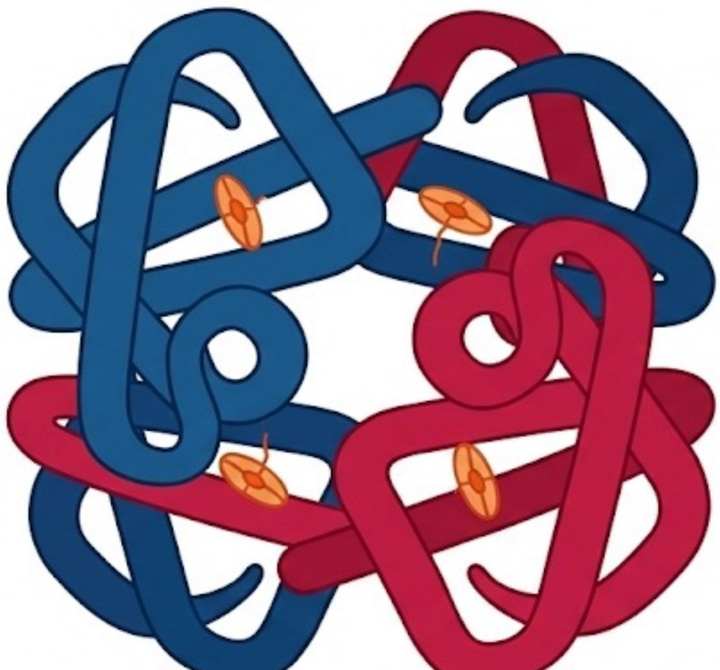
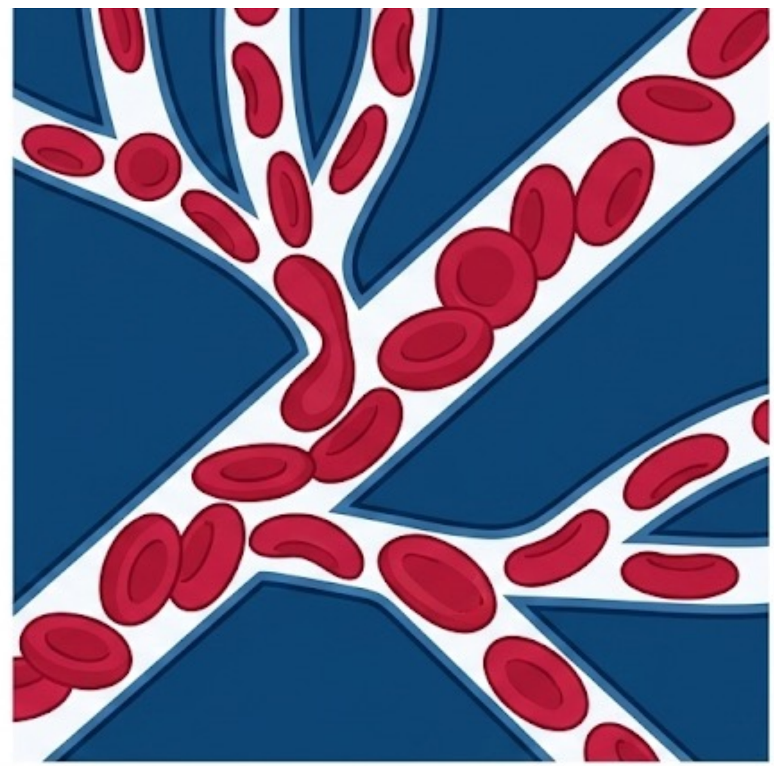
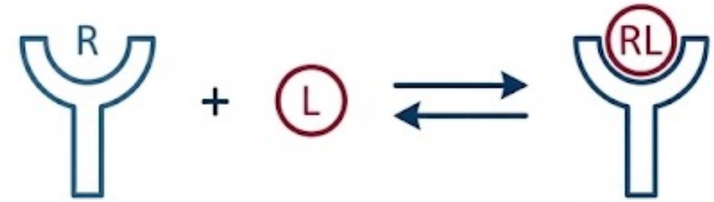
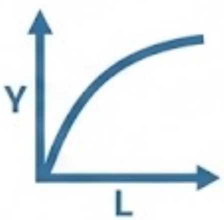
Chapter 5
Protein Binding, Molecular Recognition, and Allostery





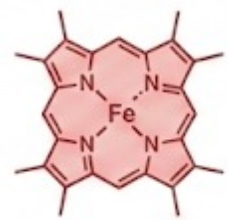


By end of lecture you can…



Binding Curve

1. Understand the qualitative relationship where ligand concentration affects receptor saturation (hyperibolic curve).

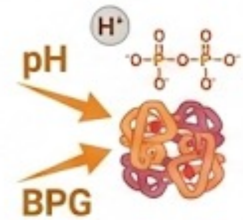


Heme Group

2. Recognize the core principle of oxygen binding to the iron within the heme prosthetic group of myoglobin and hemoglobin.


Cooperative Sigmoid

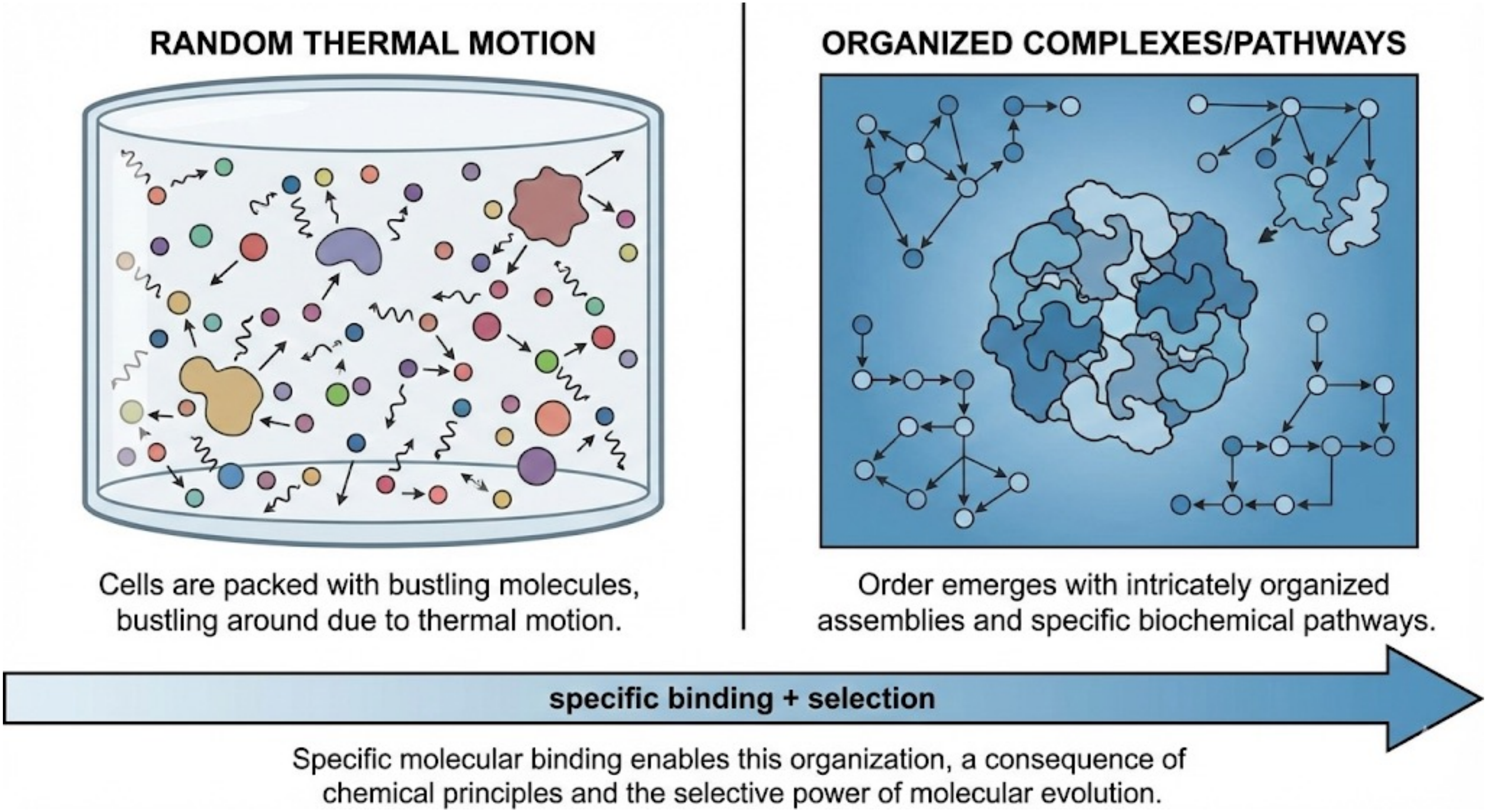
3. Explain how cooperative binding (sigmoid curve) allows hemoglobin to efficiently load and unload oxygen, enhancing its carrier function.



pH/BPG Regulator

4. Identify key allosteric regulators like pH (Bohr effect) and BPG that modulate hemoglobin's oxygen affinity.

Order is what you get when chemistry gets picky.



How do we quantify "pickiness" in a way that's useful?

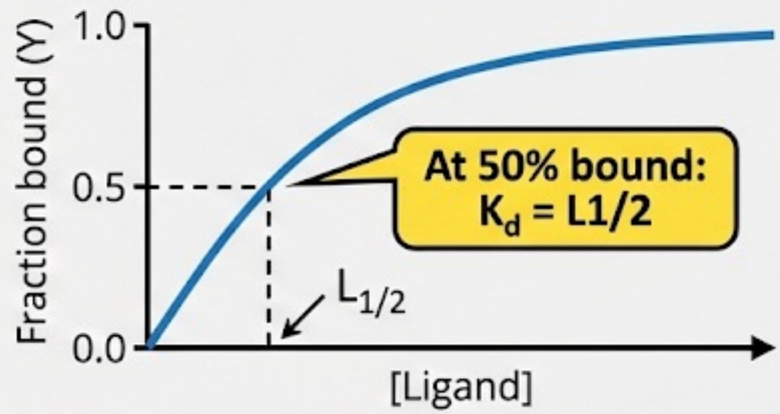
Binding basics: K_d is the "half-saturation" point

- Receptor + Ligand ⇄ Complex (R + L ⇄ RL)
- Fraction bound increases as ligand concentration increases
- $K_d = [R][L]/[RL]$ (at equilibrium)
- When half the receptors are bound, $[R] = [RL]$ so $K_d = L_{1/2}$
- Smaller K_d (or $L_{1/2}$) = tighter binding (higher affinity)

Equilibrium & K_d Formula

$K_d = [R][L]/[RL]$ (at equilibrium)

Binding Curve: Fraction bound vs [L]

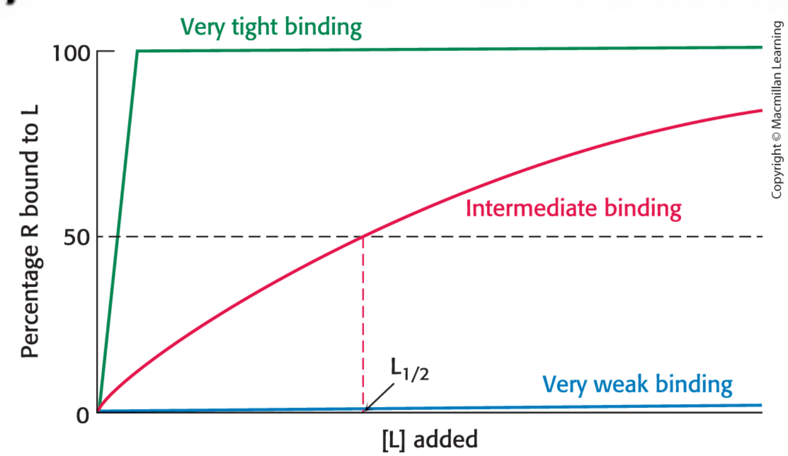


K_d is the concentration that gets you to 50% bound.

Lower K_d = higher affinity

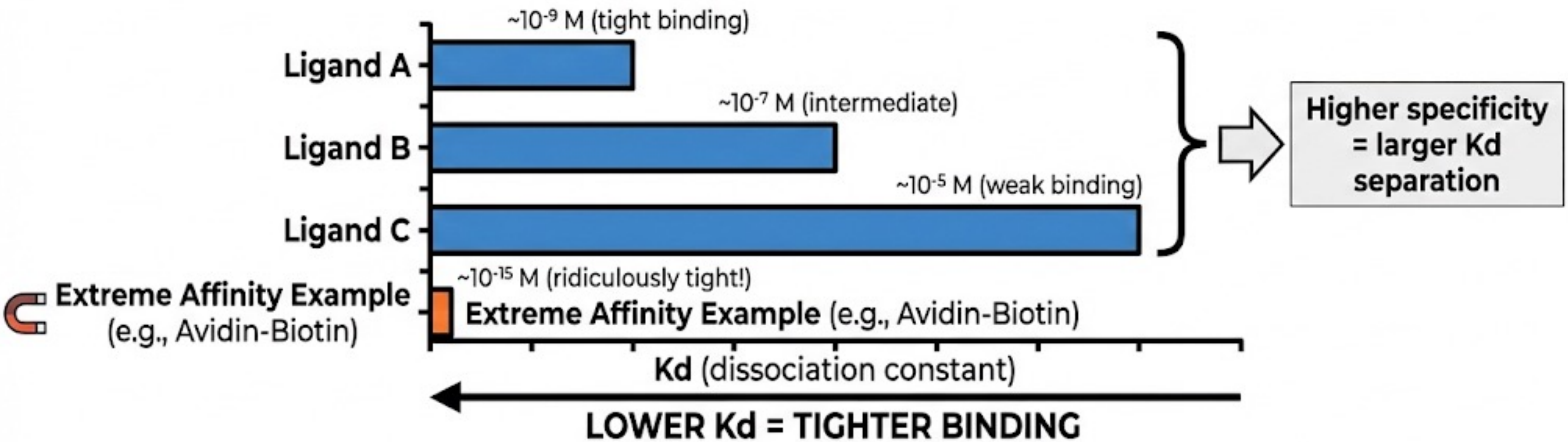
5.1 Binding is a fundamental process in biochemistry.

The ability of specific molecules to bind to one another in the face of many alternatives is referred to as **molecular recognition**.



Specificity: same protein, different K_d values

- Proteins discriminate among similar molecules via **shape** + charge complementarity
- Specificity is measured by comparing Kd values for competitors
- Big message: biology runs on relative affinities, not “perfect fits”
- Extreme affinity examples exist (used in real lab methods)

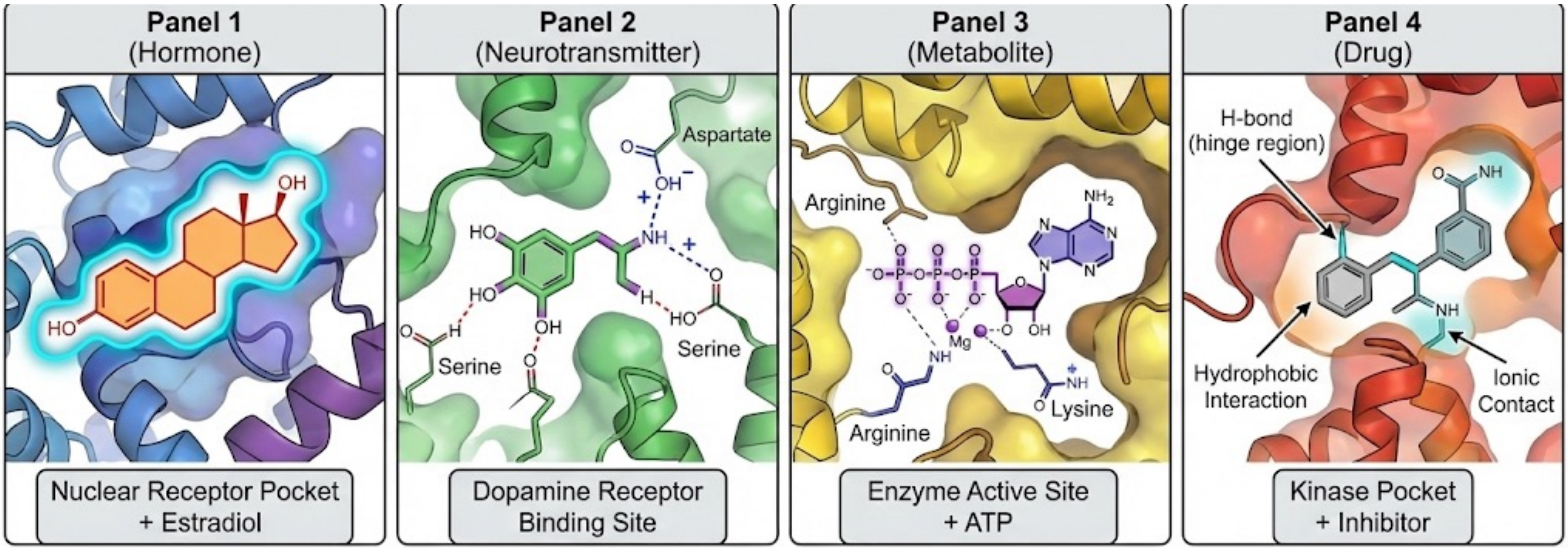


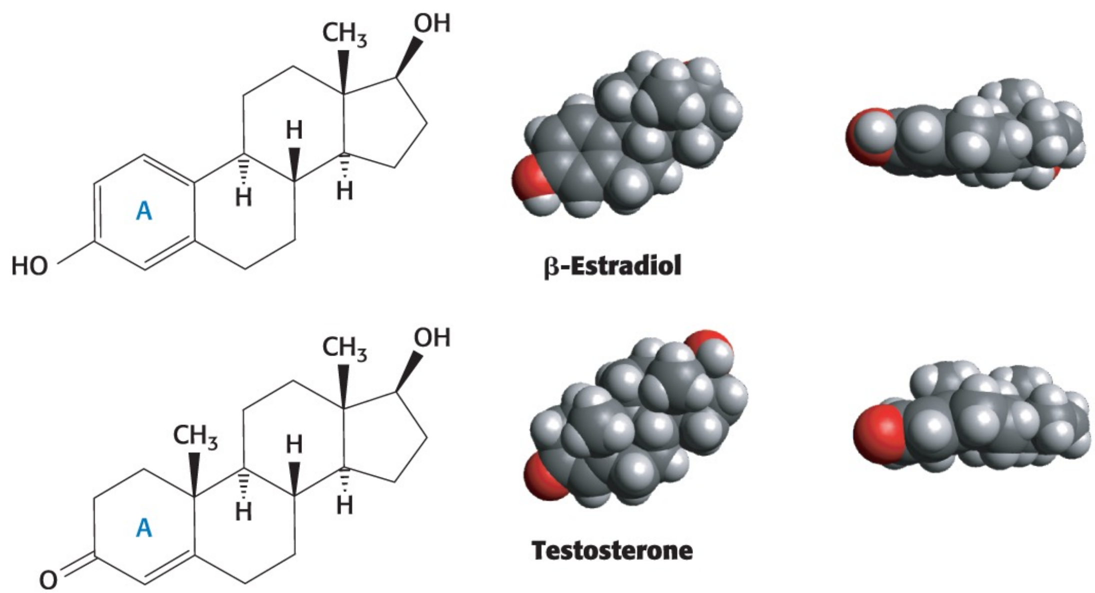
Specificity = which ligand wins at real concentrations.

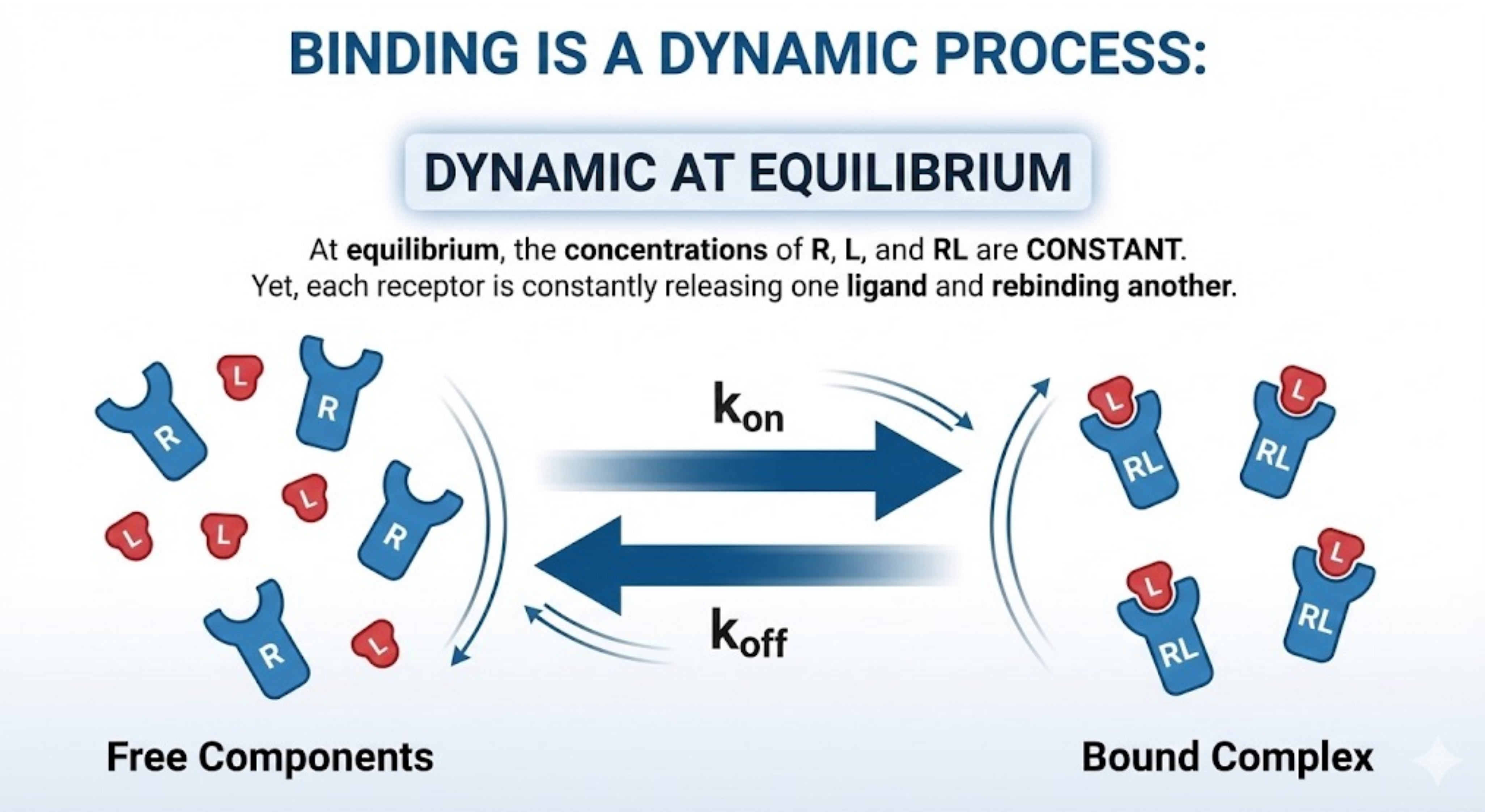
Affinity differences can be orders of magnitude.

