Chem 452 - Lecture 3
Hemoglobin & Myoglobin
111003

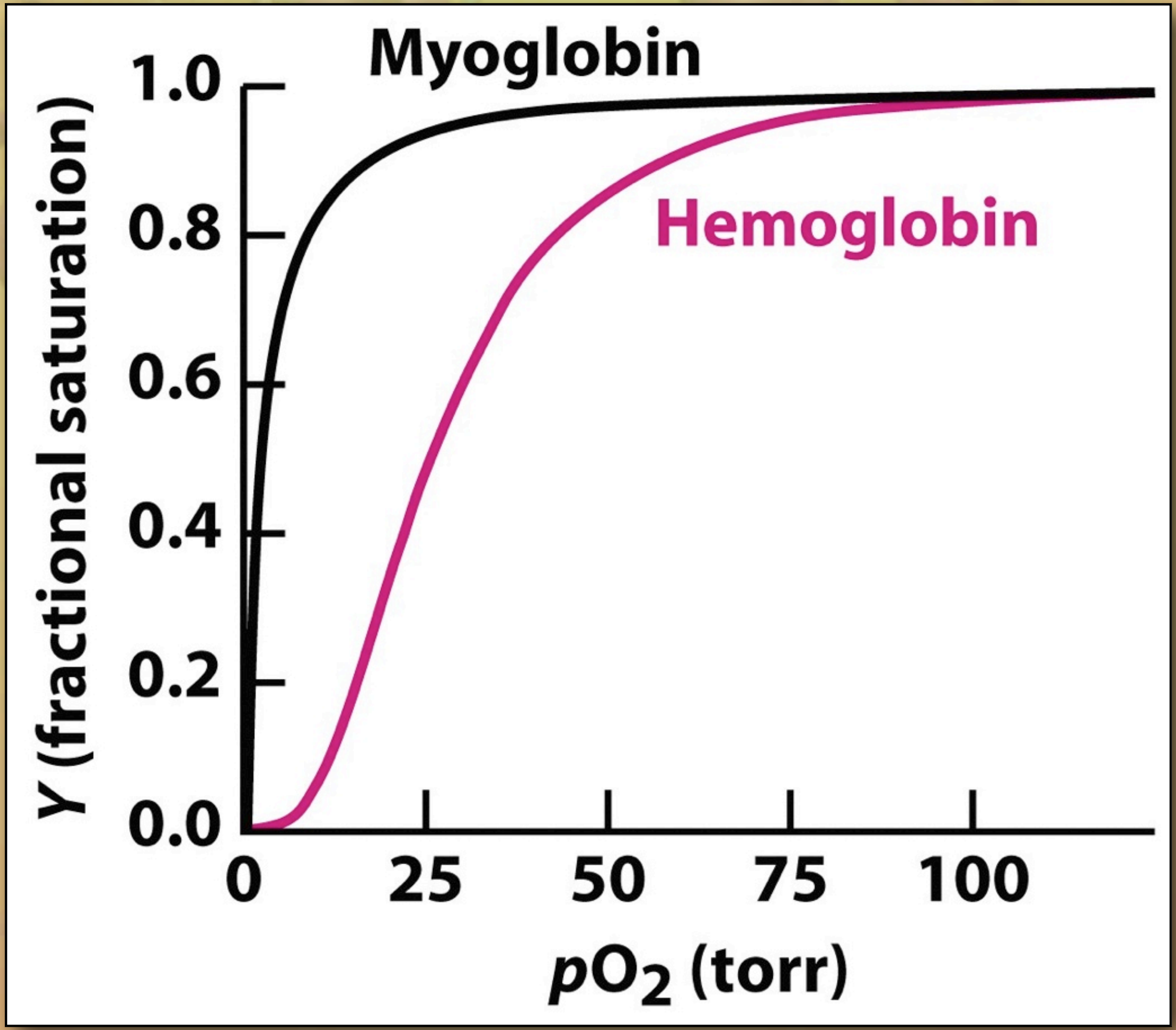
Hemoglobin (Hb) and Myoglobin (Mb) function as oxygen transport and storage molecules in higher organisms. There functions have been long studied and, together, provide a wealth of examples of how the structure and function of proteins are related.

Introduction

- Together, Hb and Mb providean excellent example of structure-function relationships in proteins.
  - They illustrate the substrate binding portion of an enzyme catalyzed reaction.
  - They illustrate **allosteric regulation.**

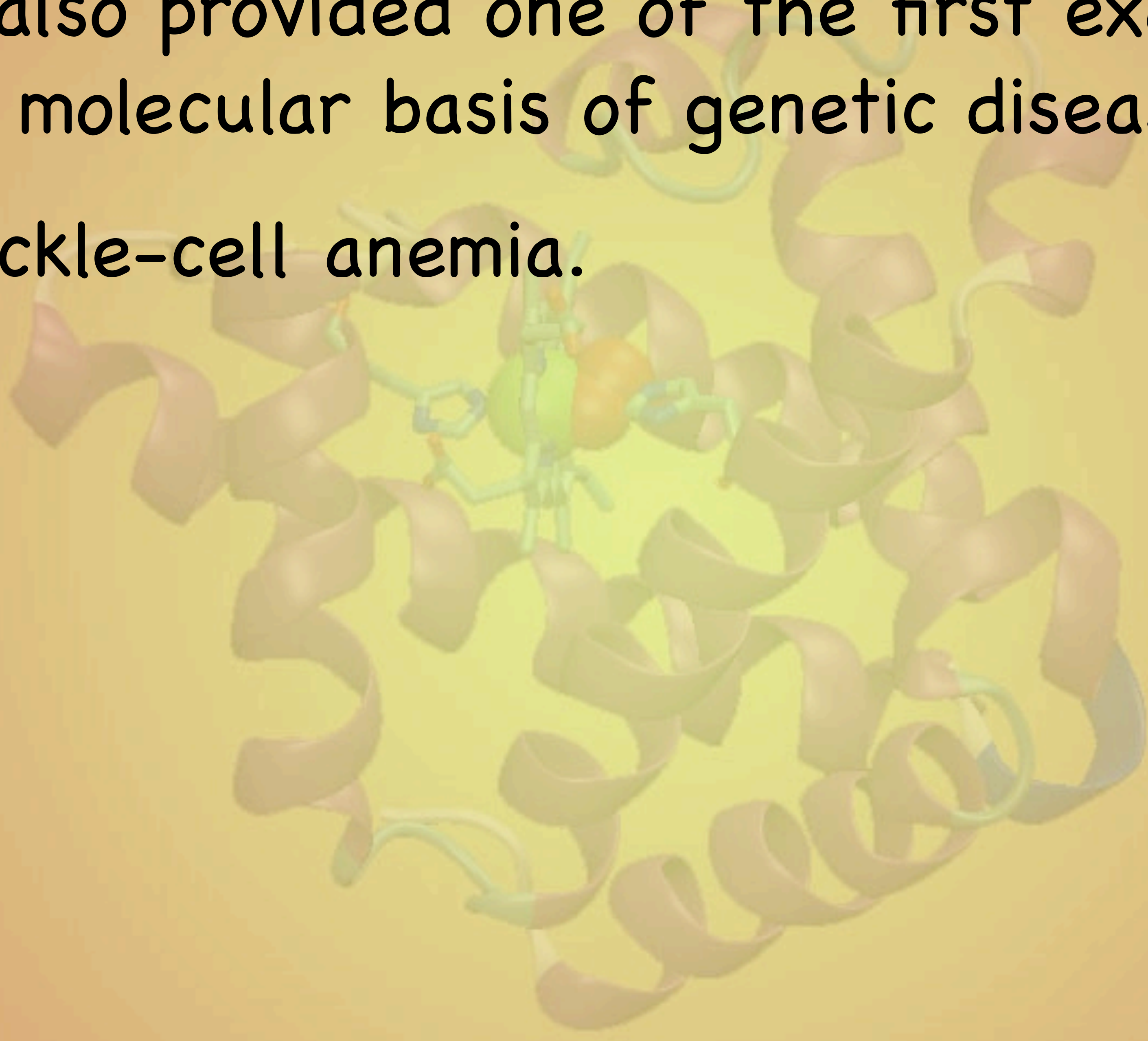
Introduction

- The cooperative binding of oxygen by Hb, compared to Mb.



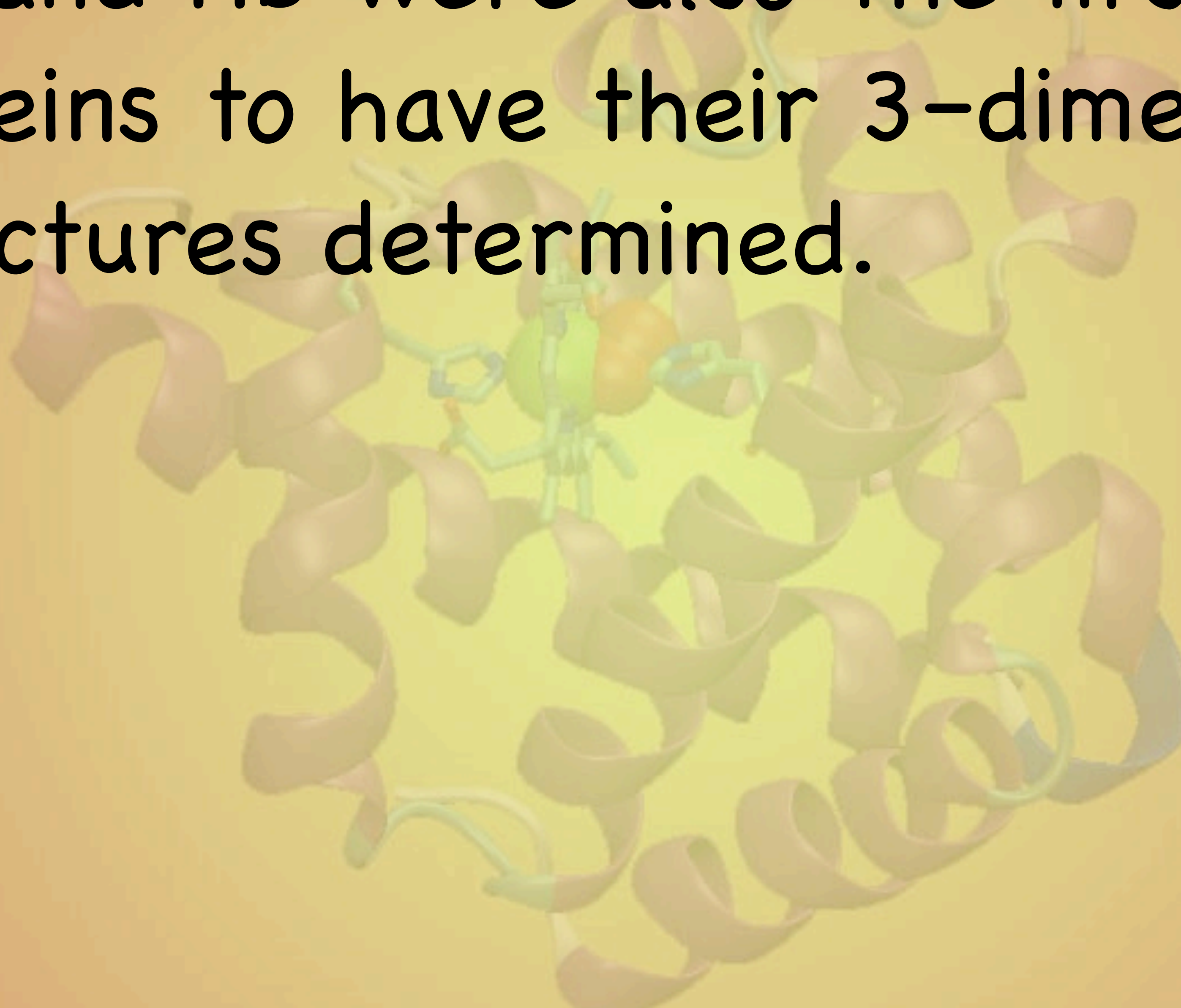
Introduction

- Hb also provided one of the first examples for the molecular basis of genetic diseases.
  - Sickle-cell anemia.



Introduction

- Mb and Hb were also the first proteins to have their 3-dimensional structures determined.



Introduction

- The crystal structure of Mb was determined by John Kendrew in 1957 using X-ray diffraction.
- This was closely followed by the crystal structure for Hb, which was determined by Max Perutz in 1958.

Introduction

John Kendrew Max Perutz

The Medical Research Council (MRC) at Cambridge University

- Kendrew
- Perutz
- Sanger
- Watson
- Crick

Nobel Prizes in 1962

Introduction

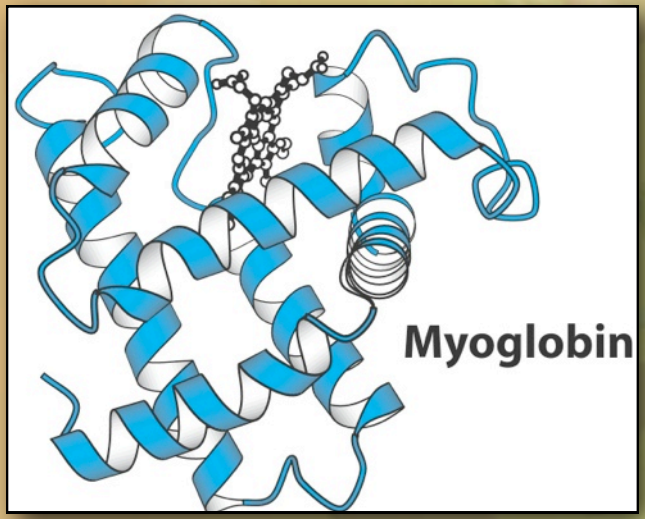
Nobel Prizes in 1962

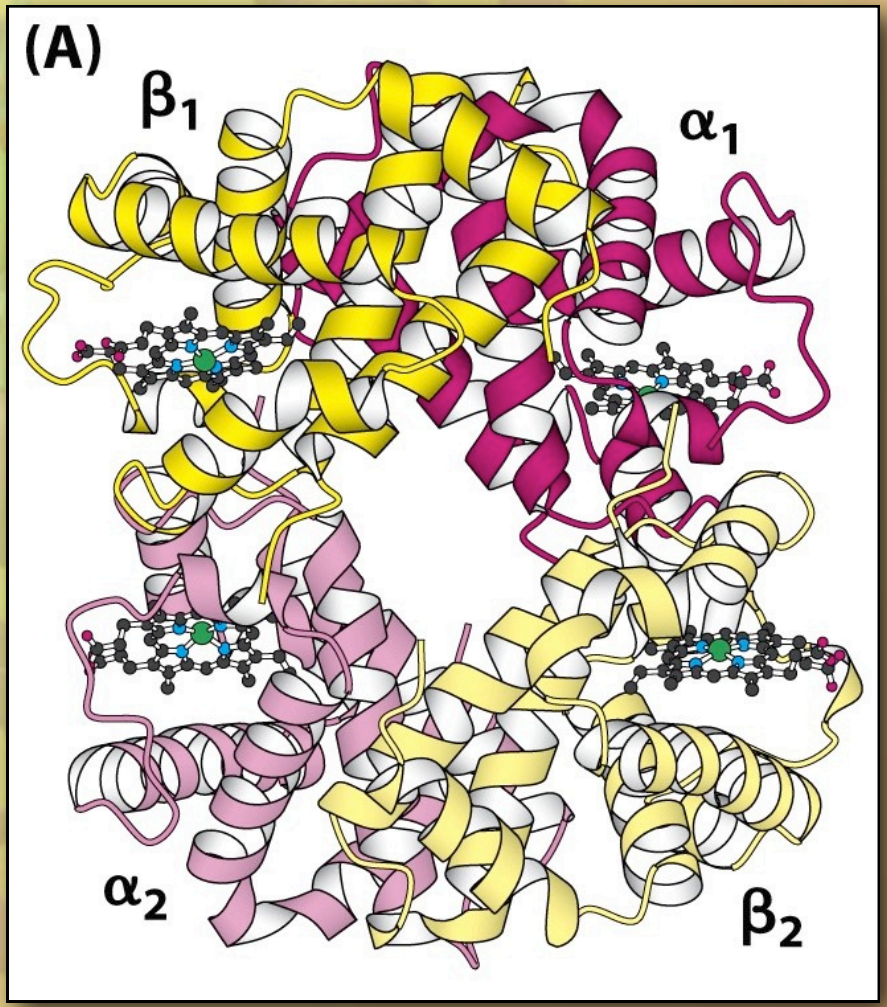
Introduction

- Hb and Mb provide an excellent example of how proteins have evolved to most efficiently carry out a particular function.
  - Hb binds oxygen in the lung, where the O_2 concentration is high, and delivers it to the tisues where the O_2 concentration is low.
  - Mb accepts the O_2 from the Hb in the tissues where the O_2 concentrations are low.

