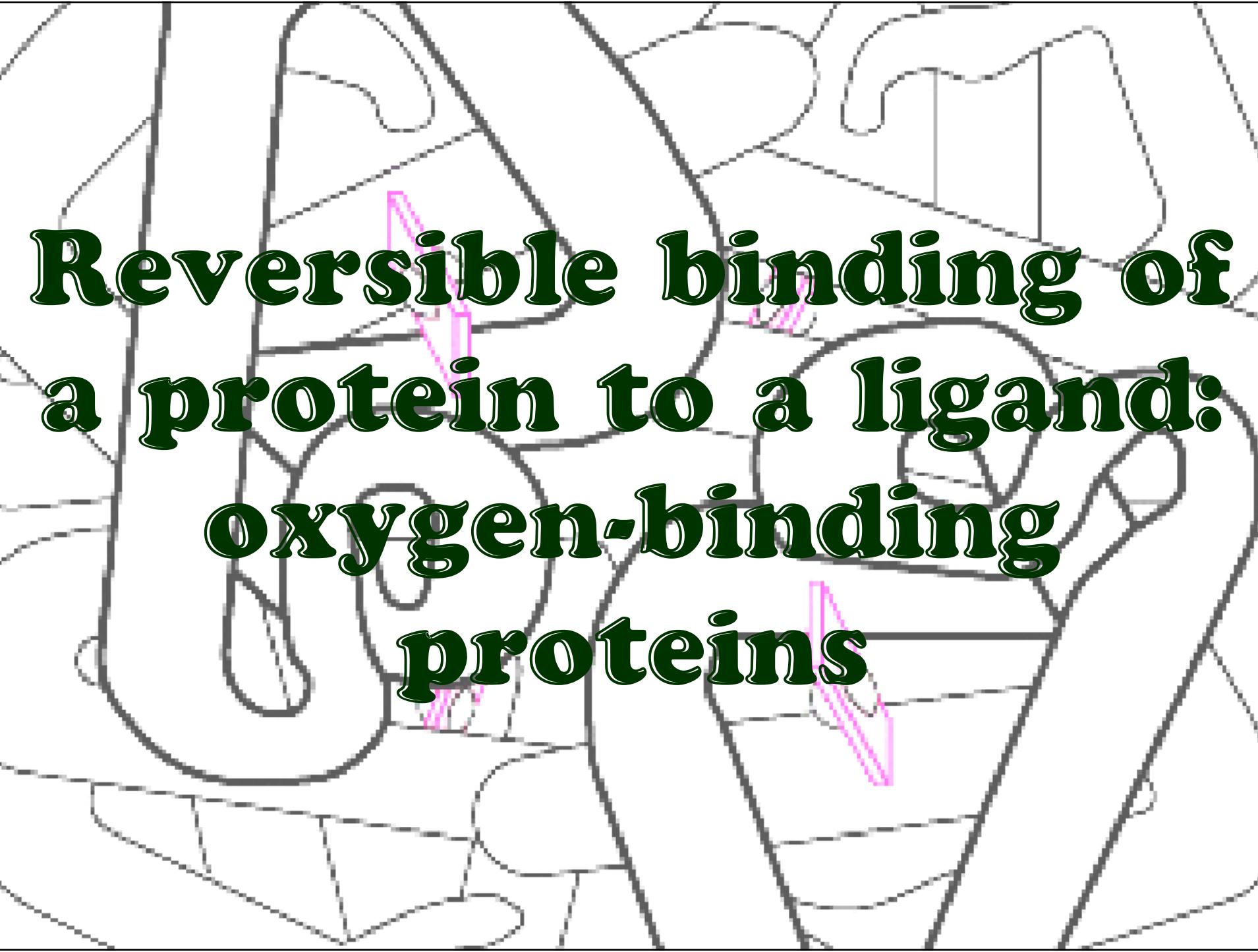


CHAPTER 5

PROTEIN FUNCTION

- Reversible binding of ligands
- Structure of myoglobin and hemoglobin
- Origin of cooperativity in hemoglobin
- Structure and function of antibodies
- Molecular mechanism of muscle movements

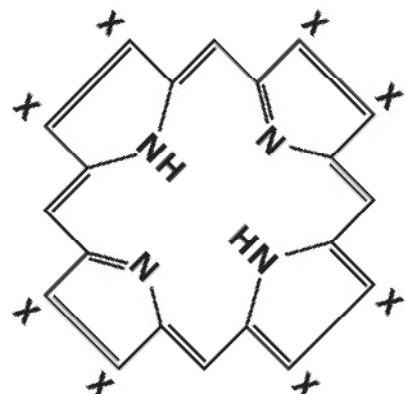


Reversible binding of a protein to a ligand: oxygen-binding proteins

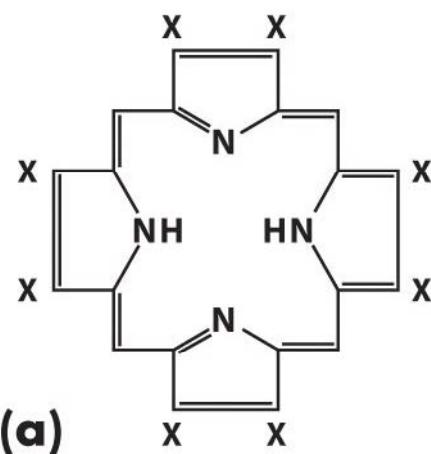
The heme group

PDB ID 1CCR

X: Modification site

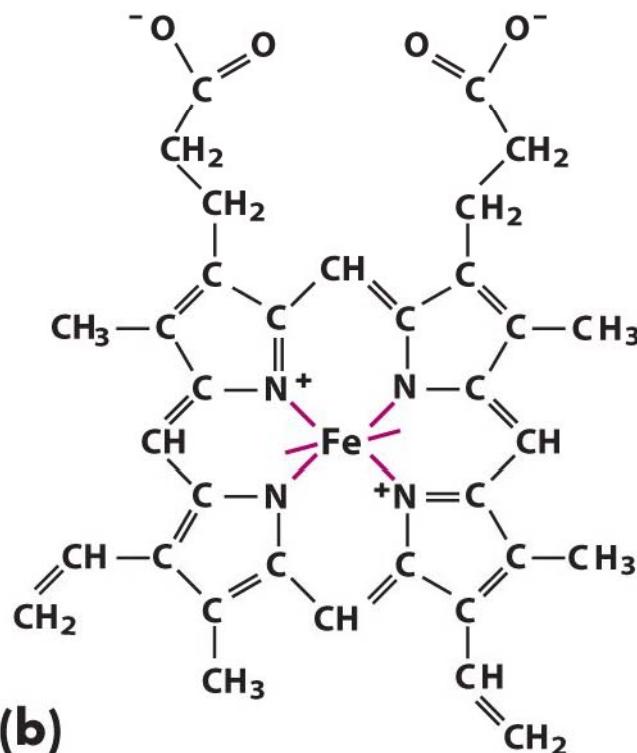


Porphyrin

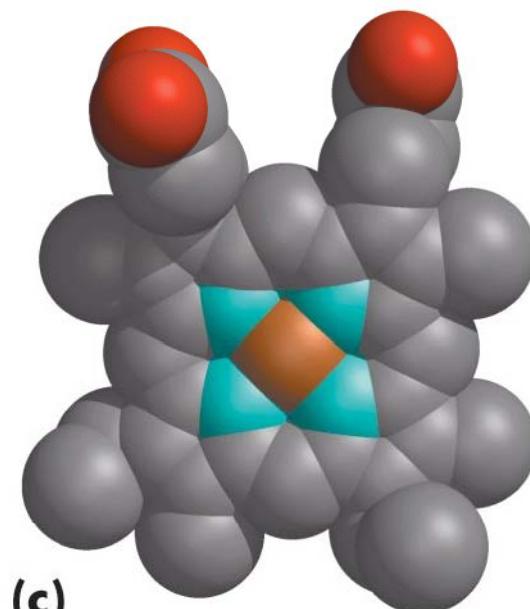


(a)

(d)



(b)

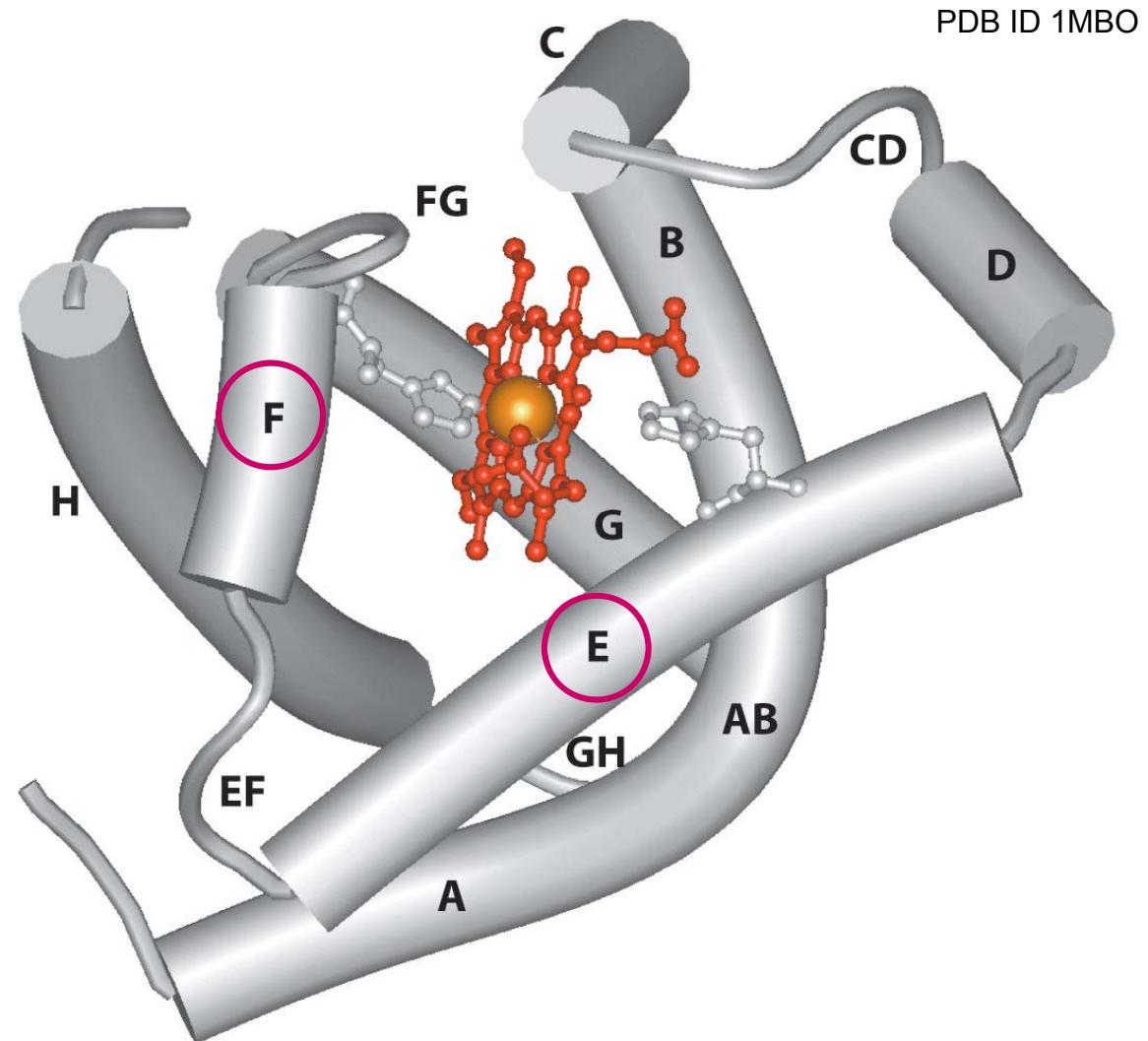
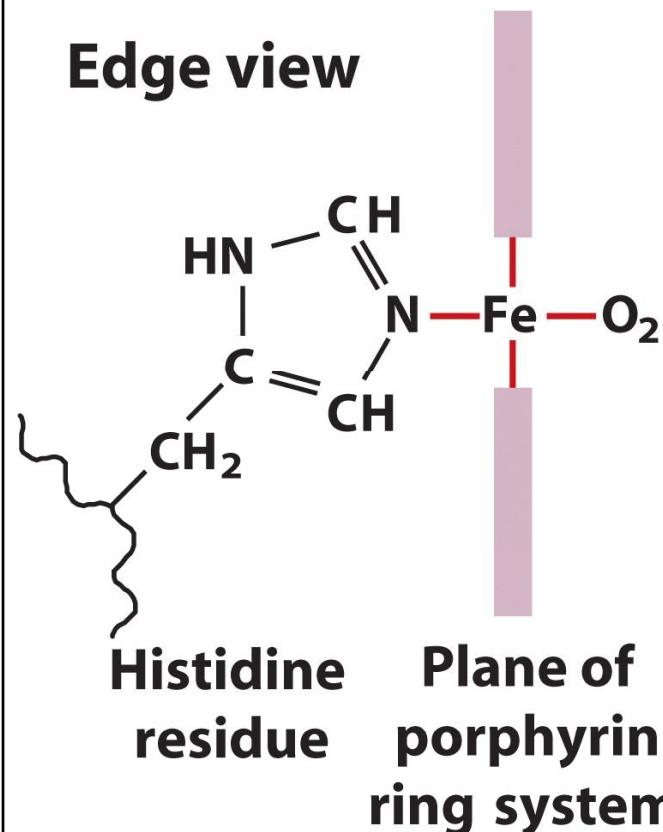


(c)

The iron atom of heme has six coordination bonds

The heme group of myoglobin

Edge view



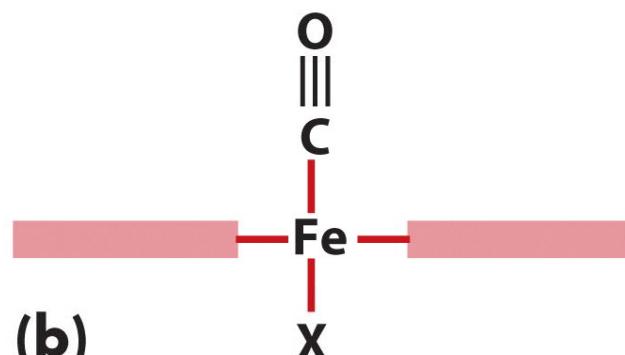
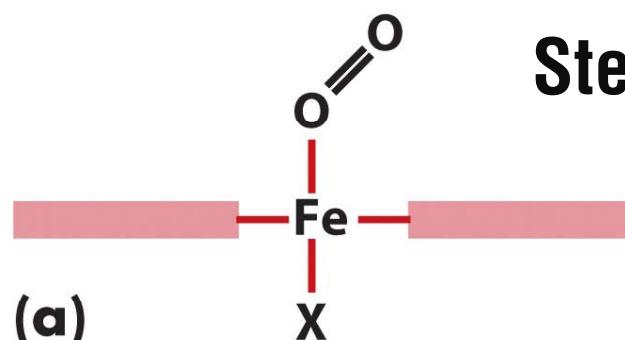
None of the amino acid side chains in proteins are suited for the reversible binding of oxygen

TABLE 3-4 Conjugated Proteins

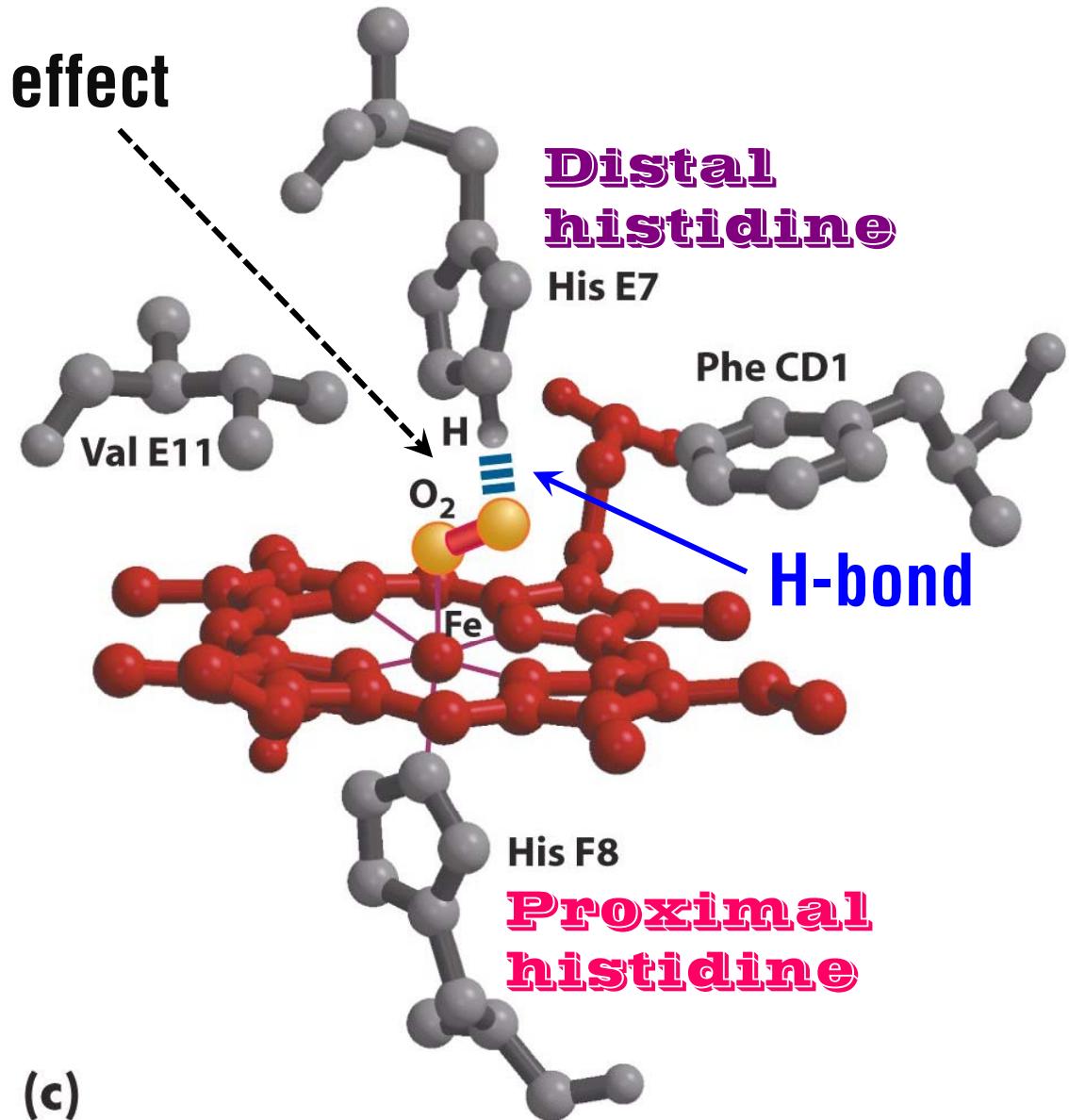
Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1 -Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

“Conjugated proteins” contain permanently associated chemical components in addition to amino acids. The non-amino acids parts of a conjugated protein is usually called its prosthetic group.

The key residues around the heme upon oxygen binding



Steric effect



Structures of myoglobin and hemoglobin

- **Myoglobin (Mb)** - monomeric protein (16.7 kDa) that facilitates the diffusion of oxygen in vertebrates
- **Hemoglobin (Hb)** - tetrameric protein (64.5 kDa) that carries oxygen in the blood
- **Heme** consists of a tetrapyrrole ring system called **protoporphyrin IX** complexed with iron
- Heme of Mb and Hb binds oxygen for transport

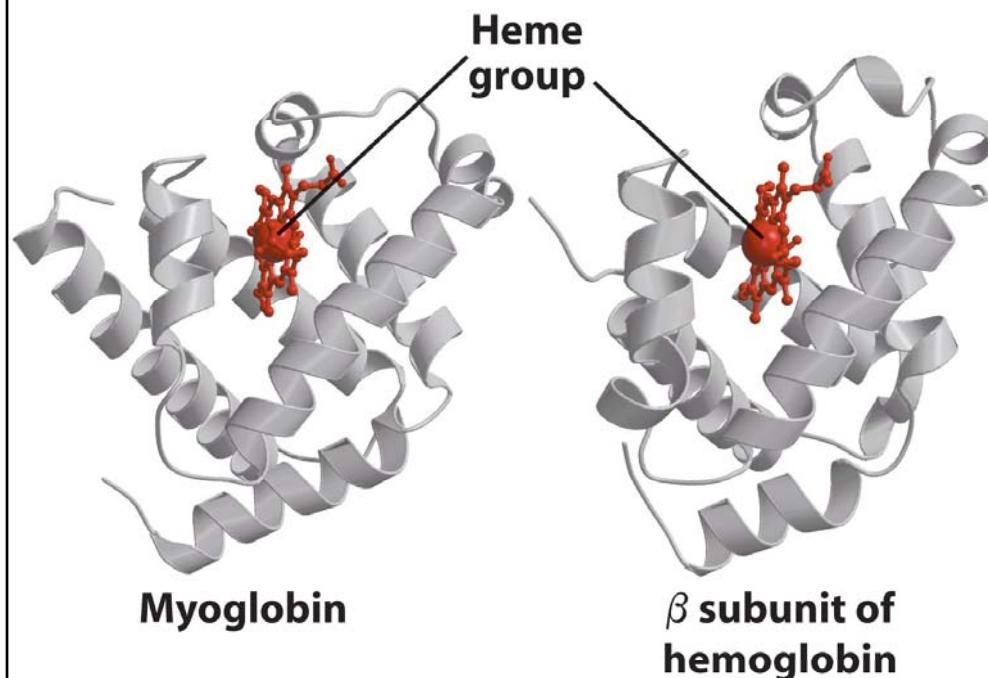
Protein component of Mb and Hb is globin

- Myoglobin is composed of **8 α helices**
- Heme prosthetic group binds oxygen
- **His-93 is complexed to the iron atom, and His-64 forms a hydrogen bond with oxygen**
- Interior of Mb contains almost all hydrophobic amino acids
- Heme occupies a hydrophobic cleft formed by three α helices and two loops

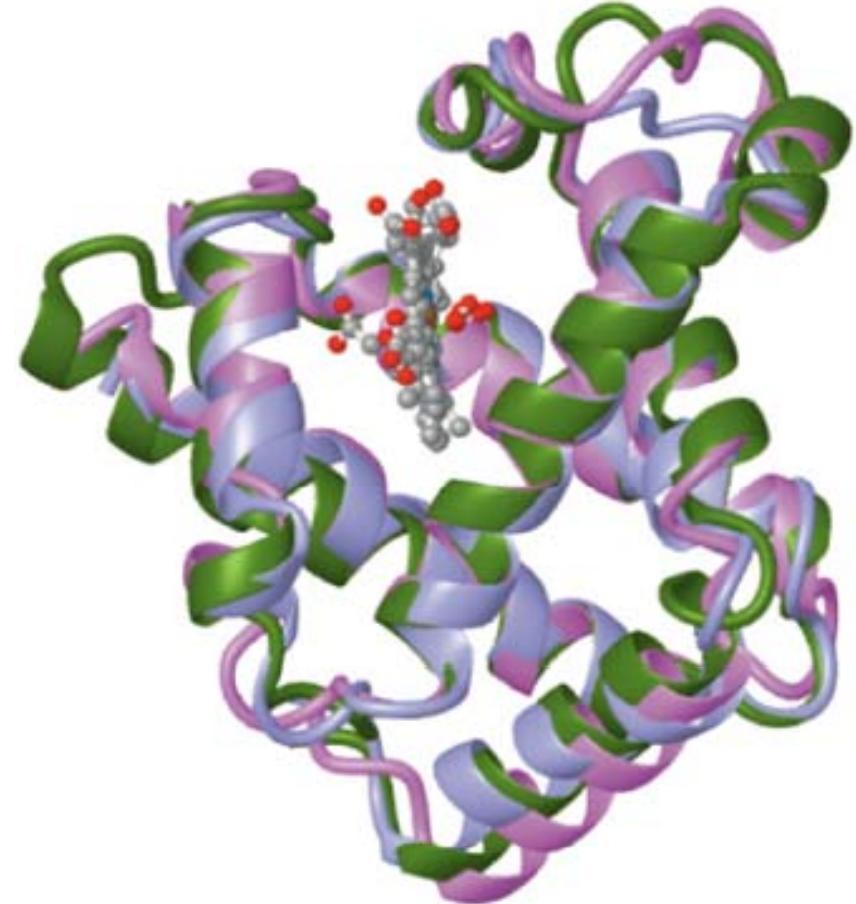
Hemoglobin (Hb)

- **Hb** is an $\alpha_2\beta_2$ **tetramer** (2 α globin subunits, 2 β globin subunits)
- **Each globin subunit is similar in structure to myoglobin**
- Each subunit has a heme group
- The α chain has 7 α helices, β chain has 8 α helices

