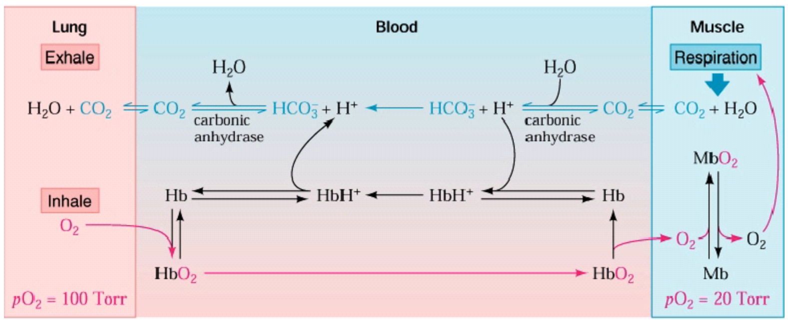
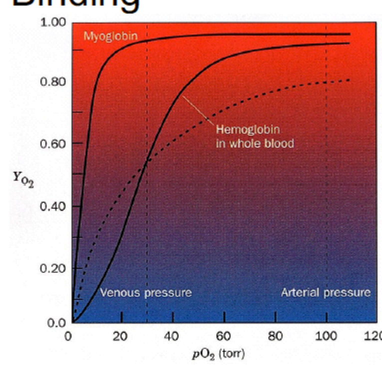
What Does Hemoglobin Do?



Oxygen Binding

	p_{50}	Y_{O_2} Arteries	Y_{O_2} Veins
Mb	2.8	0.97	0.91
Hb	26	0.95	0.55



Oxygen binding

- Hb curve is sigmoidal - cooperative
- releases significant O_2 under small changes in oxygen pressure

Related to function

- Hb is for transport from lungs to tissue
- Mb in tissue accepts O_2 from Hb, for storage (especially in aquatic mammals) and transport within the tissue

Mb and Hb

$$Mb + O_2 \rightleftharpoons MbO_2 \qquad K = \frac{[Mb][O_2]}{[MbO_2]}$$

Fractional saturation of O_2 $$Y_{O_2} = \frac{[MbO_2]}{[Mb] + [MbO_2]} = \frac{[O_2]}{K + [O_2]}$$

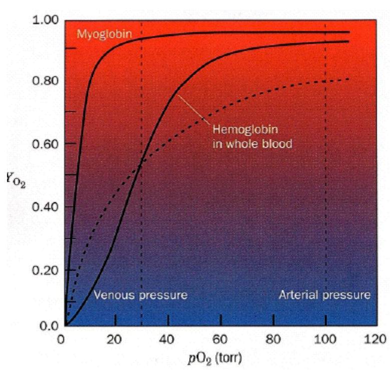
Use pO_2 instead of $[O_2]$ because it is a gas
pGas = pressure of gas if it occupied total volume by itself

$p_{50} = pO_2$ when $Y_{O_2} = 0.5$, then $K = p_{50}$ $$Y_{O_2} = \frac{pO_2}{p_{50} + pO_2}$$

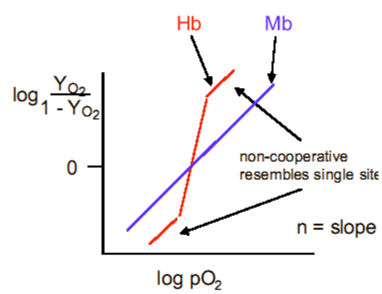
In air of 1 atm is 760 torr, pO_2 = 155 torr

Similarly, for Hb with n multiple sites: $$Y_{O_2} = \frac{pO_2^{\,n}}{p_{50}^{\,n} + pO_2^{\,n}}$$

Oxygen-Binding by Hb and Mb



Hill Plot



at $pO_2 = p_{50}$, $Y_{O_2} = 0.5$ and $\log \frac{Y_{O_2}}{1 - Y_{O_2}} = 0$

Cooperative Binding Sites

•Assume: full cooperativity - n cooperative binding sites either all filled or all empty

$$P + nL \rightleftharpoons PL_n \qquad K_d = \frac{[P][L]^n}{[PL_n]}$$

$$r = \frac{\text{ligand bound}}{\text{total protein}} = \frac{n[PL_n]}{[P] + [PL_n]} = \frac{n[L]^n}{K_d + [L]^n}$$

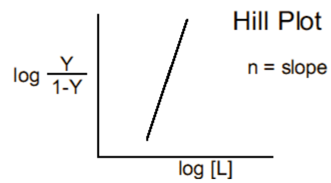
note: Book uses (moles ligand bound) / (moles sites) = r/n = Y = $\frac{[L]^n}{K_d + [L]^n}$

$$\frac{Y}{1-Y} = \frac{[L]^n}{K_d}$$

n indicates cooperativity, "Hill coefficient"
- n = 1 no cooperativity
- n < 1 negative cooperativity
- n > 1 positive cooperativity