Kd has concentration units

Protein Selective Binding—Shape + Chemistry Match



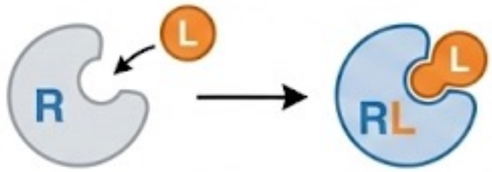



BINDING IS A DYNAMIC PROCESS:
DYNAMIC AT EQUILIBRIUM
At equilibrium, the concentrations of R, L, and RL are CONSTANT.
Yet, each receptor is constantly releasing one ligand and rebinding another.
k_{on}
k_{off}
R
L
RL
Free Components
Bound Complex

K_d: The Affinity Number (and when it equals the 50% point)

K_d is the ligand concentration (free ligand) that gives 50% occupancy under common assumptions.

ZONE 1
The binding reaction



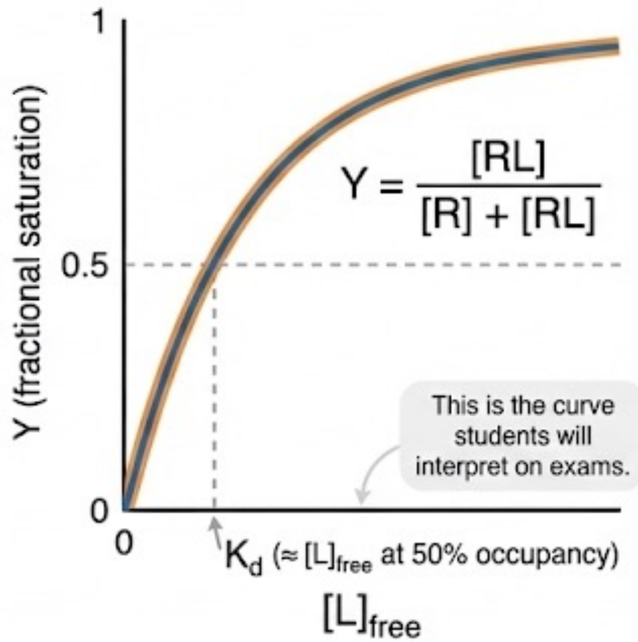
$$R + L \rightleftharpoons RL$$

$$K_d = \frac{[R][L]}{[RL]}$$

Smaller K_d = tighter binding (higher affinity)

[R] = free receptor, [L] = free ligand, [RL] = bound complex

ZONE 2
Occupancy curve + the 50% point



ZONE 3
When does K_d equal the 50% point?

The shortcut is true when…

☑ 1. Ligand is not depleted

$[L]_{total} \approx [L]_{free}$

(R is small relative to L)

☑ 2. Single binding site, simple equilibrium

No cooperativity / no multiple sites

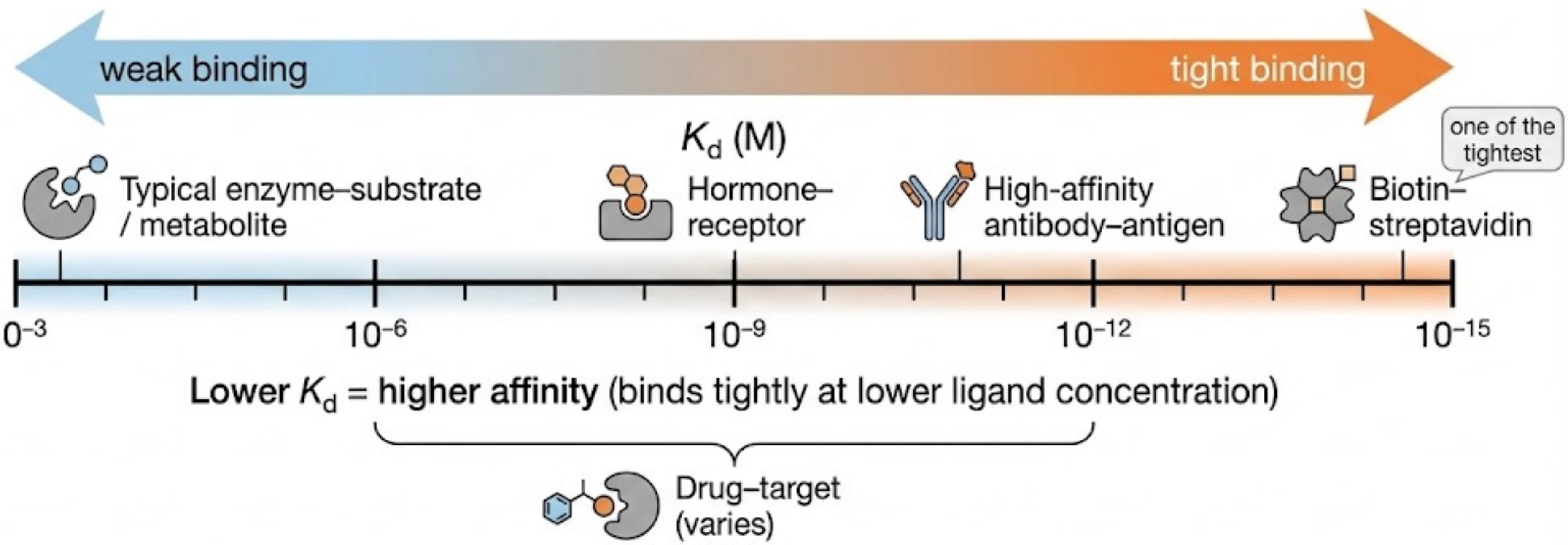
When it can fail

- High receptor concentration depletes ligand
- Multiple sites / cooperativity shifts the relationship

Exam-use translation

- ✅ Lower K_d → higher affinity
- ✅ Right-shifted curve → weaker binding (higher K_d)
- ✅ At Y = 0.5, read x-axis: ~K_d (if assumptions hold)

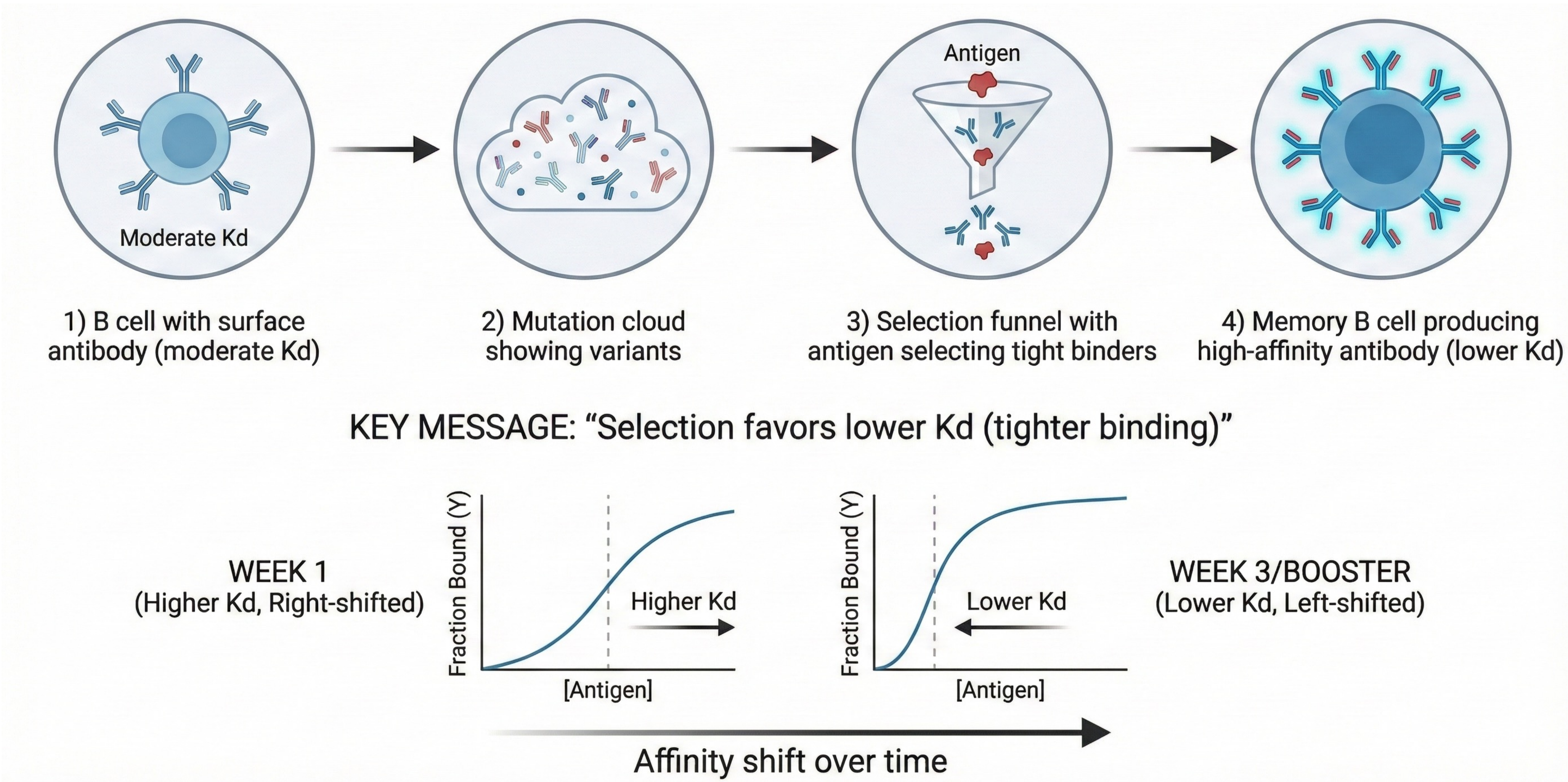
Affinity in One Glance: K_d Ranges
(What 'tight binding' means)



Exam-use translation

- ✓ Compare K_d values: **smaller wins**
- ✓ A 10× lower K_d means much **tighter binding**
- ✓ You rarely calculate K_d; you **interpret it**

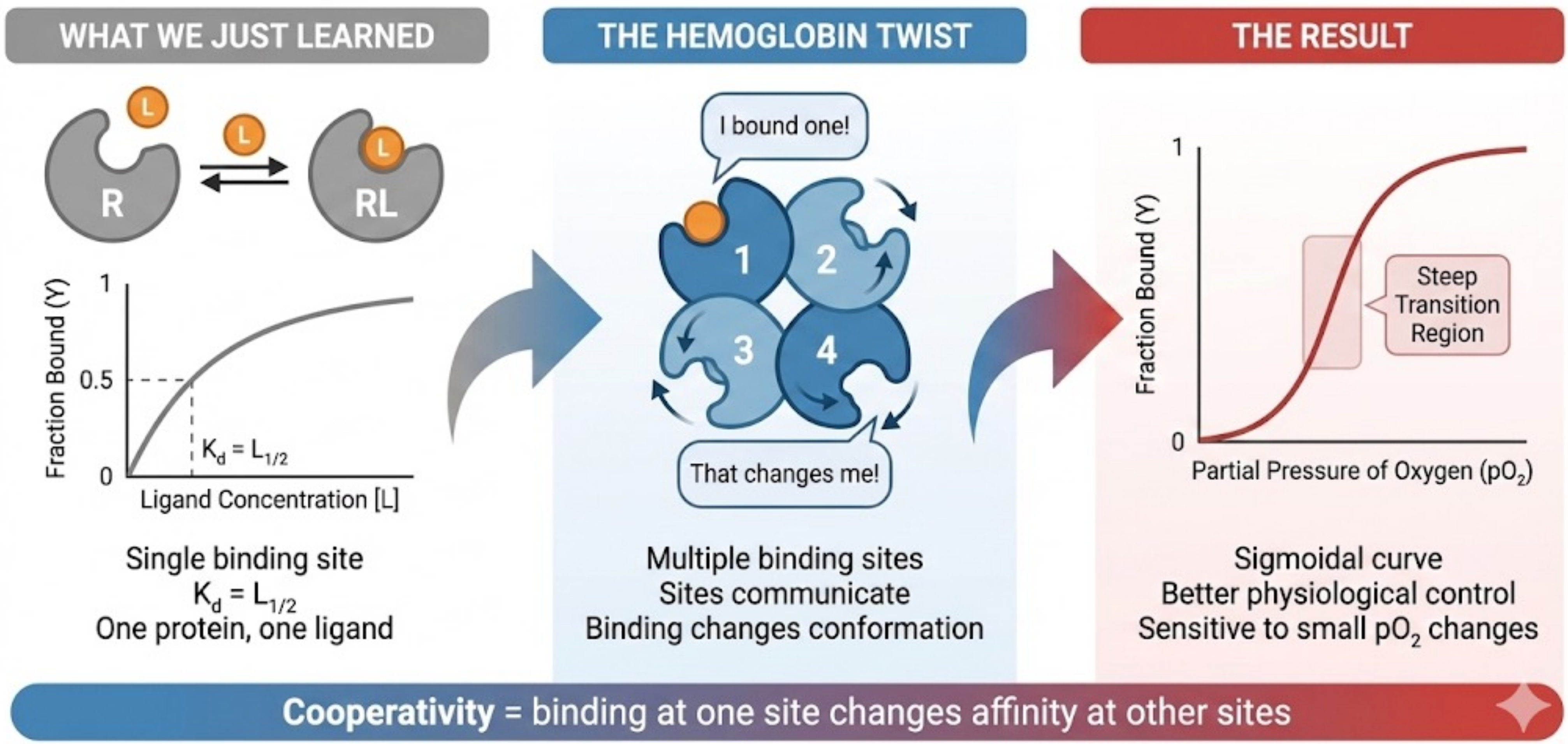
How Vaccines Generate High-Affinity Antibodies (Affinity Maturation)



True or False

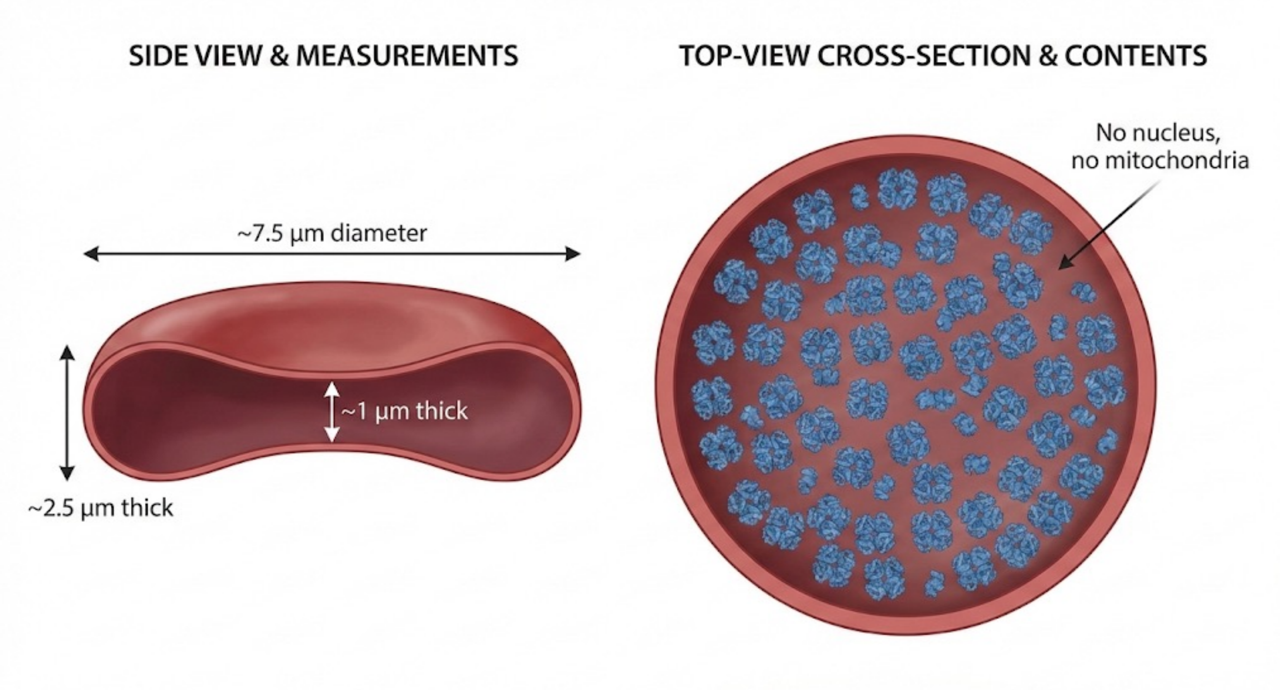
The dissociation constant of the ligand-binding reaction is equal to the concentration of L at which half of the receptor is bound to L (and half is free).

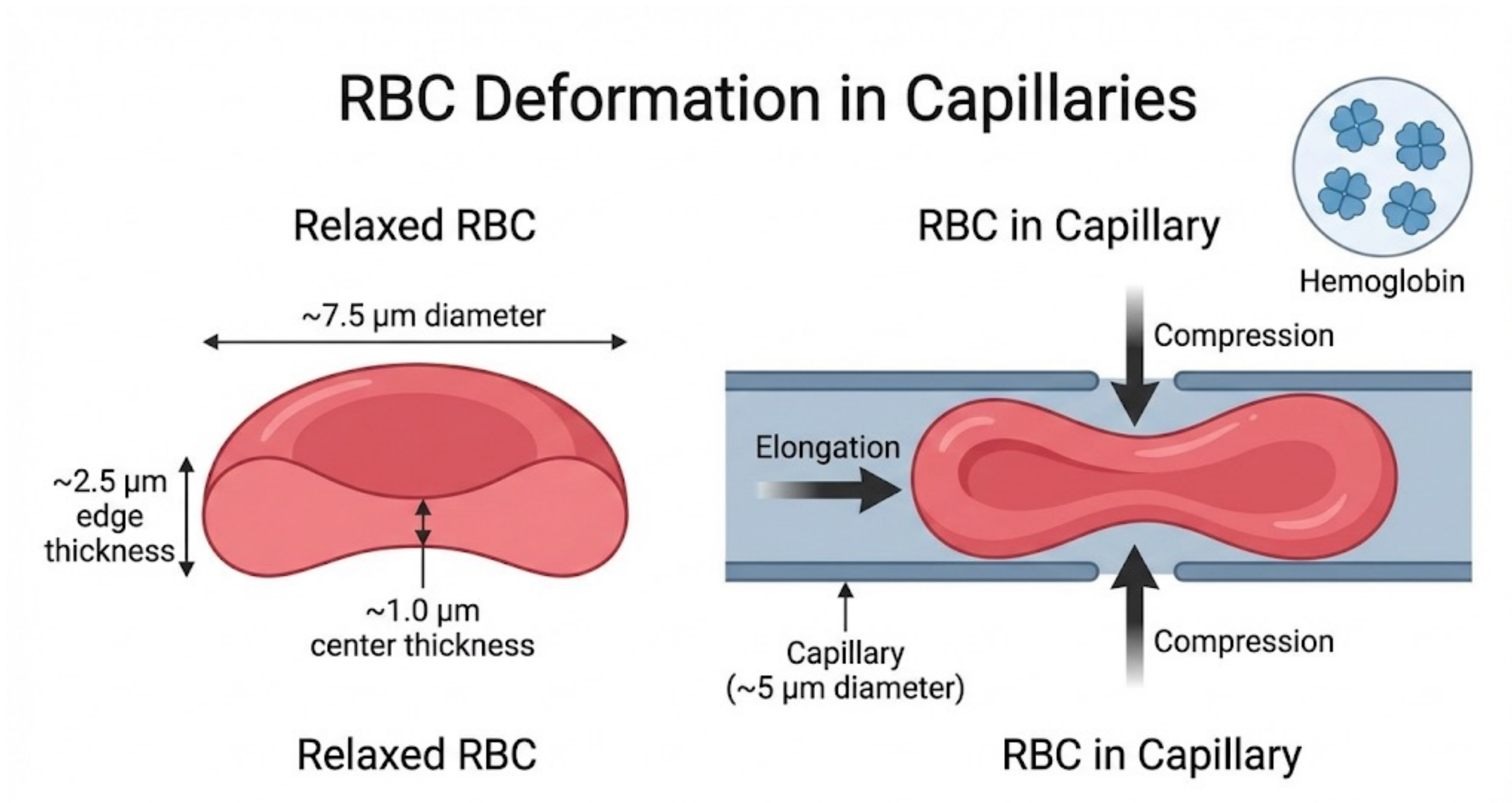
FROM SIMPLE BINDING TO COOPERATIVITY: THE HEMOGLOBIN STORY



How does hemoglobin load O_2 in lungs but unload in tissues?