Tertiary structures of myoglobin, α -hemoglobin and β -hemoglobin



Very similar



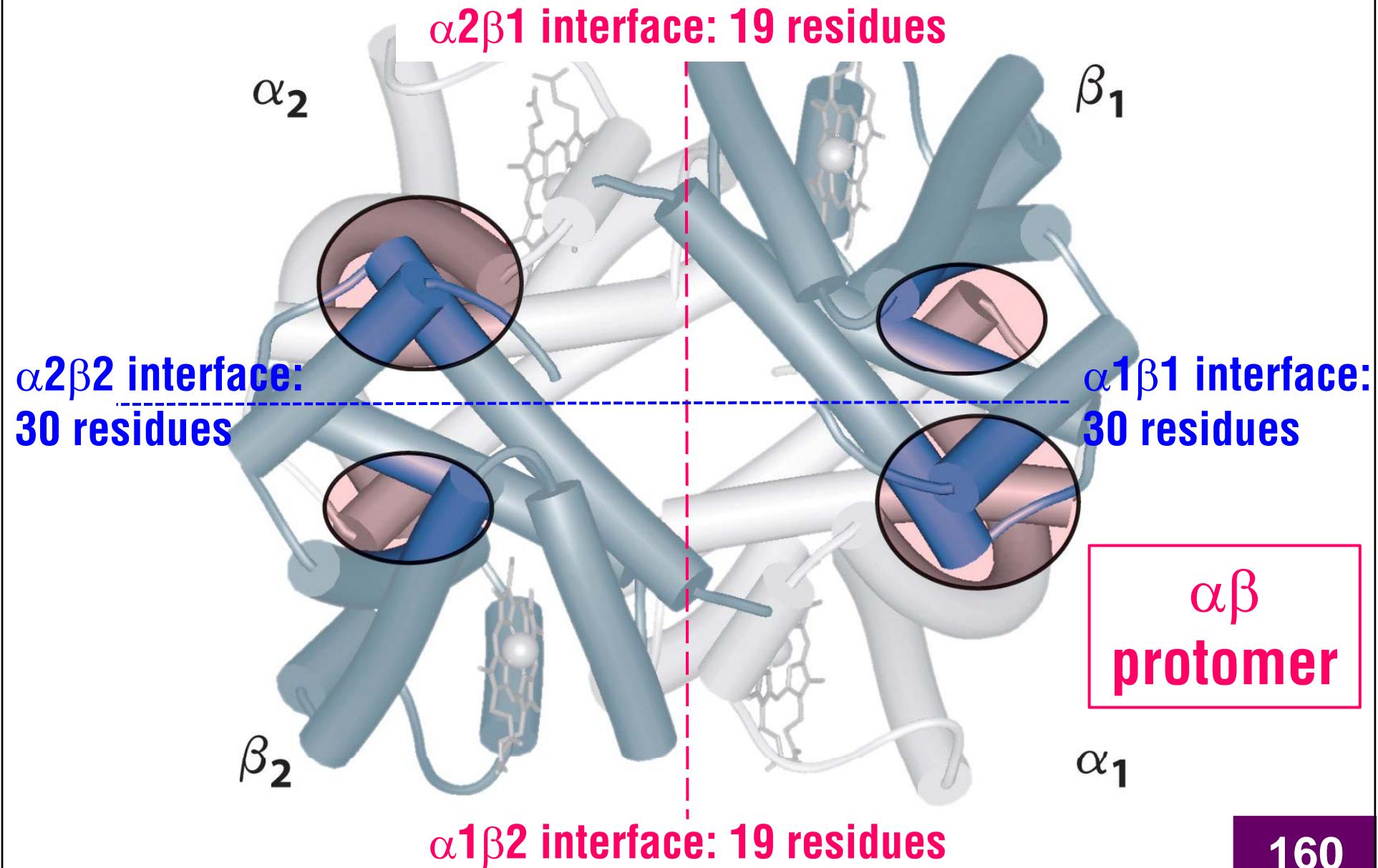
α -hemoglobin (blue)
 β -hemoglobin (purple)
Myoglobin (green)

The amino acid sequences of whale myoglobin and the a and b chains of human hemoglobin

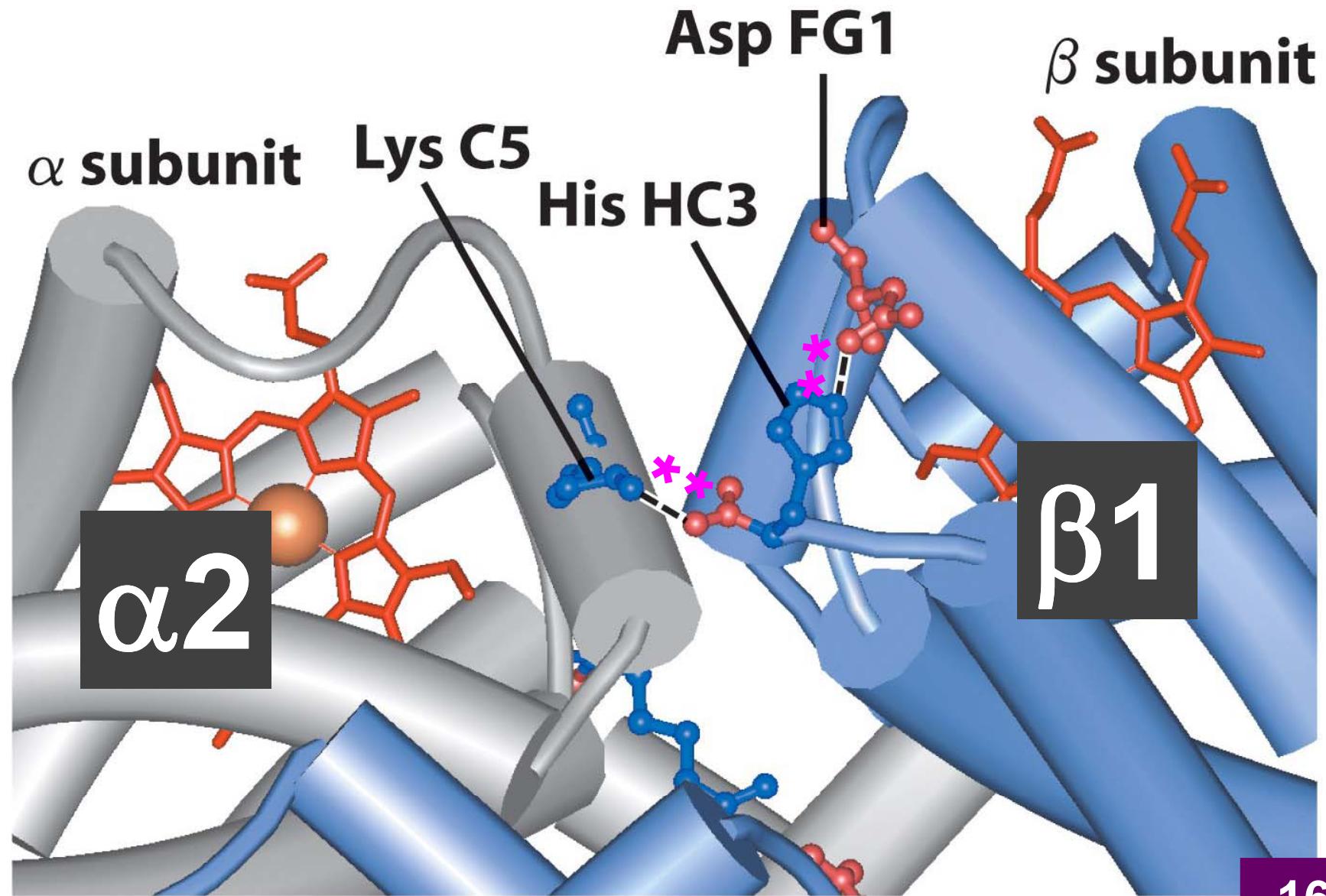
27 identical residues

	•	•	•		•	•	•
C1	---	H	F	Y	---	V	C
	P	P	P		V	T	V
	E	T	W		L	L	L
	T	T	T		H	A	A
40L	40K	Q		S	A	H	
E	T	40R		G	A	120K	
C7	---	K	Y	F	---	D	E
	F	F	F		F	F	F
	D	P	E		G	P	G
R	H	S		D	A	120P	
	F	F	F		F	F	
K	—	G		H	L	F	
H	D	D		H	A		
L	L	L		S	H		
K	S	S		A	L		
D1	---	T	H	T	---	Q	V
	E	—	P		G	A	A
A	—	D		A	S	A	
E	—	A		M	L	Y	
M	—	V		N	D	Q	
K	—	M		K	K	K	
D7	---	A	G	G	—	A	F
E1	---	S	S	N	—	L	L
	E	A	P		E	A	A
60D	Q	K		L	S	G	
L	V	60V		F	V	V	
	K	K	K	R	S	A	
	K	G	A	140K	T	N	
	H	H	H	D	V	140A	
Distal E7	---	G	G	I	L	L	
His		V	60K	A	T	A	
	T	K	K	K	K	K	Hb α
	V	V	V	Y	140Y	Y	and Hb β
	L	A	L	K	141R	146H	only
	T	D	G	—	—	—	
	A	A	A	E	—	—	
	L	L	F				
	G	T	S				
	A	N	D				
	I	A	G				
H21	---	A	S	H	—	—	—
	K	K	K	—	—	—	HC1
	Y	140Y	Y	—	—	—	HC2
	K	141R	146H	—	—	—	HC3
H26	---	L	—	—	—	—	—
	G	—	—	—	—	—	—
	Y	—	—	—	—	—	—
	Q	—	—	—	—	—	—

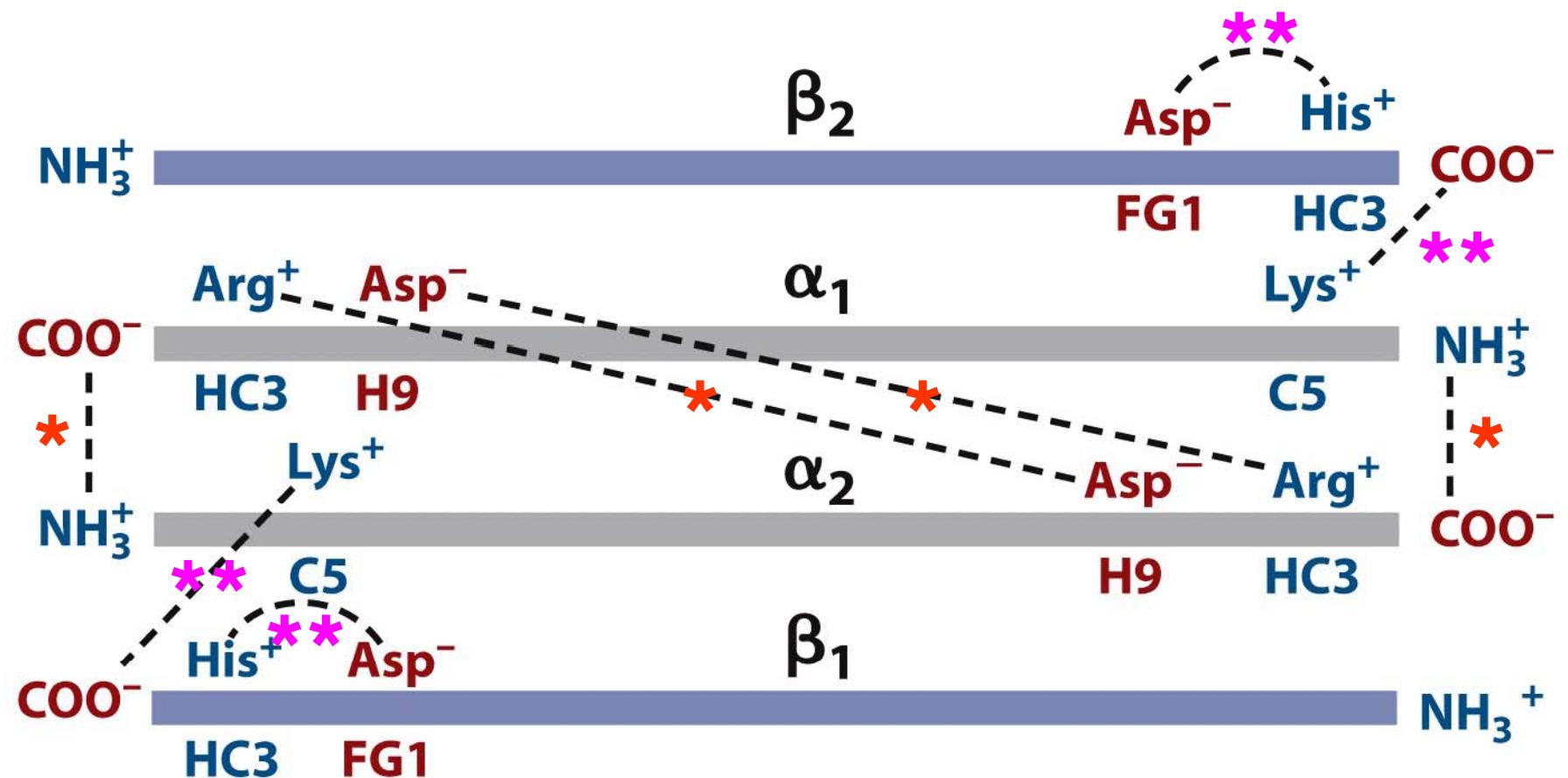
Dominant interactions between hemoglobin subunits



Some ion pairs between α 2 and β 1, and within β 1 that stabilize the T state of deoxyhemoglobin

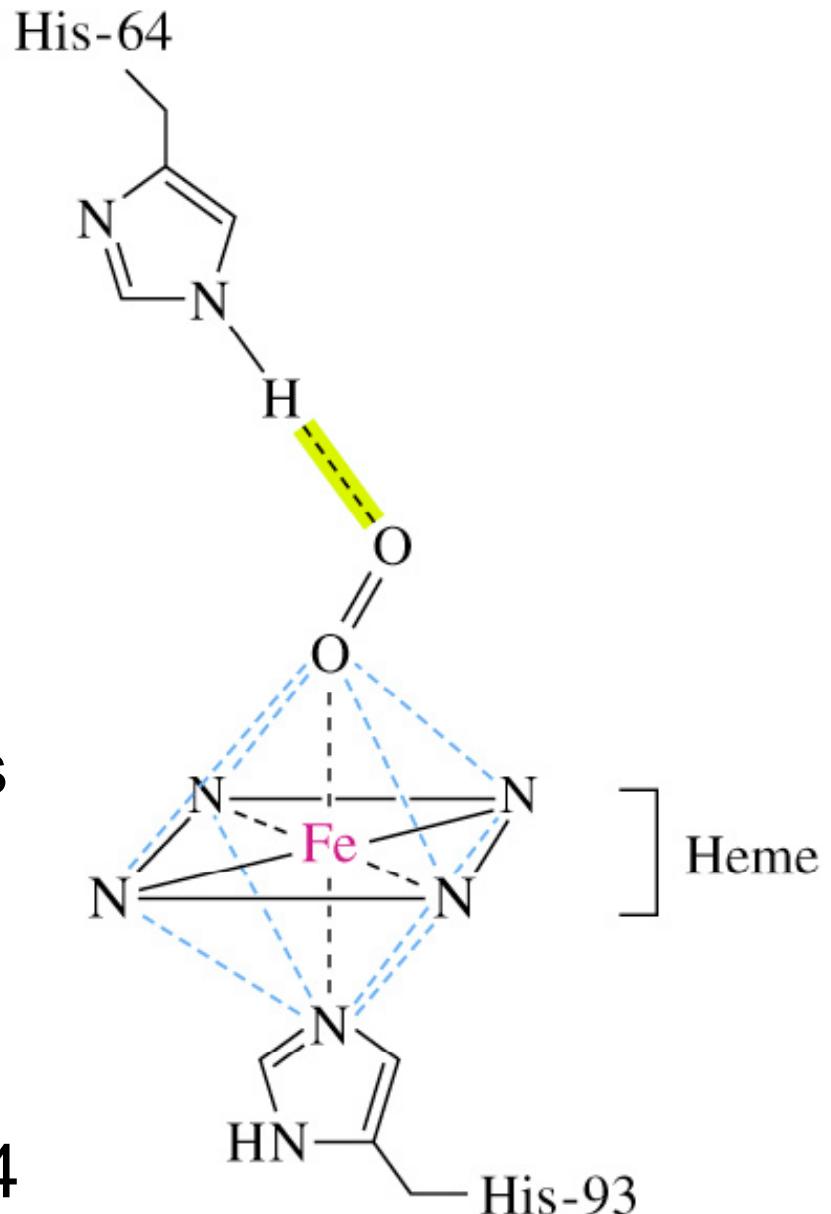


Some ion pairs that stabilize the T state of deoxyhemoglobin



Oxygen binds reversibly to heme

- **Oxymyoglobin** - oxygen bearing myoglobin
- **Deoxymyoglobin** - oxygen-free myoglobin
- In oxymyoglobin, six ligands are coordinated to the ferrous ion in octahedral symmetry
- Oxygen is coordinated between the iron and the imidazole side chain of His-64



Hemoglobin is an allosteric protein

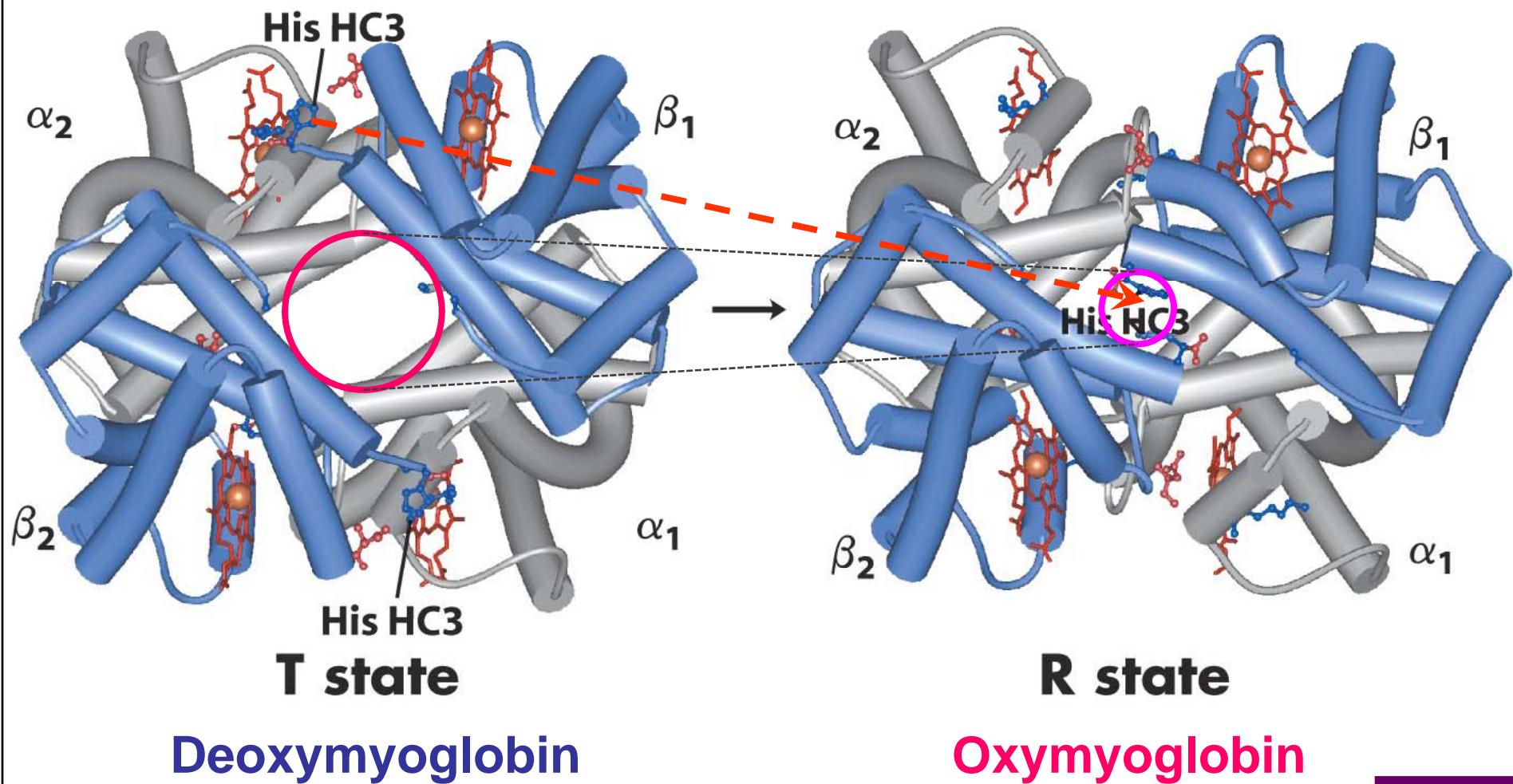
- Oxygen binding and release from Hb are regulated by **allosteric interactions**
- **Allosteric effectors (modulators)** bind to a protein **at a site separate from the functional binding site** (may be activators or inhibitors)
- An **allosteric protein** is one in which **the binding of a ligand to one site affects the binding properties of another site on the same protein**
- The activity of an **allosteric protein** is regulated by allosteric effectors

Two conformations of hemoglobin: T and R

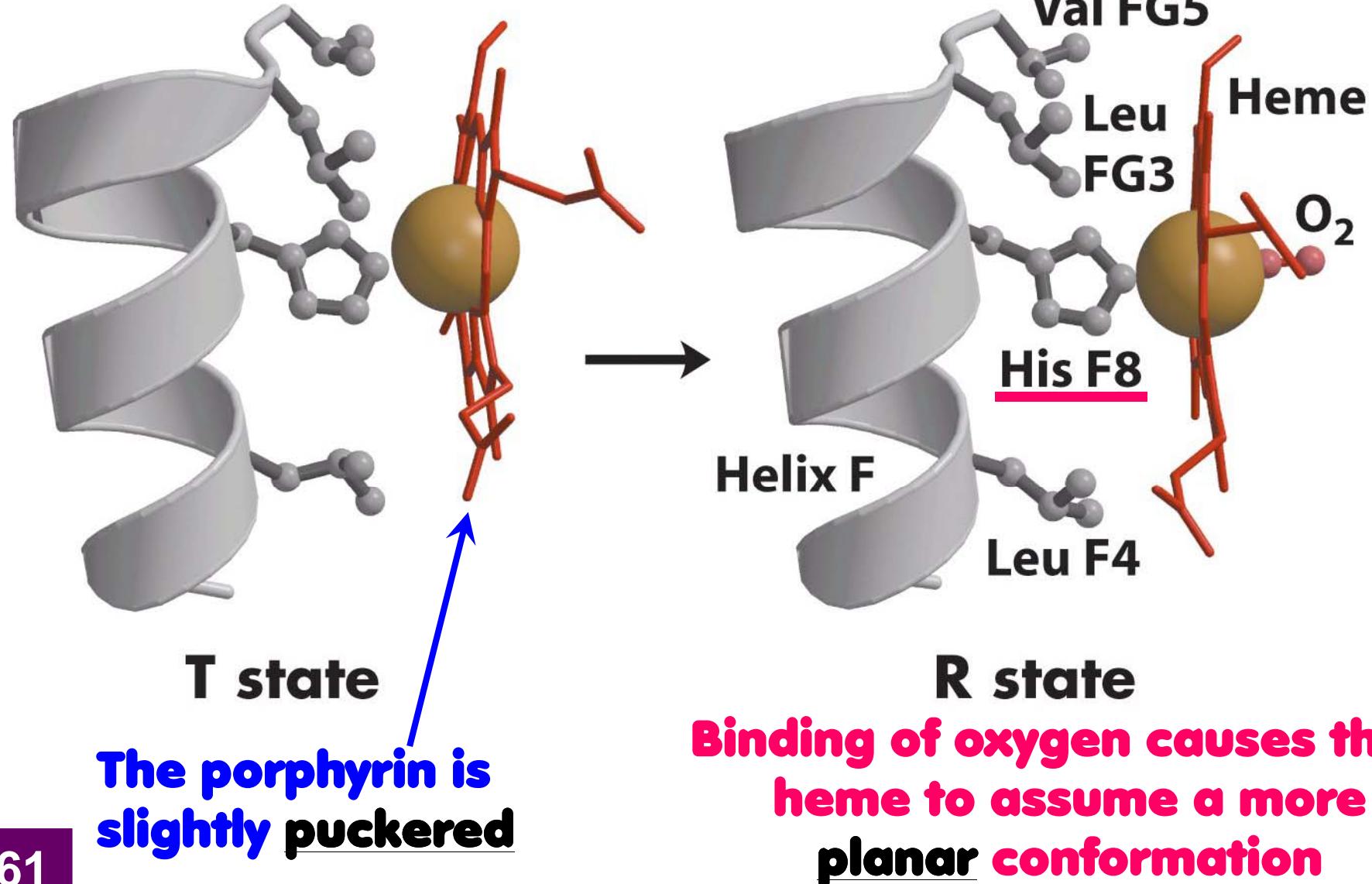
- Active (R state) and inactive (T state) forms are in rapid equilibrium in allosteric proteins
- Binding of substrates and allosteric activators stabilize the R state and shift the equilibrium in the R direction
- Allosteric inhibitors stabilize the T state and shift the equilibrium in the T direction