•Full cooperativity is ideal, normally n < number of binding sites

$$\log\frac{Y}{1-Y} = n \log [L] - \log K_d$$



Hb Conformational Changes

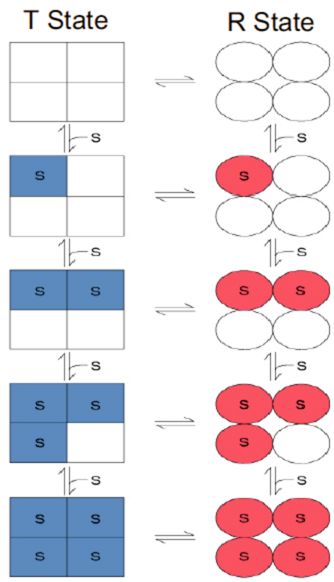
T state - stabilized by salt bridges
R state - stabilized by oxygen binding

equilibrium T ⇌ R

deoxy → T; oxy → R

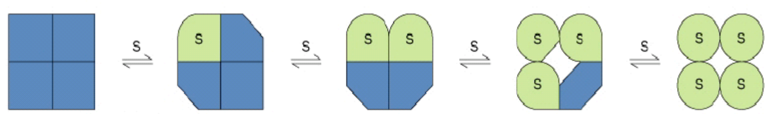
What happens when O_2 binds?

Domino effect of oxygen binding:
1. O_2 binds to heme iron
2. Fe is pulled into plane of heme
3. Proximal His (F8) is pulled closer to heme and reorients
4. Helix F shifts along with proximal His
5. Loss of salt bridges that stabilize T state
6. Contacts between $\alpha_1\beta_2$ click into new orientation
   - changes in H-bonding (switch region)
7. Causes similar changes at $\alpha_2\beta_1$ interface
8. Can't have partial changes, whole molecule snaps into R
9. Deoxy-hemes in R state have much higher affinity for O_2 - pre-organized for O_2

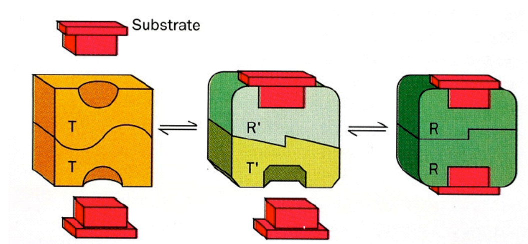


Symmetry Model

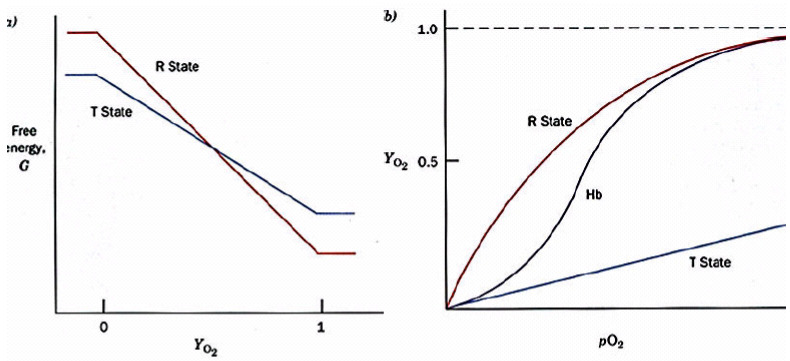
Sequential model



Induced Conformational Change



R to T State



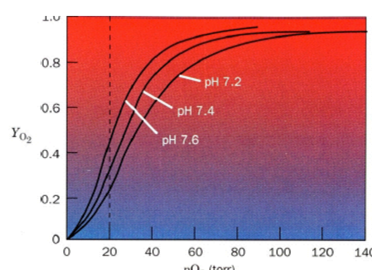
•As more oxygen binds, the R state is stabilized more than the T state

•Binding curve is a combination of the two individual states

The Bohr Effect

$HbH_x + O_2 \rightleftharpoons HbO_2 + xH^+$

x = 0.6



•Bohr Effect - OxyHb is slightly more acidic (decrease in pKa's)
- due to loss of salt bridges in R conformation
- higher pH promotes O_2 binding

•CO_2 is formed by respiration in tissues, diffuses to capillaries

In capillaries: $CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$ (carbonic anhydrase (CA))

