Chemistry 5.07SC Biological Chemistry I
Fall Semester, 2013

Lectures 4. Protein structure and function continued.

Quaternary Structure using Hb as an example and Mb for comparison. Many proteins are composed of more than one polypeptide chain. You have already seen this with the collagen. Quaternary structure can provide a basis for regulation at a distance: **allosteric regulation** and **cooperative behavior**. We will see when studying metabolic pathways, that many of the enzymes that control the flux through the pathway (rate-limiting step(s)) have quaternary structures and exhibit cooperative behavior by binding small molecule metabolites. However, there are many other proteins composed of multiple polypeptide chains where the chains act independently.

I. Big Picture: Mb and Hb have different physiological roles. Mb is a monomer and is found in tissues. It functions as a carrier of O_2 to the mitochondrial in a cell. O_2 has limited solubility (0.1 mM) and thus a carrier is required for rates of distribution to be sufficient for metabolism. O_2 is reduced to H_20 in the respiratory chain in the mitochondria and the energy released is used to make the energy currency of the cell, ATP.

Hb is a tetramer composed of two types of subunits designated α and β. These subunits are structurally homologous to each other and to Mb (Figure 2). Hb is found in erythrocytes (red blood cells (RBCs)) and also functions as an O_2 carrier. It acquires O_2 from air through the lungs and delivers it through the circulatory system to the tissues where it is picked up by Mb. The surface to volume ratio of vertebrate cells, in contrast with bacteria, often requires transporters for small metabolites and Mb, for O_2, is an example. The RBCs also retrieve CO_2 from the tissues, the end product of oxidative metabolism, and deliver it to the lungs where it is exhaled (Figure 1). The RBCs deliver CO_2 as part of the H^+/HCO_3^- equilibrium (think about Problem Set 1) and via Hb itself which is able to form carbamates ($-NHCO_2^-$) via reaction of the lysines on its surface with CO_2. These carbamates are acid labile and decompose to CO_2 and

non-modified Hb. The O_2 binding properties of Mb and Hb are distinct and dependent on quaternary structure. The distinctive bind properties (hyperbolic versus sigmoidal) are essential for their physiological function (see Figure 4).

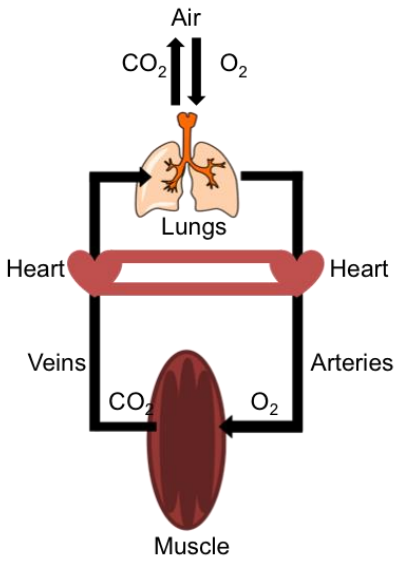

Figure 1. Cartoon of O_2 transport in vertebrates. Hb transports O_2 to the tissues (muscle). Metabolism in muscle, such as glucose oxidation, results in CO_2 production and NADH formation. NADH oxidation is coupled to reduction of O_2 in the mitochondrial respiratory chain. The energy released from H_2O formation, generates a proton gradient that can be used for ATP production. Mb transports O_2 to the mitochondria for this purpose. The resulting CO_2, the end product in metabolism, is returned to the lungs via the blood where it is exhaled.

Structures: Mb and Hb (Figure 2). Note in the case of Hb that there are multiple subunit interactions: α2β2, α1β1 and β2α1 and α2β1. The movement between subunits occurs predominantly through the second set of subunit interfaces.

For additional information, please see the Molecule of the Month article on Hemoglobin from the RCSB PDB.

