressed during office hours (Tues 11-12). I will be holding extra office hours (date and time TBA) before the first midterm.

Email: Please use email only for VERY simple yes/no questions or simple administrative matters.

Great questions, keep them coming!

Antigens & Antibodies I

Discovery of antibodies

Basic Antibody Structure

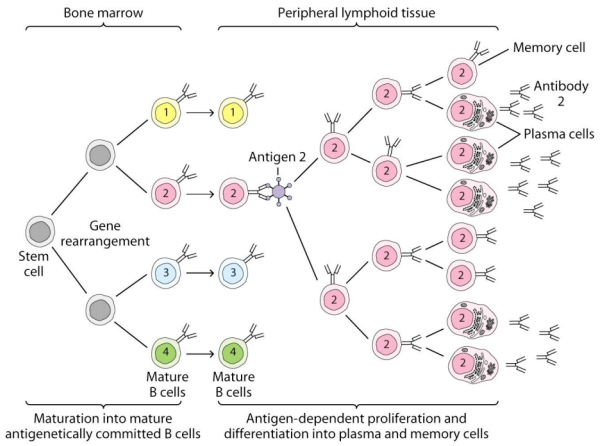
- brief review of protein structure
- disulfide linked tetramer: 2 heavy and 2 light chains
- **myeloma proteins, Ig domains, and hypervariable regions**

The antigen binding site of antibodies

Antibody isotypes: IgM, IgG, IgD, IgA, IgE

- The advantages of multivalency
- effector functions of antibody isotypes

In a normal individual, antibodies are extremely heterogeneous.

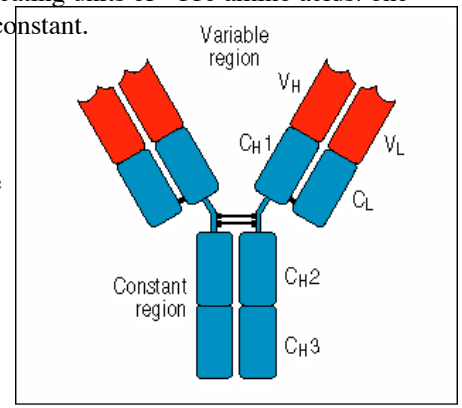


Myeloma protein: key to determining Ig structure

- Heterogeneity of antibodies makes sequencing impossible (each B cell clone produces a unique version of antibody).
- Multiple myeloma: cancer derived from an antibody producing cells (plasma B cell).
- Myeloma patients have large amounts of one particular Ig molecule in their serum (and urine)
- Many patients produce a large amount of one light chain, known as “Bence-Jones” proteins.

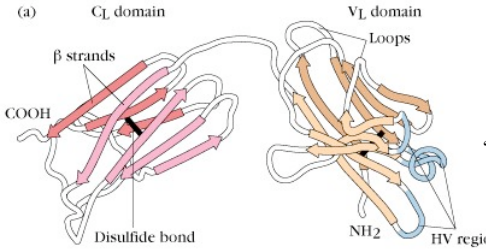
When the amino acid sequences of several different Bence-Jones proteins were compared, they were found to consist of two repeating units of ~110 amino acids: one variable and one constant.

Antibody molecules are composed of repeats of a single structural unit known as the "immunoglobulin domain"

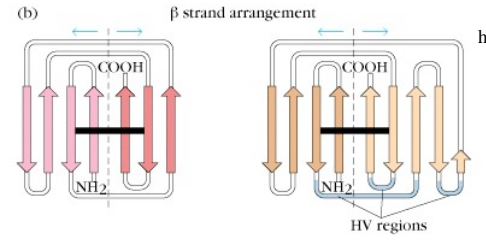


Protein homology

- Identity or similarity between domains in two or more proteins
- Most easy to see at the level of primary amino acid sequence (computer programs find it)
- Sometimes no obvious primary sequence homology but striking structural homology
- Homology can sometimes predict structure and function

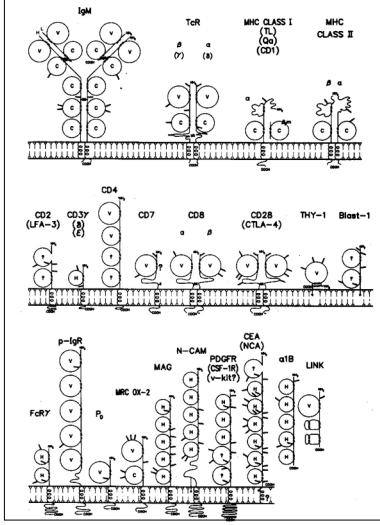


All Ig domains have a similar 3D structure known as an "Immunoglobulin Fold".



2 β-pleated sheets come together to form a sandwich, held together by disulfide bond and hydrophobic interactions.

3 flexible loops at end: correspond to hypervariable regions of primary sequence (HV).



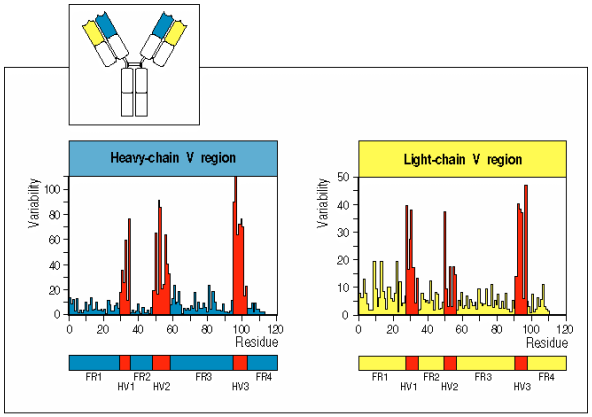
The **Immunoglobulin Fold** is a very commonly used structural motif amongst cell surface proteins

Ig domain: Genome Project Champion!

Table 25 The most populous InterPro families in the human proteome and other species

	Human		Fly		Worm		Yeast		Mustard weed		
InterPro ID	No. of genes	Rank	No. of genes	Rank	No. of genes	Rank	No. of genes	Rank	No. of genes	Rank	
IPR003006	765	(1)	140	(9)	64	(34)	0	(na)	0	(na)	Immunoglobulin domain
PR000822	706	(2)	357	(1)	151	(10)	48	(7)	115	(20)	C2H2 zinc finger
IPR000719	575	(3)	319	(2)	437	(2)	121	(1)	1049	(1)	Eukaryotic protein kinase
IPR000276	569	(4)	97	(14)	358	(3)	0	(na)	16	(84)	Rhodopsin-like GPCR superfamily
IPR001687	433	(5)	198	(4)	183	(7)	97	(2)	331	(5)	P-loop motif

The variability of antibodies occurs within 3 discrete regions of the primary sequence: hypervariable regions HV1-3



The hypervariable regions (HV1-3) are separated in primary structure, but come together in the tertiary structure where they form the antigen binding site. Alias Complementary Determining Regions or CDR1-3.

