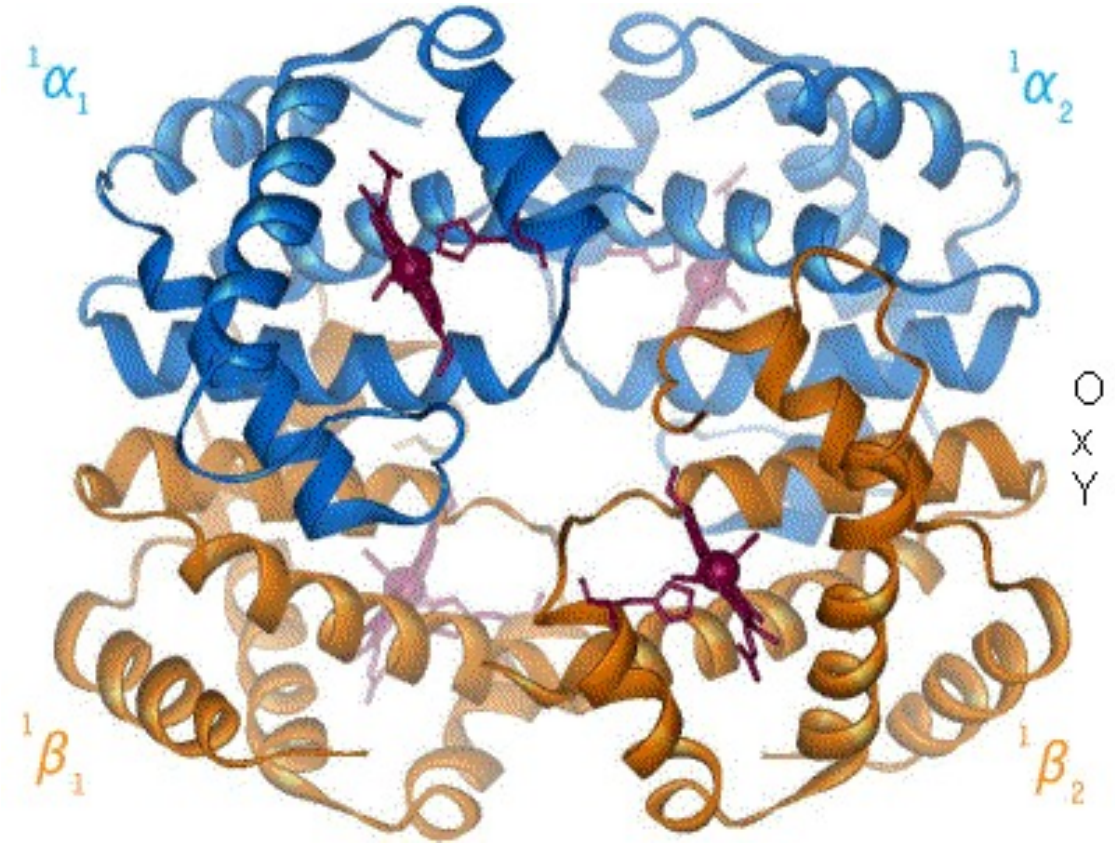


Protein structure and function

Protein-ligand interactions

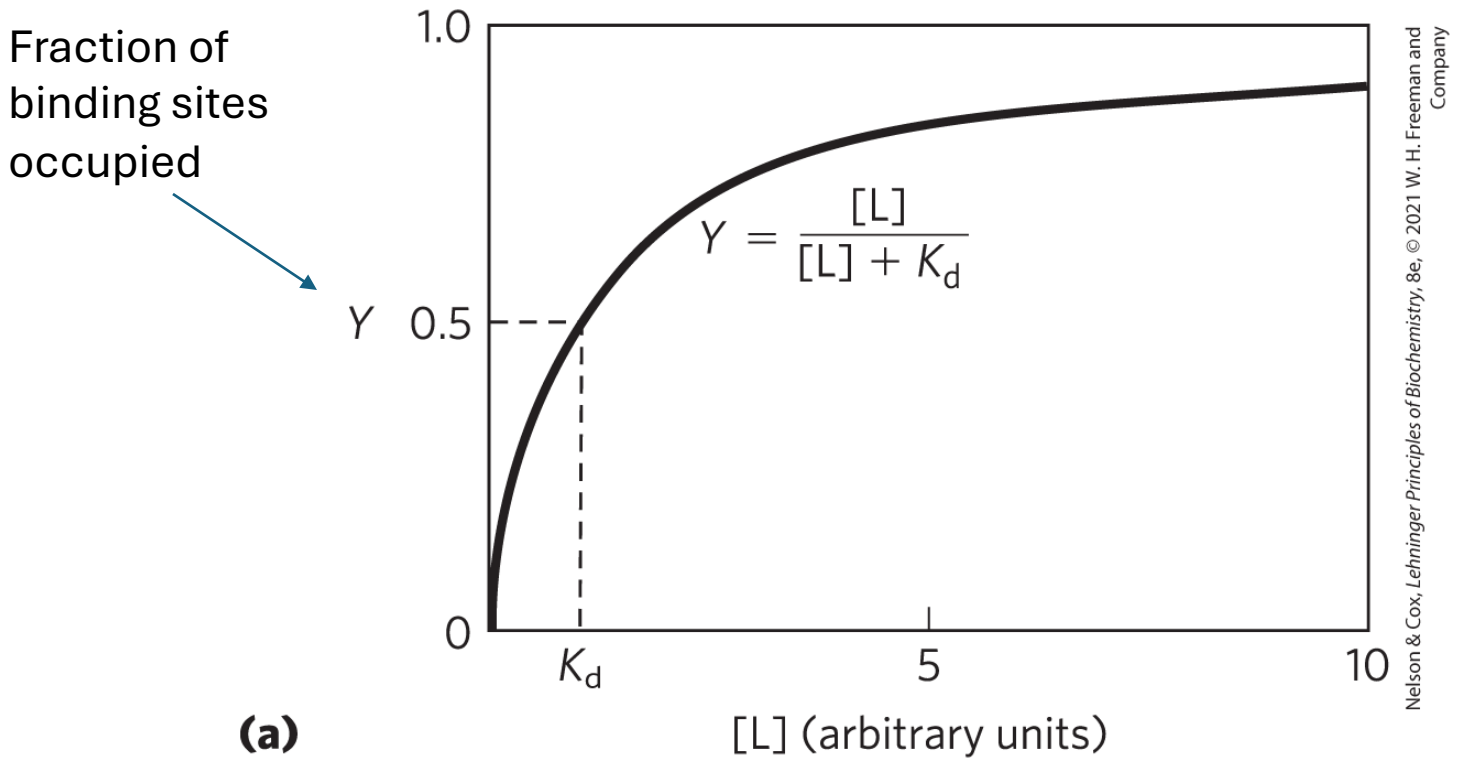
Myoglobin and hemoglobin

What do proteins do?

- Bind other proteins and/or small molecules
  - This is why we will start with an examination of ligand binding.
- Catalyze reactions
- Build structures
- Facilitate membrane transport
- Make up molecular motors
- Organize things within the cell

Graphical representation of ligand binding

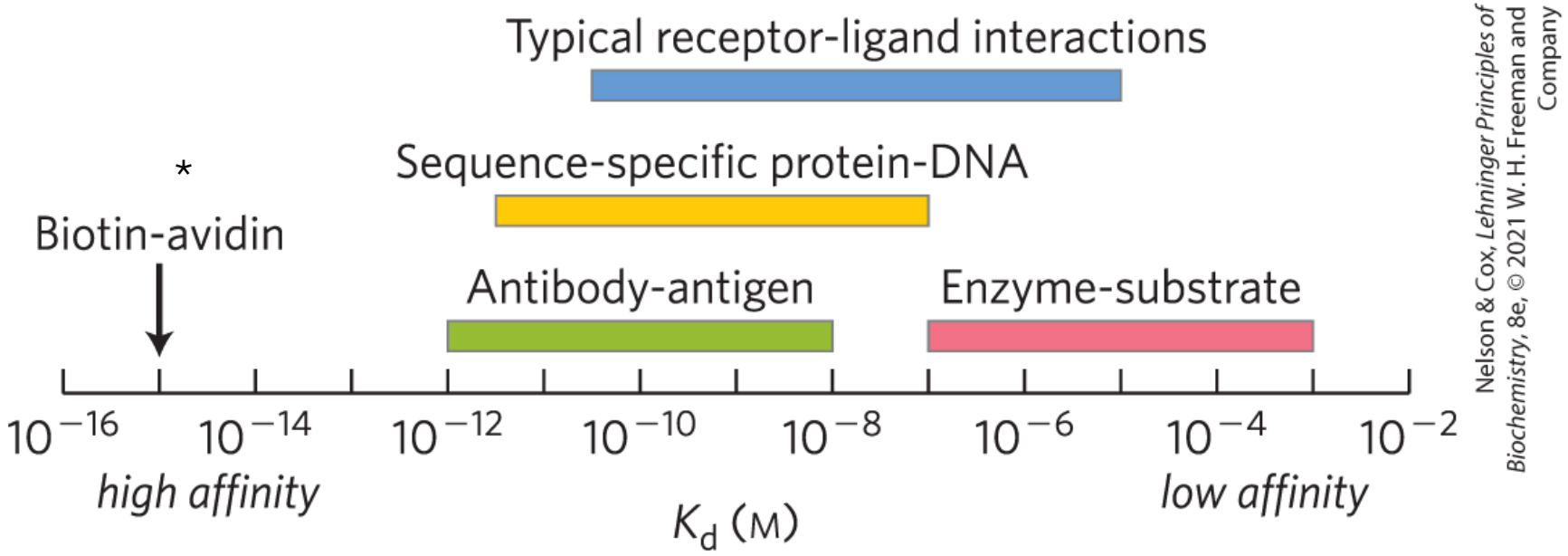
P(protein) + L(ligand)→ PL



Association constant K_a = [PL]/[P][L] = k_a/k_d

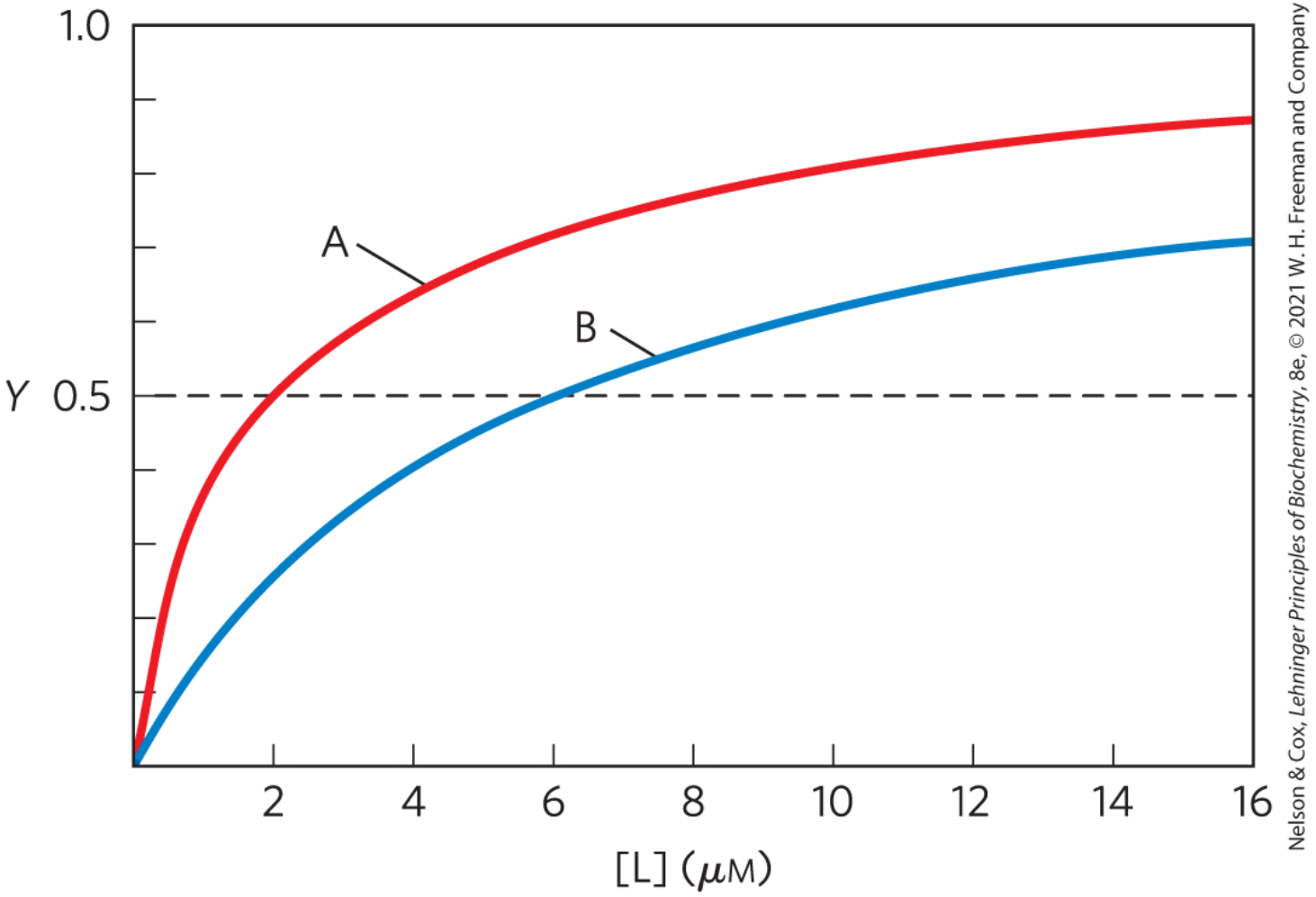
Dissociation constant K_d = [P][L]/[PL] =?