Introduction

- Structures of Mb and the α and β subunits of Hb are very similar

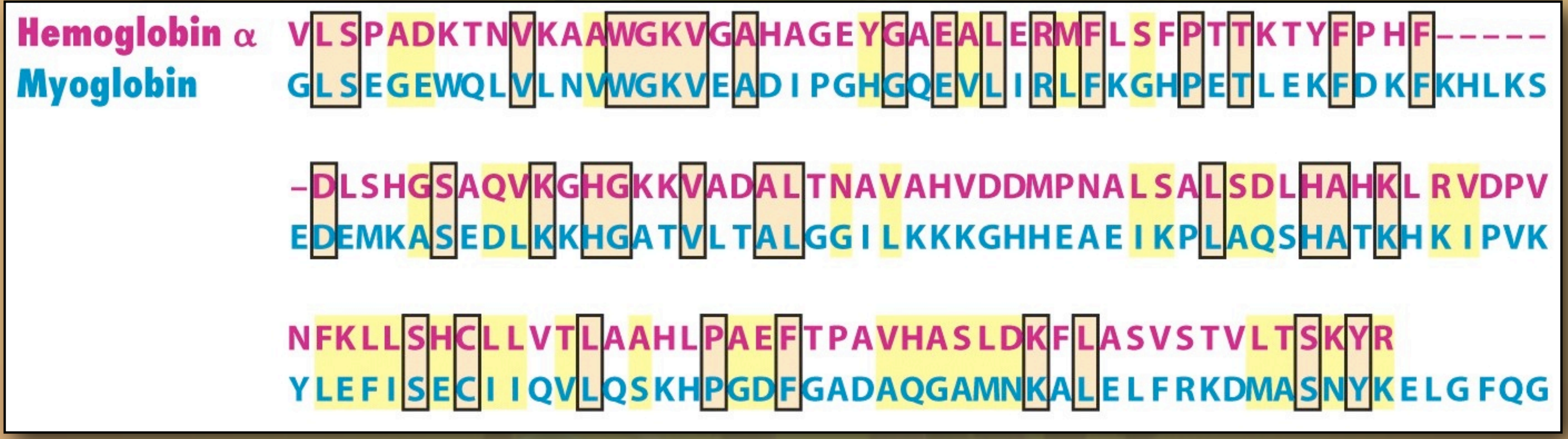




Mb Hb

Introduction

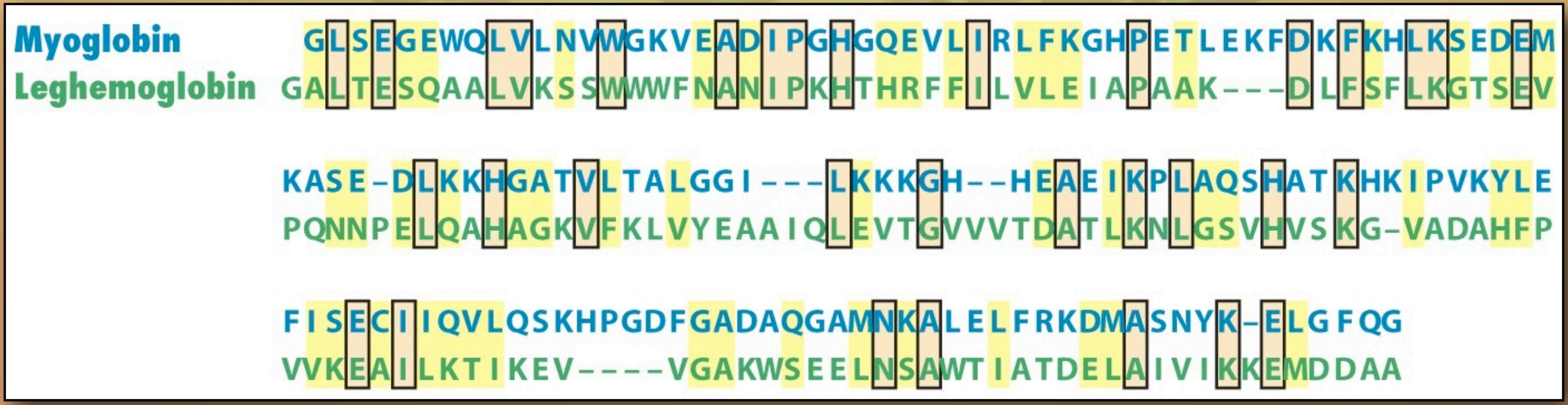
- The amino acid sequences for Mb and the α and β chains of Hb are **homologous** (Chapter 6.2-6.4)



Introduction

- The amino acid sequences for Mb is also homologous to the sequence for the plant protein leghemoglobin

25%



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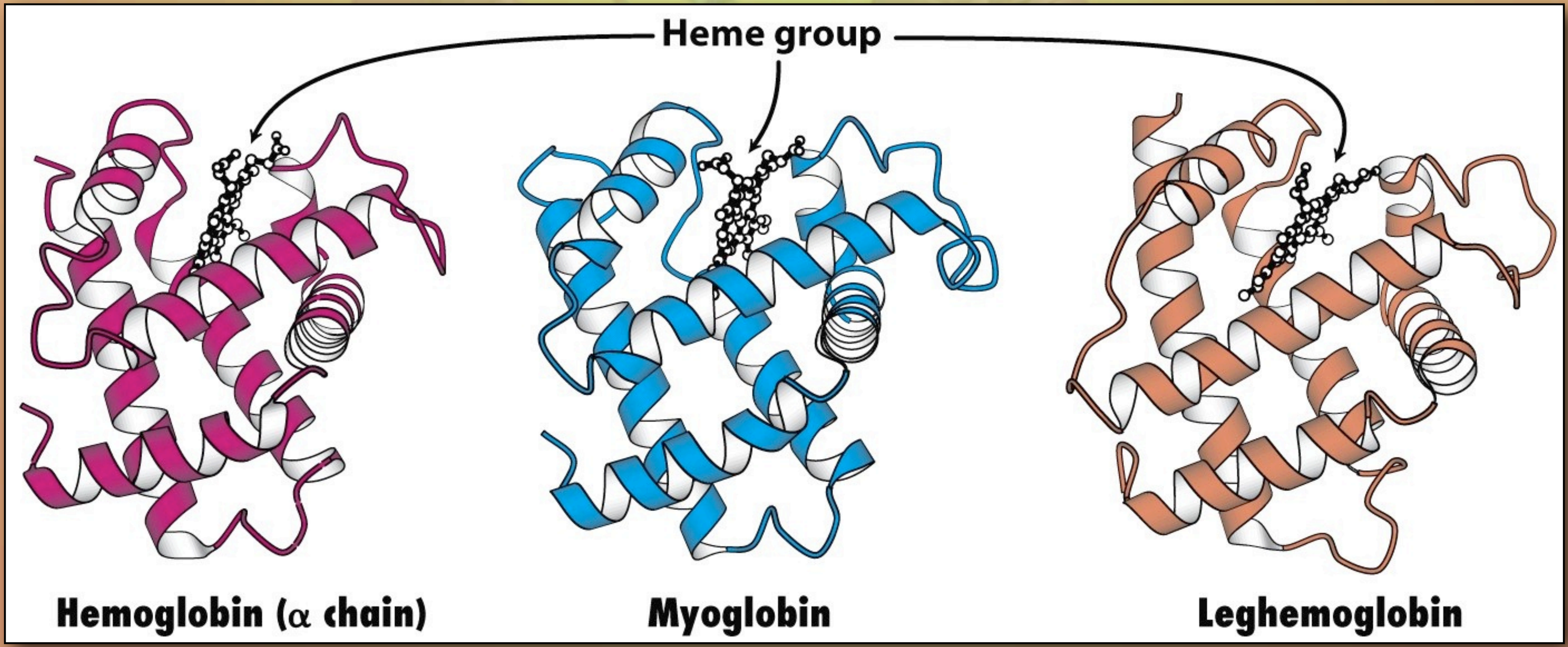
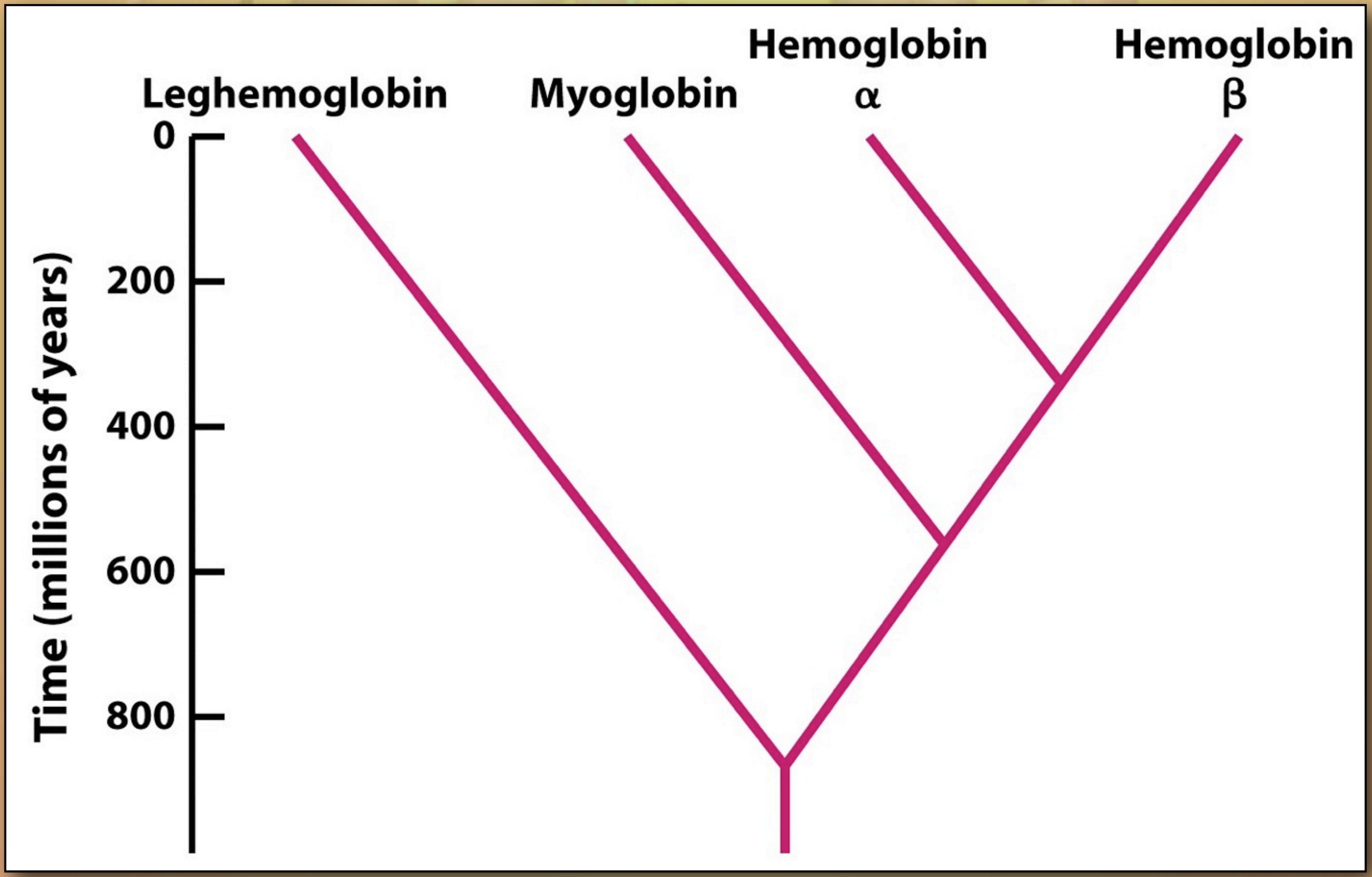
Introduction

- These three proteins also have very similar 3-dimensional structures.
  - The tertiary structure appear to be more highly conserved than the primary structure.



Introduction

- The amino acid sequences can be used to create an evolutionary tree.

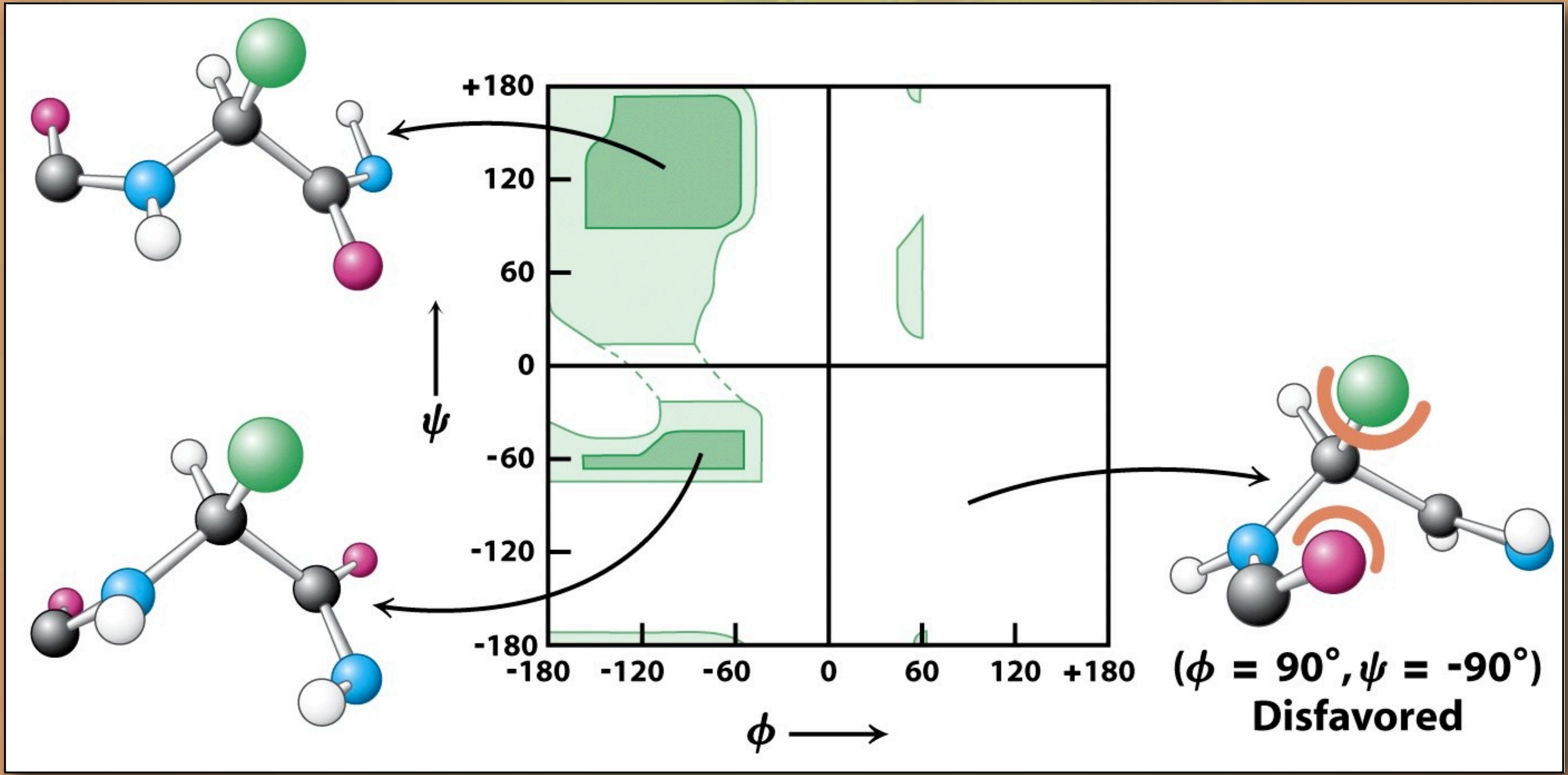


Introduction

- Number games.
  - We have seen how the Levinthal's Paradox suggests that protein folding is directed.
    - There is not enough time to fold a small protein by a brute force approach.
  - A similar numbers game with amino acid sequence reveals the same directed nature to evolution.