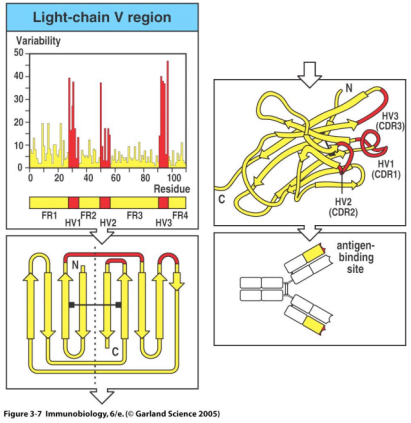
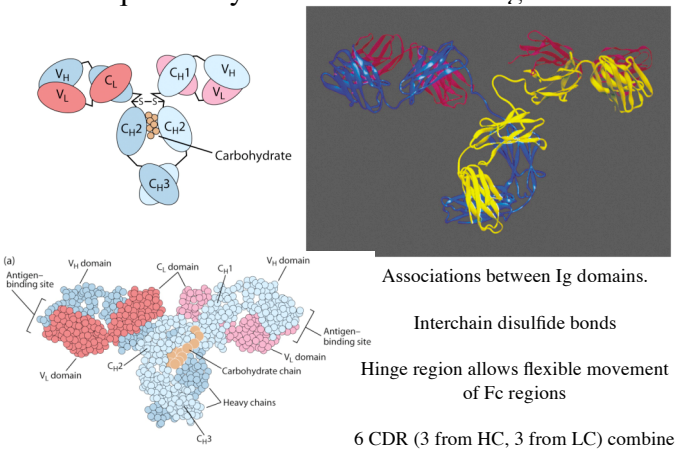


Figure 3-7 Immunobiology, 6/e.(© Garland Science 2005)

The HV regions form loops at the end of the Ig domain.

The intervening framework regions (FR1-4) make up the rest of the structure.

The quaternary structure of immunoglobulin



Associations between Ig domains.

Interchain disulfide bonds

Hinge region allows flexible movement of Fc regions

6 CDR (3 from HC, 3 from LC) combine to make up antigen binding site

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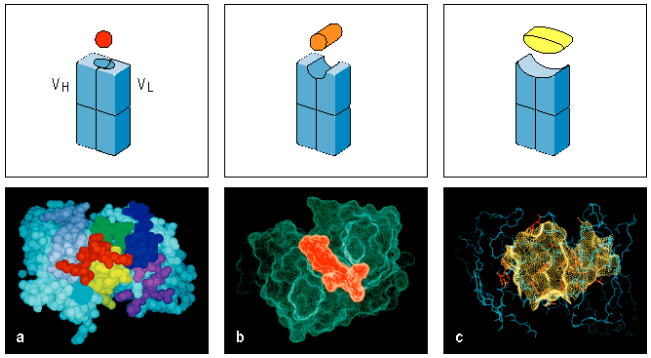
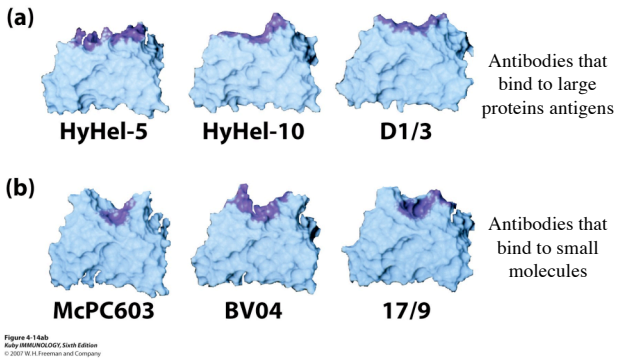
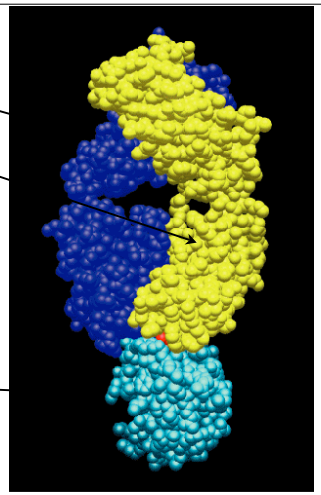
- The advantages of multivalency
- effector functions of antibody isotypes

Antigen-antibody interactions regions come in many shapes including: pockets, grooves, or extended flat surfaces.

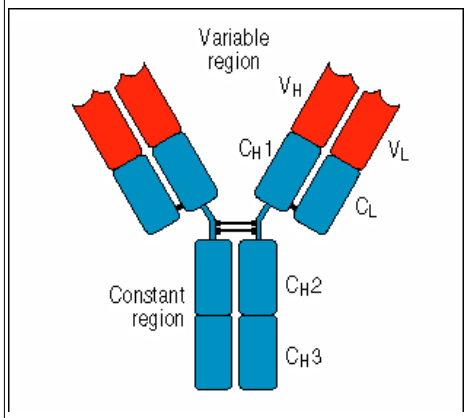


Because the CDR are highly variable, each antibody molecule has a unique antigen binding site with its own dimensions and complementarity.



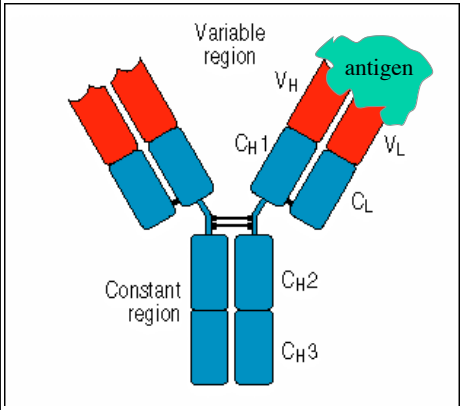


(which fragment of an antibody is this ?)



antigen

Epitope- three-dimensional face of an antigen which makes contact with the antibody

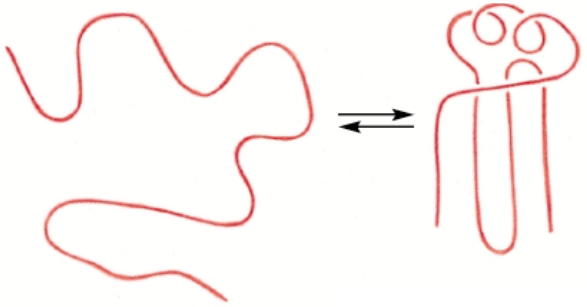


Epitope- three-dimensional face of an antigen which makes contact with the antibody