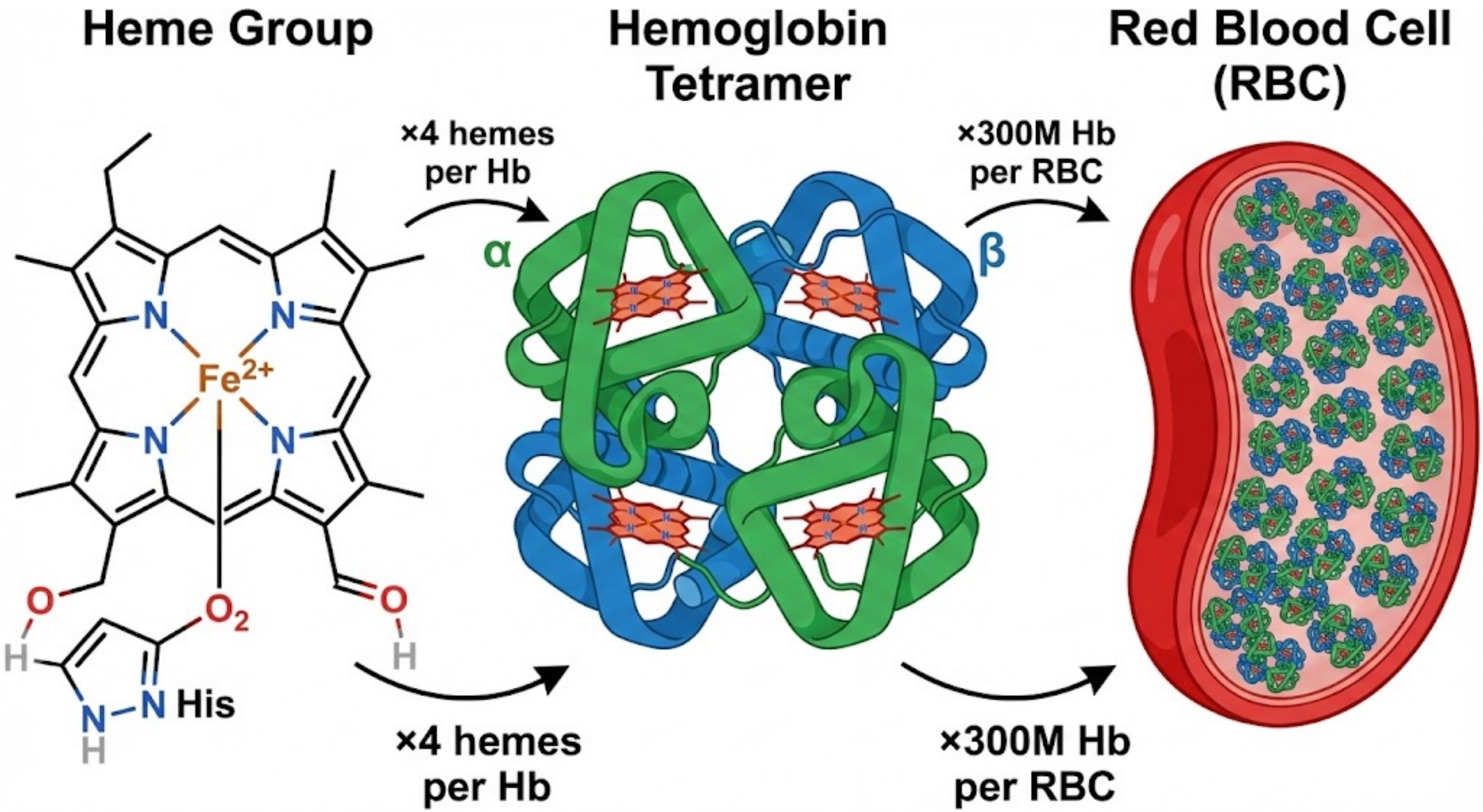
- Red blood cell:
- We have 5 million RBCs per microliter of blood
- -Biconcave---lacks some cytoskeletal elements
- Lacks several key organelles like nuclei





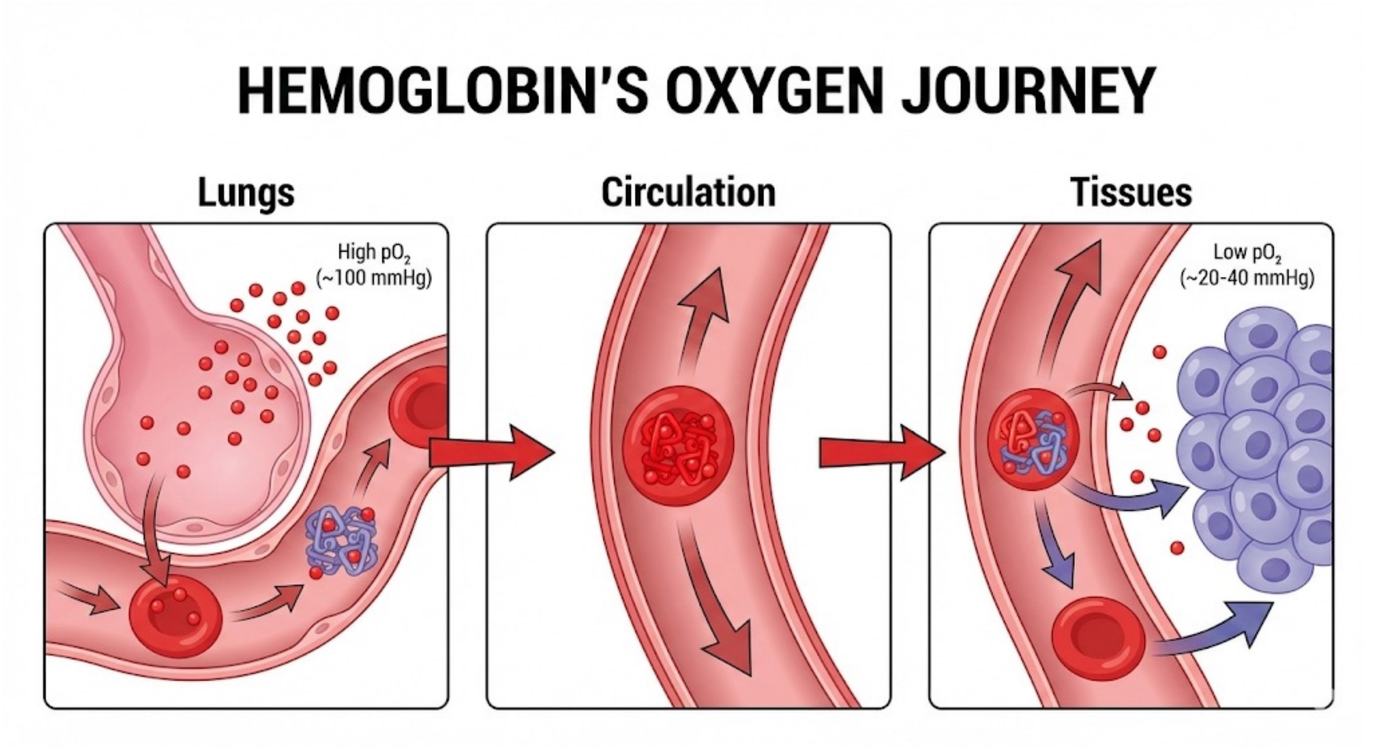
Red blood cell:
-Super flexible
-no nucleus
-can squeeze through capillaries
--the squeezing is important to maximize gas exchange
RBC lifespan (~120 days)
1 red blood cell contains about 300 million molecules of hemoglobin.

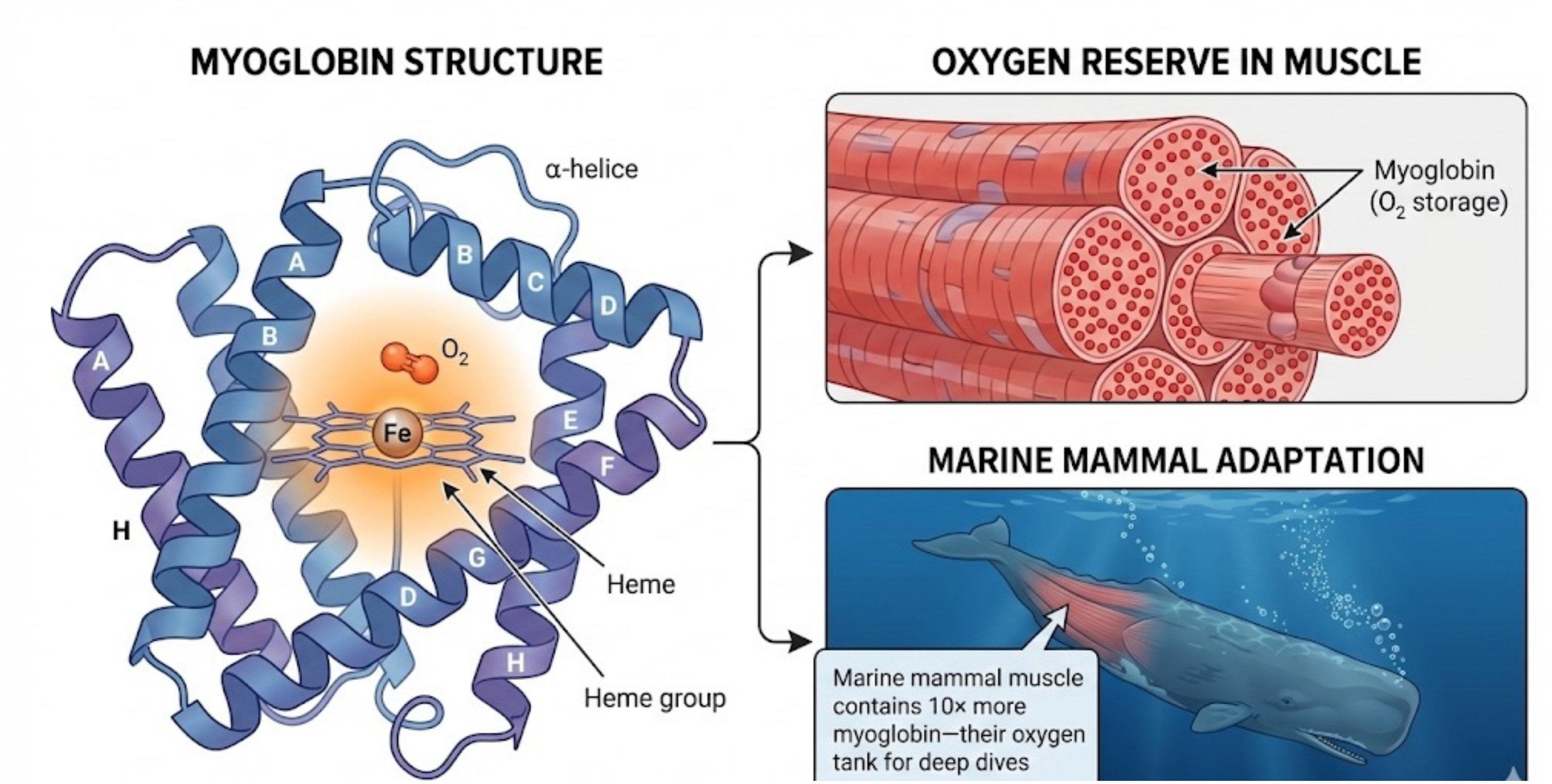
Each RBC can transport about 1.2 billion O_2 molecules.


Heme Group
Hemoglobin
Tetramer
Red Blood Cell
(RBC)
×4 hemes
per Hb
×300M Hb
per RBC
N
N
N
N
Fe2+
O
H
O2
O
H
N
N
H
His
α
β
×4 hemes
per Hb
×300M Hb
per RBC

- **Learning objective 7: Explain how <u>allosteric</u> properties contribute to hemoglobin function.**

  - Hemoglobin is a red blood cell protein that carries oxygen from the lungs to the tissues.
  - Hemoglobin is an allosteric protein that displays <u>cooperativity</u> in oxygen binding and release.
  - Myoglobin binds oxygen in muscle cells. The binding of oxygen by myoglobin is not cooperative.
  - Oxygen binding is measured as a function of the partial pressure of oxygen (pO_2).



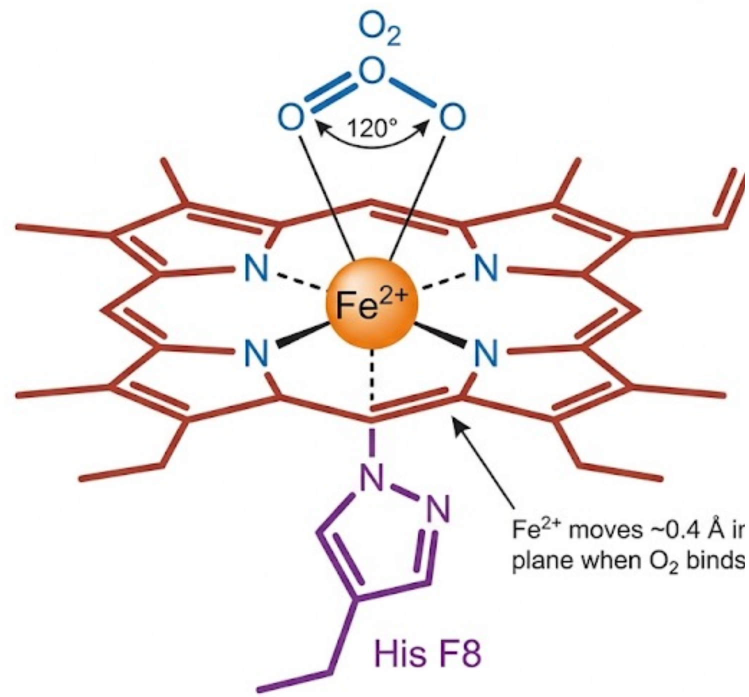


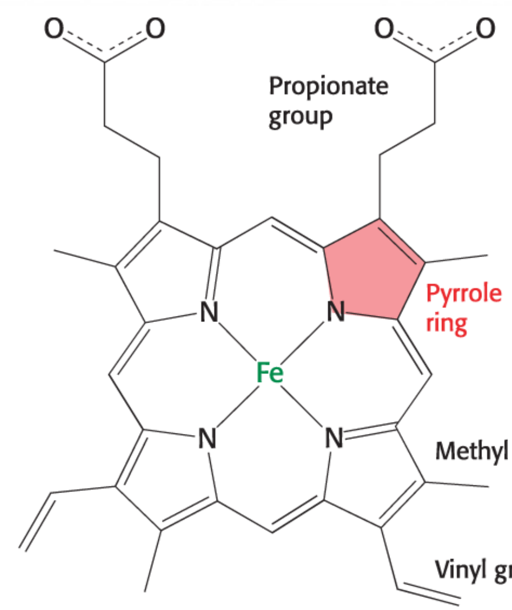
Section 5.2 Myoglobin and Hemoglobin Bind Oxygen in Heme Groups

- Myoglobin is a single polypeptide chain consisting mainly of α helices arranged to form a globular structure. This is a simple reference case.
- Myoglobin, like hemoglobin, binds oxygen at a heme, a bound prosthetic group.

Section 5.2 Myoglobin and Hemoglobin Bind Oxygen in Heme Groups (2/3)

- The heme group consists of an organic component called protoporphyrin and a central iron ion in the ferrous (Fe^{2+}) form.
- The iron lies in the middle of the protoporphyrin bound to four nitrogens.
- Iron can form two additional bonds, called the fifth and sixth coordination sites.
- The fifth coordination site is occupied by an imidazole ring of a histidine called the proximal histidine.
- The sixth coordination site binds oxygen.
- Upon oxygen binding, the iron moves into the plane of the protoporphyrin ring.

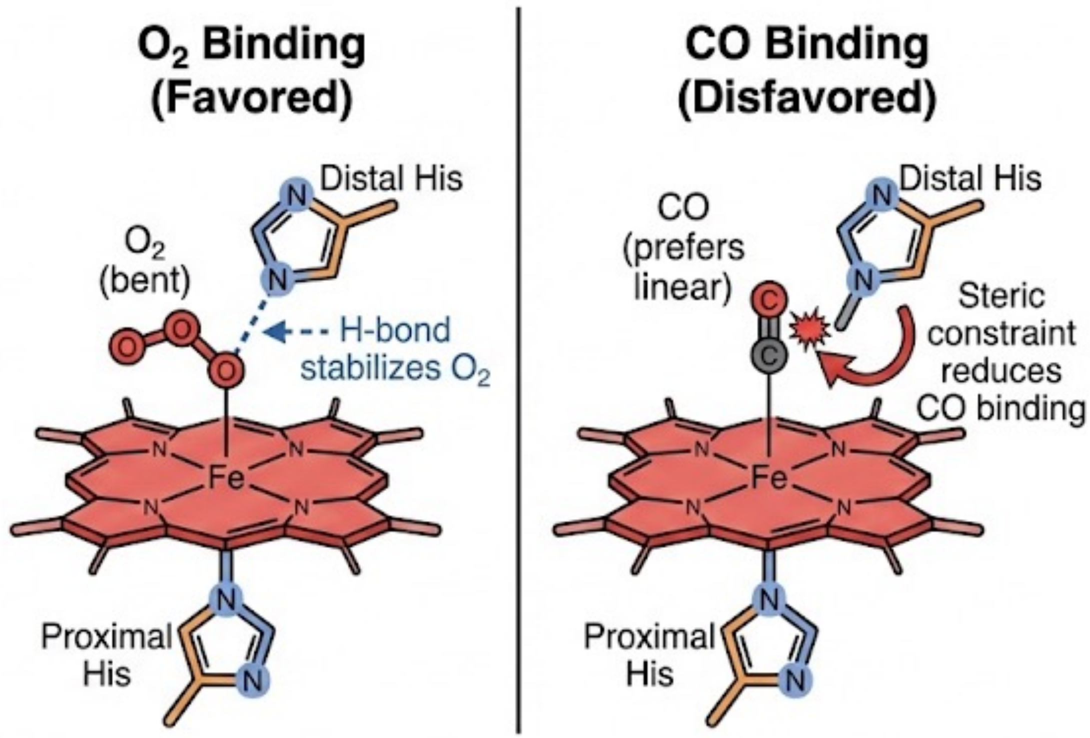




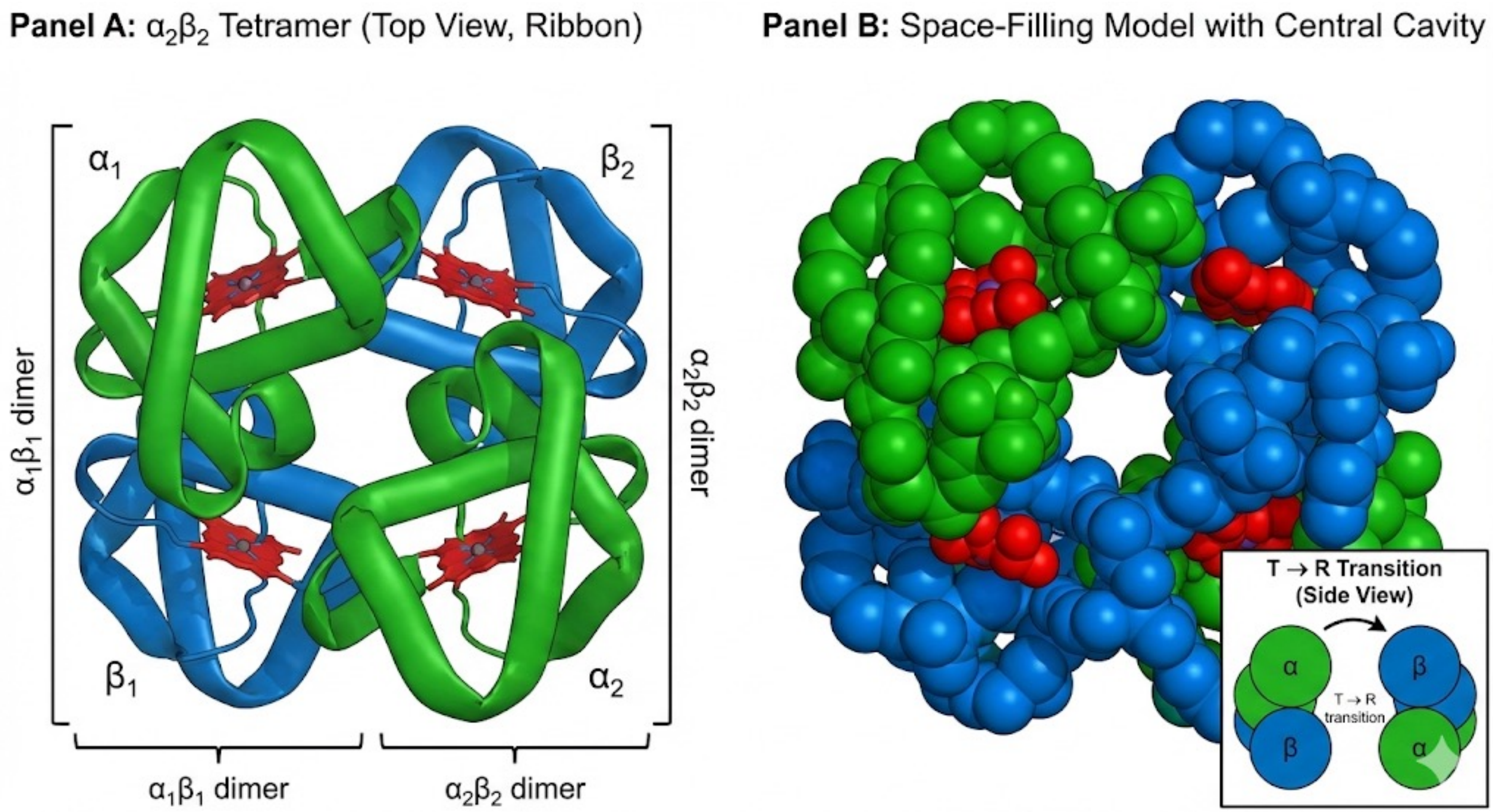
Heme
(Fe-protoporphyrin IX)

Distal histidine: the heme "bouncer" that favors O_2 over CO

- ☑ Heme iron can bind O_2 at the 6th coordination site, but CO can compete
- ☑ Distal His partially blocks straight-line binding geometries
- ☑ Distal His provides a stabilizing H-bond that favors the bent O_2 binding mode
- ☑ Net effect: boosts O_2 binding and reduces CO advantage
- ☑ Helps protect heme from undesirable chemistry (must remain functional Fe^{2+})



Protein environment, not heme alone, determines "safe" ligand binding.



Section 5.3 Hemoglobin Binds Oxygen Cooperatively

- Hemoglobin is a tetramer consisting of two α subunits and two β subunits. Each subunit has a bound heme.
- The quaternary structure is best described as a pair of identical αβ dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$).
- In deoxyhemoglobin, which corresponds to the T state of allosteric enzymes, the αβ dimers are linked by an extensive interface.