R: relaxed (high-affinity state) T: tense (low-affinity state)

The T → R transition

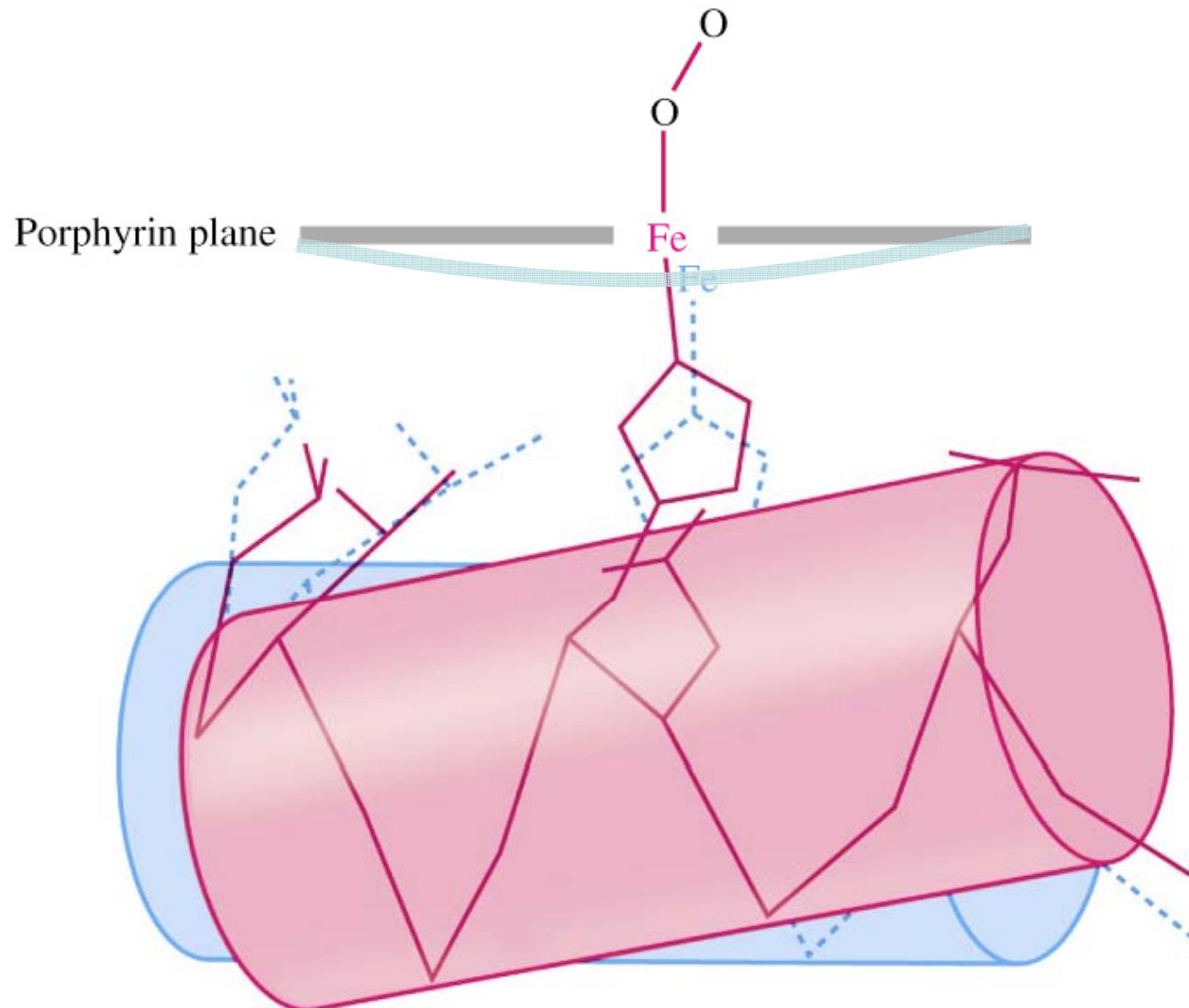


Changes in conformation near heme on O₂ binding to deoxyhemoglobin



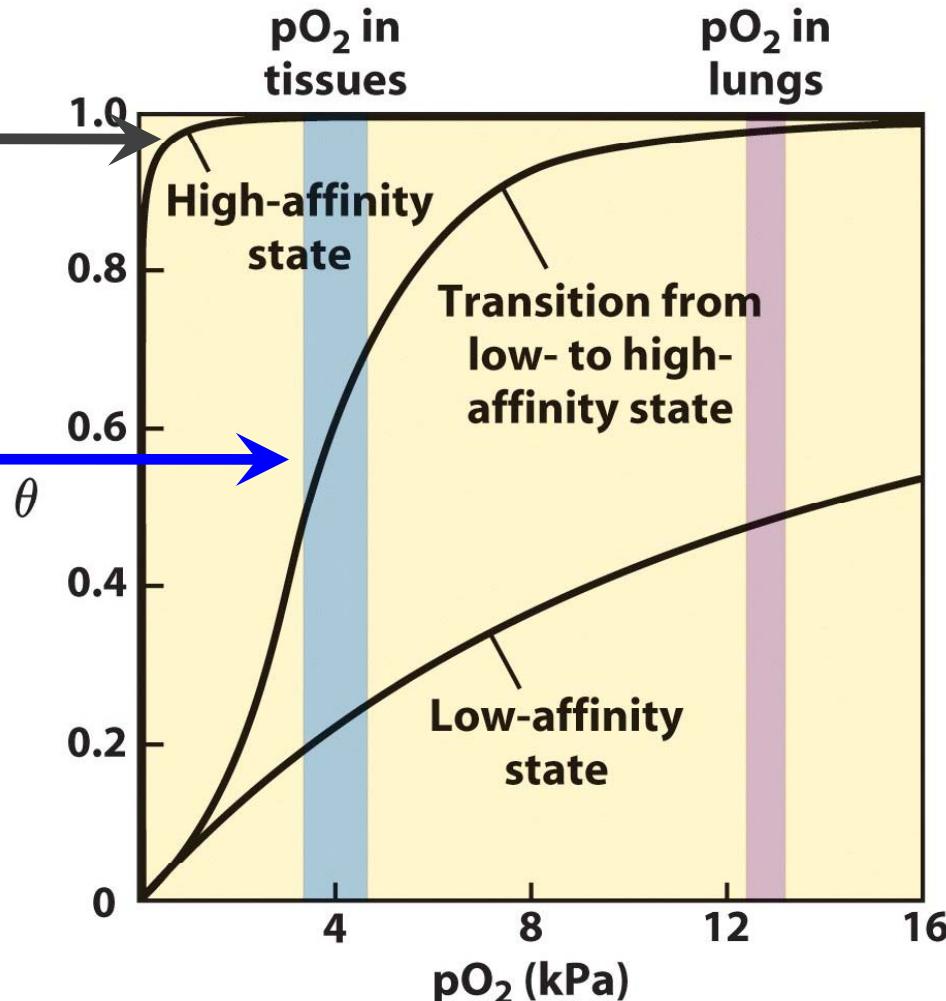
Conformational changes in a hemoglobin chain induced by oxygenation

- Oxygen binding to Fe pulls the His toward ring plane
- Helix with His shifts position, disrupting some ion pairs between subunits (blue to red position)



A sigmoid (cooperative) O₂ binding curve of Hb

Hyperbolic →
S shaped
θ: Fractional saturation



Positive cooperativity can be recognized by sigmoidal binding curves

Hemoglobin is more sensitive to the small differences in O₂ concentration between the tissues and the lungs

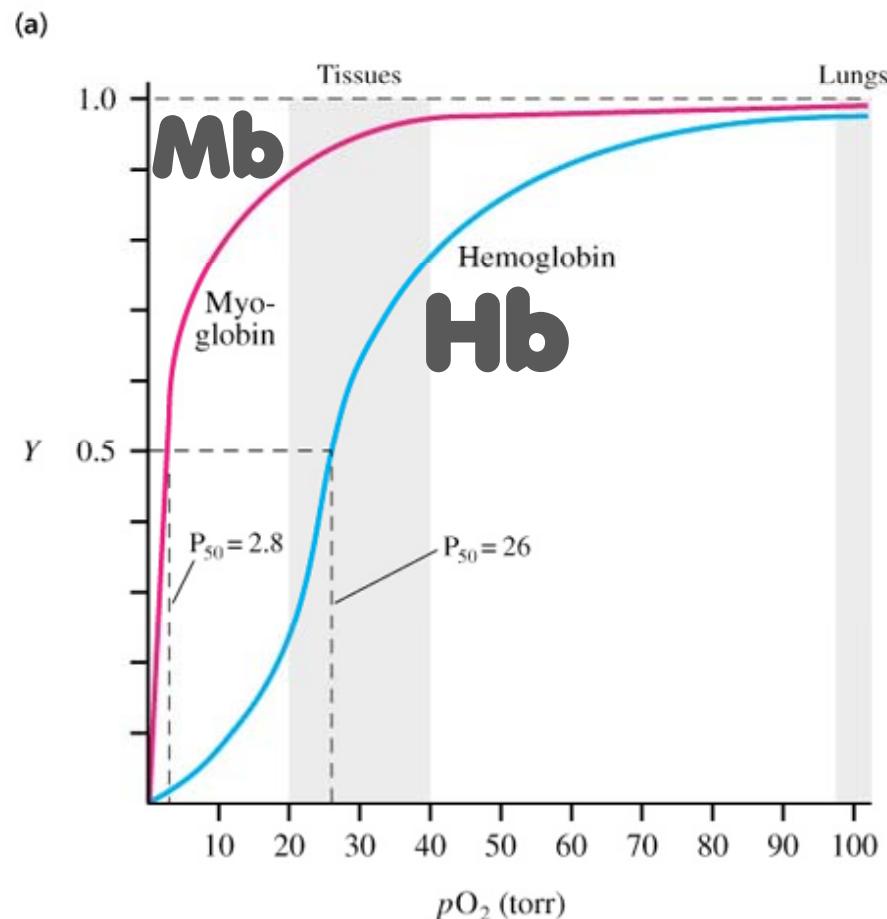
Spectroscopic Detection of Oxygen Binding to Myoglobin

- The heme group is a strong **chromophore** that absorbs both in ultraviolet and visible range
- Ferrous form (Fe^{2+}) **without oxygen** has an **intense Soret band at 429 nm**
- **Oxygen binding** alters the electronic properties of the heme, and **shifts the position of the Soret band to 414 nm**
- Binding of oxygen can be monitored by UV-Vis spectrophotometry
- **Deoxymyoglobin (in venous blood) appears purplish in color** and **oxymyoglobin (in arterial blood) is red**

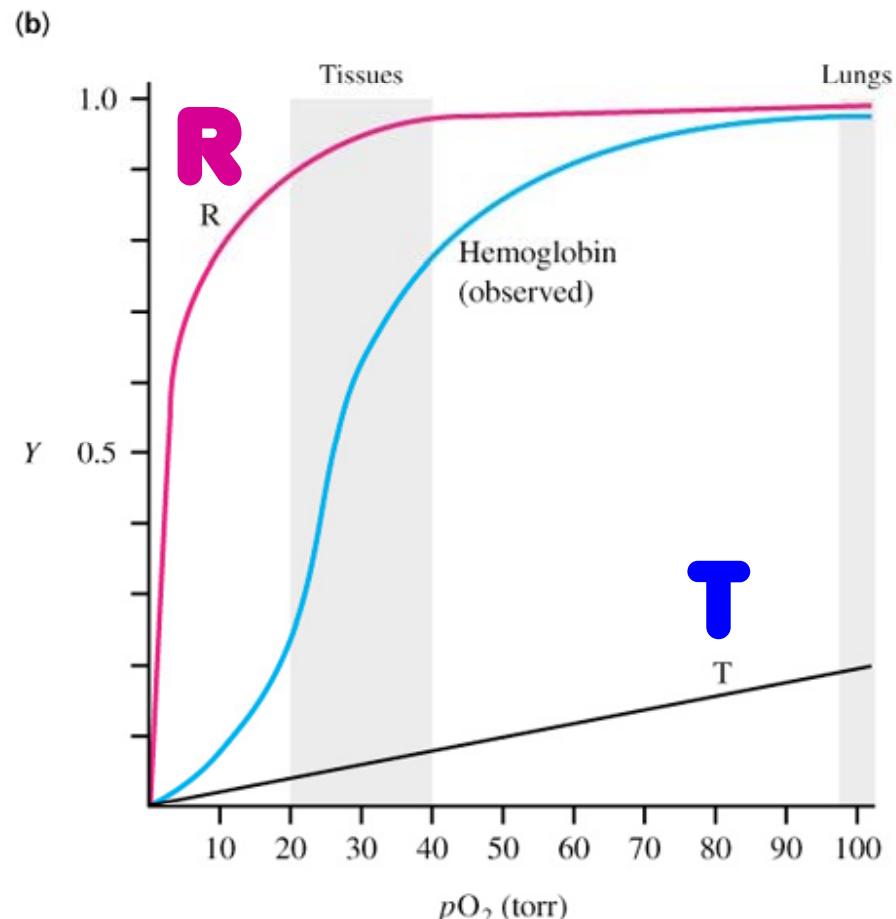
Soret band (named after its discoverer Jacques-Louis Soret): a very strong absorption band in the **blue wavelength region** of the optical absorption spectrum of a **heme protein**

Oxygen-binding curves

(a) Comparison of O₂-binding to Mb and Hb



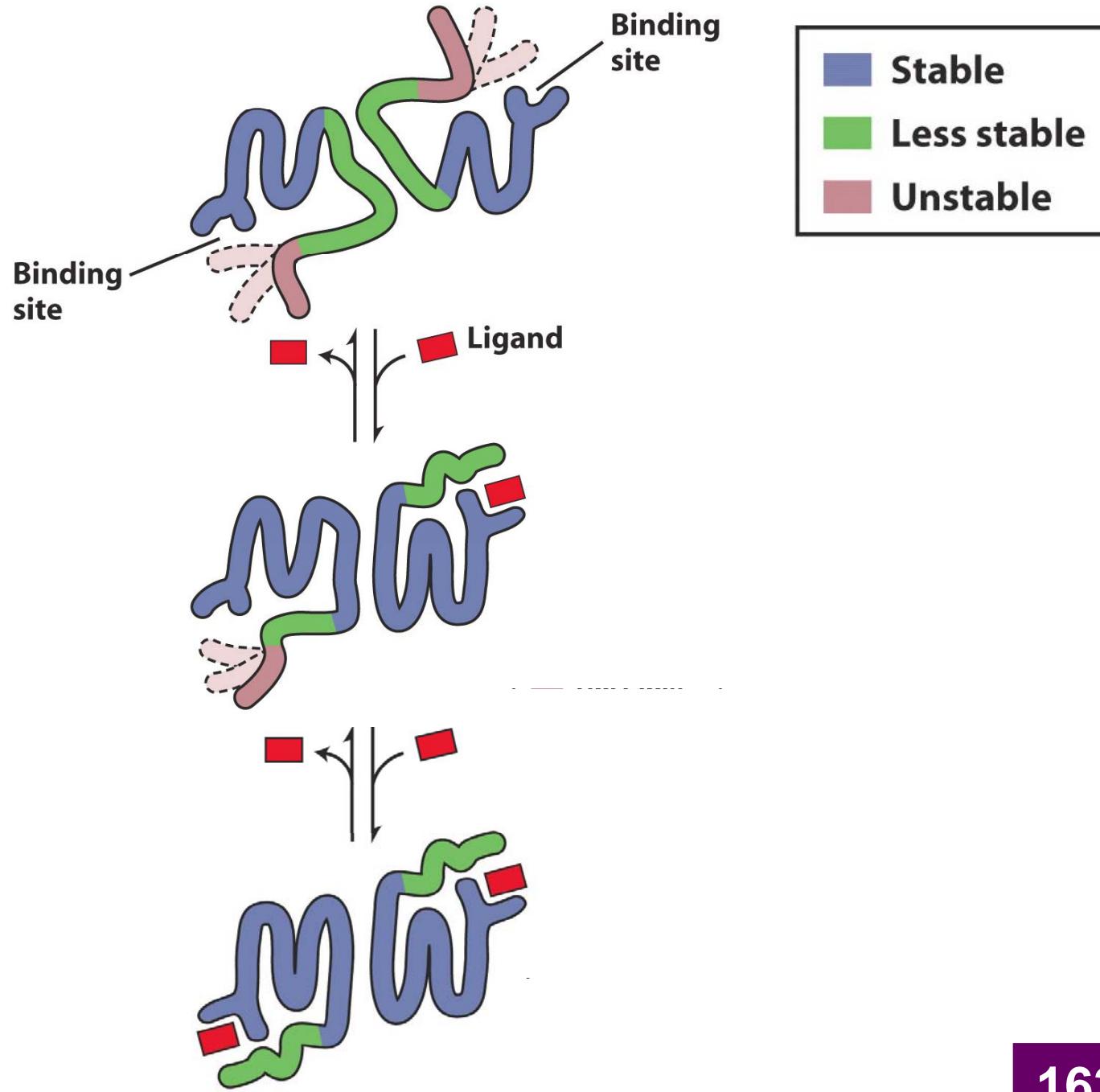
(b) Oxygen-binding curves of the R (high-affinity) and T (low affinity) forms of Hb



Oxygen-binding curves of myoglobin and hemoglobin

- Curves show reversible binding of O_2 to Mb and Hb
- The shape of the **Hb** curve shows a **positive cooperativity** in the binding of 4 O_2 molecules
- The O_2 affinity of Hb increases as each O_2 molecule is bound
- Mb- O_2 binding curve is hyperbolic, indicating a single equilibrium constant for binding O_2
- Hb- O_2 binding curve is sigmoidal, and reflects the binding of 4 molecules of O_2 , one per each heme group

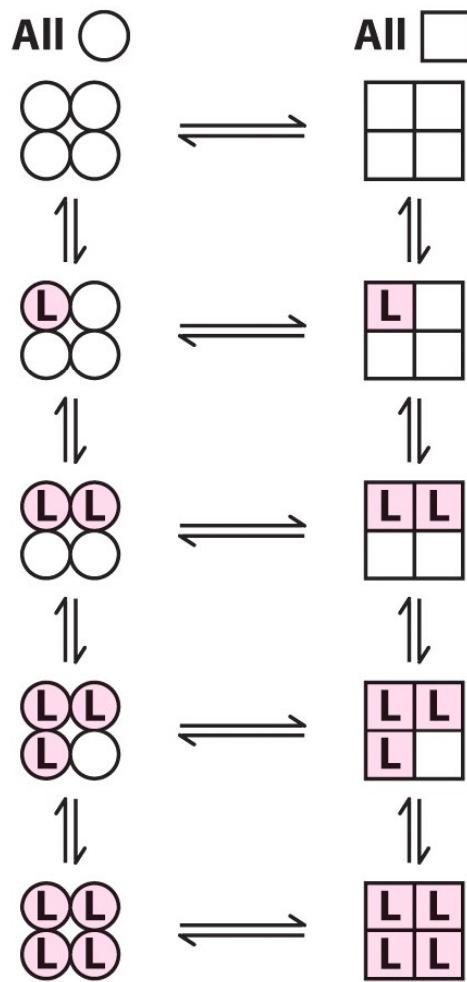
Structural changes in a multisubunit protein undergoing cooperative binding to ligand



Two models suggest mechanisms for cooperative binding

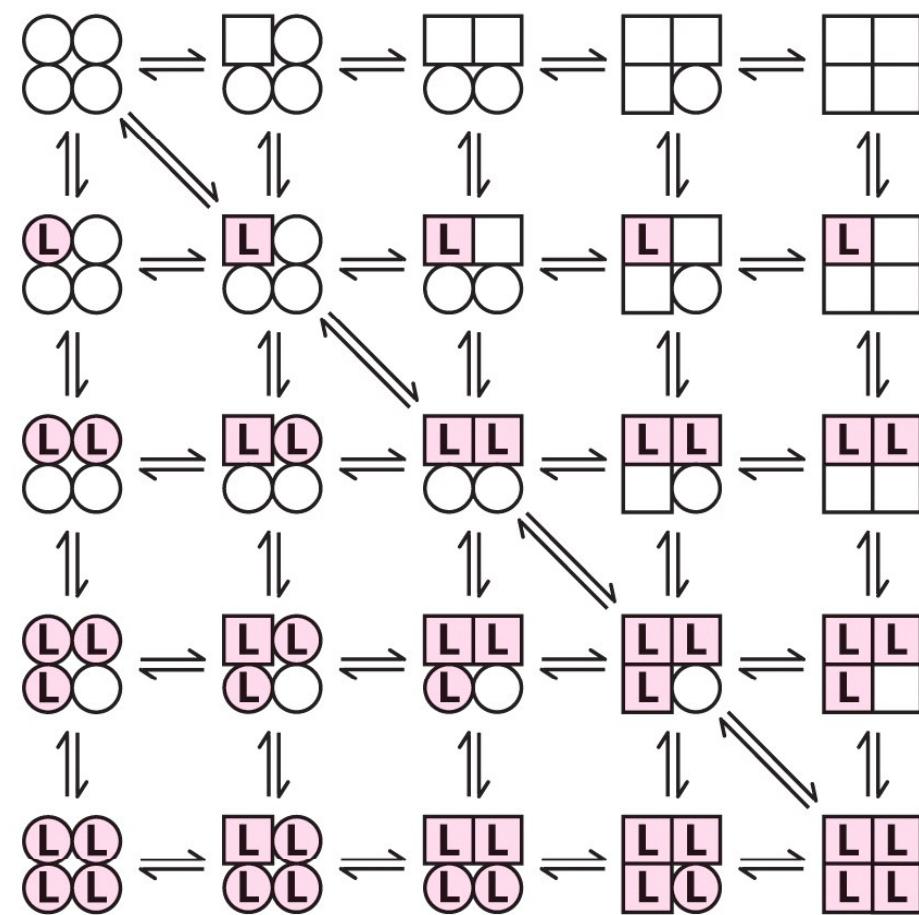
MWC/Concerted model

Proposed in 1965 by Monod, Wyman and Changeux



(a)

Sequential model (more intermediate states)

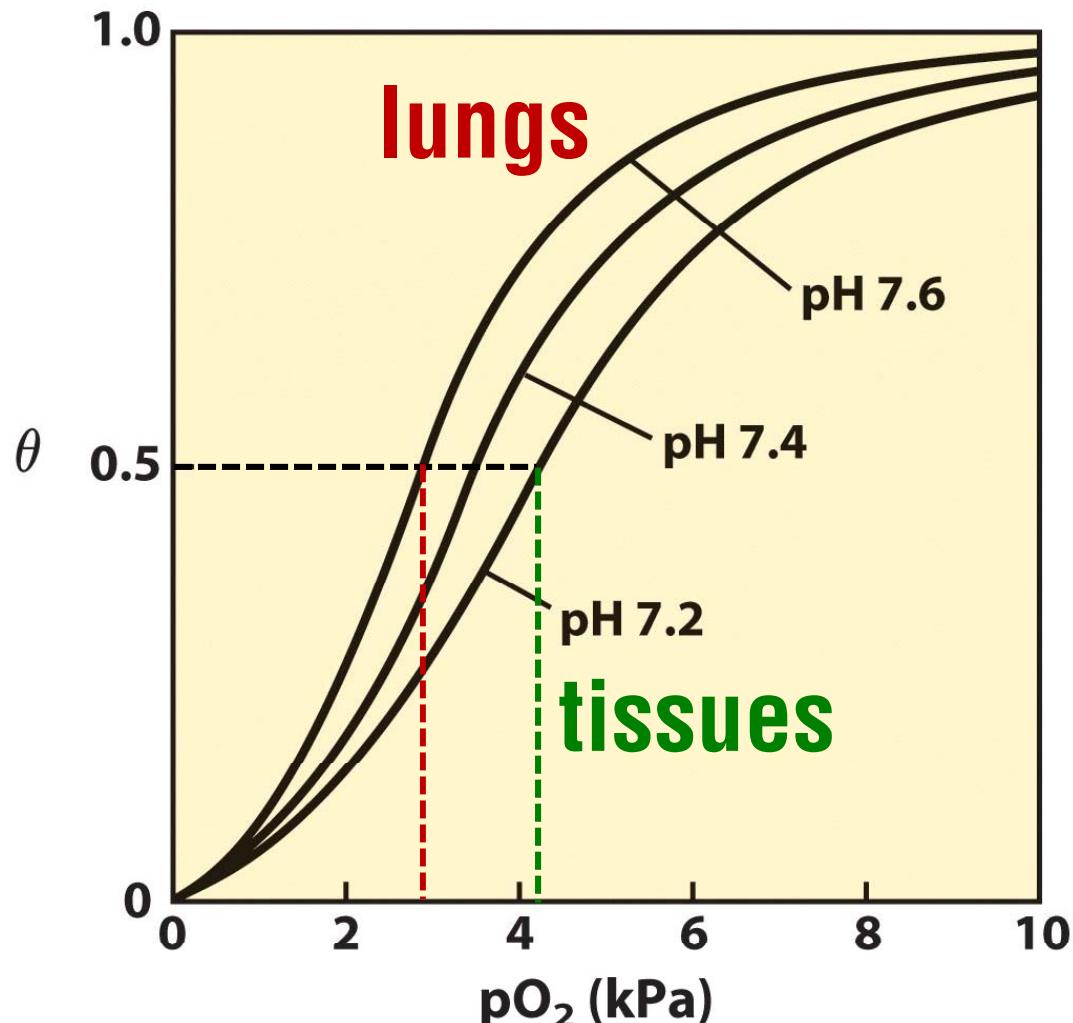


(b)

Proposed in 1966 by Daniel Koshland

Effect of pH on oxygen binding to Hb

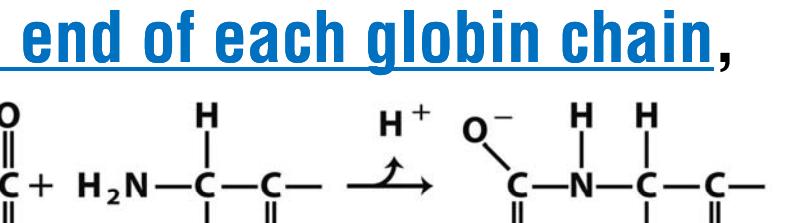
- Lowering the pH decreases the affinity of Hb for oxygen

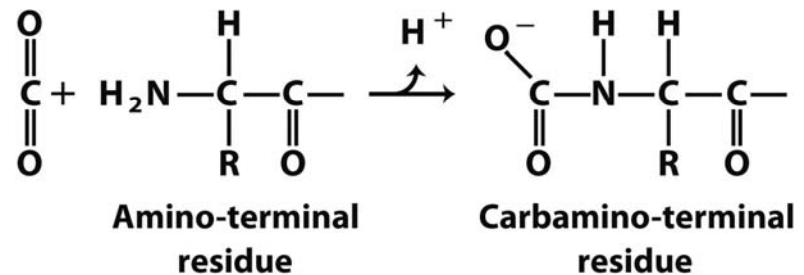


- The effect of pH and CO_2 concentration on the binding and release of O_2 by hemoglobin is called **Bohr effect**, first described in 1904 by Christian Bohr.
- The pH difference between lungs and metabolic tissues increases the O_2 transfer efficiency of Hb.