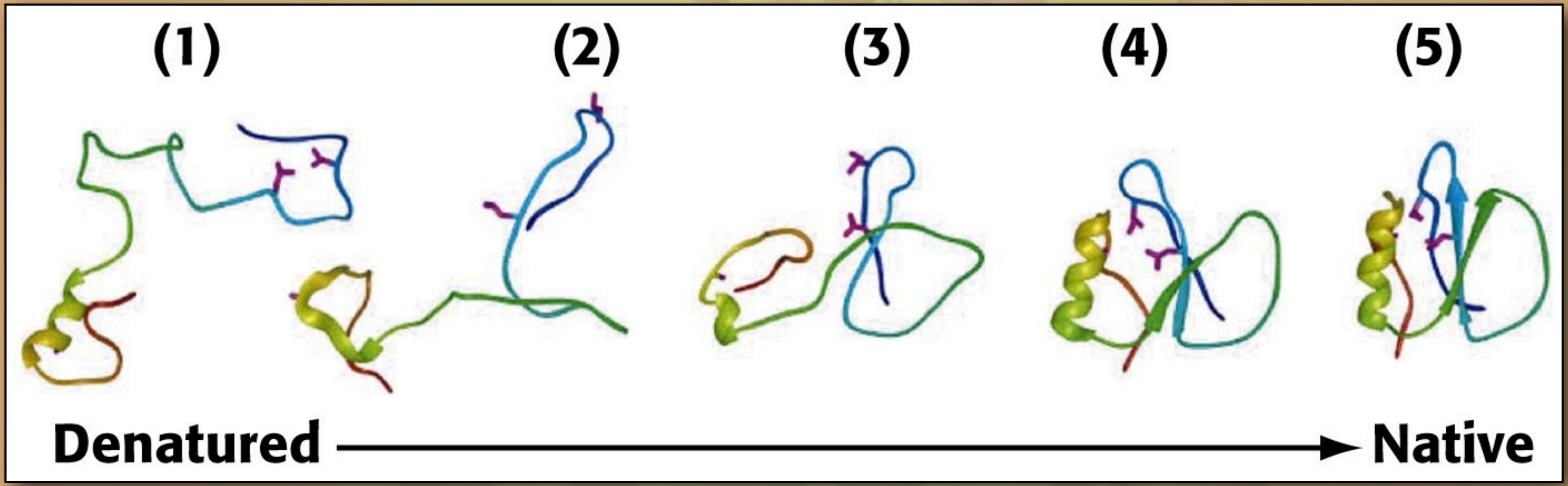
Predicting a Protein's Fold

- The Levinthal Paradox



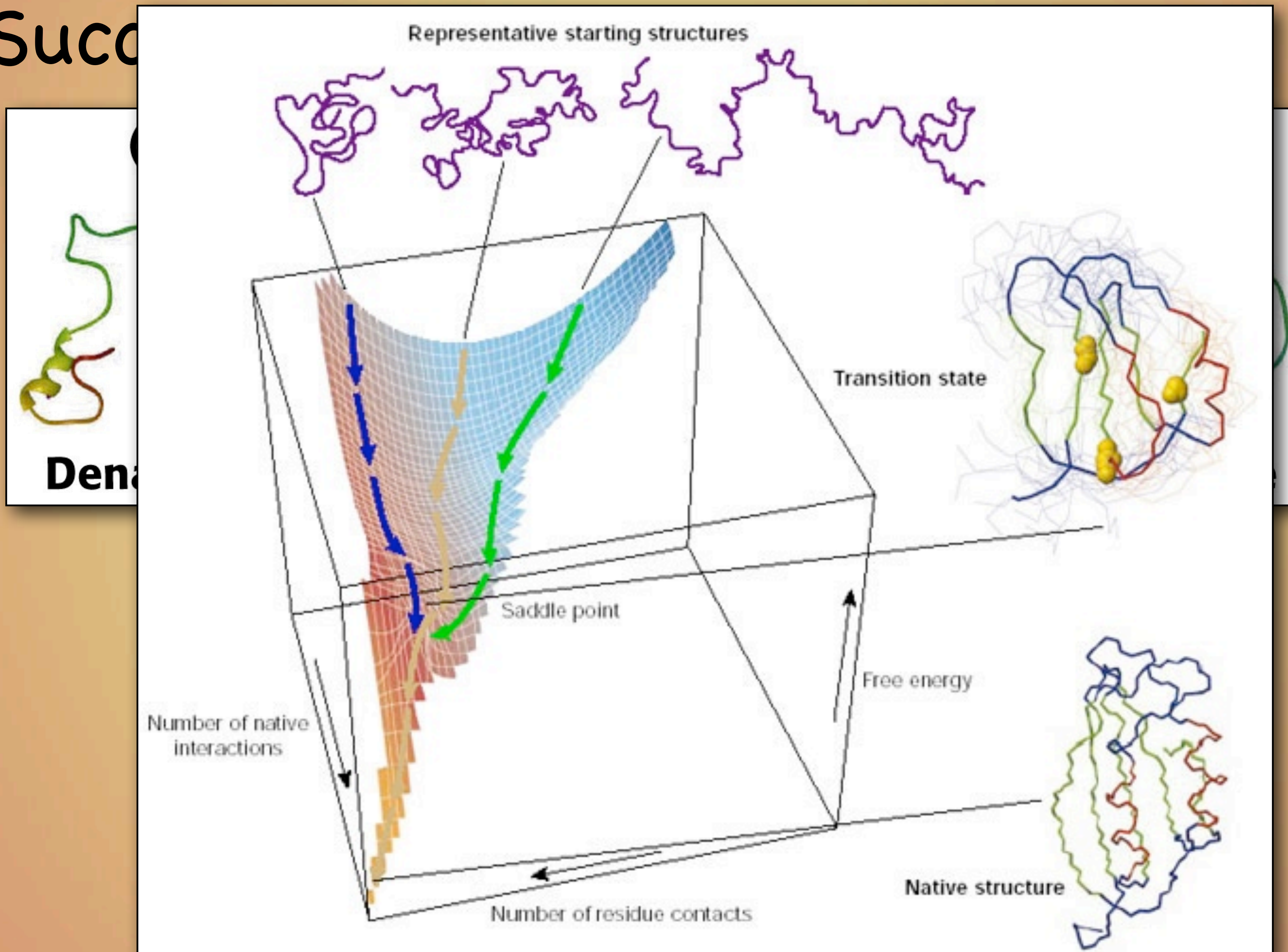
Predicting a Protein's Fold

- Successive Stabilization



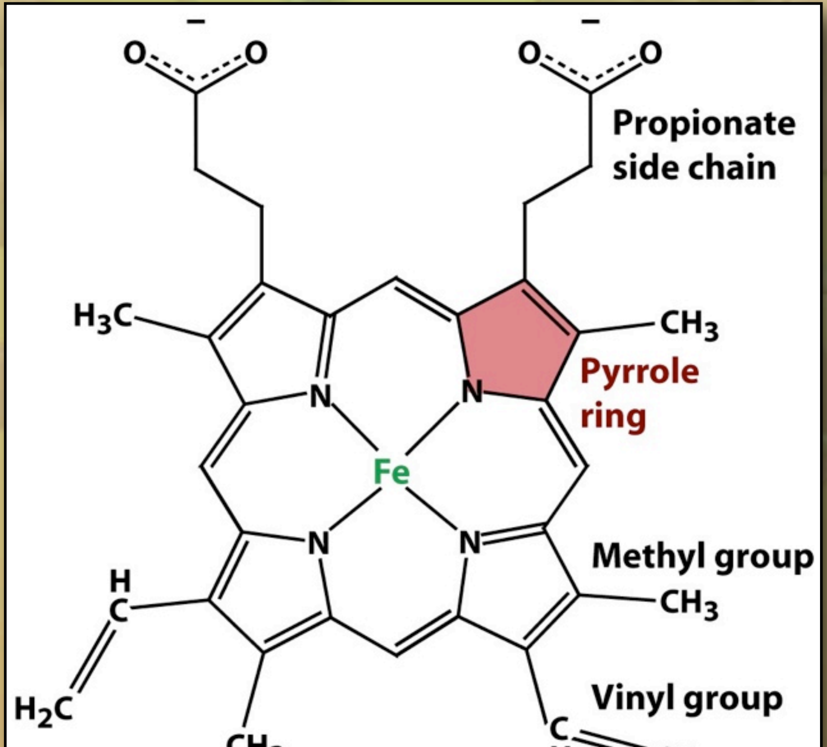
Predicting a Protein's Fold

- Succ



The O_2 binding site for Hb & Mb

- Analogous to the active site of enzymes.
  - Heme group is an example of a protein **cofactor**.



Heme
(Fe-protoporphyrin IX)

The O_2 binding site for Hb & Mb

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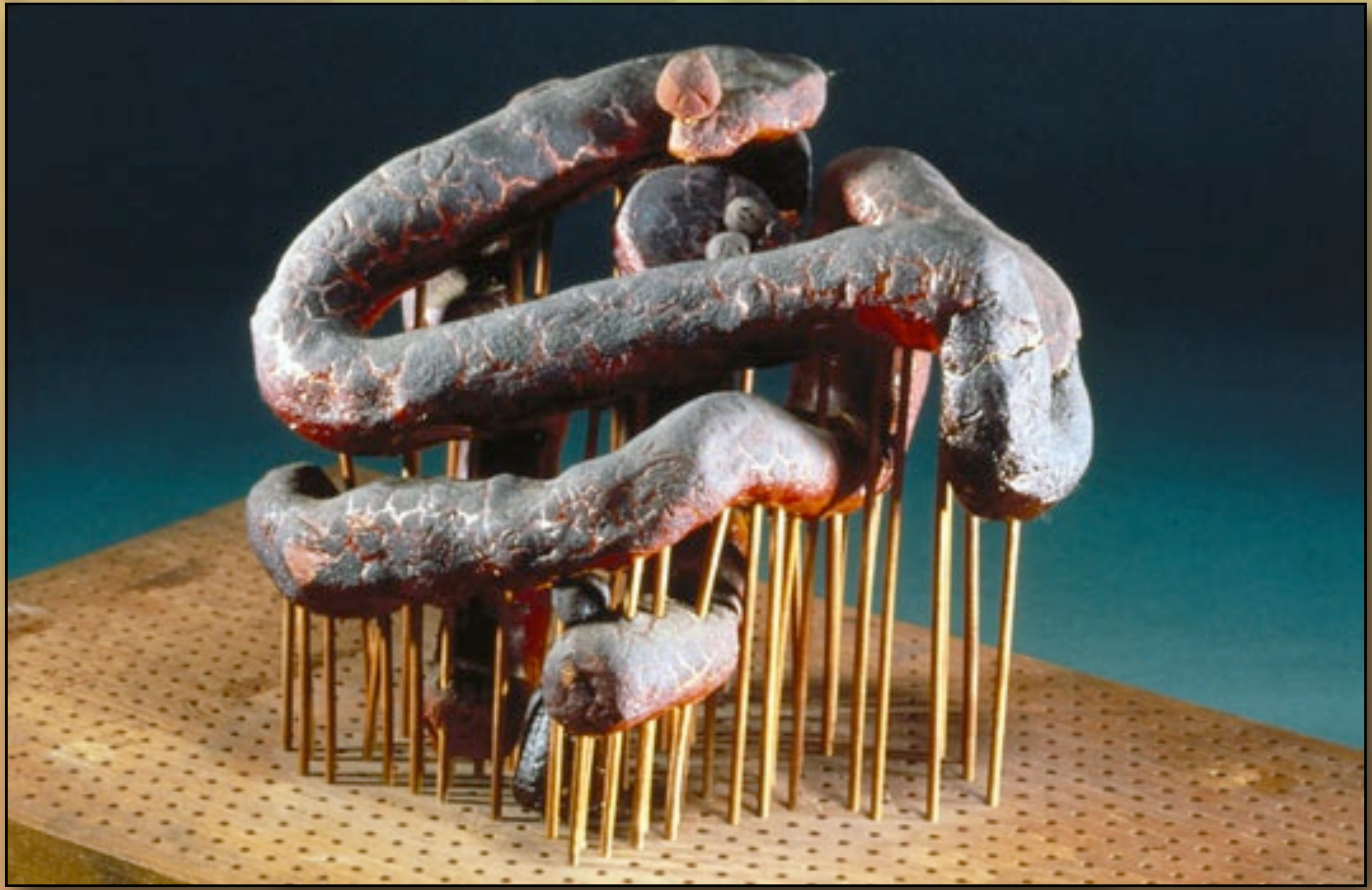

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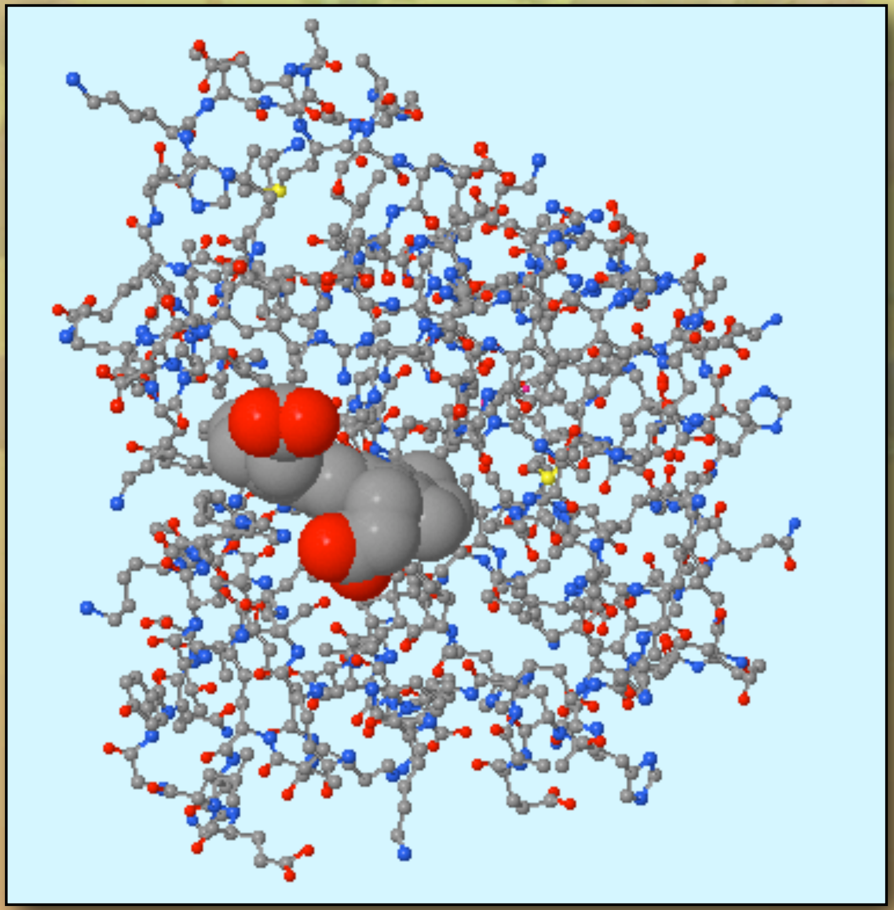
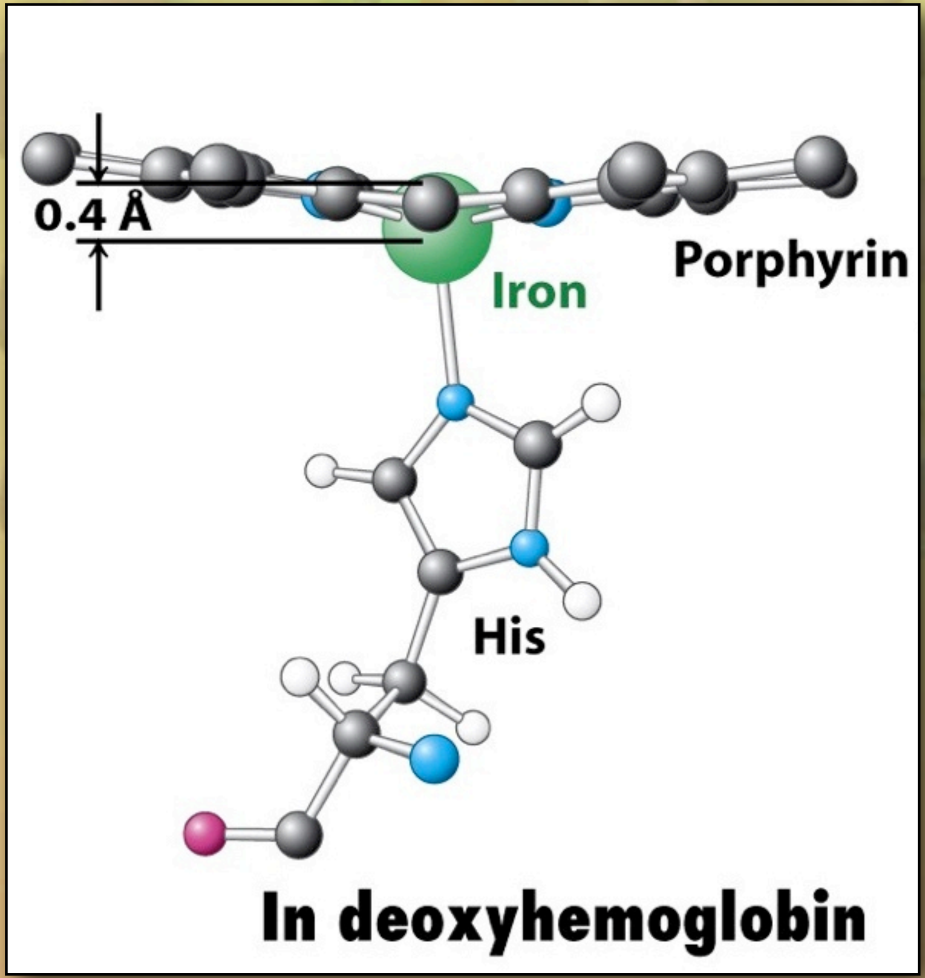


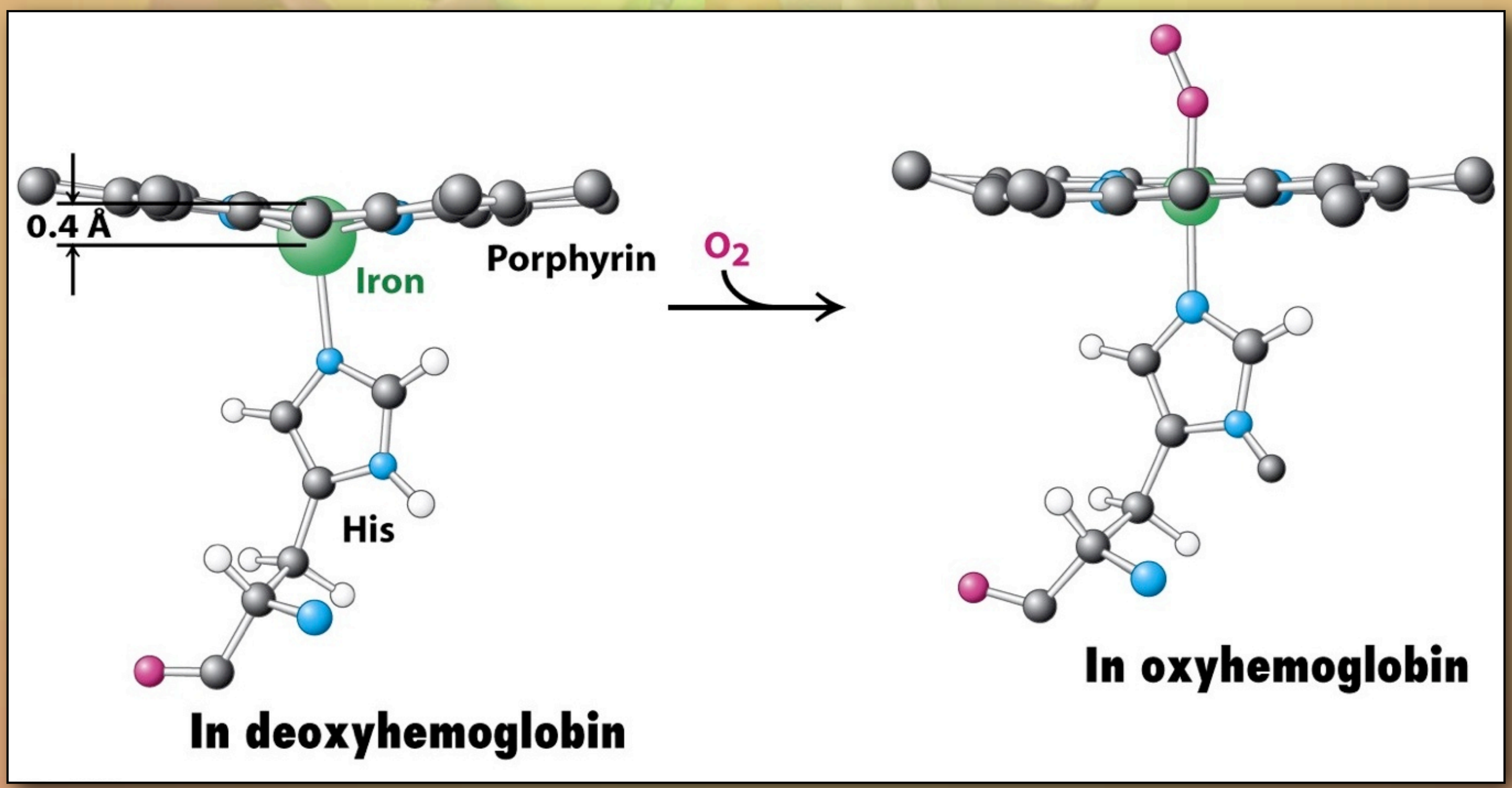
The O_2 binding site for Hb & Mb

- The heme Fe^{2+} ligated by the heme nitrogens and the nitrogen on the **proximal histidine**.