Why such a range in affinity?



*One of the strongest non-covalent interactions found in nature!

Proteins A and B bind the same ligand—how does the binding differ?



Estimate K_d from the graph for each protein.

First application of protein-ligand interaction: binding of oxygen at heme group in Myoglobin

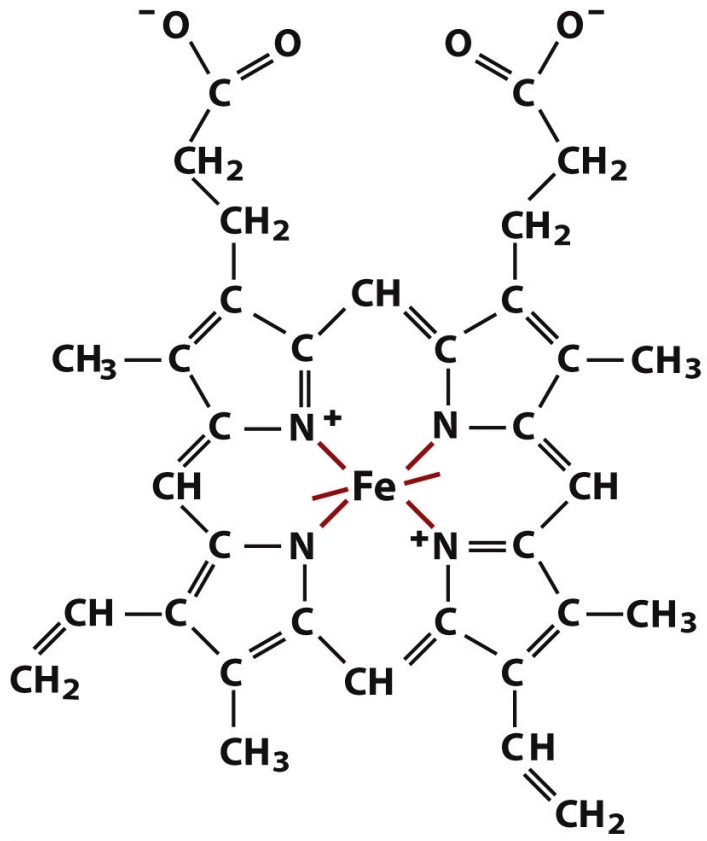


Figure 5-1b
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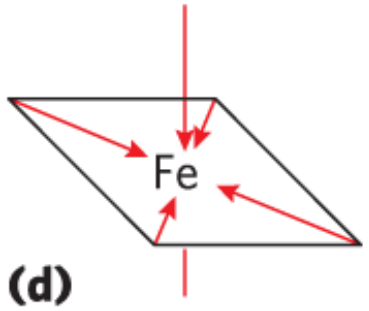
Heme is a **prosthetic group**-- an organic, tightly bound, non-proteinaceous unit required for the biological function of some proteins.

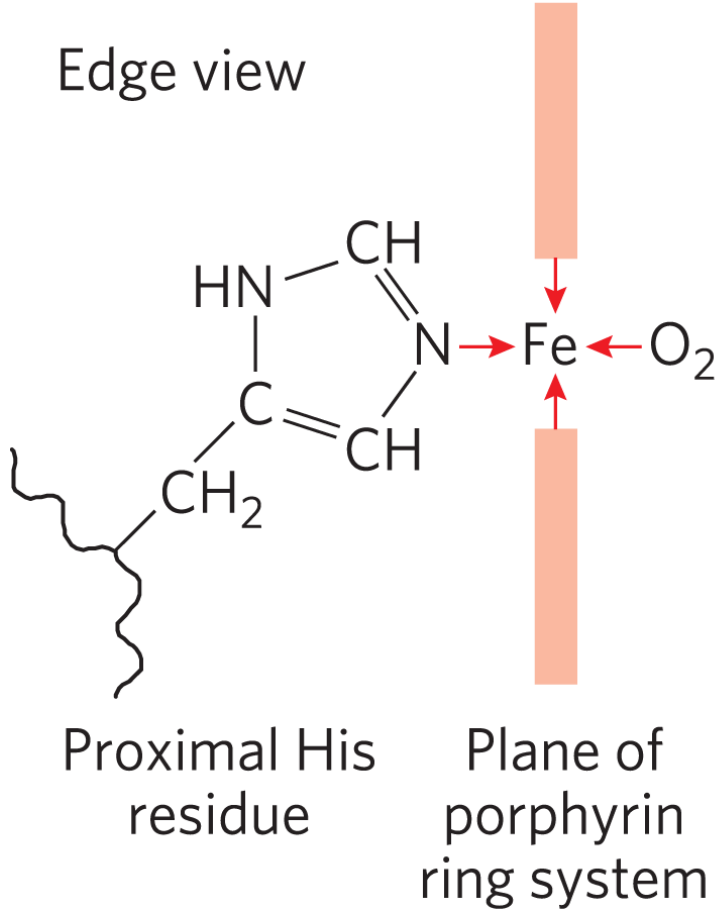
Heme is present in a variety of proteins.
(We'll see them again in electron transport.)

How does the heme group facilitate the binding of oxygen?

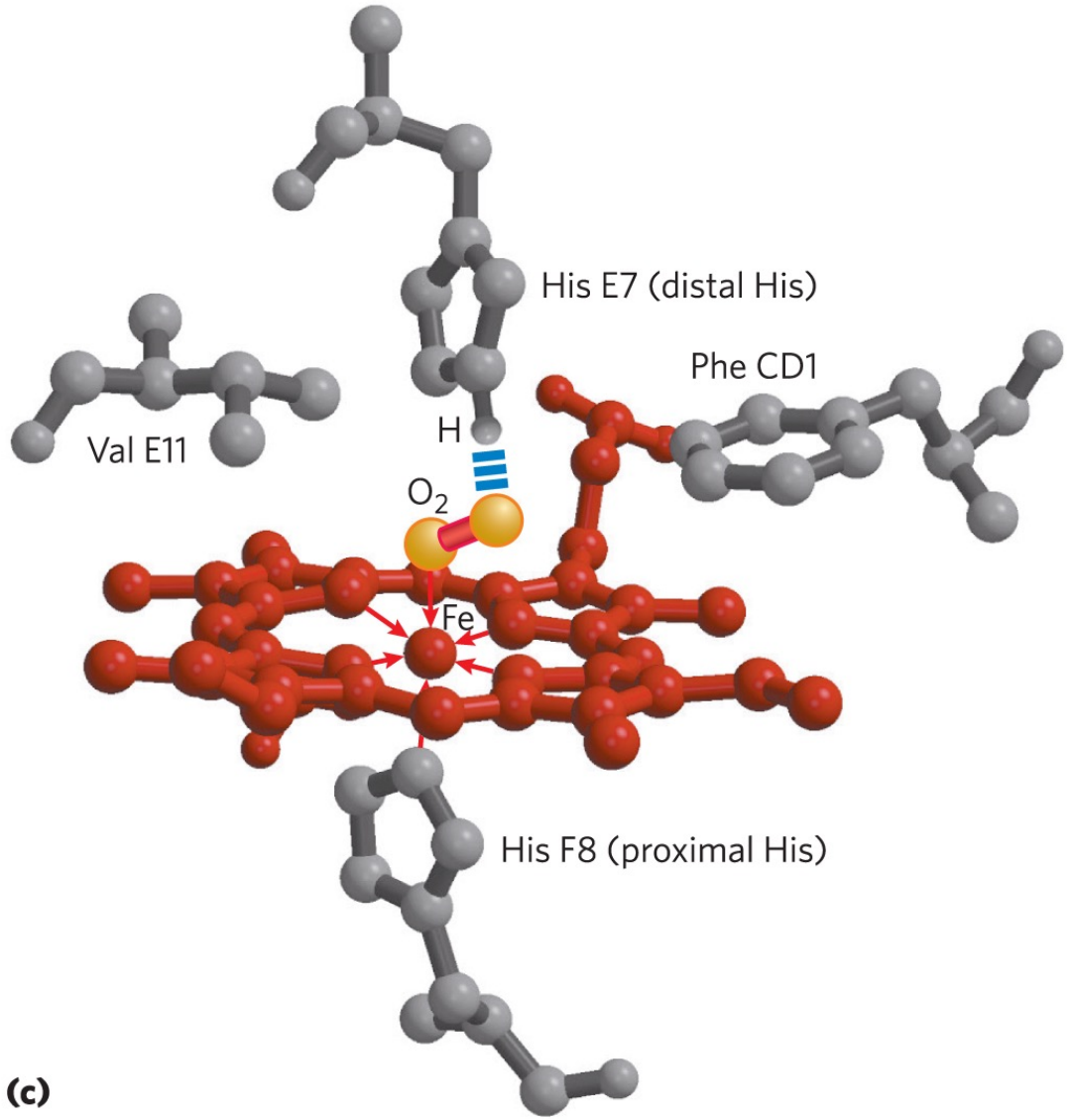
O_2 binding is coordinated by the Fe at the center of the heme and a histidine side chain from the protein.



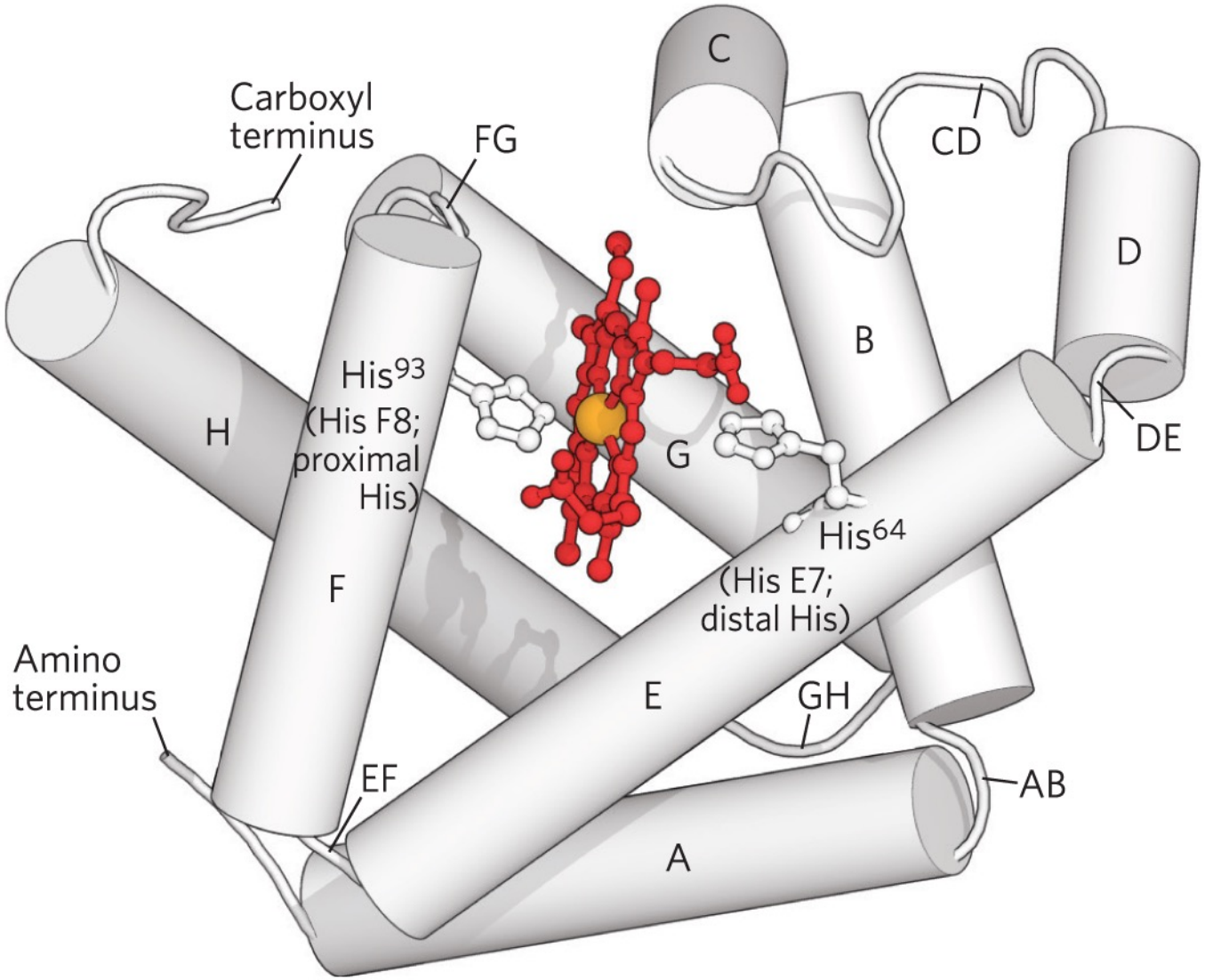
The iron atom of heme has six coordination bonds



