

CHEM 153A Week 4

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January 31, 2025

Protein Tertiary and Quaternary Structure

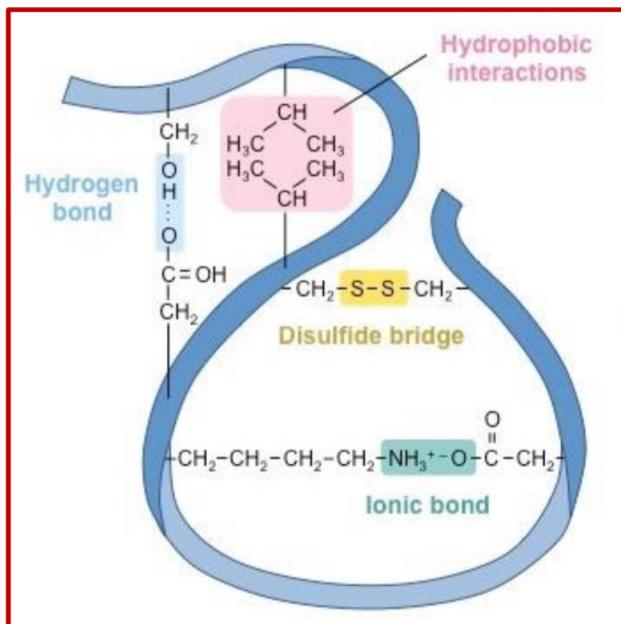
- **tertiary structure** = overall three-dimensional arrangement of all the atoms in a protein
 - weak interactions and covalent bonds hold interacting segments in position
- **quaternary structure** = arrangement of 2+ separate polypeptide chains in three-dimensional complexes

Shape → Function

Tertiary Structure - What holds it together?

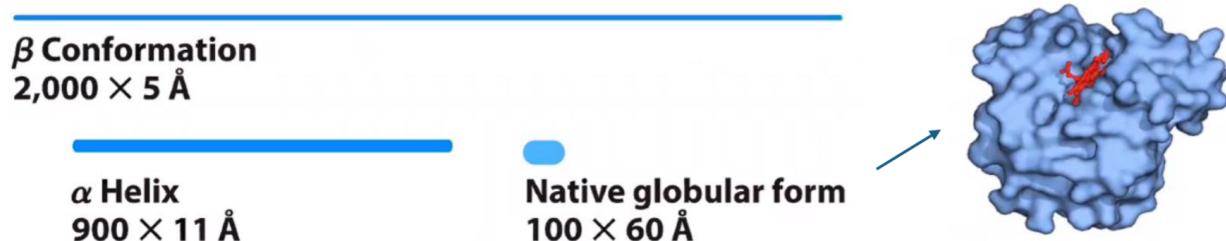
- The global interactions of tertiary structure are formed through the interaction of amino acid side chains
- Electrostatic interactions forming between charged side chains
- London dispersion forces forming between nonpolar side chains
- Hydrogen bonds forming between polar/charged side chains
- Covalent bonds forming through disulfide bridges

Type of Bond	Example	Bond Strength (kJ · mol ⁻¹)
Covalent	S—S	251
Noncovalent		
Ionic interaction	—COO ⁻ ... ⁺ H ₃ N—	86
van der Waals forces	—O—H···O—	20
Hydrogen bond	—C=O···C=O	9.3
Dipole-dipole interaction	$\begin{array}{c} \text{H} & \text{H} \\ & \\ \text{—C—H} & \cdots \text{H—C—} \\ & \\ \text{H} & \text{H} \end{array}$	0.3
London dispersion forces		



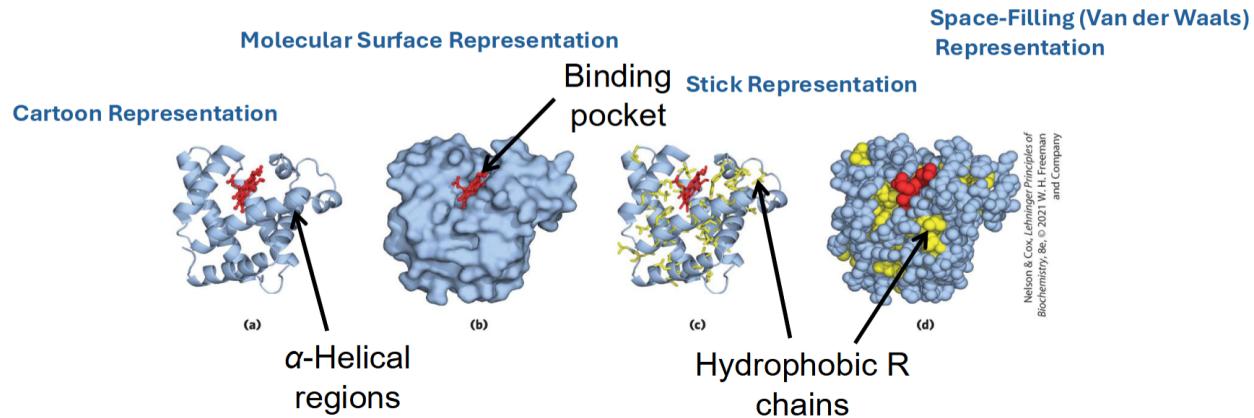
Categories of Tertiary Structure - Globular proteins

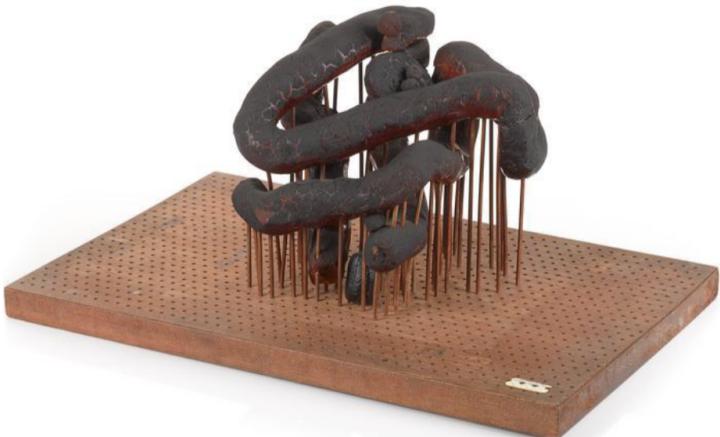
- **Globular proteins** polypeptide chains folded into a spherical or globular shape
 - fold back on each other
 - more compact than fibrous proteins
 - soluble in water
 - mixture of different secondary structures
 - quaternary structure usually held together by noncovalent forces
 - regulatory and metabolic roles (basically most proteins you can think of)
 - * enzymes
 - * transport proteins
 - * motor proteins
 - * regulatory proteins
 - * immunoglobulins



Myoglobin Provided Early Clues about the Complexity of Globular Protein Structure

- several structural representations of myoglobin's tertiary structure:





Max Perutz and John Kendrew, 1962

"I always knew it would look like that—but I never expected it to be so complicated!"
John D. Bernal

The bottom picture depicts the original model of the myoglobin molecule, constructed in plasticine in 1957.

- This was the first ever model of a protein molecule. (Won a nobel prize)
- In modern day, we have so much information about protein structure that we can train AIs to simulate protein folding.

Globular Proteins Have a Variety of Tertiary Structures

Each globular protein has a distinct structure, adapted for its biological function

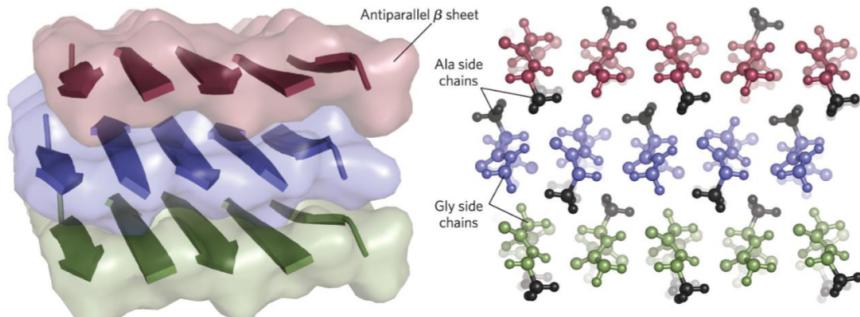
Approximate Proportion of α Helix and β Conformation in Some Single-Chain Proteins		
Protein (total residues)	Residues (%): α Helix	Residues (%): β Conformation
Chymotrypsin (247)	14	45
Ribonuclease (124)	26	35
Carboxypeptidase (307)	38	17
Cytochrome c (104)	39	0
Lysozyme (129)	40	12
Myoglobin (153)	78	0

Categories of Tertiary Structure - Fibrous proteins

- **Fibrous proteins** are long (often) rope-like proteins adapted for strength.
 - Extended structure
 - Insoluble in water
 - Simple repetitive structure (often the same secondary structure throughout)
 - Quaternary structure usually held together by disulfide bonds
 - Famously involved in a lot of extracellular structures (incl. Tendons, bones, hair, skin)

**silk, a.k.a. fibroin
(stacked β -sheets)**

enriched with Gly
and Ala (why?)



Fibrous Proteins are Adapted for a Structural Function

- give strength and/or flexibility to structures
- simple repeating element of secondary structure
- H_2O insoluble due to high concentrations of hydrophobic residues

Secondary Structures and Properties of Some Fibrous Proteins

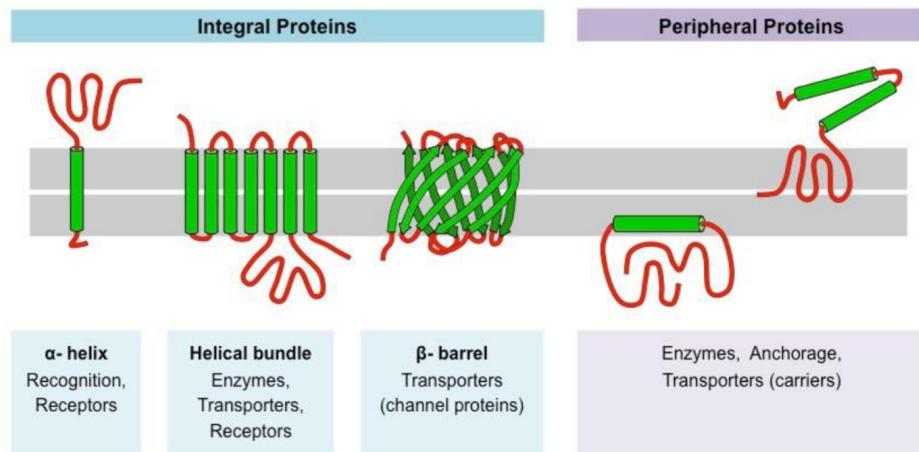
Structure	Characteristics	Examples of occurrence
α Helix, cross-linked by disulfide bonds	Tough, insoluble protective structures of varying hardness and flexibility	α -Keratin of hair, feathers, nails
β Conformation	Soft, flexible filaments	Silk fibron
Collagen triple helix	High tensile strength, without stretch	Collagen of tendons, bone matrix

Membrane Proteins

Membrane proteins are proteins with polypeptide chains embedded into lipid membranes

