

Chem 452 - Lecture 3

Hemoglobin & Myoglobin

Part 1

Question of Day: How do the differences in structure between the oxygen transport proteins myoglobin (Mb) and hemoglobin (Hb) make each more best suited for their biological roles?

Introduction

† Both Hemoglobin (Hb) and Myoglobin (Mb) are oxygen-binding proteins.

† Hb is used in mammals to transport oxygen from the the lungs to the tissues.

† Mb is used in the tissues to store the oxygen, once it gets there.

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Introduction

† Together, Hb and Mb provide an excellent example of structure-function relationships in proteins.

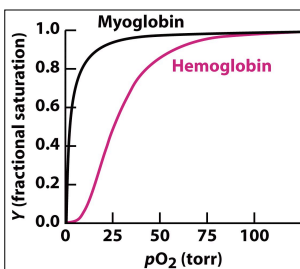
† They illustrate the substrate binding portion of an enzyme catalyzed reaction.

† They illustrate **allosteric regulation**.

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Introduction

† Hb and Mb bind oxygen differently.



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Introduction

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

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Genomics

- + Identifying cause of genetic mutation, e.g. Sickle cell anemia
- + Due to a 1 base pair change in the gene for the β subunit of hemoglobin

Hb-A: ...ATG GTG CAC CTG ACT CCT **GAG** GAG AAG TCT GCC GTT ACT...
Hb-S: ...ATG GTG CAC CTG ACT CCT **GTG** GAG AAG TCT GCC GTT ACT...

Chem 452, Lecture 1 - Introduction to Biochemistry 6

Genomics

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	U	C	A	G
U	Phe	Ser	Tyr	Cys
	Phe	Ser	Tyr	Cys
	Leu	Ser	STOP	STOP
	Leu	Ser	STOP	Trp
C	Leu	Pro	His	Arg
	Leu	Pro	His	Arg
	Leu	Pro	Gln	Arg
	Leu	Pro	Gln	Arg
A	Ile	Thr	Asn	Ser
	Ile	Thr	Asn	Ser
	Ile	Thr	Lys	Arg
	Met	Thr	Lys	Arg
G	Val	Ala	Asp	Gly
	Val	Ala	Asp	Gly
	Val	Ala	Glu	Gly
	Val	Ala	Glu	Gly

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U	Phe	Ser	Tyr	Cys
	Phe	Ser	Tyr	Cys
	Leu	Ser	STOP	STOP
	Leu	Ser	STOP	Trp
C	Leu	Pro	His	Arg
	Leu	Pro	His	Arg
	Leu	Pro	Gln	Arg
	Leu	Pro	Gln	Arg
A	Ile	Thr	Asn	Ser
	Ile	Thr	Asn	Ser
	Ile	Thr	Lys	Arg
	Met	Thr	Lys	Arg
G	Val	Ala	Asp	Gly
	Val	Ala	Asp	Gly
	Val	Ala	Glu	Gly
	Val	Ala	Glu	Gly



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Introduction

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

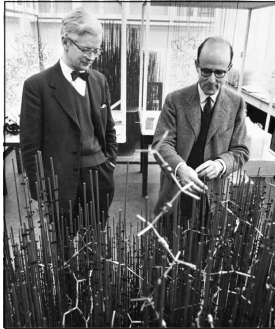
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Introduction

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

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Introduction



John Kendrew Max Perutz

The Medical Research Council (MRC) at Cambridge University

- † Kendrew
- † Perutz
- † Sanger
- † Watson
- † Crick

Nobel Prizes in 1962

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Introduction



Nobel Prizes in 1962

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Functions of Hb and Mb

- † 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 tissues, where the O_2 concentration is low.
- † Mb then accepts the O_2 from the Hb in the tissues, where the O_2 concentrations are low.

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Functions of Hb and Mb

What does this description of the roles of Hb and Mb tell you about the relative O_2 binding affinities for Hb and Mb?

- † Hb binds oxygen in the lung, where the O_2 concentration is high, and delivers it to the tissues, where the O_2 concentration is low.
- † Mb then accepts the O_2 from the Hb in the tissues, where the O_2 concentrations are low.