The Mechanical Linkage: From Iron to Quaternary Structure

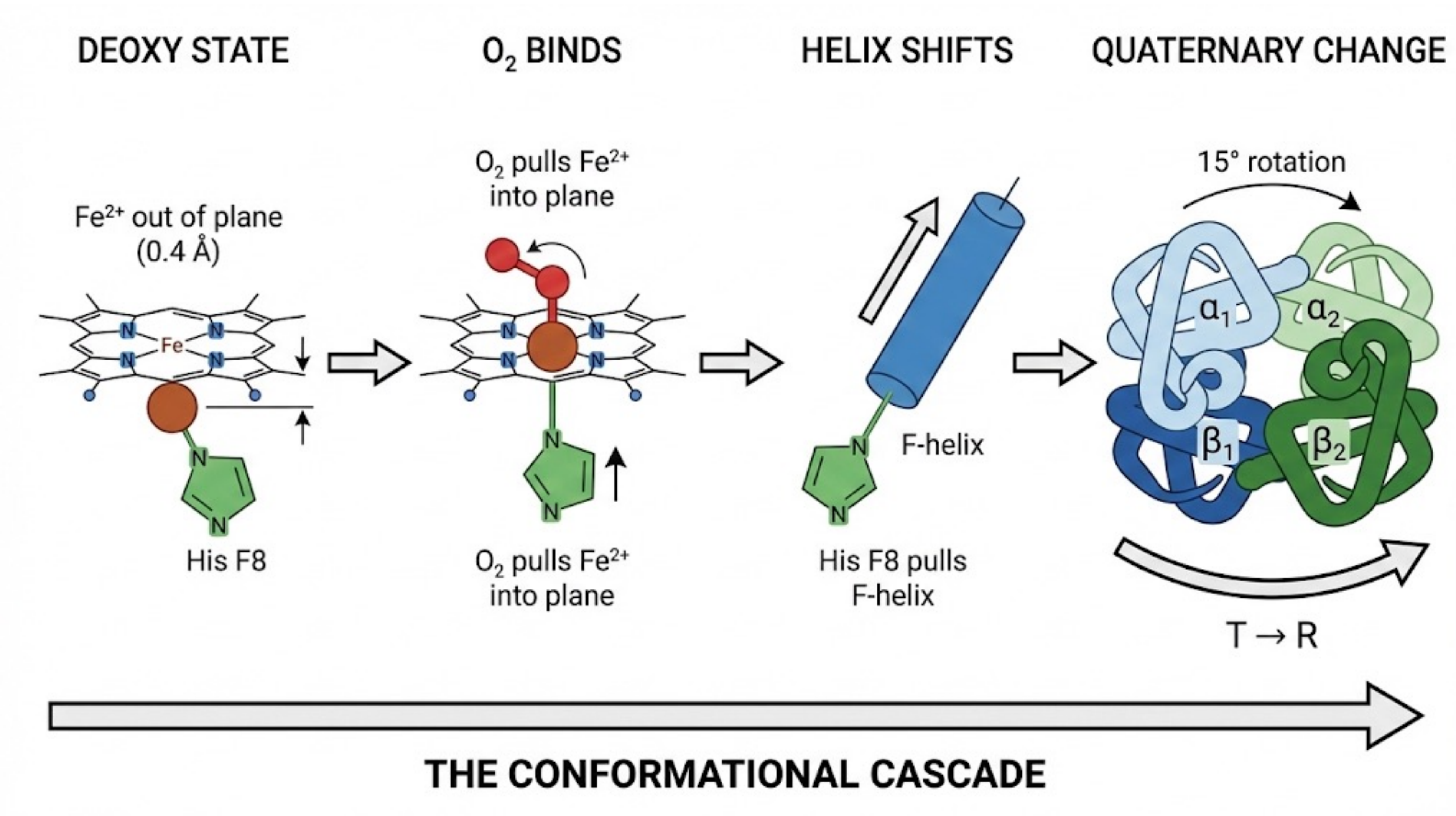
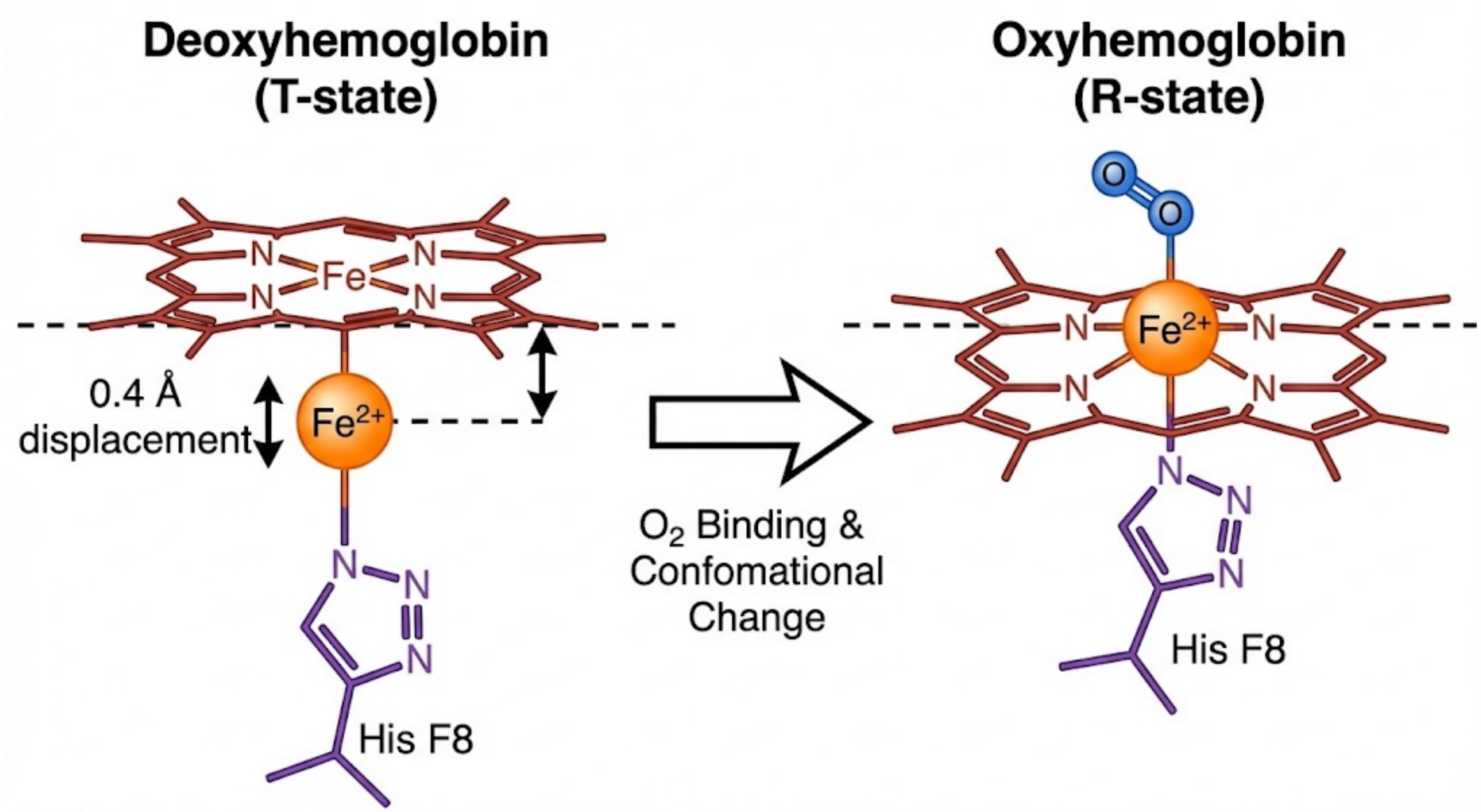
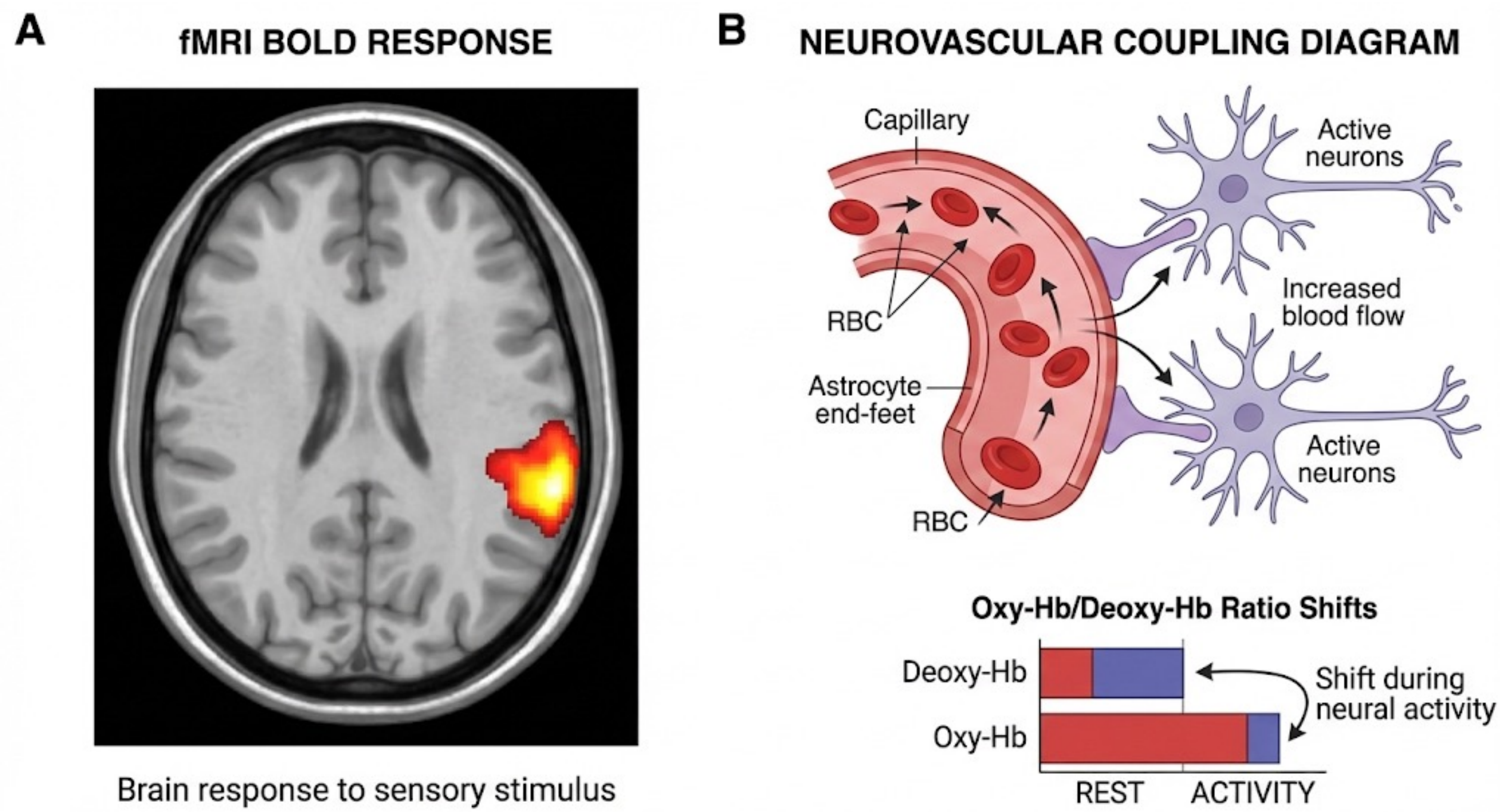


Diagram of Oxygen Binding Changing the Position of the Iron Ion



This will be the state of hemoglobin in tissues to offload O2

This will be the state of hemoglobin in lungs to onload O2



- **Clinical Insight: Functional Magnetic Resonance Imaging Reveals Regions of the Brain Processing Sensory Information**

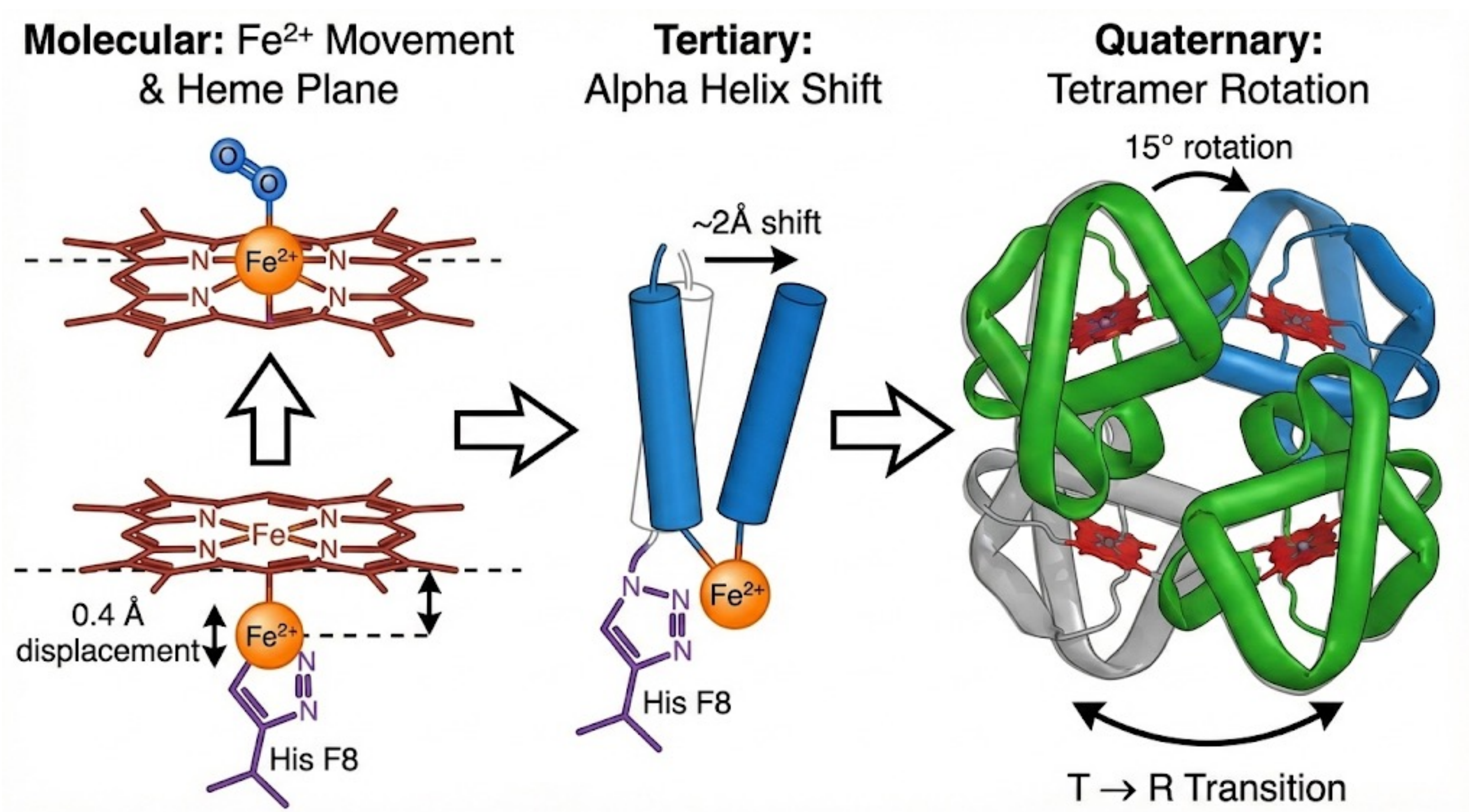
- The magnetic properties of the heme iron change when it moves into the plane of the protoporphyrin ring.

- Functional magnetic resonance imaging (fMRI) can distinguish the relative amounts of oxy- and deoxyhemoglobin.

- Functional magnetic resonance can be used to monitor activity in specific regions of the brain by measuring the increase in oxyhemoglobin.

Section 5.3 Hemoglobin Binds Oxygen Cooperatively

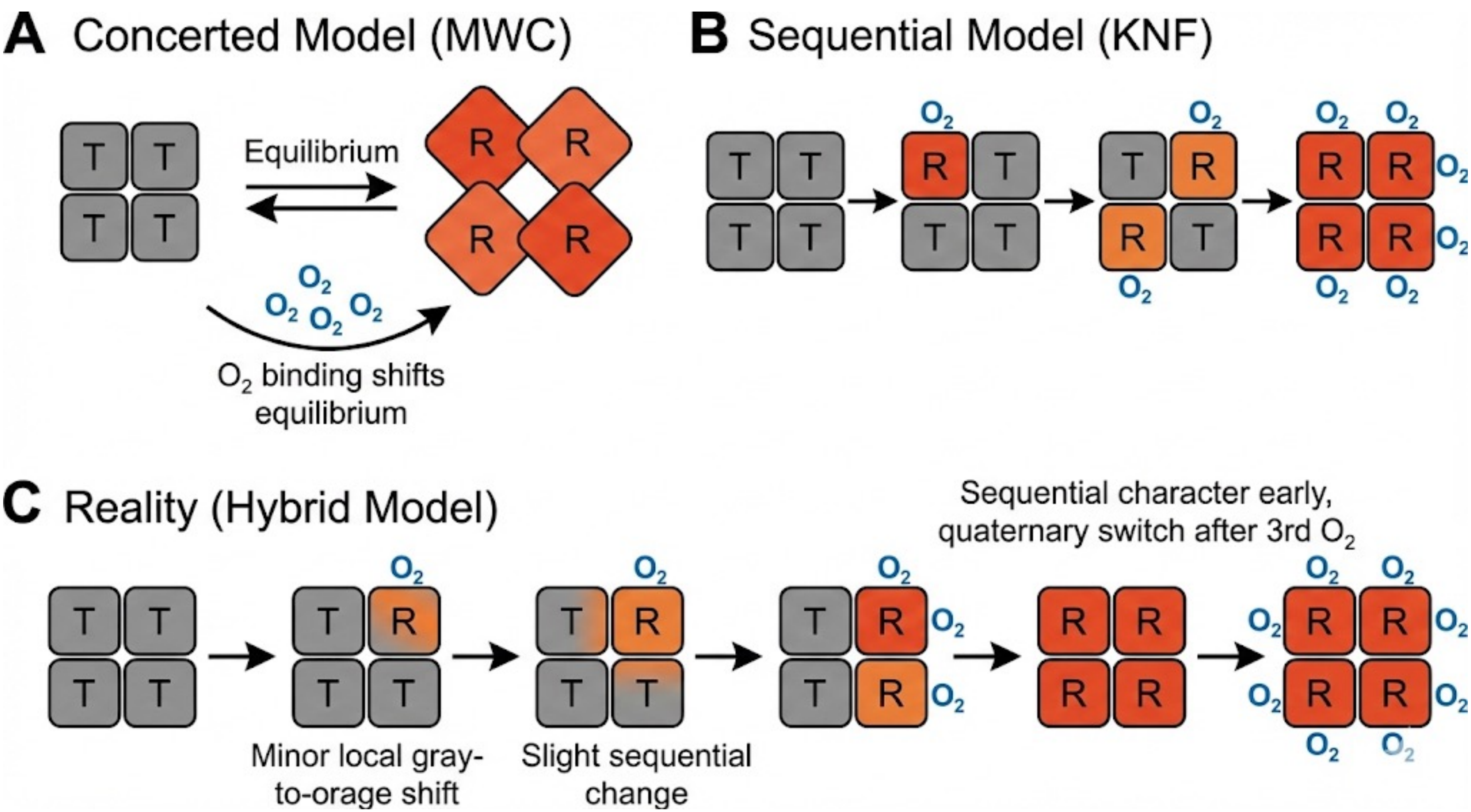
- The transition from deoxyhemoglobin (T state) to oxyhemoglobin (R state) occurs upon oxygen binding.
- The iron ion moves into the plane of the heme when oxygen binds. The proximal histidine, which is a component of an α helix, moves with the iron.
- The resulting structural change is communicated to the other subunits so that the two αβ dimers rotate with respect to each another, resulting in the formation of the R state.



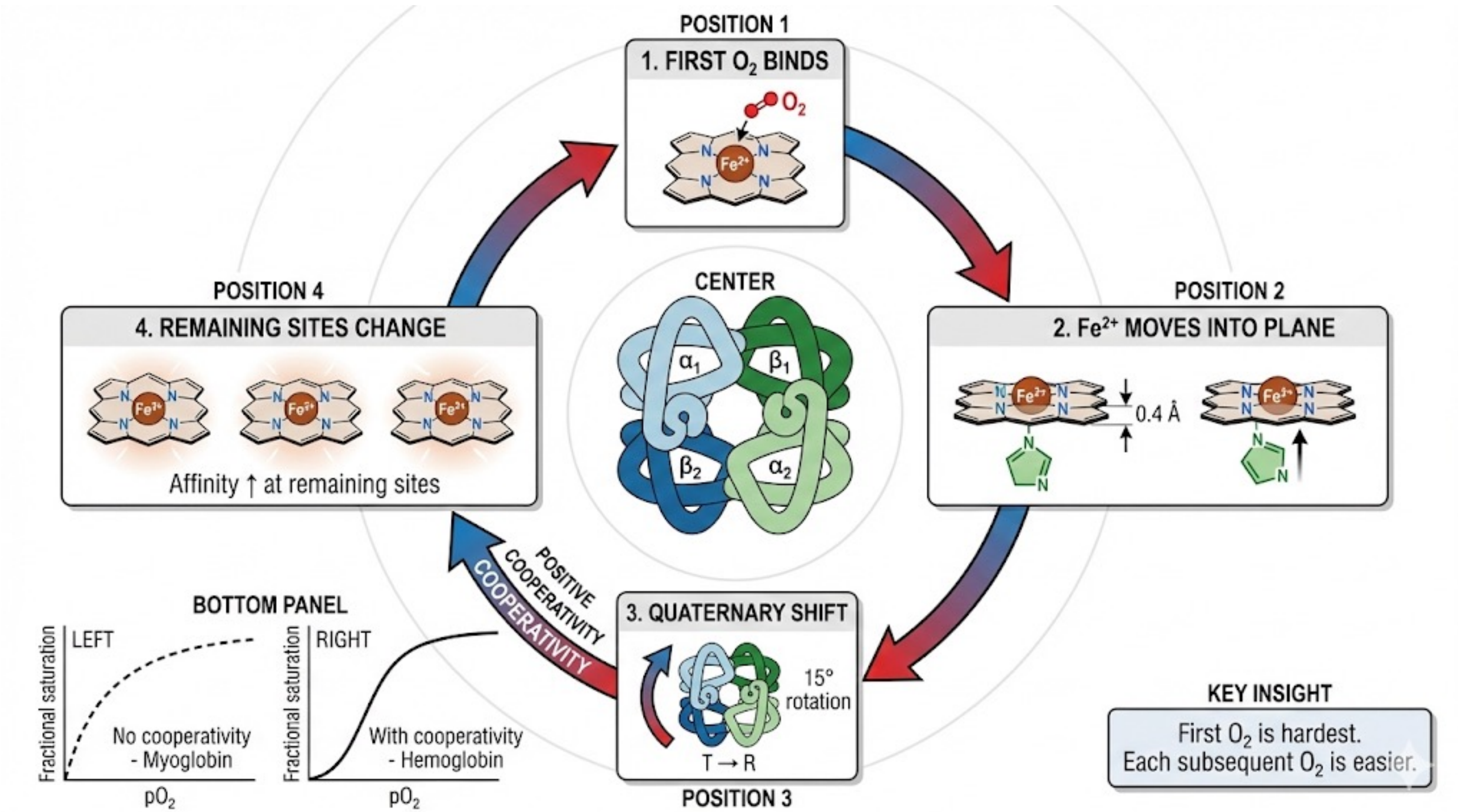
Concerted vs Sequential Models (MWC vs KNF)

low affinity → high affinity

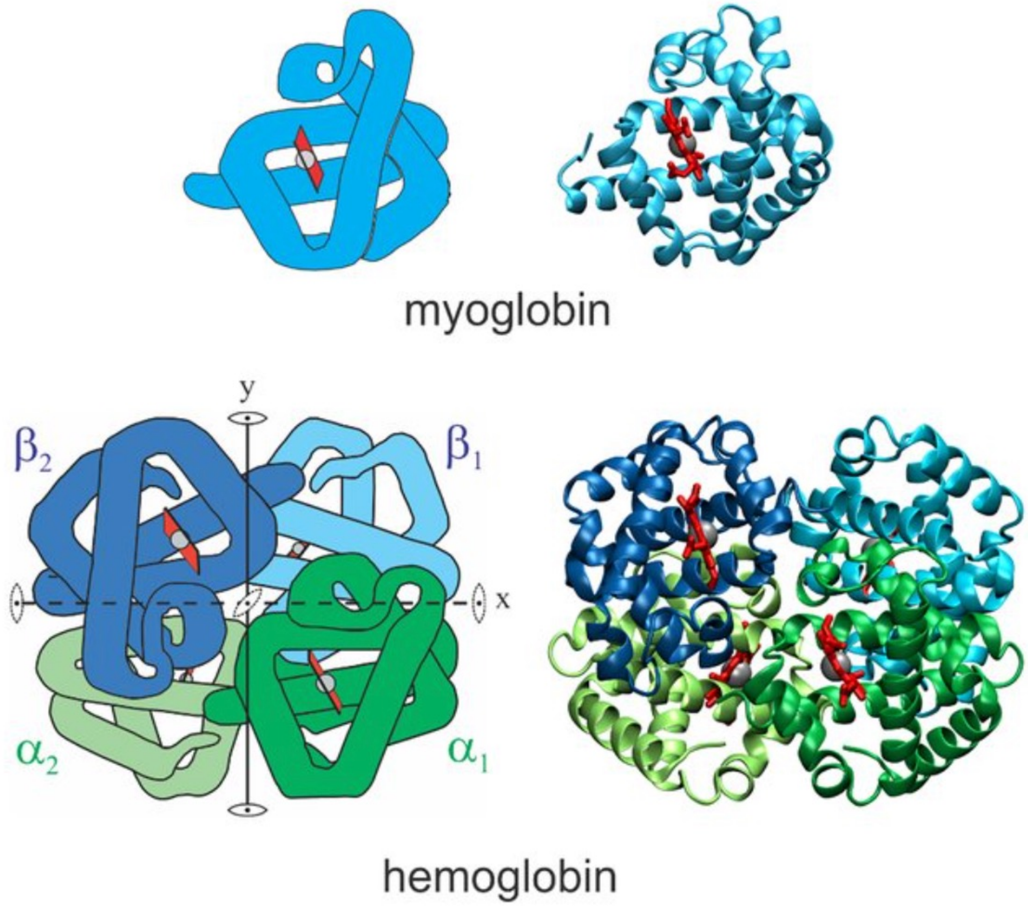
$$Hb \underset{+O_2}{\overset{K_1}{\rightleftharpoons}} HbO_2 \underset{+O_2}{\overset{K_2}{\rightleftharpoons}} Hb(O_2)_2 \underset{+O_2}{\overset{K_3}{\rightleftharpoons}} Hb(O_2)_3 \underset{+O_2}{\overset{K_4}{\rightleftharpoons}} Hb(O_2)_4$$