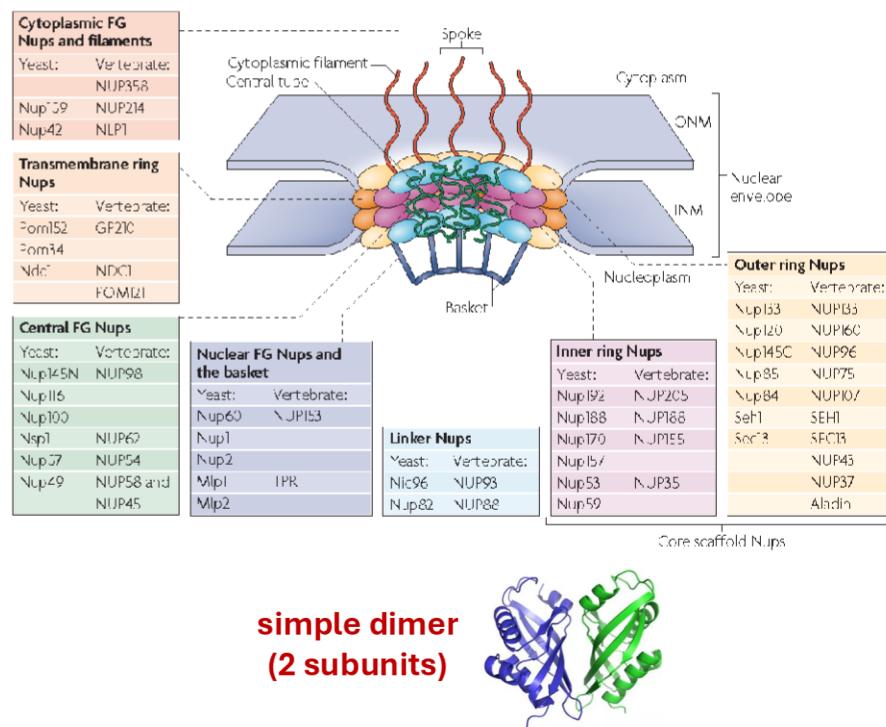
- Multiple types, commonality is that they contain hydrophobic regions so as to embed themselves
- Defined patterns of secondary structure

You don't have to know these categories or functions



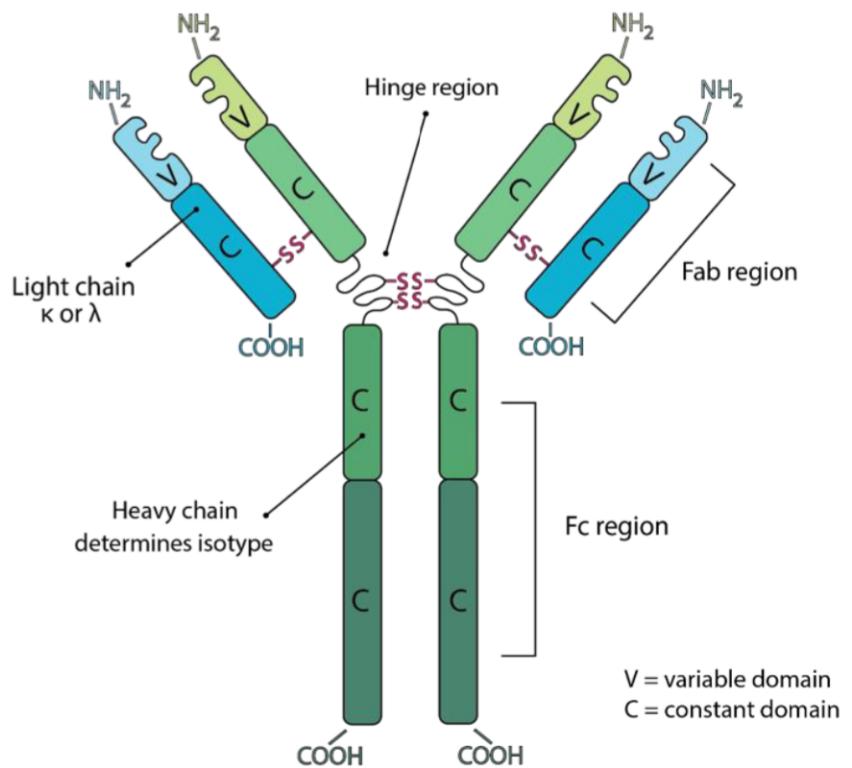
Quaternary Structure

- Folded proteins can associate forming multi-subunit complexes
 - Adds even more informational complexity to proteins as functional units
 - Variety of possibilities, anything from small oligomeric complexes to massive complexes made from many different proteins
- Subunits can be identical or different
 - Subunits are symmetrically arranged
- **oligomer = multimer = multi-subunit protein**



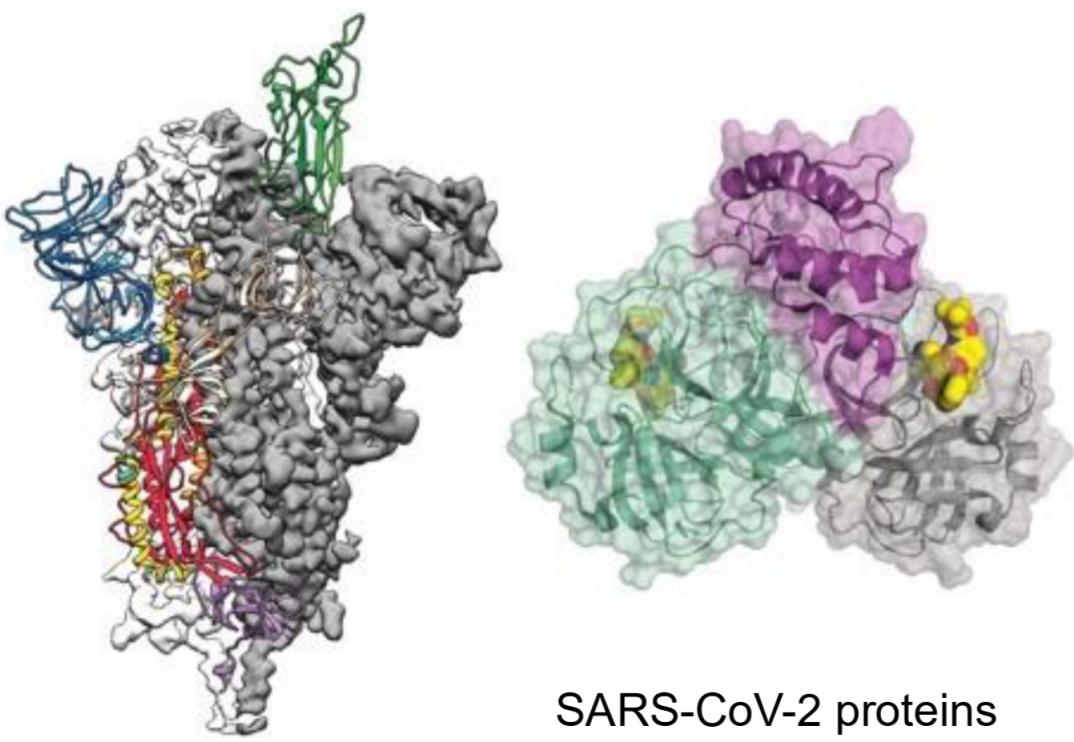
Quaternary Structure - What holds it together?

- Quaternary structure is built by interactions between protein subunits
- These take on the same characteristics as tertiary structure (this should make sense if you consider it)
- Electrostatic interactions forming between charged side chains (more prevalent in quaternary)
- London dispersion forces forming between nonpolar side chains
- Hydrogen bonds forming between polar/charged side chains
- Covalent bonds forming through disulfide bridges (less prevalent in quaternary)



Visualizing Protein Structure

Quaternary structure describes the interactions between components of a multisubunit assembly



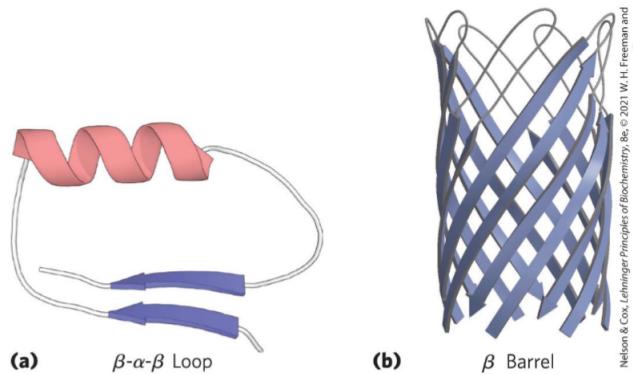
The Protein Data Bank

The Protein Data Bank (PDB): www.rcsb.org

- archive of experimentally determined three-dimensional structures
- structures assigned an identifier called the PDB ID
- PDB data files describe:
 - the spatial coordinates of each atom
 - information on how the structure was determined
 - information on its accuracy (how good the model is)
 - structure visualization software can convert atomic coordinates to an image of the molecule

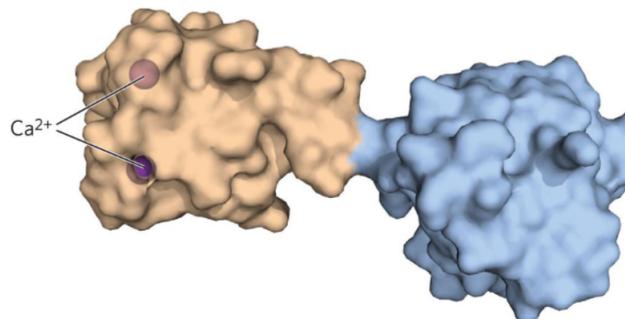
Folding Patterns of Proteins

- **motif = fold** = recognizable folding pattern involving 2+ elements of secondary structures and their connections(s)
 - can be simple, such as in a $\beta - \alpha - \beta$ loop
 - can be elaborate, such as in a β -barrel



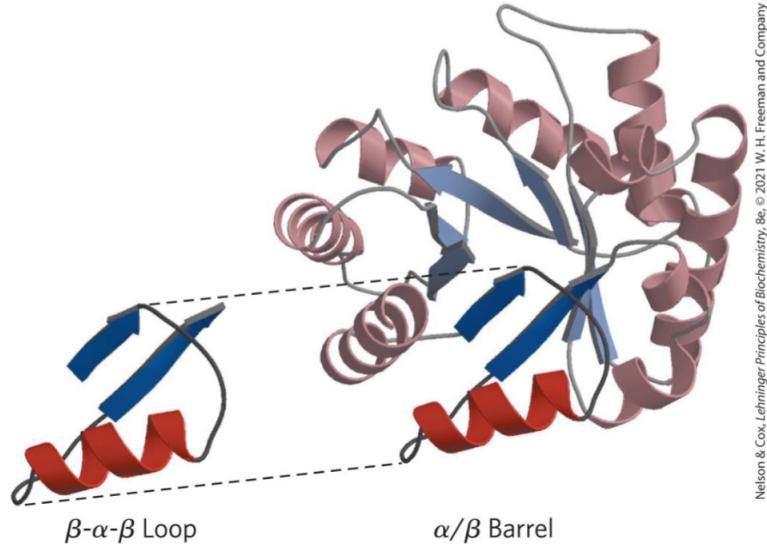
Protein Domains

- **domain** = part of a polypeptide chain that is independently stable or could undergo movements as a single entity
 - domains may appear as distinct or be difficult to discern
 - small proteins usually have only one domain



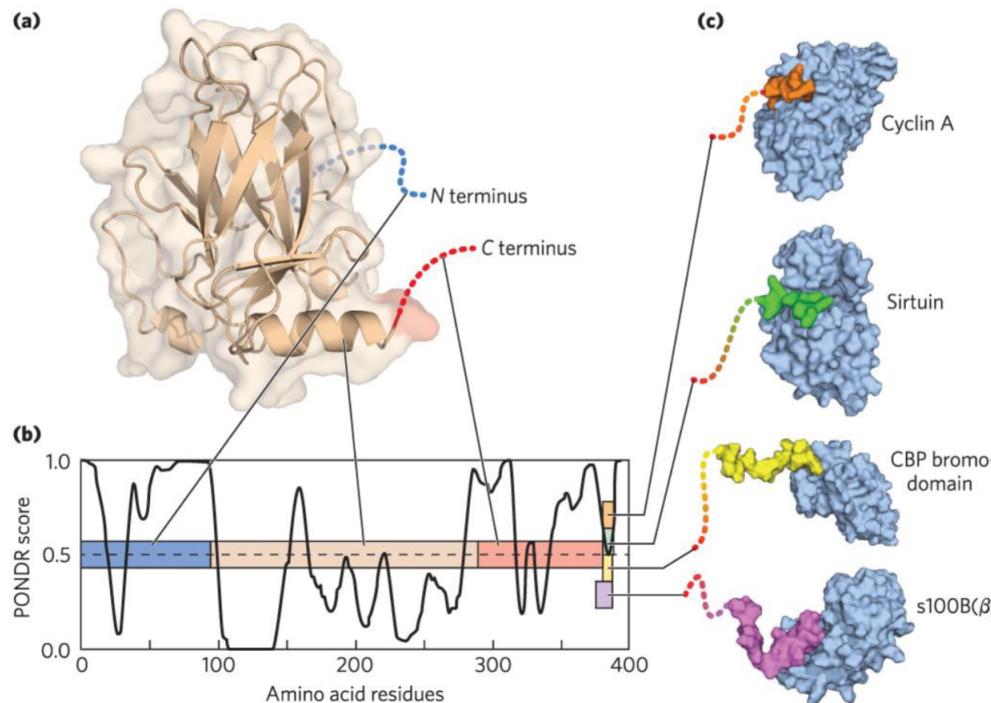
Complex Motifs are Built from Simple Motifs