A. B.

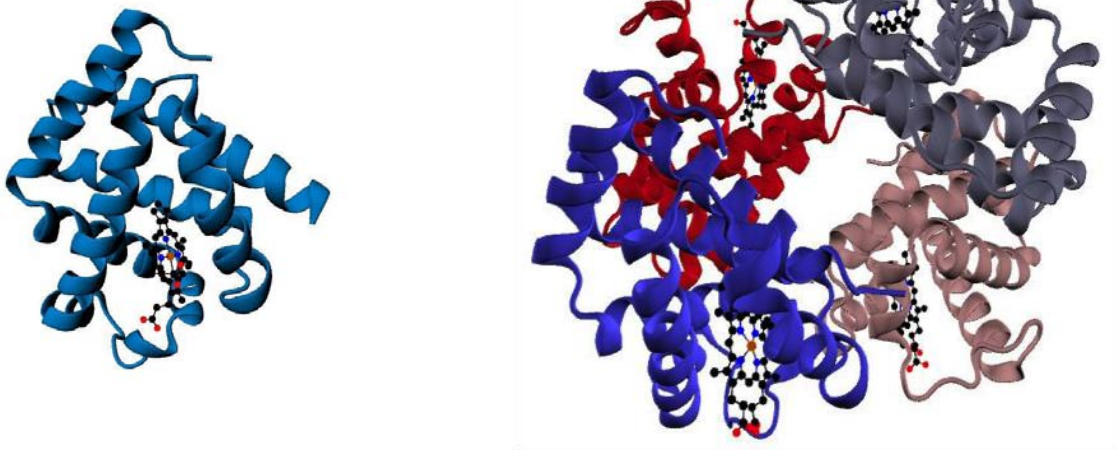

Figure by O'Reilly Science Art for MIT OpenCourseWare.

A. PDB: 3RGK
Hubbard, Stevan R., Wayne A. Hendrickson, David G. Lambright, and Steven G. Boxer. "X-ray crystal structure of a recombinant human myoglobin mutant at 2· 8 Å resolution." *Journal of molecular biology* 213, no. 2 (1990): 215-218.
B. PDB: 2HHB.
Fermi, G., M. F. Perutz, B. Shaanan, and R. Fourme. "The crystal structure of human deoxyhaemoglobin at 1.74 Å resolution." *Journal of molecular biology* 175, no. 2 (1984): 159-174.

Figure 2. Crystal structures of myoglobin and hemoglobin. Heme molecule bound is shown in grey space-filling balls. A. Structure of the monomeric myoglobin. B. Structure of tetrameric hemoglobin, showing similarity with myoglobin of the β1 subunit. Red and pink monomers are alpha. Blue and grey monomers are beta.

II. Myoglobin:

A. See the quaternary structures above. Both Mb and Hb reversibly bind to O_2 via Fe^{2+} in protoporphyrin IX (heme, see Figure 3), a key cofactor (see Lexicon). An octahedral complex is formed in which the nitrogens of the pyrrole rings are equatorial ligands and the O_2 and a His (in the F helix) are the axial ligands. The protein structure prevents oxidation of Fe^{2+} to Fe^{3+} most of the time. If oxidation does occur, there is a protein called Cytb5 (a heme protein) that reduces Fe^{3+} to Fe^{2+}.

A. B.

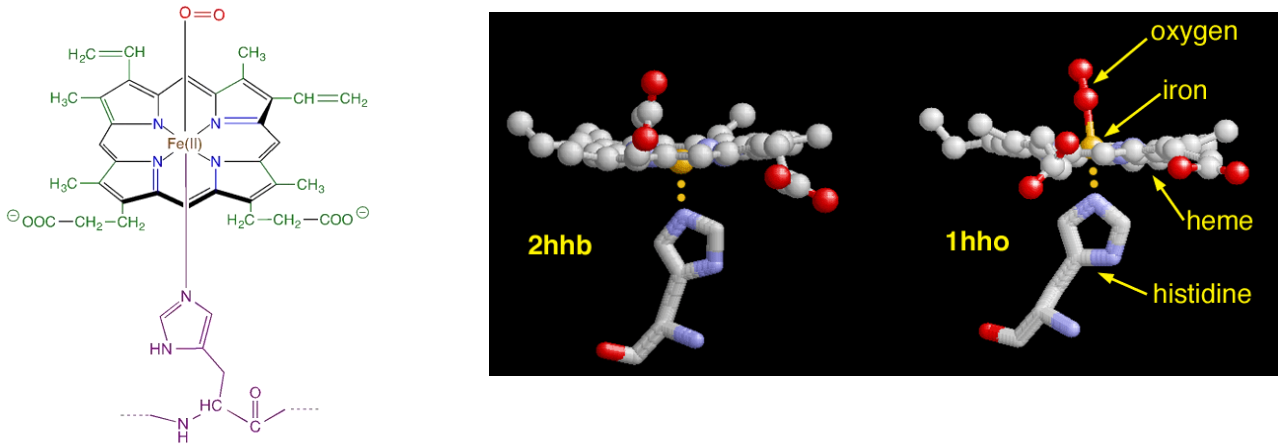

Figure 3B from S. Dutta and D. Goodsell. (May 2003) The RCSB PDB "Molecule of the Month": Hemoglobin. doi:http://dx.doi.org/10.2210/rcsb_pdb/mom_2003_5.