Hb transports 40% of total H^+ and 15~20% of CO_2 formed in the tissues to the lungs and kidneys.

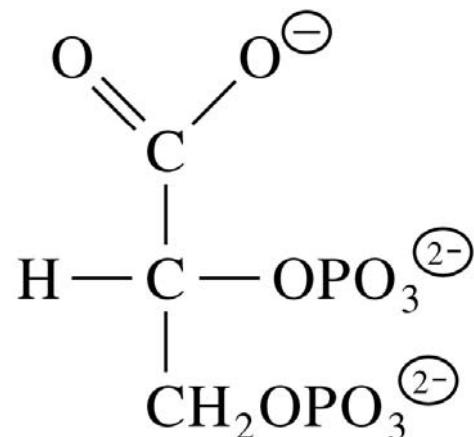
Hemoglobin also transports H⁺ and CO₂

- The CO₂, produced by oxidation of organic fuels in mitochondria, is hydrated to form **bicarbonate**: $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$
 - This reaction is catalyzed by **carbonic anhydrase**.
 - This effect of pH and CO₂ concentration on the binding and release of oxygen by hemoglobin is called the **Bohr effect**.
 - A major contribution to the Bohr effect is made by **protonated His¹⁴⁶ of the β subunits, forming ion pair with Asp⁹⁴**.
 - Hemoglobin also binds CO₂. **Carbon dioxide binds as a carbamate group to the N-terminal end of each globin chain**, forming carbaminohemoglobin.
 - H⁺ binds to several residues.



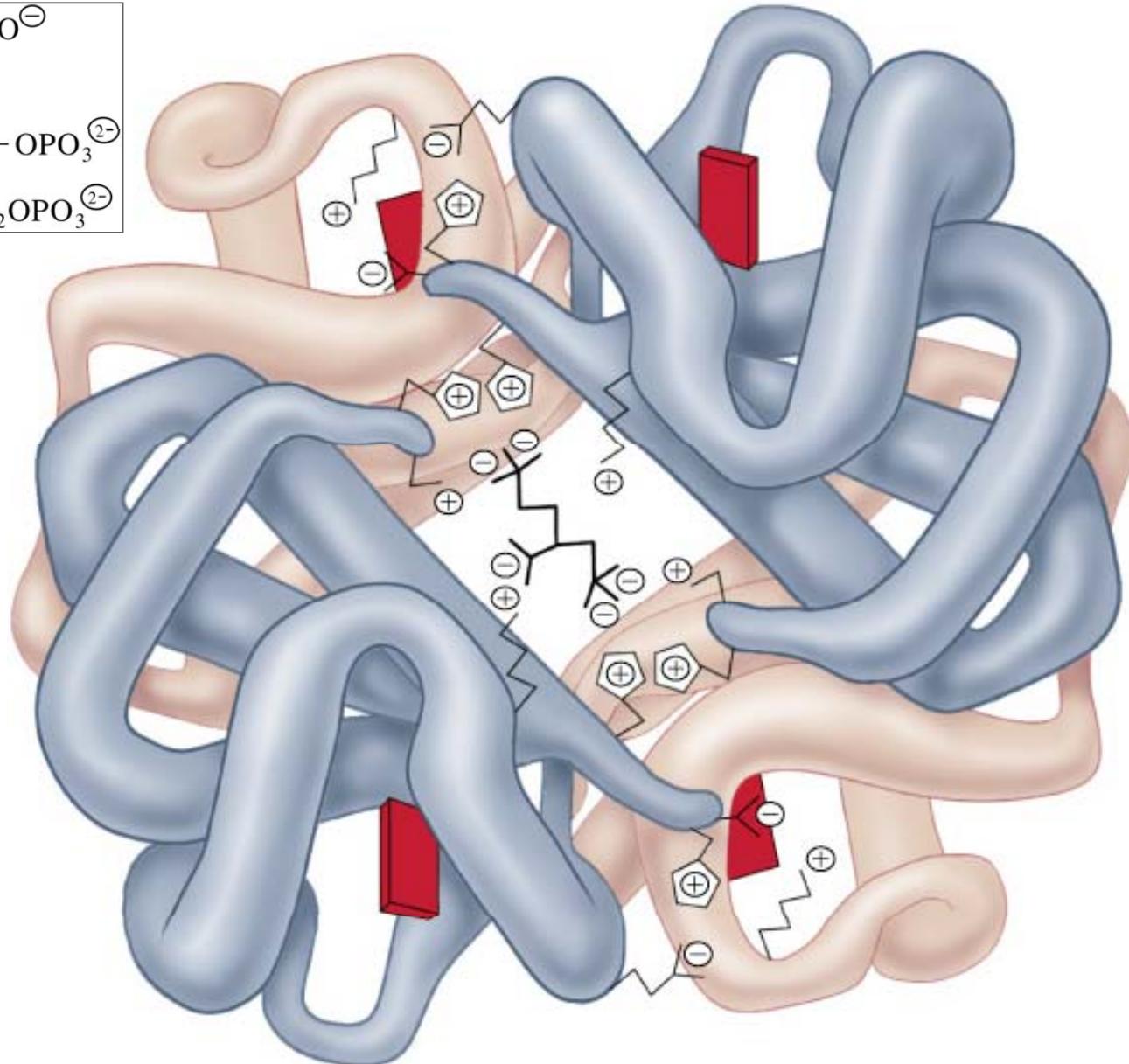
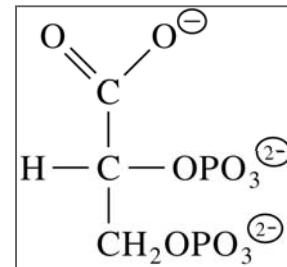
Oxygen binding to hemoglobin is regulated by 2,3-bisphosphoglycerate (2,3BPG)

- 2,3BPG is an allosteric effector of Hb
- 2,3BPG **lowers** the affinity of deoxyHb for oxygen (raises the P_{50} of Hb from ~12 to ~26 torr)
- Negatively charged 2,3BPG is bound to six (+) charged residues of deoxyhemoglobin

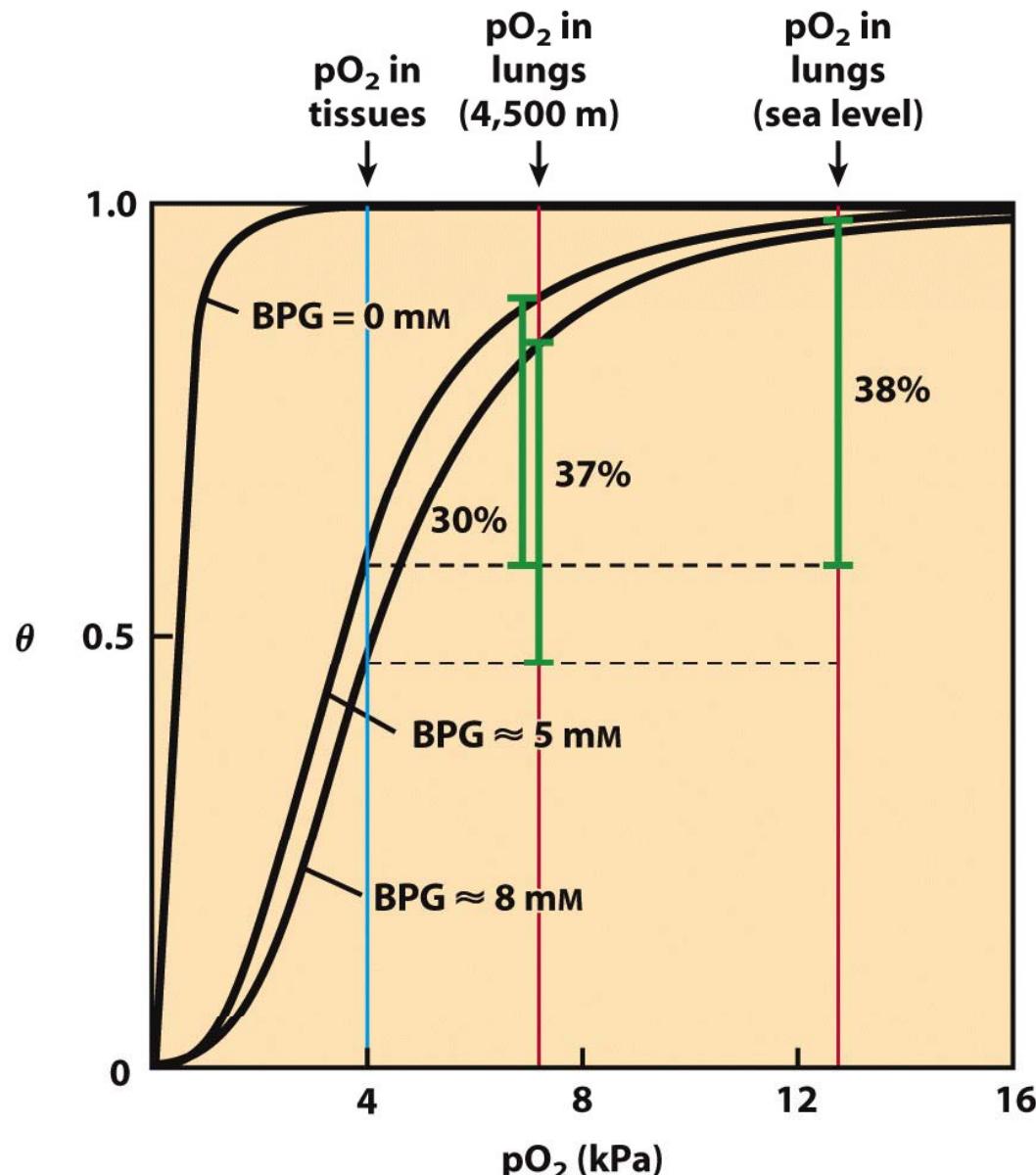


Binding of 2,3BPG to deoxyhemoglobin

- Negative charges on 2,3BPG pair with positive-charged side chains lining the central cavity of Hb, stabilizing the DeoxyHb form



Effect of BPG on oxygen binding to deoxyHb



The BPG concentration in normal human blood is about 5 mM at sea level and about 8 mM at high altitudes.

2,3BPG lowers
the affinity of
deoxyHb for
oxygen

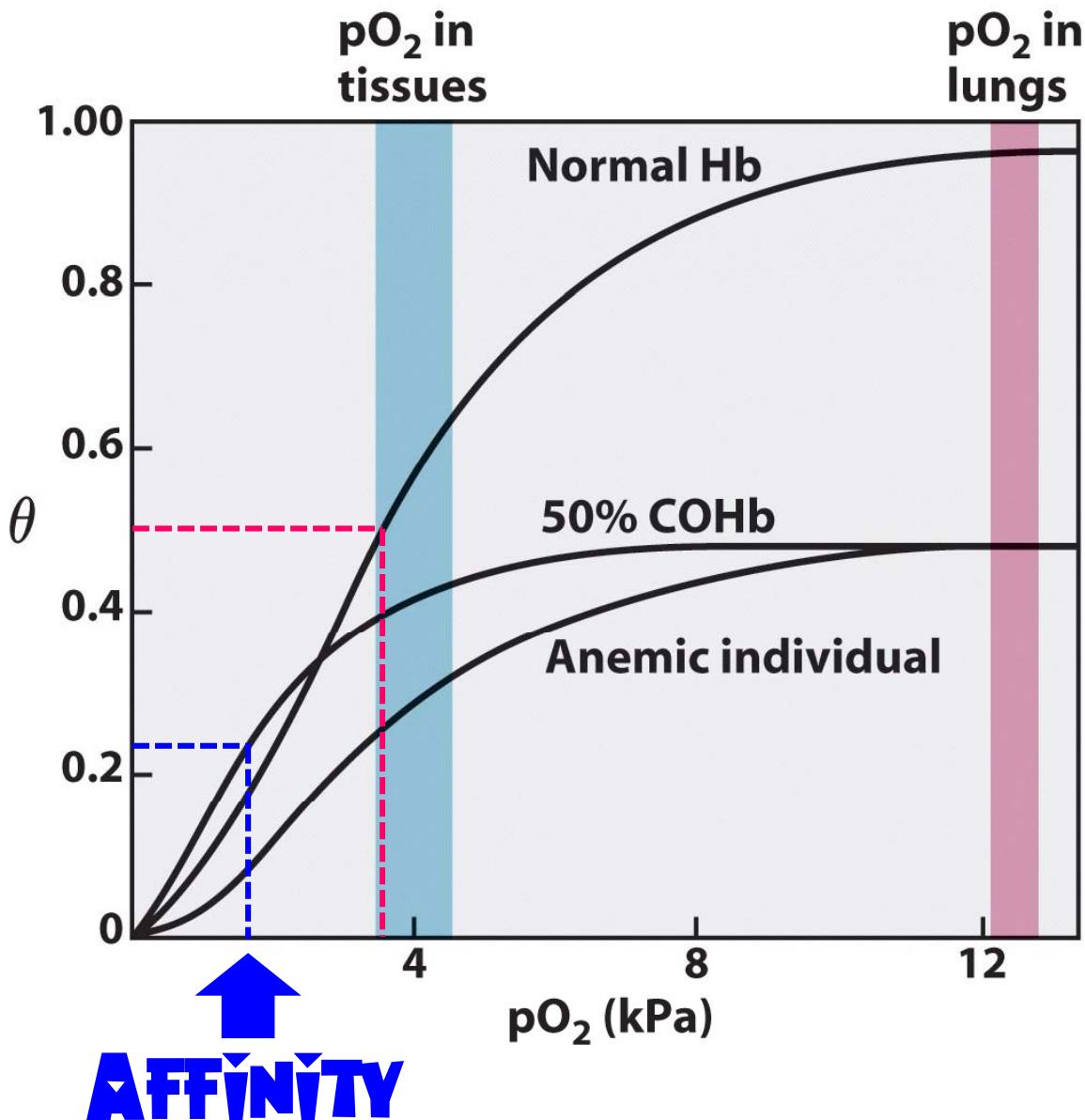
Oxygen binding to hemoglobin is regulated by 2,3-bisphosphoglycerate (2,3BPG)

- The BPG concentration in erythrocytes also increases in people suffering from hypoxia.
- The interaction of BPG with hemoglobin molecules further refines the function of hemoglobin, and provides an example of heterotropic allosteric modulation.
- The site of BPG binding to hemoglobin is the cavity between the subunits in the T state.
- Only one molecule of BPG is bound to each Hb tetramer.
- Regulation of oxygen binding to hemoglobin by BPG has an important role in fetal development. The fetus synthesizes γ subunits rather than β subunits, forming $\alpha_2\gamma_2$ hemoglobins. $\alpha_2\gamma_2$ has a much lower affinity for BPG than normal hemoglobin, and a corresponding higher affinity for oxygen.

Binding of carbon monoxide to Hb

- CO has similar size and shape to O₂; it can fit to the same binding site
- CO binds over 20,000 times better than O₂ because the carbon in CO has a filled lone electron pair that can be donated to vacant d-orbitals on the Fe²⁺
- Protein pocket decreases affinity for CO, but is still binds about 250 times better than oxygen
- CO is highly toxic as it competes with oxygen. It blocks the function of myoglobin, hemoglobin, and mitochondrial cytochromes that are involved in oxidative phosphorylation

Binding of carbon monoxide to Hb



- **Binding of CO increases the affinity of Hb for oxygen**
- **Fetal $\alpha_2\gamma_2$ Hb has a higher affinity for CO than adult Hb**

Interaction with other molecules

- A **ligand** binds at a site on the protein called the **binding site**, which is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character. Ligand binds via non-covalent forces, which enables the interactions to be transient.
- The binding of a protein and ligand is often coupled to a conformation change in the protein that makes the binding site more complementary, permitting tighter binding. The structural adaption that occurs between protein and ligand is called induced fit.
- The molecules acted upon by enzymes are called reaction substrates rather than ligands, and the ligand-binding site is called catalytic site or active site

Models for enzyme-substrate interaction

Lock and key model



Emil Fischer, Nobel Prize for Chemistry in 1902
For his work on sugar and purine syntheses

- Enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. Like a *key* into a *lock*, only the correct size and shape of the substrate (*the key*) would fit into the active site (*the key hole*) of the enzyme (*the lock*)
- First postulated by Emil Fischer in 1894 shows the high specificity of enzymes.
However, it does not explain the stabilization of the transition state that the enzymes achieve

Induced fit model

- Only the proper substrate is capable of inducing the proper alignment of the active site that will enable the enzyme to perform its catalytic function. It suggests that the active site continues to change until the substrate is completely bound to it, at which point the final shape and charge is determined.
- First suggested by Daniel Koshland in 1958, is the **more accepted model** for enzyme-substrate complex than the lock-and-key model. Unlike the lock-and-key model, **the induced fit model shows that enzymes are rather flexible structures in which the active site continually reshapes by its interactions with the substrate until the time the substrate is completely bound to it.**

APPLICATION OF A THEORY OF ENZYME SPECIFICITY TO PROTEIN
SYNTHESIS*

BY D. E. KOSHLAND, JR.†

BIOLOGY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY, UPTON, NEW YORK

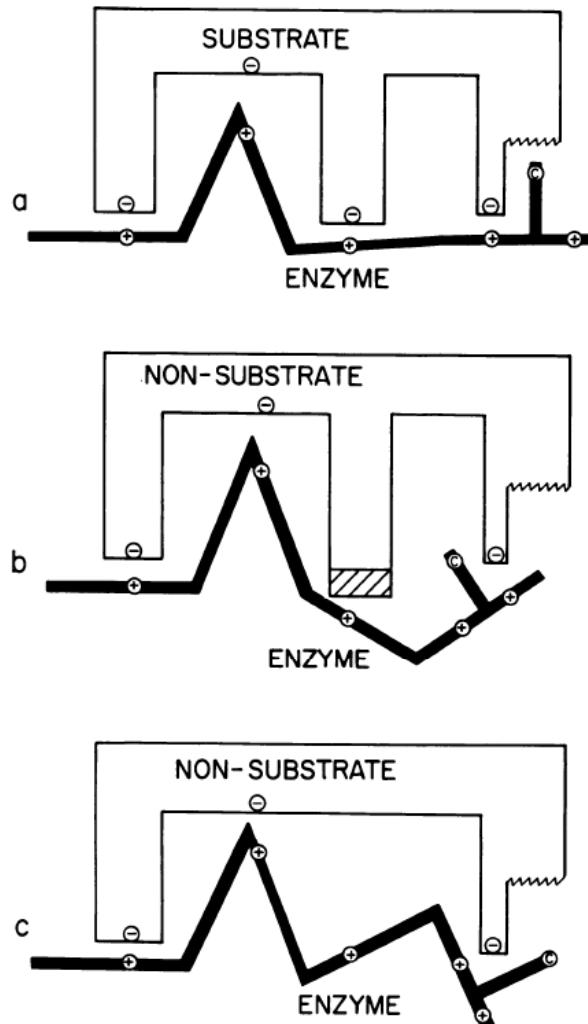
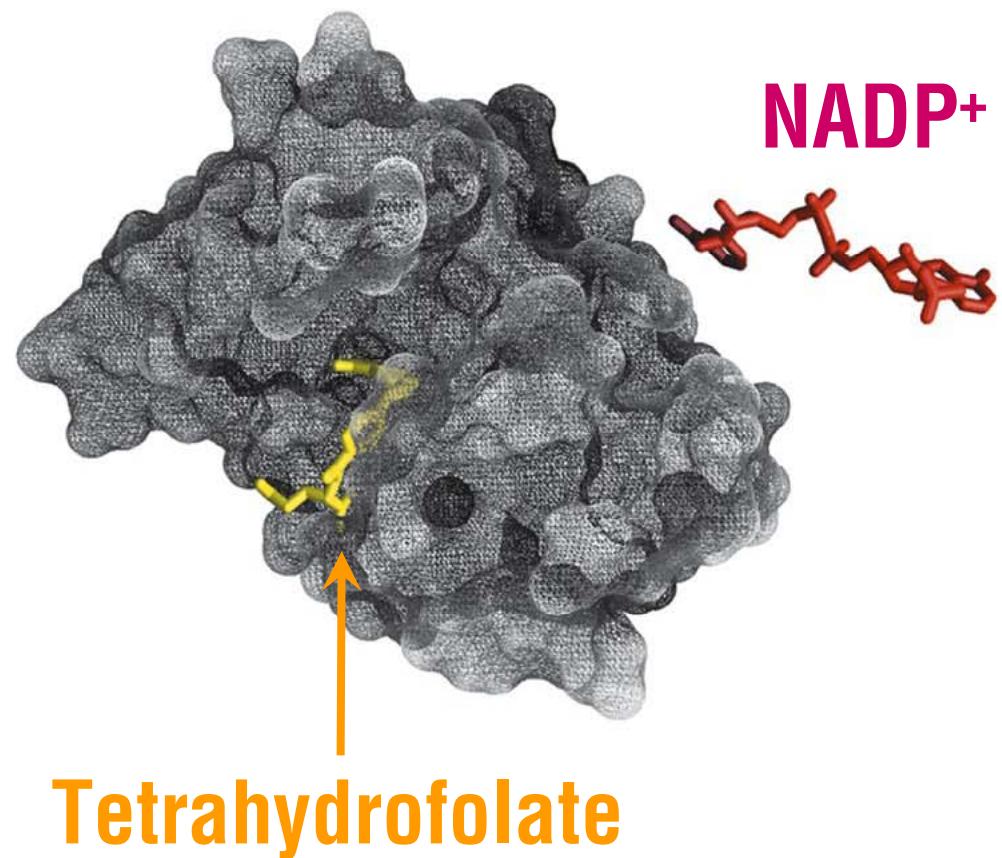
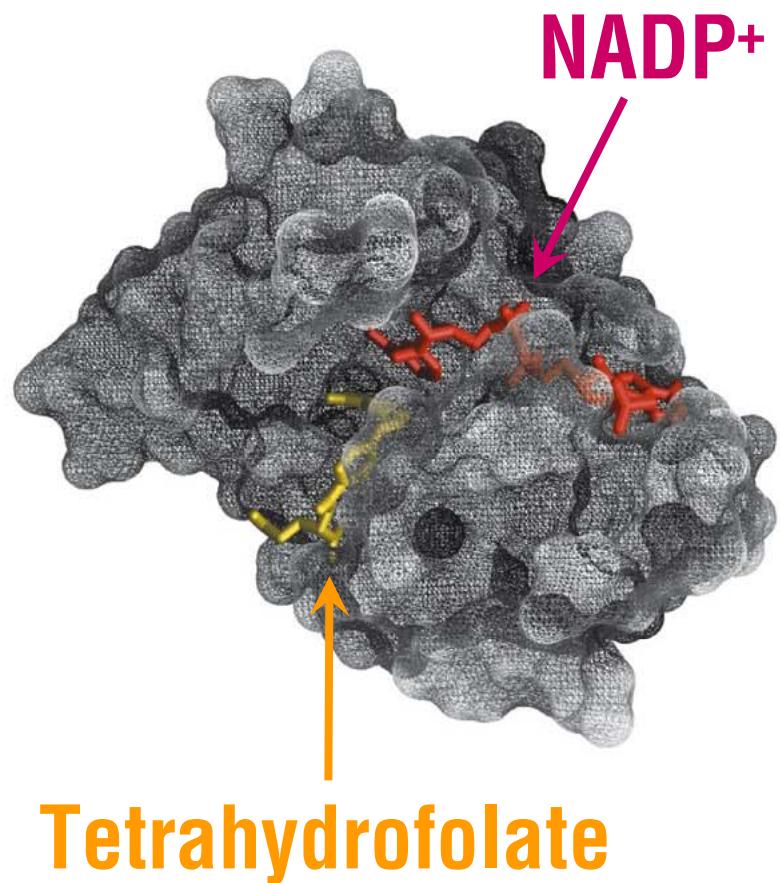


FIG. 1.—Interaction of enzyme with (a) substrate, (b) compound too large to be substrate, and (c) compound too small to be substrate. Circled pluses and minuses indicate any mutually attractive groups on enzyme and substrate. Circled C stands for catalytic group, and jagged line for bond to be broken.