α/β barrel = series of $\beta - \alpha - \beta$ loops arranged such that the β strands form a barrel



Intrinsically disordered proteins:

- lack definable structure
- often lack a hydrophobic core
- high densities of charged residues (Lys, Arg, Glu, and Pro)
- facilitates a protein to interact with multiple binding partners

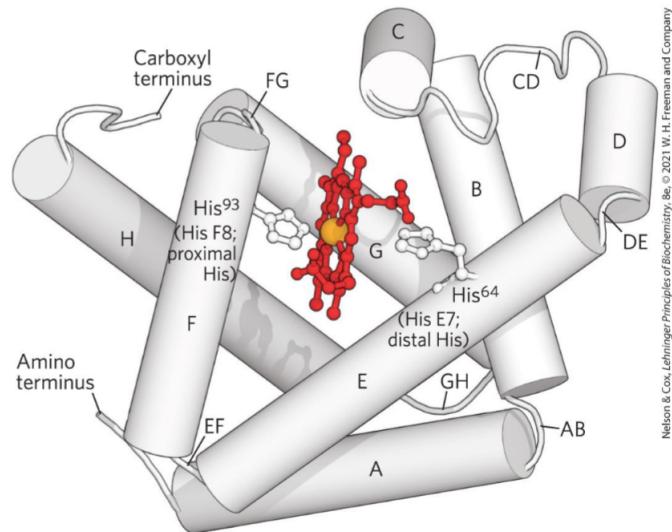


Protein Families and Superfamilies

- proteins with significant similarity in primary structure and/or tertiary structure and function are in the same **protein family**
 - ~4000 different protein families in the PDB
 - strong evolutionary relationship within a family
- **superfamilies** = 2+ families that have little sequence similarity, but the same major structural motif and have functional similarities.

Globins are a Family of Oxygen-Binding Proteins

- Globins like myoglobin and hemoglobin belong to the same protein family: **Globin Family**
- Globins are a widespread protein family:
 - highly conserved tertiary structure: eight α -helical segments connected by bends (globin fold)
 - most function in O₂ transport or storage



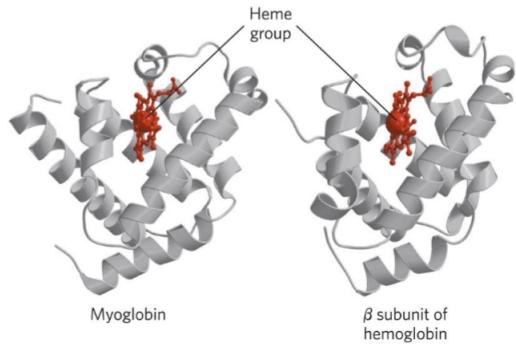
Types of Globins

- Four types in humans and other mammals:
 - **myoglobin** = monomeric, facilitates O₂ diffusion in muscle tissue
 - **hemoglobin** = tetrameric, responsible for O₂ transport in the bloodstream
 - **neuroglobin** = monomeric, expressed largely in neurons to protect the brain from low O₂ or restricted blood supply
 - **cytoglobin** = monomeric, regulates levels of nitric oxide, a localized signal for muscle relaxation

Globins and Prosthetic Groups

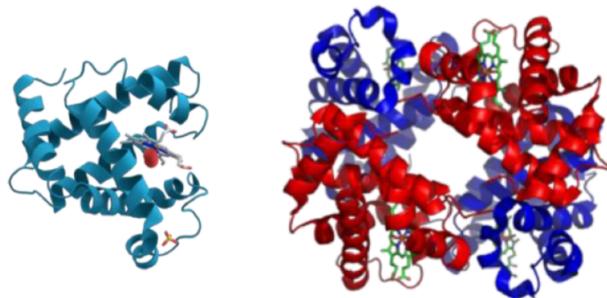
- Myoglobin and hemoglobin are examples of conjugated proteins
 - Simple proteins only have a polypeptide chain
 - **Conjugated proteins** have a non-protein component called a prosthetic group

- Myoglobin and hemoglobin have a heme prosthetic group that provides them with oxygen binding functionality (amino acids can't bind oxygen well)
- The globins are examples of **hemoproteins** (proteins with heme prosthetic groups) which are subsets of metalloproteins (proteins with metal prosthetic groups)
 - This is because heme contains ferrous iron (Fe^{2+})



Oxygen-carrying proteins

- Myoglobin (Mb)
 - O_2 acts as a ligand (can bind max 1 O_2)
 - Only one subunit
 - Acts as oxygen storage and facilitates diffusion in muscular cells
 - ≈ 64 g of Mb present in an average human body
- Hemoglobin (Hb)
 - O_2 acts as a ligand (can bind up to 4 O_2)
 - Four subunits, two subunits of α -globin, and two subunits of β -globin
 - Transports O_2 from lungs to peripheral tissues (carried in erythrocytes)
 - ≈ 775 g of Hb present in an average human body
- Both rely on the heme prosthetic group



Oxygen Can Bind to a Heme Prosthetic Group

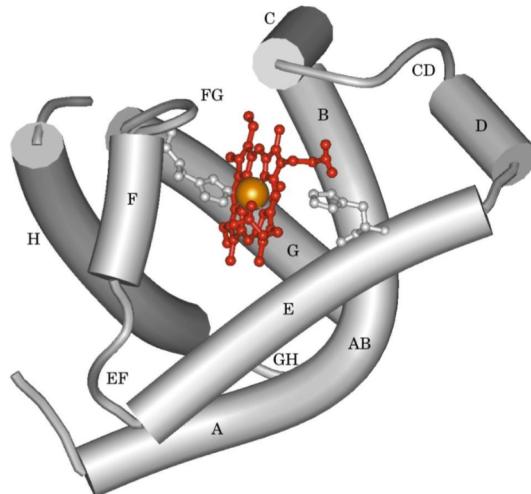
Oxygen is:

- poorly soluble in aqueous solutions
- diffusion through tissues is ineffective over large distances
- transition metals have a strong tendency to bind (iron, copper)

Heme is prosthetic group incorporated during folding

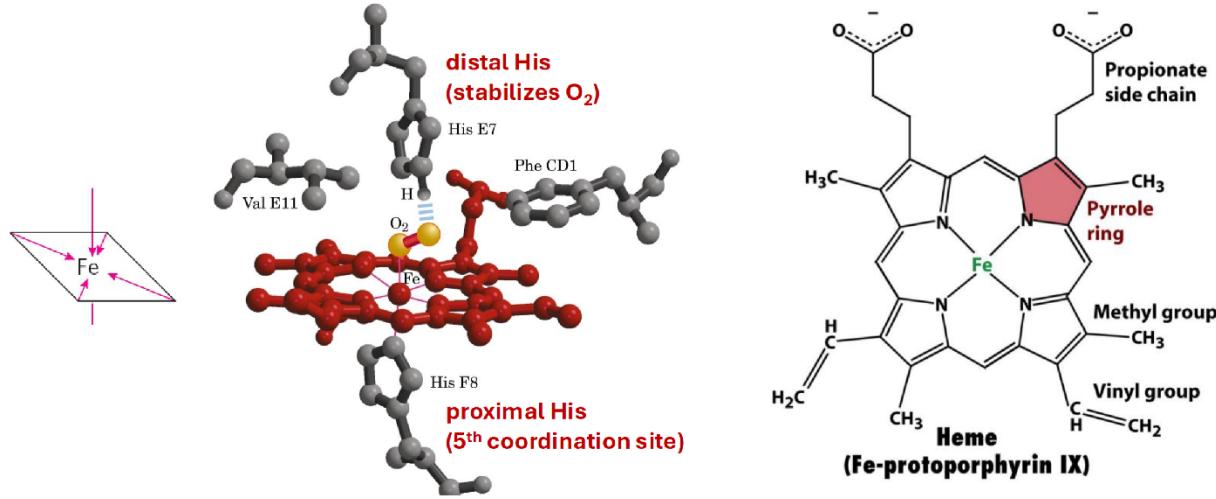
- Protoporphyrin

Heme is responsible for reversible O₂ binding.



The Heme Prosthetic Group

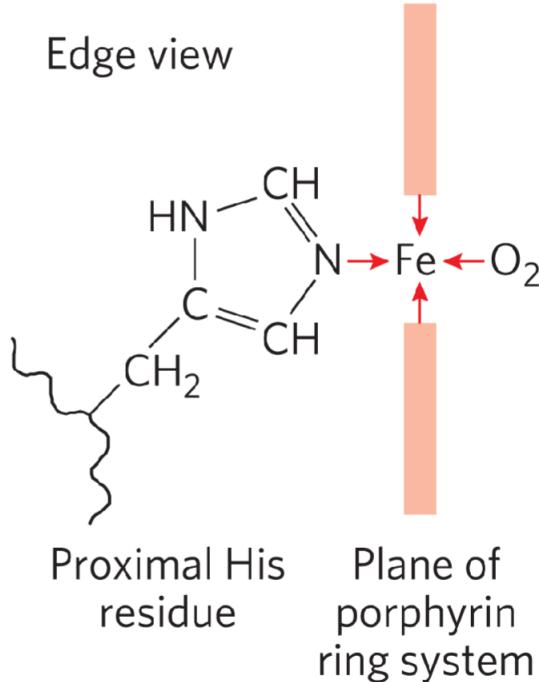
- **Heme** is prosthetic group incorporated during folding
 - Protoporphyrin
- Responsible for reversible O₂ binding
- Fe²⁺ has 6 coordination sites/bonds
 - 4 are occupied by N of pyrrole rings
 - 2 sites available perpendicular to protoporphyrin ring
 - * 1 occupied by proximal His.
 - The 6th coordination site:
 - * **Deoxyhemoglobin**: unoccupied
 - * **Oxyhemoglobin**: Occupied by O₂
 - * **Carboxyhemoglobin**: Occupied by CO



Perpendicular Coordination Bonds

Two perpendicular coordination bonds:

- one is occupied by a side-chain nitrogen of a highly conserved **proximal His** residue
- one is the binding site for molecular oxygen (O_2)
 - Fe^{2+} (ferrous iron) binds O_2 reversibly
 - Fe^{3+} (ferric iron) does not bind O_2



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Globin Contributions to O_2 Binding