Key side chains at the heme-O_2 binding site



Structure of Myoglobin

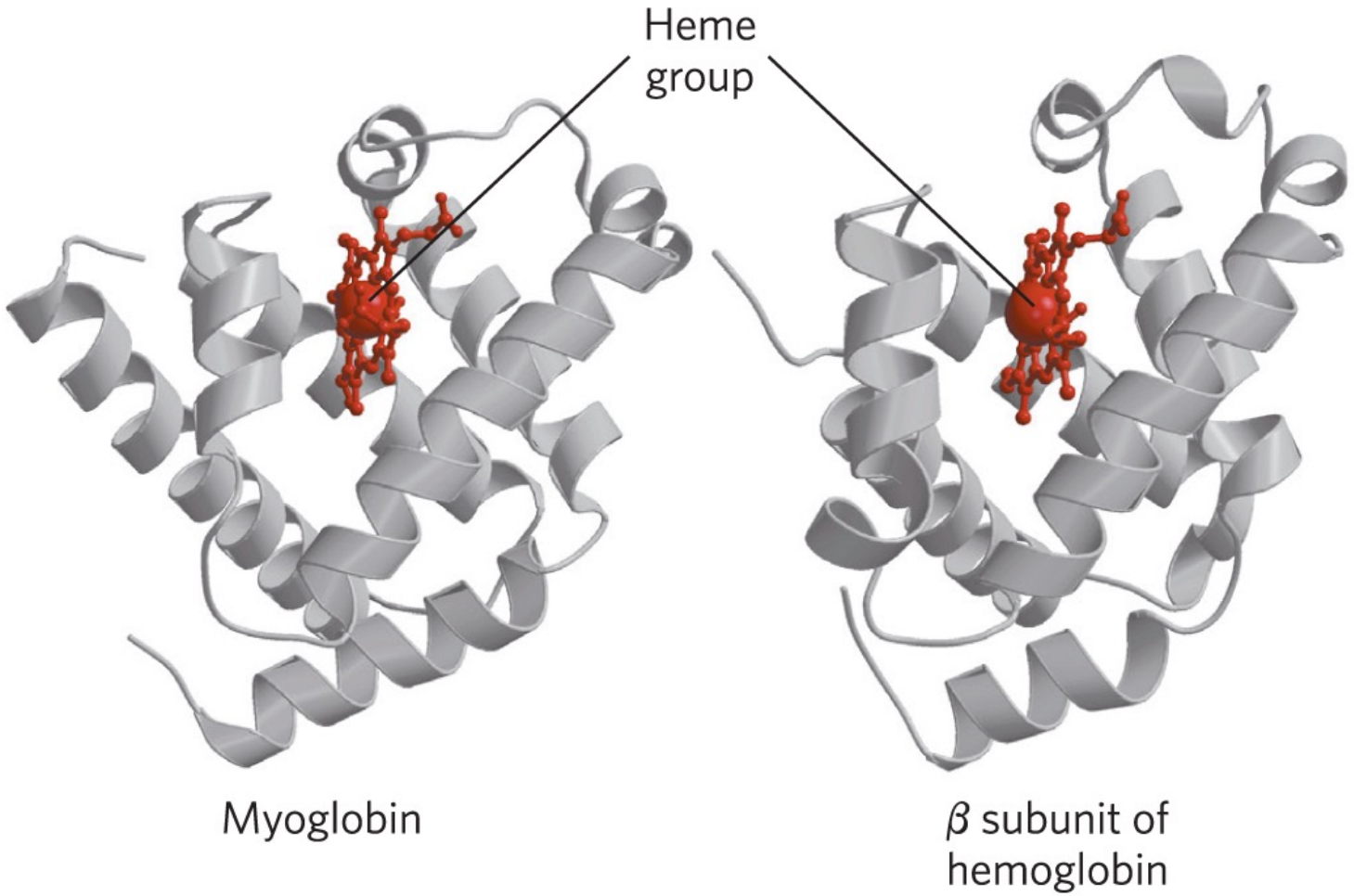


Myoglobin solubilizes O_2 in skeletal muscle. Same mechanism of O_2 binding.

Why is oxygen important?

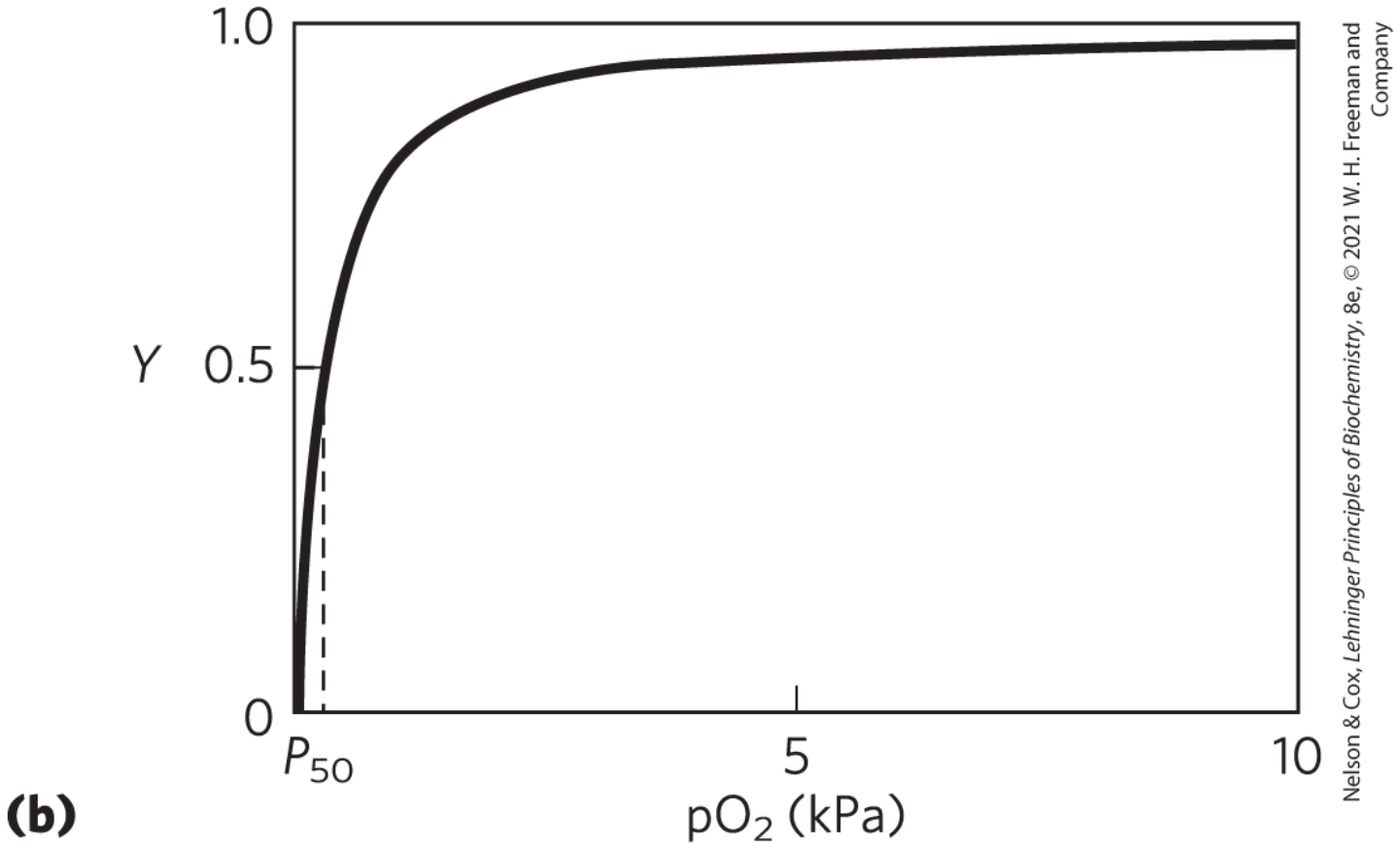
What is the role of the proximal and distal histidines?

Myoglobin vs. Hemoglobin



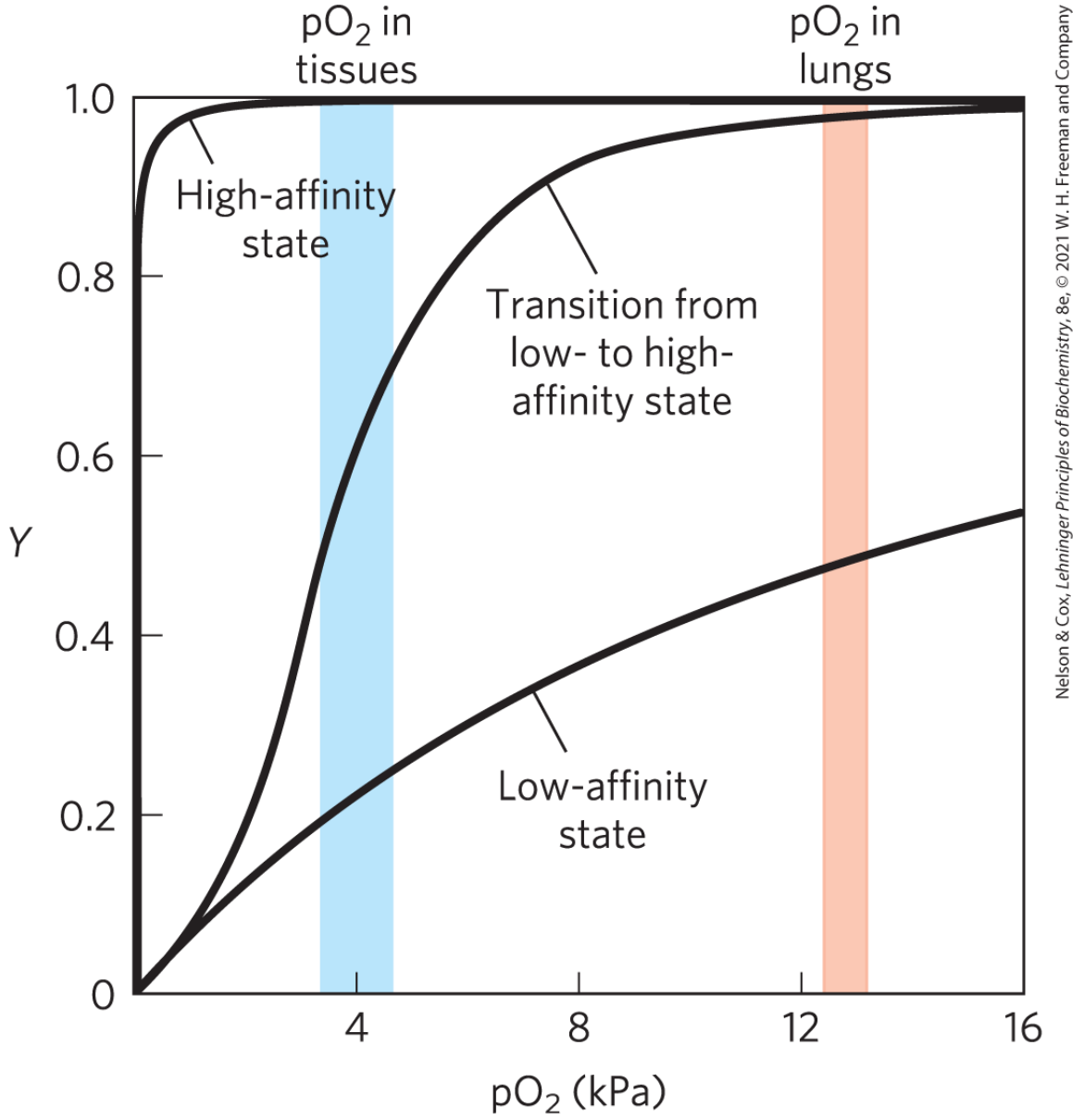
Hemoglobin is a tetramer of 2α and 2β subunits ($\alpha_2\beta_2$).
Each subunit is structurally similar to myoglobin.

What can we learn from the oxygen binding curve of myoglobin?

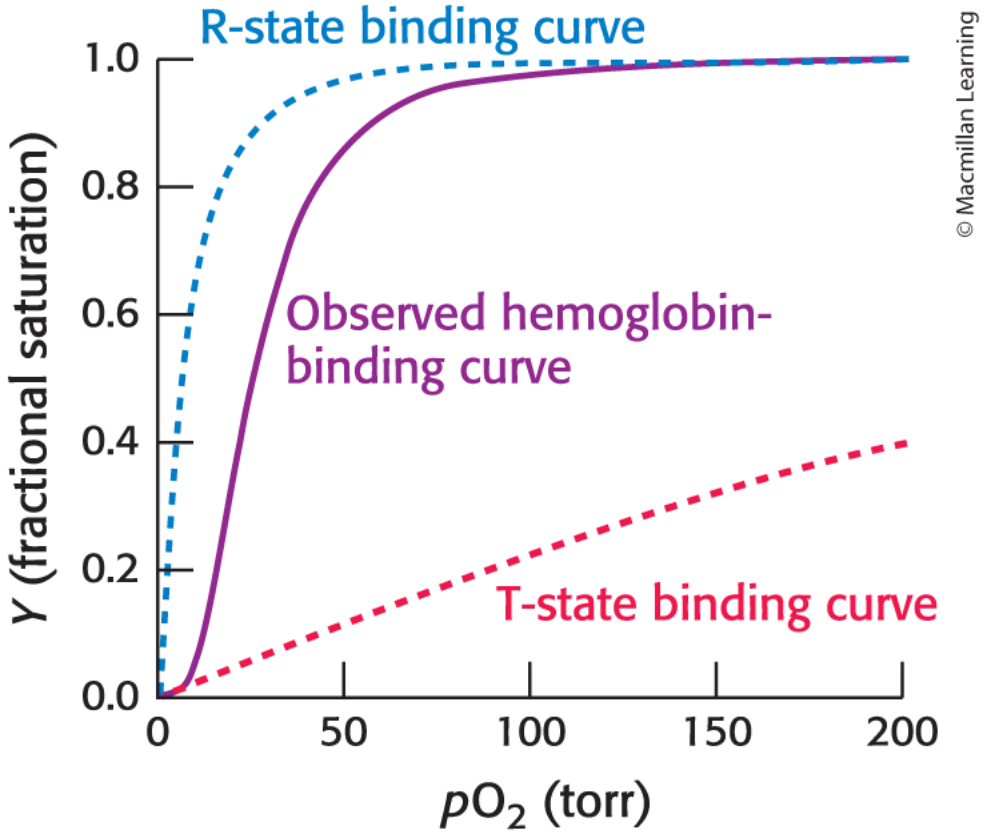


What kinds of binding can we observe?