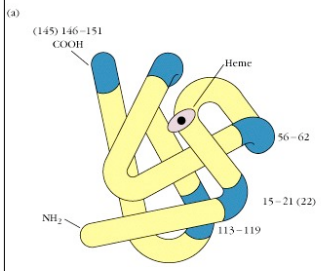
"Conformational Epitopes"

denatured

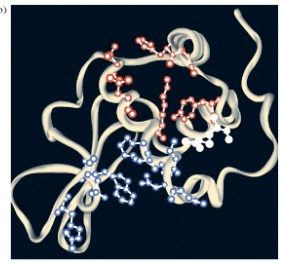
Native structure



B cell epitopes can be sequential (linear) or non sequential (conformational)

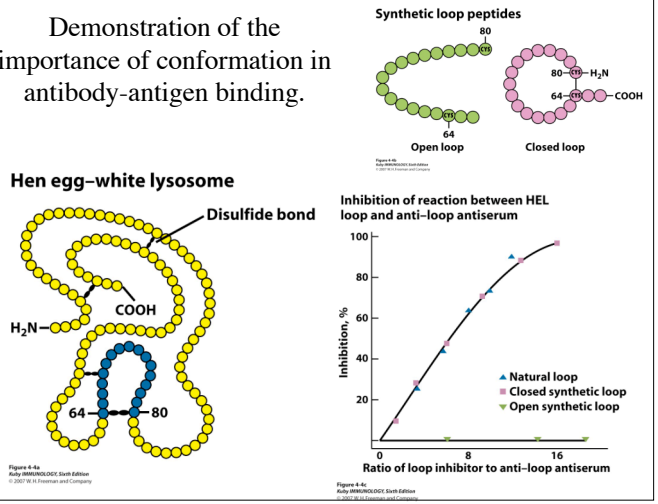


Five sequential epitopes in whale myoglobin

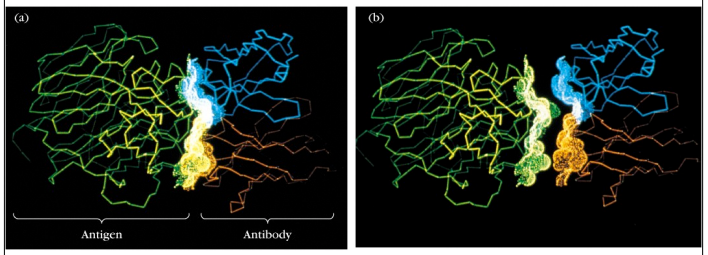


A non-sequential epitope in hen lysozyme

Demonstration of the importance of conformation in antibody-antigen binding.



Epitope and antigen binding site form complementary surfaces



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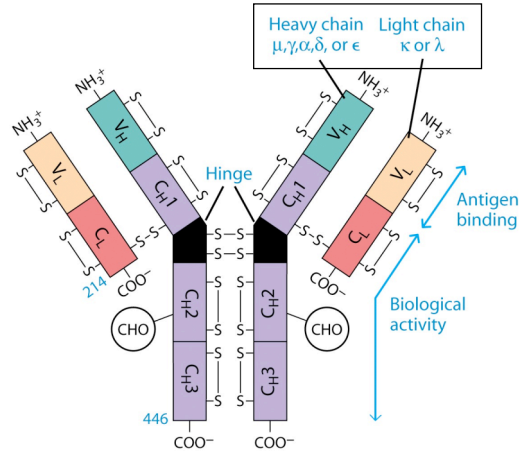
The antigen binding site of antibodies

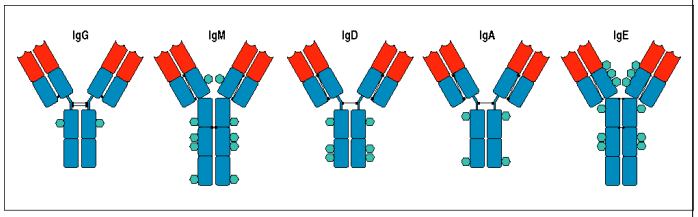
Antibody isotypes: IgM, IgG, IgD, IgA, IgE

- **Differences in valency and tissue distribution**
- effector functions of antibody isotypes

Heavy and light chains come in different types



Ig **isotypes** are due to differences in heavy-chain or light-chain constant region sequences.



Heavy chains come in 5 major types that have different tissue distributions and effector functions : γ, μ, δ, α, ε

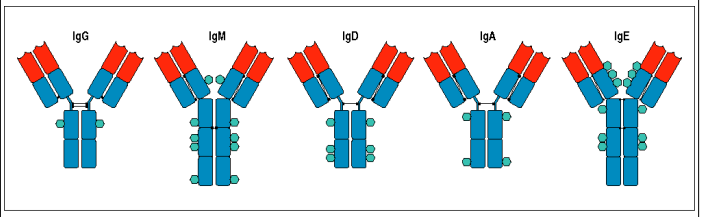
Light chains come in two major types: κ or λ

TABLE 4-1 Chain composition of the five immunoglobulin classes in humans

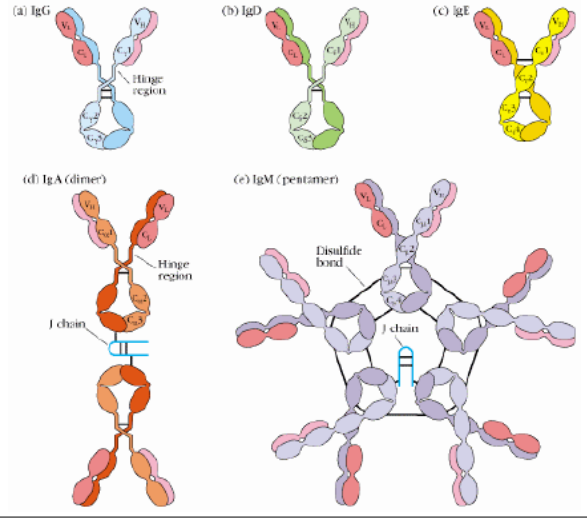
Class	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	γ1, γ2, γ3, γ4	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n = 1$ or 5
IgA	α	α1, α2	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n = 1, 2, 3,$ or 4
IgE	ε	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

Antibodies protect by recruiting other effector functions through the interaction of C_H domains with other cells and proteins of the immune system.

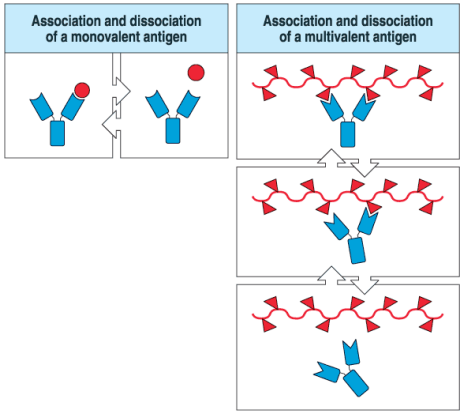
Different antibody isotypes recruit different effector functions.