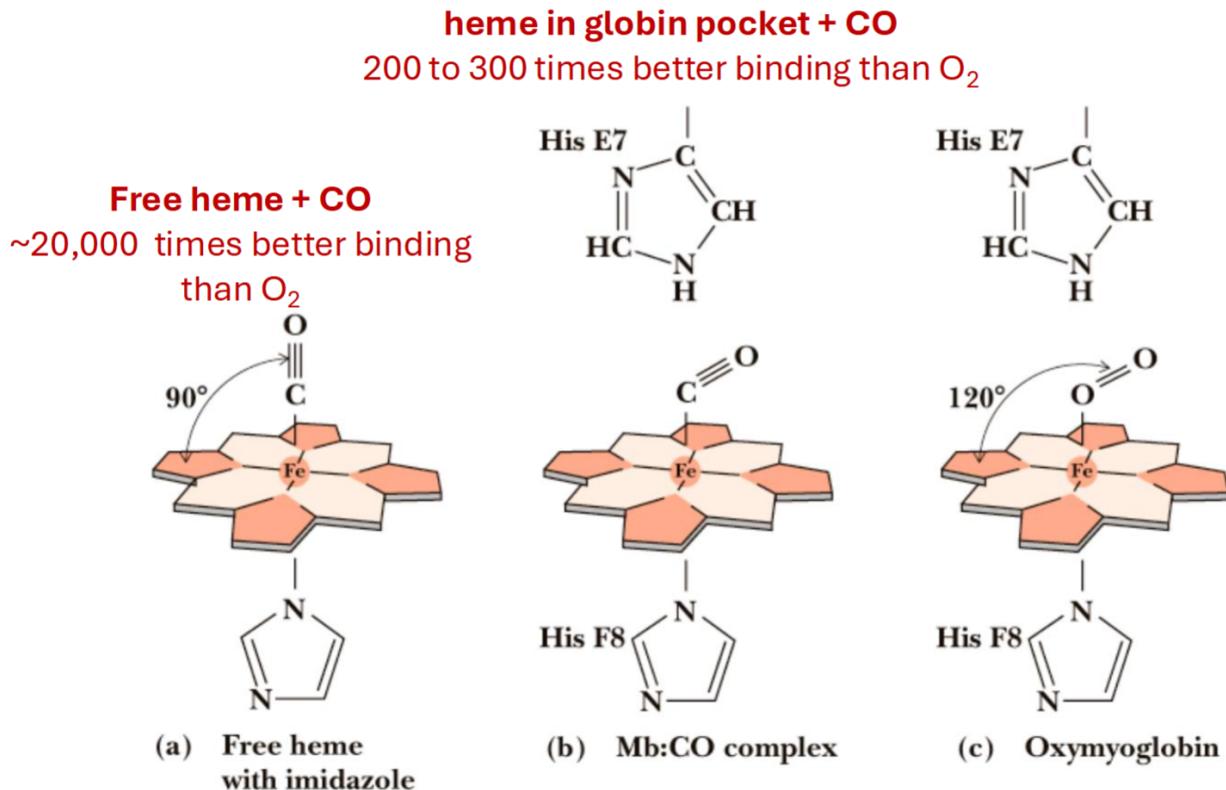
Why can't we just have heme on its own?

- Proximal histidine occupies 5th coordination site, holding heme in place (Mb and Hb)

- Other heme stabilizers - Val and Phe (Mb) or Val and Leu (Hb)
- Distal histidine stabilizes O₂ binding and acts as gate for ligand entry - encouraging specificity (Mb and Hb)
- Another contribution is specific to hemoglobin and deals with cooperativity

Role of the Distal Histidine

- For **O₂ binding**: The distal histidine stabilizes the oxygen molecule when it binds to the iron ion in the heme group via a **hydrogen bond**. This prevent the iron from being oxidized to the Fe³⁺ state, which cannot bind oxygen
- For **CO binding**: The distal histidine **hinders carbon monoxide binding** by forcing the CO molecule into a less favorable binding orientation:
 - CO prefers to bind in a straight geometry to the iron ion, but the distal histidine forces it into a bent geometry, reducing the affinity of hemoglobin for CO. While CO still binds more strongly than oxygen, the distal histidine helps prevent complete dominance by CO, providing a protective effect.



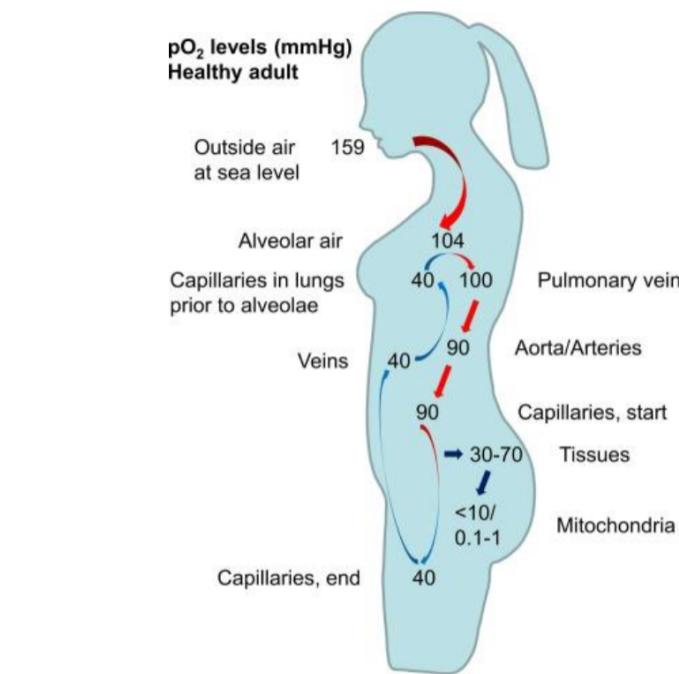
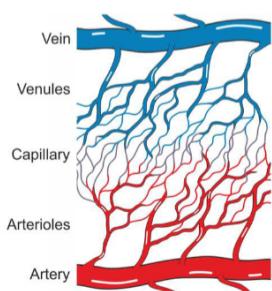
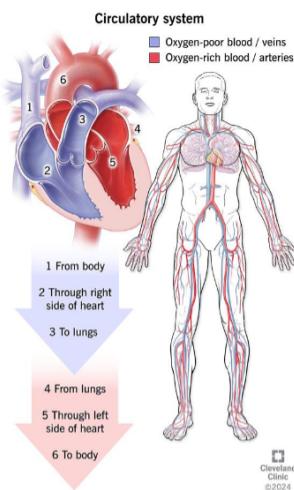
Carbon Monoxide acts as a competitive inhibitor

A **competitive inhibitor** is a compound that binds to the same site as the intended molecule (substrate or ligand), blocking the site

- Carbon monoxide acts as a competitive inhibitor to oxygen with respect to Hb

Myoglobin Has a Single Binding Site for Oxygen

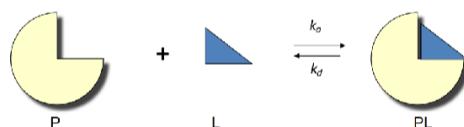
- myoglobin:
 - 153 residues + one molecule of heme
 - bends named after the α -helical segments they connect
- His⁹³ = ninety-third residue from the amino terminal end
- His F8 = eighth residue in α helix F



Venous blood (blue): low partial pressure of oxygen (pO₂), which is oxygen-poor and returning to the lungs.
Arterial blood (red): high partial pressure of oxygen (pO₂), which is oxygen-rich and delivering oxygen to tissues.

Protein-Ligand Interactions Can Be Described Quantitatively

A simple **equilibrium expression** describes the reversible binding of a protein (P) to a ligand (L):



P: Free protein

L: Free ligand

PL: Protein-ligand complex

The system will reach **equilibrium** when the rate of binding is equal to the rate of dissociation. At this point, the concentration of free protein, free ligand, and the complex remain constant

- The equilibrium state can be described with **equilibrium association constant (K_a)** and **equilibrium dissociation constant (K_d)**

Association Constant

The **association constant** (K_a) provides a measure of the affinity of the ligand L for the protein

- higher K_a = higher affinity
- equivalent to the ratio of the rates of the forward (association) and the reverse (dissociation) reactions that form the PL complex
- K_a and K_d are the forward and reverse rate constants

$$k_a[P] \cdot [L] = k_d[PL]$$

$$K_a = \frac{[PL]}{[P][L]} = \frac{k_a}{k_d}$$

Dissociation Constant

The **dissociation constant** (K_d) is the reciprocal of K_a .

- equilibrium constant for the release of ligand
- lower K_d = higher affinity.

$$K_d = \frac{[P][L]}{[PL]} = \frac{k_d}{k_a}$$

- K_d is often used to describe protein-ligand interactions in practice
- A **low K_d** means strong binding (high affinity) because the protein-ligand complex is less likely to dissociate
- A **high K_d** means weak binding (low affinity)

Understanding Protein-Ligand Binding via Equilibrium Constants

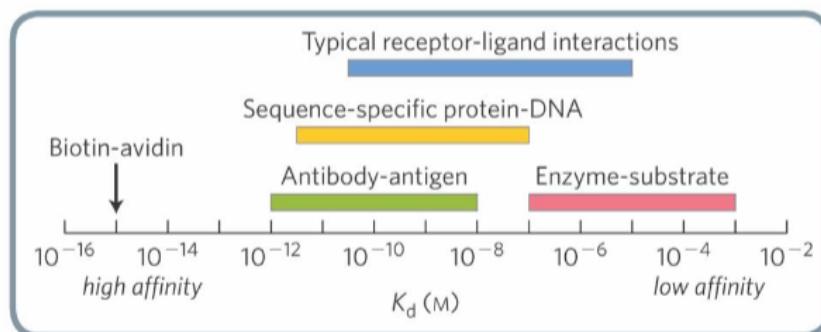
- At **equilibrium**, the ratio of bound ligand (PL) to free ligand (P and L) is constant, and this ratio is determined by K_a or K_d .
- K_a gives us an idea of how readily the protein binds the ligand, while K_d tells us how easily the complex falls apart.

Representative K_d Values

Avidin-biotin complex is the strongest known non-covalent interaction ($K_d = 10^{-15}M$) between a protein and ligand

TABLE 5-1 Protein Dissociation Constants: Some Examples and Range

Protein	Ligand	K_d (M) ^a
Avidin (egg white)	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human) ^b	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (<i>E. coli</i>)	Ni^{2+}	1×10^{-7}
Calmodulin (rat) ^c	Ca^{2+}	3×10^{-6}
		2×10^{-5}



Color bars indicate the range of dissociation constants typical of various classes of interactions in biological systems. A few interactions, such as that between the protein avidin and the enzyme cofactor biotin, fall outside the normal ranges. The avidin-biotin interaction is so tight it may be considered irreversible. Sequence-specific protein-DNA interactions reflect proteins that bind to a particular sequence of nucleotides in DNA, as opposed to general binding to any DNA site.

^aA reported dissociation constant is valid only for the particular solution conditions under which it was measured. K_d values for a protein-ligand interaction can be altered, sometimes by several orders of magnitude, by changes in the solution's salt concentration, pH, or other variables.

^bThis immunoglobulin was isolated as part of an effort to develop a vaccine against HIV. Immunoglobulins (described later in the chapter) are highly variable, and the K_d reported here should not be considered characteristic of all immunoglobulins.

^cCalmodulin has four binding sites for calcium. The values shown reflect the highest- and lowest-affinity binding sites observed in one set of measurements.

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Binding Equilibrium

Let's consider binding equilibrium from the standpoint of the fraction of binding sites on the protein that are occupied by ligand.

- **Fractional Occupancy** (Y or θ): is the fraction of the total protein bound to the ligand at any given ligand concentration (fraction of binding sites occupied)

$$Y = \frac{\text{binding sites occupied}}{\text{total binding sites}} = \frac{[PL]}{[PL] + [P]}$$

- Y ranges from 0 to 1.
 - $Y = 1$ means all binding sites are occupied (fully saturated with ligand)
 - $Y = 0$ means no binding sites are occupied (all protein is in its unbound state)

$$K_a = \frac{[PL]}{[P][L]}$$

$$K_a[L][P] = [PL]$$

substituting $K_a[L][P]$ for $[PL]$. . .

$$Y = \frac{K_a[L][P]}{K_a[L][P] + [P]}$$

$$= \frac{K_a[L]}{K_a[L] + 1}$$

$$= \frac{[L]}{[L] + \frac{1}{K_a}}$$

$$= \frac{[L]}{[L] + K_d}$$

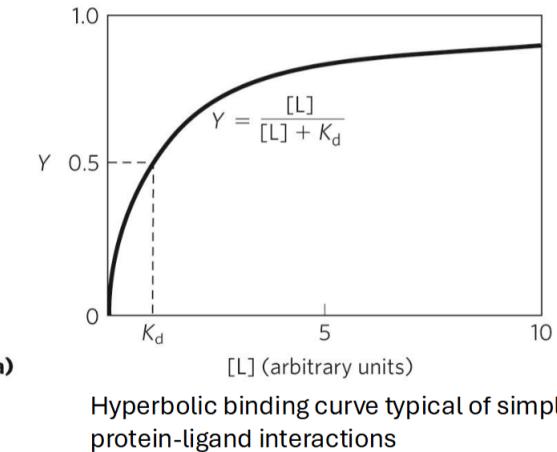
- This expression describes a hyperbola!