Figure 3. Protoporphyrin IX. A. Protoporphyrin IX with a coordinated Fe^{2+} atom. B. Deoxy Hb (left) and oxy Hb (right) forms, with movement of the iron, porphyrin ring and His upon O_2 binding.

B. O_2 binding to Mb can be measured experimentally and the results of a typical experiment are shown in the graph below. The rectangular hyperbola describes O_2 binding to Mb. The behavior of Mb (red line) is distinct from that described for Hb (blue and green lines).

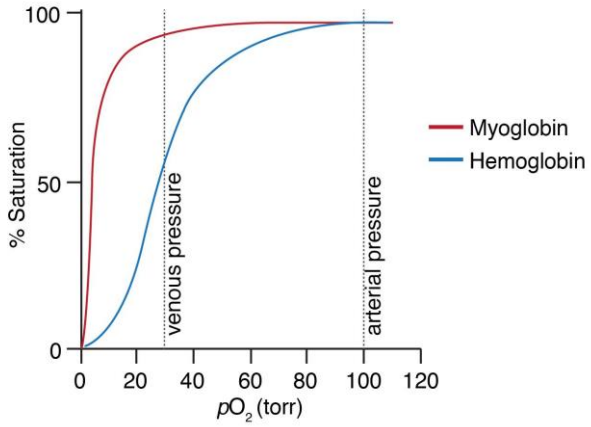

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Figure 4. O_2 dissociation curves for Mb and Hb in whole blood and their differences. Mb's O_2 dissociation curve is **hyperbolic**: Its p_{50} (the value of pO_2 when $Y_{O2} = 0.5$) is 2.8 torr where 1 torr = 1 mm Hg at 0ºC; 760 torr = 1 atm). Hb exhibits a **sigmoidal** binding curve and the amount of O_2 bound at 100 torr (arterial pressure) is substantially different from that at venous pressure (20-30 torr) where the O_2 needs to be unloaded.

C. Mb binding to O_2 is described by a standard binding analysis:

$$Mb + O_2 \leftrightarrows Mb{\bullet}O_2 \quad \text{and the}$$

$$K_d = [Mb][O_2]/[Mb{\bullet}O_2] \text{ and } Y_{O2} = Mb{\bullet}O_2/ [Mb{\bullet}O_2 + Mb]$$

The binding is described as the fraction of Mb saturated by O_2 that is,

$$(Y_{O2}) = [O_2]/ (K_d + [O_2]).$$

Since O_2 is a gas, its concentration is expressed as partial pressure of O_2 (p_{O2}). p_{50} is the partial pressure of O_2 when 50% is bound.

III. Hemoglobin

Hemoglobin has four subunits and the difference in its ability to bind O_2 relative to Mb is based on the communications between the subunits.

Historical Digression

Max Perutz (1914 – 2002) began his work on Hb in 1937, and Hb was the first protein crystallized (1949). It was nearly fifteen years later when the x-ray structure was solved (1962) and it required Perutz to invent the method of isomorphous replacement to solve the phase problem. Hb was a first in many categories, and its rich history is fascinating. Vernon Ingram (MIT Biology) established for the first time that a "single" mutation of an amino acid (E to V) is responsible for a genetic disease – sickle cell anaemia. It was Linus Pauling (again!) who proposed this theory first. Hb was the first system, and still today is the best studied system, in which cooperativity of ligand binding was established.

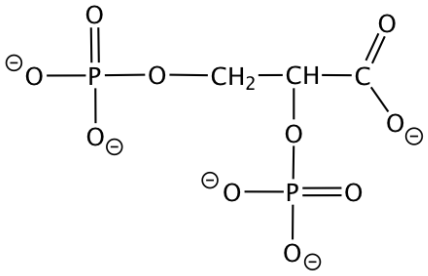
End historical digression

Binding of one ligand effects the binding of additional ligands. Based on the observation of cooperativity of binding, a number of theories (Monod/Wyman/Changeux; Koshland – covered more below) and a phenomenological description were put forth to explain the experimental observations. These theories describe a major mechanism of regulatory control for key (rate-limiting) enzymes in metabolic pathways.