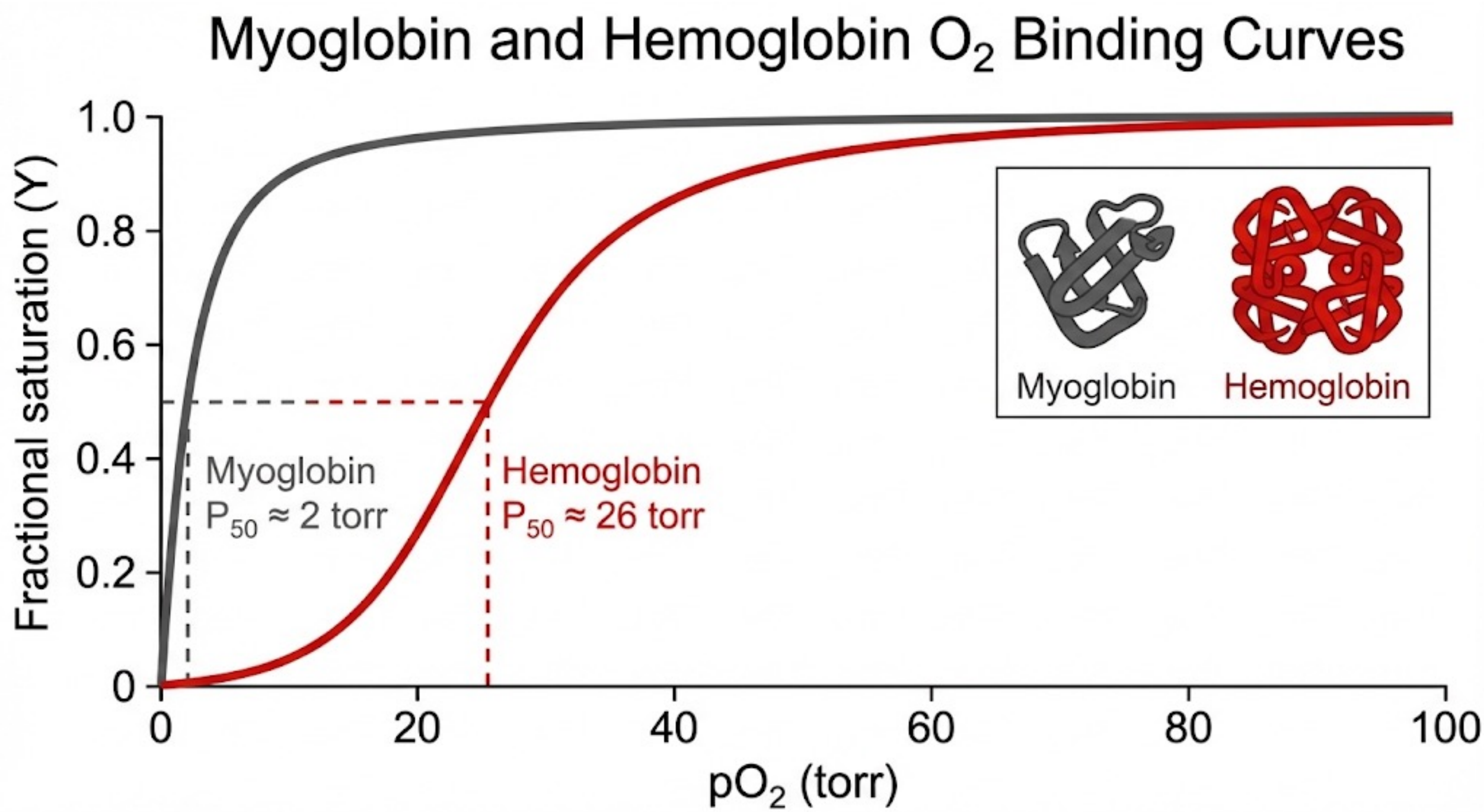
Cooperativity: The Complete Picture



What do we imagine the O2 binding curves would look like between myoglobin and hemoglobin?

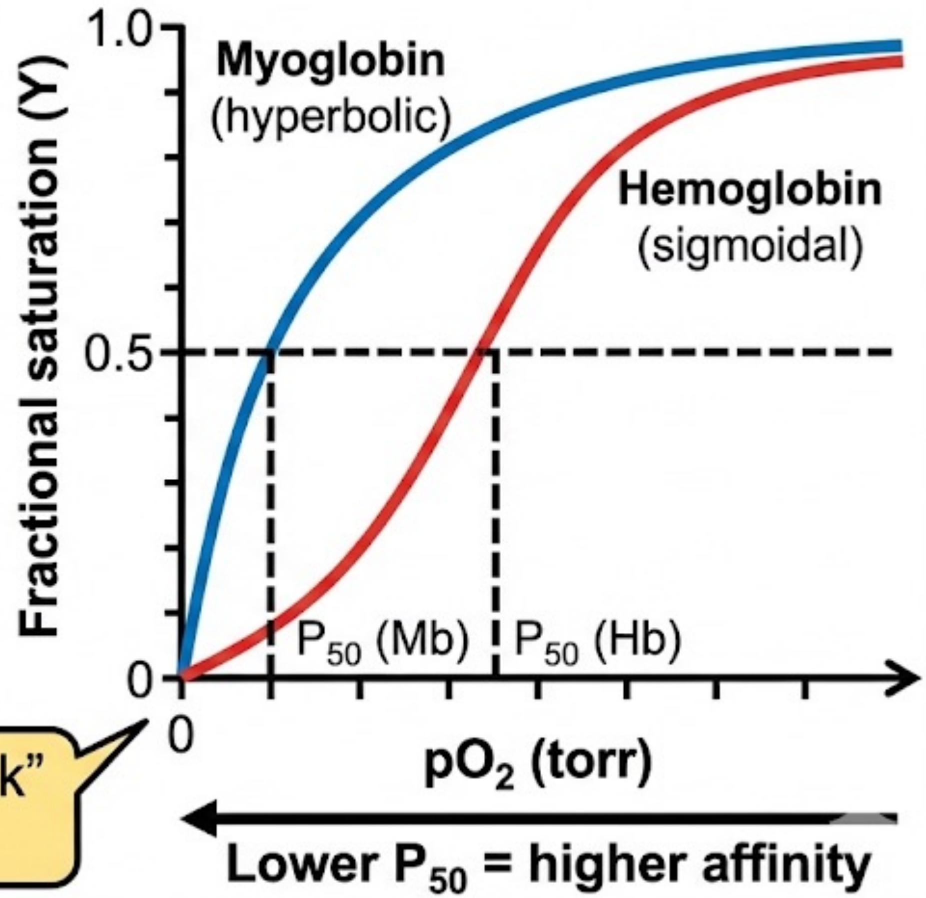


Hemoglobin Displays Cooperative Behavior



Reading O_2-binding curves: Y and P_{50}

- ☑ Y = fractional saturation (0 to 1): fraction of sites with O_2 bound
- ☑ X-axis is pO_2 (partial pressure of oxygen)
- ☑ P_{50} = pO_2 at Y = 0.5 (lower P_{50} = higher affinity)
- ☑ Myoglobin: low P_{50}, hyperbolic curve
- ☑ Hemoglobin: higher P_{50}, sigmoidal curve (cooperativity)



P_{50} is the "half-full tank" oxygen pressure.

The Hill Equation and Hill Coefficient: Quantifying Binding Cooperativity

Mathematical Framework

Hill Equation:

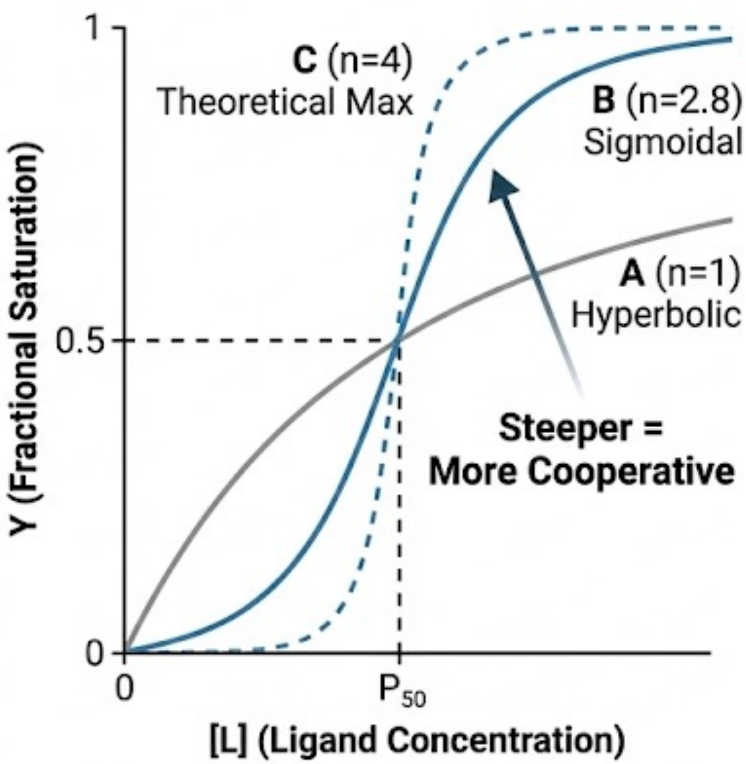
$$Y = \frac{[L]^n}{K_d + [L]^n}$$

n = 1 (Non-cooperative, hyperbolic)
- Sites are independent
- Standard binding
- Hyperbolic curve

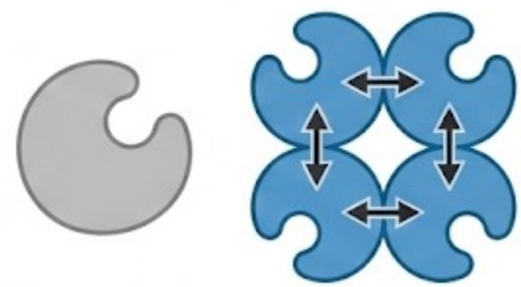
n > 1 (Positive cooperativity, sigmoidal)
- Binding increases affinity
- Sites communicate
- Sigmoidal curve

n < 1 (Negative cooperativity)
- Binding decreases affinity
- Sites communicate
- Sigmoidal curve (shallower)

Binding Curves (Y vs [L])



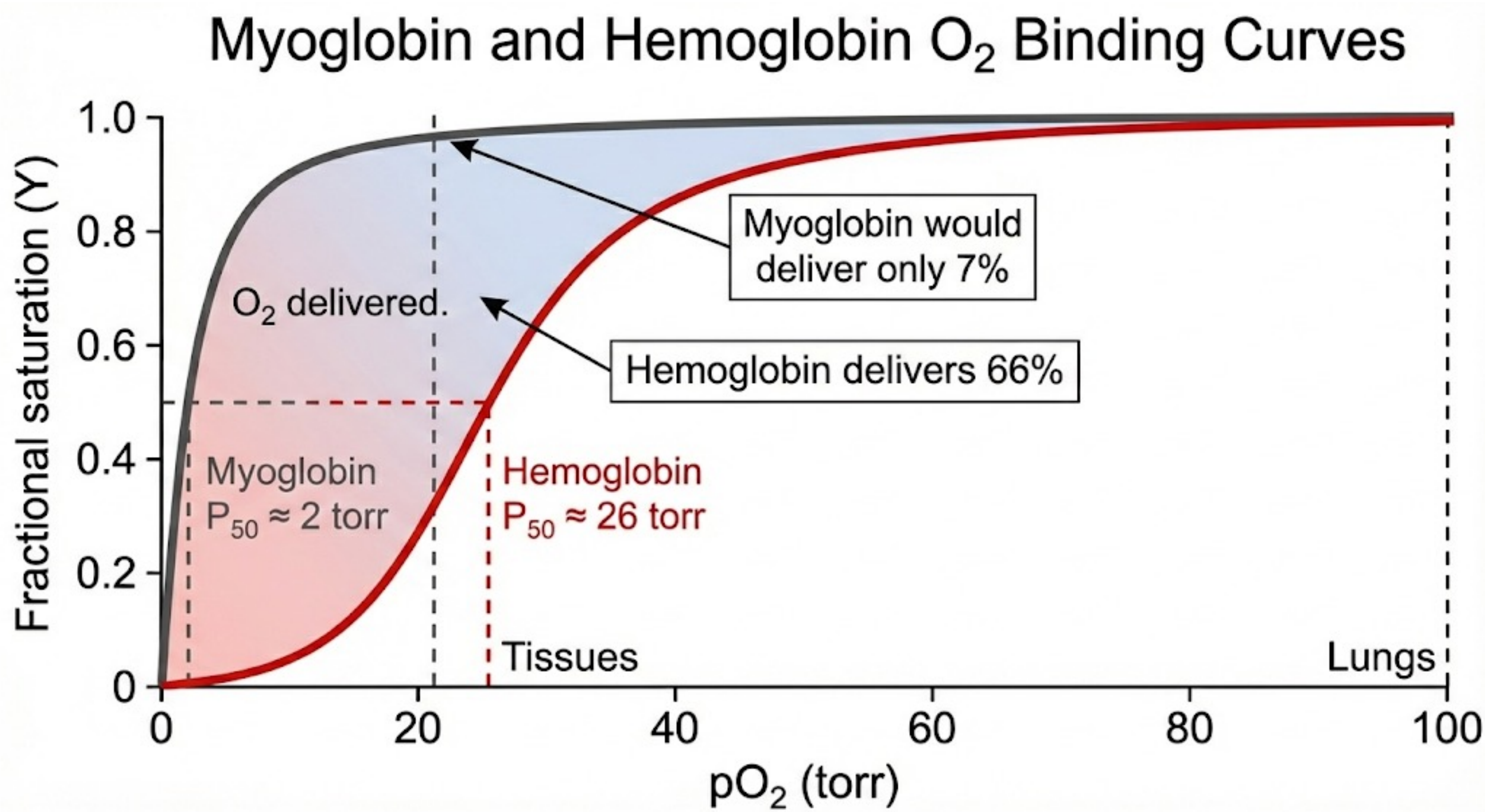
Myoglobin vs. Hemoglobin Summary



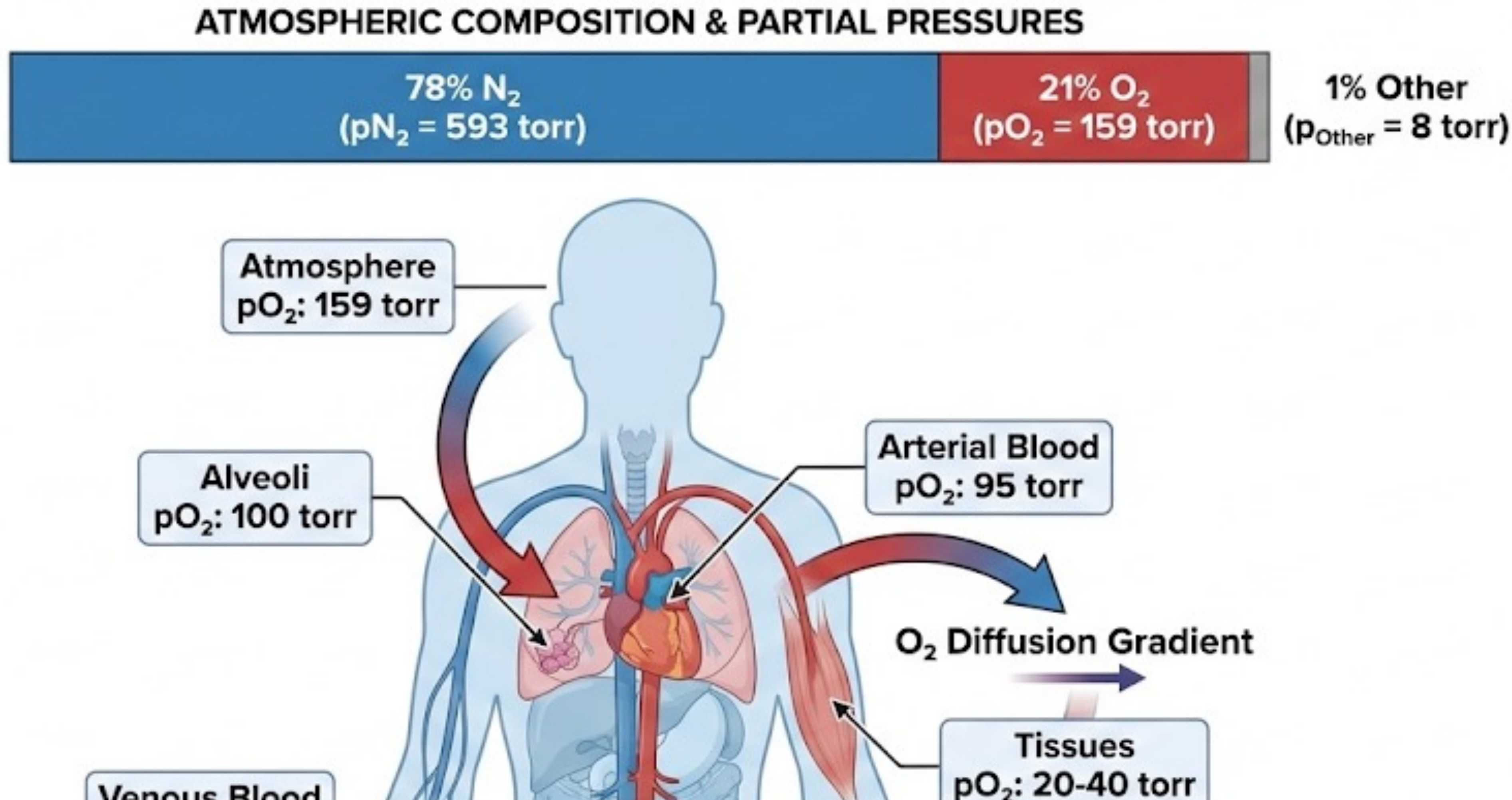
Myoglobin (Mb)	**Hemoglobin (Hb)**
n = 1	n ≈ 2.8
Independent binding	Cooperative binding

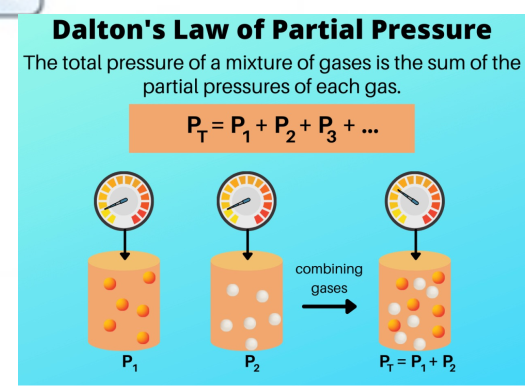
'The **Hill coefficient** quantifies how much binding sites communicate.'