Graphical Representations of Ligand Binding

$$Y = \frac{[L]}{[L] + \frac{1}{K_a}} = \frac{[L]}{[L] + K_d}$$

L at which half of the available ligand-binding sites are occupied ($Y = 0.5$) corresponds to K_d or $1/K_a$.

- Therefore, our best method for finding K_d is to find $[L]$ when $Y = 0.5$.



Binding of O₂ to Myoglobin

- substituting the [O₂] for [L]

$$Y = \frac{[O_2]}{[O_2] + K_d}$$

- K_d equals the [O₂] at which half of the available ligand-binding sites are occupied, or [O₂]_{0.5}.

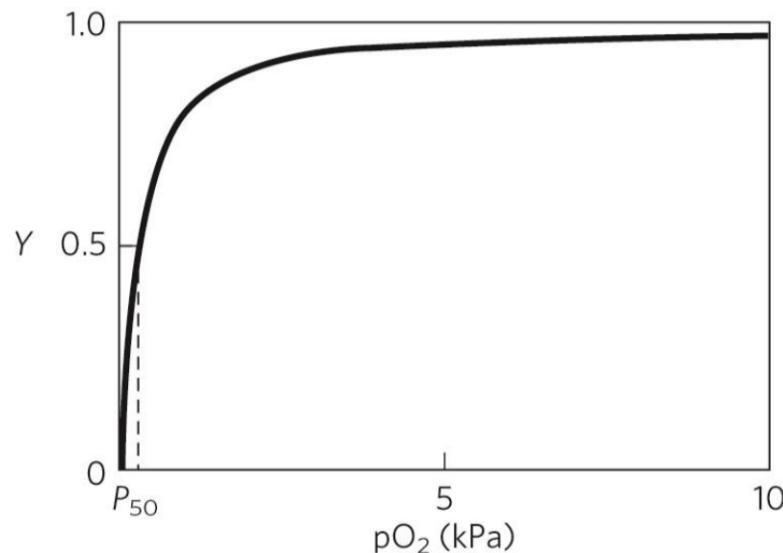
$$Y = \frac{[O_2]}{[O_2] + [O_2]_{0.5}}$$

Partial Pressure of O₂

- partial pressure of O₂ (pO_2) is easier to measure than [O₂].
- Defining the partial pressure of oxygen at [O₂]_{0.5} as P_{50} :

$$Y = \frac{[O_2]}{[O_2] + P_{50}}$$

- Thus, P_{50} refers to the **partial pressure of oxygen (pO₂)** at which the **fractional saturation (Y)** of a protein (like hemoglobin or myoglobin) is 50%. In other wordsd, P_{50} is the partial pressure of oxygen at which half of the binding sites on the protein are occupied by O₂ molecules.

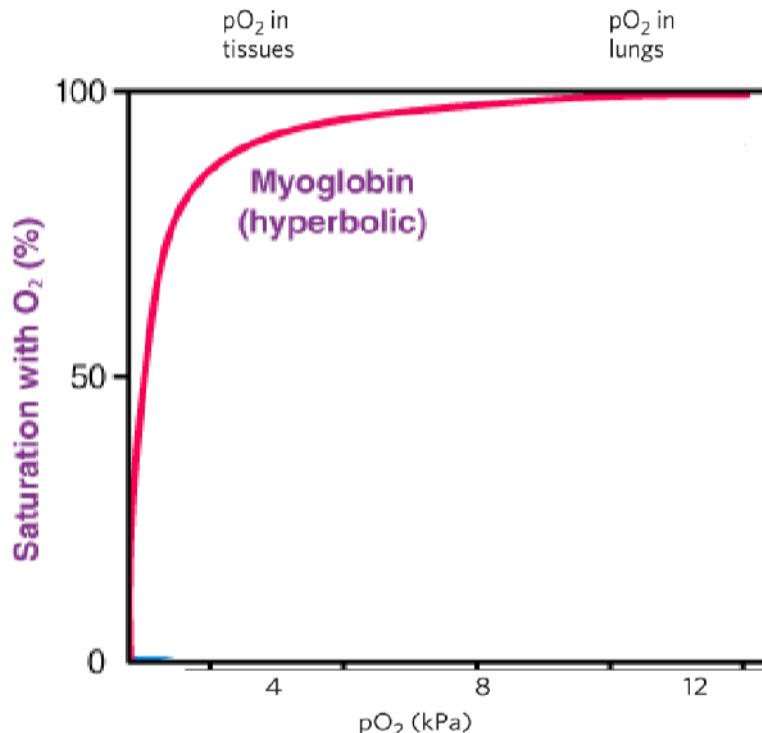


- In physiology, pO₂ is directly related to how gases exchange in the lungs and tissues. Hemoglobin saturation and oxygen transport are functions of pO₂ rather than the direct concentration of oxygen. Therefore, pO₂ is more relevant and practical to measure in the context of oxygen binding and release in biological systems, especially for processes like respiration and oxygen transport.
- **P_{50} is a measure of the affinity of the protein for oxygen.** A lower P_{50} means that the protein has a **higher affinity** for oxygen (it can bind oxygen more easily at a lower pO₂), while a **higher P_{50}** indicates a **lower affinity** (it requires a higher pO₂ to achieve 50% saturation)

Binding of O₂ to Myoglobin and Hemoglobin

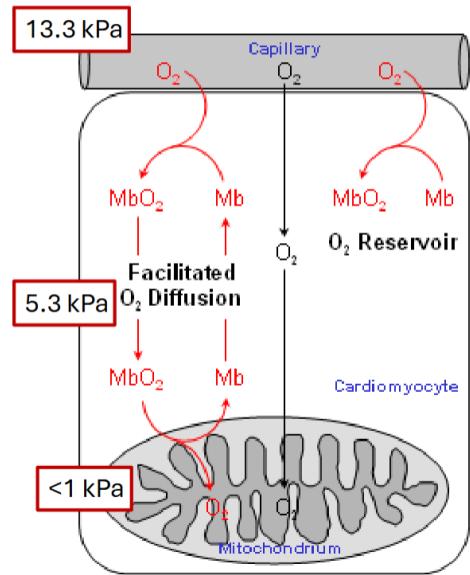
- **Myoglobin** has a **very low P₅₀** indicating a **high affinity for oxygen**. It binds oxygen tightly, even at low partial pressures, which is important for oxygen storage in muscle tissues
- **Hemoglobin**, on the other hand, **has a higher P₅₀ than myoglobin**, reflecting its lower affinity for oxygen compared to myoglobin. This allows hemoglobin to release oxygen **more easily in tissues where the partial pressure of oxygen is lower**, while still binding oxygen efficiently in the lungs, where the partial pressure is higher

Behavior of Myoglobin with respect to partial pressure of oxygen in the body

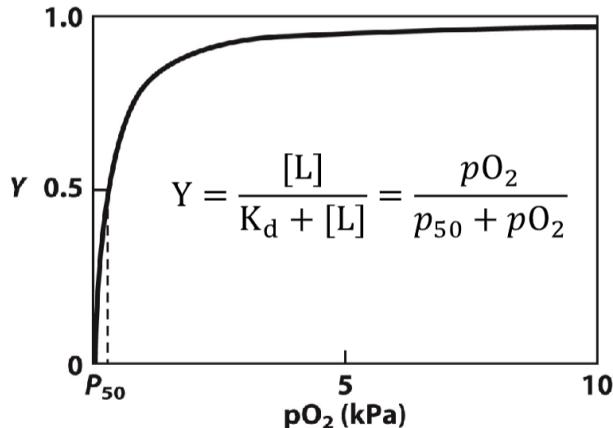


- At low pO₂, myoglobin has a high affinity for oxygen, enabling it to effectively bind oxygen in tissues even when oxygen levels are low.
- Myoglobin is primarily found in muscle tissues and serves as an **oxygen storage molecule**.
- In resting muscle, O₂ is moderate (~4-5 kPa), and myoglobin maintains a high saturation level, acting as a reservoir. In active muscle, pO₂ drops to very low levels (<1 kPa), prompting myoglobin to release its stored oxygen to support metabolic activity
- **Venous blood** (blue): low partial pressure of oxygen (pO₂), which is oxygen-poor and returning to the lungs.
- **Arterial blood** (red): high partial pressure of oxygen (pO₂), which is oxygen-rich and delivering oxygen to tissues.

Oxygen Binding to Myoglobin



Myoglobin binds oxygen tightly

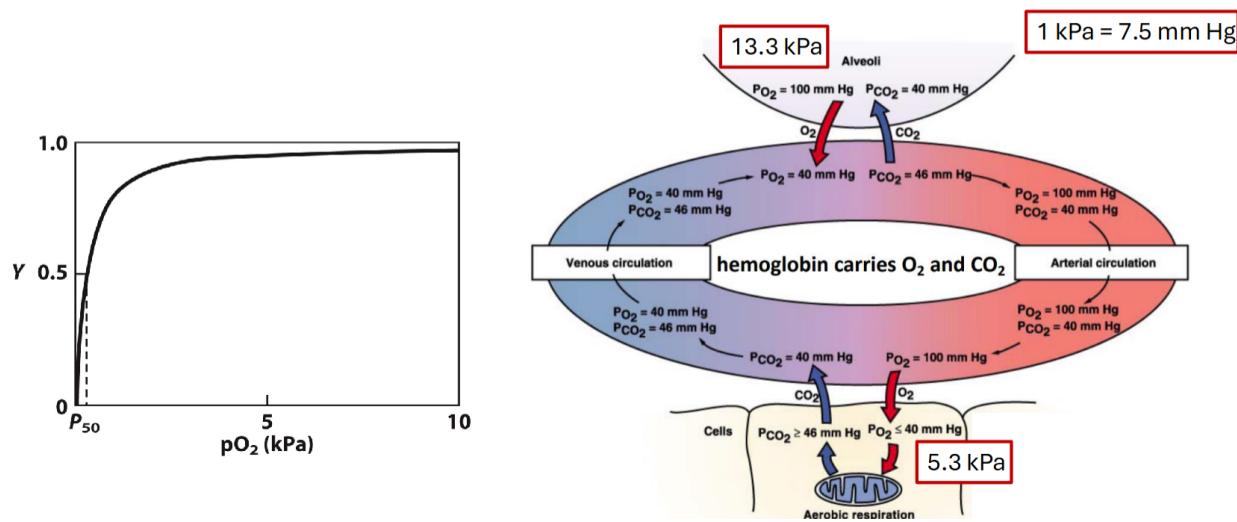


Y = fraction of sites bound, $[L]$ = ligand concentration, K_d = dissociation constant, pO_2 = partial pressure of oxygen
 P_{50} = 50% of heme groups of each myoglobin have a molecule of oxygen bound

- At 13.3 kPa (lungs/alveoli) reflects oxygen-rich conditions in the lungs
 - Myoglobin is almost fully saturated with oxygen at this high partial pressure
 - Binds oxygen very efficiently, acting as an oxygen reservoir
 - The binding curve is hyperbolic, approaching 100% saturation at these oxygen levels
- At 5.3 kPa (capillary blood/tissues) Oxygen pressure is lower, myoglobin remains highly saturated acting as an oxygen reservoir, essential for maintaining oxygen supply in tissues
- At <1 kPa (mitochondria):
 - At very low pO_2 levels inside mitochondria, myoglobin begins releasing oxygen (crucial for cellular respiration)
 - Myoglobin's high affinity ensures oxygen is available exactly where it's needed for energy production, such as in muscle cells during activity

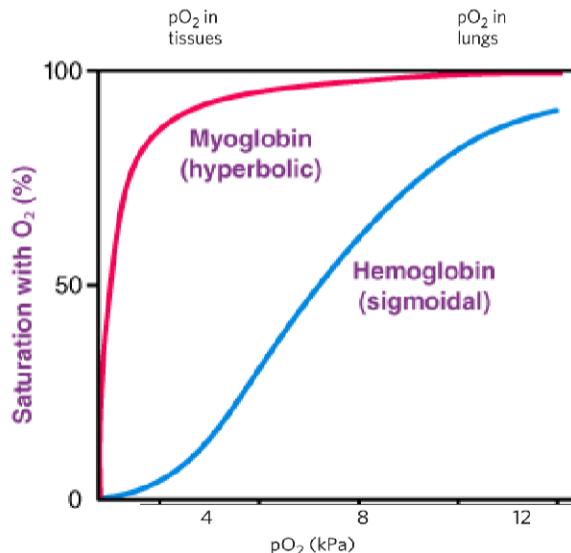
Why doesn't myoglobin carry O_2 through the body?