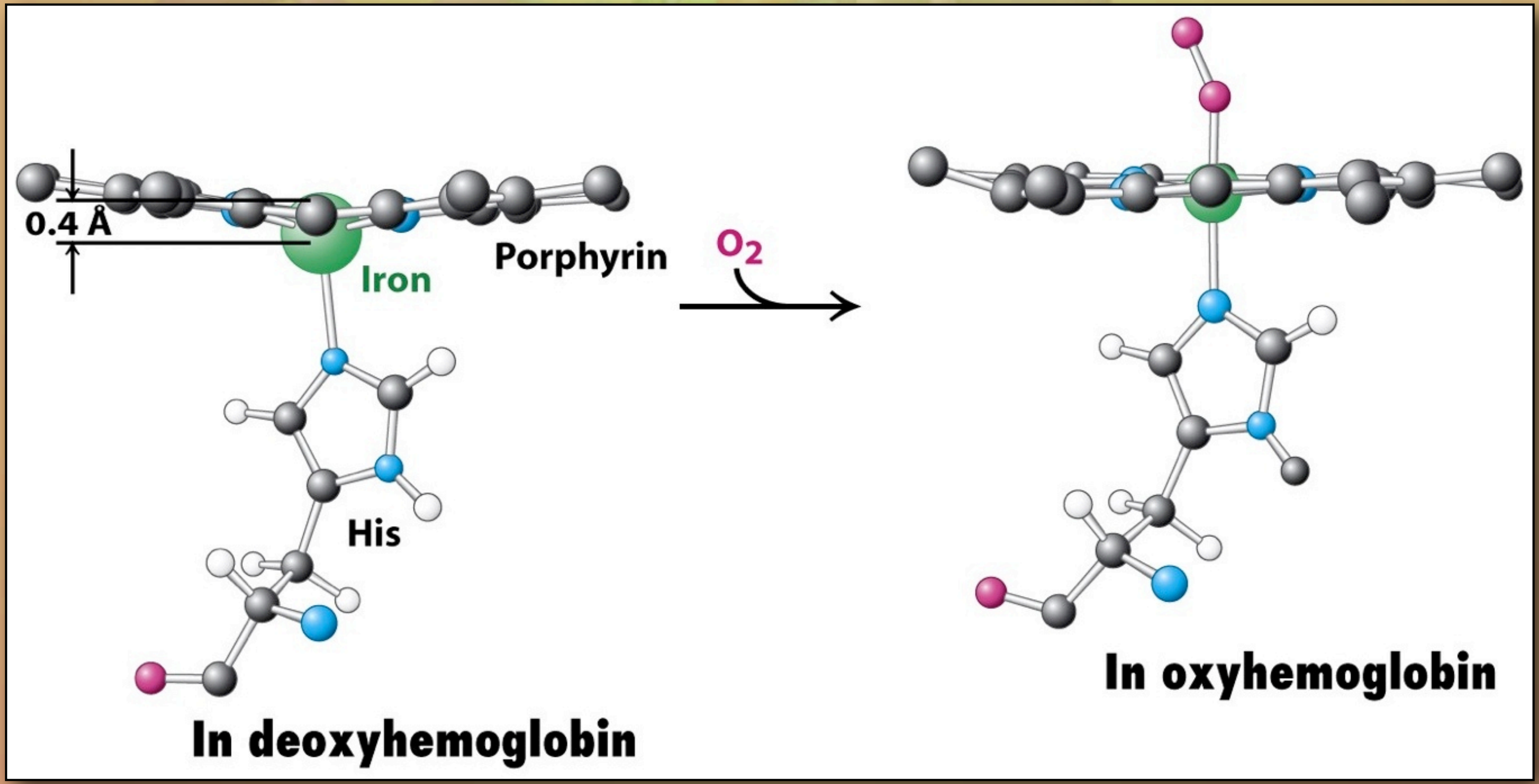
The O_2 binding site for Hb & Mb

- When bound, O_2 provides the sixth ligand for the heme Fe^{2+}



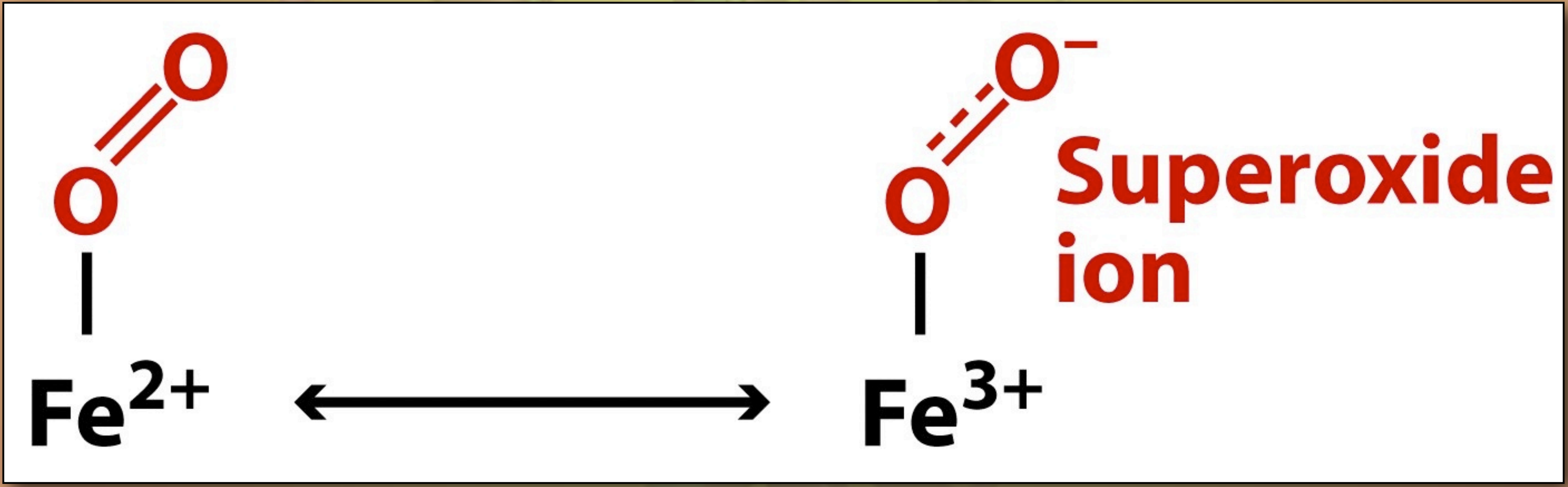
The O_2 binding site for Hb & Mb

- When O_2 binds, the heme Fe^{2+} gets smaller and moves into the plane of the heme.



The O_2 binding site for Hb & Mb

- The heme Fe^{2+} reduces the bound O_2 to a superoxide ion, O_2^-.

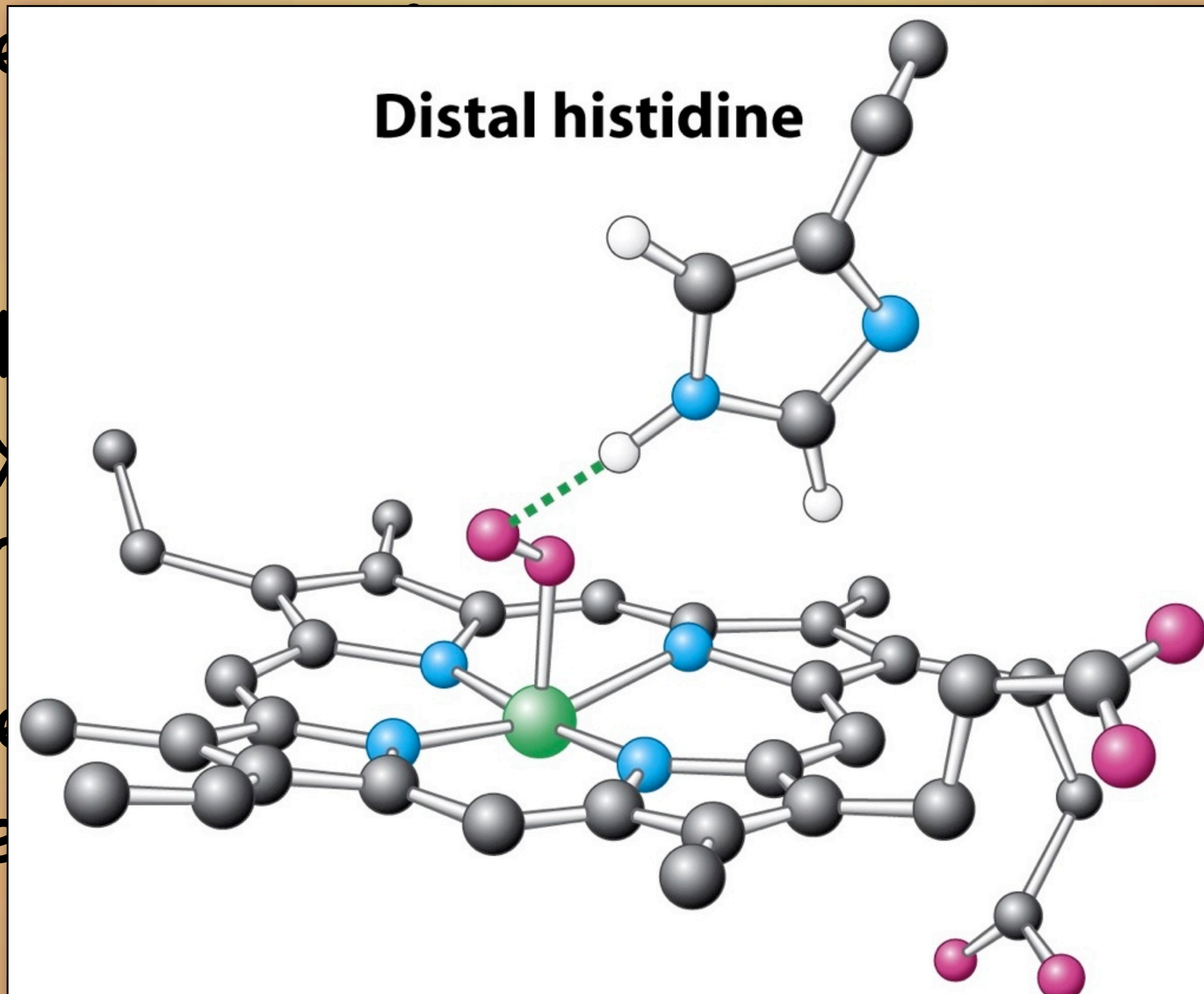


The O_2 binding site for Hb & Mb

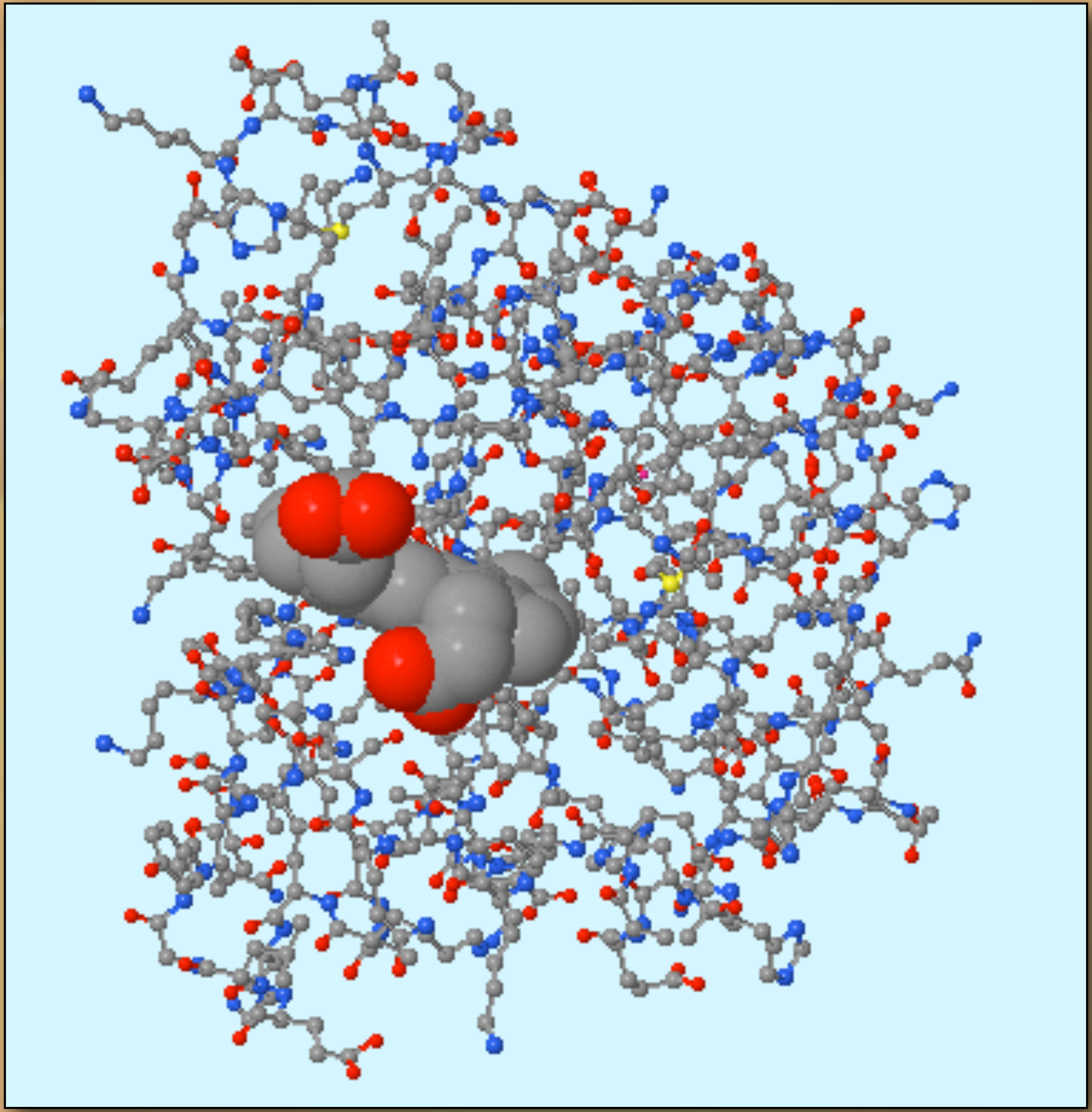
- The heme Fe^{2+} reduces the bound O_2 to a superoxide ion, O_2^-.
- Superoxide, like other reactive oxygen species (ROS's), is very damaging.
- It is the **distal histidine** that helps to prevent the release of the superoxide.

The O_2 binding site for Hb & Mb

- The … d O_2
  to …
- Su…
  oxy…
  dar…
- The…
  pre… e.



The O_2 binding site for Hb & Mb

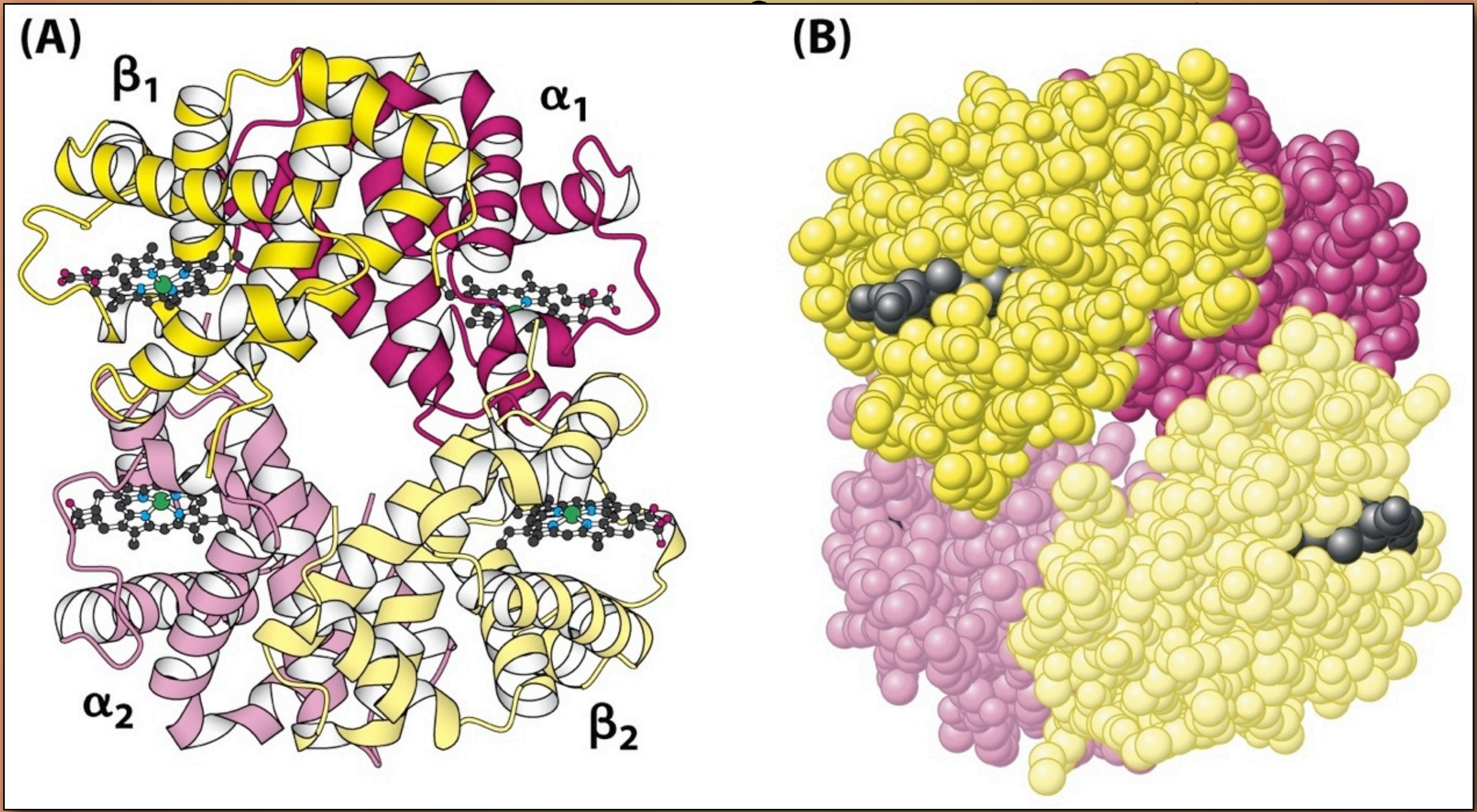


Oxymyoglobin

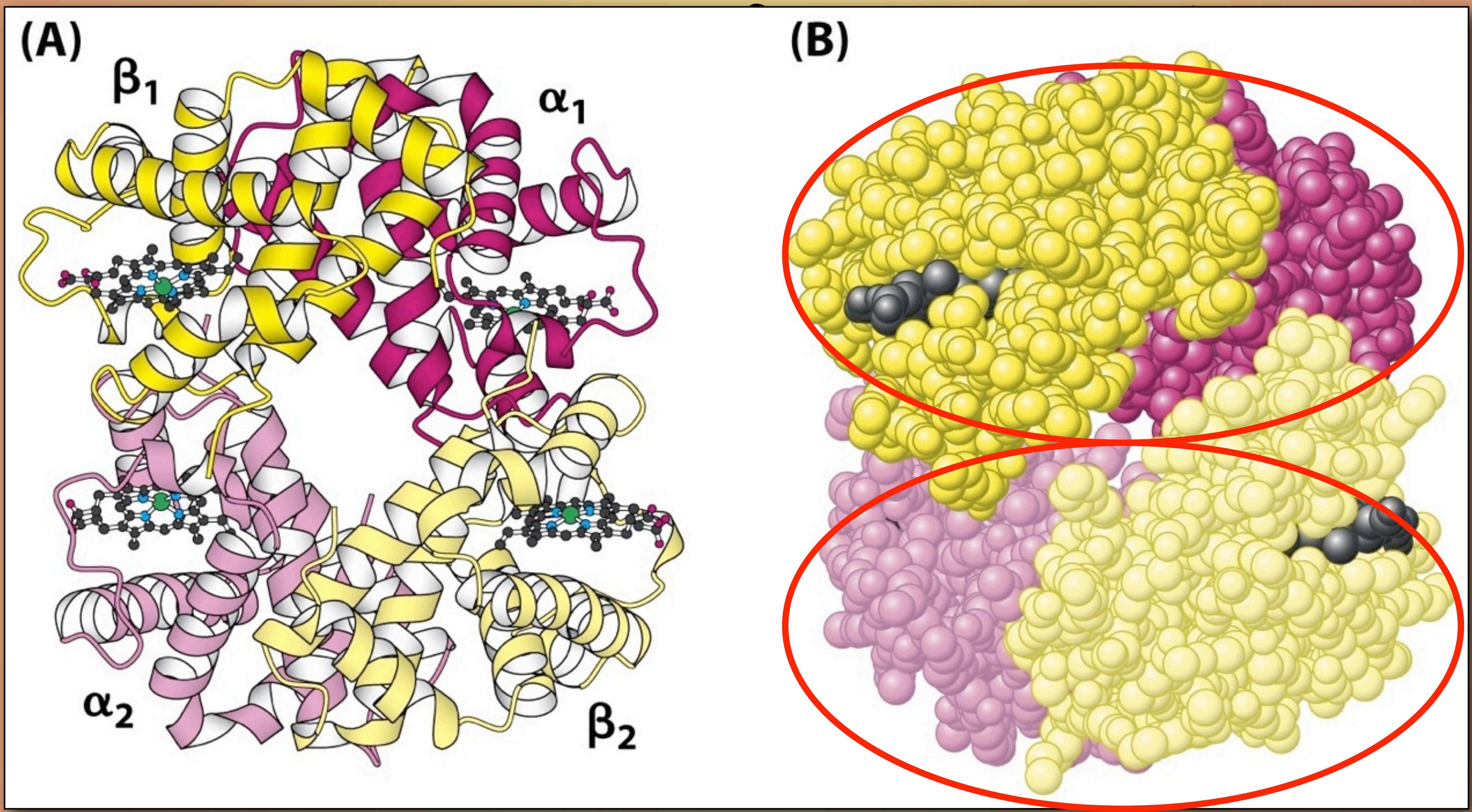
Hb is a Tetramer

- Hb's quaternary structure causes it to bind O_2 differently than Mb
- Hb is a tetramer of myoglobin-like subunits
  - Two α subunits
  - Two β subunits
- Combine as two αβ dimers
  - $\alpha_1\beta_1$ and $\alpha_2\beta_2$