CO_2: would form bubbles

H^+: bound by Hb, promotes T state release of O_2, stimulates CA

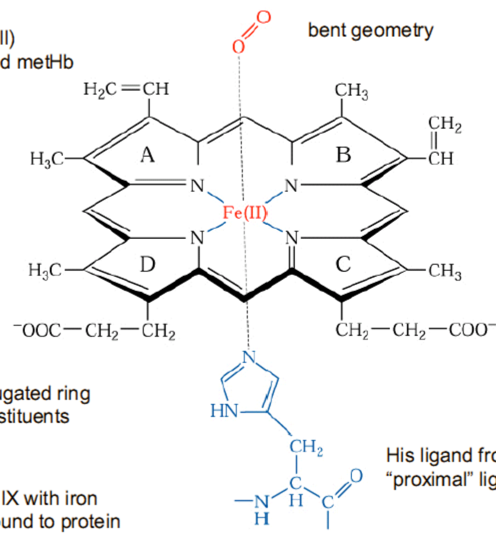
In lungs: O_2 is high, Hb releases H^+ and CO_2 is formed

In highly active muscle: acid is produced, even more is O_2 released

Heme

Heme in Mb and Hb
- usually Fe(II)
- Fe(III) called metHb

bent geometry



4 pyrrole conjugated ring
specific substituents

↓

Protoporphyrin IX with iron
Non-covalently bound to protein

His ligand from protein
"proximal" ligand

Structures of Mb vs Hb

First crystal structures of proteins

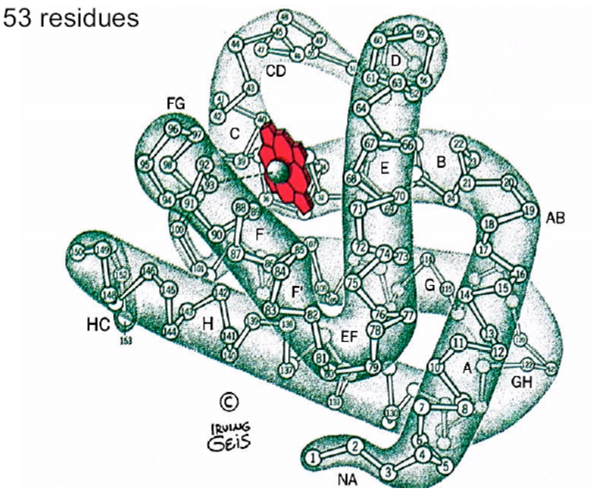
1959 Mb - John Kendrew
1968 Hb - Max Perutz

Mb
- muscle protein
- monomer, globular, ellipsoidal molecule 44 x 44 x 25 Å
- 8 helices, A-H
- heme is in a hydrophobic pocket
- 5th ligand (proximal) of heme-Fe is His F8
- in deoxy-Mb, no 6th ligand, Fe is 0.55Å out of plane towards proximal his
- protein prevents dimerization and auto-oxidation
- heme can also bind CO, NO, H_2S

Hb
- globular, 64 x 55 x 50 Å
- $\alpha_2\beta_2$ dimer of ab protomers, 2-fold axis of symmetry
- α and β subunits have very similar 3° structure to Mb and to each other
- 4 corners of Td, hole in the middle
- so interactions are $\alpha_1\beta_1$, $\alpha_2\beta_2$, $\alpha_1\beta_2$, $\alpha_2\beta_1$
- largely hydrophobic, a few H-bonds and ion pairs