We will focus on two issues (expanded on below):

A. the basis for the sigmoidal O_2 binding curve of Hb (Figure 4).

B. the mechanism of allosteric regulation by H^+ (the Bohr effect) or 2, 3-bisphosphoglyceric acid (BPG – see structure below).



BPG at pH 7 – note the **5** negative charges!

A. Cooperativity in O_2 binding to Hb is displayed in the graph above. One can observe that in the lungs where the pO_2 is 100 torr, that Hb is >90% saturated with O_2. In the tissue however, where pO_2 is 20 to 30 torr, the Hb binds O_2 at 50% saturation. Under the same conditions, the graph also shows that O_2 is tightly bound to Mb. Thus Mb is able to bind the O_2 released from the Hb.

New vocabulary used in the description of cooperative binding of ligands.

Allostery = binding of a ligand to a specific site, affects binding of the same ligand or a different ligand to an additional site.

Homotropic = both ligands are the same. In the case of Hb, the ligand is O_2.

Heterotropic = the ligands are not the same. O_2 binds and the allosteric effectors can be H^+ (Bohr effect), Cl^-, CO_2, bis-phosphoglyceric acid, BPG.

There are a number of models that describe cooperative behavior: In the case of hemoglobin, at issue is how the binding of O_2 of one subunit can increase the binding of O_2 to additional subunits. The distances between the hemes in the subunits are 35 and 27Å!

Monod/Changeux/Wyman model (1) and **Koshland/Nemethy/Filmore model (2) to explain cooperativity.** While these models are mathematically distinct, both intuitively describe the cooperative behavior and in most cases, it is experimentally difficult to distinguish between them.

1. In the Monod model described in Figure 5A, T is the tight, or taut, state (by convention) that weakly binds O_2, while R is the relaxed state that tightly binds O_2.