- Myoglobin tightly binds to O_2 at low pO_2 **and** high pO_2
- An efficient carrier of O_2 has to **let go of it** when exposed to lower pO_2



We need a different kind of curve

- Myoglobin can't let go in O_2 in tissues so why don't we lower its affinity?
 - Low affinity too inefficient
- We need an oxygen-binding protein that can flip its behavior **sigmoidally** when moving from low affinity to high affinity
- Has to have:
 - Multiple binding sites
 - Binding sites must be able to "communicate to each other"
 - * We're going to call the **cooperativity**



Graphical Representations of Ligand Binding

Fractional Occupancy (Y or θ)

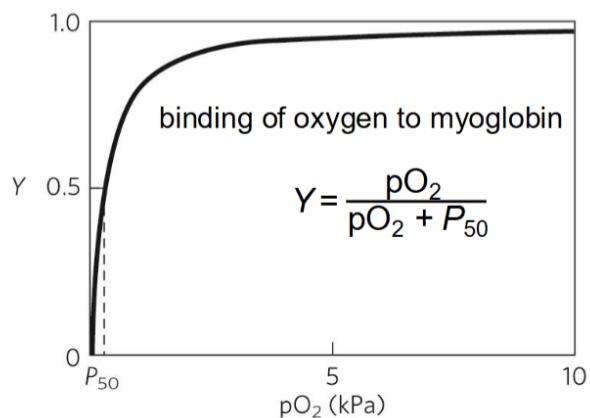
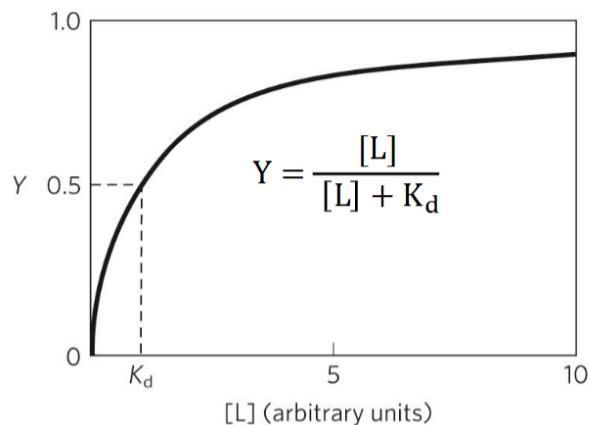
$$Y = \frac{\text{binding sites occupied}}{\text{total binding sites}} = \frac{[PL]}{[PL] + [P]} = \frac{[L]}{[L] + \frac{1}{K_a}}$$

L at which half of the available ligand-binding sites are occupied ($Y = 0.5$) corresponds to K_d .

- A lower value of K_d corresponds to a higher affinity of ligand for the protein
- The more tightly a protein binds a ligand, the lower the concentration of ligand required for half the binding sites to be occupied, and thus the lower value of K_d

•

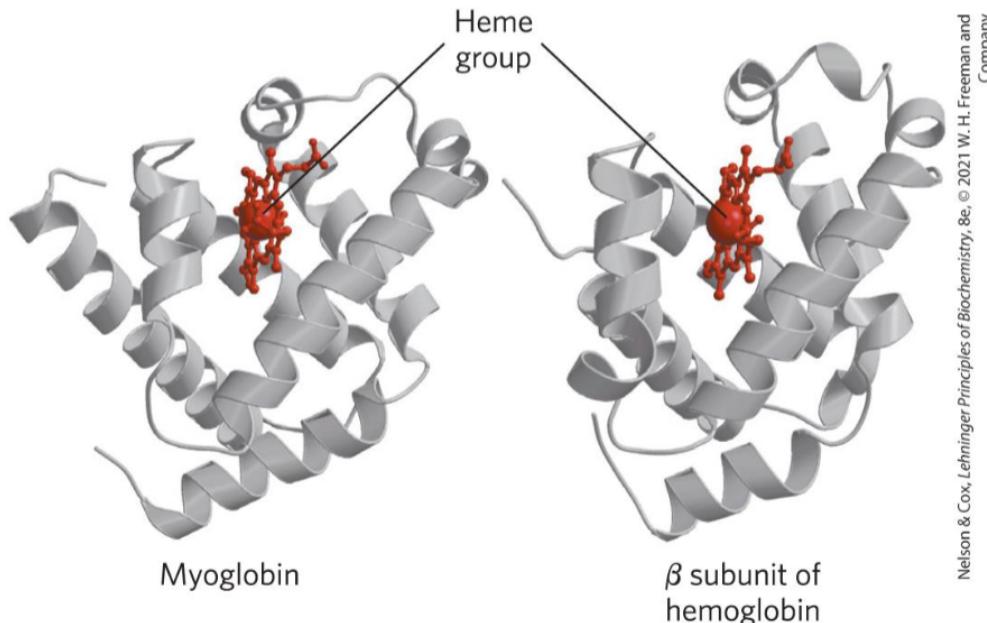
$$Y = \frac{[O_2]}{[O_2] + K_d} = \frac{[O_2]}{[O_2] + [O_2]_{0.5}}$$



Hemoglobin Subunits are Structurally Similar to Myoglobin

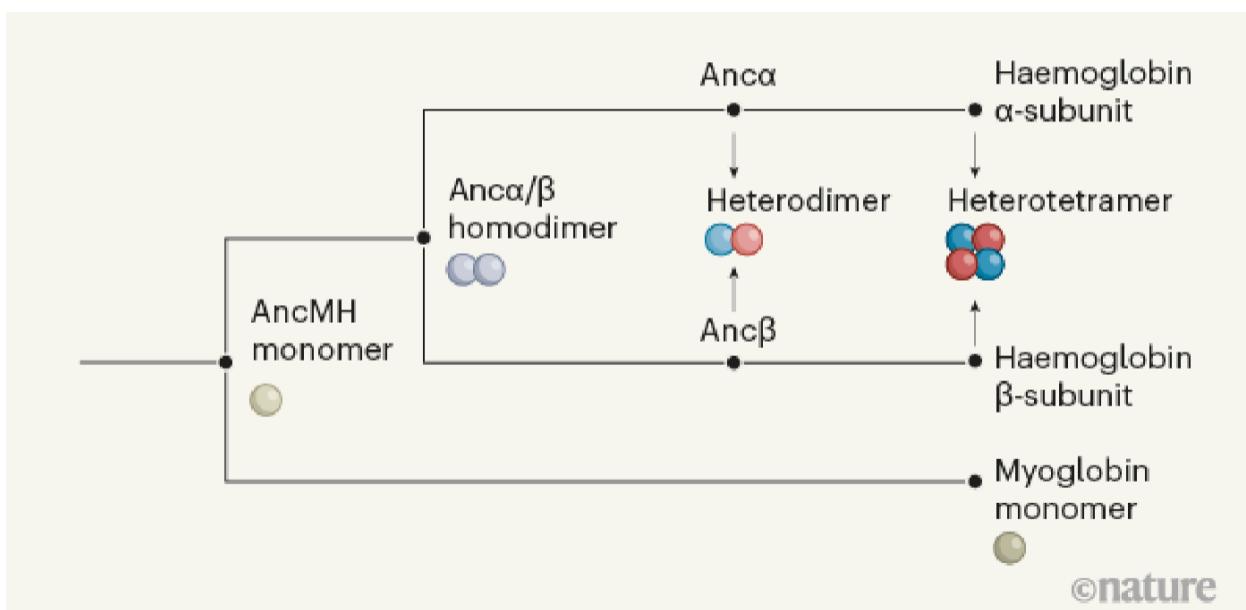
Hemoglobin:

- tetrameric protein with 4 heme groups
- adult hemoglobin has two globin types: two α chains (141 residues each) and two β chains (146 residues each)

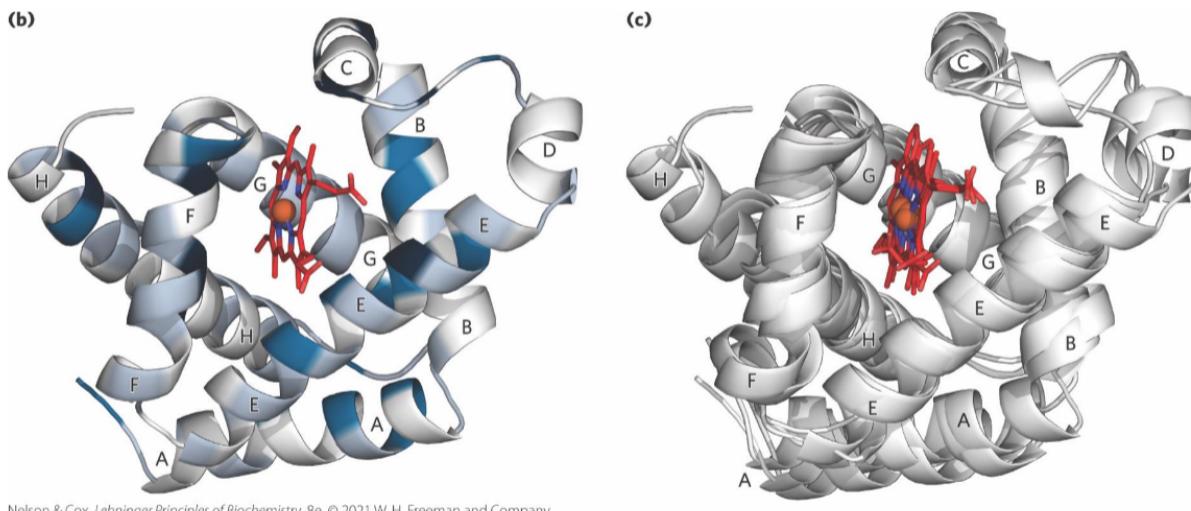


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Key steps in the evolution of the tetrameric hemoglobin protein



Structural Conversation of Globins



Low sequence similarity

High structural similarity

The Quaternary Structure of Hemoglobin

- strong interactions between unlike subunits (α and β)
 - hydrophobic effect
 - hydrogen bonds
 - ion pairs (salt bridges)
- $\alpha_1\beta_1$ (and $\alpha_2\beta_2$) interface involves >30 residues