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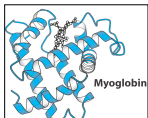
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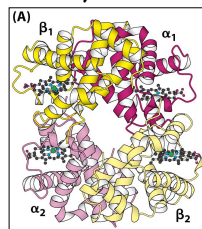
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Structures of Hb and Mb

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



Mb

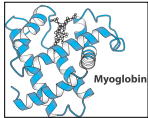


Hb

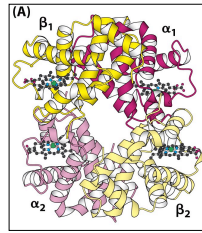
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Structures of Hb and Mb

What structural feature does Mb have that Hb lacks?



Mb

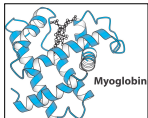


Hb

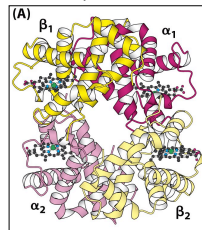
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Structures of Hb and Mb

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



Mb



Hb

Chem 452, Lecture 3 - Hb and Mb 12

Structures of Hb and Mb

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

Hemoglobin α	VLPADKTNPKAAVGGKVLCHAGEYKAELEDELSFRHKTYFPHF-----
Myoglobin	GLSEGEWQLLNIWGGKVLDPFGHEDELIIKIKGHELELEKEDKPKHLKKS
	FLSHGSAQVYRQKKNADALTNAVAHDDMPNLSALSDLFAKLRVDPVY
	EEMKASDELDKKGATLTLALGGILKKKGHHEAEIKPLAOSHATDCHKIPVK
	NFKLLSCLLVITAAHLPAEETPAVHASLDRPILASVSTVLTQKTR
	YLEFISEETIQVLSKHPDSECADAGGAMNLELFRKDMGSHKELGFQG

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Structures of Hb and Mb

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

Hemoglobin α	VLPADKTNPKAAVGGKVLCHAGEYKAELEDELSFRHKTYFPHF-----	
Myoglobin	GLSEGEWQLLNIWGGKVLDPFGHEDELIIKIKGHELELEKEDKPKHLKKS	
	FLSHGSAQVYRQKKNADALTNAVAHDDMPNLSALSDLFAKLRVDPVY	
	EEMKASDELDKKGATLTLALGGILKKKGHHEAEIKPLAOSHATDCHKIPVK	
	NFKLLSCLLVITAAHLPAEETPAVHASLDRPILASVSTVLTQKTR	
	YLEFISEETIQVLSKHPDSECADAGGAMNLELFRKDMGSHKELGFQG	

25%

Myoglobin	GLSEGEWQLLNIWGGKVLDPFGHEDELIIKIKGHELELEKEDKPKHLKKS
Leghemoglobin	GALHESGALLKLSLHWVFQMLLPHRRFLLVLEIADARK---ELLSLFTSSEI
	KASE-DEIKNGATLTALGGI---LKKKSH---HEEIRPQASATDCHKIPVKYLE
	PONNPELQALAGLQKLVYEAATQEVETVVVTTGTLIDKSSVLSGG-VADAHFF
	FISEETIQVLSKHPDSECADAGGAMNLELFRKDMGSHKELGFQG
	VVKALDKTIKEV----VGAKWSEELISQVITATDELAVIKKIDDA

23%

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Structures of Hb and Mb

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

Hemoglobin α VLSPADKTNVKAAWGKVGAHAGEYAEALDELIELSFRHTKTYDPPE----

Leghemoglobin is found in legumes. What metabolic feat are legumes known for? %

Myoglobin GLESGEWDVLIWIKKVEADITPRGGEVILRLFKGHPEYLEKFKPKHLEEDP
 Leghemoglobin GALLTESGALILKLSLHWVFVADLPLKTHRRFTLLVLEIADARK---ELSGLESTSEF
 KASE-DLNNKGAITLALGGI---LKKKQH---HEHEIRPDAQSATHKIPVKYLE
 PONNPELQALAGLQKLVYEAATIQEVTEVVVTDTLIDLSSVLSYG-VADAHFF
 FISEKTIQVLSQKHPGDFGADAQGAMNRLLEFRKDMNSNYRLELGFQGG
 VVKNALIKTKIKEV----VGAKWSEELSSQHTIATDELIVLQKNDAA