A. B.

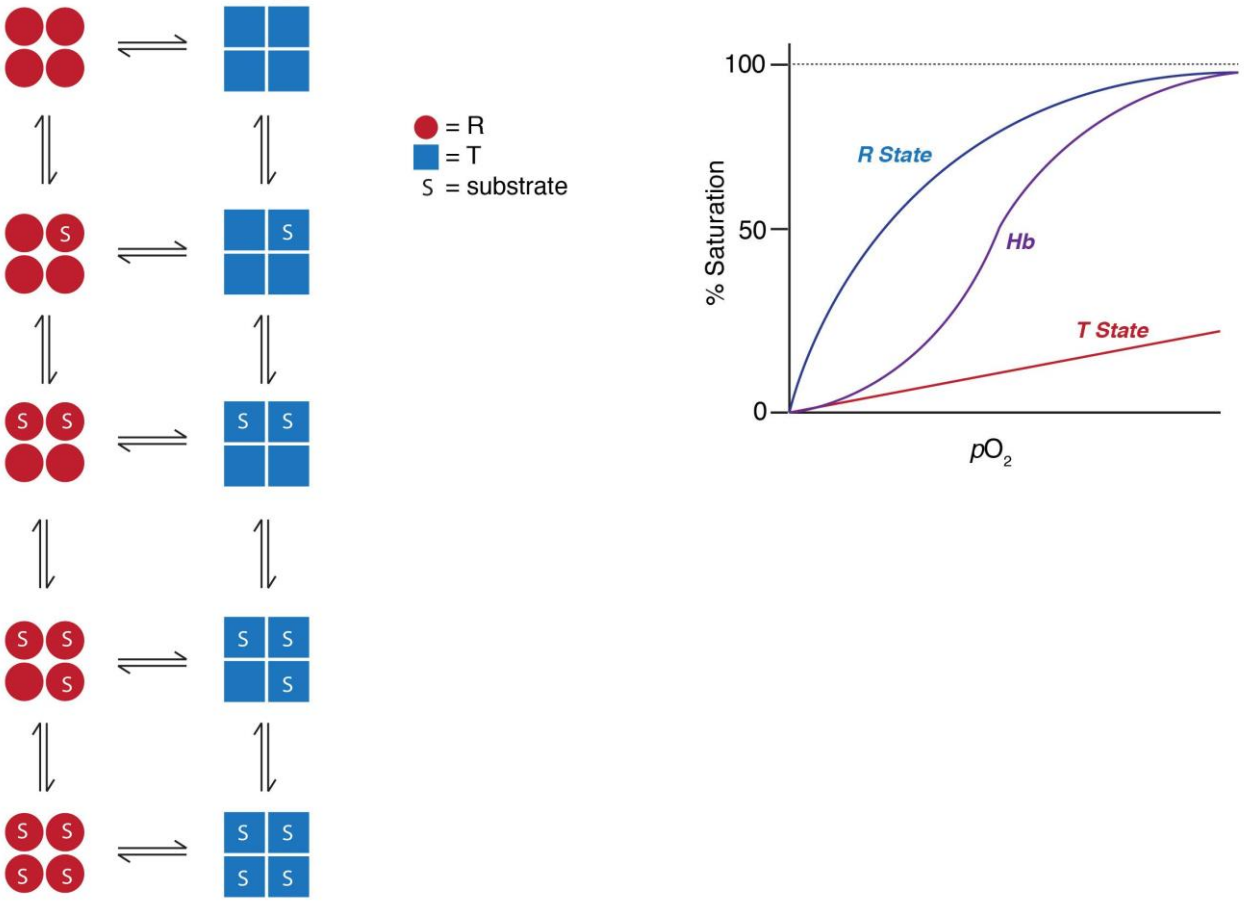


Figure by O'Reilly Science Art for MIT OpenCourseWare.

Figure 5. Monod/Changeux/Wyman model.

Assumptions of the Monod model:

a. The protein must exist in at least two conformations that are in equilibrium: the R and T states. T is the state where substrate has low affinity for Hb, while R has high affinity.

b. The states must have multiple equivalent binding sites, quaternary structure. There are 4 in the case of Hb.

c. The protein is either in the all R or all T states. There are NO mixed states.

2. Koshland/Nemethy/Filmore KNF sequential model (Figure 6).

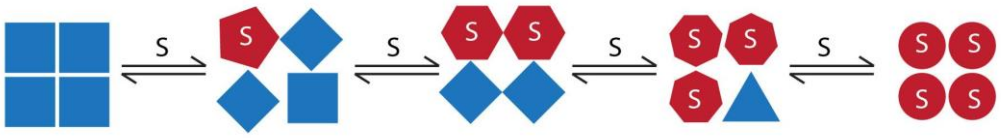


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Figure 6. Koshland/Nemethy/Filmore model.

Assumptions of the Koshland model (2):

a. The binding affinity to one site influences the binding affinity to a neighboring site.

b. This model is able to account for negative cooperativity (binding at one site, decreases the affinity at the second site) that is seen in a number of enzymatic reactions.

Neither model in its pure form accounts for the experimentally observed behavior of Hb. A combined model is required.

We have 100s of structures from different species of Hb in the oxygenated and deoxygenated states. The molecular basis for interconversion of the R and T states is understood based on structures and decades of study. The conformational change is triggered by binding of O_2 to one heme of Hb (Figure 7). Blue is deoxyHb and red is oxyHb. In the deoxyHb, Fe^{2+} is too large to fit into the opening in the porphyrin ring. Upon binding of O_2 to Fe^{2+}, the size is reduced and Fe^{2+} fits into the hole, dragging down the His (Figure 3B) axial ligand (F8) and the F helix along with it as it comes into the plane of the porphyrin ring.

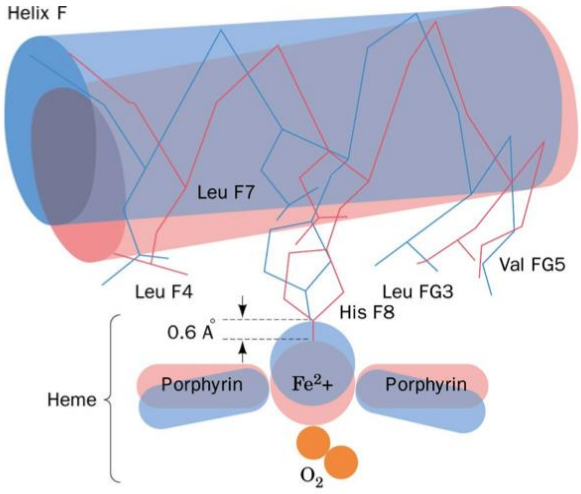

Figure 7. Conformational changes occur in both the F helix of hemoglobin and the heme upon oxygen binding. When oxygen is bound, the protein transitions from the deoxygenated structure (blue) to the oxygenated structure (pink) as the F helix moves down, pulled by its heme-coordinating His F8.