An important thing here is that hemoglobin, via the RBC, travels to and from two very different environments.



The Hb binding curve is a mash-up of the R and T state curves



R-state is a _____affinity state

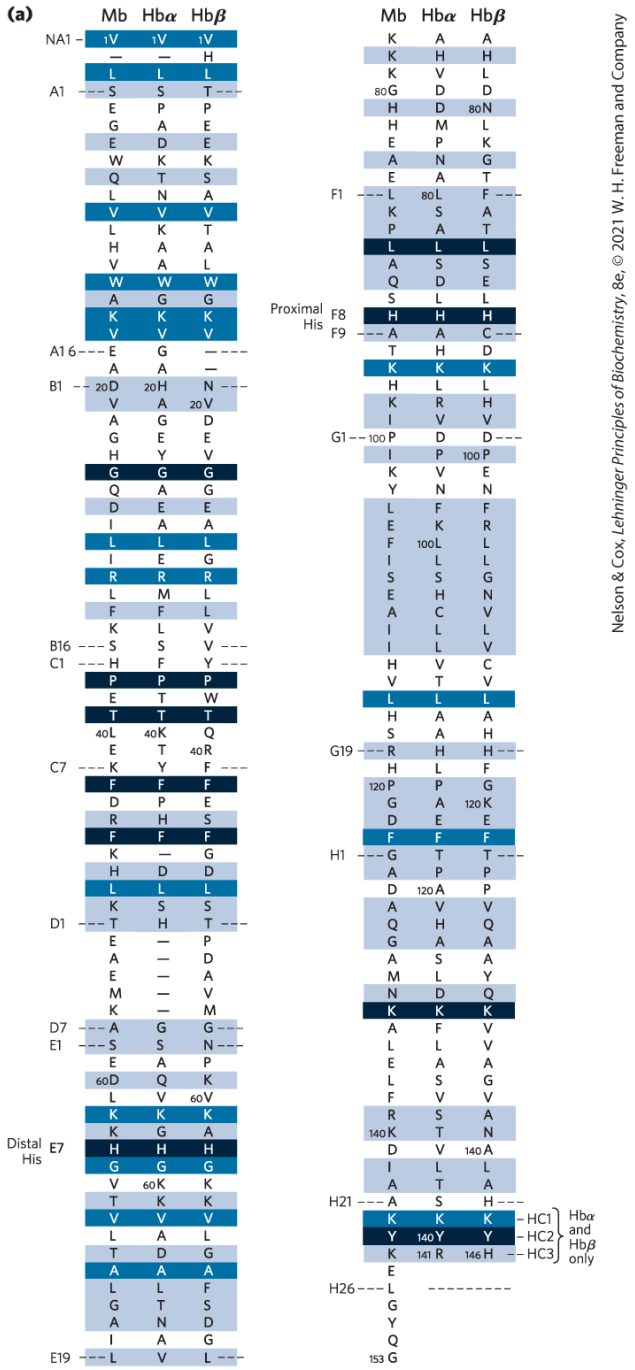
T state is a _____ affinity state

Amino Acid sequence comparison of myoglobin and hemoglobin

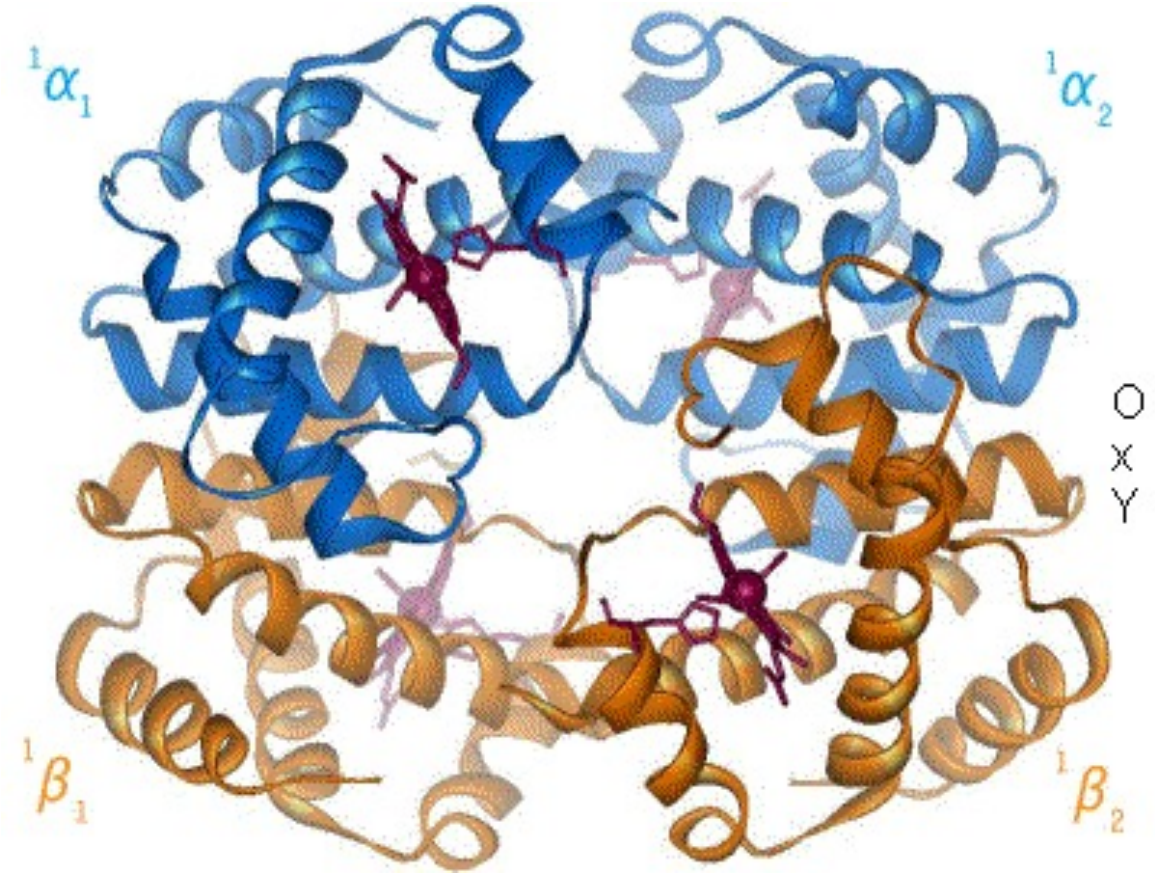
Shading key:

Black conserved in <u>all</u> known globins
Darker blue: conserved in these sequences
Lighter blue: conservative substitutions
(what does this mean?)

Which parts of the structure are most conserved?



Hemoglobin has quaternary structure



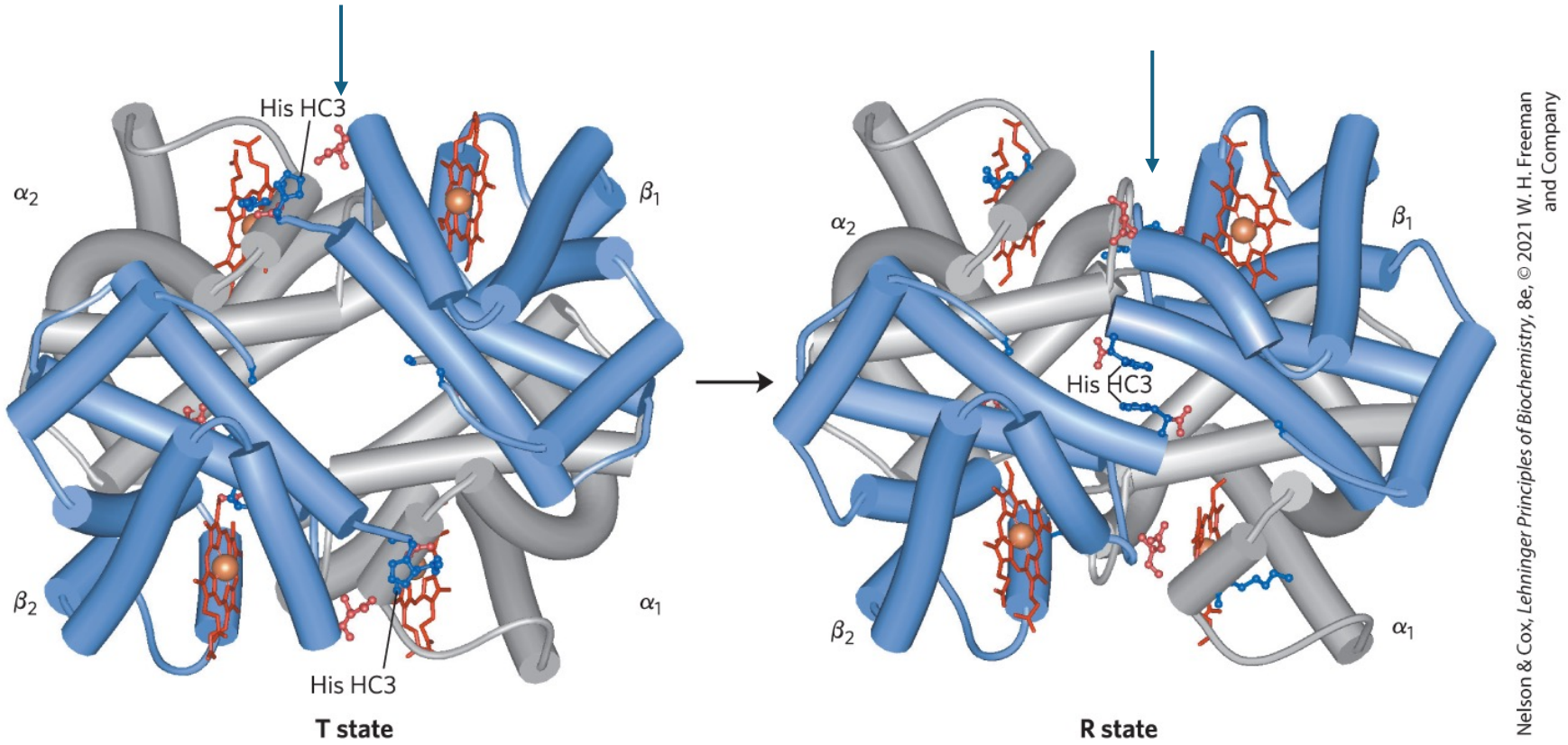
Deoxy=T
Oxy= R

Proteins with quaternary structure may/often demonstrate **cooperative** binding.
What is the consequence of the structural change we see here?

R and T States of Hemoglobin

- T = Tense state, O_2 binding triggers a T $\longrightarrow$ R conformational change.
- R = Relaxed state, this state has higher affinity for O_2 than the T state.
- Deoxyhemoglobin subunits are more stable in the T state, hence the designation “tense”.
- Conformational change from the T state to the R state involves breaking ion pairs between the α1-β2 interface.

T vs R state of hemoglobin



The transition from T to are is driven by the binding of oxygen and involves the breaking of ionic bonds

The highest affinity interactions in Hemoglobin are between α_1 and β_1 ($\alpha_2\beta_2$)

$\alpha_1\beta_1$ interactions change a bit in presence and absence of oxygen.
This perturbation drives larger changes at the $\alpha_1\beta_2$ interfaces.