Receptors that bind to the Fc portion of antibodies are called "Fc receptors".



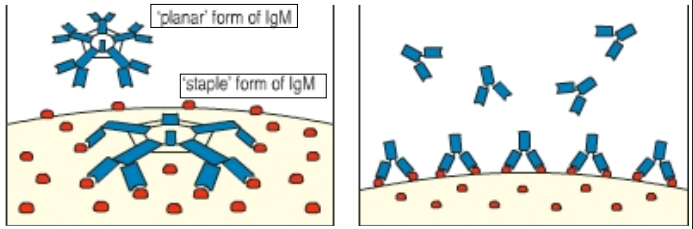
Multivalency leads to tighter binding.

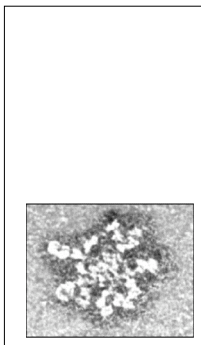


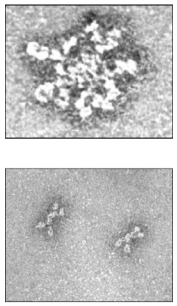
Advantage of multivalency

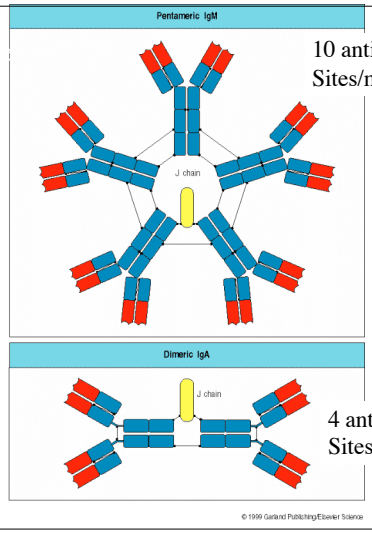
Decameric IgM

Dimeric IgG





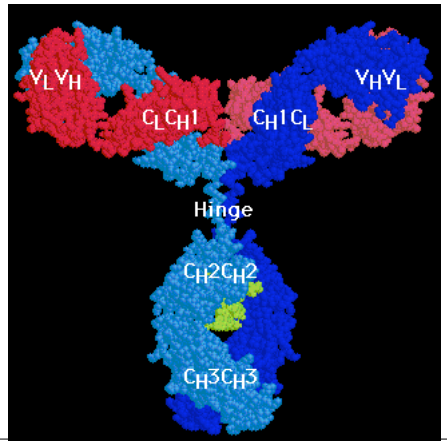




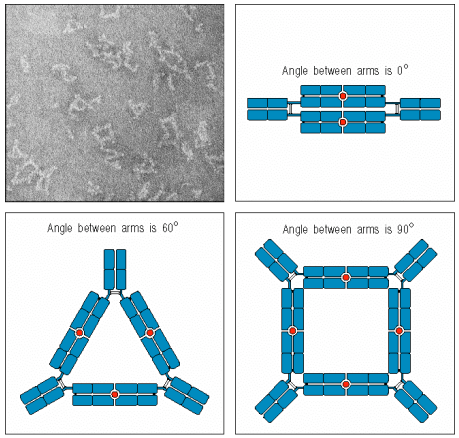
10 antigen binding Sites/molecule

4 antigen binding Sites/molecule

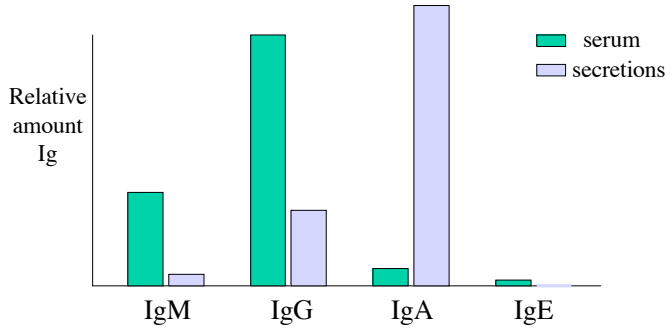
Some classes of Immunoglobulin (IgG, IgD and IgA) have a flexible, proline-rich hinge region. Flexibility of antibody arms allow for more efficient binding to multivalent antigens.



Antibody "arms" are connected by a flexible hinge



Distribution of various Ig isotypes in body fluids



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The antigen binding site of antibodies

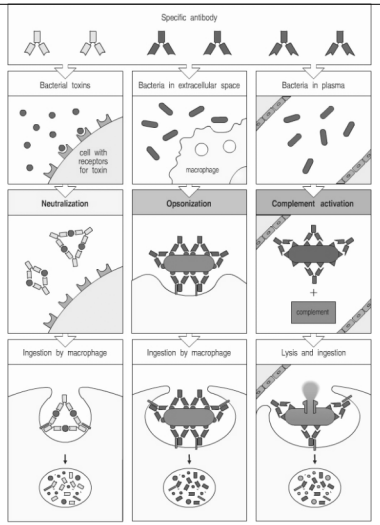
Antibody isotypes: IgM, IgG, IgD, IgA, IgE
- differences in valency and tissue distribution
- **effector functions of antibody isotypes**

TABLE 4-4 Properties and biological activities* of classes and subclasses of human serum immunoglobulins

	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM‡	IgE	IgD
Molecular weight†	150,000	150,000	150,000	150,000	150,000 – 600,000	150,000 – 600,000	900,000	190,000	150,000
Heavy-chain component	γ1	γ2	γ3	γ4	α1	α2	μ	ε	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In vivo serum half-life (days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+/−	++	−	−	−	++	−	−
Crosses placenta	+	+/−	+	+	−	−	−	−	−
Present on membrane of mature B cells	−	−	−	−	−	−	+	−	+
Binds to Fc receptors of phagocytes	++	+/−	++	+	−	−	?	−	−
Mucosal transport	−	−	−	−	++	++	+	−	−
Induces mast cell degranulation	−	−	−	−	−	−	−	+	−

*Activity levels indicated as follows: ++ = high; + = moderate; +/− = minimal; − = none; ? = questionable.
†IgG, IgE, and IgD always exist as monomers; IgA can exist as a monomer, dimer, trimer, or tetramer. Membrane-bound IgM is a monomer, but secreted IgM in serum is a pentamer.
‡IgM is the first isotype produced by the neonate and during a primary immune response.