Hb is a Tetramer

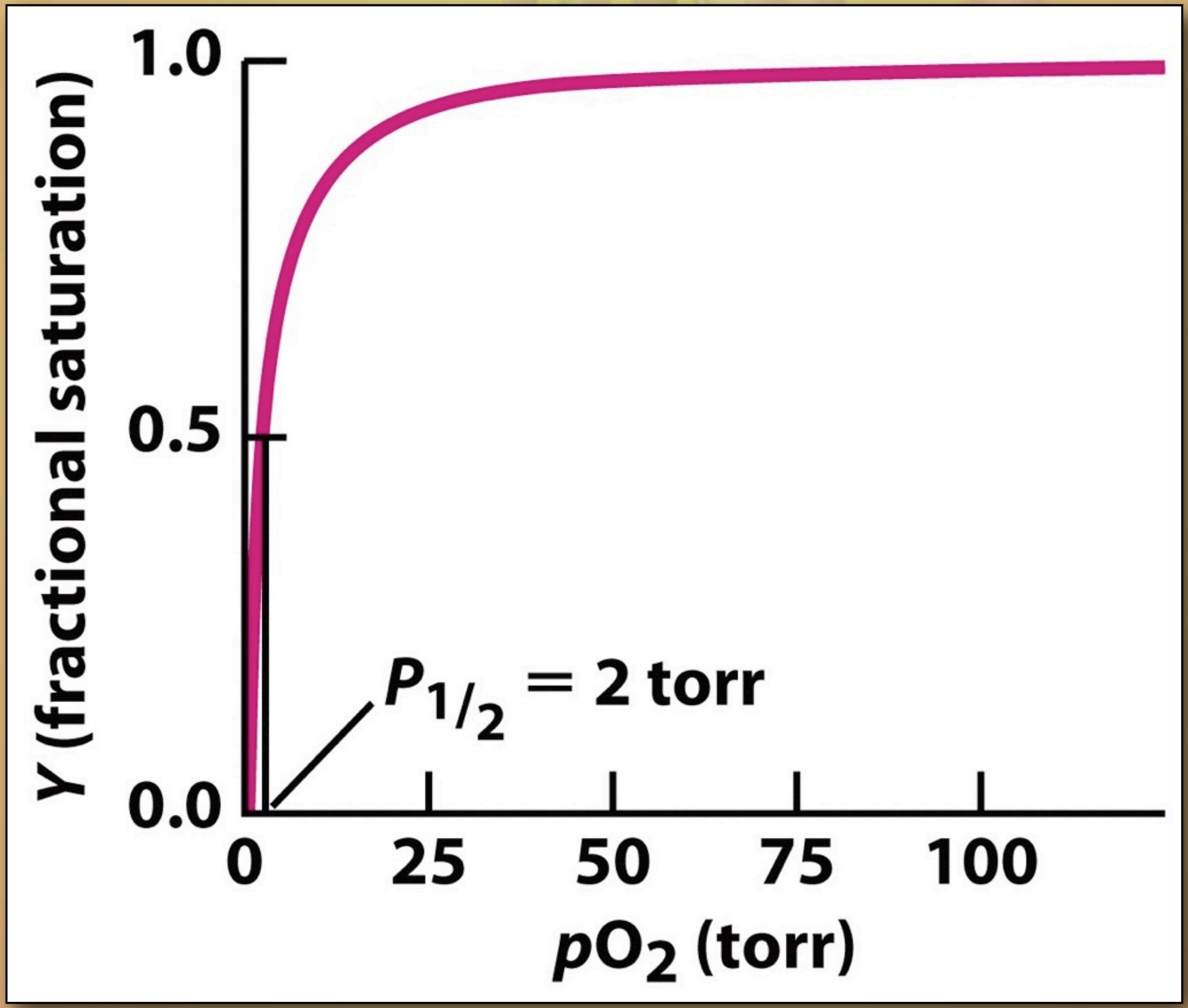


Hb is a Tetramer



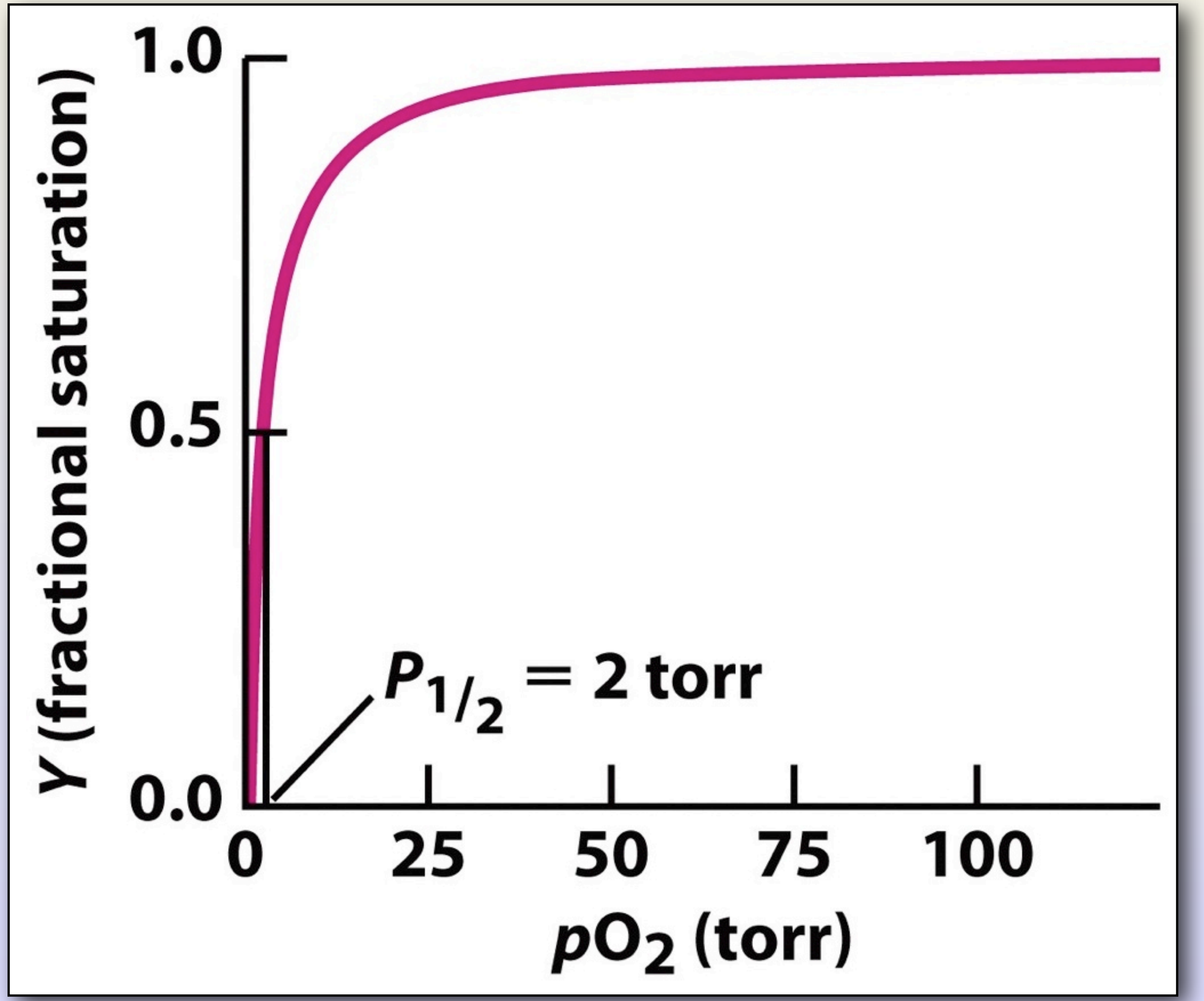
Hb Binds O_2 Cooperatively

- Mb has a P_{50} of 2 Torr



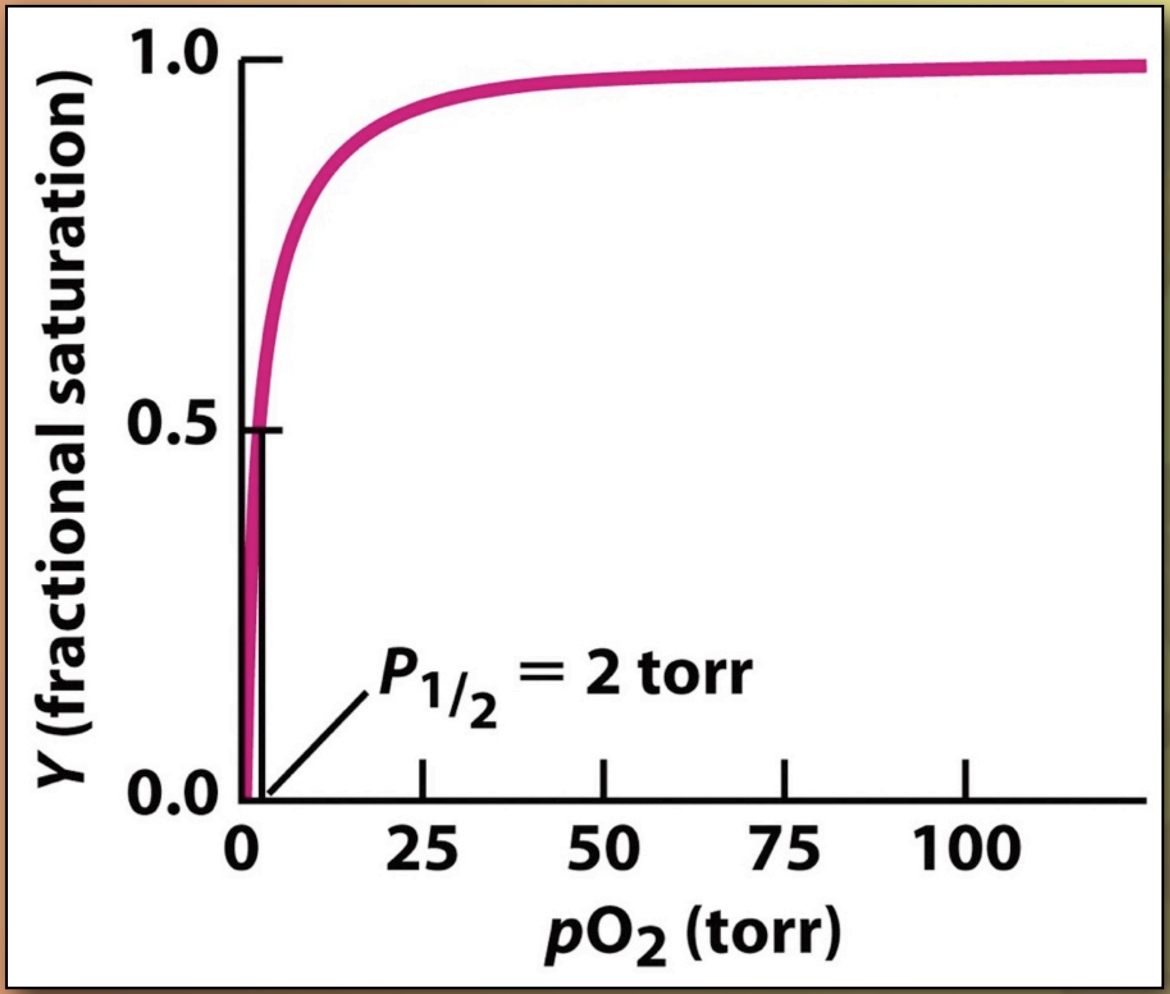
Questions

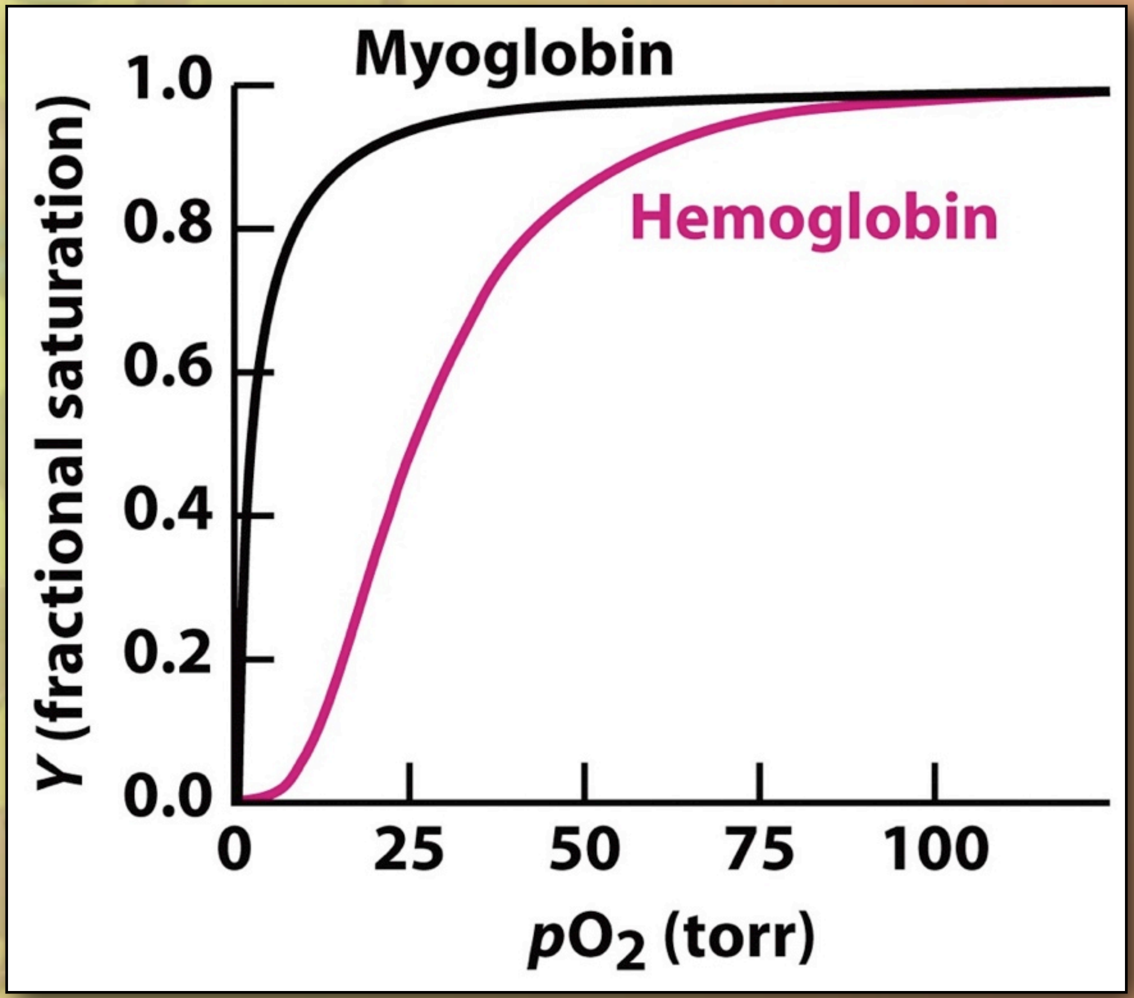
When exposed to air at 1 atm pressure, what fraction of the myoglobin molecule will be bound with O_2?