Cooperativity Enhances Oxygen Delivery

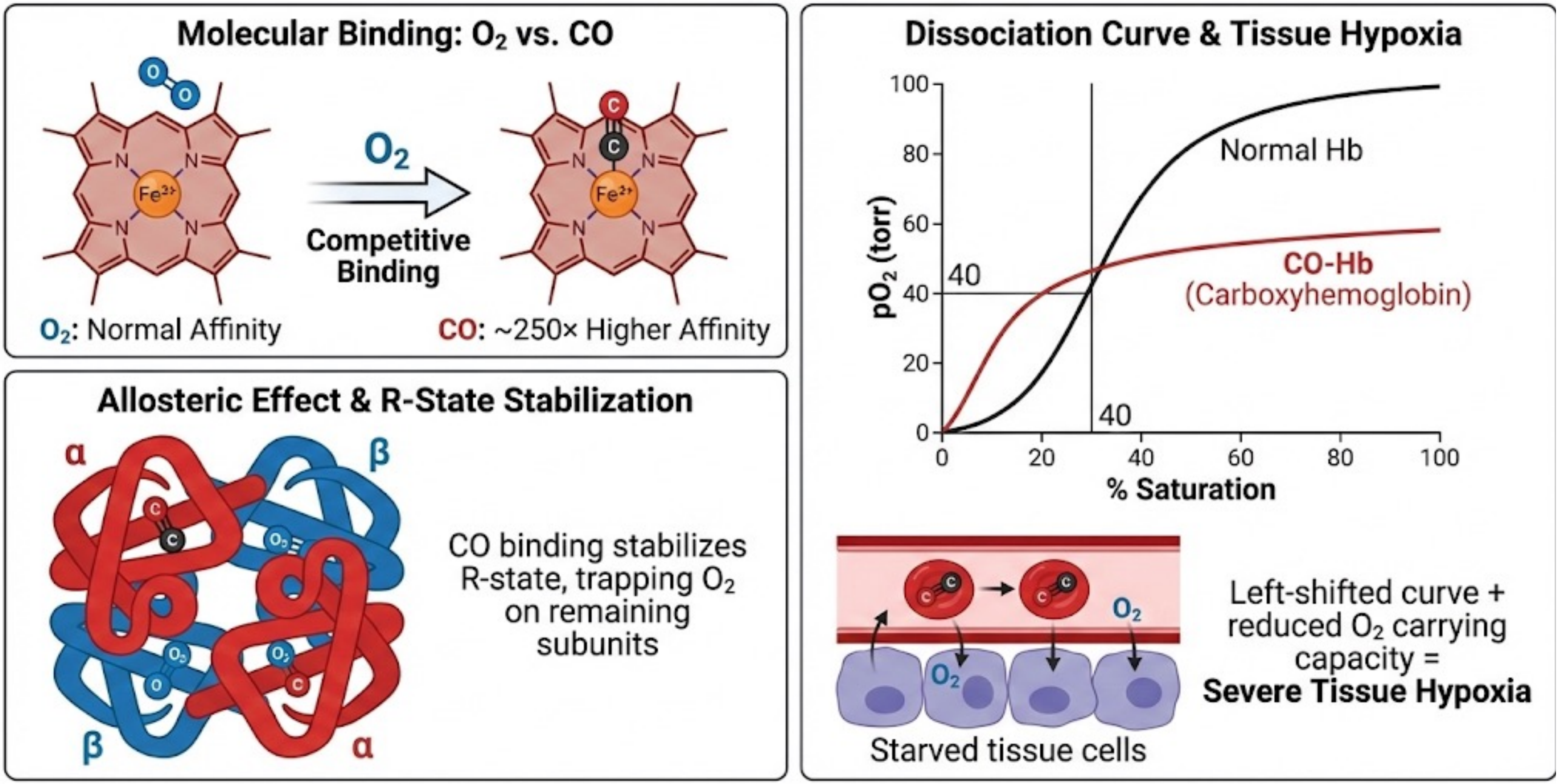


Dalton's Law & Physiological Oxygen Gradient



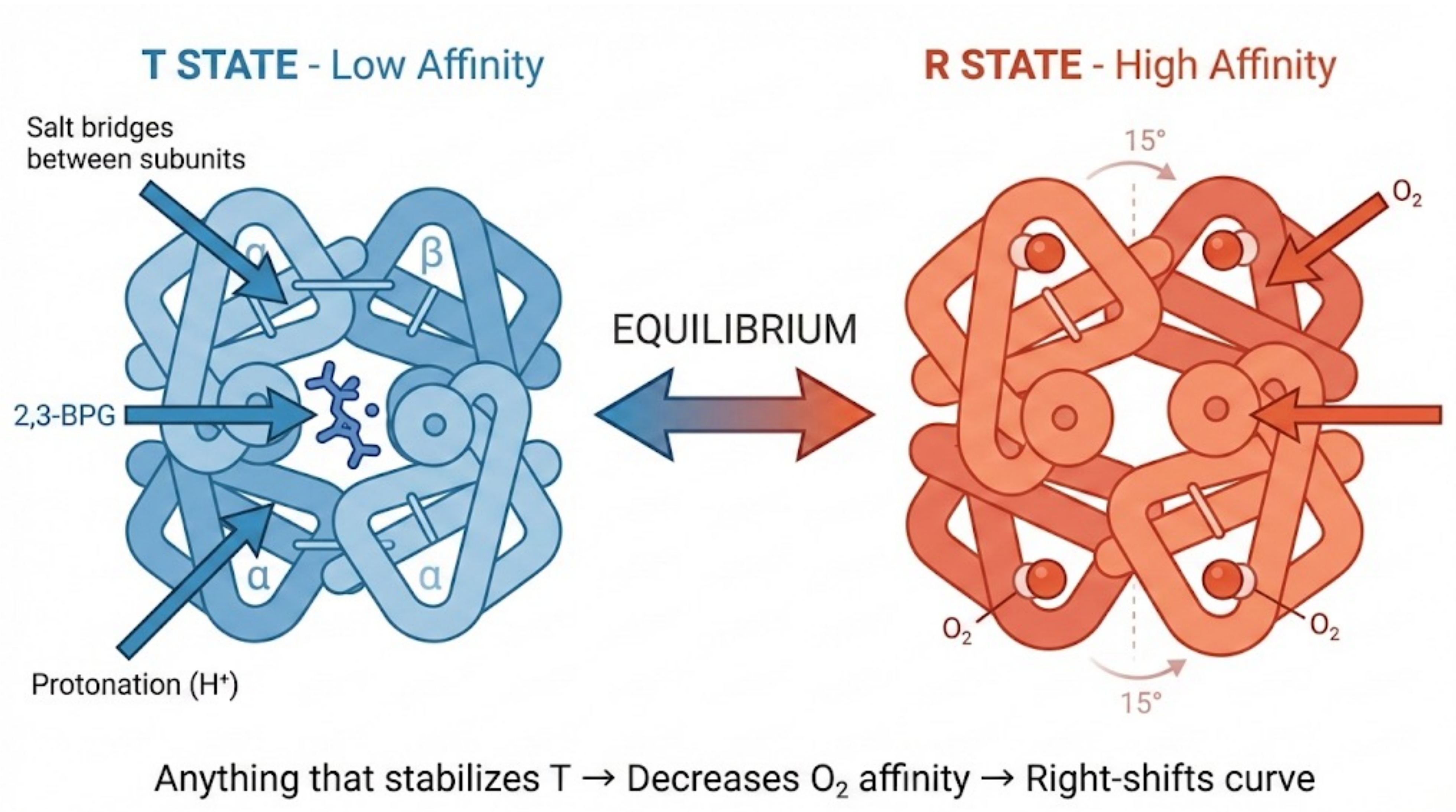


Carbon Monoxide (CO) Poisoning & Hemoglobin

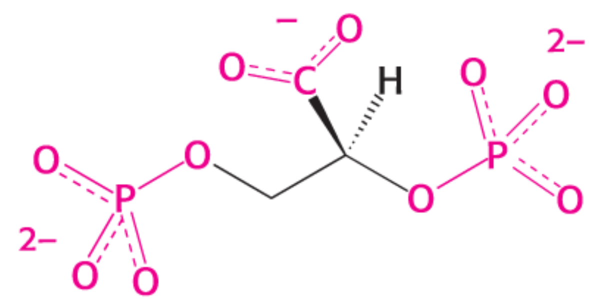


Break

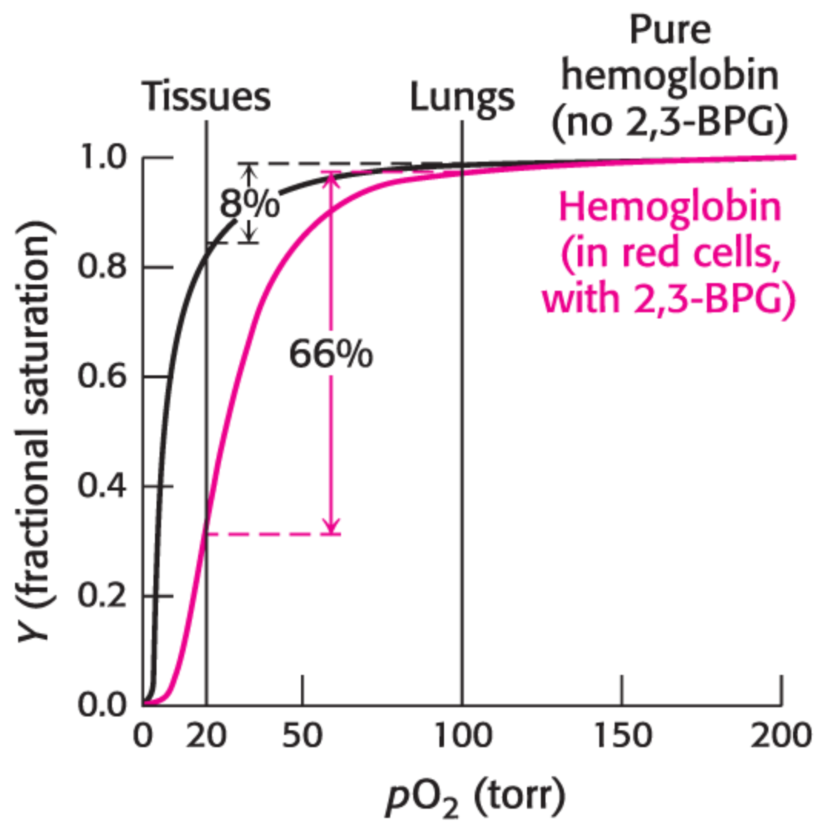
What stabilizes the T state?



Section 5.4 An Allosteric Regulator Determines the Oxygen Affinity of Hemoglobin (1/3)



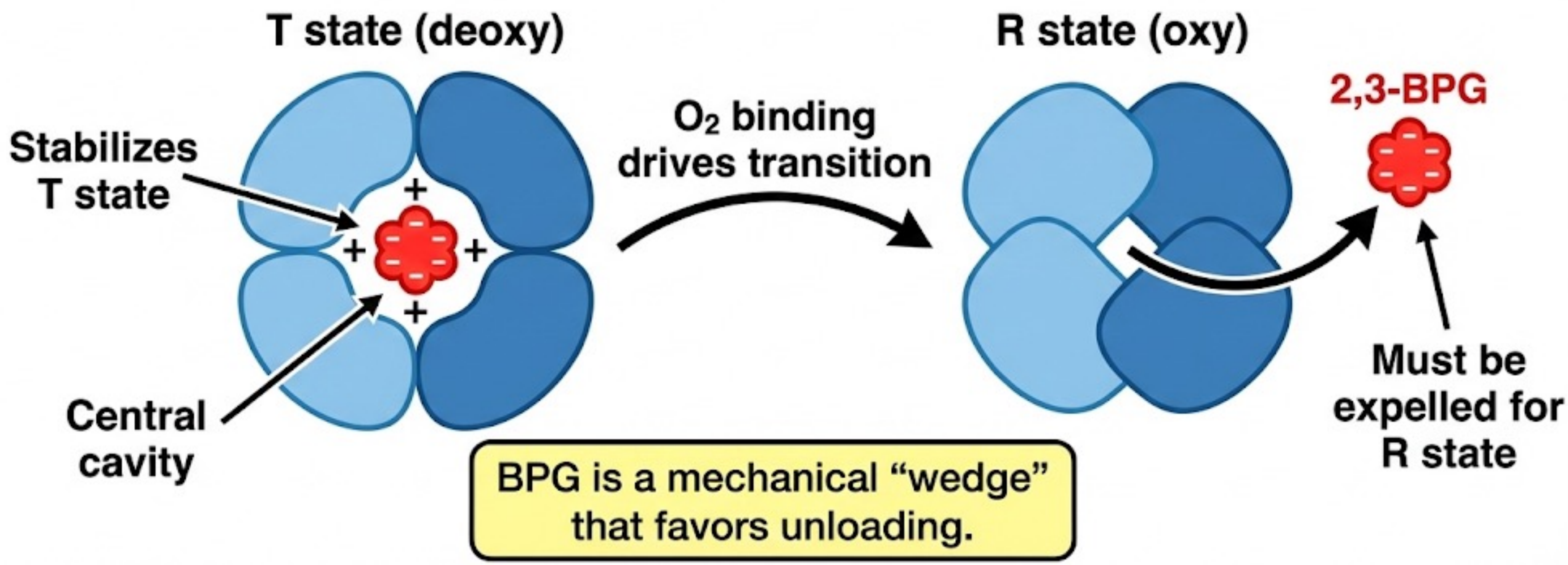
- **Learning objective 8: Identify the key regulators of hemoglobin function.**
- 2,3-Bisphosphoglycerate (2,3-BPG) stabilizes the T state of hemoglobin and thus facilitates the release of oxygen.
- 2,3-BPG binds to a pocket in the hemoglobin tetramer that exists only when hemoglobin is in the T state.
- **Tissue Specificity**: In tissues with high metabolic demand or low oxygen levels, 2,3-BPG levels are elevated. This allows hemoglobin to off-load oxygen more efficiently in these tissues.



Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company

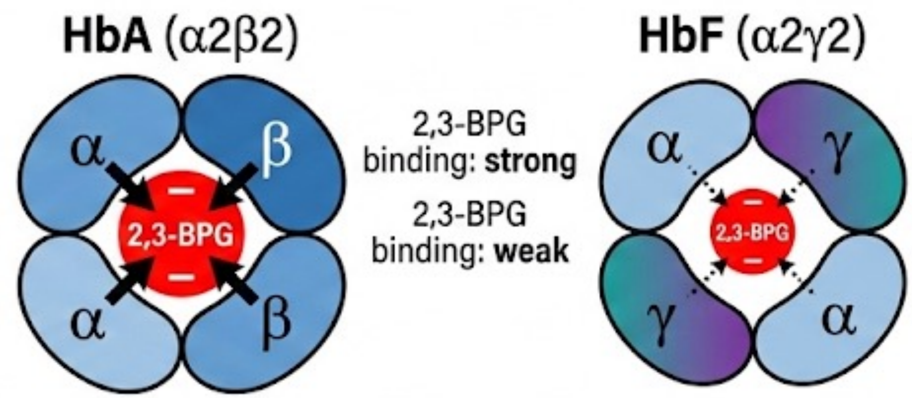
2,3-BPG locks in the T state by occupying the central cavity

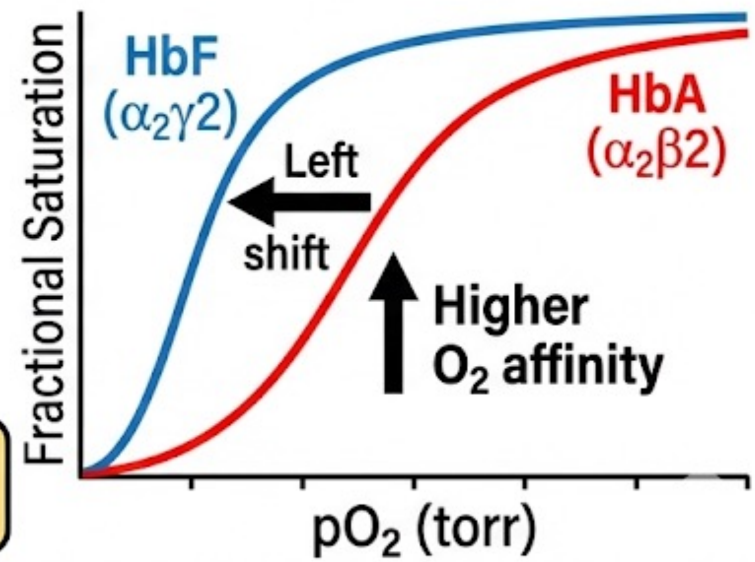
- ☑ One 2,3-BPG binds the central cavity of deoxyhemoglobin (T state)
- ☑ Electrostatic attraction: BPG is highly negative, pocket is positively charged
- ☑ Binding stabilizes T state → reduced O_2 affinity → better unloading
- ☑ To reach R state, BPG interactions must break and BPG is expelled



Fetal hemoglobin (HbF) binds O_2 tighter by binding BPG weaker

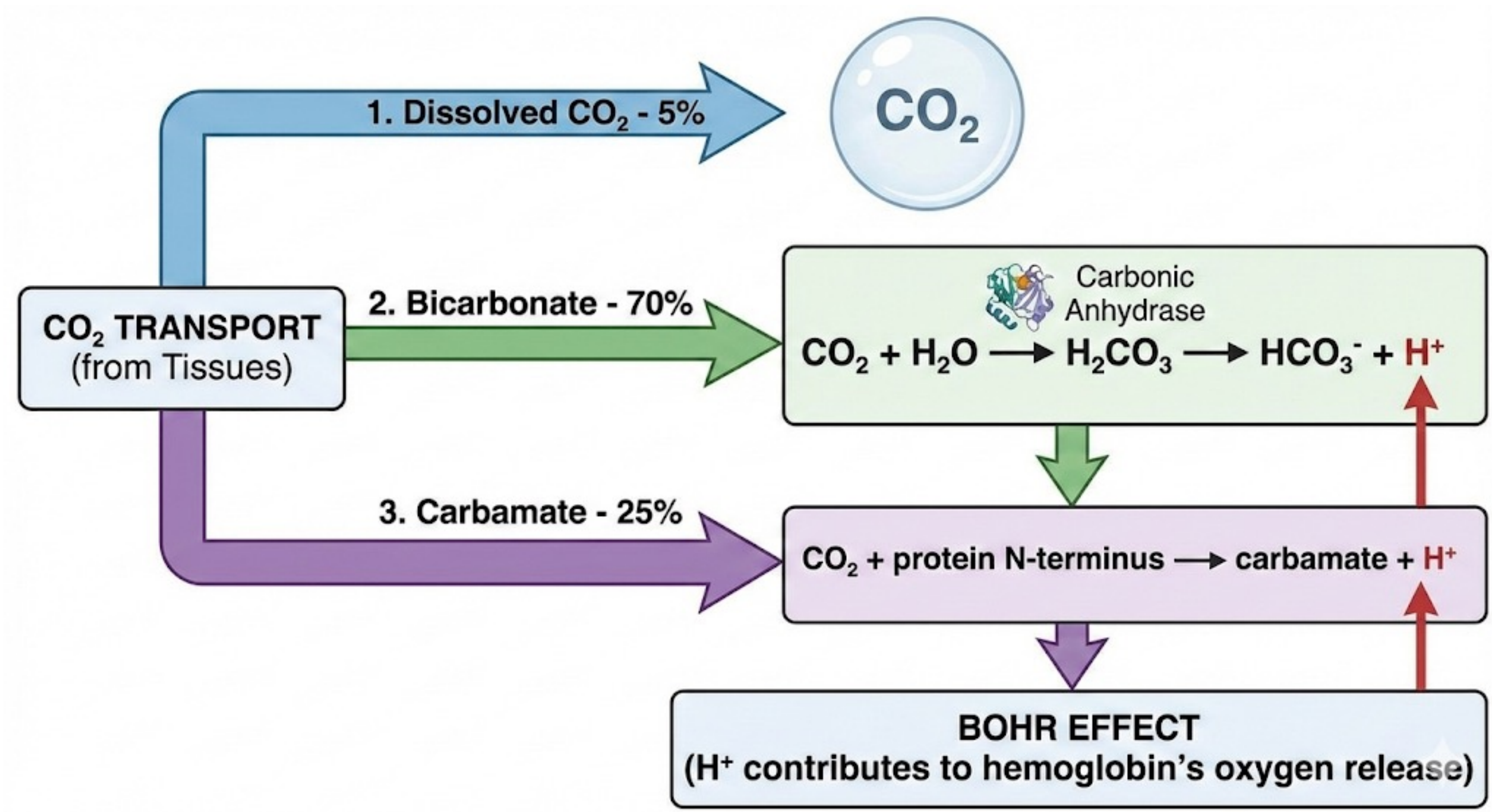
- ☑ Fetus must extract O_2 from maternal **blood** → needs higher O_2 affinity
- ☑ HbF tetramer: α2γ2 (not α2β2)
- ☑ HbF binds 2,3-BPG more weakly →
  - less T-state stabilization
- ☑ **Result: HbF curve is left-shifted relative to HbA**
- ☑ Enables efficient O_2 transfer mother → fetus



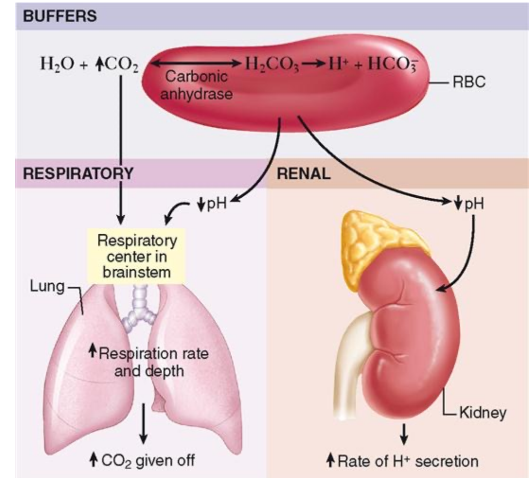


Same protein family, different subunit, different allostery.

3 modes of C02 Transport



pH Regulation



Peripheral tissues

From respiration $CO_2 + H_2O$

↓ Lower pH

H_2CO_3 **Carbonic Acid**

↓

$HCO_3^- + H^+$ **Bicarbonate**

From gylcolysis Lactic acid

Lungs

$CO_2 + H_2O$

↑ Higher pH

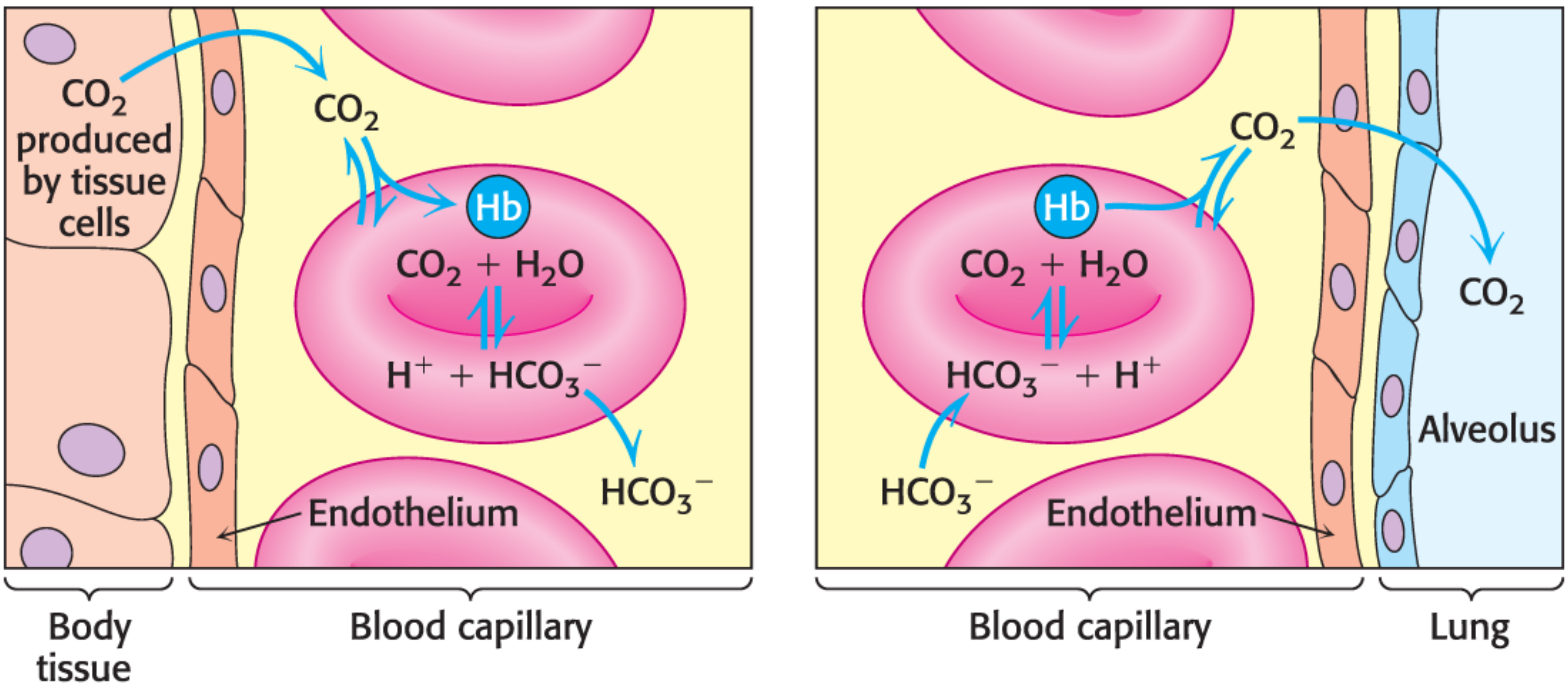
H_2CO_3

↑

$HCO_3^- + H^+$

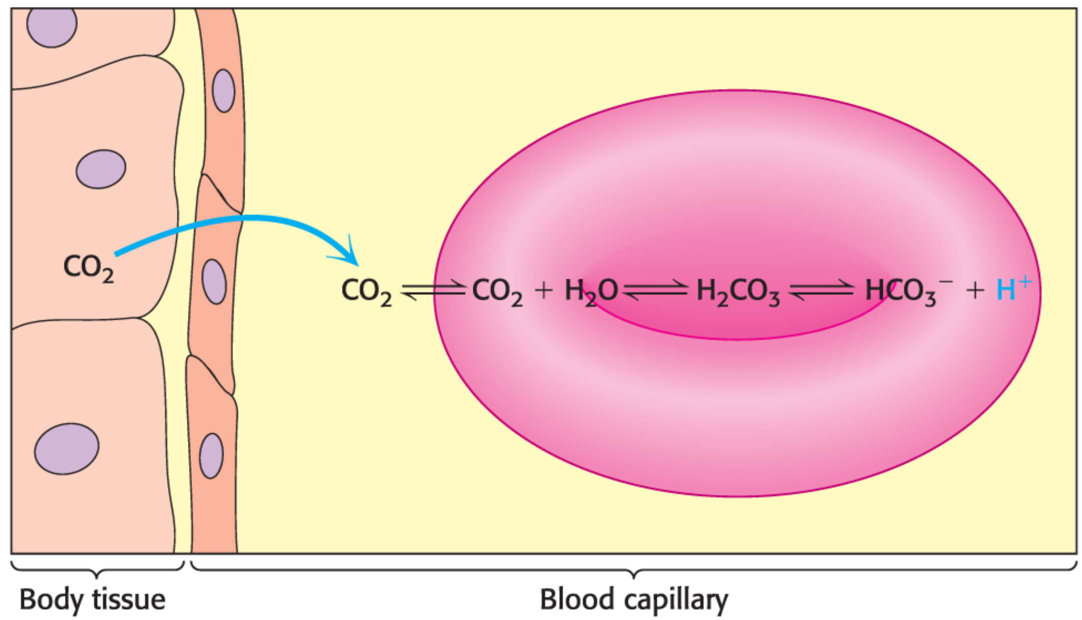
Section 5.5 Hydrogen Ions and Carbon Dioxide Promote the Release of Oxygen (3/3)

- Carbon dioxide is transported to the lungs as bicarbonate.
- Carbonic anhydrase facilitates the formation of bicarbonate ions.