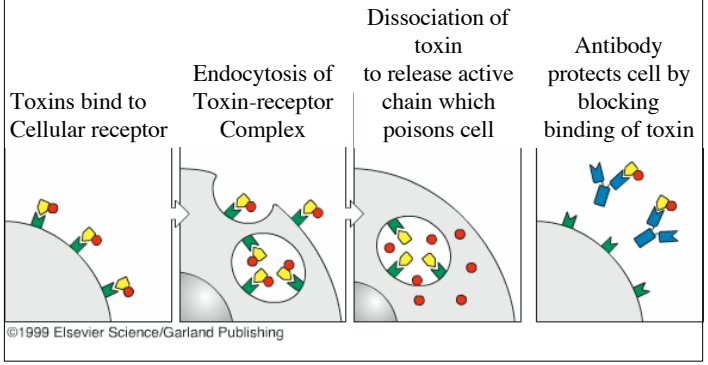
Table 4-4
Kuby IMMUNOLOGY, Sixth Edition



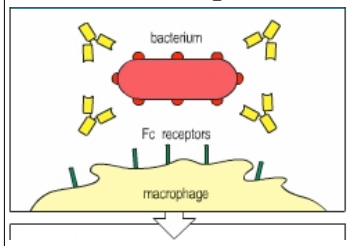
Antibody Effector Mechanisms

- Neutralization: binding itself prevents pathogenesis.
- Opsonization: enhancing phagocytosis
- Complement activation

Neutralization

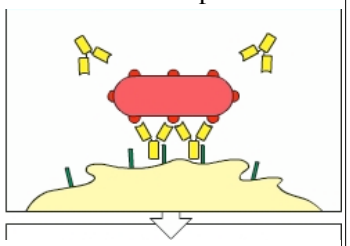


Free Ig binds poorly to Fc receptors



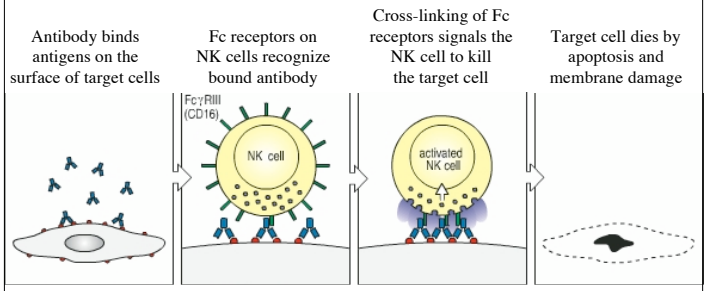
No activation of macrophage, No destruction of bacterium

Aggregation of Ig on bacterial surface promotes aggregation of Fc receptors



Activation of macrophage leading to destruction of bacterium

Antibody-Dependent Cellular Toxicity (ADCC)



IgG

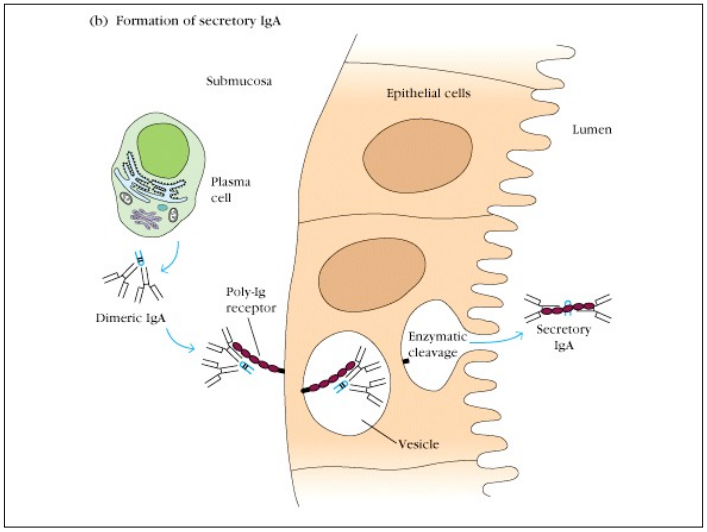
- Predominant Ig in serum.
- 4 subclasses (IgG1-IgG4)
- Important for opsonization, complement activation, ADCC,
- Crosses placenta to protect fetus

IgM

- pentameric (decavalent)
- Pentameric structure held together by J-chain and disulfide bonds.
- First Ig produced in response to infection
- Good at complement activation

IgA

- dimeric (tetravalent) predominant Ig in secretions.
- Transported across epithelial cells via poly-Ig receptor.
- 10g of IgA secreted/day, more than any other Ig!
- Found in breast milk, supplies passive immunity to baby.



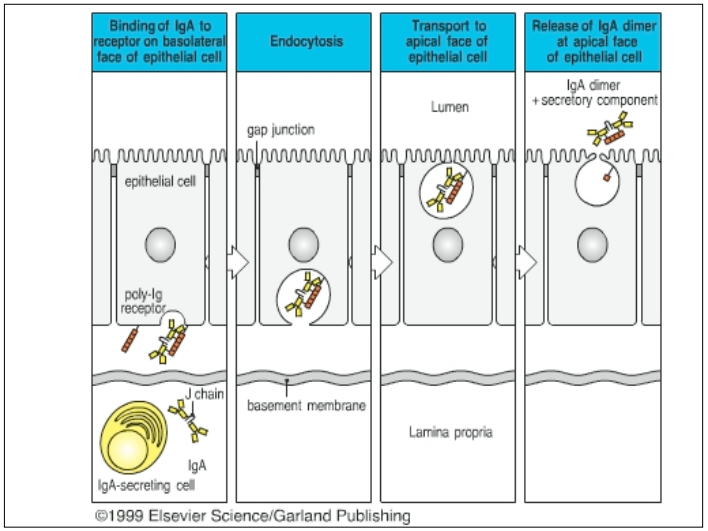
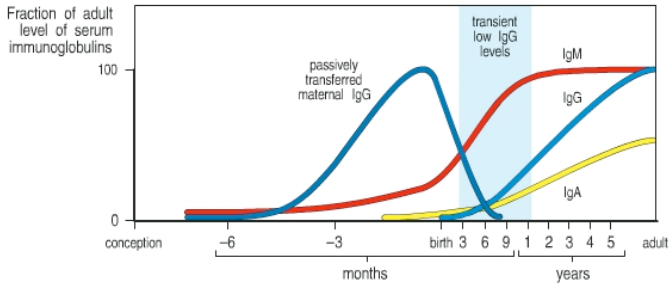