The explanation that we suggest to explain these phenomena is as follows: (a) a precise orientation of catalytic groups is required for enzyme action; (b) the substrate may cause an appreciable change in the three-dimensional relationship of the amino acids at the active site; and (c) the changes in protein structure caused by a substrate will bring the catalytic groups into the proper orientation for reaction, whereas a non-substrate will not. This set of postulates has been called "the induced fit" theory for brevity and to emphasize that, while the idea of a fit is retained from the key-lock theory, the fit in this case occurs *only after* the changes induced by the substrate itself.

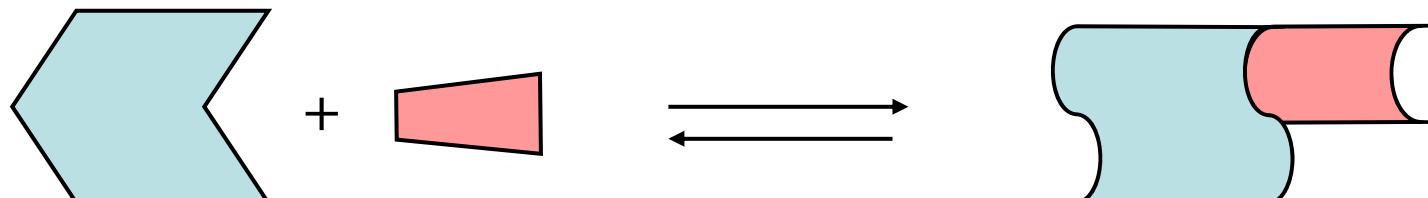
Emil Fischer's “lock and key” hypothesis

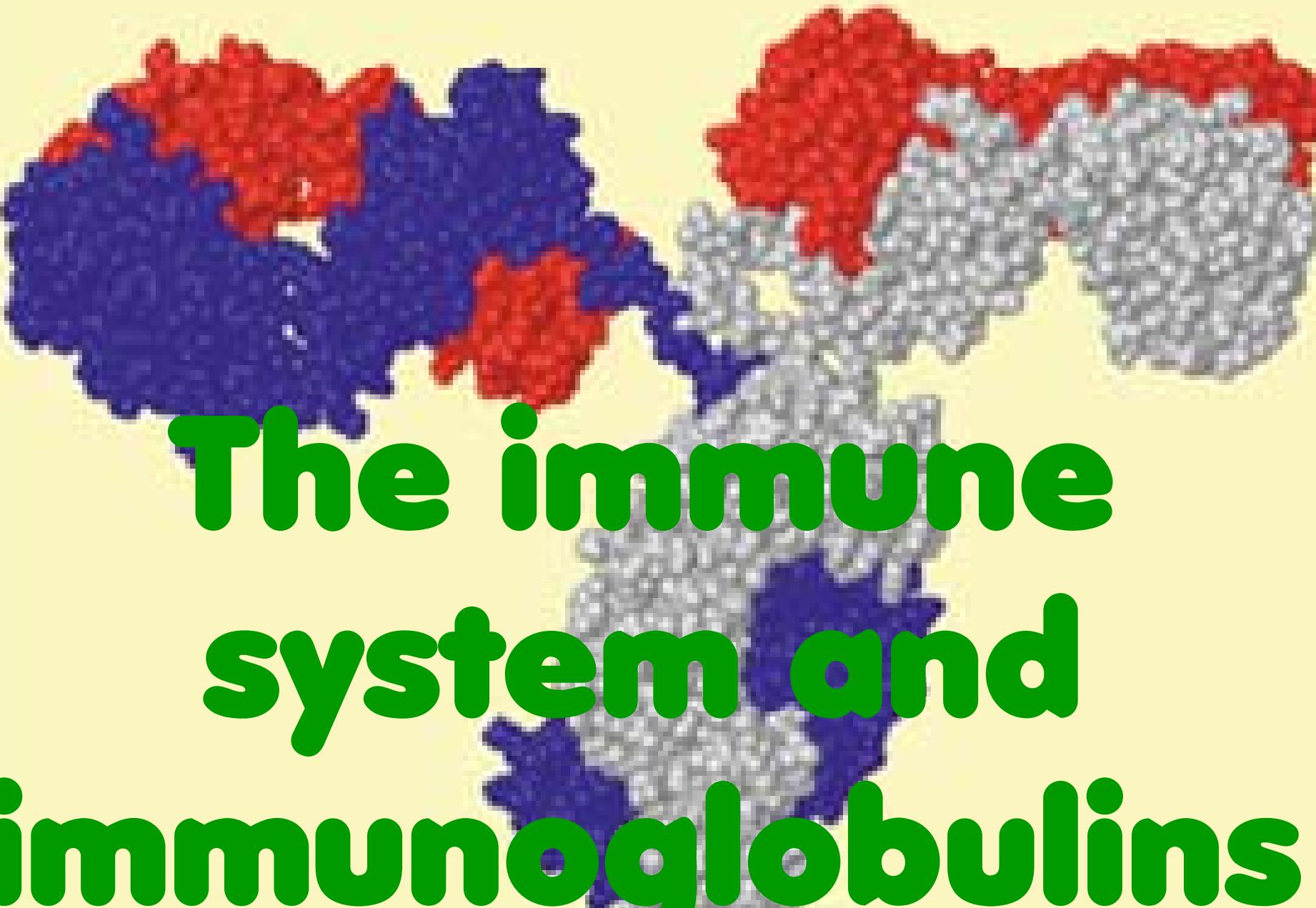
Dihydrofolate reductase with its substrate NADP⁺ (red), unbound (right) and bound (left); another bound substrate, tetrahydrofolate (yellow)



Induced fit model

- Conformational changes may occur upon ligand binding (Daniel Koshland in 1958).
 - This adaptation is called the **induced fit**.
 - Induced fit allows for tighter binding of the ligand
 - **Induced fit can increase the affinity of the protein for a second ligand**
- Both the ligand and the protein can change their conformations





The immune system and immunoglobulins

TABLE 5–2**Some Types of Leukocytes Associated with the Immune System**

Bone marrow
Thymus

Cell type	Function
Macrophages	Ingest large particles and cells by phagocytosis
B lymphocytes (B cells)	Produce and secrete antibodies
T lymphocytes (T cells)	
Cytotoxic (killer) T cells (T_C)	Interact with infected host cells through <u>receptors</u> on T-cell surface
Helper T cells (T_H)	Interact with macrophages and secrete cytokines (interleukins) that stimulate T_C , T_H , and B cells to proliferate.

T-cell receptor (TCR)

Two Types of the Immune Systems

- Cellular immune system
 - targets **own cells** that have been infected
 - also clears up virus particles and infecting bacteria
 - key players: **Macrophages**, **cytotoxic (killer) T cells (T_c)**, and **inflammatory T cells (TH_1)**
- Humoral immune system (Latin *humor*, “fluid”)
 - targets **extracellular** pathogens
 - can also recognize foreign proteins
 - makes soluble **antibodies**
 - keeps “memory” of past infections
 - key players: **B lymphocytes** and **helper T-cells (TH_2)**

Humoral immune system

- Vertebrates also fight infections with soluble **antibodies** that specifically bind **antigens**
 - **Antigens** are substances that stimulate production of antibodies
 - Typically macromolecular in nature
 - Recognized as foreign by the immune system
 - Coat proteins of bacteria and viruses
 - Surface carbohydrates of cells or viruses
 - **Antibodies** are proteins that are produced by B cells and specifically bind to antigens
 - Binding will mark the antigen for destruction or interfere with its function
 - A given antibody will bind to a small region (epitope) of the antigen
 - One antigen can have several epitopes

Antigen, epitope and hapten

- Any molecule or pathogen capable of eliciting an immune response is called an **antigen (or immunogen)**. An antigen may be a virus, a bacterial cell wall, or an individual protein or other macromolecule.
- An individual antibody or T-cell receptor binds only a particular molecular structure within the antigen, called its **antigenic determinant** or **epitope**. 抗原決定基
- Molecules of $M_r < 5,000$ are generally not antigenic. However, when small molecules are covalently attached to large proteins in the laboratory, they can be used to elicit an immune response. These small molecules are called **haptons**. 半抗原, 不完全抗原

Antigen-binding site

The diagram illustrates the structure of IgG. It shows two Fab (antigen-binding fragment) units and one Fc unit. Each Fab unit consists of a V_H domain (blue) and a V_L domain (red). The V_H domains contain antigen-binding sites with amino groups (H_3N^+). The Fc unit also contains a V_H domain and a C_L domain (red). Papain cleavage sites are indicated by yellow dashed lines between the C_H1 and C_H2 domains, and between the C_H2 and C_H3 domains. The C_H1, C_H2, and C_H3 domains are labeled with their respective abbreviations and show carboxylate groups (-COO⁻) at their C-termini.

Fab: antigen-binding fragment

C: crystallize

C = constant domain
V = variable domain
H, L = heavy, light chains

Papain cleavage sites

Antigen-binding site

IgG

150 kDa

Immunoglobin fold

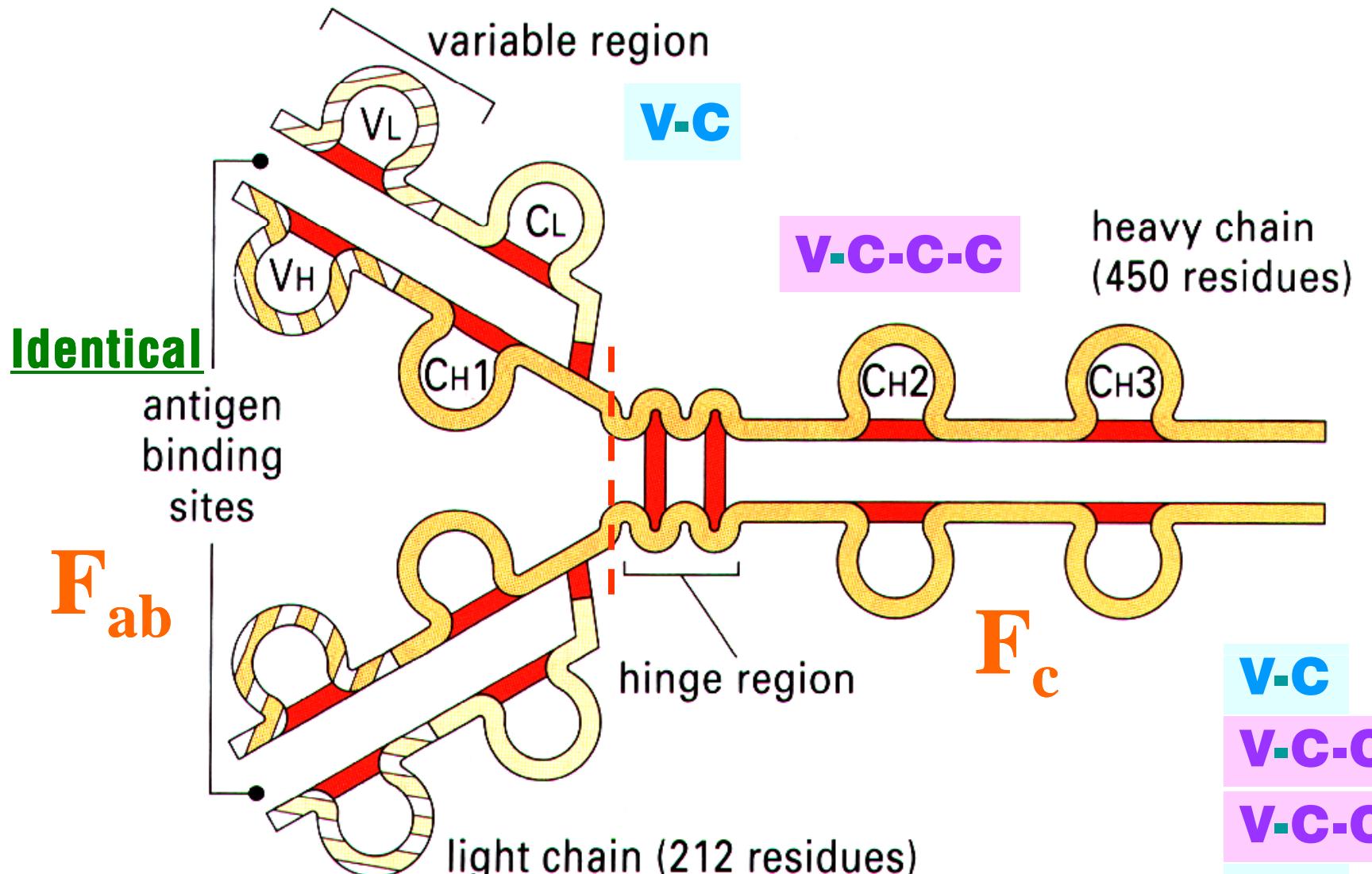
Bound carbohydrate

Constant domain: All β

171

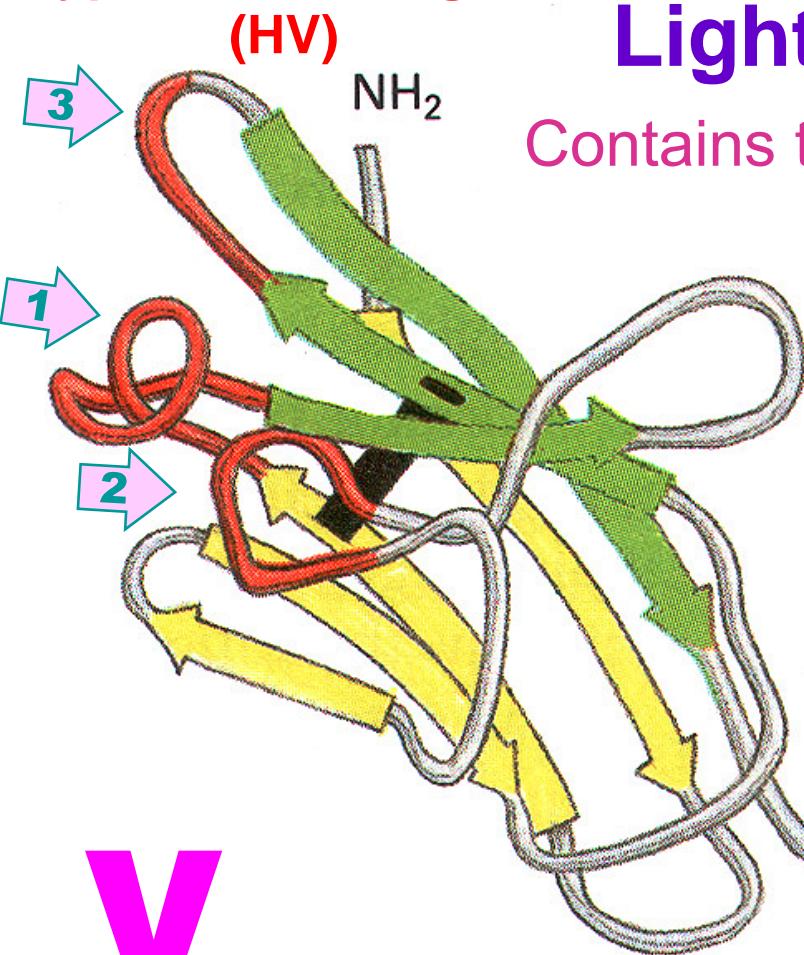
The molecular structure of the immunoglobulin

每一抗體分子有四個次單元體 (subunit) = 2H (heavy) + 2L (light)



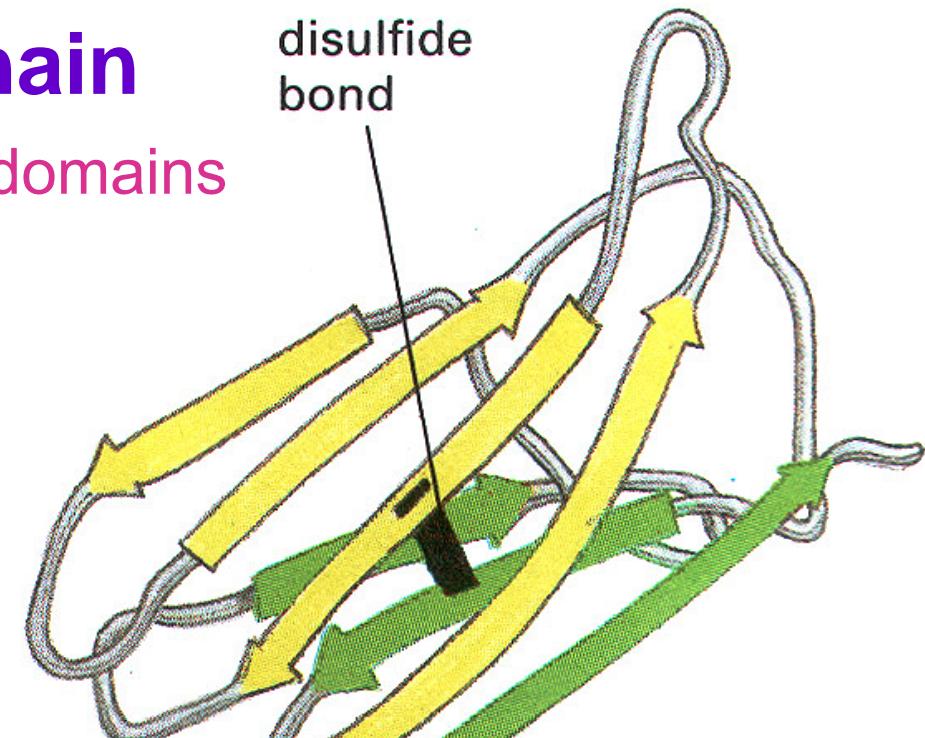
The domain structures of light chain

Hypervariable region
(HV)



Light chain

Contains two domains



V

J

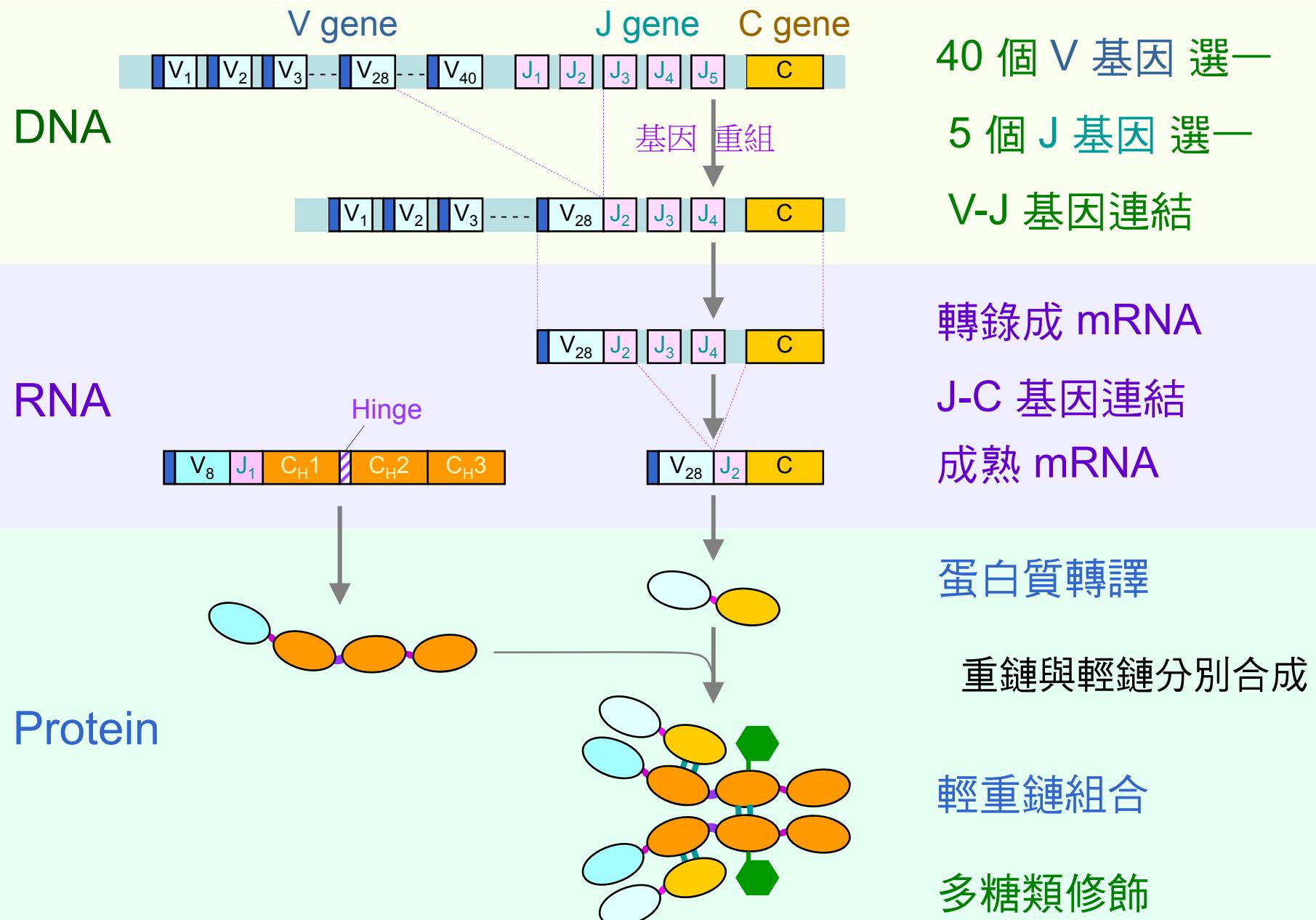
C

variable (V_L) domain
(Complementarity Determining
Regions, CDRs)