Section 5.5 Hydrogen Ions and Carbon Dioxide Promote the Release of Oxygen (1/3)

- Carbon dioxide and H^+, produced by actively respiring tissues, enhance oxygen release by hemoglobin.
- The stimulation of oxygen release by carbon dioxide and H^+ is called the Bohr effect.
- carbon dioxide reacts with water to form carbonic acid in a reaction catalyzed by the enzyme carbonic anhydrase



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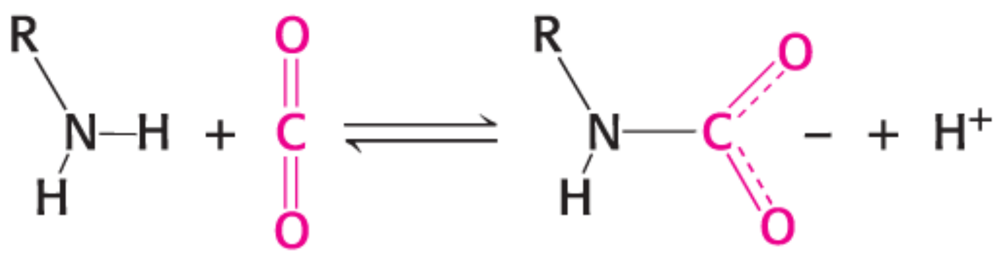
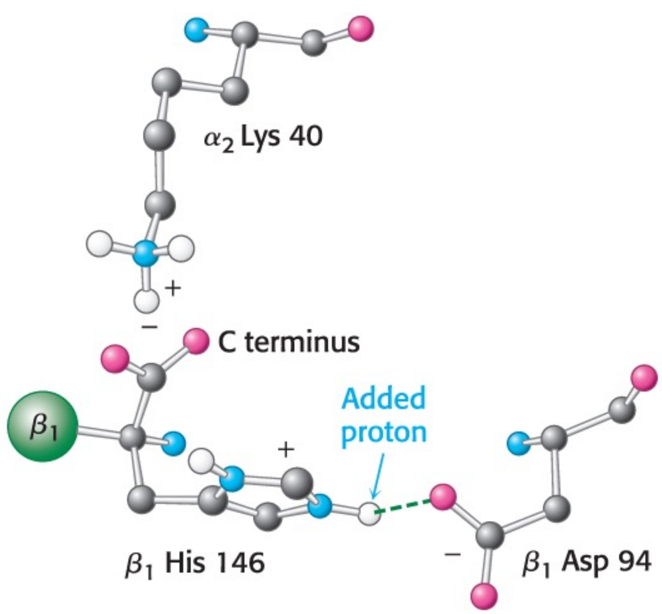
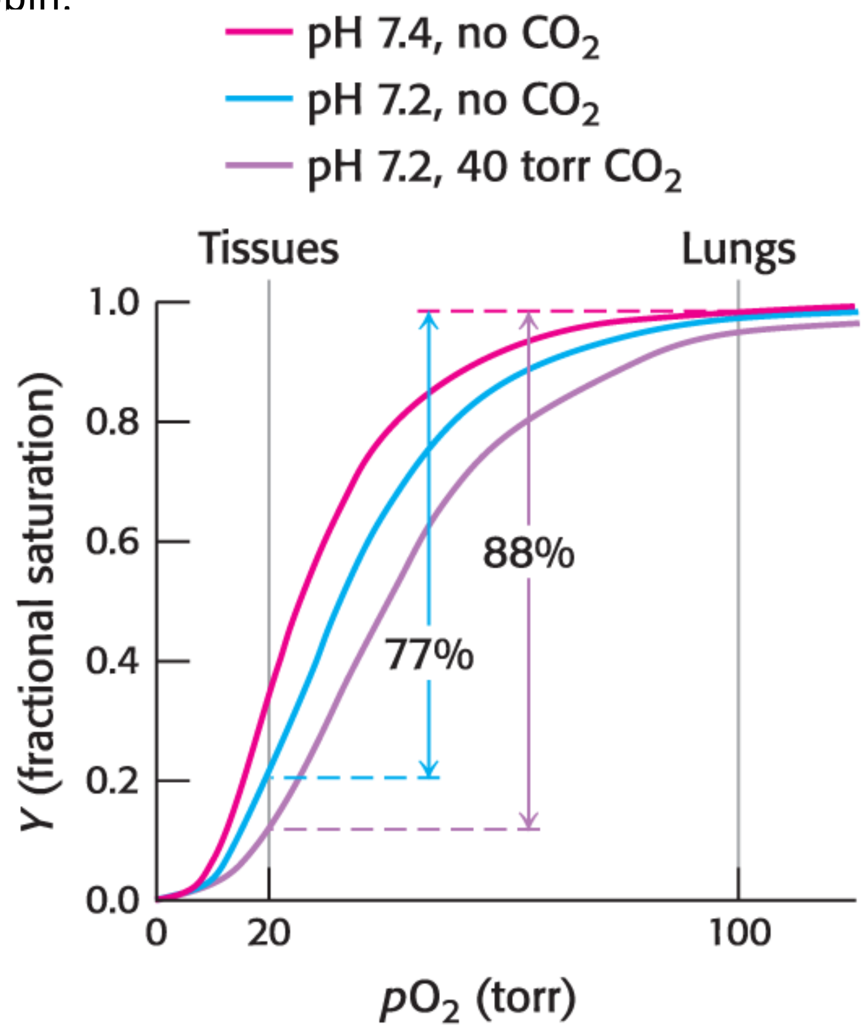
Section 5.5 Hydrogen Ions and Carbon Dioxide Promote the Release of Oxygen

- Low pH allows the formation of ionic interactions that stabilize the T state of hemoglobin, enhancing oxygen release.
- Carbon dioxide reacts with terminal amino groups to form negatively charged carbamate groups. The carbamate forms salt bridges that stabilize the T state.
- Carbon dioxide and H^+ are heterotropic regulators of oxygen binding by hemoglobin.



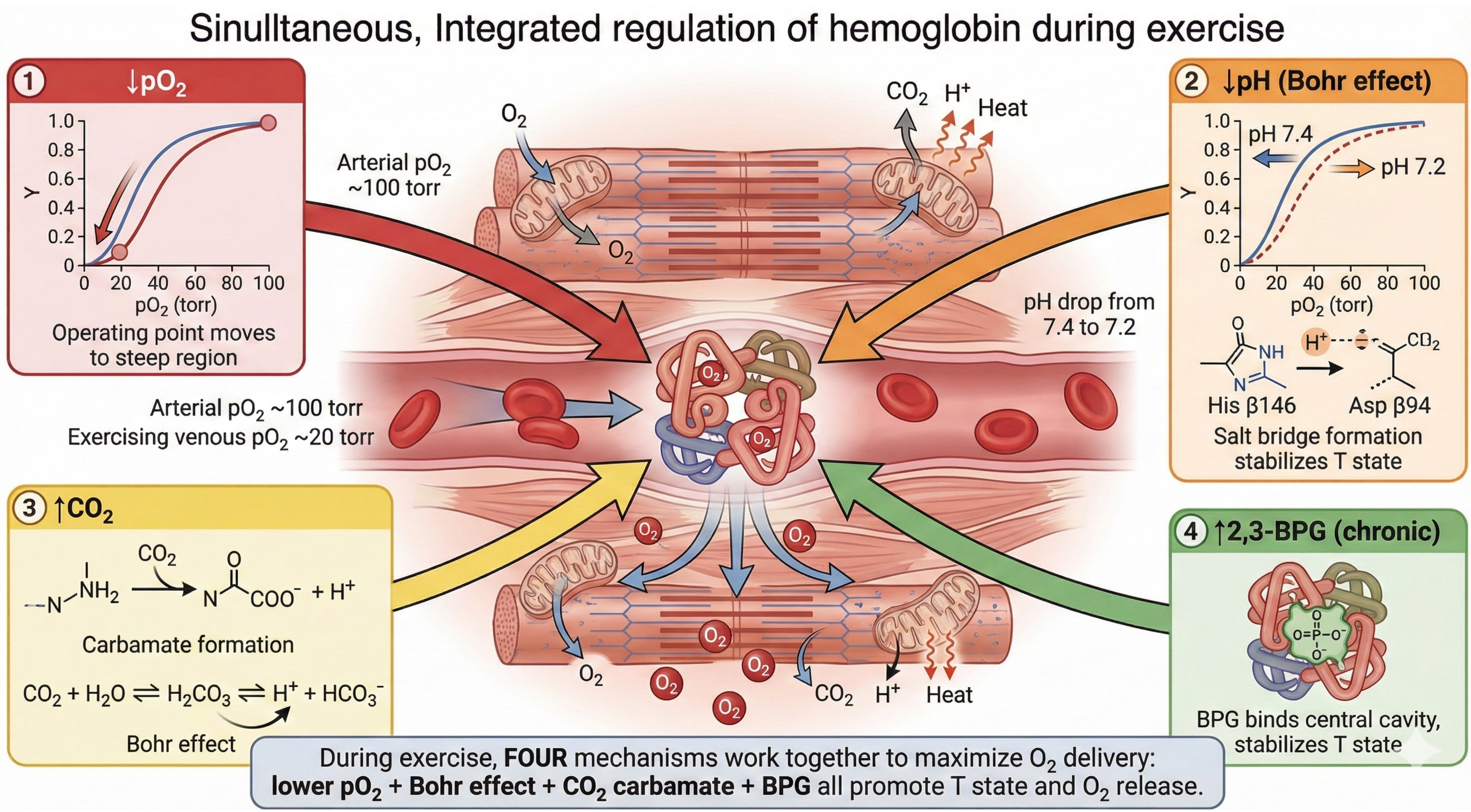
Carbamate



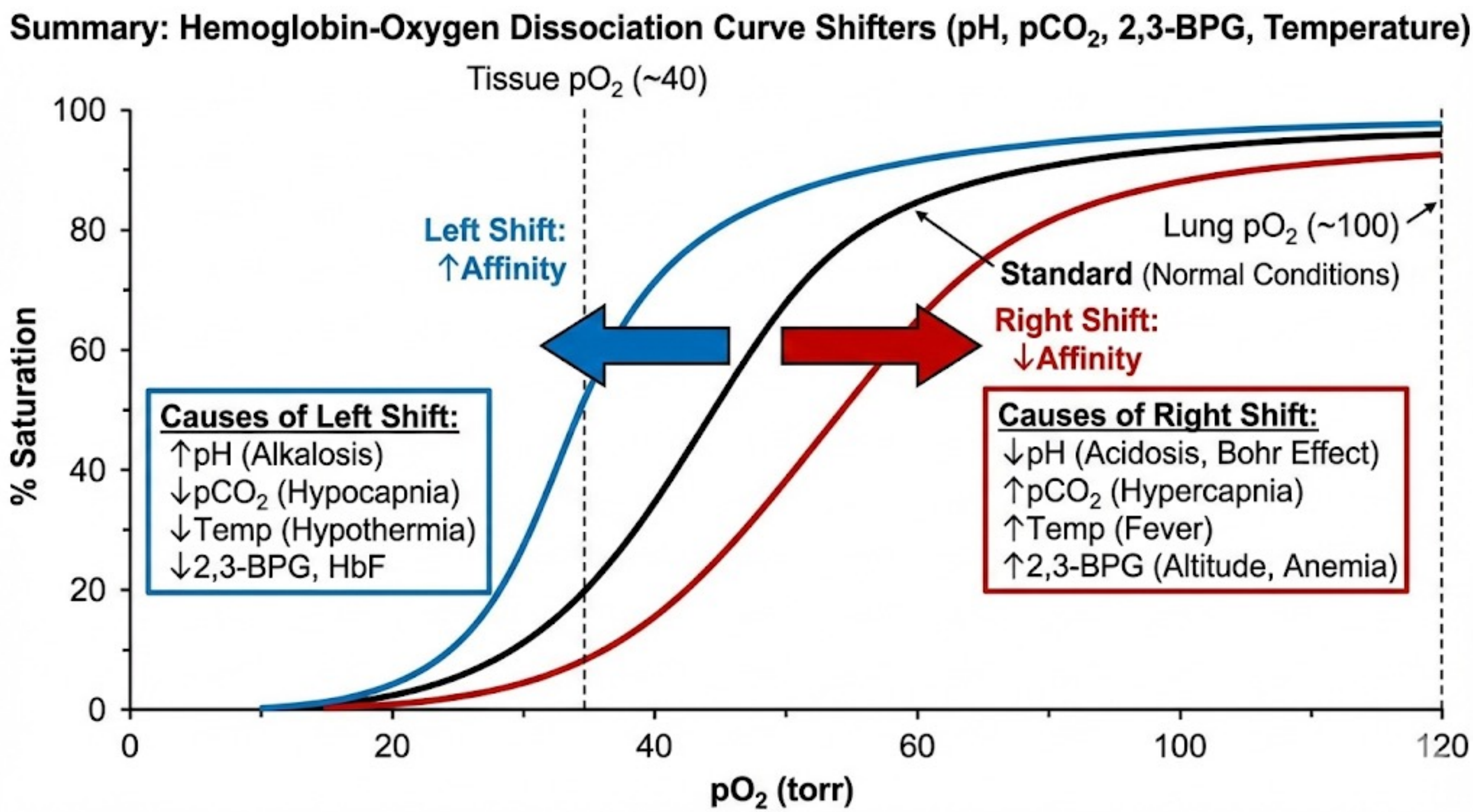


Your exercising muscle drops pO_2 to 20 torr or lower. How does hemoglobin respond—and is cooperativity enough?

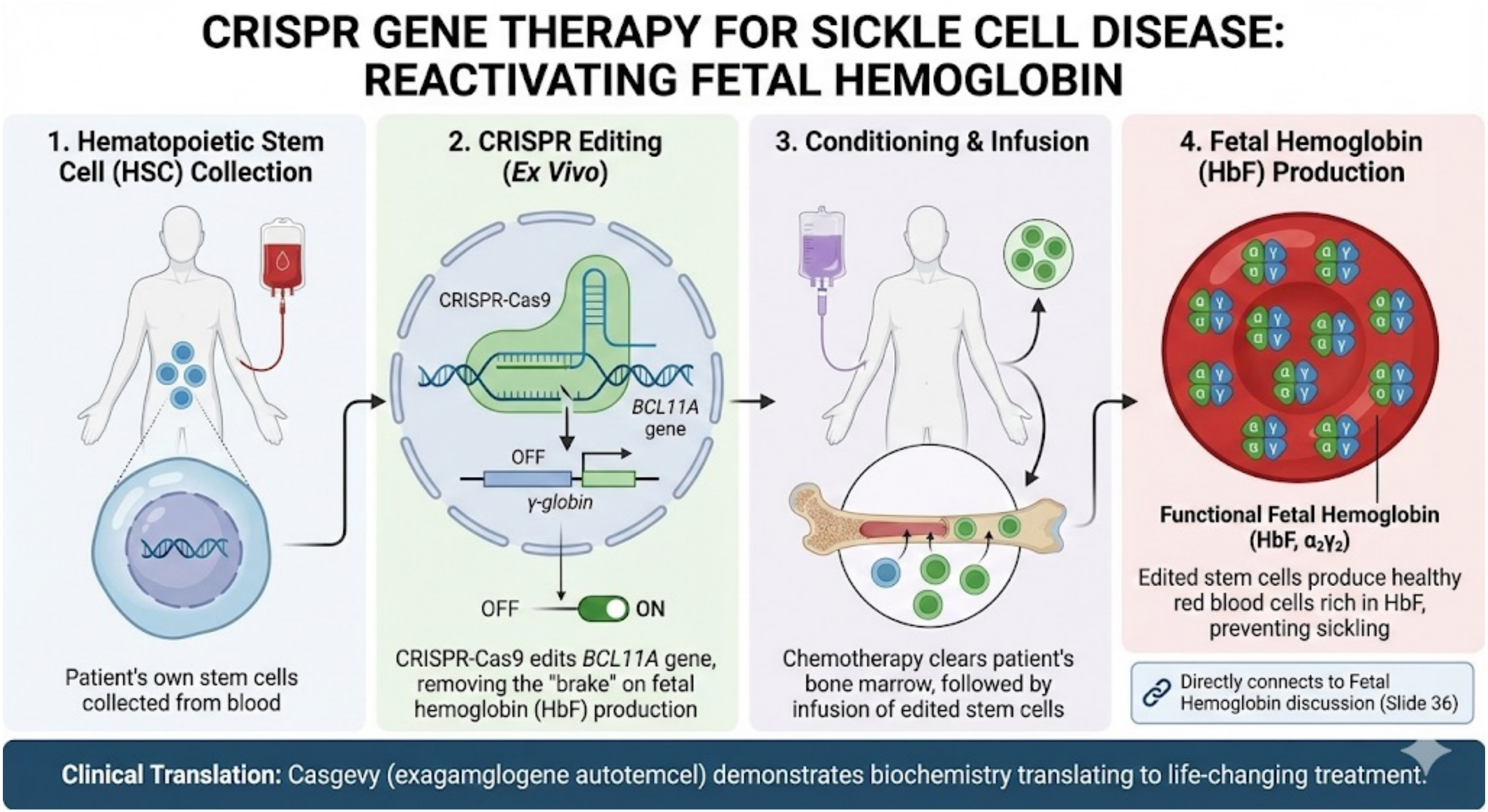
Your exercising muscles consume O_2 faster than circulation delivers it—what happens to hemoglobin?



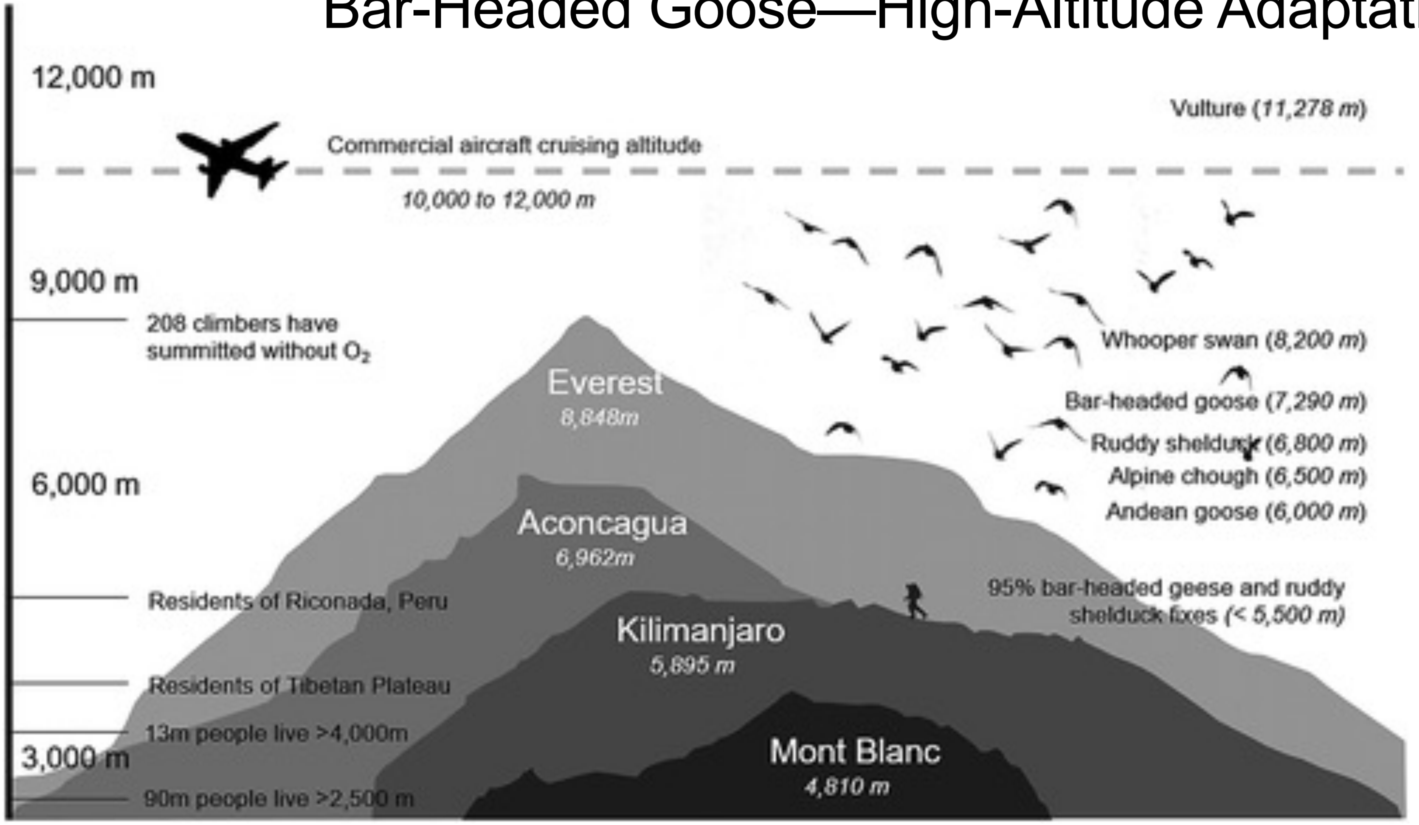
Sinulltaneous, Integrated regulation of hemoglobin during exercise
1 ↓pO2
Y
pO2 (torr)
Operating point moves to steep region
2 ↓pH (Bohr effect)
pH 7.4
pH 7.2
His β146
Asp β94
Salt bridge formation stabilizes T state
O2
CO2 H+ Heat
Arterial pO2 ~100 torr
pH drop from 7.4 to 7.2
Arterial pO2 ~100 torr
Exercising venous pO2 ~20 torr
3 ↑CO2
Carbamate formation
CO2 + H2O ⇌ H2CO3 ⇌ H+ + HCO3-
Bohr effect
4 ↑2,3-BPG (chronic)
BPG binds central cavity, stabilizes T state
During exercise, FOUR mechanisms work together to maximize O2 delivery:
lower pO2 + Bohr effect + CO2 carbamate + BPG all promote T state and O2 release.