Sperm Whale Myoglobin

153 residues



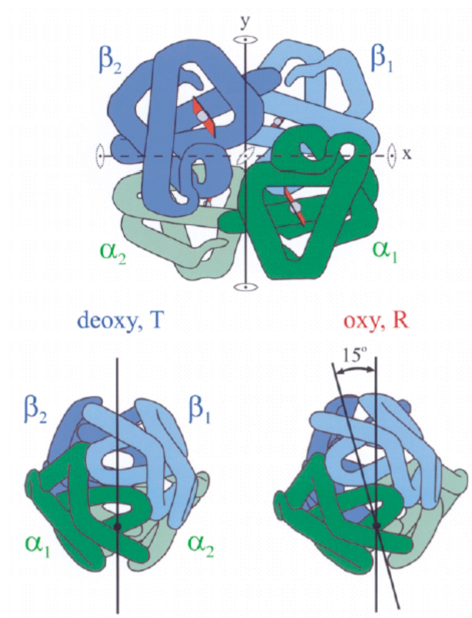
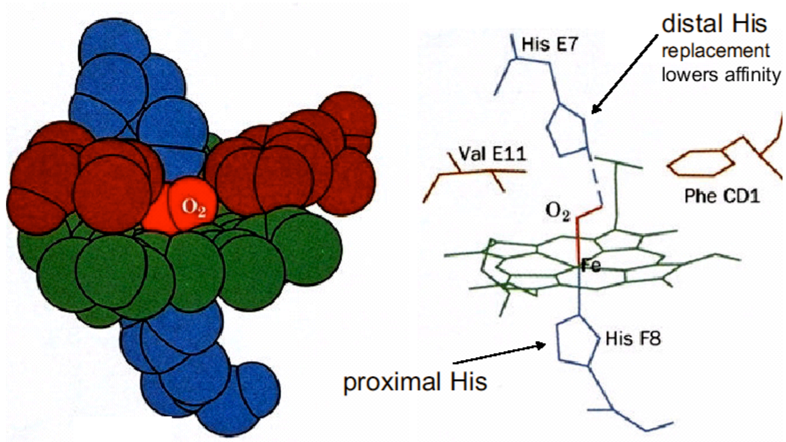
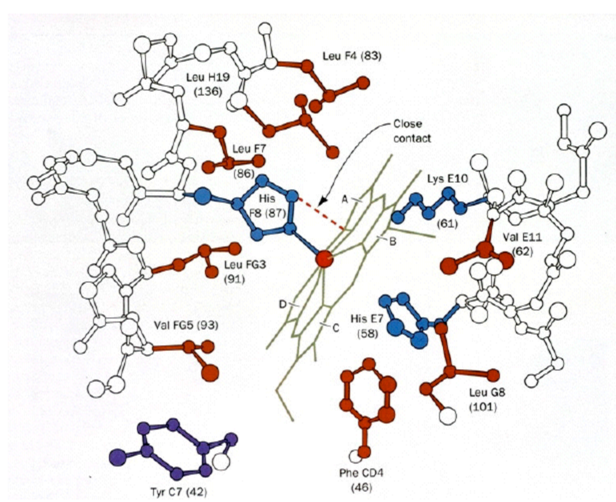


Fig. 1. Schematic structure of hemoglobin. Adapted from Dickerson and Geis [48].