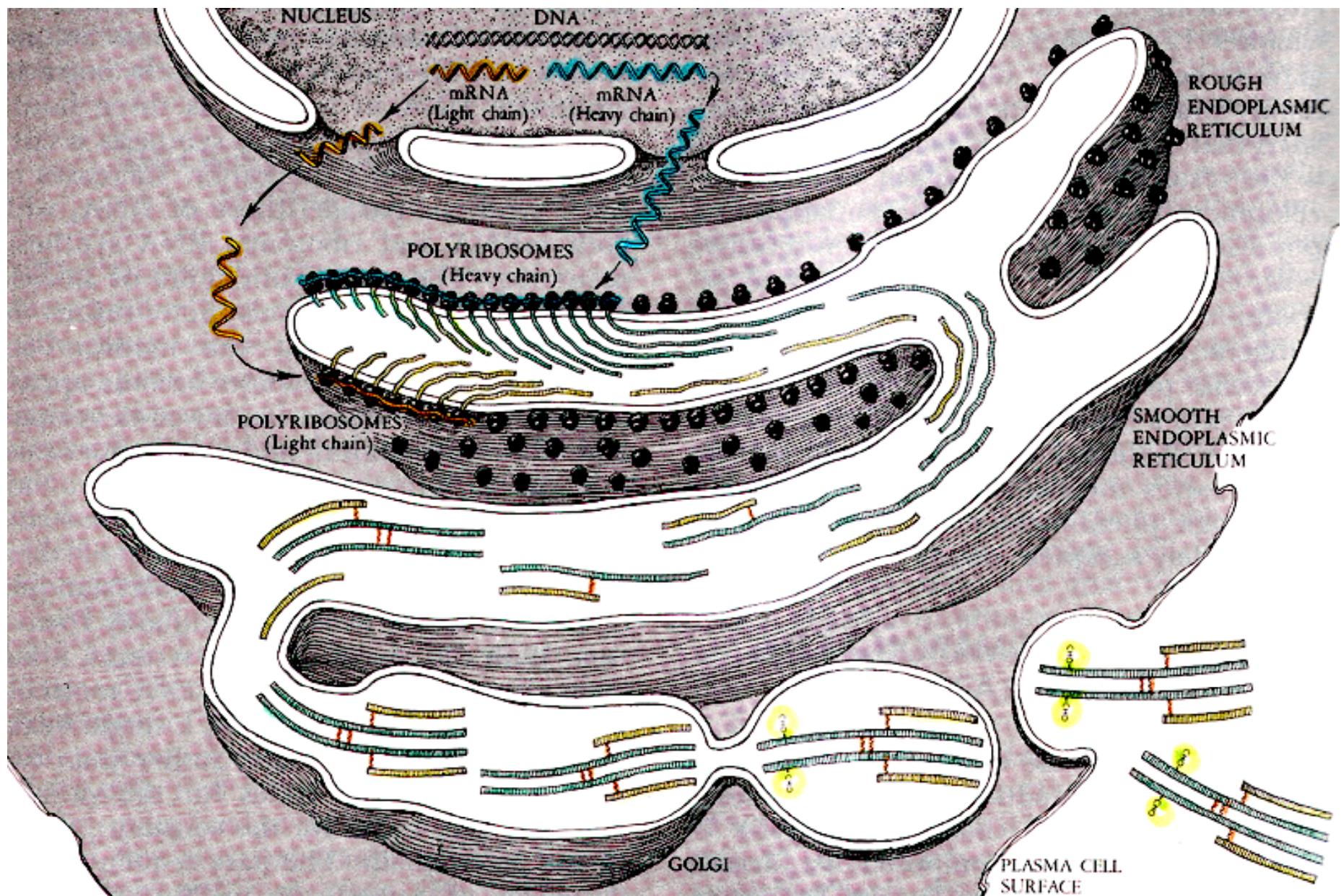
V-J-C

constant (C_L) domain

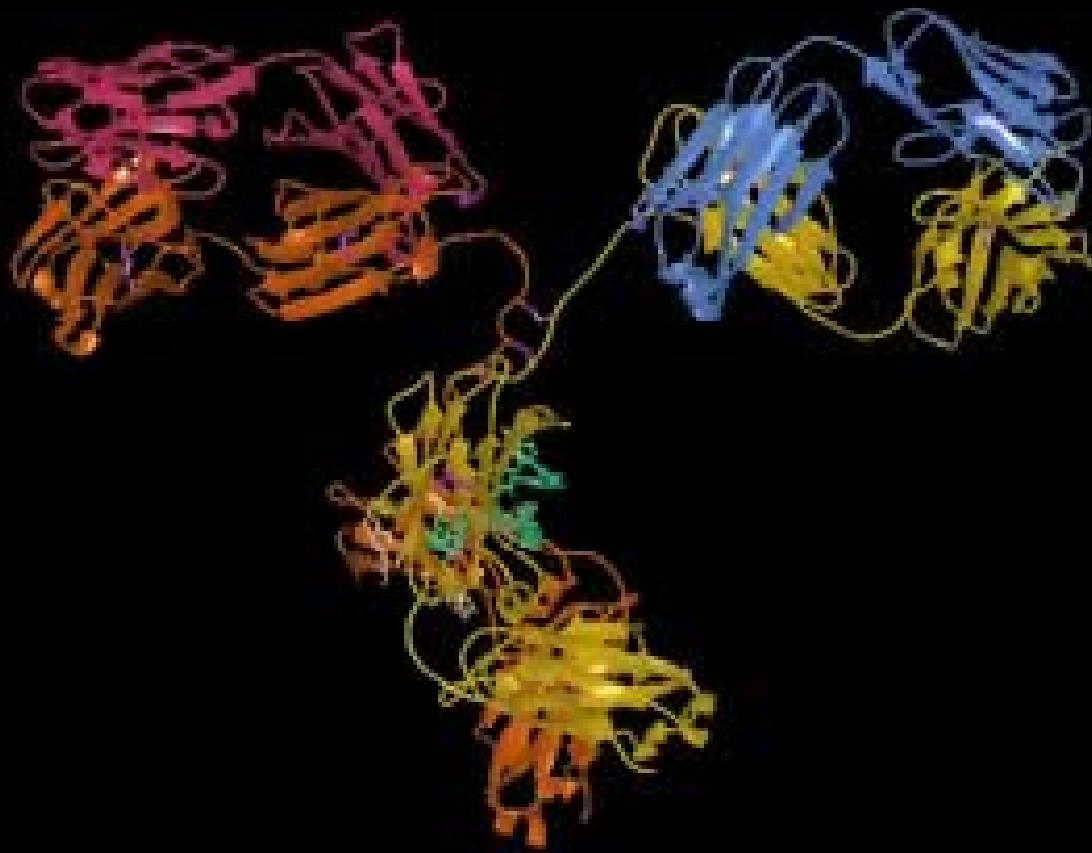
抗體基因的表現



Assembly of the antibody in the ER



抗體是一種蛋白質



Edelman and Porter (1972)

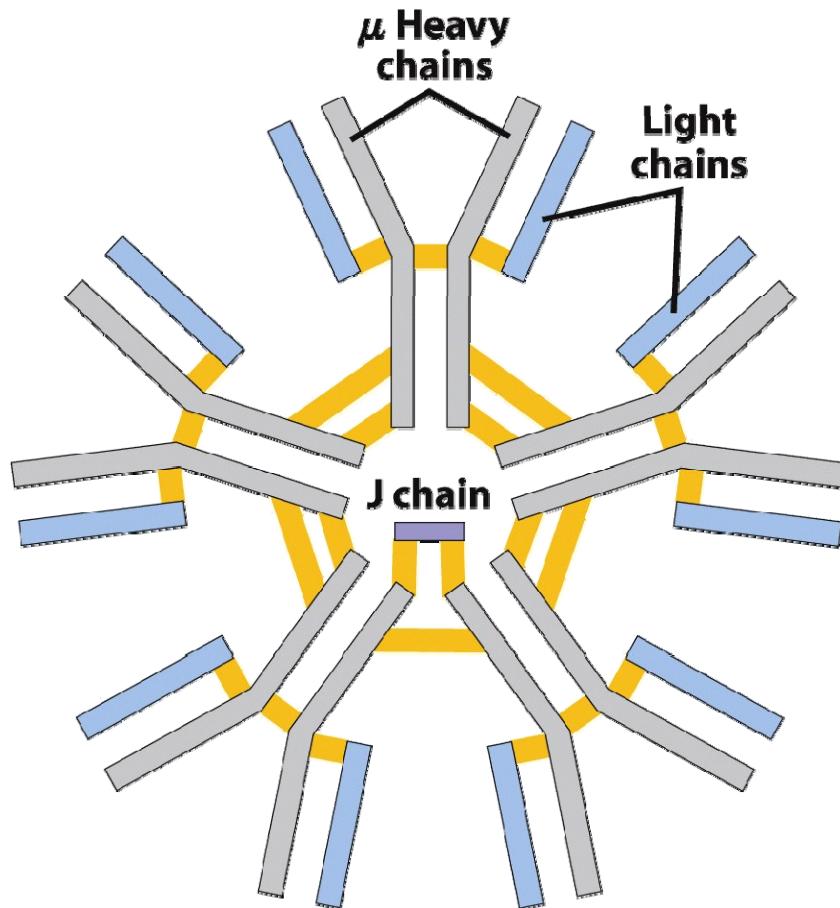
"for their discoveries concerning the chemical structure of antibodies"

Immunoglobulin

- **Immunoglobulin G (IgG)** is the major class of antibody molecule and one of the most abundant proteins in the blood serum.
- Cleavage with the protease **papain** liberates the basal fragment, called **Fc** because it usually **crystallizes** readily, and the two branches, called **Fab**, the **antigen-binding** fragments. Each branch has a single antigen-binding site.
- **Five classes of immunoglobulins: Each class has a characteristic type of heavy chain, denoted $\alpha, \delta, \epsilon, \gamma$, and μ for IgA, IgD, IgE, IgG, and IgM, respectively. Two types of light chain, κ and λ , occur in all classes of immunoglobulins.**

- The **IgG** is the major antibody in secondary immune responses.
- **IgM** occurs either in a monomeric, membrane-bound form or in a secreted form that is a cross-linked **pentamer** of this basic structure. **IgM** is the first antibody to be made by B lymphocytes and the major antibody in the early stages of a primary immune response.
- The overall structures of **IgD** and **IgE** are similar to that of **IgG**. The particular function of **IgD** is less clear.
- **IgE** plays an important role in the **allergic response**, interacting with **basophils** (phagocytic leukocytes) in the blood and with **histamine-secreting cells called mast cells**, which are widely distributed in tissues. 肥大細胞可釋放組織胺或其他參與發炎的化學物質
- **IgA**, found principally in secretions such as **saliva, tears, and milk**, can be a monomer, dimer, or trimer.

IgM pentamer of immunoglobulin units

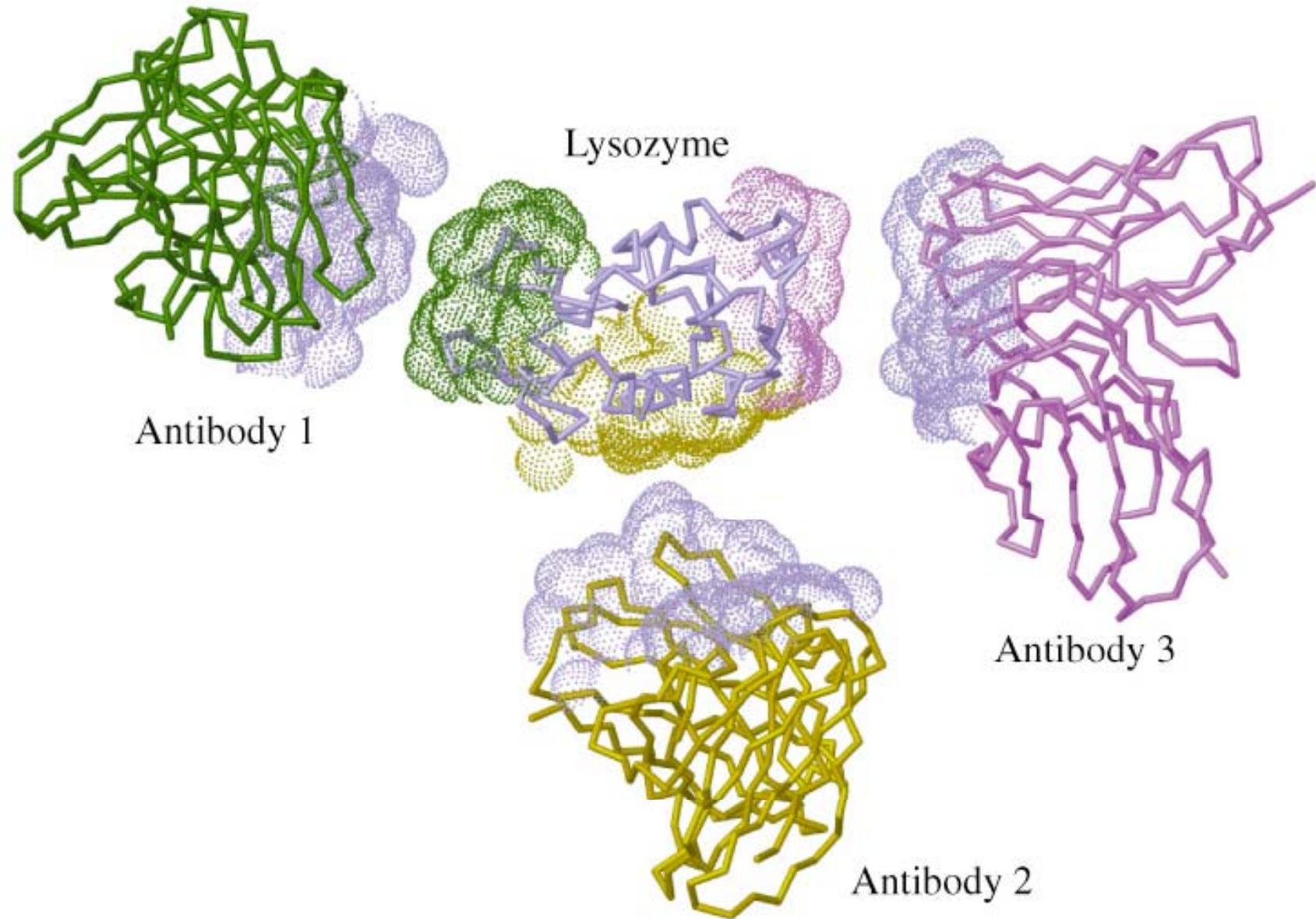


The pentamer is cross-linked with disulfide bonds (yellow). The **J chain** is a polypeptide of M_r 20,000 found in both IgA and IgM.

Antibodies bind specific antigens

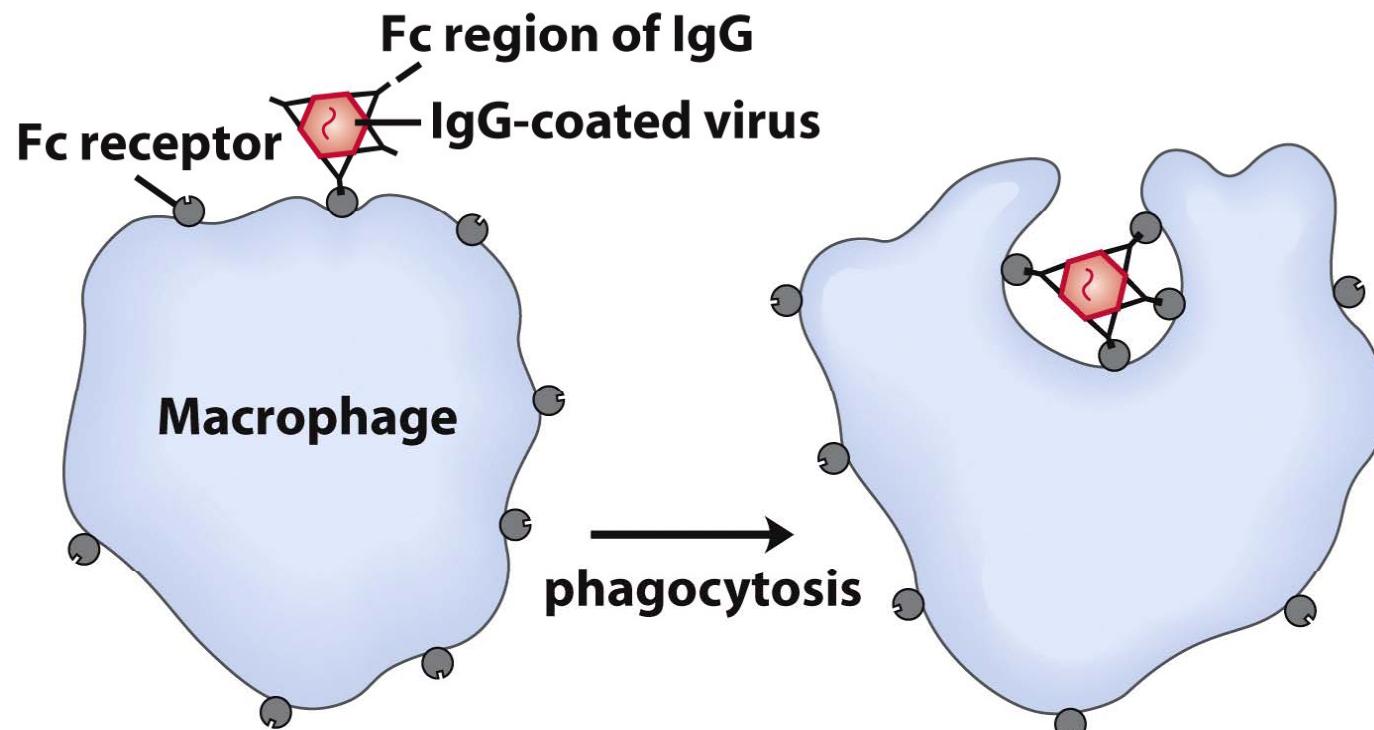
- Vertebrate immune systems synthesize protein **antibodies** (immunoglobulins) to eliminate bacteria, viruses, other foreign substances
- Antibodies specifically recognize and bind **antigens**
- Antibodies are synthesized by lymphocytes (white blood cells)
- Variable domain differs in various antibodies which are from different B-cells

Binding of three different antibodies to an antigen

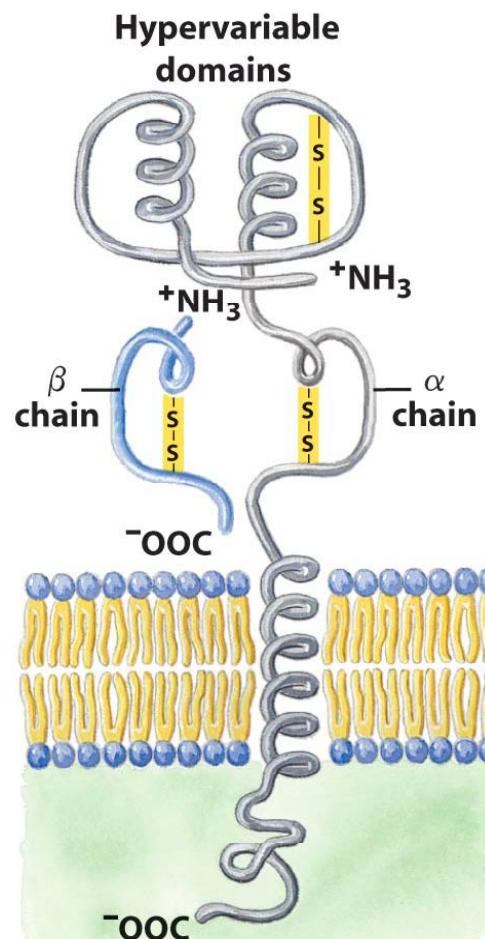


Phagocytosis of an antibody-bound virus by a macrophage

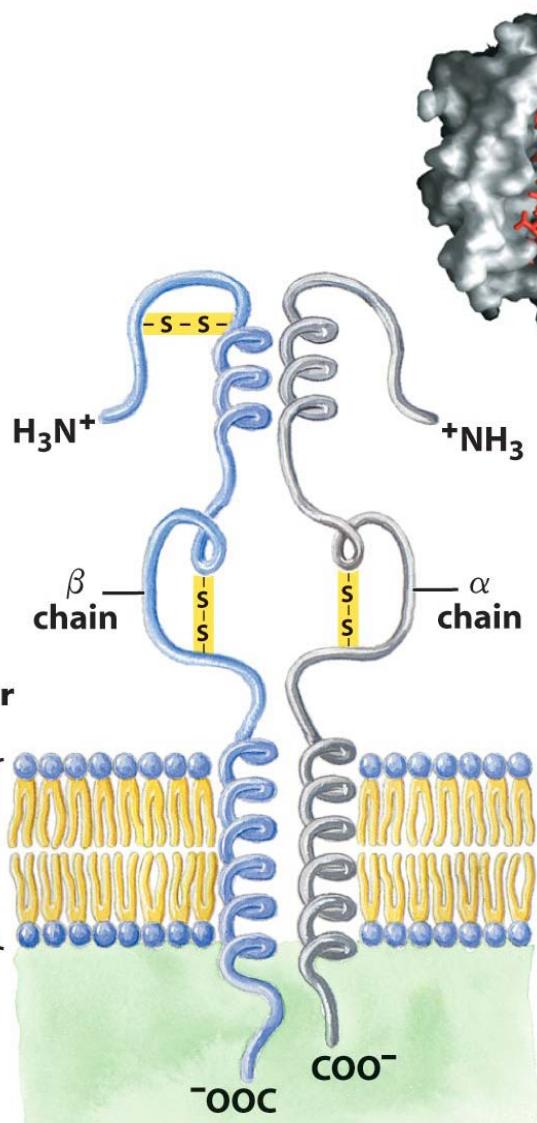
- Many infected cells display fragments of infectious particles on their surface
- Phagocytes: specialized cells that eat invaders
- Macrophages: large phagocytes that ingest bacteria



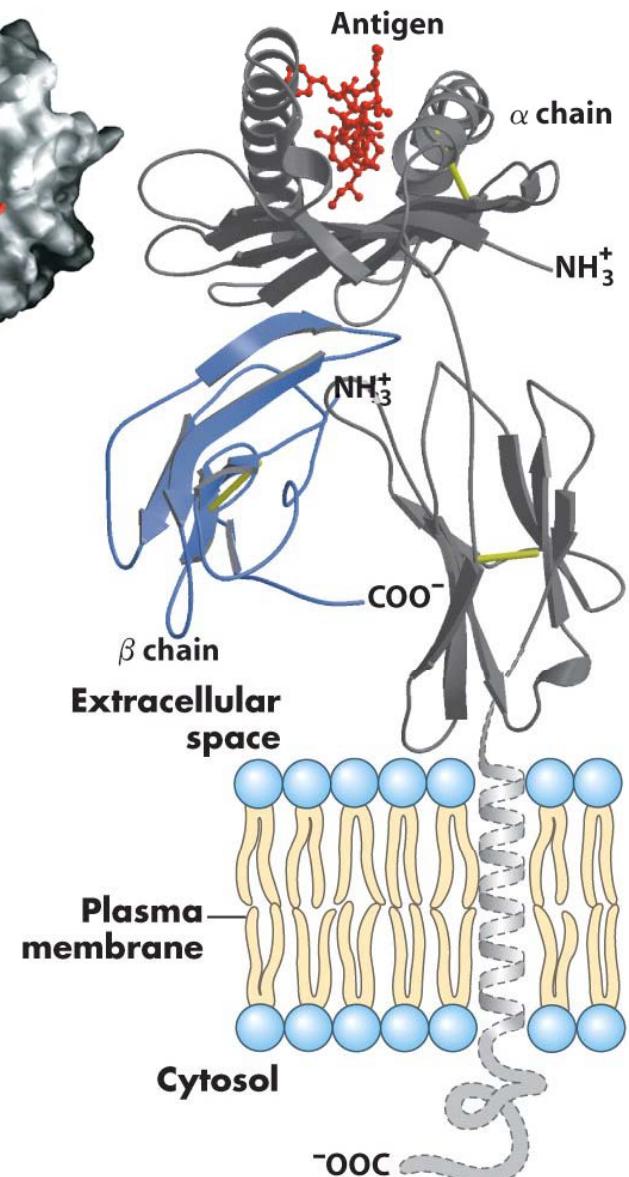
Antigen presentation on the cell surface



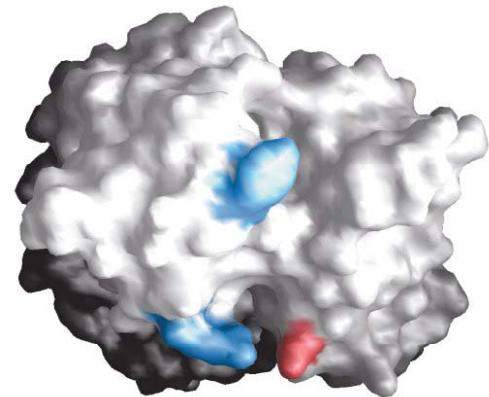
(a) Class I MHC protein



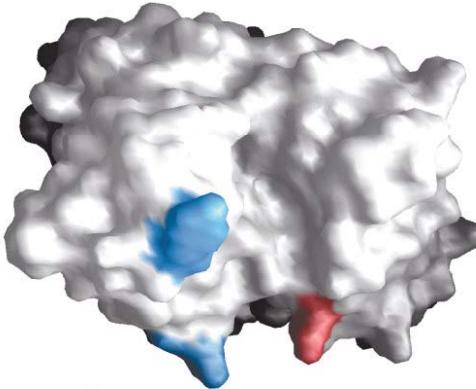
(b) Class II MHC protein



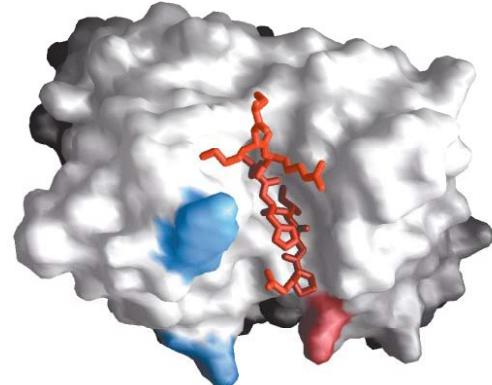
Induced fit in the binding of an antigen to IgG



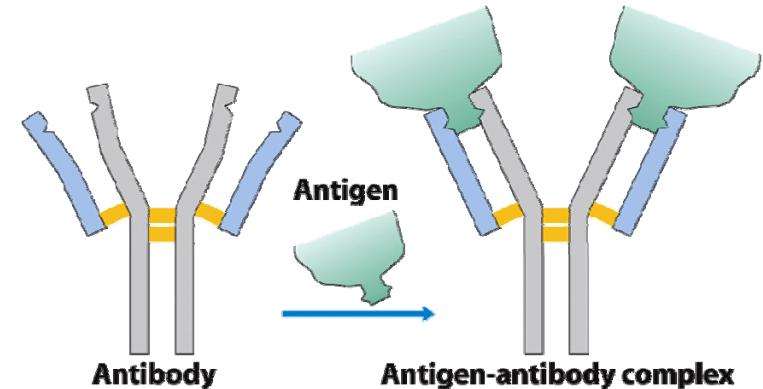
(a) Conformation with no antigen bound



(b) Antigen bound (hidden)



(c) Antigen bound (shown)



To generate an optimal fit for the antigen, the binding sites of IgG often undergo slight conformational changes.