Summary: Hemoglobin-Oxygen Dissociation Curve Shifters (pH, pCO2, 2,3-BPG, Temperature)
Tissue pO2 (~40)
Lung pO2 (~100)
Left Shift:
↑Affinity
Standard (Normal Conditions)
Right Shift:
↓Affinity
Causes of Left Shift:
↑pH (Alkalosis)
↓pCO2 (Hypocapnia)
↓Temp (Hypothermia)
↓2,3-BPG, HbF
Causes of Right Shift:
↓pH (Acidosis, Bohr Effect)
↑pCO2 (Hypercapnia)
↑Temp (Fever)
↑2,3-BPG (Altitude, Anemia)
% Saturation
pO2 (torr)
0
20
40
60
80
100
120


CRISPR GENE THERAPY FOR SICKLE CELL DISEASE:
REACTIVATING FETAL HEMOGLOBIN
1. Hematopoietic Stem Cell (HSC) Collection
Patient's own stem cells collected from blood
2. CRISPR Editing (Ex Vivo)
CRISPR-Cas9
BCL11A gene
OFF
γ-globin
OFF
ON
CRISPR-Cas9 edits BCL11A gene, removing the "brake" on fetal hemoglobin (HbF) production
3. Conditioning & Infusion
Chemotherapy clears patient's bone marrow, followed by infusion of edited stem cells
4. Fetal Hemoglobin (HbF) Production
Functional Fetal Hemoglobin (HbF, α₂γ₂)
Edited stem cells produce healthy red blood cells rich in HbF, preventing sickling
Directly connects to Fetal Hemoglobin discussion (Slide 36)
Clinical Translation: Casgevy (exagamglogene autotemcel) demonstrates biochemistry translating to life-changing treatment.

Bar-Headed Goose—High-Altitude Adaptation



- Biological Insight: Hemoglobin Adaptations Allow Oxygen Transport in **Extreme Environments**
- The bar-headed goose can fly over Mount Everest, where the oxygen concentration is low (30% that of sea level).
- Changes in hemoglobin that facilitate the formation of the R state may account in part for this remarkable ability.
- Shifts curve to the left

Apply Your Knowledge: Emergency Department Case

A 34-year-old is brought to the ED after being found in a **running car in a closed garage**. The patient is confused and **lethargic**. Pulse oximetry shows **98% O_2 saturation**, but arterial blood gas reveals **severely low tissue oxygenation**. The patient's lips appear unusually pink.

Questions

1. What molecule is likely bound to the patient's hemoglobin?
2. Why does pulse oximetry show normal saturation despite tissue hypoxia?
3. Using what you know about allosteric regulation, explain the mechanism.

Think time: 60 seconds

Click to Reveal

Hint: Consider what happens when something other than O_2 stabilizes the R state.

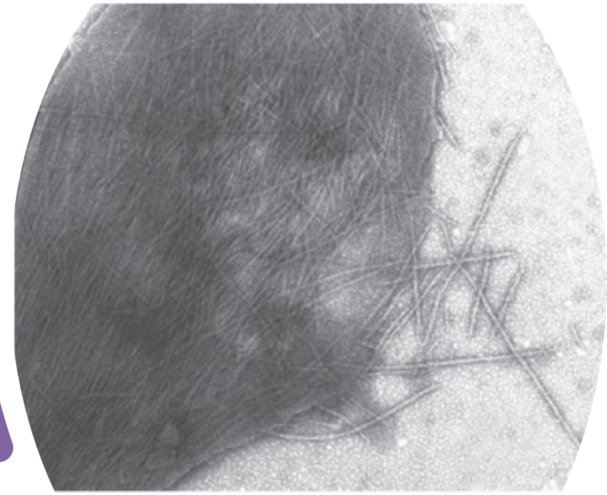
Quick Quiz

QUICK QUIZ

Name three factors that stabilize the deoxy form of hemoglobin.

Section 5.6 Mutations in Genes Encoding Hemoglobin Subunits Can Result in Disease

- **Clinical Insight: Sickle-Cell Anemia Is a Disease Caused by a Mutation in Hemoglobin**
- Sickle-cell hemoglobin is called hemoglobin S (HbS). The substituted valine is exposed in deoxyhemoglobin and can interact with other deoxy HbS to form aggregates that deform the red blood cells.
- The sickled cells clog blood flow through the capillaries, leading to tissue damage.
- Sickle-cell anemia is a genetic disease caused by a mutation resulting in the substitution of valine for glutamate at position 6 of the β chains.
- Sickle-cell anemia can be fatal when both alleles of the β chain are mutated.
- In sickle-cell trait, one allele is mutated and one is normal. Such individuals are asymptomatic. (adaptation to malaria)



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Thomas E. Wellems
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Structure of Deoxygenated Hemoglobin S

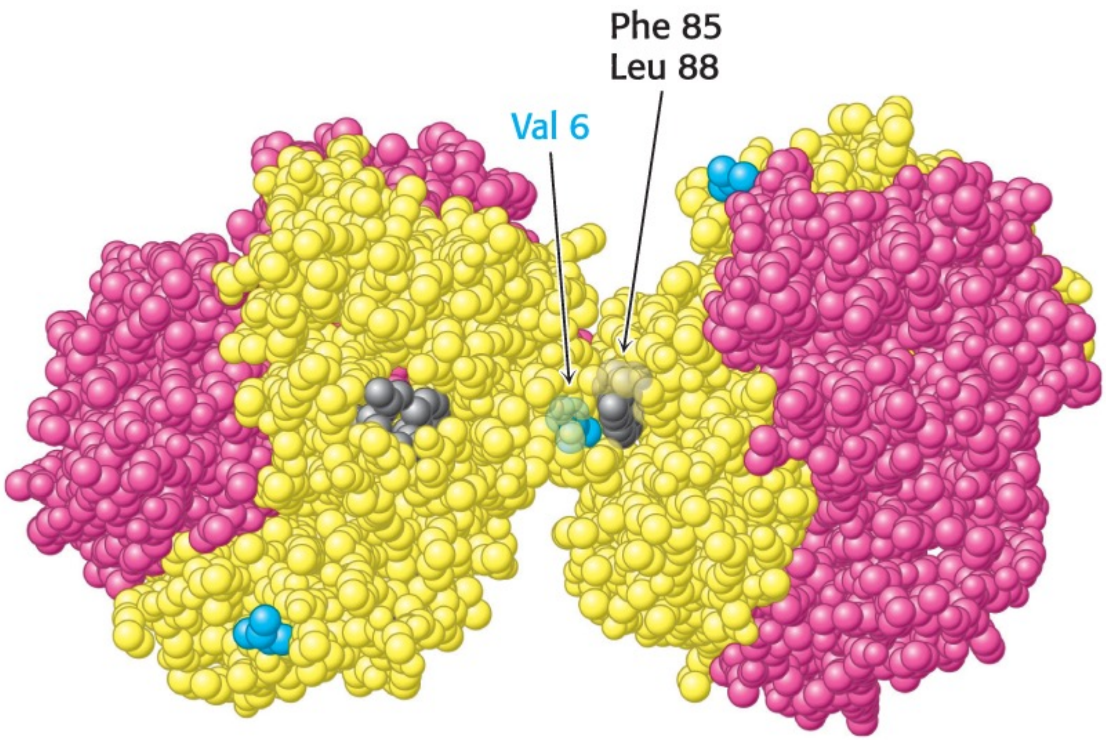

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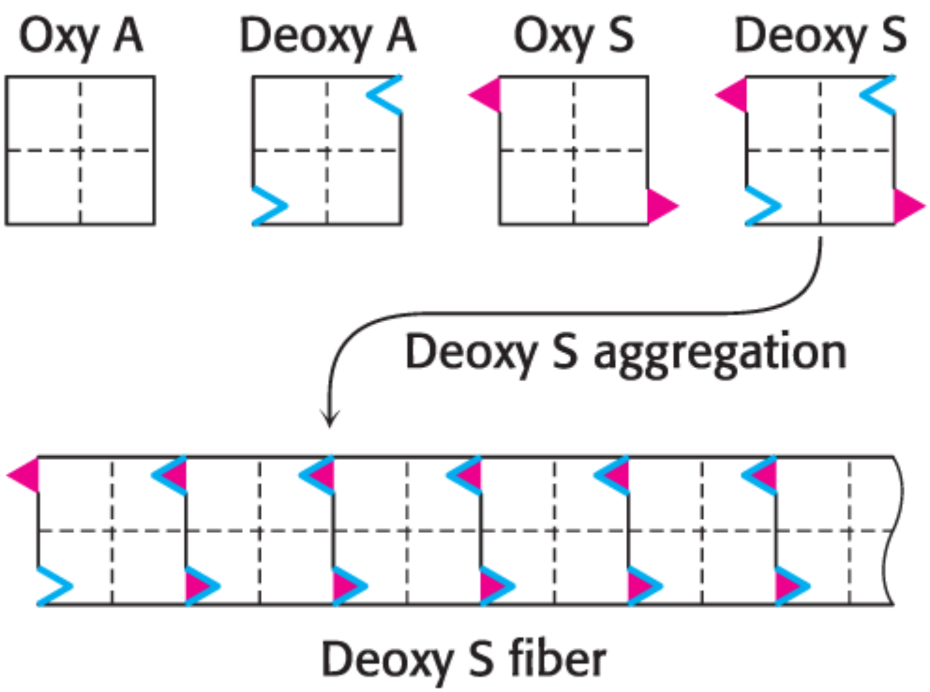
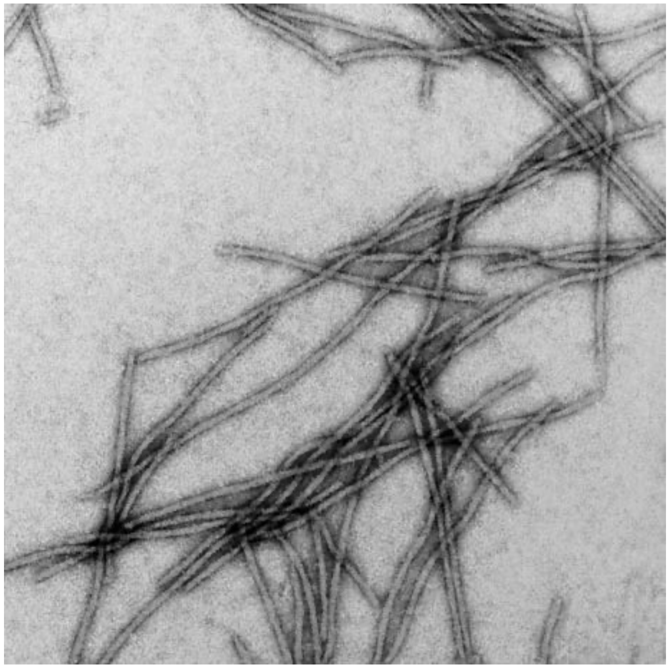
Diagram of the Formation of Hemoglobin Aggregates

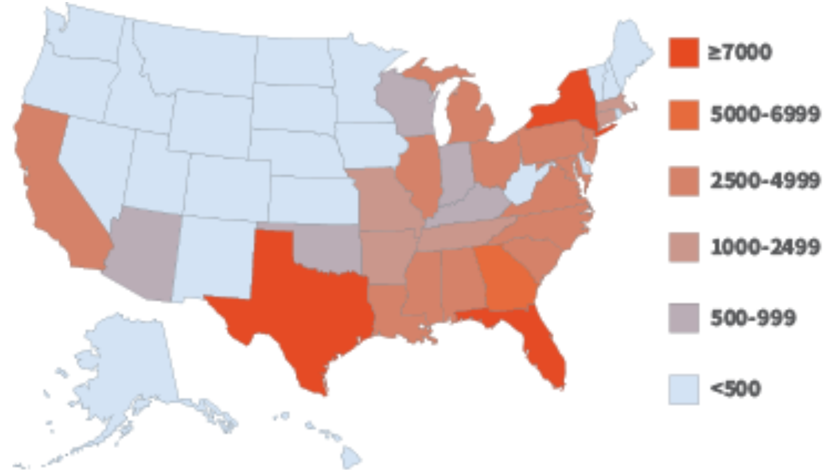




Epidemiology

FACELLY/SIPA/Newscom.

1 in 100 West Africans suffer from Sickle Cell Anemia



MORE THAN **55%** OF PEOPLE WITH SCD RESIDE IN JUST 10 STATES[2*]

Incidence of SCD[1]

~1:365
African American Births†

~1:16,300
Hispanic American Births‡

Incidence of Sickle Cell Trait[1]

~1:13 African American Births

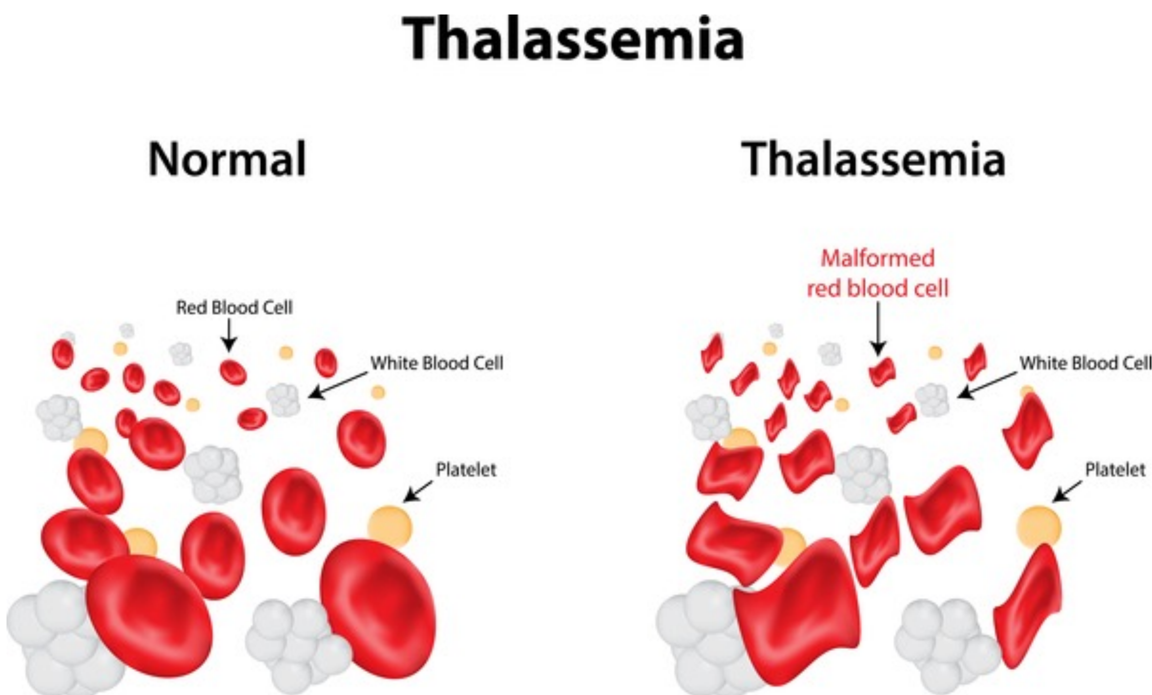
Section 5.6 Mutations in Genes Encoding Hemoglobin Subunits Can Result in Disease (3/3)

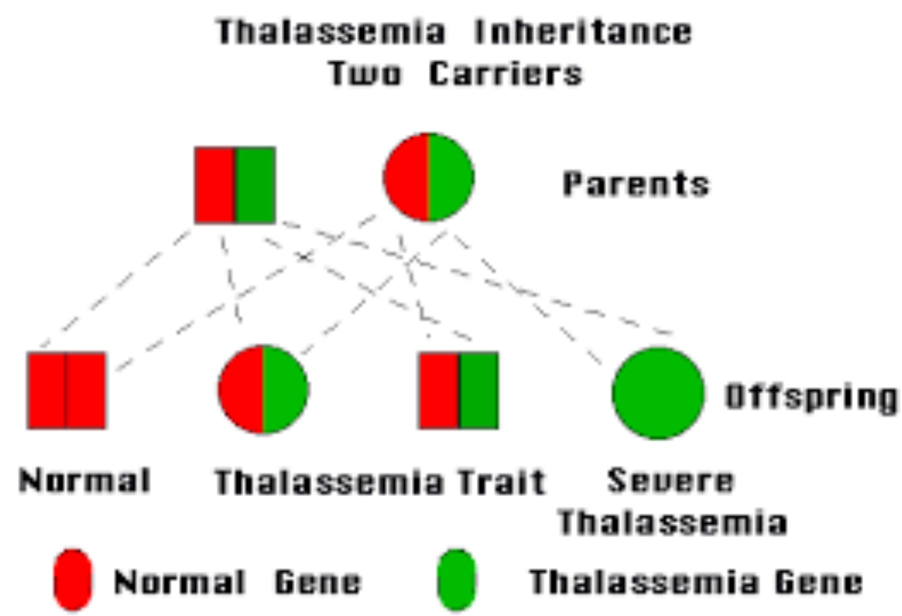
- **Clinical Insight: Thalassemia Is Caused by an Imbalanced Production of Hemoglobin Chains**

- Although sickle-cell anemia is caused by the substitution of a single specific amino acid, thalassemias are caused by a loss or substantial reduction of a single hemoglobin chain.

- In α-thalassemia, the *α* chain is not produced in sufficient quantity. Tetramers of the β chain form (HbH) and bind oxygen with high affinity but no cooperativity.

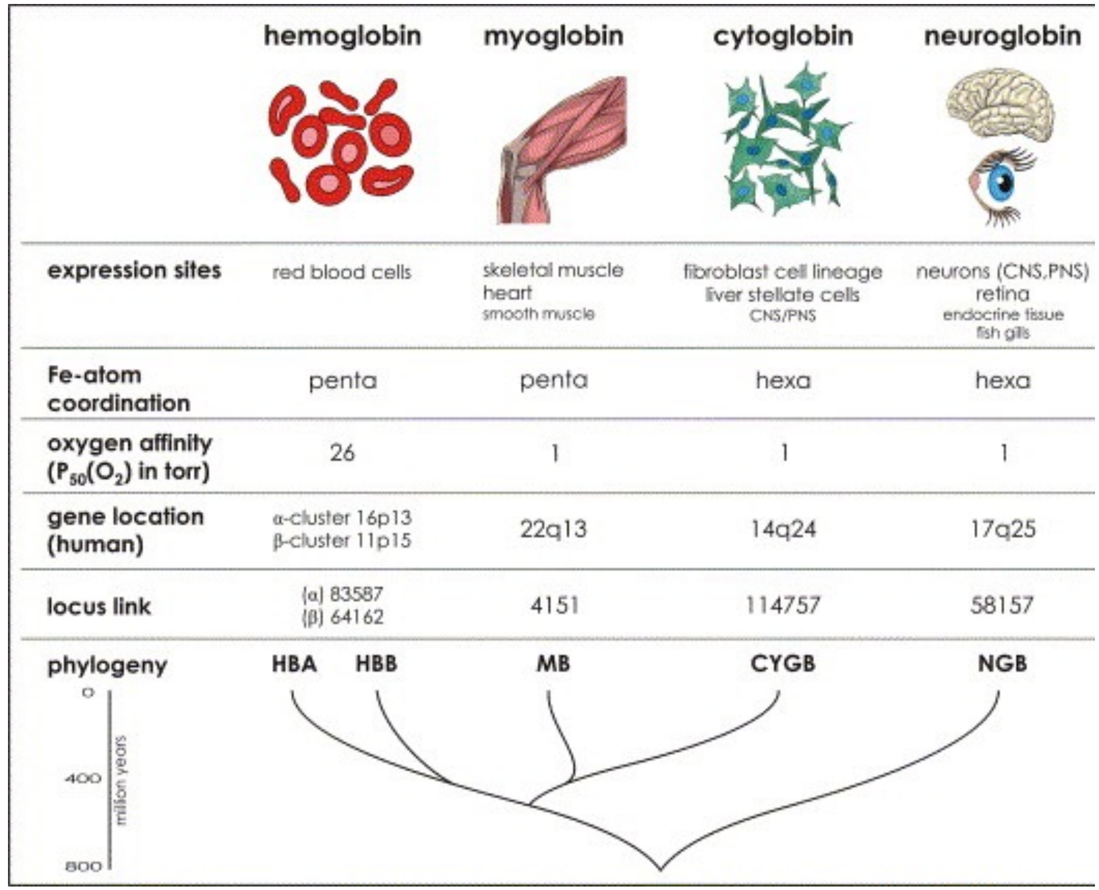
- In β-thalassemia, the β chain is not produced in sufficient quantity. The α chains aggregate and precipitate, leading to loss of red blood cells and anemia.





APPENDIX: Biochemistry in Focus

- Additional globins are encoded in the human genome
- The human haploid genome contains various globin genes, two being neuroglobin and cytoglobin.
- Neuroglobin is expressed primarily in the brain and at especially high levels in the retina. It may play a role in protecting neural tissues from hypoxia.
- Cytoglobin is expressed more widely throughout the body.
- Recent research points to an exciting therapeutic role for neuroglobin as a treatment for carbon monoxide poisoning. An altered neuroglobin binds carbon monoxide 500-fold more tightly than hemoglobin, allowing it to strip carbon monoxide from hemoglobin and then be excreted .



	hemoglobin	myoglobin	cytoglobin	neuroglobin
expression sites	red blood cells	skeletal muscle heart smooth muscle	fibroblast cell lineage liver stellate cells CNS/PNS	neurons (CNS,PNS) retina endocrine tissue fish gills
Fe-atom coordination	penta	penta	hexa	hexa
oxygen affinity ($P_{50}(O_2)$ in torr)	26	1	1	1
gene location (human)	α-cluster 16p13 β-cluster 11p15	22q13	14q24	17q25
locus link	(α) 83587 (β) 64162	4151	114757	58157
phylogeny	HBA HBB	MB	CYGB	NGB