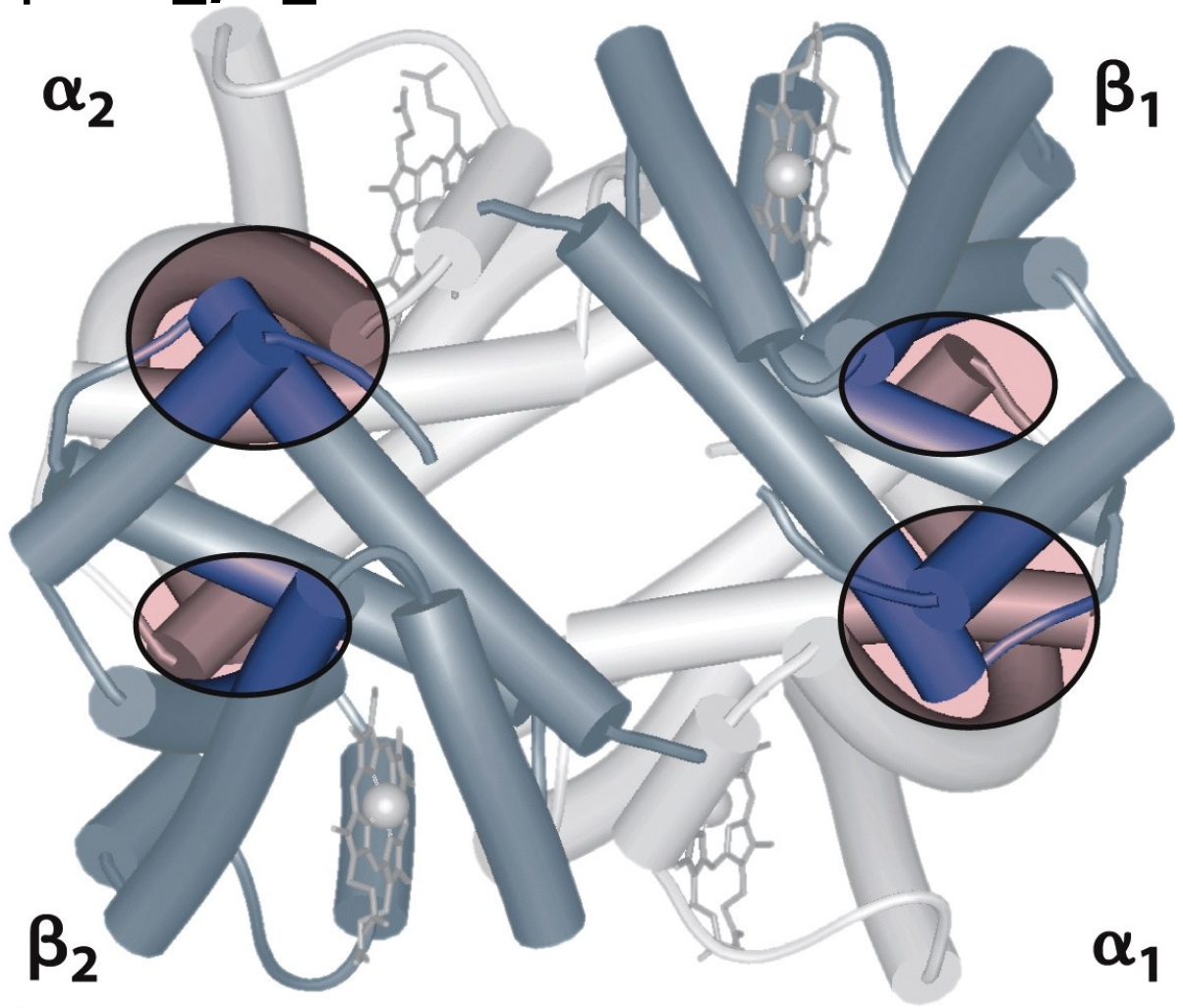


Figure 5-8
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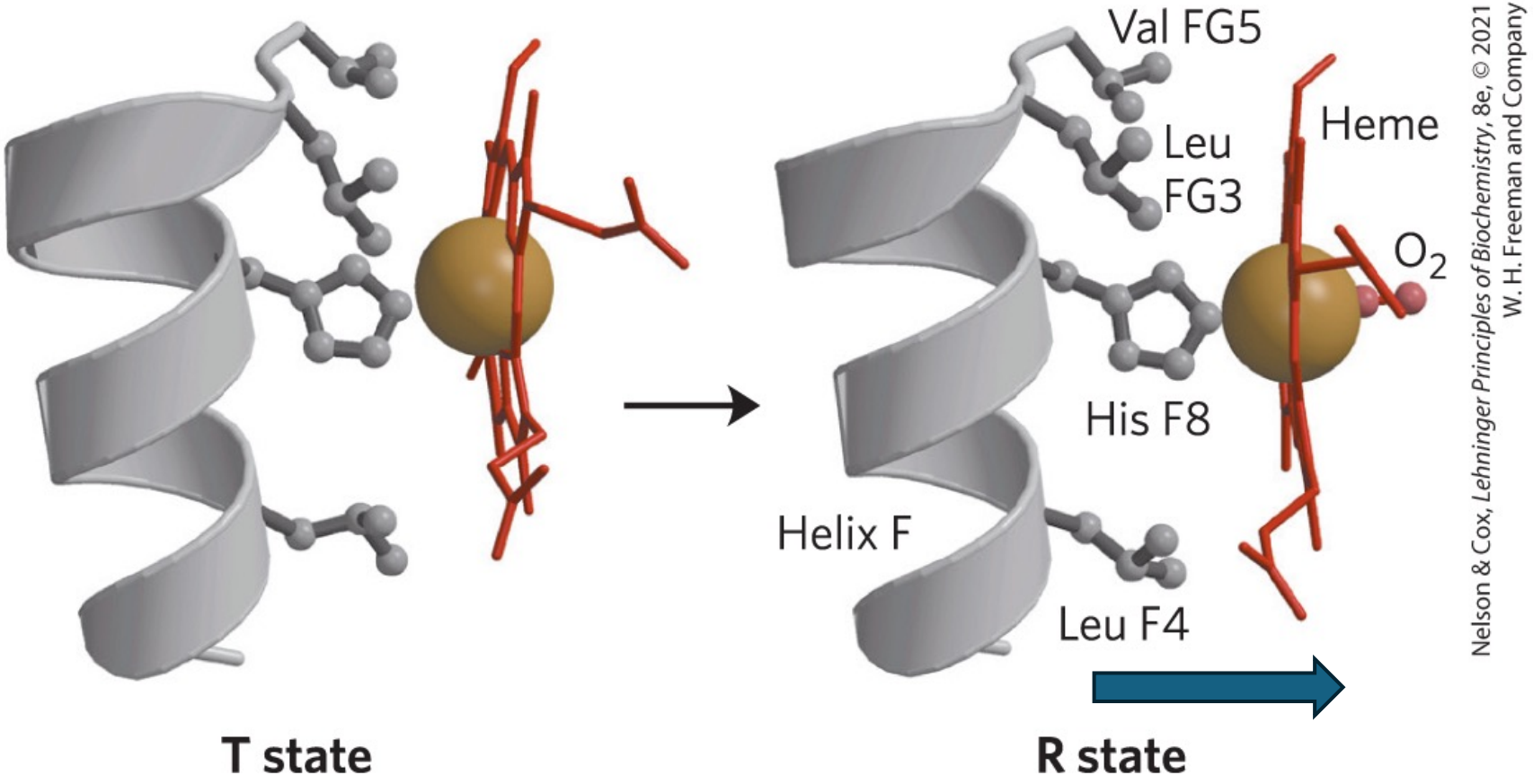
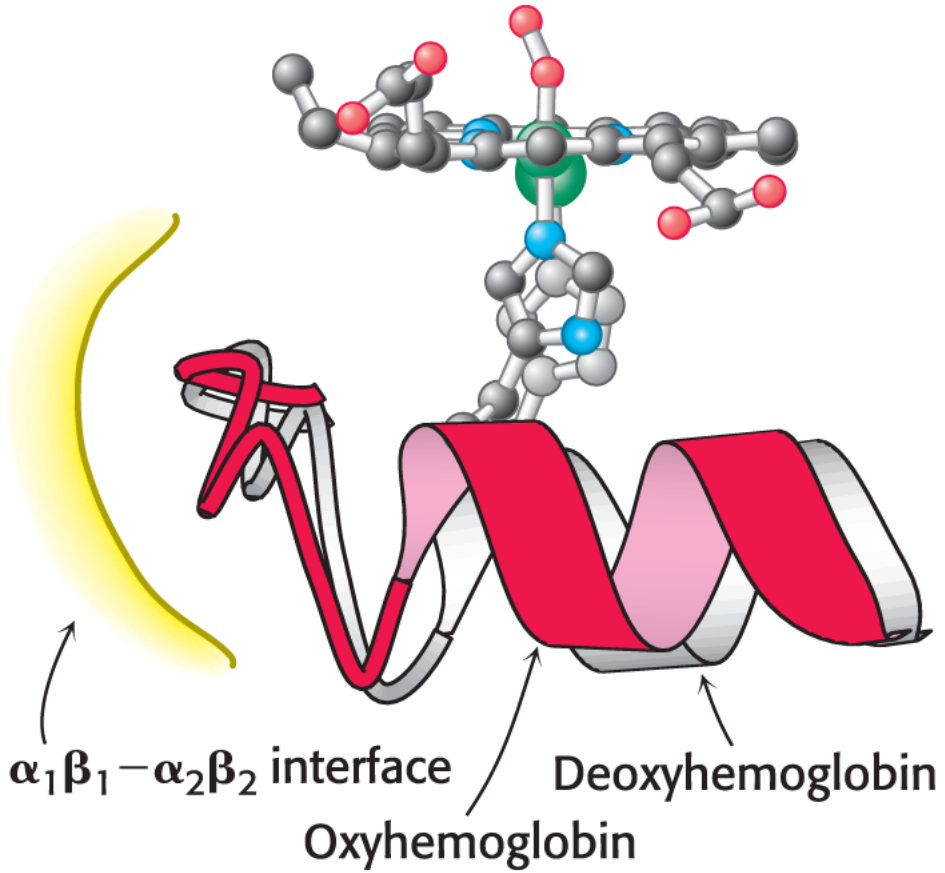
How does oxygen binding drive a conformational change?

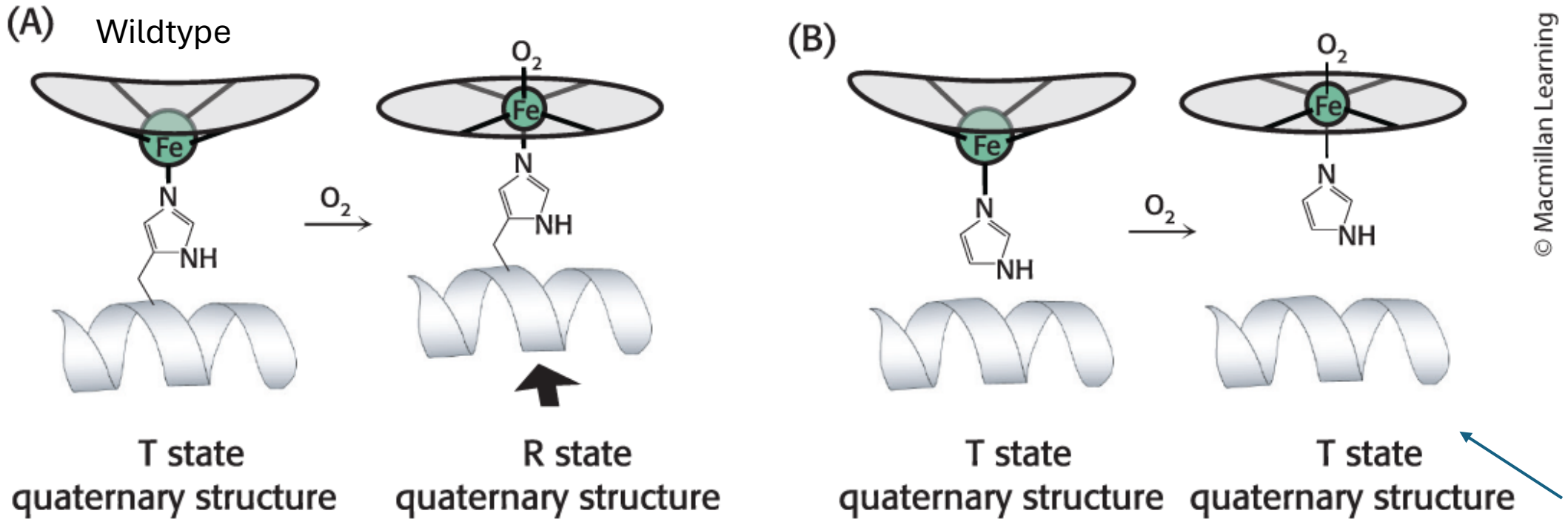


Helix F shifts towards the heme group in the presence of oxygen, as Fe moves more into the plane of the heme

Upon O2 binding, the helix shifts to the red position and perturbs the α_1 -β_1 interface



How did scientists show the key role for the proximal histidine?



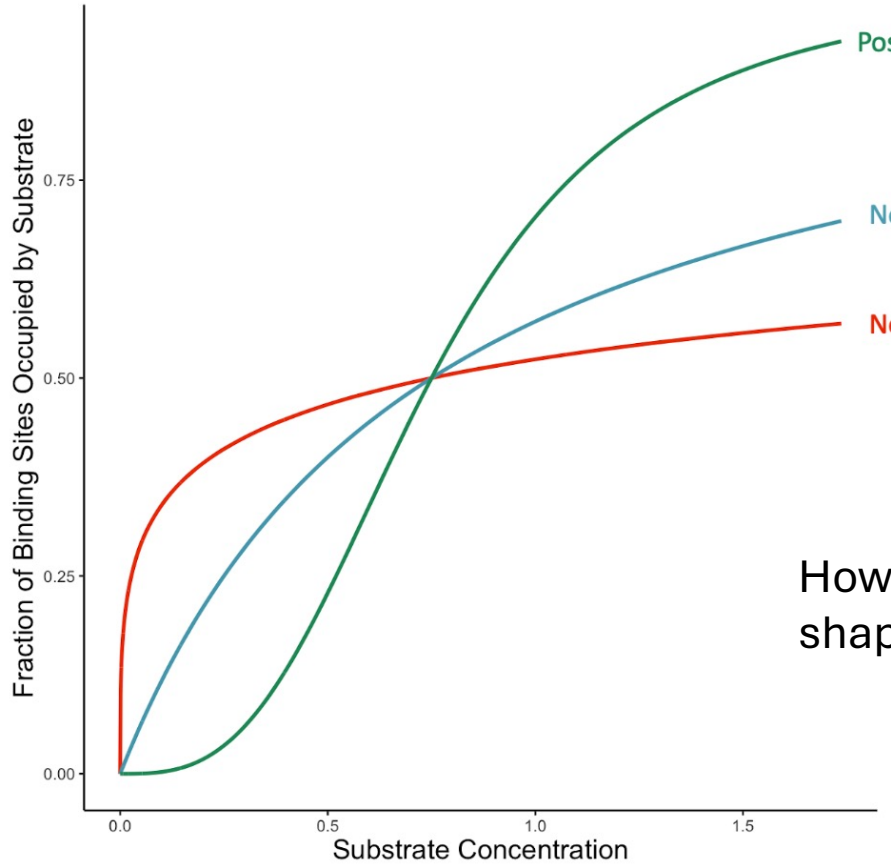
In the experiment (B), the proximal histidine was replaced with an alanine, and imidazole was added to mimic the R-group of histidine unattached to the helix.