23%

Chem 452, Lecture 3 - Hb and Mb 14

Structures of Hb and Mb

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

Hemoglobin α VLSPADKTNVKAAWGKVGAHAGEYAEALDELIELSFRHTKTYDPPE----

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 Leghemoglobin GALLTESGALILKLSLHWVFVADLPLKTHRRFTLLVLEIADARK---ELSGLESTSEF
 FLSHGDAQVLSLKKKTHALITNAVAHVDDMPNLSALSDLHAKLRVDPV
 EEMKASDELQKAGATLALGGILKKKGHHEAIEKPLAQSHATHKIPVK
 NFKLLRCLLVITAAHLPAEITPAVHASLDRKIASVSTVLTQNR
 YLEFISEKTIQVLSQKHPGDFGADAQGAMNRLLEFRKDMNSNYRLELGFQGG

25%

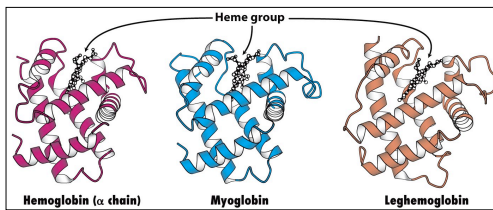
Myoglobin GLESGEWDVLIWIKKVEADITPRGGEVILRLFKGHPEYLEKFKPKHLEEDP
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 FISEKTIQVLSQKHPGDFGADAQGAMNRLLEFRKDMNSNYRLELGFQGG
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23%

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Structures of Hb and Mb

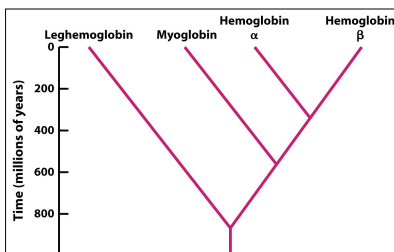
- † These three proteins also have very similar 3-dimensional structures.
- † The tertiary structure appear to be more highly conserved than the primary structure.



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Structures of Hb and Mb

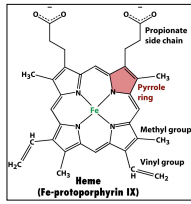
- † The amino acid sequences can be used to create an evolutionary tree.



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The O₂ binding site for Hb & Mb

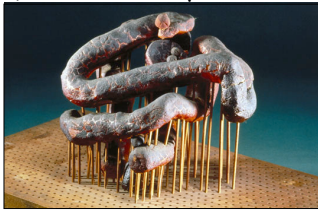
- † Both Hb and Mb contain a heme group.
- † The heme group is an example of a protein **cofactor**.



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The O₂ binding site for Hb & Mb

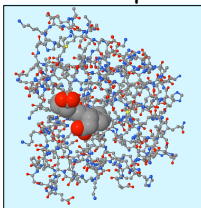
- † Kendrew's X-ray crystal structure for Mb showed the heme group inserted into a pocket produced in the tertiary fold of the protein.



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The O₂ binding site for Hb & Mb

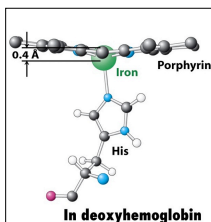
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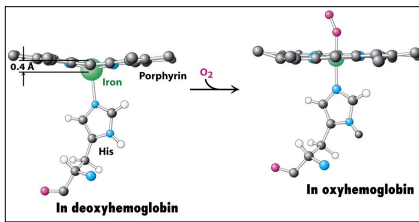
- † The heme Fe²⁺ ligated by the heme nitrogens and the nitrogen on the **proximal histidine**.