B. Regulation by H^+ or 2, 3-bisphosphoglyceric acid (BPG). We will focus briefly on the Bohr effect. The diagram below shows the effect of pH on O_2 binding to Hb. The experimental observation is that at pH 7.2 and 20 torr, more O_2 is unloaded than at pH 7.6 (Figure 8). Can we provide an explanation for this observation based on structures?

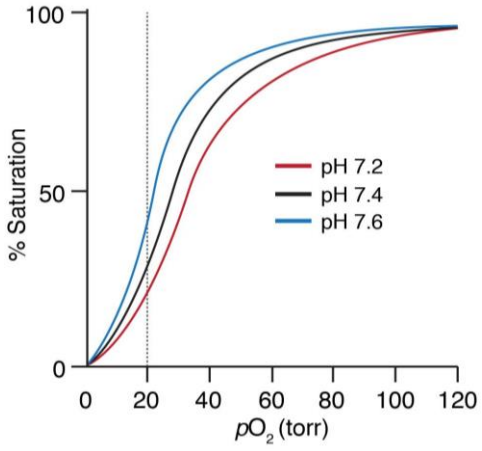

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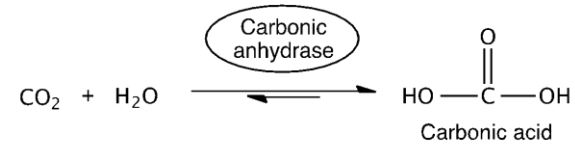
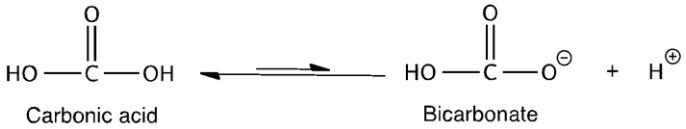
Figure 8. Binding of oxygen to the heme of hemoglobin is pH dependent.

To think about the basis of this pH effect you need to recall PS 1 and the CO_2/HCO_3^- equilibria. You also need to know that deoxyHb is a stronger base than O_2•Hb. Finally, in RBCs, there is an enzyme carbonic anhydrase, which catalyzes the hydration of CO_2 by water.





and $O_2Hb + H^+ \leftrightarrows [H^+]DeoxyHb + O_2$

Using these equations you can think about how to maximize unloading of O_2 in the tissues and unloading of CO_2 in the lungs.

First example: In muscle during exercise, glucose is converted to CO_2. The CO_2 then diffuses to the capillaries and RBCs and equilibrates to H^+ and HCO_3^-. Some of the side chains of Hb act as a buffer. The H^+s bind to deoxyHb and thus shift the equilibrium (above graph, right) to the right, releasing more O_2.

Second example: The deoxyHb is transported through the circulatory system back to the lungs where it picks up O_2. O_2 shifts the equilibrium to the left generating H^+. The protons then react with HCO_3^- to generate CO_2 and H_2O. The CO_2 is exhaled. In addition, the H^+ (transient reduction in pH) can react with the carbamylated-Hb to also release CO_2.

Medical Digression - Mutant Hemoglobins

Many mutations in Hb are associated with disease – often anaemia – a lower number of red blood cells and not enough Hb. The most common disease is sickle cell anaemia. In the US, there are 70 000 people affected by sickle cell anaemia, though about 2 million are heterologous for the sickle cell trait. Its occurrence is roughly 1 in 36 000 Hispanic Americans and 1 in 500 African Americans. The disease is recessive, requiring two copies of the sickle cell gene

containing the single mutation and is characterized by the C shaped erythrocytes that tend to get stuck in blood vessels, form clots causing damage to potentially many organs (bone pain, necrosis, skin ulcers), and are very fragile compared to wild type (normal) erythrocytes. The lifetime of sickle cell erythrocytes is about a tenth the length of the wild type cells (10-20 days instead of ~120 days). It is this sickle cell trait that resistance to malaria parasites persists. For more information on sickle cell anaemia, see the webpage on Sickle Cell Diseases at the National Heart, Lung, and Blood Institute from the National Institute of Health.

End medical digression

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