How can binding be “cooperative”?

- Must be a protein with multiple binding sites (the protein is usually but not always multi-subunit).
- Binding sites must be able to interact with each other.
- This phenomenon is called cooperativity.
  - Positive cooperativity: first binding event increases affinity at remaining sites
  - Negative cooperativity: first binding event reduces affinity at remaining sites

Positive cooperativity can be recognized by sigmoidal binding curves

Hill coefficient is a measure of cooperativity



Hill coefficient > 1

Hill coefficient=1

Hill coefficient < 1

How can we interpret the shape of each curve?

inspiraadvantage.com
contact@inspiraadvantage.com

https://www.inspiraadvantage.com/blog/hill-coefficient-mcat-test-prep-simple

What factors impact the interaction between Hb and oxygen?

- pH
- CO_2
- CO (carbon monoxide)
- Temperature
- BPG

pH Effect on O_2 binding to Hemoglobin

- Blood in tissues has slightly lower pH than blood in the lungs.
  - Metabolism results in the presence of some acidic compounds
- Affinity for oxygen depends on the pH
  - Oxygen binds well at higher pH
  - Oxygen is released well at lower pH
- The pH difference between lungs and metabolic tissues increases the O_2 transfer efficiency.
- This is known as the Bohr effect.

Bohr (pH) effect

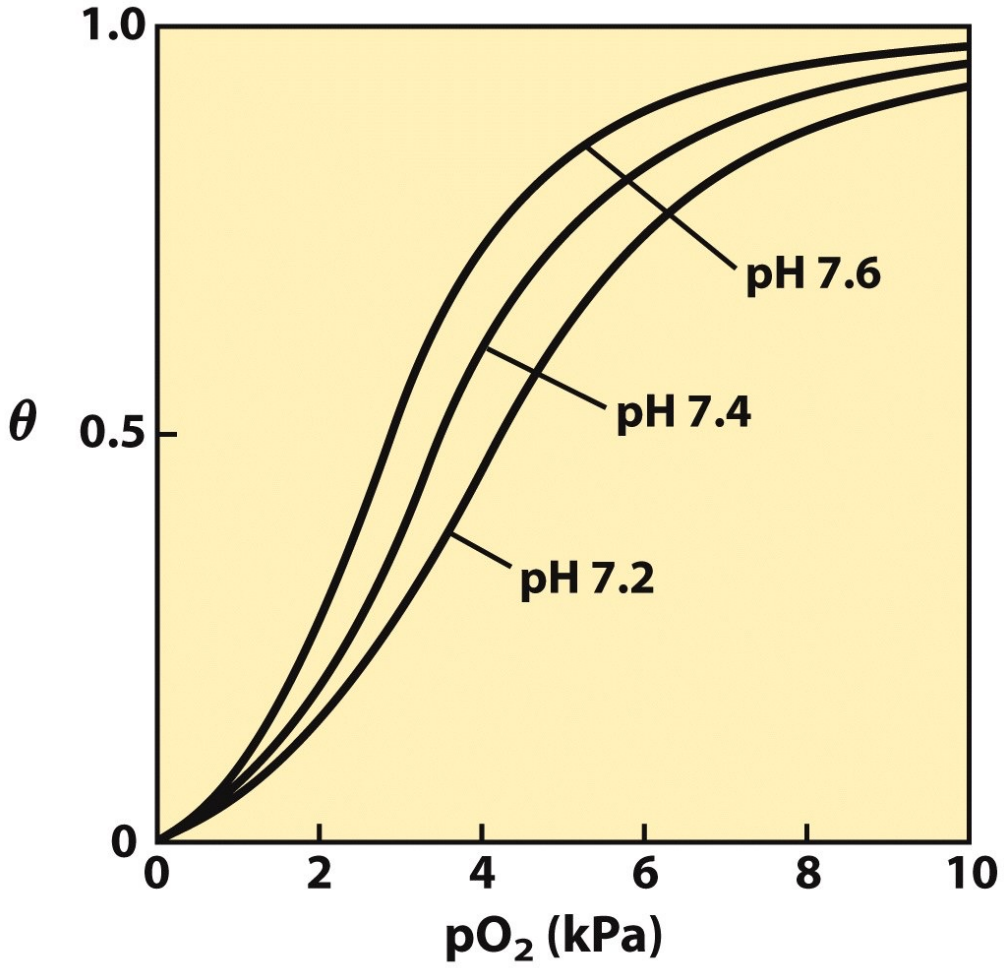


Figure 5-16
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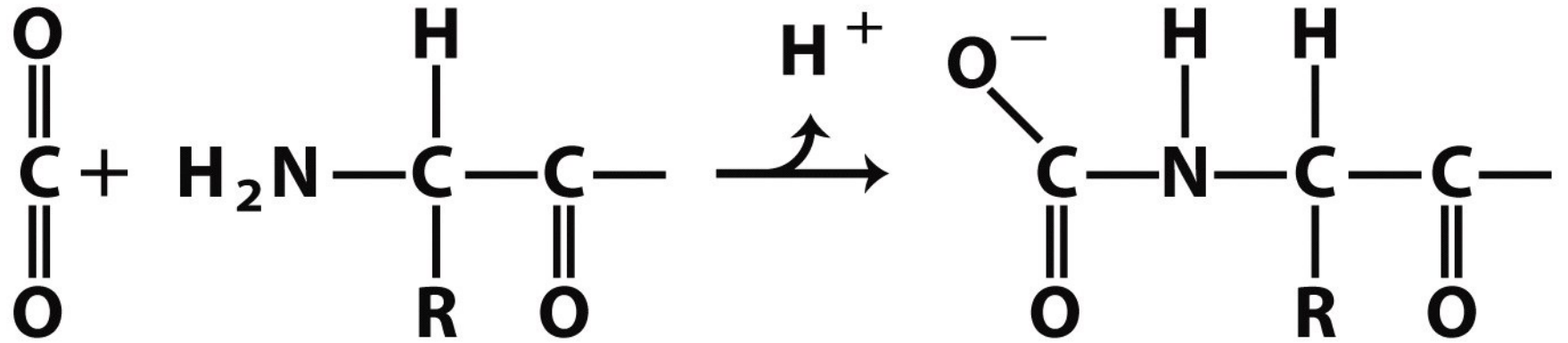
Venous blood is generally reported to be about pH 7.2 and arterial blood 7.38-7.42.

Venous blood pH may drop even lower in muscle tissue during exercise.

Hemoglobin and CO_2 Export

- CO_2 is produced by metabolism in tissues and must be exported to the lungs.
- Some CO_2 exported is dissolved in the blood plasma and most CO2 is converted to bicarbonate in the blood.
- Some CO_2 is exported in the form of a carbamate on the amino terminal residues of each of the polypeptide subunits.
- Notice that the formation of a carbamate yields a proton which can bind to hemoglobin and promote oxygen dissociation

Some CO_2 is carried covalently attached to the N terminus



Unnumbered 5 p166
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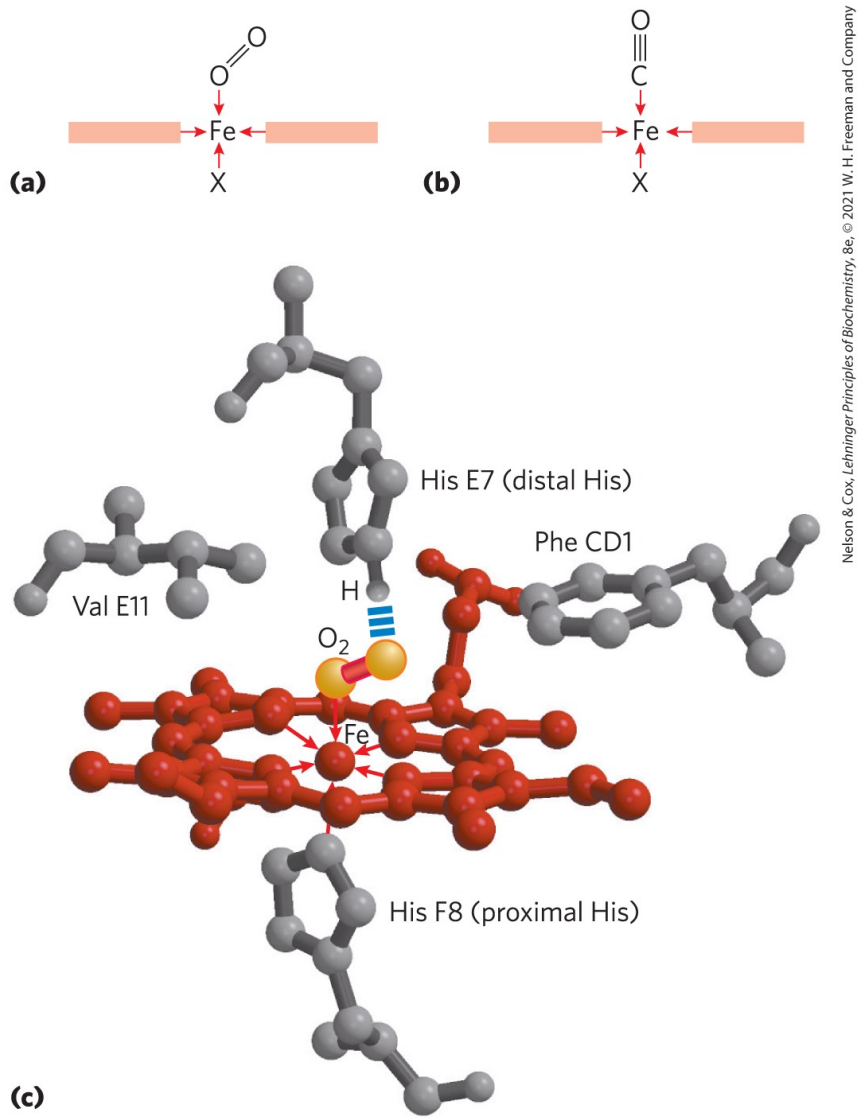
Binding of Carbon Monoxide

- CO has similar size and shape to O_2; it can fit to the same binding site
- CO binds over 20,000 times better than O_2 because the carbon in CO has a filled lone electron pair that can be donated to vacant d-orbitals on the Fe^{2+}
- 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

Carbon Monoxide locks hemoglobin in the T state



Why is this a problem in the body?

Why is CO binding hard to reverse?