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The O₂ binding site for Hb & Mb

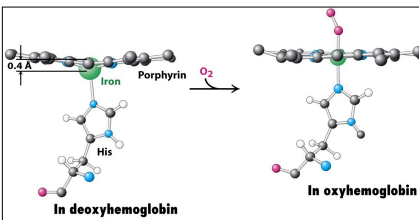
- When bound, O₂ provides the sixth ligand for the heme Fe²⁺



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The O₂ binding site for Hb & Mb

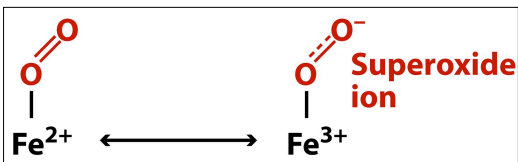
- When O₂ binds, the heme Fe²⁺ gets smaller and moves into the plane of the heme.



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The O₂ binding site for Hb & Mb

- The heme Fe²⁺ reduces the bound O₂ to a superoxide ion, O₂⁻.



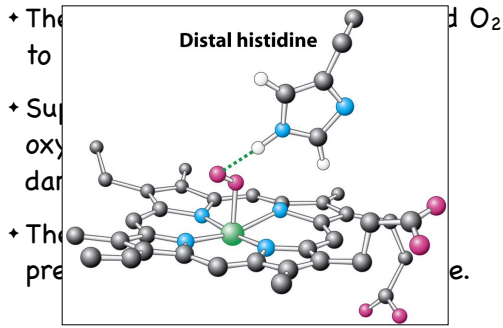
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The O₂ binding site for Hb & Mb

- The heme Fe²⁺ reduces the bound O₂ to a superoxide ion, O₂⁻.
- Superoxide, like other reactive oxygen species (ROSs), is very damaging.
- It is the **distal histidine** that helps to prevent the release of the superoxide.

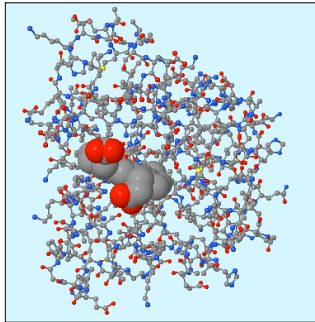
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The O₂ binding site for Hb & Mb



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The O₂ binding site for Hb & Mb



Oxymyoglobin

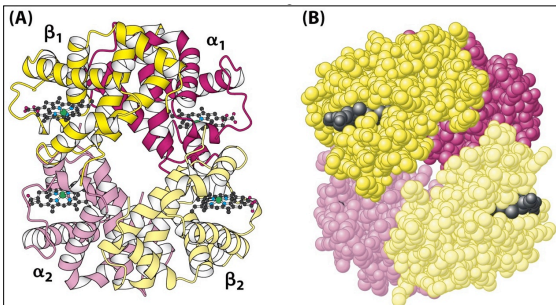
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Hb is a Tetramer

- + Hb's quaternary structure causes it to bind O₂ differently than Mb
- + Hb is a tetramer of myoglobin-like subunits
 - + Two α subunits
 - + Two β subunits
- + Combine as two $\alpha\beta$ dimers
 - + $\alpha_1\beta_1$ and $\alpha_2\beta_2$

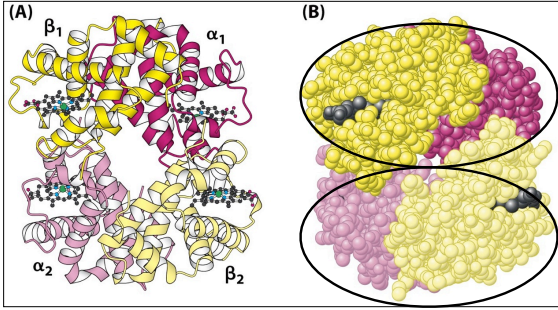
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Hb is a Tetramer



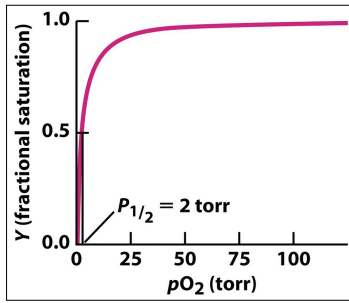
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Hb is a Tetramer