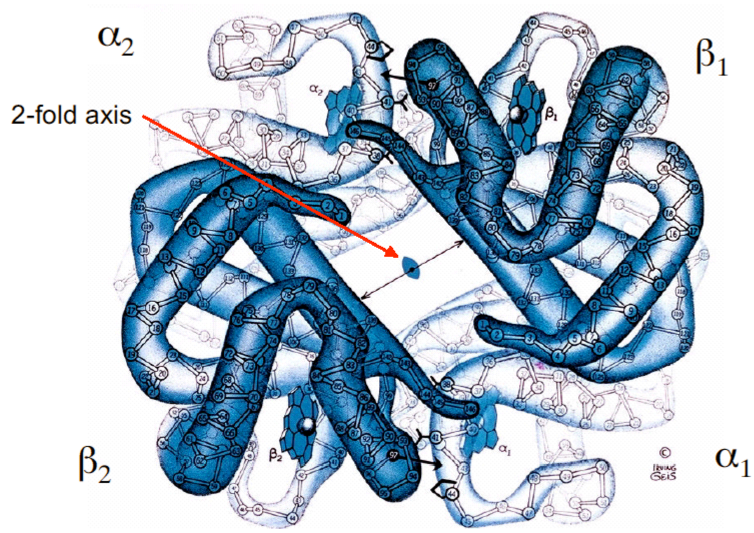
Heme Complex



Deoxy-Heme From Hb



Deoxy-Hb



Hb Conformational Changes

equilibrium T $\rightleftharpoons$ R

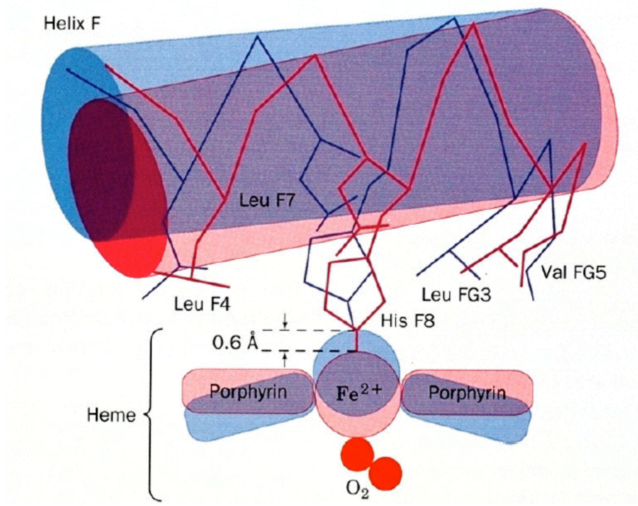
deoxy → T; oxy → R

T state - stabilized by salt bridges
R state - stabilized by oxygen binding

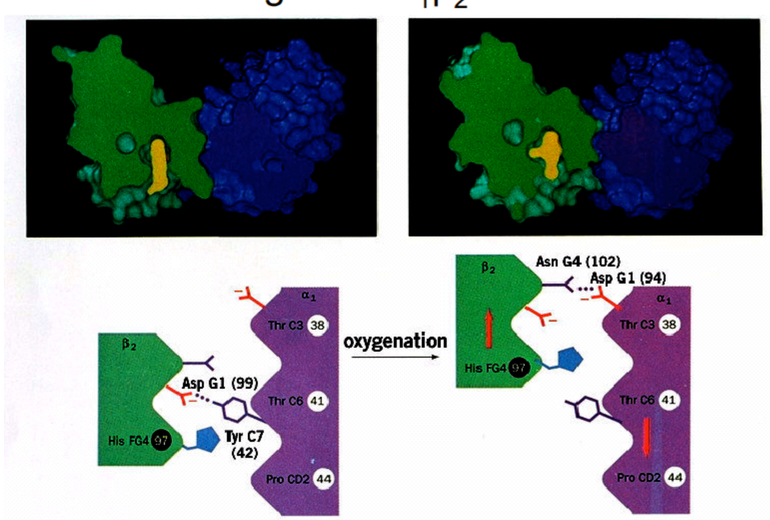
What happens when O_2 binds?

Domino effect of oxygen binding:
1. O_2 binds to heme iron
2. Fe is pulled into plane of heme
3. Proximal His (F8) is pulled closer to heme and reorients
4. Helix F shifts along with proximal His
5. Loss of salt bridges that stabilize T state
6. Contacts between $\alpha_1\beta_2$ click into new orientation
   - changes in H-bonding (switch region)
7. Causes similar changes at $\alpha_2\beta_1$ interface
8. Can't have partial changes, whole molecule snaps into R
9. Deoxy-hemes in R state have much higher affinity for O_2 - pre-organized for O_2

Effects of Oxygen Binding



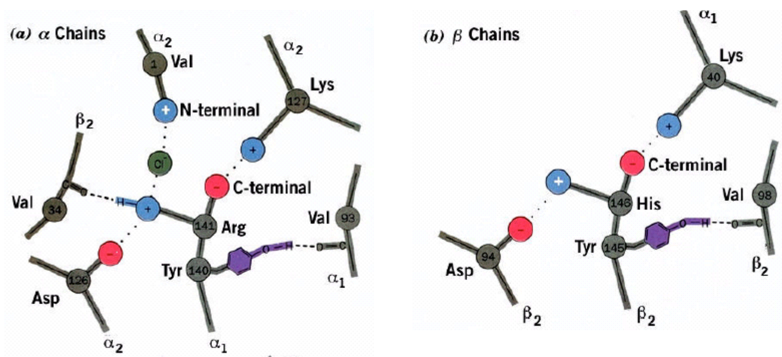
Changes at $\alpha_1\beta_2$ Interface



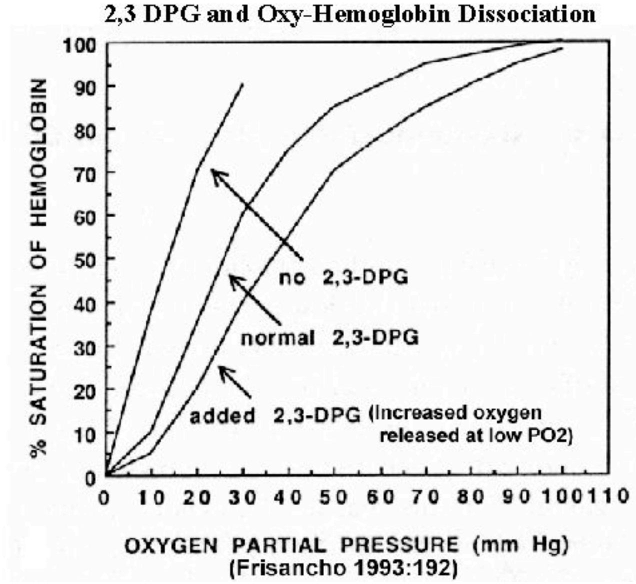
(a) **T Form (deoxy)** *(b)* **R Form (deoxy)**

T-State is Stabilized by Salt Bridges

- Also involved in Bohr Effect
- Salt bridges affect the pKa's of the functional groups