TABLE 4-3 Immune benefits of breast milk

Antibodies of secretory IgA class	Bind to microbes in baby's digestive tract and thereby prevent their attachment to the walls of the gut and their subsequent passage into the body's tissues.
B_{12} binding protein	Reduces amount of vitamin B_{12}, which bacteria need in order to grow.
Bifidus factor	Promotes growth of *Lactobacillus bifidus*, a harmless bacterium, in baby's gut. Growth of such nonpathogenic bacteria helps to crowd out dangerous varieties.
Fatty acids	Disrupt membranes surrounding certain viruses and destroy them.
Fibronectin	Increases antimicrobial activity of macrophages; helps to repair tissues that have been damaged by immune reactions in baby's gut.
Hormones and growth factors	Stimulate baby's digestive tract to mature more quickly. Once the initially "leaky" membranes lining the gut mature, infants become less vulnerable to microorganisms.
Interferon (IFN-γ)	Enhances antimicrobial activity of immune cells.
Lactoferrin	Binds to iron, a mineral many bacteria need to survive. By reducing the available amount of iron, lactoferrin thwarts growth of pathogenic bacteria.
Lysozyme	Kills bacteria by disrupting their cell walls.
Mucins	Adhere to bacteria and viruses, thus keeping such microorganisms from attaching to mucosal surfaces.
Oligosaccharides	Bind to microorganisms and bar them from attaching to mucosal surfaces.

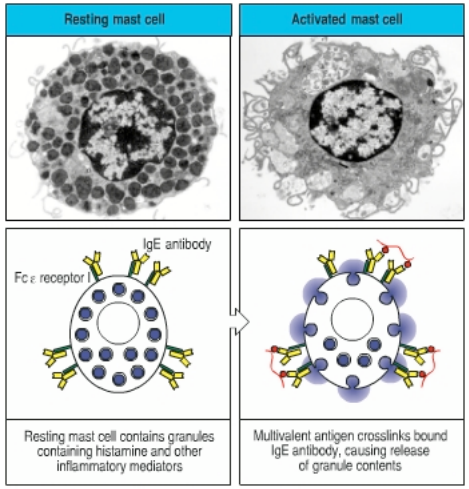
SOURCE: Adapted from J. Newman, 1995, How breast milk protects newborns, *Sci. Am.* **273(6):**76.

Antibody levels early in life



IgE

- Present in VERY LOW amounts in serum
- Binds to Fc Receptors present on mast cells and basophils
- Levels increase in setting of parasitic infection
- Can transfer allergy between individuals



Antigens & Antibodies I: focus on antibody structure

Discovery of antibodies
Basic Antibody Structure
The antigen binding site of antibodies
Antibody isotypes: IgM, IgG, IgD, IgA, IgE

→

Antigens & Antibodies II: focus on antibody-antigen interactions

Definitions
A comparison of antigen recognition by B and T cells
Factors that determine immunogenicity
Quantitating the strength of antibody-antigen interactions: affinity and avidity
Cross-reactivity of antibodies
Measuring antibody-antigen binding

Antigens & Antibodies II

Definitions

A comparison of antigen recognition by B and T cells

Factors that determine immunogenicity

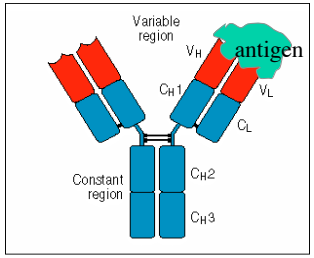
Quantitating the strength of antibody-antigen interactions: affinity and avidity
- Equilibrium constants
- equilibrium dialysis
- impact of multivalency

Cross-reactivity of antibodies

Measuring antibody-antigen binding

Definitions

- **Antibody**: a protein (immunoglobulin) that binds an antigen.
- **Antigen**: a substance that is recognized by the immune system
- **Immunogen**: a substance that elicits an immune response (not all antigens are immunogenic!)



- **Epitope**: the portion of an antigen that is recognized by the antibody (or TCR). Also called "antigenic determinant"
- **Hapten**: a small molecule that cannot by itself induce an immune response, but can be an antigen.

The antigen receptor of B cells (antibody) binds directly to antigen. Antibody exists in both a transmembrane receptor and secreted form.

The antigen receptor of T cells (TCR) binds processed antigen (peptide) on the surface of an antigen presenting cell. TCR exists only as transmembrane form.

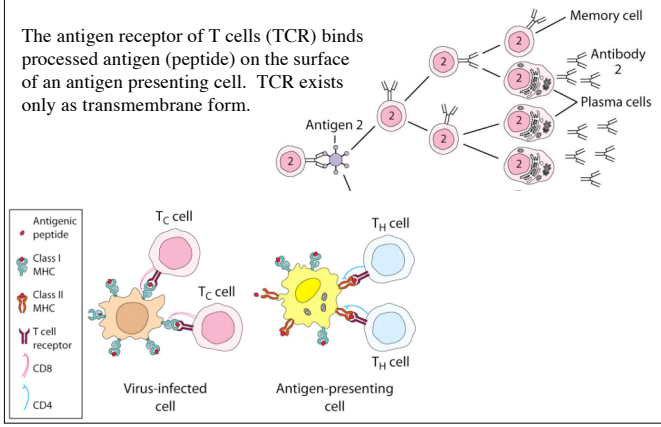


TABLE 4-2 Comparison of antigen recognition by T cells and B cells

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules

Table 4-2
Kuby IMMUNOLOGY, Sixth Edition

Immunogenicity

- **Foreignness**-- greater difference from host
- **Size**-- bigger is better
- **Complexity**- polyglycine is a poor immunogen
- **Susceptibility to phagocytosis**- particles better than soluble material
- **Genotype of host**- esp MHC types
- **Route of administration** subcu better than IV
- **Dose** - not too high, not too low

Adjuvants enhance the immunogenicity of antigens by:

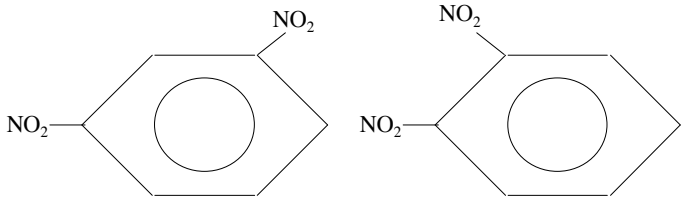
-triggering the innate immune system (many contain TLR agonists)
-slowing release of antigen
-promoting phagocytosis of antigen, others?