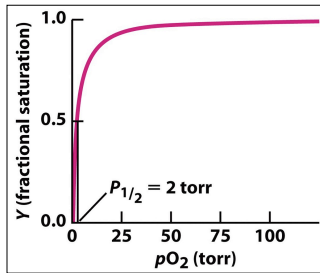
Hb Binds O_2 Cooperatively

+ Mb has a P_{50} of 2 Torr



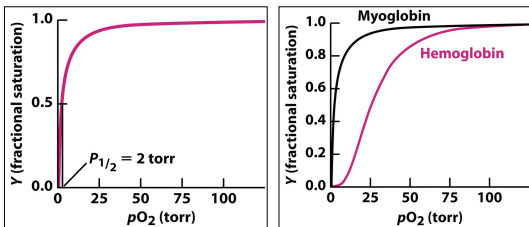
Questions

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



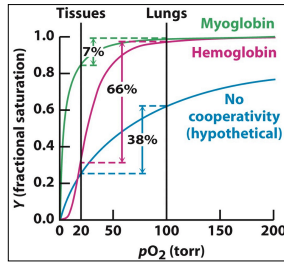
Hb Binds O_2 Cooperatively

+ Hb binds O_2 more weakly than Mb



Hb Binds O₂ Cooperatively

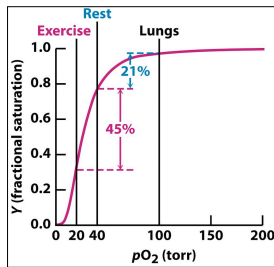
+ Cooperative binding makes Hb a more efficient transporter of O₂ than Mb.



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Hb Binds O₂ Cooperatively

+ Hb efficiently delivers O₂ to the tissues during stress or exercise.



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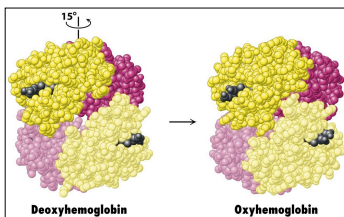
Problem 7.12 & 7.14

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

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Hb Binds O₂ Cooperatively

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



Tense (T) State Relaxed (R) State

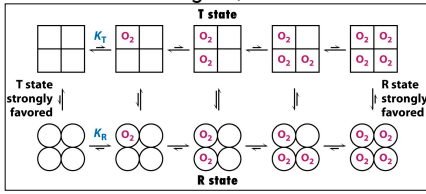
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Hb Binds O₂ Cooperatively

Models to explain the cooperativity:

MWC Model

(Jacques Monod, Jeffries Wyman & Jean-Pierre Changeux)



Concerted Model

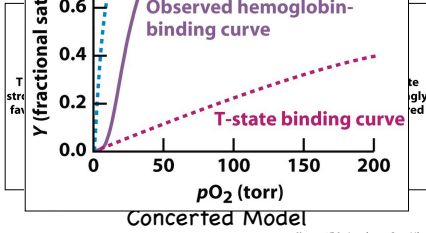
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Hb Binds O₂ Cooperatively

Models to explain the cooperativity:

MWC Model

(Jacques Monod, Jeffries Wyman & Jean-Pierre Changeux)



Concerted Model

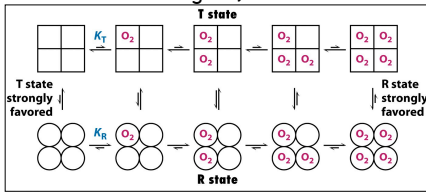
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Hb Binds O₂ Cooperatively

Models to explain the cooperativity:

MWC Model

(Jacques Monod, Jeffries Wyman & Jean-Pierre Changeux)



Concerted Model

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Hb Binds O₂ Cooperatively

Models to explain the cooperativity:

Sequential Model



Sequential Model

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Problem 7.12a & 7.14

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 primitive Hb from a lamprey eel is given

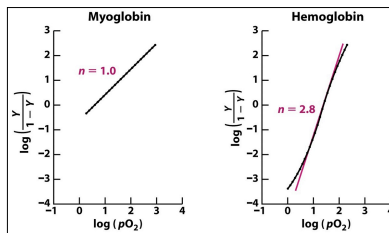
- Plot data and determine P_{50}
- Make Hill plot and determine n
- Propose model to explain cooperativity

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Hb Binds O_2 Cooperatively

† Cooperativity can be assessed with a **Hill plot**.