2,3 DPG and Oxy-Hemoglobin Dissociation



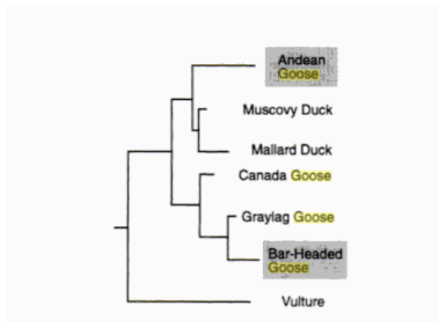
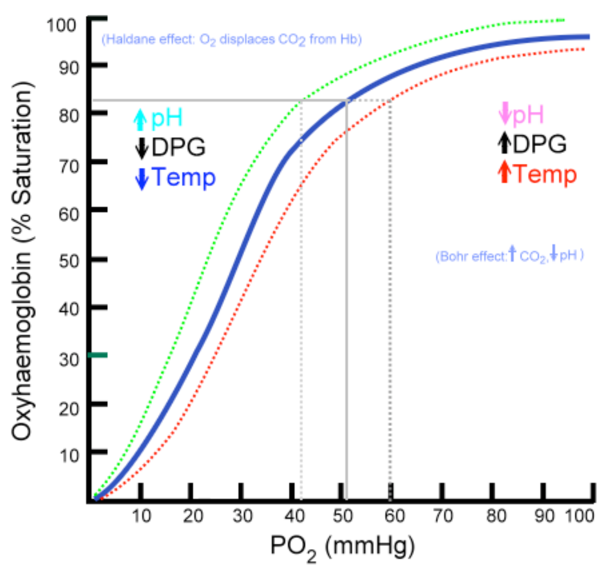


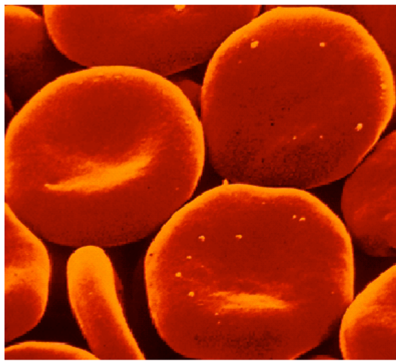
Figure 3.7. A simplified phylogenetic tree showing the distant relationship between the bar-headed goose and the Andean goose, both of which have independently evolved high-affinity Hbs. A muta-

Biochemical adaptation: mechanism and process in physiological evolution
Peter W. Hochachka, George N. Somero

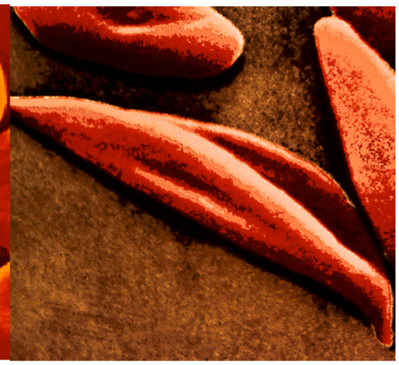


Electron Micrographs of Erythrocytes

Normal cells



Sickle cells



BLAST	Protein	Structure	PubMed	Taxonomy
Genome	Nucleotide	3D-Domains	Books	Help

Query: gi|122615 Hemoglobin beta chain
Matching gi: 29437, 22094827, 4504349, 7428621, 13937929, 29441, 183830, 442846, 442848, 455997, 532506, 532507, 532508, 532509, 532510, 532511, 532512, 532513, 532514, 532515, 532516, 532517, 532518, 532519, 532520, 532521, 532522, 1066768, 1066771, 1066774, 1066777, 1066780, 2144721, 2253432