TABLE 3-3 Postulated mode of action of some commonly used adjuvants

Adjuvant	Postulated mode of action: Prolongs antigen persistence	Enhances co-stimulatory signal	Induces granuloma formation	Stimulates lymphocytes nonspecifically
Freund's incomplete adjuvant	+	+	+	–
Freund's complete adjuvant	+	++	++	–
Aluminum potassium sulfate (alum)	+	?	+	–
Mycobacterium tuberculosis	–	?	+	–
Bordetella pertussis	–	?	–	+
Bacterial lipopolysaccharide (LPS)	–	+	–	+
Synthetic polynucleotides (poly IC/poly AU)	–	?	–	+

The first adjuvant Freund's complete adjuvant: emulsified mineral oil and mycobacterial extract.
The most effective adjuvants cannot be used in humans due to toxcity (exception: diptheria-pertussis-tetanus combined vaccine (DPT))

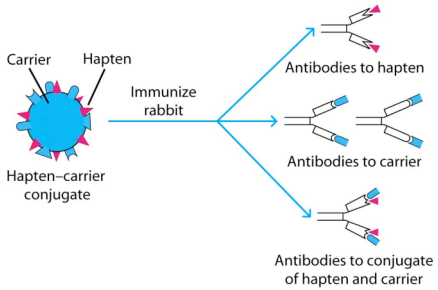
Hapten: a small molecule that cannot by itself induce an immune response, but can be an antigen.



DNP-- 1,3 Dinitrophenol DNP-- 1,2 Dinitrophenol

Even closely related haptens can be distinguished antigenically; antibodies raised against 1,2 DNP may not react with 1,3 DNP.

Haptens are not immunogenic unless they are coupled to a carrier protein.



Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti–BSA
Hapten–carrier conjugate (DNP-BSA)	Anti–DNP (major) Anti–BSA (minor) Anti–DNP/BSA (minor)

Antigens & Antibodies II

Definitions

A comparison of antigen recognition by B and T cells

Factors that influence immunogenicity

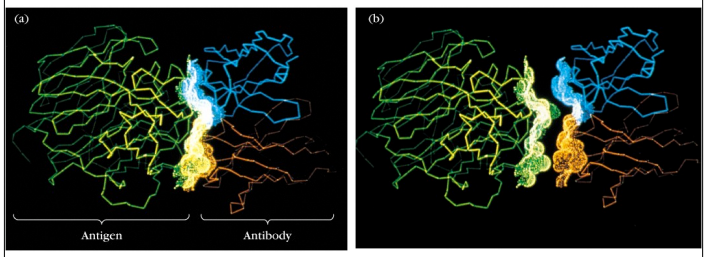
Quantitating the strength of antibody-antigen interactions

- Equilibrium constants
- equilibrium dialysis
- impact of multivalency

Cross-reactivity of antibodies

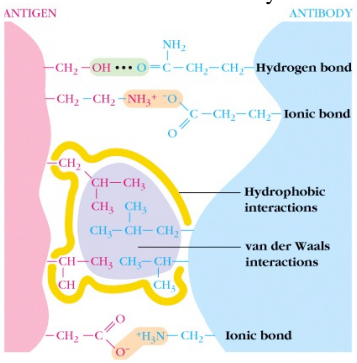
Measuring antibody-antigen binding

Epitope and antigen binding site form complementary surfaces

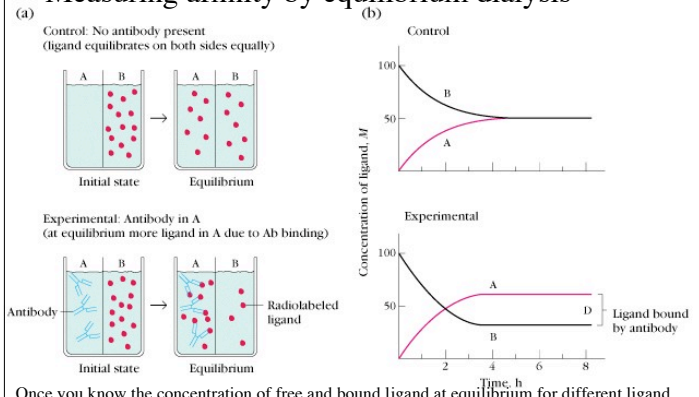


Quantitating antibody-antigen interactions:

Strength is determined by the sum of multiple non-covalent bonds. Strength of interaction between a single epitope and antigen binding site is called its affinity. Each antibody-antigen interaction has a distinct affinity.



Measuring affinity by equilibrium dialysis



Once you know the concentration of free and bound ligand at equilibrium for different ligand concentrations, you can calculate the equilibrium binding constant (K), which provides a quantiative measure of the affinity of the interaction.

Note that equilibrium dialysis is based on differential ability of ligand and antibody to pass through membrane. Can only be used when the ligand is small (e.g. a hapten).

Equilibrium binding equation

$$Ag + Ab \underset{k2}{\overset{k1}{\rightleftharpoons}} Ag\text{-}Ab$$

Free antigen — Free antibody — Antigen-Antibody complex

$$Ka = \frac{[Ab\text{-}Ag]}{[Ab][Ag]}$$

Ka is the association binding constant.
k1 or k_{on} is the association rate constant.
k2 or k_{off} is the dissociation rate contant.

Equilibrium binding equation

$$Ag + Ab \underset{k2}{\overset{k1}{\rightleftharpoons}} Ag\text{-}Ab$$

$$Ka = \frac{[Ab\text{-}Ag]}{[Ab][Ag]}$$

If binding is weak: k2 (off rate) is high, and Ka (association binding constant) will be low (equilibrium shifted to the left).

If binding is strong: k2 (off rate) is low, and Ka will be high (equilibrium shifted to the right).

Sometimes binding strength is represented by Kd (dissociation equilibrium constant) = 1/Ka

$$\text{Ag-Ab} \underset{k1}{\overset{k2}{\rightleftharpoons}} \text{Ag + Ab}$$

$$\text{Kd} = \frac{[\text{Ab}][\text{Ag}]}{[\text{Ab-Ag}]}$$

Kd (dissociation equilibrium constant) = 1/Ka
(units are moles/liter)

The ligand concentration at which 1/2 of the antibody is binding ligand at equilibrium, is close to the Kd

Stronger binding corresponds to lower Kd

The concentration at which 50% of the antibody and ligand are bound at equilibrium, is close to the K_d

$$\text{Kd} = \frac{[\text{Ab}][\text{Ag}]}{[\text{Ab-Ag}]}$$

If the concentration of total antibody and antigen (bound and free) is 2 x 10^{-7}M (moles/liter):

For an interaction whose $K_d = 10^{-7}$M:
50% of antibody and antigen are bound

$$10^{-7}\text{M} = \frac{10^{-7}\text{M} \times 10^{-7}\text{M}}{10^{-7}\text{M}}$$

For an interaction whose $K_d = 0.5 \times 10^{-9}$
95% of antibody and antigen are bound

$$0.5 \times 10^{-9}\text{M} = \frac{10^{-8}\text{M} \times 10^{-8}\text{M}}{1.9 \times 10^{-7}\text{M}}$$

TABLE 6-1 Forward and reverse rate constants (k_1 and k_{-1}) and association and dissociation constants (K_a and K_d) for three ligand-antibody interactions

Antibody	Ligand	k_1	k_{-1}	K_a	K_d
Anti-DNP	ϵ-DNP-L-lysine	8×10^7	1	1×10^8	1×10^{-8}
Anti-fluorescein	Fluorescein	4×10^8	5×10^{-3}	1×10^{11}	1×10^{-11}
Anti-bovine serum albumin (BSA)	Dansyl-BSA	3×10^5	2×10^{-3}	1.7×10^8	5.9×10^{-9}

SOURCE: Adapted from H. N. Eisen, 1990, *Immunology*, 3rd ed., Harper & Row, Publishers.