† n is the **Hill coefficient**

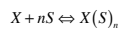


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Hb Binds O_2 Cooperatively

† Cooperativity can be assessed with a **Hill plot**.

† n is the **Hill coefficient**



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

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

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

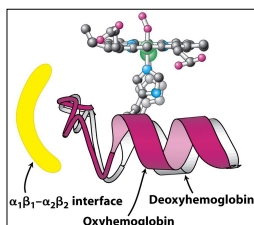
$$\log\left(\frac{Y}{1-Y}\right) = n \log(pO_2) - n \log(P_{50})$$

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Hb Binds O_2 Cooperatively

† At the molecular level.

† Conformational changes occurring upon O_2 bonding to one subunit are transmitted to other subunits

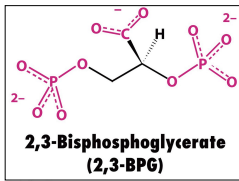


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Allosteric Regulation

• Hb provides an example of **allosteric regulation**.

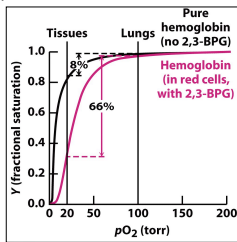
• In red blood cells (RBC's), the metabolite 2,3-Bisphosphoglycerate (2,3-BPG) alters the O_2 binding behavior of Hb.



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Allosteric Regulation

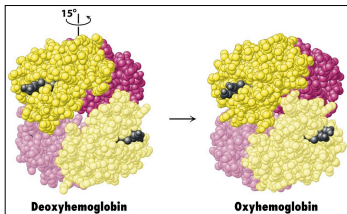
• 2,3-BPG lowers Hb's affinity for O_2 , allowing it to release O_2 more efficiently to the tissues.



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Hb Binds O_2 Cooperatively

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

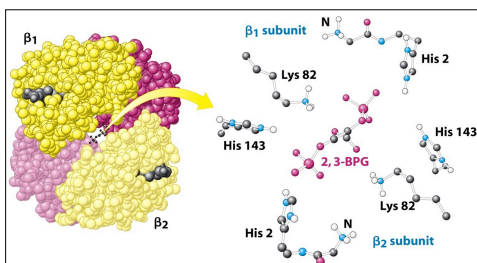


Tense (T) State Relaxed (R) State

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Allosteric Regulation

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



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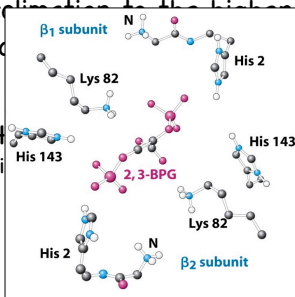
Allosteric Regulation

- † 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)

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Allosteric Regulation

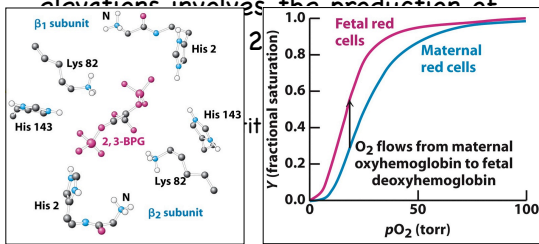
- † The acclimation to the higher elevations involves the production of higher levels of 2-BPG.
- † Fetal Hb
 - † γ chains are substituted for β chains (H143S)



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Allosteric Regulation

- † The acclimation to the higher elevations involves the production of higher levels of 2-BPG.



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To Summarize

- † **Question of Day:** How do the differences in structure between the oxygen transport proteins myoglobin (Mb) and hemoglobin (Hb) make each more best suited for their biological roles?

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Next up

+ Hemoglobin and Myoglobin (cont'd).

- + Bohr effect
- + Sickle-cell Hb

+ Enzymes (Chapter 8)