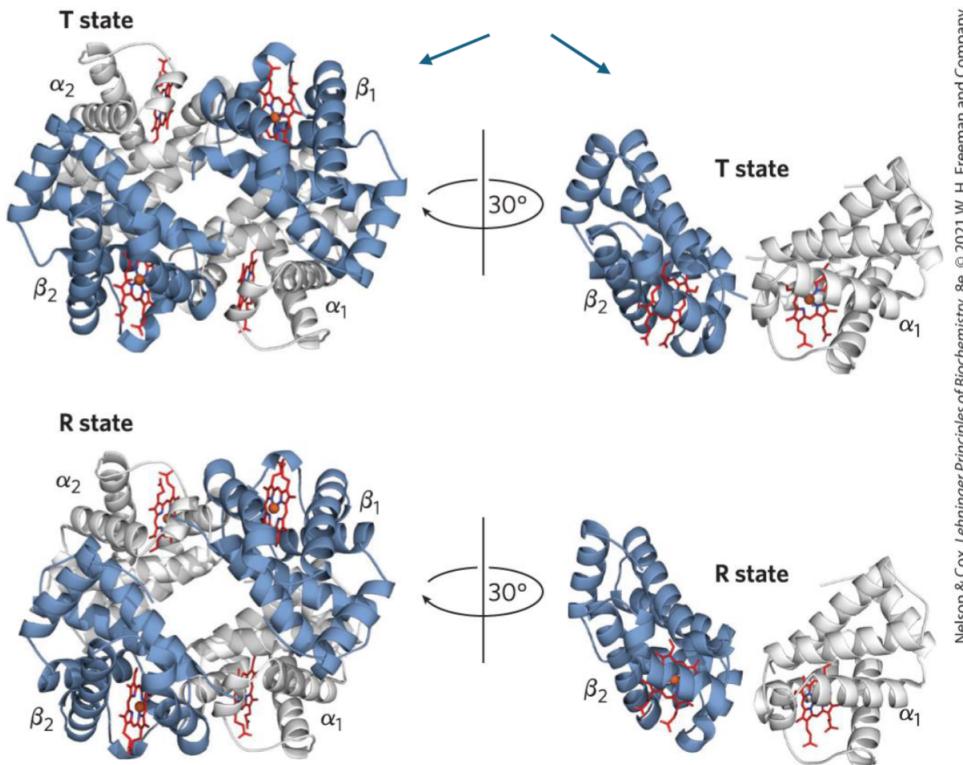
- $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface involves 19 residues

The binding of a protein and ligand is often coupled to a conformational change in the protein that makes the binding site more complementary to the ligand, permitting tighter binding. The structural adaptation that occurs between protein and ligand is called **induced fit**.

Hemoglobin Undergoes a Structural Change on Binding Oxygen

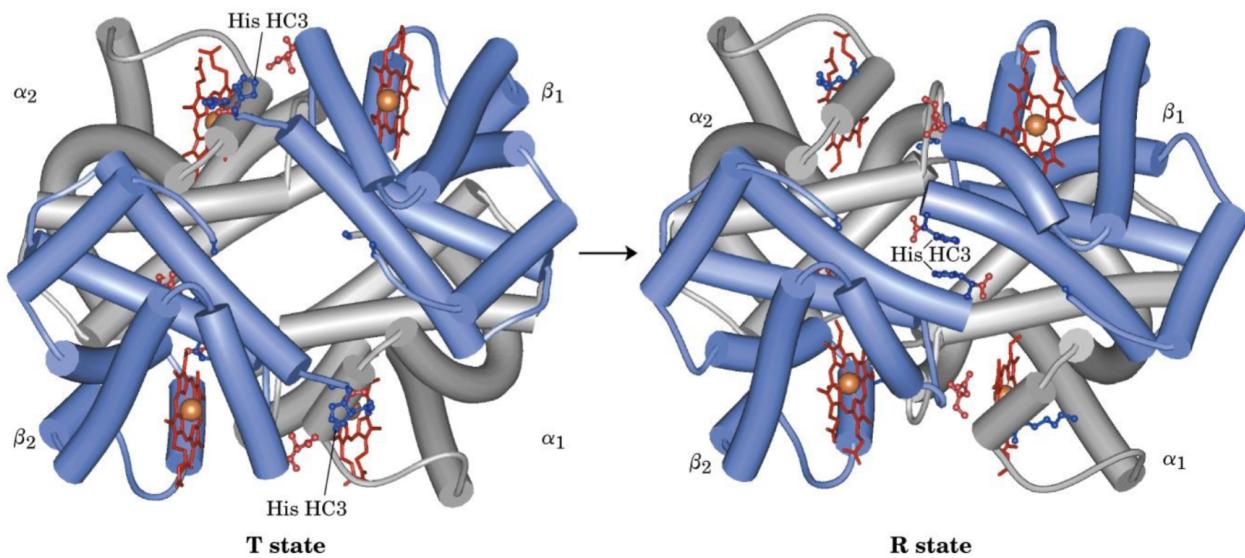
There are two conformations of hemoglobin:

- **T state** = more stable when O₂ is absent, predominant conformation of deoxyhemoglobin
- **R state** = O₂ has a higher affinity for hemoglobin



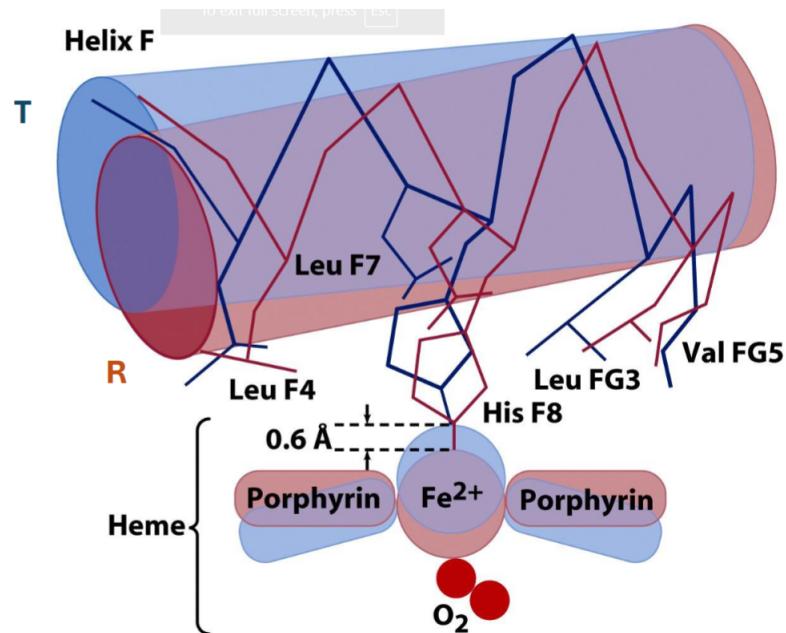
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Change in State from T → R

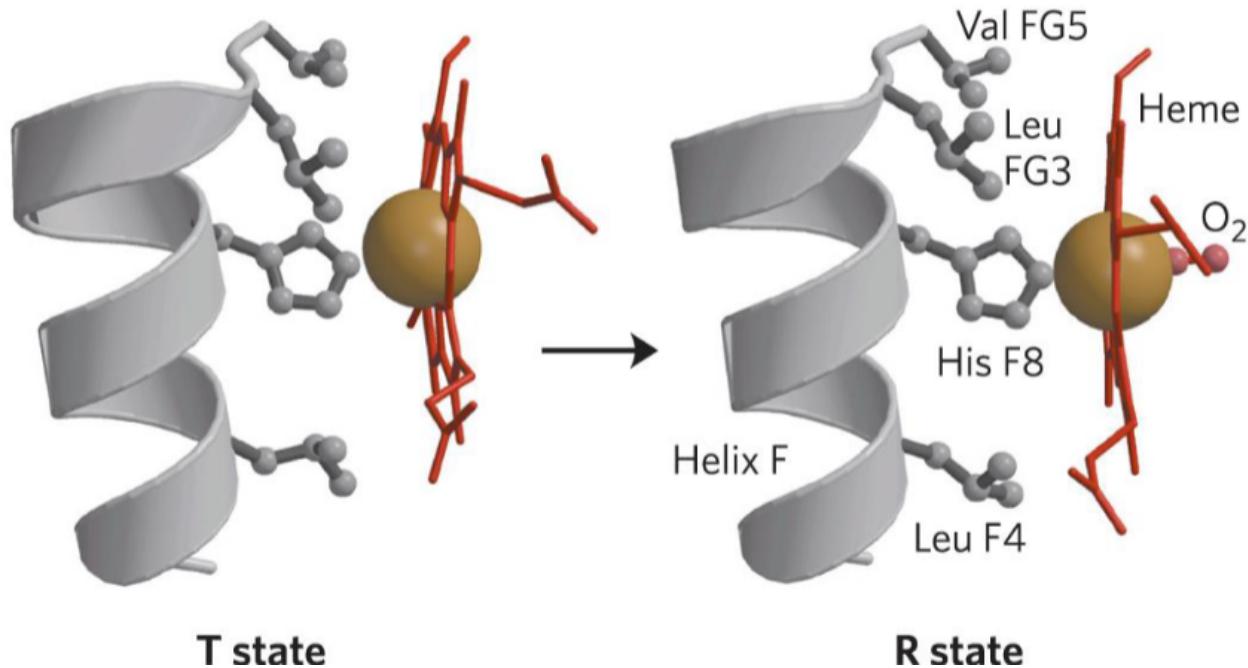


How does oxygenation promote the R state?

- In T state, Fe^{2+} sits below the plane of protoporphyrin
- When oxygen binds, this pulls the Fe^{2+} downwards, pulling the proximal His along
- This shifts the alpha helix (Helix F) that the proximal His is a part of.
- Helix F movement alters the spatial arrangement of subunits