Regulation of O_2 Binding by 2,3 BPG

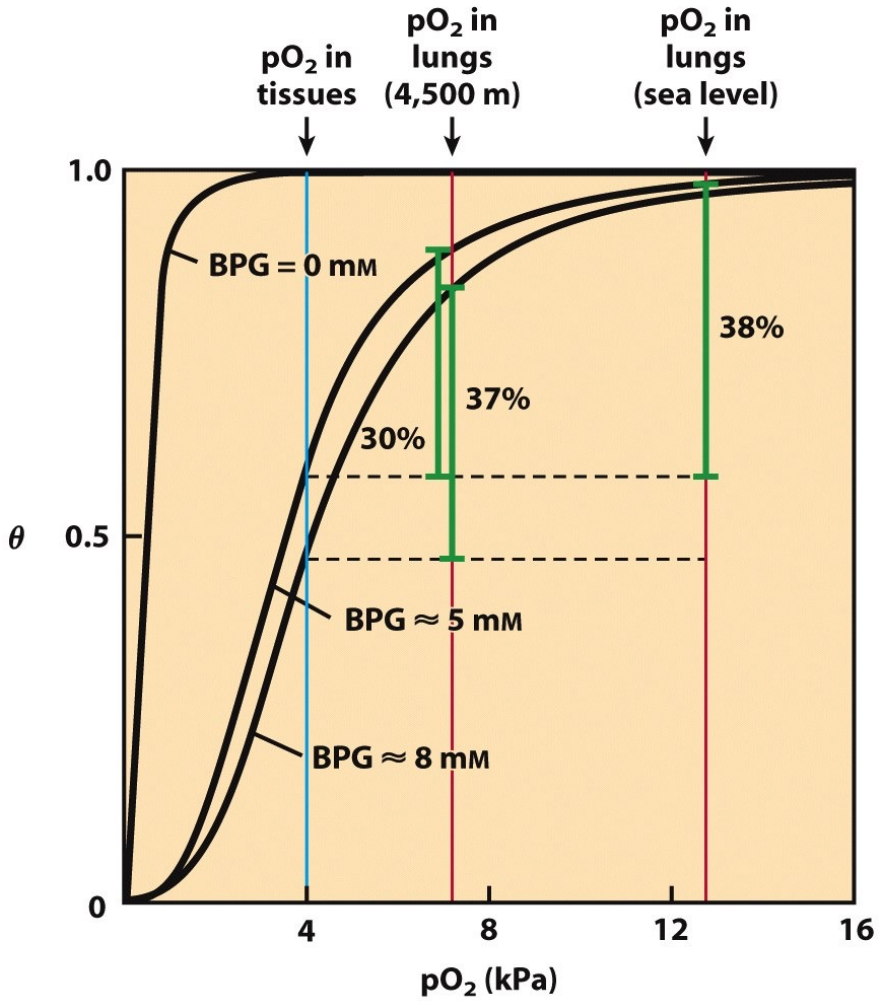


Figure 5-17
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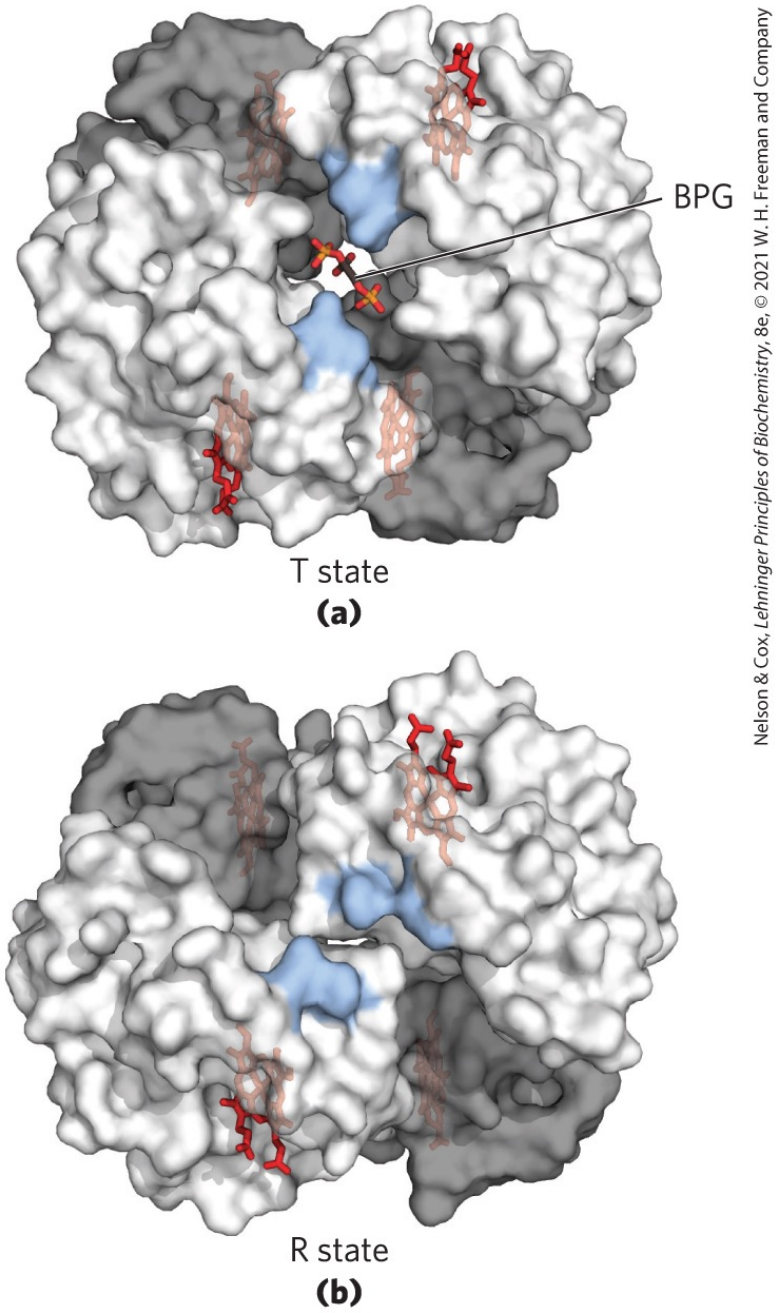
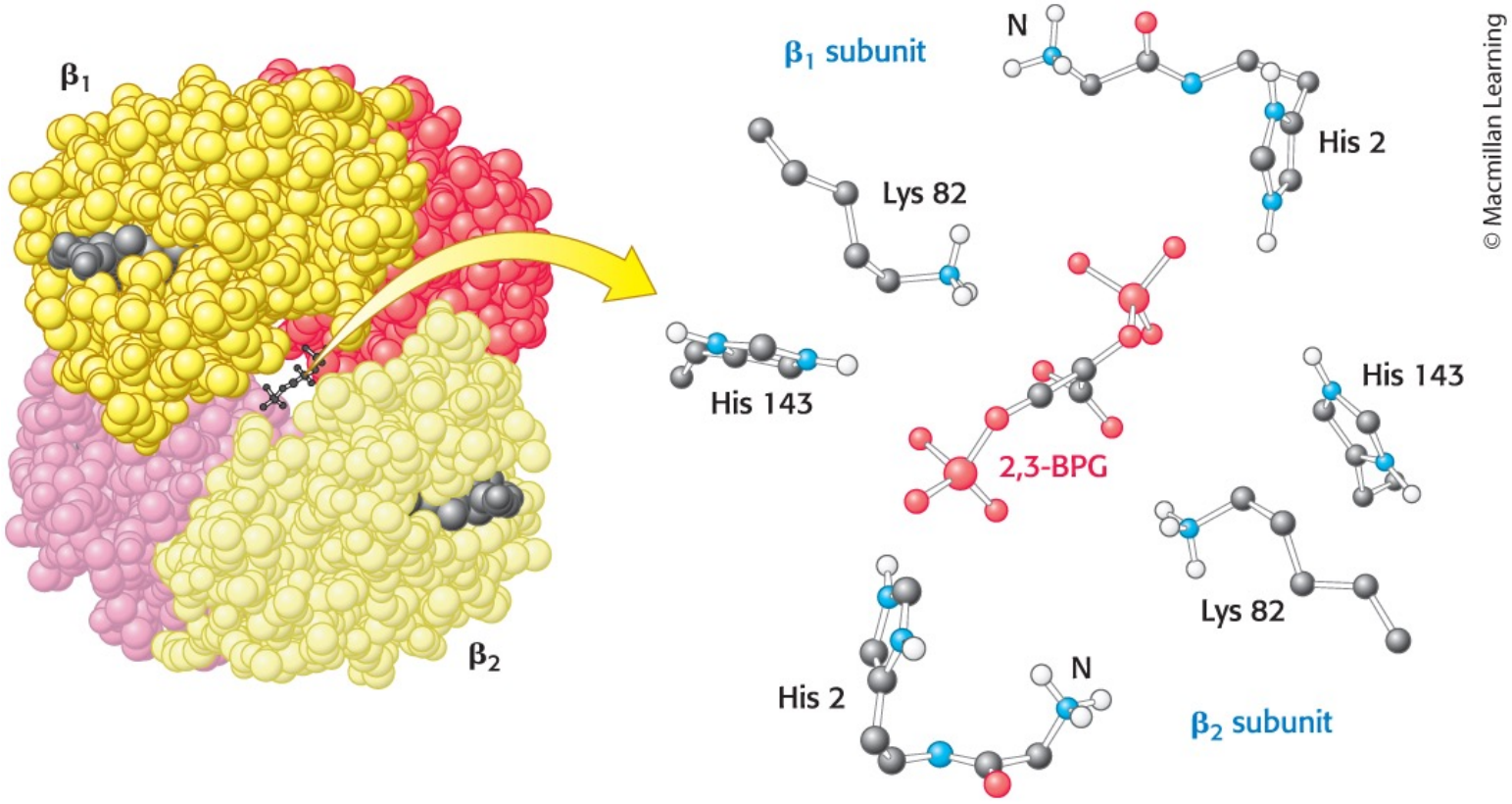
What can affect the quantity of BPG present?

BPG binds at the center of the tetramer

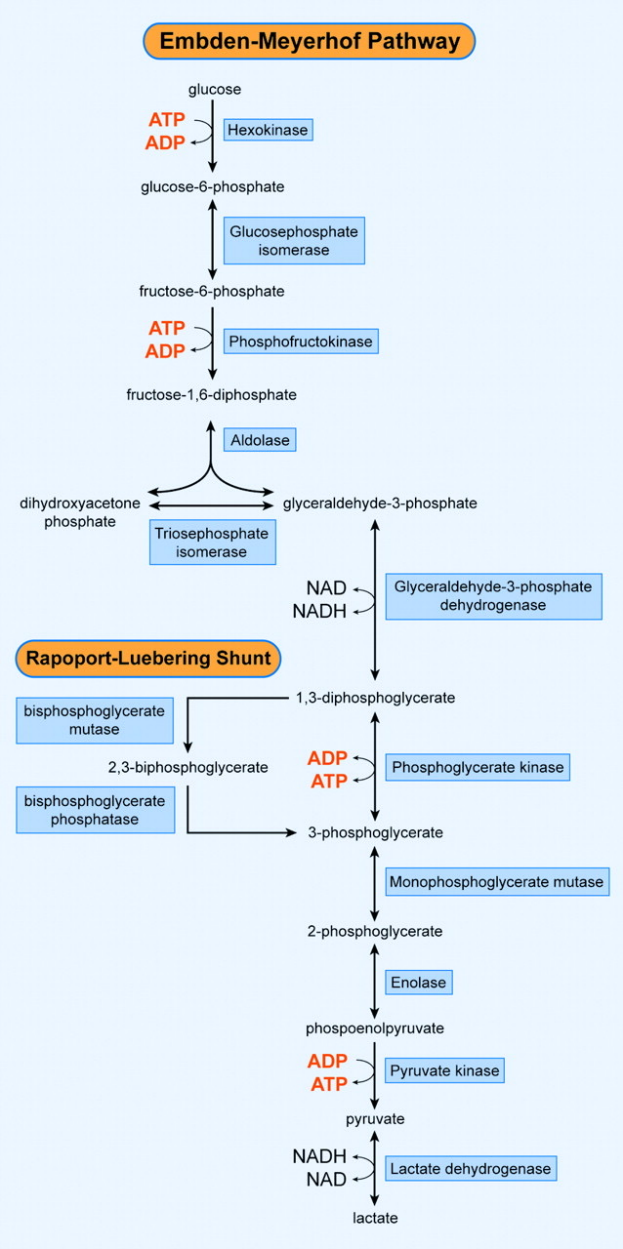
Why does this stabilize the T state?



Chemical interactions between Hb and BPG



Formation of 2,3 BPG in RBCs



Emden-Meyerhof: The anaerobic metabolic pathway by which glucose, especially the glycogen in human muscle, is converted to lactic acid.

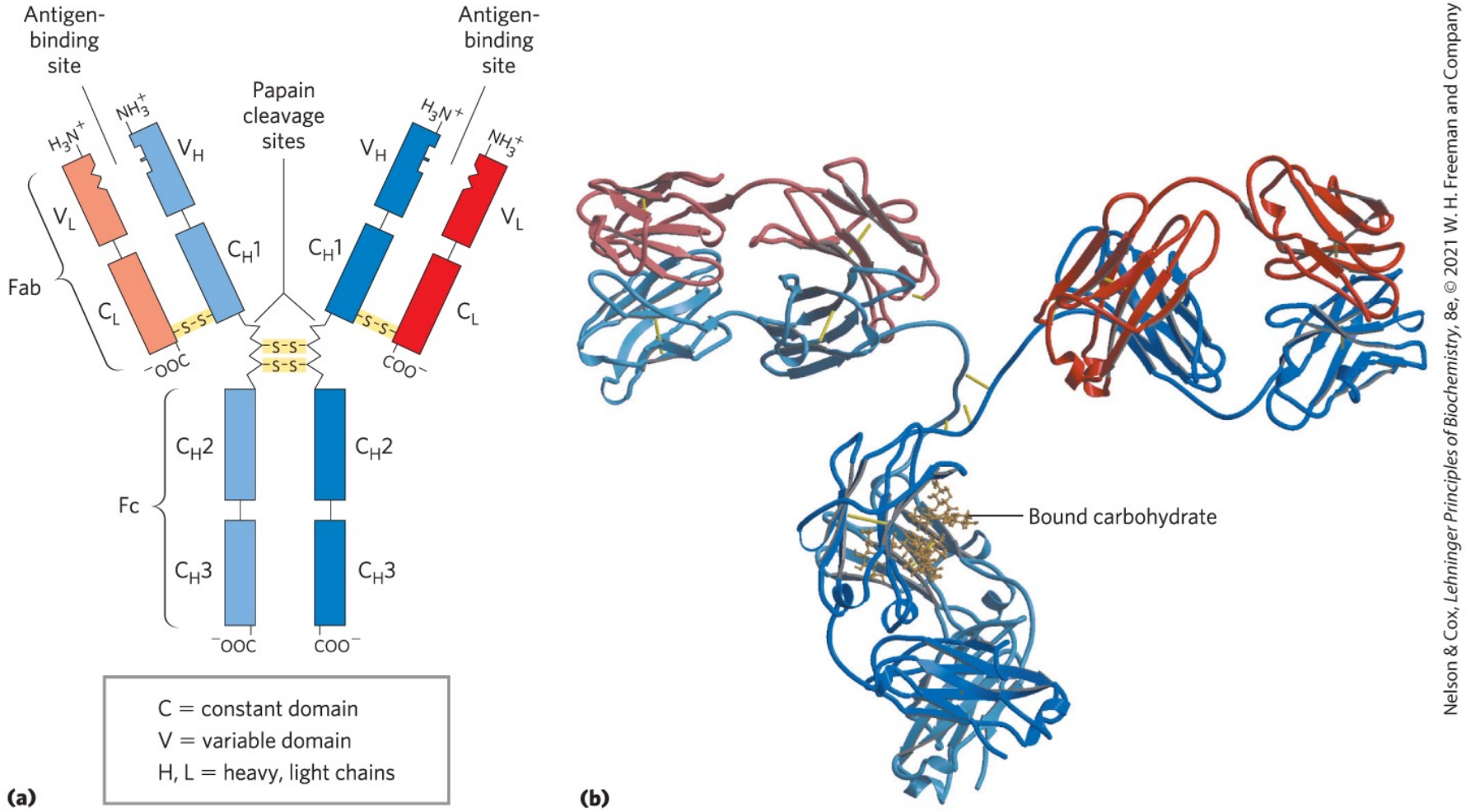
High altitude adaptations

- Short term:
  - Make more BPG in glycolysis (more anaerobic energy production)
  - Hyperventilation
  - Increased heart rate
- Longer term
  - Increase in hematocrit
  - Capillary growth (angiogenesis)