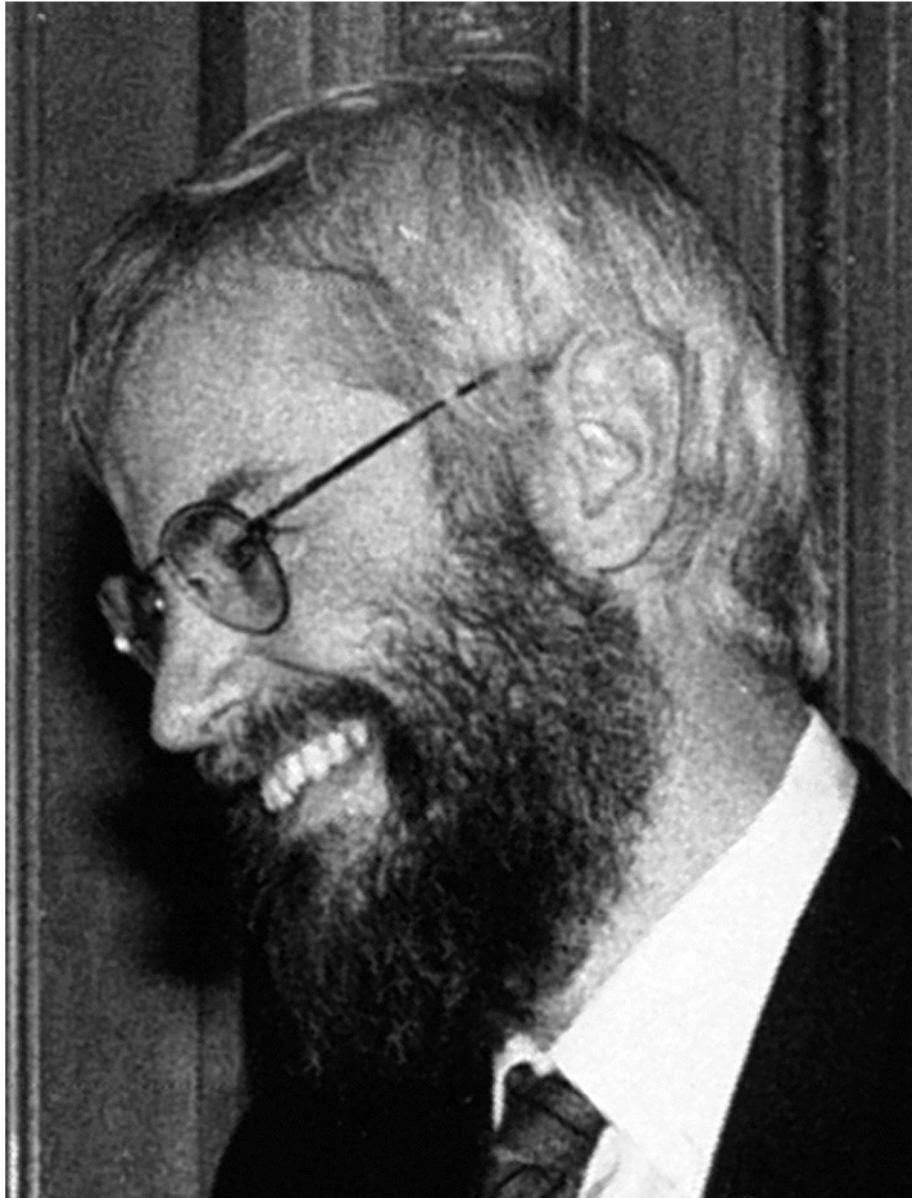
Specificity is conferred by complementarity between the antigen and its specific binding site, in terms of shape and the location of charged, nonpolar, and hydrogen-bonding groups.

The antibody-antigen interaction is the basis for a variety of important analytical procedures

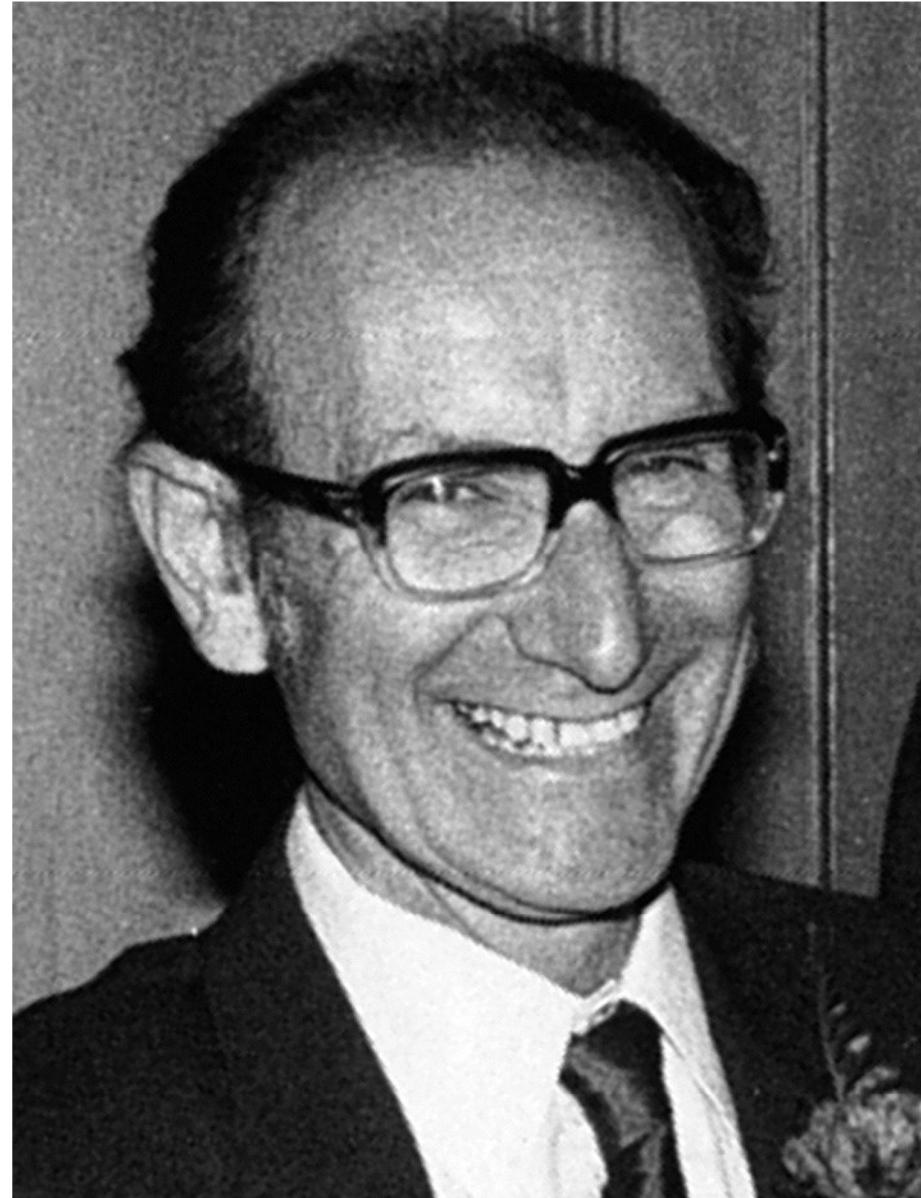
- Two types of antibody preparations are in use: polyclonal and monoclonal.
- **Polyclonal antibodies** are those produced by many different B lymphocytes responding to one antigen.
- **Monoclonal antibodies**, in contrast, are synthesized by a population of identical B cells (**a clone**) grown in cell culture.



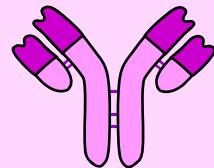
Monoclonal antibody techniques



Georges Köhler, 1946–1995

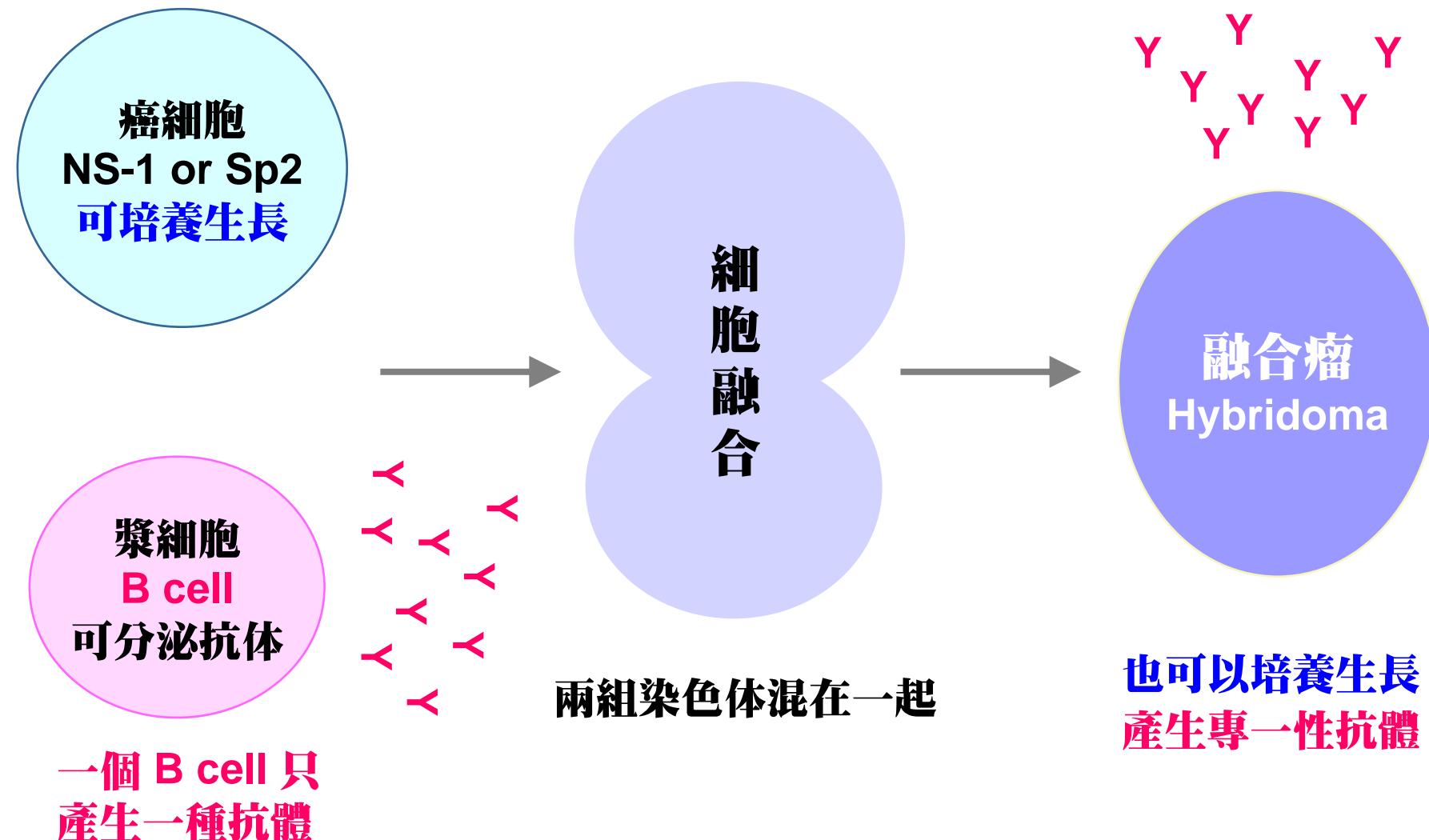


Cesar Milstein, 1927–2002

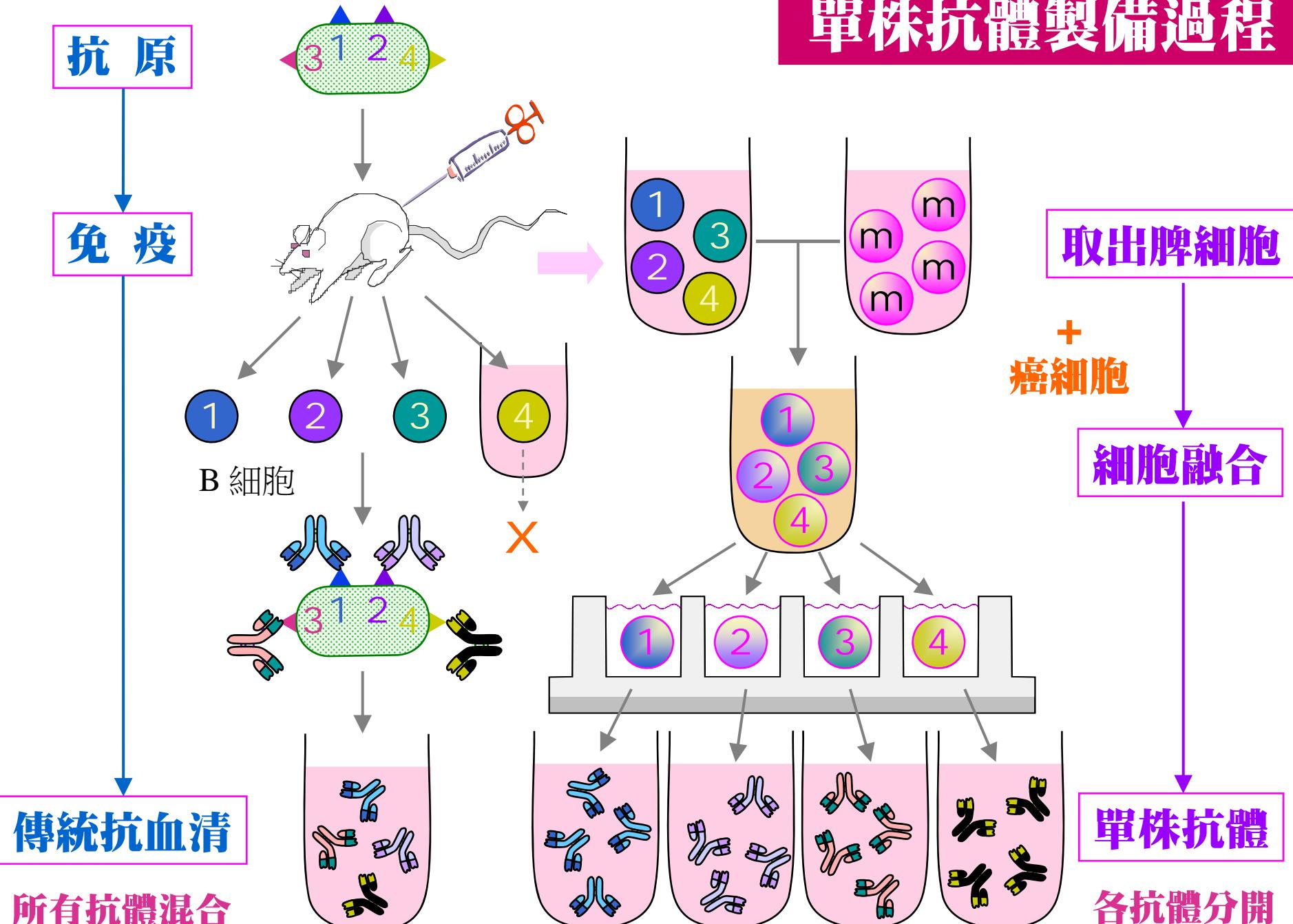


單株抗體

可生產有用抗體的 淋巴細胞 若與 癌細胞 融合，則形成穩定而可培養的細胞株。



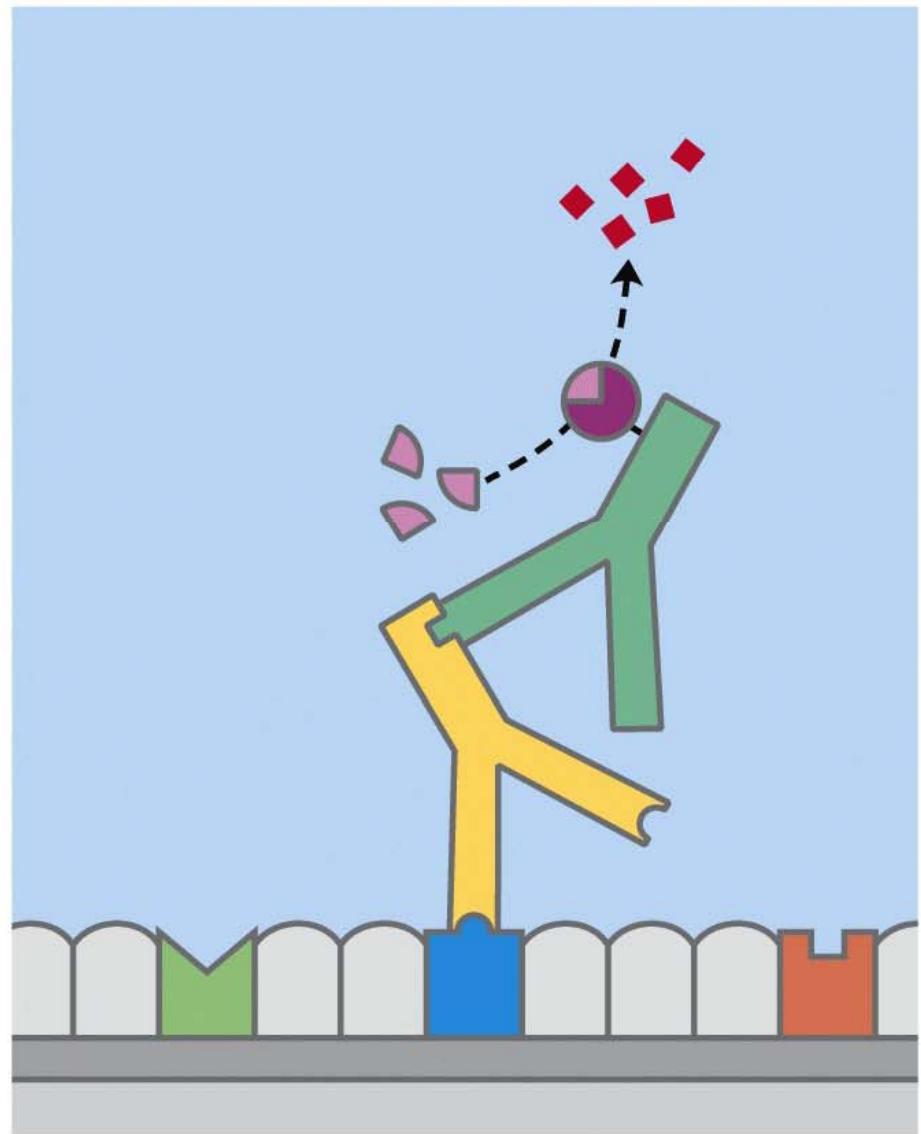
單株抗體製備過程



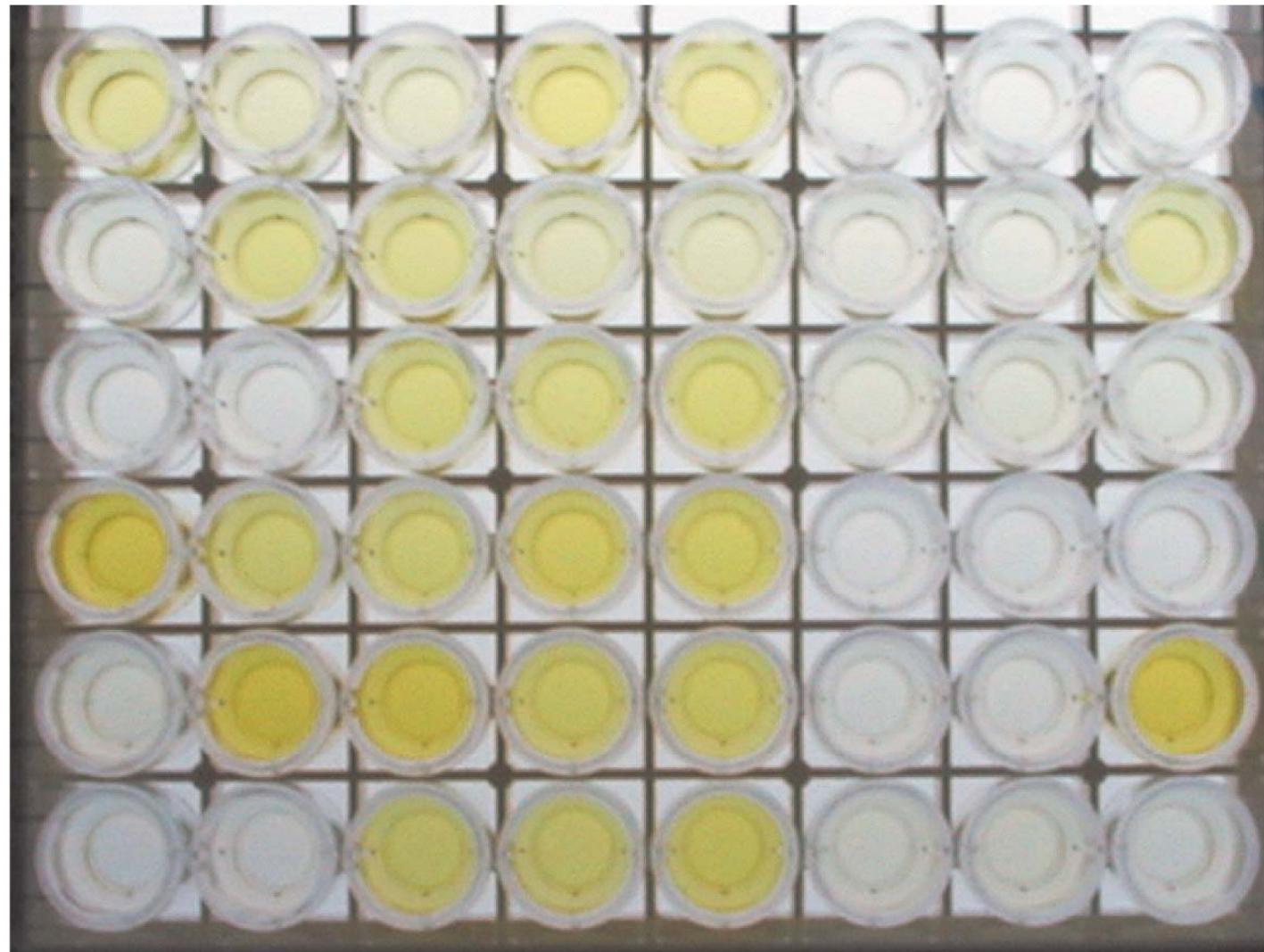
Adapted from Milstein (1980) *Scientific American*, Oct. p.58

A schematic representation of the general antibody techniques

- ① Coat surface with sample (antigens). 
- ② Block unoccupied sites with nonspecific protein. 
- ③ Incubate with primary antibody against specific antigen. 
- ④ Incubate with secondary antibody–enzyme complex that binds primary antibody. 
- ⑤ Add substrate. 
- ⑥ Formation of colored product indicates presence of specific antigen. 