Hb Binds O_2 Cooperatively

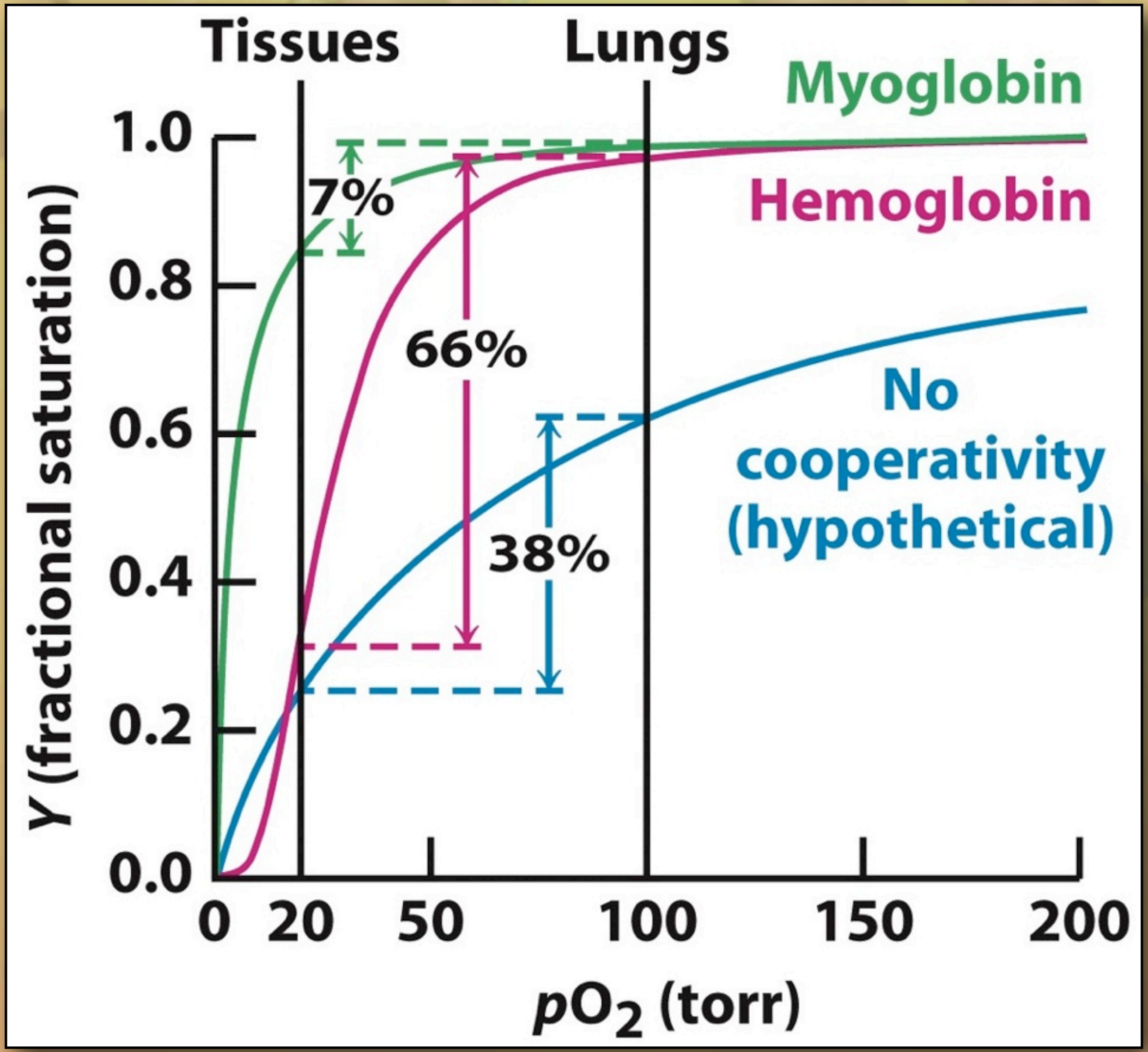
- Hb binds O_2 more weakly than Mb





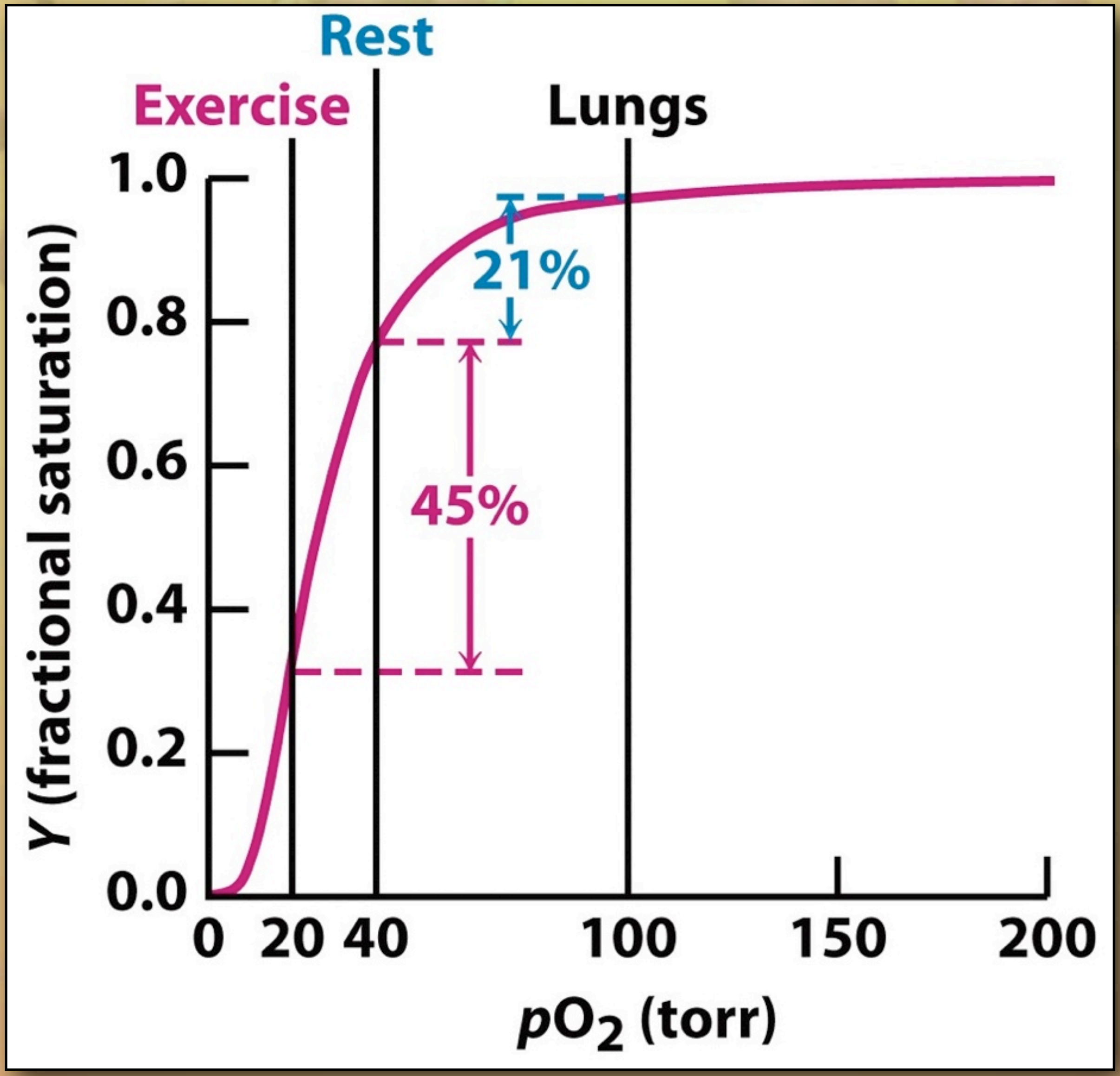
Hb Binds O_2 Cooperatively

- Cooperative binding makes Hb a more efficient transporter of O_2 than Mb.



Hb Binds O_2 Cooperatively

- Hb is efficiently delivers O_2 to the tissues during stress or exercise.

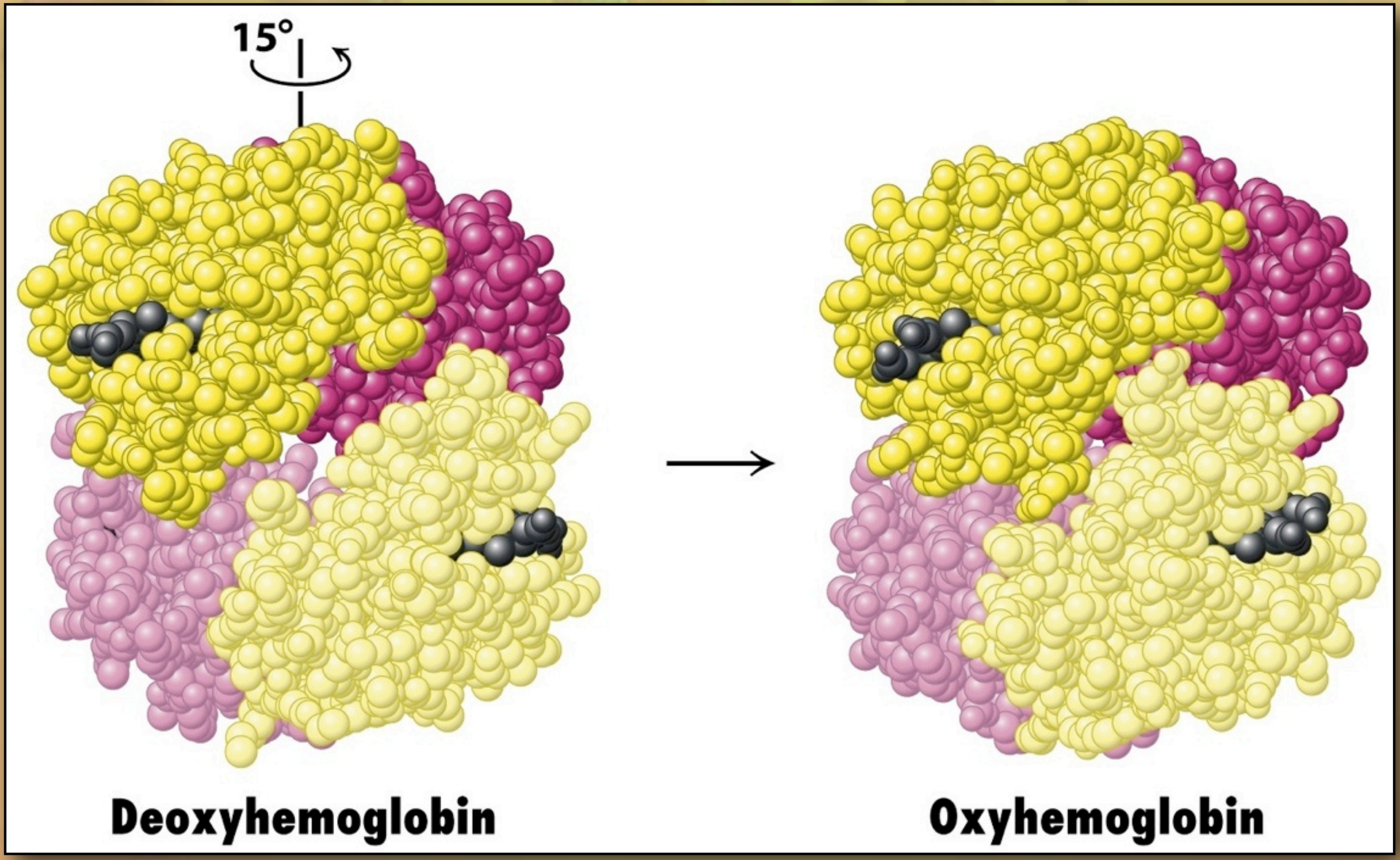


Problem 7.12 & 7.14

For Friday, work Problems 12 and 14 at the end of Chapter 7 and be ready to discuss them in class.

Hb Binds O_2 Cooperatively

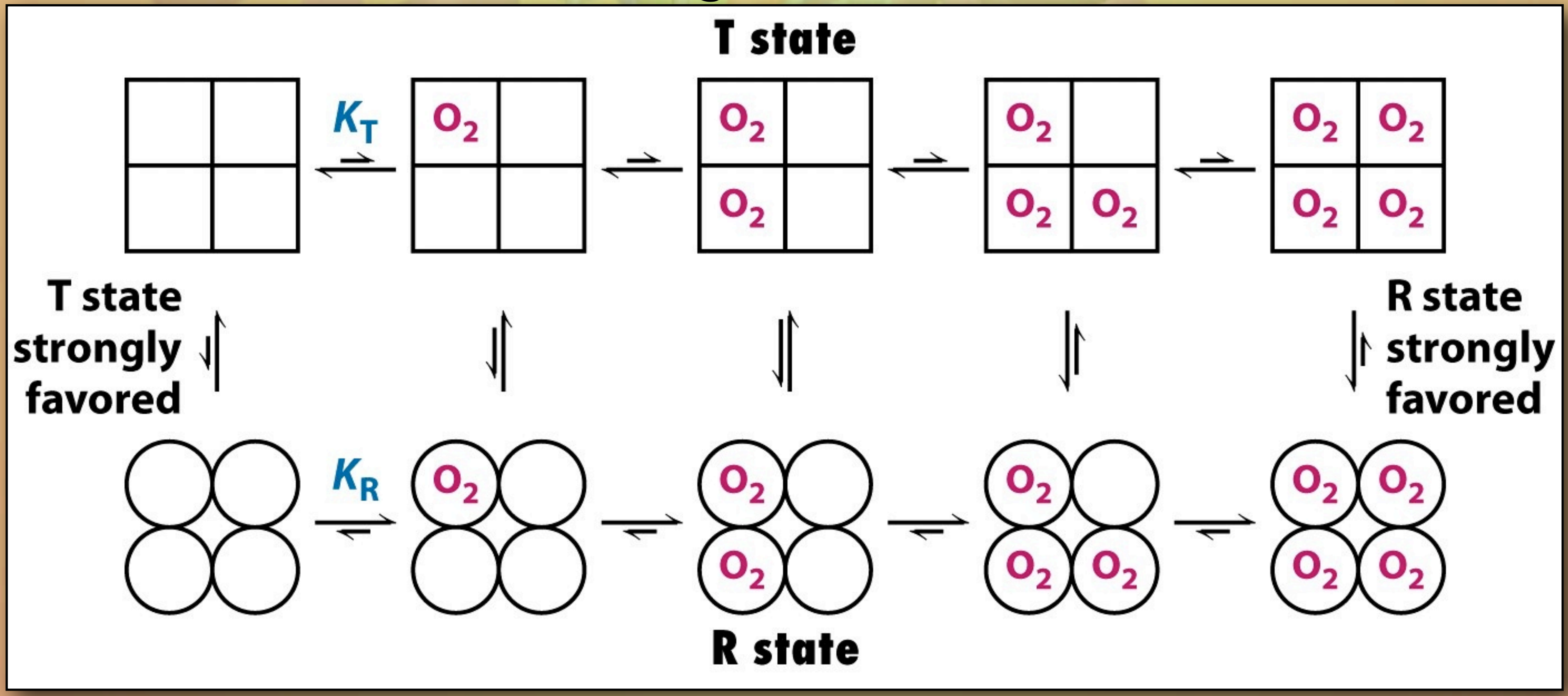
- Cooperativity is associated with changes in the quaternary structure of Hb



Tense (T) State Relaxed (R) State

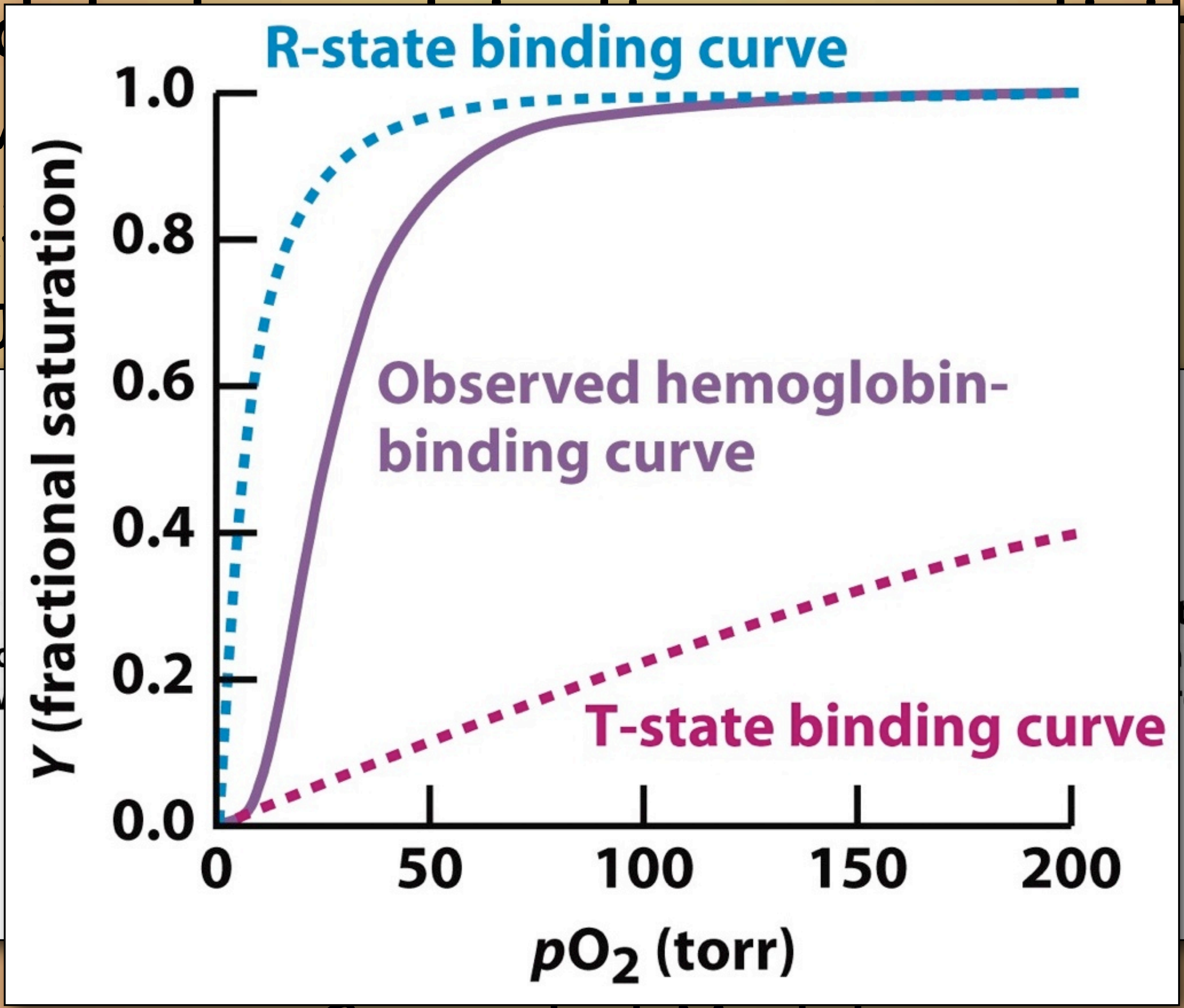
Hb Binds O_2 Cooperatively

- Models to explain the cooperativity:
  - MWC Model
    - (Jacques **M**onod, Jeffries **W**yman & Jean-Pierre **C**hangeux)