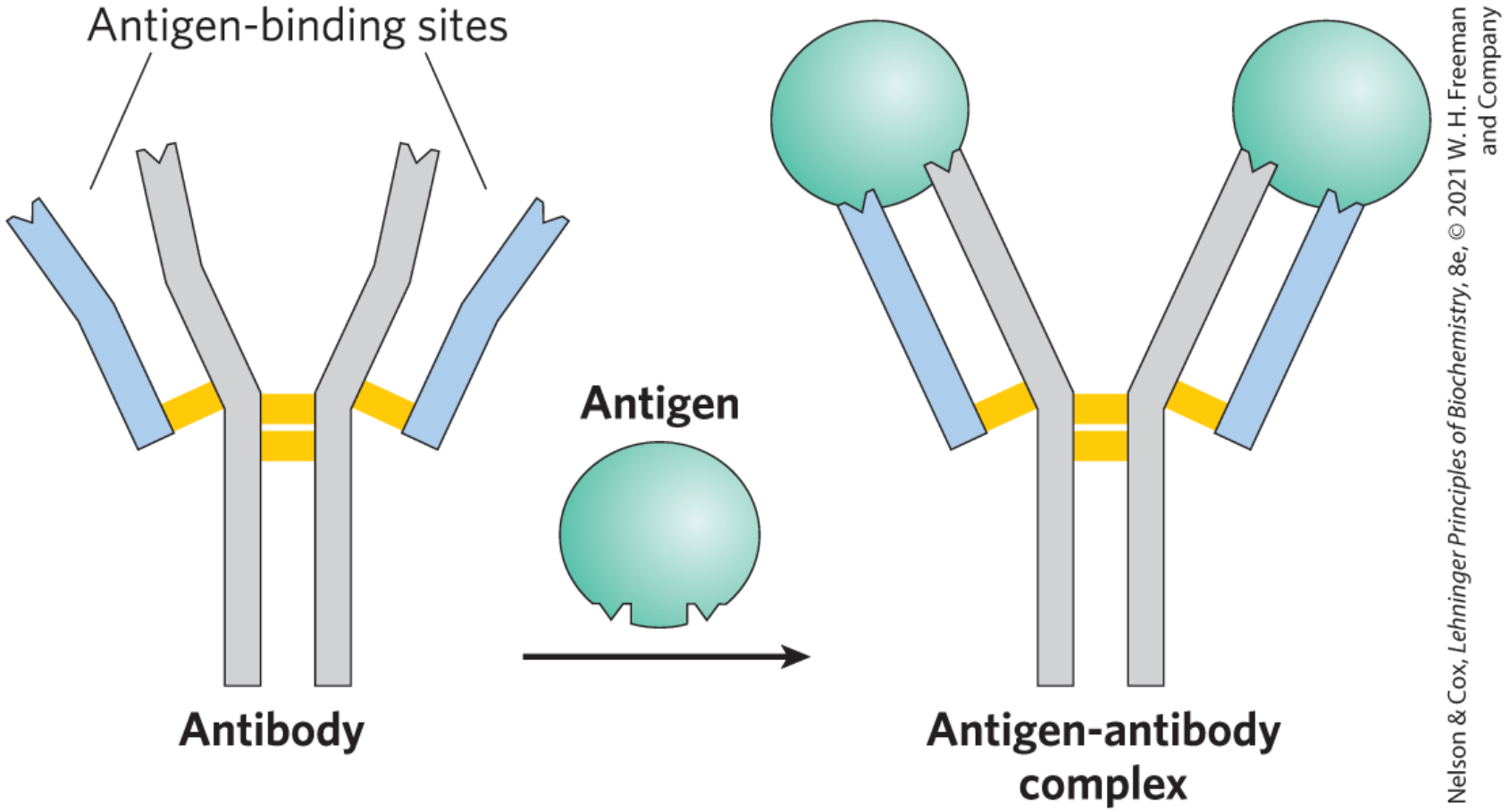
Antibodies

- Antibodies are proteins that are expert at binding a wide variety of ligands
  - The immune systems distinguishes self from non-self (in part) using antibodies
    - Antibodies tag foreign invaders for phagocytosis
  - Each antibody is specific, but there are many different antibodies we can make with different specificities
  - Antibodies are a great laboratory tool

The general structure of an IgG antibody

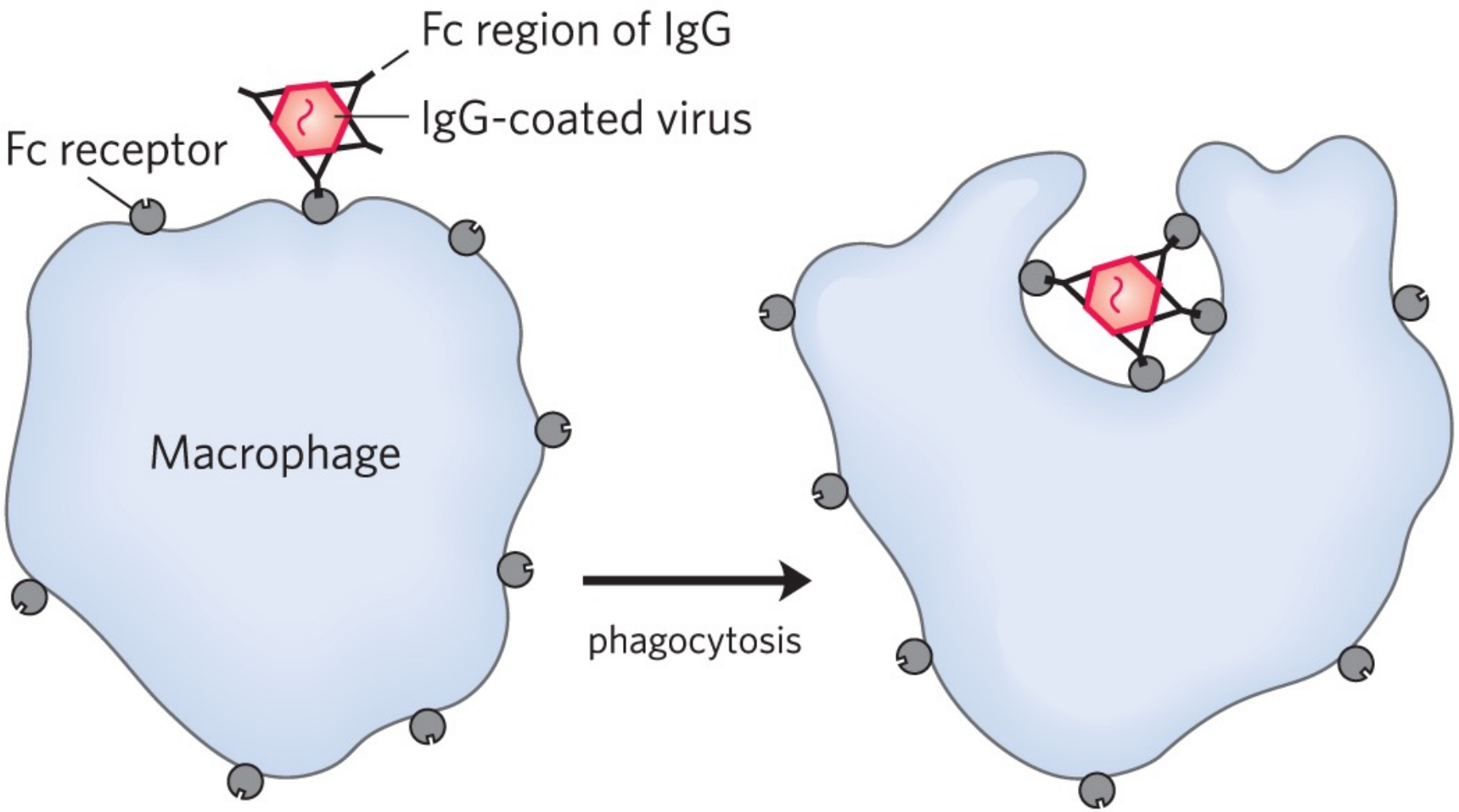


Each natural antibody has two identical antigen-binding sites

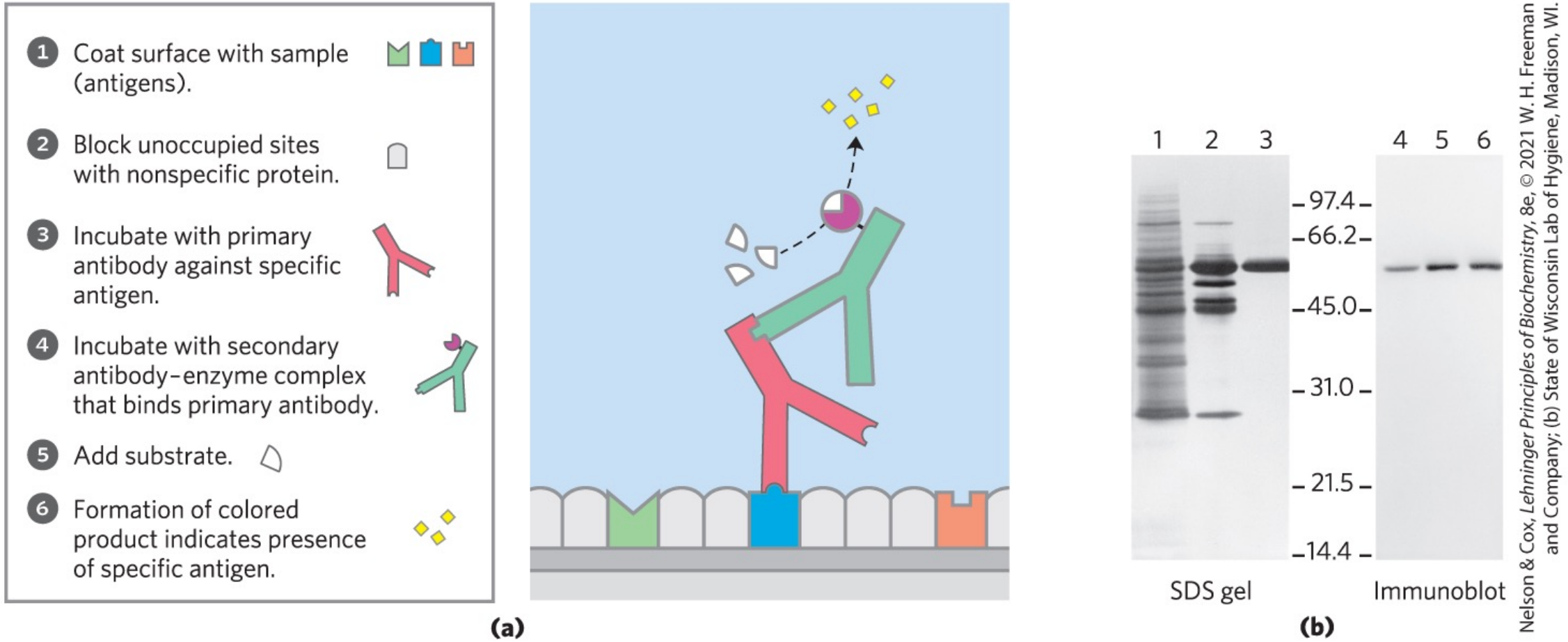


What about bi-specific antibodies?

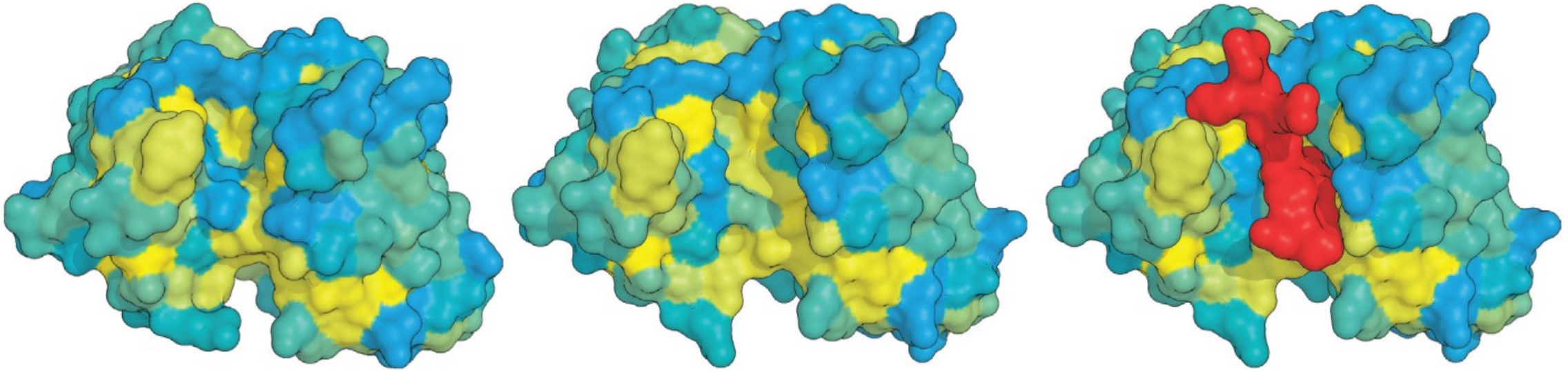
Antibodies can signal to macrophages for phagocytosis



Antibodies as a laboratory tool



Antigen-antibody binding as an example of induced fit



(a) Conformation with no antigen bound

(b) Antigen bound (but not shown)

(c) Antigen bound (shown)