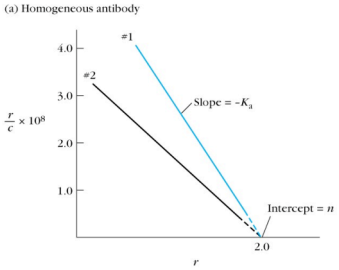
Table 6-1
Kuby IMMUNOLOGY, Sixth Edition

Note that for two antibody-ligand pairs with similar on rates (k_1), a lower off rate (k_{-1}) corresponds to tighter binding (higher K_a, lower K_d).

Note that for two antibody-ligand pairs with similar off rates (k_{-1}), a faster on rate (k_1) corresponds to tighter binding (higher K_a, lower K_d).

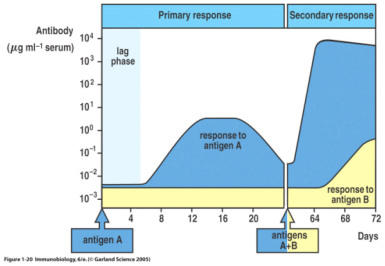
Data from equilibrium dialysis can be analyzed using **Scatchard Plot**:
r= bound ligand / total antibody
c= free ligand
n= number of binding sites per antibody molecule

Slope = -Ka
X-intercept = n



Note: this only works if the antibody is homogeneous: all antigen binding sites identical, e.g. myeloma protein or a monoclonal antibody. What happens with polyclonal antibody which consists of mixtures of many different types of antibodies?

Polyclonal antisera



Can be generated by repeated immunization of animal (rabbit) with antigen (with adjuvant).

polyclonal antibodies are a complex mixture of antibodies directed against different epitopes and that differ in their affinity for the antigen.

Polyclonal antibodies vs Monoclonal antibodies

Polyclonal antibodies: antibody preparations from immunized animals. Consist of complex mixtures of different antibodies produced by many different B cell clones

Monoclonal Antibody: homogeneous antibody preparations produced in the laboratory. Consist of a single type of antigen binding site, produced by a single B cell clone (later we'll talk about how these are made).

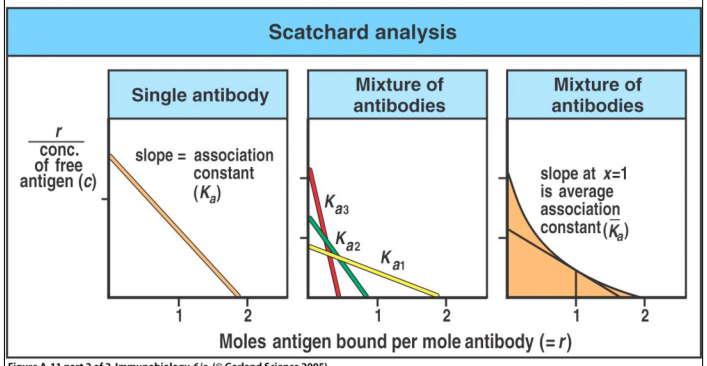
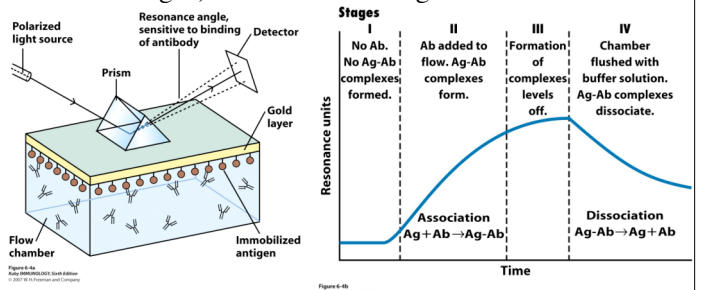


Figure A-11 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Affinity between two macromolecules (antibody and protein antigen) can measured using a biosensor.



-Resonance units are proportional to the degree of binding of soluble ligand to the immobilized receptor. *(or soluble antibody to immobilized antigen, as shown here)*
- Determining the amount of binding at equilibrium with different known concentrations of receptor (antibody) and ligand (protein antigen) allows you to calculate equilibrium constants (Ka, Kd).

-Rate of dissociation and association (k_{off}, k_{on}) can also be calculated.

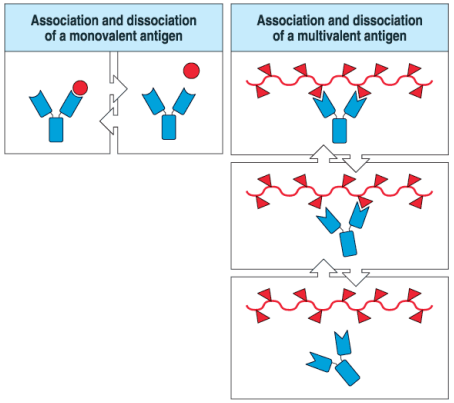
Affinity refers to strength of binding of single epitope to single antigen binding site.

But antibodies have 2 or more identical binding sites.

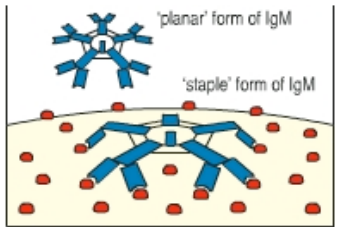
Most antigens are multimeric.

What is impact of valence on strength of binding?

Avidity (strength of binding) is influenced by both Affinity (Ka of single binding site) and the Valence of the interaction (number of interacting binding sites)



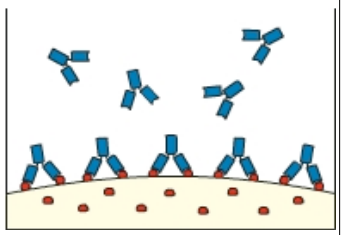
Decameric IgM



low affinity interactions can have high avidity if valence is high.

IgM tend to bind tightly, but have less specificity.

Dimeric IgG



Avid binding due to high affinity. Binding of IgG tends to be more specific. (more perfect "fit" between antigen binding site and antigen)