Best hits | Common Tree | Taxonomy Report | 3D structures | CDD-Search | GI list

200 BLAST hits to 118 unique species Sort by taxonomy proximity

0 Archaea 0 Bacteria 198 Metazoa 0 Fungi 0 Plants 0 Viruses 0 Other Eukaryotae

Keep only Cut-Off 100 Select Reset

147 aa

SCORE	P	ACCESSION	GI	PROTEIN DESCRIPTION
777	25	P02024	232230	Hemoglobin beta chain
776	25	AAN84548	26892090	beta globin chain variant [Ho
775	27	0907233B	223683	hemoglobin beta
774	25	AAD19696	4378804	hemoglobin beta chain [Homo s
773	25	AAA88054	1066765	beta-globin
772	25	1DXUB	442850	Chain B, Hemoglobin (Deoxy) M
771	25	1HDBB	1431650	Chain B, Human Hemoglobin, De
771	1	1K1KB	27065154	Chain B, Structure Of Mutant
771	25	1DXVB	442854	Chain B, Hemoglobin (Deoxy) M
769	25	1O1OB	27574248	Chain B, Deoxy Hemoglobin (A,
768	25	AAL68978	18418633	mutant beta-globin [Homo sapi
768	25	AAN11320	23268449	hemoglobin beta chain variant

```
Query:                            2    VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGD
                                       VHLTP EKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGD
Sbjct:                            1    VHLTPVEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGD
helix                             58
helix                             21                      **************
helix                             5        *************

Query:                            62   KAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLG
                                       KAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLG
Sbjct:                            61   KAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLG
helix                             101                                         *******
(HEM,   1 ) Protoporphyrin Ix G>   92                               *
helix                             85                         ********
helix                             61   ************

Query:                            122 EFTPPVQAAYQKVVAGVANALAHKYH 147
                                      EFTPPVQAAYQKVVAGVANALAHKYH
Sbjct:                            121 EFTPPVQAAYQKVVAGVANALAHKYH 146
helix                             124    *******************
```

Frequency in the population

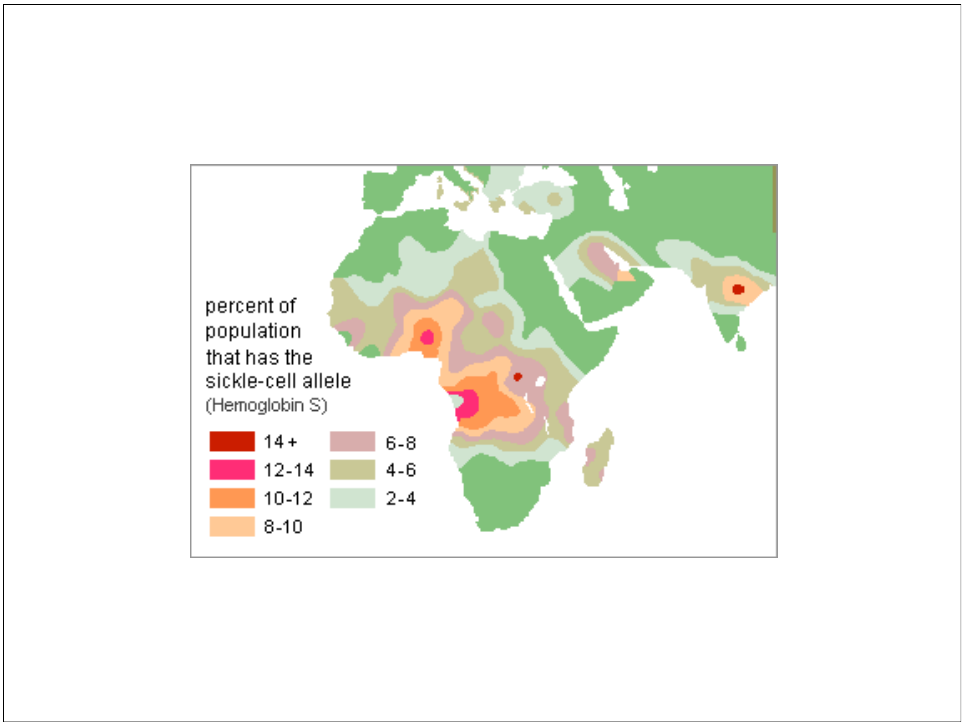
HbS hetero ~ 25% of Africans

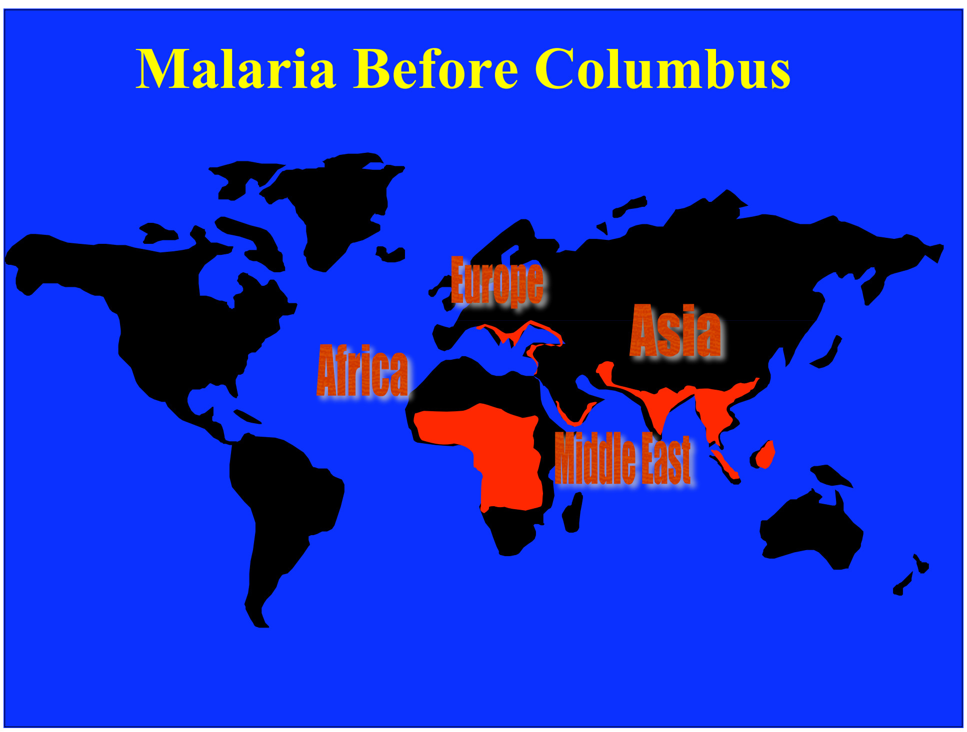
$$\Delta p = \frac{pq[p(w_{AA} - w_{Aa}) + q(w_{Aa} - w_{aa})]}{\overline{w}}$$