Concerted Model

Hb Binds O_2 Cooperatively



Concerted Model

Hb Binds O_2 Cooperatively

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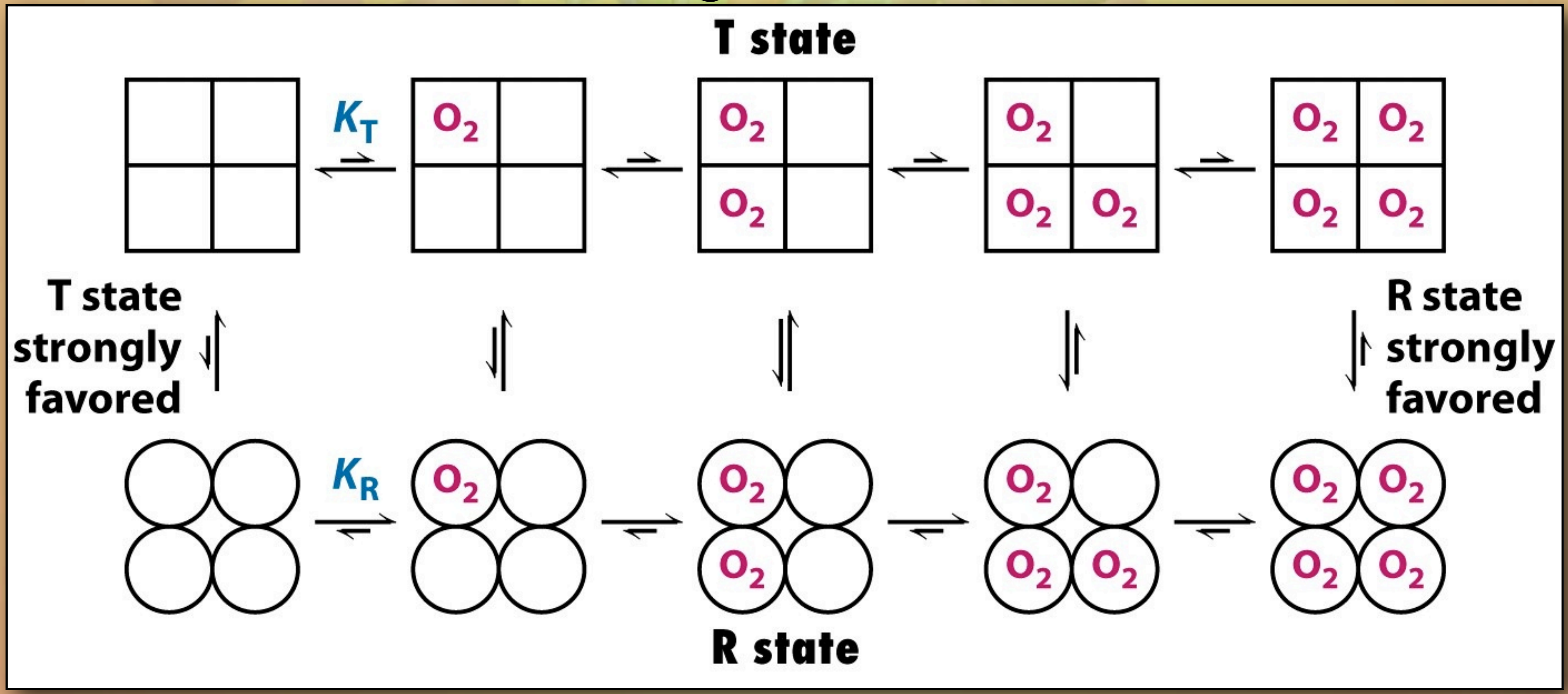
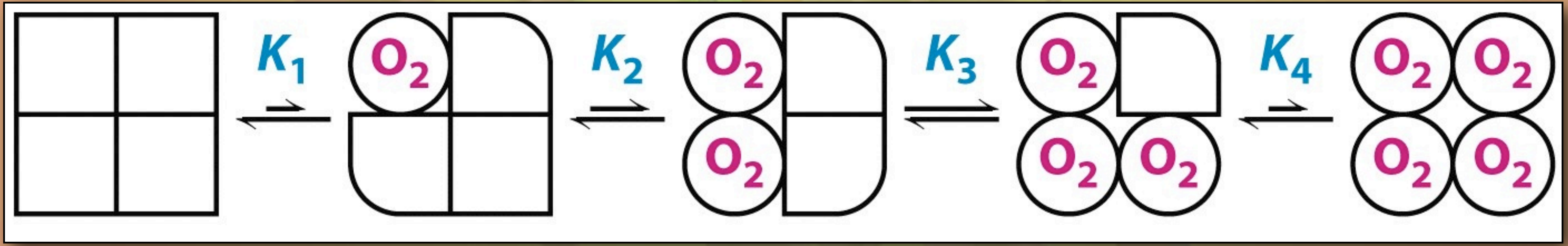


Concerted Model

Hb Binds O_2 Cooperatively

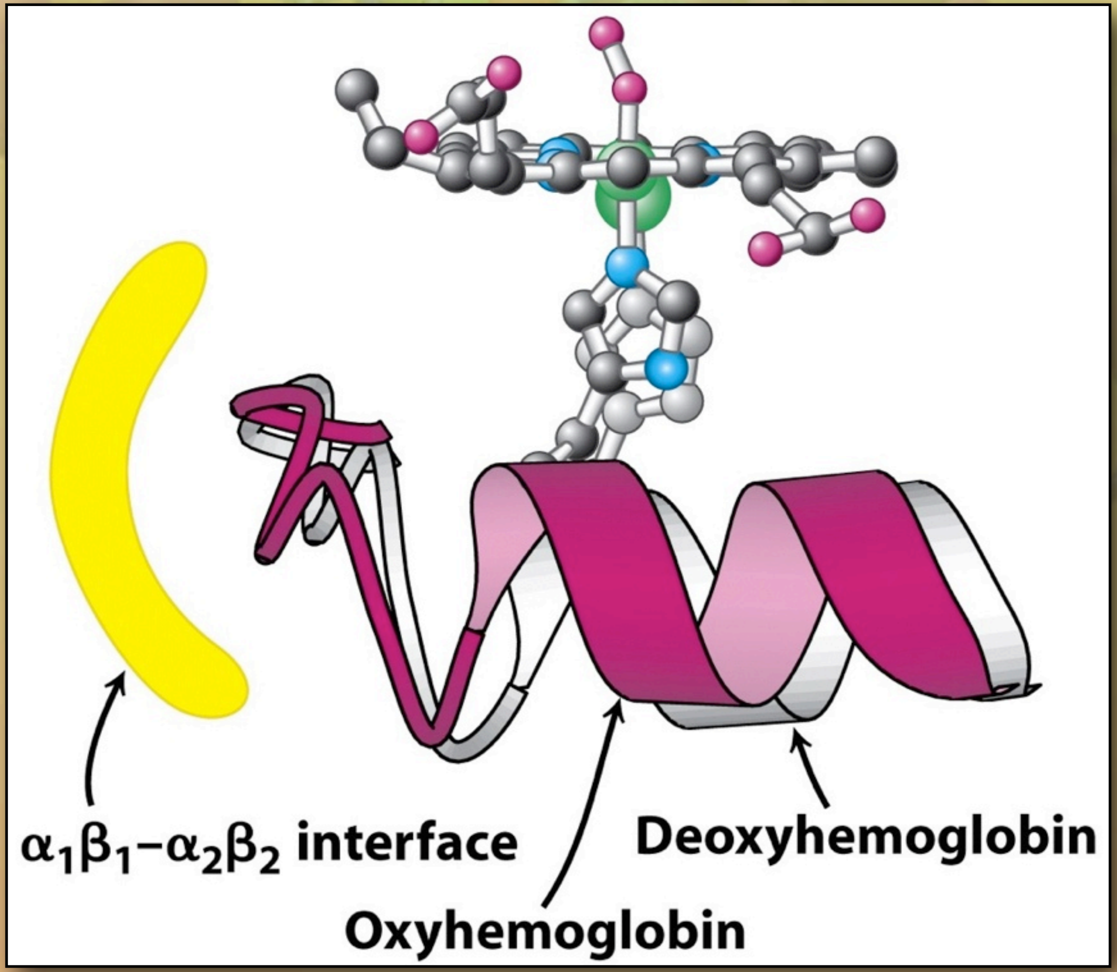
- Models to explain the cooperativity:
  - Sequential Model



Sequential Model

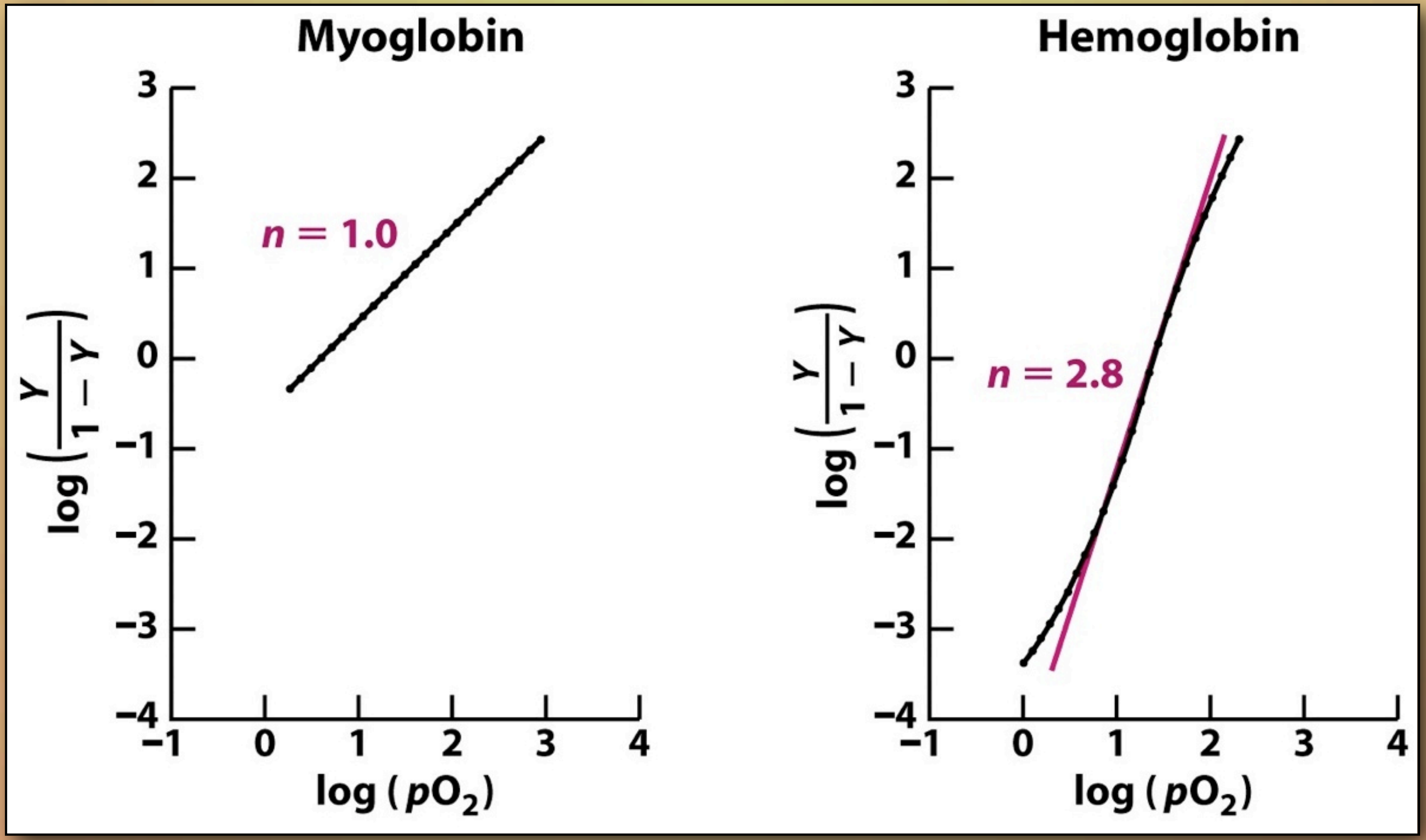
Hb Binds O_2 Cooperatively

- At the molecular level.
  - Conformational changes occurring upon O_2 bonding to one subunit are transmitted to other subunits



Hb Binds O_2 Cooperatively

- Cooperativity can be assessed with a **Hill plot**.
  - **n** is the **Hill coefficient**



Hb Binds O_2 Cooperatively

- Cooperativity can be assessed with a **Hill plot.**
  - **n** is the **Hill coefficient**

$$X + nS \Leftrightarrow X(S)_n$$

$$Y = \frac{[S]^n}{[S]^n + [S_{50}]^n}$$

$$Y = \frac{pO_2^n}{pO_2 + P_{50}^n}$$

$$\frac{Y}{1-Y} = \frac{pO_2^n}{P_{50}^n}$$

$$\log\left(\frac{Y}{1-Y}\right) = n\log(pO_2) - n\log(P_{50})$$

Problem 7.12a & 7.14

For Friday, work Problems 12a and 14 at the end of Chapter 7 and be ready to discuss it in class.

7.12.a Using the Hill equation, plot an oxygen binding curve for a hypothetical two-subunit hemoglobin with n - 1.8 and P_{50} = 10 torr.

7.14 Oxygen binding for primative Hb from a lamprey eel is given

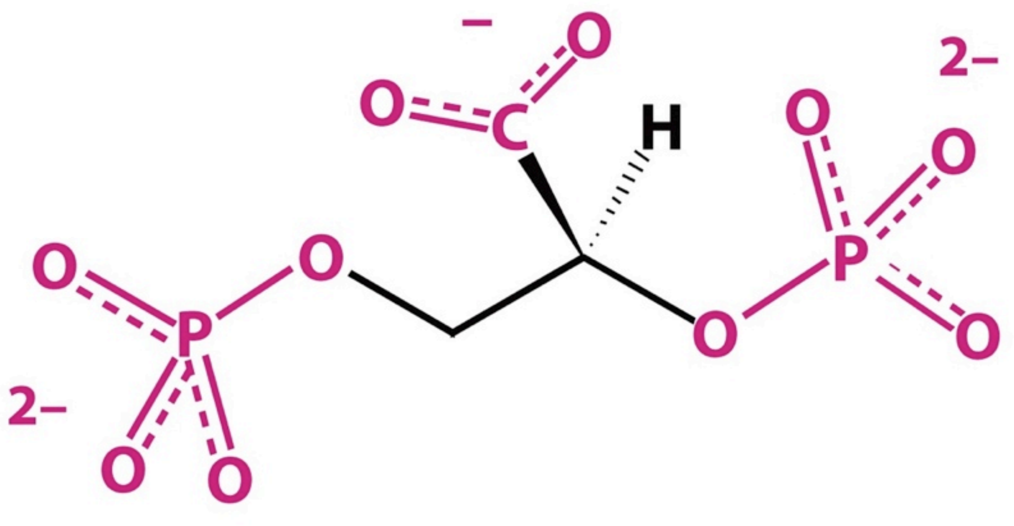
A) Plot data and determine P50

B) Make Hill plot and determine n

C) Propose model to explain cooperativity

Allosteric Regulation

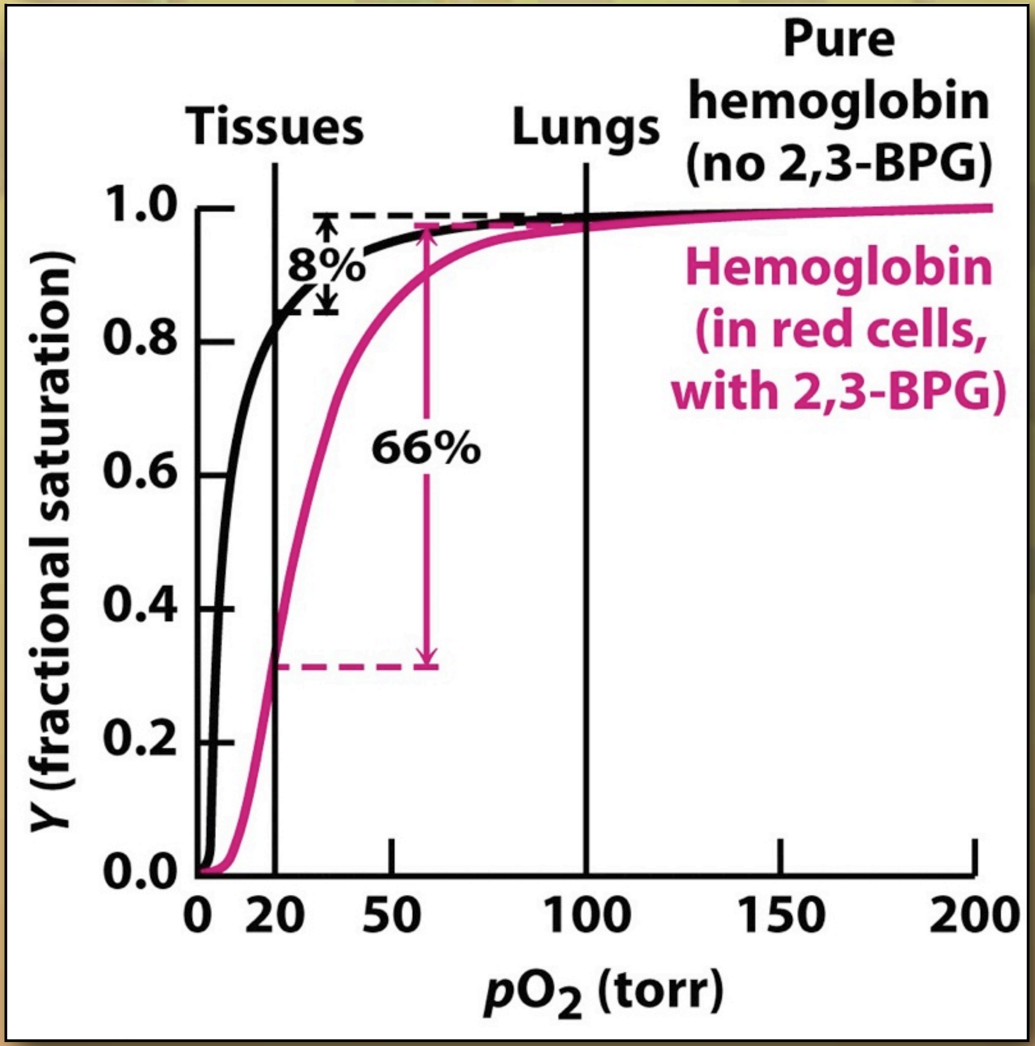
- Hb provides and example of **allosteric regulation**.
  - In red blood cells (RBC's), the metabolite 2,3-Bisphosphoglycerate (2,3-BPG) alters the O_2 binding behavior of Hb.