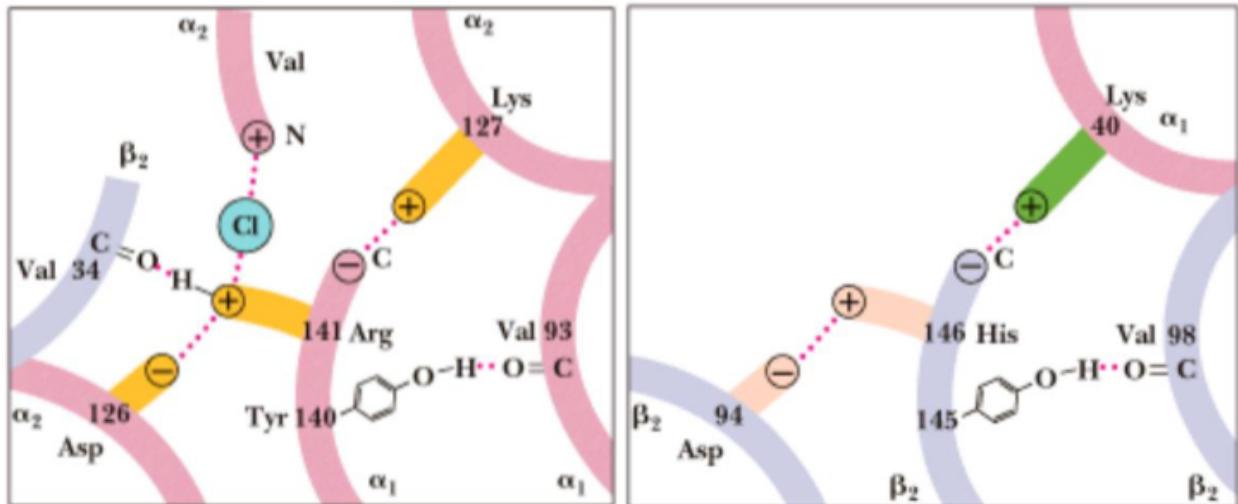
Changes in Conformation Near Heme



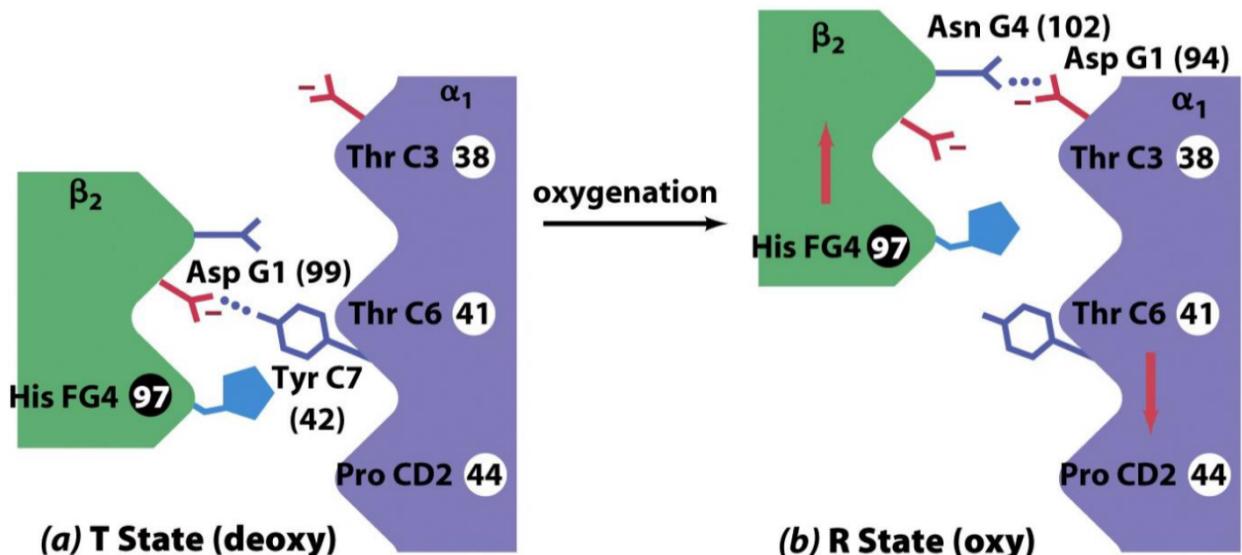
Disruption of Salt Bridges Reinforces Transition

- Shifting causes salt bridges between adjacent subunits to break
- The more bridges break, the more **Relaxed** structure you get (this is where R state name comes from
- T state is Tense)



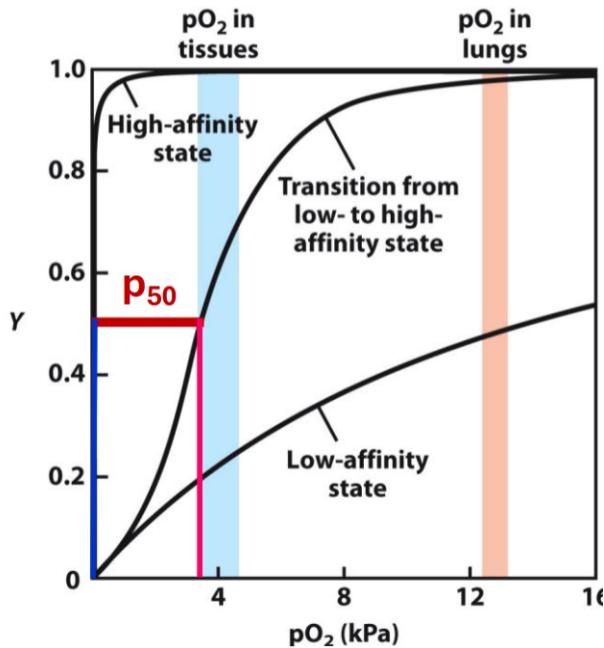


More changes at α/β interface during transition



(From Before...) We need a different kind of curve

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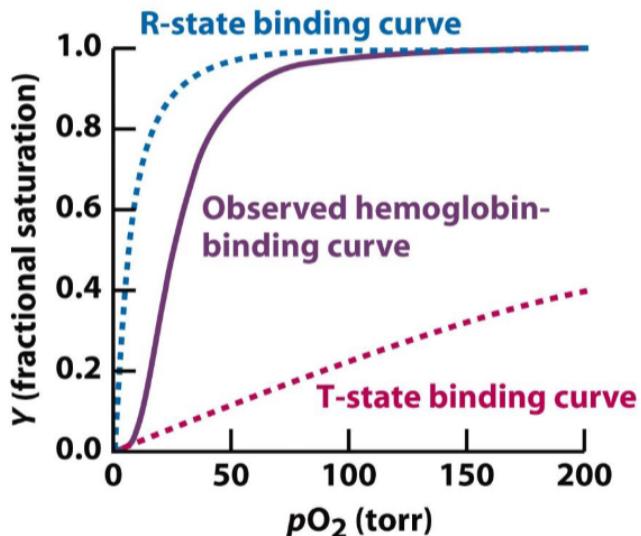
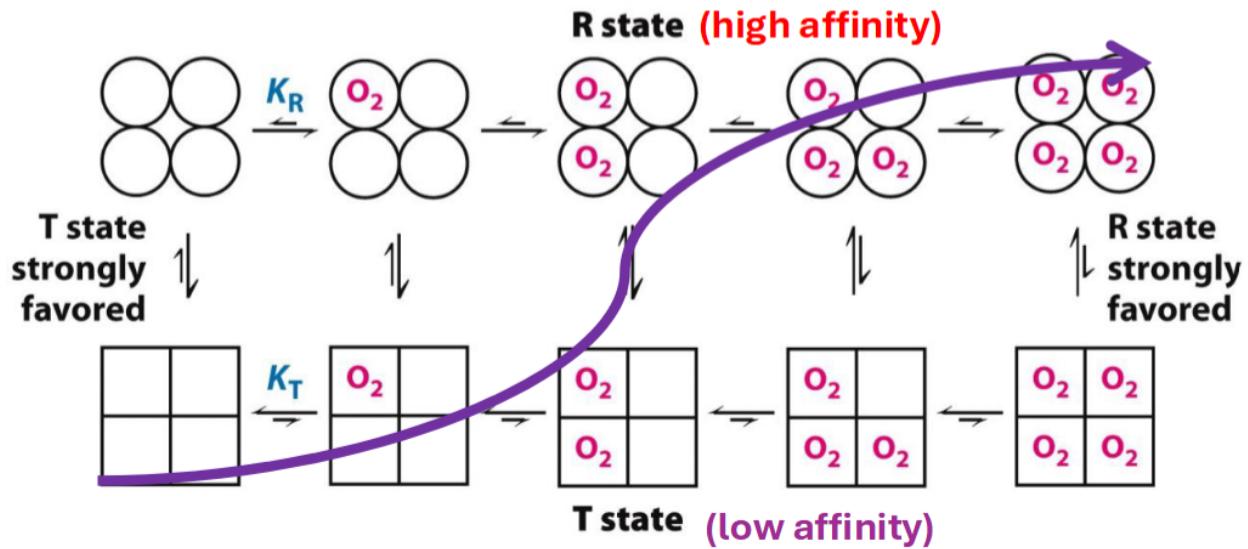


Cooperativity in Hemoglobin

- **Cooperativity** happens when the shape of one subunit (in a multi-subunit protein) is altered by the ligand, impacting the shape of a neighboring subunit
 - **Positive cooperativity** when this effect increases affinity for the ligand at the other subunits
 - * Sigmoidal binding curve is how we recognize the image below.
 - **Negative cooperativity** when this effect decreases affinity for the ligand at the other subunits
- Hemoglobin is positively cooperative its four subunits increase in affinity as oxygen binds
 - T → R state is low → high affinity

R and T states of Hemoglobin

- As pO_2 increases, oxygen binds to hemoglobin
- This increases the likelihood of transitioning to R state
 - Which increases the affinity to O_2
- Same process can occur in reverse ($\downarrow pO_2$, $\downarrow O_2$ binding, R → T, \downarrow affinity)



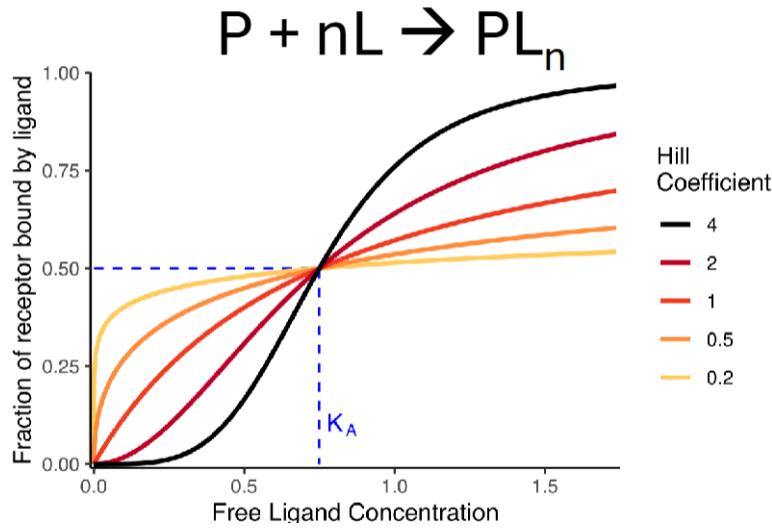
Measuring Cooperativity - Hill Coefficient

- Cooperativity can be quantified - generating our sigmoidal behavior
- The **Hill Coefficient (nH)** determines the degree of cooperativity of a protein-ligand system
- Cannot exceed the number of ligand binding sites on the protein
- Hill coefficient = n_H** = slope of a Hill plot
 - If n_H = 1, ligand binding is not cooperative
 - n_H > 1 indicates positive cooperativity
 - n_H < 1 indicates negative cooperativity

$$Y = \frac{[L]}{K_d + [L]} \quad Y = \frac{[L]^n}{K_d + [L]^n}$$

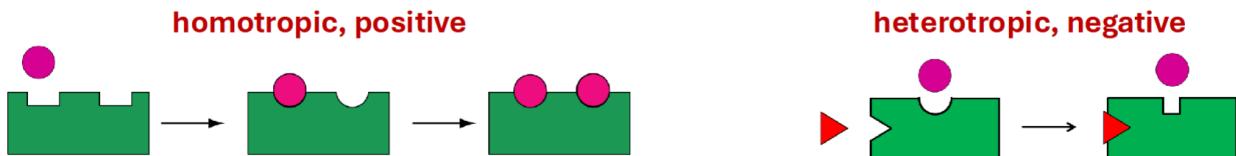
- Based on the equation, the Hill plot should have a slope of n. However, the experimentally determined slope actually reflects not the number of binding sites but the degree of interaction between them. The

slope of a Hill plot is therefore denoted by nH , the **Hill coefficient**, which is a measure of the degree of cooperativity.



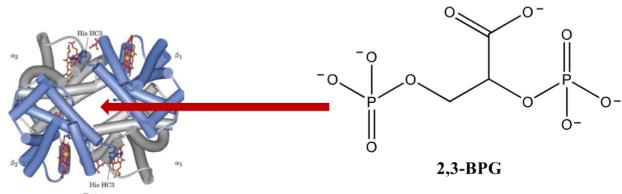
Allosteric Regulation of Protein Function

- **Allosteric regulation** is the regulation of protein function by the binding of an effector molecule at a site other than the impacted site (also a concept for enzymes)
- Allosteric regulation can be homotropic or heterotropic
 - **Homotropic regulation** is when the substrate also regulates function
 - **Heterotropic regulation** is when a different molecule than the substrate regulates function
 - Can be positive or negative
 - * **Positive** regulation increases activity/findings, **negative** decreases activity/binding



Other Allosteric Regulators of Hemoglobin: 2, 3-BPG

- Oxygen isn't the only regulator of hemoglobin's behavior
- **2,3-BPG** (2,3-Bisphosphoglyceric acid) is a heterotropic negative regulator of O₂ binding that's present in human red blood cells at high concentrations (≈ 5 mmol/L)
- It binds to the central cavity of hemoglobin, stabilizing the T state



- Behavior of BPG was discovered when researchers studied **pure hemoglobin**
- Pure hemoglobin couldn't release O₂ at low pO₂, which would create a low efficiency delivery system
 - BPG attunes hemoglobin's binding curve for maximal delivery