For $w_{AA} > w_{Aa} > w_{aa}$ stable solutions p=1 and p=0

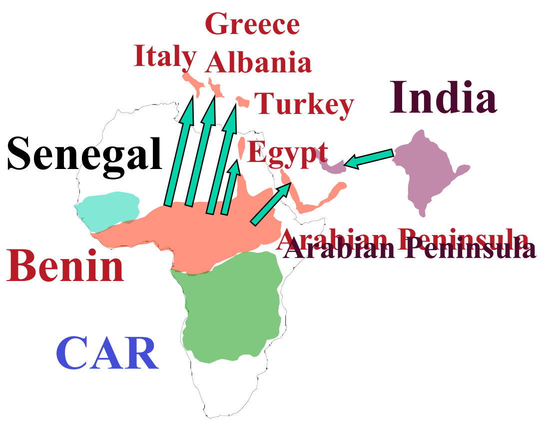
Other stable solutions arise if $w_{Aa} > w_{aa}$ $w_{Aa} > w_{AA}$

Heterozygous superiority.

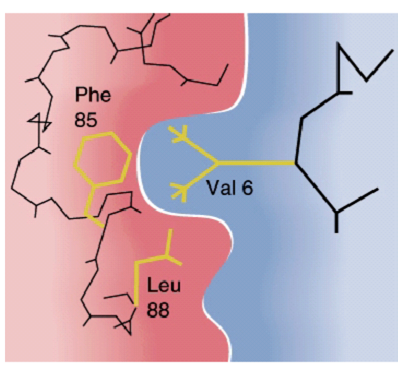

percent of
population
that has the
sickle-cell allele
(Hemoglobin S)
14+
12-14
10-12
8-10
6-8
4-6
2-4

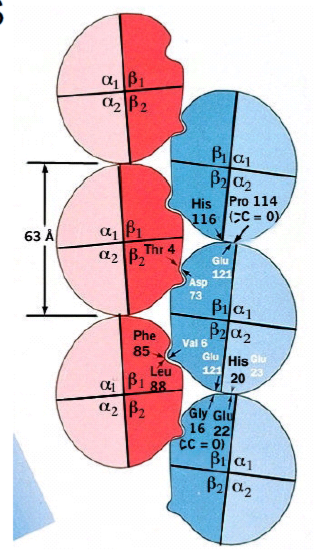

Malaria Before Columbus
Europe
Asia
Africa
Middle East

Origins of the Sickle Cell Gene



HbS





Kinetics of aggregation

$$1/t_d = k\left(\frac{c_t}{c_s}\right)^n$$

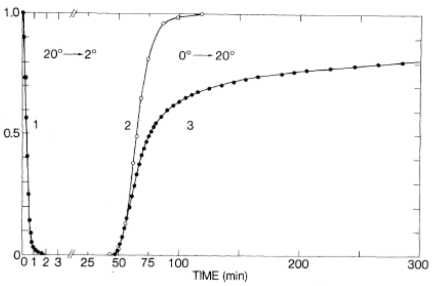


FIG. 1. Fractional extent of gelation versus time. Curve 1 results from rapidly changing the temperature of a 23.3 g % deoxyhemoglobin S gel from 20°C to 2°C in an optical experiment. Curves 2 and 3 were obtained by rapidly changing the temperature of the same sample from 0°C, where it is a nonbirefringent liquid, to 20°C in calorimetric and optical experiments, respectively. The total birefringence is taken as the birefringence at infinite time, estimated by extrapolation of the last part of the curve which approaches a limiting value exponentially.

Sickle Cell