2,3-Bisphosphoglycerate (2,3-BPG)

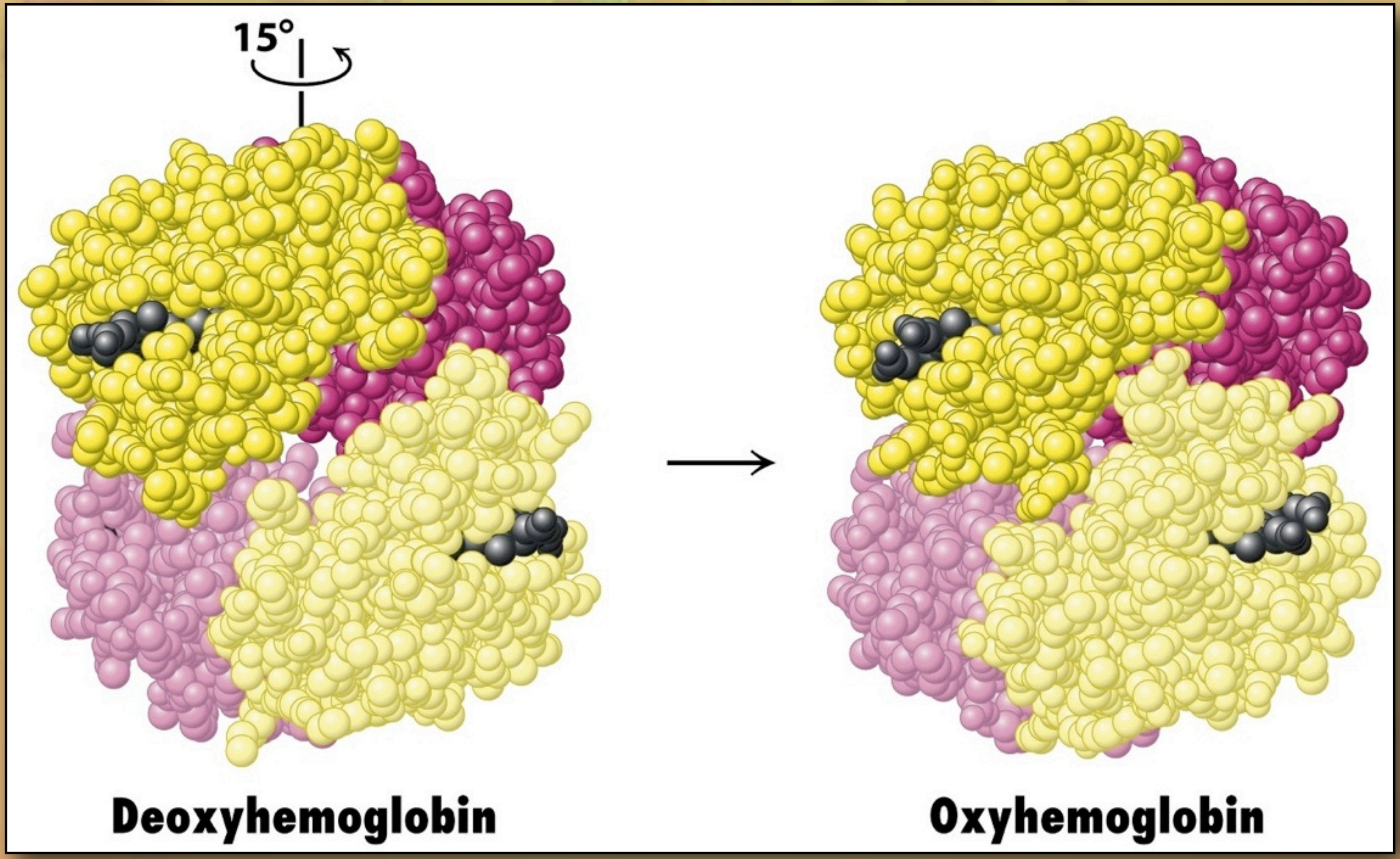
Allosteric Regulation

- 2,3-BPG lowers Hb's affinity for O_2, allowing it to release O_2 more efficiently to the tissues.



Hb Binds O_2 Cooperatively

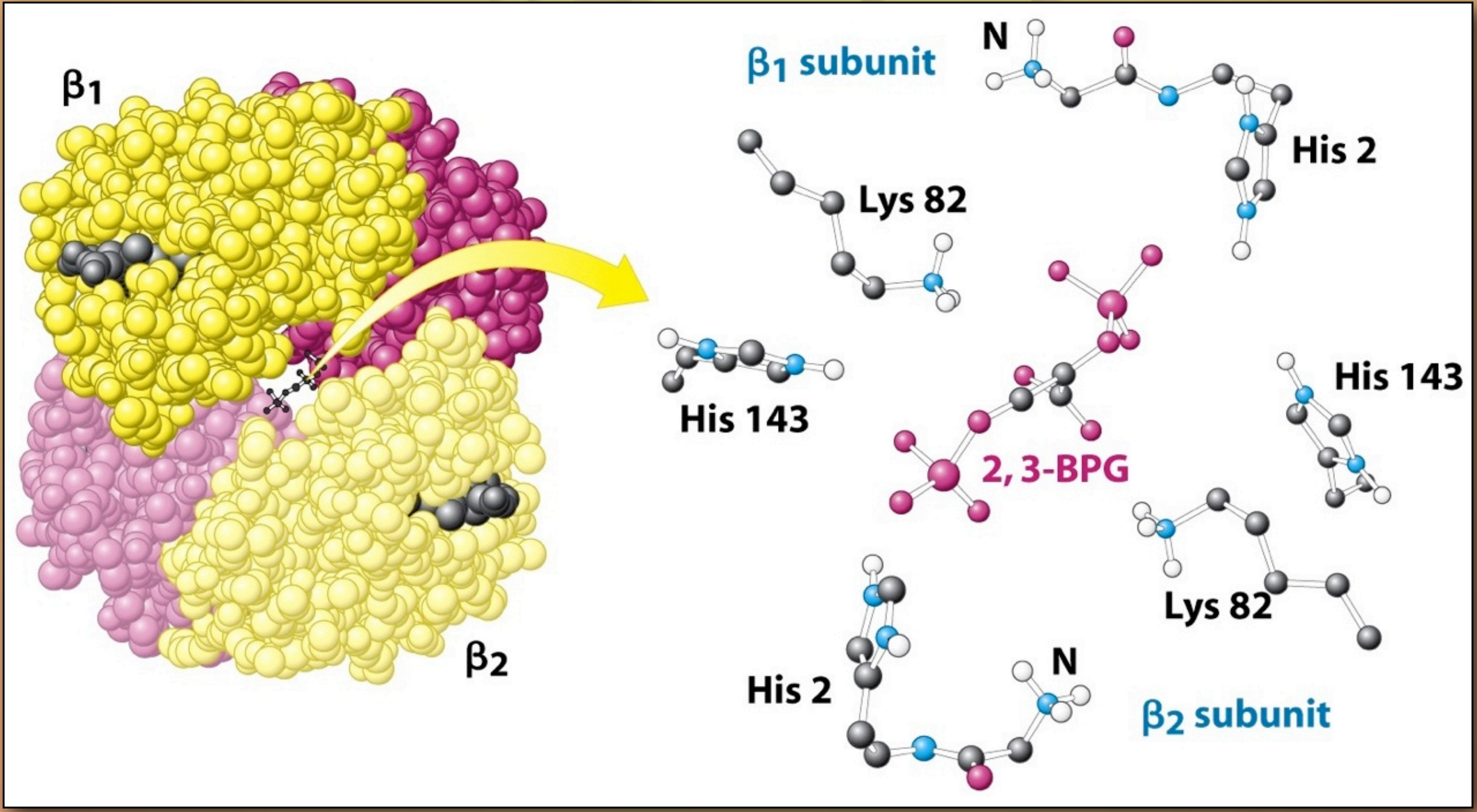
- Cooperativity is associated with changes in the quaternary structure of Hb



Tense (T) State   Relaxed (R) State

Allosteric Regulation

- 2,3-BPG binds to, and stabilizes, the T-state of Hb.

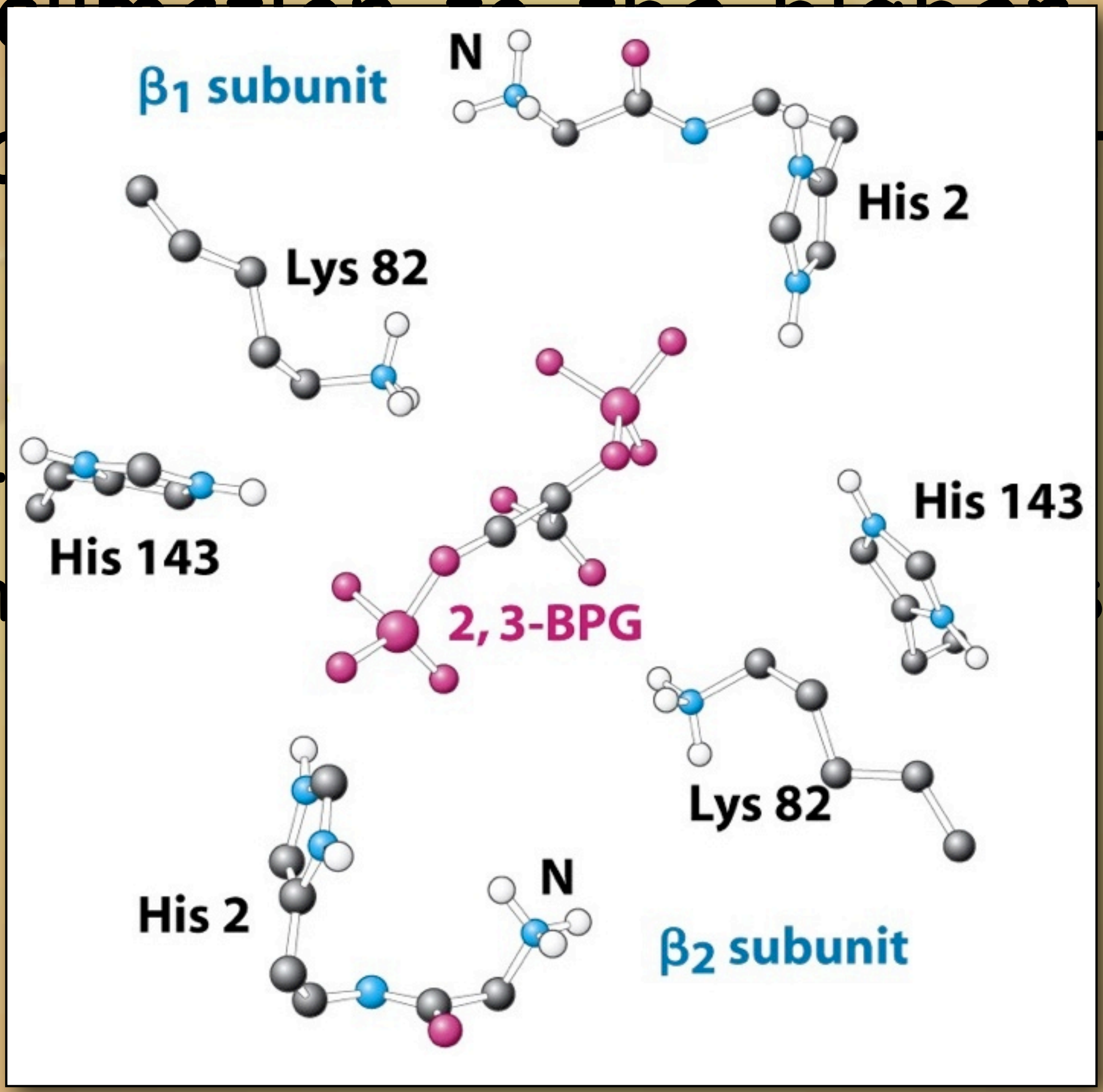


Allosteric Regulation

- The acclimation to the higher elevations involves the production of higher levels of 2-BPG.
- Fetal Hb
  - γ chains are substituted for β chains (H143S)

Allosteric Regulation

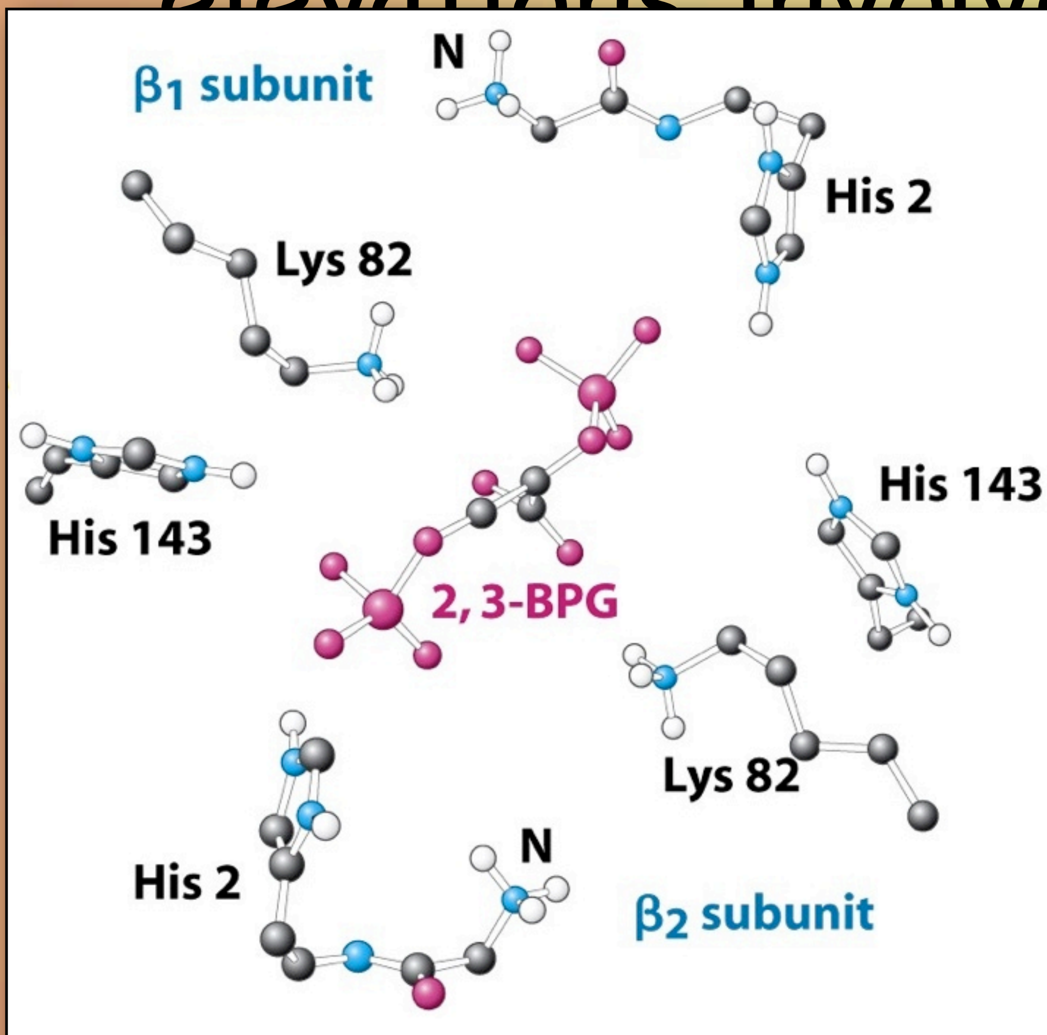
- The acc… elevati… …ion of higher…

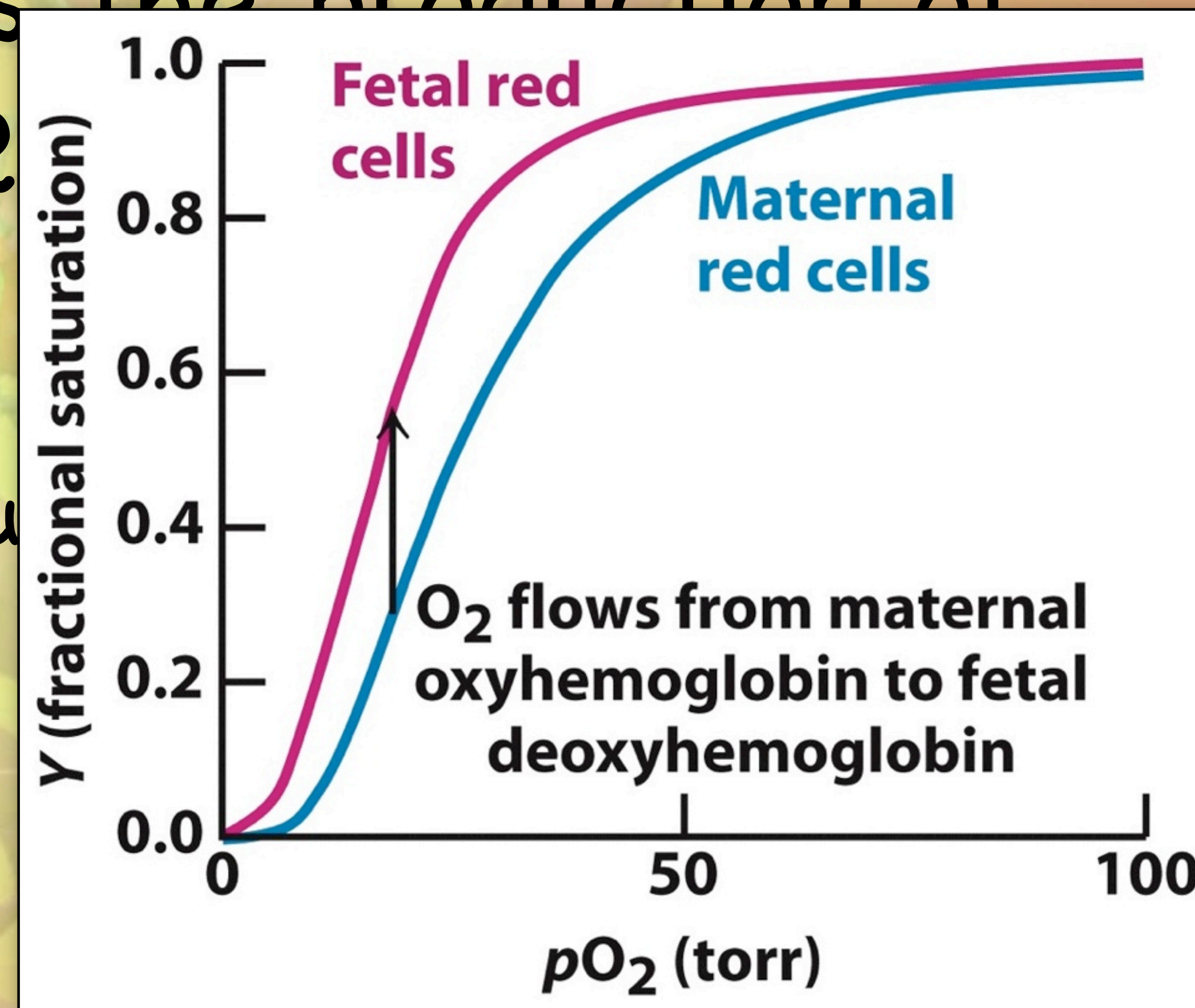


- Fetal H…
  - γ chain… (H143S)

Allosteric Regulation

- The acclimation to the higher elevations involves the production of





Next up

- Hemoglobin and Myoglobin (con'd).
  - Bohr effect
  - Sickle-cell Hb
- Enzymes (Chapter 8)