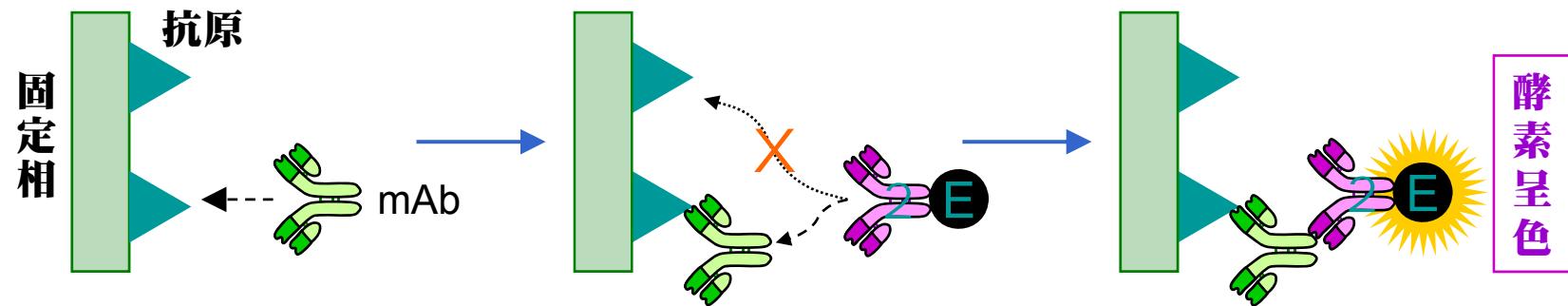
Enzyme-linked immunosorbent assay



ELISA

174

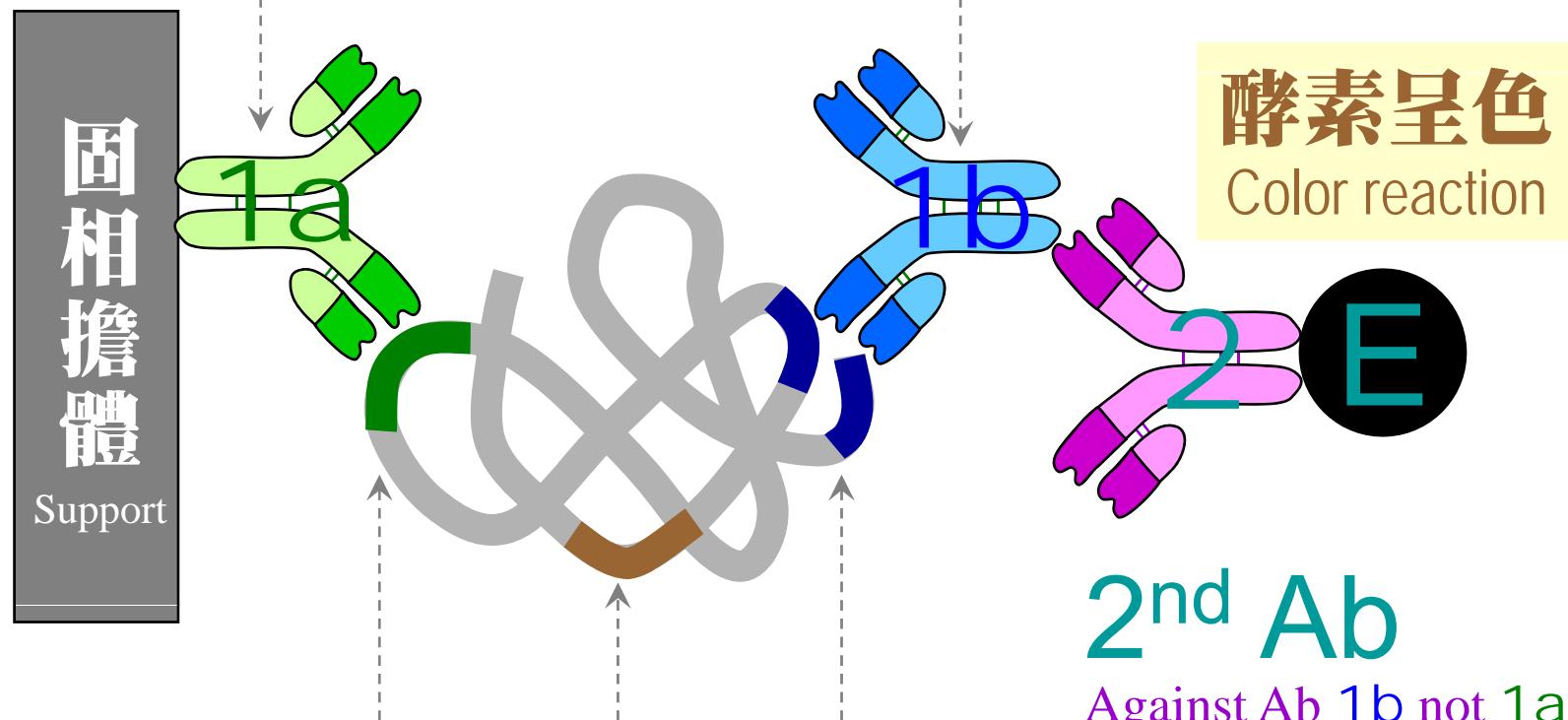
以酵素免疫分析法檢測樣本中的專一性抗體



■ 三明治免疫分析法 Sandwich ELISA method

使用不同動物來源的兩種抗體

Use two Ab from different animal sources

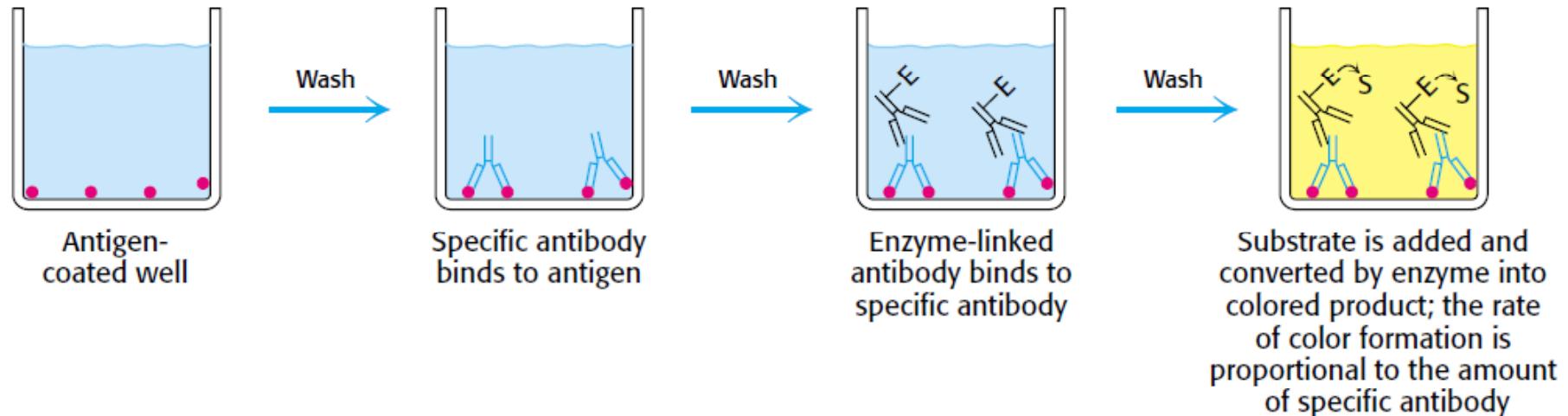


抗原要有多個抗原決定基

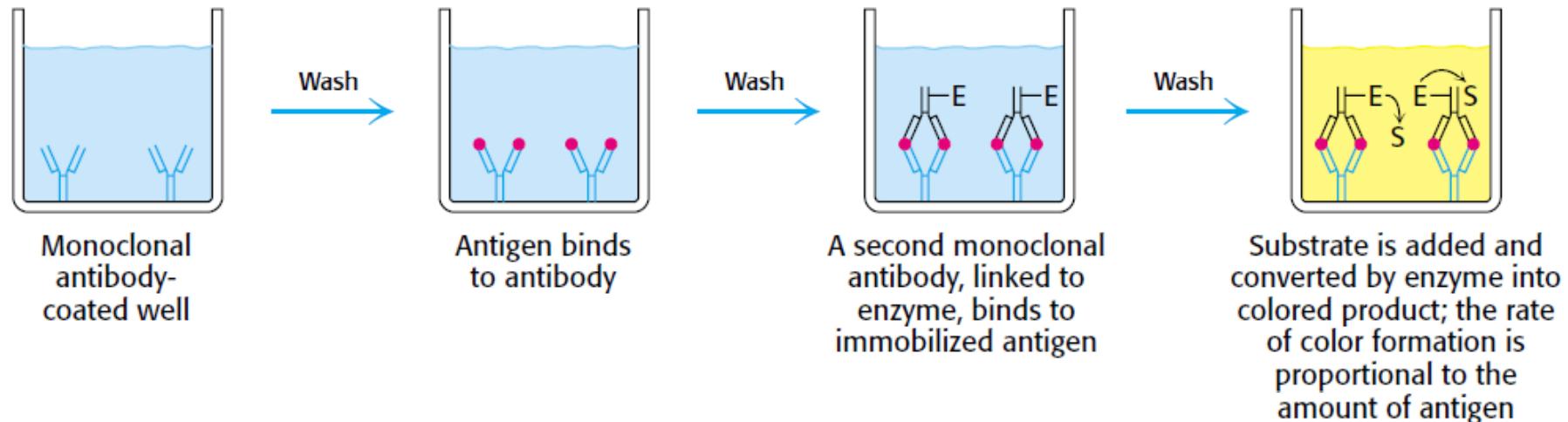
Ag contains at least two epitopes

Indirect ELISA and sandwich ELISA

(A) Indirect ELISA

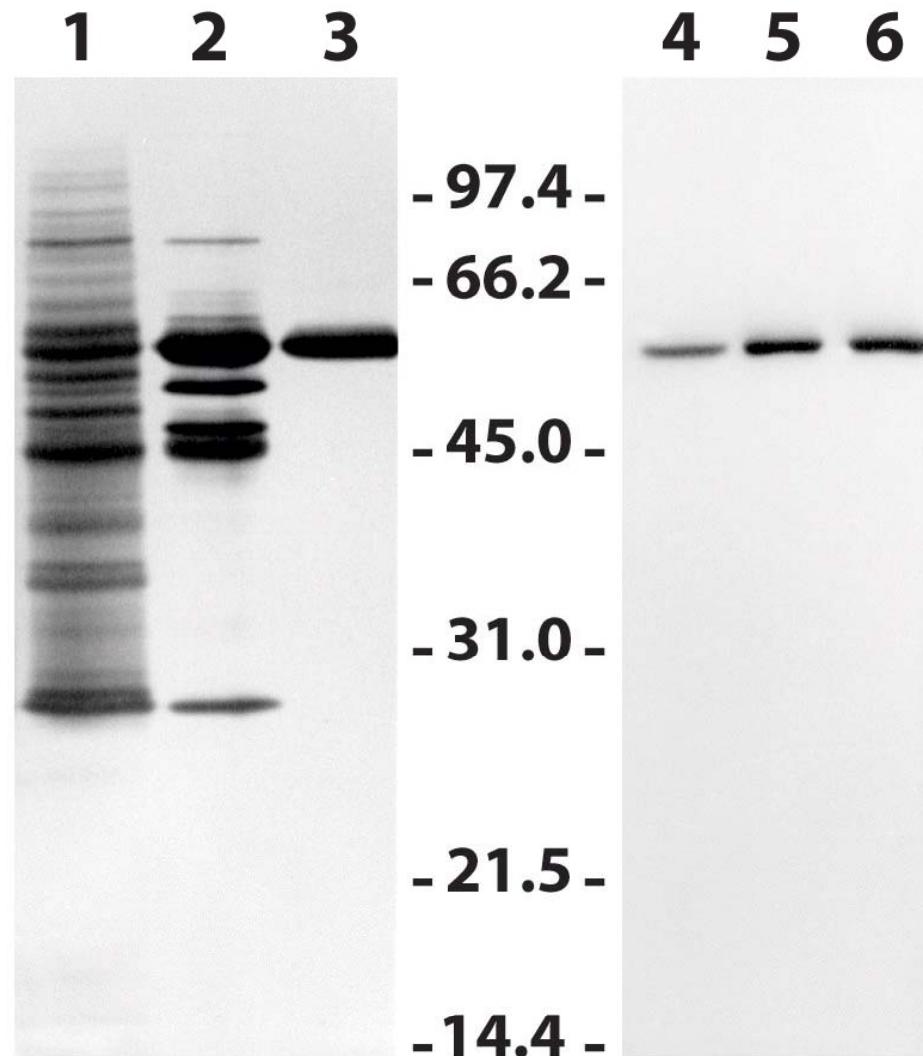


(B) Sandwich ELISA



(A) In indirect ELISA, the production of color indicates the amount of an antibody to a specific antigen. (B) In sandwich ELISA, the production of color indicates the quantity of antigen.

Immunoblot (Western blotting)



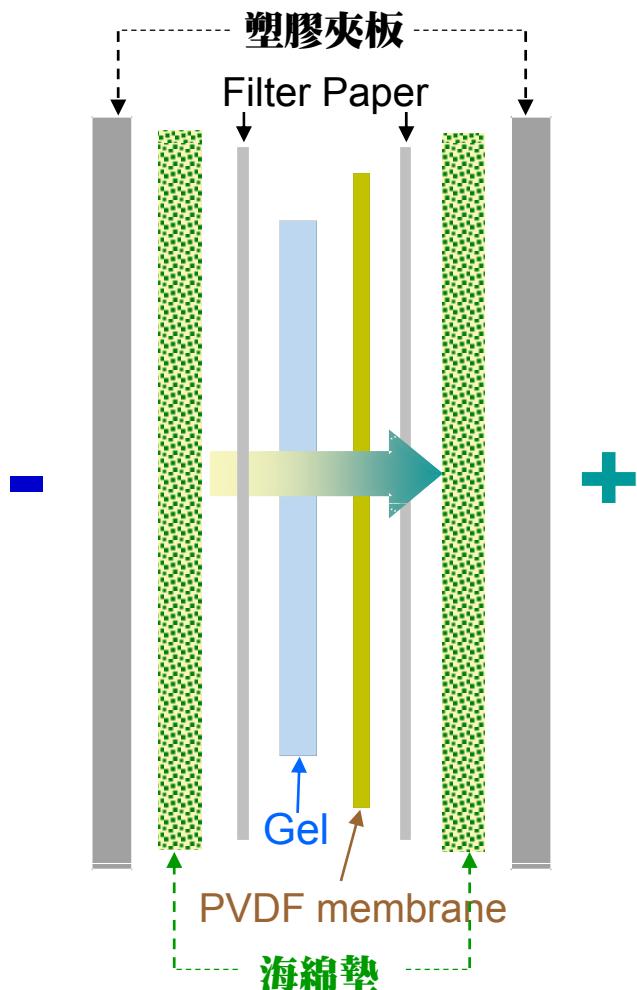
SDS gel

Immunoblot

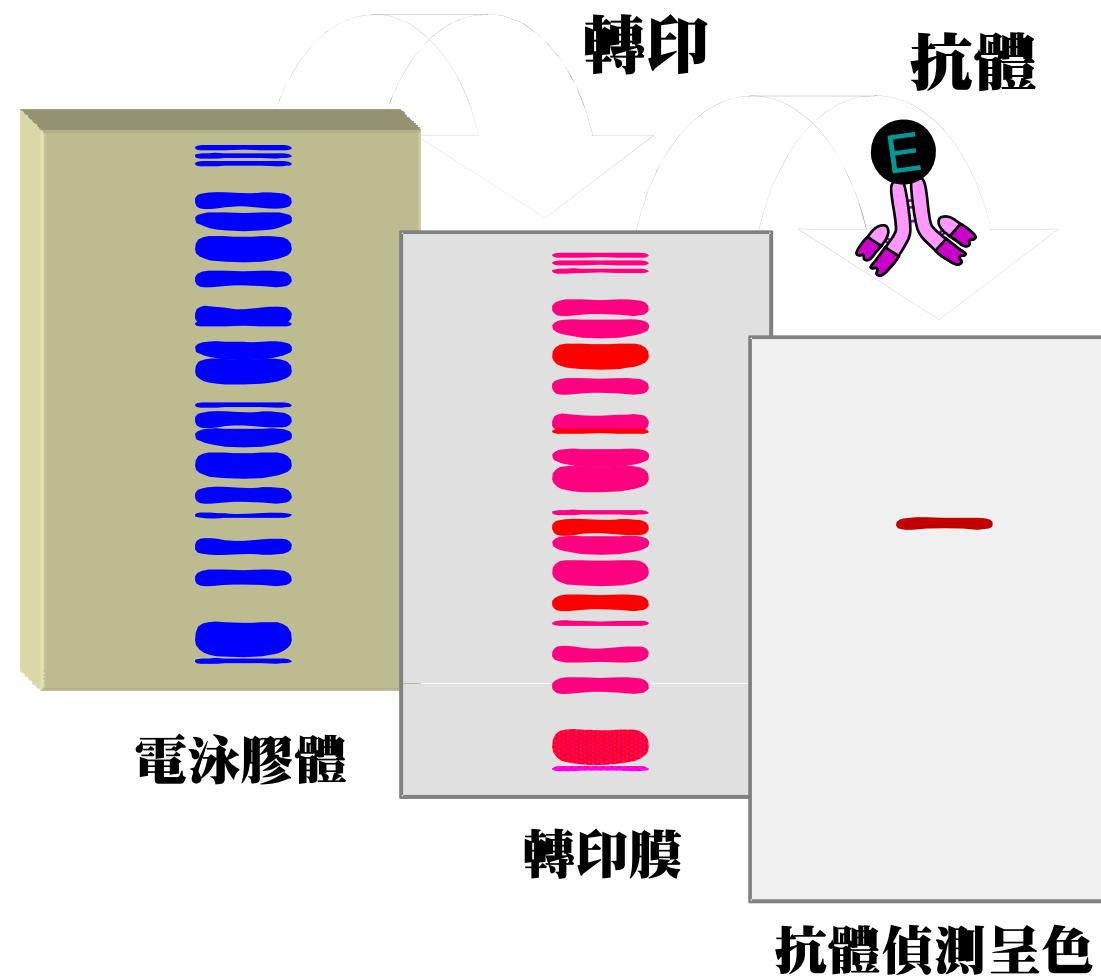
■ 轉印及免疫染色流程



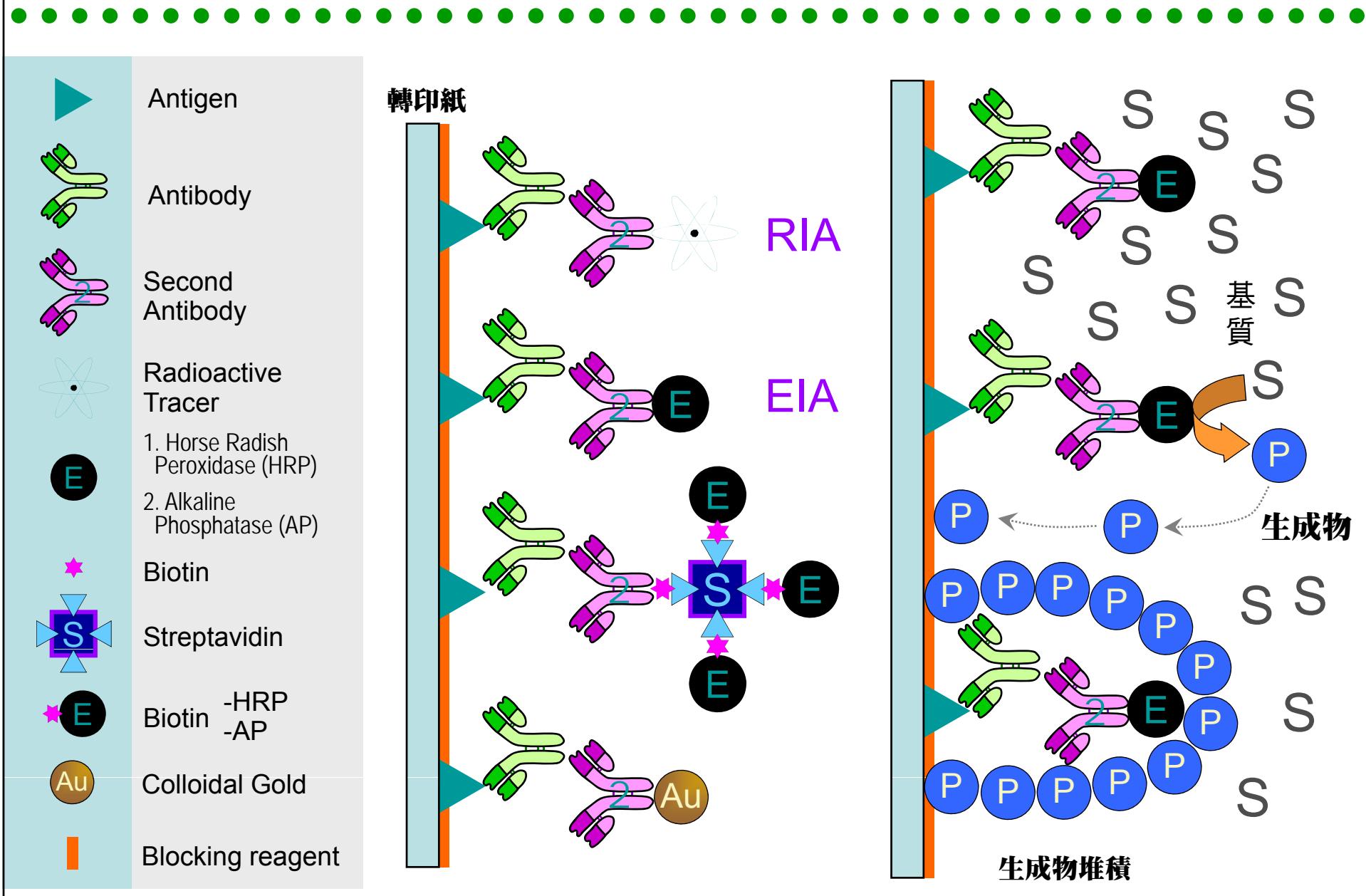
A 轉印三明治



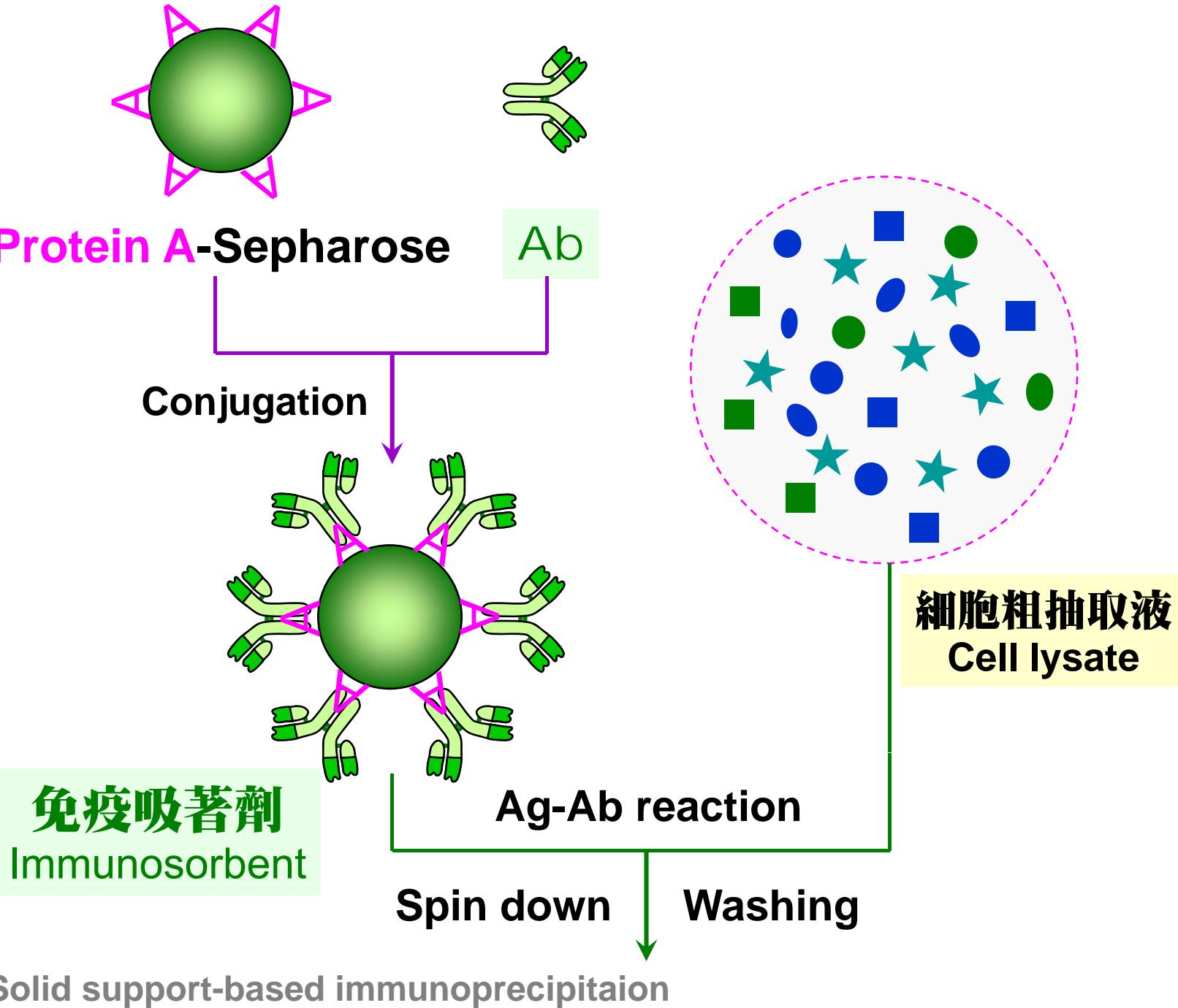
B 免疫染色流程及結果



■ 免疫轉印的種類與呈色機制



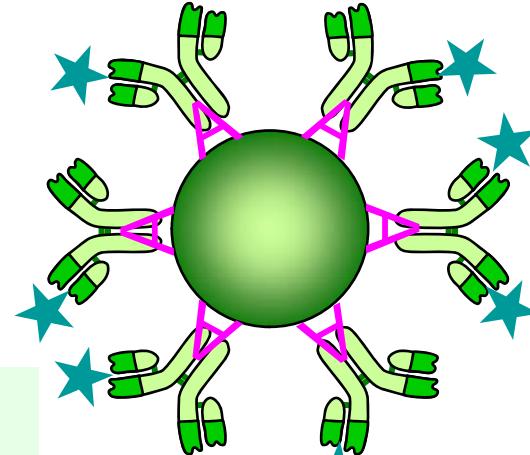
■ 擔體免疫沈澱的原理及應用



■ 擔體免疫沈澱的原理及應用

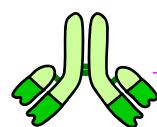
細胞粗抽取液
Cell lysate

擔體免疫沈澱 Immunoprecipitation



SDS-PAGE

抗原可能有兩個次體
Ag might contains two subunits



抗體有輕鏈及重鏈
Ab contains H & L chains

